# Effect of Aging and Obesity on Parameters of Insulin, T4/T3 Ratios, Metabolic Rates, Sirtuins and Longevity in Congenic LA/Ntul//-cp (Corpulent) Rats

Orien Tulp *

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**Article**

# Effect of Aging and Obesity on Parameters of Insulin, T4/T3 Ratios, Metabolic Rates, Sirtuins and Longevity in Congenic LA/Ntul//-*cp* (Corpulent) Rats

**Orien L Tulp**

Professor, Colleges of Medicine and Graduate Studies, University of Science Arts and Technology, Montserrat, British West Indies MSR1110; o.tulp@usat.edu / https://www.orientulp.com/

**Abstract:** Changes in thyroidal activity occur in aging, where they typically exhibit a U-shaped curve for plasma TSH concentrations, with variable responses in other parameters of thyroidal function and peripheral physiological actions and their pathophysiologic sequelae. The LA/Ntul//-*cp* rat strain, developed from a cross between a Koletsky Rat and an NIH longevity-prone LA/N strain of unknown origin exhibits one of the longest life spans of the various rodent strains that express a trait for early onset obesity in association with impairments in sympathetic and thyroidal components of non-shivering thermogenesis, insulin resistance and energy metabolism. To determine the effects of aging and obesity from adolescence through much of the projected lifespan of the obese phenotype, groups of congenic lean and obese female LA/Ntul//-*cp* rats were studied from 4 until 24 months of age. Measures of Resting Metabolic rates (RMR), fasting plasma insulin and glucose, T3, T4, plasma half-life of T4, and computation of T4/T3 ratios were determined as an indication of the capacity for deiodination of T4 to T3 in peripheral tissues and its cumulative effects on RMR. Body weight of obese >>> lean at all ages studied. Plasma Insulin was significantly elevated in the obese phenotype, and decreased with advancing age, while plasma glucose remained within the normal fasting range in all rats of both phenotypes. RMR of lean > obese at all ages studied and decreased with advancing age in both phenotypes. Liver T4-5′ deiodinase activity of lean > obese at all ages studied. Plasma T4/T3 ratios decreased with aging in both phenotypes, with the greatest decrease in the obese phenotype. The $T_{1/2}$ of $^{131}$I-T4 was over 40 % longer in the obese phenotype littermates at 4 months of age ($p=< 0.01$).These results suggest that conversion of T4 to T3 in peripheral tissues in association with attenuation of hyperinsulinemia contributes to decreases in RMR with aging and is further decreased in the obese phenotype, thereby decreasing the quantifiable availability of tissue T3 mass for thyroidal actions at the genomic level, including decreases in harmful reactive oxygen species (ROS) and other entities as products of intermediary metabolism. Thus, the progressive decreases in plasma insulin and deiodination-mediated activation of T4 may represent a longevity attribute via decreases in resting metabolic rates, and in an associated attenuation of free radical generation and other metabolic factors of aging and longevity in this strain.

**Keywords:** adiposity; obesity; thyroid hormones; sirtuins; insulin resistance; longevity; RMR; corpulent rat

## INTRODUCTION

The integrated effects of aging on thyroidal function and other hormonal and regulatory aspects of metabolism are an important element in various aspects of aging and longevity.[1] As reviewed by Taylor et al, the quantity of thyroid hormones normally released from the thyroidal epithelium is determined in large part by the actions of thyroid stimulating hormone (TSH), which stimulates the thyroid gland to release T4 and some T3 to the peripheral circulation, in response to metabolic and physiologic demands including growth and development across the lifespan. The T3 is the physiologically most active form of the hormone and is produced in peripheral tissues via the actions of T4-5′ deiodinase activity on the outer phenolic ring of the T4 moiety.[2-4] Typical effects in early childhood are directed to genomic expression of neurologic and physical growth and development,

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while in adult mammalian species the primary effects may be observed via increases in resting and catecholamine-stimulated metabolic rates, commonly measured in rodents via measures of VO2 under conditions of thermal neutrality and corrected for differences in body size and mass.[5,6] While the progression of plasma TSH concentrations typically suggest a U-shaped curve, with the highest plasma concentrations reported at either extreme of the lifespan, the corresponding plasma T4 or T3 concentrations may not always follow the same profile.[1] In addition, excursions from the normal plasma concentrations may become associated with a variety of pathophysiologic conditions including cardiovascular, musculoskeletal, cognitive and other disorders, in addition to alterations in diet and environment.[1] Thyroid hormones initiate their metabolic effects following stereospecific genomic binding to receptor domains that are highly committed to bind T3, the physiologically most active form of the iodothyronines.[3,7,8] Thus, it is instructive to assess the parameters of T3 generation in key tissues and examine their associated contributions to glycemic and metabolic parameters. This is especially important as thyroidal and other physiologic factors contribute to the efficiency of energy metabolism and storage, at a time in history where the prevalence of obesity, overweight conditions, and metabolic syndrome (MeTS) are approaching epidemic proportions in many communities.[9-13] The underlying basis for the changes in the prevalence in overweight conditions in recent generations is likely secondary to many factors including changes in common dietary practices linked to the prevailing food supply in modern society, greater consumption of preprocessed foods of greater energy density, a more sedentary lifestyle than in previous generations, and likely economic factors.[14] Some 70 years ago the US Department of Agriculture established the food pyramid, as a pictorial guide to assist individuals in selecting healthier food selections. Since the original pyramid was established however, many changes have been incorporated into the pyramid in attempts to promote decreases in saturated fat intake, while increasing the proportions of carbohydrate sources in part to improve palatability, dietary appeal and broader public acceptance. In addition, the development of manufactured sweeteners including the widely preferred high fructose corn syrup (HFCS) have now become widely accepted in many cultures and communities, thereby increasing fructose ingestion up to 5-fold or more since the introduction of HFCS in the food and carbonated beverage supply chains and thereby likely further contributing to the burgeoning prevalence of overweight and obesity and their comorbidities in adolescents and adults.[14]

The independent contributions of aging and obesity or overweight conditions on sirtuin-mediated thyroidal function have not been fully clarified. Hyperinsulinemia and insulin resistance are common hallmarks of adiposity, due in part secondary to disruption of the normal biosynthetic and intracellular transport of the glucose transporter proteins including GLUT4, essential for insulin-dependent glucose uptake in muscle and adipose tissues.[14-16] These two sources are the most prevalent tissue beds where insulin resistance is expressed, and together they contribute to the multiple comorbidities commonly associated with obesity. [15,16] The development of the congenic corpulent rat has now become an important animal model to investigate the independent contributions of obesity and aging, whereby the only difference between the obese and the lean phenotypes is the epigenetic expression of early onset obesity.[17-19] In addition, the background strain is derived from the longevity-prone LA/N rat maintained in the small animal genetics section of the NIH, and backcrossed multiple times with the Koletsky rat to acquire the congenic obesity or corpulent (*-cp*) trait by Hansen.[19] In previous studies in the corpulent rat strains have demonstrated that the capacity for non-shivering thermogenesis (NST) under conditions of thermal neutrality to be decreased typically by an average of 20% under most environmental and dietary conditions.[17,20] The decreases in NST were found to be attributed to a combination of sympathetic and thyroidal actions in the obese phenotype. [11,17,20] In addition to the impaired thermic responses to factors of diet and environment, in those studies measures of serum T3 concentrations were also often found to be lower in littermates of the obese male and female phenotype, suggestive of a physiological subclinical hypothyroid state.[11] A condition consistent with subclinical hypothyroidism has been described by several authors, where only modest deficits may account for excess weight gain.[1,12,13] Adiposity with an onset of middle age or beyond is also a common observation in aging.[10] Aging contributes additional impairments in the thermogenic responses to diet and cold environment in the obese

