Published in final edited form as:

Appetite. 2014 April ; 75: 21–29. doi:10.1016/j.appet.2013.12.004.

Analyses of meal patterns across dietary shifts

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Abstract

The direct controls of meal size can be categorized into positive signals such as those from the oral cavity and negative signals such as postoral inhibitory cues. It follows that the relative contribution of these signals, and in turn meal pattern parameters, change across periods of high-energy diet exposure. Here, we compared daily intake and meal pattern analysis in male Sprague-Dawley rats presented a high-energy diet for 6 weeks then standard chow for ~ 1 week (HE), with those of standard chow fed controls (CHOW). These measures allow for evaluation of 1) whether there are distinct dynamic and static phases of DIO and if so, how they are characterized, 2) how meal patterns change across short and long term HE experience, and 3) ingestive behavioral changes when HE-fed animals are returned to standard chow. The HE animals showed significantly higher intake primarily driven by an increase in meal size compared to CHOW controls. This was most pronounced during the first several days of high-energy diet exposure thus characterizing the dynamic phase. Intake and meal size decreased with longer exposure to the diet but remained significantly higher than those of CHOW. Increased meal size could be driven by enhanced orosensory stimulation and/or reduced sensitivity to postoral inhibitory feedback. Distribution curves derived from histogram plots of meal size revealed both larger average meal size (right shift) and spread (standard deviation) thus it is tempting to speculate that more than one type of mechanism influences increased meal size. Meal number decreased suggesting post meal inhibitory signaling is relatively intact. However, this increase was insufficient to compensate for the increased meal size. When HE animals were switched to standard chow, daily intake and meal size decreased and eventually returned to values comparable to those of the CHOW rats. Meal number remained lower suggesting altered physiological mechanism(s) that underlie the control of ingestive behavior as a function of previous high-energy diet exposure.

Keywords

high fat; meal size; meal number; palatability; postingestive; oral

Introduction

In the diet induced obesity (DIO) model, animals are presented an energy-dense highly palatable diet and consequently overeat and gain weight. This animal model provides an

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experimental analogy for the overconsumption of high fat/high energy foods that contribute to the obesity observed in the human population. From studies in which rats became obese following hypothalamic lesions, the development of obesity can be characterized by a rapid rate of weight gain, which has been referred to as the dynamic phase, followed by a static phase characterized by a plateau in weight gain change (see Brobeck, 1946). In DIO models, caloric intake and rate of body weight gain decreases if animals are returned to standard chow over the recovery phase. It follows that the changes in weight gain across the development of DIO may be mediated by changes in meal pattern attributes.

The direct controls of meal size can be categorized into positive and negative feedback that maintain and terminate eating behavior respectively (see Smith, 1996). Positive feedback is elicited by contact with gustatory, olfactory and somatosensory receptors in the oral cavity. Negative feedback is produced by contact with receptors in the oral cavity and postingestive receptors in the stomach and small intestine (Davis & Smith, 1990; Davis, Smith, & Miesner, 1993). Thus an increase in meal size may be attributed to increased orosensory stimulation and/or reduced sensitivity to postingestive inhibitory signals. For example, there is evidence of positive oral stimulation inducing an increase in meal size in human subjects (De Graaf, De Jong, & Lambers, 1999; Yeomans, 1996) and rats (Spector, Klumpp, & Kaplan, 1998; Warwick, Synowski, Rice, & Smart, 2003). Complementary to such findings, postoral inhibitory cues have also been shown to influence meal patterns (Lutz, Geary, Szabady, Del Prete, & Scharrer, 1995; Strohmer & Greenberg, 1994; West, Fey, & Woods, 1984). It follows that the changes in weight gain across the development of DIO are driven by alterations in the relative contributions of these orosensory and postoral signals, yet these issues remain poorly understood.

In hypothalamic-lesioned obese rats, meal pattern analysis reveals increased meal size during the dynamic phase when body weight gain is most pronounced, but meal size returns back to baseline levels during the static phase when body weight plateaus. In contrast, the number of meals does not significantly change across the phases (Brooks, Lockwood, & Wiggins, 1946; Teitelbaum & Campbell, 1958). In general high-fat diet driven hyperphagia also appears to be influenced by an increase in meal size (Cottone, et al., 2013; Farley, Cook, Spar, Austin, & Kowalski, 2003; Furnes, Zhao, & Chen, 2009; Melhorn, et al., 2010). Collectively, evidence in the literature points to changes in meal pattern parameters across the development of DIO but there are some conflicting findings. Thus it is unclear as to when the “dynamic” and “static” phases occur and what meal pattern changes characterize these phases.

