

## Role of fatty acids in the development of insulin resistance and type 2 diabetes mellitus

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*Insulin resistance is defined as the reduced responsiveness to normal circulating levels of insulin. It is the basic condition of type 2 diabetes mellitus, in which both experimental animals and humans accumulate lipids intracellularly in skeletal muscle and liver. Measurement of these lipids in humans, using nuclear magnetic resonance spectroscopy after lipid infusion, indicated they could cause inhibition of the glucose transporter GLUT4, thereby suppressing glucose entry into cells and inhibiting glucose oxidation and glycogen synthesis in muscle. Furthermore, it is known that the enzyme acetyl-CoA carboxylase2 (ACC2) suppresses the oxidation of fatty acids by inhibiting the entry of fatty acids into mitochondria. Further support for the lipocentric hypothesis of the pathogenesis of insulin resistance was provided by knocking out the gene coding for ACC2 in mice; this led to greater fatty acid oxidation, reduced fat mass and, in consequence, greatly enhanced insulin sensitivity. These studies suggest that a specific inhibitor of ACC2 would have therapeutic potential for type 2 diabetes mellitus.*

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### INTRODUCTION

“Insulin resistance . . . can be defined as a state of reduced responsiveness to normal circulating levels of insulin”.<sup>1</sup> It represents the basic condition in the pathology of type 2 diabetes mellitus (T2DM). So, for instance, the primary importance of insulin resistance in the pathogenesis of T2DM is supported by the observation of Warram et al.<sup>2</sup> of reduced glucose clearance and insulin resistance in non-diabetic offspring of two parents suffering from T2DM, one or two decades before T2DM was diagnosed in the children. Reduced glucose clearance was accompanied by compensatory hyperinsulinemia “suggesting that the primary defect was in peripheral tissue response to insulin”.<sup>2</sup> The connection of insulin resistance to lipids was described by Reaven,<sup>3</sup> who suggested that elevated plasma free fatty acids mediated insulin resistance. As long ago as 1965, Randle et al.<sup>4</sup> noted that insulin resistance, as part of the pathology of T2DM was closely linked to obesity. Shulman et al.<sup>1</sup> proposed the “lipocentric” hypothesis of the pathogenesis of insulin resistance,

in which lipid accumulation in skeletal muscle and liver are the basic links in the pathogenesis of insulin resistance and T2DM.

### LIPOCENTRIC HYPOTHESIS OF INSULIN RESISTANCE

The extensive investigations of Savage et al.<sup>1</sup> showed that lipid infusion into the plasma both in experimental animals and in humans, with resulting increased levels of plasma lipids, led to increased accumulation of intracellular lipids, particularly diacylglycerol and fatty acyl-CoA in skeletal muscle and liver. An ingenious and unique feature of their work was their ability to study the metabolism of glucose and fatty acids in human subjects non-invasively and quantitatively by the use of nuclear magnetic resonance (NMR) spectroscopy. This procedure was carried out in healthy persons as controls, in diabetic patients, and in non-diabetic offspring of diabetic parents. In these experiments, [1-<sup>13</sup>C]glucose was infused to study rates of glycogen synthesis in humans by [1-<sup>13</sup>C]-[<sup>12</sup>C] pulse-chase experiments and [<sup>31</sup>-P] was used

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to measure ATP or glucose-6-phosphate formation. The values obtained from the NMR assays were validated by biochemical assays of statistically adequate numbers of biopsies from the gastrocnemius-soleus muscle of normal human subjects<sup>5</sup>.

To give one example of a typical experiment,<sup>6</sup> eight men and 15 women of normal weight and in good health were infused intravenously with glucose and insulin during a hyperinsulinemic-euglycemic clamp test, where the glucose level is kept constant by intravenous glucose infusion despite elevated concentrations of plasma insulin. The right leg was positioned inside the magnetic coil of the NMR spectrometer and measurements of intracellular lipids were made over 120 min by NMR spectroscopy. This particular study showed a negative correlation between intracellular lipid in skeletal muscle and whole-body glucose uptake. Accumulation of lipid resulted in dramatically lowered rates of muscle glycogen synthesis and glucose oxidation, the hallmark of insulin resistance, as shown with [<sup>1-13</sup>C] glucose infusion.

As to a mechanism underlying this effect, Savage et al.<sup>1</sup> determined that the intracellular lipids caused inhibition of the glucose transporter, GLUT4, thus acting by inhibiting the rate-controlling step in glucose oxidation and glycogen synthesis in skeletal muscle because glucose transport into muscle cells was decreased. In liver, when the condition of T2DM was mimicked by lipid infusion, it led to greatly increased glucose production and decreased glycogen synthesis.

