*Original Article*

# Increased insulin resistance is associated with increased urinary excretion of chromium in non-diabetic, normotensive Saudi adults

**Suhad M. Bahijri and Eman M. Alissa***

Clinical Biochemistry, Faculty of Medicine, Nutrition Research Unit-King Fahd Medical Research Centre, King Abdulaziz University, PO Box 12713, Jeddah 21483, Kingdom of Saudi Arabia

(Received 25 December, 2010; Accepted 6 February, 2011; Published online 13 July, 2011)

**The role of trivalent chromium in improving glucose tolerance is well documented. Increased urinary chromium has been reported in type 2 diabetes mellitus, but it was not clear whether this had preceded diabetes mellitus, or was caused by it. Aim was to investigate the relationship between urinary chromium and the degree of insulin resistance in non-diabetic normotensive Saudi adults. 357 healthy adults aged 18–50 years were recruited randomly in a cross-sectional study design. Anthropometric and demographic information were taken. Insulin, glucose and free fatty acids were measured in fasting blood samples. Fasting urinary chromium and creatinine were also determined. Using modified QUICKI, subjects were labeled as high insulin resistant, or low insulin resistant. High insulin resistant subjects were matched for age and sex to low insulin resistant subjects. High insulin resistant subjects had higher mean BMI ($p$<0.001), mean waist circumference ($p$<0.01), and median urinary chromium ($p$<0.001) compared to low insulin resistant subgroup. Higher urinary chromium in high insulin resistant subgroup indicates a renal lesion leading to chromium deficiency and possibly diabetes mellitus eventually. Chromium supplementation might help to protect against the development of diabetes mellitus in this group of high insulin resistant non-diabetic Saudi individuals.**

*Key Words*: **chromium, urinary, nondiabetic, normotensive, Saudi**

The role of chromium in maintaining normal glucose tolerance is well documented.[1–4] A three fold increase in 24 h urinary chromium in diabetic subjects over values obtained from a normal control group was reported.[5] More recently, higher mean fasting urinary chromium was reported in Saudi non-insulin dependent diabetes mellitus (NIDDM) patients, compared to healthy controls,[6] and it was suggested that urinary chromium response to glucose load could be used as an indicator of chromium status.[7] It is not known whether the increased excretion has started before the disorder causing deficiency of the element and decreased glucose tolerance leading to diabetes, or it was due to the effect of diabetes on the kidney?

Studies have shown that insulin resistance predict the development of diabetes in many populations.[8–13] There has been no work so far on chromium status or serum and urinary levels in insulin resistant subjects who are not yet diabetic. Therefore, our aim is to investigate the relationship between the degree of insulin resistance; determined by modified Quantitative Insulin Sensitivity Check Index (QUICKI);[14,15] and urinary chromium excretion in normotensive, non-diabetic Saudi individuals in an attempt to answer the above question.

## Materials and Methods

**Subjects and study protocol.** A cross-sectional study design was implemented. Healthy subjects aged 18–50 years were recruited randomly from individuals visiting health centers during the period between July 2005 and January 2007.

Six health centers (representing the six health sectors of Jeddah) were chosen randomly. Based on earlier study of insulin resistance in Saudi diabetic individuals,[16] the sample size to detect differences between means or medians of anthropometric measurements and estimated blood indices in high insulin resistance (HIR), and low insulin resistance (LIR) subgroups was computed using Power And Precision (Version 2) statistical analysis software by Biostat, and selecting power of the study as 0.9 (90%). The calculated sample size was found to be 205. According to population density; a sample size was then calculated for each centre, and fractions rounded to nearest whole number. Exclusion criteria included: reported diabetes (or fasting plasma glucose ≥126 mg/dl (≥7.0 mmol/l) upon testing), endocrine disorders, hypertension, reported dyslipidaemia and coronary heart diseases. Hypertension was defined as a systolic blood pressure >140 mm Hg, or diastolic blood pressure >90 mm Hg,[17] or current use of antihypertensive medications. Dyslipidaemia was defined as increased cholesterol level (total cholesterol level ≥5.2 mmol/l, a low density lipoprotein-cholesterol (LDL-C) ≥3.36 mmol/l, and/or a high density lipoprotein-cholesterol (HDL-C) <1.04 mmol/l),[18] and/or increased level of triglycerides (≥1.7 mmol/l).[19]

