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ORIGINAL RESEARCH

Prevention of Hepatalin-Dependent Insulin Resistance Induced by a High Sucrose Diet Using a Synergistic Combination of S-Adenosyl Methionine, Vitamin E and Vitamin C (SAMEC) in Virgin and Pregnant Sprague Dawley Rats

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Introduction: Storage of nutrient energy from a meal is partitioned in the body approximately equally by insulin (with its known actions on the liver and adipose tissue) and hepatalin (released from the liver with its glycogenic action selectively in skeletal muscle, heart, and kidneys). During healthy pregnancy, there is a late stage mixed insulin resistance involving both insulin and hepatalin. In pregnant rats on a high sucrose diet, hepatalin-dependent insulin resistance (HDIR) develops with unaffected direct insulin action. Compensatory hyperinsulinemia maintains blood glucose level with resultant hypertriglyceridemia and adiposity. Previously, in male Sprague Dawley sucrose-supplemented rats, aged for one year, HDIR and the associated cardiometabolic consequences were prevented by using a targeted synergistic antioxidant cocktail composed of S-Adenosyl-Methionine, vitamin E and vitamin C (SAMEC). The current study tested the hypothesis that SAMEC would confer protection against sucrose-induced HDIR in virgin and pregnant rats.

Methods: Post-prandial insulin sensitivity was quantified using the rapid insulin sensitivity test (RIST). Sucrose supplementation (35% sucrose in water) was used to induce HDIR in female rats. Eight weeks of normal or SAMEC diet with or without sucrose supplementation was used as an intervention to determine the extent of protection against HDIR by SAMEC in virgin and pregnant rats.

Results and Discussion: SAMEC administered with the sucrose diet prevented the development of HDIR resulting in normal plasma glucose, insulin, and triglyceride concentrations in both virgin and pregnant groups, and attenuated sucrose-induced fat mass gain in virgin rats. The direct insulin action was unimpaired.

Conclusion: SAMEC preserves hepatalin-dependent glucose uptake in virgin and pregnant rats on sucrose supplementation, thus can be used as a preventative in obesity and gestational diabetes.

Keywords: HISS, sucrose, antioxidant, MIS, AMIS, insulin resistance, SAMEC, hepatalin, HDIR, female, prediabetes, pregnancy, gestation, diabetes

Introduction

Female sex is a distinct and powerful biological variable. Sex differences in the resistance and adaptations to stress vary significantly from males to females, making females prone to or protected in certain disease states.[1] Cardiometabolic stress in diabetes and obesity imposes increased health risks to mothers[2] and to the fetus during pregnancy.[3–5] A healthy pregnancy undergoes a series of neuroendocrine and metabolic adaptations that influence the chronological transitions associated with placentation, embryonic development, parturition and postpartum.[6,7]

The partitioning and storage of energy from nutrients is distributed between glycogen and lipids. Nutrients from a meal are partitioned in the body approximately equally by insulin (with its known actions on the liver and adipose tissue) and hepatalin

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(released from the liver with its glycogenic action selectively in skeletal muscle, heart, and kidneys).[8] The metabolic adaptations during the early stage of pregnancy manifests as an increase in insulin sensitivity attributable to hepatalin action and decrease in plasma triglyceride concentration.[9] However, insulin resistance develops during the late gestation, with characteristic hyperinsulinemia and hypertriglyceridemia.[9] The shift between nutrient use and storage as glycogen or lipids is beneficial to the fetus and postnatal female. An excessive metabolic shift in nutrient disposition during the late stages of pregnancy may result in adiposity, increased oxidative stress and gestational diabetes mellitus (GDM). The respective increase vs decrease in insulin sensitivity in the early vs late stage of gestation results from the augmentation and reduction in the action of a liver-derived hormone, called hepatalin.[9–11]

Hepatalin,[10] which was referred to as HISS (hepatic insulin sensitizing substance) in earlier literature, is synthesized in the liver with its maximal secretion after a meal, decreasing to insignificance after 24 hours of fasting.[10,12] In the postprandial state, pulses of insulin stimulate secretion of hepatalin from the liver. The action of hepatalin causes doubling of the glucose disposal response to administered insulin in rats[13,14] and more in humans.[15] The release of hepatalin from the liver facilitates a preferential postprandial nutrient partitioning by storing the nutrient energy as glycogen in skeletal muscle, heart and kidneys, but not in liver or adipose tissue.[8,16] Reduction in the glycogenic action of hepatalin causes compensatory hyperinsulinemia and a shift in nutrient partitioning of energy stored as glycogen and fat in the liver to fat in adipocytes. It results in hypertriglyceridemia, adiposity and increased free radical stress.[16] Hepatalin is proposed to be a missing link in prediabetes, obesity, and type 2 diabetes.[10]

The insulin-hepatalin interplay determines the metabolic transitions from the glycogenic early stage of gestation to the lipogenic late stage during a healthy pregnancy.[9] Chronic exposure to a high sugar diet by supplementation of 35% sucrose-water disrupts hepatalin release from the liver and results in the development of hepatalin-dependent insulin resistance (HDIR) in male,[16] female virgin[11] and pregnant[9] rats. HDIR is caused by sucrose-induced oxidative stress, which does not reverse spontaneously for at least 2 weeks even after withdrawal from the dietary insults.[16,17] Oxidative stress can affect pregnancy outcomes.[18] The redox imbalance with an increase in oxidative and nitro-oxidative stress intensifies insulin resistance in the mother's body and causes progression to gestational diabetes.[19]

Systemic oxidative stress and inflammation increase during pregnancy.[20] Reactive oxygen species (ROS) play a pivotal role in various physiological functions, including mitochondrial and endothelial activity.[21] Oxidative stress can cause detrimental post-translation modifications of biomolecules, changing their functions and cellular signaling process.[21] Oxidative stress induced by aging and sucrose supplementation in male,[16] and that induced by sucrose supplementation in female[11] Sprague Dawley rats cause development of HDIR with characteristic postprandial hyperglycemia, compensatory hyperinsulinemia, hypertriglyceridemia, and increased visceral adiposity.[11,16] Pharmacological intervention with a targeted synergistic antioxidant cocktail, SAMEC (S-adenosyl methionine + Vitamins E + C), provided metabolic protection against HDIR induced by oxidative stress from a high sucrose diet in male rats.[16]

