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Review

An overview on how components of the melanocortin system respond to different high energy diets

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ABSTRACT

High energy diets are used to model the obesity epidemic. Moreover, from a variety of genetic studies, it has become clear that the melanocortin system plays an important role in the regulation of energy metabolism. Since most dietary interventions are not standardized, fat/sugar-induced effects on the melanocortin system vary distinctly among different studies. How components of the melanocortin system are affected by high energy diets remains unclear. Therefore, in this review, we first present an overview of the effects of high energy diets on different elements of the melanocortin system in both mice and rats. The effects of a high energy diet are most consistent for agouti related protein levels which were either not affected or decreased after consumption of a high energy diet, whereas for proopiomelanocortin and the melanocortin receptor expression (and binding) it was difficult to define an overall response to a high energy diet. Because of the complexity of the melanocortin system, it is important to measure more than one element of the system. Only a few studies measured both melanocortin peptide and receptor expression and show that a high fat diet consumed for a longer period of time starting at an early age increases melanocortin signaling, whereas in adulthood a very high fat diet decreases melanocortin signaling. Finally, we review our own data on diet-induced changes in peptide expression and melanocortin binding and show that short term exposure to a free-choice high-fat high-sugar diet also decreases melanocortin signaling which supports hyperphagia observed in this model.

Contents

1. Introduction

The prevalence of obesity has dramatically increased over the last few decades which cannot solely be explained by a shift in genetics, but rather by changes in life style. Contemporary Western diets are frequently high in fats and sugars and are increasingly consumed in addition to sweetened liquid drinks, such as sodas. In fact, the consumption of sugar-sweetened soft drinks by children has been shown to be more than doubled between 1965 and 1996 (Cavadini et al., 2000). The Western Style diet, may, therefore, contribute to the increasing obesity epidemic. It is, therefore, important to understand the regulation of the intake of food containing high amounts of fat and sugar.

Multiple players are involved in the regulation of food intake. One key player is leptin, an adipose tissue-derived hormone that is

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released into the circulation proportional to increased energy stores in fat and acts via its receptors (OB-Rb) on several regulatory centers including the arcuate nucleus of the hypothalamus (Zhang et al., 1994). During a positive energy balance, concomitant with increased plasma leptin, a percentage of pro-opiomelanocortin (POMC) neurons become activated (Schwartz et al., 1997; Williams et al., 2010). The precursor POMC may be cleaved into α-melanocyte-stimulating hormone (α-MSH), β-MSH and γ-MSH, and acts as endogenous agonists for the main brain melanocortin receptors 3 and 4 (melanocortin$_3$ receptor and melanocortin$_4$ receptor respectively). Agouti-related protein (AgRP), also expressed in the arcuate nucleus neurons and activated during negative energy balance, functions as an inverse agonist. So the agonist and inverse agonist acting at the same receptor suggest a tight regulation of the melanocortin system in energy balance (Adan et al., 2006).

The underlying mechanisms by which overconsumption of energy dense foods and liquids result in overt hyperphagia, obesity and metabolic disorders still remain unclear. Several animal models have been developed to study diet-induced obesity aiming to unravel its physiological and molecular consequences. However, the dietary interventions are far from standardized and the experimental designs vary considerably. Moreover the resulting animal phenotypes and melanocortinergic expression patterns do not always correspond among the diet-induced obesity models. For instance, the energy content between 'high fat' diets vary considerably with fat contents ranging from 20 to 80%. Also the fat macronutrient can be derived from different sources as extensively reviewed (Buettner et al., 2007).

The response of the melanocortin system to a high fat diet may be dependent on type of diet, duration and composition. However, it has not been clarified in what way the type of (high fat) diet affects the responsiveness of the melanocortin system. Therefore, this review is aimed to provide an overview on the influence of high energy diets on the hypothalamic melanocortin system.

