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Pharmacologic Modulation of Luminal Carbohydrate Digestion Improves Insulinogenic, Glycemic and Lipid Parameters in Obese, Hyperinsulinemic T2DM Rats

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ABSTRACT

Atherogenic plasma lipid and glycemic profiles are a common observation in obesity and adult-onset diabetes (T2DM) and may become improved following therapeutic intervention. The effects of the luminal α-glucosidase inhibitor miglitol (MIG) on carbohydrate (CHO) digestion on plasma lipid profiles were determined in groups of adult male obese SHR/Ntul//-cp rats, a genetic model that develops early-onset obesity+T2DM independently of diet. Rats were fed a USDA-formulated, complete diet containing 54% sucrose as the CHO component (Control) or the same diet containing MIG at 150 mg/kg diet admixture ad libitum for < 8 weeks. MIG resulted in a ~15% decrease in energy intake ($p = < 0.05$), net weight gain ($p = < 0.05$), and 14% decrease in adiposity ($p= < 0.05$), in addition to significant ($p = < 0.05$) decreases in fasting glucose, insulin, glycated hemoglobin, a 20% reduction in glucose area under the curve (AUC; $p = < 0.05$) a 15% reduction in triglycerides, $p = < 0.05$) and a 20% reduction in total cholesterol, α- (LDL) and β-lipoprotein (HDL) fractions ($p = < 0.05$, all comparisons) after the MIG regimen. Liver glucokinase, malic enzyme and glucose-6-phosphate dehydrogenase were also decreased following the MIG regimen ($p = < 0.05$). In conclusion, these results indicate that therapeutic α-glucosidase inhibition via MIG resulted in improvements in multiple insulin-linked atherogenic parameters and may be a useful adjunct in the long-term clinical management of plasma lipid and glycemic profiles in the glucose intolerant states of obesity and T2DM.

Keywords

Obesity, Diabetes, T2DM, Miglitol, α-glucosidase Activity, Glycemic parameters, Lipid profiles, Liver enzymes, SHR/Ntul//-*cp* Rat.

Introduction

Recent reports indicate that the prevalence of obesity and type 2 diabetes (T2DM) now impacts up to one sixth of the populations of some Westernized countries, where the common pathophysiologic sequalae of obesity and T2DM are now approaching epidemic proportions with no clear preventative or therapeutic solutions on the horizon [1-4]. As recently as 2021, the CDC has estimated that over 38 million adults in the USA had diabetes, representing over 14% of the population and where over 90% of those were a blend of diagnosed and undiagnosed T2DM, the most common form of diabetes worldwide where it currently impacts over 800 million individuals [4,5]. Dietary, medication and lifestyle changes remain the hallmark of conventional therapeutic approaches to treat the diabetes element of the obesity syndrome to improve glycemic markers. Sadly, current approaches to reducing the burden of obesity and T2DM, although well intentioned, are often less than fully successful. The common clinical sequelae of Obesity+T2DM following long-held pathophysiologic contributing dietary and lifestyle parameters in practice by the individual may have been largely asymptomatic prior to a diagnostic clinical assessment has been accomplished, thus not immediately repairable. Historically, long-held dietary and lifestyle modifications may often prove difficult to change, and even when changed, the pathophysiologic progression of the typical sequelae may well be firmly established and incompletely reversible during the weeks and months after the therapeutic measures become initiated. While the glycemic improvement may commence soon after implementation of a

dietary or luminal therapeutic intervention, restoration of plasma lipid profiles likely took longer to become significant, and thus may resolve more gradually, typically requiring longer term intervention to restore and become normalized. In addition, once systemic inflammation and advanced states of atheroma and vascular plaque have become established full recovery including atheromatous reversal may be difficult to achieve [5,6].

Chronic elevations in plasma lipid profiles are common observations in Obesity+T2DM and represent a major contributor to a progression of cardiovascular disorders that often accompany the condition [1-4]. In addition, chronic hyperinsulinemia contributes to systemic inflammation in the CNS and other tissues, adding to the pathophysiologic burden of the disorder [4-6]. Thus, implementation of therapeutic measures that can bring about reduction in the magnitude of inflammation, along with measures to decrease the magnitude of elevated triglycerides, total cholesterol (TC) and LDL Cholesterol (LDL-C) are deemed essential and desirable long term treatment goals and often require a prolonged duration to achieve satisfactory progress [2].

The industrialization of the food supply chain has also introduced dietary changes in industrialized populations, including a greater abundance of commercially processed foods. While many processed foods retain nutritional qualities, they often contain excess salts and preservatives not usually present in more wholesome fresh foods. While the convenience of serving table-ready manufactured foods may seem an advantage to the modern family, in the longer timeframe they may be less healthful than the traditional wholesome home-prepared 'meals from scratch' of past generations [1,7]. The influx of high fructose corn syrup (HFCS) sweeteners for example, has resulted in an approximate 4- to 5-fold increase in fructose intake, and now approaches the generally accepted safe levels of 80 to 100 mg per day, comprising up to 20% or more of daily caloric intake for many individuals when consuming abundant quantities of prepared foods and beverages [8]. The excess consumption of HFCS-containing foods and beverages may result in further pathophysiologic divergences in optimal metabolic pathways during glycolysis and substrate oxidation [8]. Healthful dietary changes are often a challenge to implement in a population accustomed to the convenience and prevalence of readily available industrialized food choices in the marketplace, where the inclusion of lipids and sweeteners and ease of meal preparation adds to their palatability, popularity and convenience [3-8]. In addition, the modernization and macronutrient and micronutrient composition of the food supply may not be optimal or adequate to address chronic Insulin resistance and associated pathophysiologic systemic inflammation common in obese, T2DM states [7]. Moreover, once diagnosed, treatment of diabetes is typically long term, and often may continue for the remainder of their life [6-9].

