Modeling type 2 diabetes in rats using high fat diet and streptozotocin

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ABSTRACT
The pathology of type 2 diabetes is complex, with multiple stages culminating in a functional β-cell mass that is insufficient to meet the body’s needs. Although the broad outlines of the disease etiology are known, many critical questions remain to be answered before next-generation therapeutics can be developed. In order to further elucidate the pathobiology of this disease, animal models mimicking the pathology of human type 2 diabetes are of great value. One example of a type 2 diabetes animal model is the high-fat diet-fed, streptozotocin (HFD/STZ)-treated rat model. The present review first summarizes the current understanding of the metabolic profile and pathology involved in the different stages of the type 2 diabetes disease progression in humans. Second, the known characteristics of the HFD/STZ rat model are reviewed and compared with the pathophysiology of human type 2 diabetes. Next, the suitability of the HFD/STZ model as a model of type 2 diabetes with a focus on identifying critical caveats and unanswered questions about the model is discussed. The improved understanding of refined animal models will hopefully lead to more relevant preclinical studies and development of improved therapeutics for diabetes. Depending on the amount of residual functional β-cells mass, the HFD/STZ rat model might be a suitable animal model of the final stage of type 2 diabetes.

INTRODUCTION
Type 2 diabetes is increasing in prevalence worldwide,1,2, and it is strongly associated with obesity and insulin resistance,3,4 as well as defects in pancreatic β-cell function and mass.5,6 These metabolic disorders impede the critical regulatory influence of insulin on glucose, lipid and protein metabolism, thus precipitating a disease characterized by impairments in these physiological processes. However, it takes years to develop frank diabetes. Patients developing type 2 diabetes have often gone through a state of obesity associated with reduced insulin sensitivity along with an activated β-cell compensatory mechanism, such as excess basal insulin secretion and hyperproinsulinemia, as a part of their metabolic profile.7 These pathological conditions occur early in the disease progression of type 2 diabetes,8, and before the β-cells severely fail in late stage (insulin-dependent) type 2 diabetes.8,9

To combat type 2 diabetes, there is an urgent need for more effective treatments and therapeutic regimens. Thoroughly characterized and clinically relevant type 2 diabetes animal models are required to achieve this aim of testing new and better therapeutics. Both genetic spontaneous diabetes models and experimentally-induced non-spontaneous diabetes models exist. An example of an experimentally-induced animal model of diabetes is the high-fat diet/streptozotocin treated (HFD/STZ) rat model. This model involves a combination of a diet high in fat, and in some cases sugar, to bring about hyperinsulinemia, insulin resistance and/or glucose intolerance followed by treatment with the β-cell toxin STZ, which results in a severe reduction in functional β-cell mass.10,11 Together, these two stressors are designed to mimic the pathology of type 2 diabetes, though on a shorter timescale than found in the human condition.

The aim of the present review is to clarify and discuss critical caveats and unanswered questions regarding the HFD/STZ rat model, which have not been discussed in the literature. First, the impact of and differences between the diet regimens in relation to obesity and type 2 diabetes will be discussed. Second, the effect of the various STZ treatments, as well as the
importance of age, with respect to type 1 and type 2 diabetes, will be focused on. Finally, whether the HFD/STZ rat model mimics the early or late stages of type 2 diabetes are discussed. This disease stage classification is based on comparison of circulating metabolic measures provided in studies using the HF/STZ rat model. Classification of type 2 diabetes is an important consideration when choosing the best therapeutic intervention in patients.

In order to discuss these topics at the end of the present review, the human metabolic profile of the different stages in the disease progression of type 2 diabetes will be summarized first. Next, the history of the development of the HFD/STZ rat model will be recounted. It is beyond the scope of this review to present an overview of all existing diabetes rodent models, as many of the other models have been reviewed recently. To summarize, the aim of the present review is to provide a guide of the factors to take into account when modeling and working with the HFD/STZ rat model.