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phenotype in this strain. [17,20-21] Although the physiologic mechanisms that are operative for the impaired thermic responses in aging remain unclear they do appear to include decreases in mitochondrial oxidative activity, a primary source of oxygen utilization in mammalian tissues.[22-24] The biochemical mechanism of the NST component of thermogenesis in most warm-blooded animals including rodents occurs in large part via thermogenic activity in brown adipose tissue, where specialized mitochondria demonstrate a neuroendocrine-mediated activation and uncoupling of oxidative phosphorylation of ATP to ADP, resulting in the obligatory generation of heat that can be utilized to maintain body temperature regulation following alterations in diet and thermal environment.[23,24] Other tissues including liver and skeletal muscle also contribute to metabolic heat production, albeit via well- established biochemical mechanisms in intermediary substrate metabolism rather than the specialized mitochondrial process common to BAT.[3,11,21,]

Glucose uptake in BAT is essential for BAT thermogenesis to progress, and Marette et all demonstrated that insulin resistance is a major factor in the impaired thermogenic responses in obese rats.[25] In addition, Tatelman et al[22] reported that mitochondrial activity in liver tissues of lean Sprague Dawley rats became increased in parallel to serum T3 concentrations following a thermogenic diet and decreased with advancing age in when followed up to 4 months of age due to adaptations in $\alpha$-glycerate phosphate shuttle activity, but hepatic thermogenic activity responses to a longer duration of aging and to the obese phenotype remain unclear.[20]

Historically most related studies have been conducted in younger male animals. In previous studies in the corpulent rat strains, it was reported that factors of diet, cold exposure, and sympathomimetic responses to norepinephrine were impaired in the obese phenotype of both the LA/Ntul//*-cp*, the T2DM-prone SHR/Ntul//*-cp* and other obese strains strains.[17,18,25-28] Thus, the purpose of the present study was to determine the effects of aging and obesity on parameters of T3 generation in a key metabolic tissue and their association with the impaired thermogenesis in aging and obesity in a congenic female animal model highly predisposed to obesity without the usual comorbidities of NIDDM or hypertension.

The various roles of sirtuins in mediating thyroidal responses are undergoing active investigation in several laboratories. The sirtuins represent a recently discovered class of NAD+-deacetylases (n = 7 at last count) that function as silent information transfer factors.[29] The sirtuins are dependent upon cellular NAD+ and facilitate the deacetylation of lysine and possibly other amino acid residues of chromatin-based histones and other cellular proteins that contribute to epigenetic, genomic expression in tissues following deacetylation.[29,] The deacetylation reactions occur in response to nutritional and environmental signals likely transmitted via neural signals that likely originate in hypothalamic paraventricular nuclei and result in their expression in the pituitary and other tissues.[29-31] The NAD+ availability is ultimately derived mostly from the NAD+/NADH ratios mostly generated via heightened mitochondrial activity, thus are a generalized reflection of nutritional and environmental status and recent macronutrient ingestions.[22] In addition, overnutrition is associated with a shift from non-inflammatory M2, protective ROS generating macrophages toward proinflammatory M1 (iROS) generating macrophages differentially in visceral vs non-visceral adipose tissue depots, thereby contributing to the magnitude of systemic IL-6 and TNF$\alpha$ cytokine-mediated inflammation and their eventual pathophysiologic sequelae.[30-32] Nutritional inputs, predominantly from carbohydrate and other metabolizable energy sources, can bring about increases in insulinogenic activity and a decrease in sirtuin activity.[33,34] The decreases in sirtuin activity can result in reciprocal increases in the conversion of T4 to metabolically active T3 and in accompanying increases in resting metabolic rates. In addition, overnutrition and sustained positive energy balance contributes to increases in cell cycle replication and inflammatory M1-macrophage induced DNA damages, accompanied with a destabilization and shortening of telomeres over extended periods.[35-37] Since longer telomere length is linked to longevity, the combined effects of overnutrition on sirtuins thereby may negatively impact potential longevity.[37,38] In contrast, fasting, caloric deprivation, and starvation bring about increases in sirtuin availability, improvements in insulin sensitivity, accompanied by a shifting from outer ring to inner ring deiodinase activity.[3,4,8] The latter actions increase the diversion of T4 from T3 to formation of reverse T3 (rT3), a metabolically

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inactive iodothyronine that fails to associate with the TRβ-subunit domain, with accompanying decreases in RMR, cell cycle activity, and decreased M1 macrophage inflammatory cytokine activity including iROS-mediated DNA damage and thus an enhanced, more healthful longevity when in neutral or negative energy balance.[8,38-40] The caloric efficiency is notably more favorable in the obese than the lean phenotype of the corpulent rat strains, while the effects of the epigenetically-expressed obesity are expressed differentially in different adipose tissue depots before and following weight gain modulation.[41] Thus, the purpose of the present study was to determine the effects of aging and obesity on parameters of T3 generation in a key metabolic tissue and its association with the impaired thermogenesis often observed in aging and obesity in female littermates, in a congenic animal model that is highly predisposed to early onset obesity but without the usual comorbidities of NIDDM or hypertension.

## METHODS

Groups of post-adolescent female, lean and obese littermate LA/Ntul//-*cp* rats were fed Purina rodent chow diet throughout the duration of the study to construct a 2 x 3 experimental design consisting of 2 phenotypes (lean and obese) and 3 age points (4, 14, and 24 months of age). Thus, this design comprised the projected lifespan of the obese phenotype. The animals were maintained in plexiglass showbox cages lined with 1 inch of pine shavings, with free access to Purina chow and house water, and maintained at 20-21 °C, 50% relative humidity under a reverse light cycle (Dark phase 0800-2000 daily). Animals were routinely studied during the dark phase and were fasted briefly (approximately 4 hours) prior to measures of fasting blood glucose, insulin, RMR, or thyroidal parameters. Measures of daily food consumption were obtained over a 24-hour period as described by Vedula et al [26] and expressed as kjoules consumed/rat/day based on the manufacturer's certificate of analysis and energy density. Measures of live body weight as a measure of ongoing wellness were obtained periodically with an Ohaus animal balance and recorded to the nearest gram. Measures of resting metabolic rate were determined at thermal neutrality (30°C) in fasted, quietly resting animals via a closed-circuit Collins small animal respiration apparatus fitted with a 4-litre chamber and maintained at 30°C submersed in a circulating water bath and corrected for factors of altitude and relative humidity.[5,6.17] At 4, 14 and 24 weeks of age groups of lean and obese rats were sacrificed by cervical dislocation with a small animal guillotine, and truncal bloods collected for hormone and substrate analysis. The retroperitoneal, dorsal, and interscapular white adipose tissue depots and the liver were dissected in their entirety and weighed to the nearest mg. Approximately 100 mg aliquots of liver tissue was homogenized and prepared for assay of Type II thyroxine 5'-deiodinase activity in the presence of dithiothreitol (DTT) as described elsewhere.[27] Measures of tissue and serum T4 and T3 were determined via solid phase RIA.[27] The plasma half-life of T4 in 4 month-old male biological littermate rats was determined following the intravenous injection of 1 μCi of 1-131-T4 in the tail vein within a 1 minute duration, and collection of 100 μl aliquots of tail tip blood in heparinized microtubes via tail bleeding for up to 8 hours post-infusion and plotting the rate of decline in plasma radioactivity.[28] Data were analyzed via standard statistical procedures including descriptive statistics, ANOVA, Students t test, and Pages L test for detection of trend analysis. [42,43] The study was approved by the Institutional Animal Care and use committee.

