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Stressing the importance of choice: Validity of a preclinical free-choice high-caloric diet paradigm to model behavioural, physiological and molecular adaptations during human diet-induced obesity and metabolic dysfunction

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Abstract

Humans have engineered a dietary environment that has driven the global prevalence of obesity and several other chronic metabolic diseases to pandemic levels. To prevent or treat obesity and associated comorbidities, it is crucial that we understand how our dietary environment, especially in combination with a sedentary lifestyle and/or daily-life stress, can dysregulate energy balance and promote the development of an obese state. Substantial mechanistic insight into the maladaptive adaptations underlying caloric overconsumption and excessive weight gain has been gained by analysing brains from rodents that were eating prefabricated nutritionally-complete pellets of high-fat diet (HFD). Although long-term consumption of HFDs induces chronic metabolic diseases, including obesity, they do not model several important characteristics of the modern-day human diet. For example, prefabricated HFDs ignore the (effects of) caloric consumption from a fluid source, do not appear to model the complex interplay in humans between stress and preference for palatable foods, and, importantly, lack any aspect of choice. Therefore, our laboratory uses an obesogenic free-choice high-fat high-sucrose (fc-HFHS) diet paradigm that provides rodents with the opportunity to choose from several diet components, varying in palatability, fluidity, texture, form and nutritive content. Here, we review recent advances in our understanding how the fc-HFHS diet disrupts peripheral metabolic processes and produces adaptations in brain circuitries that govern homeostatic and hedonic components of energy balance. Current insight suggests that the fc-HFHS diet has good construct and face validity to model human diet-induced chronic metabolic diseases, including obesity, because it combines the effects of food palatability and energy density with the stimulating effects of variety and

choice. We also highlight how behavioural, physiological and molecular adaptations might differ from those induced by prefabricated HFDs that lack an element of choice. Finally, the advantages and disadvantages of using the fc-HFHS diet for preclinical studies are discussed.

KEYWORDS
choice, chronic metabolic disease, diet preference, fat, obesity, sugar

1 | DIETARY ENVIRONMENT AND DAILY-LIFE STRESS ARE KEY FACTORS DRIVING THE PREVALENCE OF OBESITY

Current-day society is characterised by a dietary environment that, as Clemmensen and colleagues recently stated, "supersedes peripherally derived satiation and adiposity signals, exploits the limbic system, is 'unnaturally' energy-dense and hyperpalatable, and comes in virtually unlimited quantities".[1] This engineered dietary environment is a major driver of the current global obesity epidemic, which, together with the comorbidities of obesity, now poses a major societal health problem with an immense social and financial burden.[2-6] Coinciding with the rise in the prevalence of obesity, substantial progress has been made in our understanding how the central nervous system receives and integrates information from a multitude of external and internal metabolic cues to generate appropriate behavioural, autonomic and endocrine output to maintain energy homeostasis.[1,7,8] As expected, obesity is hallmarked by a profound imbalance in this process.

In addition to a dietary environment rich in high-calorie foods, modern-day society is also characterised by relatively high levels of external and psychosocial stress, defined as an ongoing or anticipated threat to homeostasis or well-being. Acute or chronic exposure to stress can induce a variety of physiological responses, including activation of the neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis, which in turn has profound effects on metabolic and behavioural responses in both humans as well as experimental animals.[9,10] External and psychosocial stressors can bidirectionally impact energy homeostasis, an effect dependent on many biological and environmental factors. Although some individuals will have diminished appetite and food intake upon exposure to stress, the majority of individuals will consume more calories and shift their preference towards palatable foods.[10-17] Notably, this shift towards palatable foods even occurs in individuals that decrease their caloric intake upon exposure to stress.[13] Following exposure to various stressors, rodents also prefer intake of palatable "comfort foods" over less palatable foods, a behaviour that is associated with blunted HPA axis reactivity.[9,10,18-5]

Although the intake of palatable food items can thus induce temporal stress relief, this protective behaviour can become maladaptive upon chronic exposure to stress. Indeed, repeated cycles of stress exposure can persistently promote the consumption of palatable calorie-rich "comfort foods", which will drive the development of an obese state. Reciprocally, an obese state is associated with a greater likelihood of developing major depressive disorder.[26] This is also observed in preclinical studies, where obese rodents show increased basal activity of the HPA axis, increased HPA reactivity to stress, and increased depressive- and anxiety-like behaviour compared to normal-weight controls.[17,27,28] Thus, when exposed to stress and when given a choice between food items varying in energy density and palatability, most humans and rodents will shift their preference towards more palatable food items in an attempt to relieve stress. However, such repeated "self-medication" upon chronic stress exposure can drive excessive intake of calories and subsequently promote the development of an obese state and increase the likelihood of developing major depressive disorder.

2 | IMPLEMENTATION OF FREE-CHOICE DIETS FOR THE STUDY OF STRESS-RELATED BEHAVIOUR AND OBESITY

To investigate the molecular mechanisms underlying central dysregulation of energy balance and mental well-being during diet-induced chronic metabolic disease, such as obesity, animals are commonly given free access to a bottle of water and a container with nutritionally-complete prefabricated pellets rich in (varying amounts of) fat and carbohydrates, commonly referred to as a high-fat diets (HFDs). Although rodent strains vary widely in their susceptibility to develop diet-induced obesity, such palatable HFDs usually take 2-3 months to induce an obese state and associated metabolic disorders, including a pre-diabetic state marked by insulin resistance.[29-31] These "no-choice" HFDs (nc-HFDs), however, lack an important factor that is present in the modern-day dietary environment of humans, namely a choice between an almost unlimited number of food components varying in texture, taste and caloric content. An additional important factor that is lacking in nc-HFDs is that a substantial part of the modern human daily energy intake is consumed in fluid form.[32]

In the last two decades, many preclinical studies have demonstrated that giving an animal a choice between dietary components results in profoundly different behavioural, physiological and molecular adaptations compared to those occurring in an animal eating a nc-HFD. For example, rats with access to a free-choice diet, where the animal can choose between several (palatable) dietary components, have blunted HPA axis responsivity to a variety of stressors.[19,21,33,34] By contrast, exposure to a palatable nc-HFD,

TABLE 1 Metabolic, hormonal and behavioural adaptations with free-choice diets compared to the control diet

Diet	Body weight parameters	Hyperphagia	Meal pattern aspects	Glucose metabolism		Leptin dynamics		Central molecular adaptations after 1 week of diet
				1 week of diet	4 weeks	1 week of diet	3-8 weeks of diet	
fc-HFHS	BW ↑ WAT ↑ FFA ↑	Persistent	Size = Number ↑	Ins^{Basal} =,↑ Glu^{Basal} =,↑ EGP = Ins^{GTT} = Glu^{GTT} ↑ Hep-IS ↓ Per-IS ↓	Ins^{Basal} ↑ Glu^{Basal} ↑ Ins^{GTT} ↓ Glu^{GTT} ↑	Lep^{Plasma} ↑ LR ↓	Lep^{Plasma} ↑ LR ↓	ARC *Npy* ↑ ARC *Pomc* ↓ ARC *Agrp* =
fc-HF	BW = WAT ↑ FFA =,↑	Transient	Size ↑ Number ↓	Ins^{Basal} = Glu^{Basal} = EGP = Ins^{GTT} = Glu^{GTT} = Hep-IS ↓ Per-IS =	Ins^{Basal} = Glu^{Basal} = Ins^{GTT} = Glu^{GTT} =	Lep^{Plasma} =,↑ LR =	Lep^{Plasma} ↑ LR =	ARC *Npy* ↓ ARC *Pomc* = ARC *Agrp* =
fc-HS	BW = WAT ↑ FFA ↑	Transient	Size ↓ Number ↑	Ins^{Basal} =,↑ Glu^{Basal} = EGP = Ins^{GTT} = Glu^{GTT} = Per-IS =	Ins^{Basal} ↑ Glu^{Basal} = Ins^{GTT} = Glu^{GTT} =	Lep^{Plasma} = LR =	Lep^{Plasma} ↑ LR =,↓	ARC *Npy* = ARC *Pomc* = ARC *Agrp* =
nc-HFHS	BW = WAT =	Transient	Size ↑ Number ↓					
References	33-37,39-42, 43,47	33-41,43,47	33	36,37,41,53	36,37,53	34,35,41	34-37,39,40,54	35

Agrp, *Agouti-related peptide* expression; ARC, arcuate nucleus of the hypothalamus; BW, body weight; EGP, endogenous glucose production; fc-HF, free-choice high-fat; fc-HFHS, free-choice high-fat high-sucrose; fc-HS, free-choice high-sucrose; FFA, plasma-free fatty acids; Glu^{Basal}, basal glucose levels; Glu^{GTT}, plasma glucose excursions during glucose tolerance test; Hep-IS, hepatic insulin sensitivity; Ins^{Basal}, basal insulin levels; Ins^{GTT}, plasma insulin excursions during glucose tolerance test; Lep^{Plasma}, plasma leptin levels; LR, leptin responsivity; nc-HFHS, no-choice high-fat high-sucrose; *Npy*, *Neuropeptide Y* expression; Per-IS, peripheral insulin sensitivity; *Pomc*, *Proopiomelanocortin* expression; WAT, white adipose tissue.

even if just for a few days, can activate and increase responsivity of the HPA axis.[35,36] Thus, the element of dietary choice produces differential, sometimes even opposite, effects on HPA axis function and behavioural responding to stress compared to no-choice diets. Because humans also have a choice between dietary items and this choice clearly impacts stress-related behaviour and adaptations, researchers should keep this important aspect in mind when considering a preclinical diet model to investigate the interactions between palatable diet intake and stress-related behaviour.