The magnitude and duration of the insulin response to a meal is generally proportional to the type, quality and quantity of the carbohydrate consumed in the meal. The compound 1,5 dideoxy-1,5-[(2-hydroxyethyl) imino]-D glucitol; generic = miglitol; marketed as Glyset®) is an established competitive inhibitor of luminal starch digestion and acts within the brush border α-glucosidase receptor domains of the small intestine [10-13]. Once the compound is competitively bound to the glucosidase receptor domains, it effectively delays the rate-limiting process of starch of digestion into absorbable monosaccharide moieties and their subsequent luminal glucose uptake from the gastrointestinal tract [9]. Unlike some other α − glucosidase inhibitor agents, miglitol is also fully or nearly fully absorbed in the small intestine, but escapes hepatic P-450 metabolism and conjugation, and is cleared by the kidneys without further metabolism within a few hours of ingestion [9]. Since the usual intestinal starch digestive process occurs rapidly, absorbable monosaccharide entities including glucose become readily available for immediate luminal uptake. In addition, the generation of monosaccharide moieties also represent the rate-limiting step in glucose uptake, the post-ingestive glycemic excursions are proportional to the approximate rate of dietary post-ingestive α-glucosidase activity.

Dietary sources of fructose may occur following the luminal digestion of sucrose into its constituent glucose and fructose monosaccharide moieties, or from free fructose contained in the food and beverages consumed. The luminal uptake of fructose occurs more slowly however, via specialized GLUT monosaccharide transporters (GLUT1, GLUT2 and GLUT5 in various tissues) located along the basolateral epithelial membrane of the brush border in the gut and liver GLUT transporters respectively [10]. Fructose uptake occurs via insulin-independent GLUT transporters, in contrast to glucose. In addition, fructose uptake is a saturable, rate-limiting process with a more restrictive k_m for uptake than glucose, thereby delaying and prolonging the immediate glycemic and insulinogenic responses beyond those normally encountered following a calorically equivalent non-fructose carbohydrate meal. Numerous physicochemical factors including diet composition may also influence the interactions with the brush border enzymes and consequently the efficiency of subsequent digestive activities. Specifically, the presence of dietary fibers, gums and pectin in addition to plant-derived phytochemicals can become somewhat protective of the glycemic excursions by impeding direct access to the brush border enzymatic actions. These typical food constituents can effectively decrease the rate and efficiency of luminal brush border digestion, with a corresponding attenuation in the glycemic and secondary insulinogenic responses, a well-established attribute of the dietary fiber content [7]. The addition of natural and pharmacologic inhibitors of α-glucoidase activity can further attenuate the insulinogenic and glycemic responses, while not compromising the net digestion and further luminal monosaccharide, nutrient or micronutrient absorption that may occur more distally in the gastrointestinal tract [9-12].

Characteristic of luminal carbohydrate digestive enzymes, the α-glucosidase activity is greatest in the proximal regions of the upper intestinal track and decreases progressively as the digestive contents continue their distal movement [9]. As the rates of luminal CHO digestion become decreased, the generation of

absorbable monosaccharide moieties also occurs more slowly, thereby resulting in less extreme excursions in plasma glucose and insulin concentrations following carbohydrate ingestion and digestion. As the glycemic excursions become attenuated, plasma insulin requirements may also plateau later but often at a lower magnitude because less insulin would then likely be required to facilitate immediate peripheral monosaccharide uptake, oxidation and disposal [9,10]. The extended nature of the fructose-induced insulinogenic response may trigger a shift in insulin stimulated secondary actions including lipogenesis, glycogenesis, and plasma insulin concentrations during the late post prandial period. Thus, the insulin-lowering phenomenon of α-glucosidase activity as mono- or combined therapy may be enhanced in the presence of inhibitors of luminal starch digestive activity [9,12-15]. In addition, because the physiologic haff-life of insulin receptor activity typically extends considerably longer than that of starch digestion and subsequent monosaccharide uptake and oxidation, downstream improvements in biochemical pathways of intermediary metabolism and lipogenesis would likely follow [13-15]. Thus, dietary supplements or additives that might extend the process of luminal digestion of starches and luminal absorption of simple carbohydrates and delay the rate of glucose uptake pose an interesting prospect in modulating downstream physiological events including contributions to appetite, satiety factors, plasma insulin activation, and including the metabolic effects of insulin on lipogenic and cholesterol generating parameters [9-11]. The improvements in glycemic responses would also likely elicit favorable improvements in other hormonal activities commonly linked to the glycemic responses including thyroidal, sympathetic, and likely glucocorticoid interactions.

The Insulinogenic actions exert numerous downstream effects on several key parameters of intermediary metabolism, in peripheral tissues including modulation of the rates of glucose oxidation, protein synthesis and degradation (protein turnover), carbohydrate oxidation and storage, and lipogenesis to cite just a few responses that are pertinent to this study [16-18]. Glucose readily enters glycolysis in peripheral tissues, resulting in providing substrates for glycogen deposition in addition to providing reducing equivalents for mitochondrial high energy phosphate generation. In contrast, fructose, once absorbed in liver or intestinal tissues, is converted to fructose- 1-phosphate and ADP, and splitting into two trioses (dihydroxyacetone phosphate or DHAP and glyceraldehyde or GA,) both of which can provide preferential substrate for *de novo* insulin-stimulated lipogenesis [17]. In addition, the ADP undergoes further degradation to AMP, IMP, and eventually may be degraded to uric acid. Uric acid is less soluble in plasma than IMP, has limited capacity for competitive renal secretion, and may form painful gouty lesions due to inflammatory uric acid crystallization in tissues over time when plasma concentrations exceed solubility levels [17]. Thus, the purpose of the present investigation was to determine the effects of partial luminal α-glucosidase inhibition via inclusion of miglitol as a controlled dietary admixture on plasma glycemic and lipid profiles. The studies were conducted in an animal model where early onset obesity, hyperinsulinemia, insulin resistance and T2DM occurs in the obese phenotype as the result of an autosomal recessive inheritance of the -*cp* trait, with an age of onset during early stages of adolescence [16,19,20]. Because of the genetic predisposition, T2DM occurs reproducibly in the obese offspring of both genders without the further inclusion of high fat diets or other dietary extremes. In addition, the pathophysiologic stigmata of obesity and T2DM once expressed remain present thereafter [17-19]. As noted, the epigenetic expression of obesity and further progression to T2DM occurs via expression of an autosomal recessive trait, and becomes accompanied soon afterward with the commonly observed progression of chronic pathophysiologic sequelae including derangements in plasma cholesterol and lipid profiles. Preliminary observations suggest that the development of hyperglycemia and hyperinsulinemia of T2DM are less severe in females than males, and that F1 hybrids with the nondiabetic LA/Ntul//-*cp* strain were found to result in what appear to result of a genetic dilution impact of the obesity- linked T2DM but not the obese stigmata. Thus, the T2DM in this strain occurs reproducibly with normal, otherwise healthful diets, in contrast to the application of high-fat diets or other dietary extremes to produce diabetic symptoms as has been employed in other strains of obese rodents, somewhat analogous to the inheritance patterns for obesity and T2DM that appear to occur in humans [17-19].