Furthermore, animals that are switched from a palatable or energy-dense diet to a standard chow diet generally reduce food intake (Rogers, 1985; Rolls, Rowe, & Turner, 1980) and body weight (Stephens, 1980; Wang, et al., 2012). This has been reported to be accompanied by a number of changes including expression of genes associated with feeding and reward (Archer, et al., 2005; Sharma, Fernandes, & Fulton, 2012) and hypothalamic inflammatory markers (Wang, et al., 2012). Meal pattern analysis of this recovery period has been limited in the literature. One study in which rats were switched to standard chow following exposure to a cafeteria diet showed that animals initially reduced intake, meal size and meal frequency (Rogers, 1985). The manner in which meal patterns to standard chow alter as a function of previous exposure to a high-energy diet could be indicative of changes in the underlying physiological mechanisms of the behavior.

Here, meal patterns of rats switched to a high-energy diet were monitored across a period of several weeks and also after animals were returned to a standard chow diet. These meal pattern parameter values were compared to those of a control group that was maintained on standard chow throughout the study. These measures were systemically taken across

consecutive daily sessions to evaluate first, whether there are distinct dynamic and static phases of DIO, and if so, how meal pattern parameters characterize them. Second, the study compared how meal pattern parameters change as a function of short- and long-term exposure to a high-energy diet. Finally, meal pattern analysis addressed how ingestive behavior changes when animals are returned to a standard chow diet following several weeks on a high-energy diet. An increase in meal size would be indicative of an increase in positive feedback (e.g. oral stimulation) and/or a decrease in negative feedback (e.g. postoral inhibitory signal). The relative contributions of these signals would possibly be most robust during the initial hyperphagia observed upon presentation of a high-energy diet, less evident with continual exposure to high-energy diet and return to control levels when HE animals are switched back to standard chow.

Methods

2.1 Subjects

Sixteen male Sprague Dawley rats (Harlan) ~275–300 g in body weight upon arrival were single-housed in DietMax System food intake monitoring cages (AccuScan Instruments, Inc., Columbus, OH) (length 32 cm, width 22 cm) that have stainless steel wire mesh bottoms in polycarbonate plastic cages. Animals were presented with ad libitum access to powdered chow (3.1 kcal/g, calories from protein 24%, calories from fat 18%, calories from carbohydrate 58%; 2018 Teklad, Harlan) and water, except where noted. Rats were housed in a room with automatically controlled temperature (range 18–24°C), humidity (range 18–53%) and a 12:12-h light-dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at The Johns Hopkins University School of Medicine.

2.2 Behavioral Procedure

Following 7 days of habituation to the laboratory environment and maintenance on chow diet, animals were assigned to the CHOW or HE groups such that the groups did not significantly differ in body weight, average daily intake, number of meals per day or average meal size. On Day 0, both groups were on standard chow. On Day 1, the HE animals were switched to ad libitum access to powdered high energy diet (4.73 kcal/g; calories from protein 20%, calories from fat 45%, calories from carbohydrate 35%; D12451, Research Diets). The diet has ~7% calories from corn starch, ~10% calories from maltrodextrin and ~17% calories from sucrose that also promote intake. The CHOW controls remained on the powdered standard chow (3.1 kcal/g, calories from protein 24%, calories from fat 18%, calories from carbohydrate 58%; 2018 Teklad, Harlan). This continued for 42 consecutive days which will be referred to as the “experimental diet period”. Next, the animals that had been presented with the high-fat diet were returned to standard chow and measurements were taken across 6 consecutive days - what will be referred to as the “recovery period”. Meal pattern analysis data were lost for day 19 of the experimental diet period and day 4 of the recovery period due to computer issues, and thus are not included in the analysis.

Food intake was monitored continuously over 23-h daily test sessions. Powdered laboratory chow diet was provided ad libitum in a food jar placed on a scale in the feeding compartment of the cage. The animals had access to the food jar via an opening in the wall of the cage. A water bottle was mounted on an adjacent wall of the cage from which the rat had access to water via a spout through a hole in the cage wall.

2.3 Data Analysis

A feeding bout was operationally defined as requiring 0.2 g food. An interval of 10 min without food intake defined the termination of a meal. This bout criterion has been used

previously (Dailey, Tamashiro, Terrillion, & Moran, 2010; Dunn-Meynell, et al., 2009) and on average accounted for ~95% of the feeding data in the current study. Meal size measured in grams or calories was divided by meal length in seconds to calculate eating rate values (g/s and kcal/s). Two-way ANOVAs were conducted to compare meal pattern parameter values across groups (group x day) and for a given group across light-dark phases (phase x day). Bonferroni-corrected post hoc t-tests were conducted at each day to compare values between the groups.

