HHS Public Access

Author manuscript

Int J Obes (Lond). Author manuscript; available in PMC 2014 June 01.

Published in final edited form as:

Int J Obes (Lond). 2013 December ; 37(12): 1515–1523. doi:10.1038/ijo.2013.22.

Centrally administered urocortin 2 decreases gorging on high-fat diet in in both diet induced obesity-prone and -resistant rats

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Abstract

Objective—Obesity is a costly, deadly public health problem for which new treatments are needed. Individual differences in meal pattern have been proposed to play a role in obesity risk. The present study tested the hypothesis that *i)* the microstructure of chronic high-fat diet intake differs between genetically selected Diet-Induced Obesity (DIO) and Diet Resistant (DR) rats, and *ii)* central administration of urocortin 2 (Ucn 2), a corticotropin-releasing factor type 2 (CRF_2) agonist, decreases high-fat diet intake not only in lean DR rats, but also in obese DIO rats.

Design—Male, selectively bred DIO and DR rats (*n*=10/genotype) were chronically fed a high-fat diet. Food and water intake as well as ingestion microstructure were then compared under

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Disclosure/Conflict of interest

The authors declare no conflict of interest

Supplementary information is available at the International Journal of Obesity's website

baseline conditions and following third intracerebroventricular injection of Ucn 2 (0, 0.1, 0.3, 1, 3 μg).

Results—Irrespective of genotype, Ucn 2 reduced nocturnal food intake with a minimum effective dose of 0.3 μg, suppressing high-fat diet intake by ~40% at the 3 μg dose. Ucn 2 also made rats of both genotypes eat smaller and briefer meals, including at doses that did not reduce drinking. Obese DIO rats ate fewer but larger meals than DR rats, which they ate more quickly and consumed with 2/3rd less water.

Conclusions—Unlike leptin and insulin, Ucn 2 retains its full central anorectic efficacy to reduce high-fat diet intake even in obese, genetically-prone DIO rats, which otherwise show a "gorging" meal pattern. These results open new opportunities of investigation towards treating some forms of diet-induced obesity.

Keywords

obesity or obese; feeding or food intake; meal pattern or meal size or meal frequency or meal microstructure; satiety or satiation; appetite; high-fat diet; urocortin or corticotropin-releasing factor or corticotropin-releasing hormone or CRF or CRH receptor

Introduction

Obesity is a major public health problem that increases morbidity, mortality, and economic burdens[1, 2]. Some individuals may be susceptible to becoming obese when exposed to palatable, calorically-dense food[3–5] due to an inherited resistance to the negative feedback influence of neuropeptides and peripheral hormones[6] on energy metabolism and appetite[7–11]. Accordingly, Levin and colleagues selectively bred rats for differential weight gain responses to a high-fat/high-energy diet. The resulting diet-induced obesity-prone (DIO) and diet-resistant (DR) rat lines model the polygenic individual differences in human vulnerability to diet-induced obesity[12–14]. When fed a high-fat diet, DIO rats become fatter than DR rats, which do not gain excess weight or body fat[12–14]; in contrast, young adult DIO rats remain lean when fed low-fat food, per a gene-environment interaction[14, 15]. The effects of high-fat diet on body weight and metabolism in genetically-selected DIO rats are well-studied[4, 12, 13, 16], but it remains unclear whether DIO rats eat differently than do DR rats. The microstructure of intake[15, 17] can provide key insights into the controls of feeding[18, 19] and has been linked to body composition; in humans, increased meal size and decreased meal frequency are putative risk factors for obesity[7–11]. Still lean, but obesity-prone DIO rats show a snacking-like microstructure pattern when fed a regular chow diet, consuming more, but smaller, meals than chow-fed DR rats[15]. It is unknown, however, whether these two genetic animal lines eat differently when chronically fed a high-fat diet, a key question given that the lines' weight and adiposity differ as young adults only when challenged by high-fat diet. The first aim of the present study was to test the hypothesis that the microstructure of food intake differs between high-fat diet-fed DIO and DR rats.

Relative to lean individuals, obese individuals are resistant to the appetite suppressant and weight-loss promoting properties of several anorexigens, including leptin and central insulin[20–26]. Resistance states perpetuate obesity; potential weight-reducing

pharmacotherapies must engage substrates downstream of or parallel to the signaling resistance. The urocortin (Ucn)/corticotropin-releasing factor 2 receptor (CRF_2) system is a potential therapeutic target for overeating and obesity[15, 27–35]. Ucns and CRF_2 receptors are co-distributed in feeding-regulatory hypothalamic nuclei and the nucleus of the solitary tract[36]. Central administration of Ucn 2 and Ucn 3, endogenous CRF_2 agonists, suppress food intake at doses that do not elicit malaise- or anxiety-like behavior[15, 31–33, 37, 38]. Moreover, CRF_2 knock-out (KO) mice eat larger meals,[29] with increased nocturnal intake of sweet chow[29] and high-fat food[30] vs. wildtype mice.[16, 39, 40]. Ucn 2 retains its maximal anorectic efficacy, in chow-fed, lean DIO rats[15], unlike leptin and insulin[16, 38, 39]. The anorectic effectiveness of CRF_2 agonists in high-fat diet-fed, obese DIO rats is unknown. The second aim of the present study was therefore to test the hypothesis and microstructure mechanism by which central administration of Ucn 2 decreases intake of high-fat food similarly in obese genetically-selected DIO as in lean DR rats.

Materials and methods

Please see Supplementary Material for additional details.

Subjects

Male Diet-Induced Obesity (DIO) (*n*=10) and Diet Resistant (DR) (*n*=10) rats, descendants of the original DIO and DR rat colonies (Levin et al., 1997), were born at The Scripps Research Institute. Rats were maintained in a 12:12 hr reverse-lighting cycle in a humidity- and temperature-controlled vivarium. Rats had access to LM-485 Diet 7012 chow (65% [kcal] carbohydrate, 13% fat, 21% protein; 3.1 kcal/g; Harlan Teklad, Indianapolis, IN) and water *ad libitum* before experiments. Procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication number 85–23, revised 1996) and the “Principles of laboratory animal care” and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Drugs

Rat Urocortin 2 (Ucn 2) and angiotensin II, generously provided by Dr. Jean Rivier (The Salk Institute, La Jolla, CA), were synthesized as previously described[41, 42]. Ucn 2 and angiotensin II were dissolved in 0.5X PBS and 1X PBS (pH=7.4), respectively.

