B-E-2

Susceptibility of VLDL Subfractions to Oxidation in Patients with Type 2 Diabetes Mellitus and Their Effect on Vascular Cell Adhesion Molecule Expression in Endothelial Cells

KCB Tan, SWM Shiu
Department of Medicine, University of Hong Kong, Hong Kong

Introduction: Hypertriglyceridaemia is an independent cardiovascular risk factor in patients with type 2 diabetes mellitus and is mainly due to an increased in VLDL. The aims of this study were to determine whether VLDL subfractions from patients with type 2 diabetes mellitus were more susceptible to oxidation and to investigate the effects of VLDL subfractions on cytokine-induced vascular cell adhesion molecules (VCAM) expression in human endothelial cells.

Methods: VLDL1 and VLDL2 were isolated by density gradient ultracentrifugation. The susceptibility of individual VLDL subfraction to oxidation was determined by measuring the kinetics of conjugated diene formation during copper-mediated oxidation. To investigate VCAM expression, human aortic endothelial cells were pre-incubated with VLDL subfractions and VCAM expression determined by flow cytometry after addition of tumour necrosis factor alpha.

Results: VLDL1 and VLDL2 were isolated from 23 patients with type 2 diabetes mellitus and 11 non-diabetic controls. There were no significant differences in the lag time of VLDL1 to oxidation but the lag time of VLDL2 was significantly shorter in diabetic patients than controls (88.2 ± 37.9 minutes vs 115.2 ± 30.0 respectively, $p<0.05$). VLDL2 from diabetic patients induced a greater degree of VCAM expression than controls (2.90 ± 0.82 fluorescence units vs 2.24 ± 0.42, $p<0.05$).

Conclusion: Small dense VLDL2 in patients with type 2 diabetes mellitus is potentially atherogenic. It is more prone to oxidation and causes a greater degree of augmentation of cytokine-activated VCAM expression in endothelial cells.

B-E-3

In vivo and *in vitro* Effects of the Hepatic Glucokinase Gene Promoter –258 A Variant on Promoter Activity and Insulin Sensitivity – Controversy Revisited

NMS Wat,[1] W Zhang,[1] M Zhang,[1] ED Janus,[2] SSM Chung,[3] KSL Lam[1]
[1]Department of Medicine; [2]Clinical Biochemistry Unit and [3]Institute of Molecular Biology, University of Hong Kong, Hong Kong, China

Introduction: The –258 G-A variant of the glucokinase (GCK) gene hepatic promoter has been reported to be associated with impaired promoter activity *in vitro* and insulin resistance *in vivo* in a small cohort of African-Americans with normal glucose tolerance (NGT). In this study, we have attempted to resolve the controversy of non-reproducible *in vivo* finding in other ethnic populations.

Method: We studied 318 unrelated Chinese subjects (aged 50.7 ± 12.0 years; mean ± SD) with NGT (M:/F=43%: 57%) according to WHO (1985) diagnostic criteria. Fasting and 2-hour plasma glucose and insulin levels were measured during a 75-g oral glucose tolerance test. The Homeostasis Model Assessment of insulin resistance (HOMA-IR), was calculated. Genotype frequency was determined by AccI restriction fragment length polymorphism. PGL3 vector (with a luciferase report gene containing a 0.7 kb (–1 to –700 including the mutant site) or a 1.7 kb fragment (including an upstream liver specific enhancer –1000 to –700) of GCK promoter was transfected into HepG2 cells along with pGL3-GCK promoter clones and pCMVbeta-gal internal control plasmid. The wild-type (WT) and mutant clones were confirmed by sequencing. Cells were harvested in lysate buffer containing protease inhibitors after 72-hour transfection.

Results: The mean promoter activity was 3058 U* (100%) in the (WT) (GG) and 1788 U (58%) in the mutant (AA) for the 0.7kb fragment (n = 3); 6934 U (100%) in the WT (GG) and 6958 U (100%) in the mutant (AA) for the 1.7kb fragment (n = 2). Fasting and 2-hour insulin levels and HOMA IR were not significantly different between subjects with the A allele (allele frequency 0.24) (5.50 ± 0.32 vs 5.36 ± 0.23 mU/L for GG, 51.20 ± 5.61 vs 53.49 ± 3.82 mU/L for GG, 1.25 ± 0.07 vs 1.21 ± 0.05 for GG respectively; mean ± SEM) and those with the GG genotype.

Conclusion: These data suggest that the –258 hepatic glucokinase gene promoter variant is not associated with insulin resistance in Chinese subjects with NGT, similar to finding in Danish Caucasians. In the hepatic GCK gene, while a short fragment of promoter containing this variant has decreased promoter activity, any upstream liver specific enhancer in the promoter can lead to the negation of this effect resulting in no observable reduction in insulin sensitivity *in vivo*. (*arbitrary unit after adjustment for beta-galactosidase activity and amount of protein)