**METABOLIC PROFILE OF HEALTHY AND PREDIABETES HUMANS**

Before discussing the HFD/STZ rat model, it is important to review the stages and transitions in the progression of type 2 diabetes, which the HFD and STZ treatments are meant to emulate. The first transition is the shift from a healthy state to a prediabetes state. In prediabetes, patients have either impaired fasting glucose, impaired glucose tolerance, or both, and is often associated with insulin resistance. In healthy individuals, the adipose tissue functions as a safe storage site for lipids during a positive caloric balance. Likewise, excess circulating glucose is accommodated by the liver and muscle tissue in the form of glycogen. In the context of fully occupied glycogen stores, high glucose levels might also bring about de novo lipogenesis, occurring mainly in the liver and, to a lesser extent, in the adipose tissue. De novo lipogenesis helps maintain normal blood glucose levels by sequestering away excess glucose from the circulation. Normoglycemia in healthy individuals is maintained by the unique interplay between the almost opposing hormones, insulin and glucagon. The dialogue between these two hormones becomes perturbed with the disease progression of type 2 diabetes. The transition from a metabolically healthy state to prediabetes often includes an obese state characterized by hyperinsulinemia, insulin resistance, and dyslipidemia. However, it should be stressed that both metabolically healthy obese individuals, as well as metabolically unhealthy lean individuals, can be found in the general population. This implies that obesity might not automatically or immediately result in the development of type 2 diabetes, and highlights that type 2 diabetes is a highly polygenic and heterogeneous disease. The nutritional overload, which in the long term leads to obesity, can quickly induce insulin resistance in skeletal muscle as well as in the liver (Figure 1). Insulin resistance in skeletal muscle might reduce the occurrence of lipotoxic effects in muscle by redirecting the excess energy to the adipose tissue stores, and can thus be seen as a normal physiological function in healthy individuals.

Severe expansion of the adipose tissue is tightly associated with adipose inflammation and a distorted adipokine profile, marked by high leptin and low adiponectin levels representing dysfunctional adipocytes. Dysfunctional adipose tissue leads to ectopic fat accumulation in non-adipose tissue, such as muscle, liver, and β-cells (Figure 1). Intramyocellular lipid accumulation is associated with insulin resistance. Insulin-resistant muscles have lower glycogen synthesis and redirect glucose to the liver, where it contributes to hepatic lipid accumulation through de novo lipogenesis (Figure 1). Hepatic fat accumulation can induce hepatic insulin resistance (Figure 1), with decreased glycogen synthesis and increased gluconeogenesis. This impaired insulin-induced suppression of hepatic glucose output may contribute to hyperglycemia (Figure 1). Further inflammation of the abdominal adipose tissue may worsen the dysfunctional state of the adipocytes, leading to more ectopic fat accumulation, insulin resistance and hyperinsulinemia, in a negative feedback loop (Figure 1). However, beneficial aspects of inflammation, such as proliferation of certain classes of macrophages in the adipose tissue, has been illustrated.

In the early state of type 2 diabetes progression, β-cell compensatory mechanisms have typically adapted to preserve normoglycemia. The compensatory mechanisms might include increased β-cell mass, augmented β-cell function, or a
functions can to some extent been explained by the twin cycle suppression of basal insulin secretion. Failure of these cose-stimulated pulsatile insulin release and the appropriate and b-cell function, which includes an appropriate level of glucose-stimulated pulsatile insulin release and the appropriate suppression of basal insulin secretion. Failure of these b-cell functions can to some extent been explained by the twin cycle hypothesis. The idea behind this hypothesis is that hepatic lipid accumulation leads to b-cell lipid uptake, worsening insulin resistance, and promoting b-cell failure and death. The factors and mechanisms involved in programmed b-cell death have recently been reviewed. Importantly, the amount of physical b-cell mass left in an individual with type 2 diabetes seems to be dependent on the duration of the disease. Consequently, the metabolic profile in type 2 diabetes patients will depend on the duration of their disease (an early vs late stage of type 2 diabetes). The choice of therapeutics will differ between patients being in either an early or late state of type 2 diabetes. The type of therapeutic intervention, lifestyle vs pharmacological therapeutics, such as insulin therapy, will further affect the metabolic profiles.

Finally, it is important to note that the cell types involved in the pathogenesis of type 2 diabetes are not solely limited to adipocytes, myocytes, hepatocytes and b-cells. In the 2009 Banting lecture, Dr Ralph Defronzo stressed the pivotal role of all members of the ‘ominous octet’ in the development of glucose intolerance, which includes the brain, kidneys, alpha cells, and the gastrointestinal tract, besides the four cell types already mentioned.

