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# 4-Hydroxyisoleucine ameliorates fatty acid-induced insulin resistance and inflammatory response in skeletal muscle cells



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### ABSTRACT

The 4-hydroxyisoleucine (4-HIL), an unusual amino acid isolated from the seeds of *Trigonella foenum-graecum* was investigated for its metabolic effects to ameliorate free fatty acid-induced insulin resistance in skeletal muscle cells. An incubation of L6 myotubes with palmitate inhibited insulin stimulated-glucose uptake and -translocation of glucose transporter 4 (GLUT4) to the cell surface. Addition of 4-HIL strongly prevented this inhibition. We then examined the insulin signaling pathway, where 4-HIL effectively inhibited the ability of palmitate to reduce insulin-stimulated phosphorylation of insulin receptor substrate-1 (IRS-1), protein kinase B (PKB/AKT), AKT substrate of 160 kD (AS160) and glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ) in L6 myotubes. Moreover, 4-HIL presented strong inhibition on palmitate-induced production of reactive oxygen species (ROS) and associated inflammation, as the activation of NF- $\kappa$ B, JNK1/2, ERK1/2 and p38 MAPK was greatly reduced. 4-HIL also inhibited inflammation-in the presence of palmitate, leading to enhanced insulin-stimulated IRS-1 tyrosine phosphorylation in the presence of palmitate, leading to enhanced insulin sensitivity. These findings suggested that 4-HIL could inhibit palmitate-induced, ROS-associated inflammation and restored insulin sensitivity through regulating IRS-1 function.

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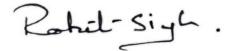
### 1. Introduction

Insulin resistance, defined as an impaired response to physiological concentrations of insulin, is strongly associated with several metabolic abnormalities such as obesity, type 2 diabetes mellitus, and the metabolic syndrome (Kahn et al., 2006; Petersen and Shulman, 2006). Insulin plays a key role in the regulation of glucose and lipid homeostasis in adipose tissue, skeletal muscle and liver. Insulin stimulates the uptake and storage of glucose in skeletal muscle and adipose tissue while simultaneously inhibiting hepatic glucose production, thus serving as the primary glucose regulator. Resistance to insulin action in these tissues results in profound dysregulation of circulatory glucose level, leading to hyperglycemia, the hallmark feature of diabetes mellitus (Saltiel and Kahn, 2001).

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Insulin resistance is induced by several molecules, including high glucose, high insulin, free fatty acids, certain cytokines, and activation of innate immune components (Houstis et al., 2006; Pickup, 2004; Reynoso et al., 2003; Roden et al., 1996; Tamrakar et al., 2010; Tilg and Moschen, 2008). Elevated plasma fatty acid levels, which often accompany obesity, play pathogenic role in the development of insulin resistance. The skeletal muscle is the major site for glucose utilization and plays a major role in whole body glucose homeostasis. Excess accumulation of saturated fatty acids or incubation of isolated muscle or cultured muscle cells with saturated fatty acids impaired insulin action (Boden, 1997; McGarry, 2002; Shulman, 2000), suggesting that deposition of fatty acids in insulin target tissues promote loss of insulin sensitivity, thus causing insulin resistance. Insulin resistance is closely associated with chronic, lowgrade inflammation (Shoelson et al., 2006). Several reports indicate that fatty acid exposure induces inflammatory response in insulin sensitive tissues characterized by enhanced production of reactive oxygen species (ROS), inflammatory cytokines, and activation of mitogen activated protein kinases (MAPK) and nuclear factor- $\kappa$ B (NF- $\kappa$ B), contributing to the development of insulin resistance (Chung et al., 2005; Hotamisligil, 2006; Sinha et al., 2004; Tilg and Moschen, 2008).

No chemical agent has yet been shown to improve insulin activity impaired by free fatty acids (FFA). Although thiazolidinediones



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