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ALTERED SENSITIVITY TO CHOLECYSTOKININ OCTAPEPTIDE (CCK-8) IN DIET-INDUCED OBESITY PRONE (DIO) AND RESISTANT (DR) RATS.

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ABSTRACT

Over the past several decades, obesity in the United States has been on the rise and has turned into a serious national health problem. In simplistic terms, the etiology of obesity arises from an imbalance between energy input and expenditure, leading to an excessive build up of stored energy in the form of fat. This imbalance between energy input and expenditure is directly correlated to meal size and frequency. The size of the meal consumed has been linked to certain digestive hormones and their interaction between the digestive system and the brain. One hormone produced by the small intestinal endocrine cells and controls meal size is cholecystokinin (CCK). The objective of the study presented in this thesis was to determine whether dietary-induced obesity leads to changes in CCK sensitivity and whether these changes were associated with alteration in food intake, body weight, adiposity and glucose impairments. The diet-induced obese prone (DIO) and diet-induced obese resistant (DR) rat models were used because they exhibit a polygenic phenotype similar to obesity in humans. The results show that, compared to DR, DIO rats were more sensitive to low doses of CCK and less sensitive to high doses of CCK. At the end of the experiment, DIO rats were heavier than DR rats, and consumed more food during 24h. However, DIO rats ate less grams of food per body weight than DIO rats. In addition, there was a significant difference in raw fat deposit weight (epididymal, retroperitoneal and visceral). The DR rats had larger fat pads as proportion of their body weight, compared to DIO rats. The findings suggest that, in addition to other reported deficits, DIO rats may have a deficit in CCK signaling leading to significant weight gain, even in the absence of energy store deficits.

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INTRODUCTION

OBESITY IN THE UNITED STATES

Obesity in the United States has risen to epidemic proportions in recent years. The United States Public Health service has ranked obesity, its related morbidities, and associated costs above problems related to smoking and alcohol consumption.

The obesity epidemic in the United States has been the subject for numerous studies and has developed into a healthcare nightmare. In today's world, it is becoming increasingly difficult to eat healthy and exercise. Since most of the country lives a fast pace lifestyle, the American diet has developed into a fast food restaurant menu, full of saturated fats, cholesterol and added sugars. Eating healthy and exercising has long been thought of as a fail safe way to lower body fat and lose excess pounds. Unfortunately, preventing excess weight gain and obesity is much more complicated than that. In fact, the scientific community has adopted the notion that obesity is encoded by a few number of genes and this genotype when placed in a certain environment can lead to the development of obesity [1]. Therefore, individuals with a certain genotype when subjected to certain foods will develop excess weight gain and possibly become obese. New findings and research into the role that genetics is playing in obesity is quickly becoming a key in understanding the basis behind the obesity epidemic.

Obesity, according to the *Centers for Disease Control and Prevention (CDC),* is defined as a Body Mass Index (BMI) of greater than or equal to 30 kg/m^2 [2]. The prevalence of obesity in the United States continues to grow and from a 2006 study, over 33% of the country has been diagnosed with obesity [3]. Coupled with a rise in health care costs, obesity also increases the risk factors for many serious diseases and health

conditions. These include, but are not limited to: hypertension, type 2 diabetes, stroke, heart disease and some cancers [2]. As illustrated in the figure below, the obesity epidemic has become widespread and has grown substantially from 1985 to 2007 [4].

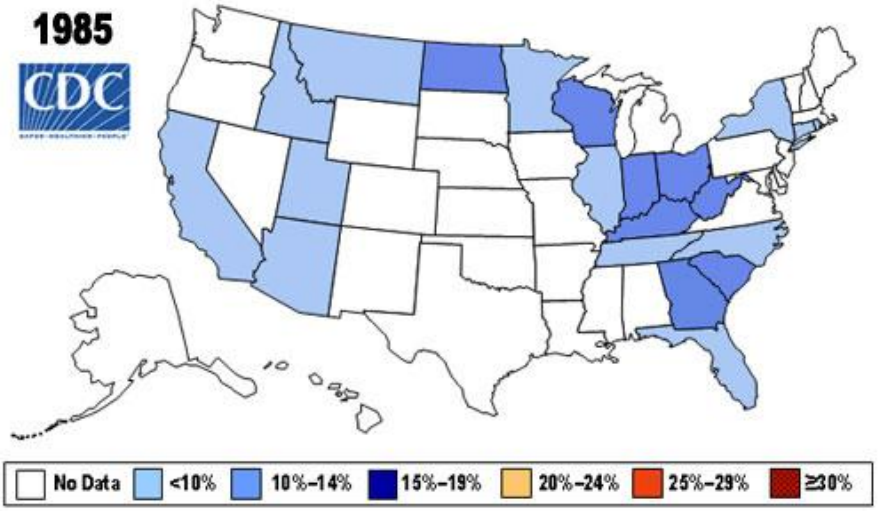

Fig.1. Prevalence of obesity in the United States in 1985. A large majority of states did not report, but the states that did report had obesity rates of either less than 10% or between 10% and 14%.