## RESULTS

The effects of aging on longevity are depicted in Figure 1 and indicate that the effects of obesity on longevity are associated with a significant decrease in typical duration in both phenotypes, with lean females projected survival the greatest at approximately 50 months of age, while obese male rats exhibit the shortest projected lifespan, seldom exceeding 25 months of age under typical laboratory conditions. The effects of obesity on decreased projected longevity averaged ~28% in both phenotypes. Body weights of the animals of this study at 4, 14 and 24 months of age are exhibited in Figure 2 and show that body weights increased in both phenotypes with advancing age, and that the final body weights of the obese phenotype far exceeded the weights of their lean littermates at each age surveyed. Adiposity, determined as a percent of final body weight in the obese phenotype also

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greatly exceeded the percent of body weight represented by the sum of three fat depots of the lean phenotype (Figure 3A,B) and indicated that the sum of the three fat depots measured in the obese phenotype greatly exceeded those of the lean phenotype. In addition, the combined fat pad mass increased progressively with aging in both phenotypes with advancing age with the greatest increases in the obese phenotype. The effects of aging on RMR are depicted in Figure 4 and indicate that the RMR of lean rats was greater than the obese rats at each age studied ($p = < 0.05$), and that the RMR tended to decrease with advancing age in both phenotypes with the greatest decrease noted in the obese phenotype (Figure 4).

The effects of aging and obesity on daily energy intake are depicted in Figure 5 and indicate that energy intake, determined by multiplying the grams of food intake per day times the energy content per gram of chow (3.34 kcal/13.975 kjoules/g) as greater in the obese phenotype than the lean phenotype, and increased in both phenotypes with aging at the 14 month age point, with a trend toward stabilizing at the oldest age studied in both phenotypes. Regardless of the trend, the consistent greater energy intake of the obese phenotype and is indicative of simple hyperphagia, albeit it to support a significantly greater body mass in the obese littermates.

The effects of ageing on fasting plasma insulin glucose and insulin: glucose ratio is depicted in Figure 6A and 6B respectively and indicate that fasting plasma insulin concentrations in the obese phenotype were significantly greater than were observed in their lean littermates at each age determined (left panel, Figure 6A). Although there was a modest trend toward greater fasting glucose concentrations in the obese phenotype, the plasma glucose concentrations remained within the normal range in both phenotypes at all ages studied.(Figure 6B, right panel). In addition, plasma insulin concentrations in the obese phenotype tended to decrease with advancing age, while the corresponding differences in fasting plasma insulin in the lean phenotype were modest by comparison (Figure 6A). The insulin to glucose ratios are depicted in Figure 6B, and indicate the I:G ratios in the obese phenotype were greater than those in their lean littermates at all ages studied, suggestive of evidence of chronic insulin resistance in the obese phenotype, albeit it decreasing in absolute magnitude in spite of the greater relative adiposity depicted in Figure 3B.

Thyroidal parameters of aging and obesity on plasma T3, T4 and T4/T3 ratios are presented in Figures 7 through 9 below. The effects of aging and obesity are depicted in Figure 7A and 7B and indicate that plasma T3 while remaining within the normal range in both phenotypes and at all three ages studied, illustrated a modest trend toward an age-related increase. Plasma T4 concentrations were greater at 4 months of age than at 14 or 24 months of age, but plasma concentrations were similar in both phenotypes in all age comparisons made. The T4 to T3 ratios are depicted in Figure 7B and indicate that the ratio decreased progressively in both phenotypes with aging, suggesting that less T3 may be being generated per unit of T4 at each age studied, and that the enzymatic peripheral T3 generation became further decreased with advanced aging in both phenotypes, with the greatest decreases noted in the obese phenotype.

The effects of aging on liver T4-5′ Type II deiodinase are depicted in Figure 8 and indicate that the activity of the enzyme was greater in the lean than the obese phenotype and decreased with advancing age in both phenotypes. The liver is a major site for generating T3 for subsequent uptake in peripheral tissues via Type-2 deiodinase. Stereospecific enzymatic conversion of T4 to T3 occurs via DI-1 an DI-2, which catalyze a deiodination reaction only at the outer ring 5′position to yield metabolically active T3, the physiologically most active form of the hormone, and which may suggest a decreased receptor-domain binding affinity for T3. The 5′-deiodinase activity appears to be substantially greater in the lean than the obese phenotype, and the 5′-deiodinase activity decreases in both phenotypes with aging. Tissue T3 concentrations are depicted in the Right panel of Figure 8 and indicate that despite the changes in deiodinase activity the tissue concentrations remained similar in both phenotypes with aging. Plasma T4 to T3 ratios are depicted in Figure 7B and indicate that the T4/T3 ratio decreased with aging in both phenotypes and that the ratio was always greater in the lean than in the obese phenotype, consistent with the differences in deiodinase activity and decreases in the rate of deiodinase activity and generation of T3 where progressively less T3 is formed per unit of available T4 throughout most of the adult lifespan in this strain. The plasma half-life of

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$^{131}$I-T4 was determined in a group of lean and obese male biological littermates and was found to be approximately 40% longer in the obese vs the lean phenotype, thereby confirming the delayed peripheral conversion of T4 to T3 *in vivo* as reflected above (Figure 9). The observation that the T4 half-life was determined in male vs female littermate rats of a similar age in this study is deemed of little consequence, since the onset of and magnitude obesity and the physiologic characteristics of thermogenesis in response to diet and environment have been found to be remarkably similar in both genders of this strain.

