

The relationship between serum total sialic acid levels and adenosine deaminase activity in obesity

Naciye Kurtul, PhD, Ersin Akarsu, MD, Sebnem Aktaran, MD.

ABSTRACT

Objective: To evaluate the relationship between serum adenosine deaminase (AD) activity and serum total sialic acid (TSA) levels in obese individuals.

Methods: We performed this study at the Department of Chemistry, Division Biochemistry, Kahramanmaraş Sutcu Imam University Arts and Science Faculty, Turkey from 2003 to 2004. Fifty obese subjects and 25 non-obese healthy controls were included in the study. The serum AD activity and TSA concentrations were measured by spectrophotometric methods.

Results: The AD activity ($p < 0.01$) and TSA concentrations ($p < 0.001$) were significantly higher in the sera of obese subjects than those of non-obese control subjects. But, there

was no statistically significant difference in the serum TSA levels and AD activity of the obese subjects with metabolic syndrome properties compared with those without metabolic syndrome properties. A significant correlation between the serum TSA and AD was found in the obese subjects ($p < 0.05$, $r: 0.33$).

Conclusion: Our findings suggest that there may be a closer interaction between the inflammatory events and obesity. However, our observations need to be confirmed by further studies to understand more regarding the underlying mechanisms.

Saudi Med J 2006; Vol. 27 (2): 170-173

The prevalence of obesity is increasing at a fast rate and obesity has become one of the major health problems in developed countries affecting over a hundred million people worldwide. Obesity is an important cardiovascular risk factor, and is frequently associated with disease, such as insulin resistance, hypertension, type 2 diabetes, and dyslipidemia.¹⁻⁵ The etiology of obesity represents a complex interaction of genetics, diet, metabolism, and physical activity level.² It has been suggested that obesity may be a low-grade systemic inflammatory disease.^{1,2} Also, it has been reported that overweight and obese children

and adults have elevated serum levels of C-reactive protein (CRP), interleukin-6, tumor necrosis factor- α , and leptin, which are known markers of inflammation and closely associated with cardiovascular risk factors and cardiovascular and non-cardiovascular causes of death.² Serum total sialic acid (TSA) has been reported as a cardiovascular risk factor, with elevated concentrations associated with increased cardiovascular mortality and cerebrovascular disease.⁶⁻⁸ It has been reported that serum TSA is related to markers of obesity and also a marker of inflammation.^{6,9} Adenosine deaminase (AD) is a purine metabolic

From the Department of Chemistry, Division of Biochemistry (Kurtul), Faculty of Science, Kahramanmaraş Sutcu Imam University, Kahramanmaraş and the Department of Endocrinology and Metabolism (Akarsu, Aktaran), Medical School, Gaziantep University, Gaziantep, Turkey.

Received 3rd July 2005. Accepted for publication in final form 12th December 2005.

Address correspondence and reprint request to: Dr. Naciye Kurtul, Department of Chemistry, Division of Biochemistry, Faculty of Science, Kahramanmaraş Sutcu Imam University, Kahramanmaraş, Turkey. Tel. +90 (344) 2191283. Fax. +90 (344) 2191242. E-mail: naciyekurtul@hotmail.com

enzymes that specifically catalyzes the deamination of adenosine to inosine to the regulation of intracellular and extracellular concentrations of adenosine, and probably modulates energy metabolism.¹⁰⁻¹² The physiological function of AD is crucial in regulating the steady state concentrations of adenosine in a variety of systems, especially immunology, neurological and cardiovascular systems.¹² Adenosine is an anti-inflammatory agent.¹³ Also, adenosine directly acts to stimulate insulin activity via several processes such as glucose transport, lipid synthesis, pyruvate dehydrogenase activity, leucine oxidation and cyclic nucleotide phosphodiesterase activity. Therefore, adenosine and AD play an important role for modulating the bioactivity of insulin.^{11,14,15} On the other hand, insulin resistance emerges as an important variable linking obesity to disease risks and outcomes.⁵ Thus, it may be useful to evaluate serum AD activity and TSA levels in obesity. The present study was undertaken to investigate the alteration of serum AD activity and also serum TSA concentrations in the obese individuals.