An ethical approval was granted by the bioethical and research committee. Informed consents were obtained from all participants after explanation of purpose, nature and potential risks of the study. Recruits were checked for hypertension, and only normotensive individuals were interviewed for demographic information, and their anthropometric measurements were taken. Abdominal obesity was defined as >88 cm in females and >102 cm in males.[20]

Selected subjects were given an appointment for blood and urine collection while fasting. Collected urine void samples were processed as described earlier[7] and frozen at –20°C for later estimation of chromium, while collected blood samples were immediately placed on ice prior to processing. Glucose was determined first in separated serum, and subjects showing hyperglycemia were excluded. Remaining samples were divided into aliquots and frozen at –70°C for later measurements of insulin and free fatty acids (FFAs).

The modified QUICKI was calculated as reported earlier [modified QUICKI = 1/[log (fasting insulin) + log (fasting blood

*To whom correspondence should be addressed.
E-mail: em_alissa@yahoo.com

doi: 10.3164/jcbn.10-148

**Table 1.** Demographic and anthropometric characteristics of the study group

| | Male | Female | Total |
|---|---|---|---|
| No. of subjects (%) | 76 (36.4%) | 133 (63.6%) | 209 (100%) |
| Age (yrs) | 33.0 ± 10.8 | 31.3 ± 10.2 | 31.8 ± 10.4 |
| Weight (Kg) | 73.2 ± 16.0 | 67.2 ± 15.8 | 69.3 ± 16.1 |
| Height (cm) | 168.0 ± 9.5 | 157.5 ± 7.6 | 161.3 ± 9.7 |
| BMI (Kg/m$^2$) | 25.7 ± 5.3 | 26.90 ± 6.5 | 26.44 ± 6.12 |
| BMI classes N (%): | | | |
| Normal (<25 Kg/m$^2$) | 37 (48.7%) | 59 (44.4%) | 96 (46.0%) |
| Overweight (25-<30 Kg/m$^2$) | 24 (31.6%) | 35 (29.3%) | 59 (28.2%) |
| Obese (≥30 Kg/m$^2$) | 15 (19.7%) | 39 (29.3%) | 54 (25.8%) |
| Waist (cm) | 87.2 ± 15.3 | 82.3 ± 16.4 | 84.6 ± 17.0 |
| Hip (cm) | 98.4 ± 15.9 | 105.1 ± 14.9 | 103.3 ± 16.1 |
| Waist: Hip ratio | 0.89 ± 0.015 | 0.78 ± 0.08 | 0.82 ± 0.15 |
| Family history of diabetes mellitus N (%) | 39 (51.3%) | 70 (52.6%) | 109 (52.1%) |

BMI: body mass index, N: number of subjects. Data are presented as mean ± SD, or number and percentage.

**Table 2.** Biochemical parameters of the study group

| | Male | Female | Total |
|---|---|---|---|
| No. of subjects (%) | 76 (36.4%) | 133 (63.6%) | 209 (100%) |
| Glucose (mmol/l) | 5.6 ± 0.80 | 5.5 ± 0.80 | 5.5 ± 0.80 |
| Insulin (mU/l) | 7.5 (4.4–14.3) | 7.8 (5.7–11.1) | 7.7 (5.3–11.5) |
| FFA (mg/dl) | 8.0 (5.3–10.8) | 8.8 (6.1–11.6) | 8.4 (5.8–11.3) |

FFA: Free fatty acids, TC: Total cholesterol, TG: Triglycerides, N: number of subjects. Data are presented as mean ± SD for normally distributed parameters and as median and (IQR) for non-normal distributed ones.

glucose) + log (fasting FFA)].[14] Individuals whose samples had a value outside limits reported for non insulin resistant healthy subjects by Perseghin *et al.*[14,15] for modified QUICK, were labeled HIR. They were matched for age and sex to individuals from the rest of the study population to obtain the LIR.

