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RESEARCH ARTICLE

Effect Of Aging on Glucose Oxidation and Insulin Resistance in Diaphragm in Congenic Obese Rats

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Abstract

The purpose of the current investigation was to determine the characteristics of glucose oxidation in diaphragm muscle in aging congenic, lean and obese rats from 4 to 24 months of age. Insulin resistance (IR) in the diaphragm muscle is a condition where the muscle may become less responsive to insulin, thereby impairing glucose uptake and potentially contributing to diaphragmatic weakness and respiratory dysfunction. Thus, IR may become a contributor to insulin resistance in muscle and adipose tissue depots particularly in conditions like obesity and type 2 diabetes (T2DM/NIDDM). While peripheral skeletal musculature groups often represents a primary locale of IR, the ongoing physiologic contractile activity of the diaphragm may escape much of the early impact of IR since it remains a more physiologically active muscle group than skeletal muscle throughout the lifespan in man and animals, and which may confer a measure of metabolic protection from more extensive features of IR. Groups of lean and obese female littermate LA/Ntul//-*cp* rats, a rodent model where obesity occurs independently of NIDDM were studied at 4, 14, and 24 months of age. Parameters of IR and glucose oxidation in diaphragm determined in the presence or absence of insulin (100 µU/ml; Medium glucose 10 mM). Relative adiposity was computed by the addition of the sum of dorsal and retroperitoneal adipose tissue depots as a proportion of final body weight. Body weight of lean animals was similar at all ages studied, while body weight of obese rats increased at each successive age studied in the obese phenotype. Fasting plasma glucose concentrations were similar in both phenotypes at all ages studied, but plasma insulin concentrations and the insulin: glucose ratios were markedly elevated in the obese phenotype. animals were markedly elevated, with only modest age-related improvement. Measures of glucose oxidation in diaphragm were consistent with age related decreases in baseline and insulin stimulated glucose oxidation in the lean phenotype. In contrast, baseline and insulin- stimulated glucose

oxidation in the obese phenotype were consistent with IR. These results suggest that the capacity for insulin-stimulated glucose oxidation becomes decreased in aging, with further decreases in the obese phenotype.

Keywords: aging, obesity, insulin resistance, diaphragm, glucose oxidation, rats

Introduction

The prevalence of obesity and overweight conditions is approaching epidemic proportions in Westernized society, currently with more than one third of individuals meeting that criterion.[1-3] Obesity and overweight conditions pose numerous health challenges to mankind, including increased risks for cardiovascular, NIDDM, Metabolic syndrome, and other significant metabolic disorders.[4,5] Among the typical contributing factors are overconsumption of sweetened beverages including refined carbohydrate and fatty foods, overly sedentary lifestyles, and likely some variable degree of genetic predisposition.[3,6] The present study utilized congenic aging female lean and obese LA/Ntul//-*cp* littermates obtained from the Drexel University colony, and where the only variable was the expression of the obese phenotype in the corpulent (-*cp*) phenotype where it exists as an epigenetically-mediated autosomal recessive trait.[7,8] This animal model was originally developed by Hansen, following 12 or more cycles of backcrossing to eliminate non-obese traits to satisfy the congenic status.[8] Obese animals demonstrate early onset obesity, hyperinsulinemia, hyperamylinemia, and disordered lipid parameters soon after weaning.[7,9] This strain is among the longest living of obese rodents strains, often exceeding 24 months of age, while lean animals may survive well beyond 4 years of age under conventional laboratory conditions.[7] Additionally, in this strain IR develops independently of progression to NIDDM. Obesity is considered a disorder with multiple etiologies since it is the cumulative sum of genetic predisposition, sedentary lifestyle, and environmental factors, including diet selection and nutritional imprinting, resulting at least in part in disordered appetite control with an onset often from dietary experiences in early, postweaning life.[2,3,6]