HISTORY OF THE DEVELOPMENT OF THE HFD/STZ RAT MODEL
In the beginning of the new millennium, Reed et al. reported a new rat model of type 2 diabetes. This model is today known as the HFD/STZ rat, as well as by other names (e.g. high energy/STZ rat). Recently, the model is most often referred to simply as a type 2 diabetes model. The aim of the study by Reed et al. was to develop a rat model simulating the natural diabetes pathology progression, from prediabetes and/or insulin resistance to a state of type 2 diabetes and hypoinsulinemia, in a condensed timeline. Reed et al. fed 7-week-old Sprague–Dawley rats a diet with 40% kcal fat for 2 weeks. The presence of insulin resistance was indicated through the observation of equal glucose clearance profiles in fat and lean rats, respectively, with an increase in glucose-induced insulin responses in the fat-fed rats. Subsequently, overnight-fasted animals were dosed (i.v.) once with STZ (50 mg/kg). A total of 3 days after STZ treatment, rats that had reached an elevated blood glucose plateau were included in the study and their response to metformin was tested. The metformin-induced lowering of blood glucose further established the HFD/STZ model to be a rat model of type 2 diabetes relevant to the human condition. Later, another HFD/STZ rat model was generated by using a low dose of STZ. The HFD/STZ model was then further modified by Zhang et al., wherein the STZ-treatment comprised multiple low doses of STZ instead of a single dose. This approach was inspired by the type 1 diabetes animal model involving multiple low doses of STZ. This approach has been reported to induce an inflammation-mediated destruction of the b-cells instead of the fast induction of the b-cell death induced by a single dose of STZ. After these three key publications, several versions of the HFD/STZ rat have appeared in the literature.

DISCUSSION OF THE HFD/STZ RAT MODEL: DISEASE MODELING
Impact of the Diet Regimen in Relation to Obesity and Type 2 Diabetes
In the HFD/STZ rat models, the state of obesity, insulin resistance and/or glucose intolerance in prediabetes is simulated by a period of a high-fat or ‘Western’ diet. Whether the rats actually reach a state of true overweight or obesity within this time period seems to depend on the duration of the fat feeding, which tends to be either relatively long (≥ 3 months) or relatively short (2–4 weeks; Table 1). Furthermore, the classification of overweight and obesity in humans is based on
Table 1 | Summary of high-fat diet-fed, streptozotocin rat studies

