Biochemical Changes and Fatigability in Albino Rats after Oral Administration of Adenosine Triphosphate

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors SVK, SB and GSK designed the study, wrote the protocol and supervised the work. Authors SB, SVK, KH and RM carried out all laboratories work and performed the statistical analysis. Author RM managed the analyses of the study. Authors SVK, RM and KH wrote the first draft of the manuscript. Authors SVK, RM and KH managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The study was undertaken to evaluate the biochemical changes and fatigability in albino rats after oral administration of adenosine triphosphate (ATP).

Study Design: Animal experimental study.

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Methods: Twelve Swiss albino rats of either gender weighing between 200 to 300 gm were taken. Animals were grouped randomly into two groups consisting of 6 rats each. Group I received distilled water (5 ml/kg body weight) and Group-II ATP orally at the dosage of 60mg/kg body weight for 8 days. On 8th day of experiment, all animals were evaluated for extent of physical fatigue by using exhaustive swimming test. The blood samples were collected and blood sugar, urea, uric acid, hsCRP, total cholesterol and triglyceride were measured.

Results: There was a significant (p<0.05) increase in serum uric acid, blood sugar and urea in Group- II as compared to Group- I.

Conclusion: Oral administration of ATP may lead to hyperglycaemia, hyperuricemia and dyslipidaemia.

Keywords: Adenosine triphosphate; hyperglycemia; hyperuricemia.

1. INTRODUCTION

It is well established that adenosine-5'-triphosphate (ATP) is involved in all aspects of biosynthesis in cells and acts as the primary intracellular energy source. Extracellular ATP and its metabolites are involved in regulating a variety of biological processes like cardiac function, neurotransmission, liver glycogen metabolism, as cofactor for muscle contraction and platelet aggregation, which are primarily mediated through purinergic membrane receptors [1,2,3]. ATP has also been proposed as a mediator of vasodilation during ischemia, hypoxia, and exercise [4,5].

Substantial concentration of ATP is present in a number of foods like meat, soya, mushrooms and in breast milk [6-12]. Furthermore, capsules containing ATP are currently available in market as oral supplementation and also used for the treatment of low back pain of muscular origin [13]. It has to face a number of challenges before being used by the human body. The large molecular weight, the negative charge at physiological pH (pH 7.4), and the lack of known nucleotide transporters makes the intact ATP absorption difficult from outer cell membrane of small intestine. The enzyme ecto-nucleoside triphosphate diphosphohydrolase present on the luminal side of enterocytes will dephosphorylate ATP via ADP (Adenosine diphosphate) to AMP (Adenosine monophosphate). AMP in turn, is further degraded by ecto-5'-nucleotidase and alkaline phosphatase to adenosine [14]. Adenosine can be taken up into the enterocytes of the intestinal wall. This occurs through concentrative or equilibrative nucleoside transporters [15] on the baso-lateral side of enterocytes. Adenosine is taken up by erythrocytes, provided adenosine is released intact into the vascular bed and will come in contact with erythrocytes [16,17,18]. Then it will quickly enter into liver. In liver, adenosine will be broken down to uric acid by the enzymes adenosine deaminase and xanthine oxidase [19].

Effectiveness of oral ATP administration in sports is not well established, but usage is common for body building. Oral ATP supplements have shown beneficial effects in some but not in all studies examining physical performance [13]. Arts ICW et al showed that, a single dose of orally administered ATP is not bioavailable [13]. Studies have shown bioavailability of oral ATP is limited. The identification of a number of nucleoside transporters in the small intestine further suggested that orally administered ATP may be absorbed and utilized by the human body [18].

On the other hand, increase uric acid after release of ATP in the proximal part of the small intestine suggest that ATP or one of its metabolite is absorbed and metabolized, similar results were also observed by Coolen EJCM et al. ATP administered orally does not increase ATP concentrations in the blood [13]. It does not appear to have dangerous side effects in healthy individuals, but an increase in uric acid in the blood could increase the risk of gout [13,20].

