Short communication

EFFECT OF CARBOHYDRATE TYPE ON BRAIN COMPOSITION AND SENESCENCE IN AGING, HYPERINSULINEMIA-PRONE OBESE LA/Ntul//-*cp* RATS

Abstract

The consumption of simple vs complex carbohydrate (CHO) sources exerts significant differences on glycemic parameters in man and animals, with the simple CHO resulting in a greater magnitude of hyperinsulinemia (HI) and glucose intolerance (IGT) in obesity than in non-obese subjects. Inflammatory cytokines originating and residing in adipose tissue of obesity have been reported to contribute to DNA damage in neuronal and other tissues, impede cell replication, and accelerate cell senescence.To determine if phenotype and the carbohydrate type resulted in alterations in brain composition in the obese phenotype of the congenic LA/Ntul//-*cp* rat, groups (n= 8 rats/group) of male littermate lean and obese rats were fed standardized isocaloric diets containing 50% (w/w) heat treated cornstarch (ST diet) or 54% (w/w)sucrose (SUC diet) from 1 until 10.5 ± 0.5 months of age. The obese phenotype of his strain develops early onset chronic hyperinsulinemia without NIDDM associated with hypertrophic-hyperplastic obesity during early postweaning growth. Brain tissues were dissected, and representative aliquots subjected to total fat, protein and DNA analysis. Body weights of obese >> lean, and were greater when fed the SUC than the ST diet in both phenotypes. Brain mass of lean > obese, and SUC was associated with modestly lower brain weights in rats fed the SUC than the ST diet. Brain total Protein and DNA content of lean rats were > obese rats and were modestly Lower in SUC than ST fed rats in both phenotypes but the percent of lipid content was proportional to brain mass. Total body fat mass of obese was significantly greater than occurred in lean littermates and was only modestly greater in SUC than ST fed rats in both phenotypes. These results indicate that brain growth and cellular development is impaired in the aging, hyperinsulinemia-prone obese phenotype of this strain, were further impaired when fed SUC than ST diets, and were likely associated with development of a chronic neuronal inflammatory syndrome common to excessive fat accretion and obesity, resulting in premature brain senescence.

Key words: Obesity, Brain development, Hyperinsulinemia, Senescence, DNA, Starch, Sucrose, Rat

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Introduction.

The prevalence of obesity is now a serious concern among Westernized societies, where it is rapidly approaching epidemic proportions. Moreover, the prevalence of obesity and overweight conditions now represents one of the most challenging issues facing the delivery of health care, in large part due to its close association with comorbidities of hypertension and non-insulin dependent diabetes.[1] In addition, chronic obesity and nutritional factors have been linked to premature dementia including Alzheimer's disease in addition to impaired neurodevelopment and DNA damage in multiple tissues when it occurs during earlier, formative life stages of growth and development and in aging.[2-5] The syndrome of insulin resistance, glucose intolerance, and chronic inflammation typically accompanies

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obesity in man and animals, often resulting in increased comorbidities and decreased life span even in the absence of the pathophysiologic impacts of hypertension or diabetes when the condition is left untreated.[4,5] The mechanisms contributing to chronic insulin resistance have been attributed to multiple factors, including overnutrition, chronic hyperphagia and other dietary macronutrient imbalances, disordered glucocorticoid metabolism and actions, impaired cellular translocation of insulin-dependent GLUT4 transporters, and immune dysfunctions.[6-8] Macrophage infiltration of adipose tissue, often resulting in disordered immune functions is one of the earmarks of obesity-mediated immune dysfunction.[9,10] Among the immunologic dysfunctions, adipose tissue tends to attract macrophages, which lead to secretion of inflammatory cytokines including IL-6 and others, that may now circulate systemically and impinge on numerous central and peripheral tissues, where they may initiate pathophysiologic sequela including vascular lesions and likely microglial contributions to premature apoptosis of neuronal cells.[9-12] In humans, the neuronal changes in obesity plus Alzheimer's syndrome include shrinkage of brain volume and cognitive deficits that accompany the increased adiposity and brain shrinkage.[13] Thus, the purpose of the current study was to determine key parameters of brain composition in older, congenic lean and hyper insulinemic obese male rats, and to correlate the changes in brain composition with the magnitude of adiposity.