On a given behavioral test day, data of every meal was plotted to derive a histogram by meal size (bins of 1.0 kcal, x-axis) by the count (number of meals, y-axis). Values from each animal were accumulated across each group and the count was converted to a frequency value (count at each meal size bin divided by total count for data of a given group on a given day). Nonlinear regression was used to fit the following Gaussian equation to the data:

$$y = \frac{1}{\delta \sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\delta^2}}$$

where y= frequency calculated from meal number, x=meal size (kcal), μ =mean meal size and δ =standard deviation. This allowed for comparisons of μ values (meal size) and δ (indication of the spread of the distribution) across behavioral testing days and groups.

Results

3.1 Body weight

The HE animals gained significantly more weight across the experimental diet period and were significantly heavier by the end of the experimental diet period than the CHOW controls. A two-way ANOVA comparing body weight gain across days, expressed as a percent of body weight on the first behavioral test day, revealed a main effect of group ($F(1,14)=13.495$, $p=0.003$), main effect of day ($F(41,574)=6.188$, $p<0.001$) and significant interaction ($F(41,574)=6.188$, $p<0.001$). Post hoc t tests revealed a significant group difference in body weight gain that was apparent from behavioral day 6 and a significant group difference in body weight that was apparent from behavioral day 35 of the experimental period (Figure 1). Although a significant group difference remained evident for body weight gain when HE animals were switched to standard chow, the significant group difference in body weight was no longer apparent by day 3 of the recovery period.

3.2 Total Intake

Caloric intake across the experimental diet period was higher for HE compared to CHOW animals (Figure 2). A two-way ANOVA revealed a main effect of group ($F(1,9)=29.518$, $p<0.001$), a main effect of day ($F(40,360)=8.831$, $p<0.001$) and significant interaction ($F(40,360)=6.884$, $p<0.001$). A two-way ANOVA comparing total calories across the recovery period also revealed a main effect of group ($F(1,14)=5.805$, $p=0.030$), main effect of day ($F(4,56)=14.110$, $p=0.001$) and significant interaction ($F(4,56)=3.017$, $p=0.025$) but unlike during the experimental diet period, when HE animals were switched back to a standard chow diet, total calories consumed by the HE group was significantly lower than that of the CHOW controls.

Daily water intake was significantly lower in the HE group compared to CHOW controls across the experimental diet period. A two-way ANOVA comparing water intake between the two groups from day 2 to the end of this period, revealed a main effect of group ($F(1,14)=22.526$, $p<0.001$), main effect of day ($F(40,560)=2.297$, $p<0.001$) and no

significant interaction ($F(40,560)=1.118$, $p=0.290$). In contrast, when HE animals were switched back to standard chow during the recovery period, a two-way ANOVA comparing water intake showed no main effect of group ($F(1,14)=1.577$, $p=0.230$), a main effect of day ($F(5,70)=2.937$, $p=0.018$) and no significant interaction ($F(5,70)=0.755$, $p=0.585$).

3.3. Meal Pattern Analysis

3.3.1 Meal size—Across the experimental diet period, the higher total calories observed in the HE group was driven by larger meals during the light and dark phases and overall (Figure 3). When meal size values in grams were compared between the groups, a two-way ANOVA revealed no main effect of group ($F(1,9)=0.250$, $p=0.629$), a main effect of day ($F(40,360)=2.119$, $p<0.001$) and a significant interaction ($F(40,360)=1.862$, $p=0.002$). For meal size in calories, a two-way ANOVA comparing values between the two groups revealed a main effect of group ($F(1,9)=37.023$, $p<0.001$), main effect of day ($F(40,360)=2.943$, $p<0.001$) and significant interaction ($F(40,360)=2.668$, $p<0.001$). Comparing meal size in kcal during the light phases between the two groups revealed a main effect of group, ($F(1,5)=9.631$, $p=0.027$), main effect of day ($F(40,200)=3.219$, $p<0.001$) and significant interaction ($F(40,200)=2.738$, $p<0.001$). Similarly, comparing meal size in kcal during the dark phases also revealed a main effect of group ($F(1,9)=31.068$, $p<0.001$), main effect of day ($F(40,360)=2.274$, $p<0.001$) and interaction effect ($F(40,360)=1.779$, $p=0.003$).