2. POMC and AgRP gene activation upon high fat diets

When consuming a diet that increases caloric intake and fat mass, the melanocortin system, in order to counter the obesity, is expected to increase its activity and thus decrease food intake and increase energy expenditure. Therefore, one would expect POMC mRNA to be increased and/or the AgRP expression to be decreased. Indeed, several studies in which rats or mice were subjected to a high fat diet ranging from 1 day to 7 weeks reported a decrease of AgRP expression, albeit no changes in POMC expression (details from different studies are depicted in Table 1) (Densmore et al., 2006; Wang et al., 2002; Ziotopoulou et al., 2000; Archer et al., 2004; Archer et al., 2007). Increased leptin levels have been proposed as a possible underlying reason for decreased AgRP levels, but do not explain the lack of POMC activation. Also, it is not clear why the response of AgRP seems to occur earlier than POMC and whether this is an important compensation for the attempted resistance to diet-induced obesity. In addition, many studies were not able to detect a difference in AgRP expression levels between the diets (Archer et al., 2005a; Clegg et al., 2003; Guan et al., 1998; Xu et al., 2010; Heijboer et al., 2005; Kinzig et al., 2005). The reason for this lack of difference is not explained by differences in species, fat content, sugar or a multiple palatable component. It could be that leptin resistance is causal to this effect, however not many studies have measured responses to leptin injections in the same animals as the expression levels were determined.

High fat diet feeding results in a non-consistent POMC gene activation pattern. Considering all studies with all time points included (Table 1), the expression was either increased (Huang et al., 2004; Ziotopoulou et al., 2000; Torri et al., 2002; Shiraev et al., 2009; Kinzig et al., 2005), decreased (Gout et al., 2008; Lin et al., 2000; Huang et al., 2003a; Kinzig et al., 2005; la Fleur et al., 2010a) but mostly unaffected (Wang et al., 2002; Densmore et al., 2006; Guan et al., 1998; Heijboer et al., 2005; Clegg et al., 2003; Archer et al., 2004, 2005a,b, 2007; Fam et al., 2007; Dziedzic et al., 2007). Because POMC is the precursor of melanocyte-stimulating hormone, a potent inhibitor of food intake, the increase of POMC may resist the effect of high-fat diet to promote increased food intake in an effort to maintain energy homeostasis and to counteract obesity. Yet, the downregulation of the POMC gene upon an obesogenic diet seems paradoxical, and might be a counterintuitive response. It is not clear what the origin is of this response, but apparently it seems specific for POMC as AgRP has not been shown to deviate from its 'expected' behavior upon caloric excesses.

The diets that showed the paradoxical POMC change lasted at least 8 weeks (Gout et al., 2008; Lin et al., 2000; Huang et al., 2003a; Kinzig et al., 2005). Although this could suggest that timing is an important part of this paradoxical finding, we observed that this paradoxical POMC response occurred already at one week in rats that were subjected to a free-choice high-fat high-sugar diet (la Fleur et al., 2010a, Fig. 1). Moreover, we showed that also neuropeptide Y expression exhibited a counterintuitive response; i.e. it was elevated after one week on a free-choice high-fat high-sugar diet (la Fleur et al., 2010a). Since we did not observe this response in rats on a free-choice sugar diet, we proposed that the choice component, and not the sugar, accounted for these paradoxical results. The other studies did not have a choice component, so this conclusion cannot be extrapolated to those studies. Still the choice component can still account for the fact that this paradoxical result was already visible at one week.

Besides our own study (la Fleur et al., 2010a), also Kinzig et al. (2005), showed this counterintuitive increase of neuropeptide Y when feeding rats a high fat diet for 8 weeks. The fat content of this diet was 80%, at the expense of the carbohydrates which were minimized to 5%, pointing to an unbalanced diet. It is debatable whether the paradoxical outcome is really imputable to the high fat content.

3. Effect of different diets on the melanocortin$_4$ receptor

Overconsumption of excess calories is expected to enhance signaling through melanocortin system, which, when activated, signals satiety and restores body weight towards normal levels. Deletion of the melanocortin$_4$ receptor results in hyperphagia and obesity (Huszar et al., 1997), and thus changes in the expression level of the melanocortin$_4$ receptor or melanocortin binding (as an indirect way to study melanocortin receptor availability) due to consuming a high energy diet could alter energy metabolism.

