Role for the urinary bladder in pathogenesis of metabolic syndrome in Wistar rats

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ABSTRACT

Introduction: Perfusion of rat urinary bladder with uric acid (UA) or 1-methyl uric acid (1-MUA) solution was reported to produce features of metabolic syndrome, viz. hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia and hypercholesterolaemia. The present study was carried out to confirm that UA or 1-MUA in the bladder can produce insulin resistance, and to demonstrate that an unknown humoral factor from the bladder is possibly involved in producing features of metabolic syndrome.

Methods: Wistar rats weighing 200–300 g were used. Two sets of protocols, perfusion study and cross-over study, were followed. For the perfusion study, urinary bladders were perfused with distilled water or solution of UA or 1-MUA, and serum levels of glucose and insulin and insulin resistance during perfusion were compared between groups. For the cross-over study, serum from distilled water or UA perfused rats (donors) was infused intravenously into rats (recipients), and serum levels of glucose, insulin, true triglyceride and total cholesterol and insulin resistance were compared.

Results: Perfusion of bladder with UA or 1-MUA solution resulted in an increase in serum levels of glucose and insulin, and insulin resistance, on comparison with distilled water perfused. Infusion of serum from donors perfused with UA resulted in hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia and hypercholesterolaemia and increase in insulin resistance in recipients when compared with recipients infused with serum from donors perfused with distilled water.

Conclusion: The present study confirms that UA and 1-MUA in the bladder produce features of metabolic syndrome and could be the result of an unknown humoral factor released from the bladder mucosa.

Keywords: insulin resistance, metabolic syndrome, methylxanthines, uric acid, urinary bladder

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INTRODUCTION

The metabolic syndrome is highly prevalent and features hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia and hypercholesterolaemia.[1] Hyperuricaemia and hyperleptinaemia have also been proposed as components of the metabolic syndrome.[2] However, the molecular basis of metabolic syndrome is not yet understood. In an earlier study in this laboratory, uric acid (UA) and its structurally related 1-methyl uric acid (I-MUA), the metabolite of methylxanthines present in coffee, tea and cocoa, were reported to play a role in the pathogenesis of the metabolic syndrome.[3] The previous study was based on reports that in laboratory rats and migratory birds on long-term fasting, a drop in the fat mass below 5% of the total body mass results in an increase in blood glucocorticoids and UA levels, followed by foraging activity and subsequent fat storage.[4-8] Glucocorticoid excess per se does not cause a dose-dependent increase in food intake or body weight in normal animals,[9] and as such, it cannot play any direct role in restarting the foraging activity following long-term food deprivation and subsequent fat storage. The increase in the plasma UA level could possibly be the afferent signal to promote fat storage. The glucocorticoids are also found to increase the UA excretion.[10]

The simultaneous increase in both glucocorticoids and UA levels in blood following long-term starvation could result in an increase in UA excretion. Hence, the site of action of UA could not be by its presence in the blood but in the urine. The possible site of action for uric acid could be the urinary bladder because urine is stored in the bladder for some time before it is voided. Accumulation of fat is also associated with hyperglycaemia, hyperinsulinaemia, hyperlipidaemia and hypercholesterolaemia as seen in the metabolic syndrome[1] and in the hibernating animals as a preparation for hibernation.[2,11] If the UA in the bladder urine is expected to stimulate fat accumulation, it can also produce an increase in the blood levels of glucose, insulin, triglyceride and cholesterol. It was shown that perfusion of urinary bladder in rats with a saturated solution of either UA or 1-MUA could produce features of metabolic syndrome, viz. hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia and hypercholesterolaemia.[3]

To prove further that UA or 1-MUA in the urinary bladder could play a role in the pathogenesis of metabolic

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syndrome, the present study was undertaken to show that UA or 1-MUA in the urinary bladder could produce an insulin resistance state. It is also hypothesised that a hitherto unknown humoral factor released from the urinary bladder mucosa in response to UA could be responsible for the pathogenesis of metabolic syndrome. To prove this hypothesis, a cross-over study was carried out in which serum from donor rats whose urinary bladders were perfused with either distilled water (DW) or UA solution was infused into recipient rats, and the effects of such infusion on the serum levels of glucose, insulin, true triglyceride and total cholesterol and insulin resistance state were studied.