Optimal nutrition has been shown to influence pre- and postnatal growth and to be positively associated with multiple developmental parameters that may follow later in life in man and animals.[14] In contrast, early malnutrition and undernutrition are often followed by reduced stature and other developmental parameters later in life, proportional to the magnitude of the nutritional depravation. Brain development can also be impacted by early nutritional factors, and in the most dire examples with reduced brain development and decreased brain DNA content.[14] In epigenetic rodent obesity, however, pre- and early postnatal nutrition are presumed to be comparable to similarly reared lean littermates in that they typically share the same lactation, equal access to solid food and nutritional experiences by weaning and thereafter when fed and housed as littermates under standard laboratory conditions.[15,16] Thus, deficits in a congenic animal model are unlikely to be associated with inadequacies in nutritional deficits, but may be associated with the hyperphagia and resulting metabolic sequela that accompany the epigenetic expression and subsequent development of hyperinsulinemia, typically followed by progressive adiposity and obesity.[14]

The epigenetic expression of obesity in the LA/Ntul//-cp (corpulent) rat strain occurs as the result of an autosomal recessive trait, and results in 25 % of the offspring of heterozygous breeding pairs demonstrating early stigmata of the progressive development of obesity by 5 to 6 weeks of age.[15-19] The obese phenotype demonstrate hyperphagia, hyperinsulinemia, hyperamylinemia, hyperlipidemia including hypertriglyceridemia, and impaired glycemic responses to a glucose tolerance within a few weeks of postweaning life.[15,16,20,21] In this strain, however, the obese littermates develop impaired glycemic responses typical of peripheral insulin resistance, but to date have remained non-diabetic throughout their lifespan.[15,20] The animals have remained specific pathogen free (SPF) in an isolated colony throughout many generations in our laboratory. Thus, the primary aspect of metabolism that remains is the chronic, lifelong hyperinsulinemia and hyperamylinemia and their pathophysiologic sequela.[15,16,21]

Methods.

Animals were housed in large plexiglass cages lined with one inch of pine shavings, maintained at 22°C and 50% RH under standard housing conditions in littermate pairs. The only known difference between the lean and obese phenotypes was the epigenetic expression of the-*cp* trait for obesity, originally obtained from the Koletsky rat[18] and backcrossed into the longevity-prone NIH LA/N background strain for 12 cycles by Hansen to establish the congenic designation at the NIH.[17,19] Rats were maintained on Purina chow (#5012) and free access to house water from weaning. At 6 weeks of age rats were placed on a nutritionally adequate diet containing 54% carbohydrate as a lower glycemic index diet containing cooked cornstarch, 20 % protein, and 16% mixed fat plus essential vitamins, minerals and fiber or a higher glycemic index diet containing 54% sucrose in place of the cornstarch, as described by Michaelis et al.[20] At 10.5 months of age animals were sacrificed by cervical dislocation with a small animal guillotine after a brief 4 hour fast and cervical blood was obtained for later analysis. The brain tissue was dissected and weighed to the nearest mg. and 50-75 mg tissue aliquots of representative samples taken for proximate analysis. The residual carcasses including the remaining brain tissues were frozen, homogenized in a Waring blender, lyophilized and subjected to gravimetric analysis for determination of protein and lipid content.[22,23] Measures of protein content of brain and residual carcass were obtained with the methods of Dole and Meinertzfor lipid content and the classic methodology described by Lowry for protein content.[22,23]The DNA analysis in brain tissue was determined by the method of Burton.[24] Data were analyzed by standard statistical procedures.[25,26] The study was approved by the Institutional Animal Care and Use Committee.