Expression of melanocortin$_4$ receptor mRNA after exposure to a diet does not provide a clear consistent view. One study found a downregulation of the melanocortin$_4$ receptor gene in the paraventricular nucleus after feeding mice a high fat diet for as little as 48 h. Also after 2 and 18 weeks this downregulation remained present (Densmore et al., 2006). On the contrary, an increase in paraventricular nucleus melanocortin$_4$ receptor gene expression was found after as little as 24 h of feeding high fat diet (Archer et al., 2005b) and the same pattern was visible in the ventromedial hypothalamus and amygdala or whole hypothalamus after 22 or 16 weeks high fat feeding, respectively (Huang et al., 2003a; Gout et al., 2008) (Table 1). In addition, some other feeding studies were not able to find any effect on the expression of melanocortin$_4$ receptor (Archer et al., 2004; Clegg et al., 2003; Shiraev et al., 2009; Xu et al., 2010). Although the fat percentage or the sugar amount within the diets used in these studies does not explain the different outcome, one interesting finding is worth to mention: rats were subjected for 8 weeks to two exact similar diets, except that 1 diet contains an additional 20% fat content (80% compared to 60%), but lower sucrose content (0% compared to 7%). This extra 20% fat increase has been shown to be sufficient to trigger a response to increase the melanocortin$_4$ receptor gene expression levels (Kinzig et al., 2005). This change in melanocortin$_4$

Table 1
Effects of high fat diets on melanocortin gene expression levels.

