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ORIGINAL ARTICLE

Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry

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BACKGROUND: The biological mechanisms that link the development of depression to metabolic disorders such as obesity and diabetes remain obscure. Dopamine- and plasticity-related signalling in mesolimbic reward circuitry is implicated in the pathophysiology and aetiology of depression.
OBJECTIVE: To determine the impact of a palatable high-fat diet (HFD) on depressive-like behaviour and biochemical alterations in brain reward circuitry in order to understand the neural processes that may contribute to the development of depression in the context of diet-induced obesity (DIO).
METHODS: Adult male C57Bl6 mice were placed on a HFD or ingredient-matched, low-fat diet for 12 weeks. At the end of the diet regimen, we assessed anxiety and depressive-like behaviour, corticosterone levels and biochemical changes in the midbrain and limbic brain regions. Nucleus accumbens (NAc), dorsolateral striatum (DLS) and ventral tegmental area dissections were subjected to SDS-PAGE and immunoblotting using antibodies against D1A receptor, D2 receptor, brain-derived neurotrophic factor (BDNF), phospho-DARPP-32(thr75), phospho-CREB and ΔFosB.
RESULTS: HFD mice showed significant decreases in open arm time and centre time activity in elevated plus maze and open field tasks, respectively, and increased immobility (behavioural despair) in the forced swim test. Corticosterone levels following acute restraint stress were substantially elevated in HFD mice. HFD mice had significantly higher D2R, BDNF and ΔFosB, but reduced D1R, protein expression in the NAc. Notably, the expression of BDNF in both the NAc and DLS and phospho-CREB in the DLS was positively correlated with behavioural despair.
CONCLUSIONS: Our results demonstrate that chronic consumption of high-fat food and obesity induce plasticity-related changes in reward circuitry that are associated with a depressive-like phenotype. As increases in striatal BDNF and CREB activity are well implicated in depressive behaviour and reward, we suggest these signalling molecules may mediate the effects of high-fat feeding and DIO to promote negative emotional states and depressive-like symptomology.

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INTRODUCTION

The increased prevalence of overweight and obesity is a serious medical and public health concern. Obesity is directly associated with increased morbidity from cardiovascular disease, type 2 diabetes and some cancers. Epidemiological data suggest that obesity is also linked to an increased risk of depressive and mood disorders.[1–3] According to the World Health Organization, depression affects about 121 million people worldwide and is among the leading causes of disability. Despite this information there is presently little information on how the development of obesity heightens the risk for depression.

Increased availability and excessive intake of energy-rich foods is a significant factor contributing to obesity. Palatable high-fat and high-sugar foods are rewarding and their consumption is associated with changes in brain reward circuitry.[4–6] Dopamine (DA) neurons originating in the ventral tegmental area (VTA) and substantia nigra of the midbrain that innervate limbic sites, including the nucleus accumbens (NAc) and dorsal striatum, are an essential component of the neural circuitry underlying motivation and reward. Corticolimbic neural processes relay important sensory, cognitive and emotional information associated with seeking out and consuming food.[7] Several lines of evidence point to decreased DA signalling in the striatum in obese humans[8,9] and in rodent models of obesity.[4,5,10,11] Apart from its role in regulating the motivational properties of different stimuli, mesolimbic DA signalling is also implicated in the pathophysiology and aetiology of depression and mood disorders.[12]

In the striatum, DA binds to receptors located on two subtypes of medium spiny neurons: (1) dynorphin neurons that mainly express D1 receptors and (2) enkephalin neurons that express high levels of D2 receptors. The behavioural, biochemical and transcriptional actions of DA are mediated by several signalling molecules. One of the early signals is DA- and cAMP-regulated phosphoprotein-32 (DARPP-32). Signals downstream of DARPP-32 include brain-derived neurotrophic factor (BDNF) and the transcription factors pCREB and ΔFosB (truncated splice variant of FosB). Increased levels of BDNF by psychostimulant drugs are implicated in drug-induced morphological changes in NAc neurons that are fundamental to synaptic plasticity. Accumulation of the highly stable transcription factor ΔFosB in the NAc is associated with enhanced sensitivity to the rewarding effects of cocaine. These neural adaptations are thought to lead to an

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individual's increased response to drugs and natural rewards, leading to escalating intake and compulsive use.[13,14] Studies have also shown that manipulations of BDNF and CREB within VTA–NAc circuit produces unique behavioural phenotypes that are directly relevant to depression.[12] While there has been more focus on the role of hippocampus and frontal cortex in aspects of depression there is limited understanding of how high-fat diet (HFD) may impact brain reward circuitry to modulate depression. We studied the influence of long-term exposure to a palatable HFD and diet-induced obesity (DIO) on anxiety and depressive-like behaviour, and signalling changes linked to depression and reward.