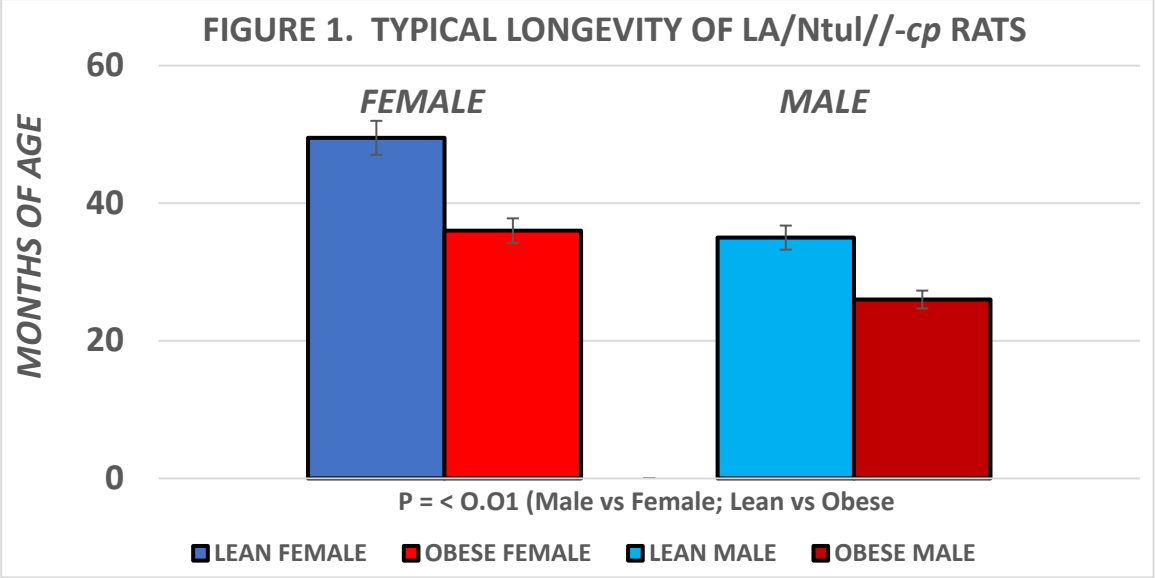


**Figure 1.** Projected longevity pf lean and obese LA/NtuL//.-cp rats. Extrapolated from ref 17.

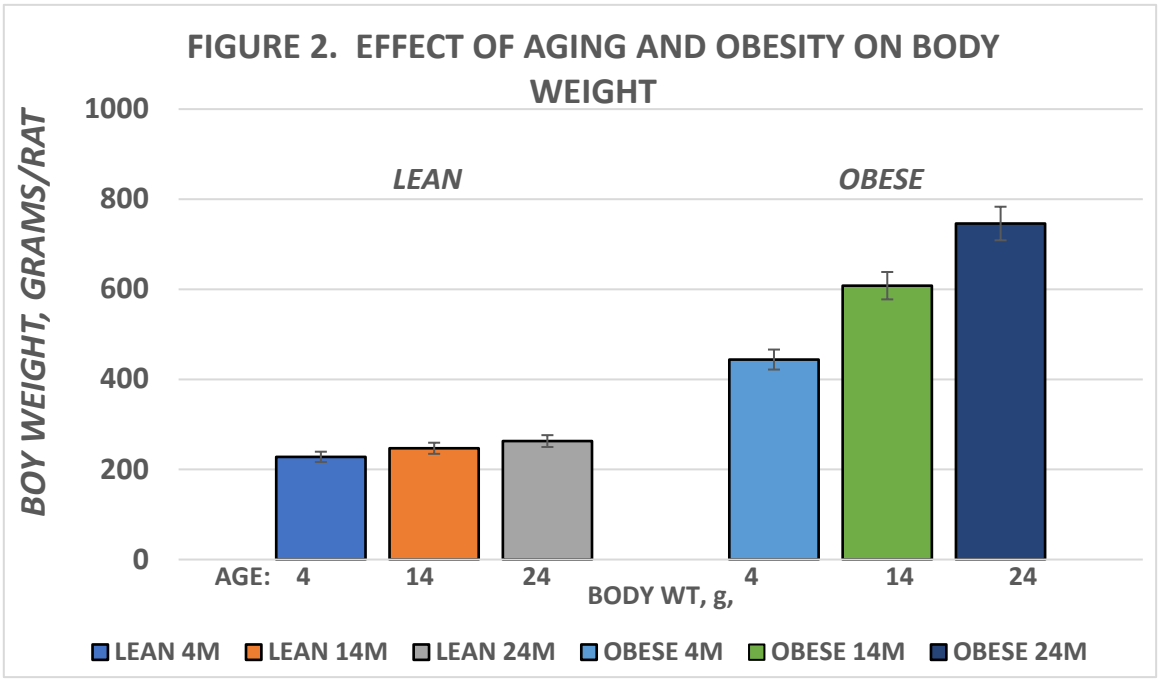


**Figure 2.** Effect of aging and obesity on body weights of female LA/Ntul//-*cp* rats.

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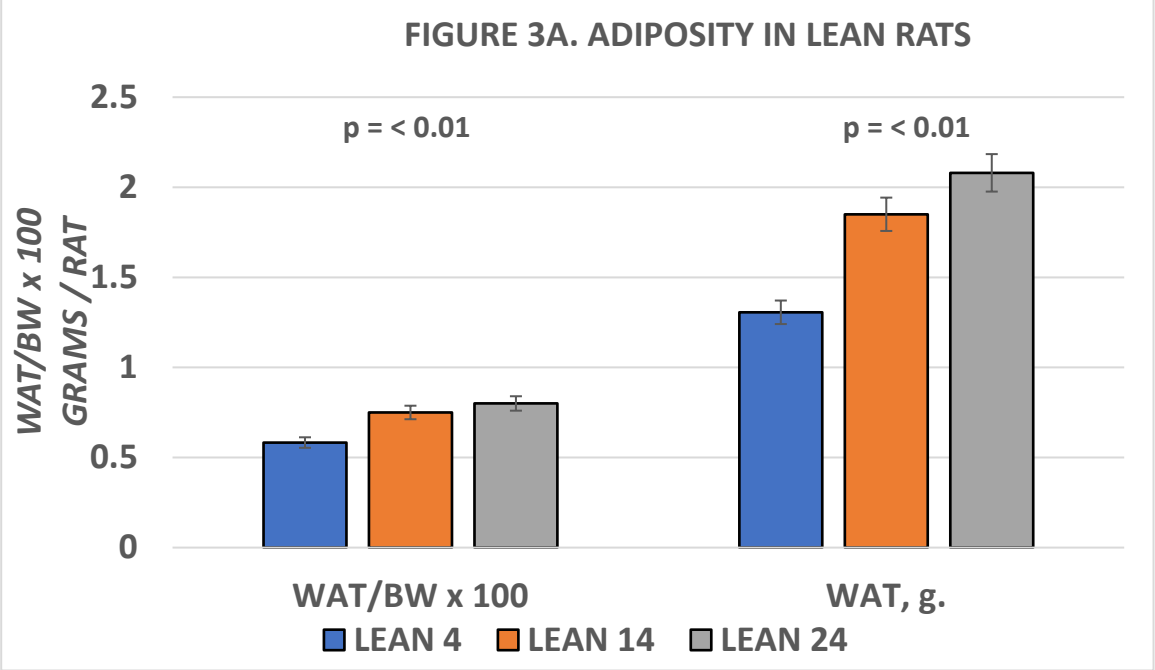


