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Effect of Acute Cold Exposure on Differential Expression of Tissue D-I and D-II Iodothyroinine Deiodinase [T4-5'-Deiodinase] Activity in Congenic Lean and Obese LA/Ntul//-*cp* Rats: Iodothyroinine Deiodinase Activity in Obese Rats

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ABSTRACT

Thyroid hormones are known to play a critical role in metabolic adaptation to chronic changes in diet and environment. The prohormone tetraiodothyronine (T4) is converted to the active form, triiodothyronine (T3) in peripheral tissues via actions of outer ring deiodination by D-I or D-II isoforms of T4-5' deiodinase activity. In contrast, T4 may also become inactivated during periods of caloric depravation via an inner tyrosyl ring D-III deiodinase to form an inactive hormone, 3'5'3 triiodothyronine, or 'reverse T3' (rT3). Measures of D-I and D-II were determined in selected tissues obtained from congenic adolescent lean and obese female LA/Ntul//-*cp* rats when 16 weeks of age following laboratory temperature exposure of 22°C or 14 hours acute cold exposure at 4°C. Circulating T4 concentrations were similar in lean and obese rats and serum T3 but not T4 concentrations increased dramatically in both phenotypes following the cold exposure, consistent with phenotype- and maximal tissue-linked changes in outer ring T4-5'-deiodinase activity / mg tissue protein and per depot. In Gastrocnemius muscle, only D-II was detected, and gastrocnemius D-II activity of obese increased modestly following cold exposure. In Liver, Kidney, and IBAT ~~and in~~ cold-induced temperature exposure linked increases in IBAT deiodinase activity / mg tissue protein in this strain, but when D-I and D-II deiodinase activity were computed /

tissue mass however further analysis indicated that D-I was the predominating adaptive deiodinase in liver, kidney, and gastrocnemius muscle, while in IBAT D-II > D-I activity / IBAT depot and was greater in obese than lean rats. Cold exposure was associated with modest increases in net deiodinase activity only in kidney. Thus, the cold induced increases in circulating T3 in lean and obese rats following cold exposure are likely attributed at least in part to modest increases in IBAT outer ring T4-5' D-II and renal D-I deiodinase activity, in addition to likely attaining maximal rates of conversion in other peripheral tissues in addition to possible combination with decreases in hormone clearance rates and enhanced receptor occupancy during cold induced stress, and where they contribute to protective measures during dietary or environmental stress.

Keywords: T4-5' deiodinase, thyroid hormones, cold adaptation, congenic rats, obesity.

INTRODUCTION

Thyroid hormones are well established entities that readily mediate numerous essential biochemically mediated elements of normal development, growth and energy metabolism (EM) in response to alterations in diet, environment and life stage of development. A family of three highly specific Iodothyronine deiodinases, consisting of a subfamily of selenocysteine iododeiodinase enzymes that exert important roles in the activation and deactivation of thyroid hormones in virtually all somatic tissues of vertebrate organisms, where they can bring about variations in the rate of metabolism including energy expenditure and/or conservation of energy utilization in peripheral tissues.[1-5] Because the iodothyronine 5'deiodinases are strategically located in the cellular membrane compartments of the outer plasma membrane (D-I, D-III) and the endoplasmic reticulum membrane (D-II), both occur in close proximity to the thyroid hormone (TH) receptor domains where they can bring about activation or inactivation of thyroidal hormone activity.[1,6] The emergence of T3' (or rT3) by removing the inner tyrosyl ring 5-iodine from the T4 substrate via D-III deiodinase (EC 1.21.99.5) represents the third type of deiodinase activity.[5] In addition, the D-III deiodinase can also inactivate hormonally active T3 to form diiodothyronine (T2) to further terminate the hormonal activity. The Iodothyronine deiodinases including Type 1 deiodinase (EC 1.21.99.4 and EC 1.21.99.3 (Type II deiodinase activation) also includes the conversion of the inactive prohormone levotetraiodothyronine (L-T4, T4) to the hormonally active levotriiodothyronine (T3) via a Type 1 deiodinase (EC 1.21.99.4) or a Type II deiodinase (EC 1.21.99.3) by removal of the outer ring 5'- iodine.[3] Conversely, T4 and T3 can be converted to an inactive form of iodothyronine (3', 5', 3 iodothyronine), commonly referred to as 'reverse deiodinase) and Type III deiodinase (EC 1.21.99.5) are the only members of the subfamily of membrane associated deiodinase enzymes found to be important in the intracellular activation and/or deactivation of thyroid hormones; D-I and D-III in association with plasma cell membrane, and D-II on the endoplasmic reticulum membrane.[1-5] The cumulative impact is geared toward regulation of the amount of thyroidal hormone activity on metabolic and epigenetic processes in response to diet, environmental and life stage growth changes. [7,8]

The molecular hormonal actions of thyroidal entities are expressed via interaction of triiodothyronine (T3) with two thyroid hormone receptors (THRs), THR-α- and THR-β, both of which reside on thyroid hormone response elements (TREs) located on TH promotor regions of their target genes.[6] In so doing, they normally mediate transcription events via both genomic

and some non-genomic mechanisms. The TH α- and β- receptor affinity for T3 is much greater than for T4, however, thereby necessitating outer ring deiodination of the L-T4 prohormone to hormonally active L-T3 to most efficiently impinge on the TREs. Synthetic D-T4 often applied to treat hypercholesterolemic conditions fails to effect the same epigenetic reactions on gene expression or metabolic actions.[9-11] This outer ring deiodination process occurs by one of two forms of T4-5'-diodinase, namely D-I deiodinase, located in association with the plasma membrane, or D-II deiodinase located on the endoplasmic reticulum membrane, both of which culminate in molecularly active L-T3.[2-5] In addition, hormonal inactivation may occur via D-III deiodinase, an inner ring deiodinase also found on the plasma membrane that results in the formation of the metabolically inactive 3'5'3-triiodothyronine (rT3), rather than the active form 3'5,3-triiodothyronine or T3 and exhibiting a profound impact on aspects of substrate and energy metabolism. [1-3,5] As depicted in Figure 1 below, D-III deiodinase can also bring about the inactivation of T3 to diiodothyronine (T2) by secondary removal of the inner ring iodine on the 5 position of T3. Both D-I and D-II tend to remain active during both the fed state and in response to environmental temperature changes, while D-III becomes activated to form 'reverse T3' (rT3) in response to fasting, starvation, and energy depravation, thereby forming a metabolically inactive form of the hormone and enabling thyroidally-mediated elements of metabolic energy conservation to occur during energy privation.[7,8] In addition, the activity of D-II has been found to be reciprocal to tissue T4 concentrations, being greatest when T4 concentrations are vanishingly low and lowest when T4 concentrations are greater. Thus, the hormonal activity of THs play important roles in both T4 deiodination activity and in energy metabolism and conservation by facilitating the epigenetic expression of aspects of cytosolic energy metabolism and utilization during periods of variable dietary availability and environmental changes. Because plasma concentrations of thyroid hormones are more reflective of the patency of feed-back signals controlling thyroidal hormone secretion than they are of end organ effects, they may not be able to adequately access intracellular metabolic actions resulting from the receptor mediated translational activity originating at the TRE level of organization, and thus may contribute to a syndrome of an apparent undetected state of subclinical hypothyroidism.[12,13]

Once considered an important contributor to life saving heat generation in newborns of mammalian species as its primary function, brown adipose tissue also plays an important role in energy balance in later stages of life via increasing heat production in man and animals in response to dietary or environmental induced increases in energy consumption and by generating additional heat during cold exposure to augment the transition from shivering-mediated thermogenesis as summarized by Himms-Hagen and others.[7,8,14-16] Activation of BAT-mediated thermogenesis occurs via the uncoupling of oxidative phosphorylation and metabolism of high energy phosphate bonds, thereby generating thermal energy.[15,16] Thyroidal mediation also requires the presence of L-T3, generated largely via intracellular outer ring D-I and D-II deiodinases.[17-19] Thus the cumulative result of activated BAT is to assist in maintaining body temperature regulation during nutritional or environmental challenges, which it accomplishes at least in part via activation of thyroid hormones.[7,8,15,19]