METHODS

Adult albino rats of Wistar strain of either gender weighing 200–300 g maintained on a standard laboratory diet and water *ad libitum* were used for the present study. After keeping them without food for an overnight period of 12 hours, the animals were anaesthetised with sodium pentobarbital ip (45 mg/kg body weight). The animal was placed on a rat-dissecting table and the rectal temperature was maintained at 37ºC. A tracheal cannula was inserted to ensure a free air way. The external jugular veins were used to obtain blood samples and to infuse normal saline or anaesthesia, if required. A midline incision of pelvis was used to expose the urinary bladder. Both the ureters near the urinary bladder were cannulated using 26G needles sleeved onto polythene tubing so that the urine from the kidneys was diverted away and was not allowed to enter the urinary bladder. The distal ends of the cut ureters were also similarly cannulated and were used for perfusing the urinary bladder with test solutions. The urethra near the urinary bladder was cannulated using polythene tubing (OD 2 mm; ID 1.5 mm) as an outlet for the perfusing solutions. The perfusing fluid temperature was maintained at 37ºC. The rate of perfusion was maintained at 0.3 ml/min. The incision was closed using crocodile clips. The Indian National Science Academy's guidelines for the care and use of laboratory animals were followed.

30 albino rats were divided into three groups of ten each. Group I served as a control group perfused with DW. Following cannulation, the bladder was perfused with DW. The perfusate was analysed for UA at 20 min and was found not to contain any UA. Blood samples of 0.5 ml each were obtained at 20-, 40- and 80-minute intervals of perfusion for serum analysis of glucose and insulin and an equal volume of erythrocytes suspended in normal saline was injected back after each sampling to prevent a fall in the blood volume and minimise stress. The sample of blood was allowed to clot, the clot to retract and the serum was separated using a refrigerated centrifuge at 3,000 rpm for 15 minutes. The serum was immediately stored at –20ºC in a deep freezer till analysis.

Group II served as the UA perfused group. The animals of this group were treated in a similar way as those of control group (Group I) except that the bladder was perfused with an aqueous solution of UA (0.005 g/100 ml) instead of DW. Group III served as the 1-MUA perfused group. The animals of this group were treated in a similar way as those in Group I except that the bladder was an perfused with an aqueous solution of 1-MUA (0.0024 g/100 ml) instead of DW.

In donor rats whose urinary bladders were perfused with either DW or an aqueous solution of UA (0.005 g/100 ml), a maximum amount of blood was collected from the common carotid artery at the end of 80 min of perfusion. The sample of blood was allowed to clot, the clot to retract, and the serum was separated using a refrigerated centrifuge at 3,000 rpm for 15 min. The serum was fully saturated with ammonium sulphate to precipitate major serum proteins, and using disposable dialysers, ammonium sulphate was then removed from the serum. The remaining serum was diluted five times with normal saline and 0.5 ml of this diluted serum was infused intravenously into the recipient rats.

20 albino rats were divided into two groups of ten each. Group IV served as the cross-over DW group. The recipient rats of this group were infused intravenously with serum from donor rats, whose urinary bladders were perfused with DW, and samples of 0.5 ml of blood was obtained at 20-, 40- and 80-minute intervals following infusion for serum analysis of glucose, insulin, true triglyceride and total cholesterol. An equal volume of erythrocytes suspended in normal saline was injected back after each sampling to prevent a fall in the blood volume and minimise stress. The sample of blood was allowed to clot, the clot to retract, and the serum was separated using a refrigerated centrifuge at 3,000 rpm for 15 min. The serum was immediately stored at –20ºC in a deep freezer till analysis. Group V served as cross-over UA group. The recipient rats of this group were treated similar to Group IV, except that the infused serum was from donor rats whose urinary bladders were perfused with the aqueous solution of UA (0.005 g/100 ml) instead of DW.

UA (SICO, Germany) was dissolved in DW to make up a solution of 0.005 g/100 ml. A saturated solution of 1-MUA (Sigma, St Louis, MO, USA) was prepared in boiling DW (2.5 mg/100 ml) and cooled to room temperature. The concentration of 1-MUA in the perfusion solution was determined by spectrophotometry at 291.2 nm (UV-visible double beam spectrophotometer, model UVIDEC-650, JASCO, Tokyo, Japan) by comparing with standard 1-MUA dissolved in 0.1 N sodium hydroxide solution.

