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Hepatalin: the missing link in prediabetes, obesity, and type 2 diabetes

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Abstract

Hepatalin is a hormone secreted by the liver in response to pulses of insulin after a mixed nutrient meal, but only if the liver receives two permissive synergistic feeding signals from the stomach. Hepatalin stimulates glucose uptake and storage as glycogen in skeletal muscle, heart, and kidney but not liver, intestines, or adipocytes. Insulin acts primarily on liver and fat. Reduced hepatalin action results in postprandial hyperglycemia, compensatory elevation of insulin secretion, and a resultant shift in partitioning of nutrient energy storage from glycogen in muscle, to fat. Chronic hepatalin suppression leads to a predictable chronology of dysfunctions, first diagnosable as Absence of Meal-induced Insulin Sensitization (AMIS) which progresses to prediabetes, adiposity, and type 2 diabetes. The focus on nutrient partitioning and the role of hepatalin allows AMIS to be diagnosed, prevented, and treated, including through the use of lifestyle interventions.

Key words: AMIS, hepatalin, HISS, prediabetes, obesity

1. Is there a missing link?

Approximately 450 million people around the world have currently been diagnosed with type 2 diabetes (International Diabetes Federation 2021), and those are only the ones with access to conventional diagnostics. Since the discovery of insulin 100 years ago, the paradigm for dealing with diabetes has focused on insulin, with blood glucose levels as the primary diagnostic and the index of therapeutic control. A multitude of pharmaceutical interventions have come and gone, but the "pandemic" of obesity and type 2 diabetes continues to accelerate, threatening to overwhelm health care capacity in even the wealthiest countries. Insulin analogs and secretagogues have been shown to lower blood glucose levels, but progression to the pathologies associated with diabetes is only delayed, not prevented. The problem is that the focus of research and intervention has not been on the initiating event. While standard screening has been based on data from the stable fasted state, the actual dysfunction is in the dynamic postprandial processing of the nutrient energy from a meal, that is, in the fed state. By the time the diagnosis of type 2 diabetes is made based on fasting glucose levels or HbA1c, the process of what we have termed the Absence of Meal-induced Insulin Sensitization Syndrome (AMISS) is well underway (Section 8). There is clearly a missing link in the understanding of this disease.

2. Postprandial focus

The current approach of screening for type 2 diabetes using the fasting metabolic status is not effective. Ceriello (2000) concluded that, "… paradoxically, the vast majority of the studies on cardiovascular disease risk factors have been conducted by measuring them in strictly fasting conditions. This simply means that most of the data available to date may not reflect the real situation". Postprandial hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and oxidative stress have all been reported to be independent risk factors for cardiovascular disease (reviewed in Lautt 2007). Similarly, these postprandial, rather than fasted metabolic markers, are also better indicators of prediabetic and diabetic dysmetabolism.

2.1. Postprandial hyperglycemia

The strongest age- and sex-adjusted risk for all-cause and cardiovascular mortality has been associated with 2 h postload plasma glucose levels to a much greater extent than with fasting plasma glucose (Simon et al. 1987; Hanefield et al. 1996; de Vegt et al. 1999; DECODE Study Group 1999; Shaw et al. 1999; Vaccaro et al. 1999; Engelgau et al. 2000; Simon and Brandenberger 2002). Postprandial plasma glucose has been shown to be a determinant of both onset and development of nephropathy in type 2 diabetic patients (Shichiri et al. 2000). Acute hyperglycemia was associated with an acute increase in clotting factor VII (Ceriello et al. 1988) and enhanced thrombin activity that was proportional to the level of hyperglycemia (Ceriello et al. 1996).

2.2. Postprandial hyperlipidemia

Postprandial hyperlipidemia is prevalent in diabetic patients even with normal fasting triglyceride concentrations (Mero et al. 1998). The postprandial triglyceride increment

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after a fat meal was paralleled by an inflammatory leukocyte increment due to an increase in neutrophils in the postprandial first 2 h (Van Oostrom et al. 2003). Low density lipoprotein oxidation increased after meals and was directly related to the degree of hyperglycemia (Ceriello et al. 1999). Postprandial hypertriglyceridemia has been recognized as an independent predictor of cardiovascular pathology (Anderson et al. 2001).

2.3. Postprandial hyperinsulinemia

Ingelsson et al. (2005) discussed postprandial hyperinsulinemia as a risk factor for cardiovascular disease and proposed several possible mechanisms. The current paradigm accounting for insulin resistance and hyperinsulinemia is based on the assumption that the whole-body glucose uptake response to insulin is due to a direct effect of insulin, and that insulin resistance represents a decrease in this direct effect. In this review the case is made that a significant proportion of glucose uptake results from an indirect action of insulin, based on insulin-mediated stimulation of secretion of a hormone from the liver that activates glucose uptake mainly in skeletal muscle (Section 3). Thus, insulin has a direct effect on glucose uptake and an indirect effect acting via this hepatic hormone. Reduction in either direct or indirect glucose uptake will result in compensatory hyperinsulinemia and subsequent metabolic consequences (Section 5.1). This paradigm allows re-interpretation of much of the literature.

2.4. Postprandial reactive oxidative stress

From his review in 2001, Brownlee concluded that hyperglycemia-induced overproduction of superoxide by the mitochondrial electron transport chain likely accounts for the main molecular mechanisms implicated in glucose-mediated vascular damage associated with blindness, renal failure, nerve damage, atherosclerosis, stroke, and limb amputation (Brownlee 2001). Ceriello (2000) suggested that increased postprandial oxidative stress may be the common pathway through which the majority of diabetic complications occur.

Although postprandial hyperglycemia, hyperinsulinemia, hyperlipidemia, and oxidative stress are well accepted cardiovascular risk factors associated with prediabetes and type 2 diabetes, the mechanism connecting them has been unknown. There is a missing link.

3. Hepatalin and the AMISS paradigm

The bold claim made in this review is that the discovery of a hepatic hormone that accounts for the majority of glucose uptake after feeding, represents the missing link in understanding the etiology and mechanism of metabolic dysfunction in prediabetes, which in turn leads to obesity and type 2 diabetes, followed by the full array of associated pathologies. Until this review, the tentatively named putative hormone has been referred to in the scientific literature as Hepatic Insulin Sensitizing Substance (HISS). Over time, the biological activity of this hormone has been quantified, and descriptions of its regulatory systems, its actions, and its role in pathology have been published. Absence of postprandial action of this hepatic hormone accounts for postprandial hyperglycemia, hyperinsulinemia, hypertriglyceridemia and increased oxidative stress. This review has the objective of explaining what is known about this new paradigm. As research continues, I introduce the new name “hepatalin” in this review.

The discovery of hepatalin and ongoing research have presented opportunities for development of novel early diagnosis, prevention, and treatment of prediabetes and type 2 diabetes.

3.1. The early science of hepatalin

Electrical stimulation of hepatic parasympathetic nerves resulted in the net fasting efflux of glucose from the liver to be completely blocked within minutes (Lautt and Wong 1978). Hepatic parasympathetic neuropathy was suggested to initiate type 2 diabetes via an ineffective parasympathetic signal and a potential therapeutic direction was proposed (Lautt 1980). In the absence of knowledge of the existence of hepatalin, this suggestion was not pursued by us or others.

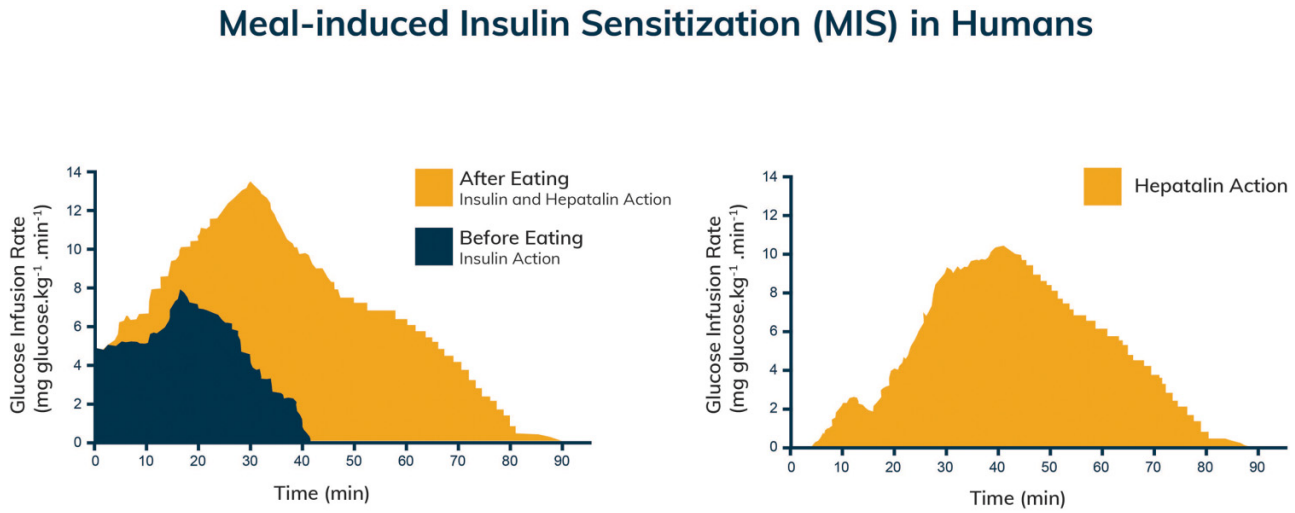
In 1993 (Xie et al. 1993), we reported that denervation of the anterior hepatic nerve plexus resulted in a decreased whole body hypoglycemic response to insulin. The initial hypothesis was that the impaired response to insulin was a consequence of impaired hepatic glucose uptake. However, in 1996 (Xie and Lautt 1996*a*), we reported that surgical denervation of the liver, or intraportal atropine administration, resulted in a reduced glucose uptake response to insulin in skeletal muscle but not liver or organs draining into the portal vein, and that the effect on the hindlimbs could be restored by intraportal infusion of acetylcholine (ACh) directly to the liver at doses too low to recirculate to the systemic circulation (Xie and Lautt 1996*b*). The neurogenic signal from the liver (Section 6.1) to the periphery was transmitted via the blood. The action of a previously unknown hormone had been revealed.

