

## ***In vivo* platelet activation and aspirin responsiveness in type 1 diabetes mellitus**

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The pathophysiology, age of onset and metabolic complications of type 1 diabetes mellitus (T1DM) are different from type 2 diabetes (T2DM). However, T1DM is also associated with a high risk of vascular diseases and the pathophysiology of the atherothrombosis in T1DM is less understood as compared to T2DM (De Ferranti et al. *Circulation*, 2014). The antiplatelet effect of low-dose aspirin (ASA) has been reported to be impaired in a fraction of T2DM subjects, and a more frequent dosing regimen seems to improve platelet inhibition as compared to the standard once daily regimen (Rocca et al. *J Thromb Haemost*, 2012). However, ASA responsiveness in T1DM remains unexplored.

Our objectives were to investigate in T1DM: *in vivo* platelet activation and its determinants, as reflected by urinary thromboxane (TX)<sub>A<sub>2</sub></sub> metabolite, 11-dehydro-TXB<sub>2</sub> (TXM); the kinetics of ASA responsiveness, as reflected by serum TXB<sub>2</sub>.

Fifty-one T1DM patients (32 M, age 37±11 years) and 10 matched healthy subjects (6 M, age 33±7 years) were studied at baseline. Ten patients were repeatedly studied to assess the intra-subject variability of urinary and serum biomarkers; 31 patients and 10 controls were given 100 mg ASA once daily for 21 days. To assess the degree of platelet COX-1 inhibition we measured serum TXB<sub>2</sub> as previously described (Patrono et al. *Thromb Res*, 1980). The rate of *in vivo* TXA<sub>2</sub> biosynthesis, was assessed by measuring the urinary excretion of TXM (Ciabattoni et al. *Biochim Biophys Acta*, 1989). TXM and TXB<sub>2</sub> were measured at baseline, 12, 24, 48, 72 hrs and 7 days after the last witnessed ASA intake. Urinary excretion of the F<sub>2</sub>-isoprostane, 8-iso-PGF<sub>2α</sub> (Wang et al. *J Pharmacol Exp Ther*, 1995), and the major urinary prostacyclin metabolite (PGIM), 2,3-dinor-6-keto-PGF<sub>1α</sub> (Cavalca et al. *Thromb Haemost*, 2014) were also measured as well as circulating inflammatory biomarkers. ASA-treated patients underwent continuous glucose monitoring (CGM) within 24 hrs post-ASA.

In 10 T1DM patients, urinary TXM and serum TXB<sub>2</sub> values were quite stable over time with intra-subject coefficient of variation averaging 17±8% and 16±6%, respectively. TXM excretion was persistently increased in T1DM patients vs. controls (1284±803 vs 535±234 pg/mg creatinine, respectively; p<0.01). Female patients had higher TXM excretion than males:1432[839-1944] vs. 836[646-1244] pg/mg creatinine, respectively (p<0.05). Multivariate analysis including urinary 8-iso-PGF<sub>2α</sub>, microalbuminuria and gender as independent variables, indicated microalbuminuria and urinary 8-iso-PGF<sub>2α</sub> as independent predictors of TXM excretion (adjusted R<sup>2</sup>=0.66 for the entire model; p<0.001). PGIM, 8-iso-PGF<sub>2α</sub>, serum TXB<sub>2</sub> and inflammatory biomarkers were similar between the two groups. Inflammatory indexes were unrelated to TX-related biomarkers, isoprostanes or PGIM. The maximal inhibition of serum TXB<sub>2</sub> at 12 and 24 hrs post-ASA (12 hrs: 99.2 [98-99.6] and 99.3 [98-99.5]% inhibition; 24 hrs: 98.6 [98.3-99.3] and 98.4 [98-99.2] % inhibition, in controls and patients, respectively; p=ns for both comparisons) and the kinetics of its recovery following ASA withdrawal were similar in patients and controls (F-test=3.2, p=0.42) and not influenced by CGM parameters. Urinary TXM excretion was reduced by ASA in both groups. Consistent with an increased baseline rate, TXM excretion remained significantly higher in T1DM subjects as compared to controls up to 7 days post-ASA. The overall kinetics of urinary TXM recovery was similar between the two groups (F-test=3.2, p=0.7).

We conclude that the enhanced platelet activation in T1DM is independent of glycemic control and possibly related to female gender, microvascular and oxidative damage. Differently from T2DM, the response to ASA is unchanged, suggesting that the metabolic disturbance *per se* is not responsible for altered pharmacodynamics. The efficacy and safety of low-dose aspirin in T1DM warrants further investigation.