Results

The results of final body weights obtained on the morning of the day of dissection are depicted in Figure 1, and indicate that by 10.5 months of age, the obese phenotype weighed more than twice the weights of their lean littermates, despite having been reared identically with respect to both diet and environment, and with *ad libitum* access to the nutritionally sound diet. In addition, the body weight of rats of both phenotypes fed the SUC diet weighed more than their ST fed companions. The brain wet weights and the brain weight to body weight ratios are depicted in Figure 2 and indicate that the brain weights of the obese rats weighed significantly less than their lean littermates at 10.5 months of age ($p<0.05$). In addition, the ratio of brain weight to body weight was also decreased in the obese phenotype. The effects of the higher glycemic index sucrose diet tended to result is yet smaller absolute brain weights when fed the SUC vs. the ST diet and resulted in a further moderate trend toward decreases in the brain weight to body weight ratios that were mostly secondary to the greater fatness in the sucrose fed obese phenotype. There appears to be a mild diet effect on final brain weights, with ST fed animals of both phenotypes tending to weigh slightly more than was recorded for their ST fed littermates. In addition, the ratio of brain weight to final body weight of obese animals is depicted in Figure 3 and indicates that brain weights were also significantly less than were observed in their similarly reared lean littermates ($p=<0.01$).

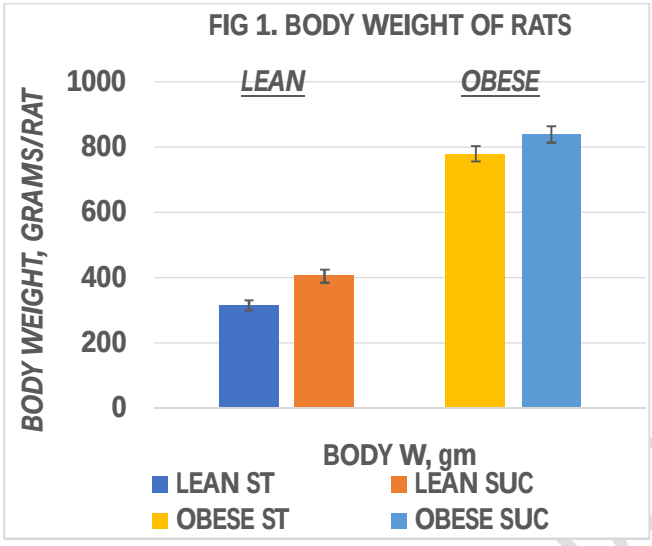

Figure 1. Effect of diet and phenotype on body weights of rats. Data are mean ± 1 SEM, n=8 rats/treatment group. P = < 0.01 for phenotype and for diet in lean but not obese (p=<0.10) .

