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Original Article

# EFFECTS OF AGING AND EPIGENETIC PREDISPOSITION OF EARLY ONSET OBESITY AND T2DM ON THE EXPRESSION OF GLYCEMIC RESPONSES INCORPULENT RAT STRAINS

Prof. Orien L Tulp, PhD, MD, FACN, CNS*
ORCID 0000-0001-6904-2573

Professor of Medicine and Graduate Studies, University of Science Arts and Technology, Montserrat, British West Indies. MSR1110

*Corresponding author. Contact: o.tulp@usat.edu.

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**ABSTRACT:**

To determine the effects of epigenetic predisposition of early onset obesity,aging and T2DM on glycemic parameters, groups of congenic male lean, obese, and obese-diabetic rats (n= 5-6 rats/group) that share the same genetic trait for obesity (the -*cp* trait) were reared under normal laboratory conditions and fed stock Purina Rodent chow*ad libitum* throughout. Rats were subjected to measures of fasting glucose, insulin, amylin, insulin:glucose ratios, and glycated hemoglobin concentrations and on the glycemic response to an oral glucose tolerance (OGT) and Area Under the curve (AUCglc) at 4 and again at 12 months of age. Obese animals weighed more than their lean littermates (p = < 0.05). Fasting plasma glucose, insulin, and amylin concentrations of obese > lean, and in increased further in NIDDM-prone animals, with the greatest increases in the oldest animals. Hemoglobin A1c trended higher in obese than in lean littermates and was markedly greater in the NIDDM-prone animals of both ages.(p = < 0.05) Oral glucose tolerance and AUCglucose of obese > lean, and was markedly greater in NIDDM-prone animals, with the greatest increase in the oldest animals. The AUCglc was greater in obese than in lean littermates and increased significantly on T2DM-prone animals at both ages. In conclusion, these results demonstrate the effects of aging on the obese trait on development and expression of insulin resistance may occur independently of T2DM in these closely related congenic strains and thattheglycemic responses deteriorate further with aging when left untreated.

**Key Words:** Obesity, Diabetes, T2DM, Congenic Rat, epigenetics

## INTRODUCTION:

The incidence of obesity and its associated co morbidities is rapidly approaching epidemic proportions in much of industrialized society,



**How to Cite**

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where it tends to occur following factors of deregulated diet and lifestyle often along familial lines.[1-3]While the development of insulin resistance and adult-onset diabetes, also commonly known as Type II diabetes (T2DM of NIDDM) rank among the most common complications of the obese state, not all individuals who develop obesity also develop T2DM.[4.5,6] This suggests that there may be independent links for the two common manifestations of obesity. Thus, the development of insulin resistance, while a hallmark of T2DM, implies factors that may not be directly linked to T2DM.[7] Insulin resistance vs. insulin sensitivity involves activities of the glucose transporter protein, GLUT4, normally formed via a genetic expression mechanism in the endoplasmic reticulum of somatic cells.[8-10] Once formed, the GLUT4 transporter undergoes intracellular cytoplasmic translocation to the plasma membrane, where it facilitates the stereo specific and insulin-dependent transmembrane uptake of molecular glucose. Hormonal factors including glucocorticoids, a common element of obesity and metabolic syndrome impair the genomic expression of GLUT4, thereby impeding eventual glucose uptake in peripheral tissues.

The cumulative effect of decreased availability of GLUT4 transporters also contributes to the metabolic parameters of hyperinsulinemia and insulin resistance. Hyperinsulinemia further contributes to the development of T2DM and obesity and neurologic dysfunction.[8]While dietary strategies remain the primary foundation of nominal early clinical management, such changes often pose challenges to implement, especially during early management stages when the pathophysiologic changes remain largely asymptomatic, but when they likely could most effectively contribute to meaningful positive change. Thus by the time their progression becomes physiologically apparent, the magnitude of metabolic disease has likely advanced, and more aggressive therapeutic measures may be necessary to arrest, resolve, or reverse further progression to attenuate or prevent more serious health issues. [7]

The LA/Ntul//-cp and SHR/Ntul//-*cp* (corpulent) rat models were developed in the small animal genetics unit at the NIH by Hansen to create a congenic rodent model for application to studies of obesity, cardiovascular and related studies.[11] The present corpulent rat strains were developed by incorporating *the -cp* trait from the Koletsky rat into a longevity-prone NIH (N) Lister-Albany train of unknown origin.[11-13] This was followed by crossing the N-*cp* strain with the spontaneously hypertensive rat (SHR), and completing 12 or more cycles of backcrossing sufficient to establish congenic status while preserving the SHR and obesity (-*cp*) traits. The hypertensive trait was preserved only in the lean phenotype while the T2DM developed soon after weaning in the obese phenotype.