#### **LOWERING INTRACELLULAR LIPID BY SWITCHING OFF EXPRESSION OF THE ACETYL COA CARBOXYLASE2 ENZYME**

The lipocentric hypothesis of Savage et al.,<sup>1</sup> concerning the role of fatty acids inducing insulin resistance, would predict that by stimulating oxidation of fatty acids specifically in skeletal muscle, intracellular lipid would decline. Lowering intracellular lipids may then prevent insulin resistance and hence T2DM. This possibility was investigated by the use of mutant mice by Choi et al.<sup>7</sup> These mice lacked the gene coding for the enzyme acetyl CoA carboxylase2 (ACC2).

ACC has two functions: 1) the synthesis of malonyl-CoA from acetyl-CoA, which is the first step in the synthesis of fatty acids; and 2) the regulation of fatty acid transfer into mitochondria for their oxidation by means of the allosteric inhibition of carnitine palmitoyl transferase-1. ACC, therefore, regulates both fatty acid synthesis and fatty acid oxidation. Two isoforms of the enzyme exist: 1) ACC1, occurring mainly in adipocytes and liver, that catalyses the synthesis of fatty acids; and 2) ACC2, found mainly in oxidative tissues, such as skeletal

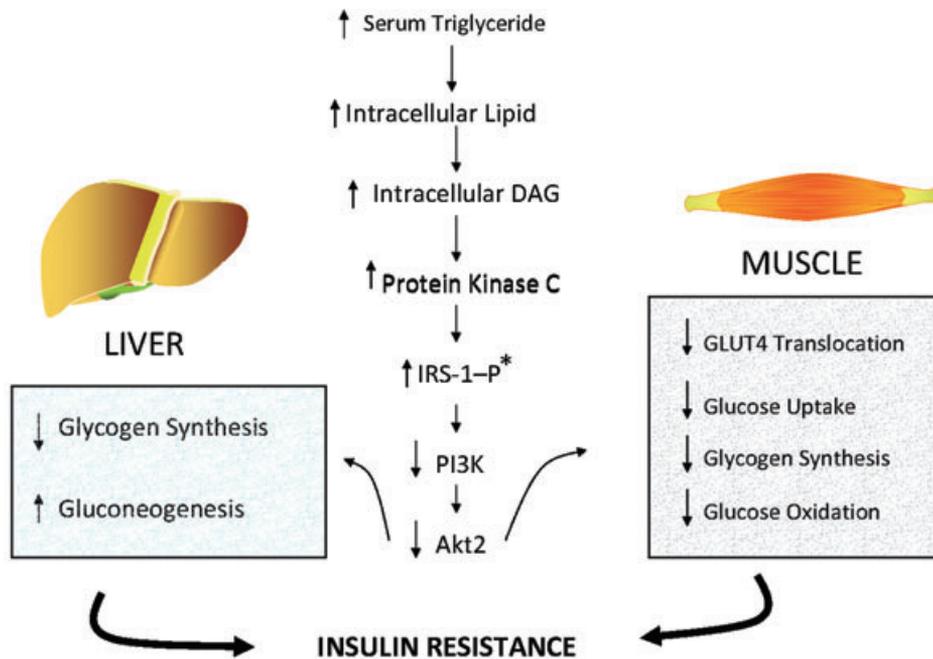
muscle, that regulates fatty acid oxidation through allosteric inhibition of carnitine palmitoyl transferase.

To explore the possibility that ACC2-deficient (ACC2<sup>-/-</sup>) mice might be less susceptible to insulin resistance, Choi et al.<sup>7</sup> proceeded to feed either a regular or a high-fat diet to ACC2<sup>-/-</sup> mice and to wild-type (WT) controls. They observed that feeding either of those diets led to lower fat mass in the mutant mice compared to the WT mice, even though food intake of the ACC2<sup>-/-</sup> mice was greater than that of the WT mice. Energy expenditure of the ACC2<sup>-/-</sup> mice was 15–19% higher than that of the WT controls. The authors measured the respiratory quotients (RQs) of the ACC2<sup>-/-</sup> and WT mice. They found that the RQ was similar in both groups, even though the energy expenditure of the ACC2<sup>-/-</sup> animals was greater. Higher energy expenditure with similar RQs signifies increased fat and carbohydrate oxidation in the ACC2<sup>-/-</sup> mice.

Insulin resistance, of course, means a decreased responsiveness of glucose metabolism to insulin. To determine the details of whole-body insulin responsiveness, the authors<sup>7</sup> fed a high-fat diet (55% fat) to ACC2<sup>-/-</sup> and WT mice for 3 weeks. This diet induced severe insulin resistance in the WT mice, as indicated by increased glucose production and lowered peripheral glucose uptake by liver. In the ACC2<sup>-/-</sup> mice, the glucose and fatty acid levels in plasma were about 30% lower than in the WT controls.

