Insulin Sensitivity in Skeletal Muscle Regulated by a Hepatic Hormone, HISS

W. Wayne Lautt

Abstract/Résumé

The current state of the HISS (hepatic insulin sensitizing substance) hypothesis is briefly outlined. In the postmeal absorptive state, 50–60% of the glucose storage action of insulin is accounted for by the actions of HISS released from the liver and acting on skeletal muscle. Hepatic parasympathetic nerves permissively regulate the ability of a pulse of insulin to release HISS, thereby potentiating the impact of insulin in the fed state. HISS release in response to insulin decreases progressively with fasting to create a physiological state of HISS-dependent insulin resistance. HISS release is regulated by parasympathetic nerves via muscarinic receptors and nitric oxide, and insulin resistance of skeletal muscle produced by hepatic denervation is reversed by intraportal but not intravenous acetylcholine or a nitric oxide donor. It is suggested that HISS-dependent insulin resistance occurs in animal models including sucrose-fed rats, spontaneously hypertensive rats, chronic liver disease, fetal alcohol effect in the adult offspring, and type 2 diabetes.

L’état actuel des connaissances concernant la prétendue substance hépatique sensibilisant à l’insuline (HISS) est présenté brièvement. Après un repas, 50–60% des actions de l’insuline pour stocker le glucose sont faites par le HISS issu du foie et agissant sur le muscle. L’influx des nerfs parasympathiques hépatiques permet à l’insuline de libérer le HISS pour potentialiser l’effet de l’insuline en période d’absorption. La libération du HISS en réponse à l’insuline diminue graduellement avec le jeûne jusqu’à créer une insulinorésistance.
dépendante du HISS. La libération du HISS est contrôlée par des fibres parasymphathiques agissant sur des récepteurs muscariniques et par le monoxyde d’azote; l’insulinorésistance du muscle squelettique obtenue après la dénervation du foie est renversée par l’acétylcholine dans la circulation portale et non par celle dans la circulation veineuse ou par le monoxyde d’azote d’un donneur. Nous stipulons que l’insulinorésistance dépendante du HISS se manifeste chez des modèles animaux dont les rats nourris au sucrose, les rats spontanément hypertendus, la maladie hépatique chronique, l’effet de l’alcool fœtal chez le descendant adulte, et le diabète de type 2.

Introduction

In this brief review, data are reported that are consistent with the working hypothesis that proposes a novel mechanism regulating insulin sensitivity in skeletal muscle which, when nonfunctional, leads to severe insulin resistance of the type seen in type 2 diabetes.

The HISS Hypothesis

Some 50 to 60% of the glucose disposal effect of an injection of insulin is actually dependent on the action of a second hormone, the hepatic insulin sensitizing substance (HISS), which is released from the liver and stimulates glucose uptake in skeletal muscle. Blocking of HISS action results in HISS-dependent insulin resistance (HDIR). HDIR may account in whole or in part for insulin resistance in many clinical conditions including type 2 diabetes, obesity, chronic liver disease, fetal alcohol effects, and chronic hypertension (Lautt, 1999, 2004). HISS release and HISS action is maximal in the immediate postprandial period and declines progressively with the duration of fasting (Lautt et al., 2001). In a manner not yet well understood, parasymathetic nerves are activated in the liver following a meal to signal the prandial status. In this condition, the insulin which reaches the liver causes pulsatile release of HISS. The HISS enters the bloodstream and stimulates glucose uptake primarily into skeletal muscle (Xie and Lautt, 1996a, 1996b). Thus, after a meal the release of HISS from the liver causes a dramatic stimulation of glucose storage in skeletal muscle and effectively doubles the glucose disposal effect of a pulse of insulin. Insulin resistance occurs when HISS action is absent, and this condition is referred to as HISS-dependent insulin resistance (HDIR).

