The elevated proinsulin-to-insulin ratio is associated with oxidative stress in Down's syndrome

To the Editor: Overloads of reactive oxygen species (ROS) induce oxidative stress in cells. Oxidative stress is associated with a number of pathological conditions such as inflammation, atherosclerosis, carcinogenesis, aging and reperfusion injury. Oxidative stress could also play a role in the pathophysiology of diabetes mellitus, because prolonged exposure to hyperglycaemia causes non-enzymatic glycation of proteins through Maillard's reaction and the resulting products such as Schiff base and Amadori products can lead to the production of ROS [1]. However, it is quite difficult to evaluate the association between beta-cell dysfunction and oxidative stress in diabetic conditions because hyperglycaemia induces dysfunction of beta cells by itself. On the other hand, Down's syndrome is known to be a genetic abnormality with increased prevalence of diabetes mellitus. Phenotypic analysis indicates that the trisomy of the subband 21q22.1 is responsible for the majority of the clinical features of trisomy 21. The Cu/Zn-superoxide dismutase (CuZnSOD), a key enzyme in the metabolism of oxygen free radicals, is encoded by the *SOD1* gene in band 21q22.1. Overexpression of the CuZnSOD gene could disturb the steady-state equilibrium of ROS within the cell, resulting in oxidative damage to biologically important molecules. Indeed, the previous study suggested that fetal neurons of patients with Down's syndrome generate increased amounts of ROS leading to neuronal apoptosis, and this increased generation of ROS could contribute to mental retardation and predispose to Alzheimer's disease [2].

We have recently reported that plasma concentrations of proinsulin are higher in non-diabetic patients with Down's syndrome, and suspected that pancreatic beta cells of the patients with Down's syndrome are oxidatively stressed, resulting in higher concentrations of plasma proinsulin and the proinsulin-to-insulin ratio (PI/I ratio) [3]. In this study, we evaluated oxidative stress in the non-diabetic patients with Down's syndrome by measuring plasma concentrations of oxidized low density lipoprotein (oxLDL), and examined the association between beta-cell dysfunction and oxidative stress by estimating the association between the PI/I ratio and plasma concentrations of oxLDL.

A total of 19 patients with Down's syndrome were studied [3]. They were in a residential home for adults patients with mental and physical handicaps, which were identified as Down's syndrome by chromosome analysis (15 patients with regular trisomy 21, four patients with mosaic trisomy 21). As control subjects, 10 patients with mental retardation caused by other diseases including unclassified disorders were studied, which were in the same residential home. None of the subjects was previously diagnosed as having diabetes mellitus. We measured fasting plasma concentrations of glucose, insulin, and proinsulin, and calculated the PI/I ratio and HOMA (insulin resistance index assessed by homeostasis model assessment). The direct measurement of immunoreactive proinsulin was done by using a sensitive enzyme-linked immunosorbent assay (Yuka Medias, Ibaraki, Japan) [3]. Concentrations of plasma oxLDL were measured by sandwich ELISA using the monoclonal antibody (DLH3) recognized oxidatively modified lipoproteins and the anti-human apolipoprotein B monoclonal antibody [4]. Data were expressed as means ± SD and compared by nonparametric Mann-Whitney U test. Correlations were estimated by Spearman's rank correlation (nonparametric). A p value of less than 0.05 was considered statistically significant.