MATERIALS AND METHODS

Animals and diets

Male adult C57Bl/6 mice (8 weeks of age; Charles River, St Constant, QC, Canada) were maintained in an environmentally controlled room (22–24 °C) for at least 10 days to acclimatize to a reverse 12-h light–dark cycle and provided with *ad libitum* access to standard chow and water. Mice ($n = 8–12$ per group) were placed on one of two diets for 12 weeks: (1) a high-fat and high-sugar diet ('HFD'; D12231, Research Diets, Inc., New Brunswick, NJ, USA) containing 58% kcal from fat in the form of hydrogenated coconut oil, 16.4% kcal from protein and 25.5% kcal from carbohydrates, and (2) an ingredient-matched, low-fat diet ('LFD'; D12328; Research Diets, Inc.) containing 10.5% kcal from fat, 16.4% kcal from protein and 73.1% kcal from carbohydrates for 12 weeks. Separate groups of individually housed mice were used to measure preference between LFD and HFD ($n = 6$ per group) and caloric intake ($n = 8$ per group). For the diet preference test, singly housed male C57BL6 mice (8 weeks of age) that were initially consuming a standard chow diet were provided with both LFD and HFD for 3 days. Powder diets were placed in food cups that were fixed inside a larger tray to catch any spillage. Food trays were fixed side-by-side at the end of each cage and the position of each diet was alternated each day. For preference and food intake studies, the amount of food consumed over 24 h was measured right before the onset of the dark cycle. All behavioural testing and sacrifices below were carried out in the dark phase of the light–dark cycle. All procedures involving the use of animals were approved by the CRCHUM Animal Care Committee.

Elevated plus maze test

In order to assess anxiety-like behaviour following the diets, one cohort of mice fed a LFD ($n = 8$) or HFD ($n = 8$) for 12 weeks were tested in both the elevated-plus maze and open field test (below). The EPM apparatus consists of two closed arms that oppose two open arms in a plus design (Med Associates, Inc., St Albans, VT, USA). Decreased time spent in the open, exposed arm is an indicator of increased anxiety-like behaviour. The apparatus is placed 60 cm above the floor and has a video camera fixed overhead. Each mouse was placed in the middle of the maze facing the open arm opposing the experimenter. Movement in the maze was recorded and tracked for 5 min by an overhead video camera connected to a PC with Ethovision XT software (Med Associates, Inc.).

Open field test

We used the open field test as an additional measure of anxiety-like behaviour. The open field test was carried out 1 day after the EPM task. The open field consisted of a Plexiglas box ($50 \times 50 \times 30$ cm) in a brightly lit room. Each mouse was placed in the middle of the arena and allowed to explore the field for 5 min. Movement in the field was recorded and tracked by an overhead video camera connected to a PC with Ethovision XT software.

Forced swim test (FST)

The FST is widely used to screen and validate antidepressants.[15,16] In this test, animals display 'behavioural despair' as indicated by increased immobility and less escape-oriented behaviours. When forced to swim in a glass cylinder filled with water in which they are confined mice eventually cease escape attempts and become immobile. The increasing immobility time reflects a state of helplessness and despair. After 12 weeks of HFD or LFD, all the mice were forced to swim in a glass cylinder (height, 15 cm; diameter, 12 cm) containing water (23 °C) at a 10-cm depth. A video camera located above the apparatus recorded each test. The duration of immobility during the last 4 min of the 6-min testing period (2 min habituation) was calculated.

Locomotor activity

Spontaneous locomotor activity was assessed over 15 min in HFD and 24 h after the FST in LFD mice. Mice were placed in metabolic cages (Accuscan Instruments Inc., Columbus, OH, USA) consisting of 16 light beams arrays in *x*, *y* and *z* axes. Distance travelled (horizontal activity) was measured by nearby computer-controlled software.

Basal and stress-induced corticosterone measures

Both basal and stress-potentiated plasma corticosterone levels were measured. To measure basal corticosterone, blood samples were collected during the sacrifice of mice used for the FST experiment 2 days following behavioural testing. For restraint stress experiments, we used mice from the EPM and open field cohort. The day following the open field test each mouse was restrained for 30 min in decapicones (Braintree Scientific Inc., Braintree, MA, USA) and blood samples obtained immediately afterwards. Plasma corticosterone was measured by an ELISA corticosterone kit (Enzo Life Sciences, Farmingdale, NY, USA).

Western immunoblotting

Mice were decapitated under Isoflurane anaesthesia. Brains were rapidly dissected and stored at −80 °C. Frozen brains were sliced into 0.5 mm coronal sections using a brain matrix. Coronal sections were mounted onto slides and maintained on dry ice. Nuclei were microdissected using brain tissue punches (Stoelting, Inc., Wood Dale, IL, USA). Bilateral punches of 0.75 mm diameter were obtained from the VTA and 1.0 mm diameter punches from the NAc and dorsolateral striatum (DLS). Microdissected tissues were homogenized on ice in 100 μl of cell lysis buffer (20 mM Tris, pH 7.5; 150 mM NaCl; 1 mM Na_2EDTA; 1 mM EGTA; 1% Triton; 2.5 mM sodium pyrophosphate; 1 mM β-glycerophosphate; 1 mM Na_3VO_4; 1 μg ml^{-1} leupeptin) with added protease (PMSF 100 μM) and phosphatase inhibitors (Sigma phosphatase inhibitor cocktails I and II) in 1.5 ml tubes using a motorized pestle. Tubes containing homogenates were centrifuged for 15 min at 14 000 *g*. Protein concentrations were measured using BCA protein assay (Pierce Biotechnology, Rockford, IL, USA). Protein samples (20 μg) were separated by electrophoresis on a 10% polyacrylamide gel and electrotransferred to a PVDF membrane (Millipore, Bedford, MA, USA). Non-specific binding sites were blocked in TBS 5% low-fat milk and 0.1% Tween-20 or 5% BSA. Membranes were rinsed in buffer (0.1% Tween-20 in TBS) and then incubated with anti-BDNF (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-pCREB (Ser133), anti-CREB, anti-Delta FosB, anti-pDARPP32, anti-DARPP32 (1:1000; Cell Signalling Technology Inc., Danvers, MA, USA), anti-DA D1A receptor, anti-D2 receptor, anti-TH (1:1000; Millipore), followed by anti-rabbit or anti goat or anti-mouse IgG horseradish peroxidase-conjugate (1:5000). After rinsing with buffer, immunocomplexes were visualized by chemiluminescence using the western lighting plus ECL kit (PerkinElmer, Waltham, MA, USA). Protein size was compared by using precision plus protein ladder (Bio-Rad, Bedford, MA, USA). The film signals were digitally scanned and then density quantified using ImageJ software (Free software available from NIH at http://rsbweb.nih.gov/ij/). GAPDH was used as an internal control for western blot such that data were standardized according to GAPDH values.