The research goal of our laboratory is to determine the molecular maladaptations in the human brain that result from frequent consumption of palatable diet components, as well as how such maladaptations drive continued overconsumption of calories, ultimately resulting in a vicious cycle that underlies the development of obesity and the deterioration of metabolic health. To increase the construct and face validity to model the development and pathophysiology of diet-induced obesity in humans, our laboratory decided to implement a free-choice high-fat high-sucrose (fc-HFHS) diet. This diet paradigm accounts for the elements of dietary choice and variety. The fc-HFHS diet also accounts for a substantial caloric intake from a fluid source. We use a fc-HFHS diet consisting of four individual diet components: (i) a container with prefabricated pellets of a nutritionally complete control diet (CD), often relatively low in caloric content; (ii) a container with saturated fat (lard or beef tallow); (iii) a bottle with tap water; and (iv) a bottle with a 30% solution of sucrose, or table sugar, which is a disaccharide consisting of glucose and fructose. Initial experiments with rats using a 10% sucrose solution, which is comparable to most sugar-sweetened beverages consumed by humans, revealed a ceiling effect on sucrose solution intake and no intake of tap water (data not shown). Therefore, we now use a 30% sucrose solution, which, despite being strongly preferred by rats over tap water, is still associated with drinking of tap water. This facilitates a choice between two water sources that differ in caloric content. In the recent years, our laboratory, as well as several other research groups, have extensively studied behavioural, physiological and molecular adaptations in response to a fc-HFHS diet. Furthermore, to control for dietary choice and/or the individual diet components, our laboratory has used several CDs, such as no-choice HFHS (nc-HFHS), free-choice high-fat (fc-HF) and free-choice high-sucrose (fc-HS) diets. The nc-HFHS diet consists of two components: a bottle of tap water and a container with prefabricated pellets with the same caloric composition as normally consumed under fc-HFHS conditions (approximately 35% kcal from fat, 15% kcal from sucrose and 50% kcal from CD).[33] The fc-HF and fc-HS diets consist of three components: a CD container, a bottle of tap water and a container with fat or a bottle with 30% sucrose solution, respectively. In this review, we highlight recent advances in our understanding how a fc-HFHS diet produces adaptations in peripheral processes and brain circuits that govern homeostatic and hedonic components of energy balance and, importantly, how these adaptations differ from those induced by other free-choice diets (fc-HF and fc-HS) and no-choice CDs (nc-HFD, nc-HFHS and CD). We will also discuss advantages and disadvantages of the fc-HFHS diet, which can help investigators decide if this diet model has construct and face validity to answer their research questions.

3 | BODY COMPOSITION AND CALORIC CONSUMPTION BEHAVIOUR

3.1 | Effects of fc-HFHS diets on body weight and white adipose tissue mass

Consumption of a fc-HFHS, for up to 6 months, increases total body weight gain compared to CD controls, and this was associated with profound increases in abdominal and subcutaneous white adipose tissue (WAT) mass.[33-42] Male Wistar or Sprague-Dawley rats with access to a fc-HF or fc-HS diet consistently show no or very limited effects on total body weight gain compared to CD controls, despite increases in terminal WAT mass.[33-37] When female Sprague-Dawley rats were maintained on a fc-HFHS diet containing a solution with maltodextrin, a polysaccharide composed of chains of glucose, for up to 36 weeks, increases in body weight gain and persistent hyperphagia were also observed.[43] In contrast to sucrose, maltodextrin, which is often added to commercial beverages and processed foods, does not contain the monosaccharide fructose. Although the widespread addition of fructose to the modern human diet is a likely contributor to the current pandemic of chronic metabolic diseases,[44] it has been demonstrated that maltodextrin can produce similar detrimental metabolic and cognitive effects to those of sucrose in male Wistar rats.[45] These observations indicate that chronic overconsumption of the fc-HFHS diet promotes overeating and weight gain and are independent of whether sucrose or maltodextrin was used in the sugar solution (Table 1).

The majority of fc-HFHS diet studies to date have utilised rat models because the historical use of rats in physiology has provided a rich and detailed literature on how diet and obesity affect behaviour, as well as vice versa. However, given the widespread availability of transgenic mouse models, the question arose as to whether mice would respond to a fc-HFHS diet in a behavioural and metabolic manner similar to rats. The fc-HFHS diet paradigm was slightly adapted to increase accurate measurement of fc-HFHS diet components in mice. First, beef tallow was melted and subsequently frozen to generate solid beef tallow pellets. Second, the concentration of the sucrose solution was lowered to 10% because we noted abnormalities in drinking behaviour when the mice were presented with a 30% sucrose dilution (data not shown). The 10% sucrose was chosen as this solution concentration promotes the highest sucrose solution intake in C57BL/6J mice during a dose-response study.[46] Similar to rats, male C57Bl/6J mice maintained on a fc-HFHS diet for up to 6 weeks, with the above-mentioned adaptations as well as containing a CD with 12% kcal from fat, demonstrated greater body weight gain and terminal abdominal WAT mass compared to CD controls.[47] Importantly, the magnitude of the increases in body weight and WAT was similar to those observed in rats.[33-37,47] This latter observation suggests that the fc-HFHS diet can be used in combination with available transgenic mouse models to specifically

target and study potential genes underlying behavioural, physiological and molecular adaptations during human diet-induced obesity and metabolic dysfunction.

3.2 | Effects of fc-HFHS diets on total caloric consumption

Consumption of a fc-HFHS diet, for up to 8 weeks, results in persistent hyperphagia compared to CD controls in male Wistar rats and C57BL/6J mice.[33-41,47] Similarly, providing female Sprague-Dawley rats with a fc-HFHS diet, containing a maltodextrin solution, for up to 36 weeks also results in persistent hyperphagia.[43] By contrast, exposure to nc-HFDs or nc-HFHS diets results in transient caloric overconsumption, with caloric intake usually returning to CD control levels between 1 and 3 weeks after initial exposure to the diet.[33,48] Importantly, consumption of a fc-HF or fc-HS diet, for up to 5 weeks, also results in transient overeating, similar to no-choice diets.[34-37] Collectively, these consistent observations indicate that simultaneous access to individual fat (eg, beef tallow) and sugar solution diet components of the fc-HFHS diet is required to induce persistent hyperphagia compared to CD controls. Notably, both rats [33-41] and mice [47] demonstrate persistent caloric overconsumption. Because rats and mice are were given access to slightly different versions of the fc-HFHS diet, these observations indicate that fc-HFHS diet-induced persistent hyperphagia is independent of the sugar source of the solution (sucrose vs maltodextrin solutions), the caloric content of the sucrose solution (30% vs 10% dilution) or the caloric fat content of the CD (18% vs 12% kcal from fat). To understand why simultaneous access to individual fat and sugar diet components drives persistent overeating, it will be relevant to determine the minimal concentration of sucrose solution necessary to drive persistent overeating during the fc-HFHS diet paradigm.

