Role of VMH ketone bodies in adjusting caloric intake to increased dietary fat content in DIO and DR rats.

Running title: Role of ketone bodies and fatty acids in DIO and DR food intake

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ABSTRACT

The objective of this study was to determine the potential role of astrocyte-derived ketone bodies in regulating the early changes in caloric intake of diet induced-obese (DIO) vs. diet resistant (DR) rats fed a 31.5% fat high-energy (HE) diet. After 3 d on chow or HE diet, DR and DIO rats, were assessed for their ventromedial hypothalamic (VMH) ketone bodies levels and neuronal ventromedial hypothalamic nucleus (VMN) sensing using microdialysis coupled to continuous food intake monitoring and calcium imaging in dissociated neurons, respectively. DIO rats ate more than DR rats over 3 d of HE diet intake. On day 3 of HE diet intake, DR rats reduced their caloric intake while DIO rats remained hyperphagic. Local VMH astrocyte ketone bodies production was similar between DR and DIO rats during the first 6 h after dark onset feeding but inhibiting VMH ketone body production in DR rats on day 3 transiently returned their intake of HE diet to the level of DIO rats consuming HE diet. In addition, dissociated VMN neurons from DIO and DR rats were equally sensitive to the largely excitatory effects of β-hydroxybutyrate. Thus, while DR rats respond to increased VMH ketone levels by decreasing their intake after 3 d of HE diet, this is not the case of DIO rats. These data suggest that DIO inherent leptin resistance prevents ketone bodies inhibitory action on food intake.
INTRODUCTION

Obesity and type 2 diabetes mellitus are major worldwide public health issues (1, 2, 4, 12, 16, 19, 37, 38). Both obesity and type 2 diabetes have important co-morbidities which make it imperative to understand the underlying mechanisms that regulate food intake. Increased consumption of highly palatable, energy-dense foods, especially those rich in fats, represents a major cause of excess caloric intake (13). Indeed, a direct relationship exists between total fat intake and obesity (8). However, the effects and the mechanisms of chronic and excessive high-fat diet (HFD) consumption in the development of obesity are still poorly understood. Towards this end, we have used selectively bred diet-induced obese (DIO) rats as a model of human obesity (26, 27, 32) to assess the underlying factors that regulate their responses to high energy (HE; 31.5% fat) diet intake. These rats are selectively bred to produce polygenically inherited diet-induced obesity or to remain diet-resistant (DR) when fed HE diet. DIO rats are larger but not fatter than DR rats when fed a low fat chow diet, but rapidly become hyperphagic, obese, and insulin resistant when fed HE diet (27, 30). Most importantly, when chow-fed DIO and DR rats are fed HE diet, both overeat for 3 d. While DR rats then reduce their intake to chow-fed levels on day 3, DIO rats continue to overeat for 6-8 wk more, despite their early and persistent increase in leptin levels (29). In addition to these defects, we have previously shown that fatty acid (FA) sensing in ventromedial hypothalamic nucleus (VMN) neurons from DIO offspring were more affected by exposure of their dams to HE diet during gestation and lactation than were those from similarly exposed DR dams (25).

Several studies have shown that food intake can be altered by ingestion of a high fat diet (HFD) (9, 11, 20, 33, 39). Based on the knowledge that astrocytes are the major source of FA
oxidation and only source of ketone body production in the brain (6), we demonstrated that
ventromedial hypothalamic (VMH) ketone body production during restricted ingestion of a very
high fat (60%) diet inhibited caloric intake over a 6 h period (23). To further assess the
importance of VMH ketone production during intake of a high fat diet during normal feeding, we
utilized the DIO/DR rat model of early intake of HE diet to determine whether there was a
differential production of or sensitivity to VMH ketone bodies that underlay the reduced intake
of HE diet in DR but not DIO rats (29).

We postulated that, since DIO rats have abnormal neuronal VMH FA sensing (25) and
fail to reduce their hyperphagia on HE diet on day 3 of intake (27, 29, 30), that they will have
defective VMH ketone production and/or neuronal ketone sensing as compared to DR rats.

