Insulin Stimulation of Hepatic Triacylglycerol Secretion and the Etiology of Insulin Resistance

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ABSTRACT The recent observations that insulin can either stimulate or inhibit triacylglycerol secretion by the liver, depending on prior metabolic (possibly insulinemic) state, have rationalized the many apparently contradictory observations, obtained over the past three decades, on the effects of the hormone on this aspect of hepatic metabolism. Extrapolation to the situation in vivo suggests that frequent stimulation of insulin secretion may result in a chronic stimulation of VLDL secretion, and increased delivery of acyl moieties to muscle, where they induce insulin resistance if provided in excess of the oxidative needs (mostly due to exercise) of the tissue. High fructose/sucrose diets, which also stimulate hepatic VLDL secretion, will have the same effect, especially if consumed frequently during the diurnal cycle. Due to the quantitative importance of muscle as a site for insulin-sensitive glucose metabolism, these effects may initiate the metabolic vicious cycle that results in the development of the metabolic syndrome, well in advance of overt obesity or the diagnosis of type-2 diabetes. J. Nutr. 131: 2074–2077, 2001.

KEY WORDS: • insulin resistance • obesity • type-2 diabetes • liver • VLDL • triacylglycerol

Insulin resistance is the metabolic feature common to several interrelated conditions collectively known as the metabolic syndrome X (1). In humans, insulin resistance can develop slowly and remain undiagnosed for years. However, under experimental conditions in both animals and humans, it can be induced (and reversed) very rapidly, for example, by feeding high fat (2) or high fructose (3) diets, or treating animals with an irreversible inhibitor of carnitine palmitoyltransferase I (4), the enzyme that catalyzes the first step that commits long-chain fatty acids to β-oxidation within mitochondria (5). Physiologic inhibition of this enzyme may also be associated with insulin resistance in muscle in vivo (6). Muscle is the major site of insulin-dependent glucose metabolism and is the most important tissue in the establishment of whole-body insulin resistance. Insulin resistance is invariably associated with an increase in intramyocellular triacylglycerol (TAG) content (7) as would be expected if uptake of fatty acids by muscle fibers exceeds their capacity to oxidize them (mostly through exercise), thus diverting fatty acid metabolism toward glyceride synthesis. However, it is thought that interference with insulin signaling is mediated by increased long-chain acyl-CoA and diacylglycerol levels. Intake of high fat diets results in adipose tissue also becoming rapidly insulin resistant, in parallel with TAG accumulation in hypertrophied adipocytes. TAG accumulation also occurs in pancreatic β-cells, where it is associated with disruption of glucose-induced insulin secretion (Fig. 1). Concomitantly, adaptive changes in gene expression accompany the induction of insulin resistance and favor the diversion of glucose to adipocytes (8). Triacylglycerol-rich lipoproteins (TRL) are an important source of fatty acyl moieties to oxidative muscle. However, the role of VLDL-TAG in the etiology of muscle insulin resistance has been difficult to quantify. Although muscle becomes resistant before adipose tissue, concomitantly with an increase in plasma concentration of VLDL-TAG rather than that of nonesterified fatty acids (NEFA) (9), there are conflicting observations on the reversal of insulin resistance when hypertriglyceridemia is alleviated (10,11).

Direct Effects of Insulin on Hepatic TAG Secretion. Insulin inhibits hepatic VLDL-TAG secretion indirectly through its antilipolytic action on adipose tissue. When adipose tissue becomes insulin resistant, the rate of lipolysis increases, resulting in the chronic elevation of NEFA concentrations in the plasma (which contribute directly to muscle insulin resistance) and an increased rate of secretion of VLDL-TAG by the liver. However, this describes the situation when adipose insulin resistance has already been established. What we would like to highlight is the causative role that hepatic VLDL secretion itself may have, when stimulated by insulin and/or fructose, in the etiology of muscle insulin resistance and its consequences under normal Western dietary conditions (12,13). However, determining the extent, and indeed, the direction of the intrahepatic effect of insulin on liver TAG secretion has been controversial because the effects of this hormone appear to depend on the type of liver preparation or in vivo model used.