mRNA	Rodent type/ strain	Type of diet	High fat diet Fat%	Control diet Fat%	Choice components	Diet length (days)	Technique	Region	Result	Refs
POMC	C57Bl/6 mice	HF	36	3,1	1	56/112	qPCR	Whole HT	↓/=	Gout et al. (2008)
	C57Bl/6 mice	HF	59	9,7	1	7 or 56	ISH	Arc	=	Lin et al. (2000)
	C57Bl/6 mice	HF	59	9,7	1	133	ISH	Arc	↓/↑	Lin et al. (2000)/ Huang et al. (2004)
	C57Bl/6 mice	HF	58	10	1	7, 14, 49 or 126	ISH	Arc	=	Wang et al. (2002) Densmore et al. (2006)
	C57Bl/6 mice	HF	45	10	1	1, 2 or 7/14	qPCR	Whole HT	=/↑	Ziotopoulou et al. (2000)
	C57Bl/6 mice	HF	19	17	1	168	ISH	Arc	=	Guan et al. (1998)
	C57Bl/6 mice	HF	43	4	1	14	qPCR	Whole HT	=	Heijboer et al., (2005)
	C57Bl/6 mice	HF-DIO	40	10	1	154	ISH	Arc	↓	Huang et al. (2003a)
	LE rats	HF	41	11	1	56	qPCR	Whole HT	=	Clegg et al. (2003)
	SD-rats	HE/HE-EN	33	12	1/2	35	ISH	Arc	=	Archer et al. (2007)
	SD-rats	EN-C/HE-EN-C	22	12	2/3	35	ISH	Arc	=	Archer et al. (2007)
	SD-rats	HE-EN	31	33	2	91	ISH	Arc	=	Archer et al. (2005a)
	SD-rats	HE	33	12	1	½, 1, 2, 14 or 35	ISH	Arc	=	Archer et al. (2004, 2005b)
	SD-rats	caf	28	8,3	Multiple	56	ISH	Arc	↑	Torri et al. (2002)
	SD-rats	HF	30	8,3	1	42	ISH	Arc	=	Torri et al. (2002)
	SD-rats	HF	60	3	1	14 or 35	qPCR	Whole HT	=	Fam et al. (2007)
	SD-rats	caf	34	14	Multiple	77	qPCR	Ventral HT	↑	Shiraev et al. (2009)
	SD-rats	HF	60/80	16	1	56	ISH	Arc	↑/↓	Kinzig et al. (2005)
	Wistar rats	HF	40	10	1	42	ISH	Arc	=	Dziedzic et al. (2007)
	Wistar rats	HFHS	35	4	3	7	ISH	Arc	↓	la Fleur et al. (2010a)
	Wistar rats	HF/HS	50/2	4	2	7	ISH	Arc	=	la Fleur et al. (2010a)
AgRP	C57Bl/6 mice	HF	36	3,1	1	56/112	qPCR	Whole HT	↓/=	Gout et al. (2008)
	C57Bl/6 mice	HF	58	10	1	7 or 49	ISH	Arc	↓	Wang et al. (2002)
	C57Bl/6 mice	HF	45	10	1	1, 7 or 14/2	qPCR	Whole HT	=/↓	Ziotopoulou et al. (2000)
	C57Bl/6 mice	HF	58	11	1	1 or 2 or 126/14	ISH	Arc	=/↓	Densmore et al. (2006)
	C57Bl/6 mice	HF	19	17	1	168	ISH	Arc	=	Guan et al. (1998)
	C57Bl/6 mice	HF	60	10	1	14 or 56/84	qPCR	Whole HT	=/↓	Staszkiewicz et al. (2007)
	C57Bl/6 mice	HF	43	4	1	14	qPCR	Whole HT	=	Heijboer et al. (2005)
	C57Bl/6 mice	HF-DIO	40	10	1	154	ISH	Arc	↓	Huang et al. (2003a)
	LE rats	HF	41	11	1	56	qPCR	Whole HT	=	Clegg et al. (2003)
	SD-rats	HE	33	12	1	½,1, 2 or 14/35	ISH	Arc	=/↓	Archer et al. (2004, 2005b)
	SD-rats	HE/HE-EN	33	12	1/2	35	ISH	Arc	=	Archer et al. (2007)
	SD-rats	HE-EN-C	22	12	3	35	ISH	Arc	=	Archer et al. (2007)
	SD-rats	EN-C	18	12	2	35	ISH	Arc	↓	Archer et al. (2007)
	SD-rats	HE-EN	31	33	2	91	ISH	Arc	=	Archer et al. (2005a)
	SD-rats	HF	60 or 80	16	1	56	ISH	Arc	=	Kinzig et al. (2005)
	Wistar rats	HF	60	5	1	84	qPCR	Whole HT	=	Xu et al. (2010)
	Wistar rats	HFHS/HF/HS	35/50/2	4	3/2/2	7	ISH	Arc	=	la Fleur et al. (2010a)
MC4R	C57Bl/6 mice	HF	36	3	1	56/112	qPCR	Whole HT	=/↑	Gout et al. (2008)
	C57Bl/6 mice	HF	58	11	1	2, 14 or 126	ISH	PVN	↓	Densmore et al. (2006)
	C57Bl/6 mice	HF-DIO	40	10	1	154	ISH	VMH/MePD	↓/↑	Huang et al. (2003a)
	LE rats	HF	41	11	1	56	qPCR	Whole HT	=	Clegg et al. (2003)
	SD-rats	HE	33	12	1	½, 2, 14 or 35/1	ISH	PVN	=/↑	Archer et al. (2005b)
	SD-rats	caf	34	14	Multiple	77	qPCR	Dorsal HT	=	Shiraev et al. (2009)
	SD-rats	HF	60/80	16	1	56	ISH	PVN	=/↑	Kinzig et al. (2005)
	Wistar rats	HF	60	5	1	84	qPCR	Whole HT	=	Staszkiewicz et al. (2007)
MC3R	SD-rats	HE	33	12	1	½, 1, 2 or 14/35	ISH	Arc	=/↓	Archer et al. (2004, 2005b)
	SD-rats	HE	33	12	1	35	ISH	VMH	=	Archer et al. (2004)

Arc, arcuate nucleus; HT, hypothalamus; VMH, ventromedial part of hypothalamus; DMH, dorsomedial part of hypothalamus; PVN, paraventricular nucleus; BST, bed nucleus of stria terminalis; MePD, medial amygdaloid nucleus; ISH, in situ hybridization; qPCR, quantitative PCR; HF, high fat; HE, high energy; HFHS, free-choice high-fat high-sugar; Caf, cafeteria; HF-DIO, animals that have been selected as prone to develop obesity (as opposed to diet resistant animals); Fat%, energy percentage derived from total amounts of fat. Inclusion criteria: solely rodent studies were included. Reports (Huang et al., 2003a,b, 2004) in which diet induced obesity (DIO i.e. prone to develop obesity) and diet resistant rats were used, only the effect in DIO animals are included. Exclusion criteria: no studies have been included of genetically modified animals, studies where interventions were performed or where animals have been chronically fasted. The data are grouped by rodent type/strain (SD = Sprague–Dawley, LE = Long Evans).

receptor is probably due to a decrease in POMC mRNA observed in the same rats whereas AgRP did not change (Kinzig et al., 2005).