**Figure 3.** A. Effect of aging and obesity on adipose tissue in lean rats. Data are mean ± 1 SEM, n = 8 rats/group.

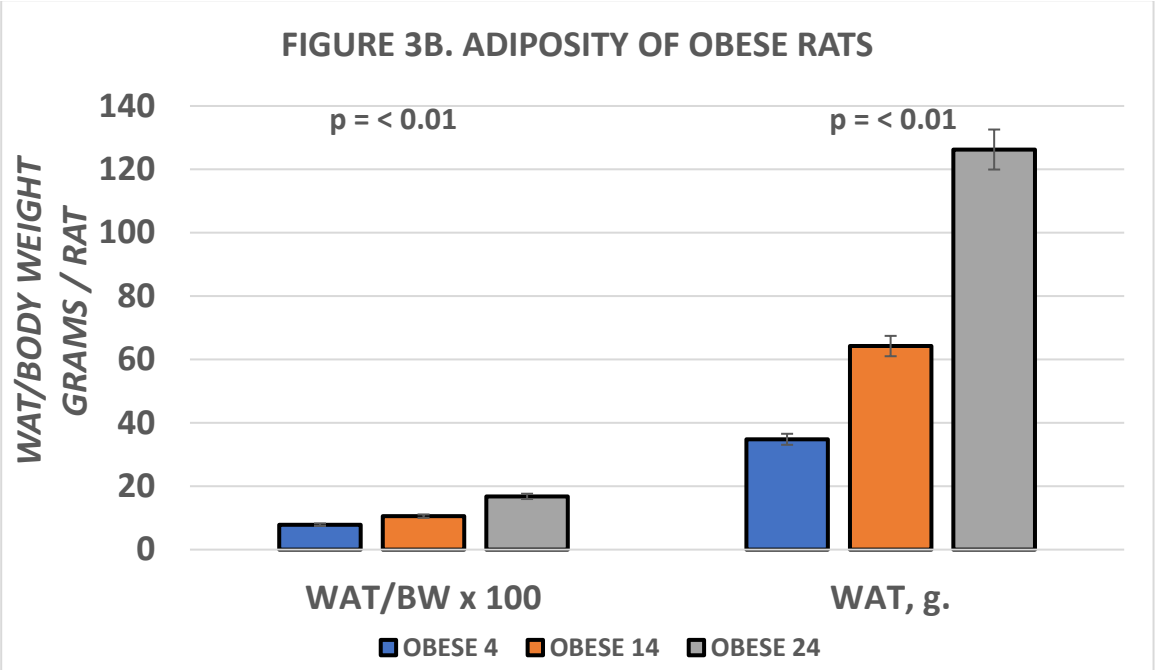


**Figure 3.** B. Effect of aging and obesity on adipose tissue in obese rats. Data are mean ± 1 SEM, n = 8 rats/group.

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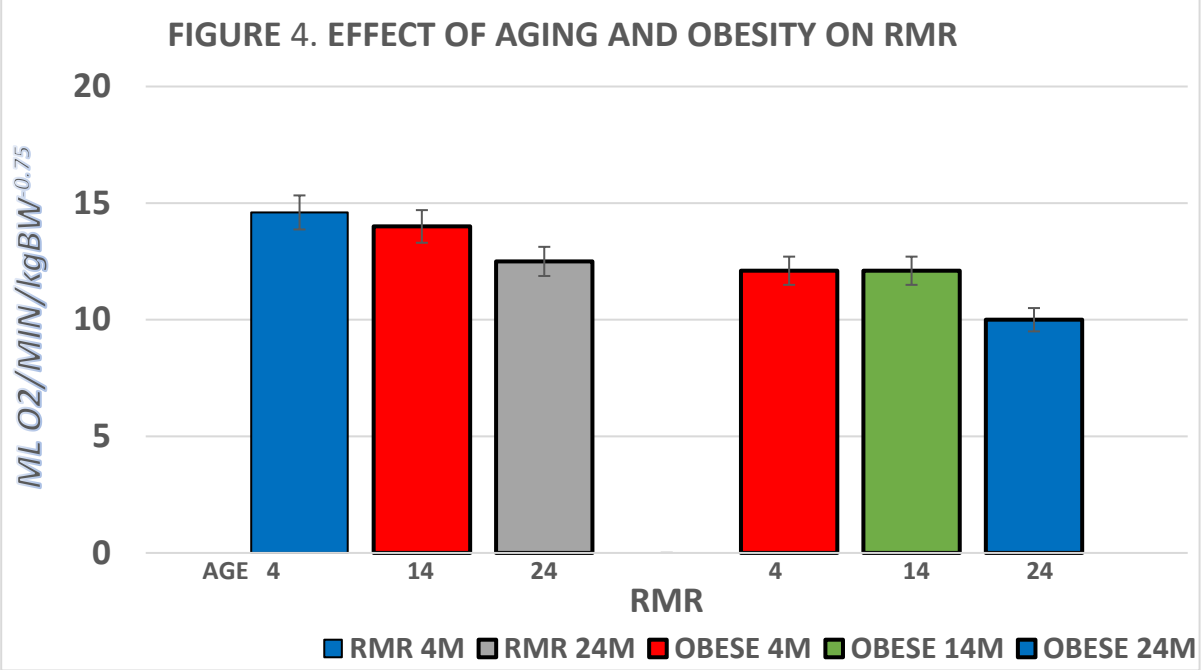


**Figure 4.** Effect of aging and obesity on RMR in lean rats. Data are mean ± 1 SEM, n = 8 rats/group.

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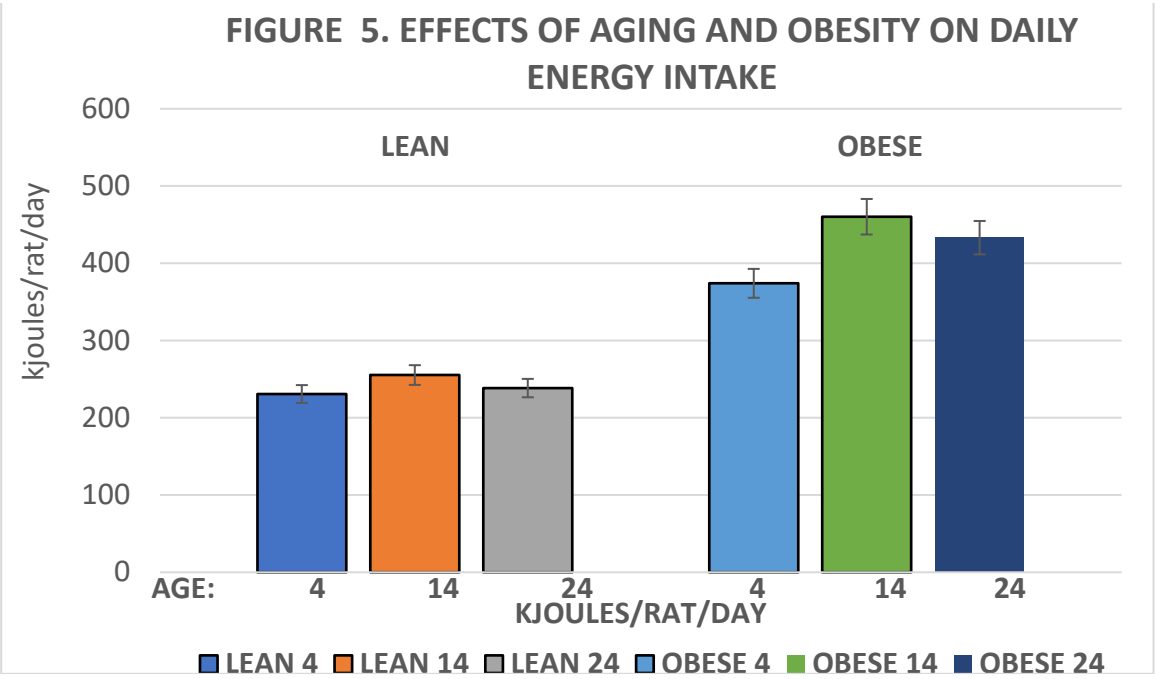