The newly developed SHR/N-*cp* strain preserved the albino coat of the SHR strain. Both phenotypes exhibit a significantly decreased lifespan due to complications of T2DM compared to their original longevity-prone NIH (N) heritage.[14]Thus, the current study was undertaken to more fully determine the pathophysiologic effects of aging on the expression of the obesity and T2RM traits. The development of T2DM is not known to occur in the obese phenotype of the congenic LA/Ntul//-*cp* strain, while is occurs reliably soon after the onset of obesity in the obese phenotype of the congenic SHR/Ntul//-*cp*strain.[15]

## MATERIALS AND METHODS:

Animal subjects and Housing. Male animals were selected from the Drexel colony of LA/Ntul//-*cp* and SHR/Ntul//-*cp* rats at 4 months of age and separated into lean and obese groups based on developing characteristics of the emerging obese or obese+T2DM/NIDDM state (n=5-8 rats/group). Early physiological changes included subtle changes in gait, stance, palpable subcutaneous fat and resting VO2 upon weaning. Selected animals were placed in shoebox cages lined with one inch of pine shavings, in a temperature (22-24 C) and humidity controlled (50-60% RH) environment from weaning and thereafter. Animals were fed standardized Purina Chow #5054 and house water ad libitum throughout the study.

The Purina chow had a reported energy density of 3.34kcal/gram (14.2kjoules/gram) based on the manufacturer's certificate of analysis and contained

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(w/w) 55.6% carbohydrate, 22.5% protein, 4.5% fat, 4.6% crude fiber, 6% ash, and 1-2 % essential vitamins and minerals and <5% moisture. Specific nutrient sources cited in the Purina Chow included ground extruded yellow corn, soybean meal, fish meal, cane molasses, wheat middling, alfalfa meal, ground oats, brewer's dry yeast, wheat germ meal, dried beet pulp, soybean oil, dicalcium phosphate , calcium carbonate, salt, and a blended vitamin supplement containing B-12, calcium pantothenate, choline chloride, riboflavin, thiamin, niacin, DL-methionine, D-activated animal sterol as a source of Vitamin D3, vitamin A, pyridoxine hydrochloride, vitamin E, calcium iodate, manganous oxide, ferrous carbonate, cobalt carbonate, copper sulfate, zinc sulfate, and zinc oxide.

This diet has been deemed a complete life cycle diet especially formulated to support the healthful growth, development, and maintenance of rats, including reproduction and lactation, and has been a standard rodent diet for biomedical research worldwide for many decades. Importantly, this diet has a relatively low fat content(~4.5%) and has not been found to independently induce characteristics of obesity or T2DM in rodents in our experience.[15]

## SUMMARY OF EXPERIMENTAL PROCEDURES:

Oral Glucose Tolerance (OGT) administration and process. Animals were administered a 5-point oral glucose tolerance test (OGT) after a brief 4-hour period of food deprivation, with free access to house water throughout. The glucose challenge containing 2.50 g glucose/kg of body weight as a 50% glucose solution and administered gently in quietly resting animals via intragastric gavage over an approximate 30 second to one minute duration during the forenoon hours (1000-1200hr). Animals became introduced to the procedures via preliminary pretreatment procedures in advance so as to minimize any potential stress-related impacts on the gavage and blood collection procedures. Bloods were obtained via tail bleeding before (i.e., fasting) and after 30, 60, 90, and 120 minutes following the intragastric glucose challenge.[16] Blood glucose concentrations were determined and recorded immediately after the blood draws with a rapid glucose oxidase method (Glucometer Elite®, Ames Laboratory, Elkhart IN) based on the original procedure of Raabo and Terkilden.[16]The glucose area under the OGT curve (AUC) was determined by the procedure originally described by Sagakuchi et al and used in our laboratory for many years.[17]

Plasma concentrations of Insulin and amylin were determined via radioimmunoassay using materials obtained from Penninsula Laboratories, Pomona CA.[18] Hemoglobin A1C was determined spectrophotometrically following rapid micro column resin separation of hemoglobin fractions using commercially prepared reagents and materials obtained from Sigma Chemical Company, St Louis, MO, USA.[19] Data were analyzed via standard statistical procedures with the Stat view statistics program, Abacus Concepts Berkley CA) and Pages 'L' Test for trend analysis where significance was suggested but not confirmed via ANOVA or Students T Test comparisons.[21,22] Graphics accomplished with the Prism program (Prism Academy, San Diego, CA). The protocol and all experimental procedures were approved by the Institutional care and use committees prior to the conduct of this study.