There is a positive association between dietary antioxidants and pregnancy outcomes, however the findings from clinical trials with the use of antioxidant supplements during pregnancy remain largely unimpressive.[22] One of the possible explanations for the failure of antioxidant supplements to provide protection during pregnancy is their inability to target the redox stress in different subcellular compartments, ie, the hydrophilic and lipophilic and mitochondrial phases in cells. S-adenosyl methionine (SAMe) targets the mitochondrial phase, vitamin E targets the lipid phase and vitamin C targets the aqueous phase of the cell, conferring protection against oxidative stress at all cellular sites in a synergistic manner.[23] All three components are required to provide the synergistic protection, but the individual components are unable to render the benefits.[23] The current protocol examines the effects of SAMEC to prevent the development of sucrose-induced insulin resistance in virgin and pregnant rats. HDIR caused by sucrose supplementation was prevented using SAMEC.

Methods

Ethics

Animals were cared for in accordance with the guidelines in *the Guide to the Care and Use of Experiment Animals*, and protocols were approved by the Protocol Management and Review Committee at the University of Manitoba. Animal use was minimized using a power calculation assuming a 50% difference between groups and an alpha-value of 5%.

Animals and Groups

Female Sprague-Dawley rats, received at 4 weeks of age from Charles River, St. Constant, QC, Canada, were housed in pairs in a climate- and light-controlled animal care facility. All groups of rats had free access to pure reverse osmosis water. Animals were randomly assigned between the study groups. There were two groups of female rats, virgin and pregnant, which were further assigned to a total of eight subgroups – 1) V-C (virgin control, N = 10): virgin rats fed normal chow with water; 2) V-C on SAMEC (virgin control on SAMEC, N = 8): virgin rats fed normal chow with water, but receiving SAMEC treatment; 3) V-SS (virgin sucrose supplemented, N = 10): virgin rats fed normal chow with water and 35% sucrose water solution; 4) V-SS on SAMEC (virgin sucrose supplemented on SAMEC, N = 8): virgin rats fed normal chow with water and 35% sucrose solution, but receiving SAMEC treatment; 5) P-C (pregnant control, N = 10): pregnant rats fed normal chow with water; 6) P-C on SAMEC (pregnant control on SAMEC, N = 8): pregnant rats fed normal chow with water, but receiving SAMEC treatment; 7) P-SS (pregnant sucrose supplemented, N = 10): pregnant rats fed normal chow with water and 35% sucrose solution; 8) P-SS on SAMEC (pregnant sucrose supplemented on SAMEC, N = 8): pregnant rats fed normal chow with water and 35% sucrose solution, but receiving SAMEC treatment.

The SAMEC diet contains 0.5g per kg diet of SAMe, 12.5g per kg diet of vitamin C and 1.5g per kg diet of vitamin E. The supplemented chow was formulated with the drugs, vacuum purged with nitrogen and sealed in foil bags (Research Diets Inc., New Brunswick, NJ). Given the average daily food consumption of 20 g in rats, the approximate daily intake for vitamin C is 250 mg/kg body weight, for vitamin E is 30 mg/kg body weight and for SAMe is 19 mg/kg body weight. All groups received their assigned water and chow treatments for 10 weeks.

Animals were 14–15 weeks of age at the time of experimentation (4 weeks of age upon arrival, 8 weeks of normal or SAMEC diet with or without sucrose supplementation, and 2 weeks of gestation). Food and water intake were recorded for the week prior to breeding to assure similar levels of consumption between groups (Table 1). Gestation occurred from weeks 8 to 10.

Male Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) were randomly assigned to females for breeding to multiple groups. Females assigned to breeding groups were bred with a male between the hours of 16:00 to 08:00. In the morning following breeding, pregnancy was confirmed by the presence of sperm on vaginal swab. Animal weights for all groups were then tracked every 3–4 days until the day of acute surgical experimentation for determination of weight gained over the gestational period. Females not displaying signs of pregnancy were re-bred with the same male for 3 more nights or until pregnancy was confirmed. If a female was unable to get pregnant, she was removed from the protocol. For daytime until the next night of breeding over the week, the breeding group females were returned to their virgin control cage-mate. Gestation day 1 was considered the day when plugs were present and/or sperm was detected on vaginal swab. After breeding was complete, gestating animals were permanently re-paired with their original virgin control until the acute experimentation.

Table 1 Water, Sucrose-Water, Standard Chow, and SAMEC Chow Intake in Pregnant Groups

	P-C (N = 10)	P-C on SAMEC (N = 8)	P-SS (N = 10)	P-SS on SAMEC (N = 8)
Water intake (mL/day)	33 ± 1**	38 ± 1**	4 ± 1	7 ± 1
Sucrose water intake (mL/day)	-	-	48 ± 3	46 ± 2
Total fluid intake (mL/day)	33 ± 1	38 ± 1	52 ± 3**	53 ± 2**
Chow intake (g/day)	17 ± 1*	-	10 ± 2	-
SAMEC chow intake (g/day)	-	21 ± 1	-	16 ± 3

Notes: The consumption of chow, water, sucrose solution, and SAMEC diet intake was recorded across the pregnant study groups. *p<0.05, **p<0.01.

Abbreviations: P-C, pregnant control group on standard chow and water; P-C on SAMEC, pregnant control group on standard chow and water but receiving SAMEC treatment; P-SS, pregnant sucrose supplemented group on standard chow, water, and sucrose solution; P-SS on SAMEC, pregnant sucrose supplemented group on standard chow, water, and sucrose solution but receiving SAMEC treatment.

