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SERVICES FOR SCIENCE AND EDUCATION

Impact of Brown Adipose Tissue Reduction and Overfeeding on Adiposity and Depotspecific Adipose Tissue Cellularity: Brown Fat Reduction and Adipose Tissue Cellularity

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ABSTRACT

Brown adipose tissue contributes to adaptive changes in metabolic energy expenditure in response to alterations in diet and environmental conditions, thereby assisting an animal to maintain thermoregulation and energy balance in various mammalian species including man and animals. Cafeteria overfeeding of normally lean rats during early postweaning growth typically results in significant hyperplasia and in an increased capacity non-shivering thermogenesis and energy expenditure in brown adipose tissue (BAT). The Interscapular BAT depot is readily surgically accessible and normally represents approximately one third of the total BAT mass in lean Sprague Dawley (SD) rats. The effects of experimental overnutrition via offering a Cafeteria feeding regimen (Café) combined with surgical reduction of the IBAT mass of adipose tissue cellularity and regional fat deposition was determined in lean SD rats during 8 weeks of postweaning growth and development to adulthood. Groups (n= 8 rats/group) of male, SD rats were fed a Purina Chow diet or the Chow diet plus the Café regimen for 52 days from weaning. An additional group of the Chow+Café regimen were subjected to surgical removal of their IBAT at 4 weeks of age and continued on the Chow+Café thereafter (Café-IBAT). At 80 days of age, measures of adiposity including anthropometrics and the mass and adipocyte cellularity in principle abdominal and subcutaneous fat depots were determined. Body weight (BW) and mid-abdominal girth were ~20% greater in Café and Café-IBAT, while linear growth was similar in all groups. The mass of all fat depots was greater in Café fed animals (p=<0.05) and increased further in subcutaneous depots and total WAT accumulation in the CaféIBAT animals (p=<0.05). Adipocyte lipid content and cell diameter of Café > controls in all depots with further increases in abdominal depots with Café-IBAT. Adipocyte number per WAT depots of Café > Control in all depots, with further increases in the Inguinal SC depot. Thus, these results are consistent with regional differences in the effects of Café feeding on postweaning adipose tissue hyperplasia, hypertrophy and depot mass and which underwent additional depot-specific differentiation in Café-IBAT. In conclusion, Café resulted in adipocyte hypertrophy in all depots studied, but the partial reduction of BAT mass in Cafe-IBAT rats resulted in only modest additional impact on overall adiposity during overfeeding, with the greatest impact in the ING SC depot, and thereby consistent with potential thermogenic compensation in other BAT depots to partially minimize the overall impact of the Café overfeeding regimen on developing adiposity in this strain.

Keywords: Obesity, Brown Adipose Tissue, Overnutrition, Adipose Cellularity, Adiposity, Rat.

INTRODUCTION

The magnitude of diet-induced hormonal effects on adipose tissue cellularity and fat accretion exert differential impacts in subcutaneous vs. abdominal depots.[1] The metabolic effects of brown adipose tissue on the expression of nonshivering thermogenesis (NST) responses to alterations in diet and environment in rodents are well established, implying a possible role of BAT on energy balance in mammalian species by dissipating excess caloric intake as heat while minimizing energy retention in adipose tissues.[2,3] Several authors have reported that surgical reduction of brown adipose tissue resulted in decreases in the capacity for NST in addition to a greater propensity to develop greater mass and fatness in white adipose tissue depots, analogous to that which occurs following pharmacologic ablation.[4-8] As noted above, the BAT has also been proposed as a potential buffer to maintain energy balance against excess weight gain following episodes of excess caloric intake or macronutrient imbalance.[9] In addition, deficits in BAT energy expenditure secondary to multiple physiological factors have been observed to occur in the obese phenotype of several rodent strains, where they are likely metabolic contributors to the excess weight gain and early onset adiposity in those animal species.[1013] The current increasing prevalence of obesity and its numerous pathophysiologic sequelae in Westernized societies represents a serious challenge to the effectiveness, availability and capacity of treatment resources to manage the disorders linked to the obesity-linked stigmata.[14,15] Adipose tissue is now known to contribute a broad range of hormonal activities, in addition to the roles of insulin, catecholaminergic, thyroidal, and glucocorticoid regulation.[16-20] The nutritional and metabolic factors that contribute to the current epidemic of obesity and overweight conditions in Western society typically include factors of diet, environment, and lifestyle, often combined with links to metabolic and genomic predispositions.[18-20] The therapeutic measures to treat the disorders linked to obesity and overweight conditions typically address one or more of the adaptive or heritable contributors but often may be only partially effective due to the complex nature of the conditions.