Methods. This study was performed at the Department of Chemistry, Division of Biochemistry, Kahramanmaraş Sutcu Imam University Arts and Science Faculty, Turkey from 2003 to 2004. Obese subjects were selected from the Department of Endocrinology and Metabolism, Gaziantep University Medical Faculty. A total of 50 obese subjects (22 males and 28 females; average age: 42.2 ± 10.8 years) were included in the study. All obese and control subjects were volunteer for the study. An informed consent was obtained from all subjects and an ethical approval was also obtained. Obesity was defined using the body mass index (BMI), calculated as weight/height^2 (kg/m^2). A participant who has a BMI of $\geq 30 \text{ kg/m}^2$ was considered to be obese. A part of the obese subjects ($n=22$) who have metabolic syndrome properties including abdominal obesity (a waist circumference ≥ 102 cm for men and ≥ 88 cm for women), hypertension ($\geq 130/\geq 85$ mm Hg), and hypertriglyceridemia (>150 mg/dL) and lower high-density lipoprotein (HDL) (<35 mg/dl) or both.¹⁶ The control group consisted of 25 age matched, non-obese, healthy subjects (14 males and 11 females; average age: 39 ± 9.5 years). Exclusion criteria were diabetes and impaired oral glucose tolerants (checked by administrated 75 g glucose before each blood sample taken), hypertension, cancer, autoimmune disease, and coronary heart disease and the use of vitamin or mineral supplements or medications such as corticosteroids and colchicines. The systolic and diastolic blood pressure of subjects was measured

by sphygmomanometer. Blood samples (5 mL) were drawn after an overnight fasting (12-14 hour) in all subjects and were stored at -40°C until assayed. Serum TSA was measured with the Denny's colorimetric method.¹⁷ Serum AD activities were estimated spectrophotometrically by the method of Giusti,¹⁸ which is based on the direct measurements of the formation of ammonia produced when AD acts in excess of adenosine. Results were expressed as units per liter of serum (U/L). One enzyme unit was the amount of enzyme necessary to convert $1 \mu\text{M}$ of adenosine to inosine and ammonia per min at 37°C . All chemicals in this study were of analytical grade and purchased from Sigma (Stockholm) and Merck Chemicals Co. (Germany). All solutions were prepared in deionized and distilled water. In addition, total cholesterol, HDL-cholesterol and triglyceride were measured in all blood samples. Routine biochemical analyses were made by an autoanalyzer (Roche-Modular System) using commercial kits. Data were analyzed by using SPSS® for Windows computing program. For simple comparisons between 2 values, the unpaired Student's t test was used. The p -values of <0.05 were regarded as statistically significant. Bivariate comparisons were examined using Pearson rank correlation coefficients (r). Results were expressed as means \pm standard deviation ($X \pm SD$).

Results. Results were given in **Table 1**. The AD activity ($p<0.01$) and also TSA concentrations ($p<0.001$) were significantly higher in the sera of obese subjects than those of nonobese control subjects. But, there was no statistically significant difference in the

Table 1 - Serum adenosine deaminase activity (ADA) and serum total sialic acid (TSA) concentrations of subjects.

Group	TSA ($\mu\text{g/mL}$)	ADA (U/L)
Control (n=25)	466.20 ± 119.65	14.58 ± 3.90
Female (n=11)	426.58 ± 74.22	13.31 ± 4.62
Male (n=14)	487.54 ± 136.03	15.27 ± 3.45
Obese (n=50)	$692.25 \pm 259.90^{**}$	$19.24 \pm 5.46^\dagger$
Female (n=28)	$706.61 \pm 283.91^{§§}$	$19.06 \pm 5.98^\ddagger$
Male (n=22)	$637.70 \pm 131.16^\dagger$	$19.88 \pm 3.15^{\dagger\dagger}$
Values represent mean \pm SD, * $p<0.01$ versus controls, ** $p<0.001$ versus controls, $^\ddagger p<0.05$ versus control females, $^\dagger p<0.001$ versus control female, $^\ddagger p<0.05$ versus control males, $^{\dagger\dagger} p<0.01$ versus control males		

serum TSA levels and also in the serum AD activity of the obese subjects with metabolic syndrome properties compared with those without metabolic syndrome properties. When considering the gender of the obese subjects, both male and female had a higher serum TSA level and AD activity than the male and female control subjects. A significant correlation between the serum TSA and AD was found in the obese subjects ($p < 0.05$, $r = 0.33$).

