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Review

Putting the diet back into diet-induced obesity: Diet-induced hypothalamic gene expression

Julian G. Mercer *, Zoë A. Archer

Division of Obesity and Metabolic Health, Rowett Research Institute, Aberdeen Centre for Energy Regulation and Obesity (ACERO), Aberdeen AB21 9SB, Scotland

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Abstract

A wealth of detailed mechanistic information relating to obesity and body weight regulation has emerged from study of single gene mutation models, and continues to be generated by engineered rodent models targeting specific genes. However, as an early step in translational research, many researchers are turning to models of diet-induced obesity. Interpretation of data generated from such models is not aided by the variety of diets and rodent strains employed in these studies and a strong case could be made for rationalisation. Differences in experimental protocol, which may deploy a single obligatory solid diet, a choice of solid diets, or liquid/solid combinations, and which may or may not allow a preferred macronutrient composition to be selected, mean that different models of diet-induced obesity achieve that obesity by different routes. The priority should be to mimic the palatability- and choice-driven over-consumption that probably underlies the majority of human obesity. Some of the hypothalamic energy balance genes apparently 'recognise' developing diet-induced obesity as indicated by counter-regulatory changes in expression levels. However, substantial changes in gene expression on long-term exposure to obesogenic diets are not able to prevent weight gain. Forebrain reward systems are widely assumed to be overriding hypothalamic homeostatic energy balance systems under these circumstances. More mechanism-based research at the homeostatic/reward/diet interface may enable diets to be manipulated with therapeutic benefit, or define the contribution of these interactions to susceptibility to diet-induced obesity.

Keywords: Neuropeptide Y; Agouti-related peptide; Leptin; (Sprague Dawley rat)

Contents

* Corresponding author. Division of Obesity and Metabolic Health, Rowett Research Institute, Aberdeen AB21 9SB, Scotland. Tel.: +44 1224 716662; fax: +44 1224 716686.
E-mail address: J.Mercer@rowett.ac.uk (J.G. Mercer).

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1. Applicability of animal models of energy balance to common human obesity

Loss-of-function mutations in a number of energy balance genes reveal their products to be essential for normal body weight regulation in mammals, including humans. For example, genes centred on the leptin-melanocortin receptor axis are essential for normal body weight regulation in both humans and laboratory rodents (Hebebrand et al., 2003; Montague et al., 1997; Clement et al., 1998; Krude et al., 1998). While this proof of principal is illuminating, the occurrence of small numbers of individuals with early-onset morbid obesity due to such mutations does not provide explanation of the underlying cause of the majority of human obesity, where no such deleterious changes are established. The general assumption is that common human obesity has its foundations in a susceptible genotype, where small changes in the activity or function of a number of genes combine to increase susceptibility to excess weight gain in what has been termed an obesogenic environment. The obesogenic environment, a term which first emerged in the literature in 1999 (Swinburn et al., 1999; Poston and Foreyt, 1999), is multi-faceted, encompassing diet, physical activity, built environment and socio-economic status. Thus, current lifestyles, where physical activity has been minimised without limiting access to contemporary diets that are palatable, highly nutritious and energy dense, contribute to just such a 'risk' environment. If we accept that inappropriate diet is a major contributory factor in the majority of human obesity in our obesogenic environment, then the case is made for incorporating diet-induced obesity into the design of mechanistic studies of obesity in laboratory rodents. Consequently, rodent models of diet-induced obesity are increasingly replacing well-characterised single gene mutation obesity models such as *ob/ob* and *db/db* mice, and pharmacological or surgical obesity models. A key question, however, is whether the precise dietary manipulations being performed to effect diet-induced obesity in rodents are the most appropriate to model common human obesity.