**Figure 5.** Effects of aging and obesity on daily energy intake. Data are mean ± 1 SEM, n = 8 rats / group.

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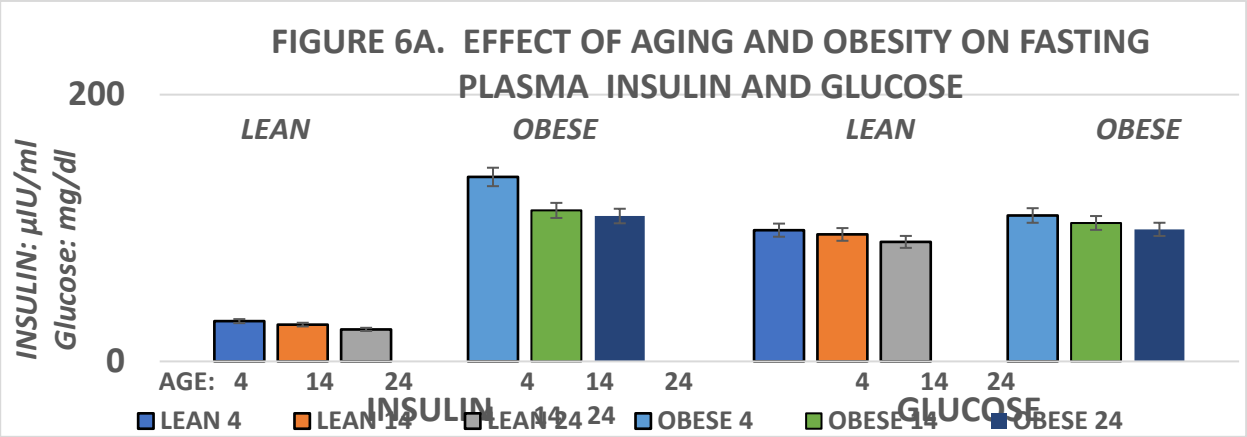


**Figure 6.** Effect of aging and obesity on plasma fasting insulin and glucose concentrations. Data are mean ± 1 SEM, n = 8 rats / group.

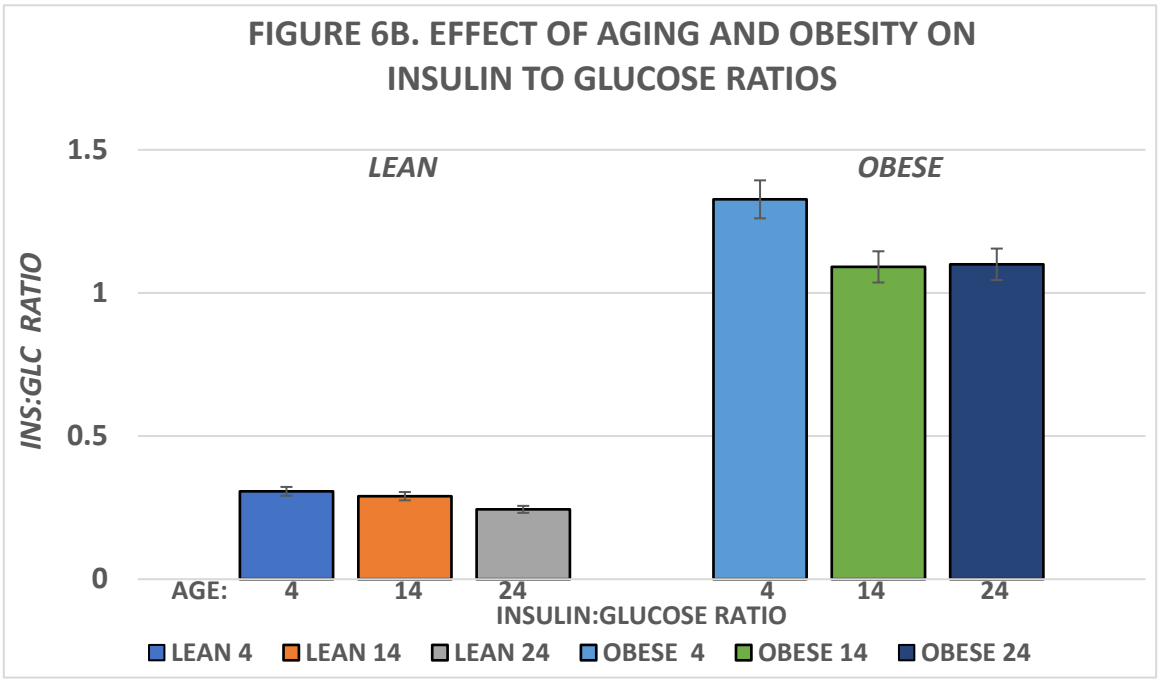


**Figure 6.** B. Effect of aging and obesity on insulin to glucose ratios. Data are mean ± 1 SEM, n = 8 rats / group.

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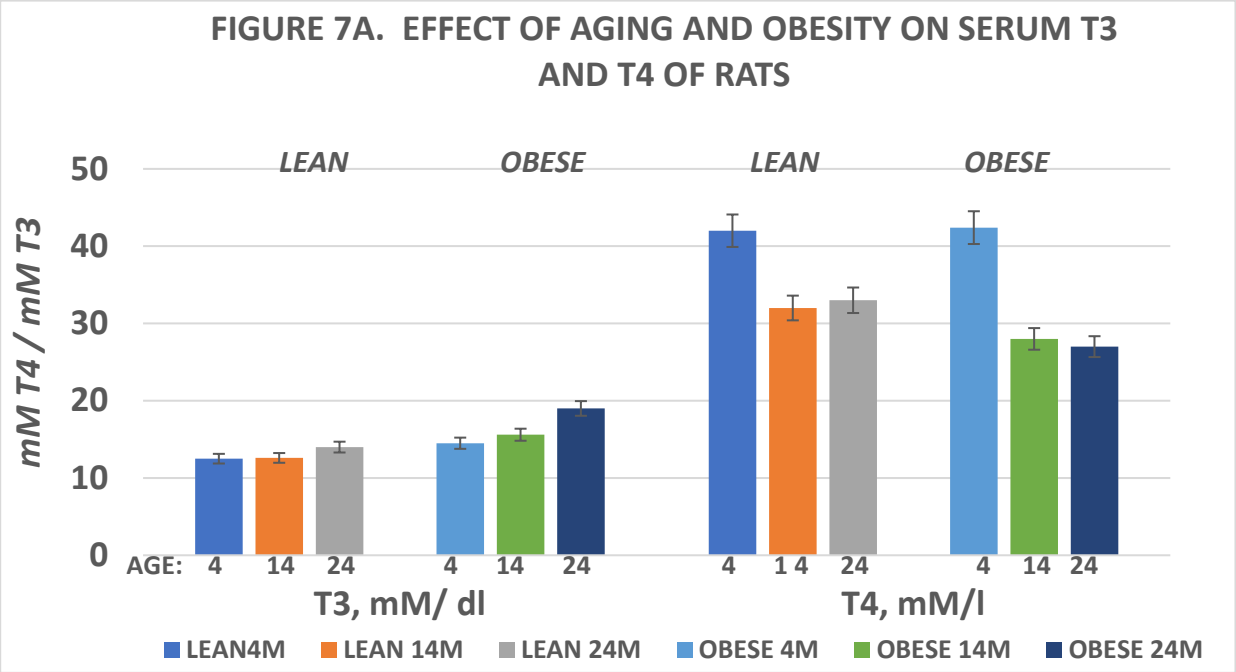