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Feed/Fast Cycle and Surgical Preparation

All rats underwent a 12-hr fast (18:00 to 06:00) with a 2-hr re-feed (06:00 to 08:00) prior to metabolic testing to ensure maximal hepatalin-dependent glucose uptake.[13] Animals were anesthetized to surgical plane by an intra-peritoneal sodium pentobarbital injection (54.7 mg/kg of pentobarbital) (CEVA Sante Animal S.A., Libourne, France). Body temperature was monitored and maintained via a rectal probe and a homeothermic temperature-controlled surgical table. Cannulation of the femoral artery and vein was conducted to establish an arteriovenous (AV) vascular shunt.[24] A side branch of the AV shunt was connected to a pressure transducer for monitoring heart rate, arterial blood pressure (upon clamping the venous side of the AV shunt), and shunt pressure. Supplemental anesthetic (sodium pentobarbital in sterile heparinized saline at a rate of 1.09 mg/100 g body weight per hour) was infused intravenously throughout the experiment.

Metabolic Markers

After a 30-minute stabilization period following surgery, a 25 μL arterial blood sample was drawn to determine the postprandial blood glucose level (glucose oxidase method) (model 27, Yellow Springs Instrument Co, Yellow Springs, OH). A stable glycemic baseline was established when three consecutive samples spaced 5 min apart fall within 3% error. A 200 μL sample of whole blood was drawn from the arterial side of the femoral AV shunt, centrifuged and the plasma was separated for determination of insulin (Insulin (Rat) Ultrasensitive EIA, Alpco Diagnostics, Salem, NH, USA) and triglyceride concentrations (Triglyceride Assay Kit, Cayman Chemical Company, Ann Arbor, MI, USA).

Rapid Insulin Sensitivity Test (RIST)

Insulin and hepatalin action can be determined with the RIST.[24] The RIST index is the amount of glucose administered per kg body weight to maintain a euglycemic state in response to a fixed bolus of intravenous insulin and it is representative of the whole-body glucose uptake. After establishment of a stable glycemic baseline, insulin (Human insulin, Humulin R, Eli Lilly & Co., Toronto, ON, Canada) was infused (50 mU/kg over 5 minutes), with the start of infusion indicating time zero. Glucose (100 mg/mL) was infused intravenously at a variable rate adjusted to maintain euglycemia beginning at time 40 seconds. The first arterial blood sample (25 uL), for glucose concentration measurement, was taken at 1 minute and every 2 minutes thereafter throughout the RIST by assessing and maintaining euglycemia, with variable adjusted glucose infusion rates. A data acquisition system (National Instruments Lab-View, Austin, TX, USA) recorded and analyzed the mean arterial blood pressure, glucose infusion rate over time, real-time adherence to baseline glycemia, time for determination of the RIST index, and graphing of the dynamic profile of insulin/hepatalin-dependent glucose uptake. If the deviation in calculated accuracy or precision was greater than 5% from the established euglycemic baseline, the RIST was excluded and required a repeat. The RIST is considered complete when the euglycemic baseline can be maintained with the glucose infusion rate returning to zero (average time = 35 minutes). Atropine (Sigma–Aldrich) was then infused intravenously (1 mg/kg) to block hepatalin release and the animal was again stabilized over 30 minutes. The glycemic baseline was re-established and the RIST was repeated. The second RIST (post-atropine blocked) was conducted using the same protocol. Atropine used in this manner has been shown to block hepatalin action with no impact on direct insulin-dependent glucose uptake.[25] The RIST is a reproducible, accurate, and reliable procedure, which can be repeated up to four times sequentially with consistency in the same anesthetised rat over an 8-h test period.[25] The RIST has been used in several species including humans.[15]

Sample Collection

Following the completion of two consecutive RISTs (control and post-atropine) the animal was euthanized by an intravenous administration of an overdose of sodium pentobarbital. Laparotomy was performed, and the three major identifiable regional fat pads (peri-renal, peri-mesenteric, and peri-uterine) were dissected, pooled, and weighed. Correlation of fat pad mass and bioelectric impedance determination of whole-body fat content has been shown.[16]

Analysis

Data are reported as the mean ± standard error (significance level $p < 0.05$). The control RIST measured both insulin and hepatalin-dependent glucose uptake. The atropine-blocked RIST measured insulin-dependent glucose uptake alone. The difference between the control RIST and the hepatalin-blocked RIST quantified the hepatalin-dependent glucose uptake. Calculation of hepatalin-dependent glucose uptake as "percentage hepatalin action" was done according to the following equation:

$$\% \text{ Hepatalin action} = [(\text{control RIST} - \text{hepatalin-blocked RIST}) / (\text{control RIST})] \times 100\%$$

The data were determined to follow a normal distribution and there was comparable variance within each group. For the analysis, 2-way ANOVAs were conducted to examine the effects of sucrose and SAMEC administration on glucose uptake, adiposity parameters and metabolic parameters. Post-2-way ANOVA analysis was conducted using the Bonferroni correction. 1-way ANOVA (with Tukey's post-test for least significant difference) and unpaired t-tests were conducted with a minimum critical value of $p < 0.05$ for parameters such as those specific to subsets of groups. Data were analyzed using GraphPad Prism, version 9.5.1, GraphPad Software Inc., San Diego, CA, USA.

Results

The average age of the study animals was 103 ± 2 days, with no differences between groups. SAMEC treatment during sucrose supplementation demonstrated prevention of visceral adiposity in V-SS group (Figure 1A). Although body weight was similar in all groups, weight gained (over the final 2-weeks of the experiment), fat pad mass and fat pad mass per kilogram body weight were significantly greater in the non-SAMEC-treated V-SS group ($p<0.01$). This demonstrates prevention of obesity and maintenance of lean body mass by SAMEC in presence of sucrose supplementation in virgin female rats. Food and water intake among all groups were tracked with no statistical difference in consumption aside from 1) V-C on SAMEC ingested significantly more chow than V-SS on SAMEC treatment group ($p<0.01$) and 2) V-SS on SAMEC ingested significantly more 35% sucrose water compared to V-SS no-SAMEC group ($p<0.01$) (data not presented). Interestingly, despite the higher caloric intake of the SAMEC-treated group, they did not gain additional fat pad mass or demonstrate a worsening of their insulin sensitivity relative to the non-SAMEC group.

