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REVIEW

You are what you eat, or are you? The challenges of translating high-fat-fed rodents to human obesity and diabetes

M Lai^{1,3}, PC Chandrasekera^{1,3} and ND Barnard^{1,2}

Obesity and type 2 diabetes mellitus (T2DM) are rapidly growing worldwide epidemics with major health consequences. Various human-based studies have confirmed that both genetic and environmental factors (particularly high-caloric diets and sedentary lifestyle) greatly contribute to human T2DM. Interactions between obesity, insulin resistance and β -cell dysfunction result in human T2DM, but the mechanisms regulating the interplay among these impairments remain unclear. Rodent models of high-fat diet (HFD)-induced obesity have been used widely to study human obesity and T2DM. With >9000 publications on PubMed over the past decade alone, many aspects of rodent T2DM have been elucidated; however, correlation to human obesity/diabetes remains poor. This review investigates the reasons for this translational discrepancy by critically evaluating rodent HFD models. Dietary modification in rodents appears to have limited translatable benefit for understanding and treating human obesity and diabetes due—at least in part—to divergent dietary compositions, species/strain and gender variability, inconsistent disease penetrance, severity and duration and lack of resemblance to human obesogenic pathophysiology. Therefore future research efforts dedicated to acquiring translationally relevant data—specifically human data, rather than findings based on rodent studies—would accelerate our understanding of disease mechanisms and development of therapeutics for human obesity/T2DM.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a rapidly growing global epidemic affecting >371 million people worldwide.¹ In the United States, >25 million people have diabetes, and it is predicted that 1 in 3 Americans will be diabetic by year 2050.² Adult-onset T2DM is a complex multifactorial metabolic disorder influenced by genetic, lifestyle and environmental risk factors. Impaired glucose homeostasis in T2DM is primarily characterized by insulin resistance and β -cell dysfunction culminating in the morbidity (nephropathy, neuropathy, retinopathy and increased risk of cardiovascular disease) and mortality. One of the major risk factors for the development of insulin resistance and subsequent T2DM is obesity, another epidemic affecting 2.1 billion individuals worldwide.³ Obese individuals often have excess central visceral adiposity, a condition that contributes to a chronic increase in circulating free fatty acids and the resulting metabolites, such as diacylglycerol and ceramide. These metabolites in turn activate various signaling cascades that interfere with insulin signaling and β -cell function, further contributing to the gluco/lipotoxicity.⁴ Obesity also increases cardiovascular risk factors, such as dyslipidemia, hypertension and atherosclerosis. Therefore much of the research efforts over the past two decades have been dedicated to delineating the etiopathogenic mechanisms of obesity and diabetes.

A large number of animal models have been generated to study obesity and diabetes using species ranging from fruit flies to primates, including dogs, cats, pigs, rabbits, hamsters and squirrels to common rodent species, rats and mice.^{5,6} In general, T2DM is

induced in experimental animals by surgical, chemical, dietary and genetic manipulations as well as combinations thereof. The most common obese models of T2DM are of spontaneous genetic origin (for example, naturally occurring mutations in leptin and leptin receptor)⁷ or experimentally induced by diet (for example, prolonged high-fat diet (HFD) feeding).^{7,8}

A simple PubMed database search (using the keywords 'high fat diet' and 'high fat diet and obesity' with the filters 'other animals' and 'publication dates') revealed that >16 000 animal-based HFD studies have been published to date since the first article appeared on PubMed in 1964. The trend for animal-based HFD studies has increased tremendously since then—of the 16 000 papers, >9800 papers were published in the past decade. Despite the wealth of information obtained from these animal-based HFD studies, the mechanisms regulating the interplay among obesity, insulin resistance and β -cell dysfunction in humans remain unclear. It appears that dietary modification in rodents has limited translatable benefit for understanding and treating human obesity and diabetes. The purpose of this review is to investigate the reasons for this translational discrepancy. This paper also addresses future directions necessary to conduct transnationally relevant human obesity and T2DM research.

HFD: DIETARY OPTIONS

The first description of HFD experiments dates back to the early 1940s when rats fed an extreme HFD (70% energy from fat) developed obesity with elevated basal and postprandial blood glucose levels.^{9,10} Another early diet was the 'cafeteria diet', a diet

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that included a variety of common, highly palatable human food that rodents were free to choose from (for example, cheese, ham, cookies, peanuts, chocolate and cakes).¹¹ The utility of this cafeteria diet has diminished over the years as it is difficult to accurately quantify the nutrient intake with substantial variations in caloric content and types.^{11,12} Most researchers now use commercially available predefined HFDs encompassing a wide range of fat content and types.⁷ For example, Harlan Laboratories and Purina TestDiet provide a number of distinct HFD formulas, where the fat content ranges from 40% to 60%. The fat types include saturated fatty acids, monosaturated fatty acids, poly-saturated fatty acids and various combinations thereof, all typically derived from ingredients such as butter, pork fat, beef tallow, lard and various oils such as corn, coconut, cottonseed, soybean, olive, peanut, sesame, cocoa butter and fish oils.^{13,14}

In addition to HFD, high-carbohydrate diets such as high-fructose and high-sucrose diets are also used to induce features of the human metabolic syndrome in rodents. High-carbohydrate diets can be used alone or in combination with a HFD. For example, a high-sucrose (10–30%) diet can be combined with high fat (20–40%) to induce metabolic perturbations, such as increased body weight, abdominal fat deposition, hyperleptinemia, hyperinsulinemia, and hyperglycemia.^{15–17} High-fructose (10–60%) is combined with high fat (20–60%) to induce the symptoms such as increased body weight and the plasma concentrations of cholesterol, triglycerides, free fatty acids and leptin.^{16,18,19}

HFD-fed rodents are often compared against control-diet-fed rodents in which a grain-based 'chow' diet made primarily from corn, wheat, oats and soybeans provides basic nutrition.²⁰ Some researchers have made HFDs by simply adding fat to grain-based chow, but this chow-based HFD is not recommended as a high percentage of fat dilutes other ingredients in the diet, thereby making the diet deficient in micronutrients and macronutrients.⁷ Given the broad range of dietary modifications now utilized in obesity and T2DM research, it has not been possible to define the 'ideal' HFD nor generate a rodent model that can accurately mimic the human disease state. As discussed in the following sections, dietary modification in rodents appears to have limited translatability due—at least in part—to divergent dietary compositions, species/strain, sex and age variability, inter- and intra-laboratory variability in disease penetrance, severity and duration and lack of resemblance to the human obesogenic pathophysiology.