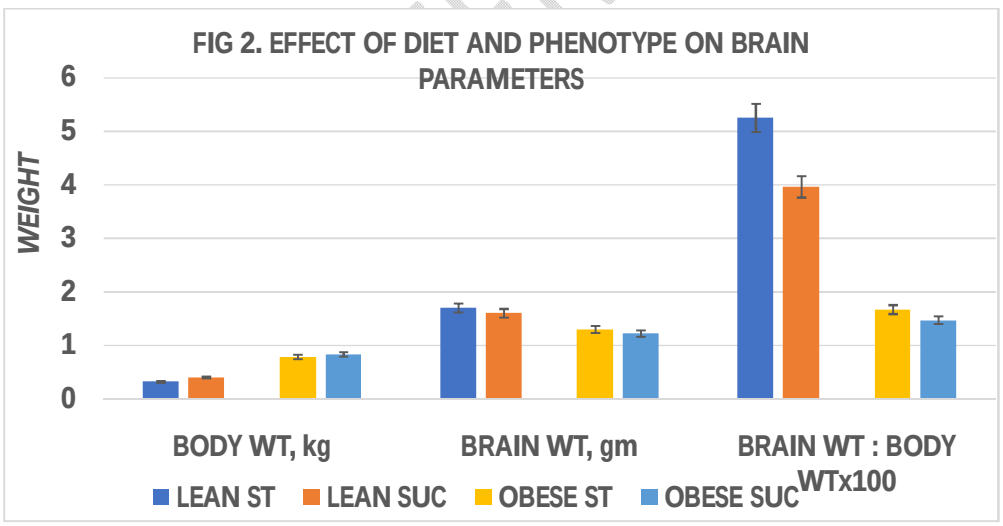

Figure 2. Effect of diet and phenotype on brain weights and brain weight: body weights of rats. Data are mean ± 1 SEM, n=8 rats/treatment group. P = < 0.05 for phenotype. Diet resulted in a significant trend toward lower brain weight in sucrose then in starch fed rats by Pages 'L' test for trend analysis. [26]

Brain total protein and DNA content are depicted in Figure 3 and indicate that measures of netprotein and DNA content per brain were both decreased in the obese phenotype. (p=<0.05 for both total protein and DNA). The effects of diet are depicted to the right of each set of bars, and while suggestive of a dfurther decrease in protein and DNA content, only the brain DNA content of the obese sucrose-fed obese animals were suggestive of a significant trend by trend analysis. Thus, the decreases in brain content are generally proportional to the decreased brain weight observed in this study.

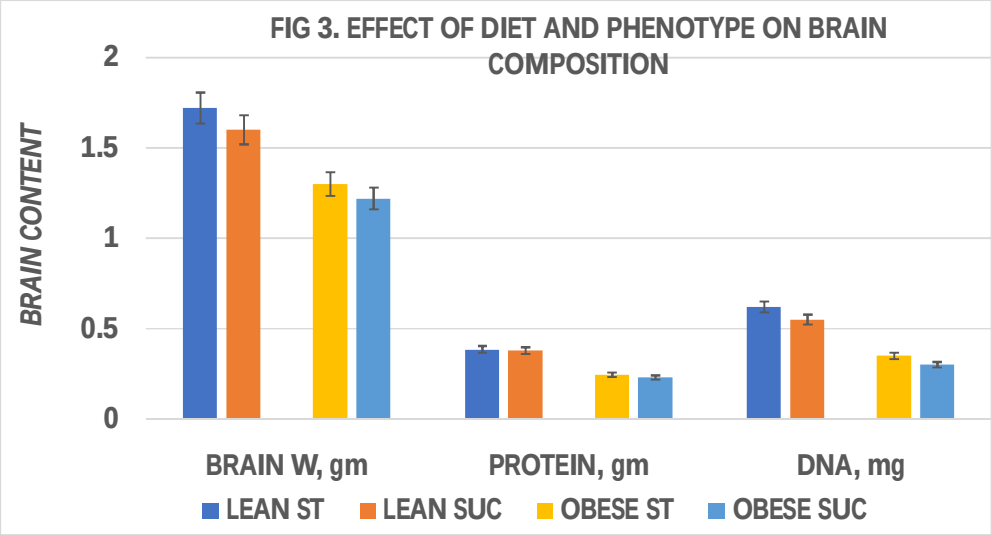

Figure 3. Effect of diet and phenotype on brain protein and DNA content of rats. Data are mean ± 1 SEM, n=8 rats/treatment group. P = < 0.05 for phenotype. Diet resulted in a significant trend toward lower brain weight in sucrose then in starch fed obese rats by Pages 'L' test for trend analysis.

Brain lipid content is depicted in Figures 4A and 4B, and indicate that although the amount of lipid per brain was less in the obese phenotype, the percentage of lipid content in the brains were similar in both phenotypes, suggestive of a proportional decrease in overall brain composition rather than a decrease in any specific chemical component. Thus, although the brain lipid mass was lower in the obese than the lean phenotype, the results are consistent with and correspond to the smaller brain mass observed in those animals. When the net brain mass is compared to final body weight, the proportion of brain tissue lipid to total body weigh was significantly less in the obese than the lean phenotype, likely reflecting the substantially greater adiposity and body mass of the obese phenotype.