In Vitro Experiments: Perfused Livers and Cultured Hepatocytes. In early work (14) it was shown that nutritional status is an important determinant of the rate of hepatic VLDL secretion, with very low secretion rates in perfused livers from food-deprived compared with fed rats, across a wide range of NEFA concentrations. That this difference relates to insulin status was shown by the strong positive correlation between TAG secretion rate and the insulinemic state of the donor animals (15,16). These data support the earlier propo-

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sition that hyperinsulinemia relates causatively to hypertriglyceridemia (17). In addition, acute stimulation of TAG secretion by insulin was demonstrated consistently for livers from normoinsulinemic, fed rats perfused with blood taken from similar animals (18–21). These findings were corroborated for livers isolated from fed, normoinsulinemic rats by Steiner (16). In another perfused liver study (22), when the rats were rendered insulin deficient by anti-insulin serum treatment for 8 h before isolation and perfusion of the livers, addition of insulin to the perfusate inhibited TAG secretion markedly. Although unrecognized at the time, this was the first evidence that prior insulinemic state determines the direction of subsequent insulin effects on hepatic TAG secretion. The data of Maves and Felts (14) were obtained with livers perfused with blood conveying high O₂ at physiologic flow rates; lowering of the oxygen delivery led to a loss of insulin stimulation of VLDL-triglyceride secretion, as well as other effects on bile flow and glucose uptake (21). These observations highlight the importance of experimental conditions in enabling the detection of certain effects of insulin.

Indeed, since the introduction of this culture system in the early 1980s, the above data have been overshadowed by repeated observations that insulin inhibits TAG secretion by cultured rat hepatocytes and hepatoma-derived cell lines [see review in (23)]. As a result of the generalization and extrapolation of these observations to the situation in vivo, it has become readily accepted that the effect of insulin on hepatic TAG secretion is inhibitory. However, it is important to note that, even in cultured rat hepatocytes, insulin becomes stimulatory for triglyceride secretion if the cells are cultured with the hormone for >48 h (24).

**In Vivo Experiments on Rats and Humans.** When insulin levels are acutely raised in rats, the rate of VLDL-TAG secretion is inhibited (25) as expected from the antilipolytic action of insulin on adipose tissue. However, in rats maintained under chronic hyperinsulinemia (with euglycemia) there is an increase in VLDL-TAG secretion (26). Moreover, chronic insulin treatment of Zucker diabetic fatty rats normalizes blood glucose but increases VLDL secretion (27). In humans too, acute hyperinsulinemia decreases the rate of hepatic TAG secretion (28). Most of this effect is due to the inhibition of adipose tissue lipolysis by insulin. However, when the plasma NEFA concentration is kept constant (29), or its fall is mimicked by administration of the antilipolytic drug acipimox (30), a net insulin-specific inhibition of hepatic triglyceride secretion still occurs, albeit to a smaller extent, indicating that there is a direct intrahepatic insulin-mediated inhibition of TAG secretion.

Therefore, there appears to be a conflict between the effects obtained on chronically hyperinsulinemic rats in vivo and those obtained on acutely insulin-treated humans. However, the rat and human studies were not performed under equivalent metabolic conditions because the humans fasted overnight before acute treatment with insulin. Only in one study were humans subjected to hyperinsulinemia either after an overnight fast or on three successive days of continuous parenteral carbohydrate supplementation (31); after the overnight fast, hyperinsulinemia inhibited VLDL-TAG secretion, whereas the effect of hyperinsulinemia switched to a markedly stimulatory one thereafter (31).

**Effect of Prior Metabolic State on the Effects of Insulin on Hepatic TAG Secretion.** These apparently conflicting observations were reconciled recently by experiments in which the effect of acute insulin treatment on TAG secretion was studied either in vitro—in perfused rat livers or isolated cultured hepatocytes—or in vivo in awake, unrestrained rats (32–34). The (donor) rats were fed and normoinsulinemic, were deprived of food for 24 h or were made severely insulin-deficient by streptozotocin treatment 10 d before being used. In both in vivo and perfused livers, it was found that the direction of insulin action depended on the prior physiologic state of the rats. Thus, although insulin stimulated TAG secretion in livers isolated from fed, normoinsulinemic rats, it inhibited it in livers isolated either from food-deprived or insulin-deficient rats (33). Similarly, when the effect of acute insulinization of the liver on the hepatic partitioning of fatty acid metabolism was studied in vivo, it was found that although insulin increased the proportion of metabolized acyl moieties that were secreted as VLDL-TAG in normoinsulinemic fed animals, it markedly lowered the same variable in the other two conditions (32). Therefore, both in perfused livers and in vivo, there is a switch in the direction of
insulin action on TAG secretion, from stimulatory in the insulin-replete state to inhibitory in the low insulin or insulin-deficient states (34). The mechanism responsible for this switch is currently under investigation, but it is suggested to involve the rapid turnover of an insulin-inducible liver protein required for the synthesis of one or more components of VLDL particles. It is not observed in cultured hepatocytes obtained from rats in the same three conditions; insulin always inhibits TAG secretion (33). Possible reasons for this discrepant behavior of isolated cells with respect to the situation in vivo have been discussed elsewhere (3,16,32). The previous observation that insulin becomes stimulatory for TAG secretion when hepatocytes are cultured for >48 h with the hormone (24) indicates that cultured hepatocytes require a considerable period of time to recover their in vivo responses, in this respect.