**Figure 7.** A. Effects of aging and obesity of plasma T3 and T4 concentrations in female PA/Ntul//-*cp* rats. Data are mean ± 1 SEM, n = 8 rats / group.

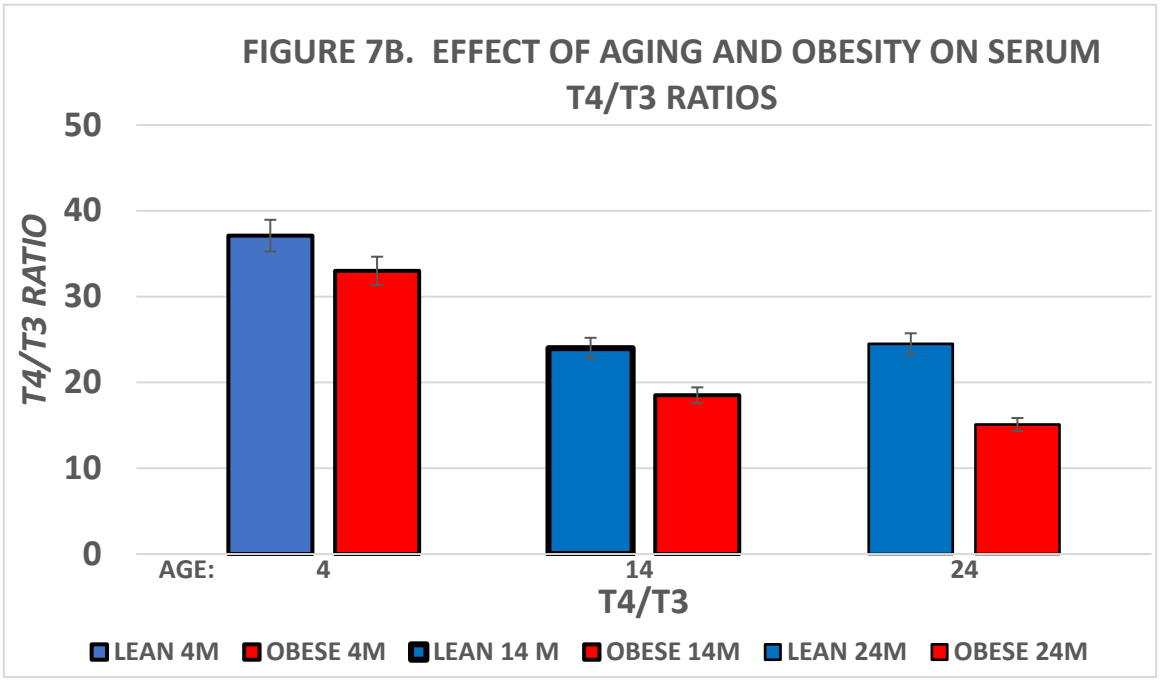


**Figure 7.** B. Effect of aging and obesity on T4/T3 ratios of female LA/Ntul//-cp rats. Data are mean ± 1 SEM, n = 8 rats / group.

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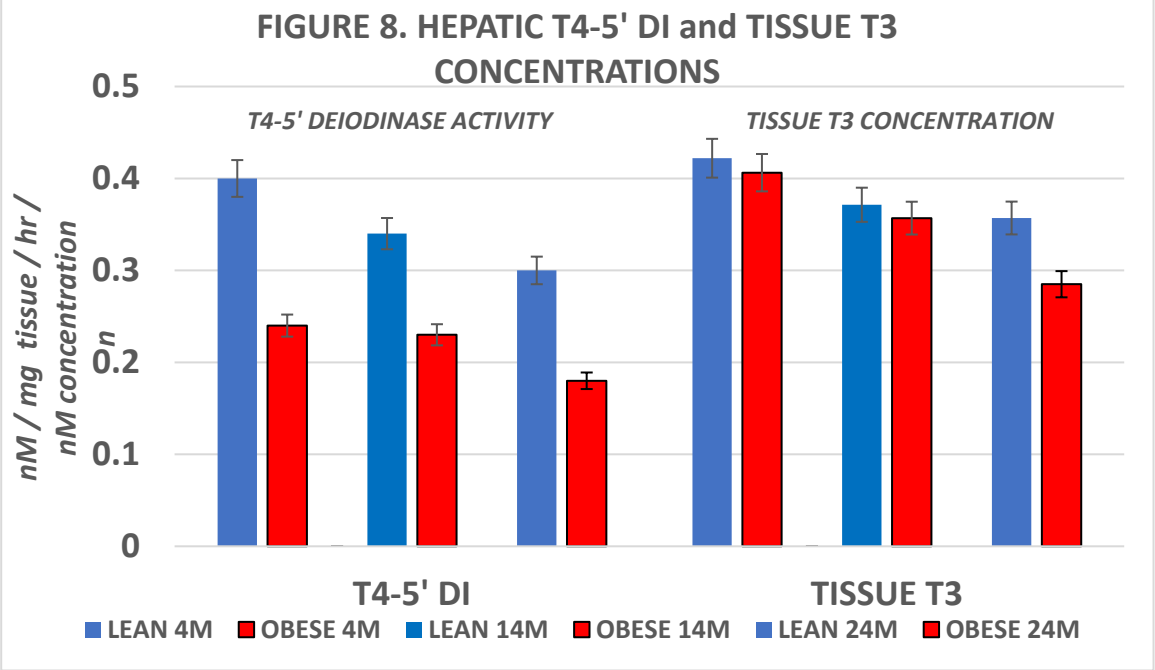


**Figure 8.** Effect of age, obesity, and phenotype of Type II T4-5′ deiodinase activity in liver. Data are mean ± 1 SEM, n= 8 rats/group. Deiodinase activity is depicted in the left panel, and tissue T3 concentrations in the right panel.