Brown adipose tissue was discovered to occur in humans and other mammalian species several centuries ago, but its physiologic role in energy metabolism has been discovered only more recently.[2,3,20-22] The morphology and functions of brown adipose tissue differ markedly from that of white adipose tissue.[15,22-25] Both tissue types occupy specific anatomic domains that are anatomically suited to their physiologic functional role in homeostasis and energy balance.[2,3] In white adipose tissue, the primary function is linked to energy storage in the form of triglycerides in both abdominal and subcutaneous locations. The triglycerides may be formed via hepatic or dietary origin, and may become deposited in mature adipocytes or preadipocytes as a single large lipid droplet, surrounded with a thin layer of cytoplasm, a flattened nucleus located in the cytoplasmic ring, accompanied with the host of cellular organelles common to somatic cells, and facilitate the processes associated with our basic cellular functions including cellular metabolism to maintain cellular viability.[23] In times of caloric excess, the lipid droplet may expand to contain up to 1 μg or more of lipid content in response to hormonal and substrate influences, while in states of caloric deprivation, the lipid droplet may be mobilized along its outer surfaces to release free fatty acids, thereby contributing to the energy demands of peripheral tissues.[3,23] Adipocytes from white adipocyte tissue may develop from

preadipocytes via hyperplasia and hypertrophy and once differentiated, remain viable thereafter. Adipocytes remain responsive to accommodating increases or decreases in energy intake and thus serve as an energy reserve from the stored triglycerides and fatty acids throughout adolescence and much of the adult lifespan. Of significant concern, the onset of overweight and obese conditions is now occurring earlier in the lifespan and remaining longer than in previous generations. Overweight and obesity now occur more often in children and adolescents where it can contribute to an earlier onset of the comorbidities commonly associated with the overweight and obese conditions.[14,15]

In contrast, the physiological functions and cellular morphology of brown adipose tissue vary considerably from those of white AT.[2,3,22-24] Brown adipocytes are typically smaller, spherical structures with a round, centrally placed nucleus and abundant specialized mitochondria. The main physiological function of brown adipose tissue is to contribute to heat generation and energy expenditure in response to alterations in diet and environment, accomplished via a large cytoplasmic density of specialized mitochondria.[23,25] BAT depots are strategically located in close proximity to abundant vascular tissues such that the heat generated my circulate to both central and peripheral tissues. Thus, brown adipose tissue can effectively dissipate the endogenous heat energy to peripheral tissues to facilitate the regulation of homeostatic body temperatures.[2,3,23-25] Brown adipocytes can mobilize the heat generation process rapidly, via specialized β-neuroadrenergic membrane-bound receptors and a dedicated sympathoneurologic presence.[3]

Because the lipid in brown adipocytes is contained in multiple small locules distributed throughout the cytoplasm, it provides a greater net metabolizable surface area to lipid content ratio. Thus, locular surface area is an important consideration that further contributes to the efficiency of their energy producing functions. The lipid locules are also strategically distributed throughout the cytoplasmic compartment in proximity to the specialized mitochondria, thereby creating a larger metabolizable surface area per unit of lipid than occurs in white adipocytes. The relatively greater metabolizable surface area thereby facilitates a more rapid mobilization of the contained lipid since lipid mobilization in both tissue types occur along the outer surface areas of the lipid droplets or locules. Additionally, because the brown adipocytes contain a centrally located spherical nucleus, surrounded by all essential organelles required to maintain cellular functions, the ease of morphologic distinction from other surrounding cells and tissues is readily discerned with or independently of histological staining or immunoreactive techniques. Brown adipose tissue develops via hyperplasia and limited hypertrophy prior to adulthood in the rat, while white adipose tissue in most depots may continue to increase by hyperplasia from preadipocytes and limited hypertrophy throughout much of the lifespan in rodents.[23,24,] Once formed, both brown and white adipocytes appear remain present thereafter and where they may continue to expand in response to caloric status. Once formed, differentiated adipocytes of either type can remain active as lipid storage depots virtually indefinitely, to accommodate the energy needs of the organism as needed during both energy privation and excess. Both tissues normally regulate their lipid stores and metabolic status via hormonal actions including those inspired by insulin and catecholamines. In contrast, pharmacologic ablation via inhibition of β-adrenergic functions results in increases in locule diameter and lipid content in isolated brown adipocytes, consistent with decreased thermogenic activity.[23]