Statistical analysis

Data were analyzed using GraphPad 5 software (http://www.graphpad.com). Data are presented as means and standard errors. A two-way ANOVA with Bonferonni *post-tests* was used to calculate preference data, and unpaired *t*-tests were used to compare HFD with LFD mice in the EPM, open field, forced swim, locomotor activity and protein expression studies. Pearson correlations were used to analyze protein expression versus immobility times. Criterion for significance was set to $P \leqslant 0.05$ in all comparisons.

RESULTS

Preference, caloric intake and body weight

To evaluate the relative palatability of the two diets, we assessed food intake in a two-diet concurrent choice task over 3 days. Mice show strong preference for the HFD we used from the first day onwards (Figure 1c; ***$P<0.001$). As illustrated in Figure 1b, mice on the HFD increase their caloric intake relative to mice on LFD over the course of the 12 week period. Finally, HFD mice show increased weight gain relative to LFD mice (Figure 1c; ***$P<0.001$).

DIO increases anxiety- and depressive-like behaviour

To determine if HFD and resulting obesity modulate anxiety, we tested mice in the elevated-plus maze, open field test and FST after 12 weeks of LFD or HFD consumption. As shown in Figure 2a, there was no difference in the percentage of entries made into the open arm between LFD and HFD mice, however, we found a significant reduction in the amount of time spent in open arms in HFD mice compared with the control LFD mice (Figure 2b, *$P<0.05$). The results of open field test show that HFD consumption significantly reduced the number of entries (*$P<0.05$) and time spent (*$P<0.05$) in the centre of the open field as compared with the control LFD mice (Figures 2c and d).

To determine if HFD and DIO increase depressive-like behaviour, we measured immobility in the FST. Immobility time was substantially elevated in HFD mice as compared with LFD mice (Figure 2e, *$P<0.05$), indicative of increased behavioural despair in HFD mice. Given that alterations in locomotor activity may account for differences in the FST, we measured this parameter in metabolic cages. As shown in Figure 2f, locomotion was similar between HFD and LFD mice, suggesting that decreased locomotor activity in HFD mice does not contribute to their increased immobility in the FST. Collectively, these behavioural results demonstrate increased anxiety and depressive-like behaviour in mice chronically exposed to a HFD.

HFD potentiates stress responses

To determine if HFD consumption is associated with increased corticosterone levels, we measured plasma corticosterone levels in LFD and HFD mice. We observed a near-significant increase in

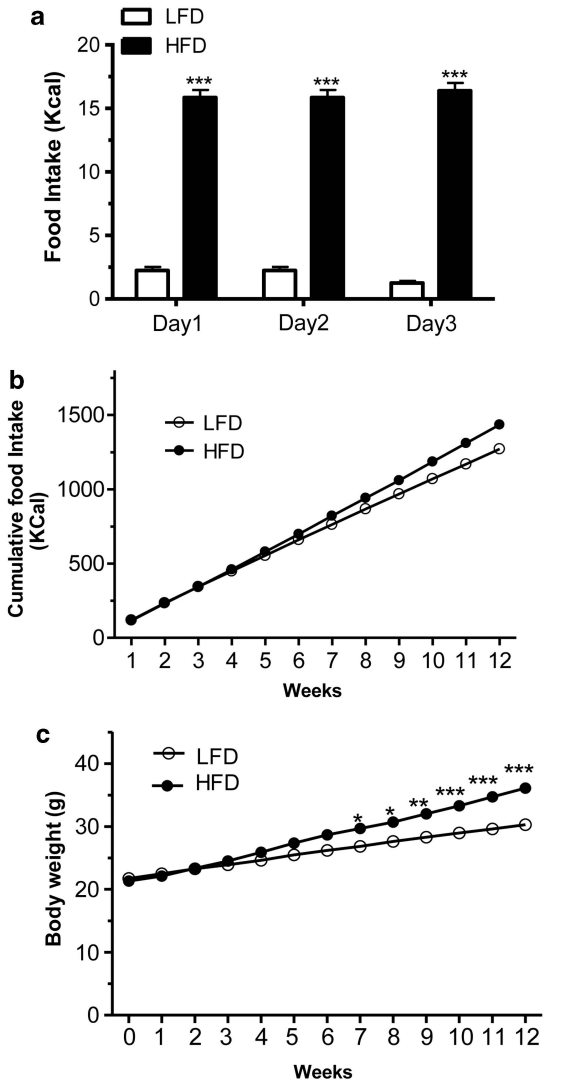


Figure 1. Increased preference and intake of a palatable high-fat diet. (**a**) Mice show a strong preference for a high-fat diet (HFD) over an ingredient-matched low-fat diet (LFD) ($n=12$). (**b**) Cumulative food intake of control LFD and palatable high-fat diet for 12 weeks. Mice rapidly increase their caloric intake on the HFD relative to LFD controls ($n=8$ per group). (**c**) HFD for 12 weeks increased body weight gain in C57Bl6 mice ($n=8$) as compared with the control LFD mice ($n=8$). *$P<0.05$, **$P<0.01$, ***$P<0.001$.