## RESULTS:

The effects of Phenotype and strain on final body weights are shown in Figure 1 and indicate that body weight of the obese phenotypes of both LA/Ntul//-*cp* and SHR/Ntul//-*cp* rats are significantly greater than lean littermates of the same respective strainsand ages, and that final body weights in the old obese+T2DM rats weighted considerably more than all other groups. The Lean, Obese, and Young Lean and Obese+T2DM were 4 months of age, while the Old Obese+T2DM rats were 12 months of age.

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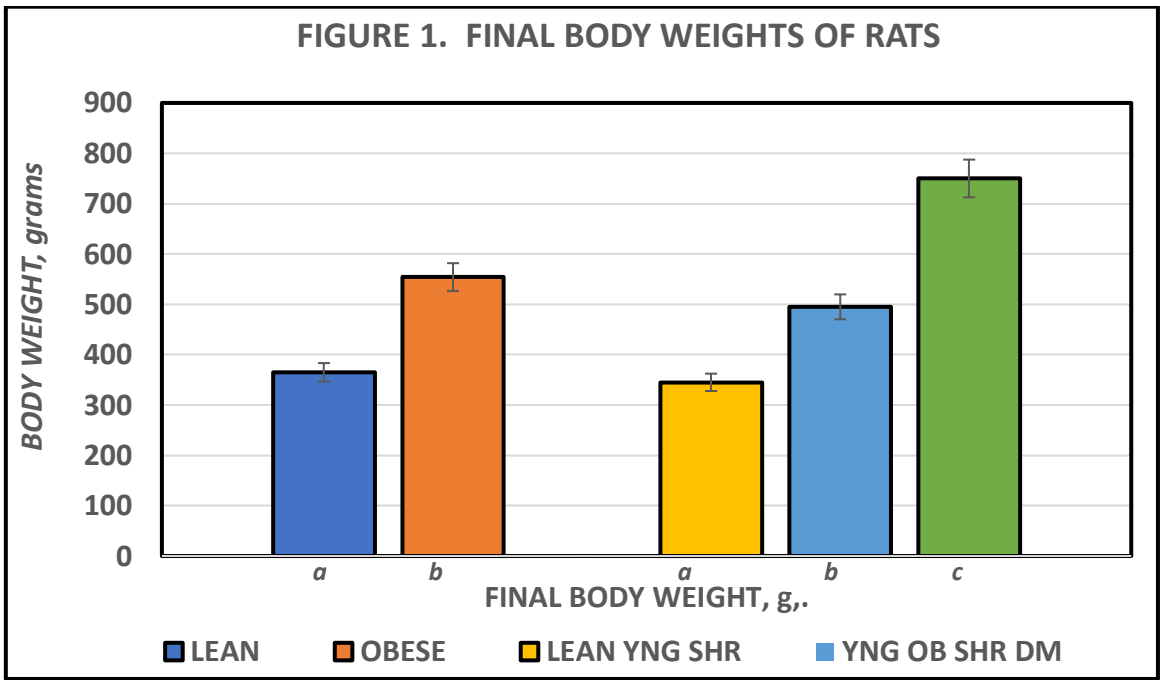


Figure 1. Effect of strain and phenotype on final body weights of rats. Data are mean ± 1 SEM, n = 5-8 rats/group. The number below each bar indicates a difference at p = < 0.05* / p = < 0.01** via ANOVA = Newman-Keuls subgroup analysis. For qualitative estimates of glycosuria, NEG = negative for urine glucose; POS = positive for urine glucose.