Rapid Insulin Sensitivity Test (RIST)

In order to quantitate HISS-dependent and HISS-independent insulin action, it is necessary to use methods that have been validated to detect these effects. The initiation of the HISS hypothesis began with studies in cats utilizing the insulin tolerance test (Xie et al., 1993), but in subsequent studies we felt the need to develop a method that avoided the hypoglycemia which occurs in the insulin tolerance test. We thus developed the RIST, which is a transient euglycemic clamp in response to a pulse of insulin administration. The timeline of the RIST is shown in Figure 1. The RIST index is simply the amount of glucose that had to be administered in order to maintain euglycemia after the bolus administration of insulin.
Typically our standard test dose of 50 mU/kg results in a requirement for approximately 230 mg glucose/kg body weight.

This method allows quantitation of HISS-dependent and HISS-independent insulin action in two dramatically different ways. The use of the RIST index demonstrates that 55% of the glucose disposal produced by insulin is accounted for by HISS by the fact that it produces a normal control RIST followed by a second RIST carried out after the blocking of HISS action. The second form of analysis involves subtraction of the two dynamic curves which reveals the dynamic action of HISS shown to begin within minutes of insulin administration, reaching a peak at about 15 min and continuing for approximately 9 min after the direct action of insulin is completed (Lautt et al., 2001).

**Figure 1.** Rapid insulin sensitivity test (RIST) time line. Three stable arterial glucose levels determined at 5-min intervals established the ideal euglycemic baseline. Intravenous insulin infusion is administered over 5 min with the glucose infusion and first arterial glucose sample beginning after 1 min of glucose infusion. A variable intravenous glucose infusion is adjusted to maintain euglycemia based on arterial samples taken at 2-min intervals throughout the test period. The RIST index is the total amount of glucose infused to maintain euglycemia over the test period, which is terminated when no further glucose infusion is required. The test period for the standard insulin dose in rats (50 mU/kg) is 30 min, but different durations may be needed for higher doses, different conditions, or different species (from Lautt, 1999).
The euglycemic clamp differs from the standard clamp in that it is transient. This was a fortuitous development; the rationale was that insulin release normally occurs in a pulsatile manner, and hormones released in a pulsatile manner are best studied by pulsatile administration. A recent description of insulin pulsatile secretion in humans indicates that very large pulses of a 10-min period occur even during sleep, with mean amplitude of oscillations reaching 50% of mean levels even in conditions with continuous enteral nutrition (Simon and Brandenberger, 2002). Whereas pulses of 50 mU/kg insulin can be shown to release HISS several times in a row, continuous administration at the rate of 1 mU/min for 50 minutes blocks the release of HISS when tested using the RIST after the completion of the clamp (Reid and Lautt, 2004).

This observation may relate to similar demonstrations in which gonadotropin hormones administered in a pulsatile manner stimulate testosterone secretion, yet the same dose administered continuously results in chemical castration and is used in treating prostate hypertrophy (Belchetz et al., 1978). It must be cautioned, however, that there may be species or other conditions when this precaution may not apply, in view of the recent confirmation of the essentials of the HISS hypothesis in the dog as demonstrated using the prolonged hyperinsulinemic euglycemic clamp (Moore et al., 2002).

The primary problem with the RIST methodology is the need for rapid sampling of arterial blood. This was overcome by the use of an arterial-venous vascular shunt. The shunt has been tested in various combinations, shunting from the femoral artery to the femoral vein or the carotid artery to the jugular vein. The A-V shunt allows for direct sampling of arterial blood through puncture into the silicone sleeve of the shunt. Further advantages include the ability to record blood pressure in the shunt continuously to determine shunt patency, and the fact that arterial pressure can be measured by brief occlusion of the venous outflow. In addition, intravenous drugs can be infused directly into the shunt.

Although the insulin tolerance test is equally effective in detecting HISS-dependent and HISS-independent actions in cats (Xie et al., 1993) and rats (Reid et al., 2002), the ITT cannot produce multiple repetitions nor can it show the dynamic actions of HISS. In contrast, the RIST can provide quantitative RIST indexes as well as dynamic patterns of HISS action. The RIST can be repeated as many as six times sequentially in the same animal. It can be carried out in the anesthetized (pentobarbital) or conscious state equally well (Latour and Lautt, 2002a).

