

Biometry, Adiposity and Mechanism of Protein Sparing Growth in Congenic Preobese LA/Ntul//*-cp* rats

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ABSTRACT

The LA/Ntul//*-cp* rat is a congenic rat strain where the obese characteristic is expressed as an autosomal recessive epigenetic trait by 5 to 6 weeks of age in 25% of the offspring of heterozygous breeding pairs. Groups of lean and preobese 6-week-old female rats were subjected to measures of biometry and adipose tissue mass and cellularity of primary adipose tissue depots, followed by measures of *in vitro* protein synthesis and degradation in auricular appendage (AA) and skeletal digitorum longus muscle (DLM). Adipose depot mass, cell lipid content and tissue cell number were already greater in the dorsal subcutaneous, abdominal retroperitoneal and gonadal depots at 6 weeks of age. The interscapular brown fat depot (IBAT) mass and IBAT mass: body weight was also greater in the obese than the lean littermates. The tissue weights of the heart of obese > lean, but DLM of obese was similar to the lean rats. In contrast, the rates of protein synthesis and degradation as determined by the rate of ¹⁴C-phe incorporation into AA and tyrosine degradation from DLM were greater in the lean than the obese, indicating an economy of the energy costs of protein turnover in the obese phenotype. At 4 high energy phosphate bonds per peptide bond formed, protein synthesis represents one of the most biochemically expensive processes of energy metabolism, especially during the active stages of early growth and development of an animal. Thus, the conservation in energy expenditure resulting from an improved efficiency of protein turnover in the obese phenotype is likely a contributing factor in the energy efficiency previously reported in the obese phenotype of this strain of rat and is likely linked to hormonal resistance factors that impact on the efficiency of energy metabolism and a propensity for energy deposition and early onset fat accretion.

Keywords: Protein turnover, Obesity, Congenic phenotype, Rats

INTRODUCTION

The incidence of obesity is rapidly approaching epidemic proportions globally, often with an onset earlier in childhood than in previous years.^{1,2} Much of this increase in prevalence has occurred since the redesign of the food pyramid some four decades ago in an attempt to wean individuals away from diets high in saturated fats with the ambitious goal of reducing dietary risks for cardiovascular disease.³⁻⁵ Thus, as the proportion of fats in the diet were reduced the proportions of carbohydrates were inadvertently increased, in part to provide a caloric

substitute for the deleted fats while improving the taste and palatability. The modifications included the placement of grains, fruits, and vegetables often containing refined carbohydrate sources at a higher prominence in the chart and implying greater proportions on the pyramid. In addition, the popularization of palatable sugary snacks and sweetened premanufactured cereals likely made further inroads into the modernization of the marketplace, all with negative impacts contributing to the burgeoning increase in the prevalence of obesity and its common pathophysiologic comorbidities, often with an earlier age of onset than in previous generations. Technological advancements in the modernization of the home and workplace and ease of public transportation have also decreased physical demands leaving more time for leisure activities and indoor activities replacing the more energetic and physically demanding regimens of our ancestors. To the extent that genetic predisposition for variations in body composition including differences in body fatness or leanness may have undergone change over the generations is unclear, but in early observations Sims and others noted the physiologic potential for the existence of the 'thrifty gene' or 'Luxuskonsumption' phenomena of Neuman and others during the Vermont Study of Experimental Obesity conducted during the 1960s, where some normally lean subjects appeared to be able to consume an abundance of excess caloric intakes while gaining only minimal excess weight and hence remaining lean.⁶⁻⁸