Binding studies, however, give information on the availability of the receptor, and may indirectly show melanocortin signaling in a better way than only receptor expression levels. Eight weeks of feeding a highly palatable cafeteria diet consisting of powdered lab chow, condensed milk and sucrose resulted in a decrease of melanocortin$_4$ receptor protein binding in multiple hypothalamic regions, including arcuate nucleus, median eminence, and ventromedial and dorsomedial part of the hypothalamus (Harrold et al., 1999b). In a separate experiment, the same result appeared to be present even after 2 weeks on a cafeteria diet (Harrold et al., 2000). Another study included melanocortin$_4$ receptor binding in the paraventricular nucleus, but found no effect on the binding capacities in this region, nor in the other hypothalamic nuclei, apart from the lateral hypothalamus, albeit comparable diet length, animal strain and a higher fat and sugar content (Irani et al., 2007) (an overview is depicted in Table 3). In addition, also our own data show that diet-induced hyperphagic rats (when on a free-choice high-fat high-sugar diet) show decreased melanocortin binding in the arcuate nucleus and ventromedial hypothalamus, whereas in the paraventricular nucleus it was not affected (Fig. 1). This change was not explained by the intake of fat in this paradigm, because on a free-choice high fat diet, rats did not show changes in melanocortin binding in these areas (however where also not hyperphagic, although obese) (Fig. 1). Thus, in models in which rats are hyperphagic, melanocortin binding

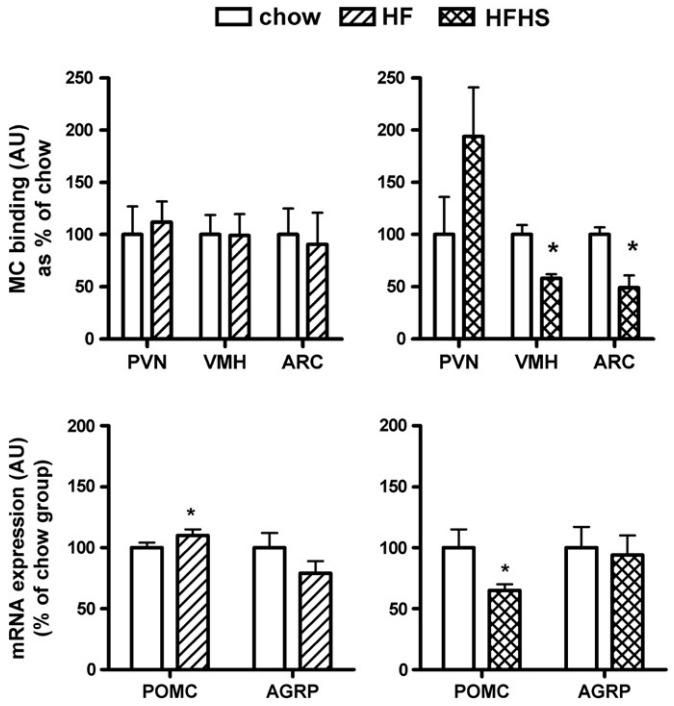

Fig. 1. Arcuate nucleus POMC and AgRP mRNA levels and melanocortin (MC) binding in rats that were subjected for one week to (gray bars) a free-choice high fat (HF) diet, or (black bars) a free-choice high-fat high-sugar (HFHS) diet compared to rats on a chow diet (white bars). Melanocortin signaling is enhanced in rats on a free-choice high fat diet (with an increase in POMC mRNA and no changes in melanocortin binding), whereas in rats on a free-choice high-fat high-sugar diet melanocortin signaling is decreased (with a decrease in arcuate nucleus POMC mRNA and a decrease in melanocortin binding). Data adapted from la Fleur et al., 2010a (POMC and AgRP mRNA). Melanocortin binding was performed as described earlier (Vrinten et al., 2000).

decreases in areas within the hypothalamus pointing towards a decrease in melanocortin signaling and thus in more feeding and less energy expenditure resulting in obesity and continued hyperphagia.