Hyperuricemia may lead to gout and it is an independent risk factor for Diabetes mellitus, Dyslipidemia, Cardiovascular complications like coronary artery disease and stroke [21]. Most of studies have shown ATP administration leads to hyperuricemia, but not mentioned about other biochemical parameters, hence the present study was undertaken to know the changes in biochemical parameters like serum uric acid, urea, random blood sugar (RBS), serum
triglyceride (TGL), serum total cholesterol (TC), high sensitive C reactive protein (hsCRP) and fatigability, in albino rats after oral administration of ATP.

2. MATERIALS AND METHODS

After obtaining clearance from Institutional Animal Ethics committee and animal regulatory body of India, twelve Swiss albino rats of either gender were selected from the central animal house of S Nijalingappa Medical College, Bagalkot, Karnataka, India.

Animals were randomly grouped into two groups, six animals in each. Distilled water 5 ml/kg body weight, was administered orally to Group I rats (Control group), and ATP 60 mg/kg body weight was administered orally for Group II rats (Experimental group). No protocol related procedures were undertaken before taking animal ethics committee approval. All procedures carried out in the study were as per CPCEA guidelines. Selected animals were examined and screened for general health condition including vital parameters. All the animals were acclimatized to laboratory for one week before starting the study. The animals were housed under standard laboratory conditions maintained at 25±5°C and exposed to 12 hr dark and 12 hr light cycle and fed with standard pellet diet and water ad libitum. Group I and group II animals received distilled water and ATP orally for seven days respectively.

On the 8th day of experiment, all animals were evaluated for extent of physical fatigue by using exhaustive swimming test, using a cylindrical glass container, containing water at 25°C. Immobility time i.e. time taken by the animal to reach a stage where it makes only those movements required to keep its head above water is measured in minutes using stop watch. Then blood samples were collected under aseptic precautions. Serum uric acid, urea, RBS, TGL, TC, hsCRP were measured. All the biochemical parameters were estimated using STATFAX 3300, semi-auto analyser and kits were supplied by the BIOSYSTEMS Pvt Ltd.

All values were expressed in mean± SD. The data was statistically analysed using unpaired 't' test, p<0.05 was considered as statistically significant.

3. RESULTS

There was no statistically significant difference in body weight (p=0.978) and swim time (p=0.204) between control rats and rats fed with oral ATP (Table 1).

| Parameter | Group | Mean±SD | t  | p  |
|-----------|-------|---------|----|----|
| Weight    | I     | 163.0±31.7 | 0.02 | 0.978 |
|           | II    | 162.5±30.7  |  |  |
| Swim time | I     | 2.5±0.7  | 1.35 | 0.204 |
|           | II    | 2.1±0.0  |  |  |

Group I = Rats administered distilled water
Group –II= Rats administered oral ATP 60 mg/kg body weight

Table 2 shows the biochemical parameters in control group and experimental group. All the biochemical parameters serum uric acid (p=0.000), Urea (p=0.004), RBS (p=0.000), were increased to statistically significant levels, in rats administered with oral ATP as compared to control albino rats. The other parameters like TGL (p=0.68), TC (p=0.89), hsCRP(p=0.798) were also increased in rats with oral ATP administration compared to control group, but it was not statistically significant.