| References | STZ* | Diet† | Initial age/WW | Strain‡ | Metabolic measures†† | T2D stage (H) |
|------------|------|-------|----------------|--------|----------------------|--------------|
| Hu et al.88 | 1 × 30–35 | 12W, 26K, 152P, 588%, % | 10–12 weeks | SD | PG = 22 (H), PI = 191 (H), HOMA-IR = (H) | Early (T2D) |
| Abo-Elnaty et al.88 | 1 × 35 | 2W, 17C, 25P, 58%, % | – | A | – | Late (T2D) |
| Gandhi et al.89 | 1 × 40 | 2W, 73ND, 25C%, 20C, | 180 ± 10 g | W | FBG = 17 (H), PI = 198 (H), TG = 17 (H), TC = 25 (H) | Early (T2D) |
| Khan et al.90 | 1 × 35 | 2W, 20C%, 10F%, 25CO, 10 | 230 ± 20 g | SD | FG = 14 (H), PI = 111 (L), LG = 14 (H), C = 57 (H), | Late (T2D) |
| Ren et al.91 | 1 × 30 | 6W, 6ND, 20C%, 10F%, 10 | 8 weeks/180–220 g | SD | – | – |
| Hou et al.92 | 1 × 25 | 20W, 30P, 59F | 200–220 g | – | TG = 18 (H) | – |
| Guo et al.93 | 1 × 30 | 7W, 6ND, 20C%, 10F%, 10 | 140–180 g | W | IS = (L) | – |
| Mahmud et al.94 | 1 × 35 | 2W, 4IC, 18P, 40F | 190 ± 10 g | RN | G = 16 (H), HbA1c = 9 (H), I = 108 (L), HOMA-IR = (H) | Late (T2D) |
| Si et al.95 | 1 × 70 | 2W, 4IC, 18P, 40F | 7 weeks, 200 g | SD | BG = (H) | NA (T2D) |
| Guo et al.96 | 1 × 30 | 6W, 6ND, 20C%, 10F%, 10 | 140–180 g | W | PG = 18 (H), PI = 206 (H), TG = 1.7 (H), TC = 3.5 (H), IRI = (H) | Early (T2D) |
| Hussein et al.97 | 1 × 35 | 2W, 3C, 74P, MP, 23P, 10 | 15–21 weeks | W | FG = 13, FI = 107, TG = 21, TC = 52, HD = 1.0 | Late (T2D) |
| Guo et al.98 | 1 × 30 | 4W, 6ND, 20C%, 10F%, 10 | 140–180 g | W | – | – |
| Sharma et al.99 | 1 × 40 | 15W, 73ND, 25P%, 20C | 170–200 g | W | BG = 17 (H), I = 123 (H), APQ = (H), TG = (H), LG = (L), HDL = (L), | NA (T2D) |
| Albersen et al.100 | 1 × 20 | 2W, ND added 10F%, 20C | 12 weeks | SD | – | Early (T2D) |
| Lu et al.101 | 1 × 30 | 8W, 30C, 22P, 12F, 30 | 8 weeks, 250 ± 20 g | W | HbA1c = 11, ¶¶ | NA (T2D) |
| Parveen et al.102 | 1 × 25 | 2W, 4IC, 18P, 40F, % | 160–220 g | W | – | – |
| Zou et al.103 | 1 × 30 | 8W, 25C, 164P, 58%, 10 | 220–30 g | SD | – | – |
| Xing et al.104 | 1 × 30 | 6W, 6ND, 20C%, 10F%, 10C, 10 | 170–200 g | SDSS | – | – |
| Zhang et al.105 | 1 × 35 | 8W, 60 P%, 10 | 180–200 g | W | – | – |
| Islam et al.106 | 1 × 40 | 2W, 4C, 35%, 20C%, 20F% | 5 weeks, 120–140 g | SD | – | – |
| Zhang et al.107 | 1 × 30 | 4W, 48C, 20P, 20F | 200–250 g | W | – | – |
| Gao et al.108 | 1 × 25 | 4W, 30C%, 15F% | 210–220 g | W | – | – |
| Sahin et al.109 | 1 × 40 | 2W, 30C%, 10F%, 40P%, 10 | 8 weeks, 200–250 g | W | – | – |
| Danda et al.110 | 1 × 31 | 5W, 60F%, % | 175–200 g | W | – | – |
| Sinivasan et al.111 | 1 × 35 | 2W, 7K, 25P, 58%, 10 | 160–80 g | W | – | – |
| Zhou et al.112 | 1 × 40 | 4W, 5C, 13P, 20F%, 50 | 4 weeks, 8 ± 5 g | SD | – | – |
| Wu et al.113 | 1 × 30 | 2W, 4IC, 18P, 41F% | 8 weeks | W | – | – |
| Zhang et al.114 | 1 × 15 | 8W, 50C, 13, 30F | 8 weeks | W | – | – |
| Yang et al.115 | 1 × 15 | 8W, 40C, 13P, 40F, 70 | 8 weeks | W | – | – |
| Reed et al.116 | 1 × 50 | 2W, 4IC, 18P, 40F | 7 weeks, 200 g | W | – | – |