3.3 | Effects of fc-HFHS diets on diet component selection

During the first 2-3 days of exposure to a fc-HFHS diet, rats commonly consume relatively large amounts of fat. Despite this temporal and relative high intake of fat, the intake of all individual diet components is generally quite stable over time for each animal.[33,35,36,40] However, rats do show substantial inter-individual variability in their preference for the different fc-HFHS diet components. As a result, it is important that experiments with free-choice diets should be based on a robust experimental design that accounts for this baseline variability to attain statistical power. One factor that appears to modulate selection behaviour is the macronutrient content of the CD. Our initial studies, using a CD that contained 9% kcal from fat, showed that rats persistently consumed approximately 50% kcal from the CD, 35% kcal from beef tallow, and 15% kcal from sucrose solution.[33,35,36,41] However, after relocating to a research facility that uses a standard CD with 18% kcal from fat, we noted that male rats on the fc-HFHS diet consumed equal or more %kcal from sucrose solution than from beef tallow[38,39] (S. E. La Fleur, unpublished observations). These indirect observations suggest that the fat percentage of the CD might influence intake of the other fc-HFHS diet components, especially the fat component. This notion is corroborated by Apolzan and Harris,[37] who directly compared CDs with different fat percentages. Apolzan and Harris[37] observed that male Sprague-Dawley rats consumed significant more lard (and total calories) when offered a CD with 4% kcal from fat compared to a CD with 10% kcal from fat. Finally, female Sprague-Dawley rats maintained on a fc-HFHS diet with a CD containing 17.2% kcal from fat also consumed more calories from a 30% maltodextrin solution than from the fat diet component.[43] Thus, these observations suggest that both male and female rats decrease their intake of the fat diet component when consuming a CD with relative high amounts of fat, indicative of homeostatic monitoring of caloric intake. However, it is interesting to note that, despite this level of homeostatic monitoring of calories, animals maintained on a fc-HFHS do persistently overconsume calories compared to nc-HFHS, fc-HF, fc-HS and CD controls. Additional studies that directly compare various fc-HFHS diet component compositions (eg, low vs high %kcal from fat in CD) will help determine how male and female rats monitor caloric intake from dietary sources varying in structure, fluency, and caloric density.

3.4 | fc-HFHS meal pattern analysis

To unravel the mechanisms underlying the persistent hyperphagia in fc-HFHS rats, meal patterns have been analysed in fc-HFHS, nc-HFHS, fc-HF and fc-HS rats (Table 1). This approach identified two behavioural changes during consumption of a fc-HFHS diet. First, we observed that rats maintained on a fc-HFHS diet consumed approximately half of their sucrose solution during the light/inactive phase,[33] a circadian phase that in nocturnal rats is normally associated with resting and very limited caloric intake. The arrhythmic consumption of the sucrose solution is also observed in mice but, in contrast to rats, mice also consume more fat during the light/inactive phase.[47] Intriguingly, rats that were allowed to consume the sucrose solution only during the last 4 hours of the light/inactive phase as well as the entire dark/active phase showed similar persistent caloric overconsumption compared to rats that had access to a sucrose solution only during the first 8 hours of the light/inactive phase or compared to rats that had unrestricted access to all components during the entire 24 hour cycle.[33] Thus, the circadian timing of sucrose solution intake does not appear to drive the persistent hyperphagia during fc-HFHS consumption. Second, we also observed that nc-HFHS and fc-HF diets were associated with larger yet fewer meals, whereas all sucrose solution-containing (fc-HFHS, fc-HS) diets were associated with more meals. Remarkably, fc-HS rats decreased their meal size accordingly to compensate for this increase in meal number, whereas fc-HFHS rats did not compensate.[33] In a recent study, Harris[49] confirmed our initial observations on snacking behaviour by demonstrating that the consumption of sucrose in a fluid but not solid form results in significant sucrose solution snacking behaviour during the light/inactive phase. Finally, it

was recently investigated whether fc-HFHS diet rats show altered sensory-specific satiety, which refers to the declining pleasure and attraction to the sensory attributes of a specific food eaten in the meal relative to other foods.[42] However, the results demonstrated that female Sprague-Dawley made obese by long-term maintenance on a fc-HFHS had intact sensory-specific satiety.[42] Together, these observations suggest that the inclusion of an individual sucrose solution source drives 'snacking' behaviour, especially during the light/inactive phase, and that the consumption of individual fat and sugar components over-rides the feedback signalling mechanisms normally preventing caloric overconsumption, without affecting sensory-specific satiety.

3.5 | Motivational behaviour during fc-HFHS diets

A factor that might underlie the hyperphagia persistently observed in fc-HFHS rats is a change in motivational behaviour. To test changes in motivational aspects of feeding behaviour, the motivation to work for sugar pellets was tested in an operant chamber under fixed-ratio and progressive-ratio schedules of reinforcement in male Wistar fc-HFHS rats and CD controls. Even when satiated, fc-HFHS rats showed increased motivation to work for sugar pellets compared to CD controls during a progressive-ratio schedule.[41] Notably, fc-HF and fc-HS rats showed normal motivation to work for sugar pellets compared to CD controls (S. E. La Fleur, unpublished observations). Together, these observations indicate that only simultaneous access to and consumption of individual fat and sugar diet components, and not just a fat or sugar component, produces profound adaptations in the reward-related brain circuitry. These adaptations are reflected by consistent hyperphagia and increased motivation for sugar pellets. Similar to fc-HFHS rats, overweight/obese female subjects also show increased motivation to work for high-calorie snacks compared to normal-weight individuals.[50] In contrast, mice made obese on a nc-HFD are less motivated to work for sugar pellets compared to normal-weight CD controls.[51] Thus, fc-HFHS diets and nc-HFDs appear to induce opposite effects on motivational behaviour, with fc-HFHS diets showing greater similarity to the human situation. It will therefore be of great interest to determine the molecular adaptations responsible for changes in motivational behaviour in fc-HFHS animals.

Using a slightly different approach to investigate motivational aspects, Pickering et al[52] demonstrated that access to a fc-HFHS diet allowed for the identification of obesity-prone and obesity-resistant male Wistar rats, and that withdrawal from the fc-HFHS diet induced enhanced motivation for sugar specifically in obesity-prone rats. Upon switching back to a CD, obesity-resistant rats demonstrated transient hypophagia, whereas obesity-prone rats remained hypophagic compared to CD controls never exposed to a fc-HFHS.[52] In addition, Wald and Myers[43] demonstrated that the subgroup of female Sprague-Dawley rats gaining the most body weight on a maltodextrin-containing fc-HFHS diet (ie, HFHS-obesity prone) acquired stronger flavour preferences associated with post-ingestive nutrient sensing compared to rats that still became obese but gained less weight on the fc-HFHS diet or CD controls. Taken together, these data indicate that the consumption of a fc-HFHS diet changes the motivation to work for palatable sugar pellets, whereas intrinsic differences in flavour-nutrient conditioning can determine behavioural responding to a fc-HFHS diet and the development of obesity.

3.6 | Summary

Persistent caloric overconsumption during the fc-HFHS diet rapidly increases WAT mass, followed by increases in body weight. By contrast, the fc-HF and fc-HS diets are associated with transient hyperphagia, as well as moderate increases in WAT mass, and are limited to no increases in body weight. Rodents generally prefer the palatable fat and sucrose solution components over the CD, an effect that also depends on the fat content of the CD. The consumption of fat generally increases meal size, whereas the consumption of sugar water increases meal number. When both items are combined (eg, in the fc-HFHS diet), increases in both meal size and number drive caloric overconsumption. This behaviour is associated with increased motivation to work for palatable sugar pellets. Obesity-prone fc-HFHS rats demonstrated increased craving for sugar upon diet withdrawal, and also show increased sensitivity to flavour-nutrient learning.

4 | EFFECTS OF FC-HFHS ON PHYSIOLOGY

4.1 | Glucose tolerance, insulin sensitivity and ß-cell function

Type 2 diabetes mellitus is a common comorbidity of obesity. It is therefore important to understand whether and how the fc-HFHS diet impacts glucose metabolism and insulin sensitivity. An initial study showed comparable caloric intake and WAT mass in fc-HFHS and fc-HF rats after 7 days of diet consumption.[35] This allowed for the analysis of the differential effects of the macronutrient intake without the confounding effects of different WAT mass. After 7 days on their respective diets, fc-HFHS but not fc-HF rats had become glucose intolerant as measured with an i.v. glucose tolerance test (ivGTT), despite both diet groups having greater WAT mass and higher circulating free fatty acid concentrations compared to CD controls.[36] Seven days of diet also reduced hepatic insulin sensitivity in fc-HFHS and fc-HF rats, whereas peripheral insulin sensitivity was uniquely decreased in fc-HFHS rats compared to CD controls.[53] During a similar timeframe, fc-HS rats, which consumed relatively more sucrose solution than fc-HFHS rats, did not become glucose intolerant compared to CD controls.[36]

After 4 weeks on their respective diets, fc-HFHS rats again showed impaired glucose tolerance during an ivGTT compared to fc-HF and CD rats.[36] Interestingly, following this duration of diet exposure, fc-HFHS but not fc-HF rats showed decreased ß-cell responsivity compared to CD controls.[36] Because the first 5 minutes during the ivGTT represent the most direct effects of glucose on

the ß-cell, we used correlation analysis with the goal to determine whether changes in adipose tissue mass determined the observed changes. However, ß-cell responsivity and increases in insulin during the first five minutes of the ivGTT were positively and negatively correlated, respectively, with total WAT mass in fc-HF and fc-HFHS rats,[36] indicating an effect independent of adipose mass. Finally, consumption of a fc-HS diet for 4 weeks resulted in higher basal insulin levels, without affecting glucose tolerance, insulin excursions or ß-cell responsivity during an ivGTT.[36] Additional studies are currently focused on the effects of the fc-HFHS diet on ß-cell responsivity.