Intra-cranial surgery and injection procedures

Because type 2 urocortins can suppress food intake via hypothalamic sites of action[15, 31], stainless steel cannulae were stereotaxically implanted in isoflurane-anesthesized rats to target the third ventricle (3v; interaural flat-skull; anterior/posterior −0.8 mm from bregma; dorsal/ventral: −3.5 mm from skull)[1543].

For testing, drug solutions or vehicle (2 μl) were injected over 90 sec with a Hamilton microsyringe linked by PE 20 tubing to a 31-gauge injector projecting 3 mm beyond the guide cannula. Injectors were left in place for 1 min. Placement was functionally verified post-study as a positive dipsogenic response (>5 ml water intake/30 min) to 3v angiotensin II (100 ng/2 μl).

Microstructural analysis of ingestion

Apparatus—To study the microstructure of ingestion, rats were individually housed in previously described Plexiglas test cages[17, 4444, 45]. Rats obtained individual 45-mg pellets from an automated, photocell-monitored dispenser (Med Associates, St. Albans VT). Rats were first trained using a chow diet (45 mg precision 5TUM: carbohydrate 65.5% (kcal), fat 10.4%, protein 24.1%, 3.7 kcal/g; Test Diet/Purina Mills, Inc., Richmond, IN, USA), but ultimately tested with a high-fat diet. The microstructure high-fat diet (F56381: fat 34.9% [kcal], carbohydrate 46.4%, protein 18.7%, 4.2 kcal/g; Bioserv, Frenchtown, NJ) was a 45-mg precision-pelleted variation of the high-fat diet that rats consumed in their home cages. Water delivery (0.1 ml) into a reservoir was governed by a response-contingent solenoid activated by nose-poke interruption. Post-reinforcement timeout intervals (3.25 and 1 sec for food and water, respectively) prevented duplicate reinforcement[15].

Study design

Beginning from 50 days of age, DR and DIO rats were provided only a high-fat diet in their home cages (D12266B) unless otherwise specified. Rats resided in and learned how to obtain food and water in the microstructure enclosures beginning from 110–120 days of age. After establishing stable daily food and water intake (12–13 sessions), rats remained in their home cages for chronic diet exposure. At 218 days of age, rats were implanted with the 3v guide cannula and allowed to recover for one week. Microstructure housing resumed at 226 days of age. After re-attainment of stable food intake (12–13 sessions), rats were provided high-fat diet in the enclosure. After high-fat diet intake stabilized (<15% food intake variation across 3 consecutive days), spontaneous baseline high-fat diet intake and meal microstructure of DIO vs. DR high-fat rats were measured at 241 and 242 days of age. To determine the effects of acute central Ucn 2 administration on high-fat diet intake, rats then received 3v doses of 0, 0.1, 0.3, 1, or 3 μg 10 min before testing. Based on previous studies[15, 32, 33], these infusions were given using a within-subject Latin square design with 1–2 intervening treatment-free days beginning from 244 days of age. Food and water intake were monitored as nose-poke responses for 23.5 hr.

Meal pattern analysis—Microstructure analysis used a meal definition that recognizes the existence of prandial drinking within meals[45] and was performed as previously described[15]. Meals were defined to contain at least 0.09 g of food (2 pellets). Parameters included meal frequency; the average size, duration and ingestion rate of meals; and the average intermeal interval (IMI).

Within-meal microstructure analysis—To identify differences between high-fat diet-fed DIO vs. DR rats in the *rate* and *regularity* of sustained eating *within* meals, analysis of the log-normal(ln)-transformed duration of consecutive (uninterrupted by drinking) within-meal interfeeding intervals (IFIs) was performed[15, 17]. The mean, standard deviation, kurtosis, skewness and histogram entropy of the ln-transformed duration of each subject's consecutive IFIs was individually determined and averaged across subjects.

Fat pad and body composition analysis

Two days after completing the Ucn 2 study, animals were euthanized (262 days of age). Frozen carcasses were shipped to the University of Alabama at Birmingham for fat pad measurement and chemical analysis of eviscerated body composition[46].

Statistical analysis

To analyze the time course of ingestion, analyses of variance (ANOVAs) were performed on incremental (1-hr bins) food intake, averaged from the two baseline days. Genotype was a between-subjects factor and Time a within-subject factor. Student's *t*-tests identified genotype differences in spontaneous meal microstructure. To compare fat measures, analysis of covariance (ANCOVA) was used[47], with the fat measure as the dependent measure, genotype as the between-subjects factor and non-fat mass as a covariate. To allow comparison with other work, fat measures also were compared as a percentage of body weight by Student's *t* test.

To assess Ucn 2 anorexia, a three-way mixed ANOVA was performed on incremental (1-hr bins) food intake. Dose and Time were within-subject factors, and Genotype was a between-subjects factor. Meal microstructure measures were analyzed by 2-way (Dose and Genotype) ANOVAs. Linear contrasts determined whether Ucn 2 exerted a log-linear, dose-dependent effect. IMI durations were ln-transformed[15, 45, 48].

To interpret main effects, *post hoc* pairwise comparisons were performed within the general linear model. The software packages used were Systat 11.0 (SPSS, Chicago, IL, USA), Excel 2003 (Microsoft, Redmond, WA, USA), SigmaPlot 11.0 (Systat Software, Inc., Point Richmond, CA, USA), and InStat 3.0 (GraphPad, San Diego, CA, USA).

Results

Spontaneous food intake in high-fat diet fed DR and DIO rats

Time course of ingestion—Time course analysis of nocturnal food intake revealed no Genotype [$F(1,18)=0.04$, $p=0.84$] or Hour*Genotype effects [$F(11,198)=1.42$, $p=0.17$] (Figure 1A). While no Genotype effect was seen on diurnal intake [$F(1,18)=0.39$, $p=0.56$], an Hour*Genotype interaction [$F(11,198)=3.26$, $p<0.001$], reflected that DIO rats ate less than DR rats during the first hour of the light cycle (M±SEM 2.2±0.6 g vs. 0.3±0.2 g, $t(18)=3.1$, $p<0.01$), but then progressively compensated and attained control levels of total intake by the end of the light cycle (Figure 1A). Thus, consistent with previous findings[49], DIO rats ultimately ate as much high-fat diet as DR rats across each phase of the day (Supplementary Table 1).