The LA/Ntvl//*-cp* rat is a congenic animal model, where the obesity trait is expressed epigenetically as an autosomal recessive trait (the *-cp* trait) that was originally obtained from the Koletsky rat and backcrossed 12 or more cycles into a longevity-prone strain of the Lister/Albany/NIH rat (the LA/N strain) at the NIH by Hansen to attain a congenic status.⁹ Thus, the only known surviving trait from the Koletsky donor strain was the retention of the recessive *-cp* trait as previously reported.^{9,10} The lean and obese members of this strain have been reported to survive among the longest of known obese rat strains under conventional laboratory conditions.¹⁰ The obese phenotype are noted to demonstrate significant hyperinsulinemia, hyperamylinemia, and abnormal lipid profiles while remaining euglycemic throughout much or all of their lifespan.^{11,120} The genetic basis for their obesity in this and other strains of obese rodents has been linked to a genetic locus on chromosome 5 in rodents.¹³ The physiologic basis for their obesity is reported to be linked to an impaired capacity for non-shivering thermogenesis associated with both thyroidal and sympathetic compromise, including decreases in T4-5' deiodinase activity in peripheral tissues.¹⁴ In addition, decreases circulating T3 and adrenergic responses following alterations in diet and environment, in association with likely diminished thyroid hormonal nuclear receptor activity.^{14,15} These hormonal changes typically result in decreases in resting metabolic rates and impaired cold tolerance throughout much or all of their lifespan.^{10,15} Thus, it is useful to determine if parameters of protein turnover might represent part of the observation of an improved efficiency of energy metabolism as a contributing factor in the progression of the adiposity, in that factors of protein synthesis and degradation are heavily influenced by thyroidal and insulin actions. In addition, at an energy cost of 4 high energy phosphate bonds per peptide bond formed, this biochemical mechanism represents one of the energetically most expensive biochemical processes in mammalian physiology, especially during the active post weaning growth phase of an animal. In the present study measures of protein synthesis and degradation were determined in an isolated section of cardiac smooth muscle (the auricular appendage) and digitorum longus skeletal muscle strips, both tissues of less than 0.5 mm diameter thinness to enable adequate tissue perfusion during *in vitro* incubation processes.

Measures of protein requirements for normal growth to occur have been known to differ in the obese phenotype of both Zucker fatty rats and LA/Ntvl//*-cp* (corpulent) rats, both strains are though to have similar genetic traits for obesity.^{16,17} When Sprague Dawley rats were offered a protein-restricted, otherwise nutritionally adequate diet in a model of protein calorie malnutrition from weaning, the growth of the animals was restricted by over 50% within a few weeks of the dietary regimen.^{18,19} However, when the obese phenotype of the Zucker fatty rat and the LA/Ntvl//*-cp* (Corpulent) rat were fed the same 8% protein restricted diet, the obese animals gained weight normally attaining body weights and linear dimensions similar to normally fed animals typical of both phenotypes.¹⁶⁻¹⁸ Thus, those observations suggested that factors of protein metabolism in the obese phenotype were likely more metabolically and energetically efficient than occurred in their lean littermates under similar conditions, and permitting typically normal parameters of linear growth and dimensions of lean body mass.

METHODS

Briefly, groups of lean and obese littermates (n=6 rats/group) were selected from the breeding colony at 5 weeks of age and maintained as littermate pairs in 1 inch of pine shavings in plexiglass shoebox cages maintained at 22 ° C and 50% RH with Purina chow # 5512 and house water *ad libitum* on a conventional light cycle until the time of the study. Measures of adipocyte size and number were determined in representative aliquots of weighed adipose tissue sections by the osmium tetroxide fixation method of Hirsch and Gallian as performed in our laboratory, and the osmium fixed particles counted on a Model B Coulter particle counter.^{17,19,20.}

Measures of protein turnover were performed in freshly excised tissues from 6-week-old female lean and obese LA/Ntvl//*-cp* rats via the method of Li and Goldberg.²¹ Thin muscle strips (0.5 mm thickness) of freshly excised auricular appendage or digitorum longus muscles (muscle strips) were weighed to the nearest 0.1 mg on a dual range balance and incubated in a Krebs physiologic buffer at 37°C containing 14C-phenylalanine for 90 minutes in a shaking incubator. At the completion of the incubation, the tissues were removed from the buffer, homogenized and solubilized in 0.2M perchloric acid, and the 14C content of the pellet determined. The protein content of the pellet was determined by the method of Lowry.²² The tyrosine content of the incubation buffer was also determined fluorometric method of Waalkes and Udenfriend²³ to determine the amount of protein degradation that occurred during the 90 minutes of incubation. Data were analyzed by Students unpaired 'T' test.²⁴ These studies were approved by the Institutional Animal Care and Use Committee.

