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Hepatic protein tyrosine phosphatase 1B (PTP1B) deficiency protects against obesity-induced endothelial dysfunction

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ABSTRACT

Growing evidence suggests that hepatic-insulin resistance is sufficient to promote progression to cardiovascular disease. We have shown previously that liver-specific protein-tyrosine-phosphatase 1B (PTP1B) deficiency improves hepatic-insulin sensitivity and whole-body glucose homeostasis. The aim of this study was to investigate the impact of liver-specific PTP1B-deficiency (L-PTP1B$^{-/-}$) on cardiac and peripheral vascular function, with special emphasis on endothelial function in the context of high-fat diet (HFD)-induced obesity.

L-PTP1B$^{-/-}$ mice exhibited an improved glucose and lipid homeostasis and increased insulin sensitivity, without changes in body weight. HFD-feeding increased systolic blood pressure (BP) in both L-PTP1B$^{-/-}$ and control littermates; however, this was significantly lower in L-PTP1B$^{-/-}$ mice. HFD-feeding increased diastolic BP in control mice only, whilst the L-PTP1B$^{-/-}$ mice were completely protected. The analysis of the function of the left ventricle (LV) revealed that HFD-feeding decreased LV fractional shortening in control animals, which was not observed in L-PTP1B$^{-/-}$ mice. Importantly, HFD feeding significantly impaired endothelium-dependent vasorelaxation in response to acetylcholine in aortas from control mice, whilst L-PTP1B$^{-/-}$ mice were fully protected. This was associated with alterations in eNOS phosphorylation. Selective inhibition of COX-2, using NS-398, decreased the contractile response in response to serotonin (5-HT) only in vessels from control mice. HFD-fed control mice released enhanced levels of prostaglandin E, a vasoconstrictor metabolite; whilst both chow- and HFD-fed L-PTP1B$^{-/-}$ mice released higher levels of prostacylin, a vasorelaxant metabolite.

Our data indicate that hepatic-PTP1B inhibition protects against HFD-induced endothelial dysfunction, underscoring the potential of peripheral PTP1B inhibitors in reduction of obesity-associated cardiovascular risk in addition to its anti-diabetic effects.

1. Introduction

Obesity incidence is reaching epidemic proportions worldwide, and is associated with an increased risk of premature death [1,2]. As a consequence, the incidence of obesity-related disorders, such as metabolic syndrome, diabetes and cardiovascular disease, is rising at an alarming rate. A common feature of these disorders is the development of insulin resistance, resulting in decreased insulin-stimulated glucose uptake, failure to suppress hepatic glucose production, and accumulation of hepatic lipids. Obesity, in particular abdominal obesity, was pointed out as a primary contributor to acquired insulin resistance, as increasing adiposity is correlated with impaired insulin action [1,3].

Growing evidence suggests that hepatic insulin resistance is sufficient to induce several components of the metabolic syndrome and promote progression to cardiovascular disease [1]. Vascular dysfunction related to obesity, in particular endothelial dysfunction in various vascular beds and in response to different

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vasodilator stimuli, might affect both peripheral vascular resistance and the delivery of substrates to metabolically active tissues, thereby contributing to both hypertension and metabolic abnormalities. Endothelial dysfunction is characterized by defects in the normal vasodilator response to mediators such as acetylcholine or to shear stress. It is considered as an independent predictor of cardiovascular events that has been consistently associated with obesity and the metabolic syndrome in a complex interplay with insulin resistance and inflammation. Deficiency of endothelial nitric oxide (NO) is believed to be the primary defect that links insulin resistance and endothelial dysfunction.

The mechanisms linking insulin resistance to endothelial dysfunction remain not well understood. There is evidence to suggest that the direct effects of insulin on the endothelium or disrupted endothelial insulin signalling may disturb endothelial function. Insulin stimulates endothelial cell production of NO [4], and, therefore, insulin resistance at the level of the endothelium might be expected to be associated with decreased insulin-stimulated NO. Duncan et al. [5] demonstrated that transgenic mice with endothelium-targeted over-expression of a dominant-negative mutant of human insulin receptor had a significant endothelial dysfunction, as evidenced by blunted aortic vasodilation in response to acetylcholine. The insulin receptor is a classic receptor tyrosine kinase and, as such, is inactivated by protein tyrosine phosphatases, notably the protein tyrosine phosphatase (PTP)1B [6]. PTP1B is also a negative regulator of leptin receptor signalling [7]. Whole-body PTP1B knockout studies in mice established PTP1B as a key negative regulator of body mass and insulin sensitivity. PTP1B$^{-/-}$ mice are lean, insulin sensitive, and have enhanced muscle and liver insulin receptor phosphorylation [8,9]. Mice with brain-specific PTP1B$^{-/-}$ deficiency exhibit a similar phenotype to the global knockouts in terms of resistance to diet-induced obesity and enhanced insulin sensitivity, mostly due to central effect on leptin signalling [10]. Muscle-specific PTP1B deficient mice exhibit marked improvement in whole-body glucose homeostasis, without changes in body mass or adiposity as well as myeloid-cell specific knockouts [11]; whilst adipocyte-PTP1B deficient mice exhibit mild glucose intolerance and increased adipocyte cell size [12,13].