The metabolic deiodination pathways of thyroid hormones are summarized in Figure 1 below, and depict the multiple membrane-associated locales for D-I, D-II and D-III actions. This figure reflects the systematic transformation of the prohormone levo-tetraiodothyronine (L-T4) to its

metabolically active and inactive forms of T3, rT3 and T2 respectively. The active T3 formation is depicted on the left side of Figure 1 via Type I Deiodinase (D-I) and Type II deiodinase (D-II), and its metabolically inactive inner ring tyrosyl ring form of rT3 via Type III deiodinase (D-III) is depicted on the Right side of the diagram. Both T3 and rT3 are further inactivated to T2 (3,3'T2) and are devoid of nuclear binding or other physiologic activity. The iodine moieties that are thereby generated may be recaptured and recycled by the thyroidal epithelium with great efficiency, where they may contribute to the iodination of additional thyroglobulin tyrosyl residues during the *de novo* formation of additional L-T4. This reuptake of iodine moieties is highly beneficial to human health as dietary iodine availability is often sparse in many remote parts of the global land masses, especially in landlocked locales distant from the oceans and in iodine unsupplemented foodstuffs, thus enabling more efficient metabolism of primary energy substrates in iodine sparse locales.[16, 17,20]

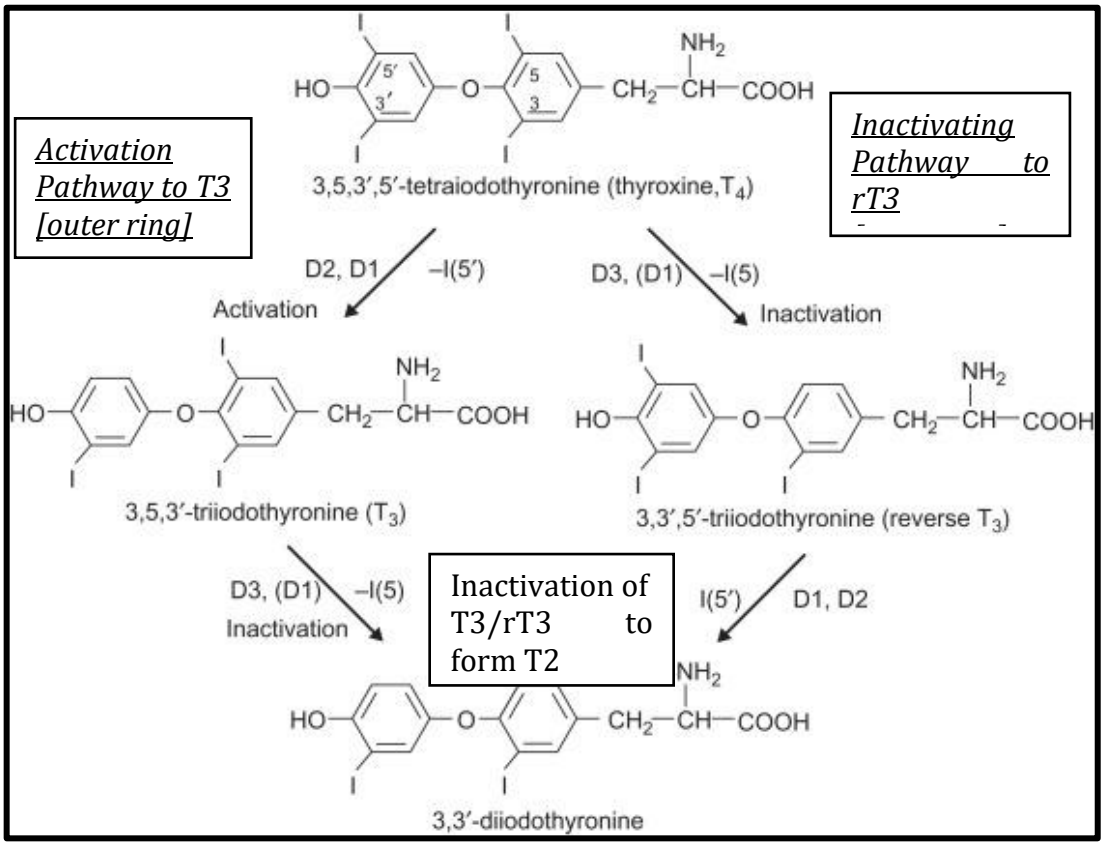


Figure 1: Schematic of iodothyronine deiodination in peripheral tissues. T4 = tetraiodothyroiine; T3 = triiodothyronine; T2 = diiodothyronine. IDI = T1 5' deiodinase; IDII = T2 5-deiodinase; IDIII = T3 deiodinase. Outer benzene ring also referred to as the β-ring, located on the left side of the molecule as indicated, while the inner tyrosyl ring on the right side as depicted is often referred to as the α-ring. -I(5') removal of outer 5' position iodine; -I(5) = removal of inner 5 position iodine; -I(5) = removal of inner ring 5' position iodine; I(5') = removal of outer ring 5' position iodine. Ref modified from multiple refs.[2,7,818]

The LA/Ntul//-*cp* rat is a unique congenic rat strain that demonstrates the epigenetic expression of obesity as an autosomal recessive trait soon after weaning. ILAR J These responses include hyperinsulinemia, hyperamylinemia, impaired glucose tolerance, caloric

efficiency resulting in excessive weight gain (WG), followed by excess fat accretion in virtually all primary white adipose tissue depots and including brown adipose tissue depots.Huang, ref, ILAR J The obese phenotype also demonstrates a lower rate of resting metabolic rate (RMR) when adjusted for the differences in body size and surface area, in addition to impairments in norepinephrine stimulated nonshivering thermogenesis (NST) in response to alterations in diet and environment. *Tulp oct 2025 ref* The impaired thermic responses occur despite significant early increases in brown adipose tissue (BAT) mass and cellularity during postweaning growth and development, possibly at least in part via responses to early onset hyperphagia, a recognized stimulus for early proliferation and hyperplasia of brown adipose tissue. (ref) . The $T_{1/2}$ for T4 has been shown to be delayed by up to 50% in the obese phenotype, also possibly contributing to the atypical responses in plasma T3 in response to changes in diet and environment. (ref) Thyroid hormones also exert actions on aspects of BAT metabolism and nonshivering thermogenesis via interacting with nuclear receptors, where they contribute an essential element in expression of the thermic responses, including decreases in metabolic rate following thyroidal depletion of thermogenic activity.(REF). In lean rats, plasma concentrations of T3 become progressively elevated soon after feeding and continue to rise as the duration of the dietary excursion continues, reaching an apparent maximum within 3 weeks of an experimental overnutritional manipulation or overfeeding regimen. (REF) The increases in circulating T3 in obese littermates fail to increase to the same magnitude. However, despite demonstrating a state of exaggerated hyperphagia and excess caloric intake, greater deposits of BAT mass and cellularity, and with an inadvertent gain in adipose weight and progressive increases in adiposity, likely due at least in part to an enhanced caloric efficiency inherent in the obese phenotype. Thus, the purpose of the present study was to determine the effect of acute cold exposure on parameters of thyroidal status, including measures of Type D-1 and D-II T4-5' deiodinase activity before and after acute cold exposure. In addition, the determination of D-1 and D-II deiodinase activity were determined in several peripheral tissues including liver, kidney, skeletal (gastrocnemius) muscle, and interscapular BAT to further characterize the deiodinase activity in those key tissues. This animal strain is deemed to represent an appropriate model in which to investigate the effects of obesity on metabolic and physiologic parameters due to the congenic status of the strain, where the only known difference between the phenotypes is the epigenetic expression of the obese phenotype in a non-diabetic, normotensive obese animal model.(ref ILAR J)