The research that followed for the next 30 years was pure basic research focused on understanding the role and control of this newly discovered hormone. The first demonstration of hepatalin action in humans was carried out by the Macedo team in Lisbon. The findings were strikingly similar to the data from several other species (Patarrao et al. 2008) (Fig. 1 in humans; Fig. 3 in rats).

3.2. Terminology—HISS, MIS, AMIS, AMISS, and hepatalin

As studies of this new paradigm progressed, new methodologies and terminology were required. HISS was the tentative name we gave to the substance that was secreted from the liver and acted on skeletal muscle to potentiate the response to insulin after feeding (Lautt 1998). The first review of the HISS story in 1999 (Lautt 1999) left the question open as to whether HISS sensitized muscle to insulin or had direct glucose uptake action, thus the term “sensitizing substance” was tentatively retained for convenient reference. It was later shown that the hepatic hormone does not interact with the insulin signal, but has independent and selective

Fig. 1. Rapid Insulin Sensitivity Test (RIST) in fasted and fed humans showing Meal-induced Insulin Sensitization (MIS). The rate of glucose infusion required to maintain baseline euglycemia is shown for healthy male volunteers after intravenous administration of a bolus of 50 mU/kg insulin in the 24 h fasted state, and 100 min after consumption of a mixed test meal. The mean RIST curves were obtained by averaging glucose infusion rates at 0.1 min intervals. The RIST index increased from a fasted level of 215 to 681 mg glucose/kg after feeding due to the action of hepatalin. Hepatalin action shown in the second panel is calculated from the difference in the curves in the first panel. (Figure modified from Patarrao et al. 2008.)



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action on glucose uptake, and is tightly regulated by different neuroendocrine signals. Accordingly, to recognize the relationship between the liver and insulin, we more appropriately named this hormone hepatalin.

As a result of hepatalin action, the whole-body glucose uptake response to administration of insulin is at least doubled after a meal. Thus, the glucose uptake response to a pulse of insulin consists of a direct action of insulin and an indirect action mediated by hepatalin that results in a "sensitization" of the whole-body glucose uptake response to postprandial insulin. This response to insulin does not occur in the fasted state. We named this response Meal-induced Insulin Sensitization (MIS). If hepatalin secretion is absent after a meal, we referred to this condition as Absence of Meal-induced Insulin Sensitization (AMIS). Finally, the predictable progression of related pathologies seen with chronic AMIS has been referred to as the AMIS Syndrome (AMISS) (Ming et al. 2009; Lautt et al. 2010).

4. Quantifying dynamic insulin and hepatalin action, the RIST

Endogenous insulin is secreted in pulses of oscillations of about 13 min in both humans and rats (Lang et al. 1979; Chou et al. 1991) and 9 min in monkeys (Goodner et al. 1977). Increased insulin secretion is associated with an increase in magnitude, not frequency. Non-pulsatile administration is not physiological and results in a linear duration-dependent inhibition of hepatalin action beginning at infusion duration of 10 min, with insignificant hepatalin action remaining after 1 h of constant infusion (Reid and Lautt 2004). Hepatalin action can, therefore, not be assessed by the classical 2–3 h of constant infusion of insulin used in the "gold standard" hyperinsulinemic euglycemic clamp.

The dynamic action of insulin and hepatalin can be determined using the insulin tolerance test based on the degree of induced hypoglycemia in the first 30 min after injection and before counter-regulatory mechanisms act to restore glucose to basal levels (Reid et al. 2002; Afonso et al. 2016; Martins et al. 2016). However, the ensuing hypoglycemia limits the use of this test. To avoid the hypoglycemia, and to detect the dynamic response to pulses of insulin, the Rapid Insulin Sensitivity Test (RIST) was developed and has been used in cats (Xie et al. 1996), anesthetized or conscious rats (Lautt et al. 1998; Latour and Lautt 2002*b*), mice (Latour and Chan 2002; Latour and Lautt 2002*b*), rabbits (Peitl et al. 2006), and humans (Patarrao et al. 2007, 2008, 2012).

The RIST is a rapidly sampled and adjusted euglycemic clamp to quantify the glucose uptake response to a pulse of insulin. The RIST provides reproducible test scores up to four consecutive times in anesthetized rats (Lautt et al. 1998). An operating procedure has been described for the RIST in animals (Lautt et al. 1998) and humans (Patarrao et al. 2008). The RIST index is the amount of glucose that is required to be infused to compensate for glucose uptake while maintaining a constant arterial glucose level after administration of a pulse of insulin. An advantage of using the RIST index is that protocols can be designed to exploit the multiple and reproducible tests in anesthetized and conscious preparations, providing both greater statistical power and economy of animal use. A disadvantage of either the RIST or insulin tolerance test is that the procedure must be conducted against a background of steady baseline glycemia; therefore, RISTs conducted after a meal are done 1.5–2 h post-feeding.

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5. Evidence of meal-induced insulin sensitization (MIS)

The concept of MIS was derived from the observation that the dynamic glucose uptake response to insulin, determined after a 24 h fast, is at least doubled when tested 100 min after administration of a mixed meal in conscious rats (Latour and Lautt 2002*b*; Sadri et al. 2006) and humans (Patarrao et al. 2007, 2008, 2012). This greatly amplified response to insulin is through the action of the hormone, hepatalin, and is regulated by parasympathetic nerves.

The effect of feeding on insulin action (MIS) was reported in 2001 (Lautt et al. 2001). This increased postprandial response to insulin was attributed to a previously unknown hormone which we tentatively named Hepatic Insulin Sensitizing Substance (HISS). An overview of its physiology was described by Lautt in 1999. Several reviews followed the progress in concepts, including: the effects of adult and fetal alcohol exposure on MIS (Ting and Lautt 2006); the effect and interactions of diet, exercise, and antioxidant supplementation on the AMIS syndrome and hepatalin action (Chowdhury et al. 2013*b*); adiposity as an early component of the AMIS syndrome (Lautt and Wang 2014); AMISS and fatty heart and liver (Lautt et al. 2015); and a review of the role of the parasympathetic nerves in metabolic regulation in health and disease (Macedo et al. 2013) (Fig. 1).

5.1. Nutrient partitioning

The nutrient energy contained in a meal must be rapidly processed and stored for use between meals. The primary storage sites are as glycogen in liver and muscle, and as lipids. Insulin action is primarily on liver (glycogen formation with excess going to lipids) and adipose tissue. Hepatalin action is primarily on glycogen formation in muscle, heart, and kidney (Section 8). A healthy balance between the actions of hepatalin and insulin results in the majority of nutrient partitioning going to glycogen in muscle. As hepatalin action decreases, the partitioning shifts from glycogen to lipids, with accumulated adiposity and development of an early prediabetic state.

Regulation of nutrient partitioning is dynamic and affected by many factors: physiological, pharmacological (Sections 6.4 and 8.6), toxicological (Section 8.10.1), and lifestyle, including stress (Section 8.5), diet (Sections 8.2 and 8.4), exercise (Section 8.3), gestation (Section 8.10.2), and age (Section 8.1) (Fig. 6).

6. Regulation of hepatalin production

Hepatalin secretion from the liver is regulated by three signals mediated by parasympathetic nerves, elevated hepatic glutathione (GSH), and pulses of insulin (Fig. 2).

6.1. The parasympathetic signal

The nerve signal is activated by the presence of a mixed-nutrient meal in the stomach or upper GI tract (Afonso et al. 2016) and travels via the vagus nerve to the hepatic branch of the parasympathetic nerves (Latour and Lautt 2002*a*). Neither pure glucose nor sucrose activate the nerve signal (Sadri et al. 2006; Afonso et al. 2016). Also, given separately, glucose, amino acids, and lipids do not activate MIS (Afonso et al. 2016).

The hepatic parasympathetic nerves release ACh, acting on muscarinic receptors (Xie and Lautt 1995*a*, 1995*b*) to activate nitric oxide synthase, leading to hepatic nitric oxide (NO) synthesis, which activates hepatic guanylate cyclase (Guarino et al. 2004). Atropine or surgical denervation of the hepatic parasympathetic nerves blocks hepatalin secretion which can be reversed by either a cholinergic agonist (Xie and Lautt 1996*a*, 1996*b*) or an NO donor (Sadri and Lautt 1999); the effect of blocking NO synthase production of NO can be reversed by an NO donor but not ACh (Guarino et al. 2001). Thus, NO production is dependent on a cholinergic signal.

The parasympathetic signal is absent in obstructive hepatitis. Chronic bile duct ligation resulted in AMIS that was reversed by intraportal infusion of ACh (Lautt and Xie 1998), suggesting that the GSH signal had not been blocked. In contrast, diet-induced AMIS results in absence of both the nerve and GSH signal (Lautt et al. 2011) (Section 6.4, Fig. 5). The earliest manifestation of diabetic autonomic neuropathy is associated with parasympathetic abnormality (Hosking et al. 1978) (Section 8.1).

