Diet-induced obesity in the Sprague–Dawley rat: dietary manipulations and their effect on hypothalamic neuropeptide energy balance systems

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Abstract
The SD (Sprague–Dawley) rat model of DIO (diet-induced obesity) is reported to exhibit a clear segregation into susceptible and resistant subpopulations shortly after transfer to a HE (high energy) diet. This does not appear to be the case for rats sourced in the U.K., where body weight gain on obesogenic HE diet is normally distributed, as might be anticipated for a polygenic trait in an outbred population. Many of the energy balance effects of dietary manipulation in this model (e.g. supplementation of HE diet with the liquid diet, Ensure; energy intake and defence of body weight following withdrawal of obesogenic diet) appear to be characteristics of the diets being manipulated rather than subject traits. The activities of energy balance-related hypothalamic signals are affected by diet and the development of DIO, but may not be able to differentiate between different diets and the relative levels of obesity that develop.

DIO (diet-induced obesity)
Human obesity has a very strong genetic component and loss-of-function mutations in individual genes can result in obesity. For example, functionally relevant mutations in the Mc4R (melanocortin-4 receptor) probably account for 2–4% of early onset extreme obesity [1]. Similarly, in a relatively small number of individuals, the cause of obesity has been ascribed to mutations in further key energy balance genes such as leptin [2], the OBR (leptin receptor) [3] and POMC (pro-opiomelanocortin; [4]). The products of these genes are essential for normal body weight regulation in both laboratory animals and humans. However, most human obesity is not the result of specific mutations in a particular gene, but rather represents the outcome of an underlying polygenic (multi-gene) predisposition or susceptibility to obesity, which leads to the expression of an obese phenotype under appropriate environmental conditions. Diet is a major factor within our current obesogenic environment, where energy dense, high fat, high sugar foods and beverages engage both homoeostatic and hedonic regulatory systems. The likelihood that contemporary dietary components, and the energy contained therein, lie at the root of the current obesity epidemic has increased interest in rodent models of DIO. A number of different types of diet have been employed to induce obesity in rodent models, including high-fat diets, HE (high energy) diets (moderately high fat and high sugar), and palatable liquid diets. Research characterizing the outbred SD (Sprague–Dawley) rat DIO model, as pioneered and driven forward by Levin and co-workers, has frequently employed an HE diet, often in combination with the complete liquid diet, EN (Ensure) [5–7]. In the present study, we review this intriguing model, focusing on the specific dietary manipulations that have been applied, and the consequences of these for energy intake, body weight and hypothalamic energy balance systems.

The SD rat model of DIO
The SD rat model of DIO has been investigated extensively by Levin and co-workers over the last decade, initially with outbred rats but lately concentrating more on selectively bred susceptible and resistant lines. The outbred model, as described, hinges on several key dietary manipulations. These are as follows: (1) transfer from chow diet to HE pellet diet, (2) supplementation of HE diet with the complete liquid diet EN and (3) withdrawal of HE or HE + EN and transfer back to chow pellet. The critical characteristics of the outbred model have been described in [5]. SD rats transferred from chow diet [Purina 5001; 3.34 kcal/g (where 1 cal ≡ 4184 J); 23% energy as protein, 12% as fat and 65% as carbohydrate] to HE diet (Research Diets C11024; 8% corn oil and 44% sweetened condensed milk and 48% Purina 5001; 4.5 kcal/g; 15% energy as protein, 33% as fat and 52% as carbohydrate) were reported to segregate into...
DIO-R (obesity-resistant) and DIO-S (obesity-susceptible) subpopulations. For experimental convenience, 40% of rats with the lowest or highest weight gain after 2 weeks on HE diet were assigned to each group. The DIO-S group remained on HE diet, whereas the DIO-R rats then received, as an additional supplement, the chocolate flavoured liquid diet, EN (Abbott Nutrition; 1.06 kcal/ml; 14% of energy as protein, 22% as fat and 64% as carbohydrate). Provision of EN induced additional weight gain in the DIO-R group, and enabled them, through sustained over-consumption of calories, to achieve a similar body weight to the DIO-S group feeding on HE diet only. The response of subsequent transfer of both groups back to chow was particularly intriguing. DIO-S rats, previously fed on HE diet, ‘defended’ their elevated body weight, i.e. were effectively weight stable. However, simultaneous withdrawal of both HE and EN resulted in a decrease in both energy intake and body weight in DIO-R rats, i.e. an apparent inability to ‘defend’ body weight; energy intake decreased immediately and substantially following transfer back to chow diet, and recovered only slowly, and body weight decreased to a level similar to that of control rats fed on chow diet throughout or DIO-R rats fed on the HE diet.