Does Preprandial Insulinemia Determine the Direction of Insulin Action on Hepatic VLDL-triglyceride Secretion? The observations in (22) and (35) suggest that prior insulin status may be an important determinant of the direction of subsequent insulin action on hepatic TAG secretion. Extrapolation to the prandial situation in vivo would imply that meal-induced insulin secretion that occurs after a long interprandial period, during which insulin is allowed to fall to low basal levels, would have the effect of directly inhibiting hepatic VLDL-TAG secretion. By contrast, frequent successive episodes of insulin secretion that accompany short interprandial intervals (in the preinsulin resistant state) would be expected chronically to stimulate hepatic VLDL-triglyceride secretion. This would exacerbate postprandial hyperlipemia and, therefore, would be expected to be proatherogenic. In this respect, there is a substantial body of evidence that insulin per se, either of endogenous or exogenous origin, is proatherogenic [see (36) for review]. Increased postprandial VLDL-TAG delivery to muscle would also be expected to contribute to postprandial hyperglycemia, a predictive factor for the development of cardiovascular disease (37).

Relevance to Western-type Dietary Patterns. In affluent societies, in addition to increased dietary fat and sucrose intake, there has also been the adoption of a pattern of frequent snacking that results in a quasi-continuous postprandial state for most of the day (12,13). This prevents the attainment of low basal interprandial insulin levels even in normal individuals. Consequently, subsequent meal-induced insulin release occurs against a background of an already high, basal insulin concentration, conditions that are now known to be conducive to further insulin-mediated stimulation of VLDL-TAG secretion (32–34). There is substantial evidence that as a result, insulin stimulation of hepatic VLDL-TAG secretion may be sufficiently chronic to induce muscle insulin resistance. Thus, insulin resistance in muscle is associated with hypertriglyceridemia before resistance develops in adipose tissue or in the liver (38,39). Similarly, insulin resistance can be detected in nonobese people before any elevation of plasma NEFA concentrations, but at a time when VLDL-TAG secretion rate is already increased (40). The development of insulin resistance does not depend on the ingestion of a high fat diet; indeed, the degree of insulin resistance is exacerbated by sucrose-containing, high carbohydrate diets (41,42). Moreover, the degree of postprandial hypertriglyceridemia in response to a standard meal is markedly increased after a short period of adaptation to a high carbohydrate diet (43). Conversely, feeding diets rich in (n-3) fatty acids, which suppress hepatic VLDL secretion relative to saturated fatty acids, prevent insulin resistance (44). It is of interest, therefore, that the stimulatory effect of insulin on VLDL-TAG secretion in livers of fed, normoinsulinemic rats is abolished when the donor animals are fed a diet enriched in fish oil (45). This suggests that (n-3) polyunsaturated fatty acids may mediate protection against insulin resistance, at least in part, by preventing the stimulation of hepatic TAG secretion by insulin. The observation that pravastatin treatment of hypertriglyceridemic patients results in a 30% decrease in the incidence of type-2 diabetes over a 10-y follow-up period (46), combined with the observation that statins inhibit apolipoprotein B secretion by the liver (47), also suggests a role for an elevated rate of VLDL-TAG secretion in the etiology of insulin resistance syndrome. In accordance with this, Steiner (10) showed that gemfibrozil treatment of insulin-resistant patients reverses, in parallel, both their pronounced hypertriglyceridemia and the severity of insulin resistance, although no such correlation was observed in more mildly hyperlipidemic patients (11). Thus, the relative importance of NEFA and TRL in delivering acyl moieties to muscle may change during the progression of the metabolic syndrome. In this respect, it is to be emphasized that although insulin resistance in muscle may be induced before that in adipose tissue (48), once it is established in the latter (and in the absence of pharmacologic intervention, e.g., with a peroxisome proliferator-activated receptor-γ agonist), resistance may not be fully reversed before plasma NEFA levels are normalized.

Summary and Conclusions. From the above, we conclude that chronically enhanced hepatic VLDL-TAG secretion may be sufficient to initiate the metabolic vicious cycle, leading eventually to the establishment of the insulin resistance syndrome. Prolonged diurnal exposure of the liver to high basal insulin per se, through its stimulatory effect on hepatic VLDL secretion, may be responsible for the initial induction of muscle insulin resistance, under conditions generated by a Western-style pattern of food intake. High fructose (sucrose) intake, would be anticipated to have the same effect, especially if frequent. It would appear that an emphasis on a low fat content of the diet may not be sufficient to avoid the development of muscle insulin resistance and its consequences. Nutritional recommendations may have to recognize this role of fructose and to encourage the adoption of a diurnal pattern of dietary intake that allows the liver to be exposed to low basal levels of plasma insulin for several hours in between meals, as part of a strategy to combat the emerging epidemic of type-2 diabetes, especially in adolescents (36).

LITERATURE CITED
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