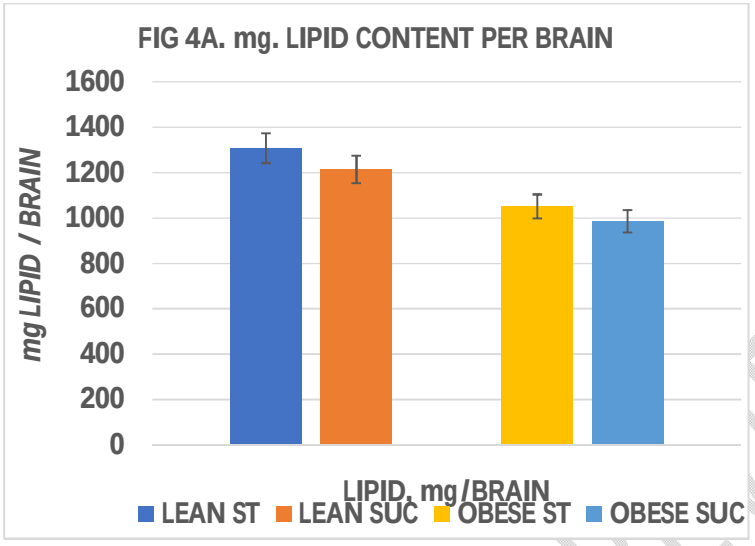

Figure 4A. Effect of diet and phenotype on brain lipid content of rats. Data are mean ± 1 SEM, n=8 rats/treatment group. P = < 0.05 for phenotype. A Diet effects on total lipid content trend (sucrose < starch) were not significant when computed as mg of lipid weight by Pages 'L' test for trend analysis.[26]

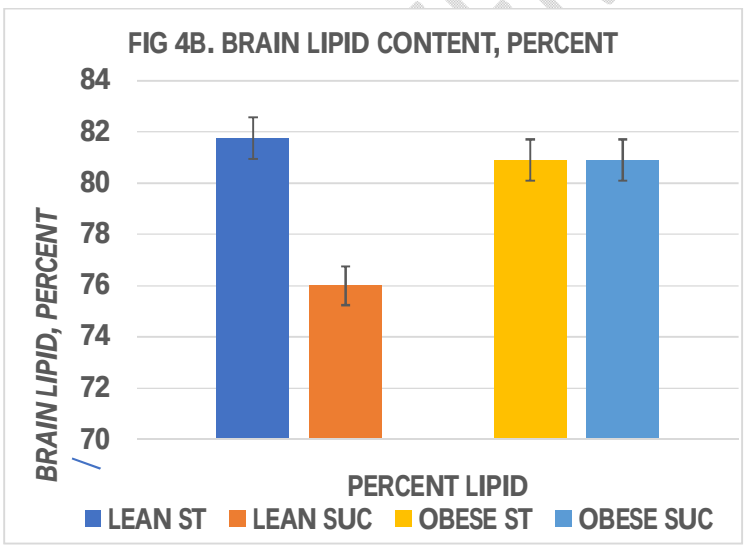

Figure 4B. Effect of diet and phenotype on percent brain lipid content of rats. Data are mean ± 1 SEM, n=8 rats/treatment group. P = n.s. for phenotype and diet. A Diet effect on percent lipid content trend (sucrose < starch) was significant in lean but not obese rats when computed as percent of of lipid weight by Pages 'L' test for trend analysis.

Discussion.