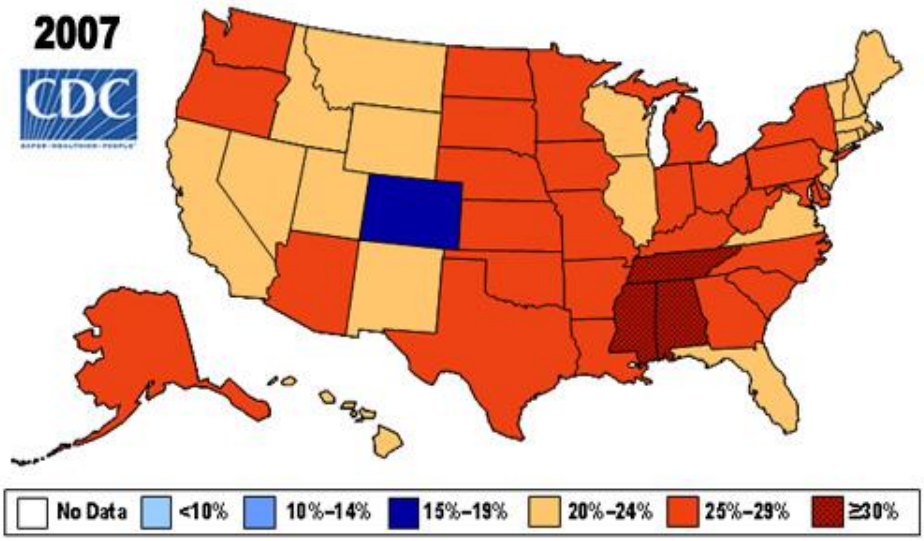

Fig.2. Prevalence of obesity in the United States in 2007. All states in the union except Colorado reported obesity rates of at least 20% to 24%, and Alabama, Mississippi and Tennessee reported obesity rates of greater than 30%.

It is clear that over the past two decades obesity rates have increased dramatically in the United States. Besides the associated health risks the cost of treating obesity and its

co-morbidities such as heart disease, type 2 diabetes and hypertension are costing the country billions of dollars every year. Below is a figure from ObesityInAmerica.org and illustrates the high financial burden put on the country by the obesity epidemic [5].

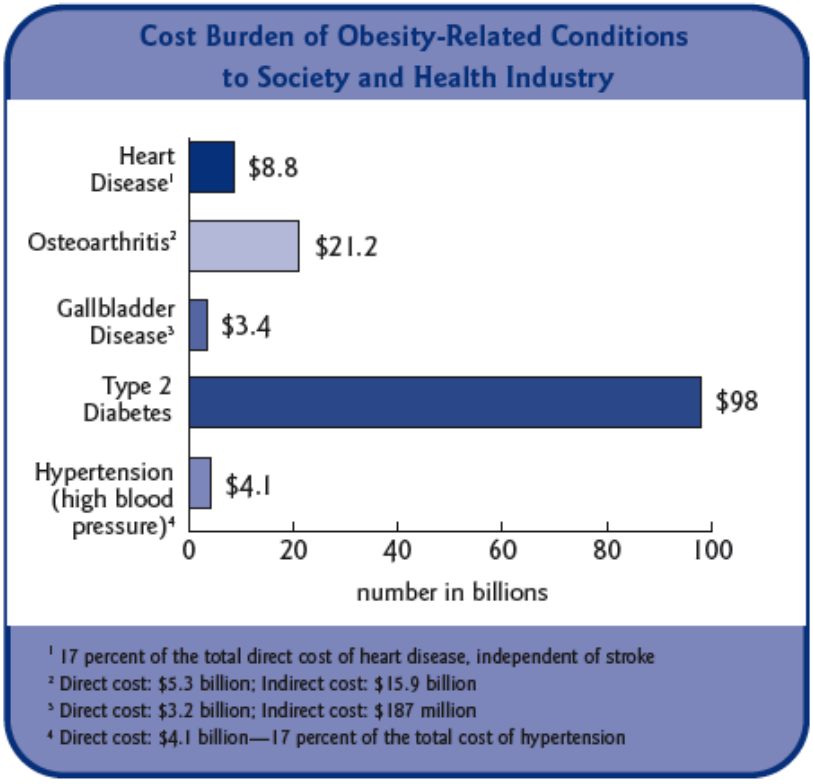

Fig. 3. The cost of obesity epidemic. The numbers are in billions of dollars. Type 2 diabetes has the highest cost related with it, while gallbladder disease has the smallest cost.

For many decades, a lack of understanding about obesity has prevented successful and affordable treatment. Obesity is a complex issue involving metabolical, neural, physiological, psychological social and environmental factors. Controlling how much an individual eats is an important part of the equation to maintain energy homeostasis. The meal size is the fundamental unit of intake. There are numerous hormonal and paracrine signals secreted in response to the presence of food in the gastrointestinal tract. These GI signals that influence the brain to stop an ongoing meal are called satiation signals.

SATIATION

Satiation is the reduction of food intake that occurs in response to ingestion of food and ultimately results in meal termination. The process of satiation is mediated by feedback signals from the gastrointestinal tract. Because meals typically end long before the majority of meal contents are absorbed, the afferent signaling that leads to cessation of eating must start earlier than this, and almost certainly arises from the gastrointestinal tract. Studies showing that upper gastrointestinal administration of nutrients and mechanical distention potently and dose dependently suppress food intake constitute a classical example of the role of the negative feedback mechanism in limiting meal size. Although there has been considerable progress in our understanding of how gastrointestinal feedback signals participate in satiation, very little experimental attention has been paid to the possibility that alteration in the response to gastrointestinal signals may result in disordered phagia associated with body weight gain. Likewise, relatively less attention has been paid to consequences or concomitants of obesity, which could contribute to maintenance or exacerbation of the obese state.