HFD: MAJOR LIMITATIONS TO HUMAN TRANSLATABILITY

HFD-feeding: effect of dietary components

A wide variety of methods are utilized in HFD studies, with no single method being comparable to the human experience. A multitude of diets with very different fatty acid compositions are included under the term 'high fat diet', and therefore the resulting phenotypes also vary considerably. Given the extensive literature on this topic, only select studies are presented below to illustrate the variable effects of dietary components and schemes on glucose regulation. For example, weight gain was reported to differ significantly between mice fed a HFD consisting of beef fat versus a HFD of canola oil (138% more weight gain with beef fat compared with canola oil) despite 40.8% of energy from fat in both diets.²¹ In another study, weight gain in mice varied with the type of fat intake from oils containing different amounts of palmitic, linoleic and oleic acids: soybean > palm > or = lard > or = rapeseed > or = safflower > or = perilla > fish oil.²² More recent studies reiterate that even when protein and carbohydrate ratios are kept constant, weight gain in the two most commonly used mouse strains (C57BL/6J and A/J) sharply contrasted between 60% HFD consisting of 100% saturated fatty acids and a 60% HFD consisting of 35% saturated, 43.4% monounsaturated and 15.9%

polyunsaturated fatty acids.²³ In addition to weight gain, the resulting metabolic manifestations can also differ with the type and amount of fat. For example, lard and olive oil have been shown to cause higher insulin resistance in rodents than coconut oil and fish oil.^{7,24} Moreover, despite similar energy intake, combination diets such as high-fat/high-sucrose diets can exert differential effects on insulin sensitivity and islet β -cell adaptation compared with HFD alone.²⁵ Variations in fat-mediated metabolic perturbations and their impact on rodent studies are discussed in detail later.

Comparative dietary effects are generally made against chow-based control diets; however, variations in control diets can seriously impair data analysis. A study that examined HFD publications in the year 2007 revealed that only 14% of the publications made the appropriate comparisons.²⁶ The vast majority of these studies made conclusions regarding dietary effects by comparing grain-based chow with a predefined HFD, neglecting the differences in nutrient content in the two diets, which can cause unintended but significant metabolic disturbances. For example, the type of protein may modulate weight gain in rodents—rats fed soy diets have less hepatic lipid deposition, no hyperleptinemia and less overall weight than those fed casein diets.²⁷ Other ingredients such as fructose can exacerbate weight gain and isoflavones (phytoestrogens) can influence fat deposition, plasma insulin, leptin, thyroid, estradiol and corticosterone levels, lipogenesis and lipolysis in rat adipocytes as well as consumptive behavior (food and water intake), learning and memory and anxiety-related behaviors in rodents.²⁶ These results suggest that the effects of HFD are confounded by other components present in each diet, further hindering accurate data interpretation and extrapolation.

Even the standard 'control' animals may not be appropriate controls for obesity research as, in the words of the study author, these mice kept in controlled environments under a sedentary lifestyle with continuous access to food are 'metabolically morbid... obese, glucose intolerant, and on a trajectory to premature death'.²⁸ Taken together, due to a broad range of dietary interventions confounded by other factors affecting overall metabolism and physiology, it has not been possible to define the 'ideal' HFD nor the ideal environment in which to conduct these experiments. Consequently, results reported among laboratories are not comparable even among the same species/strain, and reliable cross-species extrapolation is challenging.

HFD-induced metabolic perturbations: sex, age and strain-dependence

The commonly reported T2DM-related metabolic perturbations induced by HFD in rodents include weight gain and increased carcass lipid content, higher degree of insulin resistance and increased levels of plasma glucose, insulin, leptin, cholesterol and triglycerides. However, as discussed below, the development, severity and duration of these metabolic perturbations are highly variable due to several factors such as the age at which HFD feeding starts, duration of HFD feeding, sex and the rodent species as well as the strain used,²⁹ rendering data analysis and extrapolation very difficult. Given the breadth of the literature on this topic, only representative examples are discussed below.

The age of onset and the duration of diet are important parameters when studying the metabolic syndrome in rodents. For example, in the most commonly used obesity-prone mouse strain C57BL/6J, 10-week-old mice displayed smaller increases in body weight, serum glucose, cholesterol and urea levels and higher increases in high-density lipoprotein levels than 54-week-old mice following the identical HFD for 12 weeks.³⁰ When given a high-fructose or a HFD for 13 weeks, the HFD caused the largest changes in serum lipids and lipid accumulation in the liver and kidney in young (4-weeks old) rats, whereas the high-fructose diet

increased visceral lipid stores, mean arterial pressure and heart rate in adult (12-weeks old) rats, and the metabolic perturbations became evident at different stages of the dietary intervention, suggesting that metabolic effects of diet vary considerably with age.³¹ Young mice (6-weeks old) were able to maintain normoglycemia in response to a HFD by increasing β -cell mass and β -cell proliferation, whereas older mice (7–8-month old) were not able to counter-regulate by the same mechanism and became diabetic.³²