In contrast, when HE animals were returned to standard chow across the recovery period the effect on meal size was no longer apparent. Comparisons of meal size showed no main effect of group ($F(1,14)=0.161$, $p=0.694$), no main effect of day ($F(4,56)=1.729$) but did show a significant interaction ($F(4,56)=3.144$, $p=0.021$). Comparing meal sizes during the light phases revealed no main effect of group ($F(1,13)=0.190$, $p=0.670$), no main effect of day ($F(4,52)=2.107$, $p=0.093$) nor a significant interaction ($F(4,52)=0.280$, $p=0.890$). Comparing meal size during the dark phases revealed no main effect of group ($F(1,14)=0.071$, $p=0.793$), a significant effect of day ($F(4,56)=3.232$, $p=0.019$) and an interaction effect ($F(4,56)=2.788$, $p=0.035$). The interaction effects appear to be attributed to the HE group showing a smaller meal size on the first day of the recovery period on standard chow which subsequently returns to values comparable to the CHOW group. Post hoc *t* tests reveal no significant group differences across any of the recovery days.

During the experimental diet period, meal size for CHOW rats during the dark phase was larger than those taken during the light (Figures 3B and 3C). A two-way ANOVA (phase $\times$ day) comparing meal size in CHOW animals between light and dark phases revealed a main effect of phase ($F(1,10)=16.588$, $p=0.002$), no main effect of day ($F(40,400)=1.322$, $p=0.097$) and no interaction effect ($F(40,400)=1.279$, $p=0.126$). For the HE group however, comparisons of meal size across the dark-light phases revealed no main effect of phase ($F(1,4)=3.279$, $p=0.144$), a main effect of day ($F(40,160)=2.137$, $p<0.001$) and no interaction effect ($F(40,160)=1.391$, $p=0.079$). Meal size was larger during the dark phase compared to the light phase for HE animals when the group was switched back to standard chow during the recovery period. This was confirmed by a main effect of phase ($F(1,13)=4.751$, $p=0.048$), no main effect of day ($F(4,52)=0.542$, $p=0.706$) and a significant interaction ($F(4,52)=3.037$, $p=0.025$).

3.3.2 Rate of eating—The HE group showed not only larger meal size in kcal across the experimental diet period, but also shorter meal length in seconds. A two-way ANOVA comparing meal length between the two groups revealed a main effect of group ($F(1,9)=27.876$, $p=0.001$), no main effect of day ($F(40,360)=0.978$, $p=0.512$) and no significant interaction ($F(40,360)=0.798$, $p=0.806$). Thus, compared to the CHOW controls,

rate of eating was faster for HE animals. When rate of eating was expressed as grams per second, a two-way ANOVA comparing rate of eating between the two groups during the experimental diet period revealed a main effect of group ($F(1,9)=21.414$, $p<0.001$), a main effect of day ($F(40,360)=3.102$, $p<0.001$) and a significant interaction ($F(40,360)=2.686$, $p<0.001$). Similarly, a two-way ANOVA comparing rate of eating expressed as calories per second between the two groups revealed a main effect of group ($F(1,9)=41.848$, $p<0.001$), main effect of day ($F(40,360)=3.314$, $p<0.001$) and significant interaction ($F(40,360)=3.003$, $p<0.001$). The group difference in eating rate was no longer apparent when the HE group was returned to standard chow during the recovery phase. A two-way ANOVA comparing rate between the two groups revealed no main effect of group ($F(1,14)=0.394$, $p=0.540$), no main effect of day ($F(4,56)=1.291$, $p=0.285$) but a significant interaction ($F(4,56)=4.519$, $p=0.003$). Post hoc t-tests revealed a significantly higher rate for the HE group on one of the recovery days (Figure 4).

3.3.3 Meal number—In addition to an increase in meal size, when presented a high-fat diet, the HE group decreased the number of meals consumed (Figure 5). A two-way ANOVA comparing meal number between the two groups revealed a main effect of group ($F(1,9)=12.465$, $p=0.006$), a main effect of day ($F(40,360)=2.301$, $p<0.001$) and no interaction effect ($F(40,360)=0.760$, $p=0.854$). As expected, the number of meals during the light phase was low for both groups. Comparisons of meal number during the light phase revealed no main effect of group ($F(1,9)=5.058$, $p=0.051$), a main effect of day ($F(40,360)=1.944$, $p=0.001$) and no interaction effect ($F(40,360)=1.000$, $p=0.475$). The HE group took fewer meals during the dark. A two-way ANOVA comparing meal number during the dark revealed a main effect of group ($F(1,9)=5.310$, $p=0.047$), main effect of day ($F(40,360)=2.061$, $p<0.001$) and no interaction effect ($F(40,360)=0.687$, $p=0.926$).