The primary metabolic roles of Insulin, catecholamines, glucocorticoids, and macronutrient energy intake regulation in addition to numerous other secondary factors via both direct stimulatory and permissive effects regulation of energy balance in man and animals.[15-18] Insulin functions exhibit both glycemic and lipogenic actions in peripheral tissues via stimulating glucose uptake, stimulating lipogenesis and lipid uptake of preformed lipids, while impeding lipid mobilization and ketogenesis from white adipose tissue stores. Thus, insulin is a primary and highly influential hormone in the peripheral management of energy stores over time. Catecholamines can bring about the mobilization of glycogen and lipid stores during instances of duress, including negative energy balance, macronutrient imbalance, and/or food depravation. While the metabolic effects of insulin actions may persist for hours, the responses to catecholamine occur rapidly and are initiated via adrenoreceptor actions located on the plasma membrane. Accordingly, the positive effects of catecholamines on energy balance are more immediate and give rise to the initiation of the common 'fight or flight' response. Plasma glucose can become significantly elevated within minutes in response to catecholaminergic stimulation, while in the presence of insulin resistance, plasma glucose and processes of glucose disposal in peripheral tissues may persist for hours and take longer to recover after the adrenergic challenge.[26]

Brown adipocytes have specialized β3-adenoreceptors, in addition to individual sympathomimetic neural synapses to facilitate highly specialized and virtually instantaneous responses once activated .[3] In white adipose tissue, insulin typically impedes lipoprotein mobilization, thereby enzymatically limiting the mobilization of free fatty acids and diglycerides from stored triglycerides.[16] Thus, the overall integrated regulation of energy stores from carbohydrate and lipid sources is a complex and ongoing process, and which may continue throughout the healthful lifespan of the individual or animal species baring pharmacologic inactivation.

The presence of brown adipose tissue has been noted in humans for many generations. Among the earliest observations were those noted during cadaveric dissections some 1500 years ago.[21] More recently, histologic evidence in humans has been demonstrated to occur throughout much of the lifespan.[21,22,27] Thus, while the existence of BAT in mammalian species has been known for many years, the biochemical processes of energy generation in brown adipose tissue and its presence in adult humans have been established with advances technologic and diagnostic processes only more recently.[2,3,22,23,25] In rodents, the IBAT depot represents approximately one third of the total BAT mass, and represents the depot that is most conveniently accessible from a surgical perspective. Thus, the purpose of the present study was to determine if partial reduction of brown adipose tissue would result in measurable changes in adiposity and energy storage in a normally lean rat model of following induced hyperphagia by offering unlimited access to the cafeteria diet approach, typically consisting of multiple appetizing, energy rich foods common to the Western diet consumed in many developed countries.[5]

MATERIAL AND METHODS

Groups of weanling Sprague-Dawley Rats (n = 6-8 rats/group, 40-42 g BW each) were obtained from Charles River Laboratories, and acclimated to plexiglass shoebox cages in littermate pairs and fed Purina Chow and house water, as libitum. At 4 weeks of age, two groups were offered a

highly palatable Cafeteria diet (Café) in addition to the Purina chow regimen. At 4 weeks of age, one group of the Café diet regimen was subjected to surgical removal of the entire Interscapular brown adipose tissue depot (Café-IBAT) under pentobarbitalketamine anesthesia as described elsewhere [5,7-9] and continued on the Café diet regime thereafter. Recovery from the surgery was uneventful and complete within a few days. Body weights were monitored periodically throughout the study. After 52 days of the Café regimens animals were sacrificed via cervical dislocation and measures of biometry including torso length and girth diameter determined with an anthropometry tape. The epididymal, retroperitoneal, mesenteric, inguinal and dorsal adipose tissue depots dissected in their entirety, weighed to the nearest mg, and prepared for measures of adipose tissue cellularity via the osmium fixation methods of Hirsch and Gallian as performed in our laboratory.[24,27,28] Tissue lipid content was determined gravimetrically via the microchemical method of Dole and Meinertz as conducted in our laboratory.[26,30] Measures of adipocyte diameters were determined with a stage micrometer via light microscopy at 45X magnification.[23] Data were analyzed by ANOVA corrected for multiple comparisons where indicated and descriptive analysis via standard statistical procedures.[31,32] The study was approved by the Institutional Animal Care and Use Committee.

RESULTS

The measures of biometry after 52 days of the chow or café regimens are depicted in Figure 1 and 2 and show that the Café diet resulted in significantly greater final body weights in both the Café and the Café-IBAT groups. There were no significant differences in the initial or final body weight between the Café and Café-IBAT groups however, suggesting that the ablation of IBAT likely induced only a modest impact on overall parameters of adiposity. Measures of torso length were similar in all groups, indicative of an adequacy in macro- and micro- nutrient intake when fed the Café diet. Measures of mid-abdominal girth and girth to torso length ratio were greater in the Café fed group and were similar in both Café and Café-IBAT groups.