Harris and Apolzan[54] used free-choice diets and synthetic CDs to study the effects of sugar consumption, either in solid or fluid form, on glucose metabolism. Following 12 days on their respective diet, an i.p. GTT (ipGTT) in adult male Sprague-Dawley rats elicited similar glucose excursions when fed a fc-HFHS, fc-HS or fc-HF diets, or when fed a synthetic nc-HFD (containing 60% kcal from fat and 20% kcal from carbohydrates) or synthetic nc-LFD (containing 10% kcal from fat and 70% kcal from carbohydrates). However, the ipGTT revealed higher excursions in plasma insulin in all groups consuming high levels of sucrose compared to the groups consuming low levels of sucrose,[54] indicating differences in insulin sensitivity, irrespective of whether animals eat or drink the sucrose. We did not observe such effects in our fc-HS rats during an ivGTT or a hyperinsulinaemic-euglycaemic clamp.[36,53] A possible explanation for these differential observations is the difference in sucrose solution volume consumed because the fc-HS rats in the study Harris and Apolzan[54] drank twice the amount of sucrose solution compared to the fc-HS rats from our studies.[36,53,54] An alternative explanation is the methodology used to test insulin sensitivity because the glucose bolus during an ipGTT will be absorbed first in the portal system and be cleared by the liver before entering the systemic blood stream, whereas the glucose bolus during an ivGTT circulates more rapidly without liver passage.

Harris and Apolzan[54] also reported that there was no overt effect of fat consumption on insulin sensitivity as assessed by an ipGTT.[54] These observations corroborate our data showing that fc-HF rats had normal fasting plasma insulin or insulin excursions during an ivGTT after one or 4 weeks on the diet compared to CD controls.[36] However, we did observe clear hepatic insulin resistance in fc-HF rats,[53] suggesting that consumption of the fat diet component can contribute to the development of an insulin-resistant state. Furthermore, simultaneous consumption of individual fat and sucrose solution diet components had additive effects on rate of disappearance values, a measure of glucose uptake, as measured by an hyperinsulinaemic-euglycaemic clamp.[53] Finally, our data are in line with the role of dietary fat in the development of hepatic insulin resistance,[55,56] whereas studies using only dietary sugar reveal effects on hepatic and peripheral insulin resistance after longer periods (> 3 weeks) of sugar consumption.[57-60] Thus, although both fat and sugar can have detrimental effects on glucose metabolism and insulin sensitivity, the simultaneous intake of both these diet components, even for a short period of time, appears to accelerate the development of an insulin-resistant state independent of weight gain.

4.2 | Leptin dynamics and neuroinflammation

Increased adiposity, a key hallmark of obesity, is associated with elevated levels of circulating leptin.[61] Leptin, an adipocyte-derived cytokine, binds to leptin receptors in several brain areas to initiate signalling cascades via phosphorylation of signal transducer and activator of transcript 3 (STAT3).[62] Within the arcuate nucleus of the hypothalamus (ARC), neurones expressing the anorectic transcript *proopiomelanocortin* (*Pomc*) and neurones expressing the orexigenic transcripts *agouti-related peptide* (*Agrp*) and *neuropeptide Y* (*Npy*) are oppositely modulated by leptin to regulate feeding behaviour, glucose homeostasis and energy expenditure.[63] Although ARC POMC and AGRP/NPY neurones are among the first to respond to changes in circulating leptin levels, leptin receptors are widely expressed in the brain and are involved in a multitude of physiological processes, such as locomotor activity, motivational aspects of feeding behaviour and thermoregulation.[62]

Chronic high levels of circulating leptin are associated with desensitisation of the leptin receptor, commonly termed leptin resistance.[61] After 1 week of diet consumption, fc-HFHS and fc-HF but not fc-HS rats had higher plasma leptin levels compared to CD controls.[35,41] However, after 4 weeks of diet, all experimental groups (ie, fc-HFHS, fc-HF and fc-HS rats) had higher plasma leptin levels compared to CD controls.[34,36] After the initial 7 days on their respective diets, fc-HFHS rats remained hyperphagic despite high circulating leptin concentrations, whereas fc-HF rats, with similar elevated circulating leptin concentrations, showed transient hyperphagia and normalised their caloric intake to CD control levels after the first week.[34-37] Furthermore, following 7 days of diet, CD, fc-HF and fc-HS but not fc-HFHS rats significantly reduced their caloric intake in response to intraperitoneal administration of leptin.[34] Based on these observations, we investigated whether maintenance on a fc-HFHS diet was associated with altered integrity of the blood brain barrier, thus affecting whether and how leptin can reach the brain and potentially explaining the observed decreased sensitivity in response to intraperitoneal administered leptin. However, after 7 days of maintenance on a fc-HFHS diet, male Wistar rats demonstrated normal blood-brain barrier permeability compared to CD controls.[64]

After a 28-day maintenance on their respective diets, the same pattern of leptin responsivity was observed, despite equally elevated circulating leptin concentrations in the fc-HF, fc-HS and fc-HFHS groups.[34] These data suggest that the effects of simultaneous fat and sugar consumption on leptin sensitivity are independent of weight gain and/or circulating leptin concentrations (Table 1). How would this potentially work? It has been hypothesised that chronic activation of the leptin receptor results in phosphorylation of STAT3, which increases *Suppressor of cytokine signaling 3* (*Socs3*) expression and, in turn, SOCS3 inhibits phosphorylation of STAT3, thus decreasing signalling through the leptin receptor.[8] Our observations with the fc-HFHS diet suggest that additional factors are involved in this process of leptin resistance. Although we did observe normal leptin sensitivity in fc-HS rats, using different methodology, Harris and Apolzan[54] demonstrated that both fc-HS and fc-HFHS rats did not

change their caloric intake in response to i.p. leptin administration after 3 weeks of diet consumption. Harris then went on to demonstrate that the effects of sugar consumption on leptin sensitivity are independent of sweet taste, only occurring when sugar is consumed in fluid form, and not occurring when sugar is consumed in solid form by consuming prefabricated pellets enriched with sucrose.[49,65] Similar to our own observations in fc-HF and fc-HFHS rats,[33] the availability of a sucrose solution diet component in addition to a CD resulted in profound sucrose solution snacking behaviour, especially during the light/inactive phase.[49] Thus, it is possible that changes in meal patterns modulate the timing of leptin resistance development, and that a palatable diet characterised by light/inactive phase 'snacking' behaviour accelerates the onset of leptin resistance. One molecular mechanism how sugar drinking could modulate leptin responsiveness is the hexosamine biosynthetic pathway.[66,67]

Although fc-HFHS rats did not reduce their caloric intake in response to peripheral (ie, intraperitoneal) administration of leptin after 7 days of diet consumption,[34] caloric intake was similarly reduced in fc-HFHS and CD rats 24 hours following central (ie, intracerebroventricular) administration of leptin.[68] In fc-HFHS rats, this reduction in caloric intake was driven predominantly by decreases in CD and fat intake.[68] Notably, i.c.v. leptin administration decreased *Npy* and increased *Pomc* expression in the ARC of CD rats compared to vehicle-treated CD controls, a gene expression profile that fits with decreased feeding behaviour. Remarkably, *Npy* and *Pomc* expression was unaltered in the ARC of leptin-treated fc-HFHS rats compared to vehicle-treated fc-HFHS controls.[68] Furthermore, i.c.v. leptin administration decreased and increased tyrosine hydroxylase (*Th*) expression in the VTA of CD and fc-HFHS rats, respectively, compared to vehicle-treated controls.[68] Lastly, proenkephalin (*ppEnk*) expression in the NAc was unaffected in leptin-treated CD rats, although it was decreased in leptin-treated fc-HFHS rats compared to vehicle-treated controls.[68] Collectively, these data indicate that a fc-HFHS diet is associated with an ARC that is unresponsive to leptin after 1 week on the diet, whereas extrahypothalamic brain regions still remain responsive to changes in leptin. Previous findings in mice maintained on a nc-HFD have demonstrated a similar rapid onset of leptin unresponsiveness in the ARC without affecting leptin sensitivity in other hypothalamic areas.[69]

A recent study assessed leptin sensitivity in male Wistar rats prior to giving them access to a fc-HFHS diet. Interestingly, baseline leptin sensitivity on a CD, although not total caloric intake or differences in diet component preference, predicted subsequent weight gain when switched to a fc-HFHS diet.[40] In contrast to the rapid onset of leptin unresponsiveness observed in one study after 7 days of fc-HFHS diet,[34] we now observed leptin resistance after 28 but not 14 days of fc-HFHS diet consumption.[40] These differences might be explained by different methodology. In the study by de Git et al[40], leptin was administered intravenous early in the light period, after overnight access to 10 g of CD to avoid direct interference with fat and sugar. In contrast, in the study that observed rapid onset of resistance, leptin was administered i.p. in the middle of the light period after a 5-hour fast.[34] Thus, it is possible that leptin responsiveness is modulated by acute nutritional input and that overnight removal of the fat and sugar diet components is still capable of reversing leptin unresponsiveness during the first weeks on a fc-HFHS diet.