RESEARCH DESIGN AND METHODS

Animals

Animals were housed at 23-24 C on a reversed 12:12-h light-dark cycle (lights off at
0900). Male rats selectively bred to express the DR or DIO genotypes (27) were raised in our in-
house colony and used for all studies. These colonies were originally derived from outbred
Sprague-Dawley rats (Charles River Labs) following a breeding scheme as previously described
(30). Briefly, the highest and the lowest weight gainers after 2 wk on HE diet were selected as
breeding stock to produce the DIO and DR genotypes (31), respectively. These substrains have
been maintained for almost 20 y in our vivarium with essentially no change in phenotype. In the
current studies, litters were culled to 10 pups per dam on postnatal day 2 (P2) and weaned at P21
onto Purina rat chow and water *ad libitum*. Purina rat chow (#5001) contains 13.5% fat, 28.5% protein, and 58% carbohydrate as a percent of total energy content. All work was in compliance with the Institutional Animal Care and Use Committee of the E. Orange Veterans Affairs Medical Center.

**VMH β–hydroxybutyrate (βOHB) and feeding measurements**

At 10-11 wk of age, DIO and DR rats (n=8/group) had unilateral VMH guide cannulae (CMA 11, Harvard apparatus, Holliston, MA) and jugular catheter implanted followed by 2 wk of recovery. Two days before microdialysis, they were fed *ad libitum* on HE diet containing 31.5% fat, 16.8% protein and 51.4% carbohydrate as a percentage of total energy content (D12266B, Research Diet, New Brunswick, NJ). On the third day of HE diet, at 0700, microdialysis probes (3mm membrane length and 6kDa pore size (CMA 11, Harvard Apparatus, Holliston, MA)) were inserted into the guide cannulae and perfused at 1.0 µl/min for 8 h with artificial cerebrospinal fluid (aCSF) and jugular catheters were connected. Microdialysis eluates and blood samples were collected every 30 min and food intake was monitored continuously using the BioDAQ© apparatus (Research Diets, New Brunswick, NJ).

A second set of DR rats (10-11 wk old, n=8/group) were implanted with bilateral VMH guide cannulae and unilateral jugular catheters. After a 2 wk recovery period, rats were begun on HE diet. On the third day of HE diet intake, food was removed and bilateral microdialysis probes were inserted at 0700 and infused with aCSF+0.4% DMSO vehicle or 30 µmol/L hymeglusin in aCSF+0.4% DMSO at 1.0 µl/min for 2 h before light off followe with aCSF for 6 h (n=8/group). Hymeglusin is a 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase) inhibitor (23,
36). HE diet was returned at 0900 and eluates from the same microdialysis probes and blood samples were collected at 30 min intervals for 8 h and analyzed for β-hydroxybutyric acid (βOHB). Food intake was monitored continuously as above.

**Effects of glucose, oleic acid (OA) and βOHB on activity of dissociated DIO vs. DR VMN neurons**

DIO and DR rats were weaned at P21 and fed either chow or HE diet for 3 d. P24 rats were perfused with an ice-cold oxygenated (95% O2/5% CO2) perfusion buffer (in mmol/l: 2.5 KCl, 1.25 NaH2PO4, 28.0 NaHCO3, 7.0 MgCl2, 0.5 CaCl2, 7.0 glucose, 1.0 ascorbate, 3.0 pyruvate and 233 sucrose), the VMN was bilaterally punched from VMH slices and neurons were dissociated as previously described (18, 22-24). Evaluation of glucose-, oleic acid (OA-) and βOHB -induced alterations in intracellular calcium ([Ca^{2+}]_i) oscillations in individual VMN neurons was carried out using fura-2 AM (Invitrogen, Grand Island, NY), as previously described (18, 22-24). Neurons were classified first as glucose excited (GE), glucose inhibited (GI) or non-glucosensing (NG), then as OA excited (OAE), inhibited (OAI) or non-responsive (OAN) and then as βOHB-excited (βOHBE), -inhibited (βOHBI) or non-responsive using previously established criteria for changes in [Ca^{2+}]_i area under the curve (18, 23, 24). Studies began with neurons held at either 2.5 mmol/L (comparable to brain levels during a meal), glucose followed by 15 nmol/L OA and then by increasing concentrations (0.1-1000 nmol/L) of βOHB. All neurons were incubated with 20 nmol/L glutamate terminally to assess viability.