METHODS

Groups of lean and obese littermates(n= 6-8 rats/group) were obtained from the Drexel colony at 5 weeks of age and placed in plexiglass showbox cages lined with one inch of pine shavings. Rats were maintained on Purina rodent chow (formula 5012) and house water *ad libitum* in littermate pairs (1 lean plus 1 obese) from weaning throughout the study under conventional laboratory environment (22°C, 50% RH, on a reverse light cycle (light 8 PM to 8 AM). At 8 to 16 weeks of age, lean and obese littermate were subjected to determination of resting metabolic rate in a Collins small animal thermogenesis apparatus at thermal neutrality (30°C) fitted with a 1 cf plexiglass closed circuit metabolic chamber and RMR expressed / kg of Body Weight^{-} during the mid dark cycle after a brief 6 hour fast and expressed as kg $BW^{-0.75}$ to adjust for differences in body size and surface area as outlined by Klieber and Yang [21,22] before and after administration of norepinephrine (100 TO 400 µg/kg BW, s.c. on separate days). At 16 weeks of age, animals were sacrificed by acute cervical dislocation and blood, liver, kidneys,

gastrocnemius muscle and IBAT harvested, weighed to the nearest mg. The tissues were homogenized in a phosphate buffer and measures of T4-5' deiodinase determined both in the presence and absence of the D-I inhibitor propylthiouracil (PTU, 10 µg/aliquot) and in the presence of dithiothreitol (DTT, 3 µg) to determine the maximum activities of D-1 and D-II respectively. Measures of tissue T3 and plasma T3 were determined by radioimmunoassay and expressed as ng T3/dl of plasma or of homogenate after a 120-minute incubation of homogenates at 37°C in a shaking water bath at 50 cycles per minute.[18] Data were analyzed via standard statistical methods.[23,24] Tissue protein was determined according the original method of Lowry and Roseborough.[25] The study was approved by the Intuitional animal care and use committee (IACUC).

RESULTS

The effects of phenotype on principle fat pad mass are depicted in Figure 2, and indicate that body fat accretion in the obese phenotype far exceeded that in their lean littermates at 16 weeks of age, whether measured in absolute terms or as a reflection of total body weight per depot. As indicated, the majority of the excess fat accretion occurred in the retroperitoneal and dorsal depots. The physiological basis or the exaggerated fat accretion in the obese phenotype remain unclear, but are consistent with an improved efficiency of energy conservation, in addition to a likely early onset of hyperphagia as contributing factors. In normally lean rats, both thyroidal and sympathetic actions have been shown to be essential in the expression of nonshivering thermogenesis.

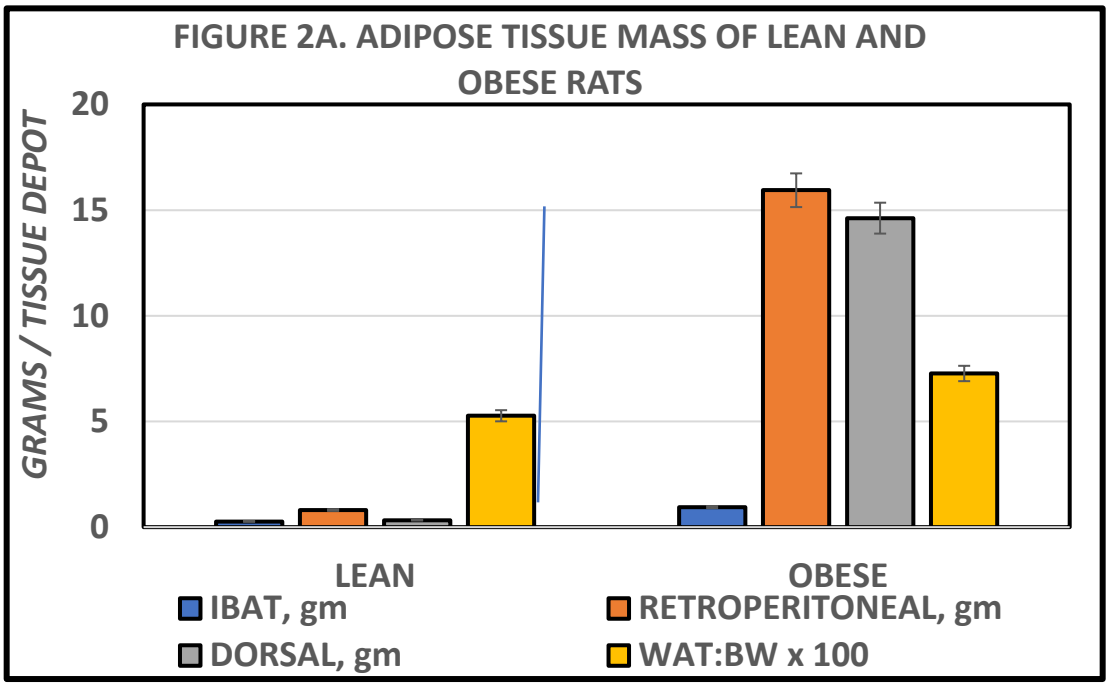


Figure 2A: Effects of phenotype on adipose tissue mass. Data are mean +/- 1 SEM, n = 6 rats/phenotype. P = < 0.01 for retroperitoneal and dorsal AT depots; p = < 0.05 for IBAT depot.

The effect of the obese phenotype on tissue weights of liver and kidney are weights are depicted in Figure 2B. and indicate that the mass of the liver was markedly greater in the obese than the lean phenotype at 16 weeks of age. Despite having consumed a diet of equal macronutrient

composition and energy density since weaning. In contrast kidney weights were only modestly greater in the obese phenotype, and gastrocnemius muscle weights were similar in both phenotypes, suggestive of a similar net rate of protein accumulation, without respect to possible differences in the efficiency of protein turnover, as in earlier studies, the efficiency of protein turnover was found to be markedly greater in obese animals of this strain, while the final maas was similar, an observation attributed to the secondary impact of chronic insulin resistance on parameters of energy metabolism among obese animals.