RESULTS

The results of biometry of lean and obese rats are shown in Table 1 and indicate that by 6 weeks of age the obese phenotype weighed modestly more than their lean littermates. The heart weight was also significantly larger while the digitorum longus muscle, torso length, and tibia length were of similar dimensions in both phenotypes at 6 weeks of age.

Table 1. Biometry of lean and preobese LA/Ntvl//*-cp* rats

Phenotype	N	Body weight, g	Heart, mg	DLM, mg	Torso Length, cm	Tibia Length, mm
Lean	6	159 ± 4	569 ± 5	67 ± 7	13.6 ± 0.2	39 ± 2
Obese	6	227 ± 6	788 ± 29	74 ± 6	14.1 ± 2.0	36 ± 2
P =		<0.05	< 0.01	n.s.	n.s	n.s.

Data are mean ± 1 SEM, n= 5-6 rats / group, littermates. Students unpaired 'T' test.

The adipose mass and cellularity are shown in Table 2 and indicate that early increases in the adipose cellularity are evident at 6 weeks of age. The fat pad mass in all three white adipose tissue depots dissected were modestly but significantly greater in the obese than the lean phenotype in all three depots dissected. The increase in adipose mass is the result of a combination of increased cell lipid content in abdominal depots and increases in cell number in the dorsal subcutaneous depot. The increases are modest however compared to the increases in adiposity that occur in the obese phenotype at later ages.^{10,15}

The Interscapular brown normally adipose tissue has a primary function of heat production in response to alterations in diet and environment in rodents and other mammalian species. The brown adipose tissue depot in the present study is indicated in the lower section of Table 2, and shows that the IBAT mass in the obese phenotype is significantly greater in the obese than the lean phenotype both in grams of brown adipose tissue per pad and as a proportion of body weight, similar to that which has been reported in other strains of obese rats that demonstrate insulin resistance and an impaired capacity for nonshivering thermogenesis in response to diet, environmental and pharmacological stimuli.²⁵⁻²⁷

The measures of protein turnover in digitorum longus skeletal muscle strips are depicted in Figure 1 below and indicate that the tissue mass is of similar mass in the obese and the lean animals. Protein synthesis however, as measured by ¹⁴C-phenylalanine incorporation into protein per hour during a 90-minute incubation was significantly greater in the lean than the obese animals, while measures of protein degradation as determined by simultaneous tyrosine release from the muscle was also greater in the lean than the obese phenotype. Thus, the rate of protein turnover is greater in the lean than the obese phenotype, despite the similarity in muscle tissue mass in the lean and obese phenotypes.

Table 2. Adipose tissue characteristics of lean and obese LA/Ntvl//*-cp* rats.

Group	N	Lean Rats	Obese Rats	p
Dorsal fat Pad				
Fat Pad Weight, mg	6	548 ± 119	3349 ± 741	<0.01
Lipid/cell, µg/cell	6	0.164 ± 0.052	0.250 ± 0.049	n.s.
Cells/depot x 10 ⁶ /pad	6	2.82 ± 0.64	4.93 ± 0.09	<0.05
Retroperitoneal Fat Pad				
Fat Pad Weight, mg	6	219 ± 28	1931 ± 399	< 0.01
Lipid/cell, µg/cell	6	0.126 ± 0.063	0.364 ± 0.054	<0.01
Cells/depot x 10 ⁶ /pad	6	1.77 ± 0.13	2.19 ± 0.26	n.s.
Gonadal Fat Pad				
Fat Pad Weight, mg	6	357 ± 59	2235 ± 237	<0.01
Lipid/cell, µg/cell	6	0.176 ± 0.044	0.313 ± 0.033	<0.05
Cells/depot x 10 ⁶ /pad	6	3.16 ± 0.53	2.93 ± 0.40	n.s.
Interscapular Fat Pad				
Fat Pad Weight, mg.	6	293 ± 33	692 ± 4	<0.05
IBAT: Body Weight x 10 ⁶	6	1.84 ± 0.19	3.09 ± 0.03	<0.05

Data are mean ± 1 SEM, n= 6 rats / group, littermates. Students unpaired 'T' test.