High-fat diet-induced leptin resistance has been hypothesised to involve an inflammatory response in the hypothalamus. Indeed, 7 days of diet consumption, when fc-HFHS rats have become leptin unresponsive,[34] was associated with increased expression of inflammation-related markers, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), in the hypothalamus of fc-HFHS rats.[70] Interestingly, overnight removal of the fat and sugar diet components at the same time as providing 10 g of CD diet, similar to the methodology used by de Git et al[40] to test leptin sensitivity, normalised expression of inflammation-related markers in the hypothalamus of fc-HFHS rats compared to CD controls.[70] Similar detrimental effects of 7-day fc-HFHS diet consumption were observed for expression of hypothalamic indicators of cellular stress.[71] Similar to inflammation-related markers, acute fc-HFHS diet withdrawal also ameliorated these effects.[71] Collectively, these data support the notion that induction of NF-κB phosphorylation, a key intracellular inflammatory response, in the hypothalamus of fc-HFHS rats could contribute to the development of leptin resistance.[72] Additional work will be needed to clarify the exact role of hypothalamic NF-κB in the development of leptin resistance during a fc-HFHS diet.

4.3 | Summary

Persistent overconsumption of calories during the fc-HFHS diet rapidly increases WAT mass, and this is accompanied by the development of insulin resistance and glucose intolerance (Table 1). Glucose intolerance was specific to the fc-HFHS diet and independent of increases in adiposity mass, whereas the fc-HF diet was also associated with hepatic insulin insensitivity. After 4 weeks of fc-HFHS diet exposure, altered glucose tolerance was accompanied by reduced ß-cell responsivity. In addition to changes in insulin sensitivity, leptin sensitivity was decreased in rats maintained on a fc-HFHS-diet for 1 week, which appears to be linked to the option and/or pattern of sucrose solution drinking. Finally, maintenance of rats on a fc-HFHS-diet for 1 week was associated with hypothalamic inflammation, an effect that was ameliorated by overnight removal of the diet or fasting.

5 | CENTRAL MOLECULAR ADAPTATIONS

5.1 | The arcuate nucleus of the hypothalamus

To identify central molecular adaptations that might drive differential caloric intake during the consumption of fc-HFHS, fc-HF and fc-HS diets, the expression of genes known to be involved in feeding behaviour has been analysed in the ARC, a key brain region involved in energy homeostasis.[1] After 7 days on the diet, fc-HFHS rats had higher plasma leptin levels, were hyperphagic, and had higher *Npy* and lower *Pomc* expression in the ARC compared to CD controls.[35] Such a hypothalamic gene expression profile reflects the

hyperphagic behaviour of the fc-HFHS rats. By contrast, after a similar time on the diet, fc-HF rats had higher plasma leptin levels and were hyperphagic, yet they had lower *Npy* and higher *Pomc* expression in the ARC compared to CD controls,[35] indicating an adaptive gene expression response in the hypothalamus to limit caloric intake. Fc-HS rats had normal leptin levels and showed limited hyperphagia and normal expression of ARC *Npy* and *Pomc* compared to CD controls.[35] Expression of *Agrp* was not altered in any of the free-choice diet groups.[35] Similar to fc-HF rats, nc-HFD-fed rodents show an adaptive gene expression response, albeit not consistently, in the hypothalamus to limit caloric intake.[73-76] On a mechanistic level, hepatic vagotomy prevented the effects of the fc-HFHS diet on ARC *Pomc* expression,[35] indicating that the hepatic vagus nerve links intake of palatable diet components to *Pomc* expression in the ARC.

Increased ARC *Npy* expression is commonly observed during a negative energy balance (eg, fasting), aiming to increase caloric intake and restore energy levels. Therefore, given the persistent hyperphagic state of fc-HFHS rats, it was surprising to observe an increase (and not the expected decrease) in *Npy* expression in the ARC of fc-HFHS rats.[35] To determine whether this was compensated by a decrease in NPY responsiveness, we assessed the efficacy of NPY to modulate caloric intake in fc-HFHS, fc-HF and fc-HS rats after 4 weeks of diet consumption. Only fc-HFHS rats showed altered responses to NPY because they were hypersensitive to i.c.v. administration of NPY compared to CD controls.[34] Notably, i.c.v. NPY administration significantly increased CD and fat intake but not sucrose solution intake (although a trend for greater sucrose solution intake was observed) in fc-HFHS rats.[34] These findings indicate that the NPY circuitry is unbalanced and hypersensitive during consumption of a fc-HFHS diet.

In addition to the effects on ARC *Pomc* expression, 1 week of fc-HFHS diet is also associated with decreased melanocortin receptor binding in the ventromedial nucleus of the hypothalamus and the ARC.[77] Therefore, we assessed whether sensitivity to the non-selective melanocortin 3 and 4 receptor agonist melanotan II (MTII) was changed during consumption of free-choice diets compared to CD controls. MTII efficiently decreased caloric intake in fc-HFHS, fc-HF and fc-HS diet rats compared to vehicle-treated controls, with fc-HF rats showing the strongest decrease in caloric intake.[78] These effects were driven by the relatively specific effects of MTII on fat intake.[78] Thus, a 7-day consumption of the fc-HFHS diet dysregulates the NPY system without inducing apparent functional changes in melanocortin 3 and 4 receptor signalling.

5.2 | The mesolimbic dopamine circuitry

Several studies have visualised adaptations and their functional consequences with respect to the mesolimbic dopamine circuitry of obese humans. One striking observation is the lower striatal dopamine D2/3 receptor ($DRD_{2/3}$) availability in obese subjects compared to lean controls.[79,80] In rats, $DRD_{2/3}$ availability in the dorsal striatum was lower in fc-HF but not nc-HFD rats compared to CD controls after 4 weeks of diet consumption.[81] These findings indicate that the element of choice might differentially affect dopamine dynamics compared to a no-choice diet during this timeframe. In addition, following a 28-day maintenance on a fc-HFHS diet, rats that consumed relatively high amounts of fat and relatively low amounts of sugar had lower $DRD_{2/3}$ availability in the NAc compared to both rats that consumed relatively low amounts of fat and relatively high amounts of sugar and CD controls.[82] Thus, both the hypothalamus and the striatum appear to be affected by the fc-HFHS diet, and these changes could point to a reciprocal interaction between the hypothalamus and striatum. We have previously shown that NPY from the ARC innervates the NAc and also that NPY, when administered in the striatum, alters neuronal activity and enkephalin expression in the striatum.[68] Thus, the functional changes in ARC NPY circuitry in fc-HFHS rats could potentially drive the molecular adaptations observed in striatal dopamine system. In turn, changes in striatal dopamine dynamics could drive feeding behaviour in animals on the fc-HFHS diet by influencing motivational drive.

6 | CONCLUSIONS AND FUTURE PERSPECTIVES

In this review, we have highlighted recent developments in the usage of preclinical free-choice diet paradigms to model behavioural, physiological and molecular adaptations during human diet-induced obesity and metabolic dysfunction. Collectively, these developments provide compelling evidence that the preclinical fc-HFHS diet paradigm has good construct and face validity to model the element of choice in human eating behaviour and, more importantly, that high-caloric free-choice diets impact caloric consumption behaviour and metabolic health in a manner different from that of high-caloric no-choice diets.