*STZ treatment: Number of doses × dose (mg/kg) of Streptozotocin. The route of administration was intraperitoneally unless otherwise indicated; intravenously (iv). †Diet (duration): Duration of diet regimen in weeks (W) before STZ treatment. ‡Diet (nutritional content): dietary Carbohydrate percentage (C): Starch (ST), Sucrose (s); dietary Fat percentage (F): Animal Fat (AF), Lard (L), Coconut Oil (CN), Vegetable Oil (VO); dietary Protein percentage (P): Casein (CA), Milk Powder (MP), Soy Bean (SB); dietary Cholesterol percentage (CO); dietary percentage of Other components than C, F, P and CO (O); Normal diet (ND); %ka is specified with %. ‡‡Strain of male rats: Sprague Dawly (SD), Wistar (W), Albino (A); **Female. ¶¶Metabolic measures: The metabolic measure was either Higher (H) or Lower (L) in the HFD/STZ rat than in lean controls, or the same. All data have been converted to SI units. The mIU Unit was used for: Glucose (G), Blood Glucose (BG), Plasma Glucose (PG), Fasting Glucose (FG), Fasting Blood Glucose (FBG), Triglycerides (TG), Total Cholesterol (TC), Plasma Triglycerides (PTG), Plasma Cholesterol (PTC), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL); The mU Unit was used for: Insulin (I), Plasma Insulin (PI), Fasting Blood Insulin (FBI), Fasting Serum Insulin (FSI); The mg/mL Unit was used for: adiponectin (APQ); Homeostasis Model of Assessment of β-cell function (HOMA-B); Homeostasis Model of Assessment of Insulin Resistance (HOMA-IR); Insulin Sensitivity Index (IS), Insulin Resistance Index (IR); ¶¶Statistics against controls were not provided, ¶¶NA lean controls were included. **Stage of type 2 diabetes (T2D): Whether the animal model mimics the Early versus the Late stage of type 2 diabetes was based on the levels of insulin and glucose provided in the study. The model was concluded be mimetic the early stage if glucose and insulin levels were higher than in controls, whereas the model was concluded to mimetic the late stage if insulin levels were lower than or the same as in controls. ‡‡Homenumeral for the HFD/STZ rat model: Type 2 diabetes (T2D); diabetes (D); according to the Diet and STZ treatments (DS); lack of metabolic parameters to classify the model or lack of nomenclature (NA).
body mass index and can also be defined as abnormal or excessive fat accumulation that might impair health (www.who.int). However, it is often unclear how one can apply this definition to rodents. Unfortunately, no standardized definition of rodent obesity exists. Hence, the actual presence of obesity in the various HFD/STZ rats should always be taken into consideration when working with this model. Besides the duration of the HFD feeding timeframe, the composition of the diet seems to greatly affect the weight gain and fat distribution. The diets used in the HFD/STZ model vary in both nutritional composition and source of the nutrients (Table 1). Some studies utilized a diet high in carbohydrates to produce a 'high energy' feeding regimen. However, the most commonly used approach is to feed rats with a diet high in fat, but with normal levels of sugar.

In our experience, 5 weeks of high-fat/high-sucrose (HF/HS) feeding induced a higher body fat percentage and impaired glucose tolerance along with hyperinsulinemia (S. Skovsø, unpublished data). In contrast, induction of insulin resistance, by even shorter high-fat diet regimens, has been reported. Furthermore, short periods of HFD feeding have been reported to simulate insulin resistance in lean patients, which is different from 'true' obese state that might take a much longer time to replicate in rats. Thus, one should carefully consider the length and nutritional composition of the diet regimen, depending on what state one wants to mimic. Short diet regimens (2 weeks) tend to just induce insulin resistance and/or glucose intolerance, whereas relatively longer diet periods (5 weeks) can also induce a higher body fat percentage. Hence, this relatively short diet feeding seems to mimic the human situation of prediabetes, including obesity and hyperinsulinemia, more appropriately than the short diet regimens. Even longer feeding timeframes (>3 months) are preferred when aiming for 'true' obesity including significant bodyweight increases.

HFD/STZ rats are often reported to be dyslipidemic, similar to the metabolic profile of type 2 diabetes in humans. Whether this is a direct consequence of the diet regimen alone is rarely reported in the literature. Data comprising the presence of hyperinsulinemia, obesity and impaired glucose tolerance, all representing the prediabetes state, are also rarely reported in the literature at the time-point before initiation of STZ treatment. Such data, before establishment of severe hyperglycemia with STZ, would be required to underscore similarities with the progression of human type 2 diabetes, with respect to the order of the main pathological events. In unpublished studies, we have found that it is possible to maintain normal fasting glucose levels while significantly increasing the levels of total body fat (magnetic resonance imaging scanning), the liver fat (computed tomography scanning), plasma C-peptide and triglyceride, as a consequence of a 5-week HFD diet regimen consisting of 4 kcal% fat (lard), 35% carbohydrate (corn starch, sucrose, and maltodextrin) and 20% protein (casein), before initiation of STZ treatment (S. Skovsø, unpublished data). Furthermore, similar to the findings of other groups, we have observed glucose intolerance in HFD-fed rats. After STZ treatment of HFD-fed rats, we and others have observed profound hyperglycemia, low levels of circulating adiponectin, and high levels of plasma alanine aminotransferase (S. Skovsø, unpublished data). In summary, the diet regimen is one of the most important factors to consider in the HFD/STZ rat model.

Does STZ Treatment Make the Model a Type 1 or a Type 2 Diabetes Animal Model?