The effects of phenotype and strain on glycemic parameters are depicted in Table 1 and indicate that Glycemic parameters in the lean phenotype of both strains remained within normal non-diabetic range at four months of age. Obese non-diabetic animals exhibited only modestly greater fasting glucose, plasma insulin, and plasma amylin concentrations, but remained non-diabetic.Glycemic parameters in both young and old Obese-Diabetic groups were significantly elevated compared to all other groups, consistent with expression of profound diabetic stigmata, and with differential effects of the expression of the obese phenotype in the SHR background strain, in that the same -*cp* trait failed to induce diabetic stigmata in the LA/Ntul//-*cp* strain. Measures of glycated hemoglobin were determined in fasting bloods obtained at the end of the study, and indicate modest glucose elevations in obese, non-diabetic LA/Ntul//-*cp* rats, and significant elevations in glycated hemoglobin in both obese+T2DM groups. In contrast, glycated hemoglobin content in the lean of both strains remained well within the normal, non-diabetic range for rodents and consistent with grazing- vs meal-feeding behavior characteristic of laboratory-reared rats .Glycosuria was determined in random morning urine via Dipstick and is consistent with diabetic stigmata in both obese+T2DM groups. The letter below each bar indicates difference at p = < 0.05.

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Table 1. Glycemic parameters in lean,obese , and obese+T2DM corpulent rats.

| Phenotype and Strain (n) | Fasting Glucose | Fasting Insulin | Fasting Amylin | HbA1c | Glycosuria |
|---|---|---|---|---|---|
| LA/Ntul//-*cp*<br>Lean (6) | 4.97±0.16a | 0.62±0.10a | 5.2±0.08a | 7.03±0.81a | Negative |
| Obese (6) | 6.06±0.22b | 14.7±1.5b | 23.72.6b | 8.60±1.26a | Negative |
| SHR/Ntul//-*cp*<br>Young Lean rats (6) | 5.48±0.50a | 10.1±1.0c* | N.D. | 6.57±1.83a | Negative |
| Young Obese-Diabetic (6) | 12.50±1.60c | 39.3±3.0c | 50.5±5.5c | 11.00±1.1b | Positive (4+) |
| Old Obese-Diabetic (5) | 14.90±2.00c | 78.4±4.1d | 66.7±4.9d | 12.50±1.1b | Positive (4+) |

Table 1. Glycemic characteristics of lean, obese, and obese-diabetic rats. Data are mean± 1 SEM, N = 4-6 rats/group.
OGT and AUC 4month of age in lean and T2DM/NIDDM rats and old (12months) obese T2DM/NIDDM rats. Glucose reported in mM, Insulin as ng/ml, Amylin as pM, and HbA1c as %. The letter following each set of values indicate difference at p=<0.05 from other groups (ANOVA w/Newman-Keuls subgroup analysis).* n=4. N.D. = analysis not performed.