4. DISCUSSION

In the present study there was significant increase in serum uric acid, urea and random blood glucose in albino rats fed with ATP, but there was no significant increase in strength. In rats chronic oral administration of ATP at 5 mg/kg/day increased portal vein ATP concentration and nucleoside. uptake by erythrocytes which resulted in an increase in ATP synthesis in the erythrocytes [22]. Animal and human study conducted by Jäger R et al. concluded that oral ATP administration can increase post-exercise blood flow, hence can be effective during exercise recovery [13]. Long-term oral administration of ATP has been shown to increase both the uptake and synthesis of ATP in the erythrocytes of rodents [22]. Animal studies reporting alterations in cardiac, vascular and pulmonary function after 30 days of oral ATP supplementation, also found no increase in systemic concentrations of plasma or erythrocyte ATP [22,23]. However, the concentration of ATP in plasma taken from the portal vein of rats increased rapidly up to a 1000-fold after instillation of ATP in the small intestine [22]. Oral ATP administration has been shown to improve muscular function. This is linked to accelerate recovery in people with acute and subacute lower back pain by improving muscular cell.
function and increased blood flow [24]. Rathmacher JA et al. [25] in their study, supplementation with 400 mg ATP/d for 15 days tended to reduce muscle fatigue and improved participant’s ability to maintain a higher force output at the end of an exhaustive exercise bout. In the study by Jordan et al. [26] no changes in whole blood and plasma ATP concentrations were detected, but the dosages administered were 225 mg or less. Wilson JM et al in their 3-phase randomized, double-blind, and placebo-and diet-controlled study showed that, oral ATP supplementation may enhance muscular adaptations following 12-weeks of resistance training, and prevent decrements in performance following overreaching. No statistically or clinically significant changes in blood chemistry or haematology were observed (Wilson JM et al. [27]). In an experimental study, to investigate anaerobic performance by Jordan et al.[26] three groups of nine healthy men received enteric coated ATP (150 or 225 mg) for 14 days. Physical performance and muscular strength were positively affected, although no significant differences were observed in whole blood and plasma ATP concentrations between the ATP and placebo groups [26].

Arts ICW et al. [13] in their study showed that the ATP is not orally bioavailable; also they found increased levels of serum uric acid. Hyperuricemia may also lead to progressive renal insufficiency, which leads to increase in urea also. Similar results were found in the present study. In animal studies by Kichenin et al. rats were injected with 10 mg/kg/day of ATP into the jejunum. Some of the molecules of ATP which are regenerated can be absorbed from the intestinal lumen and secreted into the portal vein [22,23]. ATP, adenine, inosine, adenosine, AMP, ADP and uric acid concentrations were increased in plasma [12]. Whereas, in humans [20], no changes in plasma ATP or metabolites were detected in the systemic circulation, the concentration of adenine, inosine, adenosine, adenosine monophosphate (AMP) and uric acid in plasma from the portal vein were increased. The authors concluded that these purine nucleosides can be absorbed from the intestinal lumen and secreted into the portal bloodstream [18].

In Maastricht University pilot-studies, experiments were performed to investigate the effects of oral ATP administration when ATP was ingested either dissolved in water or as enteric coated capsules [12]. These pilot studies showed a possible rise in blood uric acid concentration, but not ATP after oral ATP administration [12]. In a study by FoxI H et al. neither ATP nor adenosine concentrations were increased, suggesting that instead of being used for ATP synthesis in the erythrocytes, orally administered ATP is degraded to uric acid by xanthine oxidase, an enzyme which is expressed mainly in the liver and in endothelial cells of blood vessels [12,19], in the current study also there was no difference in swimming time between control and orally ATP fed rats but there was significant rise in serum uric acid in rats fed with oral ATP.

Hyperuricemia has been proposed as one of the risk factors for diabetes, but the epidemiologic studies have mixed results [28]. Elevated serum uric acid on its own has been linked to other gout-related conditions such as metabolic syndrome, insulin resistance, renal disease and hypertension [29,30]. Hyperuricemia has also

### Table 2. Biochemical parameters in control and experimental groups

| Biochemical parameter | Group | Mean±SD       | t    | p    |
|-----------------------|-------|---------------|------|------|
| Uric acid (mg/dl)     | I     | 2.2±0.9       | 19.22| 0.000|
|                       | II    | 4.5±0.1       |      |      |
| Urea (mg/dl)          | I     | 30.3±2.1      | 3.74 | 0.004|
|                       | II    | 37.3±3.9      |      |      |
| TGL (mg/dl)           | I     | 69.8±7.3      | 0.14 | 0.68 |
|                       | II    | 71.0±1.1      |      |      |
| TC (mg/dl)            | I     | 45.7±1.5      | 0.13 | 0.89 |
|                       | II    | 46.0±5.4      |      |      |
| RBS (mg/dl)           | I     | 73.1±6.0      | 14.24| 0.000|
|                       | II    | 192.1±19.5    |      |      |
| hsCRP (µgm/L)         | I     | 1.3±0.8       | 0.26 | 0.798|
|                       | II    | 1.4±0.9       |      |      |