The variability of metabolic perturbations among different rodent strains has been reported since the 1970s. Schemmel *et al.*³³ found that among seven different rat strains, HFD-induced weight gain ranged from 50% weight gain in Osborne-Mendel and Wistar-Lewis rats to 12% weight gain in S5B/P1 males to no weight gain in S5B/P1 females, indicating both strain- and sex-related differences. Similarly, the common mouse strains also range between obesity-prone and obesity-resistant.³⁴ A more in-depth comparison between the two most common strains of mice (C57BL/6J and AKR/J) indicated that insulin sensitivity and regulation of glucose homeostasis differ markedly between these two strains: C57BL/6J mice display rapid weight gain (14.78 vs 3.74 g in control), hyperglycemia (132 vs 117 mg dl⁻¹ in control), and insulin resistance (twofold less sensitive), whereas AKR/J have increased carcass lipid content (2916 vs 1006 mg in control), severe impaired glucose tolerance and insulin resistance (5.5-fold less sensitive) but remained euglycemic (100 vs 96 mg dl⁻¹ in control).³⁵ There were no factors that could account for this greater insulin resistance in AKR/J strain given that non-adipose tissue triglyceride accumulation was similar in both strains (143 in C57BL/6J vs 171 mg dl⁻¹ in AKR/J, $P > 0.01$).³⁵ Of note, it has been shown that some strains have higher fasting plasma glucose levels and lower fasting plasma insulin levels regardless of dietary content.³⁶ This is not so much an issue considering human T2DM patients also display a wide range of glucose intolerance and insulin resistance; however, the major limitation is that these data are not reproducible among research groups and therefore cannot be reliably extrapolated across species.

Levin *et al.*³⁷ reported that, in an outbred adult male Sprague-Dawley population, weight gain displayed a bimodal distribution pattern even though the rats were given the same HFD. Only half the rat population developed obesity, while the remainder showed no difference from control group. Similarly, female Wistar rats also showed a bimodal weight gain pattern on a HFD.³⁸ As both Sprague-Dawley and Wistar rats are outbred strains, one might argue that the metabolic heterogeneity is due to the ill-defined genetic nature of outbred colonies.³⁹ However, similar metabolic variability is also found in C57BL/6J, the most commonly used inbred mouse strain used for HFD studies. Burcelin *et al.*⁴⁰ found that when C57BL/6J mice were on a HFD for 9 months, the development of obesity and diabetes was not uniform within the population—both lean and obese phenotypes developed on the HFD. More strikingly, it was found that mice that remained lean and non-diabetic after 9 months of HFD feeding actually had a significantly higher rate of glucose clearance than those mice in the control group. The wide scattered phenotypic differences cannot be explained by the variations in genetic background, because each C57BL/6J mouse has the identical genetic background. The variability in metabolic perturbations within a single population compromises any conclusions drawn from HFD studies, but no solution exists in the research community to overcome this critical issue.

Some researchers consider the variability among rodent strains to be similar to the ethnic diversity in the human T2DM population and argue that multiple phenotypic variations provide a selection pool for finding suitable models.⁴¹ However, in reality, this variability among strains causes more detriment than presumed benefit owing to irreproducible results. For example, even with the same C57BL/6J strain as a comparison baseline,

DBA/2 mice were observed as obesity-prone in one study³⁶ but obesity-resistant in another.⁴² Similar discrepancies have been observed in FVB/N mice as well.^{43,44} To date, only a few comprehensive studies using multiple strains have been published, and the vast majority of HFD studies are based on a single strain and single sex. It is unclear whether most researchers select their 'most suitable strain' based on thorough examination of the phenotypic variations reported. What is clear is that no single strain can represent a reliable model for human obesity and T2DM.

HFD-feeding: a comparative analysis of intra- and inter-laboratory variability

As discussed, HFD-induced metabolic syndrome in rodents is plagued by many confounding factors, including, but not limited to, the type of diet (and control diet), duration of exposure to diet, animal species and strain, age and gender of the animals, and the clinical manifestations developed and their definitions. Therefore it is inevitably challenging to find studies suitable for direct comparison. Nevertheless, it is important to determine whether HFD data from different laboratories (and within the same laboratory) can be reliably compared and extrapolated. In this regard, Table 1 illustrates comparisons made with six studies that used the same mouse strain, C57BL/6J.^{23,40,45–48}

Among the six studies, the studies of Winzell and Ahren⁴⁵ and of Reimer and Ahren⁴⁷ have the most similar protocols: both studies used female wild-type C57BL/6J and started HFD at the age of 4 weeks with the same diet composition. However, even in studies from the same lab, presumably under the same protocol (that is, 58% calories from fat), different observations have been made regarding the development of hyperinsulinemia (see 1 week versus 8 weeks in Table 1). It is difficult to explain this disparity, because these two experiments are from the same lab, presumably with the same protocols, with the only difference being the duration of study (1 year versus 8 weeks on HFD). Considering the duration of study, Sone and Kagawa⁴⁶ conducted their study for 1 year, which is the same as Winzell and Ahren.⁴⁵ However, C57BL/6J mice used in the two studies were of different sexes, age of onset of HFD feeding and diet compositions. The two studies reported inconsistent results regarding the progression of weight gain and hypersinsulinemia (Table 1), but it is difficult to explain the disparity because the two protocols differ in many aspects except for the duration of study. Considering that dietary effects vary greatly within species and between closely related rodent species such as mice and rats, it is challenging to extrapolate to a distantly related species like *Homo sapiens*.