**Assays of βOHB.**

βOHB levels were analyzed using a colorimetric assay (Wako, Richmond, VA).
Statistics

Using Systat (Chicago, IL) and GraphPad Prism software, La Jolla, CA), one-way and two-way ANOVA and one-way ANOVA for repeated measures with post-hoc Bonferonni corrections were carried out for the in vitro and in vivo studies. T-tests were also performed for 2 group comparisons. No more than 2 outliers per group were removed if necessary as utilizing Systat software.

RESULTS

Dietary effects on blood and VMH ketone levels and food intake

We postulated that DR rats reduce their intake of HE diet after 3 d on HE diet due to an increase in VMH ketone body production which overrides normal FA sensing, as seen in outbred rats, while DIO rats have reduced ketone body production. To test this hypothesis, DIO and DR rats were fed chow diet from weaning and, beginning at 10 wk of age, were fed HE diet ad libitum for 4 d, with serum and VMH levels of βOHB determined on day 3 (Fig. 1, 2). DIO rats increased their caloric intake of HE diet by 31% above their previous intake of chow after 1 d and consumed more calories over all 4 d after being switched to HE diet (F<sub>1,8</sub>=17.126, P=0.003; Fig. 1A) with no significant change in intake on days 3 or 4. On the other hand, DR rats significantly increased their intake of HE diet after 1 d by 41% above their previous intake of chow and then reduced their caloric intake by 42% of day 2 intake on the 3rd day of HE diet intake (Fig. 1A). On day 3, DIO caloric intake was significantly greater than DR rats’ intake during both 3 h intervals after feeding onset and over the entire 24 h period (Fig. 1B, C). After
feeding onset on day 3, serum βOHB levels peaked at 1.5 h to 2.5 h and 4.5 h to 5.5 h in DR rats (Fig. 1D), while VMH βOHB levels were transiently higher in DIO rats at 1 h after feeding onset (Fig. 1E). This resulted in VMH/serum ratio spikes between 1.5 and 2 h, 4.5 and 5.5h in DIO rats compared to DR rats with a transiently higher ratio in DR rats at 4h (Fig. 1F). However, overall cumulative serum, VMH and VMH/serum βOHB levels did not differ between DIO and DR rats, over the first and second 3 h intervals after feeding onset (Fig. 2).

To test the hypothesis that the generation of ketone bodies by VMH astrocytes exposed to the 31.5% fat HE diet was responsible for the decrease in DR rats caloric intake on day 3, DR rats underwent bilateral VMH reverse dialysis of hymeglusin to inhibit local ketone body production (23) for 2 h prior to feeding onset on day 3 of HE diet intake. Hymeglusin decreased the production of VMH ketone bodies over the first 3 h period ($F_{1,13} = 29.14$, $P=0.041$) as compared to vehicle in DR rats (Fig. 3B). VMH/serum βOHB levels were also decreased during the first 1.5h (Fig. 3C). In parallel with the decrease in VMH/serum βOHB levels in hymeglusin-treated DR rats, there was an increase in caloric intake by 219% over the first 3 h period and by 195% over the second 3h period after feeding onset as compared to vehicle controls (Fig. 3D). This VMH βOHB production inhibition resulted in cumulative caloric intake over the first 3 h interval that equaled that in DIO controls (Fig. 3D). As expected with the use of a self-limited pharmacological inhibitor of ketone body production, the increased food intake of DR rats treated with VMH hymeglusin was restored to control DR levels during the second 3 h interval of feeding on day 3 (Fig. 3E), as well as on the fourth day when no hymeglusin was provided (Fig. 3D). Finally, for uncertain reasons, serum βOHB levels were transiently decreased from 2-3h after feeding onset in DR rats given VMH hymeglusin (Fig. 3A).
Taken as a whole, these data suggest that local VMH astrocyte ketone body production plays an important role in the reduction of caloric intake that occurs on the third day of *ad libitum* HE diet intake in DR rats, an effect which does not occur in DIO rats. However, since there were no major differences in VMH ketone body production between DR and DIO rats during the first 6h of day 3, this suggested that there might have been differences in the sensitivity to ketone bodies in VMH metabolic sensing neurons between DR and DIO rats.