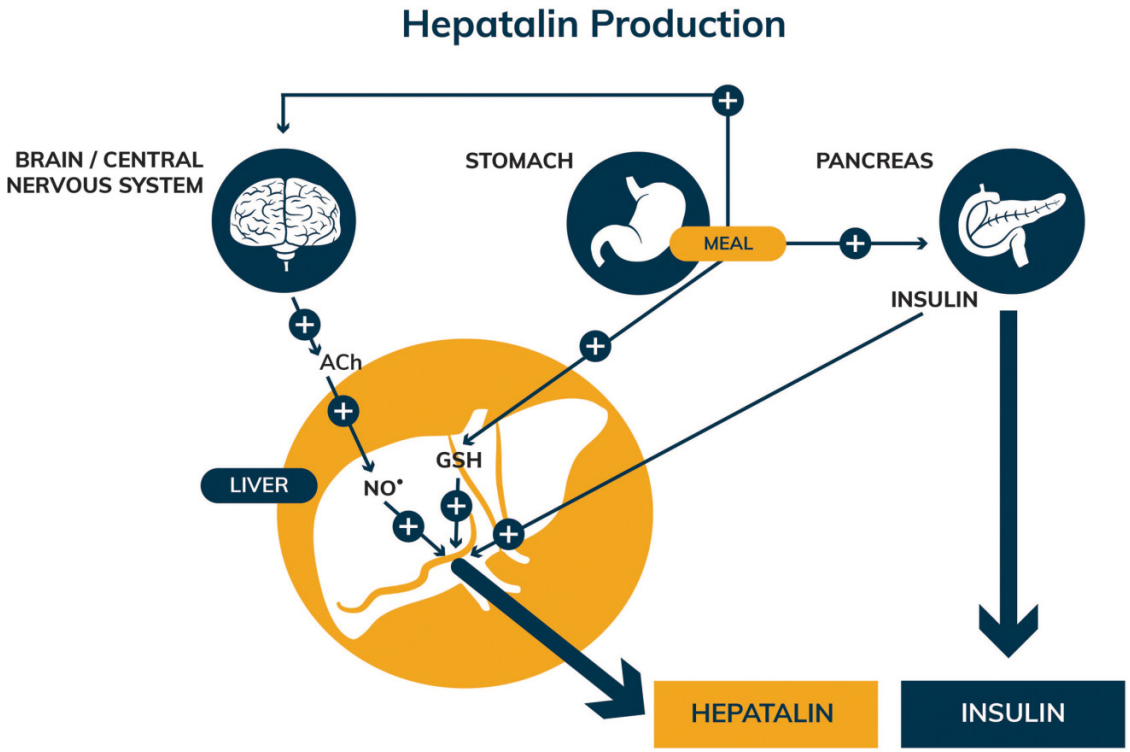
For at least 6 h after a meal, the response to insulin in the anesthetized state does not decrease. However, fasting in the conscious state results in a decrease of hepatalin secretion in response to insulin by about 10% per hour until becoming insignificant after a 24 h fast (Lautt et al. 2001; Schafer et al. 2010). The reduced AMIS seen after 8 h of fasting can be restored to fed levels by potentiating the parasympathetic signal using an acetylcholinesterase antagonist (Schafer et al. 2010), suggesting that at this time point GSH levels have not been decreased, and that the early reduction in hepatalin secretion after fasting is regulated by the nerve signal (Fig. 3).

6.1.1. Atropine

Atropine is a useful tool to block hepatalin secretion in a postprandial steady state (which is required before a valid RIST can be carried out (Lautt 2003)). The use of atropine as a tool to differentiate hepatalin and insulin action has been controversial because of the suggestion that atropine at an appropriate dose and at an appropriate time, blocks the ability of insulin to stimulate hepatalin secretion without having other major metabolic consequences relevant to the acute dynamic glucose uptake response to a pulse of insulin (Fig. 4).

The glucose-uptake response to insulin in a 24 h fasted conscious unrestrained rat doubled when measured 90 min after gastric injection of a test meal. Atropine administration eliminated this potentiated response resulting in a RIST index identical to that seen in the fasted state despite other maintained postprandial conditions (Sadri et al. 2006). Atropine has no effect on insulin action in fasted rats, where physiological suppression of hepatalin release already exists (Lautt 2003). Similarly, if hepatic nerves are cut to eliminate the permissive neural feeding signal, hepatalin release is blocked in the fed state, and the remaining direct response to insulin is not altered by atropine and is not different from the

Fig. 2. Hepatalin synthesis hypothesis. Hepatalin production is regulated by the synergistic permissive action of feeding signals from the stomach activating hepatic cholinergic muscarinic stimulation of nitric oxide synthase to produce NO and activate generation of cGMP. Hepatic glutathione levels increase by about 50%. In the presence of both synergistic, permissive feeding signals, pulses of insulin stimulate secretion of pulses of hepatalin. The degree of production of hepatalin determines the ratio of partitioning of nutrient energy storage as glycogen or lipids.



fasting response (Lautt et al. 2001). Atropine has no effect on the RIST index if hepatalin release has already been blocked by chronic dietary sucrose (Ribeiro et al. 2005; Ming et al. 2009) or fat supplementation (Afonso et al. 2007, 2010) or stress (Seredycz et al. 2006).

A precautionary note is that any drug used to block the hepatalin pathway must be administered at a stable postprandial glycemic state to avoid neuroendocrine involvement with breakdown and absorption of nutrients from the guts.

6.2. The glutathione (GSH) signal

Hepatic GSH levels increase by ~40%–50% after a meal (Lautt et al. 2011; Guarino et al. 2004). Cholinergic mimicking of the parasympathetic signal does not reverse diet-induced AMIS unless GSH levels are also raised to normal fed levels (Section 6.4). The regulation of postprandial hepatic GSH is unclear but blocking production blocks hepatalin release (Guarino et al. 2004).

To reverse chronic sucrose-induced AMIS, GSH levels must be elevated to postprandial levels using, for example N-acetyl cysteine (Lautt et al. 2011). The role of GSH in this signaling pathway is unclear. However, nitrosylated GSH can trigger guanylate cyclase to stimulate cyclic GMP synthesis (Hogg 2002) which in turn suggests that nitrosylated GSH may be an intermediate between NO synthesis and guanylate cyclase in the hepatalin synthesis pathway. Nitrosylated GSH can also trigger cyclic GMP independent S-nitrosylation of proteins (Guarino et al. 2004; Hogg 2002).

The GSH signal is subject to many insults and regulations. Glucagon may be a physiological regulator of hepatic GSH (Patarrao et al. 2015). Glucagon levels are increased during fasting when hepatic GSH levels are low. Feeding results in reduced glucagon and elevated GSH. Glucagon suppression of hepatalin secretion is mediated by cyclic adenosine monophosphate (cAMP) (Patarrao et al. 2015). A cAMP analog (DBcAMP) and glucagon produced a decrease of insulin sensitivity in a dose-dependent manner correlated with a reduction of hepatic GSH content. The response to insulin was restored by administration of a GSH analogue (Patarrao et al. 2015). When fed rats had the nerve signal blocked, thus blocking the ability of insulin to cause hepatalin secretion, the RIST index decreased by 54.2%, and addition of glucagon produced no further inhibition (54.6%). Glucagon at doses that did not affect blood pressure or glucose levels resulted in GSH levels in the fed state decreasing from 3.0 to 1.7, similar to the levels seen in the fasted state (2.1 μmol/g liver). The data are consistent with GSH mediating hepatic GSH levels relative to the feed/fast status.

Acute alcohol also results in AMIS associated with reduced hepatic GSH (Lautt et al. 2005; Ting and Lautt 2006). Fasting and diets high in sugars reduce the GSH feeding signal (Lautt et al. 2011). The GSH signal is absent after a 24 h fast but appears intact after an 8 h fast, as potentiating the nerve sig-

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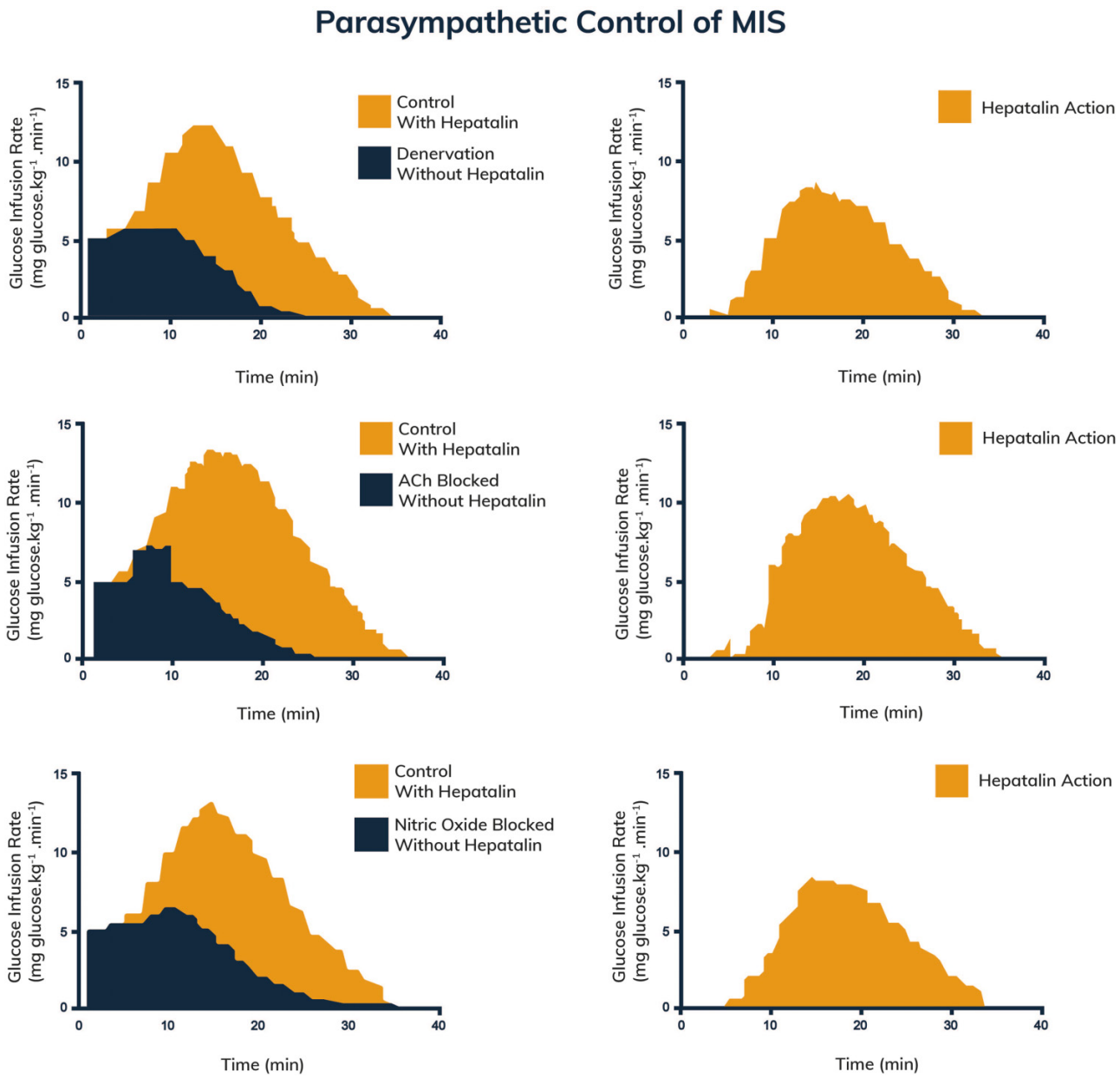
Fig. 3. Blocking hepatalin secretion via the nerve signal by three methods. Elimination of hepatalin action in fed rats was done by blocking the parasympathetic feeding signal by surgical denervation, blocking cholinergic muscarinic receptors (atropine 3 mg/kg), and prevention of production of nitric oxide by blocking nitric oxide synthase (L-NMMA 0.73 mg/kg). The mean Rapid Insulin Sensitivity Test (RIST) curves were obtained by averaging glucose infusion rates at 0.1 min intervals throughout the test response to 50 mU/kg insulin. The control RIST was done in the stable fed state and included the combined action of hepatalin and insulin. Blocking the nerve feeding signal by the three methods reduced the RIST index by 53%–63% by blocking hepatalin action. The dynamic hepatalin-dependent component of insulin action is calculated from the difference between the curves on the left. Hepatalin action commenced at 3.1 min after the onset of insulin administration and continued for 9–10 min after completion of hepatalin-independent direct insulin action. (Figure modified from Lautt et al. 2001.)



nal by blocking ACh catabolism with an acetylcholinesterase antagonist (neostigmine) fully restores MIS to fed levels (Schafer et al. 2010).