The diaphragm represents a unique muscle, as it typically remains more physically active than skeletal muscles throughout the lifespan of the animal due to its essential role in systemic respiratory functions including the process of glucose disposal and oxidation.[10] While diaphragm can develop insulin resistance, this resistance is often linked to increased lipid accumulation, inflammation, and decreased sensitivity to membrane associated GLUT4 glucose transporters.[10] Collectively the various mechanisms contribute to altered mitochondrial function within the diaphragm, which can exacerbate respiratory issues by reducing rate and force of contractile and oxidative energetic processes impacting muscle function. Evidence of systemic insulin resistance in the obese of this strain occurs by 6 weeks of age in the obese phenotype, characterized by decreases in the economy of protein degradation and synthesis, and protein turnover, without apparent decreases in net muscle mass or net energy intake.[11] Since the process of *de novo* protein synthesis in muscle is dependent on the availability of free amino acids in the cellular pool, the presence of hyperinsulinemia in the obese animals brings about an attenuation in the rate of protein degradation, thereby extending overall protein turnover, along with significant improvement in the energy demands of protein synthesis, and ATP expensive process typically consuming 4 ATPs/per peptide bond formed in the new protein. The extent to which this economy of protein turnover also occurs in diaphragm muscle has not been confirmed.[10] (Biometry)

The contributions of adaptive or nonshivering thermogenesis in brown adipose tissue (or NST) in the expression of obesity has also been suggested as a potential energy buffer, and when impaired by IR or other factors, may contribute to overweight and obesity via energy conservation processes.[12] Glucose uptake in BAT requires GLUT4-mediated glucose uptake and oxidation for the expression of nonshivering thermogenesis, and is impaired in the presence of insulin resistance.[13] Brown adipose tissue has often been implicated as an additional contributory factor in energy metabolism and adiposity. Defective mechanisms in brown adipose tissue metabolism have been implicated as a contributory factor in the etiology of obesity and overweight conditions [ref], and in some studies are linked to insulin resistance. In the corpulent rat strains, NST has been reported to decrease among obese rats from early in postweaning growth and continuing throughout their lifespan.[14] (Debolt). (ref) The activation of NST requires both sympathetic and thyroidal actions, both of which hormonal entities have been shown to be decreased in the obese phenotypes leading to a syndrome of subclinical hypothyroidism and likely linked to development of insulin resistance from early postweaning life in this strain via actions mediated at least in part via cellular thyroid hormone receptor elements. (ref). The insulin resistance is linked to increased lipid accumulation, systemic inflammation, and altered mitochondrial function within the diaphragm and other tissues, which can exacerbate

respiratory issues by reducing respiratory activity during muscle function.[15-18]

Methods

Groups of congenic lean and obese female littermate rats (n = SPF pathogen free, 12-20 rats/group) were obtained from the Drexel University colony at 6 weeks of age. Animals were maintained on a reverse light cycle (dark 0800-2000 hr. daily), maintained in plastic cages lines with 1 inch of fresh pine shavings, in a temperature and humidity-controlled environment (22+/- 1 degree C; 40-60 RH) with laminar air flow to minimize respiratory and communicable disease exposure. Rats were offered a Purina stock diet # 5012 and house water *ad libitum* from weaning throughout the study, and body weights obtained to the nearest gram with an Ohaus live animal balance at periodic intervals to characterize growth patterns in aging. At 4 14, and 24 weeks of age lean and obese rats were humanely sacrificed by cervical dislocation after a short fast in accordance with current guidelines of the AVMA and Institutional guidelines.

Blood and tissues were collected in their entirety from the Interscapular brown adipose tissue (IBAT), and retroperitoneal adipose depots, and from diaphragm muscle. Tissues were weighed to the nearest 0.1 mg, and diaphragm prepared for incubation in Krebs-Ringer solution at 50 shakes/minute fin a shaking water bath or 60 minutes in the absence or presence of insulin (100 µU/ml and glucose at 10 mM) at 37 °C. to determine baseline and insulin stimulated oxidation of glucose. A ^{14}C-glucose tracer was incorporated and ^{14}CO2 generated collected in the presence of 0.5 ml of KOH for an additional 60 minutes in conventional collection cups suspended from the rubber septum upon termination of the incubation. The congenic LA/Ntul//-*cp* rat is an excellent rodent model for this investigation, since the only variable is the presence of the obese (-*cp*) trait expressed as an autosomal recessive trait and which appears in 25% of the offspring of breeder stock that is proven heterozygous for the obese trait. Animals were studied at 4, 14, and 24 weeks of age as the oldest animals were approaching their maximum expected lifespan.[7,14] Data were analyzed via standard statistical procedures.[19]