DR and DIO rats also were similar in their duration of nocturnal feeding (Supplementary Table 1). In contrast, DIO rats drank less water than DR rats in both quantity [~1/3; $t(18)=4.6$, $p<0.001$] and duration [~1/2; $t(18)=3.3$, $p<0.01$] during the dark cycle. During the light cycle, despite eating the same amount, DIO rats spent less time eating [$t(18)=2.3$, $p<0.05$] and drank less water [$t(18)=3.5$, $p<0.01$] than DR rats (Supplementary Table 1).

Meal pattern—Although DR and DIO rats consumed the same amount of high-fat diet across 24 hr, their microstructures of food intake profoundly differed. DIO rats ate ~1.2–1.4 g more food per meal [Dark Cycle: $t(18)=2.2$, $p<0.05$; Light: $t(18)=2.5$, $p<0.001$, Figure 1C] and ate faster within meals [Dark: $t(18)=2.9$, $p<0.01$; Light: $t(18)=2.5$, $p<0.05$, Figure 1D], yielding meals of similar duration [Dark: $t(18)=0.4$, *n.s.*; Light: $t(18)=1.3$, *n.s.*, Figure 1E]. DIO rats ate fewer meals than DR rats [Dark: $t(18)=3.0$, $p<0.01$; Light: $t(18)=2.9$, $p<0.01$, Figure 1F], with ~40 min longer nocturnal post-meal intervals [Dark: $t(18)=2.9$, $p<0.01$; Light: $t(18)=0.3$, *n.s.*, Figure 1G].

Rate and regularity of eating within meals—Within meals, DIO rats had ~1.2–1.5 sec faster pellet-to-pellet intake than did DR rats [$t(18)=3.1$, $p<0.01$, Table 1]. In Figure 1B, the faster mean rate of eating by DIO rats is seen as a disproportionate left-shift towards briefer IFIs, resulting in a significantly more positive skew [$t(18)=2.3$, $p<0.05$] and decreased kurtosis vs. DR rats; [$t(18)=2.7$, $p<0.02$]. The genotypes did not differ significantly in their regularity of eating, as measured by the standard deviation or histogram entropy of IFIs [standard deviation: $t(18)=1.8$, *n.s.*; entropy: $t(18)=2.0$, *n.s.*].

Body weight, fat pad and body composition analysis

Table 2 shows the body weight progression of DR and DIO rats, their rate of weight gain, daily energy intake, and feed efficiency during the first month of high-fat diet feeding, and their terminal adiposity. Similar to previous findings[14], DIO rats ate more, gained more weight and were more feed efficient vs. DR rats during the first 8 days of high-fat access. During the subsequent ~3 weeks, daily food intake no longer differed significantly between the two genotypes, yet DIO rats still gained weight faster than DR rats, reflecting increased feed efficiency. At study end, DIO rats had heavier fat pads and more total carcass fat (Supplemental Table 3). ANCOVA analyses controlling for non-fat mass indicated that, other than for gonadal fat ($p=0.58$), the relative fat pad and whole carcass fat masses of high-fat diet-fed DIO rats were disproportionately increased vs. DR rats (see Table 2 for adjusted means, covarying for non-fat mass). The sometimes problematic[47], but popular, method of expressing fat mass as a % of body weight (Table 2) also indicated greater relative adiposity. Adiposity values of DR and DIO rats were consistent with lean and obese states[13, 15], respectively.

Ucn 2 anorexia in high-fat diet fed DR and DIO rats

Effects of Ucn 2 on high-fat food and water intake: Time course—Figure 2A shows that third ventricle injection of Ucn 2 reduced nocturnal food intake of high-fat diet-fed DR and DIO rats [Dose: $F(4,72)=11.12$, $p<0.001$]. Although visual inspection of Figure 2A might suggest that Ucn 2 tended to reduce food intake more potently in DR rats compared with DIO rats, statistical analyses consistently indicated a main effect of Ucn 2 across genotypes, with no significant (or even trends for) Dose X Genotype interactions. Ucn 2 retained its full central anorectic efficacy in obese DIO rats; the highest dose injected (3 μg) induced similar anorexia in both genotypes (% reduction after 12-hr: $M\pm$SEM, DR 38.5%±7.0, DIO 41.3%±10.4). Cumulative anorexia was greatest at the end of the dark cycle (Figure 2A, inset), so microstructure analyses were performed across this period.

Cumulative anorexia persisted through the light cycle (not shown), with no compensation or rebound.

As Table 3 shows, Ucn 2 reduced the quantity [Dose: $F(4,72)=10.46$, $p<0.001$] and duration [Dose: $F(4,72)=7.26$, $p<0.001$] of prandial nocturnal food intake irrespective of genotype [Dose*Genotype: $F(4,72)<0.64$, *n.s.*]. Effects were dose-dependent per linear contrast ANOVAs. The minimum effective dose (MED) that reliably reduced the quantity and duration of food intake was 0.3 μg. Irrespective of Ucn 2 treatment, DIO rats ate the same amount of food as DR rats [Genotype: $F(1,18)=0.40$, *n.s.*], but in less time [Genotype: $F(1,18)=13.40$, $p<0.01$].

Table 3 shows that Ucn 2 also potently (MED=0.1 μg) decreased the duration [Dose: $F(4,72)=2.75$, $p<0.05$] and quantity [Linear contrast Dose ANOVA: $F(1,18)=5.75$, $p<0.05$] of water intake, irrespective of genotype [Dose*Genotype: $Fs(4,72)<1.39$, *n.s.*]. Genotype effects again showed that DIO rats drank less [$F(1,18)=19.15$, $p<0.001$] and spent less time drinking than DR rats [$F(1,18)=6.64$, $p<0.02$].