Table I. Serum glucose levels following perfusion of the urinary bladder with DW, UA or 1-MUA at 20, 40, and 80 min.

Perfusion duration (min)	Serum glucose (mmol L^{-1}) DW	UA	1-MUA
20	5.00 ± 0.18	6.07 ± 0.29 ‡	5.49 ± 0.17
40	5.10 ± 0.17	6.96 ± 0.26 †	8.01 ± 0.80 ‡
80	5.27 ± 0.13	7.80 ± 0.44 †	7.36 ± 0.51 †

DW: Group I (control group); UA: Group II (uric acid perfused group); 1-MUA: Group III (1-methyl uric acid perfused group). Values are mean ± SE; n = 10 in each group; groups compared: DW vs. UA at 20, 40 or 80 min; DW vs. 1-MUA at 20, 40 or 80 min.
‡ $p < 0.01$, † $p < 0.001$.

Table II. Serum insulin levels following perfusion of the urinary bladder with DW, UA or 1-MUA at 20, 40, and 80 min.

Perfusion duration (min)	Serum insulin (µU ml^{-1}) DW	UA	1-MUA
20	41 ± 4	49 ± 2	49 ± 1
40	42 ± 4	62 ± 4 ‡	73 ± 3 †
80	32 ± 3	51 ± 4 ‡	56 ± 6 ‡

DW: Group I (control group); UA: Group II (uric acid perfused group); 1-MUA: Group III (1-methyl uric acid perfused group). Values are mean ± SE; n = 10 in each group; groups compared: DW vs. UA at 20, 40 or 80 min; DW vs. 1-MUA at 20, 40 or 80 min.
‡ $p < 0.01$, † $p < 0.001$.

Table III. HOMA-IR index following perfusion of the urinary bladder with DW, UA or 1-MUA at 20, 40, and 80 min.

Perfusion duration (min)	HOMA-IR index DW	UA	1-MUA
20	9.2 ± 0.8	13.2 ± 0.9 +	11.9 ± 0.4 +
40	9.6 ± 1.1	19.4 ± 1.6 †	26.1 ± 3.2 †
80	7.4 ± 0.7	18.3 ± 2.3 ‡	18.7 ± 2.6 ‡

DW: Group I (control group); UA: Group II (uric acid perfused group); 1-MUA: Group III (1-methyl uric acid perfused group). Values are mean ± SE; n = 10 in each group; groups compared: DW vs. UA at 20, 40 or 80 min; DW vs. 1-MUA at 20, 40 or 80 min.
+ $p < 0.02$, ‡ $p < 0.01$, † $p < 0.001$.

Table IV. Serum glucose levels in recipient rats at 20, 40 and 80 min following infusion of serum from donor rats of urinary bladders perfused with DW or UA.

Infusion duration (min)	Serum glucose (mmol L^{-1}) Cross-over DW	Cross-over UA
20	4.41 ± 0.12	5.99 ± 0.19 †
40	4.91 ± 0.13	6.04 ± 0.14 †
80	5.10 ± 0.04	6.42 ± 0.17 †

Cross-over DW: Group IV (recipient rats infused with serum from donor rats with urinary bladders perfused with DW). Cross-over UA: Group V (recipient rats infused with serum from donor rats with urinary bladders perfused with UA). Values are mean ± SE; n = 10 in each group; groups compared: cross-over DW vs. cross-over UA at 20, 40 or 80 min following serum infusion.
† $p < 0.001$

The concentration was found to be 0.0024 g/100 ml of solution. The uric acid in the perfusate was determined enzymatically using the diagnostic kit (catalogue no. Autopak Uric 6689, Miles India Ltd, Vadodara, India) based on the uricase-peroxidase system. The total cholesterol in serum was determined enzymatically using the diagnostic kit (catalogue no. 352–20 and C0284 Sigma, St Louis, MO, USA) based on the cholesterol esterase–cholesterol oxidase–peroxidase system. The serum true triglyceride was determined enzymatically using the diagnostic kit (catalogue no. 337–B and G 1394 Sigma, St Louis, MO, USA) based on the lipoprotein lipase–glycerol kinase–glycerol phosphate oxidase–peroxidase system. The serum glucose was determined enzymatically using the diagnostic kit (catalogue no. 510 – DA, Sigma, St Louis, MO, USA) based on the glucose oxidase–peroxidase system.