2. Rodent models of diet-induced obesity and diets employed

Assessment of the wide range of studies that might fall under the general heading of 'diet-induced obesity' readily highlights the difficulty in translating the outcomes of experiments into cause, effect and mechanism. Some consensus positions exist, but clarity is being lost in the welter of different experimental designs. As reviewed recently for high fat diets (Buettner et al., 2007), the dietary interventions themselves are far from standardised, and the resulting animal phenotypes vary considerably. For example, 'high fat' diets have been employed where fat contributes between 20% and 60% of energy, where the fat macronutrient itself may be derived from either animal fats or plant oils, and where diets may be defined and manufactured, or, at the other extreme, may consist simply of additional fat being mixed with normal rodent chow with obvious consequences for macronutrient and micronutrient balance (Buettner et al., 2007).

Broadening these arguments out from high fat diets to other obesogenic diets and to the experimental animals involved in these studies serves to place even more emphasis on the desirability of the scientific community refining experimental protocols and, preferably, agreeing on a smaller number of model systems (animals and diets) for intensive mechanistic study. Some standardisation of experimental details and manipulations should lead to the generation of datasets that are more readily comparable between studies than they are at present. At present, the animals subjected to dietary manipulation can come from different species, most frequently rats or mice, and within a species from numerous different strains. Even where a specific strain/diet combination is selected, the duration of exposure to the diet inevitably varies between different studies. A further unhelpful source of heterogeneity arises from animals ostensibly of the same strain and supplier, but sourced from different breeding colonies (see below). Furthermore, the generic state of diet-induced obesity has been examined using a number of distinct experimental strategies that may employ the same strain of rodent, the same diet, or both. Thus, studies have employed a single strain of rodent fed either an obesogenic diet or a control diet, two strains of rodent with differential susceptibility fed the same obesogenic diet (e.g. Madiehe et al., 2000), or a single strain of rodent, populations of which exhibit a range of body weight trajectories on a specific obesogenic diet. The outbred Sprague Dawley rat is an example of the last type of manipulation, where heterogeneity in response to an obesogenic diet may have parallels with differential susceptibility in the human population (Levin et al., 1989; Lauterio et al., 1994; Archer et al., 2003).

The majority of the obesogenic diets employed to generate diet-induced obesity are commercially produced pellet diets that are fed as the sole source of nutrition. This may be adequate if the objective of the study is limited to the production of an obese animal. However, by obliging the animal to consume an unbalanced diet, without any element of choice, relevance to contemporary Western diets is strictly limited. More subtle manipulations that provide for dietary choices, or include calories provided in liquid form will be required to address the mechanistic basis of over-consumption, macronutrient drive, and how diets interact with peripheral and central (brain) energy balance systems to induce obesity. The provision of liquid calories, for example, is relevant to the contribution soft drinks, shakes, yoghurt drinks and fruit puree products make to total dietary intake (Stubbs and Whybrow, 2004), and to the mechanisms whereby energy-containing fluids may induce less satiety than solids of equivalent energy content and carbohydrate load (DiMeglio and Mattes, 2000; Sclafani and Xenakis, 1984; Ramirez, 1987). Another major gap in our knowledge lies in the responses of juvenile, rapidly growing, animals to obesogenic diets, where there may be relevance to the mounting problem of obesity in childhood.

One area where the plethora of rodent models and diet manipulations makes it particularly difficult to discern robust overall outcomes is in the effect of diets and diet-induced obesity on energy balance signalling systems, both peripheral and central, where a number of studies have addressed hypothalamic neuropeptide systems. A brief summary of these systems, which are then addressed in the context of our own studies of diet-induced obesity, is provided below. A critical issue here is whether changes in the activity of these systems in diet-

induced obesity are always secondary to changes in body composition, or whether there are more direct impacts of diet.