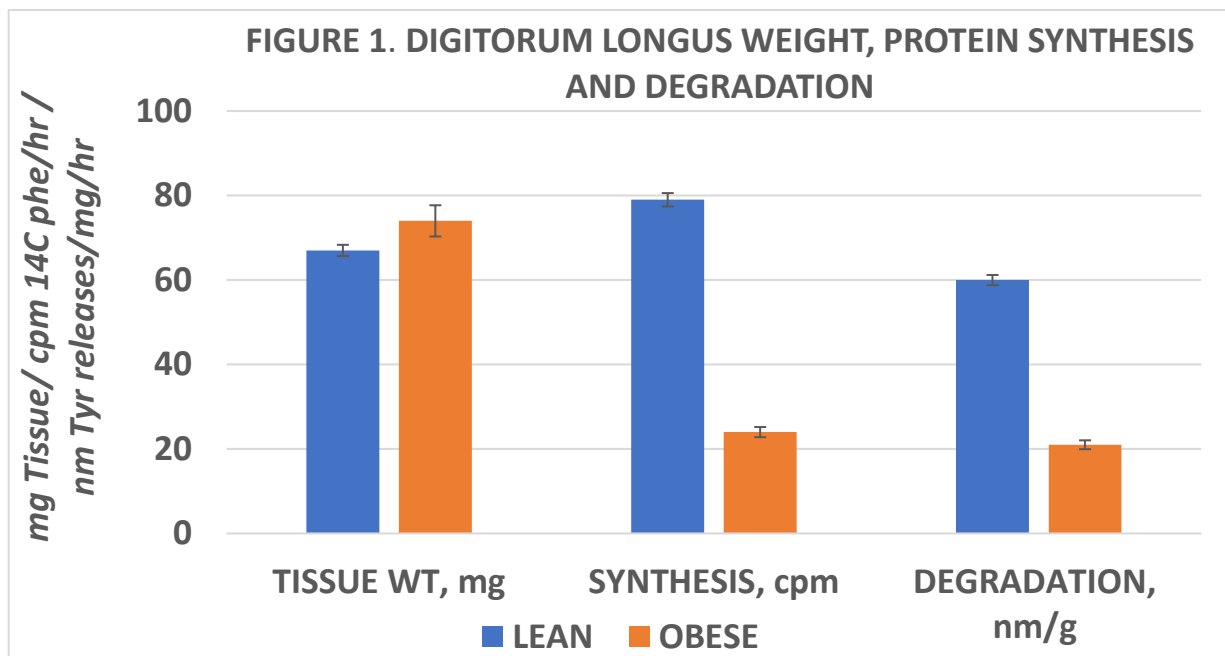


Figure 1. Digitorum longus muscle weight, protein synthesis and degradation. Data are mean $\pm$ 1 SEM, n=6 rats/group. Tissue weights are n.s.; Rates of synthesis and degradation are significant at $p < 0.01$ (Student's "T" test).²⁴

The measures of protein turnover in cardiac smooth auricular appendage muscle are depicted in Figure 2 and indicate that the tissue mass is significantly greater in the obese than the lean animals. Protein synthesis however, as measured by ^{14}C -phenylalanine incorporation into protein per hour during a 90-minute incubation was greater in the lean than the obese animals, while measures of protein degradation as determined by simultaneous tyrosine release from the muscle was also greater in the lean than the obese phenotype. Thus, the rate of protein turnover is greater in the lean than the obese phenotype, despite the greater accumulation of muscle tissue mass in the obese phenotype. It is of interest that the rate of synthesis in the cardiac tissue is greater than that which was observed in the skeletal muscle under the same conditions, while the rates of muscle degradation were similar in both tissues.