During the recovery period when the HE group was switched to standard chow, although meal size returned to values comparable to CHOW controls, meal number in the HE group remained lower than in the CHOW group. A two-way ANOVA comparing meal number between the two groups during the recovery period revealed a main effect of group ($F(1,14)=10.869$, $p=0.005$), no main effect of day ($F(4,56)=2.158$, $p=0.086$) and no interaction effect ($F(4,56)=1.673$, $p=0.169$). Similar to what was observed during the experimental diet period, comparisons of meal number during the light phase showed no main effect of group ($F(1,14)=0.654$, $p=0.432$), no main effect of day ($F(4,56)=0.935$, $p=0.451$), and no interaction effect ($F(4,56)=0.833$, $p=0.510$). Comparisons of meal number during the dark phase revealed a main effect of group ($F(1,14)=5.045$, $p=0.041$), main effect of day ($F(4,56)=2.921$, $p=0.029$) and no interaction effect ($F(4,56)=1.151$, $p=0.342$).

3.3.4 Distribution of meal size—For the CHOW group, both average meal size (indicated by the μ values) and spread of the distribution of meal size (indicated by the δ values) remained relatively consistent across behavioral testing days in both experimental diet and recovery periods (Figures 6 and 7). Across the experimental diet period when HE group were presented the high-energy diet, both μ and δ values were higher than those of the CHOW controls (Figure 6). During the first three days of high-energy diet exposure, not only was average meal size (μ) larger but the distribution spread (δ) was also larger (Figure 7D). Average meal size shifted to the left and was more narrowly distributed across the remaining days of the experimental diet period (Figure 7E). Upon presentation of standard chow, the meal size distribution of the HE group decreased both μ and δ values to those comparable of the CHOW controls (Figure 7F).

Discussion

Upon presentation of the high-energy diet, the HE group increased caloric intake which was driven by increased meal size. Meal number decreased but not sufficiently to compensate for the increase in meal size. The increase in meal size was particularly evident across the first several days but was maintained throughout HE diet access. After 6 weeks of exposure to the high-energy diet, the HE group was switched to standard chow. Consequently, caloric intake of the HE group decreased and soon returned to levels comparable to that of the CHOW rats. This was driven by a reduction in meal size but interestingly, meal number remained low. Collectively these findings suggest that increased positive signals from oral stimulation and/or dampened inhibitory feedback are most robust across the first few days upon high-energy diet presentation. With longer-term exposure to a high-energy diet, the contributions of these signals decrease yet remain sufficient to produce a group difference compared to chow-fed controls. Furthermore, although total intake returns to values comparable to those of the control group when switched to chow, sustained differences in meal pattern parameters indicate alterations in mechanism(s) underlying the control of ingestive behavior as a function of high-energy diet exposure.

A marked increase in total calories was observed across the first several days upon presentation of a high-energy diet. This was attributed to an increase in meal size which resembled the spike observed in total intake. Furthermore, the distribution curves fit to meal size histograms across the first several days were shifted to the right and had wider spreads. Similar to what was observed in the current study in response to high-energy diet, genetically obese Zucker fatty rats have been demonstrated to not only show a right-shift of meal size distribution (when plotted against meal frequency) but also greater variability compared to Zucker controls (Becker & Grinker, 1977). Hypothalamic-lesioned obese animals have also been shown to increase meal size during the dynamic phase (Brooks, et al., 1946; Teitelbaum & Campbell, 1958). Taken together, the present observations appear to characterize what has been described as the dynamic phase of obesity development (Brobeck, 1946).

These findings suggest that the high-energy diet induces increased oral stimulation which may include taste, trigeminal and olfactory cues. In line with this possibility, previous studies in rodents have shown a preference for high-fat diets over standard chow or diet with less fat (Hamilton, 1964; Harris, 1994; Rockwood & Bhathena, 1990). Here, the diet composition of the high-energy diet is substantially different to that of the standard chow. This may have contributed to the lower water intake observed in HE rats compared to CHOW water intake in both these and other findings (Corbit & Stellar, 1964). The different diet compositions likely influence both oral (e.g. sucrose and maltodextrin content) and postoral (e.g. fiber and fat content) controls which in turn attribute to the hyperphagia observed. There is possibly an adjustment period to the novelty of the dietary components in the HE group across the dynamic phase but nevertheless, these alterations result in an increase in total calories.