**Permissive Nature of the Parasympathetic HISS Release**

In the fed state, insulin action can be reduced by 55–60% by elimination of the hepatic parasympathetic nerve functions (Lautt et al., 2001) and is referred to as HISS-dependent insulin resistance (HDIR). HDIR can be achieved by surgical denervation of the liver in cats (Xie and Lautt, 1996a; Xie et al., 1993), rats (Xie and Lautt, 1996b), and dogs (Moore et al., 2002). Denervation can be carried out at the anterior hepatic plexus as shown for all three species, or at the hepatic branch of the vagus or cervical vagus nerves as demonstrated in rats (Latour and Lautt, 2002b).
The permissive nature of the parasympathetic signal has been demonstrated by the methods used to reverse denervation-induced insulin resistance. HISS-dependent insulin resistance (HIISR) is produced by surgical denervation of the liver and reversed to normal levels by intraportal administration of acetylcholine. Acetylcholine infusion is started prior to administration of the insulin bolus for the RIST and shows no significant alterations in glucose metabolism. Against this background of continuous intraportal Ach infusion (2.5 µg/kg/min) in both rats and dogs, the effect of insulin administration on HISS action is restored. Thus the parasympathetic nerves appear to provide a feeding signal that permits insulin to release HISS. The importance of this permissive signal can be recognized by noting that this signal serves as a method of controlling the release of HISS according to the prandial status of the animal.

**Skeletal Muscle as the Resistant Tissue**

Insulin resistance in type 2 diabetes and several other models of insulin resistance are mainly restricted to the skeletal muscle. The HISS hypothesis proposes that the insulin resistance in these cases is caused by lack of HISS action, not insulin, on skeletal muscle. This is consistent with the observation that mice with a knockout of the insulin receptor in muscle show normal glucose tolerance (Bruning et al., 1998). The demonstration that HISS action occurs at the skeletal muscle site has been shown in cats (Xie and Lautt, 1996b) and dogs (Moore et al., 2002).

I propose that HDIR results in an inappropriate nutrition partitioning, such that glucose storage in skeletal muscle is reduced and the resulting hyperglycemia and hyperinsulinemia results in the nutrients being partitioned as increased lipid production by the liver and accumulation in peripheral adipose tissues. In this regard, the focus on fasting metabolism in assessing diabetic status seems inappropriate.

**Prandial Status**

In rats that have been fasted and then re-fed for a 2-hr period, HISS action accounts for 50–60% of the glucose disposal effect of insulin. The HISS-dependent action decreases progressively to become insignificant after 24 hours of fasting in rats. The species differences in this regard appear to be major in that studies with cats (Xie and Lautt, 1996a; Xie et al., 1993) and dogs (Moore et al., 2002) were carried out after 18-hr fasts and still demonstrated 25–35% HISS-dependent insulin action. The rat responds quite differently to fasting in that after 24 hours its glycogen stores are fully depleted, whereas both the cat and dog have quite different feeding patterns and different metabolic responses to fasting, with the glycogen levels in dogs remaining normal after a 24-hr fast (Moore et al., 2002). The temporal relationship of fasting and physiological development of HDIR in humans is unknown.

The current paradigm for insulin resistance focuses on peripheral defects in insulin signaling with the majority of studies being carried out in the fasted state. While there can be no question that diabetes imparts an enormous risk factor for
the development of cardiovascular disease, a continued focus on the fasting state appears misdirected. It has been suggested that hyperglycemia-induced overproduction of superoxide by the mitochondrial electron-transport chain accounts for the four main molecular mechanisms implicated in glucose-mediated vascular damage associated with blindness, renal failure, nerve damage, atherosclerosis, stroke, and limb amputation (Brownlee et al., 2001).

The importance of the postmeal rather than the fasting metabolic status is amply demonstrated in several recent studies. The relationship between HBA1c and plasma glucose in patients with type 2 diabetes was determined at four time points during the day and found to be significantly predicted by plasma glucose levels measured only at postlunch and extended postlunch (5 hours) time points (Avignon et al., 1997). The strongest age- and sex-adjusted relative risk for all-cause and cardiovascular mortality were associated with 2-hr postload plasma glucose levels (deVegt et al., 1999). Increased mortality risk has been associated with 2-hr postload plasma glucose levels to a much greater extent than with fasting plasma glucose (DECODE Study Group, 1999; Hanefeld et al., 1996).

