Acute effects of hyperglycaemia on asymmetric dimethylarginine (ADMA), adiponectin and inflammatory markers (IL-6, hs-CRP) in overweight and obese women with metabolic syndrome

M. SIERVO*, M. CORANDER†, A. P. MANDER*
L. M. BROWNING* and S. A. JEBB*

*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge; and
†Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK

Metabolic syndrome (MetSyn) is associated with a state of insulin resistance, characterised by an elevation of plasma insulin levels and higher post-prandial glycaemic peaks.[1] Endothelial cells are not resistant to the systemic effects of high circulating glucose levels and asymmetric dimethylarginine (ADMA), an inhibitor of the enzyme endothelial nitric oxide synthase (eNOS), seems to be an important contributor to the pathogenesis of endothelial dysfunction.[2] Metabolic syndrome is frequently characterised by an elevated inflammatory status, which has been linked to the development of insulin resistance as well as to an increased risk for cardiovascular events.[1]

A common cause of endothelial and inflammatory dysfunction seems to derive from the increased generation of free radicals.[3] These can raise ADMA levels through an increase in methylation of the protein arginine residues as well as via an impairment of the activity of the enzyme dimethylarginine dimethyl aminohydrolase responsible for the degradation of ADMA.[2] Free radicals also modify the structure of several macromolecules and the function of several cellular pathways that can induce a defensive inflammatory response.[3]

This study explores the acute effect of moderate hyperglycaemia during an oral glucose tolerance test (OGTT) on plasma biomarkers of metabolic risk (ADMA, interleukin-6 [IL-6], adiponectin, high-sensitivity C-reactive protein [hs-CRP]) in overweight and obese women with MetSyn.

Eighty-eight overweight and obese female subjects (body mass index [BMI] range: 26.2–47.6 kg/m^2; age range: 21–69 years) were recruited. The study was approved by the local research ethics committee and all subjects gave written informed consent. Subjects were excluded if they were smokers, pregnant or breast feeding, had known diabetes, chronic inflammatory conditions, treated dyslipidaemia, liver disease or malignancy and any other disorder or medication interfering with the study outcomes. Future details of the study protocol and exclusion criteria have been reported previously.[4]

Weight and height were measured and BMI calculated. Waist circumference (WC) was measured to the nearest 0.5 cm. Systolic and diastolic blood pressures were measured using an automated machine (Critikon, Hampshire, UK).

Correspondence to: Dr Mario Siervo
MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK
Email: mario.siervo@mrc-hnr.cam.ac.uk

An OGTT was performed in the morning following overnight fast. Blood samples were then collected at 30, 60, 90 and 120 min and insulin and glucose levels were measured. Concentrations of hs-CRP, IL-6, adiponectin and ADMA were measured at 0, 60 and 120 min.

Metabolic criteria were as follows: WC ≥88 cm; triglycerides ≥1.7 mmol/L; high-density lipoprotein (HDL) ≤1.3 mmol/L; systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg; and glucose ≥6.1mmol/L.[5] Subjects were diagnosed as having MetSyn if they satisfied at least three of these criteria.

Plasma insulin concentration was analysed on an Access immunoassay system (Sanofi Pasteur Diagnostics). Plasma glucose concentrations were analysed on a Dade Behring Nephelometer II (Germany). Serum CRP was measured using a high-sensitivity assay (Dade-Behring, Walton, UK). Serum adiponectin was measured using a radioimmunoassay (LINCO Research, St. Charles, Missouri, USA). Interleukin-6 was measured by an enzyme-linked immunosorbent assay (ELISA; Diaclone, France), and ADMA was measured by ELISA (Immunodiagnostik, Belsham, Germany).

Data were analysed using SPSS version 16.0. Q-Q plots were used to check normality of distribution. Variables were log-transformed if not normally distributed. The two groups based on MetSyn diagnosis were compared at baseline using linear regression analysis adjusted for age. Subjects were matched for smoking and menopausal status.