**Biochemical measurements.** Glucose was estimated using automated enzymatic methods (Dade Behring Inc., Deerfield, IL) (CV was 1.8% and 3.9% for intra- and inter-batch respectively). Insulin was estimated in one batch using the 'electro chemiluminescence immunoassay' 'ECLIA' on Modular Analytics E 170 (Elecsys module) immunoassay analyzer, supplied by Roche Diagnostics GMbH (R&D Systems, Inc., Minneapolis, MN USA) (CV was 9.7%). All measurements were carried out at the university hospital biochemistry laboratory. FFAs were estimated manually in serum using an enzymatic method (Wako Chemicals GMbH, Neuss, Germany), with intra-, and inter-batch CV being 5.2%, and 9.8% respectively. Urinary chromium was estimated using a SOLAAR M5 (Thermo Electron, Cambridge, UK) with a deuterium background corrector and a GF95 graphite furnace with auto-sampler as described earlier.[21] The intra-assay and inter-assay coefficients of variation for urinary chromium were found to be <8% and <12%, respectively. Urine creatinine levels were measured by a Jaffe based reaction[22] using a commercial kit (Crescent Diagnostics, Jeddah, KSA).

**Statistical methods.** Descriptive statistics such as mean ± SD for normally distributed data or median and inter quartile range (IQR) for non-normally distributed variables were calculated for all parameters in the two subgroups. Statistical analysis were performed using unpaired *t* test and Mann Whitney *U* test for comparison of normally distributed and non normally distributed parameters respectively, while $\chi^2$ test was used to compare categorical parameters. A statistical computer programme (SPSS) was used to analyze the data. Significance was assigned at $p<0.05$.

## Results

Three hundred and fifty seven subjects were recruited. Only 209 subjects (76 males and 133 females) satisfied the criteria and provided required samples. The demographic and anthropometric characteristics of the selected group are presented in Table 1, while their biochemical parameters are presented in Table 2.

Using the modified QUICKI and a cut-off point of <0.419, 97 individuals (i.e. 46.4% of total) were identified as having HIR, including 34 males (35.1%) and 63 females (64.9%). The division between sexes was not significantly different to that in the group as a whole ($p>0.05$).

Matching for age and sex could be done for 90 IR subjects only. Anthropometric and demographic characteristics of both groups are presented in Table 3, while biochemical parameters are presented in Table 4.

The HIR group had significantly higher mean weight, higher percentage of obese individuals, and higher percentage of subjects with abdominal obesity.

Despite the fact that samples with high glucose value were excluded from the start, the mean glucose level of HIR group as a whole was found to be significantly higher than the corresponding mean of the LIR group ($p<0.05$). This did not prevent the significant increase noted in the median insulin value of HIR group ($p<0.001$). Further more, the median FFA value, and the median urinary chromium level were both significantly higher for the HIR group ($p<0.001$ in both cases).

## Discussion

Several methods are available to assess insulin sensitivity in humans, the "gold standard" being the euglycemic hyper-insulinemic clamp (IS clamp) because it directly measures the

**Table 3.** Anthropometric and demographic characteristics of high insulin resistant (HIR) and low insulin resistant (LIR) subgroups using modified QUICKI

| | HIR ($n$ = 90) | LIR ($n$ = 90) | $p$ |
|---|---|---|---|
| Age (years) | 32.4 ± 9.9 | 31.7 ± 9.5 | 0.66 |
| Weight (Kg) | 74.1 ± 17.9 | 66.5 ± 14.0 | 0.002 |
| BMI (Kg/m$^2$) | 29.0 ± 7.1 | 25.6 ± 4.6 | 0 |
| BMI classes N (%): | | | |
| Normal (<25 Kg/m$^2$) | 26 (28.89%) | 41 (45.6%) | |
| Overweight (25–29.9 Kg/m$^2$) | 25 (27.78%) | 34 (37.8%) | $4.29 \times 10^{-3}$ |
| Obese (≥30 Kg/m$^2$) | 39 (43.33%) | 15 (16.7%) | |
| Waist (cm) | 88.5 ± 16.7 | 82.2 ± 14.8 | 0.008 |
| Hip (cm) | 105.7 ± 15.9 | 99.3 ± 14.5 | 0.006 |
| Waist: Hip ratio | 0.84 ± 0.10 | 0.83 ± 0.11 | 0.68 |
| Waist >88 cm (F) or >102 cm (M) N (%) | 37 (41.1%) | 19 (21.1%) | $4.3 \times 10^{-3}$ |
| Family history of diabetes mellitus N (%) | 48 (53.3%) | 51 (56.7%) | 0.69 |

BMI: body mass index, N: number of subjects. Continuous variables were compared by $t$ test for normally distributed and Mann Whitney $U$ test for non-normally distributed parameters. Categorical data were compared by $\chi^2$ test.