6.3. The insulin signal

To elicit maximal hepatalin release in response to insulin, the dose must be administered as a pulse over a duration of less than 10 min. Administration of 50 mU/kg insulin over 30 s or 5 min resulted in similar dynamic responses, but infusions over a longer period resulted in a linear duration-dependent suppression of hepatalin release that was complete by 1 h of constant infusion (Reid and Lautt 2004; Reid et al. 2002). The prolonged constant administration of insulin used in the "gold standard" hyperinsulinemic euglycemic

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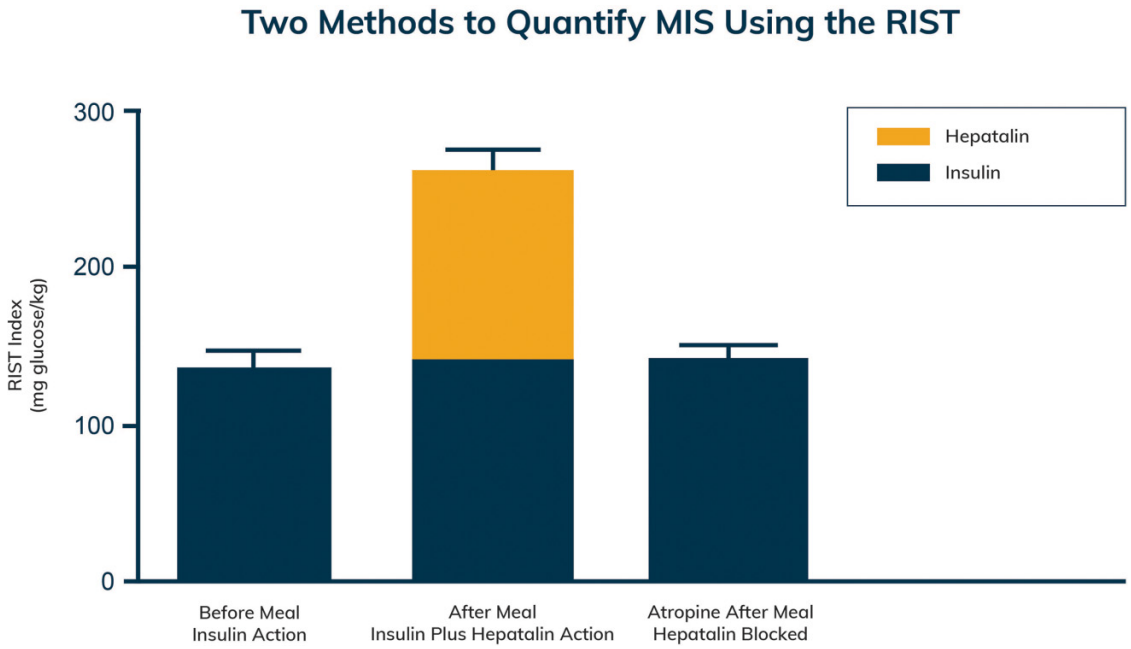
Fig. 4. The hepatalin component of the response to insulin can be quantified in two ways using the Rapid Insulin Sensitivity Test (RIST). This example is from 24 h fasted, conscious, unrestrained rats showing both approaches. To show the development of MIS, the first RIST is done before a meal (left panel) and the second after a stable glycemic baseline has been determined after the meal (center panel). The RIST index was increased by 90%, representing combined insulin and hepatalin action (this procedure was used in the human RISTs (Fig. 1)). The second method is to do the first test in the fed stable state (center panel), and then block hepatalin secretion using atropine (right panel) or blocking nitric oxide production or surgically denervating the liver (Fig. 3). Atropine completely reversed MIS with no effect on hepatalin-independent insulin action. (Figure modified from Sadri et al. 2006. Reprinted with permission from BJN.)



clamp is not capable of detecting hepatalin action, but actually blocks hepatalin secretion.

Hepatalin action is linearly related to the dose of insulin with approximately 56% of the postprandial response to insulin being hepatalin-dependent at doses from 10 to 100 mU/kg in rats (Lautt et al. 2001). The glucose uptake response to insulin has a direct component and an indirect component mediated by hepatalin. The ratio of relative activity remains constant over a wide range of insulin doses.

The background steady state insulin concentration does not affect the response to pulses of insulin, as shown by similar RIST indexes in fasted rats during intravenous or intraportal infusions that raised background insulin levels eightfold (Peitl et al. 2009). The RIST index is the same in the fasted state and in the fed state after atropine although insulin levels are elevated in the fed state (Fig. 4). Insulin release is pulsatile and must be duplicated as such in relevant research.

6.4. Therapeutic mimicking of the nerve and GSH feeding signals

Both the nerve and GSH feeding signals are absent in the diet-induced model of diabetes and can be mimicked by drugs. After 9 weeks of access to a 35% sucrose supplemented drink, rats showed complete absence of hepatalin action. A single pre-meal combination of bethanechol and N-acetylcysteine (BENAC) fully restored hepatic GSH levels and the hepatic cholinergic signal and the ability of a pulse of insulin to stimulate secretion of a pulse of hepatalin. The signals are synergistic and permissive in that they do not result in direct action on glucose uptake in the absence of a pulse of insulin. Dose-response curves indicate a maximal response for both signals that are not potentiated by higher doses (Lautt et al. 2011) (Fig. 5).

6.5. Redundant control systems

Positive redundant control systems in the liver are known. The hyperglycemic response to hemorrhage is maintained in the absence of hepatic sympathetic nerves or adrenal catecholamine secretions. However, absence of both signals prevents the hyperglycemic response to hemorrhage (Lautt et al. 1982; Lautt and Cote 1977). Similarly, the important hepatic vascular compensation for blood loss that occurs by activation of the hepatic capacitance vessels can be regulated in the absence of hepatic sympathetic nerves, adrenaline, noradrenaline, and angiotensin, all of which independently can contract the capacitance vessels. The capacitance response is particularly redundantly controlled in that elimination of the active regulators also results in a greater degree of pressure drop for the same amount of blood loss and therefore the passive contraction of the liver is also increased (Lautt et al. 1980). These are examples of positive hepatic redundant control systems whereby absence of one signal is compensated by another signal to ensure activation of the response.

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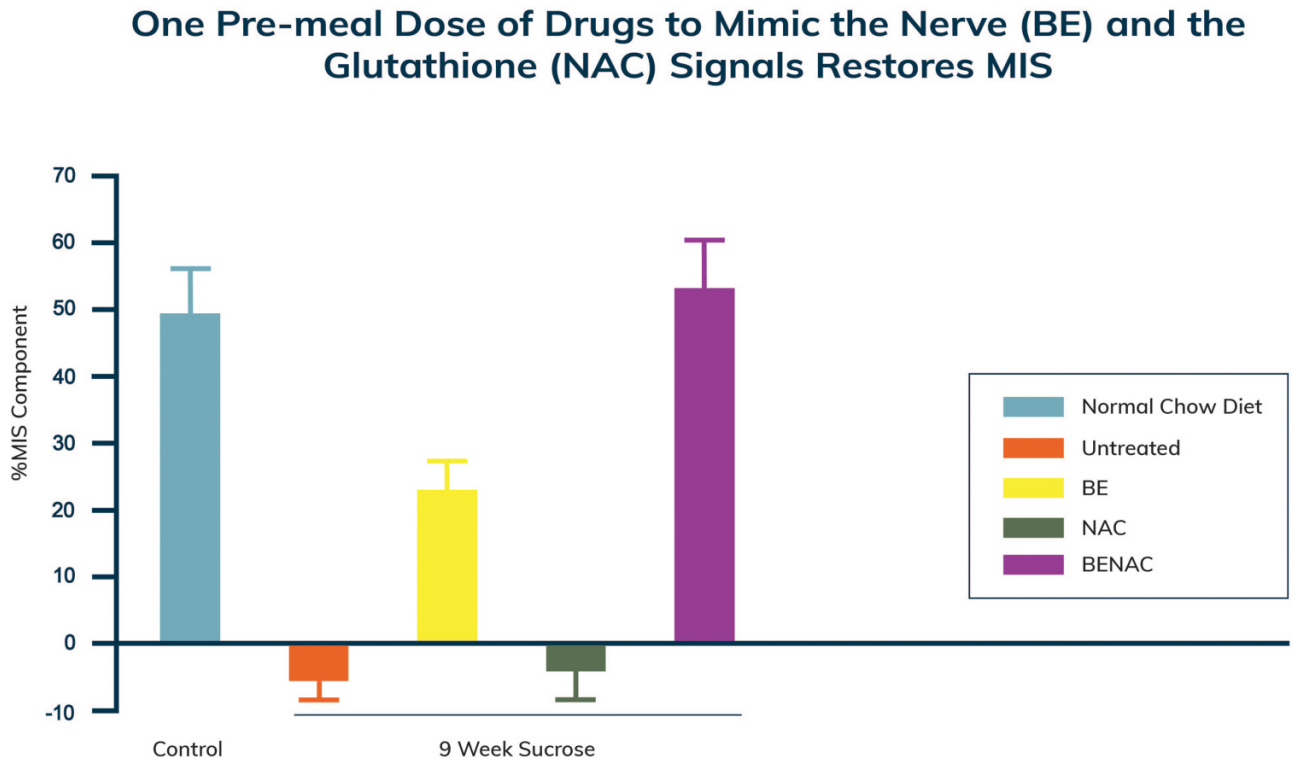
Fig. 5. Bethanechol and N-acetylcysteine (BENAC) synergistic treatment of Absence of Meal-induced Insulin Sensitization (AMIS). The %MIS (hepatalin/insulin action × 100%) calculated from the Rapid Insulin Sensitivity Test (RIST) index in 24 h fasted anesthetized rats determined before and 90 min after intragastric injection of a mixed liquid meal in a normal control group and in four groups that had been treated with 9 weeks of sucrose supplementation. The sucrose diet completely blocked MIS. Bethanechol (BE) produced a modest restoration of MIS in the diabetic model. NAC elevated glutathione (GSH) levels to that seen after feeding but had no effect on the RIST index. The combination of the two feeding signals (BENAC) resulted in a full restoration of MIS. Increasing the doses of either NAC or BE restored MIS to levels seen after feeding but not above. This model demonstrates that in sucrose-induced AMIS, both the nerve and GSH signals were absent but were able to be restored by mimicking these signals with pharmaceuticals. The synergistic permissive nature of the feeding signals is demonstrated. (Figure modified from Lautt et al. 2011.)