The effects of the glucose challenge on adiponectin, ADMA, IL-6, glucose, insulin and hs-CRP levels were analysed using univariate analysis of variance for repeated measures. Absolute values at each time point (baseline, 60 min, 120 min) and changes compared to baseline (60 min, 120 min) were entered in two separate analyses as the within-subject factors (time [T]). Metabolic syndrome was entered as the between-subject factor and the interaction term between MetSyn diagnosis and time (MetSyn*T) was built.

The Bonferroni test was used to test differences between the two MetSyn groups (M), and significance was set at $P \leq 0.05$.

The MetSyn group was older, had a higher WC ($P=0.009$), systolic blood pressure ($P<0.001$), triglycerides ($P<0.001$), lower HDL ($P=0.02$) and higher insulin ($P=0.005$) and glucose ($P<0.001$) values. Body mass index, fat mass and fasting plasma levels of IL-6, hs-CRP, ADMA and adiponectin were not significantly different between the groups.

The absolute and relative changes in glucose and insulin concentrations after the glucose oral challenge were more pronounced in the MetSyn group ($P<0.001$). The changes for glucose were different in the two groups as showed by the significance of the interaction term whereas the insulin kinetics showed similarity in both groups (Table 1).

After the glucose challenge there was a significant increase in IL-6 level which was only evident at 120 min; however, hs-CRP level was not modified by the glucose challenge. The increase in glucose level induced a significant drop in plasma ADMA level and absolute adiponectin level. The observed changes in IL-6, adiponectin and ADMA were not related to the diagnosis of MetSyn (Table 1).

This study shows an unexpected decrease in plasma ADMA concentration during a standard two-hour OGTT and

Table 1. Acute response of asymmetric dimethylarginine, adiponectin and biomarkers of inflammation (hs-CRP, IL-6) to a 75 g oral glucose tolerance test (OGTT) in overweight and obese women with (n=46) and without (n=42) metabolic syndrome.

		OGTT Baseline	OGTT 60 min	OGTT 120 min	MetSyn	Time	MetSyn*Time
Glucose (mmol/L)	No MetSyn	5.4±0.4	7.3±1.9 (+35±34%)	6.3±1.1 (+17±22%)	<0.001 (0.11)	<0.001 (<0.001)	0.002 (0.02)
	MetSyn	6.1±0.7	9.4±2.7 (+51±35%)	7.3±2.0 (+18±28%)			
Insulin (μu/mL)	No MetSyn	72.2±32.6	528.3±373.3 (+629±324%)	350.5±277.4 (390±292%)	0.04 (0.81)	<0.001 (<0.001)	0.24 (0.64)
	MetSyn	97.0±46.2	642.4±323.4 (+600±278)	481.2±351.7 (396±257)			
ADMA (μmol/L)	No MetSyn	1.00±0.23	0.90±0.23 (–8±15%)	0.84±0.23 (–14±18%)	0.35 (0.17)	<0.001 (<0.001)	0.31 0.30
	MetSyn	0.92±0.27	0.89±0.28 (–0.2±27%)	0.80±0.23 (–10±24%)			
hs-CRP (mg/L)	No MetSyn	4.8 [2.7–4.8]	4.8 [2.6–4.8] (–2±6%)	4.9 [2.5–8.3] (+2±44%)	0.4 (0.22)	0.06 (0.68)	0.39 (0.45)
	MetSyn	5.2 [3.7–9.3]	5.1 [3.5–9.2] (–4±6%)	5.0 [3.2–9.0] (–5±18%)			
IL-6 (pg/mL)	No MetSyn	1.39±1.06	1.33±1.21 (+3±77%)	2.01±1.15 (+113±168%)	0.04 (0.98)	<0.001 (<0.001)	0.07 (0.69)
	MetSyn	1.89±1.49	1.66±1.39 (–4±54%)	2.80±1.58 (+121±198%)			
Adiponectin (μg/mL)	No MetSyn	3.7 [2.7–5.0]	3.7 [2.7–5.1] (–1±4%)	3.4 [2.8–5.1] (–2±6%)	0.35 (0.21)	<0.001 (0.33)	0.27 (0.36)
	MetSyn	4.4 [3.1–6.3]	4.1 [3.0–6.1] (–4±10%)	4.1 [3.0–6.4] (–4±6%)			

Values shown as mean±SD for normally distributed variables and as median and [IQR] for non-normally distributed variables. The latter were log-transformed before analysis. The change relative to baseline was calculated and expressed as (mean±SD). $P<0.05$ was regarded as significant.