**Table 4.** Biochemical parameters of high insulin resistant (HIR) and low insulin resistant (LIR) groups

| | HIR ($n$ = 90) | LIR ($n$ = 90) | $p$ |
|---|---|---|---|
| Urinary chromium (ngm/mg creatinine) | 2.92 (1.5–10.28) | 0.55 (0.27–1.38) | 0 |
| Glucose (mmol/l) | 5.7 ± 0.8 | 5.4 ± 0.7 | 0.025 |
| Insulin (mU/l) | 11.2 (8.3–14.2) | 6.1 (4.2–8.4) | 0 |
| Free fatty acids (mg/dl) | 10.7 (8.4–13.1) | 6.5 (4.8–9.3) | 0 |

FFA: Free fatty acids, N: number of subjects, TC: Total cholesterol, TG: Triglycerides. Continuous variables were compared by $t$ test for normally distributed and Mann Whitney $U$ test for non-normally distributed parameters. Categorical data were compared by $\chi^2$ test.

insulin action on glucose utilization under steady—state conditions.[23] However, this technique is laborious and only applicable to a small number of subjects.[23] There are a number of other more practical methods used to evaluate insulin sensitivity in research and clinical larger scale settings. The most popular measures; especially among health practitioners in Saudi Arabia; are the Homeostasis Model Assessment (HOMA-IR), and the QUICKI [derived from fasting plasma glucose (FPG) and fasting plasma insulin (FPI) concentrations]. Both correlate reasonably with the clamp technique,[24,25] but both have limitations also,[25–27] as both reflect hepatic IR only, not IR at peripheral tissues.[28]

More recently, Perseghin *et al.*[14] by incorporating fasting plasma free fatty acid (FFA) concentration into QUICKI, improved its correlation to the IS clamp and its discriminatory power in cases of mild insulin resistant states.[15] Insulin resistance at adipose tissue will lead to elevated plasma FFA. Therefore, inclusion of FFA into the QUICKI formula can be beneficial and increase its detection power by including those subjects with peripheral IR, especially that lipolysis is more sensitive to insulin than glucose utilization,[29] and dysfunctional regulation of lipolysis was established in insulin resistant subjects.[30] Furthermore, a small increase in plasma FFA concentration in healthy individuals is reported to induce insulin resistance.[31] For these reasons, this method was chosen as a measure of insulin resistance in our population, and the earlier reported cut off point[14,15] was adopted.

**No significant difference was found between the two groups in the family medical history.** Even though a high percentage of subjects (>50%) in both study groups reported a family history of diabetes, finding no significant difference between the percentages of the two study groups was surprising. Higher insulin resistance is reported for off springs of diabetic parents,[10] therefore it was expected to find a higher incidence of diabetes in families of HIR group. Other factors must have been behind the increased IR in the HIR group.

**More obese and overweight individuals in the Insulin resistant group.** Insulin resistance generally rises with increasing body fat content.[32] This was noted in our study, as individuals in the high insulin resistant group had significantly higher mean weight, mean BMI, mean waist and hip circumference (Table 3) compared to LIR group. Furthermore, a higher percentage of HIR subjects suffered from abdominal obesity, as indicated by waist circumference >88 cm for females, and >102 cm for males. Thus, it can be suggested that obesity in the Saudi population is one of the main factors in the pathogenesis of insulin resistance.

**The biochemical profiles of the two study groups differed significantly.** The means or medians of almost all biochemical parameters were significantly higher in the selected HIR group. This is expected if the method of selection is efficient, which justifies our use of the modified QUICKI equation. More work is needed to determine appropriate cut of point for Saudi subjects with different degrees of obesity.

**Significantly higher median urinary chromium in HIR group is a very important finding.** Increased urinary excretion of chromium is reported in stress,[33] following exercise,[34] as well as after the ingestion of glucose load.[35] Increase excretion could lead to deficiency, and hence impaired glucose tolerance. In fact, diabetes caused by the stress of corticosteroids treatment was reported to be reversed by chromium supplementation.[33] Increased excretion is also reported in diabetic patients compared to control subjects.[5,6] Further more, a correlation between 24 h chromium excretion and 24 h glucose excretion was reported in type I diabetic patients.[36] The question to be asked is whether the increased excretion has started before the onset of the disorder, thus causing deficiency of the element and decreased glucose tolerance leading to diabetes, or it was due to the effect of diabetes


doi: 10.3164/jcbn.10-148

on the kidney?