3. Hypothalamic energy balance systems and interactions

The neuroendocrine hypothalamus, at the base of the forebrain, plays a key role in the regulation of energy balance through the integration of peripheral signals and onward signal transmission. Peripheral signals conveying information about meal processing, gastrointestinal activity, and changes in energy stores, access the brain via a number of routes, crossing or by-passing the blood-brain barrier from the systemic circulation, or changing the firing rate of vagal or other sensory nerve fibres. Knowledge of the molecular and neuroanatomical components of the energy balance systems and their regulation and interactions is now growing rapidly (Friedman and Halaas, 1998; Kalra et al., 1999; Schwartz et al., 2000; Berthoud, 2002). In addition to these homeostatic hypothalamic mechanisms, it is also clear that hedonics play a major role in food consumption in both humans and laboratory mammalian species, and may be especially important in diet-induced obesity (Saper et al., 2002), where obesogenic diets engage with the reward systems in the forebrain. The hedonic systems involve molecular components such as the opioid peptides, cannabinoids, and the dopaminergic system, and brain regions including the amygdala and the nucleus accumbens. Despite their likely significance for energy balance, interactions between the homeostatic and hedonic systems are not well understood, although it is widely speculated that forebrain reward systems are involved in overriding signalling emanating from the hypothalamic homeostatic systems.

The hypothalamic energy balance-related neuropeptide systems are best characterised in the context of responses to imposed negative energy balance. Reductions in blood levels of key metabolic hormones such as insulin and leptin as a result of the rationing or withdrawal of food lead to changes in gene expression many of which are centred on the hypothalamic arcuate nucleus. Expression of orexigenic neuropeptides, such as neuropeptide Y and agouti-related peptide is increased, and correspondingly, expression of anorexigenic genes such as pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript is decreased, changes that combine to counteract energy deficit by both conserving energy and inducing hyperphagia once food becomes available again. It is easy to envisage how such regulatory systems might have evolved to minimise the risk of starving to death (Mercer and Speakman, 2001). However, although evolutionary pressures could also have acted to limit excessive storage of surplus energy as fat (Speakman, 2007), the evidence of effective neuroendocrine systems fulfilling this role is quite limited (Mercer and Speakman, 2001). Nevertheless, forced overfeeding by tube increased expression of the anorexigenic genes, corticotrophin-releasing factor and pro-opiomelanocortin by 50% and 80% in the paraventricular nucleus (Seeley et al., 1996) and arcuate nucleus (Hagan et al., 1999), respectively. Whereas the limited data from forced feeding studies suggests the recruitment of counter-regulatory activity changes, regulatory events accompanying *voluntary* over-feeding leading to diet-induced obesity are likely to be much more relevant to human obesity. The remainder of this review focuses on our studies of the interaction of diet and diet-induced obesity with hypothalamic energy balance systems in our chosen model, the outbred Sprague Dawley rat.

4. The Sprague Dawley rat model of diet-induced obesity: dietary manipulations

A number of interesting dietary manipulations have been described by Levin and co-workers using the Sprague Dawley rat, whereby rats were transferred from a stock pellet to a high energy pellet, and depending on body weight trajectory then had this high energy pellet supplemented with the complete liquid diet, chocolate Ensure™. Utilising the high energy pellet diet, there have been a number of reports originating in the USA of the emergence of sub-populations of rats that are either susceptible to obesity or are resistant (Levin and Dunn-Meynell, 2000; Levin and Keesey, 1998; Levin, 1999; Lauterio et al., 1999). However, in our own laboratory, using the same high energy diet and Sprague Dawley rats sourced from the same commercial supplier, but through their facility in the UK, we consistently observe a normal distribution of body weight gain, and thus of susceptibility to excessive weight gain (Archer et al., 2003), as might be anticipated for a polygenic trait in an outbred population, rather than the emergence of distinct sub-groups. The second intriguing manipulation performed by Levin and co-workers with the Sprague Dawley rat was the provision of chocolate Ensure. This supplement was originally employed to produce obesity in obesity resistant rats, i.e. those with low weight gain on the high energy pellet diet (Levin, 1999), but we have since demonstrated that all rats in the population exhibit sustained caloric over-consumption and weight gain with the Ensure supplement (Mercer and Archer 2005), and irrespective of whether the Ensure is provided on a background of high energy pellet or stock pellet (Archer et al., 2007). The chocolate flavour was not required to stimulate caloric over-consumption. We tested four flavours of Ensure — chocolate, vanilla, coffee and asparagus, and each one induced a sustained increase in daily energy intake of approximately 15% on a background of stock pellets (Archer et al., 2006). There was a short-lived effect of flavour on initial consumption, with coffee and asparagus flavours being consumed less avidly than vanilla or chocolate, but no effect on body weight gain. Other attributes that might explain the stimulatory effect of Ensure supplements on total energy intake include its liquid formulation, macronutrient composition, and post-ingestive consequences.