A variety of peptides termed satiety factors are released from the gut during feeding which reduce meal size upon exogenous administration [6]. These gastrointestinal satiation peptides include cholecystokinin (CCK), bombesin (BBS), and glucagon-like peptide (GLP-1), to name a few. They have been shown to exert control on a variety of functions that control ingestion [6, 7, 8, 9]. These peptides are secreted by peripheral tissues and organs, they all reduce food intake by reducing meal size when administered exogenously, are secreted in response to nutrients, and interact with nutrients to reduce intake [7,8,9]. For these peptides, the signals that ultimately cause

satiety can be generated in the peripheral nervous system and relayed to the brain where they become integrated with other determinants of meal size. Defects in the functionality of their systems result in alterations in short term food intake as well as in long term signals leading to obesity. Finally, mounting evidence supports synergistic interactions between some of these peptides to control feeding behavior. These observations provide a mean to integrate input from short term, meal-related signals into the long-term control of energy balance. Over the past two decades the proposition that deficits in response to satiation peptides may lead to hyperphagia and obesity has been a potent prescription for research. In this thesis we elected to focus on one peptide, cholecystokinin.

CHOLECYSTOKININ

Gibbs, Young and Smith [10] discovered that exogenous administration of CCK causes a dose-dependent decrease in meal size. Subsequent studies have defined CCK as one of the most biologically potent satiety peptides. CCK reduces food intake by acting on vagal sensory neurons [11, 12, 13, 14]. Surgical [15, 16] as well as chemical [12, 17] destruction of vagal sensory fibers abolishes CCK-induced reduction of food intake. Furthermore, CCK binding sites (receptors) are transported by vagal sensory neurons [18, 19]. CCK receptors have been identified and the genes coding for these receptors have been cloned [20, 21, 22, 23]. Both systemic capsaicin [24] and nodose ganglionectomy [25], reduce CCK binding in the nucleus of the solitary tract, the site where vagal sensory afferents terminate. Finally, results of electrophysiological experiments indicate that vagal sensory fibers innervating the gastrointestinal tract can be activated by exogenous CCK [17, 26, 27, 28, 29]. Studies using selective CCK-1 and CCK-2 receptor antagonists

indicate that reduction of food intake by injections of exogenous CCK is mediated by CCK-1 receptors [30, 31, 32, 33].

RAT MODELS OF OBESITY

Obesity is a phenotypic trait expressed by a variety of rodent strains, including rats and mice with spontaneous genetic mutations, and mice with experimentally produced gene deletions [34]. Remarkably, obesity is a concomitant of alterations in a surprising variety of disparate genes. A short and non-exhaustive list includes deletions or mutations of leptin receptor genes, the GRP receptor gene, the 5-HT2c receptor gene, a transcription factor gene, and the CCK-1 receptor gene (for review see [34, 35, 36, 37, 38]). The variety of mutant and transgenically modified alleles associated with obesity probably reflects the complexity and diversity of processes that impinge on control of body energy balance and the number of points at which the balance can be upset.

DIETARY OBESITY

Most obesities are caused by chronic overeating. However, some individuals respond strongly (prone) and others weakly or not at all (resistant) to a given factor. The most extensively used DIO rat model is the outbred Sprague Dawley (SD) rat. When outbred SD rats are placed on a HE/HF diet, there is a wide distribution in body weight gain; a subset of animals become very obese (DIO), whereas others remain as lean as animals fed a low-fat diet [diet-resistant (DR)] The physiological aspects of DIO in this model replicate many of the features observed with the human obesity syndrome: a polygenic mode of inheritance, a persistence of the phenotype once it is established, and

dysregulated glucose homeostasis. Prior work in DIO rats has shown that hyperphagia and increased energy efficiency often accompany the persistent obesity produced by long-term, high fat feeding. Mechanisms underlying this persistent change in the metabolic and motivational regulation of food intake are not fully understood. However, in addition to deficits in responses of hypothalamic systems to dietary obesity, there is circumstantial evidence that these rats have decreased peripheral sensitivity to food stimuli. Specifically, rats that are adapted to HF diet also express decreased sensitivity to the suppressive effects of CCK. Thus, it is plausible that the over consumption and subsequent weight gain in DIO rats may be, in part, due to a maladaptive feedback mechanism that develops in response to sustained over consumption of HE/HF diets in obese individuals.