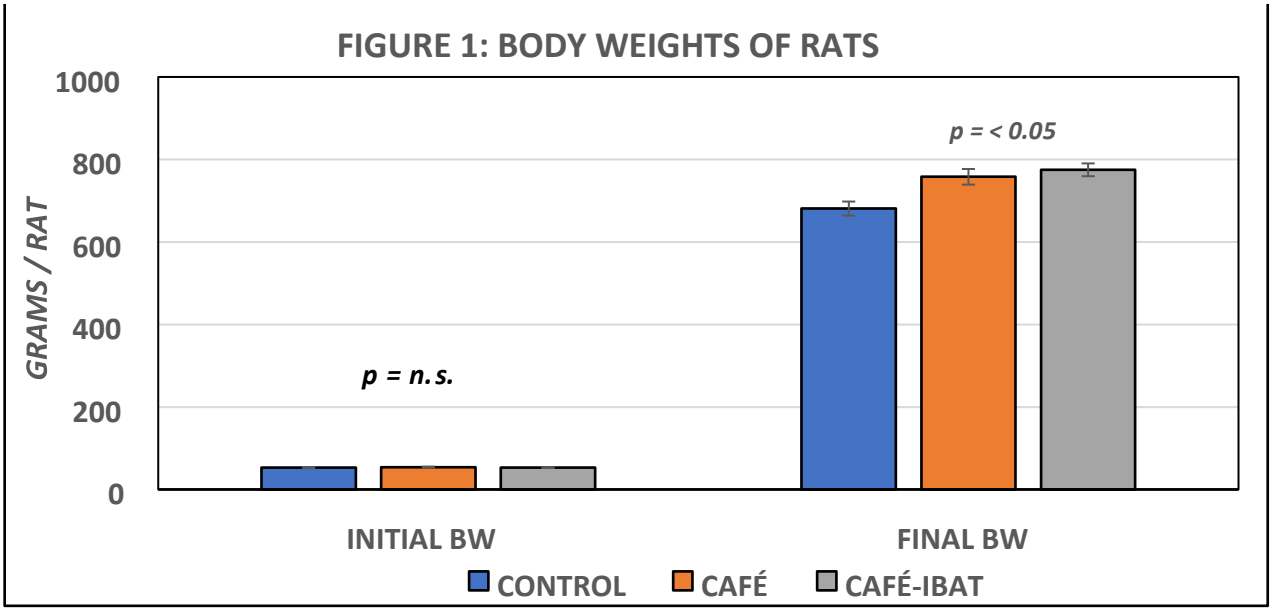

Figure 1: Effects of diet and IBAT removal on initial and final body weight of rats. Data are the mean ± 1 SEM, N= 8 rats/group.