5. The Sprague Dawley rat as a model of juvenile diet-induced obesity

The focus of most studies of diet-induced obesity to date has understandably been on adult rodents. Very little work has been done on juvenile animals (Peckham et al., 1977; Kanarek and Marks-Kaufman, 1979; Hirsch et al., 1982; Levin et al., 1986; Archer et al., 2004), despite the obvious benefits that could be derived from examination of the mechanisms underlying the development of obesity at an equivalent life stage to children

and adolescents in the human population. This prompted us to investigate the effect of one of the dietary manipulations detailed above, feeding a high energy diet, on Sprague Dawley rats 1 week after weaning (Archer et al., 2004). This manipulation resulted in the unexpected phenotype of normal or slightly reduced body weight gain over the 5 week trial (Fig. 1 upper), but increased terminal body fat and serum leptin concentration. Assessment of hypothalamic gene expression revealed that juvenile rats fed the high energy diet had lower levels of neuropeptide Y, agouti-related peptide, leptin receptor and melanocortin-3 receptor mRNAs in the arcuate nucleus (Fig. 1 lower; Archer et al., 2004). This profile of hypothalamic gene expression as a consequence of feeding on the high energy diet and/or the development of diet-induced obesity on that diet was consistent with studies of young mice (Lin et al., 2000; Guan et al., 1998) using high fat diets, and consisting, as it does, of a down-regulation of orexigenic neuropeptide genes, is suggestive of a counter-regulatory response to the state of positive energy balance signalled via serum leptin from the enlarged body fat stores.

A plausible interpretation of the obese but normal weight phenotype observed when young rats were fed high energy diet was that the relatively low percentage of energy contributed by protein in this diet may have resulted in a relative protein deficiency, during a time of rapid growth. To examine this possibility and to develop a better model of juvenile obesity, i.e. one with both additional weight gain and excess fat deposition, we then tested different combinations of the diets referred to above, namely pelleted stock and high energy diets, and liquid chocolate Ensure. Experimental groups were fed on stock pellet, high energy pellet, or were provided with a choice between these two pellet diets. Three further groups had these pellet diets or the combination supplemented with Ensure for the 5-week study (see

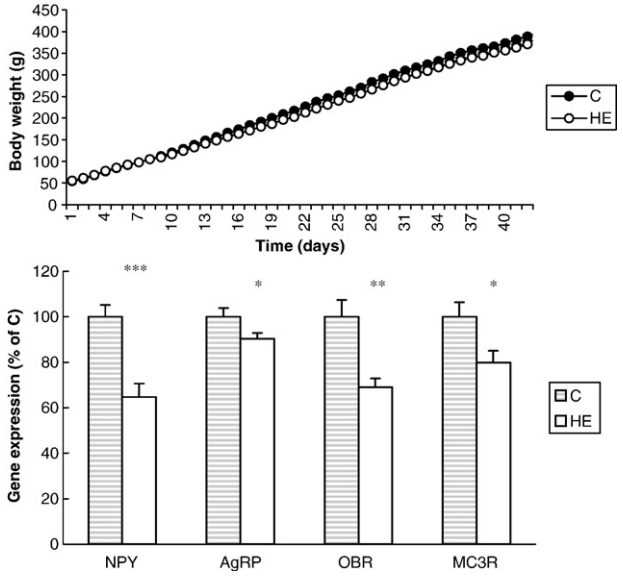

Fig. 1. Upper graph: Body weight of juvenile rats fed a control diet (C) for 1 week and then that diet or a high energy (HE) diet for 5 weeks. Lower graph: Hypothalamic neuropeptide or receptor gene expression in the hypothalamic arcuate nucleus. Asterisks indicate different from control: *$P<0.05$, **$P<0.01$, ***$P<0.001$. Values are means±S.E.M ($n=30$).