TGL: Triglyceride; TC: Total cholesterol; RBS: Random blood sugar; hsCRP: High sensitive C-reactive protein
been linked to atherosclerosis and diabetes [31]. Several studies have observed a positive association between serum uric acid levels and diabetes [28,32-37]. Pathophysiologic links between hyperuricemia, insulin resistance, and prediabetes have not been clearly established and are under investigation [28,38]. Krishna E et al. assessed the association between serum uric acid level and incidence both of type 2 diabetes and prediabetes, and their study suggested that hyperuricemia can be a useful predictor of diabetes mellitus and concluded urate concentration as an inexpensive marker for assessing the risk of future incident type 2 diabetes and diabetes-related outcomes in nonobese individuals [28]. Krishna E et al. in their cohort study among male US veterans with gout and no prior evidence of diabetes showed that in the descriptive analysis hyperuricemia among gout patients were associated with a significantly higher risk of developing diabetes compared to controls. They identified that hyperuricemia as a significant risk factor for diabetes, and medical interventions for hyperuricemia have the potential to reduce the risk of diabetes among patients at risk [39]. In a prospective cohort study of middle-aged and elderly Chinese patients, elevated serum uric acid was associated with a significantly increased risk of diabetes [40]. Moreover, a large meta-analysis of 11 cohort studies found that serum uric acid levels were associated with a higher risk of developing type 2 diabetes [41]. In the present study also, there was significant increase (p<0.000) in random blood glucose in rats fed with oral ATP, who have elevated uric acid level, than control group. Some studies have suggested that diabetics, especially those recently diagnosed, tend to have lower serum uric acid than prediabetic and normoglycemic patients [28,35].

Increased serum uric acid levels have been reported to be associated with dyslipidemia [42], and cardiovascular diseases [43-45]. There is positive correlation between serum uric acid, total cholesterol, and triacylglycerol [46]. Conenet et al. [47] and Schachter [48] research data indicated that serum triglyceride was markedly associated with hyperuricemia. In the current study there is statistically significant increase in uric acid in rats fed with oral ATP but the increase in TC and TGL, is not statistically significant.

Increased serum uric acid in humans is also associated with systemic inflammation [49], endothelial dysfunction [50], hypertension [51], CVD, and CVD mortality [52]. S. Kaptoge et al. demonstrated that hsCRP is also an inflammatory marker and independent predictor of CVD such as coronary heart disease, ischemic stroke, and vascular and non-vascular mortality [53]. In the present study there is significant increase in uric acid in rats fed with oral ATP and also increase in hsCRP but it is not statistically significant.

High uric acid concentrations have also been associated with beneficial health effects [12]. Epidemiological studies have shown that healthy subjects with high uric acid concentrations are at a reduced risk for developing Parkinson’s disease [54,55]. Furthermore, patients with multiple sclerosis are known to have lower uric acid concentrations than healthy persons, and raising the uric acid concentration by pharmacological means has been the subject of recent investigation [56]. Although increasing the uric acid concentration pharmacologically using ATP pellets might have benefits for certain individuals, these have to be weighed against increased risks of gout and possibly DM and cardiovascular disease [57-59].

The limitations of the present study were the short duration, and less biochemical parameters were estimated due to difficulty in collecting the blood sample. Further long term human studies are required to emphasize and substantiate the current results.