Effects of Ucn 2 on high-fat diet intake: meal pattern and within-meal microstructure—Figure 2C, D, E and Supplementary Table 2 show the effects of Ucn 2 on the microstructure of feeding. Irrespective of genotype [Dose*Genotype: $Fs(4,72)<0.85$, *n.s.*], Ucn 2 dose-dependently made rats eat smaller [Dose: $F(4,72)=6.87$, $p<0.002$] and briefer meals [Dose: $F(4,72)=4.95$, $p<0.002$] during which they ate more slowly [Dose: $F(4,72)=7.37$, $p<0.001$] (Figure 2). In contrast, Ucn 2 did not affect the post-meal interval [Dose: $F(4,72)=0.77$, *n.s.*; Dose*Genotype: $F(4,72)=0.64$, *n.s.*] or meal frequency [Dose: $F(4,72)=1.87$, *n.s.*; Dose*Genotype: $F(4,72)=0.20$, *n.s.*] (Supplementary Table 2). Genotype effects again showed that DIO rats ate fewer [$F(1,18)=15.85$, $p<0.001$], but larger [$F(1,18)=5.26$, $p<0.05$], meals with longer post-meal intervals [$F(1,18)=4.34$, $p=0.05$] than their diet-resistant counterparts. DIO rats also again ate more rapidly [$F(1,18)=15.91$, $p<0.001$] within meals, which were of the same duration as those of DR rats [$F(1,18)=0.05$, *n.s.*]. But, Ucn 2-treated DIO rats ate meals that no longer differed from those normally taken by obesity-resistant DR rats under vehicle-treated conditions (DIO 1 ug and 3 ug = 2.8±0.6 and 3.3±0.5 g vs. DR-vehicle 3.0±0.3 g, ps>0.57).

In contrast, Ucn 2 did not alter the amount or duration of water intake within a meal [Dose: $Fs(4,72)<1.50$, *n.s.*; Dose*Genotype: $Fs(4,72)<0.79$, *n.s.*] and even tended to increase the speed of drinking, irrespective of genotype [Dose*Genotype: $F(4,72)=1.10$, *n.s.*], at intermediate (0.3, 1.0 μg) doses [Dose: $F(4,72)=3.36$, $p<0.02$] (Supplementary Table 2). Genotype effects indicated that DIO rats again drank less water within each meal [$F(1,18)=8.89$, $p<0.01$] and drank more slowly than did DR rats [$F(1,18)=20.05$, $p<0.001$] (Supplementary Table 2).

As shown in Table 4, Ucn 2 treatment (1 μg) increased the mean duration of inter-pellet intervals in within-meal microstructure analysis [Dose: $F(4,72)=4.09$, $p<0.006$]. Moreover, Ucn 2 infusion (1 μg) also reduced the categorical regularity of pellet-to-pellet responding as revealed by increased histogram entropy [$F(4,72)=3.82$, $p<0.01$]. In contrast, Ucn 2 did not reliably alter the standard deviation of inter-pellet intervals or the skewness or kurtosis of

their distribution [*Fs*(4,72)<1.96, *n.s.*]. No effects of Ucn 2 on within-meal microstructure differed reliably by Genotype [Dose*Genotype: *Fs*(4,72)<1.80, *n.s.*]. Genotype main effects again indicated that DIO rats had disproportionately faster pellet-to-pellet intake, reflected in a briefer mean IFI [*F*(1,18)=4.49, *p*<0.005] and more positive skewness [*F*(1,18)=14.19, *p*<0.003], with no reliable difference in the other within-meal parameters [*Fs*(1,18)<1.18, *n.s.*].

Discussion

The major findings of the present study were as follows: 1) 3v administration of the CRF_2 agonist Ucn 2 retained anorectic activity in obese, high-fat diet fed DIO rats. The peptide potently reduced nocturnal food intake in both genotypes at a minimum effective dose (MED) of 0.3 μg, with similar maximal efficacy between genotypes; 2) 3v Ucn 2 reduced high-fat diet intake by making rats of both genotypes eat smaller meals that they ate more slowly; and 3) obese, high-fat diet fed DIO rats, showed a “gorging pattern” of food intake, characterized by few, but very large and more quickly eaten, meals and comparatively little water intake.

Ucn 2 retains central anorectic activity in obese high-fat diet fed DIO rats

Ucn 2 infused into the third ventricle dose-dependently decreased high-fat diet intake not only in lean DIO rats, but also in obese DIO rats. Irrespective of genotype, the peptide reduced nocturnal food intake at a minimum effective dose of 0.3 μg (~ 64 pmol). Although we cannot rule out that a larger study might have revealed subtle differences in dose sensitivity between the genotypes, the maximal efficacy of Ucn 2 was unimpeded in the DIO line, with similar maximal suppression of food intake (~40%) seen at the 3 μg dose. Consistent with previous observations in chow-fed rodents[15, 32], intraventricular Ucn 2 administration elicited a slightly delayed (~2 hours), but prolonged, anorectic action. Ucn 2 did not change the timing of when meals were taken, but rather reduced the gorge-like nature of high-fat meals, making rats eat smaller meals that were eaten more slowly. Because Ucn 2 treatment similarly influenced both genotypes, the overall meal pattern of DIO rats still differed from that of DR rats following Ucn 2 treatment. But, Ucn 2 treatment normalized the meal size of DIO rats to that normally eaten by obesity-resistant DR rats.

The mechanism by which 3v Ucn 2 reduced food intake was not explored in the present study, but accumulated results indicate behaviorally-specific actions. Ucn 2 hypodipsia[15, 32] was dissociable from anorexia because some Ucn 2 doses that reduced high-fat diet intake in DIO rats (e.g., 0.3, 1 μg) did not reduce their concurrent water intake. Similarly, as in previous studies[15, 36], reductions in drinking rate were not seen. Furthermore, the present intraventricular Ucn 2 doses do not promote anxiety-like behavior or the formation of malaise-like behavior, such as pica or a conditioned taste aversion in outbred rats[36]. Local administration of CRF_2 agonist into the CRF_2-rich ventromedial hypothalamus (VMH), a key brain site that controls food intake and energy metabolism[50, 51], also reduces food intake[31, 34]. Thus, the present results may reflect actions of 3v Ucn 2 at VMH CRF_2 receptors, but we cannot exclude roles for other brain sites at which Ucns can reduce food

intake and/or slow gastric emptying[38, 52], including the paraventricular nucleus of the hypothalamus[31], the lateral septum[53], or the dorsal vagal complex[31, 53, 54].