Discussion. This study demonstrates that obesity is associated with increased serum AD activities and increased serum TSA levels. This study is the first assessing inflammatory related parameters-serum AD and serum TSA that were investigated together in obesity. Among the individuals with obesity of unknown etiology, there is a large group of people whose obesity is connected with inflammation. Inflammation is responsible for tissue injury in pathological conditions ranging from myocardial infarction to rheumatoid arthritis.¹³ Many obese people have elevated levels of CRP which are a known sensitive marker for systemic inflammation.^{1,19,20} It is interesting to note that a positive association between BMI and CRP has been observed in healthy adults.^{2,21-23} Adenosine has been suggested to be critical regulator of inflammation and increased adenosine release could be utilized to diminish inflammation.¹³ The AD catalyzes the deamination of adenosine to inosine contributing to the regulation of intracellular and extracellular concentrations of adenosine, and probably modulates energy metabolism. Systemic administration of an AD inhibitor produce clear anti-inflammatory effects.^{24,25} This action may result from the local tissue elevations of adenosine with activation of higher affinity peripheral adenosine A2a receptors on inflammatory cells. Activation of adenosine receptors (A1, A2a, A2b, A3) on a number of vascular and immune cells can modify multiple aspects of the inflammatory process.²⁴ We have observed that serum AD activities were higher in obese subjects than those in the nonobese healthy subjects. The finding suggests that there may be an interaction between obesity and inflammation. In addition, increased AD activity may be related to inflammatory process in obesity. In the literature, there were limited studies encountered on serum AD activity in obesity. It has been reported that AD activity, probably through modulation of adenosine concentration, may have a significant effect on the BMI.²⁶ Another inflammation marker is TSA.^{7,9,27} In addition, elevated concentrations of serum TSA were suggested as a potent cardiovascular risk factor in the general population. Moreover, raised serum TSA concentrations have been shown

to predict cardiovascular and cerebrovascular mortality.^{7,27} Large epidemiologic studies have shown that obesity is a risk factor for cardiovascular disease (CVD).²⁸ The reason for the association of TSA with CVD is unclear. But, a plausible explanation is that serum TSA has been shown to be a good marker acute phase response.^{6,27} Recently, there have been reports suggesting that an acute phase response mediated by cytokines may be involved in obesity, insulin resistance and metabolic syndrome X.^{6,29} But, we did not measure the acute phase reactants, in this study. However, elevated serum TSA levels might reflect the CVD risk in obese subjects. Furthermore, it has been shown that serum TSA is related to markers of obesity and adipose tissue metabolism which may help to explain why it is a reputed cardiovascular risk factor and also it has been shown that serum TSA is positively correlated with individual BMI.⁶

Our study shows that serum TSA levels and serum AD activities were increased in obese individuals. In addition, there was a significant correlation between the serum TSA and AD in the obese subjects. But, there was no statistically significant difference in the serum TSA levels and also in the serum AD activity of the obese subjects with metabolic syndrome properties compared with those without metabolic syndrome properties. Therefore, the results of the present study suggest that increased serum AD activity and TSA concentration are directly related to obesity but not metabolic syndrome properties.

In conclusion, our findings suggest that there may be a closer interaction between the inflammatory events and obesity. However, our observations need to be confirmed by further studies to understand more regarding the underlying mechanisms.

References

1. Wlodek D, Gonzales M. Decreased energy levels can cause and sustain obesity. *J Theor Biol* 2003; 225: 33-44.
2. Das UN. Is obesity an inflammatory condition? *Nutrition* 2001; 17: 953-966.
3. Traupe T, D'Uscio LV, Muentner K, Morawietz H, Vetter W, Barton M. Effects of obesity on endothelium-dependent reactivity during acute nitric oxide synthase inhibition: modulatory role of endothelin. *Clin Sci Lond* 2002; 103 Suppl 48: 13S-15S.
4. Hall JE, Louis K. Dahl Memorial Lecture. Renal and cardiovascular mechanisms of hypertension in obesity. *Hypertension* 1994; 23: 381-394.
5. Egan BM, Greene EL, Goodfriend TL. Insulin resistance and cardiovascular disease. *Am J Hypertens* 2001; 14 (6 Pt 2): 116S-125S.
6. Crook MA, Miell J, Ameerally P, Lumb P, Singh N, Russell-Jones D, et. al. Serum sialic acid, a reputed cardiovascular risk factor, is related to serum leptin concentrations in Fijians. *Clin Chim Acta* 2003; 331: 1-5.