5. CONCLUSION

In conclusion ATP supplementation may lead to hyperuricemia, hyperglycemia and their complications without much beneficial effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Agteresch HJ, Dagnelie PC, Van den Berg JW, Wilson JH. Adenosine triphosphate: established and potential clinical applications. Drugs. 1999;58(2):211-32.
2. Kushmerick MJ, Conley KE. Energetics of muscle contraction: The whole is less than the sum of its parts. Biochem Soc Trans. 2002;30(2):227–31.
3. Burnstock G, Knight GE, Greig AV. Purinergic signaling in healthy and diseased skin. J Invest Dermatol. 2012;132(3 Pt 1):526–46.

4. Bergfeld GR, Forrester T. Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia. Cardiovasc Res. 1992;26:40–7.

5. Ellsworth ML, Forrester T, Ellis CG, Dietrich HH. The erythrocyte as a regulator of vascular tone. Am J Physiol 1995;269:H2155–61.

6. Jäger R, Roberts MD, Lowery RP, Joy JM, Cruthirds CL, Lockwood CM, et al. Oral adenosine-5'-triphosphate (ATP) administration increases blood flow following exercise in animals and humans. JISSN. 2014;11:28.

7. Duchen K, Thorell L. Nucleotide and polyamine levels in colostrum and mature milk in relation to maternal atopy and atopic development in the children. Acta Paediatr. 1999;88:1338–43.

8. Carver JD, Pimentel B, Cox WI, Barness LA. Dietary nucleotide effects upon immune function in infants. Pediatrics. 1991;88:359–63.

9. Carver JD. Dietary nucleotides: Effects on the immune and gastrointestinal systems. Acta Paediatr Suppl. 1999;88:83-8.

10. Carver JD, Stromquist CI. Dietary nucleotides and preterm infant nutrition. J Perinatol. 2006;26:443-4.

11. Pickering LK, Granoff DM, Erickson JR, Masor ML, Cordle CT, Schaller JP, et al. Modulation of the immune system by human Milk and infant formula containing nucleotides. Pediatrics. 1998;101:242–9.

12. Mols GMF. Kinetics and effects of orally administered ATP. Maastricht; Maastricht University The Netherlands; 2011.

13. Arts ICW, Coolen EJCM, Martijn JL, Bours MJL, Huyghhebaert N, Stuart MAC, et al. Adenosine 5'-triphosphate (ATP) supplements are not orally bioavailable: A randomized, placebo-controlled cross-over trial in healthy humans. JISSN. 2012;9:16.

14. Synnestvedt K, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, et al. Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. J Clin Invest. 2002;110:993-1002.

15. Molina-Arcas M, Casado FJ, Pastor-Anglada M. Nucleoside transporter proteins. Curr Vasc Pharmacol. 2009; 7:426-34.

16. Gorman MW, Feigl EO, Buffington CW. Human plasma ATP concentration. Clin Chem. 2007;53:318–25.

17. Ngo LY, Patil SD, Unadkat JD. Ontogenic and longitudinal activity of Na(+)-nucleoside transporters in the human intestine. Am J Physiol Gastrointest Liver Physiol. 2001;280:G475-81.

18. Pastor-Anglada M, Errasti-Murugarren E, Aymerich I, Casado FJ. Concentrative nucleoside transporters (CNTs) in epithelia: from absorption to cell signaling. J Physiol Biochem. 2007;63:97–110.

19. Fox IH. Metabolic basis for disorders of purine nucleotide degradation. Metabolism 1981:30:616-34.

20. Hendrick JA, Trienet R, Leon GM, J William O. van den Berg, Paul Wilson JH, et al. Beneficial effects of Adenosine Triphosphate on nutritional status in advanced lung cancer patients: A randomized clinical trial. Journal of Clinical Oncology. 2002;20(2):371-78.

21. Murgod R, Soans G. Hyperuricemia and its implications. Intl J Chem Pharm Res. 2013;2(2):66-76.

22. Kichenin K, Seman M. Chronic oral administration of ATP modulates nucleoside transport and purine metabolism in rats. J Pharmacol Exp Ther. 2000;294:126–33.

23. Kichenin K, Decollogne S, Angignard J, Seman M. Cardiovascular and pulmonary response to oral administration of ATP in rabbits. J Appl Physiol. 2000;88:1962–68.