### Pharmacology of HISS Release

Although the chemical identity of HISS has not yet been determined, HISS action can be clearly quantitated. The RIST index is decreased by 50–60% in fasted rats by blocking any of several steps in the pathway of regulation. Surgical denervation has already been described. A similar degree of HDIR is shown by administration of atropine in cats (Xie and Lautt, 1995) and rats (Lautt et al., 2001). Surprisingly, insulin resistance produced by atropine is not altered by the addition of hepatic denervation, nor does the insulin resistance induced by hepatic denervation show further impairment by atropine. The surprise is based on the fact that the denervation of the liver will result in interference with both afferent and efferent signals from the liver, and the anterior plexus denervation will also result in sympathetic denervation. In contrast, the high doses of atropine used will certainly result in blocking the action of muscarinic receptors throughout the body.

Because many biological effects of acetylcholine appear to be mediated by nitric oxide release, we tested the hypothesis that the parasympathetic reflex release of HISS in the liver was also mediated through the generation of nitric oxide (Sadri et al., 1997). Administration of the nitric oxide synthase antagonist L-NAME, at a dose of 2.5 mg/kg (i.v.), produces full but transient insulin resistance whereas a dose of 5 mg/kg leads to full insulin resistance lasting at least 2 hours (Sadri et al., 1997). The nitric oxide action depends on cGMP and adequate (fed) levels of hepatic glutathione (Guarino et al., 2003).

The possibility that the insulin resistance was produced by a direct effect on skeletal muscle was countered by the demonstration that a smaller dose of L-NAME (1 mg/kg) administered intravenously results in an insignificant effect on insulin sensitivity, whereas the same dose of L-NAME administered intraportally results in a dramatic insulin resistance (Sadri and Lautt, 1999). The approach of using comparative responses to intravenous vs. intraportal drug administration allows the site of action to be assessed as hepatic or extrahepatic. If the liver is the target
organ, intraportal administration will have a greater effect than the same dose administered intravenously; if extrahepatic tissues are the target, intraportal administration will have a lesser effect.

Reversal of insulin resistance produced by the blocking of nitric oxide synthase using L-NMMA also confirmed the hepatic site of NO action. The NO donor is administered prior to the insulin and does not result in glucose uptake, but the ability to restore HISS release in response to insulin confirms the permissive nature of the parasympathetic signal. The NO donor, SIN-1, reversed the insulin resistance produced by L-NMMA when administered into the portal vein, but not intravenously. In addition, SIN-1 is able to reverse the insulin resistance produced by surgical denervation of the liver (Sadri and Lautt, 1999). SIN-1, but not nitroprusside, is able to reverse HDIR produced by NOS antagonism (Guarino et al., 2001). Thus, while NO may produce some modest vascular responses to very prolonged infusion of high levels of insulin, the ability of NO synthase antagonists to produce insulin resistance is not a direct effect on skeletal muscle but an indirect effect caused by the blocking of insulin-mediated release of HISS from the liver.

**Conclusion**

Postprandial storage of glucose is largely achieved through glycogen in skeletal muscle. Under permissive control of the hepatic parasympathetic nerves, insulin causes the release of HISS from the liver to stimulate glucose uptake into skeletal muscle. HISS release is decreased: physiologically in response to fasting; by ablation of the nerve actions using denervation or pharmacological blockers of hepatic muscarinic receptors, cyclooxygenase or nitric oxide synthase; and by disease processes including fetal alcohol exposure (Minuk et al., 1998; Sadri et al., 2003), sucrose feeding (Ribeiro et al., 2001a), and hypertension (Ribeiro et al., 2001b). Absence of HISS action leads to a state of insulin resistance typical of type 2 diabetes. Current approaches include attempts to reverse HDIR through drug therapy.

**References**


DECODE Study Group, on behalf of the European Diabetes Epidemiology Group. (1999).


*Received June 19, 2002; accepted in final form December 3, 2002.*