SMC were isolated via enzymatic dissociation of endarterectomy specimens and using immunomagnetic beads (Miltenyi). Commercially available aortic medial SMC from six healthy donors were purchased from Promocell. Cells were used consistently at passage 3. We used two-dimensional electrophoresis with digital image analysis (SameSpots, Non-linear Dynamics) and tandem mass spectrometry to detect changes in proteome of atherosclerotic SMC.

**Results** Analysis of 2D gel images revealed 29 proteins with a statistically significant difference in expression between medial and plaque SMC (p<0.05). Plaque SMC had decreased expression of mitochondrial protein ATP synthase subunit β but an increase in the oxidised form of peroxiredoxin-4, suggesting decreased mitochondrial function, possibly owing to oxidative stress. Glycolytic enzyme pyruvate kinase was also increased by 50% in plaque SMC. Furthermore, differences in protein expression between SMC from symptomatic and asymptomatic patients were also found. Plaque SMC from symptomatic patients exhibited increased expression of heat shock protein-60 and decreased levels of the anti-inflammatory protein annexin I (p<0.05), compatible with a proinflammatory behaviour. These findings were confirmed by immunoblotting.

**Conclusions** Our data demonstrate that plaque-derived SMC are exposed to higher levels of oxidative stress than control SMC. Differences between SMC from symptomatic and asymptomatic patients appear to reflect proinflammatory changes associated with plaque instability.

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**INCOREASED PLASMINOGEN ACTIVATOR INHIBITOR-1 MAY EXPLAIN DEXAMETHASONE-INDUCED THROMBOSIS AS SITE OF INTRALUMINAL WIRE INJURY**

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We have previously shown that high-dose glucocorticoid treatment reduces neointimal proliferation following arterial injury in mice, but is associated with increased local thrombosis. Clinically, glucocorticoid excess, either in Cushing’s syndrome or with chronic treatment, is associated with increased coagulation and decreased fibrinolysis. The few animal (rat) studies which have investigated these effects support the conclusion that glucocorticoid treatment reduces fibrinolysis. This study aimed to determine the influence of glucocorticoid administration on the thrombotic potential in mice.

Male C57Bl/6j mice (aged 10–12 weeks) received either vehicle or dexamethasone (dx; 0.1 or 0.8 mg/kg/day) orally for 5 weeks (n=8/group). Tail tip bleeding time was reduced by high-dose dx (52.9±5.6 s) compared with vehicle (57.1±15.6 s; p<0.05). High-dose dx increased plasminogen activator inhibitor-1 (PAI-1; 159.5±15.1% vs vehicle 100.0±10.7%; p<0.05) and decreased tissue plasminogen activator (tPA; 55.9±4.9% vs vehicle 100.0±10.3%; p<0.05) mRNA levels in the heart. In addition, high-dose dx increased total PAI-1 (5.55±1.09 ng/ml vs vehicle 0.96±0.17 ng/ml; p<0.01) and active PAI-1 (0.92±0.09 ng/ml vs vehicle 0.31±0.07 ng/ml; p<0.001) plasma antigen levels. High-dose dx did not alter platelet activation as measured by p-selectin expression using flow cytometry. Low-dose dx had no effect on any parameters described.

Dexamethasone-induced thrombosis at the site of intraluminal wire injury may be attributable to alterations in the endogenous fibrinolytic system rather than changes in platelet activity. These results suggest that beneficial inhibition of neointimal proliferation mediated by systemic glucocorticoid administration is negated in part by changes in fibrinolysis leading to large, semi-occlusive thrombi formation at the site of injury.