**Effect of HE diet on fatty acid and ketone sensing in DIO and DR rats**

To test the hypothesis that DR and DIO rats’ VMH metabolic sensing neurons display differential sensitivities to ketone bodies, we assessed the effects of a range of βOHB concentrations on the activity of dissociated DR vs. DIO VMN neurons at concentrations of glucose seen in the VMH under fed (2.5 mM brain glucose level) conditions from rats fed chow or HE diet for 3 d. These assessments were made using calcium imaging in the presence of 15 nM OA to specifically target neurons responsive to glucose, FA and βOHB.

First, 3 d of HE diet intake led to 31% fewer GE neurons and 57% more GI neurons in DR rats, while it did not affect the number of DIO glucosensing neurons (Table 1). In the equivalent of the fed state (2.5mM glucose, 15nM OA) the major effects of prior intake of HE diet were seen in both the neuronal responses to FA and βOHB primarily in DIO rats. While there were equivalent percentages of VMN neurons that were excited and inhibited by OA in chow-fed DR and DIO rats, after 3d on HE diet, only DIO rats had a 59% increase in the percent of GI neurons that were excited by OA (Table 1).

VMN neurons were next assessed for their responses to a range of βOHB concentrations as a function of their FA sensing properties (Table 2). Overall, βOHB had a predominantly
excitatory effect with VMN neurons being 2-3 times more excited than inhibited by βOHB (Table 2, Fig. 4). When taking in account their FA sensing properties, chow-fed DIO rats had significantly fewer OAE neurons that were excited by βOHB than all other groups and but this deficit was essentially corrected by 3d intake of HE diet. On the other hand, 3d of HE diet intake in DIO rats reduced the percent of OAI neurons excited by βOHB compared to the other groups (Table 2). Next the effect of increasing concentrations of βOHB (0.1 nM to 1 µM) on VMN neuronal FA sensing was assessed at 2.5 mM glucose and 15 nM OA. Most importantly, neither OAE nor OAI neurons demonstrated a concentration-response to βOHB (Fig. 4). However, some individual effects were observed. At 0.1 nM βOHB, neurons excited by OA from DIO rats fed HE diet were 10-fold more excited by βOHB than those from chow-fed DIO rats and 2-3-fold more excited than those from DR rats fed either chow or HE diet (Fig. 4A). The same effect was also observed in OAI neurons which were inhibited by 1 nM βOHB (Fig. 4D). This suggests that VMN neurons from DIO rats fed HE diet actually became more sensitive to the effects of FA and βOHB than did those from DR rats.