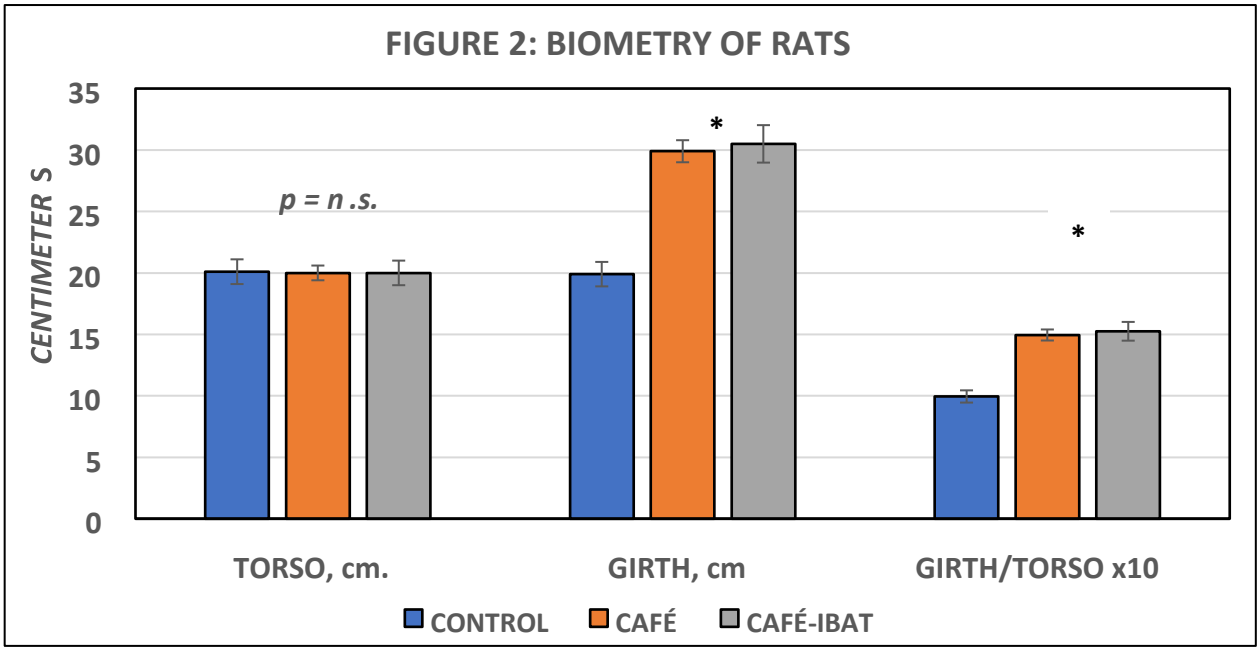

Figure 2: Effects of diet and IBAT removal on anthropomentric measures. Data are the mean ± 1 SEM, N= 8 rats/group.

Measures of the mass of principle far depots is depicted in Figure 3, and indicated that the mass of the abdominal (Epididymal (EPI), Retroperitoneal (RP), and Mesenteric (MES), and the subcutaneous (Dorsal (DOR), and Inguinal (ING)) fat pads were greater in Café fed than control rats, with further increases in depot mass in the DOR, ING, and total fat pad mass in the Café-IBAT group. The AT cell number of Café > CHOW in subcutaneous (DOR and ING) and abdominal RP, and unchanged in EPI. Adipocyte diameter and cellular lipid content of Café > CHOW in all depots. Cell number increased further in DOR, EPI and RP, and the overall differences in cell diameter corresponded to the measures of cell lipid content. In addition, surgical reduction of IBAT resulted in further increases in the mass, cell size and cell lipid content particularly in the ING depot. In the Café diet, IBAT cellularity determinations resulted in greater depot cell numbers in the dorsal, retroperitoneal and inguinal depots. The café-IBAT group demonstrated greater cell numbers in the Café-IBAT inguinal depot, while depot cell numbers in the Café-IBAT retroperitoneal group were also elevated at a level intermediate between the chow and Café groups.

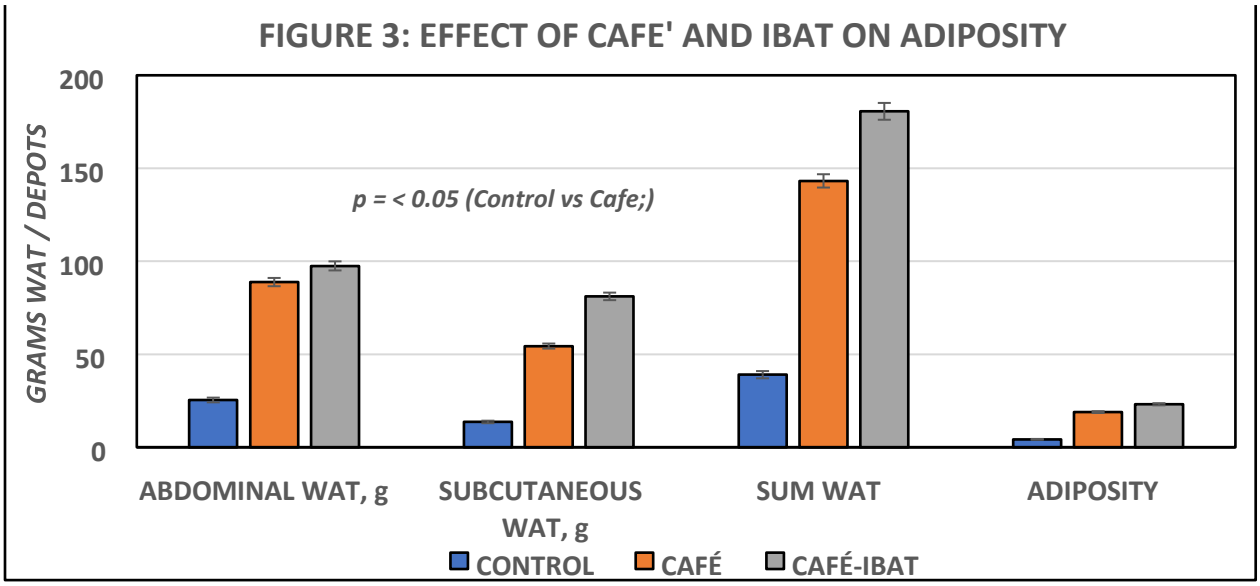

Figure 3: Effects of diet and IBAT removal on adipose tissue mass. Data are the mean ± 1 SEM, N= 8 rats/group. Abdominal represent the arithmetic sum of the epididymal, mesenteric and retroperitoneal depots, and subcutaneous represents the sum of the Dorsal and inguinal depots. The SUM represents the arithmetic sum of the abdominal and subcutaneous depots, and the Adiposity represents the sum of the fat pad mass divided by the final body weights of the rats.