Table 1
Percentage contribution of each individual diet to total caloric consumption, and macronutrient intake as a percentage of energy intake, in juvenile male Sprague Dawley rats offered various combinations of three diets

	Diet			Macronutrient		
	C	HE	EN	Fat	Carbohydrate	Protein
Group	% of total intake			% energy		
C	100	–	–	12	65	23
HE	–	100	–	33	52	15
C+HE	41.8%	58.2%	–	24.2	57.5	18.3
C+EN	45.1%	–	54.9%	17.5	64.4	18.1
HE+EN	–	77.4%	22.6%	30.5	54.7	14.8
C+HE+EN	33.7%	28.7%	37.6%	21.8	60.9	17.4

Four-week old male Sprague Dawley rats were allocated to 6 weight matched groups and fed either chow (C), a high energy diet (HE), C+HE, C plus the liquid supplement Ensure (C+EN), HE+EN or all three diets (C+HE+EN) for 5 weeks. Values are means, where $n=10$.

Table 1). These studies highlighted the issue of whether we should be using diets that induce obesity simply due to obligatory consumption of unbalanced macronutrient combinations or whether experimental animals should be able to express dietary preferences and select a more optimal macronutrient profile whilst retaining the capacity for palatability-driven over-consumption. The study successfully identified diet combinations that resulted in the desired phenotype. Maximum weight gain (Fig. 2 upper), and fat (Fig. 2 middle) and lean tissue accretion, was achieved by rats in the groups that were able to select a diet that contained at least 17% protein (i.e. where stock pellet was provided), and where Ensure was also available (i.e. C+EN and C+HE+EN in Table 1; Archer et al., 2007). The high energy and Ensure diets provided less than 15% of energy from protein, and experimental groups limited to these diets, and thus obliged to consume an energy dense diet with a relatively low protein content, had the lowest lean tissue weight by MRI, again suggesting a marginal protein deficiency. These and other diets of similar protein content are probably inappropriate for mechanistic investigation in such young rats. Dietary preferences were heavily influenced by the combination of diets on offer (Table 1). Provision of dietary choice is clearly also a more realistic manipulation in the context of the human food environment, if mimicking that environment is an objective.

Rats fed the C+EN diet had numerically the lowest blood NEFA, triglyceride and leptin concentrations of the 5 obesogenic-diet groups (leptin — Fig. 2 lower; Archer et al., 2007), despite having the highest weight gain and body adiposity. Similar profiles of dietary influence were observed for each of the three parameters. Circulating leptin levels were increased by all 5 'obesogenic' diets relative to stock diet. Leptin levels within each group correlated positively with body fat content (Fig. 3A), but C+EN rats had the lowest serum leptin relative to body adiposity. Grouping the leptin data by presence or absence of Ensure revealed an overall depressive effect of Ensure on serum leptin concentrations (Fig. 3B); Ensure affected the association between adipose tissue and leptin by reducing the relative leptin level by about 1.3 μg/L at a fixed level of adipose tissue. Dissociation between adipose tissue weight and circulating leptin has been reported previously (Ainslie et al., 2000), in