**DISCUSSION**

The objective of this study was to determine the potential role of astrocyte-derived ketone bodies production in regulating the early changes in caloric intake of DR vs. DIO rats fed a 31.5% fat HE diet. Based on our previous finding that VMH astrocytes utilize FA to produce ketone bodies which reduce caloric intake of a high fat (60%) diet (23), we predicted that differences in VMH neuronal ketone and FA sensing would underlie differences in intake of 31.5% fat HE diet between DR and DIO rats during their first 3 days of intake of this diet. To
address these issues, we used microdialysis to assess VMH $\beta$OHB levels in DR and DIO rats after 3 d on HE diet while their food intake was monitored simultaneously. As we demonstrated previously (29), DIO rats ate more than DR rats over the initial 3 d of HE diet intake and, on day 3, DR rats reduced their caloric intake back to control, chow-fed levels, while DIO rats continued their increased intake of HE diet. Contrary to our initial hypothesis, local VMH astrocyte ketone bodies production was similar between DIO and DR rats during the first 6 h after dark onset feeding. In addition, dissociated VMN neurons from DIO and DR rats were equally sensitive to the largely excitatory effects of $\beta$OHB. Nevertheless, while DIO rats had continued hyperphagia, the increase in VMH $\beta$OHB levels seen in DR rats was sufficient to reduce HE diet intake in DR rats on day 3. This was supported by their increased intake when VMH $\beta$OHB production was inhibited with hymeglusin. Thus, while DR rats do seem to respond to increased VMH local ketone body production by decreasing their intake after 3 d of HE diet, this is not the case of DIO rats which are equally as responsive to both the individual neuronal effects of ketone bodies and VMH astrocyte ketone body production on HE diet. This suggests that something else overrides what should be an inhibitory effect of VMH ketone bodies on HE diet intake in DIO rats.

Our previous studies suggest that the inherent leptin resistance of DIO rats (15, 26, 27, 29) might override the otherwise powerful inhibitory effect of VMH ketone production on HE diet intake seen in DR rats. We previously showed that despite a major increase in leptin after 3 d on HE diet, DIO rats do not respond to this by decreasing their food intake (29). However, the overriding effect of leptin on ketone bodies sensing in DIO rats is hypothetical and needs to be further assessed. Regardless of the reason for the resistance of DIO rats to the inhibitory effect of VMH ketone bodies on HE diet intake, it is important to recognize the impressive role that they
do play in reducing intake in the DR rats. At the single neuron level, βOHB mostly overrode the
actions of glucose and OA with a predominantly excitatory over inhibitory effect in both DIO
and DR rats. Importantly, and contrary to our previous findings with both glucose (17) and FA
(24), βOHB produced its effects without a concentration-dependent responsiveness in
dissociated neurons assessed in the absence of surrounding glial cells. Thus, even very small
concentration (100 pmol/L), produced an almost maximal effect in many cases. This suggests
that neuronal uptake of ketone bodies via MCT2 transporters (3, 10, 34, 35) is not a key
regulatory step and that production of ATP and/or ROS from these ketone bodies in neurons is
likely to be the main factor overriding both glucose and FA sensing (5, 24). On the other hand, 3
d of prior HE diet intake significantly altered VMN neuronal glucosensing in GI neurons of DIO
rats while it had less to no impact in DR rats. HE diet intake also altered responsiveness to FA
and βOHB in DIO rats but not in DR rats. Yet, despite this increased responsiveness, DIO rats
still failed to reduce their intake of HE diet supporting a role for other factors besides VMN
neuronal metabolic sensing as regulators of the short-term intake of HE diet in DIO rats.

We do know that neuronal FA sensing in the arcuate plus VMN (ventromedial
hypothalamus; VMH) is important during chronic intake of a high fat diet in DIO but not DR
rats. Inhibiting FA sensing by depletion of the FA sensor, CD36, causes DIO rats to become
hyperphagic and obese on 45% fat diet, while VMH CD36 depletion has no effect on long-term
intake of high fat diet in DR rats, even though they still become obese on such diets (21). Again,
these data support the contention that factors other than VMH neuronal metabolic sensing or
responses to ketone bodies determine the short-term intake of HE diet in DIO rats, while local
VMH ketone body production plays a major role in the early intake of HE diet in DR rats. On the
other hand, VMH FA sensing, as mediated by CD36, appears to be an important regulator of the long-term intake of high fat diet in DIO but not DR rats. This may possibly be due to the fact that high fat intake selectively alters the responsiveness of VMH neurons to FA in DIO but not DR rats.