Our results further implicate Ucns as promising pharmacological tools to treat obesity or overeating, putatively via CRF_2 activation[36]. For example, Ucn 1 administration reduced food intake not only in lean, but also in *ob/ob* obese mice[55, 56], and Ucn 1 potentiated molecular responses to leptin, providing a potential means of surmounting leptin resistance in obesity[57]. ICV Ucn 2 administration reduced the overeating of palatable cafeteria diet under an intermittent access schedule[33]. Finally, mice deficient in Ucn 3 or VMH CRF_2 expression are hyperphagic[34].

Genotype differences in high-fat meal microstructure of DR and DIO rats

Meal patterns associate with body composition in humans and may play a causal role in obesity[7–9]. Consistent with previous findings, DIO rats only transiently overate high-fat diet, with intake levels eventually declining to those of DR rats; yet they continued to gain excess weight and become obese[49]. Microstructure analysis revealed profound differences in how the two genotypes ate, however. Relative to their obesity-resistant counterparts, DIO rats ate ~1.2–1.4 g more food per meal and ate more quickly within meals (~1.2–1.5 sec faster pellet-to-pellet intake). These gorging-like meals were ~40 min longer apart. One interpretation of these findings is that, relative to DR rats, obese DIO rats may show decreased within-meal satiation for high-fat diets, leading to larger meals that sustain an increased post-meal interval. Obese DIO rats also drank ~2/3 less water during meals than did lean DR rats. Perhaps the decreased prandial water intake of DIO rats contributes to their decreased within-meal satiation. Accordingly, in humans, drinking water reduces test meal intake and promotes weight loss[58, 59].

Unlike results from the present study with high-fat diet, we previously observed that chow intake in lean DIO rats was characterized by more, but smaller, meals as compared to chow-fed DR rats, resembling human “snacking” behavior[15, 60]. If results from the two studies are combined, high-fat diet induced a gorging-like pattern of food intake in both genotypes. Specifically, high-fat diet doubled the meal size of DR rats (from ~9 to ~17 kcal) and tripled that of DIO rats (from ~7 to ~22 kcal). Conversely, meals were taken less frequently (DIO: from ~11 to ~4 meals; DR: from ~8 to ~ 6 meals,) with longer post-meal intervals (DIO: from ~59 to ~153 min; DR: from ~90 to ~113 min; Supplementary Table 4). The results support reports that high-fat diets increase meal size, but decrease meal frequency, as compared to a balanced diet[7, 61, 62] and descriptively suggest that this effect may be especially pronounced in obesity-prone DIO rats.

The gorge-like meal pattern induced by high-fat diet may be more relevant to the development of obesity in the DIO line than the snacking-like pattern previously seen with chow access because DIO rats do not become obese when fed chow diet. Accordingly, eating infrequent, large meals has been hypothesized to promote obesity, perhaps due to effects of this meal pattern on metabolism[7–11]. In humans, intentionally consuming more frequent, but smaller meals, reduced body weight as compared to consuming the same calories in larger and infrequent meals[63]. Conversely, intentionally decreasing the number of

meals facilitates fat mass accumulation[9]. These causal observations are consistent with correlational findings in animal models[7, 61, 64].

Meal patterns in the present study were measured after chronic high-fat diet exposure, which resulted in obesity in DIO rats. The relative contributions of DIO genotype vs. obesity to the meal pattern differences are thereby uncertain. Because high-fat diet rapidly increases meal size and decreases meal frequency[7], we believe that high-fat diet, interacting with genotype, underlies the present meal pattern findings. Future studies that compare the high-fat diet intake patterns of still lean DIO rats vs. obese DIO rats can address the contribution of the obese state.

Obese individuals exhibit *resistance* to many anorectic agents, including leptin, insulin, cholecystokinin and mu-opioid receptor antagonists[20–26, 65–70]. Analogously, DIO rats are resistant to leptin and insulin both in a pre-obesity state and after the development of obesity[16, 39, 40]. Unlike other anorexigens, Ucn 2 retains its full central anorectic efficacy to reduce high-fat diet intake even in obese, genetically-prone DIO rats. These results open new potential opportunities of investigation towards treating some forms of diet-induced obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This is manuscript 21959 from The Scripps Research Institute. We recognize the editorial assistance of Michael Arends, the administrative assistance of Mary Gichuhi, and the technical assistance of Dahai Jung, Jeanette Helfers, Molly Brennan, and Bob Lintz. We thank Jean Rivier (The Salk Institute) for generously providing the peptides. This research was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK; DK026741, DK070118, DK076896 (EPZ) and DK30066 (BEL)), the National Institute on Drug Abuse (NIDA; DA023680 and DA030425 (PC)), the National Institute on Alcohol Abuse and Alcoholism (NIAAA; AA016731 (VS)), the National Institute of Mental Health (NIMH; MH091945 (PC) and MH093650 (VS)), the University of Alabama at Birmingham Small Animal Phenotyping Core (P30DK056336 and P60DK079626 (TRN)) and the Research Service of the Veterans Administration.