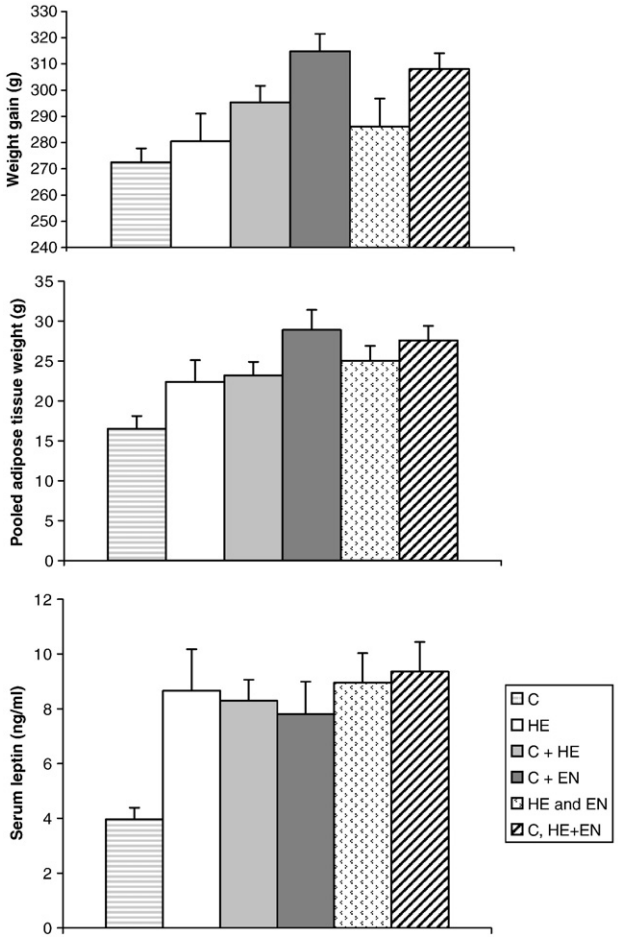

Fig. 2. Upper graph: Cumulative body weight gains of male Sprague Dawley rats allocated to 6 weight matched groups and fed either chow (C), a high energy diet (HE), C+HE, C plus the liquid supplement Ensure (C+EN), HE+EN or all three diets (C+HE+EN) for 5 weeks. Middle graph: Pooled weight of 5 dissected white adipose tissue depots at the end of the 5-week trial. Lower graph: Terminal serum leptin concentrations. Values are means± SEM, $n=10$.

female Wistar rats fed a high-fat diet, and may contribute to weight gain on high fat diets.

We assessed expression levels of a panel of energy balance genes by in situ hybridisation in a number of different hypothalamic structures. Statistical analysis revealed effects of Ensure supplementation for four genes, neuropeptide Y (arcuate nucleus; Fig. 4 upper), agouti related peptide (arcuate nucleus; Fig. 4 lower), dynorphin (paraventricular nucleus, supraoptic nucleus, supraoptic retrochiasmatic nucleus; Archer et al., 2007), and cocaine- and amphetamine-regulated transcript (supraoptic retrochiasmatic nucleus; Archer et al., 2007). In each case, mRNA level was decreased by Ensure supplementation. At least in the case of neuropeptide Y and agouti related peptide, Ensure appeared to engage the same energy balance systems as the solid high-energy diet (Archer et al., 2004), suggesting that there is recognition of developing obesity at the hypothalamic level, even though the pathways to obesity clearly differ between diets. However, despite down-regulation by 40–50% of the expression of individual genes, the obese phenotype continued to develop.

6. The Sprague Dawley rat as a model of adult-onset diet-induced obesity

The experimental design of three solid diet combinations with or without Ensure supplementation was successful in demonstrating effects of the liquid diet on hypothalamic gene expression in juvenile rats. However, an earlier study of more mature rats had failed to highlight an additional effect of Ensure, despite the sustained hypercaloric intake and additional weight gain (c.80 g) achieved by the rats fed the supplement (Archer et al., 2005). In this study, rats were fed high energy pellet for 3 weeks after which half received the chocolate Ensure supplement for 10 weeks. Half the rats in each group were then returned to stock pellet for 3 weeks. On average, rats provided with the Ensure supplement obtained 82% of their calories from this source, a much higher proportion than was recorded for juvenile rats on any of the dietary combinations investigated (Table 1). The additional energy intake in the Ensure supplemented group increased the weight of pooled dissected white adipose tissue depots by over 30% (23 g), and increased serum leptin concentrations by 50%. Despite this, the effects of Ensure supplementation on hypothalamic gene expression were very limited. Only one of the genes analysed, tyrosine kinase B, was affected by Ensure supplementation, with higher levels of expression observed in the ventromedial hypothalamic nucleus in Ensure-fed rats. TrkB is the receptor for the anorexigenic peptide, brain-derived neurotrophic factor, and is a human obesity gene (Yeo et al., 2004). More widespread changes