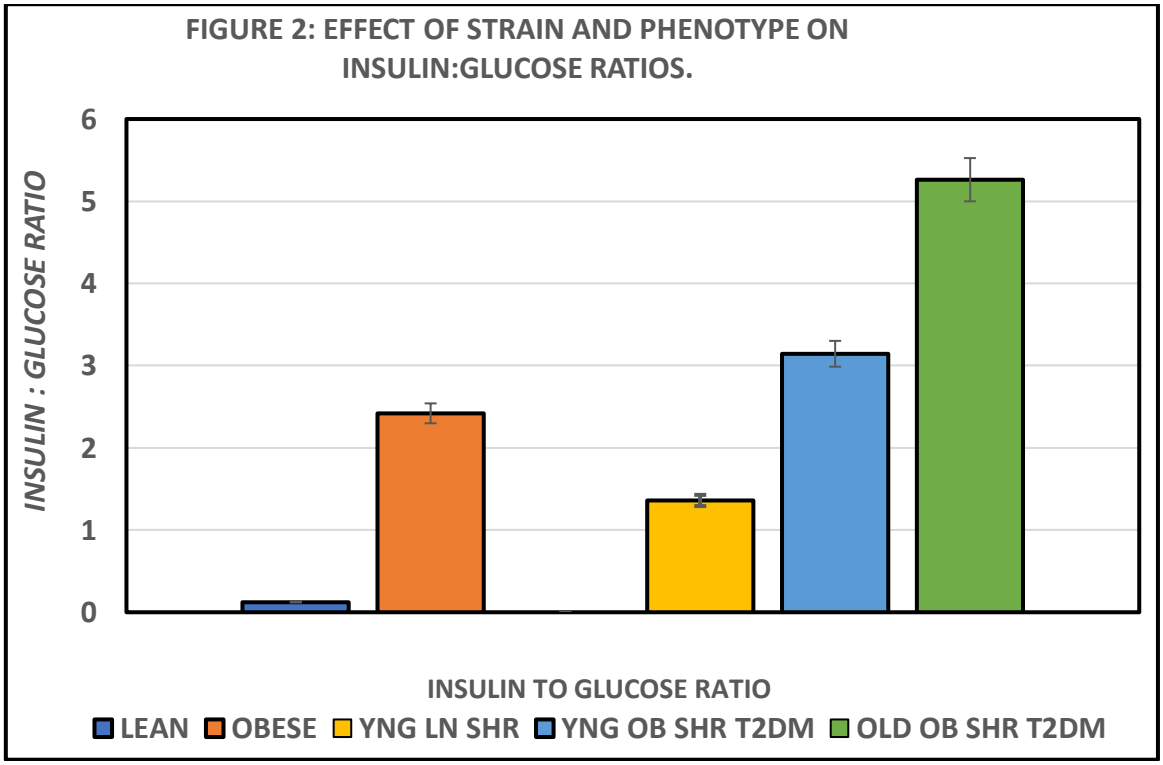


Figure 2. Effect of strain and phenotype on insulin to glucose rations as an index of insulin resistance. Insulin to glucose ratio determined by dividing insulin concentration in ng/ml by glucose as mg/dl. Glucosuria determined by random morning urine dipstick. The number below each bar indicates a difference at p = <0.05* via ANOVA = Newman-Keuls subgroup analysis. YNG = young, aged 4 months; LN = Lean phenotype; OB = Old, aged 12 months.

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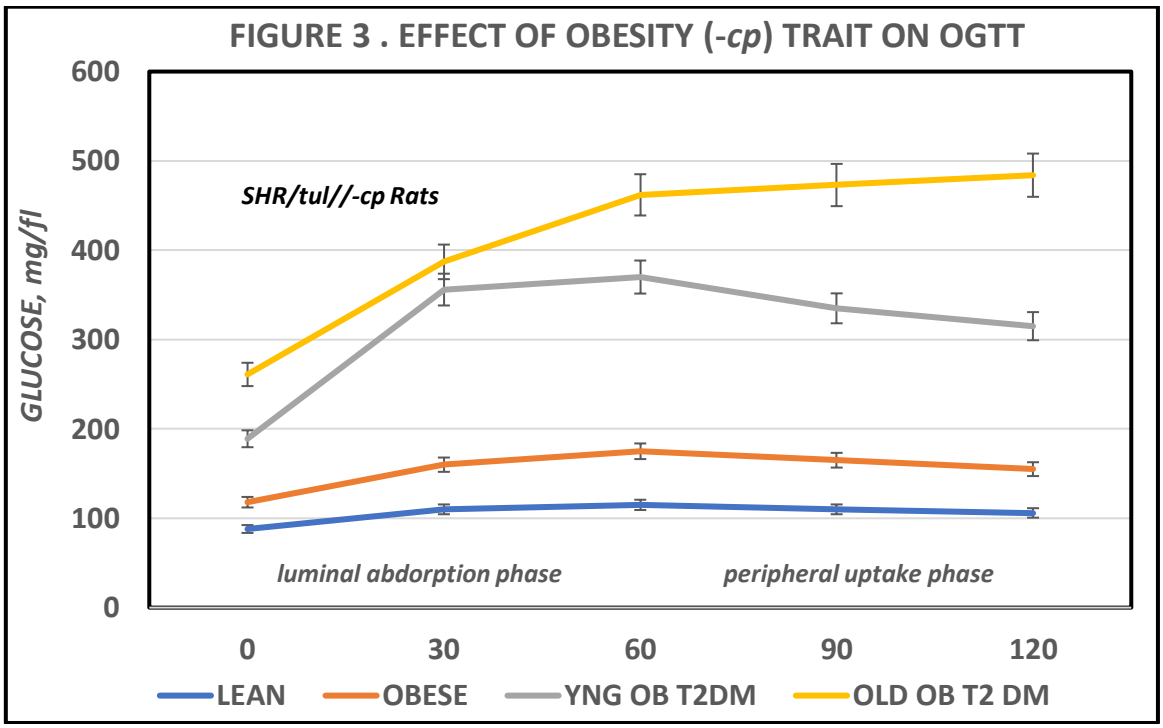


Figure 3. Effect of phenotype and age on oral glucose tolerance (OGT). Data are mean ± 1 SEM, n=5-8 rats/group.
P = < 0.05* for phenotype in obese; p = < 0.01 **for phenotype in T2DM.