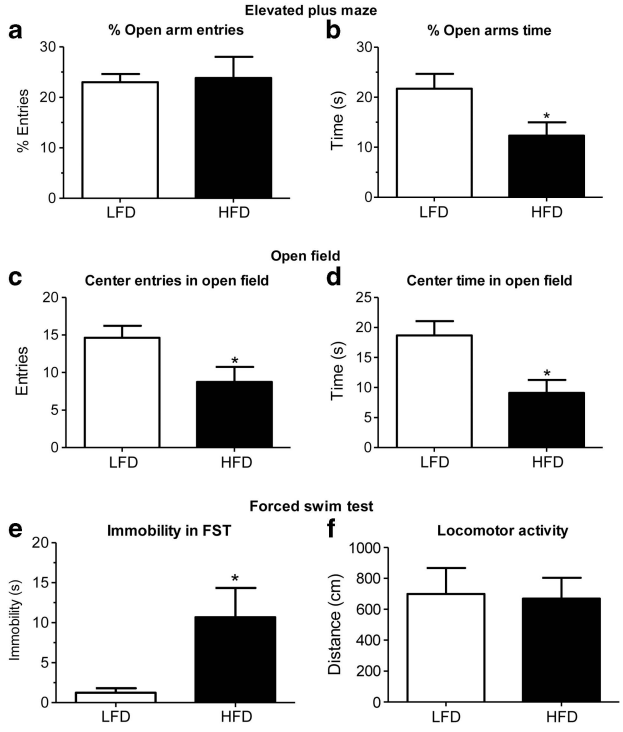


Figure 2. High-fat diet increases anxiety and depressive-like behaviours. (**a**) Similar number of open arm entries in HFD and LFD mice open arms entries as compared with the control LFD mice ($n=8$ per group). (**b**) Percentage of time spent in open arms of elevated plus maze was significantly reduced in HFD mice as compared with the control LFD mice. (**c**) Significant decrease in the entries made to centre of an open field arena in HFD mice ($n=8$) as compared with the control LFD ice ($n=8$). (**d**) Significant decrease in time spent in centre of open field in HFD mice compared with the control LFD mice. (**e**) Effect of chronic consumption of high-fat diet on depressive behaviour expressed as immobility time in FST conducted in a separate cohort of mice. (**f**) Effect of chronic HFD on spontaneous locomotor activity expressed as total distance travelled during the test in C57Bl6 mice ($n=6$ each group) as compared with the control LFD mice ($n=6$). *$P<0.05$.

basal plasma corticosterone levels in HFD as compared with LFD mice in the basal (non-stressed) state (Figure 3a, $P=0.057$). To assess whether or not HPA stress reactivity is enhanced in HFD mice, we measured plasma corticosterone levels following 30 min restraint stress. Plasma corticosterone levels were substantially potentiated in HFD relative to LFD mice in response to restraint stress (Figure 3b, $^{**}P<0.01$).

HFD alters plasticity and DA-related molecules in the NAc

We observed a twofold increase in the levels of BDNF protein in the NAc after 12 weeks of HFD as compared with the control LFD mice (Figure 4a; $P<0.05$). Levels of ΔFosB protein are increased by 1.5-fold in the NAc after 12 weeks of HFD as compared with LFD (Figure 4a; $^{*}P<0.05$). HFD lead to significantly increased activation of CREB as indicated by the ratio of levels of p-CREB/total CREB in the NAc as compared with LFD (Figure 4a; $^{*}P<0.05$).

We observed a significant decline in the protein levels of tyrosine hydroxylase (TH), the rate-limiting enzyme for DA biosynthesis, in the NAc after HFD as compared with LFD (Figure 4b; 58.88%; $^{***}P<0.001$). HFD resulted in significant increases in the levels of NAc DA D2R protein levels as compared with LFD (Figure 4b; 180%; $^{***}P<0.001$). On the other hand, the levels of DA D1AR protein levels were diminished in HFD mice as compared with the LFD (Figure 4b; 39.91%; $^{***}P<0.001$). Finally, we observed a non-significant trend for higher levels of p-DARPP32 protein in the NAc (Figure 4b; $P=0.0746$).

HFD alters plasticity and DA-related molecules in the VTA

We observed a significant increase in the levels of BDNF protein in the VTA after 12 weeks of HFD as compared with the control LFD (Figure 4c; 190.9%; $^{*}P<0.05$). The levels of ΔFosB protein were increased twofold in the VTA after 12 weeks of HFD as compared with LFD (Figure 4c; $^{**}P<0.01$). HFD resulted in significant increases in protein levels of phospho-CREB in the VTA as compared with LFD (Figure 4c; $^{*}P<0.05$).