The serum estimations of total cholesterol, true triglyceride, and glucose using the diagnostic kits were carried out in an autoanalyzer (Model SEAC CH-100, Miles India Ltd, Vadodara, India). The serum insulin was determined by radioimmunoassay using the radioimmunoassay kit for insulin supplied by Bhabha Atomic Research Centre, India. The estimation was carried out using a I-125 gamma counter (Electronics Corporation of India Ltd, Mumbai, India). The insulin resistance index was assessed using the homeostasis model assessment (HOMA-IR) originally described by Matthews et al, in which HOMA-IR (mmol/L × µU/ml) = fasting glucose (mmol/L) × fasting insulin (µU/mL)/22.5.[13,14] Values are presented as mean ± SE. Differences between the mean values were assessed by Student's *t*-test. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

The serum levels of glucose and insulin and HOMA-IR index of Group I at 20, 40 and 80 min of perfusion were compared with those of Group II or Group III. Perfusion of the urinary bladder with UA solution showed an increase in the serum glucose level on comparison with perfusion of the urinary bladder with DW ($p < 0.01$ at 20 min, $p < 0.001$ at 40 min, $p < 0.001$ at 80 min). Perfusion of the urinary bladder with 1-MUA solution showed an increase in the serum glucose level on comparison with perfusion of the urinary bladder with DW ($p < 0.01$ at 40 min, $p < 0.001$ at 80 min) (Table I).

Perfusion of the urinary bladder with UA solution showed an increase in the serum insulin level on comparison with perfusion of the urinary bladder with DW ($p < 0.01$ at 40 min, $p < 0.001$ at 80 min). Perfusion of the

Table V. Serum insulin levels in recipient rats at 20, 40 and 80 min following infusion of serum from donor rats of urinary bladders perfused with DW or UA solution.

Infusion	Serum insulin (μU ml^{-1})	
duration (min)	Cross-over DW	Cross-over UA
20	37 ± 2	66 ± 7 ‡
40	37 ± 2	56 ± 7 *
80	37 ± 2	53 ± 14

Cross-over DW: Group IV (recipient rats infused with serum from donor rats of urinary bladders perfused with DW). Cross-over UA: Group V (recipient rats infused with serum from donor rats of urinary bladders perfused with 0.005% aqueous uric acid solution). Values are mean ± SE; n = 10 in each group; groups compared: cross-over DW vs. cross-over UA at 20, 40 or 80 min following serum infusion.
*$p < 0.05$, ‡ $p < 0.01$.

Table VI. HOMA-IR index in recipient rats at 20, 40 and 80 min following infusion of serum from donor rats of urinary bladders perfused with DW or UA solution.

Infusion	HOMA- IR index	
duration (min)	Cross-over DW	Cross-over UA
20	7.1 ± 0.4	17.6 ± 2.3 ‡
40	8.0 ± 0.5	14.9 ± 1.7 ‡
80	8.4 ± 0.5	15.1 ± 4.0

Cross-over DW: Group IV (recipient rats infused with serum from donor rats of urinary bladders perfused with DW). Cross-over UA: Group V (recipient rats infused with serum from donor rats of urinary bladders perfused with 0.005% aqueous uric acid solution). Values are mean ± SE; n = 10 in each group; groups compared: cross-over DW vs. cross-over UA at 20, 40 or 80 min following serum infusion.
‡ $p < 0.01$.

urinary bladder with I-MUA solution showed an increase in the serum insulin level on comparison with perfusion of the urinary bladder with DW ($p < 0.001$ at 40 min, $p < 0.01$ at 80 min) (Table II). Insulin resistance expressed as HOMA-IR index showed a significant increase following perfusion of the urinary bladder with UA solution when compared with that of perfusion of the urinary bladder with DW ($p < 0.02$ at 20 min, $p < 0.001$ at 40 min and $p < 0.01$ at 80 min). When HOMA-IR index during I-MUA perfusion was compared with that of DW perfusion, it showed a significant increase ($p < 0.02$ at 20 min, $p < 0.001$ at 40 min and $p < 0.01$ at 80 min) (Table III).