Adiposity parameters in pregnant groups are depicted in Figure 1B. The pregnant animals that were supplemented with sucrose (with/without SAMEC) experienced increased weight gain over the gestational period, fat pad mass, and fat pad per kilogram body weight versus the non-sucrose groups. Adiposity due to sucrose supplementation was not impacted by SAMEC during pregnancy in this animal model.

Glucose uptake measurements derived from the RIST in virgin and pregnant groups are presented in Figure 2. The insulin resistance caused by the chronic 35% sucrose supplementation was completely attributable to loss of hepatalin action. There were no differences in direct insulin action between virgin groups nor between pregnant groups (Figure 2). SAMEC supplementation prevented loss of hepatalin-dependent insulin action in both virgin (Figure 2A) and pregnant (Figure 2B) rats.

Sucrose supplementation caused hyperinsulinemia and hypertriglyceridemia (Figure 3), as seen in previous studies.[11] In the sucrose supplemented group treated with SAMEC, the prevention of HDIR resulted in prevention of hyperinsulinemia and hypertriglyceridemia (Figure 3).

Glucose levels were not significantly difference between groups. Blood glucose levels were determined after at least 2 hours from the end of the feeding period, after anesthesia and surgical preparation, and after they had become stable for at least 15 minutes prior to the first RIST. By the time of sampling, postprandial hyperinsulinemia had restored glucose levels to baseline for all groups Accordingly, the extent of dynamic postprandial hyperglycemia cannot be assessed from this protocol.

In terms of pregnancy parameters, all pregnancy groups had a similar time-to-conception (2.1 ± 0.3 days). Average litter sizes were similar across all groups (15.5 ± 0.5 pups per litter).

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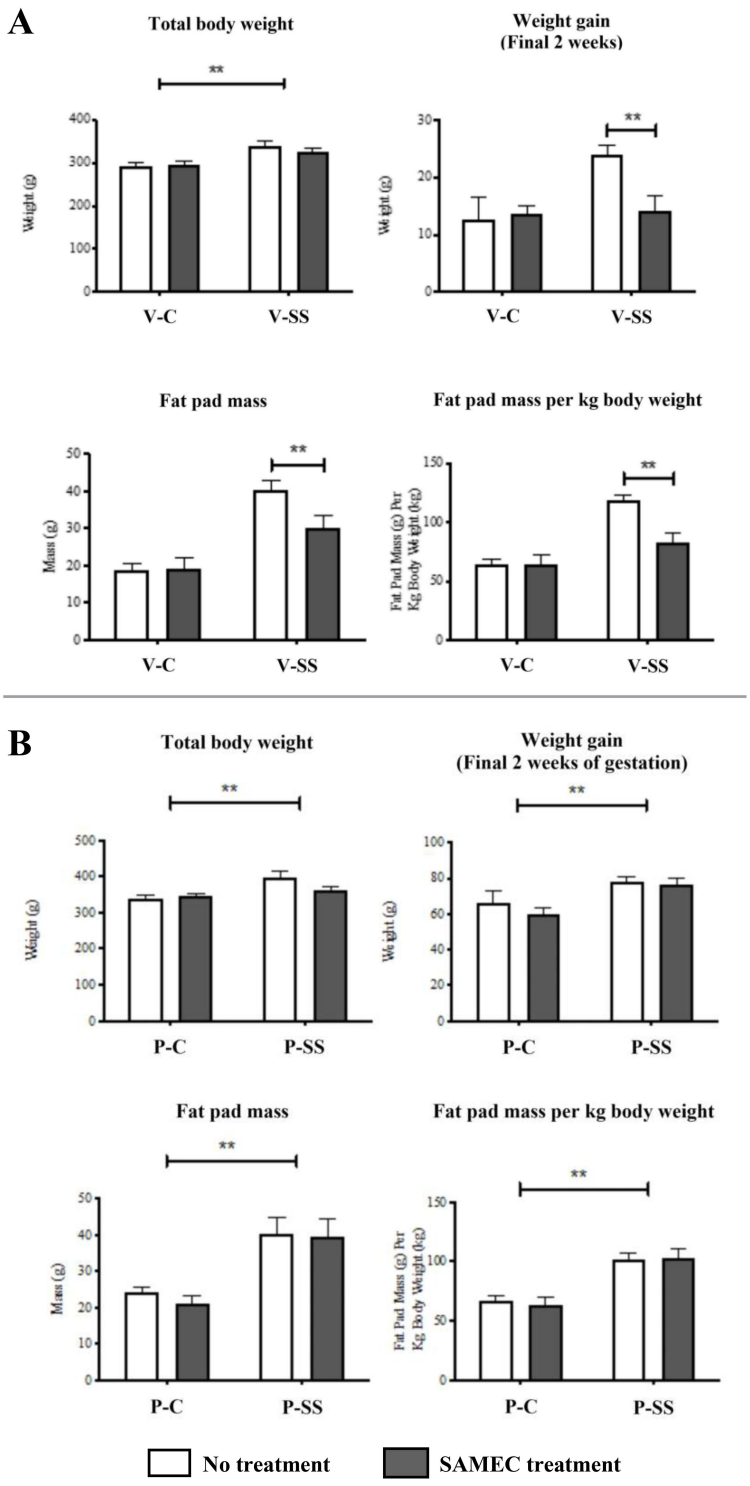

Figure 1 Adiposity parameters: Total body weight was similar across all groups, but sucrose supplementation caused increased adiposity. (**A**) When sucrose supplemented virgin animals were treated with SAMEC, weight gained over the final two weeks of experimentation was attenuated attributable to lower fat pad mass. This resulted in reduced fat pad mass per kg body weight, ie, reduced adiposity. (**B**) Weight gain in pregnant rats over the final 2-week gestational period was higher in the sucrose-fed groups. Visceral fat content was higher in the sucrose groups. SAMEC treatment did not cause any significant reduction in body weight, weight gain during gestation and visceral adiposity during pregnancy. (*p<0.05, **p<0.01).