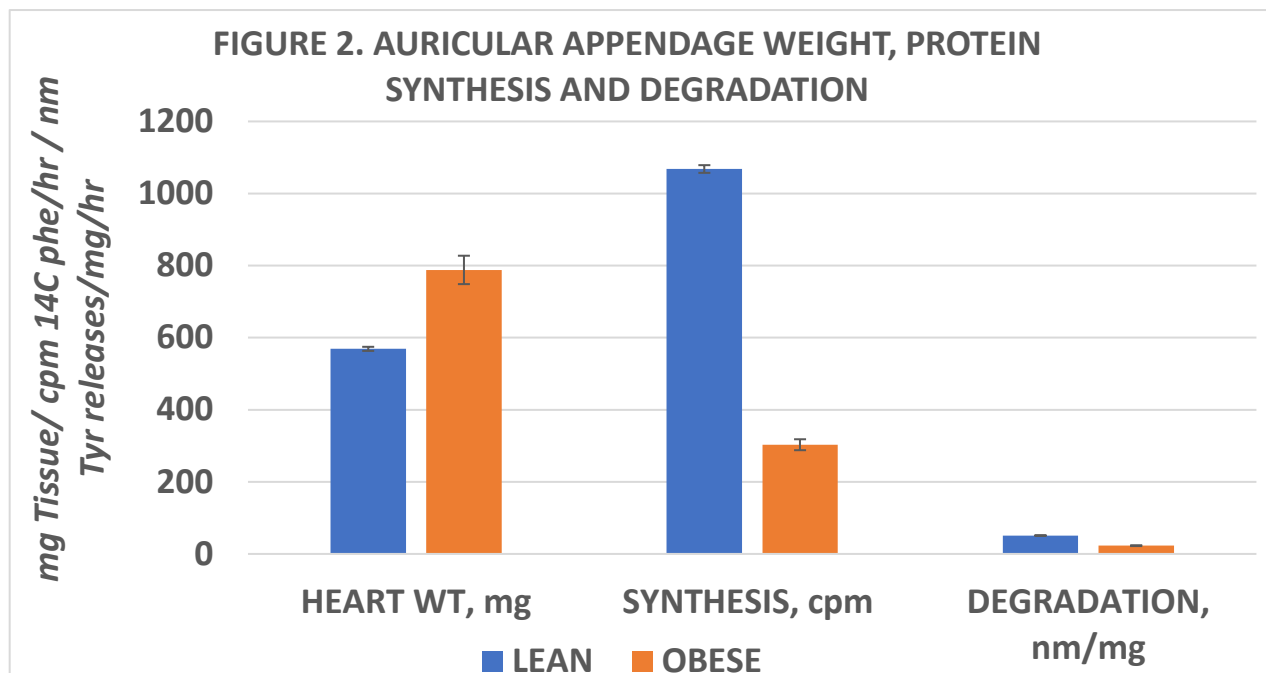


Figure 2. Heart weight, and auricular appendage synthesis and degradation. Data are mean $\pm$ 1 SEM, n=6 rats / group. $P < 0.01$ for heart weight and synthesis, and $p < 0.05$ for degradation (students 'T' test).²⁴

Plasma insulin concentrations are depicted in Figure 3 and indicate that plasma insulin concentrations were significantly elevated approximately 4-fold in the obese phenotype by 6 weeks of age. The effects of plasma insulin, the insulin to glucose ratio and the molar T4 to T3 ratios are depicted in Figure 3, extracted from a study of like animals from a similar study, where the animals were studied periodically throughout aging. In that study hyperphagia and insulin resistance was found in the obese phenotype in the absence of hyperglycemia at all ages up to and beyond two and a half years of age. In addition, evidence of resistance to the metabolic effects of thyroid hormones in that the T4:T3 ratio and the VO2 were observed and continued to deteriorate with aging in the obese phenotype. The decreased T4:T3 ratios are consisted with a decreased hormone receptor affinity at the nuclear receptor level or organization previously reported in the obese phenotype of this strain.¹⁴ The complex molecular interconnections between the insulin resistance and impaired T4:T3 ratios remain unclear. Thus, thus represents the earliest age to date at which insulin resistance has been demonstrated in the obese of this strain.