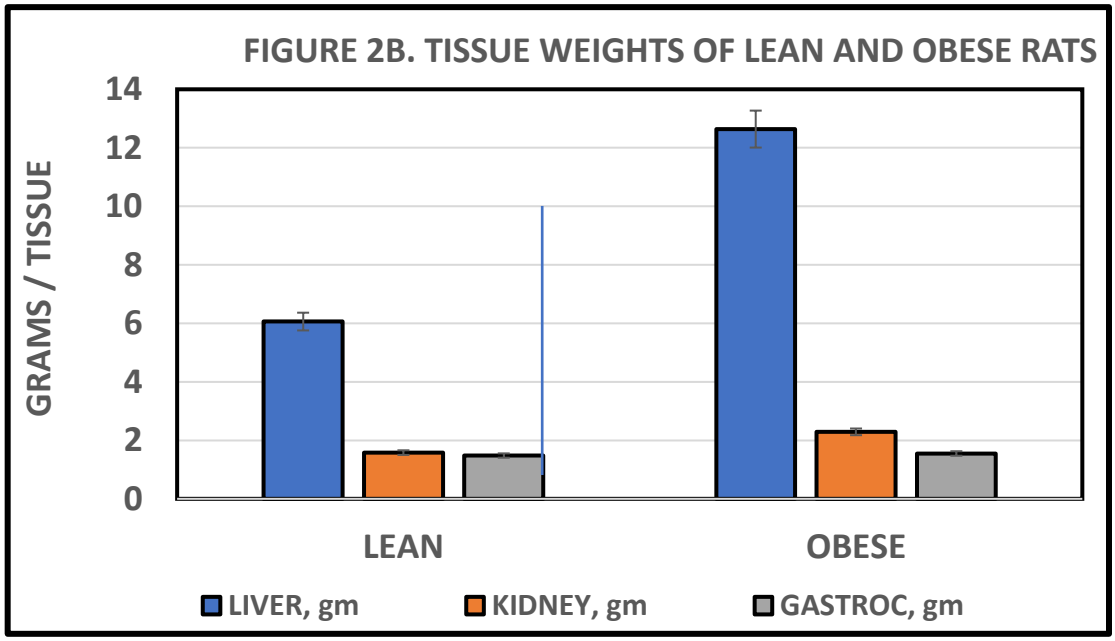
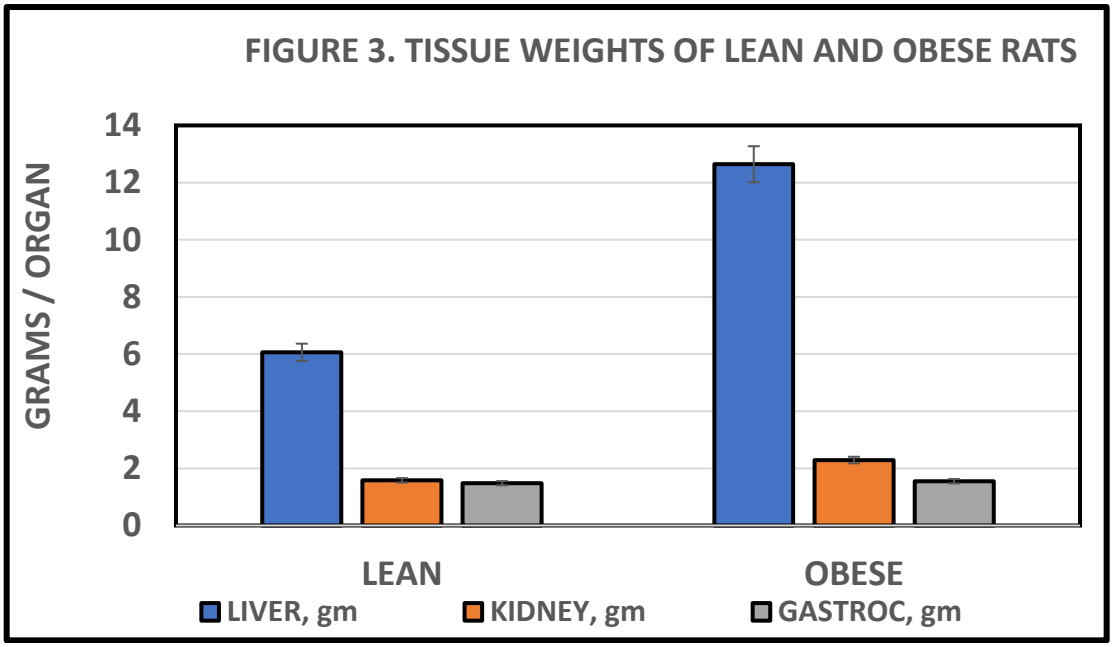


Figure 2B: The impact of the obese phenotype on individual tissue weight in lean and obese animals. Data are mean +/- 1 SEM, n = 6 rats / phenotype. P = < 0.01 for liver weights.

The effects of phenotype on resting and norepinephrine-stimulated VO2 are depicted in Figure 3A and indicate that resting VO2 in the obese phenotype when corrected for differences in mass, body weight, and surface area were lower than those obtained in their obese littermates. In addition, the thermogenic response to maximal dosages of norepinephrine were also of less magnitude in the obese phenotype, despite the greater mass of brown adipose tissue present commonly observed in genetically obese rodents.[26,27] The BAT depots in obese rodents typically far exceed those found in their lean littermates, although the physiologic mechanism(s) that result in the exaggerated BAT depots remains speculative. In the lean phenotype, BAT normally responds robustly to NE whether administered via exogenous or endogenous mechanisms, since brown adipocytes are normally endowed by thermogenic β-adrenergic receptors on their plasma membranes. In Figure 3B, the effects of acute cold exposure on thermogenesis are depicted and indicate that the maximal increase in VO2 after 5 minutes was decreased by approximately 45% and remained at a lower magnitude thereafter. The total area under the thermogenesis curves averaged over 30% less than in their lean littermates throughout the 45 minutes duration of the 4°C cold exposure.



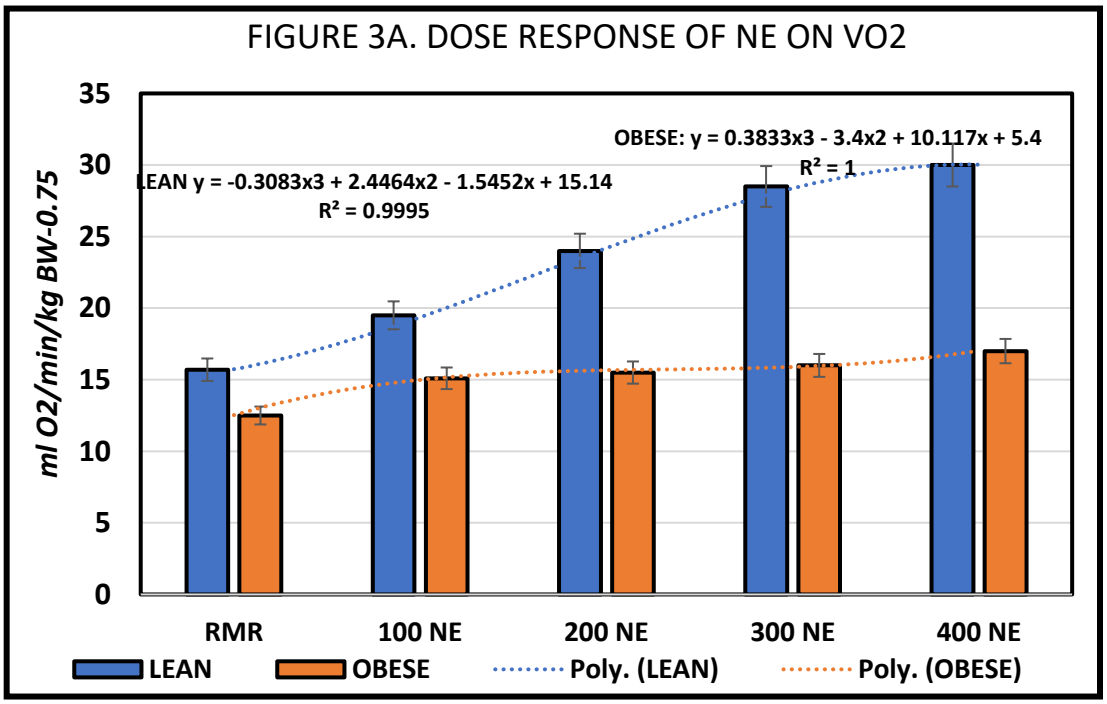


Figure 3A: The effects of phenotype and norepinephrine administration on VO2. Data are 4-6 rats/group. P = < 0.05 at all dosages for Lean vs obese at both 22 °C and 4° C temperature. R^2 are as noted for both phenotypes.