Agonist binding to G-protein-linked receptors may down-regulate that receptor. Therefore an explanation for the diminished melanocortin$_4$ receptor density is increased or decreased levels of α-MSH or AgRP protein, respectively. However, most reports that determined α-MSH or POMC protein levels in response to high fat feeding in either whole hypothalamus or separate hypothalamic regions, including anterior, arcuate and paraventricular hypothalamus, did not find any difference between diets (Harrold et al., 1999b; Torri et al., 2002; Hansen et al., 2005). Yet, one study (Hansen et al., 2001) that used much younger rats (5 weeks old) and fed for a longer time (20 weeks) compared to the other mentioned studies, found selectively reduced α-MSH content in the paraventricular nucleus. Such a reduction after 20 weeks on a high fat diet was also observed in the arcuate nucleus of mice (Enriori et al., 2007). Since this reduction in the normal endogenous melanocortinergic inhibitory tone was not visible until 20 weeks of diet feeding, this change in α-MSH peptide content likely reflects long-term adaptive responses to increased weight rather than an acute mechanism to restore energy balance (Hansen et al., 2001). Despite a lack of α-MSH response, Harrold et al. (1999a) were able to show an effect on AgRP, whereby the protein was increased after both 2 and 8 weeks of cafeteria diet feeding (Table 2). This is apparently paradoxical, as this orexigenic peptide is not expected to be elevated after increased caloric intake. Moreover, increased AgRP availability at the melanocortin$_4$-receptor would be expected to upregulate the receptor, rather than downregulate it. The most likely explanation, as was proposed by Harrold et al. (1999a) is reduced synaptic release of AgRP leading to increased storage in nerve terminals. Overall, altered energy balance leads to a downregulation of melanocortin$_4$ receptors, in the absence of changes in α-MSH concentrations, but with increased availability of the endogenous melanocortin$_4$ receptor antagonist, AgRP.

4. The role of melanocortin$_3$ receptor in diet-induced obesity

Melanocortin$_3$ receptors are expressed in the brain and are involved in energy homeostasis. In contrast, melanocortin$_3$ receptor deficient mice are not hyperphagic, have a normal metabolic response to increased energy consumption, and do not develop diabetes, thus the role of melanocortin$_3$ receptor in regulating food intake remains controversial (Butler and Cone, 2002). Only a few studies have investigated the effect of a high fat diet on the melanocortin$_3$ receptor gene expression levels or receptor binding activities. With respect to gene activation, to our knowledge solely the group of Archer et al. (Archer et al., 2004, 2005b), studied the effect of a high energy diet on melanocortin$_3$ gene expression. Whereas the mRNA levels of melanocortin$_3$ receptor in the arcuate nucleus were unaffected in rats subjected to a high energy diet fed for up to 2 weeks, feeding the rats the same high energy diets for 5 weeks resulted in a downregulation of melanocortin$_3$ receptor in the arcuate nucleus with leaving the melanocortin$_3$ receptor in the ventromedial hypothalamus unaffected (see also Table 1). Regarding protein binding, a high energy diet for 7 weeks in rats which are genetically predisposed to develop diet-induced obesity did not lead to any change in the receptor density in any of the hypothalamic regions except the lateral hypothalamus (Irani et al., 2007). A cafeteria diet (recognized as highly palatable) for 8 weeks was not able to affect protein binding in either of the hypothalamic regions, although in this study the lateral hypothalamus region was not included (Harrold et al., 1999b) (Table 3). Based on these limited studies, it does not seem likely that melanocortin$_3$ receptor plays a major role in the response to a high caloric diet.