Results

The effects of aging and phenotype are depicted in Figure 1 and indicate that final body weights of the lean phenotype were minimally impacted by aging, increasing from a mean of 227 grams/rat at 4 months of age to 264 grams at 24 months of age. In contrast the final body weights of the obese phenotype were more than twice those of their lean littermates at each age studied and increased further at each age studied; Final body weights were 2x, 2.6x, and 2.8x greater than their lean littermates of the same age, despite having consumed the same diets throughout their projected lifespan. The effects of age and phenotype on relative adiposity are depicted in Figure 2 and reflect an increase in adiposity of 5.84x, 7.85x, and 11.53x respectively in the obese vs the lean phenotype, suggesting the increase in the obese phenotype was largely attributed to increases in the expression and magnitude of obesity beginning early in their lifespan and increasing progressively thereafter at each age studied. The metabolic factors that contributed to the greater adiposity likely includes glycemic and hormonal factors outlined at least in part below.

The effects of fasting plasma glucose, a primary energy substrate in muscle, are depicted in Figure 3 and indicate that plasma glucose remained within the normal range at each age studied in both phenotypes. Thus, all animals of both phenotypes remained euglycemic without further progression to NIDDM throughout their lifetime with the diet fed. To date, NIDDM has not been observed in either phenotype of this strain of rats regardless of the diet fed.[7,20,21] The effects of aging and phenotype on plasma insulin are depicted in Figure 4A and indicate that plasma insulin concentrations remain within the normal physiological range in the lean phenotype at each age studied. In contrast, fasting plasma insulin was markedly increased in the obese phenotype at all ages studied, but the increases were less prominent as the animals aged. Fasting plasma insulin concentrations averaged 5 x, 3.44x, and 3.87x fold greater in the obese phenotype with aging, indicating an age-related decrease in absolute values over the age spectrum, with further decreases at each age studied. These observations are consistent with development of an obesity-linked insulin resistance in the obese phenotype that persisted throughout the projected age spectrum in this strain but decreased in magnitude with advancing age. The effect of age and phenotype on Insulin: Glucose ratios, a measure of insulin resistance, are depicted in Figure 4B and reflect significant insulin resistance in the obese phenotype at all ages studied, but which magnitude also decreased progressively by nearly 50% with each advancing age when 24 months of age. Indeed, the magnitude of insulin resistance at 4 months of age was profound, while the magnitude of insulin resistance at 24 months remained only modestly lower than that observed at 14 months of age.

The effects of aging and phenotype on glucose oxidation in diaphragm are depicted in Figure 5. Baseline glucose oxidation in lean and obese animals was similar at all ages studied. Inulin typically stimulates the rate of glucose uptake and disposal in most tissues.