EXPERIMENTAL PURPOSE

In this experiment the diet induced obese prone (DIO) and diet induced obese resistant (DR) rats were used to asses their sensitivity to CCK when presented with chow. Unlike DR rats, the DIO rats become obese when placed on a high fat diet [39].The DIO model resembles the polygenic phenotype of human obesity. The relationship between the environment and genes is key in understanding obesity [40]. The DIO rat model has become the best model to represent obesity in humans because the high fat diet used to selectively outbreed the rats to develop obesity, is similar to the American, or Western style diet [41]. We know from previous studies that an intraperitoneal administration of CCK suppresses food intake in a variety of species and animal models [41]. We also know that chronic exposure to a high fat diet will cause significant weight gain in the DIO rats as well as the development of obesity, while the DR rats are seemingly

unaffected [41]. Previous studies from Dr. Covasa's laboratory has shown that when rats were maintained on a high fat diet, the satiation effects of CCK were significantly decreased [45]. Similarly, humans maintained on a high fat diet report decrease in appetite ratings, eat more and exhibit acceleration in gastric emptying [34].The potential participation of satiation deficits in obesity has been highlighted with the demonstration that OLETF rats, which do not express CCK-A receptors, overeat and become obese [46]. Several laboratories, including Dr. Covasa's [47], have demonstrated that OLETF rats do not reduce their food intake in response to systemic injections of CCK. In addition, another genetically obese strain, the Zucker fatty rat, exhibits reduced satiation in response to exogenous CCK, compared to lean controls [48]. This rat also exhibits apparent reduced responses by other systems that also are controlled by CCK [48]. There is only one report examining the effects of CCK on food intake in DIO rats, with the DIO rats being more sensitive to the suppressive effects of CCK when placed on a high fat diet. However, only one dose of CCK was tested (3.0 μg) and the correlates of weight gain were not examined. Therefore, the present study was conducted in order to systematically assess differences in CCK sensitivity, food intake, body weight and adiposity between the DIO and DR rats maintained on a regular chow diet.

METHODS

SUBJECTS

Preselected and inbred DIO and DR rats (n = 12 per strain) weighing 190 – 210 g at the beginning of the experiment were purchased from Charles River Laboratory (Wilmington, MA) and used throughout the study. Rats were kept in hanging wire bottom cages in a temperature controlled vivarium with a 12:12-h light cycle (lights on at 0600 h). Before any experiments began, rats were adapted to housing conditions for two weeks.

DIET AND DRUGS

Rats were fed standard rat chow (Purina 5001, New Brunswick, NJ), which has an energy density of 3.3 kcal/g. Cholecystokinin octapeptide (CCK-8), the biological active fragment of CCK, was purchased from American Peptides (Sunnyvale, CA) and was dissolved in a vehicle of 0.9% saline (control vehicle).

CCK SENSITIVITY TEST

Rats were fasted overnight (1700 – 0900 h) before each test. Drugs were administered every other day and bracketed by a saline control vehicle. To find the lowest dose to reduce chow intake over 30 and 60 minutes, CCK was administered in the following order of doses: 0.1, 0.2, 0.3, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 μg/kg. Premeasured chow was presented five minutes post-injection with trays under cages accounting for any spillage. At 30 and 60 minutes, chow was removed and weighed to determine intake during the experiment.

BODY WEIGHT

Rats were weighed before the experiment on each test day. Average body weight was calculated daily as well as weekly.

24 HOUR CHOW INTAKE

Premeasured chow was presented to the rats. Grams of chow consumed was recorded over a 24 hour period.

ORAL GLUCOSE TOLERANCE TEST

After an overnight fast (minimum 16 h), glucose (2 g/kg, 500 g/l) was administered orally using latex intragastric gavage, and tail blood was taken by tail nips without anesthesia at 0, 30, 60, and 120 min. Blood glucose was determined with a glucometer (Elite Glucometer, Bayer, Elkhart, IN). Animals were classified as diabetic if the peak level of plasma glucose at any time point was 16.8 mmol/l (300 mg/dl) or glucose level at 120 min > 11.2 mmol/l (200 mg/dl).

FAT PAD COLLECTION

Following tissue fixation and brain collection for subsequent immunohistochemistry studies, epididymal, retroperitoneal and visceral fat pads from each individual rat were excised and weighed.

STATISTICAL ANALYSES

All statistics were computed using Statistical Analysis Software (SAS, version 9.1.3, Cary, NC). Weekly body weights were analyzed through two-way student unpaired T-Test. 24 hour food intake was analyzed through one-way (strain) repeated measures Analysis of Variance (rmANOVA). Suppression of food intake by CCK-8 was analyzed by two-way rmANOVA with strain and treatment as independent variable. Blood glucose levels were analyzed by one-way rmANOVA. Gross fat pad weight as well as fat pad percentage based on body weight were both analyzed by two-way unpaired student T-Test. All data are expressed as means ± the standard error of the mean. Significance was determined by a P-value < 0.05.

RESULTS

ALTERED SENSITIVITY TO CCK-8

Two-way repeated measures ANOVA showed that there was a fixed effect of treatment but not strain on food intake and there was a significant interaction between strain and treatment [$F(5.624)=2.84$, $P=0.015$].

CCK-8 (1.0 μg/kg)

Significant suppression of food intake occurred following administration of 1.0 μg/kg CCK-8 in the DIO and DR rats. DIO rats consumed 6.46 ±0.40 g of chow when 1.0 μg/kg of CCK-8 was administered, while 8.98±0.15 g of chow was consumed after saline administration ($P<0.0001$). DR rats consumed 7.96±0.47 g when CCK-8 1.0 μg/kg was administered compared to 9.61±0.16 g when saline was administered ($P=0.0010$).