That animals are taking larger but less frequent meals also raises the possibility that inhibitory cues that signal satiety and meal termination are weakened upon access to a high-energy diet. The effect of satiation signals such as CCK, however, have been shown to be less effective in reducing feeding in rats that have been maintained on high fat diet for a longer period of time (Covasa & Ritter, 1998; De Lartigue, Barbier De La Serre, Espero, Lee, & Raybould, 2012) suggesting increased insensitivity as a result of longer high-fat diet exposure. Thus during the dynamic phase, the relative contribution of postoral inhibitory influences may be less pronounced. These possibilities are not mutually exclusive. If the hyperphagia were driven primarily by one type of signal, we would predict that the

distribution curve of meal size would resemble that of the CHOW controls in shape, but be shifted to the right reflecting a larger meal size. In fact, not only is the curve shifted but the spread of the distribution (indicated by the standard deviation δ values) also increases, thus it is tempting to speculate that more than one categorical mechanism is driving the hyperphagia observed.

With continual exposure to the high-energy diet, although the initial hyperphagia decreased after several days, total intake in HE animals remained significantly higher than that of the CHOW controls. This increase continued to be attributed to larger meal size in the HE group. When measured in grams, intake and meal size across the two groups are comparable but given the higher caloric density of the high-energy diet, these values were also calculated in calories whereby group differences emerge. Due to different experimental design parameters such as time points for measurements, diet composition and group differences in body weight, there is some variability in the literature as to the changes that occur with longer exposure to high-fat diet but overall, other studies have also reported an increase in meal size with high-fat diet access (Farley, et al., 2003; Furnes, et al., 2009; Melhorn, et al., 2010; Synowski, Smart, & Warwick, 2005; Warwick, Mcguire, Browen, & Synowski, 2000; Warwick, et al., 2003). In a study that compared meal patterns across changes in diet density, it was reported that meal length but not meal number is more sensitive to such changes (Levitsky & Collier, 1968). Studies with animals maintained on a 60% high-fat diet report a change in meal parameter values that return to those similar of controls with longer exposure to the diet (Furnes, et al., 2009). In rats maintained on a cafeteria diet, initial increase in intake was driven by both meal size and meal number but over several months, only meal size remained higher (Rogers & Blundell, 1984).

Particularly with longer exposure to the energy-dense diets, positive postoral controls of feeding play a role. In the literature, compared to naïve rats, previous exposure to a taste stimulus has been shown to generally result in increased intake upon subsequent presentations (see Hill, 1978) thus also suggesting a learning component in the control of meal size. Taken together, it is plausible that both positive oral and postoral cues, as well as the high-caloric density of the diet are necessary to maintain changes in meal pattern parameters over time. In the current study, following the initial robust hyperphagia, increased total intake, larger meal size, fewer meals and the right-shifted and wider meal size distribution curves observed in the HE animals remained remarkably consistent across the experimental diet period.

Within meals, eating rate, whether expressed as calories or grams per second, was significantly higher in HE rats compared to the CHOW controls. Thus the group difference in rate was not accounted for by the difference in caloric density of the two diets. These findings are consistent with previous reports that eating rate for fat or a high-fat diet are higher than that for other macronutrients or a chow control (Furnes, et al., 2009; Melhorn, et al., 2010; Miller, Hrupka, Gietzen, Rogers, & Stern, 1994). Furthermore, whereas total intake and meal size robustly increased across the first several days upon exposure to the high-energy diet, a similar spike was not observed for eating rate. Instead, compared to standard chow, exposure to the high-energy diet appeared to induce an enhanced rate of eating expressed as calories per second, regardless of day of diet presentation. On the first day the HE group was switched back to standard chow, eating rate returned to values similar to those of the CHOW controls. Thus it appears that eating rate is primarily driven by more immediate signals such as orosensory cues, rather than longer-term influences such as previous diet exposure. Together these findings demonstrate that the HE animals are not only taking larger meals but consuming these calories at a faster rate.