The results of this study indicate that at 10.5 months of age, total brain weight and absolute lipid, protein and DNA content were decreased in the obese phenotype, despite having been reared since birth with biological littermates via the same nutritional and environmental conditions with the exception of a low glycemic index starch vs a higher glycemic index sucrose diet. The sucrose diet resulted in greater fat accretion and greater final body weights in the sucrose fed than the starch-fed animals of both phenotypes. Although the study did not monitor daily caloric intake, numerous previous reports have demonstrated hyperphagia and hyperinsulinemia to occur throughout much if not all of the lifespan in the obese phenotype of this strain, and where the typical duration of the lifespan of the obese phenotype is about one third shorter than its similarly reared and housed lean littermates.[15,20,27-29]Moreover, Michaelis et al reported that the hyperinsulinemia of sucrose-fed rats remained greater than occurred in starch fed rats of this strain.[20]Previously, brain size and cellularity have been shown to be decreased under conditions of severe malnutrition, and in humans that develop Alzheimer's disease, but only limited reports tend to identify obesity as an isolated contributor to premature brain shrinkage or brain dysfunction. The decreases in brain DNA content in the present study indicate decreased neuronal cellularity, and presumably decreased cognitive functions as well and may accompany the decrease in cell number. Although the animals were not subjected to cognitive evaluation, and the decreased brain size and cellularity could not be directly correlated with cognitive decline in the present study. They do however permit an assumption of decreased overall cognitive potential, and one where the presence of inflammatory cytokines common to obesity may be considered as a contributing factor in the neurologic decline. Of interest, the substitution of sucrose for starch in the diets resulted in a trend effect toward decreased DNA and protein content, and it seems likely that with a larger number of animals per treatment group or a longer duration of the dietary regimen that the diet induced results may have been more pronounced.

In addition, since brain measurements were only undertaken at age 10.5 months, the chronologic timeline of the progression of neurologic decline or more profound effects of a high glycemic diet could not be firmly establishedin this study. Unlike dietary induced forms of obesity, where return to the normal diet may often be associated with weight loss, the obese phenotype in this epigenetic strain has to date proven to be remarkably refractory to significant weight loss, or a return to the bodily habitus of their lean littermates following dietary intervention.[27] To date, only excess daily T3 but not T4 administration has demonstrated significant increases in plasma T3, VO2 and in weight loss in the obese phenotype, with adrenalectomy resulting in partial but incomplete recovery.[27-29]

The physiologic or metabolic basis for the differences in brain weight and composition are unclear but are highly suggestive of chronic hyperinsulinemia as contributing factors. Michaelis et al has previously reported impaired glucose tolerance and hyperinsulinemia in this strain from postweaning to 10 months of age.[20] Thus, elevations in plasma insulin have been reported to occur through much if not the entire lifespan of the obese phenotype of this strain, and which contribute to the impaired thermogenesis in response to factors of diet and environment, including insulin inhibition of UCP1 actions in brown adipose tissue (BAT).[28-31] The BAT is a primary tissue in expressing nutritionally and environmental increases in thermogenesis, and when impaired due to metabolic aspects of insulin resistance in BAT

and other peripheral tissues, results in increased rates of lipogenesis, metabolic efficiency and excess body fat accretion in both visceral and subcutaneous adipose tissue depots.[29-33]

In lean animals, white adipose tissue (WAT) is enriched with Type 2 immune cells, which interact with each other to generate Type 2 cytokines including IL-4, IL-5, and IL-13 to maintain a healthy type 2 immunologic protective physiologic environment. In contrast, with the development of obesity and in the presence of chronic hyperinsulinemia,[9,10] and may promote development of a Type 1 inflammatory response, including the generation of inflammatory cytokines IL-6 and others which may produce damaging effects on neuronal DNA and neuronal survival. The type 1 inflammatory response includes metabolically activated macrophages, T-cells, B cells and others which release inflammatory cytokines, resulting in chronic systemic inflammation. The activated macrophages and other immune cells can collectively induce a Type 1 inflammatory environment. The inflammatory cytokines can now migrate to virtually all somatic and neuronal tissues, where they may contribute to cellular senescence. Dietary factors including excess fatty acids common to hypertriglyceridemia and obesity in concert with the low gradehypoxia that occurs in WAT have been shown to contribute to a chronic low-grade inflammation that can induce the pathophysiologic sequela including premature apoptosis among neuronal tissues, similar to that which appears to occur in Alzheimer's disease.[9,13]