We previously demonstrated that outbred rats fed a very high (60%) fat diet on a restricted 3 h/d schedule, also had delayed reduction in intake accompanied by a peak of VMH βOHB at 1 h after feeding onset (23). Furthermore, we demonstrated that local inhibition of ketone bodies production with hymeglusin ablated this delayed intake of the high fat diet (23). One of the most important findings of the current set of studies is that freely feeding DR rats fed a diet of only moderate fat concentration (31.5% HE diet) appear to utilize this same mechanism of VMH astrocyte production of ketone bodies to downregulate their intake of the HE diet. Since we examined VMH βOHB levels only at 3 d, there is no way to know if levels were raised during the first 2 d on HE diet and/or whether they continue to be elevated after the 3rd day on the diet. Such issues will require further studies.

In conclusion, this study show that, in DR rats, local ketone body VMH production associated to normal ketone body sensing after 3 d on HE diet is sufficient to decrease HE diet intake to the levels of chow diet intake. However in DIO rats, even though their ketone bodies VMH levels and sensing are similar to DR rats, it is not sufficient to override their inherent leptin resistance that could prevents them from decreasing their HE diet intake.

**PERSPECTIVE AND SIGNIFICANCE**
The increased consumption of palatable, high fat diet contributes to the excess caloric intake that leads to the development of obesity. Thus it is important to understand the mechanism underlying the relationship between high fat diet consumption and the regulation of feeding. We have shown that specialized hypothalamic metabolic sensing neurons respond to changes in ambient brain levels of substrates such as glucose, FA and ketone bodies as signaling molecules to alter their activity (17, 22, 23). Using a restricted feeding schedule (3 h/d) of a 60% fat diet in outbred rats, we previously demonstrated that there was a delayed inhibition of intake and that this inhibition was reversed by transiently inhibiting local VMH astrocyte production of ketone bodies (23). The current studies were initiated to examine the potential role of VMH ketone body production in modulating ad libitum intake of a diet of much lower (31.5%) fat content. We chose the selectively bred DIO/DR model because of our previous finding that DR, but not DIO rats reduce their intake of this diet to low fat control levels after only 3 d (29). Our hypothesis was that DR rats might either have increased levels of VMH ketone body production and/or that their VMH metabolic sensing neurons were more sensitive to the largely excitatory effects of ketone bodies than were those in DIO rats. In fact, neither of these postulates was correct, even though inhibiting VMH ketone production clearly did prevent DR rats from reducing their intake of 31.5% fat HE diet to control levels on day 3. Since we know that DIO rats are inherently leptin resistant (7, 14, 26, 28) and fail to reduce their intake for up to 6-8wk after onset of HE diet intake, despite very high leptin levels (29), our findings here strongly suggest that VMH ketone bodies and fatty acid sensing neurons clearly are important regulators of feeding, energy and glucose homeostasis in rats with normal leptin sensitivity (22, 23). However, with or without obesity the presence of underlying leptin resistance appears to override normal VMH fatty acid
and ketone body sensing in the regulation of feeding in the early response to increase in dietary fat content. Thus, there appears to be a hierarchy of control mechanisms regulating feeding in which normal metabolic sensing is dependent upon normal leptin sensitivity.

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GRANTS

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REFERENCES


**FIGURE LEGENDS**

**Figure 1:** DIO and DR rats were fed HE diet (31.5% fat; n=10/group) ad libitum for 2 d and then on the third day had food intake, serum and VMH microdialysis βOHB levels assessed every 30 min during the first 6 h after food was introduced at dark onset. **A:** Daily food intake before and after the microdialysis; **B:** Cumulative food intake over the 6h and 24h period; **C:** Food intake in kcal during the 6 h of microdialysis; **D:** Serum βOHB levels; **E:** VMH βOHB levels; **F:** VMH/serum βOHB ratios x100; *P<0.05 by t-test for A, B. *P<0.05 One way ANOVA C-F.

**Figure 2:** DIO and DR rats were fed HE diet (n=10/group) ad libitum for 2 d and then on the third day had serum and VMH microdialysis βOHB levels assessed every 30 min during the first 6 h after food was introduced at dark onset. **A:** Cumulative serum βOHB during 0-3h and 3-6h period; **B:** Cumulative VMH βOHB during 0-3h and 3-6h period; **C:** Cumulative VMH/ Serum β-OHB ratio during 0-3h and 3-6h period.