In addition to the biological variability, technical variability inherent in the assays and techniques used for measuring various parameters of the metabolic syndrome in rodents further complicates data acquisition, analysis and interpretation. Assays such as glucose and insulin tolerance tests and hyperglycemic and hyperinsulinemic–euglycemic clamps have inherent technical difficulties confounded by procedural factors affecting glucose metabolism, such as anesthesia, site and volume of blood sampling, fasting duration and even ambient lighting and time of day.⁴⁹ For example, anesthesia can induce hyperglycemia in mice and influence the assessment of glucose metabolism.⁵⁰ Tail-bleed blood sampling of 100 μ l can cause stress and lead to increased catecholamine and basal glucose levels compared with same volume of artery blood sampling.⁴⁹ In addition, rodent genetic background and environmental interactions (for example, housing facilities) can affect clinical chemical and hematological parameters, such as glucose, cholesterol, triglycerides, creatinine, uric acid, hemoglobin, red and white blood cell counts and platelet counts.⁵¹ Fasting duration, especially an 18-h overnight fast, can result in the loss of total body, lean and fat masses as well as hepatic glycogen levels while a 5-h fast can result in increased

Table 1. Inter- and intra-laboratory variability among six HFD studies using C57BL/6J mouse models

HFD in C57BL/6J	(a) Winzell and Ahren ⁴⁵	(a) Reimer and Ahren ⁴⁷	(b) Sone and Kagawa ⁴⁶	(c) Wu <i>et al.</i> ⁴⁸	(d) Gallou-Kabani <i>et al.</i> ²³	(e) Burcelin <i>et al.</i> ⁴⁰
Sex	Female	Female	Male	Male	Both sexes	Male
Age of onset of HFD feeding	4-week old	4-week old	6-week old	8-week old	Not specified	4–5-week old
Control diet	11.4% fat	11.4% fat	4% fat	4.3% fat	10% fat	12% fat
HFD: diet	58% fat	58% fat	40% fat	35.2% fat	60% fat	72% fat
HFD: oil	Lard	Not specified	Beef tallow	Lard	Various ^a	Corn oil and lard
HFD: duration	1 year	8 weeks	1 year	40 weeks	20 weeks	9 months
Weight gain	Weight increased	Weight increased	Weight loss observed the last 2 weeks.	Weight increased	Weight increased	Variable weight gains
Hyperglycemia	Hyperglycemia evolved after 1 week on HFD	Hyperglycemia evolved after 1 week on HFD	Hyperglycemia	Mild hyperglycemia. Lost at week 40	Hyperglycemia	Heterogeneous responses
Hyperinsulinemia	Hyperinsulinemia developed after 1 week on HFD	Hyperinsulinemia developed after 8 weeks on HFD	Hyperinsulinemia at month 4 but no difference from control at month 12	Hyperinsulinemia	Hyperinsulinemia only found in males	Heterogeneous responses

Abbreviation: HFD, high-fat diet. Wu *et al.*⁴⁸ used transgenic C57BL/6J while others used wild-type C57BL/6J. (a) Presumably with the same protocol, Winzell and Ahren⁴⁵ observed both hyperglycemia and hyperinsulinemia after 1 week on HFD, in which blood glucose concentration increased by $1.8 \pm 0.2 \text{ mmol l}^{-1}$ and serum insulin increased by $78 \pm 15 \text{ pmol l}^{-1}$; both $P < 0.001$. However, Reimer and Ahren⁴⁷ only observed hyperglycemia after 1 week (increased by $2.1 \pm 0.2 \text{ mmol l}^{-1}$) as the insulin level dropped from 106 ± 13 to $71 \pm 6 \text{ pmol l}^{-1}$ in HFD-fed mice, which did not develop hyperinsulinemia until week 8 on a HFD ($308 \pm 59 \text{ pmol l}^{-1}$); (b) With the same duration of study, Winzell and Ahren⁴⁵ reported steady weight gain, hyperglycemia and hyperinsulinemia progression, while Sone and Kagawa⁴⁶ reported weight loss at the last 2 weeks, and the progression of hyperinsulinemia stopped near the end of the study; (c) Wu *et al.*⁴⁸ used genetically modified mice (low-density lipoprotein receptor knockouts), and altered genetic background further contributed to the HFD-induced phenotypic variability; (d) Gallou-Kabani *et al.*²³ used a different protocol and found significant sex-related difference where only male C57BL/6J mice developed overt type 2 diabetes mellitus but not female C57BL/6J mice; (e) Burcelin *et al.*⁴⁰ used yet a different protocol compared with the other studies discussed here and found the development of obesity and diabetes was not uniform in the population. ^aVarious: 35.0% of saturated fatty acid, 43.4% of monounsaturated fatty acids and 15.9% of polyunsaturated fatty acids.

insulin sensitivity.⁵² In humans, prolonged fasting impairs insulin-stimulated glucose utilization, but the opposite is true for rodents.⁵³ Metabolic studies in rodents have shown that changes to circadian rhythm can also affect glucose metabolism.⁵⁴ It is clear that various factors associated with the technical procedures for assessing glucose metabolism in rodents significantly contribute to inconsistent results, rendering inter-species extrapolation an arduous task.

HFD-feeding: in combination with chemical, surgical and genetic manipulations

HFD is often used in combination with chemical, surgical and genetic modes of T2DM induction, but limitations inherent in these methods have severely restricted the ability to interpret data in conjunction with HFD feeding. For chemical induction, the cytotoxic glucose analogs alloxan and streptozotocin are commonly used to induce irreversible pancreatic β -cell destruction.⁵⁵ High-dose streptozotocin destroys most of the endogenous β cells, thereby decreasing insulin secretion substantially (a condition similar to T1DM), whereas low-dose streptozotocin induces mild impairment of insulin secretion (a condition similar to T2DM).^{56,57} Chemical induction of a T2DM-like state has its own disadvantages and combining that with HFD feeding creates further complications hindering reliable data interpretation and extrapolation. For example, the dosage and the number of streptozotocin injections considerably vary among studies: in rats, dosage can range from 90 mg kg^{-1} (Eiki *et al.*⁵⁸) to 30 mg kg^{-1} (Cao *et al.*⁵⁹) for single injections while the number of injections can range from two (Ding *et al.*⁶⁰) to four (Li *et al.*⁶¹) with the dosage ranging from 5 mg kg^{-1} (Li *et al.*⁶¹) to 30 mg kg^{-1} (Zhang *et al.*⁶²). This in turn leads to highly variable fasting blood glucose levels (from 5 to 25 mmol l^{-1}), indicating different degrees of pancreatic β -cell destruction.⁶² Such discrepancies make it difficult to compare data even among rodent studies.