The insulin to glucose ratios, indicative of insulin resistance and T2DM are depicted in Figure 2 below and support the glycemic characteristics reported in Table 1 above. Of interest, the Insulin: glucose ratio of helean SHR/Ntul//-*cp* rats was modestly greater than those of lean LA/Ntul//-cp rats, suggestive of a diabetogenic trait but remaining within non diabetic range. In contrast, the insulin to glucose ratios of obese LA/Ntul//-cp rats was greater than their lean littermates, indicative of moderate insulin resistance.

The insulin to glucose ratios of obese+T2DM rats of both ages were significantly greater than in all other groups, reflecting significant insulin resistance and glucose intolerance. Moreover, the magnitude to insulin resistance and hyperglycemia became greaterin the aging rats. The oral glucose tolerance in lean, obese and obese T2DM rats of both ages are depicted in Figure 3, and indicate that the OGT of obese, non-T2DM rats reflect a moderate degree on glucose intolerance, while remaining largely euglycemic. In contrast, the OGT of young T2DM rats weas markedly elevated, and like non-diabetic animals, demonstrated a peak response at the 60 minute time point, and decreased during the second hour of the OGT toward but not attaining a full return to fasting glucose concentrations as occurred in both groups of lean and obese LA/Ntul//-*cp* rats. The OGT in old, 12 month old untreated was markedly impaired, and blood glucose concentrations continued to increase throughout the entire 2 hours of the OGT, suggestive of greater magnitude of progressive insulin resistance and impaired glucose disposal in peripheral tissues occurred with aging. The effect of phenotype and strain on the Area under the OGT curve is depicted in Figure 4, and indicate that the AUCglucose of obese rats was significantly greater than that of their lean littermates, while the AUCglucose of the young and older T2DM-obese rats was markedly greater than both non-T2DM groups, and deteriorated yet further in the older group. The slopes of the absorptive phases of the OGT response, indicative of the luminal glucose uptake in each 30 to 60 minute phase of the OGT responses are depicted in Figure 5.

The initial (0 to+30 minutes) of obese rats was approximately twice a rapid as occurred in their lean littermates, while the 0 to +30 minute post absorptive slopes of the obese+T2DM rats was ~7.5 times greater than that of similar age lean rats, and ~4 fold greater than occurred in non-diabetic obese

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rats and remained greater in the older obese+T2DM rats. Of interest, the final post-absorptive phase (60 to 120 minutes post ingestion) reflected a negative slope in all lean, obese and younger obese+T2DM rats, consistent with euglycemia in lean and non-T2DM obese rats. However, the slope during the late post absorptive phase remained positive in the older obese T2DM rats, consistent with a greater magnitude of insulin resistance in addition to possible neurogenic delay in gastric emptying.