CCK-8 (2.0 μg/kg)

Significant suppression of food intake occurred following administration of 2.0 μg/kg of CCK-8 in the DIO and DR rats. DIO rats consumed 7.21±0.31 g of chow when 2.0 μg/kg CCK-8 compared to 8.98±0.15 g when saline was administered ($P<0.0001$). DR rats consumed 8.28±0.32 g of chow when 2.0 μg/kg CCK-8 was administered, compared to 9.61±0.16 g when saline was administered ($P<0.0001$).

CCK-8 (4.0 μg/kg)

Administration of 4.0 μg/kg CCK-8 resulted in a significant suppression of food intake in the DIO rats, but not the DR rats. DIO rats consumed 6.85±0.43 g when CCK-8 was administered, compared to 8.98±0.15 g when saline was administered ($P<0.001$). DR rats consumed 8.5±0.52 g when 4.0 μg/kg CCK-8 was administered, compared to 9.61±0.16 g when saline was administered ($P=0.2340$).

CCK-8 (8.0 μg/kg)

Significant suppression of food intake was found when 8.0 μg/kg CCK-8 was administered in the DR rats, but not the DIO rats. DR rats consumed 7.2±0.54 g when CCK-8 was administered, compared to 9.61±0.16 g when saline was administered ($P<0.0001$). There was no significant suppression of food intake in the DIO rats when 8.0 μg/kg CCK-8 was administered, 7.87±0.53 g, compared to 8.98±0.15 g when saline was administered ($P=0.3438$).

CCK-8 (16.0 μg/kg)

There was significant suppression of food intake in both strains when 16.0 μg/kg CCK-8 was administered. DIO rats consumed 6.94±0.53 g of chow when CCK-8 was

administered, compared to 8.98±0.15 g when saline was administered (P<0.0001). DR rats consumed 7.27±0.53 g when CCK-8 was administered, compared to 9.61±0.16 g when saline was administered (P<0.0001).

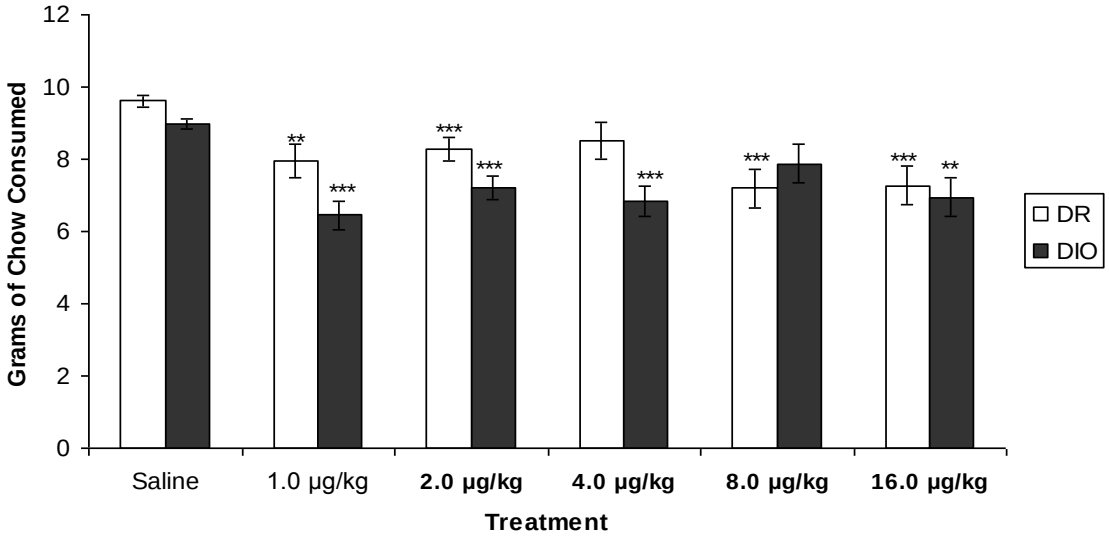

Fig. 4. Suppression of chow intake (± SE), CCK-8 dosages of 1.0 µg/kg, 2.0 µg/kg, 4.0 µg/kg, 8.0 µg/kg and 16.0 µg/kg. DIO rats exhibited increased sensitivity to CCK-8 at lower doses (1.0 µg/kg, 2.0 µg/kg and 4.0 µg/kg), while the DR rats showed increased sensitivity to CCK-8 at higher doses (8.0 µg/kg and 16.0 µg/kg).
** denotes P<0.001, *** P<0.0001, compared to respective saline baseline.