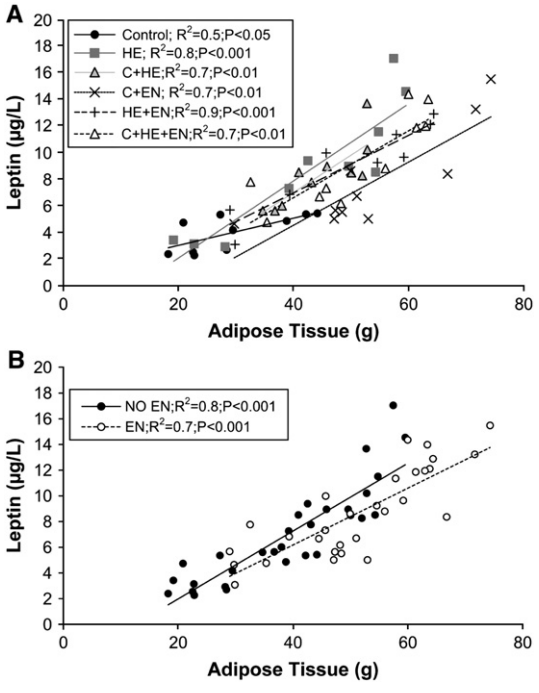

Fig. 3. [A] Correlation of serum leptin concentrations (µg/L human equivalents) with total body fat assessed by MRI scanning of male Sprague Dawley rats allocated to 6 weight matched groups ($n=10$) and fed either chow (C), a high energy diet (HE), C+HE, C plus the liquid supplement Ensure (C+EN), HE+EN or all three diets (C+HE+EN) for 5 weeks. [B] Correlation of serum leptin concentrations with total body fat assessed by MRI scanning for groups with no EN (C, HE and C+HE; $n=30$) or with EN (C+EN, HE+EN and C+HE+EN; $n=30$).

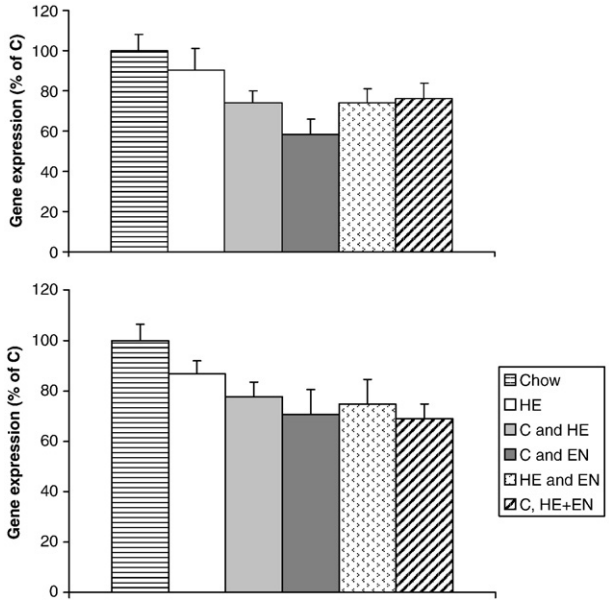

Fig. 4. Hypothalamic arcuate nucleus NPY (upper graph) and AgRP (lower graph) gene expression of male Sprague Dawley rats allocated to 6 weight matched groups and fed either chow (C), a high energy diet (HE), C + HE, C plus the liquid supplement Ensure (C + EN), HE + EN or all three diets (C + HE + EN) for 5 weeks. Values are means ± SEM, $n = 10$.