Liver-specific PTP1B deletion (L-PTP1B$^{-/-}$) improves whole-body glucose and lipid homeostasis, independently of changes in body mass/adiposity [14,15]. Liver-specific PTP1B$^{-/-}$ mice exhibit increased hepatic insulin signalling, enhanced insulin-induced suppression of hepatic glucose production in clamp studies and decreased expression of gluconeogenic genes. L-PTP1B$^{-/-}$ mice are also protected against HFD-induced increase in serum and liver triglyceride and cholesterol levels, associated with decreased expression of lipogenic genes [14,15]. Hepatic PTP1B may affect lipid metabolism via a pathway distinct from the insulin signalling where its location within the endoplasmic reticulum (ER) membrane appears critical. This was mainly attributable to L-PTP1B$^{-/-}$ mice being protected against obesity-induced ER stress in the liver [14,15]. ER stress has been reported to play a crucial role in insulin resistance and lipid accumulation [16].

Considering that in vivo liver-PTP1B deficiency improves hepatic insulin sensitivity and both global glucose homeostasis and lipid metabolism independently of changes in adiposity and body mass, we hypothesized that liver-specific PTP1B deficiency would also lead to protection against obesity-induced endothelial dysfunction and reduction of cardiovascular risk.

2. Materials and methods

2.1. Animal studies

All animal studies were performed under a project licence approved by the Home Office under the Animals (Scientific Procedures) Act 1986. Mice were maintained on a 12-h light/dark cycle in a temperature-controlled barrier facility, with free access to water and food. L-PTP1B$^{-/-}$ mice were described previously and were achieved using an Albumin-Cre promoter [14,15]. All mice studied were age-matched littermate males on the mixed 129Sv/C57Bl6 background. Genotyping for the PTP1B floxed allele and the presence of Albumin-Cre was performed by PCR [14,17]. Mice were placed either on standard chow pellet diet (Rat and Mouse Breeder and Grower, Special Diets Services, Horley, UK) or high-fat diet (HFD) (Adjusted Calories Diet, 55% Fat, Harlan Teklad, Belton, UK or Research Diets, 45% Fat) at weaning (21 days old), and weights were monitored weekly.

2.2. Metabolic measurements

Glucose from tail blood was assessed using a glucometer (Accu-Check, Burgess Hill, UK). Serum insulin was determined by ELISA (CrystalChem, Downers Grove, USA). Glucose tolerance tests (GTTs) were performed as described previously, following an overnight fast [12,14].

2.3. Blood pressure

Each mouse was trained to the tail-cuff technique for 2 days before each measurement was recorded with a Physiograph Desk Model and an Electro-Sphygmomanometer (LE 5001 non-invasive blood pressure meter, Panlab, Barcelona, Spain). Five separate measurements were made on conscious mice for systolic and diastolic blood pressure (mmHg) and heart rate determinations.

2.4. Echocardiographic examination

In vivo transthoracic echocardiography was performed using a Vevo770 high frequency ultrasound machine (Visual Sonics, Toronto, Canada) in mice anesthetized with isoflurane (induction 5%, maintenance 2%) inhalation. Briefly, a two-dimensional short axis view of the left ventricle was obtained at the level of the papillary muscle in order to record M-mode tracings. Left ventricular end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were measured by the American Society of Echocardiology leading-edge method from at least 3 consecutive cardiac cycles. Doppler cardiac output was calculated cardiac index was calculated by normalizing the cardiac output to the animal body weight as previously performed [18].

2.5. Vascular reactivity

Mice were sacrificed and aorta removed and carefully cleaned of adhering fat and connective tissue and then cut into rings (1.5–2 mm long) that were mounted on a wire myograph filled with physiological salt solution (PSS), as previously described [18–20]. Endothelium-dependent vasodilatation in response to acetylcholine (ACh, 1 nM to 10 μM, Sigma–Aldrich, St. Quentin, Fallavier, France) was studied in aortas with functional endothelium pre-contracted with the thromboxane A2 agonist (9,11-dideoxy-11a,9a-epoxymethanoprostaglandin F2-α) (U46619; Sigma–Aldrich, St. Quentin, Fallavier, France) at 80% of their maximal response in the presence or absence of the superoxide dismutase (SOD) mimetic, Mn(III)tetrakis (1-Methyl-4-pyridyl)porphyrin Pentachloride (MnTMPyP; Sigma–Aldrich, St. Quentin, Fallavier, France).

Contractile response was assessed by concentration–response curves by cumulative application of serotonin (5-HT, 1 nmol/l to 10 μmol/l; Sigma–Aldrich, St. Quentin, Fallavier, France) to aortas