The serum levels of glucose, insulin, HOMA-IR index, true triglyceride and total cholesterol of Group IV at 20, 40 and 80 min of serum infusion were compared with those of the Group V. The serum glucose levels of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with UA solution showed a significant increase over the serum glucose levels of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with DW at 20, 40 and 80 min of infusion ($p < 0.001$ at 20, 40 and 80 min) (Table IV).

The serum insulin levels of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with UA solution showed a significant increase over the serum insulin levels of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with DW at 20 and 40 min of infusion ($p < 0.01$ at 20 min and $p < 0.05$ at 40 min) (Table V). The HOMA-IR of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with UA solution showed a significant increase over the HOMA-IR of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with DW at 20 and 40 min of infusion ($p < 0.01$ at 20 and 40 min) (Table VI).

The serum true triglyceride levels of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with UA solution showed a significant increase over the serum true triglyceride levels of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with DW at 20 and 40 min of infusion ($p < 0.05$ at 20 min and $p < 0.02$ at 40 min) (Table VII). The serum total cholesterol levels of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with UA solution showed a significant increase over the serum total cholesterol levels of the recipient rats infused with serum from donor rats whose urinary bladders were perfused with DW at 20 and 40 min of infusion ($p < 0.05$ at 20 min and $p < 0.01$ at 40 min) (Table VIII).

DISCUSSION

The present study is a follow-up of an earlier study in this laboratory in which perfusion of the urinary bladder in rats with a saturated solution of either UA or I-MUA, the metabolite of methylxanthines present in coffee, tea and cocoa, was demonstrated to produce features of metabolic syndrome, viz. hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia and hypercholesterolaemia.[3] In the present study, insulin resistance could also be produced on perfusion of the urinary bladder with UA or I-MUA solution. Insulin resistance is the hallmark of the metabolic syndrome and type 2 diabetes mellitus. In the cross-over study, intravenous infusion of serum from donor rats whose urinary bladders were perfused with UA solution resulted in hyperglycaemia, hyperinsulinaemia, increased insulin resistance, hypertriglyceridaemia and hypercholesterolaemia in recipient rats. The cross-over study of the present work brings to light the involvement of a hitherto unknown humoral factor from the urinary bladder mucosa in response to uric acid in producing the

Table VII. Serum true triglyceride levels in recipient rats at 20, 40 and 80 min following infusion of serum from donor rats of urinary bladders perfused with DW or UA solution.

Infusion duration (min)	Serum true triglyceride (mmol L^{-1})	
	Cross-over DW	Cross-over UA
20	0.48 ± 0.03	0.67 ± 0.06 *
40	0.45 ± 0.02	0.62 ± 0.05 +
80	0.33 ± 0.04	0.43 ± 0.06

Cross-over DW: Group IV (recipient rats infused with serum from donor rats of urinary bladders perfused with DW). Cross-over UA: Group V (recipient rats infused with serum from donor rats of urinary bladders perfused with 0.005% aqueous uric acid solution). Values are mean ± SE; n = 10 in each group; groups compared: cross-over DW vs. cross-over UA at 20, 40 or 80 min following serum infusion.
* $p < 0.05$, + $p < 0.02$.

Table VIII. Serum total cholesterol levels in recipient rats at 20, 40 and 80 min following infusion of serum from donor rats of urinary bladders perfused with DW or UA solution.

Infusion duration (min)	Serum total cholesterol (mmol L^{-1})	
	Cross-over DW	Cross-over UA
20	1.07 ± 0.07	1.37 ± 0.09 *
40	0.97 ± 0.04	1.16 ± 0.03 ‡
80	0.91 ± 0.06	1.05 ± 0.04

Cross-over DW: Group IV (recipient rats infused with serum from donor rats of urinary bladders perfused with DW). Cross-over UA: Group V (recipient rats infused with serum from donor rats of urinary bladders perfused with 0.005% aqueous uric acid solution). Values are mean ± SE; n = 10 in each group; groups compared: cross-over DW vs. cross-over UA at 20, 40 or 80 min following serum infusion.
* $p < 0.05$, ‡ $p < 0.01$.

features of the metabolic syndrome. From the present study, it can be construed that UA and its structurally-related 1-MUA might be involved in the pathogenesis of metabolic syndrome.