Abbreviations: V-C, Virgin control group on standard chow and water; V-C on SAMEC, Virgin control group on standard chow and water but receiving SAMEC treatment; V-SS, Virgin sucrose supplemented group on standard chow, water, and sucrose solution; V-SS on SAMEC, Virgin sucrose supplemented group on standard chow, water, and sucrose solution but receiving SAMEC treatment; P-C, pregnant control group on standard chow and water; P-C on SAMEC, pregnant control group on standard chow and water but receiving SAMEC treatment; P-SS, pregnant sucrose supplemented group on standard chow, water, and sucrose solution; P-SS on SAMEC, pregnant sucrose supplemented group on standard chow, water, and sucrose solution but receiving SAMEC treatment.

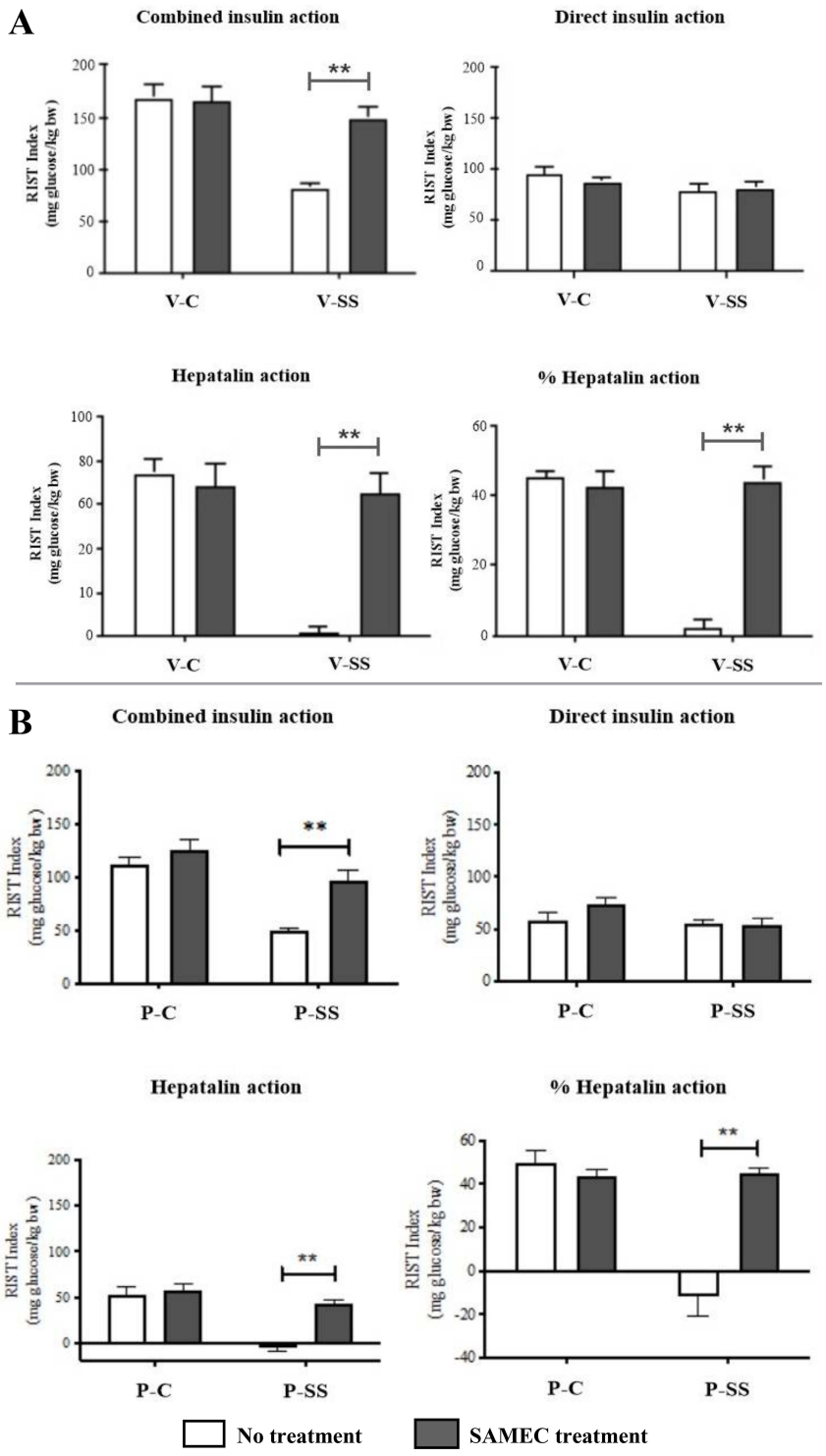

Figure 2 RIST measurements of insulin and hepatalin action: The postprandial glucose uptake response to insulin (insulin plus hepatalin) was reduced when animals received chronic sucrose with normal chow in both virgin (**A**) and pregnant (**B**) animals. Direct insulin action was unaffected. Hepatalin action was lost with sucrose supplementation but was preserved in animals treated with SAMEC. Sucrose induced HDIR (hepatalin-dependant insulin resistance) but did not affect direct insulin action. When sucrose supplemented animals were treated with SAMEC, HDIR was prevented, and their combined insulin action was preserved. This protective effect was entirely attributable to the preserved hepatalin-dependent insulin action. (**p<0.01).

Abbreviations: V-C, Virgin control group on standard chow and water; V-C on SAMEC, Virgin control group on standard chow and water but receiving SAMEC treatment; V-SS, Virgin sucrose supplemented group on standard chow, water, and sucrose solution; V-SS on SAMEC, Virgin sucrose supplemented group on standard chow, water, and sucrose solution but receiving SAMEC treatment; P-C, pregnant control group on standard chow and water; P-C on SAMEC, pregnant control group on standard chow and water but receiving SAMEC treatment; P-SS, pregnant sucrose supplemented group on standard chow, water, and sucrose solution; P-SS on SAMEC, pregnant sucrose supplemented group on standard chow, water, and sucrose solution but receiving SAMEC treatment.