From the intriguing responses to dietary manipulation outlined above, it is clear that the SD-DIO model has the potential to illuminate a number of related issues of importance in obesity research. These include mechanisms of susceptibility to obesity, body weight defence, and interaction of diet with homoeostatic and reward-based energy balance systems. However, a clearer picture might emerge of the interaction of diet with energy balance systems if these manipulations were applied to a whole population of rats irrespective of body weight trajectory.

A reassessment of dietary manipulations
Using outbred rats from Charles River (Margate, Kent, U.K.), we applied the dietary manipulations (1)–(3) outlined above, and observed many of the characteristics of the SD-DIO model reported previously, but also some major differences.

Dietary manipulation (1) – chow to HE
We observed a range of body weight trajectories in rats transferred to HE diet, but also in rats maintained on chow diet (Figure 1; [8]). Maximal rates of body weight gain were observed in rats fed HE diet, but this diet did not always induce obesity; some rats on chow diet gained more weight than individuals on HE diet (Figure 1). Although differential susceptibility to DIO clearly exists within outbred populations of SD rats, in our hands, there was no evidence of a bimodal distribution of body weight gain, i.e. of the emergence of subpopulations of susceptible and resistant individuals on feeding HE diet (Figure 2A; [8]). In fact, applying published selection criteria [5] to a large group of rats in order to generate arbitrary groups on the basis of weight gain over the first 14 days on HE diet (upper 40% and lower 40% of weight gain) gave rise to groups with a weight difference of approx. 44 g at 14 days (Figure 2C; [8]). The difference in body weight gain between high (DIO-S) and low (DIO-R) weight gain ‘groups’ of U.K.-sourced animals was if anything more substantial than between equivalent groupings in the U.S.A. rats [5,8]. However, not only was weight gain during this 14 day period normally distributed (Figure 2A; [8]), but so was weight gain and tissue weight at later time points. Analysis of these rats as arbitrary subgroups may be inappropriate.

Dietary manipulation (2) – supplementation of HE with EN
In the now classical study by Levin and Keesey [5], only rats from the group with lowest rate of weight gain were given EN. Our identical dietary manipulation of a large outbred population induced sustained elevated energy intake and increased weight gain, with the whole population mounting this characteristic response [9]. By again imposing the DIO-S/DIO-R categorization on to these results, it is clear that the incremental increases in energy intake and body weight gain with EN are very similar between the groups (Figures 2B and 2C), although longitudinal changes in energy intake are apparent.

Dietary manipulation (3) – withdrawal of HE or HE + EN and transfer back to chow
In confirmation of reports in the literature [5,7,10], rats defend the body weight that they attain on HE diet when returned to chow diet (Figure 1), although rats fed chow throughout may continue to increase in body weight [8]. Transferring rats back to chow caused a transient hypocaloric intake in rats previously fed HE diet (Figure 2B), but this effect was overshadowed by the outcome of withdrawal of HE + EN, as recorded elsewhere [5–7]. Analysis of our results following DIO-S/DIO-R assignment demonstrates that the magnitude of the hypocaloric intake response to transfer back to chow diet is independent of early body weight trajectory, i.e. DIO phenotype. Furthermore, at least
Figure 2  (A) Weight gain distribution (g) of outbred male SD rats fed HE diet for 2 weeks, (B) energy intake (kcal) and (C) body weight (g) of outbred male SD rats designated relatively susceptible (DIO-S) or relatively resistant (DIO-R) to DIO (upper and lower 40% of weight gainers after 2 weeks on HE diet).

Rats were all fed chow for 7 days, followed by HE diet for 21 days \((n=80)\), after which half the animals remained on HE diet (HE group) and half had HE diet supplemented with EN (HE + EN group) for 10 weeks. In each group, 20 animals were killed and the remaining animals were transferred back to chow for 3 weeks. Vertical lines on (B) show points of dietary manipulation – (1) transfer from chow to HE diet, (2) supplementation of HE diet with EN, and (3) withdrawal of HE or HE + EN and transfer back to chow.

Partial loss of additional weight acquired through EN feeding is also common to DIO-S and DIO-R groups.