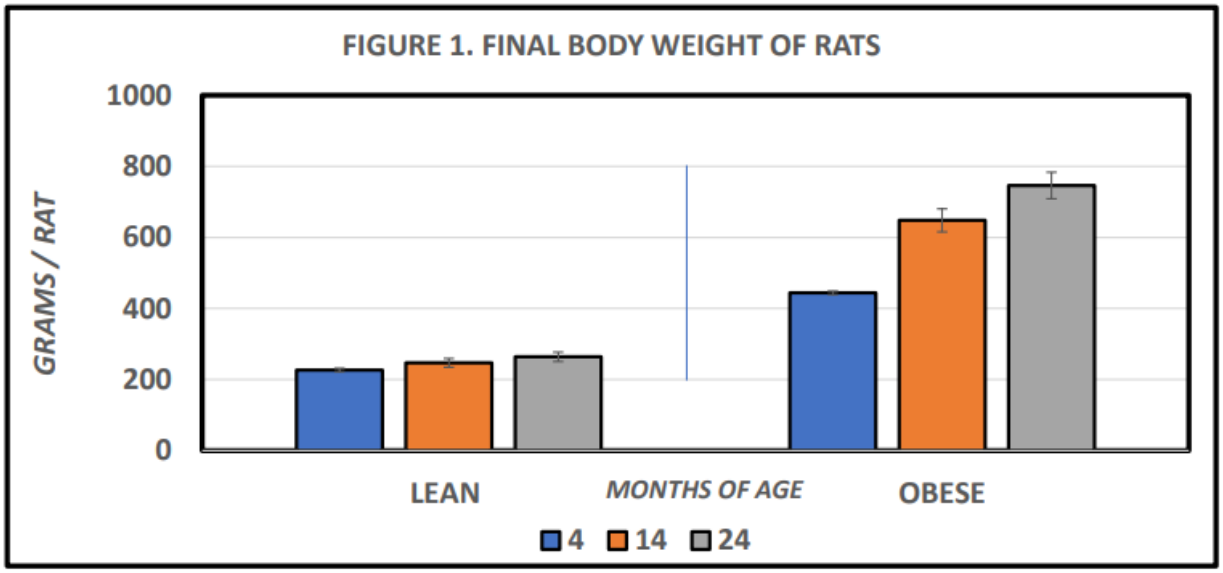

Figure 1. Effect pf age and phenotype on body weight. Data are mean ± 1 SEM, n = 6-11 rats/phenotype/treatment group. p = <.0.01 Obese vs lean at all ages.

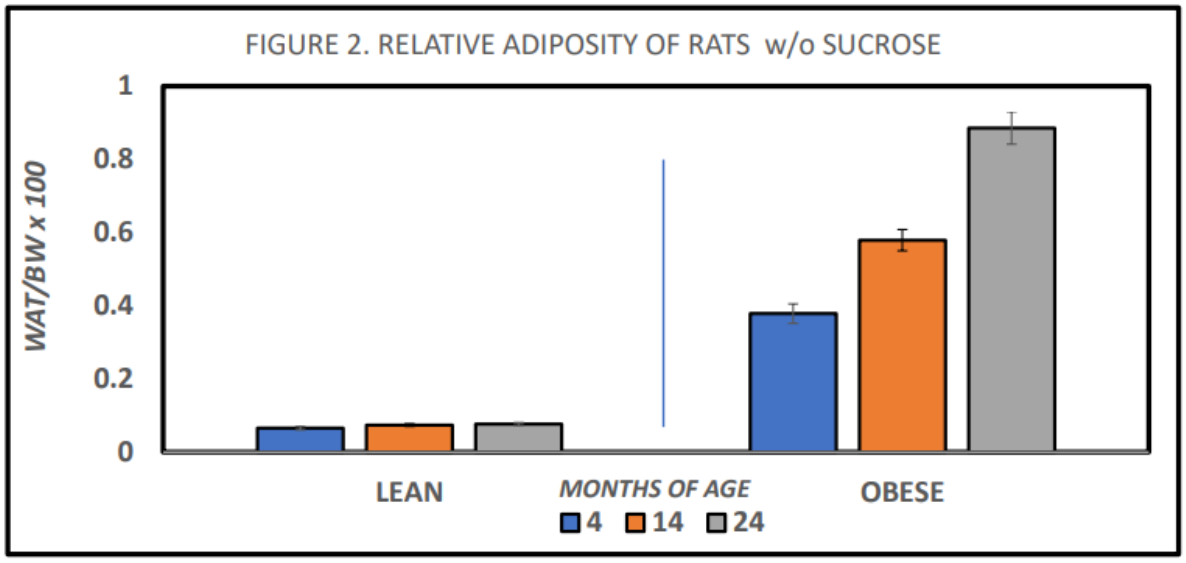

Figure 2. Relative adiposity of rats. Data are mean ±1 SEN, n = 6-10 rats/group. Data represent the sum of the dorsal and retroperitoneal adipose tissue depots as a proportion of final body weight. P = O 0.01 (Obese vs lean at each age studied.