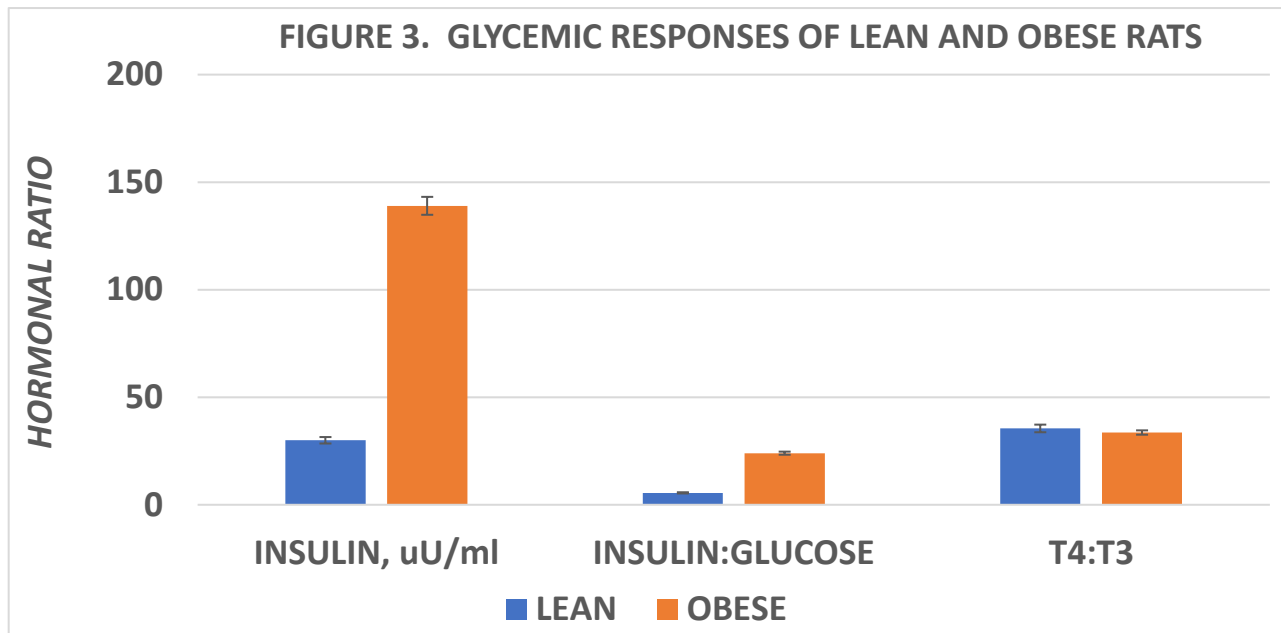


Figure 3. Glycemic and hormonal responses of young female LA/Ntvl//cp rats, n- 6 rats/group. Data are mean $\pm$ 1 SEM. Extracted from ²⁵. Insulin reported as μ U/ml, glucose as molar units, and thyroid hormones as nM units. $P = < 0.01$ for Insulin and < 0.05 for insulin:glucose ratio (Lean vs obese, Students t test); $p = \text{n.s.}$ for T4:T3 ratio.

DISCUSSION

The results of this study indicate that parameters of protein metabolism, including the energy expensive processes of protein synthesis at four high energy phosphate bonds per peptide bond formed are significantly decreased in the obese phenotype of the LA/Ntvl//cp rat compared to that which occurs in its lean littermates when 6 weeks of age. Measures of protein degradation were also significantly decreased in the tissues from the obese phenotype, indicative of an overall conservation of amino acid substrates during protein synthesis, and which may have contributed to the limitations in the activity of protein synthesis. The amino acids typically generated via *in vivo* proteolysis during tissue growth and remodeling occur throughout the lifespan would be expected to provide much of the amino acid substrate for protein resynthesis, with the carbon skeletons of the unused amino acid balance becoming available for entry into and subsequent oxidation in the Krebs cycle in oxidative processes of muscle tissues. Dietary protein intake is assumed to have been equal in both phenotypes, considering that the animals were housed as littermate pairs (one lean plus one obese with 24/7 equal access to Purina 5012 rodent chow and house water, *ad libitum*), thus eliminating availability of dietary protein intake as a likely primary influential variable. This feeding regimen is a highly respected and nutritionally fortified dietary formulation that is well known to support the optimal growth and development of rodents of the age studied.

The economy of energy metabolism was associated with an early onset of adiposity as a result of adipocyte hyperplasia and hypertrophy in primary abdominal and subcutaneous adipose tissue depots. The increase via hyperplasia was more evident in the dorsal subcutaneous than the abdominal sites as the increase in adipocyte number was significant in that site by 6 weeks of age. In contrast, the increases in the depot mass in the retroperitoneal and gonadal site were more prominently associated with significant increases in adipocyte lipid content than

increases in adipocyte number, suggesting not only that lipid deposition may occur differentially in different depots, but that different depots may convey different metabolic or even pathophysiologic implications as well.