There has been no work so far on chromium status or serum and urinary levels in insulin resistant subjects any where in the world. Therefore, our work is the first, as far as we know. Finding significantly higher median urinary chromium in HIR group might answer the above question, as our results prove that the increased excretion exists well before the development of diabetes in our HIR subjects. As mentioned earlier, insulin resistance predicts the development of diabetes in many populations.[8–13] However, many insulin resistant individuals will never develop diabetes, and their metabolic characteristics may well differ from those who do. Identifying metabolic abnormalities that predispose to diabetes, and following the time course with which these abnormalities change as glucose tolerance worsens is expected to help in instituting preventative measures against the world wide epidemic of diabetes. Therefore, our findings are very important, and might help to change the management of insulin resistance and the metabolic syndrome in our population.

To conclude, obesity as well as increased urinary chromium excretion are associated with higher insulin resistance in studied Saudi population. The use of chromium supplements might be recommended to prevent; or at least delay; the onset of diabetes.

## Acknowledgment

This study was supported by a grant number (429/094) as part of the Saudi Diabetes Study Research Group activities, King Abdulaziz University, Jeddah, Saudi Arabia.

## Abbreviations

| | |
|---|---|
| BMI | body mass index |
| FFA | free fatty acids |
| FPG | fasting plasma glucose |
| FPI | fasting plasma insulin |
| HDL-C | high density lipoprotein cholesterol |
| HIR | high insulin resistant |
| HOMA-IR | homeostasis model assessment insulin resistance |
| IQR | inter quartile range |
| IR | insulin resistant |
| LDL-C | low density lipoprotein cholesterol |
| LIR | low insulin resistant |
| NIDDM | non-insulin dependent diabetes mellitus |
| QUICKI | Quantitative Insulin Sensitivity Check Index |
| SD | standard deviation |