There was a significant decline in the levels of TH protein in the VTA after HFD as compared with LFD (Figure 4d; 80.87%; $^{*}P<0.05$). We observed a non-significant trend for higher levels of phospho-DARPP32 (thr75) protein in the VTA after 12 weeks of HFD as compared with LFD (Figure 4d). The levels of DA D2R protein were not different in the VTA of HFD mice as compared with LFD mice (Figure 4d).

HFD alters plasticity and DA-related molecules in DLS

We observed a significant increase in the levels of BDNF protein in the DLS after 12 weeks of HFD as compared with the control LFD (Figure 4e; $^{***}P<0.001$). The levels of ΔFosB protein were not different after 12 weeks of HFD mice (Figure 4e), however, HFD elevated phospho-CREB levels in the DLS (Figure 4e; $^{**}P<0.01$).

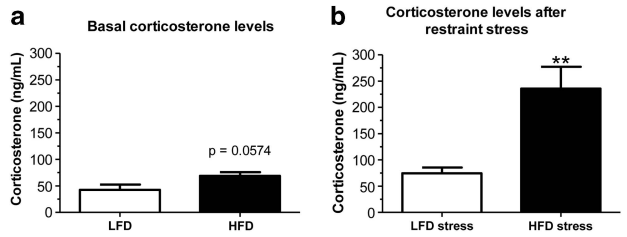


Figure 3. High-fat diet heightens corticosterone stress response. (**a**) Trend for increased corticosterone levels in HFD mice ($n=6$) as compared with the control LFD mice ($n=6$). (**b**) Significant increase in plasma corticosterone levels after restraint stress in HFD mice ($n=8$) compared with the control LFD mice ($n=8$). $^{**}P<0.01$.

Levels of TH protein in the DLS were similar between HFD and LFD mice (Figure 4f) and there was no significant difference in the levels of DA D2R protein following long-term HFD in DLS (Figure 5b). However, we observed that 12 weeks of HFD led to significantly higher levels of p-DARPP32 protein in the DLS as compared with LFD (Figure 4f; $^{***}P=0.001$).

HFD-induced depressive behaviour is positively correlated with striatal BDNF and pCREB

To determine if a relationship exists between the expression of the signalling molecules we examined and behavioural despair, we correlated protein levels with immobility times in the FST. There were significant positive correlations between BDNF protein levels in NAc (Figure 5a; $r=0.4228$, $P=0.02$) and levels of phospho-CREB ($r=0.6018$, $P=0.003$) and BDNF ($r=0.5111$, $P=0.009$) proteins in DLS (Figure 5c and d). The expression of NAc D2 receptor shows a modest and near-significant positive correlation with immobility times ($r=0.32$, $P=0.053$, Figure 5b)

DISCUSSION

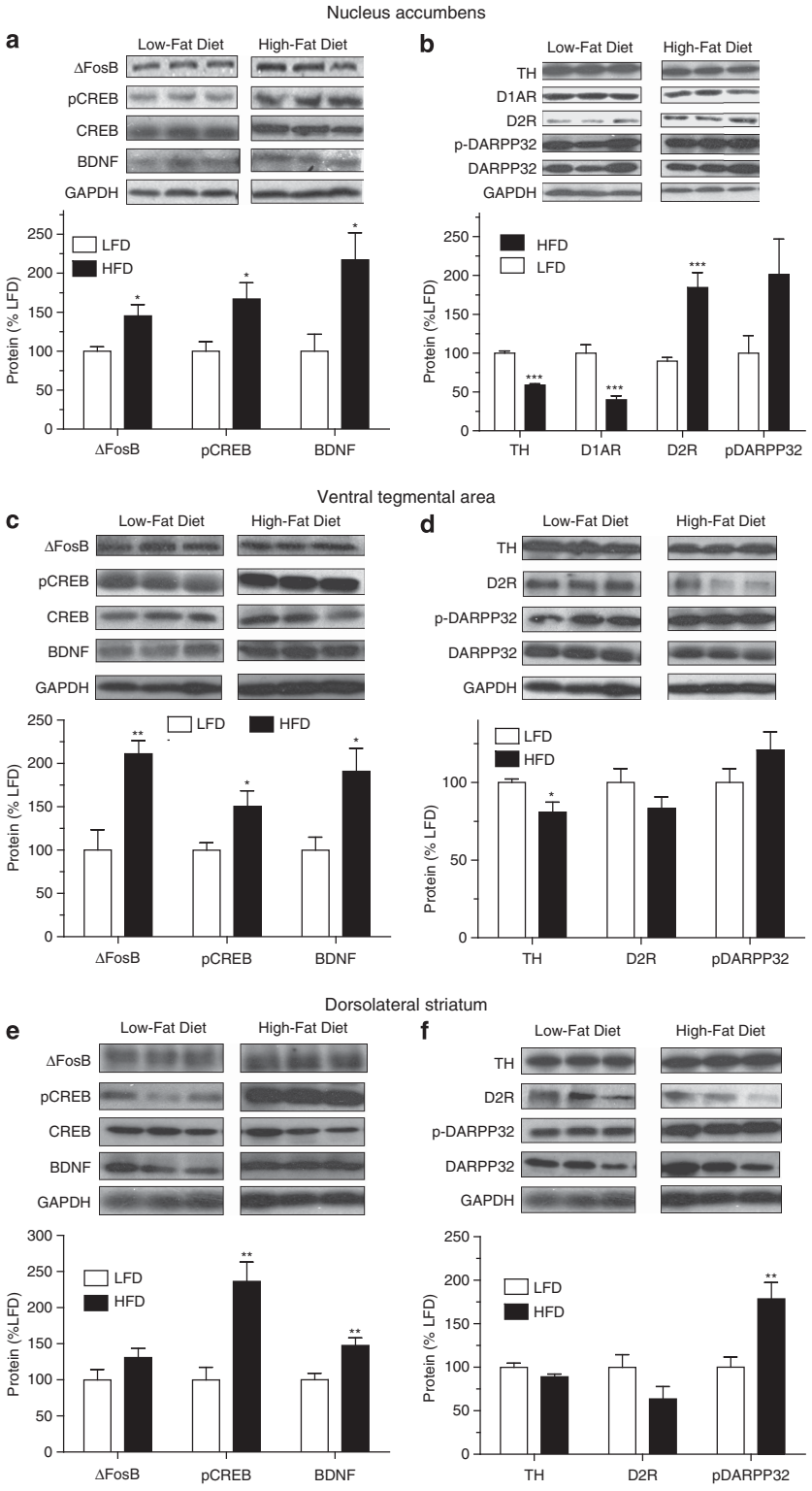
Epidemiological data suggest that obesity is associated with increased risk of developing depression,[17,18] yet there is little understanding of the neural mechanisms and brain reward pathways that underlie the link between DIO and vulnerability to depression. In the current study, we found that chronic consumption of a palatable HFD increases anxiety- and depressive-like behaviour, heightens the HPA response to stress and is responsible for several biochemical modifications in brain reward circuitry. Our study demonstrates for the first time that chronic consumption of palatable HFD has pro-depressive effects that are associated with increases in BDNF and phospho-CREB in the striatum, two signals that are well implicated in behavioural plasticity and reward. In view of these findings, we propose a model whereby high-fat feeding and obesity increase levels of BDNF and pCREB in the striatum (NAc and DLS) that contribute to negative emotional states and depressive-like symptoms (Figure 6). As illustrated in Figure 6, our data also show that chronic intake of HFD and DIO is linked with several other neural adaptations that may promote depressive-like behaviour and excessive caloric intake on a HFD. These changes include decreases in TH in the VTA and NAc that may lead to reduced DA tone in the mesolimbic pathway and elevated pCREB, BDNF and ΔFosB in the NAc and VTA, which may modulate behavioural plasticity in favour of elevated palatable food consumption.