In related studies, parameters of daily caloric intake, plasma glucose concentrations, insulin concentrations, , insulin to glucose ratios, and T4 to T3 ratios were indicative of both significant insulin resistance and thyroid hormone resistance.^{10,25} In previous studies, the magnitude of the insulin and thyroidal resistance factors became more pronounced as the obese state progressed with advancing age.^{10,12,14,15,25} In the cited studies, both of the hormonal measures likely contributed to a greater feed efficiency in the obese phenotype and a consequent propensity for a greater metabolic capacity for fat accretion. Despite the development of insulin resistance, the obese phenotype of this strain has remained euglycemic albeit with an impaired glucose tolerance indicative of impaired rate of glucose disposal in peripheral tissues as would be anticipated in states of insulin resistance. While the significance of insulin resistance on glucose disposal may not be equal in all peripheral tissues, the impact on muscle and adipose tissue depots likely reflects the majority of the impaired peripheral glucose uptake observed, with possible secondary effects of insulin resistance occurring in brain, where secondary effects including brain atrophy may be observed over time in this strain of rats.²⁶⁻³¹

The economy of protein metabolism may be a common observation among obese rodent strains, as in several studies, when obese rats were fed a protein restricted diets containing only one third (8% w/w) of the dietary protein offered to their controls, parameters of linear growth and lean tissue growth occurred at the same rates as when fed diets containing 24% (w/w) protein, while growth and development in the lean littermates fed the protein restricted diet was markedly impaired by 50% or more.¹⁶⁻¹⁸ In those obese strains, all are reported to develop insulin resistance, while the commonality of impaired thyroid hormone parameters while similar were not as clearly delineated. A common denominator of all the obese rodent strains studied to date however is an impaired capacity for non- shivering thermogenesis and decreased cold tolerance, thermogenic parameters that require essential participation from both sympathetic and thyroidal contributions.^{26,28}

SUMMARY AND CONCLUSIONS

Regardless of the biomolecular mechanisms involved, the results clearly indicate that the processes of protein turnover were remarkably more efficient in the obese than the lean phenotype of this strain, contributing to an economy in energy metabolism but without notable effects on limiting lean tissue growth. This energetic efficiency occurred in support of the protein sparing effects of feeding protein restricted diets on survival and body composition in the obese phenotype previously reported in this and other obese rodent strains. In this instance, the protein sparing effects were likely due to a combination of the insulin resistance which tends to favor lysosomal stability and decreased proteolysis, resulting in a longer half life for tissue proteins, in combination with decreases in thyroidal activation mechanisms at the nuclear level of hormonal mRNA receptor activation on thyroidal nucleosomes. Moreover, the direct effects of insulin resistance exert secondary decreases in the formation of GLUT4 transporters at the level of the endoplasmic reticulum, in addition to decreases in their intracellular translocation to the plasma membrane where they can facilitate the cellular uptake of glucose moieties for cellular glycolysis, mitochondrial oxidation, electron transport support of glucose supported ATP generation.^{32,33} The extent to which these intracellular

activities may contribute to the decreases in protein turnover and its ATP-dependent peptide bond formation remains unknown and speculative at best. Increases in aerobic exercise of up to 2 hours per day, predicted to increase mitochondrial activity and glucose oxidation in skeletal muscle were found to be only partially effective in ameliorating the progression of obesity in this strain.³⁴ Thus the impact of chronic insulin resistance that likely occurs with an onset at or soon after weaning (21 days in the rat) would appear to represent a primary element of metabolic dysregulation that contributes to multiple long term pathophysiologic sequela in this and other strains of obese rats, and in the expression of a 'thrifty gene' of luxuskonsumption in this strain of rats similar to that which Neumann referenced over 100 years ago in humans and was rediscovered by Sims et al observed in the majority of normally lean subjects when overfed during the Vermont Study of Obesity.^{6,35,36} Finally, the proposed energy conservation mechanisms of protein sparing events in the obese phenotype likely represents but one of multiple constituents in the metabolic processes resulting in the propensity for the epigenetic expression of obesity vs. evidence in support of a 'thrifty gene' of luxuskonsumption in this strain of rats.³⁷

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