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The regulatory systems controlling hepatalin secretion represent a negative redundant control system whereby absence of either signal blocks the response. The nerve and GSH signals are both required for pulses of insulin to stimulate the secretion of pulses of hepatalin from the liver. These redundant negative signals are also unique in that they are permissive only. Activation of the feeding signals in the absence of insulin pulses does not result in the secretion of hepatalin (Lautt et al. 2011).

7. Absence of Meal-induced Insulin Sensitization (AMIS)

AMIS results from diminished hepatalin action following a meal. A healthy MIS involves the actions of insulin and hepatalin, with the result being tight glycemic control and storage of nutrient energy primarily as glycogen in muscle and liver. The partitioning and storage of nutrient energy via glucose is essentially complete by 2–3 h in the healthy state. Acute AMIS results in increased and prolonged postprandial hyperglycemia (Lautt et al. 2011) with compensatory hyperinsulinemia. The elevated insulin acts on the liver to form glycogen and triglycerides and on adipose tissues throughout the body to store nutrient energy as lipids resulting in hyperlipidemia, elevated free radical production, and a shift in nutrient storage from glycogen to fat.

The presence of hepatalin also accounts for the vasodilation in skeletal muscle traditionally associated with insulin (Fig. 8). The absence of hepatalin is associated with lack of an increased blood flow response to insulin administration (Ming and Lautt 2011). Chronic AMIS results in a progressive predictable series of metabolic and cardiovascular dysfunctions that can be increased or decreased by manipulating hepatalin release (Ming et al. 2009, 2011).

A sucrose-supplemented diet for nine weeks resulted in complete elimination of MIS. Intraportal bethanechol (nerve signal) produced a modest restoration of MIS, whereas N-acetylcysteine alone (GSH signal) conferred no improvement. The synergistic effect of the two feeding signals is shown by the full restoration of MIS in response to mimicking both feeding signals with one dose prior to the meal (Fig. 5). AMIS that occurred with age and was potentiated by a sucrose

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supplement was attenuated by an antioxidant cocktail (Ming et al. 2009).

8. Hepatalin and the AMIS syndrome (AMISS)

The AMISS paradigm proposes that the initiating metabolic defect that progressively leads to type 2 diabetes is an absence of postprandial hepatalin action. Postprandially, hepatalin acts in concert with insulin and accounts for the majority of glucose uptake following a meal: 55% in rats (Lautt et al. 2001), 45% in mice (Latour and Chan 2002), and 67% in humans (Patarrao et al. 2008). Hepatalin acts selectively on skeletal muscle, kidney, and heart to form glycogen (Fernandes et al. 2011), but not liver, the gastrointestinal system, or fat (Xie and Lautt 1996*a*).

Absence of hepatalin secretion results in AMIS. AMIS for one meal results in elevated postprandial hyperglycemia with compensatory hyperinsulinemia and resultant hyperlipidemia and elevated free radical generation. The vasodilator action of hepatalin is also absent. If AMIS becomes chronic, a predictable sequence of pathologies comprising the AMIS Syndrome progresses from prediabetes to obesity and type 2 diabetes (Ming et al. 2009, 2011). Based on this paradigm of postprandial nutrient energy processing, diagnosis of prediabetes is possible at a much earlier stage than previously available.

The clustering of pathologies and dysfunctions associated with AMIS has previously been given multiple names which simply reflect a clustering of symptoms. These names have included syndrome X, metabolic syndrome, insulin resistance syndrome, dysmetabolic syndrome, plurimetabolic syndrome, cardiometabolic syndrome, dyslipidemic hypertension, hypertriglyceridemic waist, and deadly quartet (Grundy et al. 2005), with the most recent recommendation by the American Diabetes Association to use the term, cardiometabolic risk (Stern and Izkhakov 2006). However, none of the past names reflects knowledge of the mechanistic basis for the syndrome. Equally importantly, these previous descriptive categorizations do not include the concept of chronological progression. We have proposed the term AMIS syndrome (AMISS) to define the predictable chronology of pathologies that is initiated by the acute absence of hepatalin action. Chronic AMISS progresses from early-stage prediabetes associated generally with cardiovascular alterations and body fat accumulation, to dysfunctions in every organ system including heart, blood vessels, kidneys, nerves, eyes, endocrine organs, and the central nervous system (Fig. 6).

8.1. Age and AMISS

Ageing is associated with a generalized reduction in parasympathetic nerve function demonstrated for the cardiovascular system (O'Brien et al. 1986; Ingall et al. 1990), gastrointestinal tract (Phillips and Powley 2007), urinary bladder (Schneider et al. 2005) and eyes (Fitzgerald et al. 2005). Parasympathetic dysfunction can be identified before the development of diabetes (reviewed Hosking et al. 1978) and is later associated with the metabolic syndrome (Britton et al. 2007) and obesity (Skrapari et al. 2007; Von Kanel et al. 2007). Parasympathetic dysfunction in the liver results in the blocking of hepatalin release and resultant AMIS as the earliest stage of AMISS.

The free radical (oxidative stress) theory of ageing was first proposed by Harman (1956). According to this theory, endogenously produced free radicals continuously and progressively cause permanent DNA and tissue damage, finally leading to ageing. Since many free radicals are produced from the mitochondria, the current version of this theory is the mitochondrial free radical theory of ageing. Oxidative damage to proteins has been found in association with age in a variety of tissues and cells including fibroblasts, brain, liver, heart, and skeletal muscle (reviewed Merker et al. 2001). Free radicals are involved with, or possibly even trigger, the process of ageing (Biesalski 2002).

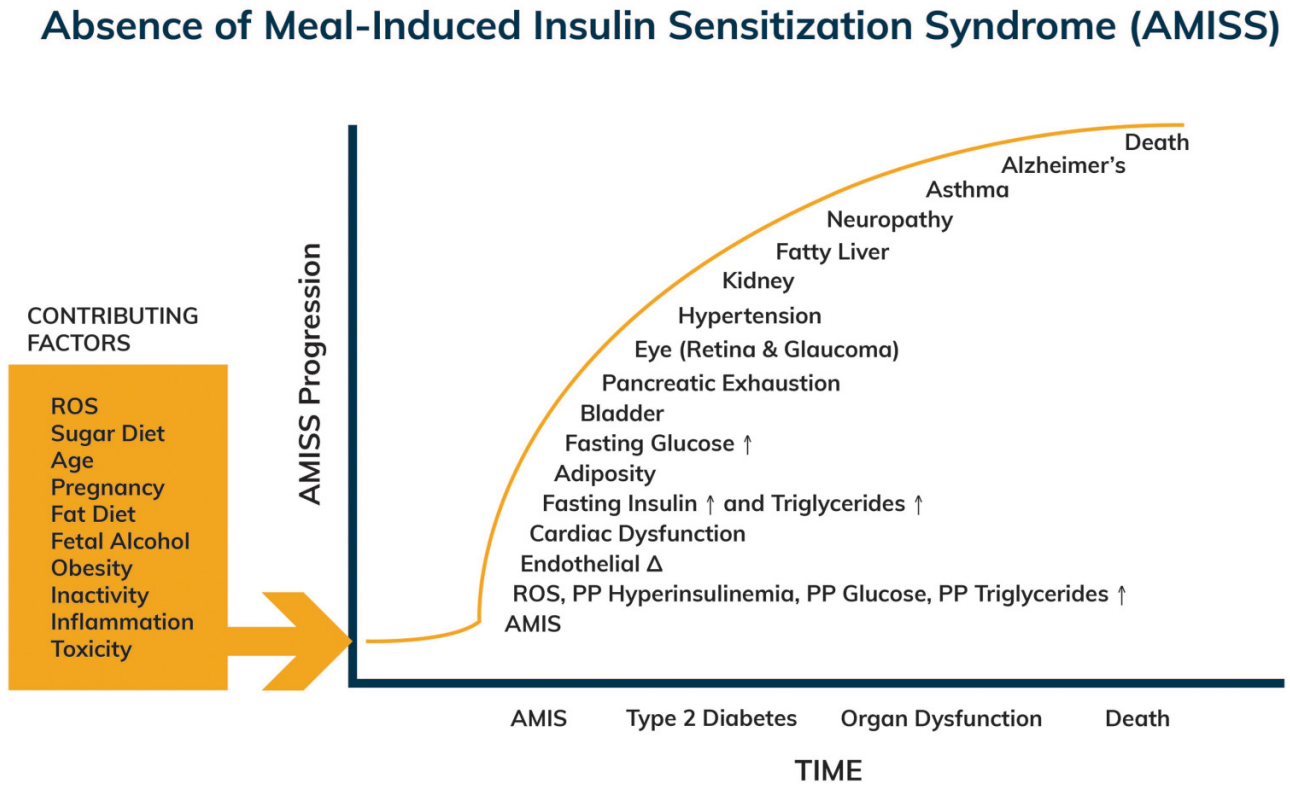
Insulin action in rats decreased until nine weeks of age and remained unchanged thereafter, whereas the hepatalin-dependent component decreased from nine weeks of age throughout ageing (Ribeiro et al. 2008). Figure 7 shows the % of glucose uptake response to insulin that is attributable to hepatalin action, decreasing dramatically with age. Sucrose supplementation increased AMIS and accelerated AMISS, whereas SAMEC (a combination of antioxidants as described in Section 8.2) protected partially against age- and completely against diet-induced AMIS (Ming et al. 2009).