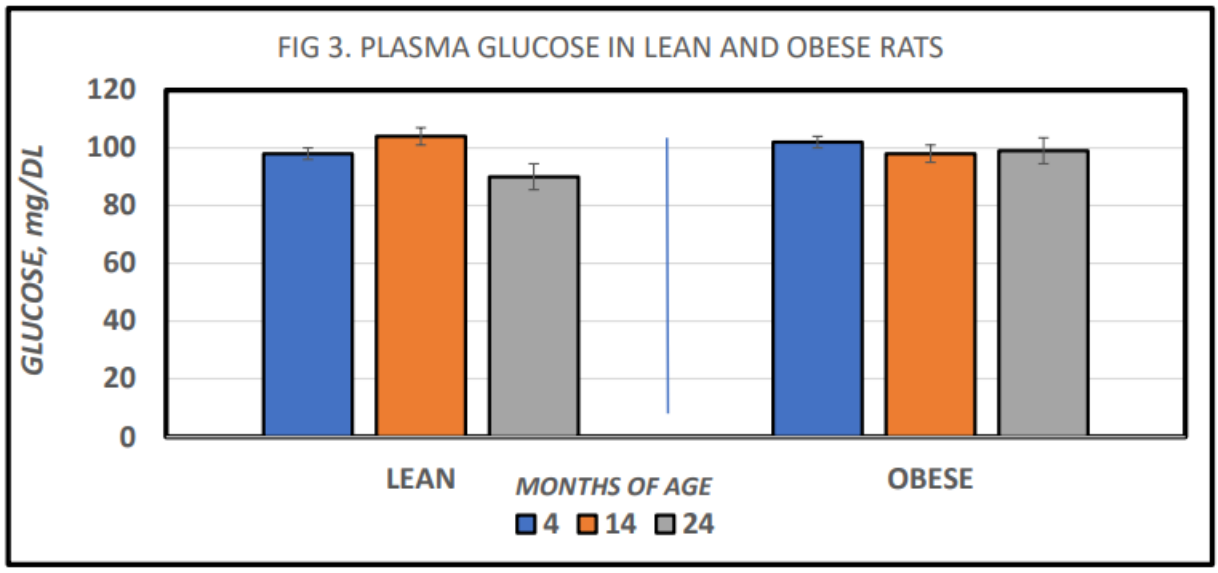

Figure 3. Fasting plasma glucose in lean and obese rats. Data are mean ±1 SEM, n = 6-10 rats/group. p = n.s. (lean vs obese at each age.