ADMA: asymmetric dimethylarginine, hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin 6; MetSyn: metabolic syndrome.

the observed decline was independent of the diagnosis of MetSyn. Asymmetric dimethylarginine levels in this population are higher than those reported in healthy subjects of normal weight[6] and are comparable to ADMA plasma levels measured in insulin-resistant subjects.[7] However, the observed decrease in ADMA following a glucose challenge is counterintuitive considering the role of ADMA as an established biomarker of endothelial dysfunction.

The acute changes in ADMA in response to hyperglycaemia have been tested previously and the results have been divergent. Eid *et al*.[8] reported a decrease in ADMA levels during acute hyperinsulinaemia in male volunteers, aged 21–24 years, with borderline hypertension, whereas Konukoglu *et al*.[9] showed that ADMA increased significantly two hours after a glucose load in normal, impaired and diabetic glucose-tolerant patients.

The paradoxical decrease in ADMA in response to moderate hyperglycaemia could characterise ADMA more appropriately as a biomarker of chronic metabolic risk, thereby explaining the lack of an acute response to hyperglycaemia. However, this hypothesis does not fully explain the decline of ADMA level, which should be investigated in more controlled studies.

Changes in IL-6 and adiponectin were consistent with the results published in the literature, whereas hs-CRP levels in the present study did not change during the OGTT and were significantly higher in subjects with MetSyn. Other studies have found CRP levels to respond rapidly to post-challenge glycaemia.[9,10] In contrast, IL-6 was responsive to the glucose challenge at two hours, which suggests an association between hyperglycaemia and an increased release of inflammatory cytokines.[11] This effect may be linked to increased generation of reactive oxygen species during hyperglycaemia.

A negative association between hyperglycaemia and adiponectin level has been reported recently in subjects with impaired fasting glucose.[12] The changes in adiponectin showed significant association with changes in glucose but not with IL-6 and insulin levels, which may be related to an independent effect of hyperglycaemia on adipokine release.

While the prediction of metabolic risk was confirmed by the rapid and predicted responses of IL-6 and adiponectin to acute elevation in plasma glucose, the decrease in ADMA was unexpected. The clinical and laboratory interpretation of these findings and the evaluation of ADMA as an acute biomarker of endothelial dysfunction should be investigated in more controlled studies. □

References

1 Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006; **444** (7121): 840–6.

2 Krzyzanowska K, Mittermayer F, Wolzt M, Schernthaner G. ADMA, cardiovascular disease and diabetes. *Diabetes Res Clin Pract* 2008; **82** (Suppl 2): S122–6.
3 Grattagliano I, Palmieri VO, Portincasa P, Moschetta A, Palasciano G. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. *J Nutr Biochem* 2008; **19** (8): 491–504.
4 Krebs JD, Browning LM, McLean NK *et al.* Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women. *Int J Obes (Lond)* 2006; **30** (10): 1535–44.
5 Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005; **365** (9468): 1415–28.
6 Sydow K, Fortmann SP, Fair JM *et al.* Distribution of asymmetric dimethylarginine among 980 healthy, older adults of different ethnicities. *Clin Chem* 2010; **56** (1): 111–20.
7 Stuhlinger MC, Abbasi F, Chu JW *et al.* Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. *JAMA* 2002; **287** (11): 1420–6.
8 Eid HM, Reims H, Arnesen H, Kjeldsen SE, Lyberg T, Seljeflot I. Decreased levels of asymmetric dimethylarginine during acute hyperinsulinemia. *Metabolism* 2007; **56** (4): 464–9.
9 Konukoglu D, Firtina S, Serin O. The relationship between plasma asymmetrical dimethyl-L-arginine and inflammation and adhesion molecule levels in subjects with normal, impaired and diabetic glucose tolerance. *Metabolism* 2008; **57** (1): 110–5.
10 Festa A, D'Agostino R Jr, Tracy RP, Haffner SM. C-reactive protein is more strongly related to post-glucose load glucose than to fasting glucose in non-diabetic subjects; the Insulin Resistance Atherosclerosis Study. *Diabec Med* 2002; **19** (11): 939–43.
11 Esposito K, Nappo F, Marfella R *et al.* Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 2002; **106** (16): 2067–72.
12 Ozeki N, Hara K, Yatsuka C *et al.* Serum high-molecular weight adiponectin decreases abruptly after an oral glucose load in subjects with normal glucose tolerance or impaired fasting glucose, but not those with impaired glucose tolerance or diabetes mellitus. *Metabolism* 2009; **58** (10): 1470–6..