In the HE rats, meal size not only increased compared to CHOW but variability was also greater. In the literature, the variability in meal size has been shown to be distributed across a wide meal size range in genetically obese rats in contrast to a tighter distribution observed in non-obese controls (Becker & Grinker, 1977). Similarly, meal pattern analysis of obese Zucker rats reveal evidence for three distinct classes of meal size ranging from “very small” to “super-meals” compared to the graded meal size distribution observed in lean rats (Castonguay, Upton, Leung, & Stern, 1982). Furthermore, in the current study, when HE animals were returned to standard chow, meal size and group variability decreased to levels similar to those of controls. Taken collectively, the high-energy diet induced variability in meal size is suggestive of a disruption in the control(s) of both meal maintenance and termination.

As expected, both groups took most of their meals during the dark rather than the light hours. In the control CHOW group, average meal size taken during the dark was significantly larger than meals taken during the light. This dark-light difference was not observed for the HE animals except during the recovery period when the animals were switched to standard chow. In other studies, the size of meals taken in the dark or light were comparable for both chow-fed and high-fat diet fed rats (Furnes, et al., 2009; Melhorn, et al., 2010) or dark meal size was larger than light meal size for both chow-fed and high-fat fed rats (Cottone, et al., 2013; Cottone, Sabino, Nagy, Coscina, & Zorrilla, 2007). In contrast, consistent with the findings of the current study, average meal size of chow pellets taken during the dark cycles were larger than those taken in the light cycles for both Otsuka Long-Evans Tokushima Fatty (OLETF) rats that do not express CCK-A receptors and Long-Evans Tokushima (LETO) controls (Moran, Katz, Plata-Salaman, & Schwartz, 1998). Similarly, average meal size taken by a group of animals that did not gain as much weight on a high-fat diet (“diet resistant” DR) and chow-fed controls were larger in the dark compared to meals taken in the light cycles. Yet, this light-dark effect on meal size was not as evident for the cohort of animals that gained the most weight on the high-fat diet (DIO) (Farley, et al., 2003). Furthermore, it has been reported that Zucker fatty rats show comparable average intake across the light and dark cycles (Becker & Grinker, 1977). Together, these data suggest disordered light-dark meal pattern parameters across a number of, but not all obesity and energy-dense diet animal models. In the current findings, while high-energy fed animals take most meals during the dark cycle, it appears that high-energy diet induced meal size is not heavily influenced by whether the meals are during the dark or light.

When HE animals were returned to standard chow, total caloric intake decreased but after a few days returned to levels comparable to those of CHOW controls. This transient hypophagia was accompanied by a decrease in average meal size but meal number remained largely unchanged. Previous studies have also reported an initial hypophagia when animals are switched from a palatable and/or energy-dense diet to a less energy-dense diet (Geary, Scharrer, Freudlsperger, & Raab, 1979; Rogers, 1985; Rolls & Rowe, 1979; Rolls, et al., 1980; Stephens, 1980). Here, several days after HE animals had been returned to standard chow, total caloric intake returned to levels similar to those of controls but the number of meals remained significantly lower. Similar to what was observed here, when the diet of cafeteria-fed animals was changed to standard chow, an initial decrease in total intake was driven by a reduction in meal size and meal frequency (Rogers, 1985). Further monitoring in the current study may have revealed that meal number would also normalize although after several weeks, intake and meal size returned to values similar to those of controls but meal number remained low in a previous report (Rogers, 1985). Animals switched from a high-fat to a high-carbohydrate diet also display hypophagia characterized by a decrease in meal size but with little change on meal number (Prete & Scharrer, 1995). The initial hypophagia may, at least in part, be explained by incentive contrast (see Flaherty, 1982). In this case, the high-energy diet is replaced with a less energy-dense and orally stimulating standard chow diet

thus setting up a negative contrast effect. This does not however, necessarily account for the sustained decrease in meal number when total calories return to control levels.

Switching from a preferred to a regular chow diet has consequences beyond alterations in meal patterns. In one study, switching a cafeteria-fed group to a chow diet was sufficient to return body weight, adipose tissue and insulin sensitivity to levels similar to controls (Goularte, Ferreira, & Sanvito, 2012). In contrast, a number of changes are not rescued by switching from a palatable diet to a standard chow including gene expression of neuropeptide Y (NPY), brain-derived neurotrophic factor (BDNF) and cocaine-and-amphetamine regulated transcript (CART) in areas including the arcuate nucleus and ventromedial hypothalamus in rats (Archer, et al., 2005) and corticosterone release factor type 1 receptor (CRF-R1) and BDNF in certain brain areas of mice (Sharma, et al., 2012). High-fat diet induced changes in a number of gastric vagal afferent function measures was only partially reversed when mice were switched to a diet of a lower fat content (Kentish, et al., 2013). Switching to standard chow from a high-fat diet has also been shown to improve insulin sensitivity but not affect expression of inflammatory-related genes (Wang, et al., 2012). When rats were switched from a high-fat high-sugar diet to a standard chow, rats categorized as obesity-prone reduced time spent in the center during an open field test and had lower chow intake, compared to rats categorized as obesity-resistant (Pickering, Alsio, Hulting, & Schioth, 2009). Also, excessive fat cell production as a function of high-fat diet exposure has been put forth as an explanation as to why high-fat-fed animals maintained a higher body weight compared to a chow-fed control group, even when returned to chow (Faust, 1980).