## References

1 Glinsman WH, Merz W. Effect of trivalent chromium on glucose tolerance. *Metabolism* 1966; **15**: 510–519.
2 Brown RO, Forloines-Lynn S, Cross RE, Heizer WD. Chromium deficiency after long term total parenteral nutrition. *Dig Dis Sci* 1986; **31**: 661–664.
3 Freund H, Atamian S, Fischer JEP. Chromium deficiency during total parenteral nutrition. *JAMA* 1979; **241**: 496–498.
4 Jeejeebhoy KN, Chu RC, Marliss EB, Greenberg GR, Bruce-Robertson A. Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation in a patient receiving long-term total parenteral nutrition. *Am J Clin Nutr* 1977; **30**: 531–538.
5 Morris BW, Kemp GJ, Hardisty CA. Alterations in plasma and urine chromium in diabetes mellitus. *J Endocrinology* 1986; **108**: 298.
6 Bahijri SM, Mufti AMB, Mira SA, Ghafouri H, Ajabnoor MA. Serum and urinary chromium in diabetic and normal adults and children. *Arab J Lab Med* 1997; **23**: 359–374.
7 Bahijri SM, Mufti AM. Beneficial effects of chromium in people with type 2 diabetes and urinary chromium response to glucose load as a possible indicator of status. *Biol Trace Elem Res* 2002; **85**: 97–109.
8 Lillioja S, Mott DM, Spraul M, *et al*. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 1993; **329**: 1988–1992.
9 Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR. Role of glucose and insulin resistance in development of type 2 diabetes. Results of a 25-year follow-up study. *Lancet* 1992; **340**: 925–929.
10 Warram JH, Martin BC, Krolewski AS, Soeldner JS, Kahn CR. Slow glucose removal rate and hyperinsulinemia precede the development of type 2 diabetes in the offspring of diabetic parents. *Ann Intern Med* 1990; **113**: 909–915.
11 Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Charles MA, Bennett PH. A two-step model for development of non-insulin dependent diabetes mellitus. *Am J Med* 1991; **90**: 229–235.
12 Charles MA, Fontbonne A, Thibult N, Warnet JM, Rosselin GE, Eschwege E. Risk factors for NIDDM in white population: paris prospective study. *Diabetes* 1991; **40**: 796–799.
13 Chen KW, Boyko EJ, Bergstrom RW, *et al*. Earlier appearance of impaired insulin secretion than of visceral adipocity in the pathogenesis of NIDDM. 5-Year follow-up of initially nondiabetic Japanese-American men. *Diabetes Care* 1995; **18**: 747–753.
14 Perseghin G, Caumo A, Caloni M, Testolin G, Luzi L. Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. *J Clin Endocrinol Metab* 2001; **86**: 4776–4781.
15 Rabasa-Lhoret R, Bastard J, Jan V, *et al*. Modified quantitative insulin Sensitivity check index is better correlated to hyperinsulinemic glucose clamp than other fasting-based index of insulin sensitivity in different insulin-resistant states. *J Clin Endocrinol Metab* 2003; **88**: 4917–4923.
16 Mira SA, Akbar DH, Hashim IA, Salamah SH, Zawawi TH. The insulin resistance syndrome among type—2 diabetics. *Saudi Med J* 2002; **23**: 1045–1048.
17 Chobanian AV, Bakris GL, Black HR, *et al*. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension* 2003; **42**: 1206–1252.
18 Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA* 2001; **285**: 2486–2497.
19 World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO Consultation. Part 1: diagnosis and classification of diabetes mellitus. Geneva, Switzerland: World Health Organization, 1999; 4–7.
20 World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation on obesity. Geneva, Switzerland: World Health Organization, 1998; 9–10.
21 Alissa EM, Bahjri SM, Ahmed WH, Al-Ama N, Ferns GA. Chromium status and glucose tolerance in Saudi men with and without coronary artery disease. *Biol Trace Elem Res* 2009; **131**: 215–228.
22 Husdan H, Rapoport A. Estimation of creatinine by the jaffe reaction. A comparison of three methods. *Clin Chem* 1968; **14**: 222–238.
23 Defronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; **237**: E214–E223.
24 Katz A, Nambi SS, Mather K, *et al*. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; **85**: 2402–2410.
25 Hanson RL, Pratley RE, Bogardus C, *et al*. Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. *Am J Epidemiol* 2000; **151**: 190–198.
26 Abbasi F, Reaven GM. Evaluation of the quantitative insulin sensitivity check index as an estimate of insulin sensitivity in humans. *Metabolism* 2002; **51**: 235–237.
27 Mather KJ, Hunt AE, Steinberg HO, *et al*. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *J Clin Endocrinol Metab* 2001; **86**: 5457–5464.
28 Abdul-Ghani MA, Tripathy D, Defronzo RA. Contributions of β cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. *Diabetes Care* 2006; **29**: 1130–1139.
29 Stumvoll M, Wahl HG, Machicao F, Häring H. Insulin sensitivity of glucose disposal and lipolysis: no influence of common genetic variants in IRS-1 and

CAPN10. *Diabetologia* 2002; **45**: 651–656.
30 Groop LC, Bonadonna RC, Delprato S, *et al*. Glucose and free fatty acid metabolism in non-insulin dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. *J Clin Invest* 1989; **84**: 205–213.
31 Roden M, Price TB, Perseghin G, *et al*. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 1996; **97**: 2859–2865.
32 Abbasi F, Brown BW Jr., Lamendola C, McLaughlin T, Reaven GM. Relationship between obesity, insulin resistance, and coronary heart disease risk. *J Am Coll Cardiol* 2002; **40**: 937–943.
33 Ravina A, Slezak L, Mirsky N, Bryden NA, Anderson RA. Reversal of corticosteroid-induced diabetes mellitus with supplemental chromium. *Diabet Med* 1999; **16**: 164–167.
34 Anderson A, Bryden NA, Polansky MM, Deuster PA. Acute exercise effects on urinary losses and serum concentrations of copper and zinc of moderately trained and untrained men consuming a controlled diet. *Analyst* 1995; **120**: 867–870.
35 Morris BW, Blumsohn A, Mac NS, Gray TA. The trace element chromium—a role in glucose homeostasis. *Am J Clin Nutr* 1992; **55**: 989–991.
36 Morris BW, Griffiths H, Kemp GJ. Correlations between abnormalities in chromium and glucose metabolism in a group of diabetics. *Clin Chem* 1988; **34**: 1525–1526.


doi: 10.3164/jcbn.10-148