The role of glucocorticoids in the development of obesity also merits some discussion. Dysregulation of glucocorticoid actions may follow several lines of evidence. Glucocorticoids can induce both anti-inflammatory and inflammatory immune responses particularly from macrophages, which can generate both Type 1 (secretes IL-6, inflammatory) and promote type 2(anti-inflammatory) responses.[2,3,31-35]The physiologic response of glucocorticoids tend to be counterregulatory to those of insulin, creating a somewhat vicious cycle that may only become further aggravated in the presence of a chronic hyperinsulinemia state and which help to distinguish the impaired modulation of immune responses.[2,7,28] Among other processes, dysregulation of glucocorticoid actions impede the formation and cellular translocation of GLUT4 glucose transporters from the endoplasmic reticulum of somatic cells, resulting in impaired cellular glucose uptake, thereby spiking further increases in insulin release mechanisms to insulin dependent tissues including skeletal uscle and adipose tissue, while promoting lipogenesis in liver and other receptive tissues.[7,8] Indeed a hallmark characteristic of genetically-obese rats if the progressive development of a fatty liver with advancing age, and which were visually apparent in all of the obese rats of the present study that were dissected.[15-16,17] Although glucocorticoids can induce cell death and reduce cell survival in immune cells such as T and B cells, macrophages in most tissues tend to be relatively resistant to glucocorticoid-induced apoptosis. Overall, while glucocorticoids act to suppress systemic inflammation and are frequently prescribed to treat chronic inflammatory condition involving lymphocytes, they are less effective in macrophage-mediated diseases, such as chronic obstructive pulmonary disease and the chronic inflammation and hypoxia of obesity where an elevated release of inflammatory cytokines may occur differentially in different adipose tissue depots.[32-36] Regardless of the cellular processes implicated, the brain size, absolute composition, and cellularity based on DNA content was reduced in the aging adult obese non-diabetic phenotype of the LA/Ntul//-cp rat.

Summary and Conclusions.

The results of the present study indicate that brain mass and cellular content at 10.5 months of age is decreased in the hyperinsulinemia-prone obese phenotype of this strain, and are likely associated with a chronic inflammatory syndrome and cytokine expression that are common to hyperinsulinemia and obesity. Substitution of the higher glycemic index sucrose diet resulted in a trend toward further decreases in brain DNA and protein content, but the trends were not highly significant by conventional statistical analysis. The results could not determine the chronology of development the decrease or confirm an etiologic or developmental origin in brain size or composition as the data reflect only a single time point taken during late adulthood, in animals that typically often only survive for 12 to 15 months due to pathophysiologic complications of their obesity. In contrast the lean littermates have been observed to survive for 2 years or longer under similar environmental conditions, with obese females exhibiting a lesser magnitude of hyperinsulinemia and glucose intolerance and surviving longer than males.

The decreased brain size was characterized by proportionate decreases in total lipid, brain protein and brain DNA content, in association with marked body fat accretion, obesity and a decreased brain to body weight ratio in the epigenetic obese (*-cp/-cp*) phenotype of this congenic rat strain. The decreases in brain size and cellularity are also consistent with the brain shrinkage that occurs in Alzheimer's Disease and other states of dementia where chronic exposure to inflammatory cytokines may prevail and that occur in aging humans.The biological mechanisms or chronology through which the changes in brain composition occurred in the present study could not be determined from the single data point obtained. Inflammatory cytokines of the macrophage-generated Type 1 category include IL-6 and others and have been reported to generate free radical damage to DNA and contribute to neuronal senescence, and thus remain an interesting causal speculation from the data obtained. Regardless of the biological mechanism involved, the brain mass and apparent cellularity was significantly decreased in the obese phenotype of this rodent strain, and the more highly refined high glycemic index sucrose diet tended to exaggerate the damaging impact on brain composition in the obese phenotypic of this strain.

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