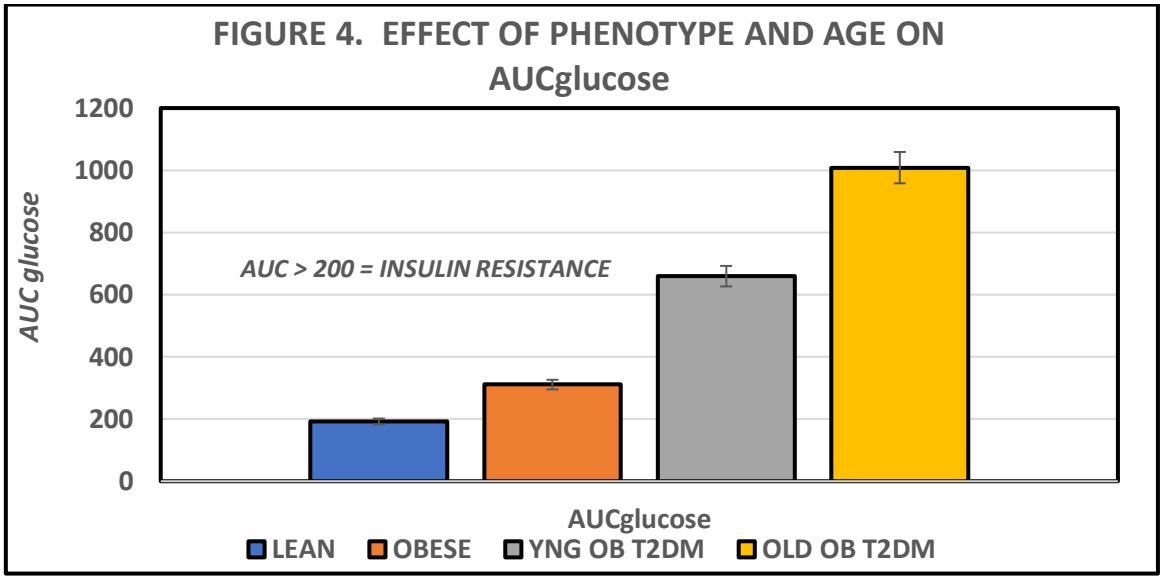


Figure 4. Effect of phenotype and age on AUC glucose. Data are mean ± 1 SEM, n=5-8 rats/group. p= < 0.05* forphenotype in obese; p = < 0.01** for phenotype in T2DM.

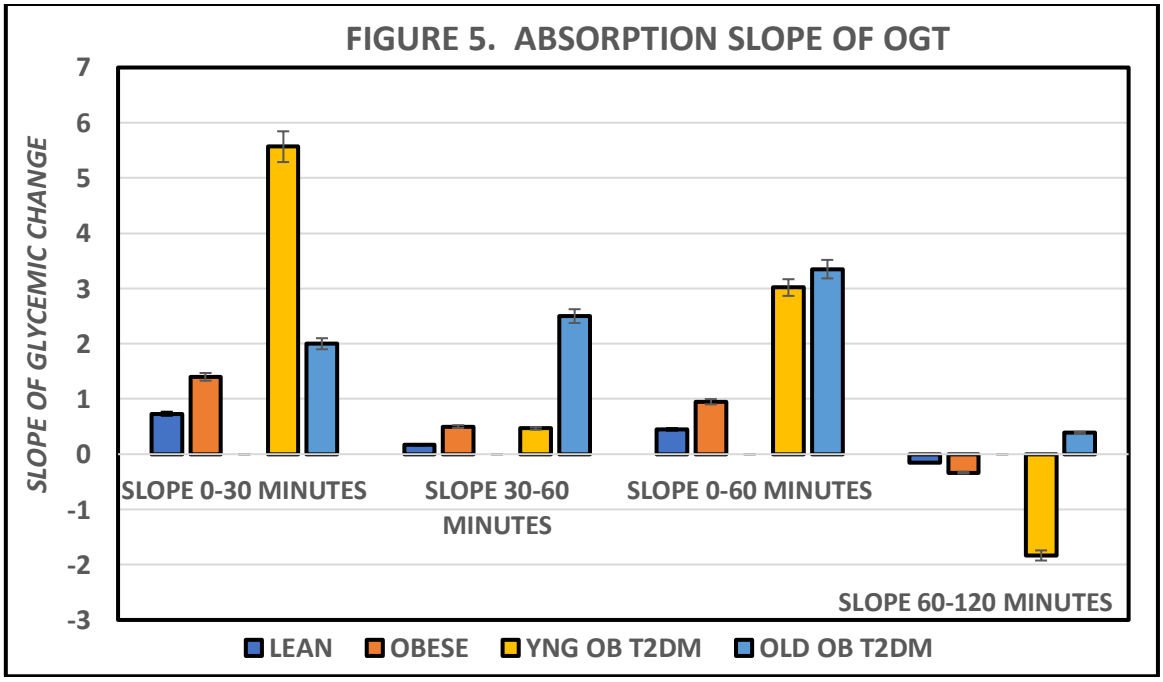


Figure 5. Glycemic Absorption Slope of OGT Response Phases. Data are mean ± 1 SEM, N = 5-6 rats/group.
Slope determined by formula m = (Y2 – Y1 / X2- S1).

**DISCUSSION:**

The prevalence of obesity, overweight conditions, and their progression to T2DM are approaching

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endemic proportions throughout much of industrialized society.[2,5-7] This prevalence has persisted despite marked advances in understanding the etiology and therapeutic options to remedy and resolve the disorder.The disorders tend to include some socioeconomic, environmental and familial patterns, with a remarkably strong heritable contribution. The application of animal models has proved valuable in contributing to understanding the pathophysiologic and biochemical processes implicated in the development of both excess adiposity and expression of T2DM.