One consistent observation is that the fc-HFHS diet persistently drives overconsumption of calories, a key behavioural aspect associated with the development of diet-induced obesity in humans. Another key observation is that the addition of a bottle of sucrose solution to a free-choice diet paradigm (ie in fc-HS or fc-HFHS diets) produces substantial snacking behaviour in rats and mice. This snacking behaviour appears to be a behavioural trait that is commonly observed with free-choice but not nc-HFD diet paradigms in rats.[33,47,49,83,84] In mice, circadian shifting of caloric intake during nc-HFD consumption is more commonly observed.[85-87] Maintenance on a fc-HFHS diet, with access to a sucrose solution (which promotes snacking behaviour) and a separate source of fat, results in a remarkable increase in meal number without a compensatory reduction in meal size. Collectively, this drives persistent caloric overconsumption. Whether snacking behaviour is associated with the development of obesity in humans is currently a matter of debate because studies have reported evidence both in favour and against this association.[88-91] Although snacking is not always easily defined in humans, we recently validated the metabolic effects of the fc-HFHS diet in a translational study.[92] In this clinical study, a dietary approach similar to a fc-HFHS diet was provided to lean

healthy young men and the metabolic and cerebral response to this diet intervention was assessed. In line with the rodent studies, we demonstrated that a hypercaloric HS or HFHS diet increased meal frequency (ie, snacking behaviour) and increased fat accumulation in the liver, whereas isocaloric increases in meal size (ie, representing big meals) did not change liver fat accumulation.[92] Notably, only those subjects who consumed both sugar drinks and fat, and not the subjects who consumed only sugar drinks, showed signs of lower hepatic insulin sensitivity.[92] Furthermore, serotonin transporter availability in the diencephalon was reduced in subjects who consumed both sugar drinks and fat, suggesting an independent effect of diet composition and/or meal pattern on function of the serotonin brain circuitry, which regulates eating behaviour and interacts with the melanocortin system. Thus, a diet rich in separate sources of fat and sugar produces snacking behaviour and alters brain function in both humans and rodents.

It is intriguing that addition of a sucrose solution component to a free-choice diet results in snacking behaviour that occurs frequently during the light/inactive phase.[33,47,49,83] This time of day is when nocturnal rodents normally sleep and consume relatively low amounts of calories. For nocturnal animals, eating during the light/inactive phase can be considered as eating at the "wrong" time of day. This notion is derived from observations that caloric intake during the light/inactive phase has negative metabolic consequences, including accelerated development of weight gain, hyperinsulinaemia and hepatic steatosis.[85,93] We observed similar negative consequence of circadian misalignment of caloric intake. For example, rats with ad libitum access to all components of a fc-HFHS diet, rats with access to a sucrose solution during either the entire dark/active phase and 4 hours of the light/inactive phase, and rats with access to a sucrose solution for just 8 hours of the light/inactive phase showed equal hyperphagia compared to CD controls.[33] These observations indicate that sucrose solution drinking during the light/inactive phase is not necessary to induce fc-HFHS diet-associated hyperphagia. However, despite similar hyperphagia, rats with access to a sucrose solution during just 8 hours of the light/inactive phase gained significantly more body weight than ad libitum-fed fc-HFHS rats and rats with access to a sucrose solution during the entire dark/active phase and 4 hours of the light/inactive phase.[33] These findings support the notion that consumption of calories during the light/inactive phase has greater negative impact on energy homeostasis than consumption of the same number of calories during the dark/active phase.

Consumption of fc-HFHS diets or nc-HFDs results in several similar metabolic consequences, including increases in body weight and WAT mass, caloric overconsumption, and an increase in meal size. However, nc-HFDs or fc-HFHS diets also appear to have differential effects. First, fc-HFHS rats had higher *Npy* and lower *Pomc* in the ARC of the hypothalamus compared to CD controls after 1 week on the diet. Such a gene expression profile is normally only observed during a negative energy balance (eg, fasting) to stimulate caloric intake. This expression profile is also opposite to the hypothalamic gene expression profiles commonly observed during short-term consumption of a fc-HF diet [35] or nc-HFDs.[73-76] Additional work is required to determine why there is such a misalignment between apparent nutritional status, as reflected by gene expression in the ARC, and actual caloric intake when consuming a fc-HFHS diet. Second, fc-HFHS rats show increased motivation to work for sugar pellets, similar to overweight/obese female subjects,[50] whereas mice made obese on a nc-HFD are less motivated to work for sugar pellets compared to normal-weight CD-fed controls.[41,51] Identification of the responsible molecular adaptations will be crucial for understanding how these opposite changes in motivational behaviour occur. Third, obesity is associated with a greater risk of developing major depressive disorder.[94] Similarly, mice made obese by feeding them a nc-HFD for 12 weeks or transgenic leptin-deficient obese mice demonstrate anxiety-like behaviour and altered behavioural responding to an acute swim stressor compared to lean wild-type controls.[28,95] However, other murine studies have reported increased resilience to stress following nc-HFD consumption.[22,96] It has to be noted, however, that these studies often rely on results from the forced-swim test, a paradigm that lacks construct and face validity to model depressive-like behaviour.[97] To date, no studies have investigated how free-choice diets and subsequent metabolic maladaptations modulate the development of a depression-like state. However, based on the observation that fc-HFHS rats often can become "jumpy", a recent study did assess anxiety-like behaviour in fc-HF-, fc-HFHS- (containing a sucrose solution) and fc-HFHG- (containing a glucose solution) rats compared to CD controls.[98] Both sugar-containing diets induced hypoactivity in the open-field test (intermixed though with bursts of high speed running) and anxiety-like behaviour in the open-field test and elevated-plus maze paradigm.[98] Additional studies will be necessary to determine whether and how free-choice diets modulate the development of anxiety- and/or depression-like states in response to (chronic) stress, an accepted general risk factor to develop major depressive disorder, and also to identify the underlying molecular mechanisms. Lastly, free-choice diets will facilitate the identification of factors that mediate changes in diet preference during voluntary wheel running, a rodent model that mimics aspects of aerobic physical exercise training, which traditionally has been investigated by offering various nutrients [99] or by comparing nc-HFDs with different energy densities.[100]

Although rodents on a fc-HFHS diet persistently overconsume calories, they do often show substantial variability in their preference of the individual diet components. Because differences in diet component selection drive variability in behavioural, physiological and molecular adaptations to the fc-HFHS diet, a robust experimental design must account for this baseline variability. If not designed properly, a low group size will limit statistical power to interpret the data. Furthermore, for reasons still unknown, it is not uncommon to note differences in baseline preference for the individual diet components between experiments performed at different times of year or in different research facilities. As mentioned, the fat content of the CD diet appears to be a modulator of intake of the other diet components. It should also be noted that the rapid increase in WAT mass coinciding with the persistent caloric overconsumption complicates the disentanglement of the effects of overeating and adiposity.

A disadvantage of the free-choice diets is that they are more labour-intensive to work with compared to a nc-HF. The latter can be rapidly weighed and replenished in the diet holder of the animal cage, whereas frequent weighing of multiple food types and frequent switching out individual food sources to prevent spoilage can take substantially longer. Although this could refrain investigators from adapting free-choice diet paradigms in their laboratory, we strongly encourage the widespread usage of the cheap and versatile free-choice diet models in the preclinical field of neurobiological adaptations underlying diet-induced obesity. Free-choice diet paradigms have strong construct and face validity to model palatable diet-induced obesity in humans and this should ensure their greater translatability with respect to observations in humans. At the very least, when interested in behaviour underlying palatability-driven hyperphagia, researchers should replicate key findings obtained with no-choice diet paradigms in free-choice diet paradigms to increase both scientific insight and general reproducibility.

The studies conducted to date utilising free-choice diets have provided compelling evidence that the element of choice in palatable diet paradigms produces different behavioural, physiological and molecular responding to caloric overconsumption than diets without an element of choice (ie nc-HFDs). Given the complex nature of human eating behaviour, especially in our modern-day dietary environment, it is remarkable that many preclinical studies still investigate behavioural elements of palatable diet-induced obesity using no-choice diets. Although many and even very complex variations are possible with free-choice diets, the fc-HFHS diet is a relatively simple and easily applicable diet paradigm. Free-choice diets facilitate the controlled investigation of how simultaneous access to palatable fat and/or sugar diet components, in addition to healthier options, interacts at the behavioural and molecular level, resulting in a persistent and negative impact on metabolic health and brain function. Understanding how this occurs might help identify the neuronal pathways that can be targeted with respect to the development of therapeutic treatments for diet-induced obesity and associated comorbidities.