7. Lindberg G, Eklund GA, Gullberg B. Serum sialic acid concentration and cardiovascular mortality. *BMJ* 1991; 302: 143-146.
8. Lindberg G, Rastam L, Gullberg B, Eklund GA. Serum sialic acid concentration predicts both coronary heart disease and stroke mortality: multivariate analysis including 54,385 men and women during 20.5 years follow-up. *Int J Epidemiol* 1992; 21: 253-257.
9. Gavella M, Lipovac V, Car A, Vucic M, Sokolic L, Rakos R. Serum sialic acid in subjects with impaired glucose tolerance and in newly diagnosed type 2 diabetic patients. *Acta Diabetol* 2003; 40: 95-100.
10. Bottini E, Gerlini G, Lucarini N, Amante A, Gloria-Bottini F. Evidence of selective interaction between adenosine deaminase and acid phosphatase polymorphisms in fetuses carried by diabetic women. *Hum Genet* 1991; 87: 199-200.
11. Hoshino T, Yamada K, Masuoka K, Tsuboi I, Itoh K, Nonaka K, et. al. Elevated adenosine deaminase activity in the serum of patients with diabetes mellitus. *Diabetes Res Clin Pract* 1994; 25: 97-102.
12. Singh LS, Sharma R. Alloxan diabetes regulates adenosine deaminase activity in mice: tissue- and age-specific correlation. *Biochem Mol Biol Int* 1998; 46: 55-61.
13. Cronstein BN. Adenosine, an endogenous anti-inflammatory agent. *J Appl Physiol* 1994; 76: 5-13.
14. McLane MP, Black PR, Law WR, Raymond RM. Adenosine reversal of in vivo hepatic responsiveness to insulin. *Diabetes* 1990; 39: 62-69.
15. Rutkiewicz J, Gorski J. On the role of insulin in regulation of adenosine deaminase activity in rat tissues. *FEBS Lett* 1990; 271: 79-80.
16. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C; American Heart Association; National Heart, Lung, and Blood Institute. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004; 109: 433-438.
17. Denny PC, Denny PA, Allerton SE. Determination of sialic acid using 2-thiobarbituric acid in the absence of hazardous sodium arsenite. *Clin Chim Acta* 1983; 131: 333-336.
18. Giusti G. Adenosine Deaminase. In: Bergmeyer HV, editor. *Methods of enzymatic analysis*. 2nd ed. New York: Academic Press; 1974. p. 1092-1099.
19. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 1999; 282: 2131-2135.
20. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Low-grade systemic inflammation in overweight children. *Pediatrics* 2001; 107: E13.
21. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, et. al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation* 1999; 99: 237-242.
22. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. *BMJ* 1996; 312: 1061-1065.
23. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-Reactive protein levels in overweight and obese adults. *JAMA* 1999; 282: 2131-2135.
24. Sawynok J, Liu XJ. Adenosine in the spinal cord and periphery: release and regulation of pain. *Prog Neurobiol* 2003; 69: 313-340.
25. Adanin S, Yalovetskiy IV, Nardulli BA, Sam AD 2nd, Jonjev ZS, Law WR. Inhibiting adenosine deaminase modulates the systemic inflammatory response syndrome in endotoxemia and sepsis. *Am J Physiol Regul Integr Comp Physiol* 2002; 282: R1324-1332.
26. Bottini E, Gloria-Bottini F. Adenosine deaminase and body mass index in non-insulin-dependent diabetes mellitus. *Metabolism* 1999; 48: 949-951.
27. Sillanaukee P, Ponnio M, Jaaskelainen IP. Occurrence of sialic acids in healthy humans and different disorders. *Eur J Clin Invest* 1999; 29: 413-425.
28. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Chayama K, Oshima T. Effect of obesity on endothelium-dependent, nitric oxide-mediated vasodilation in normotensive individuals and patients with essential hypertension. *Am J Hypertens* 2001; 14: 1038-1045.
29. Crook M, Lumb P, Andrews V, Swaminathan R. Serum total sialic acid, a reputed cardiovascular risk factor, and its relationship to lipids, plasma fasting insulin, blood pressure and body mass index in normal individuals. *Clin Sci Lond* 1998; 95: 53-57.