BODY WEIGHT

There was no significance difference between the average body weights of DIO and DR rats at arrival: 5wks: DIO 136.33±1.59 g, DR 135.75±2.53 g; P=0.839. However, there was a significant difference starting at weeks 6 and through 12, with the DIO rats exhibiting a higher average body weight than the DR rats; 6wks: DIO 153±1.86 g, DR 142±2.79g; P=0.0014;, 7wks: DIO 189±2.14 g, DR 162 ±3.17 g; P<0.0001;, 8wks: DIO 219±1.99 g, DR 180±3.20 g; P<0.0001;, 9wks: DIO 247±1.94 g, DR 199±3.79 g; P<0.0001, 10wks: DIO 269±1.94 g, DR 211±3.81 g; P<0.0001, 11wks: DIO 290±2.25 g, DR 226±4.34 g; P<0.0001, 12wks: DIO 305±2.52 g, DR 236±4.37 g; P<0.0001. (Fig. 5)

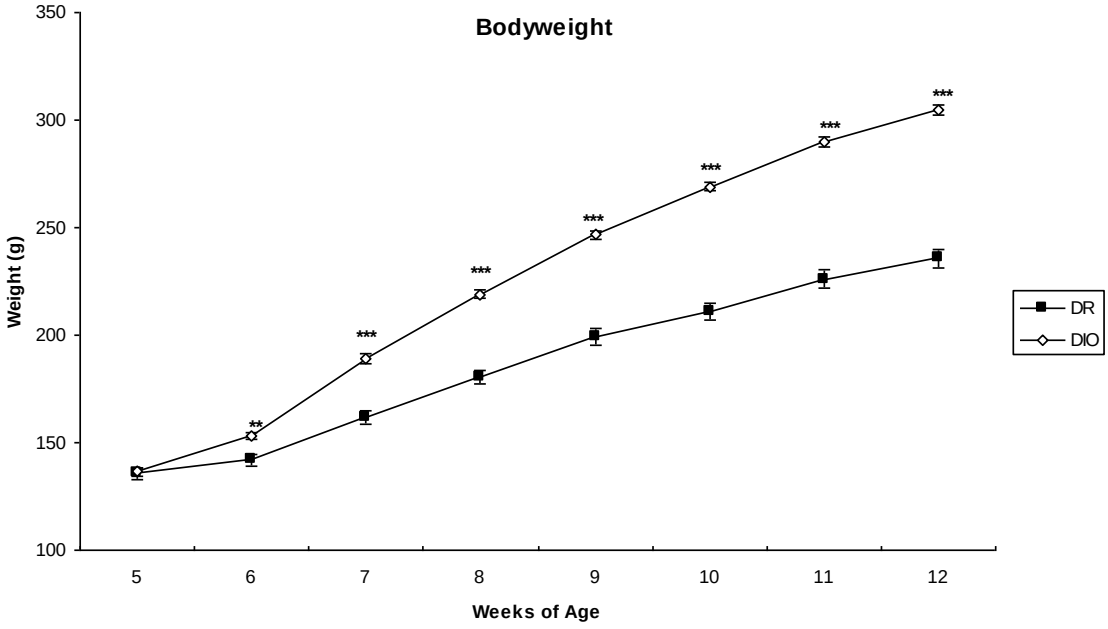

Fig. 5. Average body weight (± SE) throughout the experiment in DR and DIO rats. DIO rats exhibited significantly higher average body weight than the DR rats.
** denotes P<0.001, *** P<0.0001, between strains.

24 HOUR CHOW INTAKE

Based on 24 hour chow intake data there was a significant difference in the amount of chow consumed between the DIO and DR rats (DIO: 21.1±0.11 g; DR: 19.4±0.27 g, (P=0.0231)) (Fig. 6).

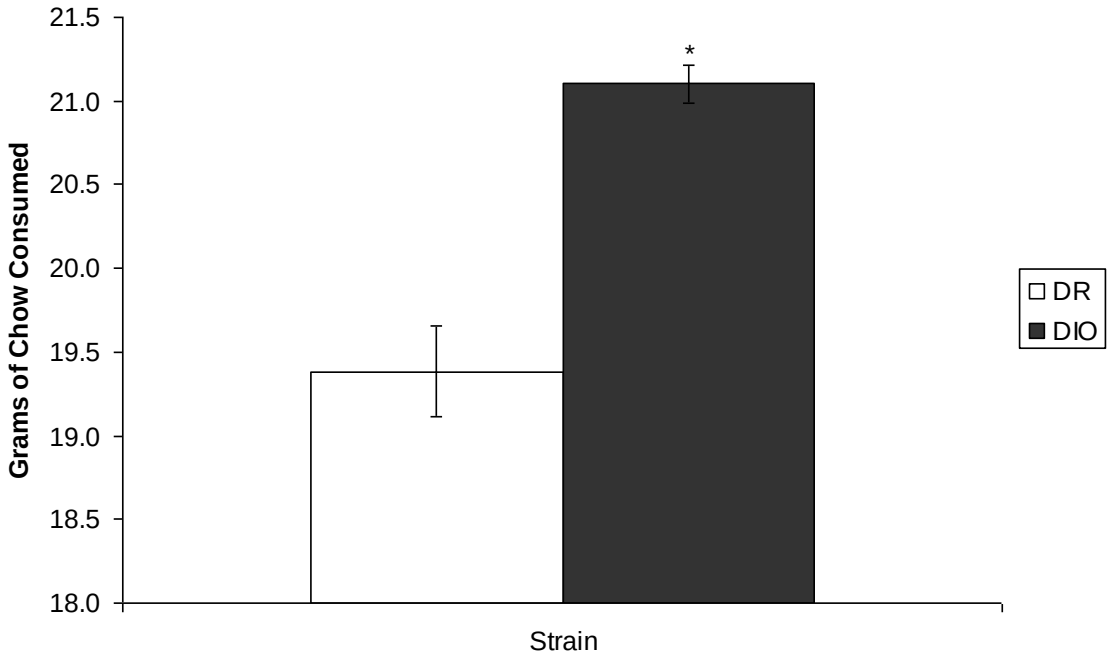

Fig.6. Grams of chow consumed (± SE) in 24 hours. The DIO rats consumed significantly more chow than the DR rats during the 24 hour intake period.
* denotes P<0.05, compared to DR.