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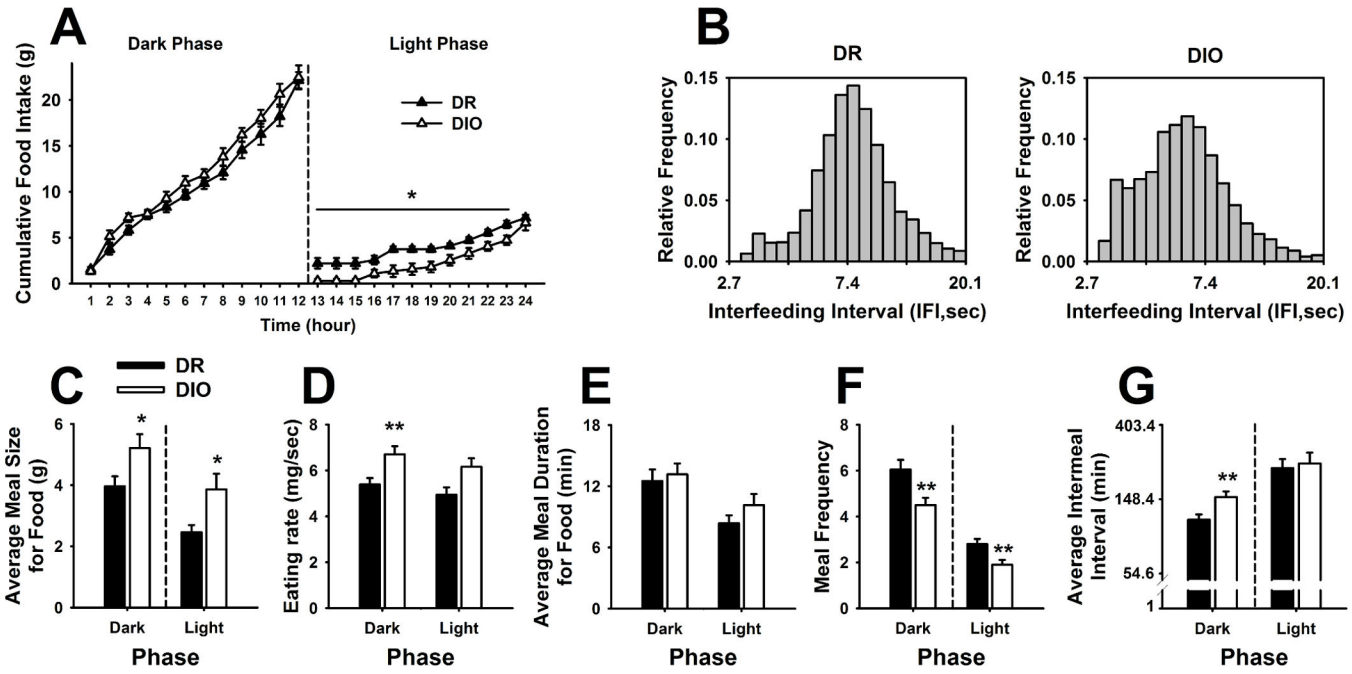

Figure 1.

Spontaneous food intake, within-meal microstructure and meal pattern differences between genetically-selected diet-induced obesity-resistant (DR) and susceptible (DIO) male rats (n=10/genotype), fed a high-fat diet. Data represent the M±SEM. (**A**) Cumulative nocturnal (left panel) and diurnal (right) food intake. (**B**) Relative frequency histogram of the ln-transformed duration of consecutive, within-meal interfeeding intervals (IFI's) in male genetically-selected diet-induced obesity-resistant (DR) (left panel) and susceptible (DIO) rats (right panel) during the dark cycle. The frequency histogram shows consecutive interfeeding intervals that were between e^1 and e^3 sec in duration (2.7–20.1) with a bin width of $e^{0.1}$. This time scale focuses on the intervals of *sustained* eating, as represented in the peak. The tail that extends to the right of the distribution putatively represents within-meal pauses. Note ln-scale of x-axis. (**C–G**) Spontaneous meal microstructure differences (**C**) average meal size for food, (**D**) eating rate, (**E**) average meal duration for food, (**F**) meal frequency, and (**G**) average intermeal interval (note ln scale of y-axis for intermeal interval duration, reflecting their time scale). Symbols denote significant genotype differences,* p<0.05, ** p<0.01 (Student's t-test).