Potential risk of patient misclassification using a point-of-care testing kit for urine drugs of abuse

J. C. MATTHEWS and W. S. WASSIF
Clinical Biochemistry Department, Bedford Hospital NHS Trust, Kempston Road, Bedford

Many commercial kits are available that provide a rapid method to screen for drugs of abuse. The Quantum Diagnostics One-Step Multi-Drug Screen Panel kit (Quantum Diagnostics, Waltham Abbey, Essex EN9 3BZ, UK) is provided for use in a limited number of locations in the authors' hospital to provide a quick screening test for drugs of abuse in urine. Approximately 200 kits are used per year. In the emergency setting, this kit is used as a quick screening method to assess unconscious patients or those who have a head injury or unexplained symptoms where the use of drugs may account for symptoms.[1,2] In the neonatal and maternity unit, the kit is used to monitor babies of mothers who are known to use drugs of abuse, to ensure the health and wellbeing of the baby, which is particularly important when the mother is breast feeding.

A large number of urine dipsticks are available on the market. Most people who perform point-of-care (POC) analysis are familiar with the principle that a positive sample will generate a coloured line, and in a negative sample the coloured line will be absent. However, with the Quantum Diagnostics' screening test[3] the opposite is found, whereby a negative sample will generate a coloured line and a positive sample will not.

As with other drugs-of-abuse screening kits, the Quantum kit is based on a lateral flow chromatographic immunoassay,[3] the results of which are read visually without the aid of any instrumentation. The immunoassay is based on competitive binding, and when a drug is present in the urine sample it competes with the immobilised drug complex for binding to the antibody-coated particles. In the absence of a drug, the antibody-coated particles will bind to the immobilised drug complex and a visible coloured line is produced in the test region on the strip. When a drug is present at a level above the detection limit, it will saturate the binding sites on the coated particles and no coloured line will form in the test region. A second test region acts as a control whereby a coloured line will always be produced if a proper volume of sample has been used, the urine has migrated by capillary flow to the test regions and the appropriate time interval for reading the results is allowed.

As staff only perform the test on an occasional basis, concern was expressed about whether or not users of the Quantum Diagnostics' kits were adequately aware of how the kit operated and were generating accurate results. A short questionnaire was circulated to the six locations in the hospital where the kit is known to be used. Three locations have their own supply (neonatal unit [NNU], delivery suite/maternity ward [MW] and the psychiatric unit [PS]) whereas critical care (CC), the acute assessment unit (AAU) and emergency department (ED) borrow kits as and when required.

The questionnaire covered the interpretation of example test strips and how the test should be performed. Participants were encouraged to complete the questionnaire in the same way they would handle a urine sample. Instructions on how to use the kit and interpret the results were made available with the questionnaire.

It proved difficult to conduct this audit as responses to the questionnaire were not readily forthcoming. Visits were made to senior staff in the different units (in some cases on several occasions) along with telephone calls and reminder letters. A total of 31 responses were received and therefore the data obtained were limited; however, they proved sufficient to cause alarm at the standard of analysis.

Four responses were obtained from staff who had not used the kit: two did not attempt the section concerning interpretation of results and were excluded from the analysis. The other two respondents, who had never used

Correspondence to: Dr W. S. Wassif
Clinical Biochemistry Department, Bedford Hospital NHS Trust, Kempston Road, Bedford MK42 9DJ
Email: ws.wassif@bedfordhospital.nhs.uk