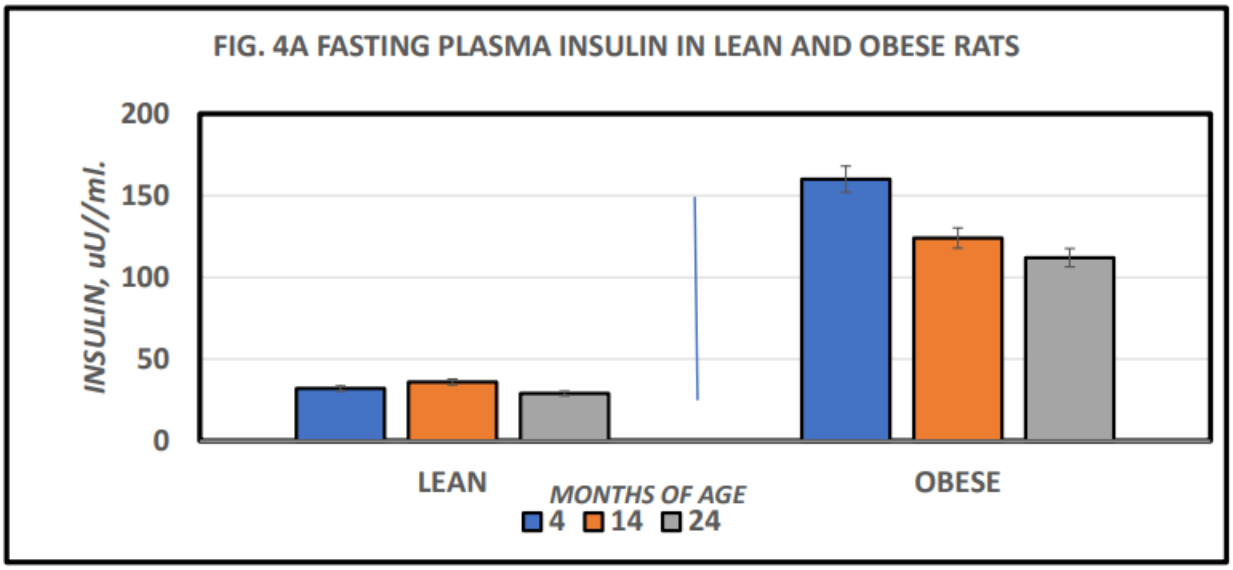

Figure 4A. Fasting insulin concentrations in lean and obese rats. Data are mean ±1 SEM, n = 6 rats/group/ p = < 0.01, lean vs obese at each age studied.

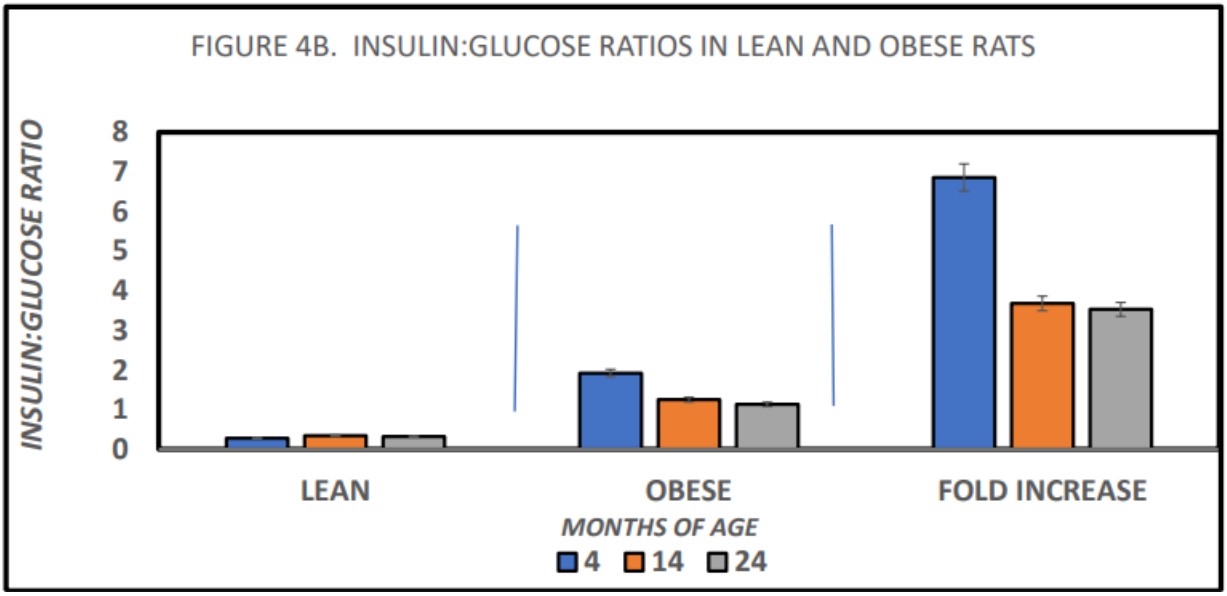

Figure 4B. Effects of age and phenotype on Insulin: glucose ratios. Data are mean ± 1 SEM n= 6-10 rats/group. p = < 0.05 (Obese vs Lean) at each age studied. The fold increased computed arithmetically by dividing obese via corresponding lean age group.