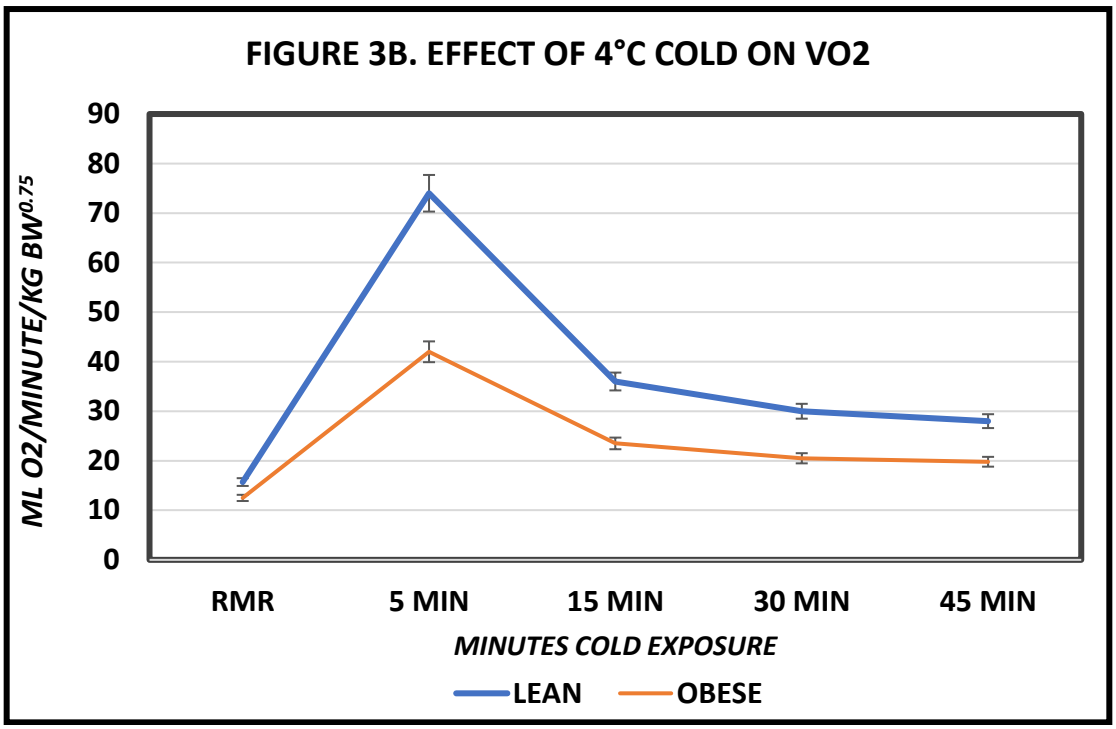


Figure 3B: Effect of acute 4°C cold on VO2. Data are mean +/- 1 SEM, n = 6 rats/group. Data are corrected to $^{0.75}$ of body weight to correct for differences in mass and body surface area between lean and obese phenotypes.[21,22]

The effects of cold exposure on plasma T3 are depicted in Figure 4A and indicate that resting plasma T3 concentrations were greater in the lean than in the obese phenotype. When the cold induced an increase in plasma T3 concentrations in both lean and obese animals however, the magnitude of the increase was greater in the obese than in the lean phenotypes (mean = 61 % in obese vs 35 % in lean littermates). The effects of cold exposure on plasma T4 are depicted in Figure 4B and indicate that a modest decrease in plasma T4 occurred in the lean phenotype, while in the obese phenotype plasma T4 increased following the cold exposure, suggesting a greater magnitude of dehydration or increased thyroidal release of T4 may have occurred.

The effects of net 5'deiodinase activity in the presence of dithiothreitol (DTT) are depicted in Figure 6 and indicate that net deiodinase activity was not significantly impacted in liver, kidney, or gastrocnemius muscle homogenates, while net deiodinase activity in interscapular brown adipose tissue (IBAT) homogenates were increased in both lean and obese phenotypes. In addition, the cold exposure resulted in greater net T4-5' deiodinase activity in both phenotypes, with the greatest net increase occurring in the obese phenotype. Thus, the thyroidal responses were significant regardless of whether expressed per unit of cellular protein, or as per total depot mass of the thermogenic tissues examined and as expressed in Figures 6A-D.

The effects of D-I and D-II 5'isoforms of deiodinase activity in liver homogenates are depicted in Figure 6A and indicate that net D-1 deiodinase activity was greater than D-II activity, and both enzyme activities tended to decrease further following the acute cold exposure. In contrast, in the obese phenotype, D-I activity was similar to that of its lean littermates and remained high following cold exposure. The Net D-II activity was lower than D-I activity and

reflected only a modest increase following cold exposure. The differences between D-I and D-II activity were significant at p=<0.05 in both phenotypes, but phenotype differences in liver homogenates were not observed with the exception of D-II in the cold environment between the lean and obese phenotypes (p=<0.05).

The effects of kidney homogenates on D-1 and D-II activity are depicted in Figure 6B, and indicate that renal T4-5' deiodinase activity exceeded T4-5' deiodinase activity in both thermal cold settings, and that in both phenotypes the duration of cold exposure failed to further increase to cold settings. Measures of net Type I and Type II deiodinase activity in kidney are depicted in Figure 6B and indicate that activity of Type I was greater than Type II deiodinase in both phenotypes, and that cold exposure was associated with only modest further increases in Type II endoplasmic reticulum-associated deiodinase in activity in both phenotypes.

The effects of gastrocnemius muscle homogenates on D-1 and D-II activity are depicted in Figure 6C and indicate the only D-II activity increased following cold exposure, while D-I activity remained nil in both lean and obese phenotypes despite the cold exposure and likely inclusion of some shivering thermogenesis during the initial stages of cold exposure.

Finally, the effects of Interscapular brown adipose tissue homogenates on D-1 and D-II activity are depicted in Figure 6D. These data indicate that measures Type 1 deiodinase exceeded that of T4 5'- deiodinase in the IBAT depot when expressed as pMol of T3 generated / depot per hour of incubation. In the homogenates, both D-1 and D-II membrane components are both reflected under the conditions of the incubation, and are suggestive of a hormone receptor subunit effect in the obese phenotype, thereby impeding full expression of the thyroidal actions. The role of insulin resistance has recently been identified as a contributing factor in mediating actions of hormonal activity and contributing to multiple pathophysiologic aspects of insulin actions.[28]

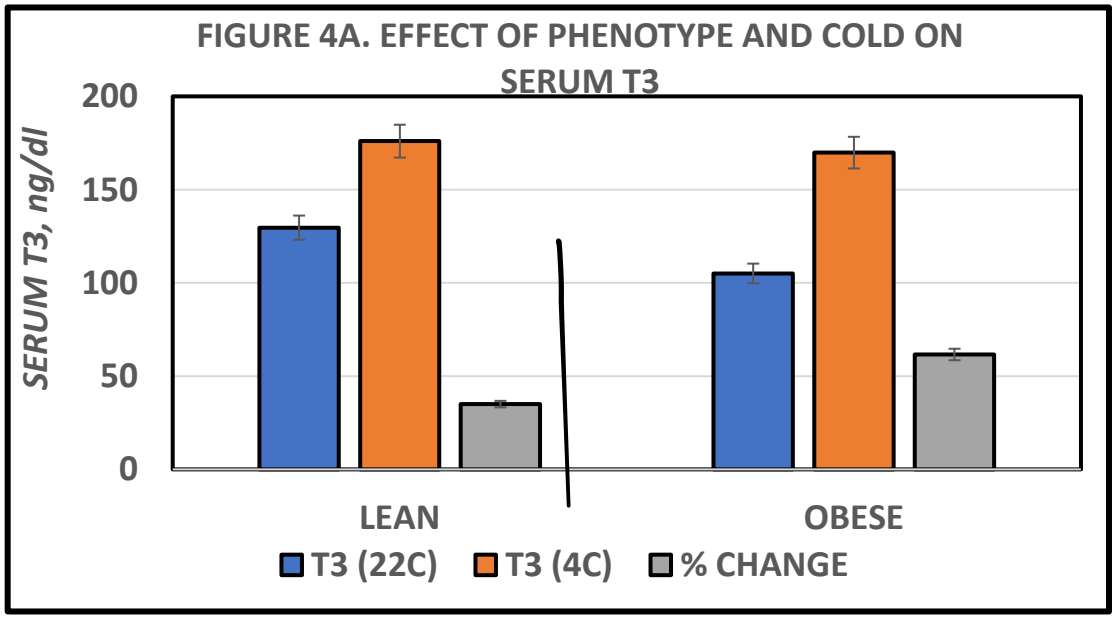


Figure 4A: Effect of 14 hours of cold exposure on plasma T3 concentration. Data are mean +/- 1 SEM, n = 6 rats/group. P = < 0.05 for lean vs obese at 22°C, n.s at 4°C.