Mice that were exposed to long-term HFD showed symptoms associated with depression in three behavioural tasks: elevated-plus maze, open field and forced swim. These findings are consistent with those of a recent study reporting increased depressive-like behaviour in HFD mice when using the forced swim task and sucrose preference test.[19] We cannot rule out the possibility that the increased adiposity of HFD mice may impair their swimming ability and thereby contribute to increased immobility times in the forced swim task. However, as we did not observe any correlation between body weight and immobility times (data not shown) and did not find differences in general locomotor activity, we feel it is unlikely that reduced swimming capacity accounts for the observed differences. Furthermore, our observation of increased anxiety behaviour using two additional tasks provides support for the depressive-like symptoms we observe in HFD mice.

Other studies demonstrate that palatable high-fat food produces an anxiolytic and anti-depressant effect when preceded by stressful experiences.[20] It is important to note, however, that acute or chronic consumption of HFD may have opposing effects

on anxiety and depressive symptoms. In the above-mentioned study, the mice were tested 2 weeks after exposure to HFD, whereas we used a 12-week regimen that was accompanied by significant increases in adiposity. We also observed that HFD consumption heightens responses to an acute stressor. These data suggest that not only does HFD increase stress-related behaviour but that it also enhances sensitivity to stressors and are thus are consistent with data showing increased corticosterone levels following HFD and elevated stress response in obesity.[21,22]

Our results show enhanced phosphorylation of CREB in both the NAc and DLS after chronic intake of a palatable HFD. CREB is activated in brain reward circuitry in response to variety of stimuli including stress and drug exposure.[23–28] Both chronic and acute psychostimulant drugs increase CREB phosphorylation, whereas



modulation of CREB by other drugs of abuse is a bit more complicated.[29–34] Elevations of CREB within the rat NAc produce anhedonia-like signs, reduces the rewarding effects of cocaine and sucrose and increase immobility time in FST.[24,35,36] We observed similar increase in levels of CREB in NAc after chronic consumption of HFD, indicating that enhanced activation of CREB in mesolimbic circuitry could be a risk factor for depressive-like behaviour and increased food intake during DIO.

CREB binds to CRE-binding sites that are located on several gene promoters including BDNF, identifying BDNF as a downstream target of CREB.[37,38] Additionally, upregulation of BDNF transcription has been shown to be CREB dependent.[38,39] Just as altering CREB can lead to changes in BDNF levels, manipulations of BDNF have been shown to alter CREB phosphorylation.[40,41] We observed that chronic HFD exposure significantly elevated BDNF levels in the NAc, VTA and DLS in these mice. Interestingly, elevated BDNF expression in the NAc by psychostimulant drugs is implicated in drug-induced morphological changes in NAc neurons that are fundamental to synaptic plasticity.[42] Several studies have shown that altered BDNF signalling is linked to hyperphagia and obesity in mice and humans.[43–47] BDNF has been widely studied in the hippocampus and frontal cortex for its role in depression, where a decline in BDNF levels is associated with depression. In contrast, increasing BDNF levels in NAc or VTA produces a depression-like phenotype and animals with selective knockout of BDNF in VTA are protected from depressive effects produced by social defeat stress.[48,49] A key study by Krishnan and colleagues demonstrates that increased BDNF signalling in the NAc mediates susceptibility to the depressive effects of social defeat and that depressed humans display increased BDNF levels in the NAc. As we found a significant positive relationship between depressive-like behaviour in the FST and BDNF levels in the NAc and DLS, we speculate that DIO exerts its pro-depressive effects through increasing BDNF in these limbic sites.