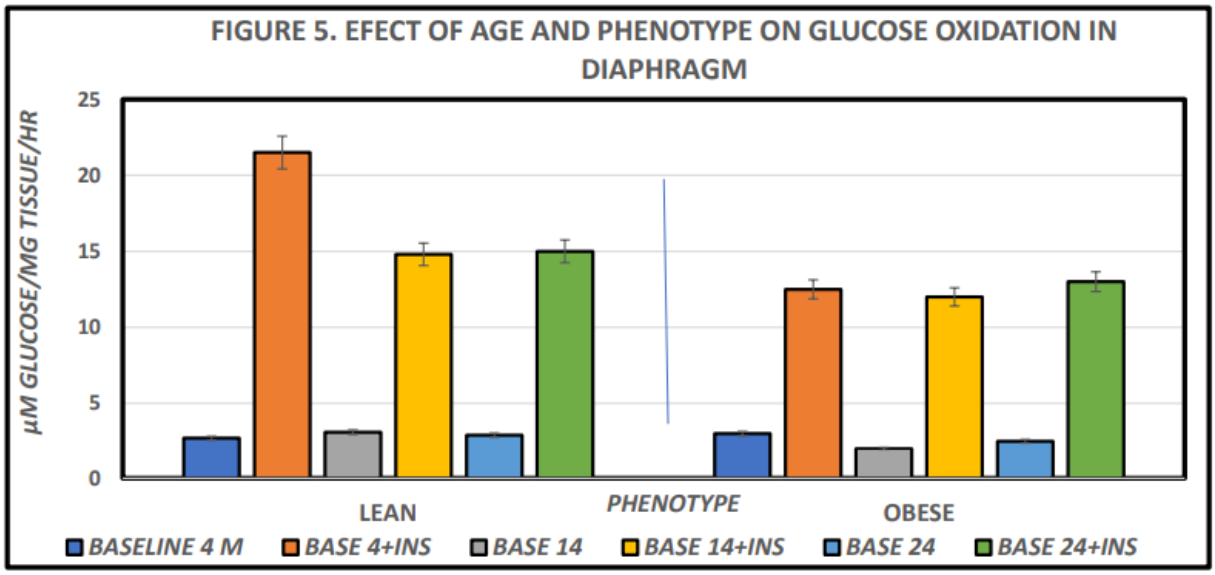

Figure 5. Effects of age and phenotype on glucose oxidation in lean and obese rats. Data are mean ±1 SEM, n = 6-10 rats/group. p = < 0.05 (insulin vs baseline) at each age studied; p = < 0.05 (Obese vs lean insulin stimulated oxidation at 14 months of age; n.s at 14 and 24 months of age, trend only.

The addition of insulin resulted in a marked (8.25x) stimulation of glucose oxidation at 4 months of age and dropped to an average 5 x stimulation at 14 and 24 months of age respectively in the lean phenotype. The effects of insulin on glucose oxidation in the diaphragm in the obese phenotype are depicted in the right panel of Figure 5 and indicate that the rates of insulin-stimulated glucose oxidation increased by 4-fold at 14 months of age and averaged 5-fold greater than their baseline at 14 and 24 months of age. The decreases in fasting plasma insulin concentration with are consistent with the decreasing fasting plasma insulin concentrations with aging in both phenotypes and indicate the development of more modest elevations in insulin resistance in the obese phenotype.

