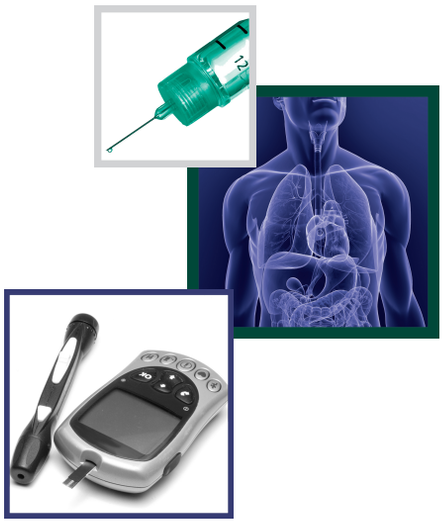
EDITORIAL



Insulin resistance caused by a redox-associated hepatokine

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Type 2 diabetes is a multifactorial disease that is caused by the disruption of interorgan networks. These disruptions lead to absolute and/or relative deficiencies in the actions of insulin due to either a genetic disposition or environmental factors. Recent studies have unraveled humoral and nutritional factors, and a neuronal pathway that may govern such interorgan networks [1]. The disruption of these interorgan networks leads to insulin resistance. Several organ-derived bioactive mediators, such as reactive oxygen species, fatty acids, plasminogen activator-1 and cytokines/hormones secreted from the liver and adipose tissue, may cause oxidative stress and thereby promote insulin resistance and vascular complications [2]. Insulin resistance is an underlying feature involved in the pathogenesis of Type 2 diabetes and its related vascular complications. Specifically, the liver plays a central role in energy homeostasis and is a major source of bioactive secretory proteins that contribute to the pathophysiology of diabetes and subsequent complications. Therefore, comprehensive gene expression analyses of the liver are important steps for understanding the molecular signature of Type 2 diabetes. By using an in-house cDNA microarray, we found that hepatic expression of genes encoding angiogenic factors, fibrogenic factors and redox-associated factors are altered in people with Type 2 diabetes compared with those without diabetes [3–6]. This differential expression may contribute to the pathophysiology of Type 2 diabetes and its clinical manifestations. Based on these findings, we hypothesized that in a manner analogous to adipose tissues [7] the liver may also contribute to the development of diabetes and insulin resistance through the production of secretory proteins, termed hepatokines. To identify a novel hepatokine, we first applied a serial analysis of gene expression (SAGE) technique that makes it possible to compare tag levels among independent libraries and to identify previously unrecognized genes encoding hepatokines that may regulate the pathophysiology of diabetes [8]. Samples for SAGE were obtained from five patients with Type 2 diabetes

"Insulin resistance is an underlying feature involved in the pathogenesis of Type 2 diabetes ... Specifically, the liver plays a central role ... Therefore, comprehensive gene expression analyses of the liver are important steps for understanding the molecular signature of Type 2 diabetes."

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and five subjects with normal glucose tolerance. A total of 37,054 genes were unique in the two libraries (normal glucose tolerance: 27,622 genes; Type 2 diabetes: 15,337 genes) [9]. Most abundant mRNAs in the liver were genes for secretory proteins, suggesting that the liver is a major source of secretory proteins [8]. We identified 117 genes encoding putative secretory proteins with expression levels at least 1.5-fold higher in diabetic patients as compared with normal subjects. Second, of these candidate genes, DNA chip methods were used to identify genes whose hepatic expression levels were significantly correlated with glycemic control (HbA1c), obesity (BMI) or insulin resistance (HOMA-R and metabolic clearance rate). Third, we referred the expression of the genes to the various animal models of diabetes, obesity and fatty liver [10–13]. Based on these approaches, we isolated 62 candidate genes for hepatokines associated with insulin resistance, hyperglycemia and obesity [8]. Of these, we identified a gene encoding selenoprotein P (SeP), the expression levels of which were positively correlated with insulin resistance and hyperglycemia [14]. Serum levels of SeP are elevated in people with Type 2 diabetes, and significantly correlated with fasting plasma glucose and HbA1c levels.

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Selenoprotein P is a 50 kDa secretory protein mainly produced in the liver, and has been known to be a selenium-carrier protein that distributes selenium to peripheral tissues such as the brain and testis. However, its metabolic actions have remained unknown.

In a hyperinsulinemic euglycemic clamp study, administration of purified SeP enhanced hepatic glucose production and suppressed glucose uptake into the skeletal muscle in C57BL mice [14]. Insulin-stimulated Akt phosphorylation was impaired in the liver and skeletal muscle of mice pretreated with SeP. Hepatic expression of *Sepp1* and serum levels of SeP were increased by approximately 1.5-fold in Type 2 diabetic model OLETF rats and KKAy mice compared with the controls. A liver- and blood-specific 30% reduction of SeP protein levels by delivery of *Sepp1*-specific siRNAs into KKAy mice using a hydrodynamic transfection method improved both glucose intolerance and insulin resistance in the mice, suggesting that SeP can be a therapeutic target for insulin resistance. Indeed, mice in which SeP was deleted were insulin sensitive. Insulin-induced phosphorylation of Akt was enhanced in the liver and skeletal muscle of SeP-knockout mice. These findings indicate that liver-derived SeP causes insulin resistance in the liver and skeletal muscle.

Concerning the regulation of SeP by nutrients, glucose and palmitate upregulate and insulin downregulates *Sepp* expression in H4IIEC hepatocytes. Hepatic SeP mRNA was elevated during fasting in mice. Therefore, the regulation of SeP by nutrients seems consistent with glucose homeostasis in the body (i.e., in the fasting state) – SeP reduces insulin sensitivity leading to the maintenance of circulating glucose levels.

We examined the mechanisms through which SeP causes insulin resistance and found that treatment with SeP reduces phosphorylation of AMP-activated protein kinase (AMPK) and expression of fatty acid β-oxidation-related genes in H4IIEC hepatocytes. AMPK is a key player in anti-aging as it activates PGC-1a and FoxO to enhance energy expenditure and longevity. Constitutively active AMPK prevented SeP-induced insulin resistance in H4IIEC hepatocytes, suggesting that SeP causes insulin resistance, at least partly, by inactivating AMPK [14]. Specifically, SeP acts as a redox protein by activating glutathione peroxidase. How does antioxidant SeP induce insulin resistance? Our findings seem paradoxical because these are against the recent concept that oxidative stress causes insulin resistance. However, in concert with our experimental findings, selenium supplementation was paradoxically associated with an increased risk for diabetes in humans [15]. Furthermore, in a previous study, the antioxidant reagent *N*-acetyl-L-cysteine rescued palmitate-induced insulin resistance only partly in cultured hepatocyte cell lines, whereas it effectively suppressed palmitate-induced activation of JNK [16]. In addition, mice lacking one of the selenoproteins involved in the elimination of physiological reactive oxygen species, glutathione peroxidase 1, are reported to be protected from high-fat-diet-induced insulin resistance [17]. Collectively, these findings and our study [14] suggest that there may be a pitfall in antioxidant supplements for the treatment of diabetes/obesity-associated diseases. Future diabetes research may involve possible reductive stress, rather than oxidative stress, to cause insulin resistance.

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