Choi et al.<sup>7</sup> then subjected the high-fat fed ACC2<sup>-/-</sup> and WT mice to a hyperinsulinemic-euglycemic clamp and studied the metabolism of [<sup>14</sup>C]-glucose by measuring the infusion rate of glucose needed to maintain the plasma glucose at a normal level (euglycemia). They found a twofold increase in the glucose infusion rate to maintain euglycemia with hyperinsulinemia in the high-fat-fed ACC2<sup>-/-</sup> compared to the WT mice, indicating greatly increased insulin responsiveness. This effect was reflected in a 78% induced suppression of liver glucose production, a 42% insulin-stimulated whole-body glucose uptake, a 26% increase in whole-body glycolysis, a 75% increase in glycogen synthesis, a 66% increase in glucose uptake into skeletal muscle, and a 100% increase of glucose uptake into white adipose tissue. Peripheral lipolysis was suppressed by 42%, and plasma insulin concentration was 26% lower due to increased insulin clearance in the high-fat-fed ACC2<sup>-/-</sup> compared to the WT mice.

Intracellular concentrations of triglycerides and long-chain acyl CoAs in liver and skeletal muscle in the high-fat-fed ACC2<sup>-/-</sup> animals were suppressed compared to the corresponding WT mice, no doubt as a consequence of the deficiency of ACC2. Moreover, diacylglycerol levels in liver and the membrane/cytosol ratio of skeletal muscle diacylglycerol were lowered by 50%.



•SERINE-THREONINE PHOSPHORYLATION OF IRS-1 BLOCKS TYROSINE PHOSPHORYLATION AND ACTIVATION OF IRS-1  
 •PI-3-KINASE ACTIVITY IS DECREASED LEADING TO DECREASED Akt2 ACTIVATION

**Figure 1 Proposed molecular mechanism by which ectopic lipid accumulation leads to insulin resistance.** Increased intracellular lipid in muscle and liver cells leads to an increase in intracellular diacylglycerol (DAG). DAG is a potent stimulator of protein kinase C, especially PKC $\theta$  in muscle and PKC $\epsilon$  in liver. Activation of a serine/threonine phosphorylation cascade leads to the phosphorylation of insulin receptor substrate-1 (IRS-1) and inhibits the activation of IRS-1 by tyrosine phosphorylation, which normally would activate phosphatidylinositol-3-kinase (PI3K). Lower PI3K activity leads to lower Akt2 activation, which then reduces GLUT4 translocation to the plasma membrane and reduces glucose uptake into muscle with resultant reduced net glucose utilization by muscle. In the liver, reduced Akt2 results in increased glucose production by reducing glycogen synthesis and increasing gluconeogenesis. These changes in liver and muscle result in the characteristic signs of insulin resistance.

The experiments of Choi et al.<sup>7</sup> showed that, by switching off the expression of the enzyme ACC2, the resulting increased oxidation of fatty acids led to improved insulin sensitivity. This finding is in support of the lipocentric hypothesis<sup>1</sup>: “ectopic lipid accumulation” is the cause of increased insulin resistance in T2DM. Similar, though less complete, results had been obtained earlier by Abu-Elheiga et al.<sup>8</sup>

#### ECTOPIC LIPID ACCUMULATION AND INCREASED INSULIN RESISTANCE IN T2DM: A POSSIBLE Akt2-MEDIATED MECHANISM

The authors<sup>7</sup> then investigated the molecular mechanism whereby accumulation of lipid in muscle and liver would cause insulin resistance. Increased serum triglycerides resulted in intracellular diacylglycerol (DAG) accumulation in muscle and liver. DAG is a strong activator of protein kinase C, in particular protein kinase- $\theta$  in muscle and protein kinase- $\epsilon$  in liver. The activated protein kinases initiate a serine/threonine

phosphorylation cascade that phosphorylates insulin receptor substrate-1 (IRS-1), inhibiting tyrosine phosphorylation of IRS-1 and activating phosphatidylinositol-3-kinase that, in turn, reduces phosphorylation and activation of Akt2. Lowered Akt2 activity affects translocation of the glucose transporter, GLUT4, leading to decreased glucose uptake into muscle cells and, hence, less glucose oxidation and glycogen synthesis. In liver, reduced Akt2 activity lowers liver glycogen synthesis and increases gluconeogenesis (Figure 1). The end result, then, would be the characteristic signs of insulin resistance: less glucose oxidation and glycogen synthesis in skeletal muscle and less glycogen synthesis and more gluconeogenesis in liver.

#### CONCLUSION

As the work reviewed here has shown, insulin sensitivity could be raised substantially by means of knocking out the gene coding for enzyme ACC2, through the resulting increase in fat oxidation.<sup>7</sup> Therefore, a therapeutic poten-

tial for combating T2DM is suggested: find an inhibitor of ACC2, which would relieve the suppression of the entry of fatty acids into mitochondria and thereby increase fatty acid oxidation. The outcome would be increased insulin sensitivity.

However, attempts to construct such inhibitors have so far failed,<sup>9</sup> mainly because of the presence of ACC1, an isozyme of ACC2. ACC1 knockout mice do not survive the embryonic stage.<sup>10</sup> Isozyme-selective inhibitors for ACC2 have not been developed as yet and remain a challenge to pharmaceutical research.

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