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Effect of rosuvastatin on insulin sensitivity in an animal model of insulin resistance: Evidence for statin-induced hepatic insulin sensitization

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Abstract

Statin-treatment of fructose-fed/insulin resistant hamsters was recently shown to ameliorate metabolic dyslipidemia and hepatic VLDL overproduction. Here, we provide evidence that rosuvastatin treatment of insulin resistant hamsters can induce improvements in hepatic and whole body insulin sensitivity. Treatment with 10 mg/kg/day rosuvastatin for 10 days significantly reduced fasting insulin (−59%) and triglyceride (−50%) levels in fructose-fed hamsters ($p < 0.05$). Following an intraperitoneal (IP) glucose challenge, rosuvastatin-treated hamsters exhibited enhanced glucose clearance compared to untreated hamsters maintained on the high-fructose diet (area under curve (AUC) = 1772 ± 223 mM min vs. 2413 ± 253 mM min, respectively; $p < 0.002$) with a significant reduction in 2 h post-challenge glucose ($n = 5$, $p < 0.02$). Rosuvastatin-treatment also significantly improved sensitivity to an IP insulin challenge (AUC = 314 ± 39 mM min vs. 195 ± 22 mM min for rosuvastatin-treated and fructose-fed hamsters, respectively; $p < 0.04$, $n = 3$). At the molecular level, significant increases in tyrosine-phosphorylation of the hepatic insulin receptor and IRS-1 were observed for rosuvastatin-treated hamsters (+37% and +58%, respectively) compared to fructose-fed controls following an intravenous (IV) bolus of insulin ($p < 0.05$). Increases in insulin receptor and IRS-1 phosphorylation were also observed in muscle and adipose tissue. Analysis of hepatic Akt phosphorylation and mass revealed a small (25%) increase in serine phosphorylation of Akt with no significant change in Akt mass, although serine-phosphorylation and mass of Akt2 were significantly increased (+32%, $p = 0.03$, and +42%, $p = 0.01$, respectively). Interestingly, expression of PTP-1B, a key negative regulator of insulin signaling, showed a non-significant trend toward reduction in liver and was significantly reduced in adipose tissue (−20% and −37%, respectively). Taken together, these data suggest that statin-treatment increases whole body and peripheral tissue insulin sensitivity via improved cellular insulin signal transduction.

Keywords: Insulin resistance; Rosuvastatin; HMG-CoA reductase; Syrian golden hamster; Insulin receptor; Insulin receptor substrate-1; Protein tyrosine phosphatase-1B; Akt

1. Introduction

The metabolic syndrome has emerged in recent years as a major public health concern due to its increasing global prevalence. By 2002 it was estimated that as many as 24% of American adults suffer from the metabolic syndrome ([1] as defined by the ATPIII criteria). The implications of this disorder are profound, as afflicted individuals have been demonstrated to be at increased risk of the development of hypertension, atherosclerosis, type II diabetes and cardiovascular disease [2,3]. The term ‘metabolic syndrome’ refers to a cluster of metabolic abnormalities associated primarily

Abbreviations: apoB, apolipoprotein B; FF, fructose-fed; FFR, fructose-fed + rosuvastatin; HDL, high density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IR, insulin receptor; IRS-1, insulin receptor substrate-1; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonylfluoride; PTP-1B, protein tyrosine phosphatase-1B; SDS, sodium dodecyl sulfate; TG, triglyceride; VLDL, very low density lipoprotein.

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with obesity including elevated plasma triglycerides (TG) and very low-density lipoprotein (VLDL) cholesterol, reduced high-density lipoprotein (HDL) cholesterol, and elevations in blood pressure and fasting glucose [4]. Clinical repercussions of the metabolic syndrome include alterations in glucose and lipid homeostasis in which insensitivity to the actions of insulin is a key feature. This 'resistance' to insulin action is thought to promote a metabolic dyslipidemic state in which hepatic gluconeogenesis, glucose output, and VLDL secretion are enhanced. Furthermore, normal post-prandial suppression of adipose tissue lipolysis is compromised in the insulin resistant state leading to a persistent elevation in circulating free fatty acid (FFA) levels [5]. Enhanced FFA mobilization triggers a variety of metabolic deficiencies including further decreases in insulin sensitivity and subsequent development of hyperinsulinemia [6]. Current ATPIII guidelines for the treatment of patients with the metabolic syndrome encourage therapies that lower LDL-cholesterol and TG, and raise HDL-cholesterol [4]. Primary intervention often involves treatment with statins to improve the lipid profiles of these patients.