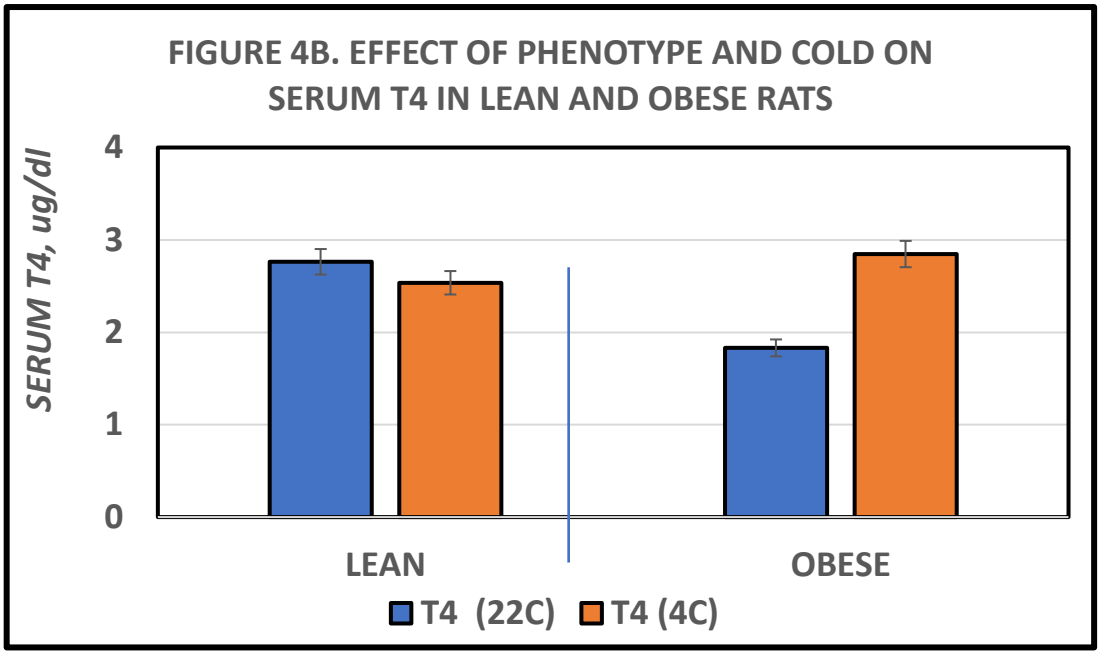


Figure 4B: Effect of 14 hours of Cold exposure on plasma T4 concentrations. Data are mean +/- 1 SEM, n = 6 rats/group. P = < 0.05 for lean vs obese at 22°C, n.s. at 4°C.

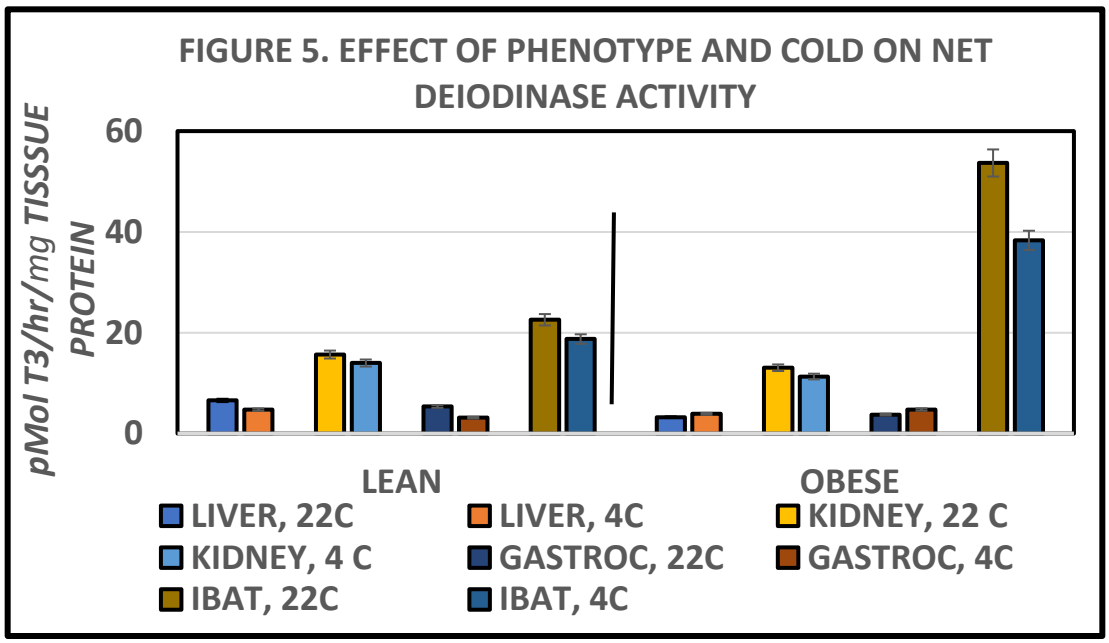


Figure 5: Effect of phenotype and cold exposure on tissue 5'deiodinase activity / mg protein. Data are mean +/- 1 SEM, n = 6 rats / group.

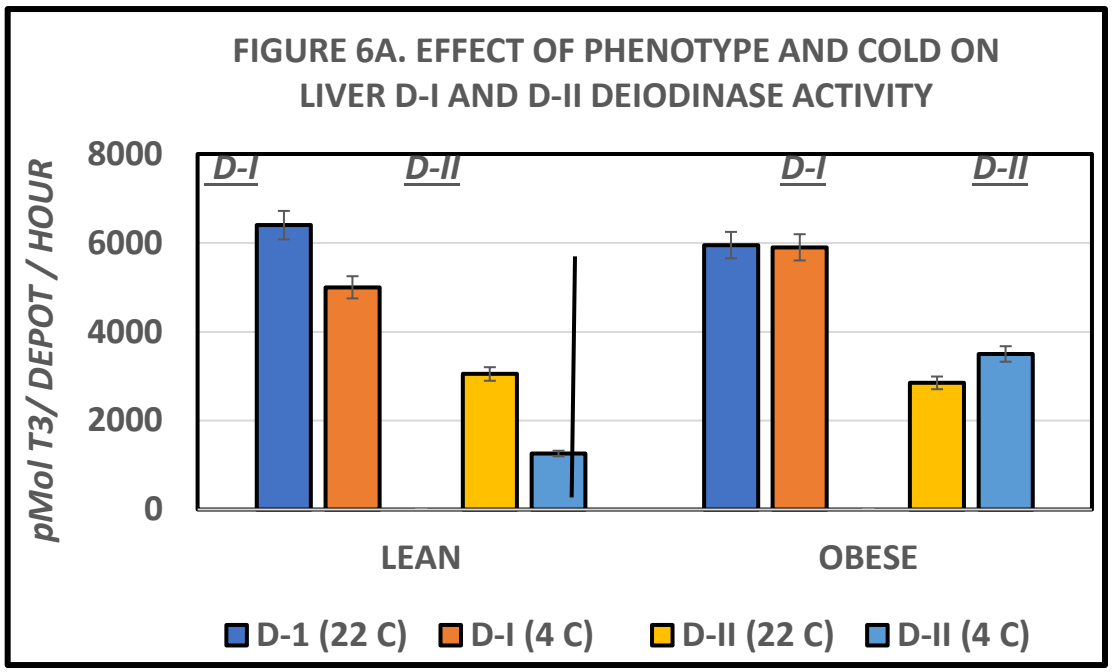


Figure 6A: Effects of phenotype and cold on 5' deiodinase activity in liver. Data are mean +/- 1 SEM, n = 6 rats/group. P = < 0.05 for D-I vs D-II in both phenotypes.