One of the common reasons put forward for the high prevalence of metabolic syndrome is a westernised diet.[2] The western diet contains a larger proportion of fructose in the form of high fructose corn syrup (HFCS), and the consumption of HFCS had gone up by 35% over the past 35 years in USA. Fructose rapidly raises the UA level as a consequence of activation of fructokinase with ATP consumption, intracellular phosphate depletion, and stimulation of AMP deaminase. Along with an increased intake of fructose in the form of HFCS, the intake of methylxanthines in the form of cocoa, in soft drinks and candies/sweets in USA had also gradually gone up from 0.45 kg to 2.18 kg per capita yearly consumption in the last 100 years.[15,16] Cocoa contains the methylxanthines, viz caffeine, theophylline and theobromine, and one of the metabolites of these methylxanthines is 1-MUA.

Recently, Nakagawa et al, using fructose-fed rat models of metabolic syndrome, had shown that UA is a possible causative factor for the high prevalence of metabolic syndrome. According to Nakagawa et al, nitric oxide (NO) induces vasodilatation and augments insulin-mediated glucose uptake in insulin target tissues, and an increase in blood UA level (hyperuricaemia) by its ability to reduce bioavailability of NO plays a possible role in the pathogenesis of metabolic syndrome.[16-18] However, drawing evidences from nuclear magnetic resonance spectroscopic studies in his laboratories and in others, Shulman had pointed out that the decreased delivery of insulin or its substrate, glucose, to the tissue bed is not responsible for the insulin resistance in patients with type 2 diabetes mellitus, and as such, the role of a decreased insulin-mediated vasodilatation could not be the primary defect for the insulin resistance state seen in metabolic syndrome.[19] Thus, the hyperuricaemia-induced decrease in glucose disposal in fructose-fed rats explained on the basis of reduced bioavailability of NO by Nakagawa et al could only be an additional factor for the insulin resistance seen in the fructose-fed rat model of metabolic syndrome.

Using the confirmatory factor analysis technique, Pladevall et al proved that the components of the metabolic syndrome are manifestations of a single common factor, genetic or environmental,[2] which remains unknown to date. From a careful analysis of the available literature and from the results of our present work, it can be construed that the metabolic syndrome is the result of an exaggerated physiological response to seasonal variations that could be remnant in human beings. The hibernators accumulate body fat during summer and autumn as a preparation for hibernation during winter. The increase in body weight in the hibernators as a result of accumulation of body fat is always accompanied by an insulin resistance state along with hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia and hypercholesterolaemia.[11,12] Therefore, a metabolic syndrome-like state is seen in pre-hibernators in the absence of any large intake of fructose or methylxanthines. The remnants of this physiological response is expected to be present in all mammals, as Srere et al have pointed out that hibernation is a retained ancestral trait in modern mammals and the genes required to specify the hibernating phenotype are common among the genomes of all mammals.[20]

These studies further support our hypothesis that the metabolic syndrome is the result of an exaggerated physiological response to an increase in UA and/or 1-MUA level as a result of a large intake of fructose in the form of HFCS and/or a large intake of methylxanthines in the form of cocoa, tea or coffee. The UA and the 1-MUA could in turn

contribute to the epidemic of metabolic syndrome as per our hypothesis. The existence of blood-borne substances that induce or inhibit hibernation had been reported in the literature. Earlier works of Dawe and Spurrier had proved the presence of a dialysable fraction (< 10,000 daltons) that trigger hibernation and a non-dialysable fraction that is responsible for the prehibernation activities in hibernating animals.[21] The non-dialysable fraction could be responsible for the increased food intake and body weight and subsequent obesity-associated changes, like hyperglycaemia, hyperinsulinaemia, insulin resistance, hypercholesterolaemia and hypertriglyceridaemia in pre-hibernators. However, the chemical natures of these dialysable and non-dialysable fractions reported by Dawe and Spurrier have not been elucidated to date. It can be conjected that the humoral factor from the urinary bladder mucosa producing insulin resistance, hyperglycaemia, hypertriglyceridaemia and hypercholesterolaemia in the recipient rats could be similar to the non-dialysable fraction of Dawe and Spurrier. Further purification and characterisation of this putative humoral factor from the urinary bladder mucosa will throw more light on the pathogenesis of the metabolic syndrome.

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