The final event involved in the development of type 2 diabetes is β-cell failure/death. This is also the case in type 1 diabetes. Hence, the β-cell toxin, STZ, has been used in both type 1 and type 2 diabetes animal models. The STZ dose will greatly affect the β-cell mass remaining in the rats. Likewise, variation in the amount of β-cell mass lost in both type 1 and type 2 diabetes exists in humans. Despite the lack of non-invasive measurement techniques, it has been suggested that 60–80% of the functional β-cell mass is lost by the time of diagnosis of type 1 diabetes. In contrast, only a 24% reduction has been observed in patients with a <5 years’ history of type 2 diabetes compared to controls. However, another study has reported a 54% reduction in β-cell mass 15 years after diagnosis of type 2 diabetes. Collectively, these data suggest a similarity in β-cell mass, when comparing early type 1 and late stage type 2 diabetes.

This observation suggests that the HFD/STZ rat model could mimic the case of early type 1 diabetes coexisting with obesity. However, obesity is more often seen in patients after their type 1 diabetes diagnosis, whereas obesity is often seen decades before the diagnosis of type 2 diabetes. Thus, the order of the pathological events, obesity followed by β-cell failure, seen in HFD/STZ rats favors a mimicking of type 2 diabetes rather than type 1 diabetes, despite the observed similarity between early type 1 diabetes and late type 2 diabetes. Furthermore, the loss of β-cell mass in the pathogenesis of type 1 diabetes occurs mainly as a result of an autoimmune reaction, which is not the case in HFD/STZ rats. In contrast, the events leading to β-cell compensatory mechanisms and subsequent β-cell failure in type 2 diabetes involve lipotoxicity and/or glucolipotoxicity, insulin resistance, hyperinsulinemia, and stress, with a modest contribution from low-level inflammation. In other words, the different causality that induce β-cell death in type 1 and type 2 diabetes cannot be mimicked to perfection by STZ treatment in animal models.

Despite the lack of the autoimmune component, HFD-fed rats treated with just a single high dose of STZ show clear features of type 1 diabetes, such as hyperglycemia, insulin deficiency, drastic weight loss and resistance towards insulin-sensitizing therapeutics. Furthermore, STZ treatment of both lean-STZ (a frequently used model of type 1 diabetes, 3 × 30 mg STZ/kg bodyweight, once daily for 3 days) and HFD/STZ rats (3 × 20 mg STZ/kg bodyweight, once daily for 3 days) are often associated with an initial weight
loss (S. Skovsø, unpublished data), whereas both models respond with a weight gain after 3 weeks of insulin therapy (S. Skovsø, unpublished data). An insulin-induced weight gain is commonly seen during insulin therapy in type 1 and type 2 diabetes patients. In contrast to type 1 diabetes, a clear and sudden weight loss is not observed on diagnosis of type 2 diabetes. However, patients with undiagnosed and/or non-insulin-treated overt type 2 diabetes would inevitably also face a significant weight loss with time. Thus, the STZ-induced weight loss and gain in weight on insulin therapy, which we have observed in the HFD/STZ rat model, does not make the model a better model of type 1 diabetes than of type 2 diabetes, as they are phenomena potentially occurring in both diseases.

Confusion has been added into the literature by the fact that some high-fat fed rat models, treated with the same high dose of STZ used when modeling type 1 diabetes, have also been referred to as models of type 2 diabetes in other studies. However, when the STZ dose is changed from a single high dose to a single low dose or multiple lower doses of STZ, researchers tend to agree on the HFD/STZ rat as a suitable model of type 2 diabetes (Table 1). Thus, the dose of STZ in itself obviously has a significant impact on the phenotype of HFD-fed rats. STZ treatment induces robust (but not absolute) \( \beta \)-cell ablation in a manner that depends on the dose, the number of doses, the time interval between doses, the route of administration, the fed/fasted state upon STZ administration, and the rat strain/vendor. There are great variations in the STZ treatments, which affect the level of \( \beta \)-cell depletion. Variations among these parameters also exist in studies working with the HFD/STZ rat model (Table 1).