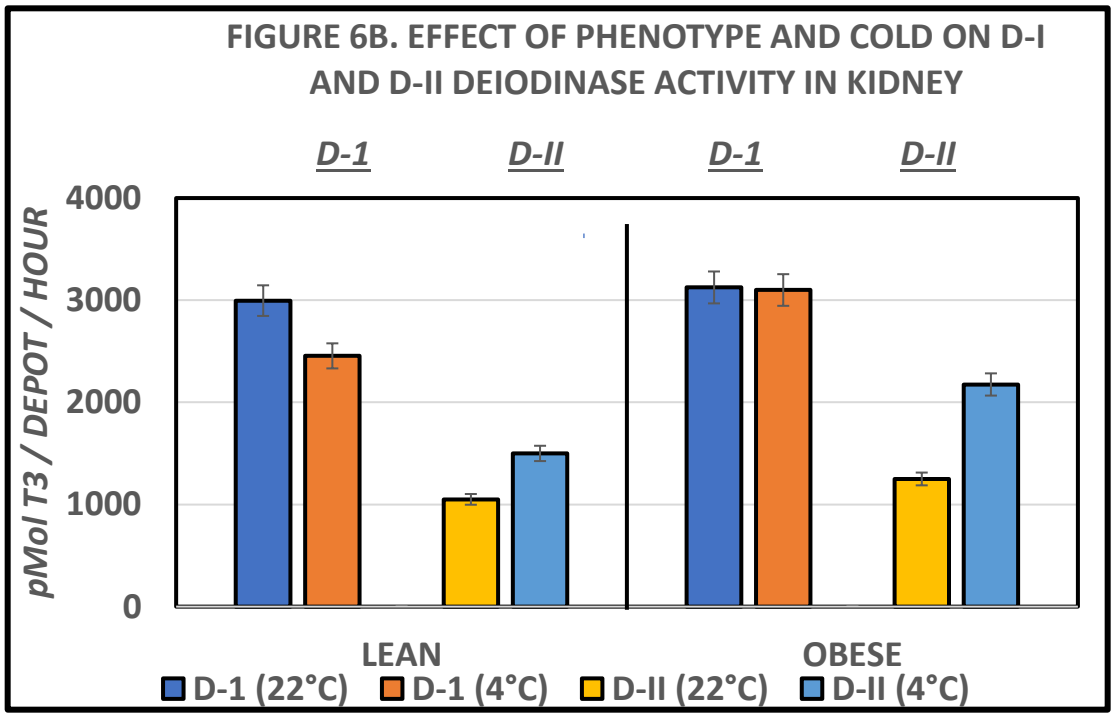


Figure 6B: Effect of phenotype and cold on expression of T4 5' deiodinase activity in kidney. Data are mean +/- 1 SEM, n = 6 rats/group. D-I > D-II at p = < 0.05 in both phenotypes.

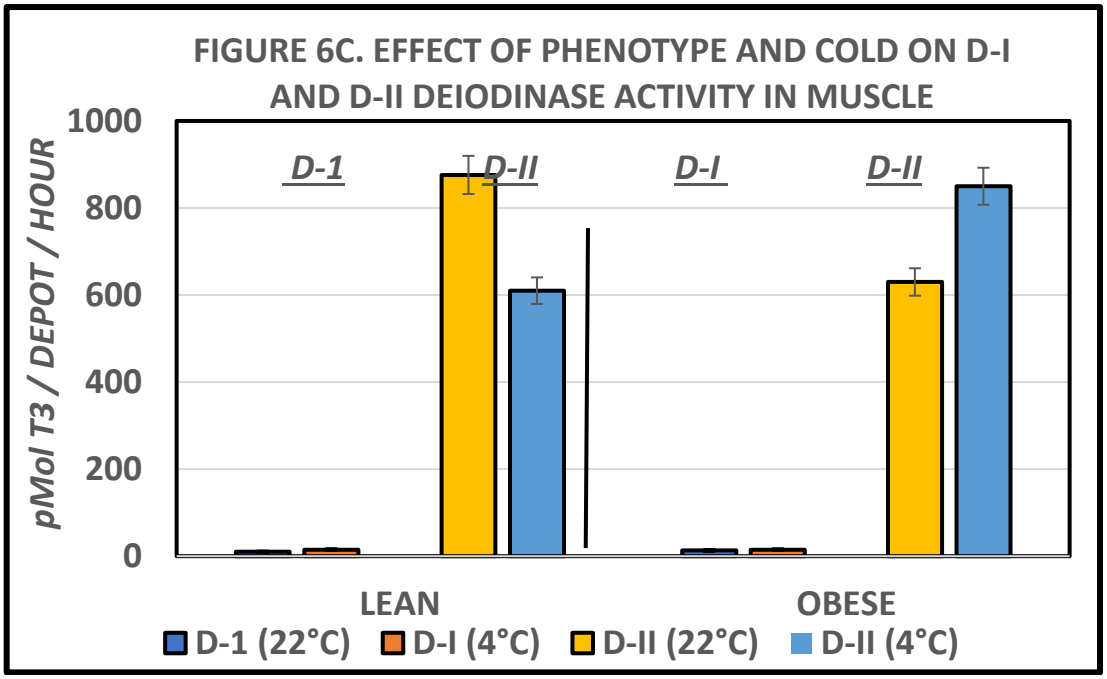


Figure 6C: Effect of phenotype and cold on 5-deiodinase activity in gastrocnemius muscle. Data are mean +/_ 1 SEM. N = 6 rats/group. P = < 0.01 for D-II vs D-I in both phenotypes.

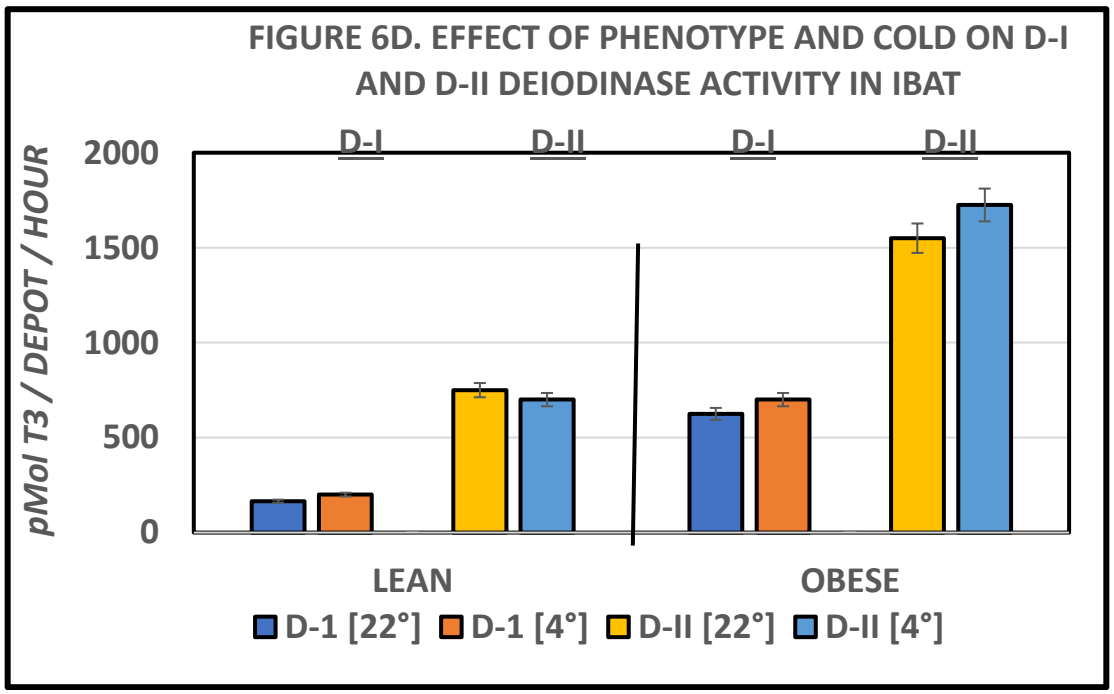


Figure 6D: Effect of phenotype and cold on 5-deiodinase activity in IBAT. Data are mean, +/- 1 SEM, N = 6 rats/group. P = < 0.05 for obese vs lean phenotype.

DISCUSSION

Multiple studies indicate that the thermic responses to diet and cold exposure may be impaired in obese rodents and are likely secondary to the combined contributions of sympathetic and thyroidal components of nonshivering thermogenesis (NST), in addition to the cumulative

impact of chronic insulin resistance in the obese phenotype.[26-29] The prohormone T4 is normally deiodinated via outer ring and intracellular deiodinase activity by D-1 and/or D-2 deiodinase activity to form hormonally active T3 during fed and cold environments, or inner ring deiodinase activity via D-3 to form reverse T3 (rT3) during periods of food or energy deprivation. The effect of 14 hours of 4°C cold exposure on the obese phenotype of the LA/Ntul//-*cp* rats was determined in normally reared young female animals at 16 weeks of age and fed the Purina Chow #5012 *ad libitum* throughout. Body weights and adipose tissue mass were greater in the obese than the lean phenotype (p=<0.05). RMR and the dose related thermic responses to norepinephrine (NE, < 400 ug., s.c.) were greater in lean than in obese. In other studies, acute cold exposure at 4°C resulted in decreases in rectal but not core temperature in obese rats, and the thermic responses to 45 minutes of 4°C cold exposure on VO2 were typical but were significantly greater in lean than obese animals at all time points measured. REF RDIT paper.