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REFERENCES

1. Clemmensen C, Muller TD, Woods SC, Berthoud HR, Seeley RJ, Tschop MH. Gut-brain cross-talk in metabolic control. *Cell*. 2017;168:758-774.
2. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA*. 2014;311:806-814.
3. Ogden CL, Carroll MD, Lawman HG, et al. Trends in obesity prevalence among children and adolescents in the United States, 1988-1994 through 2013-2014. *JAMA*. 2016;315:2292-2299.
4. Cowie CC, Rust KF, Byrd-Holt DD, et al. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health and Nutrition Examination Survey 1999-2002. *Diabetes Care* 2006;29:1263-1268.
5. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation*. 1983;67:968-977.
6. Hammond RA, Levine R. The economic impact of obesity in the United States. *Diabetes Metab Syndr Obes*. 2010;3:285-295.
7. Kim KS, Seeley RJ, Sandoval DA. Signalling from the periphery to the brain that regulates energy homeostasis. *Nat Rev Neurosci*. 2018;19:185-196.
8. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. *Nature*. 2006;443:289-295.
9. Dallman MF, Pecoraro N, Akana SF, et al. Chronic stress and obesity: a new view of "comfort food". *Proc Natl Acad Sci USA*. 2003;100:11696-11701.
10. Ulrich-Lai YM, Fulton S, Wilson M, Petrovich G, Rinaman L. Stress exposure, food intake and emotional state. *Stress*. 2015;18:381-399.
11. Macht M. How emotions affect eating: a five-way model. *Appetite*. 2008;50:1-11.
12. Gibson EL. Emotional influences on food choice: sensory, physiological and psychological pathways. *Physiol Behav*. 2006;89:53-61.
13. Oliver G, Wardle J. Perceived effects of stress on food choice. *Physiol Behav*. 1999;66:511-515.
14. Adam TC, Epel ES. Stress, eating and the reward system. *Physiol Behav*. 2007;91:449-458.
15. Epel E, Jimenez S, Brownell K, Stroud L, Stoney C, Niaura R. Are stress eaters at risk for the metabolic syndrome? *Ann NY Acad Sci* 2004;1032:208-210.
16. Stone AA, Brownell KD. The stress-eating paradox - multiple daily measurements in adult males and females. *Psychol Health*. 1994;9:425-436.
17. Hryhorczuk C, Sharma S, Fulton SE. Metabolic disturbances connecting obesity and depression. *Front Neurosci*. 2013;7:177.
18. Packard AE, Ghosal S, Herman JP, Woods SC, Ulrich-Lai YM. Chronic variable stress improves glucose tolerance in rats with sucrose-induced prediabetes. *Psychoneuroendocrinology*. 2014;47:178-188.
19. Pecoraro N, Reyes F, Gomez F, Bhargava A, Dallman MF. Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. *Endocrinology*. 2004;145:3754-3762.
20. Dallman MF, Pecoraro NC, la Fleur SE. Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav Immun*. 2005;19:275-280.
21. la Fleur SE, Houshyar H, Roy M, Dallman MF. Choice of lard, but not total lard calories, damps adrenocorticotropin responses to restraint. *Endocrinology*. 2005;146:2193-2199.
22. Finger BC, Dinan TG, Cryan JF. High-fat diet selectively protects against the effects of chronic social stress in the mouse. *Neuroscience* 2011;192:351-360.
23. Finger BC, Dinan TG, Cryan JF. The temporal impact of chronic intermittent psychosocial stress on high-fat diet-induced alterations in body weight. *Psychoneuroendocrinology*. 2012;37:729-741.
24. Maniam J, Morris MJ. Palatable cafeteria diet ameliorates anxiety and depression-like symptoms following an adverse early environment. *Psychoneuroendocrinology*. 2010;35:717-728.
25. Ulrich-Lai YM, Christiansen AM, Ostrander MM, et al. Pleasurable behaviors reduce stress via brain reward pathways. *Proc Natl Acad Sci USA*. 2010;107:20529-20534.
26. Luppino FS, de Wit LM, Bouvy PF, et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch Gen Psychiatry*. 2010;67:220-229.

27. Sharma S, Fernandes MF, Fulton S. Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. *Int J Obes (Lond)*. 2013;37:1183-1191.
28. Sharma S, Fulton S. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. *Int J Obes (Lond)*. 2013;37:382-389.
29. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. *Nutr Res Rev*. 2010;23:270-299.
30. Panchal SK, Brown L. Rodent models for metabolic syndrome research. *J Biomed Biotechnol* 2011;2011:351982.
31. Buettner R, Scholmerich J, Bollheimer LC. High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring)*. 2007;15:798-808.
32. Kit BK, Fakhouri TH, Park S, Nielsen SJ, Ogden CL. Trends in sugar-sweetened beverage consumption among youth and adults in the United States: 1999-2010. *Am J Clin Nutr*. 2013;98:180-188.
33. la Fleur SE, Luijendijk MC, van der Zwaal EM, Brans MA, Adan RA. The snacking rat as model of human obesity: effects of a free-choice high-fat high-sugar diet on meal patterns. *Int J Obes (Lond)*. 2014;38:643-649.
34. van den Heuvel JK, Eggels L, van Rozen AJ, et al. Neuropeptide Y and leptin sensitivity is dependent on diet composition. *J Neuroendocrinol*. 2014;26:377-385.
35. la Fleur SE, van Rozen AJ, Luijendijk MC, Groeneweg F, Adan RA. A free-choice high-fat high-sugar diet induces changes in arcuate neuropeptide expression that support hyperphagia. *Int J Obes (Lond)*. 2010;34:537-546.
36. la Fleur SE, Luijendijk MC, van Rozen AJ, Kalsbeek A, Adan RA. A free-choice high-fat high-sugar diet induces glucose intolerance and insulin unresponsiveness to a glucose load not explained by obesity. *Int J Obes (Lond)*. 2011;35:595-604.
37. Apolzan JW, Harris RB. Differential effects of chow and purified diet on the consumption of sucrose solution and lard and the development of obesity. *Physiol Behav*. 2012;105:325-331.
38. Blancas-Velazquez AS, Unmehopa UA, Eggels L, et al. A free-choice high-fat high-sugar diet alters day-night Per2 gene expression in reward-related brain areas in rats. *Front Endocrinol (Lausanne)*. 2018;9:154.
39. Oosterman JE, Foppen E, van der Spek R, Fliers E, Kalsbeek A, la Fleur SE. Timing of fat and liquid sugar intake alters substrate oxidation and food efficiency in male Wistar rats. *Chronobiol Int*. 2015;32:289-298.
40. de Git KCG, Peterse C, Beerens S, et al. Is leptin resistance the cause or the consequence of diet-induced obesity? *Int J Obes (Lond)*. 2018;42:1445-1457.
41. la Fleur SE, Vanderschuren LJ, Luijendijk MC, Kloeze BM, Tiesjema B, Adan RA. A reciprocal interaction between food-motivated behavior and diet-induced obesity. *Int J Obes (Lond)*. 2007;31:1286-1294.
42. Myers KP. Sensory-specific satiety is intact in rats made obese on a high-fat high-sugar choice diet. *Appetite* 2017;112:196-200.
43. Wald HS, Myers KP. Enhanced flavor-nutrient conditioning in obese rats on a high-fat, high-carbohydrate choice diet. *Physiol Behav* 2015;151:102-110.
44. Ter Horst KW, Serlie MJ. Fructose consumption, lipogenesis, and non-alcoholic fatty liver disease. *Nutrients*. 2017;9:pii: E981.
45. Kendig MD, Lin CS, Beilharz JE, Rooney KB, Boakes RA. Maltodextrin can produce similar metabolic and cognitive effects to those of sucrose in the rat. *Appetite* 2014;77:1-12.
46. Lewis SR, Ahmed S, Dym C, Khaimova E, Kest B, Bodnar RJ. Inbred mouse strain survey of sucrose intake. *Physiol Behav*. 2005;85:546-556.
47. Blancas-Velazquez A, la Fleur SE, Mendoza J. Effects of a free-choice high-fat high-sugar diet on brain PER2 and BMAL1 protein expression in mice. *Appetite* 2017;117:263-269.
48. Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P. A controlled high-fat diet induces an obese syndrome in rats. *J Nutr*. 2003;133:1081-1087.
49. Harris RBS. Source of dietary sucrose influences development of leptin resistance in male and female rats. *Am J Physiol Regul Integr Comp Physiol*. 2018;314:R598-R610.
50. Giesen JC, Havermans RC, Douven A, Tekelenburg M, Jansen A. Will work for snack food: the association of BMI and snack reinforcement. *Obesity (Silver Spring)*. 2010;18:966-970.
51. Finger BC, Dinan TG, Cryan JF. Diet-induced obesity blunts the behavioural effects of ghrelin: studies in a mouse-progressive ratio task. *Psychopharmacology*. 2012b;220:173-181.
52. Pickering C, Alsio J, Hulting AL, Schioth HB. Withdrawal from free-choice high-fat high-sugar diet induces craving only in obesity-prone animals. *Psychopharmacology*. 2009;204:431-443.
53. Diepenbroek C, Eggels L, Ackermans MT, et al. Differential effects of hypercaloric choice diets on insulin sensitivity in rats. *J Endocrinol*. 2017;232:49-57.
54. Harris RB, Apolzan JW. Changes in glucose tolerance and leptin responsiveness of rats offered a choice of lard, sucrose, and chow. *Am J Physiol Regul Integr Comp Physiol*. 2012;302:R1327-R1339.
55. Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH. Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes*. 1991;40:1397-1403.
56. Samuel VT, Liu ZX, Qu X, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem*. 2004;279:32345-32353.
57. Pagliassotti MJ, Prach PA. Quantity of sucrose alters the tissue pattern and time course of insulin resistance in young rats. *Am J Physiol*. 1995;269:R641-R646.
58. Pagliassotti MJ, Prach PA, Koppenhafer TA, Pan DA. Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. *Am J Physiol*. 1996;271:R1319-R1326.
59. Chicco A, D'Alessandro ME, Karabatas L, Pastorale C, Basabe JC, Lombardo YB. Muscle lipid metabolism and insulin secretion are altered in insulin-resistant rats fed a high sucrose diet. *J Nutr*. 2003;133:127-133.
60. Santure M, Pitre M, Nadeau A, Bachelard H. Effect of troglitazone on vascular and glucose metabolic actions of insulin in high-sucrose-fed rats. *Metabolism*. 2003;52:978-986.
61. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med*. 1995;1:1311-1314.
62. Pan WW, Myers MG Jr. Leptin and the maintenance of elevated body weight. *Nat Rev Neurosci*. 2018;19:95-105.
63. Xu J, Bartolome CL, Low CS, et al. Genetic identification of leptin neural circuits in energy and glucose homeostases. *Nature*. 2018;556:505-509.
64. Rijnsburger M, Unmehopa UA, Eggels L, Serlie MJ, la Fleur SE. One-week exposure to a free-choice high-fat high-sugar diet does not disrupt blood-brain barrier permeability in fed or overnight fasted rats. *Nutr Neurosci*. 2017; 1-10. https://doi.org/10.1080/1028415x.2017.1418727. [Epub ahead of print].
65. Harris RBS. Development of leptin resistance in sucrose drinking rats is associated with consuming carbohydrate-containing solutions and not calorie-free sweet solution. *Appetite* 2018b;132:114-121.
66. Harris RB, Apolzan JW. Hexosamine biosynthetic pathway activity in leptin resistant sucrose-drinking rats. *Physiol Behav* 2015;138:208-218.
67. Zimmerman AD, Harris RB. In vivo and in vitro evidence that chronic activation of the hexosamine biosynthetic pathway interferes with leptin-dependent STAT3 phosphorylation. *Am J Physiol Regul Integr Comp Physiol*. 2015;308:R543-R555.
68. van den Heuvel JK, Eggels L, Fliers E, Kalsbeek A, Adan RA, la Fleur SE. Differential modulation of arcuate nucleus and mesolimbic