The same STZ treatment has been applied to rats on different diet regimens, and thus rats with potentially different body compositions might also result in different phenotypes. Data supporting this concept are found in studies comparing lean STZ rats with HFD/STZ rats treated with the same amount of STZ. These rats do not show the same phenotype in respect to blood glucose levels. This might be related to the fact that STZ has been shown not to interact with lipids. Another possibility could be varying levels of glucose transporter 2, required for STZ entry into \( \beta \)-cells, in the two models. Another caveat to remember when treating HFD fed animals with STZ is that diabetes induced by STZ treatment can lead to increased insulin sensitivity when compared with controls. In contrast, type 2 diabetes in humans is characterized by insulin resistance. Finally, when discussing the effect of STZ treatment with respect to the HFD/STZ model, it should be stressed that the STZ treatment leads to a transition from an insulin-resistant state to a state of type 2 diabetes in a very fast and unnatural way. This means that the time aspect of the disease progression/transition is not mimicked ideally in this animal model. In summary, the design of the STZ treatment superimposed on the choice of the diet regimen will greatly affect the phenotype of the HFD/STZ rat model. No absolute agreement of the STZ treatment approach exists in the literature when it comes to modeling of type 2 diabetes in the HFD/STZ rat model, though some tendencies appear (Table 1).

Impact of Age in HFD/STZ Models When Deciding on the Type of Diabetes Model

Type 2 diabetes remains mainly a disease of older humans. Thus, another important factor in modeling the HFD/STZ diabetes rat model is age. The vast majority of HFD/STZ rats used in the literature are young rats (< 6 months; Table 1). The young age of the rats makes them a potential disease model for diabetes present in young human individuals. It is arguable that young HFD/STZ rats with a massive loss of functional \( \beta \)-cell mass mimic type 1 diabetes in children who are obese, but without the autoimmune component hallmarking of type 1 diabetes. In contrast, young HFD/STZ rats bearing a somewhat lower depletion of \( \beta \)-cell mass mimic obese children with type 2 diabetes. Notably, the prevalence of type 2 diabetes in young children and adolescents has increased with a rapid pace. Importantly, the pathogenesis of type 2 diabetes in young vs older individuals has indirectly been shown to be different from one another; where young type 2 diabetes patients have a tendency to be insulin deficient, the elderly have a tendency to be more insulin resistant. This fits with knowledge from genome-wide association studies pointing to genetic defects in \( \beta \)-cell function, and the general concept that earlier diagnosis would be associated with a greater genetic contribution. This point also favors the young HFD/STZ rats to be a model for type 2 diabetes in young individuals, as STZ treatment brings about insulin secretion deficiency rather than insulin resistance. It is important to mention that young rodents, like young people, have the capacity to increase \( \beta \)-cell mass. Older rodents (aged >1 year) and older people (aged >30 years) do not seem to have this capacity. This partly explains why it can be so challenging to administer the correct dose of STZ to induce the state of diabetes intended for the investigation. Collectively, these observations stress the importance of choosing the age of the rats when modeling type 2 diabetes in the HFD/STZ rat model.

Early vs Late Stage Type 2 Diabetes: STZ Treatment and \( \beta \)-Cell Functionality in High-Fat Fed Rats

So far, different ways of modeling type 2 diabetes, in the HFD/STZ rat model, have been discussed in the present review. However, another important question centers around whether the HFD/STZ rat mimics an early or late stage of type 2 diabetes. This is an important issue because of the principal metabolic differences present in subjects having had type 2 diabetes for either a shorter or longer period, including the level of remnant functional \( \beta \)-cell mass. This emphasizes the importance of characterizing the amount of, and more importantly, the function of the remaining \( \beta \)-cells and the level of insulin resistance when working with the HFD/STZ rats. This is not reported consistently in preclinical studies. In contrast, clinical studies have stressed the importance of dividing type 2
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CONCLUSIONS

In the present review, the metabolic profile of the different stages of type 2 diabetes progression in both humans and HFD/STZ rats are reviewed, and some similarities and differences are highlighted. The evolution of this model is reviewed in the context of efforts to more accurately model human type 2 diabetes. The specific variations in the dietary regimen, STZ treatment and age used in HFD/STZ rat models are discussed thoroughly. Finally, the importance of considering whether a specific HFD/STZ rat model mimics an early or late stage of human type 2 diabetes is considered. It is clear that more basic characterization needs to be carried out on this model, and this review has provided some guidance on how to proceed. Finally and most importantly, it is the opinion of this author that, despite its limitations and the wide variety of both the high-fat fed regimen and the STZ treatment, the HFD/STZ is a reasonable animal model of type 2 diabetes mainly representing the later stage of the disease depending on the amount of residual β-cell mass.

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