The congenic SHR/Ntul//-*cp* rat model was developed in the small animal genetics unit by Hansen at the NIH by incorporating the (-*cp*) trait from the Koletsky rat into a longevity-prone NIH (N) strain of unknown origin [20]. This was followed by crossing the N-*cp* (NIH) strain with the spontaneously hypertensive and diabetes-prone SHR rat and completing 12 or more cycles of backcrossing. This level of backcrossing was deemed sufficient to establish a congenic status in which 99.9% or more of the original genome remained authentic while preserving the SHR and -*cp* traits. With this model, all offspring are deemed genetically identical and the -cp trait will occur in 25% of the offspring of breeding pairs that are heterogenous for the -*cp* trait. The hypertensive trait was preserved only in the lean phenotype while the T2DM developed soon after weaning as a recessive trait in the obese phenotype. The newly developed SHR/N-*cp* strain also preserved the albino coat characteristic of the donor SHR strain, but not the agouti coat of the LA/N-cp strain. While in contrast, the F1 hybrids discussed above retained elements of both coat characteristics. Both phenotypes exhibit a significantly decreased lifespan due to complications of T2DM compared to their longevity-prone NIH (N) heritage [18]. The independent contributions of the obesity and T2DM traits may be further assessed in the nondiabetic LA/Ntul-*cp* vs the SHR/Ntul//-*cp* strains, where the development of T2DM has not been observed to occur in the LA/Ntul//-*cp* strain to date regardless of the diets offered [17-20]. Thus, the purpose of this investigation was to determine the efficacy of therapeutic luminal inhibition of α-glucosidase activity via miglitol on established insulin-linked parameters of metabolism including glycemic and lipid profiles in a unique, congenic animal model of early onset obesity and T2DM and in the absence of other confounding variables. In this animal model, the onset of the comorbidities of obesity and T2DM are expressed soon after weaning in the obese phenotype of the strain, and in this authors' experience, occur spontaneously

via an unknown mechanism presumed to be linked to a genetic predisposition, and occur independently of additional factors of diet and/or environment.

Materials and Methods

Groups of congenic obese male SHR/Ntul//-*cp* rats (n= 8 rats/group) housed under standard laboratory conditions of temperature (21-22 degrees C/ 50% RH) on a reverse light cycle (dark 0800-2000 daily) in adjacent hanging steel cages with individual occupancy. Animals were fed Purina Chow and house water *ad libitum* from weaning to 8 weeks of age, at which time early stages of obesity and T2DM were clearly established and glycosuria and T2DM confirmed. It is noteworthy that in this animal model, the onset of obesity and T2DM occur spontaneously soon after weaning via a genetic predisposition mechanism likely resulting from a Tyr763Stop mutation in the extracellular domain of the leptin receptor, and independent of specific dietary or environmental interactions [21-49]. Thus, specific metabolic pathways involved that progress to the metabolic sequelae of obesity +T2DM are currently unclear or unknown beyond their genomic origin. All rats demonstrated glycosuria via test strips by 8 weeks of age, and which continued thereafter (Urine Glucose Test Strips, OneStep). When 8 weeks of age, rats were switched to a semi-purified control diet developed at the Carbohydrate Nutrition Laboratories of the USDA that contained 54% carbohydrate as sucrose, 20% protein as equal parts casein and lactalbumin, 5.9 % cellulose, 16% fats as equal parts beef tallow, lard, corn oil, and hydrogenated coconut oil, 3.1% AIN vitamin salt mix, and 1% Teklad vitamin fortification mix (Control diet), and provided to the investigators from the carbohydrate nutrition laboratory of the USDA, Beltsville, MD USA [20]. The energy content of the diet was computed to provide 48.2 % of calories from CHO, 33.3 % of calories from fats, and 18.5% of calories from protein respectively, and provided 4.4 kcal/gram as described elsewhere [20]. The semi-purified diet was fed *ad libitum* for up to 8 weeks. In addition, additional quantities of the control diet were fortified with 150 mg of the α-glucosidase inhibitor (1,5 dideoxy-1,5-[(2-hydroxyethyl)-imino]-D glucitol; generic= miglitol, MIG diet) per kg. diet (equal to ~ 2.5 mg of miglitol/rat/day) and was fed to the α-glucosidase inhibitor treatment group for up to 8 weeks duration. Thus, the diet consumed by both treatment groups contained the same proportions of essential nutrients and caloric density, +/- miglitol.

Body weights were monitored periodically throughout as an indicator of wellness. At the end of the study, rats were fasted overnight and blood obtained via tail bleeding in heparinized tubes for plasma glucose and insulin area under the glucose tolerance curve via a glucose oxidase method on a YSI glucose analyzer and in-house radioimmunoassay, respectively [50,51] (AUC, 250 mg / 0.1 kg BW, via gavage). Insulin resistance computed as HOMA was determined as described by Mathews et al. [52] Glycated hemoglobin (HbA1c, GHb), and lipid analysis. Plasma triglycerides, cholesterol and the α- lipoprotein (LDL) and β-lipoprotein (HDL) fractions were determined spectrophotometrically (Beckman AU480) following affinity chromatographic separation via the procedure of Bentzen et al. and triglycerides by the enzymatic method of Bucolo and David, with all reagents acquired from Fischer Scientific and prepared on site [20,21]. Measures of glycolytic enzyme activity including glucokinase (GK), malic enzyme (ME) and glucose-6-phosphate dehydrogenase (G6PD) were determined spectrophotometrically and expressed as μmoles of product produced per minute per mg of protein per liver in a sucrose-EDTA- phosphate buffered liver homogenate as described by Freeland and tissue protein content determined as described by Lowry et al. [43,44] Data were analyzed via standard statistical procedures including application of Pages 'L' test for trend analysis with correction for covariables where statistical significance via the 't' test was suggestive but not confirmatory [23,24]. The study was approved by the Institutional Animal care and Use Committee.