5. Palatability among obesogenic diets

The different diets described in this review all differ in macronutrient content (specifically, in the relative amounts of fat and carbohydrate), and they may also differ with respect to palatability or hedonic components. POMC is posttranslationally not only cleaved in α-MSH but also γ-MSH and β-endorphins, where β-endorphin signals the positive hedonic value of food. Moreover melanocortin receptors are expressed in brain centers that relay information on

Table 2
Effects of high fat diets on melanocortin protein levels.

Protein	Rodent type/strain	Type of diet	High fat diet Fat%	Control diet Fat%	Choice components	Diet length (days)	Technique	Region	Result	Refs
POMC	Wistar rats	HE	13	9	1	14 or 56	RIA	Whole HT	=	Harrold et al. (1999b)
AgRP	Wistar rats	HE	13	9	1	14 or 56	RIA	Whole HT	↑	Harrold et al. (1999b)
α-MSH	C57Bl/6 mice	HF	45	12	1	140	RIA	Arc	↓	Enriori et al. (2007)
	SD-rats	caf	28	8	Multiple	56	IHC	PVN	=	Torri et al. (2002)
	SD-rats	caf	30	5	Multiple	14 or 84/140	RIA	PVN	=/↓	Hansen et al. (2001, 2005)
	SD-rats	caf	30	5	Multiple	14, 84 or 140	RIA	Arc or anterior HT	=	Hansen et al. (2001, 2005)
	Wistar rats	HE	13	9	1	14 or 56	RIA	Whole HT	=	Harrold et al. (1999b)

Table 3
Effects of high fat diets on melanocortin receptor binding.

Binding	Rodent type/ strain	Type of diet	High fat diet Fat%	Control diet Fat%	Choice components	Diet length (days)	Technique	Region	Result	Refs
MC4R	Wistar rats	HE	13	9	1	14 or 56	Autoradiography	ARC, VMH, DMH or ME	↓	Harrold et al. (1999a, 2000)
	SD-rats	HE-DIO	31	4,5	1	49	Autoradiography	PVN, Arc, VMH or DMH/LH	=/↓	Irani et al. (2007)
MC3R	Wistar rats	HE	13	9	1	56	Autoradiography	ARC, VMH, DMH or ME	=	Harrold et al. (1999a)
	SD-rats	HE-DIO	31	4,5	1	49	Autoradiography	PVN, Arc, VMH or DMH/LH	=/↓	Irani et al. (2007)

Arc, arcuate nucleus; HT, hypothalamus; VMH, ventromedial part of hypothalamus; DMH, dorsomedial part of hypothalamus; PVN, paraventricular nucleus; BST, bed nucleus of stria terminalis; MePD, medial amygdaloid nucleus; ISH, in situ hybridization; qPCR, quantitative PCR; HF, high fat; HE, high energy; Caf, cafeteria; HF-DIO, animals that have been selected as prone to develop obesity (as opposed to diet resistant animals); Fat%, energy percentage derived from total amounts of fat. Inclusion criteria: solely rodent studies were included. Reports (Enriori et al., 2007; Irani et al., 2007) in which diet induced obesity (DIO i.e. prone to develop obesity) and diet resistant rats were used, only the effect in DIO animals are included. Exclusion criteria: no studies have been included of genetically modified animals, studies where interventions were performed or where animals have been chronically fasted. The data are grouped by rodent type/strain (SD = Sprague–Dawley, LE = Long Evans).

taste and palatability, such as the amygdala, nucleus of the tractus solitarius and parabrachial nucleus (Adan et al., 2006). Palatability consists of several factors, including fat and sugar content, and variability and result in a higher hedonic impact. An obesogenic diet comprising a choice and/or liquid component is proposed to be highly palatable, yet only a few studies used such diet types. An example of a choice diet is the cafeteria diet in which rats are subjected to sweetened condensed milk, sucrose, sausages, biscuits and cakes. A limitation of this type of diet however may be the perhaps too heterogeneous and variable composition, which makes it difficult to accurately measure the caloric and macronutrient intake (Mercer and Archer, 2008). A solution is providing a choice diet of defined dietary constituents (Archer et al., 2005a; la Fleur et al., 2007, 2010a). Part of these choice diets is a liquid component representing the contemporary increased consumption of sugar solutions such as sodas.