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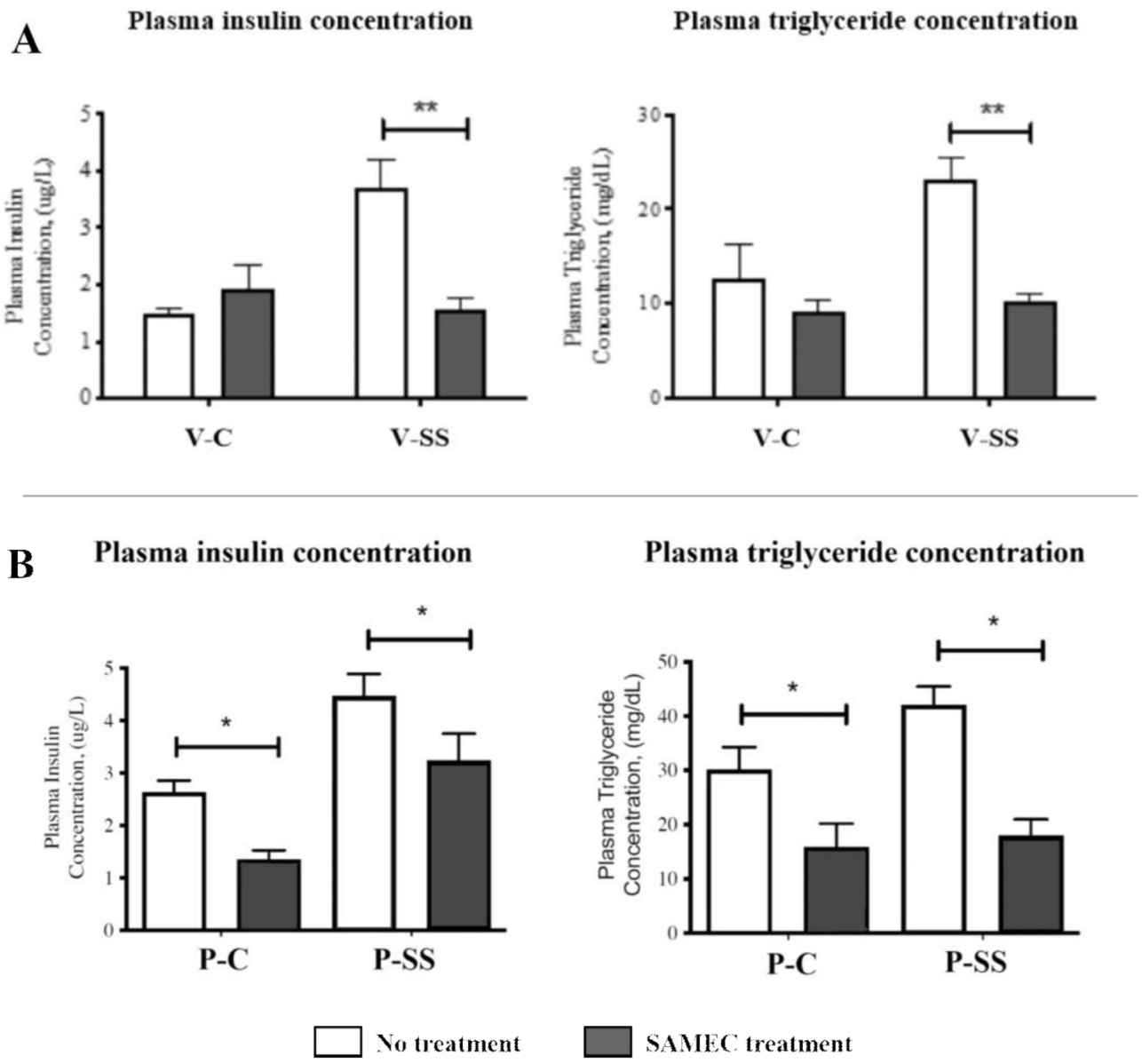

Figure 3 Metabolic parameters: Sucrose supplemented virgin (**A**) and pregnant (**B**) animals fed a standard diet had elevated insulin and triglyceride plasma concentrations. When sucrose supplemented animals were treated with SAMEC, prevention of hyperinsulinemia and hypertriglyceridemia occurred, consistent with prevention of HDIR. (*p<0.05, **p<0.01).

Abbreviations: V-C, Virgin control group on standard chow and water; V-C on SAMEC, Virgin control group on standard chow and water but receiving SAMEC treatment; V-SS, Virgin sucrose supplemented group on standard chow, water, and sucrose solution; V-SS on SAMEC, Virgin sucrose supplemented group on standard chow, water, and sucrose solution but receiving SAMEC treatment; P-C, pregnant control group on standard chow and water; P-C on SAMEC, pregnant control group on standard chow and water but receiving SAMEC treatment; P-SS, pregnant sucrose supplemented group on standard chow, water, and sucrose solution; P-SS on SAMEC, pregnant sucrose supplemented group on standard chow, water, and sucrose solution but receiving SAMEC treatment.

Discussion

Gender disparities in biomedical research tend to impose study findings from a male model to females broadly, without validation in female test subjects.[26] The female physiology is complex and has multiple hormonal fluctuations not seen in males. Additionally, pregnant females metabolically program the next generation[27–29] and the metabolic adaptations during pregnancy can be impaired by various stress factors. For instance, metabolic stress in obesity and insulin resistance during pregnancy are known to trigger adverse outcomes for both mother and fetus and are associated with increased oxidative stress and lipid peroxidation.[30,31] Elevated oxidative stress in pregnancy is naturally counterbalanced by a progressive increase in the activity of cellular antioxidant systems.[30,32] The activity of glutathione peroxidase (GPx)

and superoxide dismutase (SOD) is increased until the third trimester of a healthy pregnancy.[30,33] A decrease in the status of the enzymatic antioxidants (eg, GPx and SOD) and non-enzymatic (eg, ascorbic acid, α-tocopherol, and carotenoids) is evident in late gestation,[30] which precipitates insulin resistance, leading to gestational diabetes mellitus (GDM).