8.2. Antioxidants and AMISS

The effects of antioxidants or free radical scavengers have been widely tested for the prevention and treatment of acute and chronic diseases. However, the efficacy of pharmaceutical antioxidant treatment in clinical trials has been generally unimpressive. Although the production of free radicals is widely spread out throughout the different tissues and cellular components, the chemical property of an individual antioxidant can only allow it to scavenge the free radicals located in a limited cellular component, for example, the lipid or aqueous phase.

We have reported a unique synergistic interaction among three antioxidants selected to specifically target different cellular components: the combination of *S*-adenosyl-L-methionine and vitamins E and C (referred to for convenience as SAMEC). All three components play an important and different but interacting role in scavenging free radicals and in cell health. The water-soluble property of vitamin C makes it the first order antioxidant to protect cell components from free radical-induced damage by quenching various water-soluble radicals, for example, superoxide anion. Vitamin E is a lipid soluble molecule and can transfer its phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acid, thereby breaking the radical chain reaction, and thus preventing the lipid peroxidation in cellular and subcellular membrane phospholipids especially those of mitochondria and microsomes. *S*-adenosyl-L-methionine is a natural regulator of GSH and is involved with transmethylation and sulfation reactions. GSH is the main intracellular defense against free radicals. GSH levels are significantly depleted in liver injury induced by oxidative stress.

Fig. 6. The Absence of Meal-induced Insulin Sensitization Syndrome (AMISS) is the progressive, predictable, chronological accumulation of signs and symptoms of homeostatic disturbances caused by absence of hepatalin action after each meal. Absence of hepatalin action in the fasted state is appropriate, but if it is not rapidly activated after a meal, leads to postprandial increases in glucose, insulin, lipids, and reactive oxygen species (ROS). AMISS progresses to prediabetes, obesity and type 2 diabetes, and associated dysfunctions in major organ systems. By the time fasting glucose is elevated, the AMISS is well underway. The specific order of appearance of dysfunctions is an estimate that requires verification. A variety of contributing factors lead to suppression of hepatalin secretion. Treatment options vary with the duration and extent of AMISS. PP = postprandial. (Figure modified from Lautt et al. 2010.)



Administration of *S*-adenosyl-L-methionine is an effective means of restoring intracellular GSH stores in mitochondria, thus improving the cellular ability to scavenge free radicals (Lieber 1999).

The unique synergistic activity of SAMEC was demonstrated by the absence of any beneficial effect of vitamins E and C taken together or by *S*-adenosyl-L-methionine administered separately. In contrast, when administered together, the three conferred very significant synergistic protection against liver damage and the development of hepatalin-dependent insulin resistance that results from acute exposure to the free radical generating hepatotoxin, thioacetamide (Ming at al. 2006).

8.3. Exercise and AMISS

Voluntary exercise using a running wheel for seven days led to an improvement in the response to insulin in both healthy, and sucrose-induced, and high fat diet-induced prediabetic rats (Chowdhury et al. 2013*c*). The improvement was attributable to an increase in hepatalin action with no significant improvement in direct insulin action. The AMIS that was diet-induced was significantly reversed by seven days of access to a running wheel (Chowdhury et al. 2013*a*). Hepatalin action correlated strongly with the distance run. The animals that benefited the most per distance run had the lowest pre-training hepatalin action (Chowdhury et al. 2011). Antioxidant supplementation with SAMEC did not impair or improve the beneficial effect of exercise on AMIS. Clinical testing of the effect of exercise on AMIS has not been done.

8.4. Diet and AMISS

Rat chow supplemented with sucrose (Ribeiro et al. 2005; Lautt et al. 2011) or lipids (Afonso et al. 2007, 2010) resulted in a reduction of MIS that is attributable to absence of the feeding signals. A limited low dose of sucrose supplementation (50 mL of 5% solution shared with two cage mates) resulted in a progression of the AMIS syndrome with hepatalin action being essentially absent after 52 weeks (Ming et al. 2009). A 35% sucrose solution, in contrast, results in complete AMIS after only two weeks with accumulation in body weight being seen by six weeks (Ribeiro et al. 2005). The sucrose-induced prediabetic state was prevented by daily supplementation with SAMEC (Ming et al. 2009).

Diet also affected gestational nutrient partitioning in rats by affecting hepatalin action. A sucrose supplement shifted

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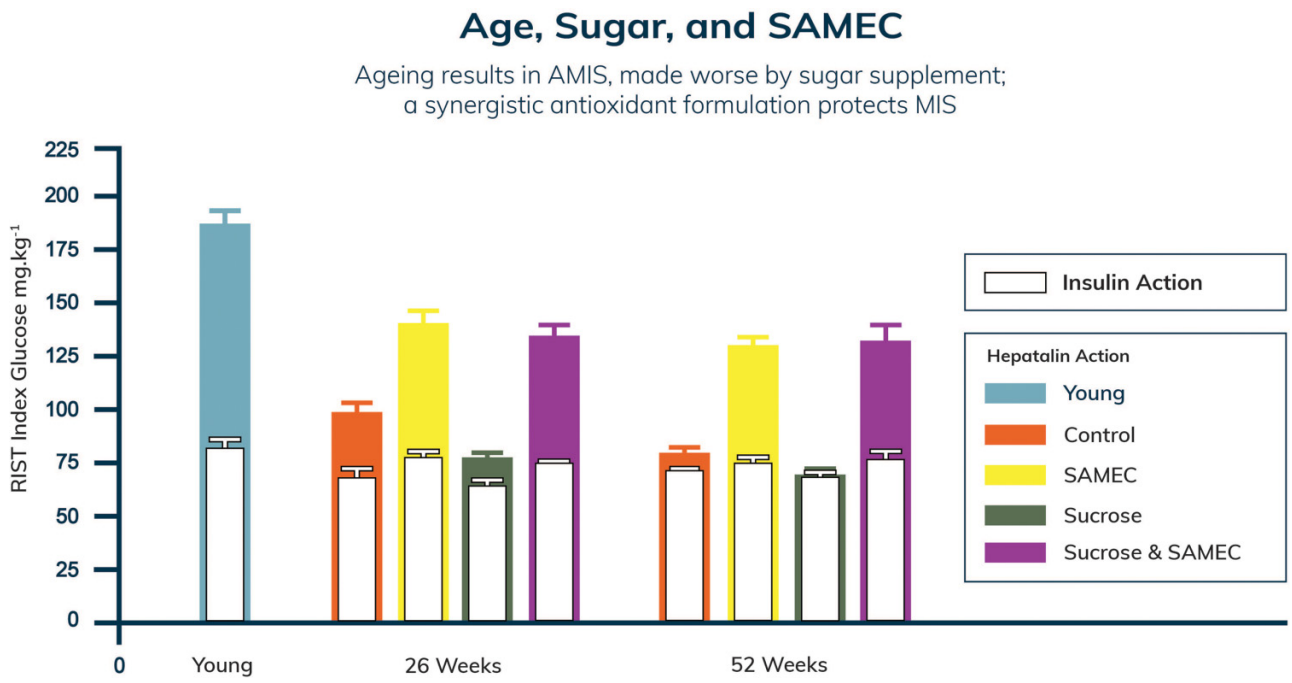
Fig. 7. The Rapid Insulin Sensitivity Test index (amount of glucose required to maintain a glycemic baseline in response to a 5 min pulse of 50 mU/kg insulin) was determined in the fed state before (insulin plus hepatalin action) and after atropine (direct glucose uptake insulin action only), to determine the relative glucose uptake response to insulin that is attributable to insulin or hepatalin. The ratio of insulin and hepatalin in partitioning of storage of nutrient energy was manipulated by interacting normal ageing (9, 26, and 52 weeks) with sucrose-induced AMIS, and the effect of a synergistic antioxidant combination (SAMEC = *S*-adenosyl-L-methionine, vitamin E and vitamin C) on both conditions. Normal ageing resulted in a severe decline in hepatalin action that was strongly protected by SAMEC. A 5% sucrose water supplement potentiated the age-related decline, which was also protected by SAMEC. The direct glucose uptake action of insulin in the absence of hepatalin action remained unchanged in these conditions. The variation in response to insulin can be attributed to hepatalin action. This model allowed monitoring the development of components of the AMIS syndrome. (Figure modified from Ming et al. 2009.)



the balance of hepatalin and insulin so that fat accumulation occurred early during gestation (Lovat et al. 2021).

8.5. Stress and AMISS

Acutely stressful physical or psychological events are often associated with development of diabetes. The link between stress and AMIS is unclear and possibly mediated by several independent mechanisms.

8.5.1. Acute stress and AMISS

As with all factors that can trigger AMIS, the acute response to stress, such as blood loss, is overlaid by other homeostatic adjustments. In response to blood loss, the hepatic sympathetic nerves and adrenal catecholamines activate glycogen breakdown in the liver that can double glucose levels within minutes (Lautt et al. 1982). The secretion of insulin in response to the hyperglycemia is blocked by somatostatin secreted by the pancreas (Lautt et al. 1983).

Acute blood loss leads to complete AMIS that was not prevented by blocking either the alpha- or beta-adrenergic receptors. However, the somatostatin receptor antagonist, cyclosomatostatin, completely blocked the development of hemorrhage-induced AMIS. Exogeneous somatostatin produced AMIS that was prevented or reversed by blocking somatostatin receptors (Seredycz and Lautt 2006). The hemorrhage experiments were done under full anesthesia, yet the stress of extensive surgical intervention did not activate these responses, as shown by the RIST index in response to intragastric injection of a liquid test meal being similar in conscious unrestrained or anesthetized rats (Sadri et al. 2006).