Modelling DIO in humans

It is clear that in humans the development of obesity on a high fat, HE diet is not inevitable [11], and that a range of susceptibilities exist. In our rat experiments, weight gain on HE diet was normally distributed. This outcome accords with the expected distribution of a polygenic trait in an outbred population more precisely than a bimodal segregation, reported elsewhere [5,6,12]. Consequently, the SD rat represents a useful model of obesity on a Western diet. There is no clear explanation of the fundamental difference in response of U.K. and U.S.A. rat populations to the same dietary manipulation. Selective breeding of DIO-S and DIO-R lines from an outbred population [13] would not depend upon the existence of a bimodal distribution, but demonstrates that it is possible to concentrate susceptibility and resistance traits. These lines have been studied in considerable detail (see publications by Levin and co-workers). We have confirmed the critical characteristics of the SD-DIO model in terms of caloric intake and body weight change following introduction and withdrawal of the obesogenic diets, HE and HE + EN, and conclude that the majority of these are diet effects rather than subject traits, e.g. failure to defend EN-induced weight gain. To improve our ability to navigate through our obesogenic environment, we need to further our understanding of how the body responds to a Western-style diet (solid and liquid), and why our energy balance regulatory systems do not protect us from dangerous weight gain.

Regulation of energy balance and body weight

The hypothalamus has a critical role in the regulation of energy balance and body weight. A network of anatomically distinct hypothalamic structures, and signalling systems therein, receive neural, metabolic and hormonal feedback from the periphery and are involved in the maintenance of an appropriate body weight and composition. Throughout the last two decades we have gained detailed knowledge of the neuronal populations involved in regulating energy balance, the orexigenic (anabolic) and anorexigenic (catabolic) molecular substrates involved, and their regulation and integration [14–18]. However, most of our knowledge of the functioning of hypothalamic peptide systems comes from the study of mutations leading to obesity, transgenic knockouts, in vivo responses to peptides injected directly into the brain, and the effect of energy deficit on gene expression and peptide concentrations. Much less well understood is the sensitivity of energy balance systems to dietary manipulation and more subtle changes in body weight over longer periods. In short, how do the energy balance systems interact with diet in the regulation of body weight in the normal animal? With this goal in mind, we are examining the interaction of diet with hypothalamic energy balance systems in the SD-DIO model.

Hypothalamic gene expression

We used panels of cloned neuropeptide and receptor genes to investigate the consequences of feeding obesogenic diets and the development of DIO.
We performed dietary manipulation (1) in outbred juvenile SD rats [19]. Gene expression was assayed after 5 weeks on HE diet, a manipulation which in young rats resulted in a small reduction in weight gain, but increases in fat pad weight and blood metabolites and hormones, such as leptin and insulin. Selected genes were analysed in key hypothalamic nuclei: ARC (arcuate nucleus), VMH (ventromedial nucleus), DMH (dorsomedial nucleus) and PVN (paraventricular nucleus). Rats fed with the HE diet had lower levels of NPY (neuropeptide Y), AgRP (agouti-related protein), OBR and Mc3R mRNA in the ARC (Table 1). NPY and OBR mRNAs were also lower in the DMH and VMH respectively. Reduced expression of orexigenic peptides (NPY and AgRP) in the ARC, the major site of leptin and other hormonal and metabolic feedback to the hypothalamus, is consistent with published results in mice [20,21], and could represent a compensatory response counteracting the state of positive energy balance. Down-regulation of OBR expression was consistent with the elevated circulating leptin concentrations that accompanied DIO.

Changes in gene expression after 5 weeks on HE diet could be the consequence of excessive accumulation of adipose tissue. In contrast, an individual’s immediate appetite for food during the initial period following introduction of a novel diet (see Figure 2B), and thus over-consume calories of the more energy-dense HE diet. The increase in CART gene expression induced by positive energy balance at the end of the first 12 h of HE-feeding may reflect the recruitment of compensatory adaptations, via this anorexigenic peptide, intended to reduce food intake and increase energy expenditure.

In order to assess the effects of dietary manipulations (2) and (3) on gene expression, we undertook the trial portrayed on Figures 2(B) and 2(C), where rats were killed at the point of withdrawal of obesogenic diet and transfer back to chow, and 3 weeks after this manipulation [9]. We hypothesized that hypothalamic energy balance systems would both distinguish between the effects of feeding on HE or HE + EN diets, i.e. the long-term effect of manipulation (2), and reflect the consequences of withdrawal of these obesogenic diets and transfer back to chow, i.e. the effect of manipulation (3). It was surprising that only TrkB (tyrosine kinase B) mRNA in the VMH was affected by EN supplementation; higher levels of expression were observed in EN-fed rats (Table 1). Despite the increased adiposity and leptin levels in HE + EN animals, this was not reflected in the gene expression of any of the classical orexigenic or anorexigenic neuropeptides. The strongest influence on hypothalamic gene expression was the withdrawal of obesogenic diet, i.e. transfer back to chow; dietary manipulation (3) caused changes in expression levels of four of the panel genes, NPY, CART, DYN (dynorphin) and BDNF (brain-derived neurotrophic factor).