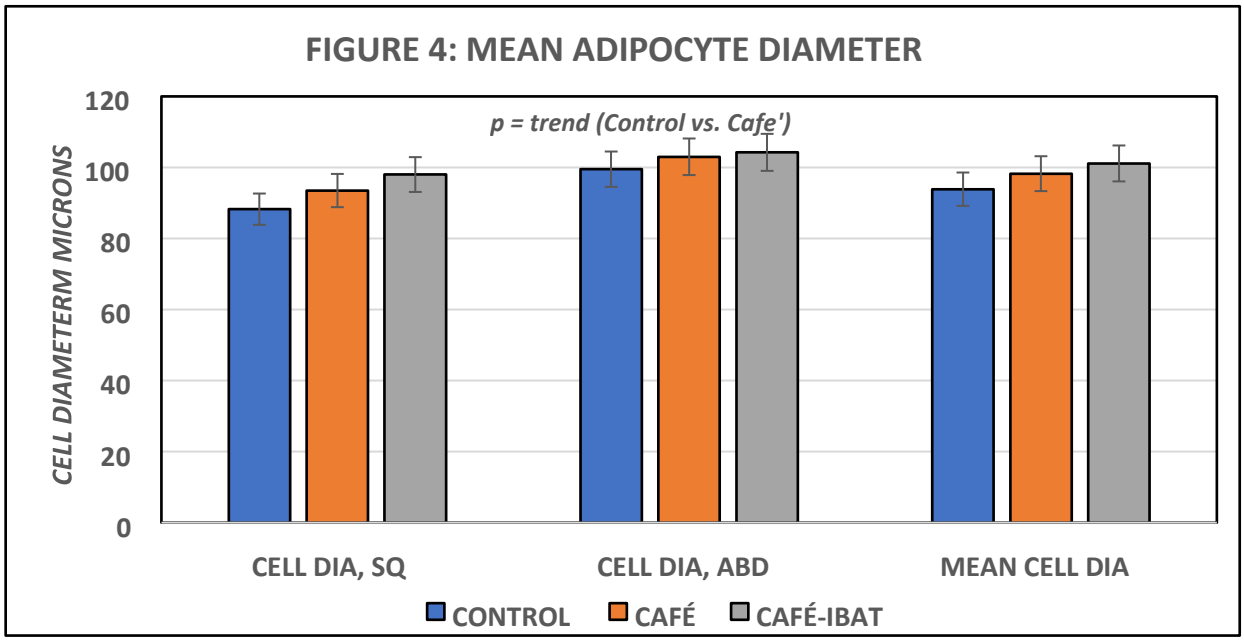

Figure 4: Effects of diet and IBAT removal on adipocyte diameter. Data are the mean ± 1 SEM, N= 8 rats/group. Abdominal cell diameters represent the arithmetic sum of the epididymal, mesenteric and retroperitoneal depots, and subcutaneous cell diameters represent the sum of the mean of the dorsal and inguinal adipocytes. The mean cell diameter represents the arithmetic mean of the abdominal and subcutaneous depots.