Results

Initial and final Body weights, net weight gain, and dietary energy intake of rats over <8 weeks of observations are depicted in Figure 1A to 1F and Figure 2 respectively. Daily energy intake are depicted in Figure 1A and indicate that control animals consumed more energy per day from week 1 to week 8. The cumulative energy intake over 8 weeks is depicted in Figure 1B and confirms that net energy intake over the 8-week course of observation was also greater in control than the miglitol group. Thus, these data indicate that net energy intake of control rats was ~ 13% greater overall than that recorded when fed the Miglitol regimen for 8 weeks (Figure 1B, left panel), with a modest improvement in feed efficiency when fed the miglitol regimen (Figure 1B, right panel).

The effects of Miglitol on fasting glucose and insulin at 15 weeks of age are depicted in Figures 1C and 1D, respectively, and indicate that Miglitol was associated with lower fasting glucose and insulin concentrations at the end of the study. The AUC for glucose during a 120-minute oral glucose tolerance test after 7 weeks of treatment is depicted in Figure 1E and indicates that the AUC of Miglitol treated rats averaged ~20% less than the control rats, consistent with the data of energy intake in those animals during the same timeframe. The HOMA score decreased only modestly, however, indicating that insulin resistance while marginally improved, remained present after the drug treatment (values cited in Legend to Figure 1C, 1D]. The improvement in the percentage of glycated hemoglobin (R panel Figure 1E) although still above the normal range, also reflected a 24% reduction following only < 8 weeks of the Miglitol regimen, a highly significant finding indicating that the Miglitol was effective both in lowering fasting insulin concentrations and in controlling the post-prandial glycemic responses to feeding. In addition, since rodents are generally considered to be grazers as opposed to meal eaters, the results in AUC glucose reduction are even more significant considering the short duration of the study. It is likely that a longer duration of miglitol treatment may have resulted in further improvement toward normalization of the percent HbA1c. This is an important consideration, as glycated hemoglobin moves the oxygen saturation curve of hemoglobin to the left and therefore impedes the release of oxygen from the glycated moieties, thereby decreasing the net efficiency of oxygen delivery to myoglobin where it can contribute to oxidative metabolism.

The effects of miglitol on liver glycolytic enzyme activity are depicted in Figure 1F and indicate that glucokinase and malic enzyme activity were lower in rats fed the miglitol regimen, while measures of Glucose-6-phosphate in miglitol fed rats reflected a significant trend only, consistent with improvements in insulin-linked elements of glycolytic and lipogenic activity.

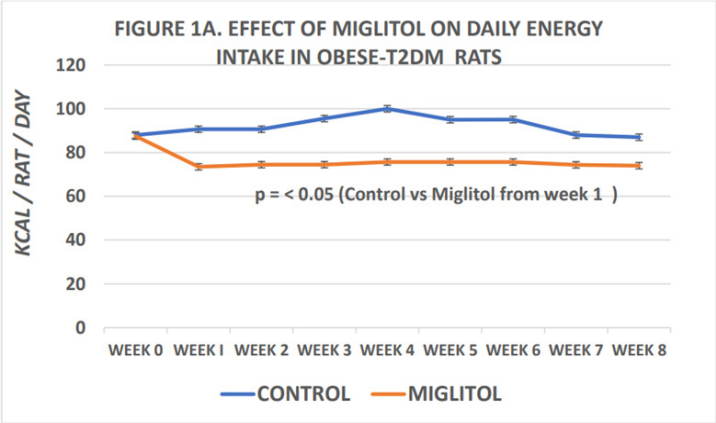


Figure 1A: Effect of miglitol on weekly food intake in T2DM rats. Data are mean ± 1 SEM, n = 6-8 rats/group. P = < 0.05.
Control vs Miglitol from week 1 to week 8. (Students t test, individual comparisons, Control vs Miglitol.).

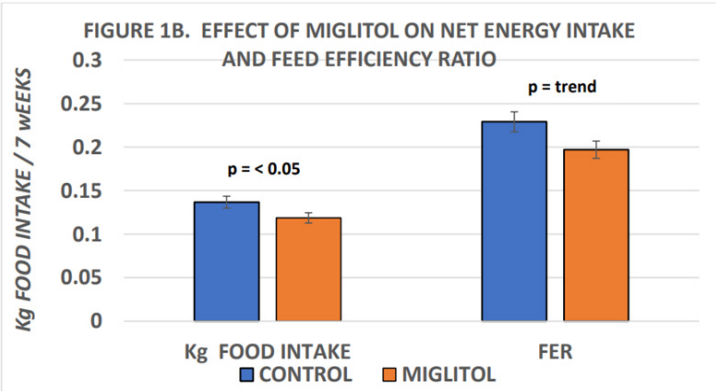


Figure 1B: Effect of α-glucosidase inhibitor on food intake and feed efficiency ratio. Data are mean ± 1 SEM, n = 8 rats/group. P = < 0.05 via students t test; p = < 0.05 (trend) via Pages L test for trend analysis.

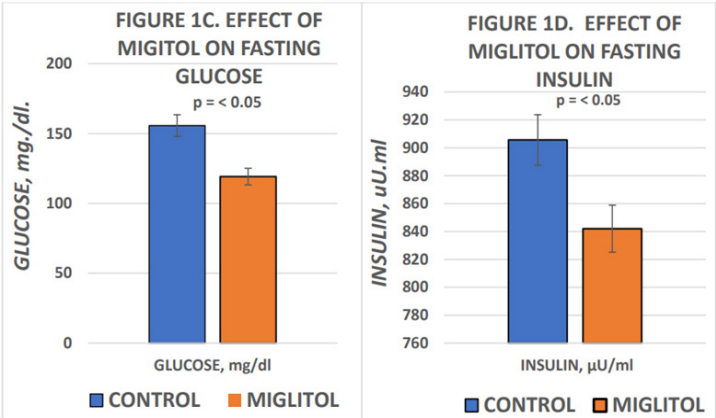


Figure 1C and 1D: Effect of miglitol on fasting glucose (Left panel) and insulin (Right Panel) at end of study. Data are mean ± 1 SEM, n = 6 rats/group. P = < 0.05 (Students t Test). This resulted in a modest 5% decrease in mean HOMA score from 2.6 ± 0.1 (Control) to 2.4 ± 0.1 (miglitol) (p = n.s.).