Pregnancy shows chronological changes in postprandial insulin sensitivity throughout gestation, regulating the disposition, utilization, and storage of nutrient energy as glycogen or fat.[9] The post-meal elevation in nutrients is managed through enhancement of the response to insulin induced by the action of hepatalin, the secretion of which is dose-dependently determined by pulses of insulin secretion. This postprandial enhancement of insulin action is termed meal-induced insulin sensitization (MIS). MIS results through the release of hepatalin from the liver under the influence of two feeding signals; activation of the hepatic parasympathetic nerves and elevation of the hepatic glutathione level. With provision of the two feeding signals, pulses of insulin from the pancreas cause a pulsatile release of hepatalin from the liver.[34] The insulin and hepatalin actions stimulate postprandial glucose uptake approximately equally in rats. Insulin predominantly facilitates the storage of glucose as fat in adipose tissue and as glycogen and fat in the liver. In contrast, hepatalin action selectively stimulates the glucose partitioning into glycogen in skeletal muscle, heart, and kidneys but not in the liver or adipose sites.[8] In early pregnancy, the increase in postprandial insulin sensitivity is manifested by an increase in hepatalin action. However, the late stage of a pregnancy is characterized by development of postprandial insulin resistance secondary to the decrease in both insulin and hepatalin action and resultant hyperinsulinemia and elevated triglyceride levels.[9] Supplementation with 35% sucrose-water induced gestational obesity and hepatalin-dependent insulin resistance,[11] possibly by increasing oxidative stress in pregnant rats. Testing the ability of a balanced antioxidant cocktail, SAMEC, to prevent insulin resistance and gestational obesity in pregnancy, as compared to that in virgins, is therefore important. Additionally, the study findings may have significant therapeutic implications.

SAMEC as a Preventative of HDIR (Hepatalin-Dependant Insulin Resistance): The Role of SAMEC in Virgin and Pregnant Females

The metabolic challenges resulting from the glycemic variations during fasting, post-meal and between meals are effectively regulated by the dynamic actions of insulin and hepatalin. Hepatalin secretion does not occur in response to insulin in the fasted state. However, in the fed state, insulin stimulates hepatalin secretion, which then accounts for 55% of the glucose uptake response to a pulse of insulin in rats[34] and 67% in humans.[15] The vasodilatory response to insulin appears to be entirely accounted for by hepatalin action.[35] Chronic dysfunctions associated with the release of hepatalin from the liver cause the development of an array of cardiometabolic dysfunctions associated with obesity, prediabetes, and type 2 diabetes.[16,36] The metabolic dysfunctions induced by 5% sucrose supplementation in ageing male rats can be prevented by SAMEC.[16]

In both virgin and pregnant rats on 35% sucrose supplementation (V-SS and P-SS), there was a significant increase in body weight and visceral fat mass compared to the control groups (V-C and P-C) (Figure 1A and B). Note that sucrose-treated groups had access to tap water in addition to 35% sucrose-water solution. SAMEC intervention decreased the body weight gain and visceral fat pad weight in virgin, but not in pregnant rats (Figure 1A and B). Postprandial insulin sensitivity was improved by SAMEC in the virgin (Figure 2A) and pregnant (Figure 2B) groups.

The rapid insulin sensitivity test (RIST) was used to measure insulin sensitivity. The RIST protocol allows up to four reproducible tests in a single rat and renders the ability to measure the direct insulin-dependent and insulin-independent (ie, hepatalin-dependent) glucose uptake.[24] The improvement in RIST index in sucrose plus SAMEC-treated groups (V-SS on SAMEC and P-SS on SAMEC) was secondary to the preservation of hepatalin action, without any changes in the direct insulin action (Figure 2). Other metabolic markers, including plasma insulin and triglyceride concentrations, were decreased by SAMEC intervention in the sucrose supplemented virgin and pregnant groups (Figure 3). The synergistic action of the ingredients in SAMEC provided protection against HDIR, possibly by mitigating the oxidative stress-induced metabolic deterioration in sucrose-treated virgin and pregnant rats.

Oxidative Stress in Pregnancy: The Role of Antioxidants

Oxidative stress is implicated in the development of many pregnancy complications.[37–39] There is an increase in free radicals via mitochondrial respiration with hyperglycemia during pregnancy. The mitochondrial synthesis of reactive oxygen species (ROS) and the subsequent increase in lipid peroxidation as well as systemic inflammation is associated with insulin resistance in pregnancy.[38] Cardiovascular adaptations to the enhanced embryo-maternal demand result in an increase in heart rate, stroke volume, cardiac output, plasma volume and the left ventricular muscle mass.[40] These adaptations are largely driven by increased production of endothelial NO,[38] which can promote the production of reactive nitrogen species (RNS) by reacting with the superoxide radicals ($O^{2\bullet-}$).[41] The vicious cycle of increased oxidative stress and gestational insulin resistance, accompanied with hyperglycemia, hyperinsulinemia, and obesity, can initiate a cascade of cardiometabolic complications in pregnancy.