In addition, streptozotocin and alloxan can cause extrapancreatic genotoxic and cytotoxic effects, including the disruption of the hypothalamic–pituitary–gonadal axis^{63,64} and changes in hyperglycemia-unrelated hepatic gene expression,⁶⁵ making it difficult to distinguish the effect caused by pancreatic cytotoxicity from those caused by extrapancreatic sites. The addition of age, sex and strain-dependent HFD feeding atop already complicated chemical T2DM induction renders intra- and inter-species extrapolation impossible.

The most common mode of surgical induction of T2DM is pancreatectomy, and ventromedial hypothalamus (VMH) lesions are sometimes used as well. The standard partial (up to 90%) or complete pancreatectomy often produces non-obese diabetic models where the extent of pancreatectomy governs the severity of diabetes.⁵ Data interpretation and extrapolation from pancreatectomy models are limited by many confounding factors. First of all, human T2DM is not caused by a sudden loss of the pancreas; rather it is actually due to progressive loss of β -cell function in combination with insulin resistance. Second, in rodents, pancreatectomy often evokes robust cellular responses that lead to β -cell regeneration giving full recovery of β -cell mass even after 40–60% partial pancreatectomy^{66,67} and up to 200% increase in β -cell number in the pancreatic remnants following 90% pancreatectomy in rats.⁶⁸ Unlike in rodents, partial pancreatectomy does not result in β -cell regeneration in humans,⁶⁹ due to differences between human and rodent β -cell replication mechanisms.⁷⁰ Although pancreatectomy is a common surgery used to induce T2DM in rodents, combining it with HFD only adds to the complexity—removal of the exocrine pancreas substantially reduces the production of most enzymes required for intestinal lipid absorption and lipolysis, making it difficult to obtain reliable results in HFD-fed pancreatectomized animals.⁷¹ These models also lack counter-regulatory mechanisms imposed by glucagon, thereby adding to the pathophysiological complexity.

VMH lesions are generally induced by administration of monosodium glutamate or direct electrical shock to create bilateral destruction of the ventromedial and arcuate hypothalamic nuclei. This leads to obesity primarily by hyperphagia and lack of control between energy absorption and expenditure governed by leptin, neuropeptide Y and insulin feedback mechanisms.⁷² Phenotypic variability in VMH rodent models is exacerbated by HFD feeding. For example, VMH rats fed the same HFD develop hyperglycemia at different rates.⁷³ Given the unnatural induction of hyperglycemic conditions by VMH lesions and the variable metabolic perturbations present even within a single rodent strain on a HFD,³⁷ it is difficult to extrapolate these data to human obesity and diabetes.

HFD is often combined with genetically modified models (transgenic, knockout, knock-in and overexpression models), where an overt diabetic phenotype may not appear until obesity is induced by HFD.⁷⁴ Regardless of the reason for dietary modification, genetically modified mice also display age-, sex- and strain-dependent variability in disease phenotype with or without HFD induction (Table 2). A prime example of this comes from knockout mouse models of an islet-enriched zinc transporter, ZnT8, thought to reside in insulin secretory granules to modulate proper insulin maturation, storage and secretion. Three independent studies published in 2009 alone provided evidence that mice lacking the gene encoding ZnT8 develop an increased risk for diabetes; however, there were intriguing differences among these animal models. As shown on Table 2, these studies reported age-, sex-, strain- and diet-dependent phenotypic variability (for example, glucose tolerance, insulin secretion, body weight, etc.),^{75–77} even when two colonies of the same ZnT8 null were maintained in two separate laboratories with the primary difference being the number of times the knockouts were backcrossed into C57BL/6J background (three times in the Toronto, Canada colony and two times in the London, UK colony).⁷⁵ Because of the highly variable results observed from the mixed genetic backgrounds in these studies, another group later generated ZnT8 knockouts on a pure genetic background (C57BL6/J) and concluded that ZnT8 does not have a substantial impact on mouse physiology.⁷⁸ In addition, even the *in vitro* insulin secretion from ZnT8 knockout mouse islets varied from no effect⁷⁶ to decreased effect⁷⁷ to enhanced insulin secretion.⁷⁵ Furthermore, global knockouts of ZnT8 were shown to be more susceptible to diet-induced obesity compared with tissue-specific knockout mice.⁷⁹ Following all these efforts to generate and characterize ZnT8 mouse models, a recent study involving 150 000 people carrying ZnT8 mutations revealed that mouse results are not congruent with the phenotype observed in humans: in marked contrast to the animal models where gene ablation resulted in increased risk for T2DM, protein-truncating loss-of-function mutations actually protect humans from developing T2DM.⁸⁰

There are many other examples of highly disparate results arising from the combination of HFD feeding with genetic modification. For example, loss of GPR40 (a G-protein-coupled receptor expressed predominantly in pancreatic islets mediating free fatty acid-induced insulin secretion) protects mice from HFD-induced diabetes,⁸¹ whereas the same gene ablation fails to protect mice from HFD-induced diabetes in another study.⁸² Although disparity in GPR40 studies arose on a C57BL/6J background, metabolic phenotypes appear to differ between strains of transgenic/knockout mice with identical genetic mutations (for example, insulin receptor and insulin receptor substrate-1 knockout mice).^{83,84} When HFD is combined with genetic modification in both sexes, the disparate phenotypes seen in males and females have forced studies to draw conclusions mainly from one sex^{85–88}—many HFD studies only include one sex in their experiments to avoid sex-related differences. There have even been instances where effects of a HFD diminished with time,

as has been shown for low-density lipoprotein receptor knockout mice on a C57BL/6J background who lost their lard-based HFD-induced mild hyperglycemia after 40 weeks.⁴⁸ This is rather unexpected as C57BL/6J is reported to develop more severe metabolic disturbances over other strains, such as AKR/J and A/J mice.³⁵ These studies beg the question, if a model using one rodent age, sex, strain and diet type cannot be extrapolated to another cohort of the same species, how much confidence can there be in extrapolating across the species barrier to humans?