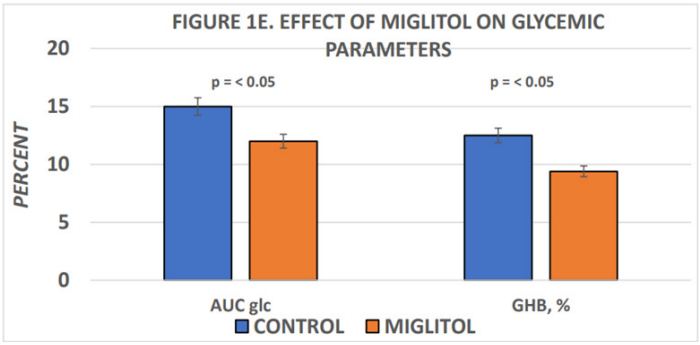


Figure 1E: Effect of miglitol on glycemic parameters at 15 weeks of age. Data are mean ± 1 SEM, n= 6-8 rats/group. p = < 0.05 as determined by Students t test.

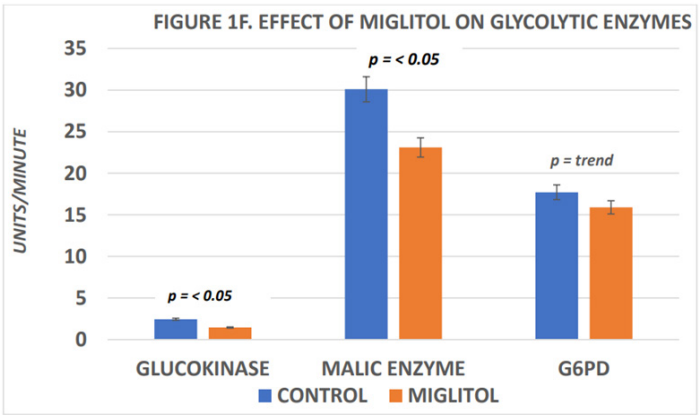


Figure 1F: Effects of miglitol on enzyme parameters at 15 weeks of age. Data are mean ± 1 SEM, n = 6 rats/group, recorded as units, where 1 unit = 1 µmole/minute/mg protein of liver homogenate. P = < 0.05 via Student's t Test; Trend = Pages L test for trend analysis.

The body weights and weight gain are depicted in Figure 2. The initial weights were similar in both treatment groups (left panel, 263 ± 11 g. vs. 263 ± 12 g). In addition, the final body weights of the control rats were modestly (~6%) greater than those fed the miglitol regimen, with a final mean weight gain that was 12% less than occurred in the control fed rats. Of interest, the weight gain per gram of protein consumed was similar in both groups (Mean grams gain/gram protein consumed = 8.65 ± 0.18 g/g in control vs 8.74 ± 0.21 g/g in the miglitol-fed rats, thereby suggestive but not conclusive of an improved economy of energy utilization when fed the Miglitol regimen (p = n.s.) Thus, the α-glucosidase inhibitor miglitol resulted in modestly (~12-13%) lower rates of weight gain and in similarly lower final body weights during the 8 weeks of observation of the study. (Control vs. Miglitol FBW: p. = < 0.05 via trend analysis; Control vs. Net Gain: p=<0.05 via trend analysis.) The energy intake of rats is depicted in Figure 2 and shows that energy intake of control rats over the entire course of the study was 13% greater than in the miglitol treated animals

(p=< 0.05, Students t test). Thus, the net efficiency of weight gain over the duration of the study was similar in both phenotypes and generally proportional to the nutrient intake.

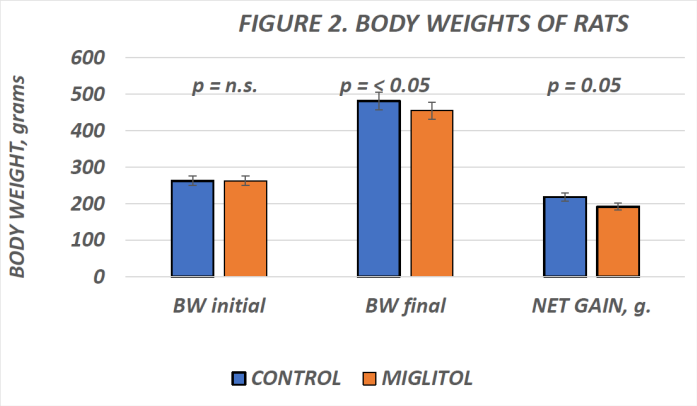


Figure 2: The body weights of rats. Data are mean ± 1 SEM, n = 8 rats/group. P = < 0.05 as indicated by students t test. Thus, mean gain/g protein consumed was proportionate to protein intake; 8.6561 g gain/g protein consumed vs MIG = 8.7532 g gain/g protein consumed.

The effects of miglitol on adipose tissue depots are depicted in Figure 3 and indicate that miglitol depicted in the near right panel was associated with a 15% decrease in the sum of the epididymal, retroperitoneal and dorsal adipose tissue depots, but not all individual adipose tissue depots depicted in the left panels were decreased proportionately. The most significant decrease in depot mass was observed in the retroperitoneal depot, while epididymal and dorsal depots were similar with only a modest downward trend in both groups. The dorsal depot and the WAT:Body weight ratio reflected only a downward trend following the miglitol regimen, suggestive of depot-specific (visceral vs non-visceral depots) effects on lipid accretion. When the sum of the WAT depots was computed as a percentage of body weight, the net decrease in WAT mass in the miglitol treated rats depicted in bars to far right of each panel Figure 4 averaged 10% of final body weights, and 18.5% of weight gain. Thus, the cumulative decreases in total combined WAT mass were of similar magnitude to the decreases in net energy intake and weight gain.

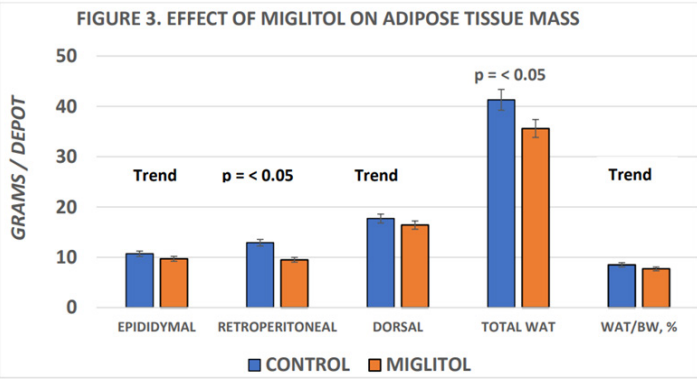


Figure 3: Effect of miglitol on adipose tissue mass at 15 weeks of age. Data are mean ± 1 SEM, n = 6-8 rats/group. p = < 0.05 for retroperitoneal and total WAT depots. Epididymal, Dorsal, and percent WAT/BW = trend = < 0.05 via Pages L test for trend analysis.