Circulating T4 concentrations were similar in lean and obese rats and serum T3 but not T4 concentrations increased dramatically in both phenotypes following the cold exposure, consistent with phenotype- and maximal IBAT-linked changes in T4-5'-deiodinase activity / mg protein or per depot in kidney, liver and IBAT and in temperature linked increases in IBAT deiodinase activity / mg tissue protein in this strain. When D-1 and D-2 deiodinase activity was computed / tissue mass, further analysis indicated that D-1 was the predominating deiodinase in liver, kidney, and gastrocnemius muscle, while in IBAT D-2 > D-1 activity / IBAT depot and was greater in obese than lean rats. Cold exposure was associated with modest increases in net kidney deiodinase activity only in kidney. Thus, the cold-induced increases in circulating T3 in lean and obese rats are likely attributed at least in part to modest increases in IBAT T4-5' D-2 and renal D1 deiodinase activity, in addition to maximal rates of conversion in other tissues in possible combination with decreases in hormone clearance rates during cold induced stress. The contributions to chronic insulin resistance in the obese phenotype adds yet an additional factor to the observed differences in deiodinase activity, and the failure to elicit optimal thermogenic responses among obese animals, where the phenomenon of insulin resistance has an early onset and is known to continue well into adulthood in this and other genetically obese rodent strains..[14, 26] While early hyperphagia is a likely contributor to the insulin resistance, additional factors suggest an inadequate response among thyroid hormone receptor actions at the intracellular or molecular level. Regardless of the mechanisms involved, the results presented above indicate that key parameters of thyroidal action are deranged in the obese phenotype of this strain, and are likely contributors to the ease and efficiency of adipose tissue expansion and contributory to the epigenetic expression, energetic efficiency and early onset obesity in the obese phenotype in this strain. The net T4 activation to T3 via outer ring T4-5'-deiodinase activity and prolonged T4 disappearance from plasma are consistent with an intracellular syndrome of subclinical hypothyroidism, which could also contribute to an improved efficiency of substrate metabolism, including key parameters associated with protein synthesis and protein turnover. In other studies, measures of net protein turnover, at a cost of 4 high energy phosphate bonds per new peptide bond formed, represents a significant contributor to the economy of daily energy requirements, and a significant component of the energy costs of maintaining resting metabolic rates.[9,27] Indeed, the energy costs of protein synthesis rank among the most expensive aspects of resting energy requirements and are decreased by the presence of insulin resistance. Insulin resistance of obesity facilitates a

decrease in the rate of protein degradation during protein tissue remodeling, which serves as a primary source of amino acid moieties for *de novo* protein biosynthesis.[27]

In addition, the decreases in hepatic T3 nuclear receptor binding studies also support the above findings.[30,] REF Nuclear T3 receptor binding was found to be substantially decreased in the obese phenotype throughout both the physiologic and supraphysiologic concentration ranges examined. This observation is indicative of a syndrome of thyroid hormone resistance, also common in the presence of insulin resistance.[12,28] Whether this phenomena occurs as a reflection of the plasma membrane actions of collocated outer ring deiodinases remains unclear. The D-II deiodinase is also strategically located internally on the endoplasmic reticulum (ER) membrane, in close intracellular proximity to epigenetic receptor domains to enhance the geometric transit to the nuclear receptor binding events. Overall, T4 deiodinase activities in several tissues are decreased in the obese phenotype, and despite the increase in IBAT mass, the decreases in thyroidal actions are likely contributors to the decreased capacity for resting and NE-stimulated thermogenesis and greater adiposity in the obese phenotype of this strain. Disordered elements of thyroid hormone actions are often a consideration in diagnosing obesity, but most often, the plasma concentrations of the respective hormonal entities are a reflection of glandular feedback mechanisms, and thus fail to discern insight into the disordered elements of cellular energy metabolism and thus are sometimes referred to as a syndrome of subclinical hypothyroidism.[12,]

The congenic LA/Ntul//-*cp* rat model is an excellent animal model to investigate the above parameters, as the only difference between the lean and the obese phenotypes as biological littermates is the epigenetic expression of early onset hypertrophic-hyperplastic obesity in the obese phenotype of the strain, thereby minimizing potentially extraneous research variables.[19] The model demonstrates hyperinsulinemia, hyperamylinemia, and other metabolic sequala of obesity soon after weaning., [19,29] The obese littermates demonstrate hyperphagia soon after weaning, followed by hyperinsulinemia, hyperamylinemia, moderate glucose intolerance, and insulin resistance but not overt hyperglycemia.[27,29] In addition, mechanisms of protein turnover, and both thyroidal and sympathetic parameters of non-shivering thermogenesis become impaired during the preadolescent growth and developmental stages by 6 weeks of age.[9,12,27.] While a central factor supporting all of the above metabolic changes could not be determined from the present studies, the overriding presence of insulin resistance in concert with impaired mechanisms of thermogenesis are likely residing close to the origin of the findings in this and other studies of thyroidal actions in obese rodents, similar to that which appears to occur in syndromes of human obesity.[30-32,]

CONCLUSIONS

The results of this study indicate that key parameters of thyroidal actions including T3 generation in peripheral tissues in addition to possible T3 receptor domain binding in peripheral tissues are impaired in the obese phenotype of this unique rodent strain. While the direct causation of thyroidal dysfunctions could not be unequivocally stated, the comorbidity of insulin resistance common to the obese phenotype of this strain is a likely contributor. Insulin resistance, occurring with or in the absence of overt NIDDM results in a greater economy of energy expenditure, via diminished thermogenic responses to diet and environment, and in a conservation of energy metabolism often in the form of excess fat

accretion. The rates of protein turnover have been reported to be conserved from an early age, reflecting a decrease in ATP generation require for peptide bond formation and proteolysis during normal protein remodeling actions.[14, 27] The LA/Ntul//-*cp* rat is a unique rodent model demonstrating the phenomena of obesity-linked insulin resistance in that this model typically doesn't develop the confounding effects of NIDDM, as occurs in some other genetic forms of obesity among rats. In addition, the congenic status of the model minimizes internal variables, since the only differences between lean and obese littermates is the development of early onset obesity and its metabolic comorbidities soon after weaning. Breeding pairs that are heterozygous for the autosomal recessive -*cp* trait historically produce 25% of offspring that are homozygous for obesity, 25% that are homozygous for lean phenotype, and the remaining 50% that are heterozygous for the -*cp* trait but remain with a lean habitus virtually indistinguishable from their homozygous lean littermates throughout their lifespan. Parameters of thyroid function contribute a major role in the determinants of the efficiency of metabolic energy balance in man and animals, and when impaired, impact secondary effects on other endocrine systems including those that modulate glycemic and lipogenic responses, in addition to the sympathetic contributions to nonshivering thermogenesis in brown adipose tissue and other organs and tissues. In the present study, despite a significant increase in the mass of brown adipose tissue, the thermogenic responses to both resting and noradrenergic stimulated thermogenesis were impaired, in agreement with previous studies in this and other reports of decreased capacity for the unimpaired expression of parameters nonshivering thermogenesis in rodents and including those related to the conservation of energy expenditure during metabolic aspects of protein turnover. While the results of the study cannot be directly applied to human metabolism, the overall mechanisms of carbohydrate and protein metabolism, and of lipid biosynthesis and fat accretion are similar in both human and rodent species, thereby inviting speculation that similar hormonally-mediated effects may occur in mankind.

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Disclaimer (Artificial Intelligence)
Author(s) hereby declare 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 there are no competing interests.

Disclosures

The authors have no disclosures

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