In addition to the effect of dietary modification, primary and secondary effects of the genetic manipulation itself may heavily influence the observed phenotype. For example, genetic manipulation does not result in an observable phenotype due to the presence of compensatory mechanisms (for example, SUR1 knockout mice), or presents a more exaggerated phenotype than what may be present in the polygenic T2DM state (for example, glucose transporter 2 null mice).⁷⁰ Gene inactivation is effective throughout development, and therefore it is difficult to distinguish phenotypes arising from developmental defects from those directly resulting from impaired glucose homeostasis. Without the ability to confidently differentiate between knockout effect, compensatory effect and developmental effects, genetically manipulated models offer limited value when combined with HFD feeding, where all of these issues are further exacerbated by HFD-dependent sex, age and strain variability. Taken together, HFD feeding with chemical, surgical and genetic manipulation further limits the ability of rodent models to accurately mimic human obesity and diabetes.

HFD-induced obesity and diabetes in rodents: relevance to human pathophysiology

In order for a rodent model to have relevance to human disease, it should effectively recapitulate the natural history, pathophysiology and complications in a manner similar to what is observed in humans. However, this does not appear to be the case for HFD-fed rodents trying to mimic human obesity and diabetes. First of all, the large number of diets used for HFD studies in rodents may not appropriately represent the general human diet, particularly the 'obesogenic' Western diet. For example, the US population on average consumes 30–40% dietary fat regardless of weight (whether normal, overweight or obese).^{89,90} Therefore, using 40–60% fat (that is, values significantly >40%) in rodent dietary interventions is too extreme compared with the average Western diet. Moreover, human diets are much more complex than the carefully formulated rodent HFDs. According to the US National Cancer Institute, human foods and beverages can be divided into 97 categories, such as grain-based desserts, pasta and pasta dishes, beef and beef-mixed dishes and so on, with many subcategories.⁹¹ Humans also consume alcohol and excessive sodium, which are not found in rodent feed, but affect human pathophysiology. Therefore rodent diets differ from the diet that lead to human obesity and diabetes and associated complications.¹⁶

Apart from the actual diet, consumptive behavior also differs between rodents and humans. Humans tend to have stress, emotions and cultural factors that affect their access to food, selection of diet and feeding behavior.⁹² For example, uncontrolled food intake can lead to overeating due to complex psychological factors in humans,⁹³ which cannot be accurately mimicked in rodent models. In some rodent models, disruption of leptin signaling is the primary cause of hyperphagia rather than feeding behavior governed by emotional factors. Furthermore, unlike humans, mice consume most of their food at night.⁹⁴ As a result, the routine overnight fasting of 16–18 h is an unhealthy long period of time that provokes a catabolic state capable of nearly depleting liver glycogen stores, a state more akin to starvation in mice.⁹⁵ In lean mice, overnight fasting can reduce

Table 2. Variability among studies in which genetic modification is combined with HFD

<i>Genetic model</i>	<i>Experimental findings</i>
ZnT8 ^{-/-} : Colony derived in France on a mixed 129SeVe/C57BL/6J background and maintained in Toronto and London ⁷⁵	Age- and sex-dependence: in the Toronto colony, elevated FPG and GI in male mice at 6 weeks of age, but not at 12 weeks; normal FPG, but GI at both ages in females; in the London colony, male mice had GI at both ages, but female mice had normal glucose tolerance at 12 weeks Strain-dependence: mixed 129SvEv/C57BL/6J background; Toronto colony was backcrossed 3 × and the London colony was backcrossed 2 × on to C57BL/6J (possible explanation for age- and sex-dependence) Diet-dependence: HFD increased body weight in ZnT8 ^{-/-} compared with wild type; increased fasting plasma insulin levels <i>In vitro</i> : increased basal insulin release in ZnT8 ^{-/-} islets
ZnT8 ^{-/-} : Colony derived in France on a mixed 129SeVe/C57BL/6J background and maintained in Leuven ⁷⁶	Age-dependence: FPG and insulin levels unchanged at ages 6, 12, 25 weeks and 1 year. No difference in insulin sensitivity at 12 weeks Sex-dependence: No apparent sex differences; only slight (but statistically significant) change in glucose tolerance at 6 weeks in female ZnT8 ^{-/-} Strain-dependence: mixed 129SvEv/C57BL/6J background; overall metabolic abnormalities mild compared with Nicolson <i>et al.</i> ⁷⁵ Diet-dependence: normal glucose tolerance on standard diet; HFD increased body weight by 10%; mild GI at 10 weeks on HFD; overt diabetes (blood glucose > 14 mM) in 50% of ZnT8 ^{-/-} <i>In vitro</i> : no change in glucose-induced insulin secretion in ZnT8 knockout islets
ZnT8 ^{-/-} : Mixed 129SeVe/ C57BL/6J background ⁷⁷	Age-dependence: no change in body weight; blood glucose unchanged at 16 weeks of age Sex-dependence: compared with females, metabolic parameters such as plasma insulin, glucose, triglycerides and cholesterol levels were higher in males, but glucagon levels were lower in males Strain-dependence: mixed 129SvEvBrd/ C57BL/6J background; FPG unaltered; decreased insulin levels; no impairment in glucose clearance; overall mild metabolic phenotype Diet-dependence: experiments conducted only on standard control diet <i>In vitro</i> : glucose-induced insulin secretion markedly decreased in ZnT8 knockout islets
ZnT8 ^{-/-} : Pure C57BL/6J background ⁷⁸	Age-dependence: normal glucose tolerance at ~20–22 weeks, but a small impairment in glucose tolerance in younger (~4 weeks old) male mice; HFD studied only at 40–50 weeks of age Sex-dependence: normal fasting insulin levels in males; reduced fasting plasma insulin levels, but no change in FPG levels in females Strain-dependence: pure C57BL/6J background; no change in glucose tolerance; female phenotype appears to be less dependent on 129SvEv-specific modifier genes while male phenotype appears to be heavily influenced by 129SvEv-specific modifier genes Diet-dependence: decreased plasma insulin levels in males; no diabetic phenotype—40–50-week-old mice protected from HFD-induced obesity <i>In vitro</i> : no change in glucose-induced insulin secretion
ZnT8 ^{-/-} : Mixed 129SeVe/ C57BL/6J backcrossed 6 times onto C57BL/6J ⁷⁹	Global and β-cell-specific deletions of ZnT8—global knockouts more susceptible to HFD-induced obesity compared with tissue-specific knockouts. Global knockouts became obese, hyperglycemic, hyperinsulinemic, insulin resistant and glucose intolerant compared with littermate controls; in contrast, β-cell-knockouts had impaired glucose tolerance, though similar body weights, compared with littermate controls