We found that chronic exposure to HFD significantly elevated the levels of ΔFosB in NAc and VTA. ΔFosB is the truncated form of FosB that following repeated exposure to rewarding stimuli is believed to help convert short-term reactions into long-term adaptations underlying neural plasticity and reward learning.[50] ΔFosB is also induced after prolonged psychostimulant drugs[51] and is reported to mediate resilience to stress and antidepressant actions in brain reward circuitry.[52] Interestingly, ΔFosB overexpressing mice show increased reward sensitivity and reduced DA signalling but 6 weeks of a palatable HFD exposure in these mice completely ameliorated these differences revealing the potent rewarding capacity of a palatable diet.[53] Thus, our

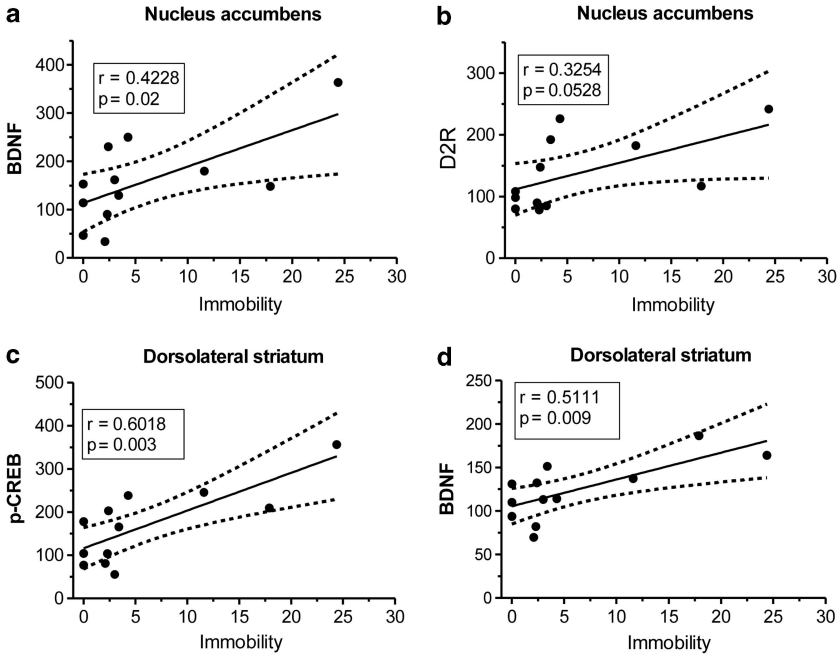


Figure 5. Correlation between depressive-like behaviour and reward-related signalling molecules. (**a**) Significant positive correlation between immobility time in FST and BDNF protein levels in NAc. (**b**) Moderate and near-significant positive correlation between immobility time in FST and dopamine D2R protein levels in NAc of HFD and LFD mice. (**c**) Immobility time in FST and p-CREB protein levels in DLS were positively correlated. (**d**) Significant positive correlation between immobility time in FST and BDNF protein levels in DLS of HFD and LFD mice.

Figure 4. Changes in reward signalling pathway in nucleus accumbens, ventral tegmental area and dorsolateral striatum after chronic consumption of HFD. (**a**) Significant increase in the ΔFosB, BDNF and p-CREB protein levels in nucleus accumbens of C57Bl6 mice ($n=6$ each group) as compared with the control LFD mice following 12 weeks of HFD. (**b**) Significant reduction in tyrosine hydroxylase, dopamine D1AR and protein levels after 12 weeks of HFD and LFD consumption in C57Bl6 mice as compared with the control LFD mice. Significantly enhanced levels of dopamine D2R protein levels after 12 weeks of HFD and LFD consumption in C57Bl6 mice ($n=6$) as compared with the control LFD mice ($n=6$). (**c**) Significant increase in the ΔFosB, BDNF and p-CREB protein levels in ventral tegmental area of C57Bl6 mice ($n=6$ each group) as compared with the control LFD mice after 12 weeks of HFD. (**d**) Significant reduction in expression of dopaminergic molecules tyrosine hydroxylase, whereas non-significant increase in dopamine D2R protein levels after 12 weeks of HFD and LFD consumption in C57Bl6 mice ($n=6$) as compared with the control LFD mice ($n=6$). (**e**) Significant increase in the BDNF and p-CREB protein levels in the dorsolateral striatum of C57Bl6 mice ($n=6$ each group) as compared with the control LFD mice after 12 weeks of HFD. (**f**) No change in expression of tyrosine hydroxylase (TH), non-significant reduction of dopamine D2R and a significant increase in the p-DARPP32 (Thr 75) protein levels after 12 weeks of HFD and LFD consumption in C57Bl6 mice ($n=6$) as compared with the control LFD mice ($n=6$). $^{*}P<0.05$; $^{**}P<0.01$, $^{***}P<0.001$.