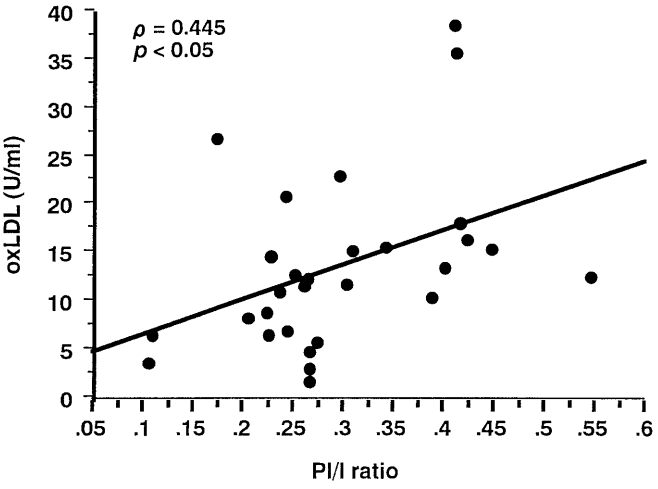

Fig. 1. Relation between plasma concentrations of oxLDL and PI/I ratio

There were no significant differences in age, BMI, and HOMA, and in plasma concentrations of glucose and insulin between in control subjects and patients with Down's syndrome. The fasting plasma glucose for each of all subjects was no more than 6.0 mmol/l. The PI/I ratio was significantly higher in patients with Down's syndrome than in the control subjects as previously reported [3]. Plasma concentrations of oxLDL were higher in the patients with Down's syndrome (15.1 ± 7.9 U/ml) than in the controls (10.7 ± 10.2 U/ml; $p < 0.05$). There were no differences in plasma concentrations of total cholesterol (5.30 ± 0.72 mmol/l vs 4.68 ± 1.00 mmol/l) and LDL cholesterol (3.25 ± 0.63 mmol/l vs 2.76 ± 0.77 mmol/l) between the patients and the control subjects. The PI/I ratio showed positive correlations with plasma concentrations of oxLDL in all subjects ($\rho = 0.445$, $p < 0.05$) (Fig. 1).

The involvement of lipid peroxidation in a number of pathophysiological conditions, such as atherosclerosis, ischaemia-induced cerebral injury, chronic renal dysfunction, and diabetes mellitus has been well documented. In particular, oxLDL has been showed to be associated with coronary artery diseases [5]. The present study demonstrates that plasma concentrations of oxLDL were significantly higher in adult patients with Down's syndrome, suggesting that the patients with Down's syndrome are oxidatively stressed. The increased plasma concentrations of oxLDL in patients with Down's syndrome seem to be caused not by environmental factors but by genetic factors (trisomy 21), because both the patients and control subjects lived in the same residential home and shared a similar lifestyle. Previous studies reported that a higher PI/I ratio is one of indicators of beta-cell dysfunction in Type II (non-insulin-dependent) diabetes mellitus and non-obese elderly subjects [6, 7]. This study shows that the higher PI/I ratio was associated with the increased plasma concentrations of oxLDL in the patients with Down's syndrome, suggesting that beta-cell dysfunction in Down's syndrome is associated with oxidative stress. Investigators reported that the pancreatic beta cells of GK rats, a model of non-obese Type II diabetes, are oxidatively stressed, and that dietary supplementation of antioxidant α-tocopherol ameliorates glycaemic control and improves insulin secretion of GK rats [8]. These observations could suggest that antioxidant supplementation in Down's syndrome has beneficial effects on the prevention of diabetes. In conclusion, we show that plasma concentrations of oxLDL were higher in the patients with Down's syndrome and that

the higher PI/I ratio was associated with the elevated plasma concentrations of oxLDL, suggesting that beta-cell dysfunction in Down's syndrome is associated with oxidative stress.

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References

1. Brownlee M, Vlassara H, Cerami A (1984) Nonenzymatic glycosylation and the pathogenesis of diabetic complications. Ann Intern Med 101: 527–537
2. Busciglio J, Yankner BA (1995) Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. Nature 378: 776–779
3. Ohyama Y, Utsugi T, Ito H, Hanaoka T, Tomono S, Kurabayashi M (2000) Elevated plasma levels of proinsulin in adult patients with Down's syndrome. Diabetes Care 23: 1030–1031
4. Itabe H, Yamamoto H, Imanaka T et al. (1996) Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. J Lipid Res 37: 45–53
5. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D (1998) Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 98: 1487–1494
6. Williams DRR, Borne C, Clark PMS (1991) Raised proinsulin concentration as early indicator of β-cell dysfunction. BMJ 303: 95–96
7. Shimizu M, Kawazu S, Tomono S et al. (1996) Age-related alteration of pancreatic b-cell function. Increased proinsulin and proinsulin-to-insulin molar ratio in elderly, but not in obese, subjects without glucose intolerance. Diabetes Care 19: 8–11
8. Ihara Y, Yamada Y, Toyokuni S et al. (2000) Antioxidant α-tocopherol ameliorates glycemic control of GK rats, a model of type 2 diabetes. FEBS Letters 473: 24–26

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