gene expression levels by central leptin in rats on short-term high-fat high-sugar diet. *PLoS ONE*. 2014b;9:e87729.
69. Munzberg H, Flier JS, Bjorbaek C. Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology*. 2004;145:4880-4889.
70. Belegri E, Eggels L, Unmehopa UA, Mul JD, Boelen A, la Fleur SE. The effects of overnight nutrient intake on hypothalamic inflammation in a free-choice diet-induced obesity rat model. *Appetite* 2018;120:527-535.
71. Belegri E, Rijnsburger M, Eggels L, et al. Effects of fat and sugar, either consumed or infused toward the brain, on hypothalamic ER stress markers. *Front Neurosci*. 2017;11:270.
72. de Git KC, Adan RA. Leptin resistance in diet-induced obesity: the role of hypothalamic inflammation. *Obes Rev*. 2015;16:207-224.
73. Archer ZA, Mercer JG. Brain responses to obesogenic diets and diet-induced obesity. *Proc Nutr Soc*. 2007;66:124-130.
74. Dziedzic B, Szemraj J, Bartkowiak J, Walczewska A. Various dietary fats differentially change the gene expression of neuropeptides involved in body weight regulation in rats. *J Neuroendocrinol*. 2007;19:364-373.
75. Lin S, Storlien LH, Huang XF. Leptin receptor, NPY, POMC mRNA expression in the diet-induced obese mouse brain. *Brain Res*. 2000;875:89-95.
76. Ziotopoulou M, Mantzoros CS, Hileman SM, Flier JS. Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice. *Am J Physiol Endocrinol Metab*. 2000;279:E838-E845.
77. van den Heuvel JK, van Rozen AJ, Adan RA, la Fleur SE. An overview on how components of the melanocortin system respond to different high energy diets. *Eur J Pharmacol*. 2011;660:207-212.
78. van den Heuvel JK, Eggels L, van Rozen AJ, et al. Inhibitory effect of the Melanocortin Receptor Agonist Melanotan-II (MTII) on feeding depends on dietary fat content and not obesity in rats on free-choice diets. *Front Behav Neurosci* 2015;9:358.
79. Wang GJ, Volkow ND, Logan J, et al. Brain dopamine and obesity. *Lancet*. 2001;357:354-357.
80. de Weijer BA, van de Giessen E, van Amelsvoort TA, et al. Lower striatal dopamine D2/3 receptor availability in obese compared with non-obese subjects. *EJNMMI Res*. 2011;1:37.
81. van de Giessen E, la Fleur SE, de Bruin K, van den Brink W, Booij J. Free-choice and no-choice high-fat diets affect striatal dopamine D2/3 receptor availability, caloric intake, and adiposity. *Obesity (Silver Spring)*. 2012;20:1738-1740.
82. van de Giessen E, la Fleur SE, Eggels L, de Bruin K, van den Brink W, Booij J. High fat/carbohydrate ratio but not total energy intake induces lower striatal dopamine D2/3 receptor availability in diet-induced obesity. *Int J Obes (Lond)*. 2013;37:754-757.
83. Martire SI, Holmes N, Westbrook RF, Morris MJ. Altered feeding patterns in rats exposed to a palatable cafeteria diet: increased snacking and its implications for development of obesity. *PLoS ONE*. 2013;8:e60407.
84. Hariri N, Thibault L. Dietary obesity caused by a specific circadian eating pattern. *Chronobiol Int*. 2011;28:216-228.
85. Hatori M, Vollmers C, Zarrinpar A, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab*. 2012;15:848-860.
86. Kohsaka A, Laposky AD, Ramsey KM, et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab*. 2007;6:414-421.
87. Pendergast JS, Branecky KL, Yang W, Ellacott KL, Niswender KD, Yamazaki S. High-fat diet acutely affects circadian organisation and eating behavior. *Eur J Neurosci*. 2013;37:1350-1356.
88. Field AE, Austin SB, Gillman MW, Rosner B, Rockett HR, Colditz GA. Snack food intake does not predict weight change among children and adolescents. *Int J Obes Relat Metab Disord*. 2004;28:1210-1216.
89. Hampl JS, Heaton CL, Taylor CA. Snacking patterns influence energy and nutrient intakes but not body mass index. *J Hum Nutr Diet*. 2003;16:3-11.
90. Keast DR, Nicklas TA, O'Neil CE. Snacking is associated with reduced risk of overweight and reduced abdominal obesity in adolescents: National Health and Nutrition Examination Survey (NHANES) 1999-2004. *Am J Clin Nutr*. 2010;92:428-435.
91. Berteus Forslund H, Torgerson JS, Sjostrom L, Lindroos AK. Snacking frequency in relation to energy intake and food choices in obese men and women compared to a reference population. *Int J Obes (Lond)*. 2005;29:711-719.
92. Koopman KE, Caan MW, Nederveen AJ, et al. Hypercaloric diets with increased meal frequency, but not meal size, increase intrahepatic triglycerides: a randomized controlled trial. *Hepatology*. 2014;60:545-553.
93. Arble DM, Ramsey KM, Bass J, Turek FW. Circadian disruption and metabolic disease: findings from animal models. *Best Pract Res Clin Endocrinol Metab*. 2010;24:785-800.
94. Roberts RE, Deleger S, Strawbridge WJ, Kaplan GA. Prospective association between obesity and depression: evidence from the Alameda County Study. *Int J Obes Relat Metab Disord*. 2003;27:514-521.
95. Yamada N, Katsuura G, Ochi Y, et al. Impaired CNS leptin action is implicated in depression associated with obesity. *Endocrinology*. 2011;152:2634-2643.
96. Del Rio D, Morales L, Ruiz-Gayo M, Del Olmo N. Effect of high-fat diets on mood and learning performance in adolescent mice. *Behav Brain Res* 2016;311:167-172.
97. Molendijk ML, de Kloet ER. Immobility in the forced swim test is adaptive and does not reflect depression. *Psychoneuroendocrinology*. 2015;62:389-391.
98. Peris-Sampedro F, Mounib M, Schele E, et al. Impact of free-choice diets high in fat and different sugars on metabolic outcome and anxiety-like behavior in rats. *Obesity (Silver Spring)*. 2019;27:409-419.
99. Oudot F, Larue-Achagiotis C, Anton G, Verger P. Modifications in dietary self-selection specifically attributable to voluntary wheel running and exercise training in the rat. *Physiol Behav*. 1996;59:1123-1128.
100. Scarpace PJ, Matheny M, Zhang Y. Wheel running eliminates high-fat preference and enhances leptin signaling in the ventral tegmental area. *Physiol Behav*. 2010;100:173-179.

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