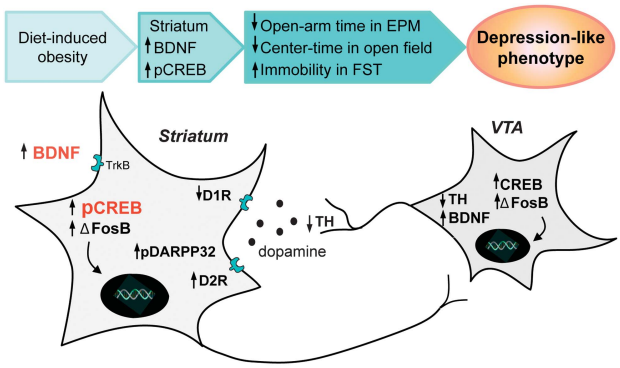


Figure 6. Proposed model for pro-depressive effects of diet-induced obesity and underlying neuroadaptations in brain reward circuitry. Long-term consumption of palatable high-fat diet and obesity promote negative emotional states of anxiety and despair that underlie depressive behaviour. These depressive symptoms arise from neural adaptations in brain reward circuitry by diet-induced obesity. Most notable are increased CREB activity in the dorsolateral striatum and increased protein levels of BDNF in the nucleus accumbens and dorsolateral striatum (shown in red) that were associated with the magnitude of HFD-induced despair and have been linked to depression in other conditions.[23–25,28,35,48,49] BDNF, brain-derived neurotrophic factor; CREB, cyclic AMP response element-binding protein; DARPP-32, dopamine- and adenosine-regulated phosphoprotein-32; EPM, elevated-plus maze; FST, forced swim test; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

observations of enhanced levels of ΔFosB in reward-related brain areas following 12 weeks of palatable HFD are consistent with these findings.

ΔFosB is known to induce expression of cyclin-dependant kinase 5 (Cdk5),[54] which in turn phosphorylates the protein DARPP-32 at Thr 75.[55] Presentation of a novel, palatable food can increase DA and cyclic adenosine monophosphate regulated phosphoprotein with a molecular mass of 32 kDa (pDARPP-32 thr75) expression in the NAc.[56] DARPP-32 regulates the transcription factor CREB, and viral-mediated decreases in CREB in the NAc are reported to increase the rewarding effects of cocaine.[28] It is known that phosphorylation of DARPP-32 (Thr 75) attenuates D1 DA receptor activity via direct inhibition of protein kinase A and inhibits phosphorylation of DARPP-32 at Thr 34.[57] We also observed reduced levels of DA D1A receptor in NAc of HFD mice. Perhaps chronic HFD alters the levels of striatal ΔFosB that in turn alters the levels of p-DARPP-32 (Thr75) resulting in reduced DA D1A receptor protein levels and dysregulation of DA signalling. A similar dietary regimen involving consumption of high-fat, high-sugar food for 12 weeks has been reported to decrease DA D1 receptor gene expression in NAc.[58] On the other hand, we observed a significant increase in D2 receptor protein levels in NAc, which may be surprising when considering that reduced D2 receptor binding is associated with human obesity.[8,9] However, we measured total protein expression of D2R, which could be quite different from the binding state of the receptor. Finally, our observations of reduced level of TH in the VTA and NAc of HFD mice are consistent with the previous reports of reduced DA tone in dietary obesity[4,5] and suggests that DA biosynthesis is involved.

Stress and inflammation have been postulated to increase the incidence of obesity and depression and alterations in neuronal plasticity and behaviour that underlie depression. Data also have demonstrated that inflammatory cytokines can interact with multiple pathways known to be involved in the development of depression, including monoamine metabolism, neuroendocrine function, synaptic plasticity and neurocircuits relevant to mood regulation.[59] However, the interaction between stress, inflammation and metabolic dysfunction in relation to the development of obesity and mood disorders remain to be elucidated. The other important aspect involved in DIO and depression is the ratio of n-6 to n-3 PUFA and recent observations describing beneficial role of omega-3 fatty acids in metabolic and nervous system disorders. It may be important to know apart from quantity of fat in the diet how the *quality* of fat affects reward pathways.

It was recently shown that leptin decreases depressive-like behaviour in mice and that leptin-overexpressing transgenic mice exhibit less depressive behaviour than non-transgenic mice.[19] In contrast, leptin-deficient *ob/ob* mice showed more severe depressive behaviour in the FST than normal mice, and leptin administration substantially ameliorated this depressive behaviour.[19] On the other hand, palatable HFD consumption has been reported to ameliorate anxiety and depression-like symptoms and improve stress responses in rats.[60,61] Similarly, HFD consumption has been shown to selectively and robustly protect against some of the negative behavioural aspects of chronic unpredictable social stressors.[62] It is not clear how to reconcile these different results, as leptin is elevated in DIO, however, the findings from Yamada *et al.*[19] suggests that central leptin resistance that appears with chronic HFD intake and DIO may be involved.

The present results demonstrate the effects of high-fat feeding and obesity to increase depressive-like behaviour in a manner that is positively associated with BDNF and phospho-CREB levels in limbic reward sites. Several lines of evidence tying elevated BDNF in the NAc to behaviourally relevant plasticity and depression in rodents and humans further support a potential role for striatal BDNF in the potentiation of anxiety and depression by high fat feeding and DIO. Further studies involving cell-specific interventional approaches hope to identify the direct contribution of BDNF and determine how high fat feeding and weight gain increase striatal BDNF to modulate emotions and relevant behavioural outputs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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