The increase in total intake upon HE presentation was driven by an increase in meal size. Meal number however, decreased thus indicating longer intermeal intervals (IMI). These reductions in meal number may be a reflection of partial compensation for the increased meal size. It has been shown that minimizing postingestive signals by sham-feeding shortens IMI (Kraly, Carty, Resnick, & Smith, 1978; Kraly, Carty, & Smith, 1978) and conversely, under some circumstances, administration of cholecystokinin (Kraly, F. Scott, et al., 1978) or gastrin-releasing peptide (Rushing, Henderson, & Gibbs, 1998) prolong IMI. Thus here, it appears that postingestive inhibitory cues controlling post-meal behaviors to HE diet are relatively intact. Furthermore, after HE animals are returned to standard chow, meal number remains low suggesting changes to post-meal satiation cues as a function of long-term high-energy diet experience.

Overall, the current findings show that upon presentation of a high-energy diet, the “dynamic” phase is characterized by a robust hyperphagia driven by increased meal size, likely a consequence of increased oral stimulation and decreased postoral inhibitory feedback. These influences decrease with continual high-energy diet exposure over what can be characterized as the “static” phase, but the lasting effects on meal pattern suggest that they remain significantly different from those in chow-fed controls. When HE animals are returned to standard chow over the “recovery” phase, following initial hypophagia, total daily intake returns to levels comparable to those of controls, yet there appear to be changes in meal patterns suggesting sustained changes in the physiological mechanisms underlying meal patterns.

Acknowledgments

We would like to acknowledge Alexander Amir Moghadam for his technical support in this study. Supported by NIH DK019302 (T.H.M.).

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Highlights

- Rats fed high-energy diet (HE) increased total daily intake primarily by larger meal size
- Distribution of meal size of HE is larger (shifts to right) and spread (standard deviation) is wider
- When switched to chow, HE rats decreased meal size but meal number remained low
- The HE group showed larger meal size in kcal eaten at a faster rate compared to CHOW controls

Figure 1.

(A) Change in body weight expressed as a percent of body weight on the first day of behavioral testing of CHOW (black symbols) and HE (white symbols) groups. Vertical dashed line indicates when HE animals were switched to standard chow. * indicates statistical significance ($p < 0.05$).

Figure 2.

(A) Total caloric intake for CHOW (black symbols) and HE (white symbols) groups. Vertical dashed line indicates when HE animals were switched to standard chow. * indicates statistical group difference, ^x indicates comparisons that were not statistically significant following Bonferroni correction ($p < 0.05$).

Figure 3. Meal size across (A) daily 23-h sessions, (B) during the light cycle and (C) during the dark cycle for CHOW (black symbols) and HE (white symbols) groups. Vertical dashed line indicates when HE animals were switched to standard chow. * indicates statistical group difference, x indicates comparisons that were not statistically significant following Bonferroni correction (p 0.05).

Figure 4.

Eating rate expressed as kcal per s for CHOW (black symbols) and HE (white symbols) groups. Vertical dashed line indicates when HE animals were switched to standard chow. * indicates statistical group difference, x indicates comparisons that were not statistically significant following Bonferroni correction ($p < 0.05$).

Figure 5. Meal number across (A) daily 23-h sessions, (B) during the light cycle and (C) during the dark cycle for CHOW (black symbols) and HE (white symbols) groups. Vertical dashed line indicates when HE animals were switched to standard chow. * indicates statistical group difference, x indicates comparisons that were not statistically significant following Bonferroni correction (p < 0.05).

Figure 6. (A) μ -values (the meal size that corresponds to the peak of the function) and (B) δ -values (standard deviation of the function), for CHOW (black symbols) and HE (white symbols) groups.

Figure 7.

Functions that were fit to the meal size distributions for CHOW (top row) and HE (bottom row) groups across days 1–3 of the experimental diet period (left column), remaining experimental diet period (middle column) and when HE animals were switched back to standard chow during the recovery period (right column)