The association between oxidative stress and pregnancy complications introduced significant interests in the use of antioxidants as a possible preventative against the negative pregnancy outcomes. There are observational studies indicating a positive correlation between the higher maternal antioxidant levels and better birth outcomes; however, the results from most randomized controlled trials testing antioxidants' ability to prevent pregnancy complication remain disappointing.[22] The failure of antioxidant supplements to provide an adequate redox protection could be due to their inability to sequester free radicals from different subcellular compartments. The benefits of S-adenosyl methionine, vitamins E and C are demonstrated with SAMEC's ability to provide protection against free radical-induced insulin resistance caused by thioacetamide,[23] aging, and sucrose supplementation.[16] The synergistic antioxidant actions in SAMEC have been demonstrated by the lack of protection provided by its individual ingredients, in contrast to the cardiometabolic benefits obtained with the three components combined.[16]

Postprandial Focus in Pre-Gestation and Pregnancy: The Future Hepatalin Research Directions

The post-meal metabolic markers, including postprandial hyperglycemia, hyperinsulinemia, and hypertriglyceridemia act as better predictor of cardiovascular pathologies, as compared to that in fasting conditions.[25,42,43] Prevention of postprandial metabolic dysfunctions in pregnancy has broad therapeutic implications for obese, metabolically deranged and insulin resistant pregnant women who ingest a high sugar diet. Given the high prevalence of obesity (and sucrose intake) in women and the risks associated with obesity,[2,44] SAMEC could provide a much-needed low-risk pharmacological tool in mitigating these morbidities in the face of the consumption of a high-sucrose diet. Since pre-pregnancy BMI and obesity are strong predictors of weight gain during gestation and metabolic risk to mother and fetus,[45] SAMEC may provide a means of managing pre-gestational insulin resistance and obesity in females of childbearing age. Additionally, by interrupting the vicious cycle of oxidative stress and gestational insulin resistance, the metabolic programming resulting in obesity in the offspring could be blunted.[28,29,46,47]

Chronic suppression of hepatalin by aging,[16] sucrose supplementation,[16,17] high-fat diet,[48] and physical inactivity[49,50] causes a predictable chronology of cardiometabolic dysfunctions. A focus on hepatalin in postprandial nutrient partitioning may allow early diagnosis, prevention and better treatment of gestational diabetes. Pharmacological manipulation of the two feeding signals for hepatalin release can reverse HDIR[12] and the use of such intervention as a possible treatment option against HDIR in female rats yet needs to be examined.

Technical Considerations and Study Limitations

SAMEC was used as a balanced antioxidant cocktail to protect the mitochondria, the lipid phase and the aqueous phase of cells. A synergistic effect was demonstrated by its ability to protect against thioacetamide-induced hepatotoxicity and blockade of hepatalin release.[23] An unbalanced antioxidant, consisting of only S-adenosylmethionine or the combination of vitamin E and C, was without benefit. The complexity of selecting specific biomarkers is seen by the observation that no antioxidant delivered alone provided protection, yet one or more markers would have been affected by each component of SAMEC, so it is unclear which of the very many possible biomarkers would be useful for detection. Showing a positive or negative effect of any specific oxidative biomarker would not be proof of the mechanism. The

study met our primary objective to test whether or not SAMEC can reverse hepatalin-dependent insulin resistance (HDIR) in animal models of pregnancy. Contributions of SAMEC in neutralizing specific oxidative stress biomarkers, finding its impact in preventing insulin resistance in an animal model of gestational diabetes (GDM), and finally correlating the observations to clinical pathologies are interesting future projects yet to be designed and tested.

RIST was used to differentiate between insulin- and hepatalin-dependent glucose uptake in rats. RIST can be repeated up to 4 times sequentially, with a low coefficient of variance in the same test rat over a 6–8 h test period.[24] Evaluation of insulin sensitivity determined by HOMA-IR and QUICKI, calculated from fasting plasma insulin and glucose, is consistent with the RIST index. HOMA-IR correlated well with the RIST index considering that HOMA-IR calculation utilized data from the 8-hour fasted conscious rats 2 weeks prior to the experiment, whereas RIST was performed in 2 hour postprandial state in anesthetized rats.[16] However, HOMA-IR does not differentiate between insulin- and hepatalin-dependent glucose uptake.[16] In the current protocol, animals were all fed before the samples were taken.

The decline in direct insulin action by sucrose and age correlates with the decrease in hepatalin action, but the insulin action drops at a relatively slower rate. Hepatalin action decreases approximately 4 times the rate of reduction in direct (or hepatalin-independent) insulin action.[16] When hepatalin dependent glucose uptake is completely abolished, direct insulin action is decreased by only one-third of the normal.[16]

One limitation of this study is the absence of chemical identification of hepatalin. Research on hepatalin activity has been conducted across various animal models and human trials, demonstrating that hepatalin is released from the liver in response to insulin pulses. Its endocrine nature is evident through studies where blocking the hepatic nerve signal via surgical denervation inhibited its action, while local intraportal - but not systemic - administration of acetylcholine, or nitric oxide donors restored its effect on skeletal muscle. Both preventive and therapeutic agents have been developed based on the hepatalin pathway of glucose uptake. A comprehensive review of what is known about hepatalin has been published and chemical identification of the molecule is under investigation.[10] A study of the mechanism of action of hepatalin to stimulate glucose uptake in skeletal muscle will follow once the chemical entity of the hormone is confirmed.

Conclusion

The relative roles of insulin and hepatalin in female rats in response to a sucrose supplemented diet[9] and the changes in the ratio of insulin-to-hepatalin action that occurs during gestation[11] have been reported. The present study demonstrates the impact of a high sugar diet and the induction of HDIR as well as the ability of a synergistic antioxidant formulation, SAMEC, to protect against the diet-induced metabolic dysfunction in virgin and pregnant rats. SAMEC, by preserving the hepatalin-dependent insulin action, helps to maintain normal postprandial plasma glucose, insulin and triglyceride concentrations without impacting maternal body fat reserves in the late gestation. These findings support the investigation of SAMEC as a preventative against the development of HDIR during pre-gestation and pregnancy in females.

Abbreviations

AMIS, Absence of meal-induced insulin sensitization; HDIR, Hepatalin-dependent insulin resistance; HISS, Hepatic insulin sensitizing substance; MIS, Meal-induced insulin sensitization; RIST, Rapid insulin sensitivity test; SAMEC, S-adenosyl methionine + vitamins E + C; SS, Sucrose supplement.

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Disclosure

WWL is the co-founder and chief scientific officer of SciMar Ltd which funded the project and holds a patent for a formulation based on SAMEC, the theme of the paper. In addition, WWL reports a patent “Use of S-adenosyl

methionine, vitamin C and vitamin E for the treatment of oxidative liver injury and insulin resistance" issued to SciMar Ltd. The authors report no other conflicts of interest in this work.

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