Statins, competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol biosynthesis pathway, have been shown to be highly effective hypolipidemic agents in patients with the metabolic syndrome [7]. The use of statin therapy for treatment of diabetic dyslipidemia in type 2 diabetic subjects has also been investigated in several recent trials [8]. Rosuvastatin is a new member of the statin family with higher efficacy in reducing LDL cholesterol than other statins at comparable doses [9,10]. Rosuvastatin has been shown to have an increased number of binding interactions with HMG-CoA reductase, compared to other statins. This enhanced binding by the drug may cause a stronger inhibition of the enzyme and thus result in a greater therapeutic efficacy [11]. As well as the benefit of having a very high affinity for HMG-CoA reductase, rosuvastatin also possesses a relatively long half-life and a high degree of selectivity for liver cells (the main site of cholesterol synthesis) compared with non-hepatic cells [12]. Rosuvastatin (10 mg) has been shown to improve dyslipidemia in patients with the metabolic syndrome in a subanalysis of a study of 194 hypercholesterolemic patients [13]. In this study, rosuvastatin enabled 64% of metabolic syndrome subjects to achieve their ATPIII non-HDL goals suggesting it has significant potential as a treatment for insulin resistant states. Shepherd et al. [14] have also shown that 71% of hypertriglyceridemic subjects with triglyceride >5 mmol/l met their LDL-c and non-HDL-c goals with rosuvastatin-treatment [15].

Although there is ample evidence for hypolipidemic effects of statins in individuals with the metabolic syndrome and type 2 diabetes, it is still unclear whether statins can directly influence insulin sensitivity and ameliorate whole body and hepatic insulin resistance. Statins are known to possess several pleiotropic effects beyond their antihyperlipidemic action [16,17], including direct effects on vascular tissue, kidney, bone, and glucose metabolism. A potentially important pleiotropic effect is insulin sensitization [18]. Evidence from several clinical trials suggests statin-induced improvement in insulin sensitivity among type 2 diabetic patients [19–21]. Of particular interest, post hoc analysis of the West of Scotland Coronary Prevention Study (WOSCOPS) indicated a significant reduction in the development of diabetes with statin therapy [19]. However, in the larger LIPID trial, the pravastatin and placebo groups did not differ in the rates at which they developed diabetes [22]. A very recent clinical study of atorvastatin in patients with the metabolic syndrome showed statin-induced reductions in the homeostasis model assessment (HOMA) index, glucose (AUC during during oral glucose tolerance test) and fasting C-peptides [23]. Conversely, other clinical studies of diabetic subjects on statin therapy have failed to demonstrate any beneficial effect of statins on insulin action [24,25]. Despite these conflicting human data, recent animal model studies in atorvastatin-treated insulin resistant/fructose fed hamsters and atorvastatin-treated Zucker lean and fatty rats suggest significant amelioration of hepatic insulin resistance by atorvastatin [26,27].

We recently reported that rosuvastatin-treatment can induce considerable amelioration of metabolic dyslipidemia in the insulin resistant/fructose-fed hamster model and normalize hepatic VLDL-apolipoprotein B (apoB) secretion [28]. Despite the widespread use of statin therapy, the cellular and signaling mechanisms involved in insulin sensitization have not been explored in any model. We propose that the fructose-fed model of insulin resistance may be an appropriate model for exploring such questions. In the present study, we provide physiological and molecular evidence for rosuvastatin-induced improvements in whole-body and/or hepatic insulin sensitivity and provide some insight into putative insulin sensitization mechanisms of rosuvastatin.

2. Materials and methods

2.1. Fructose feeding and administration of rosuvastatin

Male Syrian Golden hamsters (*Mesocricetus aureus*) weighing 110–120 g were purchased from Charles River (Montreal, PQ, Canada). Following an acclimatization period of 3–5 days, hamsters were fed a high-fructose diet (60% fructose; Dyets Inc., Bethlehem, PA) for 10 days to induce insulin resistance. After this initial feeding period, hamsters were randomly divided into two equal groups. All hamsters were maintained on the high-fructose diet (FF) but half were also given daily doses of 10 mg/kg rosuvastatin (AstraZeneca, UK) by oral gavage for an additional 10 days (FFR). This dose of rosuvastatin has previously been shown to improve plasma lipids in the fructose-fed/insulin resistant hamster [28]. Control animals (FF) were given vehicle (water) by oral gavage. Body weight and food consumption of FF and FFR hamsters