This is especially relevant in those models where there is similarity in the metabolic pathways andglycemic parameters to those which occur in humans.The effects of epigenetic predisposition of early onset obesity on T2DMand aging on glycemic parameters were determined in congenic lean, obese, and obese+T2DM rats, where the obese phenotype of both strains share the same epigenetic trait ( the -cp trait) for obesity, and the metabolic pathways share the same biochemical and pathophysiological pathways as occur in human forms of the disorder.[11-15]

The obese of both corpulent rat strains demonstrated significant insulin resistance, but T2DM occurred only in the SHR/Ntul.//-*cp* strain. This investigation demonstrated that the greatest magnitude of insulin resistance and glucose intolerance of the two strains occurred in the SHR/Ntul//-*cp* strain. These observations are consistent with metabolically-linked but independent genomic contributions to the development of obesity-linked T2DM in this animal model. In addition, the expressions of the T2DM stigmata are consistent with the observation of genetic dilution of the T2DM trait in F1 hybrid offspring of the two strains reported herein.

[23]In addition, when left untreated in an attempt to enable demonstration of age-related progression in the characterization of the glycemic parameters of T2DM as measured in this study, the magnitude of peripheral insulin insensitivity and glucose intolerance became greater and metabolic parameters became further deranged in the aged T2DM rats.[8-10,24,25]Thus, the demonstration obesity-linked insulin resistance, while a significant contributor to T2DM, may occur independently in the two disorders depending on the genomic background in which the epigenetic trait for obesity has been expressed. Of interest, the progression to obesity and T2DM occurred while feeding a healthful, non-diabetogenic ration for up one year, in contrast to the high fat and other dietary extremes reported in other studies.

It remains unclear if consumption of a diabetes-prone diet would have progressed to T2DM in the LA/Ntul//-*cp* strain, but to date no evidence of definitive diabetogenic changes have been reported in the lean or obese phenotype of the LA/Ntul//-*cp* strain. While no diabetogenic investigations have yet been conducted in the lean phenotype of the SHR/Ntul//-cp strain, the lean phenotype of LA/Ntul//-cp x SHR/Ntul//-cp F1 hybrids remained euglycemic and non-diabetic, while the magnitude of T2DM expressed in the obese hybrids was of lesser magnitude than occurred in authentic, congenic obese+T2DM-SHR/Ntul//-*cp* rats, suggestive of a dilution effect on the expression of the T2DM trait.[23]

## CONCLUSION:

The effects of aging and early onset obesity on the progression of glycemic parameters in aging obese T2DM rats and the genetic predisposition for T2DM were determined instrains of congenic male lean, obese, and obese-diabetic (T2DM) rats that share the same genetic trait for obesity (the -*cp* trait) but are epigenetically expressed differently in the two genetic backgrounds studied.[11-15] While early onset obesity and insulin resistance occurred in both strains, the further development of T2DM occurred only in one strain, suggesting that the traits for obesity and T2DM while often linked, may occur independently when expressed in genetically different backgrounds. In the LA/Ntul//-*cp* strain, the healthful, longevity-prone NIH strain of Lister Albany rats provided the background genome to accommodate the -*cp* trait, while in the SHR/Ntul//-*cp* strain, the Spontaneously hypertensive rat (SHR) provided the diabetes-prone background genetics for the -*cp* trait to progress to T2DM. The lean, obese and young obese+T2DM rats were all of the same age (4 months) and all four treatment groups were housed and reared under identical feeding and housing conditions. Of interest, neither background strain investigated in

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the present study had been noted for expression of early onset T2DM, nor which symptoms and obese or obese+T2DM stigmata became readily evident in the present investigation only in the SHR/Ntul-*cp* background.

Both strains consumed a standard, healthful rodent diet after incorporation of the obesity (-*cp*) trait and throughout their lifetime. In addition, the stigmata of T2DM became further deranged when the SHR/Ntul//-*cp* animals were one year versus 4 months of age, and were nearing the maximum endpoint of their expected longevity. In conclusion, the progression of early onset obesity and T2DM is consistent with independent but linked metabolic parameters in this strain of corpulent rats. Indeed, much progress has been accomplished in the clinical characterization, diagnosis and treatment strategies for obesity, diabetes, and the complex metabolic state of diabesity since origination of the historic Vermont Study of Obesity over a half century ago.[26]The application of unique, congenic genetic models as reported in this study continue to refine and expand the genomic constituents and therapeuric options for the disordered metabolism in new and emerging diabetogenic states.

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## USE OF ARTIFICIAL INTELLIGENCE (AI):

No applications of AI were utilized in the preparation of this manuscript.

## CONFLICT OF INTEREST:

The author declares no conflict of interest in the generation of this manuscript.

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