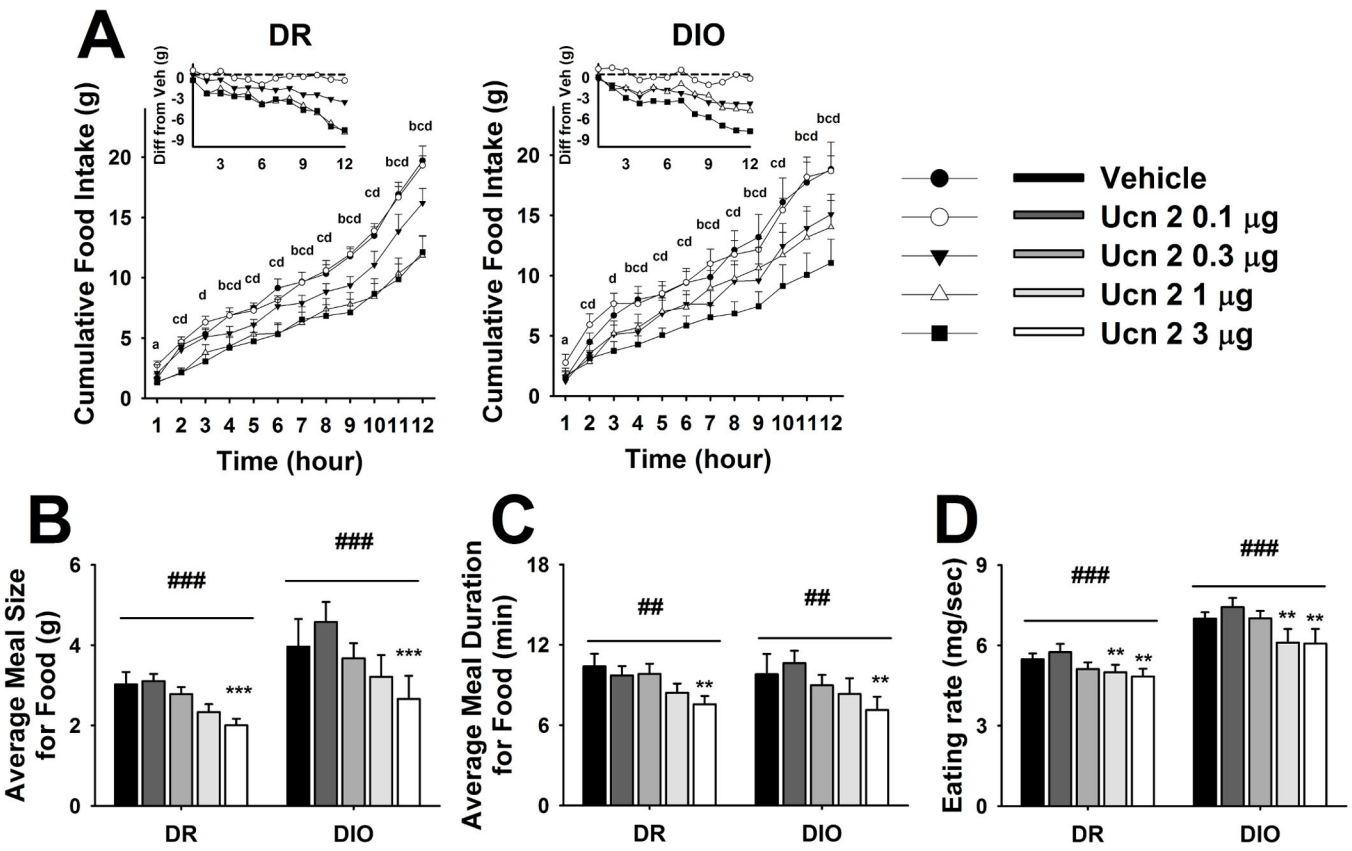

Figure 2.

Dose-dependent effects of third ventricle Ucn 2 administration on the *M*±SEM: (**A**) cumulative nocturnal food intake of genetically-selected (left panel) diet-induced obesity-resistant (DR) and (right panel) susceptible (DIO) rats fed a high-fat diet; (**B**) average meal size for food, (**C**) average meal duration for food and (**D**) eating rate. Adult male rats (*n*=10 rats/genotype) were pretreated (−10 min) with Ucn 2 in a balanced Latin square design with test sessions beginning at the onset of the dark cycle. Inset depicts mean the cumulative difference from vehicle condition. In (**A**) scale in inset differs from that of main panel. Symbols denote significant differences of the vehicle condition from (a) 0.1 µg, (b) 0.3 µg, (c) 1 µg, (d) 3 µg doses. #Overall Dose effect $p<0.05$, ##$p<0.01$, ###$p<0.001$. $Linear Dose effect $p<0.05$, $$$p<0.01$, $$$$p<0.001$; *differs from vehicle condition $p<0.05$, **$p<0.01$, ***$p<0.001$ (within-subjects ANOVA *post hoc* contrast for that dose).

Table 1

Differences in the spontaneous rate and regularity of food pellet consumption within meals in high-fat diet-fed genetically-selected DR and DIO rats

Interfeeding Interval Parameter	Dark Phase		Light Phase	
	DR	DIO	DR	DIO
Number of IFIs	475±20	488±28	150±8	143±18
Mean duration, ln sec (sec)	2.04 (7.68) ±0.07	1.81 (6.12) ±0.05 **	2.07 (7.95) ±0.07	1.91 (6.73) ±0.06
Standard deviation	0.30±0.01	0.33±0.01	0.32±0.01	0.34±0.01
Skewness	−0.04±0.23	0.55±0.13 *	−0.18±0.30	0.40±0.19
Kurtosis	1.87±0.37	0.71±0.21 *	2.03±0.47	0.75±0.36 *
Entropy	0.39±0.01	0.41±0.01	0.48±0.01	0.53±0.03

Rate and regularity of eating within meals in genetically-selected diet-induced obesity-resistant (DR) and susceptible (DIO) rats (n=10/genotype) fed a high-fat diet. Statistical parameters (expressed as M±SEM) describe the log-normal distribution of consecutive, within-meal interfeeding intervals (IFI's) studied on two consecutive days at 241–242 days of age. Parameters were calculated from the ln-transformed duration of interfeeding interval durations. Therefore, the mean and SEM are expressed in ln(sec) units; the parenthetical value for the "Mean" parameter represents the back-transformed average (sec) to facilitate interpretation. For analysis, histograms were constructed from log-transformed IFI that fell from e^1 to e^3 sec (~2.7–20.1 sec), with a bin width of $e^{0.1}$.

Symbols denote significant differences: * p<0.05 compared to DR rats, p<0.01, (Student's t-test).

Table 2

Body weight, food intake, feed efficiency and adiposity in high-fat diet-fed genetically-selected DR and DIO rats

Parameter			DR	DIO
Body weight, g				
	Day 50, First measurement		204.1±4.1	207.3±10.3
	Day 58		252.4±2.8	276.3±13.0 *
	Days 50–58	Daily body weight gain, g	6.0±0.3	8.6±0.5 ***
		Daily food intake, kcal	80.5±1.3	95.2±4.4 **
		Feed efficiency, mg weight gain/kcal	75.2±4.3	90.6±3.6 *
	Day 82		332.2±4.9	408.0±15.2 ***
	Days 59–82	Daily body weight gain, g	3.3±0.1	5.5±0.3 ***
		Daily food intake, kcal	70.6±4.6	77.9±2.1
		Feed efficiency, mg weight gain/kcal	48.8±3.5	70.7±3.9 **
	Day 104, Pre-training		387.2±9.1	486.7±16.8 ***
	Day 241–242, Baseline		463.8±11.3	612.6±20.0 ***
	Day 262, Study completion		477.5±13.2	619.8±18.6 ***
Fat Pads				
	White fat pad, g			
	Total		37.2±15.4	113.4±15.4 *
		Inguinal	10.2±3.7	31.0±3.7 **
		Gonadal	8.3±1.7	14.2±1.7
		Retroperitoneal	5.4±5.0	30.0±5.0 *
		Mesenteric	8.2±2.2	16.0±2.2 *
		Subcutaneous	5.2±3.3	22.1±3.3 **
	Brown fat pad, g		0.60±0.07	0.85±0.07 *
	White fat pad, % body weight			

Parameter			DR	DIO
	Total		9.2±0.5	16.9±2.0 ***
		Inguinal	2.5±0.1	4.7±0.5 ***
		Gonadal	1.7±0.1	2.3±0.2 *
		Retroperitoneal	1.9±0.1	4.1±0.7 **
		Mesenteric	1.5±0.1	2.7±0.3 ***
		Subcutaneous	1.6±0.2	3.1±0.5 ***
	Brown fat pad, % body weight		0.12±0.01	0.14±0.01 *
Whole Carcass	**Adiposity**			
	Fat, g		42.0±17.2	126.7±17.2 *
	Fat, % body weight		10.3±0.6	19.9±2.3 ***

Body weight, body weight gain, food intake, feed efficiency and adiposity in high-fat diet fed DR and DIO rats (n=10 rats/genotype). Feed efficiency was calculated as mg body weight gained/kcal energy intake. Weights (g) of fat pad and whole carcass fat (g) reflect the adjusted least squares means, controlling for non-fat mass. Percent (%) fat pad and whole carcass percent (%) fat values reflect the fat masses expressed as a percent of body weight. Raw fat masses, uncorrected for lean body mass, are shown in Supplementary Table 3. Values are M±SEM.