8.5.2. Psychological stress and AMISS

Mental stress suppresses MIS, which is an appropriate response preserving blood glucose levels for emergency use in a fight or flight situation. The relation between stress and metabolic dysfunction was defined as a component of the General Adaptation Syndrome by the Canadian physiologist, Hans Selye (1950), primarily focusing on the pituitary and adrenal hormonal roles. Diabetes and change in body composition were components. Similar to the general adaptation syndrome, the acute induction of AMIS is useful in the immediate fight or flight situation. However, chronically maintained, both responses will lead to pathologies. Personal observation revealed that rats experiencing loud noises showed behavioral evidence of anxiety, and consistently resulted in

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the rats having AMIS. Chronic inflammation was also associated with AMIS.

8.6. Glucagon and AMISS

A cAMP analog or glucagon produced a dose-dependent AMIS that correlated with decreased hepatic GSH content and was reversed by administration of a GSH donor. Neither hepatic nor plasma nitric oxide levels were affected, suggesting that only the GSH feeding signal had been impacted (Patarrao et al. 2015). Glucagon levels are increased during normal fasting and diabetes; and may account for the reduced GSH levels seen in the fasted and diabetic state.

8.7. Heart function and AMISS

Parasympathetic dysfunction leads to AMIS. Cardiac parasympathetic dysfunction is seen in early-stage prediabetes as a reduced heart rate variability coupled with the respiratory cycle. In health, inhalation and exhalation result in increases and decreases in heart rate that is mediated by parasympathetic nerves. This variability is seen in young and healthy subjects and is absent in the elderly and diabetics (Svensson et al. 2016; Benichou et al. 2018; Hoshi et al. 2019).

Cardiac performance in rats was evaluated using a Millar pressure volume conductance catheter system. Cardiac dysfunctions and AMIS developed with age and were increased by sucrose supplementation and inhibited by the antioxidant (SAMEC) treatment. Cardiac dysfunction correlated with AMIS and showed as reduction in cardiac index, heart rate, maximal rate of contraction and relaxation, reduced ejection fraction and increased left ventricular end diastolic pressure. Total peripheral vascular resistance also increased. These dysfunctions occurred prior to accumulation of cardiac lipids (Ming et al. 2011).

8.8. Vasculature and AMISS

Hindlimb blood flow and the metabolic response to insulin were tightly coupled and attributable to hepatalin action. Severing the hepatic nerves in the fed state resulted in a loss of hepatalin action as demonstrated by reduced hindlimb glucose uptake and blood flow in response to a pulse of insulin. Intraportal infusion of ACh to mimic the permissive nerve signal, fully restored the metabolic and vascular response to a pulse of insulin (Ming and Lautt 2011). Hepatalin action is seen only in the fed state, accounting for much of the debate as to the existence of a vascular response to insulin (Fig. 8).

Myocardial blood flow was similar in fasted diabetic and healthy subjects but increased significantly in response to a mixed meal in the control subjects but did not in the diabetic patients (Scognamiglio et al. 2005). The superiority of a mixed meal test verses a pure sugar test was shown by the inability of the oral glucose tolerance test to elicit increased cardiac blood flow that was seen in response to a mixed meal. Oral glucose does not activate the feeding signals and does not result in hepatalin secretion (Sadri et al. 2006; Afonso et al. 2016).

8.9. Obesity and AMISS

It is widely suggested that adiposity causes insulin resistance and type 2 diabetes. But guidance from this new paradigm suggests instead that reduced or absent hepatalin action leads to adiposity.

The glucose uptake response to a bolus of insulin in the fasted state is potentiated by 232% after feeding in humans (Patarrao et al. 2008). MIS was significantly less in subjects with a mild degree of adiposity (466 mg/kg glucose uptake, BMI = 22.7 verses 211 mg/kg glucose uptake in the overweight group, BMI = 27.7). The glucose uptake in response to insulin was less in the overweight group although the insulin concentration profiles were not different (Patarrao et al. 2012). The difference was attributable to reduced hepatalin action, equivalent to very mild AMIS.

Adiposity does not precede insulin resistance. Rather, the absence of hepatalin action leads to adiposity. Several studies show a correlation of reduced hepatalin action and generalized obesity (reviewed in Lautt and Wang 2014).

Postprandial elevations in glucose, insulin and triglycerides are a first sign of AMIS. Blood glucose levels may not change significantly in early AMISS due to compensatory insulinemia. Complete AMIS results after two weeks of a 35% sucrose supplemented diet, whereas adiposity was detected only after 6 weeks (Ribeiro et al. 2005), suggesting that adiposity follows, not precedes AMIS. Liver and plasma levels of lipids were elevated in the 2 week sucrose model of diabetes (Martins et al. 2016).

To test the hypothesis that AMIS precedes obesity, several chronic protocols known to have a predictable effect on hepatalin action were used to produce chronic, graded, slow onset impairment of MIS related to age; a chronic sucrose supplement to potentiate AMIS (Ribeiro et al. 2005, 2008; Lautt et al. 2008); and an antioxidant cocktail to attenuate AMIS (Lautt et al. 2008; Ming et al. 2006). By both hastening and attenuating the rate of decline in MIS, the intention was to produce a sufficient range of dysfunctions to permit interpretable correlations. Adiposity increased as hepatalin action decreased. The strong correlation is similar if adiposity is assessed by individual fat pads, or whole-body adiposity determined by bioelectric impedance (Ming et al. 2009) (Fig. 9).

Petersen et al. (2007) showed that insulin resistance in lean subjects was associated with a 60% increase in plasma triglyceride and reduction of muscle glycogen synthesis by 60% that occurred prior to the development of abdominal obesity

The data show that AMIS leads to adiposity. However, the effect of adiposity resulting from overeating on AMIS is yet unknown. Chronic accumulated fat stores and increased lipid metabolism is inflammatory (Sun et al. 2012) which may suppress hepatalin secretion.

8.10. Sex and AMISS

To say that the physiology of the human male has more similarity to that of lab rats than it does to that of the human female is perhaps overstated, but the lifelong shifting milieu of female hormones must be assumed to impact nutrient partitioning. It cannot be assumed that data on

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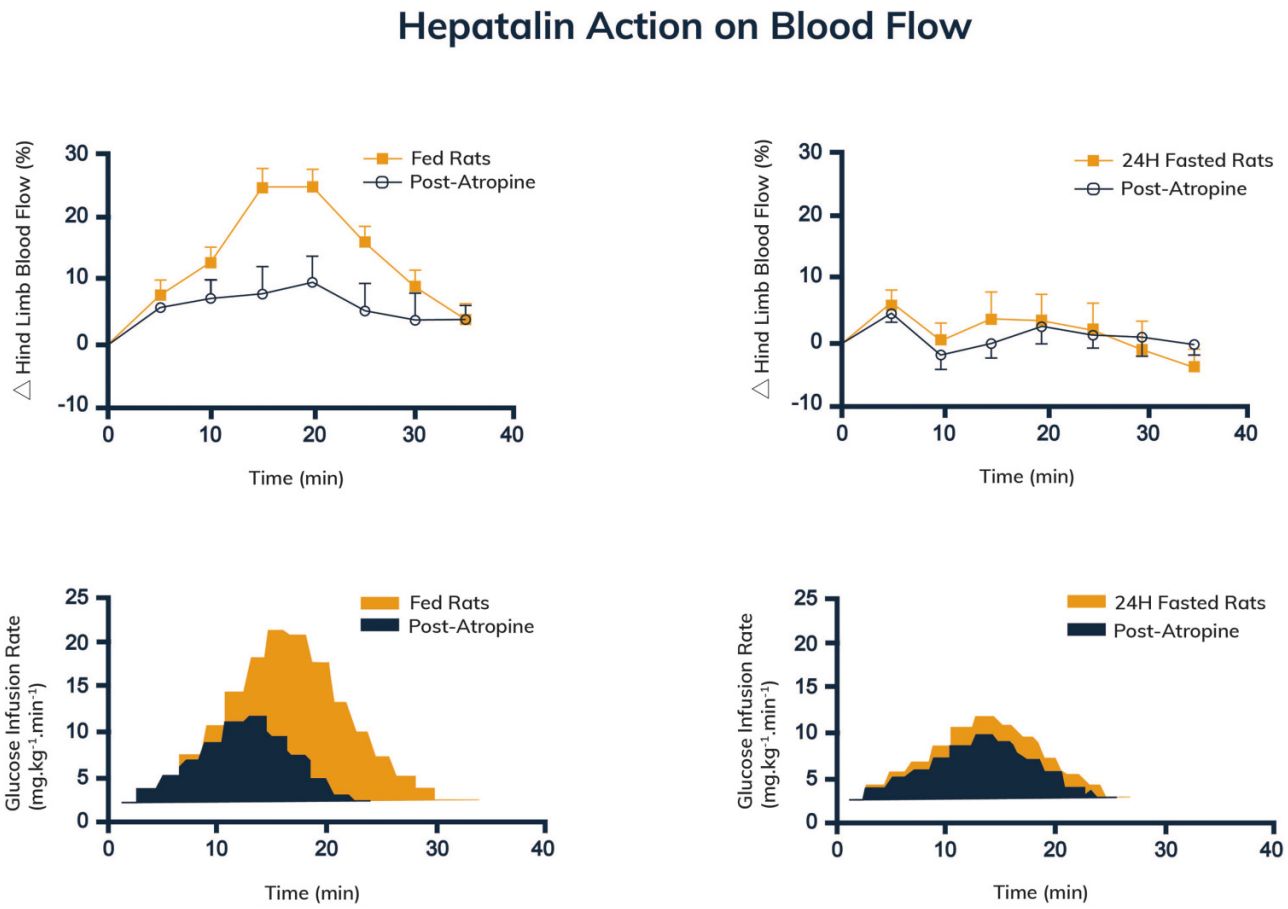
Fig. 8. The effect of a pulse of insulin quantified by the Rapid Insulin Sensitivity Test showing the combined action of insulin (black) and hepatalin (orange) in the fed state on hindlimb blood flow and glucose uptake, and inhibition of both effects by atropine. In contrast, in the 24 h fasted rats, hepatalin secretion was already physiologically suppressed and, therefore, atropine had no additional effect on insulin action. In the same study, not shown in the figure, denervation of the liver eliminated the vascular and metabolic action of hepatalin, which was restored by intraportal infusion of acetylcholine. (Data from Ming and Lautt 2011.)



nutrient partitioning obtained in males can be conflated with female biology. Although the physiology of hepatalin in healthy young virgin female rats is similar to that in males (Ribeiro et al. 2002; Lovat et al. 2020), the effect of fetal alcohol exposure and the role of hepatalin during gestation suggest that the female biology may partition nutrient energy quite differently from the male.