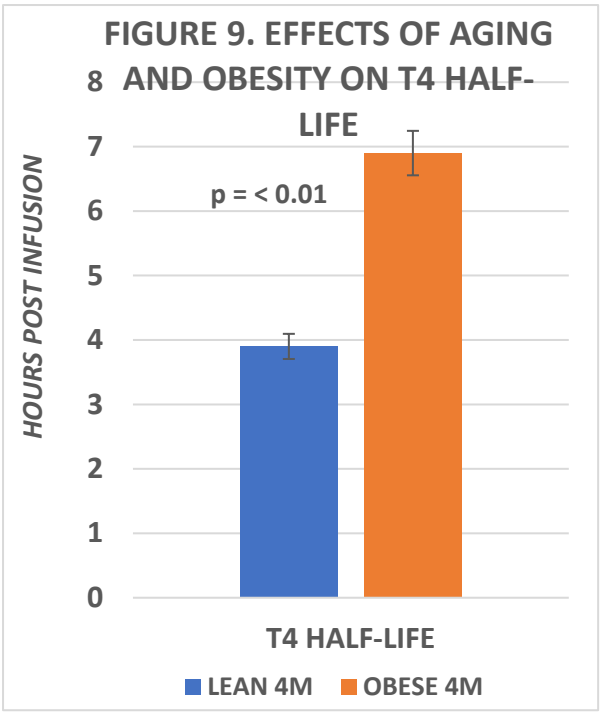


**Figure 9.** Effects of aging and obesity on T4 half-life in littermate male LA/Ntul//-cp rats at 4 months of age. Data are mean ± 1 SEM, n = 4 male rats/phenotype. Reflected as hours post-infusion of 1 μCi of $^{131}$I-T4 *(n=4 rats/group; 1 μCi/rat, dispersed in 50 μl physiologic saline and administered over a <1 minute timeframe via tail vein).*

## DISCUSSION

The various contributions of thyroid hormones to growth, development and aspects of metabolism have been well documented in man and animals.[1,17,20,21] As discussed, the regulatory

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process of thyroid hormone secretion begins in the thyrotrope cells of the anterior pituitary, where TSH is released in response to hypothalamic generated thyrotrope releasing hormone TRH, and targets the thyroglobulin stored in the thyroid gland to release its stored T4 and small amounts of T3 into the peripheral circulation.[1] Once in circulation, the T4 may be converted to T3 via D-1 and D-2 deiodinase enzymes that target the 5' iodine position outer phenolic ring to bring about increases in circulating T3 when needed to support growth, development, and when the effects of nutritional and environmental conditions dictate such need.[3,,8,44-47] In contrast, under conditions of starvation or dietary privation, the T4 is further inactivated by T4-5-DI-3 to remove the iodine from the inner ring 5-position, located on the inner tyrosyl ring, and thereby rendering the T4 to become a physiologically inactive hormone.[8] In the present study, the T4 journey to metabolic activation has been established and indicates that less T3 becomes available to peripheral tissue receptor domains that are dependent on an active T3 hormone and have been found to be decreased in the obese phenotype as the animal undergoes the aging process.[3128,44]

As the peripheral generation of T3 is conserved and hyperinsulinemia prevails in the obese phenotype of this strain, the interaction of the two hormones presumably contributes to an improved efficiency of energy conservation and storage, decreases in resting metabolic rate, and in at least partial contributions to the longevity demonstrated in both phenotypes of this strain.[19,] The T4 deiodination reactions have been suggested as a link to survival and longevity, due at least in part to the broad metabolic functions facilitated by the iodothyronine hormones, in concert with complimentary actions on aspects of energy metabolism and storage that are exerted by insulin, catecholamines, and other entities.[44,] Hyperinsulinemia is a hallmark of obesity, and is linked to energy conservation by decreasing the rates of protein turnover and ATP utilization in muscle and in lipogenesis in liver and adipose tissues.[3,11,45,46] Dysregulation of glucocorticoid actions may also contribute to energy conservation, via impaired endoplasmic reticulum generation and intracellular transport of insulin-dependent GLUT glucose transporter proteins, essential factors in cellular glucose uptake in skeletal muscle and adipose tissues, two of the major locations which constitute the bulk of insulin resistance in man and animals.[15,16,29,32]

Factors of diet and environment are well established factors in the modulation of thyroidal and insulinogenic activity in man and animals. In animals, both dietary excursions and cold exposure have been shown to bring about increases in plasma concentrations of T3, and an apparent response to increases in thyroidal release of T4 in combination with increases in peripheral iodothyronine deiodination in liver, brown adipose tissue and likely other tissues, where selective deiodination of outer- vs inner-ring iodine moieties can bring about an activation or an inactivation of thyroidal actions in peripheral tissues.[8] Outer ring deiodination results in T3 generation, while inner ring deiodination results in the formation of 'reverse' T3, or rT3, a physiologically inactive entity which when present, fails to associate with the stereospecific genomic receptor domains that mediate thyroidal actions in most if not all thyroid-receptive tissues.[3,5,33,34] In the present study, the peripheral generation of T3 was decreased and plasma insulin concentrations significantly increased in the obese phenotype at all ages studied, resulting in energy conservation as indicated by excess lipid deposition and age associated progressive decreases in resting oxygen consumption with aging. While the greater daily energy intake in the obese phenotype also represents an additional factor in the development or obesity in this strain, the impaired thermic responses to diet and environment in the obese phenotype reported elsewhere are consistent with impaired physiological responses to caloric intake in the obese phenotype of this and other genetically obese strains. Whether the presence of chronic insulin resistance may play a role in iodothyronine deiodination in the obese phenotype could not be determined from the present experimental design of this study, but the association of insulin resistance is likely a confounding and contributing factor via its biochemical effects on multiple enzymatic stages in intermediary metabolism, and their likely contributions to obesity and longevity in this strain.[15,16,31-39]

## SUMMARY AND CONCLUSIONS

The results of this study indicate that the rate of liver T4-5′-deiodinase activity is greater in the lean than the obese phenotype and decreases progressively with aging in both phenotypes in parallel to the decreases in fasting plasma insulin concentrations and in gradually decreasing RMR in both phenotypes. The significantly greater plasma half-life of T4 in the obese phenotype is consistent with the deceased hepatic conversion of T4 to T3. A major effect of T3, the active form of the thyroid hormone is to modulate the rates of intermediary metabolism and oxidation of substrates, a primary source of inflammatory ROS generation and of maintaining the rate of resting oxygen consumption.[39] Thus, the age -related decreases in T4-5′-deiodinase activity and *in vivo* peripheral conversion may contribute to the longevity in both phenotypes via influencing the generation of inflammatory ROS downward, and thereby minimizing their deleterious and damaging effects on pathogenic mechanisms in the lean and obese phenotypes when formed.[44] Excess adiposity contributes to inflammatory ROS generation, and may be a contributor to the decreased life span in the obese phenotype.[39,48] In addition, the longevity impact demonstrated in the obese phenotype while resulting in a significant decrease in longevity when compared to their lean littermates, still enabled the obese phenotype to outlive the obese of other genetically obese rat strains, most of which appear to suffer their demise soon after one year of age. These observations suggest that genetic factors may also be at play in longevity in this strain, since the background LA/N strain has been noted for its healthful longevity.[17,19] The autosomal recessive inheritance of the *-cp* trait is present in both phenotypes of the strain.[17-19] In clinical studies, modest decreases in thyroidal activity have been suggested as a contributing factor in obesity and premature longevity, presumably via decreasing the rate of age-linked contributors to the pathophysiology of thyroidal-mediated changes in aging.[31-35,44,48]

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