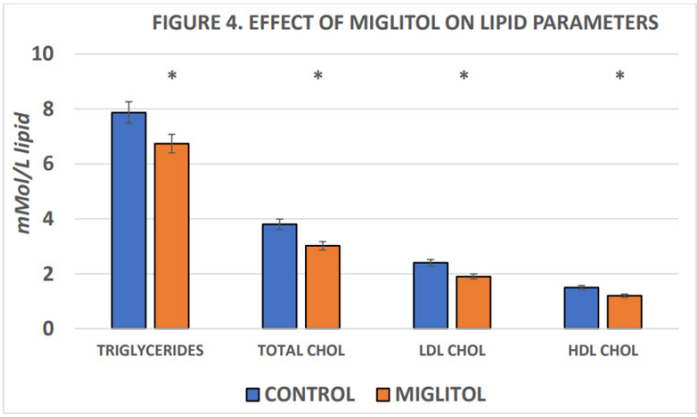
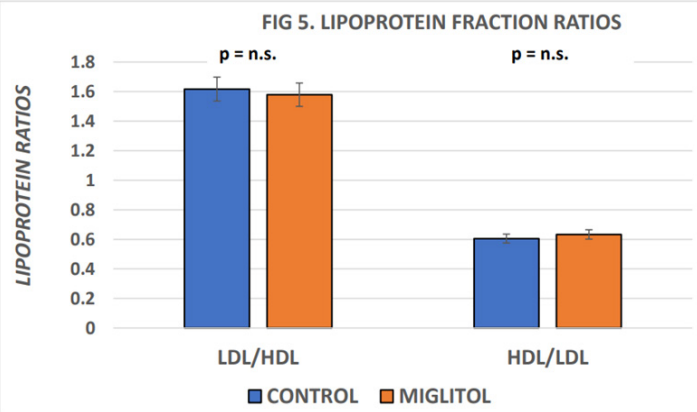


Figure 4: Effect of miglitol on lipid parameters at 15 weeks of age. Data are mean ± 1 SEM, N = 6-8 rats/group. * = p = < 0.05 by Students t test.

The effects of luminal α-glucoside inhibition on plasma triglycerides and total cholesterol concentrations are depicted in Figure 4 and indicate that α-glucosidase inhibition resulted in an approximate 20% decrease in total plasma concentrations of plasma triglycerides, cholesterol and lipoprotein cholesterol fractions after < 8 weeks of the dietary and pharmacologic treatment. In addition, the final concentrations of both the LDL and the HDL lipoprotein fractions were both decreased by an average of ~18-20% following the α-glucosidase treatment, and the effects were nearly evenly distributed across both LDL and HDL fractions. In addition, the LDL/HDL and HDL/LDL lipoprotein ratios are depicted in Figure 5 and further indicate evidence that the pharmacologic treatment of luminal α-glucosidase activity with miglitol while demonstrating improvement in all lipid fractions, was without significant effect on lipoprotein ratios. This suggests that the effects of the α- glucosidase inhibitor agent were equally distributed across all triglyceride and lipoprotein fractions, consistent with a predicted global effect of improvements in insulin actions on lipid and cholesterol biosynthesis and metabolism.

Figure 5: Effect of miglitol on Lipoprotein ratios at 15 weeks of age. Data are mean ± 1 SEM, n = 6-8 rats/group. p = n.s. for all comparisons via students t test.



Discussion

In the present study, feeding of an admixture of miglitol in a high carbohydrate, nutritionally complete diet to obese T2DM rats for up to 8 weeks resulted in decreases in energy intake, weight gain, adiposity, key glycemic parameters, and improvement in plasma lipid profiles despite only modest decreases in insulin resistance [52]. The decreases in weight gain and depot specific adiposity associated with the α-glucosidase inhibition were generally proportional to the decreases in total energy intake over the duration of the study and were suggestive of favorable contributions from gastrointestinal satiety factors due to the delayed digestion of the dietary carbohydrate. Luminal digestion of sucrose occurs rapidly in the brush border projections located mostly in the proximal regions of the duodenum where the greatest digestive activity of the glucosidase enzymes are located, and glucosidase activity decreases gradually as the food digestive progresses distally [9,10]. Any residual carbohydrate that may remain undigested in the small intestine for any reason can then likely become energy substrates for the colonic microbiota, but little carbohydrate typically escapes undigested under normal digestive processes. Regardless of where in the small intestine the carbohydrate becomes digested, the luminal uptake of monosaccharide moieties occurs straightforward, thereby accounting for the magnitude and duration of increase in plasma glucose following a carbohydrate meal. Since sucrose was the only carbohydrate contained in the research diet, luminal glucosidases would normally be capable of a complete sucrose digestion within the first 30 to 45 minutes post prandially depending on meal size, with luminal absorption of the fructose entity soon afterward. Thus, any delay in the luminal carbohydrate digestion would be predicted to decrease the magnitude and intensity of the glycemic excursions including both plasma glucose and insulin following a carbohydrate containing meal. The significant reduction in the AUCglucose in concert with modest improvement in HOMA provides further evidence of the efficacy of miglitol in modulating carbohydrate digestion in the upper regions of the small intestine, soon after gastric emptying had occurred, likely secondary to the decreases in plasma insulin concentrations. Further evidence for improvements in insulin sensitivity is supported by the decreases in glucokinase, an enzyme that functions as the β-cell sensor for insulin release in response to rising plasma glucose levels, and malic enzyme and glucose-6-drhydrogenase, which contributes to lipogenesis by catalyzing NADPH generation. The availability of NIDPH is deemed essential for *de novo* fatty acid biosynthesis in liver and adipose tissue [17,44-48]. In addition, the improved insulin sensitivity also may contribute to reducing oxidative stress in addition to potential enhancements in the economy of protein utilization [17,44-47].