8.10.1. Fetal alcohol effect and AMISS

The metabolism of offspring is affected by maternal alcohol exposure (Taylor et al. 1982; Kelly et al. 1991; Weinberg 1992). The RIST index and % hepatalin contribution are similar in male and virgin female rats (Ribeiro et al. 2002; Lovat et al. 2020). Pregnant rats fed alcohol develop AMIS with normal insulin action but impaired hepatalin action, and the offspring are pre-diabetic in young adulthood depending on the dose of maternal alcohol (Minuk et al. 1998; Sadri et al. 2003). The female offspring are more severely impacted by the fetal alcohol exposure. Females had greater hepatalin-dependent insulin resistance at all maternal doses of ethanol, and had increased perinatal mortality (Sadri et al. 2005).

8.10.2. Gestation and AMISS

Maternal obesity is among the most prevalent risk factors in pregnancy (Ladyman et al. 2010). It is associated with macrosomia, delivery complications, neural tube defects, as well as increased perinatal mortality (Begum et al. 2011; Ladyman et al. 2010; Nelson et al. 2010; Olson and Blackwell 2011). Macrosomic infants have significantly more adipose tissue, with maternal hyperinsulinemia fueling a large portion of this excess fetal growth (Ferraro et al. 2012). Cord blood triglycerides are elevated, reflecting enhanced hepatic and adipose tissue fat synthesis in fetal tissues (Zhu et al. 2010). In the mother, obesity and insulin resistance result in increased risks for preeclampsia, hypertension, and diabetes (Begum et al. 2011; Levin 2006; Olson et al. 2003).

The relative predominance of hepatalin and insulin shift during gestation with hepatalin action being more critical in early gestation when muscle and tissue growth are max-

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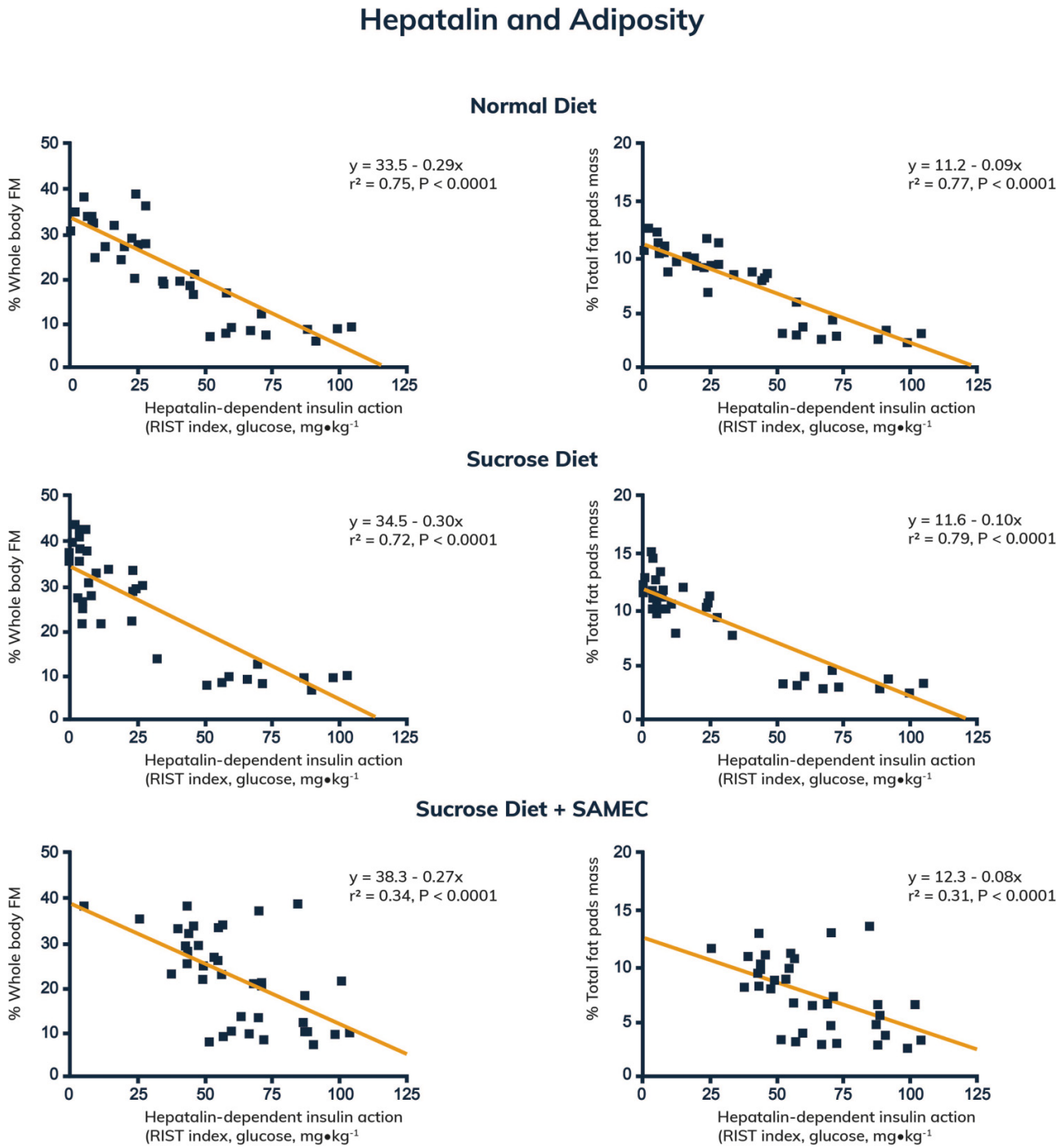
Fig. 9. The relation between hepatalin-dependent insulin action on % whole body fat mass calculated from bio electrical impedance, and from total weighed fat pad masses as % of body weight combined from rats at 9, 26, and 52 weeks of age. Hepatalin action is strongly and negatively correlated with both indices of adiposity. The correlation remains similar in the control groups on normal diet, the 5% sucrose supplemented groups, and the groups on normal and sucrose diets that had been chronically treated with SAMEC (*S*-adenosyl-L-methionine, vitamin E, and vitamin C). The data points move along the regression line with lower hepatalin action and increased fat moving to the left, and the data from the treated group with improved hepatalin action moving to the right. RIST = Rapid Insulin Sensitivity Test. (Figure modified from Ming et al. 2009.)



imal. By the latter part of gestation, the nutrient partitioning shifts away from glycogen formation to lipid metabolism and fat accumulation in both the fetus and mother, a condition similar to prediabetes but of value in storing lipid reserves for both mother and the newborn during the postpartum period (Lovat et al. 2020). A sucrose-supplemented diet shifted the timing of the appearance of AMIS during gestation and resulted in increased and earlier postprandial elevations

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in insulin and lipids and accumulated adiposity (Lovat et al. 2021).

The timing of the shift from hepatalin to insulin dominance determines the degree of accumulated adiposity and components of AMISS over the gestation term.

8.10.3. Human female data

At this point there are no clinical studies that would allow assessment of the changes in nutrient partitioning or hepatalin action that occur throughout the complex hormonal life of the human female. Females experience puberty very differently from males. The monthly hormonal changes that occur thereafter in females involve a flux of hormones that individually have metabolic impact. The role of hepatalin versus insulin throughout this cycle is unknown.

Although the obvious change in body composition in elderly humans from muscle to fat is consistent with the animal data, the effect of age on AMIS has not been specifically verified in humans, and female ageing is also made more complex by the appearance of menopause. To complicate matters further, the effect of hormonal contraceptives and hormone replacement therapy on hepatalin action and nutrient partitioning has not been examined.

9. Current status

Before settling on the name, insulin, followed by its formal introduction in May of 1922 by Banting, Best, Collip and McLeod, the hormone had two previous names, insuline and iletin, (insuline p126, iletin p147 in Bliss 1984). But the structure of insulin was not described until a half century later (Sanger 1959). The diabetes pandemic continues to increase. The existence of hepatalin action and its regulation and consequences in health and disease is shown by many preclinical studies cited here dating back to 1996 (Xie and Lautt 1996*a*) and some early clinical data from 2008 (Patarrao et al. 2008), but the chemical structure remains unknown at this time. If hepatalin is the missing link in our understanding of prediabetes, obesity, and type 2 diabetes, the discovery of new tools through focusing on the new paradigm to diagnose, prevent, and treat hepatalin-dependent insulin resistance should accelerate the growth of knowledge in this area at a much faster rate than it has developed over the past 100 years.

This new paradigm provides the scientific basis for individuals and groups to make quantitatively evaluated wellness choices, and to take control of their own health. With new understanding, early diagnosis and intervention, the pandemic of obesity and type 2 diabetes can be curbed.

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This is a review article of previously published data and no new data is presented. The science described in this review directly related to hepatalin and MIS was a gradual accumulation (1991–2021) of pieces in the missing link contributed to by undergraduate, graduate, and postdoctoral students in Winnipeg, Canada (Lautt and collaborators) and Lisbon, Portugal (Macedo and collaborators), and visiting scientists from Japan, China, Ukraine, and Portugal, and several technologists trained by my long-term collaborator, Dallas Legare. The science is being brought to market by Mick Lautt and John West (SciMar.ca). Manuscript was prepared by Wayne Lautt, with assistance from Melanie Lautt and Karen Sanders.

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Competing interests

Lautt is co-founder and Chief Scientific Officer of SciMar Ltd, a Canadian biotechnology company (SciMar.ca), that has developed clinical products based on the hepatalin paradigm.

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