Symbols denote significant differences: * p<0.05 compared to DR rats, ** p<0.01, *** p<0.001, (Student's t-tests, except fat pad weights [g], which reflect ANCOVA, covarying for non-fat mass).

Table 3

Effects of 3v Ucn 2 and genotype on nocturnal prandial intake in high-fat diet-fed genetically-selected DR and DIO rats

Parameter		DR	DIO
Feeding			
	Intake (g)		
	0 μg	20.9±0.9	18.8±2.3
	0.1 μg	19.7±0.9	18.7±1.3
	0.3 μg	16.2±5.1 *	15.1±1.7 *
	1 μg	13.6±1.3 ***	14.4±2.3 ***
	3 μg	12.4±1.3 ***	11.7±2.2 ***
	Duration (min) ##		
	0 μg	64.0±3.2	44.5±5.3
	0.1 μg	58.2±3.5	42.4±3.1
	0.3 μg	53.9±17.0 *	36.4±4.1 *
	1 μg	45.8±3.5 ***	36.7±5.4 ***
	3 μg	44.0±4.9 ***	30.6±4.5 ***
Drinking			
	Intake (ml) ###		
	0 μg	17.4±2.9	4.9±1.0
	0.1 μg	13.8±1.9 *	3.8±0.8 *
	0.3 μg	14.8±4.7	4.8±0.6
	1 μg	11.4±1.8	4.9±0.9
	3 μg	10.9±1.9 *	4.0±0.7 *
	Duration (min) #		
	0 μg	30.3±3.2	18.2±3.7
	0.1 μg	23.8±2.9 *	15.8±3.6 *
	0.3 μg	21.1±6.7 *	13.1±2.7 *
	1 μg	19.6±3.3 *	15.4±2.9 *
	3 μg	22.3±3.5 *	11.9±2.2 *

Effect of third ventricle Ucn 2 treatment and genotype on nocturnal prandial intake of genetically-selected diet-induced obesity-resistant (DR) and susceptible (DIO) rats (n=10/genotype) fed a high-fat diet. Data express the M±SEM quantity or duration of food and water intake within meals of adult male DR and DIO rats during the first 12 hr of the dark cycle following Ucn 2 pretreatment. Subjects were pretreated (−10 min) with Ucn 2 in a balanced Latin square design with test sessions beginning at the onset of the dark cycle.

Symbols signify: # Genotype main effect p<0.05, ## p<0.01, ### p<0.001 *differs from vehicle condition p<0.05, ***p<0.001 (within-subjects ANOVA *post hoc* contrast for that dose).

Table 4

Effects of 3v Ucn 2 and genotype on the rate and regularity of food pellet consumption in high-fat diet-fed genetically-selected DR and DIO rats

Interfeeding Interval Parameter	DR	DIO
Mean duration, ln sec (sec) ##		
0 μg	2.01 (7.5) ±0.04	1.77 (5.9) ±0.04
0.1 μg	1.96 (7.1) ±0.04 *	1.71 (5.5) ±0.05 *
0.3 μg	2.02 (8.2) ±0.04	1.77 (5.9) ±0.04
1 μg	2.10 (7.5) ±0.03 *	1.93 (6.9) ±0.12 *
3 μg	2.02 (7.5) ±0.05	1.81 (6.1) ±0.05
Standard deviation		
0 μg	0.33±0.01	0.29±0.02
0.1 μg	0.31±0.02	0.31±0.01
0.3 μg	0.33±0.02	0.32±0.01
1 μg	0.38±0.02	0.32±0.01
3 μg	0.36±0.02	0.36±0.03
Skewness ##		
0 μg	−0.04±0.17	0.61±0.16
0.1 μg	−0.02±0.19	0.77±0.10
0.3 μg	−0.17±0.18	0.66±0.08
1 μg	−0.22±0.18	0.50±0.19
3 μg	−0.03±0.22	0.53±0.15
Kurtosis		
0 μg	0.90±0.18	0.85±0.25
0.1 μg	1.22±0.28	0.56±0.14
0.3 μg	0.96±0.36	0.60±0.21
1 μg	0.37±0.18	0.63±0.20
3 μg	0.61±0.24	0.35±0.29
Entropy		
0 μg	0.41±0.01	0.47±0.06
0.1 μg	0.40±0.01	0.42±0.01
0.3 μg	0.42±0.01	0.44±0.01
1 μg	0.49±0.03 **	0.49±0.06 **
3 μg	0.46±0.01	0.49±0.03

Effect of third ventricle Ucn 2 treatment and genotype on the rate and regularity of eating within meals in genetically selected diet-induced obesity-resistant (DR) and susceptible (DIO) rats (n=10/genotype) fed a high-fat diet during the first 12 hr of the dark cycle following Ucn 2 pretreatment. Subjects were pretreated (−10 min) with Ucn 2 in a balanced Latin square design with test sessions beginning at the onset of the dark cycle. Statistical parameters (expressed as M±SEM) describe the log-normal distribution of consecutive, within-meal interfeeding intervals (IFI's) studied on two consecutive days at 241–242 days of age. Parameters were calculated from the ln-transformed duration of interfeeding interval durations. Therefore, the mean and SEM are expressed in ln(sec) units; the parenthetical value for the "Mean" parameter represents the back-transformed

average (sec) to facilitate interpretation. For analysis, histograms were constructed from log-transformed IFI that fell from e^1 to e^3 sec (~2.7–20.1 sec), with a bin width of $e^{0.1}$.

Symbols signify: ## Genotype main effect $p<0.01$ *differs from vehicle condition $p<0.05$, ** $p<0.01$ (within-subjects ANOVA *post hoc* contrast for that dose).