Because type-2 diabetes mellitus (T2DM) in association with obesity and overweight conditions including metabolic syndrome are now emerging as one of the most prevalent metabolic disorders in the world, it is imperative that a productive therapeutic and cost-effective strategy be developed and applied if attempts to resolve the phenomena are to be obtained [1-7]. Indeed, currently more than a third of the population throughout Westernized society is now impacted by an overweight or obese condition, with a high prevalence among those with the syndrome of developing T2DM [4]. Treatment of T2DM and obesity is often lifelong, thereby imposing a significant burden on healthcare resources due to the large number of patients who may present with comorbidities that may accompany the disorder, and the extended duration of their treatment. Discontinuation of an effective strategy typically enables the condition an opportunity to return within the weeks and months following cessation of the therapeutic intervention with an expected resurgence of the previous comorbidities [26].

The prevalence of obesity and T2DM has placed an enormous economic burden on the available health care resources of many communities [1-4]. The disorder also contributes to economic losses in workplace productivity when individuals are unable to complete their workplace obligations in a timely or effective manner due to illnesses that are linked to their obesity-T2DM status [1-3]. Modernization in the industrialization of food processing and distribution has also brought with it greater safety of the food supply, in addition to emerging changes in diet preferences and nutritional practices from those of past generations [6]. The changes in food availability and practices have also inadvertently contributed to less successful attempts by individuals to maintain energy balance in a more sedentary society. In addition, the modernized biological energy and labor-saving innovations now common to daily life in Westernized nations may also be a significant contributor, as the current standards for home and workplace efficiency allow more time for leisure and relaxation from the physical hardships of past generations. Thus, it is important to explore novel approaches to combat the emerging trends in disordered energy balance and their contributions to pathophysiologic sequelae which may result at least in part from technological advancements. While no single strategy has yet been demonstrated to combat the emerging trends in the obesity+T2DM dilemma, several relevant animal models have been developed, including the SHR/Ntul//-*cp* rat, the LA/Ntul//-*cp* rat, the Wistar Fatty Rat, the Zucker fatty rat and others [18,25-29]. These models may be applied to provide insight into effective environmental and pharmacologic strategies to address the issues and to further elucidate the pathophysiologic mechanism or mechanisms involved in the epigenetic expression of traits that contribute to obesity and T2DM.

The ingestion of high carbohydrate, overly caloric, and high glycemic index diets common to Western society are typically contraindicated in Obesity+T2DM, where they are commonly associated with unwelcome elevations in weight gain, adiposity, and in increases in fasting plasma lipid profiles and other stigmata of obesity+T2DM [1-3]. Current therapeutic strategies often include changes toward a more healthy life style, including diet planning that includes a complex carbohydrate, modest fat diet, with special attention to ensure adequacy in fiber and micronutrient intake, combined with episodes of greater physical activity. The cumulative effects of dietary and lifestyle changes are projected to bring about in a lower calorie, lower glycemic index diet, while more closely matching nutrient intake to the projected energy requirements of the individual to maintain neutral energy balance. The added incorporation of starch blocker agents such as

acarbose, miglitol or other natural inhibitors of starch digestion may be a welcome additive to the luminal effects of the lower glycemic index – high fiber complex carbohydrate diet regimen [1-3]. Incorporation of such a regimen may bring about lasting measured weight loss with corresponding improvements in the common pathophysiologic stigmata of obesity+T2DM, which may contribute to improvements in the metabolic profile of the individual. Luminal modulation of CHO digestion and monosaccharide absorption via α-glucosidase inhibitors combined with naturally occurring food components (often from vegetarian sources) may also bring about similar effects on α-glucosidase digestive activity [9]. Considering that the primary mechanism of action of most α-glucosidase and sucrase inhibitors is competitive inhibition, and is typically limited to the luminal brush border region, with little if any post-ingestive absorption or systemic distribution, considerations of hepatic or other organ toxicity become minimal. Miglitol, the focus of this study, is a complex oligosaccharide that acts as a competitive, reversible inhibitor of membrane-bound intestinal α-glucoside hydrolase activity [9,25-29]. Luminal modulators of starch digestion including acarbose and miglitol have been found to be useful agents in treating mild to moderate severity T2DM [9,25-30]. In a previous animal study of <8 weeks duration with the miglitol analog acarbose, the HbA1c and glycemic responses typically demonstrated improvement toward normalization over time [9]. The results of this study are also consistent with previous clinical findings in the Wistar Fatty Rat, and further confirm that the 8-week trial of feeding a highly palatable high carbohydrate sucrose-laden diet to obese adult Wistar Fatty Rats with well-established T2DM is an adequate duration to bring about improvements in glycemic parameters including excess adiposity and weight gain. The metabolic similarity in the development of insulin resistance between the obese phenotypes of the Wistar fatty rat, the SHR/Ntul//-*cp* rats and other similar models contribute to the development of the obese+T2DM stigmata in those animal models. All similar animal models demonstrated significant improvements in glycemic parameters when offered the glucosidase inhibitor regimen including an attenuation of the chronic hyperphagia commonly associated with the obese phenotype [9,10,37]. As reported by Boque et al. [31] when lean male Wistar rats were fed a similar high carbohydrate diet those authors also reported an increase in fasting triglyceride concentrations from the high glycemic index diet. In the present study, the miglitol treatment was associated with improvements in glycemic parameters, plasma triglyceride, total cholesterol, LDL-cholesterol and HDL cholesterol fractions, and an attenuation in the sucrose linked weight gain in addition to a decrease in the glucose AUC and percent HbA1c.