Discussion

Obesity occurs in the obese phenotype of the LA/Ntul//-cp rats as the result of an epigenetically-mediated autosomal recessive trait with an onset soon after weaning.[7,20] A common observation in the obese phenotype of this strain is the development of systemic insulin resistance without progression to T2DM, also soon after weaning, and which likely represents the common denominator of the metabolic features of hyperinsulinemia, hyperamylinemia, and glucose intolerance and their sequelae noted. The biochemical mechanism of the caloric efficiency and excess fat accretion are incompletely defined, but are associated with impairments in nonshivering thermogenesis, cold adaptation, and in impairments in thyroidal, neuroendocrine and neurodevelopmental actions in response to factors of diet and environment.[7,11,16] The obese phenotype exhibits hyperphagia from an early age, proportional to the greater adiposity and body weights of the obese phenotype. Among the metabolic sequelae are impairments in glucose disposal, lipogenesis, T4/T3 ratios, ATP regeneration, and in the process of protein synthesis and degradation without apparent deficits in lean body mass.[11] Feeding of protein restricted but otherwise balanced diets to obese rats of this and other obese strains was without effect on lean body mass of the obese phenotypes while the same diets fed to their lean littermates resulted in decreased lean tissue mass, increased VO2, and elevations in T3 but not T4, and where the common denominator was consistent with insulin resistance.[7,22,23] The improved efficiency of protein turnover, at 4 ATPs/peptide bond formed, represents an additional contribution to the metabolic efficiency in the obese phenotype of this strain, and enables the diversion of energy substrates to *de novo* lipid formation and greater fat accretion.[11] In addition, the insulinogenic metabolic pathways that normally facilitate the diaphragm to utilize glucose in response to insulin become disrupted, thereby impeding optimal processes of glucose uptake and subsequent energy production, while the ongoing physiologic activity of the muscle is consistent with an attenuation in the magnitude of IR in this tissue.

The results of this study indicate that diaphragm muscle of normally fed and reared obese animals demonstrated variable magnitudes of insulin resistance with aging, consistent with earlier reports of similar impairments in the diaphragms of mice, and in the metabolic actions in cardiac auricular appendage and digitorum longus muscle of this strain of rats.[11] The body weights of the obese phenotype increased progressively at each age studied, attaining final body weights 2- to 3-fold greater than occurred in similarly reared lean littermates. In the lean phenotype body weights remained stable with little additional impact from the aging process, while in older lean rats of this strain, age related modest decreases in body weight were noted in animals over 3 years of age. Both phenotypes responded to the presence of insulin to the muscle incubates, with the most robust response in lean animals when 4 months of age. At 4 months of age, measures of glucose oxidation the lean animals manifested a 42% greater response to insulin than their obese littermates, while overall, the cumulative 4-to-24-month age-related increases in the lean phenotype averaged an average 30% greater response than in their obese littermates. The differences in insulinogenic responses are consistent with energy conservation of oxidative metabolism, including *de novo* ATP generation. These results are consistent with age related decreases in the insulin-stimulated capacity for glucose disposal in both phenotypes, with the most dramatic magnitude in the lean animals. The observations are also consistent with the exaggerated Insulin: glucose ratios in the obese phenotype, and which likely contributed to the progressive development of obesity via enhanced pathophysiologic mechanisms of energy deposition and lipid accretion in those animals.[22-24]

Conclusion

The results of this study indicate that the onset of obesity has an early, likely prepubertal pathophysiologic onset and which remains prominent and progressive throughout the projected lifespan of the animals. In addition, in previous studies, the caloric efficiency, measures of prolonged T1/2 of protein turnover, and impaired non-shivering thermogenesis in response to parameters of diet and environment were also contributory to greater energy conservation and enhanced fat accretion in the obese phenotype. The demonstration of hyperinsulinemia consistent with insulin resistance was apparent at all ages studied in the obese phenotype. Insulin resistance has

been reported to contribute to an impaired efficiency of mitochondrial function, which would attenuate processes of *de novo* ATP generation in affected tissues. Accordingly, decreases in overall diaphragmatic glucose disposal in the obese phenotype are consistent with modest impairments in glucose disposal, energy conservation, and in enhanced body fat accretion including the diaphragm muscle of the obese phenotype of the LA/Ntul//-*cp* rat. These physiological actions are also consistent with the greater physiological activity of this muscle when compared to other skeletal muscle groups. The physiological activity of diaphragm muscle remains 100% active throughout the lifespan of the animal, while most other muscle groups linked to bodily movement have the luxury of intermitted periods of rest in between contraction events. The common denominators of the metabolic excursions in the obese phenotype are consistent with progressive development of insulin resistance soon after weaning, and which then persist throughout the remaining projected lifespan of the obese phenotype in this strain.[10,23,14]

Disclaimer (Artificial Intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Consent

It is not applicable.

Ethical Approval

The study was approved by the Institutional Animal Care and Use Committee of USAT.

Competing Interests

Author has declared that no competing interests exist.

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