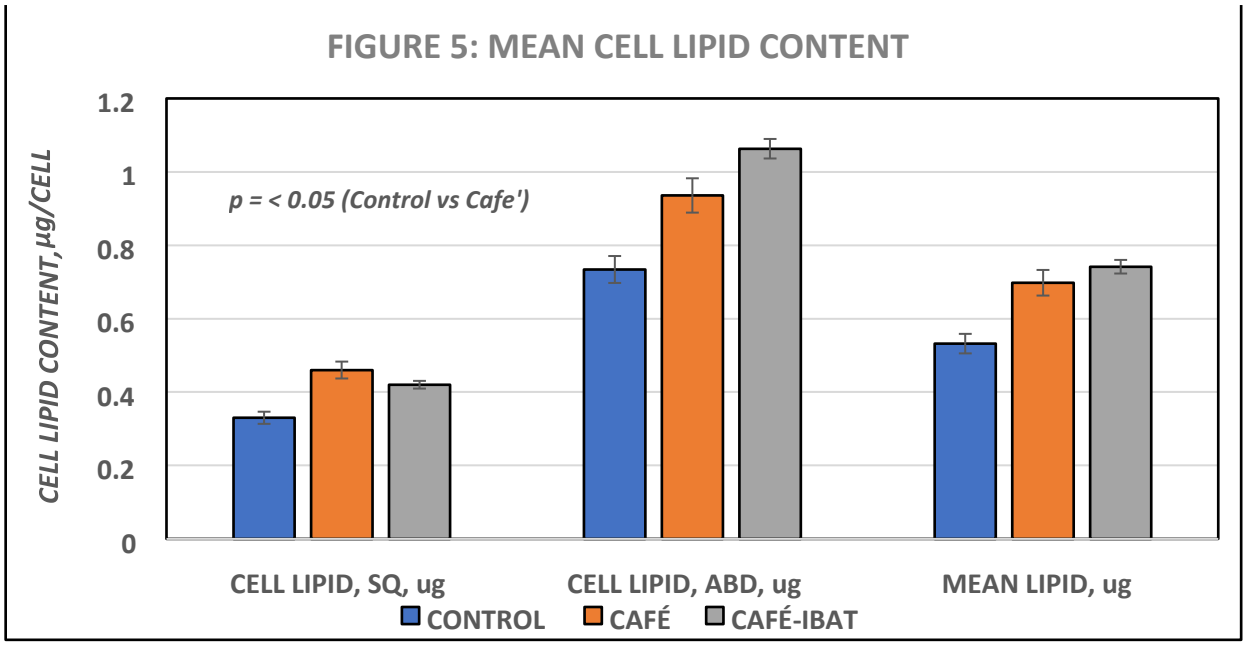

Figure 5: Effects of diet and IBAT removal on adipocyte lipid content. Data are the mean ± 1 SEM, N= 8 rats/group. Abdominal cell lipid content represents the mean of the epididymal, mesenteric and retroperitoneal depots, and subcutaneous cell lipid content represents the mean of the dorsal and inguinal adipocytes. The mean cell lipid content represents the arithmetic mean of the abdominal and subcutaneous depots.

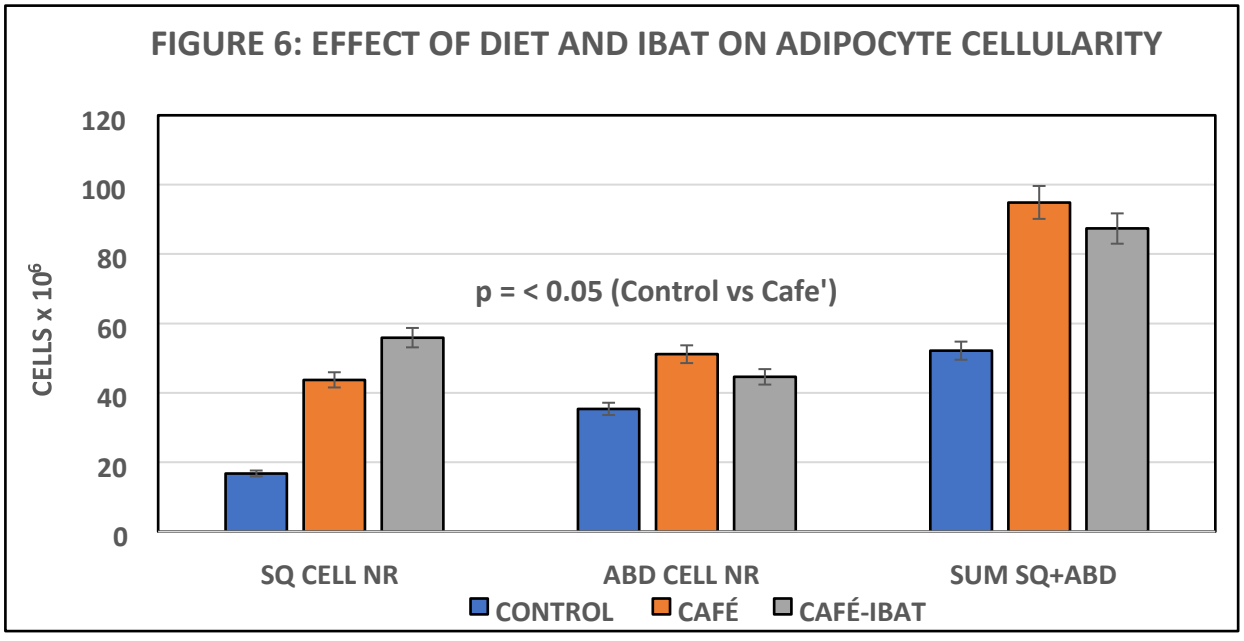

Figure 6: Effects of diet and IBAT removal on adipocyte number. Data are the mean ± 1 SEM, N= 8 rats/group. Abdominal cell lipid content represents the mean cell number/depot of the epididymal, mesenteric and retroperitoneal depots (right panel), and subcutaneous cell number (Left panel) represents the mean of the dorsal and inguinal adipocytes. The mean cell number in the far-right panel represents the arithmetic mean of the cell number in the abdominal and subcutaneous depots.