24. Bannwarth B, Allaert FA, Avouac B, Rossignol M, Rozenberg S, Valat JP. A randomized, double-blind, placebo controlled study of oral adenosine Triphosphate in subacute low back pain. J Rheumatol. 2005;32:1114–7.

25. Rathmacher JA, Fuller JC, Baier SM, Abumrad NN, Angus HF, Sharp RL. Adenosine-5'-triphosphate (ATP) supplementation improves low peak muscle torque and torque fatigue during repeated high intensity exercise sets. Journal of the International Society of Sports Nutrition. 2012;9(48):1-7.

26. Jordan AN, Jurca R, Abraham EH, Salikhova A, Mann JK, Morss GM, et al. Effects of oral ATP supplementation on anaerobic power and muscular strength. Medicine & Science in Sports & Exercise. 2004;4:983-90.

27. Wilson JM, Joy JM, Lowery RP, Roberts MD, Lockwood CM, Manninen AH, et al.
Effects of oral adenosine-5′-triphosphate supplementation on athletic performance, skeletal muscle hypertrophy and recovery in resistance-trained men. Nutrition & Metabolism. 2013;10:57. DOI: 10.1186/1743-7075-10-57

28. Krishnan E, Pandya BJ, Chung L, Hariri A, Dabbous O. Hyperuricemia in young adults and risk of insulin resistance, prediabetes, and diabetes: A 15-year follow-up study. Am J Epidemiol. 2012;176:108–16.

29. Charles BA, Shriner D, Doumatey A, Chen G, Zhou J, Huang H, et al. A genome-wide association study of serum uric acid in African Americans. BMC Med Genomics. 2011;4:17.

31. So A, Thorens B. Uric acid transport and disease. J Clin Invest. 2010;120:1791–9.

32. Bhole V, Choi JW, Kim SW, de Vera M, Choi H. Serum uric acid levels and the risk of type 2 diabetes: A prospective study. Am J Med. 2010;123:957–61.

33. EK10) Chien KL, Chen MF, Hsu HC, Chang WT, Su TC, Lee YT, et al. Plasma uric acid and the risk of type 2 diabetes in a Chinese community. Clin Chem. 2008;54:310–6.

34. Dehghan A, van Hoek M, Sijbrands EJ, Hofman A, Witteman JC. High serum uric acid as a novel risk factor for type 2 diabetes. Diabetes Care. 2008;31:361–2.

35. Kramer CK, von Muhlen D, Jassal SK, Barrett-Connor E. Serum uric acid levels improve prediction of incident type 2 diabetes in individuals with impaired fasting glucose: The Rancho Bernardo study. Diabetes Care. 2009;32:1272–3.

36. Nakanishi N, Okamoto M, Yoshida H, Matsuo Y, Suzuki K, Tatara K. Serum uric acid and risk for development of hypertension and impaired fasting glucose or type II diabetes in Japanese male office workers. Eur J Epidemiol. 2003;18:529–30.

37. Niskanen L, Laaksonen DE, Lindstrom J, Eriksson JG, Keinanen-Kiukaanniemi S, Ilanne-Parikka P, et al. Serum uric acid as a harbinger of metabolic outcome in subjects with impaired glucose tolerance: The Finnish Diabetes Prevention Study. Diabetes Care. 2006;29:709–11.

38. Facchini F, Chen YD, Hollenbeck CB, Reaven GM. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. JAMA. 1991;266(21):3008-11.

39. Krishnan E, Akhras KS, Sharma H, Marynchenko M, Wu EQ, Tawk R, et al. Relative and attributable diabetes risk associated with hyperuricemia in US veterans with gout. Q J Med. April 24, 2013. DOI: 10.1093/qjmed/hct093

40. Wang T, Bi Y, Xu M, Huang Y, Xu Y, Li X, et al. Serum uric acid associates with the incidence of type 2 diabetes in a prospective cohort of middle-aged and elderly Chinese. Endocrine. 2011;40:109–16.