ORAL GLUCOSE TOLERANCE TEST

There were no differences in fasting blood glucose levels between strains (DIO: 51±3.49 mg/dl; DR: 51±2.21 mg/dl, P=1.0). At 30 and 60 minutes post gavage the DR rats' blood glucose (30 min: 132±5.12 mg/dL; 60 min: 123± 2.80 mg/dL) was significantly higher than the DIO's (30 min: 109±4.58 mg/dL, P=0.0036; 60 min: 101 ±3.88 mg/dL, P=0.003). At 90 minutes post glucose gavage, there was no significant difference between DR (102 ±3.60 mg/dL) and DIO (94±3.75 mg/dL) blood glucose (P=1.0). At 120 minutes post glucose gavage, there was no significant difference between (DR 86±2.85 mg/dL) and (DIO 86±2.69 mg/dL) blood glucose levels (P=1.0) (Fig. 7).

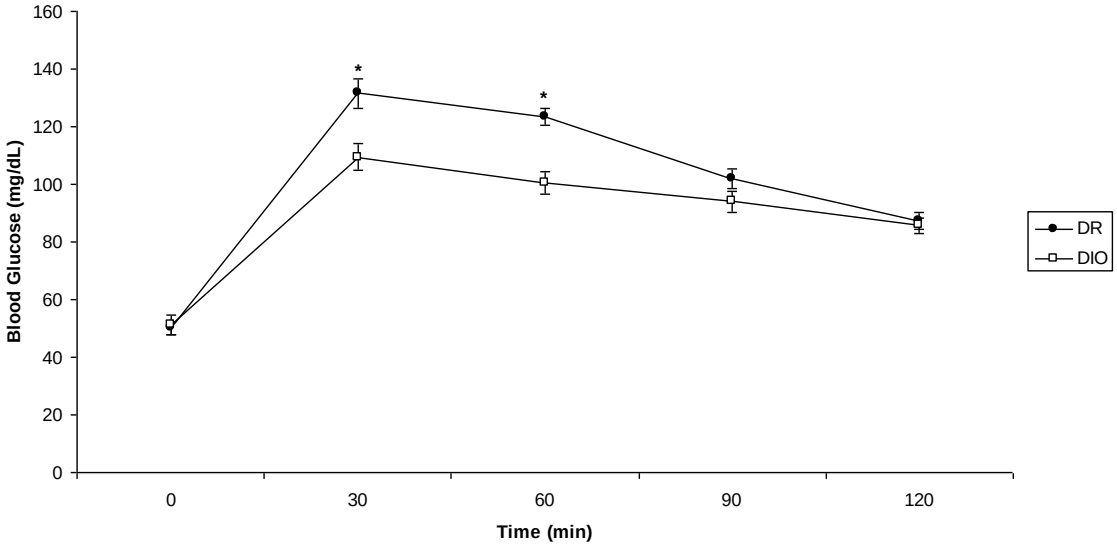

Fig. 7. Average blood glucose levels (± SE) in DR and DIO rats. At 30 and 60 minutes post glucose gavage the DR rats blood glucose was significantly higher than the DIO rats (P<0.05). At 0, 90 and 120 minutes there was no significant difference between the DR and DIO blood glucose levels.
* denotes P<0.05, compared to average DIO rat blood glucose.

FAT PAD COLLECTION

There was a significant difference in gross fat pad weight in the epididymal (DR: 3.84±0.14 g, DIO: 4.50±0.17 g; P=0.007)), visceral (DR: 4.00±0.16 g, DIO: 4.76 ±0.18 g; P=0.005) and total fat pad weight (DR: 12.9±0.63 g, DIO: 14.8±0.31 g; P=0.015) with heavier fat pads in the DIO compared to DR rats. There was no significant difference in the retroperitoneal fat pad weight between the strains (DR: 5.02±0.38 g, DIO: 5.53±0.19 g (P=0.245). When the weight of fat pads were expressed as percentage of total body weight, there were no significant difference between total fat (DR:4.30±0.22%, DIO: 3.78±0.07%; P=0.387), retroperitoneal (DR:1.67±0.13%, DIO: 1.41±0.05%; P=0.077) or visceral (DR: 1.33±0.05%, DIO: 1.22±0.05% (P=0.1031)) fat pads. However, there was a significant difference between the epididymal fat pads as percent of bodyweight between the DR and DIO rats (DR:1.28±0.04%, DIO: 1.15±0.04%; P=0.029).(Table 1).

Fat Pad (g)	DR	DIO	% Bodyweight (g fat/g BW)	
			DR	DIO
Epididymal (g)	3.84	4.50*	1.28	1.15*
Retroperitoneal (g)	5.02	5.53	1.67	1.41
Visceral (g)	4.00	4.76*	1.33	1.22
Total (g)	12.9	14.8*	4.30	3.78

Table 1. Fat pad weight in grams (± SE) from DR and DIO rats. The DIO had significantly larger levels of epididymal, visceral and total fat pad deposits compared to the DR rats (P<0.05). Based on % bodyweight, the DIO rats had significantly less epididymal fat than the DR rats (P<0.05).
* denotes P<0.05, compared to DR rat fat pad.