Table 1 | Sensitivity of hypothalamic energy balance genes to dietary manipulation in the SD rat model of DIO

<table>
<thead>
<tr>
<th>Gene</th>
<th>Hypothalamic nucleus</th>
<th>Approx. % change in gene expression and experimental summary</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY</td>
<td>ARC</td>
<td>↓35% – 5 weeks on HE diet</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>ARC</td>
<td>↑25% – Transfer back to chow for 3 weeks after 10 weeks on HE or HE + EN</td>
<td>[9]</td>
</tr>
<tr>
<td>DMH</td>
<td></td>
<td>↓35% – 5 weeks on HE diet</td>
<td>[19]</td>
</tr>
<tr>
<td>AgRP</td>
<td>ARC</td>
<td>↓10% – 5 weeks on HE diet</td>
<td>[19]</td>
</tr>
<tr>
<td>CART</td>
<td>ARC</td>
<td>↑20% – 12 h on HE diet</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>ARC</td>
<td>↓25% – Transfer back to chow for 3 weeks after 10 weeks on HE or HE + EN</td>
<td>[9]</td>
</tr>
<tr>
<td>OBR</td>
<td>ARC</td>
<td>↓30% – 5 weeks on HE diet</td>
<td>[19]</td>
</tr>
<tr>
<td>VMH</td>
<td></td>
<td>↓20% – 5 weeks on HE diet</td>
<td>[19]</td>
</tr>
<tr>
<td>DYN</td>
<td>ARC</td>
<td>↓20% – Transfer back to chow for 3 weeks after 10 weeks on HE or HE + EN</td>
<td>[9]</td>
</tr>
<tr>
<td>VMH</td>
<td></td>
<td>↓25% – Transfer back to chow for 3 weeks after 10 weeks on HE or HE + EN</td>
<td>[9]</td>
</tr>
<tr>
<td>BDNF</td>
<td>VMH</td>
<td>↓10% – Transfer back to chow for 3 weeks after 10 weeks on HE or HE + EN</td>
<td>[9]</td>
</tr>
<tr>
<td>Mc3R</td>
<td>ARC</td>
<td>↓20% – 5 weeks on HE diet</td>
<td>[19]</td>
</tr>
<tr>
<td>Mc4R</td>
<td>PVN</td>
<td>↑60% – 24 h on HE diet</td>
<td>[23]</td>
</tr>
<tr>
<td>TrkB</td>
<td>VMH</td>
<td>↑10% – 10 weeks on HE supplemented with EN</td>
<td>[9]</td>
</tr>
</tbody>
</table>
obesogenic diet. Consistent with its anorexigenic properties, BDNF gene expression in the VMH was decreased following transfer back to chow, suggesting a role in energy homeostasis similar to that of CART. The parallel down-regulation of DYN gene expression in both the ARC and the VMH on transfer back to chow indicates that this system is up-regulated by the obesogenic diets employed, but that there is no additional effect of supplying the palatable liquid EN. The effect of EN supplementation of HE diet on expression levels of selected hypothalamic genes has been examined independently in selectively bred DIO-S rats following a series of dietary manipulations [6,7], although it is difficult to make direct comparison with these results [9].

Conclusions
It may be useful to select and analyse groups of animals on the basis of susceptibility to DIO, but the distribution of weight gain observed in our studies suggests that this approach should be adopted with caution. The outcome of dietary manipulations in our unselected outbred populations complements earlier studies with both outbred rats selected for relative resistance to DIO, and selectively bred DIO-susceptible and DIO-resistant animals [5–7], and suggests that many of the energy balance effects of dietary manipulation are characteristic of the diet being manipulated rather than the phenotype of the experimental subject. A growing body of evidence indicates that the activities of energy balance-related hypothalamic and peripheral signals are affected by diet and the development of DIO. However, it is less clear whether these signalling systems can differentiate between different diets and the relative levels of obesity that develop as a consequence of feeding upon these diets. The evidence we have collected suggests that this may not be the case. A more thorough understanding is required of the interaction of different diets, and in particular liquid diets, with energy balance systems.

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References

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