Abbreviations: FPG, fasting plasma glucose; GI, glucose intolerance; HFD, high-fat diet; ZnT8^{-/-}, homozygous knockout of zinc transporter type 8 encoded by *Slc30A8* gene. This table summarizes a representative example of the variability commonly found in studies in which a genetic manipulation is combined with HFD feeding. Although there are many other studies that would fit these criteria, due to length limitations, only select examples are described in this review.

lean body mass by ~15%.⁵² In addition, prolonged fasting in mice enhances insulin-stimulated glucose utilization, whereas it is impaired in humans.⁴⁹ Moreover, mice can undergo reduced metabolic rate (physiological state of torpor), whereas humans cannot.⁹⁴

These metabolic stresses are further exacerbated by the ambient temperature in general laboratory housing (18–22 °C), which is well below their critical thermoneutrality point of ~30 °C. This chronic thermal stress causes mice to increase their metabolism by increasing food intake by as much as 50%,^{96–99} further skewing data. Controlled light/dark cycles adapted for human convenience (that is, typical 0800–1700 work hours) could have negative influences on mouse physiology, as changes to circadian rhythms can affect glucose metabolism.⁴⁹ In general, rodents have higher metabolic rates governed by much higher heart rates ranging from 350 to 550 beats min⁻¹, whereas humans have lower metabolic rates accompanied by lower heart rates around 70 beats min⁻¹.

Translatable rodent models of T2DM should accurately mirror the etiopathology of the human condition; however, this is rarely the case with the vast majority of rodent models, including HFD

models. In humans, a complex genetic background interacts with environmental factors leading to progressive disease development, which occurs over a long period of time on the order of years to decades.¹⁰⁰ In contrast, most rodent HFD studies last only a few weeks (and a few months at most) as rodents can develop obesity and insulin resistance after 2 weeks on a HFD, albeit to varying degrees.⁷ Because of rapid unnatural disease induction, HFD models often lack (or incorrectly mimic) T2DM-associated vascular complications, which develop over many years, usually starting long before the clinical diagnosis of overt hyperglycemia in humans. Macrovascular complications (primarily described as cardiovascular complications) are the leading cause of morbidity and mortality, which occur predominantly via accelerated atherosclerosis in humans. By contrast, rodents show resistance to high low-density lipoprotein cholesterol, atherosclerosis, platelet aggregation and hypertension.⁷⁰ In a study examining the association between atherosclerosis and T2DM in a HFD rodent model, no difference exists between the control and the HFD group in all indicators of atherosclerosis despite the presence of metabolic perturbations such as hyperinsulinemia, hyperglycemia and obesity.⁴⁸

The main microvascular complications that occur in humans are nephropathy, neuropathy and retinopathy; however, reliable rodent models that can effectively recapitulate these human complications are still lacking, especially with HFD-induced models. For example, advanced proliferative diabetic retinopathy characterized by intravascular neovascularization—the leading clinical feature causing blindness in humans worldwide—has not been successfully displayed in a single animal model of T2DM, much less HFD-fed rodents.¹⁰¹ For diabetic nephropathy, only early signs of renal damage are usually observed even in the long-term diet-induced models of T2DM.¹⁰² In terms of diabetic neuropathy, rodents may develop some peripheral nerve functional abnormalities but do not develop structural abnormalities seen with human diabetic neuropathy.^{70,103,104} Taken together, HFD rodent models display variable and partial features, often resembling only the early stages of human vascular complications. Therefore, these models have limited translatability benefit, and overestimation of these data can lead to inaccurate delineation of disease mechanisms in humans. Furthermore, drugs tested to be effective in models displaying only early signs of vascular complications may not work as effectively for the general human population that routinely manifests advanced disease phenotypes.

It appears that even functional genomics may not be reliably translated from HFD models to the human state. In a functional genomics study, comparing differential gene expressions in HFD rats and obese humans indicated that there is only minimal overlap of the differentially expressed genes between the HFD versus control rats and obese versus non-obese human comparisons. The study found that genes in both fatty acid metabolism and oxidation showed different regulation patterns between obese human and rats.¹⁰⁵ The discrepancies in differential gene expression between HFD rats and obese humans, especially in the metabolic pathways, further decrease the applicability of HFD rodent models to human obesity and T2DM research. In addition, species differences at every level of glucose regulation (from gene expression to the maintenance of whole-body glucose homeostasis) further restrict the ability to extrapolate data from rodent HFD models to human obesity and diabetes.⁷⁰