Because only animals of the obese-T2DM phenotype were included in this study, if it was not possible to determine if the final weight and metabolic profiles might have equated to those of their lean littermates when fed similar diets in the 8-week duration of the observations. However, since the obese rats were already significantly heavier than their lean littermates at the onset of the study (avg lean = 235±6 g. vs obese = 264±19 g. at 8 weeks of age; $p = < 0.05$), and the T2DM stigmata were already well established, it is likely that longer treatment has been necessary for full resolution. This is especially relevant since hyperinsulinemia and atheroma development have been found to commence soon after weaning in this and other similar strains [18-20,26-31]. In the present study, the decreases in net energy intake and weight gain both averaged 15% following the miglitol feeding regimen, indicative of a favorable correlation between the dietary impact and metabolic sequala, with no apparent rebound effects, metabolic complications, or adverse side effects noted. Indeed, during necropsy, no pathophysiological aberrations were noted in the miglitol-treated animals. In an earlier study, it was noted that the pattern of decreased food intake differed similarly when the glucosidase inhibitor acarbose was fed as an admixture to lean and obese non-diabetic rats, resulting in a comparable improvement in glycemic responses, adiposity and plasma lipid parameters [32]. Indeed, the presumption of evidence from the present study suggests that the dietary regimen linked improvements in the glucose AUC, HbA1c, lipid profiles and weight gain are consistent with improvements in insulinogenic actions in peripheral tissues, including skeletal muscle and adipose tissue, major sources of peripheral insulin resistance in obesity and T2DM [35-36].

Although not studied in the current investigation, the contributions of inflammatory cytokines including tumor necrosis factor alpha (TNFα), Interleukin 6 (IL6), C-Reactive Protein (CRP) and nuclear factor Kappa B (NF- kB) and others were not determined. Numerous studies in rodents and cell culture techniques have established links between elevated plasma lipids and fatty acids as likely triggers for the generation of the inflammatory entities, which are associated with disordered endothelial function, common in T2DM and its pathophysiologic comorbidities [37-41]. While the molecular mechanisms of the above cytokines is unclear, insulin resistance is a common observation when plasma levels of monocyte derived inflammatory cytokines are elevated, thereby posing an increased contributory risk for T2DM. Because T2DM ranks among the most common metabolic disorders worldwide, and miglitol is cost effective, it is a readily administrable agent that could decrease plasma lipid levels, improve glycemic status, and decrease the trend toward greater adiposity. These pharmacologic benefits could make a significant impact on this disorder, analogous to the favorable and well established anti- inflammatory and cholesterol-lowering effects of statins on cardiovascular diseases, and are now safely prescribed virtually worldwide with minimal side effects.[42] In future studies of α-glucosidase inhibitors it will be of interest to determine if such a regimen could attenuate or forestall the onset of T2DM and its sequelae in man and animals, as in most therapeutic venues, prevention is preferable to the burdens of later management.

Summary

In summary, the improvements in plasma lipid and glycemic profiles in the obese+T2DM phenotype of this and other strains following luminal glucosidase inhibition have been clearly established, despite only modest improvements in insulin resistance as computed via HOMA analysis. Left untreated, increases in biochemical markers for free radical development and are clearly

consistent with atherogenic lipid profiles, including elevations in serum triglycerides, cholesterol, and including the LDL lipoprotein fraction, which are consistent with senescent, atherogenic alterations in the vascular intima [4-6]. Although the glycemic and lipid parameters were improved but not completely normalized in the obese+T2DM rats following the miglitol treatment, it is likely that a longer duration of treatment may have resulted in a more complete recovery. While a conclusive determination of the physiological mechanism for the lipid lowering effects could not be determined from this study, since all are insulin-linked parameters, the likelihood of an improved insulin sensitivity and insulinogenic actions in peripheral tissues including the liver must be entertained. As evidence for this conclusion, the partial recovery of the precent HbA1c in miglitol treated rats is noteworthy, considering that a) the glycation reaction is considered to be non-reversible and non-enzymatic reaction correlated with the average plasma glucose concentrations, b) once the glycation reaction has occurred, is dependent on the typical four-month lifespan of the normal erythrocyte, c) the miglitol treated rats exhibited an approximate 60% recovery in the glucose AUC after only 8 weeks of study, and d) the hepatic glycolytic and lipogenic enzymes studied all demonstrated improvement following miglitol treatment. Thus, had the study been extended to months duration, the percent HbA1c decrease would likely have been closer to that obtained from non-diabetic animals, at around 7 % or less, since laboratory rats are considered grazers, and tend to eat more frequently during their feeding cycle than some other mammalian species.

Determination of percent HbA1c is considered a reliable marker for monitoring clinical diabetes therapy and thus was an important consideration for assessing the effectiveness of α-glucosidase actions on luminal monosaccharide generation and insulinogenic actions in peripheral tissues in the present study. Thus, the use of α-glucosidase inhibitors for the treatment of glucose intolerant conditions is deemed a useful clinical approach in attenuating the insulin-dependent, hyperglycemic sequelae of the obese+T2DM phenotype of this strain and supports its usefulness in the treatment of T2DM in humans [35,36]. In conclusion, while the effects of miglitol and other luminal glucosidase inhibitors on lipid parameters have sometimes been inconclusive, those differences are likely due to differences in experimental models, patient populations, duration of pre-existing illness, dosages and agents employed, and duration of the treatment regimens, the improvements noted in the present study are likely attributable to an improved economy of insulin sensitivity, glucose utilization and lipid metabolism in peripheral tissues.

Conclusion

The short-term administration of a modest dosage of the α-glucosidase inhibitor miglitol in the presence of a high glycemic index sucrose-enriched diet was found to be useful in attenuating the excess weight gain and increases in plasma cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations in the adult male obese SHR/Ntul//-*cp* Rats. These observations are consistent with typical dietary recommendations for consumption of complex carbohydrate, fiber rich diets for a variety of glucose-intolerant conditions. and suggest that miglitol when administered as a dietary admixture may be a useful therapeutic adjunct in the treatment of obesity+T2DM, and in an attenuation in the chronic systemic inflammation and pathophysiologic sequelae associated with the obese-diabetic state. Moreover, the clinical effectiveness of luminal glucosidase inhibitors on lipid parameters may be enhanced by the addition of a cholesterol lowering agent or other therapeutic adjunct, as has now been demonstrated in multiple clinical trials. The clinical efficacy of miglitol has now been demonstrated in numerous clinical trials and it's demonstrated effectiveness in treating obesity associated T2DM is supported [30-36]. While a direct correlation between rodent and human studies cannot be made from the present investigation, the remarkable similarity in processes of carbohydrate metabolism and digestive processes in rodents and humans lend credibility in that the characteristics of T2DM and its pathophysiologic comorbidities combined with the metabolic effects of miglitol are demonstrability similar in both examples. Thus, the results of this investigation may lend credence to the concept of luminal modulation of starch digestion as a pharmacotherapeutic adjunct for the treatment of obesity, T2DM and insulin resistance conditions.

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