41. Kodama S, Saito K, Yachi Y, Asumi M, Sugawara A, Totsuka K, et al. Association between serum uric acid and development of type 2 diabetes. Diabetes Care. 2009;32:1737–42.

42. Millionis HJ, Kakafika AI, Tsouli SG, Athyro VG, Bairaktari ET, Seferiadis KI, et al. Effects of statin treatment on uric acid homeostasis in patients with primary hyperlipidemia. Am Heart J. 2004;148:635-40.

43. Li D, Yu XM, Zhou XQ, Siriamornpun S, Wahlqvist ML. Uric acid status and its correlates in Hangzhou urban population. Asia Pac J Clin Nutr. 2006;15:102-6.

44. Chien KL, Hsu HC, Sung FC, Su TC, Chen MF, Lee YT. Hyperuricemia as a risk factor on cardiovascular events in Taiwan: The Chin-Shan Community Cardiovascular Cohort Study. Atherosclerosis. 2005;183:147-55.

45. Lippi G, Montagnana M, Franchini M, Favalaro EJ, Targher G. The paradoxical relationship between serum uric acid and cardiovascular disease. Clinica Chimica Acta. 2008;392:1-7.

46. Lohsoonthorn V, Dhanamun B, Williams MA. Prevalence of hyperuricaemia and its relationship with metabolic syndrome in Thai adults receiving annual health exams. Arch Med Res. 2006;37:883-9.

47. Conen D, Wittesbach, V, Bovet, P, Shamiye C, Riesen W, Paccaud F, Burnier M. Prevalence of hyperuricemia and relation of serum uric acid with cardiovascular risk factors in a developing country. BMC Public Health. 2004;4(1):9. [DOI: 10.1186/1471-2458-4-9]

48. Schachter M, Uric acid and hypertension. Curr Pharm Des. 2005;11(32):4139-43.

49. Ruggiero C, Cherubini A, Ble A, Bos AJ, Maggio M, Dixit VD, et al. Uric acid and
inflammatory markers. Eur Heart J. 2006;27:1174–81.

50. Zoccali C, Maio R, Mallamaci F, Sesti G, Perticone F. Uric acid and endothelial dysfunction in essential hypertension. J Am Soc Nephrol. 2006;17:1466-71.

51. Sundstro¨m J, Sullivan L, D’Agostino RB, Levy D, Kannel WB, Vasan RS. Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. Hypertension. 2005;45:28–33.

52. Meisinger C, Koenig W, Baumert J, Do¨ring A, Uric acid levels are associated with all-cause and cardiovascular disease mortality independent of systemic inflammation in men from the general population: the MONICA/KORA cohort study. Arterioscler Thromb Vasc Biol 2008;28:1186–92.

53. Emerging Risk Factors Collaboration, S. Kaptoge, E. Di Angelantonio, G. Lowe, M.B. Pepys, S.G. Thompson, R. Collins, J. Danesh, C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: An individual participant meta-analysis. Lancet. 2010;375:132–40.

54. Zimmermann H. Extracellular purine metabolism. Drug Dev Res. 1996;39: 337-52.

55. Al-Khalidi UAS, Chaglassian TH. The species distribution of xanthine oxidase. Biochem J. 1965;97:318-20.

56. Watts RW, Watts JE, Seegmiller JE. Xanthine oxidase activity in human tissues and its inhibition by allopurinol (4-hydroxypyrazolo[3,4-d] pyrimidine). J Lab Clin Med. 1965;66(4):688-97.

57. Stocchi V, Cucchiarelli L, Canestrari F, Piacentini MP, Fornaini G, Anal Biochem. 1987;167:181-90.

58. Ryan JW, Smith U. Metabolism of adenosine 5-monophosphate during circulation through the lungs. Trans Ass Amer Physicians. 1971;84:297-306.

59. Coade SB, Pearson JD. Metabolism of Adenine Nucleotides in Human Blood Circ Res. 1989;65(3):531-37.