SUMMARY AND FUTURE PERSPECTIVES

A combination of several techniques has enabled the creation of a large number of animal models with varying obesogenic/diabetogenic phenotypes. However, it is clear that the natural history and metabolic characteristics of the human condition cannot be effectively recapitulated in a single model or even a combination of these animal models. Induction of T2DM-like features with HFD feeding is very common in research, but major limitations significantly reduce the translatability of rodent-based dietary intervention data to human disease mechanisms and treatment options. Despite the current literature describing many thousands of diet-induced rodent studies, the question of which HFD is best to model the metabolic syndrome seen with human obesity still remains unanswered. Based on this body of literature that provides ample evidence showing even the same rodent (for example, Sprague-Dawley rat) from three different vendors (for example, Harlan, Charles River and Simonsen) can display substantial phenotypic differences with respect to metabolism,¹⁰⁶ it is not possible to define a recipe for the 'ideal' HFD, dietary components and control diet capable of yielding the best and most reproducible results in rodents, nor the characterization of metabolic perturbations based on an exact dietary composition, duration of diet and age of onset of diet due to many confounding factors such as species, strain, age, sex, housing environment and husbandry practices and biochemical/physiological assessment techniques (Figure 1). Moreover, animal studies are biased with respect to experimental design and data reporting, with excess significance and overoptimistic translational

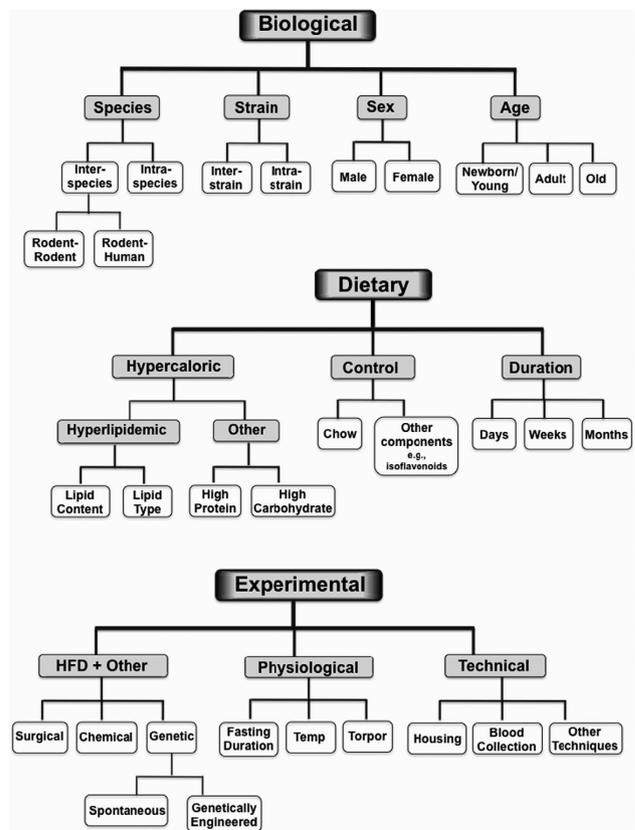


Figure 1. Primary factors contributing to the variability observed in HFD-fed rodent models. Variability observed within and among laboratories using HFD-based rodent models can be broadly classified into biological, dietary and experimental variability primarily arising from factors such as species, strain, sex, age, HFD fat content and type, other dietary components, control diet and duration of HFD feeding. This variability is further exacerbated when combined with chemical, surgical and genetic manipulations as well as physiological and environmental factors affecting data acquisition.

efficacy widely reported in the literature.^{107,108} Specious extrapolations and overstated significance of rodent studies have hindered the outcome of human therapeutics. Consequently, only a few anti-diabetic drugs are currently available on the market despite the substantial amount of information acquired thus far from animal studies of obesity and diabetes.⁷⁰ It seems clear that research efforts must be redirected to studying human T2DM using human-based methods in order to acquire human-relevant information.

The limitations of animal models suggest the value of studying human glucose biology directly. Obesity and T2DM can be studied from gene expression to whole-animal physiology to environmental level using human-based methods.⁷⁰ Utilization of a broad range of human-based methods would enable the researcher to reliably capture the whole spectrum of the polygenic, multifactorial human metabolic syndrome and T2DM. Acute and long-term effects of diet composition can be directly studied in humans on a specific dietary regime. For example, the identity of the molecular defect(s) underlying obesity-induced insulin resistance can be studied from skeletal muscle biopsy samples, and whole-body insulin sensitivity can be studied via hyperinsulinemic–euglycemic clamp from human subjects on a given dietary regime.^{109,110} Proteomic analysis of human skeletal muscle¹¹¹ presents a dynamic pattern of protein abundance in insulin

resistance, serving as a basis of novel hypothesis testing for molecular mechanistic studies.

Understanding disease etiopathogenesis is crucial for the development of new therapeutic interventions, but human environmental influence on disease ontogeny cannot be recreated in a laboratory setting nor can they exert the same effect on another species. Therefore studies in epigenome-wide DNA methylation profiling of pancreatic islets can help link epigenetics to T2DM and identify differential methylation in several T2DM-associated genes.¹¹² A number of human epidemiological studies have resulted in the identification of dietary risk factors in various human populations, such as Pima Indians, Chinese and other ethnic groups.^{113–117} Recent studies have shown that diet and obesity may even have a microbial component. For example, intestinal microbiome gene profile has been shown to differ between lean and obese individuals on different diets,¹¹⁸ and the human gut microbial profile has been linked to human insulin action.¹¹⁹ Given that diet can modify factors like gut microbiota and subsequent influence on host metabolism, it is crucial to obtain data directly relevant to the human species, as such factors can differ significantly between rodents and humans. Further refinement of existing methods and the development of novel human-based research methods will transform obesity and T2DM research in a human-relevant manner.

Dietary modification in rodent models has limited translatable benefit for understanding the pathogenesis of human obesity and diabetes. The information obtained from the HFD studies are often confined to the species and strain, and even to simply one sex, rather than being applicable to the human disease state. It is, therefore, necessary to dedicate future research efforts to obtaining transnationally relevant data, specifically human data, rather than findings based on rodent studies. Redirecting biomedical research back to humans is clearly the way to efficiently deal with the current obesity and diabetes epidemics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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