in gene expression were, however, observed when rats were returned to the stock pellet diet after 10 weeks on either high energy pellet or high energy pellet plus Ensure. Return to stock pellet for 3 weeks up-regulated neuropeptide Y gene expression in the arcuate nucleus and down-regulated expression of cocaine- and amphetamine-regulated transcript and brain-derived neurotrophic factor in the arcuate nucleus and ventromedial hypothalamic nucleus, respectively. In addition, there was down-regulation of dynorphin gene expression in both these hypothalamic centres following transfer back to stock pellet diet indicating that this system was up-regulated by obesogenic diet, as reported elsewhere (Welch et al., 1996). A plausible interpretation of these changes in gene expression on return to the stock pellet diet is of an integrated attempt to oppose further weight gain while being fed the obesogenic diets. The absence of any differential effect of Ensure on hypothalamic gene expression may be due to some degree of leptin insensitivity. The rats used in this study had considerably more adipose tissue in dissectible depots and much higher leptin levels that the juvenile rats discussed above, and serum concentrations in the former Ensure-supplemented group are nearly halved on return to stock pellet diet (Archer et al., 2005). Hypothalamic systems may not be exposed to, or sensitive to, blood concentrations of leptin over and above those produced by the consequences of ingesting the high energy pellet diet.

7. Conclusion

The ultimate goal for rodent models of diet-induced obesity should be to mimic the palatability-driven over-consumption that probably underlies the majority of human obesity. It might be possible to do this with a single obesogenic diet, but such diets may not actually induce over-consumption by weight or calories, or may oblige experimental animals to over-consume calories in order to meet their other requirements, such as for protein. We do not get fat eating a monotonous and homogeneous diet that may have an unbalanced macronutrient profile. One option is to provide a cafeteria-type diet, but an issue here may be the variable and heterogeneous composition of some of the foodstuffs supplied, which may prevent an accurate assessment of caloric and macronutrient intake. A better solution may be to provide a choice of defined dietary constituents, giving the experimenter the ability to monitor exactly what is being consumed, and the experimental subject a choice of diets that allow selection of a balanced macronutrient profile and caloric over-consumption from preference. Studies in our own laboratories have employed commercial pellet diets and the complete liquid diet, Ensure, although simple sugar solutions could be a better option in modelling Western human diets. Consideration of the diversity of dietary manipulations that are used to induce obesity suggests that although the end point may be superficially similar, i.e. an obese animal, the metabolic and physiological routes to this phenotype may be rather different. It would clearly be desirable for the scientific community to reduce the number of variables in experimental design to allow direct comparison of data (Buettner et al., 2007), and to adopt more realistic dietary regimes.

The data from our long-term feeding studies with obesogenic diets suggest an impact of body composition, at least for some hypothalamic energy balance genes, but that this is not necessarily a simple graded response. Furthermore, such relationships may be dependent on the growth trajectory of rats at the time of diet manipulation. Energy balance systems may also be sensitive to attributes of the diets themselves. Whatever the precise mechanisms involved, some of the hypothalamic homeostatic genes change in activity in a manner consistent with attempts to oppose further weight gain. However, the compensatory gene expression changes observed, some of them quite substantial, are apparently ineffective at countering the developing obese phenotype. Does this reflect the simple inability of these hypothalamic systems to regulate positive energy balance? The limited evidence from forced-feeding studies suggests this is not the case. If the hypothalamic systems do have regulatory capability in positive energy balance, albeit weaker than in negative energy balance, then it may be presumed that this capability is overridden by other brain systems. The forebrain reward systems are often discussed in this context, and certainly in the case of the liquid diet, Ensure, there is evidence of interaction with reward circuits (Kelley et al., 2003). However, in the absence of palatability driven over-consumption, this argument begins to break down. Indeed, the interaction of hypothalamic homeostatic and forebrain reward systems, although often presumed, is poorly described. In particular, the impact of diet, a risk factor in the development of obesity, on these two systems, and their co-regulation, has not been systematically studied, but variability in such interactions could be a determinant of susceptibility to diet-induced obesity. Greater understanding of these interactions might also enable diets to be manipulated with therapeutic benefit.

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