DISCUSSION

The presence of brown adipose tissue in hibernating and other animals had previously been reported for many years, but its relationship to energy balance and diet-induced adaptive thermogenesis in mammalian species and humans remained unknown or controversial for many years. [2,3] In the 20th Century, Sims et al were among the first in recent history to describe the phenomena of 'luxus consumption' in humans, which they had observed in human volunteers in the Vermont Study of Obesity.[33] In that study, healthy, normal weight and predominantly sedentary volunteers consumed up to 10,000 excess calories/day without becoming obese, and most subjects quickly lost the modest extra weight they had gained upon return to normal energy intakes and without significant changes in daily physical activity.[32] Rothwell and Stock later demonstrated the apparent presence of brown fat activity in humans following adrenergic activation, thereby further linking the thermogenic response to dietary and environmental factors.[22] While the presence of brown adipose tissue in hibernating animals and in humans had been known, the biochemical mechanisms implicated and the physiologic contributions have now been elucidated by Himms-Hagen and others.[2,3] The thermogenic mechanism was found to revolve around specialized mitochondria in BAT that are capable of generating heat from the hydrolysis of high energy phosphate bonds from ATP, a process sometimes referred to as 'uncoupled' oxidative phosphorylation.[2,3] The process results in the generation of ~ 7 kcal/mole of high energy phosphate bonds as heat and typically occurs without being linked to biosynthetic processes.[3] The effects of pharmacologic inhibition of BAT thermogenesis via β-blockade resulted in microscopic enlargement of the lipid locule diameters indicative of decreased thermogenic activity.[23] Attempts to document excess weight gain and greater adiposity following surgical removal of IBAT have yielded variable results.[5-7, 9,10,24]

In conclusion, the results of this study indicate that café- induced overfeeding resulted in depot specific increases in adiposity including increases in body weight, abdominal circumference, and in adipose tissue cellularity in abdominal and subcutaneous depots. The results obtained herein are qualitatively similar to those that have been reported by other authors. [6,7,9,10,14,24,35-37] In the present study, the increases in adipose tissue cellularity occurred by a combination of hyperplasia and hypertrophy of adipocytes that were expressed differently in different depots. In the epididymal depot, adipocyte hyperplasia is likely complete by puberty, and increased in depot mass beyond puberty occur via limited hypertrophy. Epididymal cell diameters and cell lipid content exhibited only a modest trend toward greater lipid content following the Café diet in either Café group or Café-IBAT were without additional effect. Likewise, adipocyte number in the dorsal, retroperitoneal and inguinal depots increased significantly when fed the café diet, but the cell number in the Café-IBAT group was greater only in the Inguinal depot. The reasons for the differential effects of overfeeding on differential depot specific alterations in adipose tissue cellularity are unclear but may be secondary to depot specific differences in insulin sensitivity, lipogenic actions, and chronological differences in lipid accretion in the different depots. Effects on linear growth were not observed, suggesting that the Café diet as offered in combination with their chow regimen likely provided adequate nutrition to accommodate lean tissue growth and development. In contrast, the differences in adiposity correlated more with net energy intake, including refined carbohydrates and processed food items of less nutritional value. While measures of adipocyte number in the IBAT were not measured in the present study, parallel studies in both rats and mice reported an approximate 3-fold increase in IBAT mass and cellularity after an analogous 52-day or shorter feeding regimen.[7,8,24,29,34-37]

SUMMARY AND CONCLUSIONS

The results of this study showed that feeding a Café diet supplement to normally lean rat during the postweaning growth period resulted in depot-specific increases in mass and cellularity characteristics in lean, Sprague-Dawley rats. Partial surgical reduction of BAT mass by removing the Interscapular depot in its entirety resulted in modest additional depot-specific increases in adiposity, but the net increases were not proportionate to the proportion of BAT removed. Whether other BAT depots may have compensated by additional increases in cellularity or thermogenic functions remain unclear, however in an earlier study, resting and norepinephrine simulated thermogenic responses were only modestly decreased following a similar surgical reduction of IBAT.[5,6] Thus, overfeeding via the Café regimen in a strain of normally lean rats is an effective method to bring about modest physiological changes in adiposity and adipose tissue cellularity in white adipose tissue depots via differential depot-specific effects on adipocyte hyperplasia and hypertrophy.

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Application of AI (Artificial Intelligence) Disclaimer

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

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