DISCUSSION

The results show that responses to CCK-8 differed among the DIO and DR rats while maintained on a regular chow diet. Specifically, the DIO rats were more sensitive to lower doses (1.0 μg/kg, 2.0 μg/kg and 4.0 μg/kg) of CCK-8, while the DR rats were more sensitive to higher doses (8.0 μg/kg and 16.0 μg/kg) of CCK-8. We also found that compared to DR, the DIO rats consumed more chow during the 24 hour intake period, had higher average body weights and significantly higher amount of total fat pads. Finally, although the DR rats had higher blood glucose levels than the DIO's in response to an OGTT test at 30 and 60 min, neither strain were in a hyperglycemic state or prediabetic stage.

There is only one report in the literature examining CCK responses in DIO and DR rats [41]. In this study, Chandler et al. found that a threshold dose of CCK (0.3 μg/kg) had no inhibitory effects when rats were maintained on a regular chow diet, but significantly suppressed food intake in DIO, but not DR rats when placed on a high fat diet. However, in this study, only one dose of CCK was used and the degree of sensitivity to CCK was only assessed during maintenance on a high fat diet. Our study as well as others have shown that the DIO rats not only have an accelerated weight gain rate when put on a high energy high fat diet, but they also gain weight on a regular chow diet. Therefore, it was of interest to examine whether the changes in food consumption and weight gain are related to a reduced in potency of CCK. Our results are similar to Chandlers et al. showing that DIO rats were more sensitive to the suppressive effects of CCK compared to DR rats. However, the potency of the CCK doses used varies greatly between the two studies. First, the threshold dose of CCK capable of reducing intake in

our study was higher than in Chandlers' et al. study. Secondly, doses below 4 μg/kg produced a stronger effect in DR compared to DIO rats, while an opposite effect was found when 8 μg/kg was administered. It is well established that CCK inhibits food intake in a dose dependent manner and that sensitivity to CCK differs based on the feeding regimen. For example, when rats were maintained on a high fat diet, they were less sensitive to the suppressive effects of CCK, compared to rats maintained on a low fat diet. Similarly, some obese models also display a reduction in CCK sensitivity. CCK is released by the enteroendocrne "I" type cells when ingesta, in particular fats and proteins, come in contact with the intestinal mucosa. Most of the CCK action is local, trough a paracrine and vagal, neural mode. It is possible that the differential response in DIO rats may result from an attenuated vagal responses. This is in agreement with our previous studies showing reduced Fos expression in response to CCK in rats adapted to a high fat diet. Furthermore, CCK interacts with other short term satiation signals as well as long term adiposity signals. The increased in total adiposity measured by the weight of fat pads suggests an increase in circulating leptin. CCK has been shown to enhance the suppressive effects of leptin. Therefore, at some doses, CCK, may act by interacting with leptin to enhance the suppression of food intake in DIO rats.

The DIO rats also gained significantly more than the DR rats while consuming more food. However, when food intake was expressed as percent of their body weight, there was no significant difference in 24h food consumption between the strains. This significant increase in bodyweight was not related to the diet since both strains were subjected to a low fat regular chow diet. Other studies that have concluded the high fat diet causes the significant weight gain in the DIO rat and not the DR rat [41]. Therefore

we cannot conclude that excessive chow consumption during the 24 hour intake period by the DIO rat's results in excessive weight gain and increased adiposity. The DIO rats also contained a significant amount more adipose tissue deposits than the DR rats, (Fig. 7). Once again we cannot conclude that an excessive consumption of chow leads to the increase in adipose tissue deposits, most notably in the visceral and epididymal regions of the DIO rats. This suggests that, in addition to the peripheral CCK deficits, the DIO rats have also metabolical deficits. In fact, it was previously shown by Levin and others that the DIO rats are more sensitive to leptin. When the DIO rats were peripherally injected with leptin they exhibited a decreased sensitivity to its anorectic effects. There appears to be abnormalities in the sympathetic nervous system of obese animals and humans compared to animals and humans of normal weight, which is similar to the DIO and DR rat model used in this experiment. Levin et. al also mention that one of the results related to deficit in sympathetic activity of obese subjects is hyperglycemia because glucose metabolism is directly related to the activity of the sympathetic nervous system.

CONCLUSION

Our results show that DIO rats were more sensitive to lower doses of CCK, while DR rats were more sensitive to larger doses. As expected the DIO rats gained significantly more weight, consumed more chow during a 24 hour intake and exhibited larger amounts of adiposity than the DR rats. These results also supported the claims that DIO and DR rats are resistant to the development diabetes.

The reduced sensitivity to exogenous CCK in DIO rats placed on a chronic high fat diet has been the subject of many studies. On the other hand, the role of CCK in DIO

rats maintained on a standard chow diet is not well understood. Reduced sensitivity to a major satiation signal, such as CCK in obesity prone animal model maintained on a regular chow diet, may be responsible for hyperphagia and weight gain. Therefore, studies examining changes in short term satiation signal responses may be fruitful in understanding the physiology behind overeating.

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