The free-choice high-fat high-sugar diet has been shown to be a very efficient obesity diet resulting after one week in increased food motivated behavior (la Fleur et al., 2007) and promoting hyperphagia by a paradoxical increase in neuropeptide Y and decrease in POMC mRNA (la Fleur et al., 2010a, Fig. 1). Whereas the liquid component used in the former diet consists of a 30% sugar solution, the liquid component used by Mercer and Archer (2008) represents a complete liquid diet (Ensure). The supplementation of Ensure to a high energy diet resulted in increased weight gain and body weight, but did not affect the central melanocortin system. Nevertheless, subsequently transferring rats back to a chow diet triggered neuropeptide responses, but this was independent of previous dietary history (Archer et al., 2005a), a response also observed in rats on a free-choice high-fat high-sugar diet when they are transferred back to chow (la Fleur et al., unpublished data).

The majority of reports investigating diet induced obesity provide commercial pellet diets with all the components in one pellet, preventing choice and may oblige the animal to consume an unbalanced diet or consume fat at a time of the day that it is not preferred. The commercial pellet diets are available with a wide range of macronutrient compositions including control diets for the high energy diets. In these control diets the fat has been replaced by carbohydrates, which not only consists of polysaccharides as starch but also comprises monosaccharides as sucrose. Therefore, although these low fat/control diets have a much lower fat content, the sucrose content may be substantially higher instead.

Examples are diets from 'Research Diets' with number D12451 and D12450B. The former diet (serving as the experimental high fat diet) contains 17% sucrose, the latter diet (control diet) although containing less fat, contains as much as 35% sucrose which makes the control diet probably nearly as palatable as the fat diet itself. This high sugar content clearly also effects the development of obesity and probably hyperinsulinemia as we have shown in our free-choice high sucrose diet (la Fleur et al., 2010b). Thus, the majority of studies focus on the fat content in the diet, although the palatability of a diet also depends on a sugar component. Therefore caution should be taken in not underestimating the contribution of sugar to the diet.

Lastly, not only the obesogenic diets differ in composition, also chow/control diets itself differ substantially. Whereas the majority of chow/control diets consist of about 4% fat, chow diets of 16% fat have been described as well. Apart from the fat content, protein and carbohydrate contents also vary considerably. As the effects of the melanocortin system upon high fat diets are compared with chow diets with varying fat degrees, the outcome may be changed using another control diet. In other words, the control diets might almost be as important as the high fat diet itself and for accurate and reliable results a well chosen control diets should be taken into account.

6. The overall melanocortin system in response to a high fat diet

Taken together, the effects of a high energy diet are most consistent for AgRP levels which were either not affected or decreased after consumption of a high energy diet, whereas for POMC and the melanocortin receptor expression (and binding) it was difficult to define an overall response to a high energy diet. One of the reasons might be the heterogeneity within the melanocortin system where each of the several components such as the receptor types, agonist, reverse agonists exerts a distinct role in regulating the melanocortin signaling. Moreover, only a subpopulation of the melanocortin system, as opposed to the melanocortin system as a whole, may respond to a high energy diet treatment, since for instance only 35% of POMC neurons are activated by leptin, as has been recently shown (Williams et al., 2010). In addition, considering the complexity of the melanocortin system, it is important to measure more than one element of the system. Only a few studies measured both melanocortin peptide and receptor expression and show that a high fat diet consumed for a longer period of time starting at an early age increases melanocortin signaling (either by decreasing AgRP and/or melanocortin$_4$ receptor and/or increasing POMC) (Gout et al., 2008; Densmore et al., 2006; Archer et al., 2004, 2005b; Shiraev et al., 2009; Kinzig et al., 2005), whereas in adulthood a very high fat diet decreases melanocortin signaling (Kinzig et al., 2005). This same inhibition of melanocortin signaling was observed in rats on a free-choice high-fat high-sugar diet. This paradoxically reduced melanocortin signaling could well contribute to the observed hyperphagia and increased food-motivated behavior (la Fleur et al., 2007, 2010a).

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