**Figure 3:** DR rats were fed ad libitum HE diet (n=8-10/group) and, on day 3, they had 30 µmol/L hymeglusin (n=8) or vehicle (0.4% DMSO; control; n=8) reverse dialyzed bilaterally in the VMH for 2 h before food was introduced. Food was introduced at dark onset. βOHB levels assessed every 30 min for 6 h after food was introduced. **A:** Serum βOHB levels; **B:** VMH βOHB levels; **C:** VMH/serum βOHB ratios x100; **D:** Cumulative food intake in kcal during 0-3h and 3-6h period; **E:** Daily food intake before and after the microdialysis. *a,b* Data points with differing superscripts differ from each other at the P<0.05 level after two-way ANOVA, followed by Bonferroni test.*P<0.05 one way ANOVA.
Figure 4: Dissociated VMN neurons from P24 DIO and DR rats, fed chow or HE diet (n = 8-10 rats/group) for 3 d, were categorized using calcium imaging as being OAE (A, B) or OAI (C, D) using 15 nmol/L OA at 2.5 mmol/L glucose. They were then categorized as being excited (βOHBE) (A, C) or inhibited (βOHBI) (B, D) and their sensitivity to βOHB assessed by the alteration in [Ca^{2+}]_i oscillation produced by successive exposure to 0.1 nmol/L, 1 nmol/L, 10 nmol/L, 100 nmol/L and 1 μmol/L OA. Data are expressed as percent neuron ± SEM tested in each category. Data points with differing superscripts at each βOHB concentration differ from each other by P < 0.05 or less by Bonferroni post hoc test after intergroup differences were found after two-way ANOVA.
Table 1. Effects of 2.5 mM glucose and 15 nM oleic acid (OA) on dissociated VMN neurons from P24 male DR and DIO rats fed chow or HE diet for 3d.

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<th>Glucose/FA Category</th>
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<th>DR Chow OAI</th>
<th>DR HE OAE</th>
<th>DR HE OAI</th>
<th>DIO Chow OAE</th>
<th>DIO Chow OAI</th>
<th>DIO HE OAE</th>
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Dissociated VMN neurons from DR and DIO rats were assessed by fura-2 calcium imaging at P24. Neurons were first classified by glucosensing categories as glucose was changed from 2.5 to 0.5 to 2.5 mmol/l and then for FA sensing to 15 nmol/l oleic acid (OA) at 2.5 mmol/l glucose. Neurons were classified as OA excited (OAE) or inhibited (OAI). Data are mean ± SEM percent of total neurons tested in each category. GE=glucose excited, GI=glucose inhibited. Total=total percent of each category of neurons at each glucose concentration, irrespective of their glucosensing properties with the number of neurons tested in each group divided by the total number tested in parentheses. N=8-10 rats/group. a,b Data with differing superscripts within the same category differ from each other in at the P<0.05 level after two-way ANOVA, followed by Bonferroni test.
Table 2. Effect of 2.5 glucose and 15 nM of oleic acid and a range of β-hydroxybutyrate (βOHB) concentration on dissociated VMN neurons from P24 DR and DIO rats fed chow or HE diet for 3d.

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<td>βOHBI</td>
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</table>

At 2.5 mmol/L glucose, VMN neurons were classified as OA excited (OAE) or inhibited (OAI) by alterations in [Ca^{2+}]_i oscillations produced by exposure to 15 nmol/L OA. They were then exposed to a range of concentration β-hydroxybutyrate (βOHB) from 0.1 nmol/L to 1µmol/L in the presence of 15 nmol/L OA and were then classified as βOHB excited (βOHBE) or inhibited (βOHBI). Data are in percent of total neurons ±SEM tested in each category. Total = total percent of each category of neurons for each βOHB category, irrespective of their OA sensing properties, with the number of neurons tested in each group divided by the total number tested in parentheses. N=8-10 rats/group. a,bData with differing superscripts within the same category differ from each other in at the P<0.05 level after two-way ANOVA, followed by Bonferroni test.