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Review

A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin

Sevda Gheibi[a,b], Khosrow Kashfi[c], Asghar Ghasemi[a,*]

[a] Endocrine Physiology Research Center, Research institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Neurophysiology Research Center and Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Department of Molecular, Cellular and Biomedical Sciences, Sophie Davis School of Biomedical Education, City University of New York School of Medicine, New York, USA

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ABSTRACT

Prevalence of diabetes, a serious public health problem is rapidly increasing worldwide. Type-2 diabetes is the common form of diabetes characterized by insulin resistance and abnormalities in insulin production. Despite the current development of therapeutic agents, there is no effective treatment without side effects; it is therefore necessary to find new prevention strategies and better treatments. For this purpose animal models of diabetes are appropriate tools, of which rodents due to the short generation time and economic considerations are the first choice. The aim of this review is to present features of a frequently used model of type-2 diabetes in rat, induced by a high fat diet and streptozotocin, taking into account its advantages/disadvantages and presenting a practical guide.

1. Introduction

Prevalence of diabetes is increasing and in addition to 415 million adults who have diabetes, 318 million also have impaired glucose tolerance; it is estimated that approximately 642 million people worldwide will have diabetes by the year 2040 [1].

Type-2 diabetes (T2D) which used to be referred to *adult-onset* or *non-insulin-dependent diabetes*, accounts for over 90–95% of all diabetes; T2D is a complex metabolic disorder essentially characterized by alterations in lipid metabolism, insulin resistance and pancreatic β-cell dysfunction [2]. Obesity is the most common risk factor for the development of T2D [3]; this may lead to elevated serum triglycerides, hypertension, and insulin resistance [4]. Unfortunately, currently there are no effective treatments available for T2D, although there have been many developments in the therapeutic arena [5]. Hence the urgent need for developing new preventative and/or therapeutic strategies to combat T2D.

Owing to the complexity of this disease, preclinical animal models that accurately reflect the pathogenesis of the human disease are essential [2]. Most animal models that are used for induction of diabetes make use of toxic chemicals, which target the pancreatic β-cells; for example, alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) (ALX) which was first used in 1943 (reviewed in [6]) generates free radicals that lead to fragmentation of the β-cells; however, ALX can cause kidney toxicity because it has a very narrow effective dose range [7], thus it is seldom used. The most common chemical used to induce diabetes is streptozotocin (STZ) which was first used in 1963; it can be used for induction of both type-1 and T2D [6]. High-doses of STZ severely impair insulin secretion, a feature that is similar to type-1 diabetes; moreover, this dose of STZ may also lead to ketone body formation and spillage into the urine, a characteristic of uncontrolled type-1 diabetes [8]. On the other hand, low-doses of STZ causes mild impairment in insulin secretion that is more closely resembled in the later stages of T2D [9,10]. However, the low dose STZ model does not address the insulin resistance axis typically seen in T2D [11]. Of note, some previous studies have indicated that animals that were overfed a

Abbreviations: AKT/PKB, AKT/protein kinase B; AS160, Akt substrate of 160 kDa; BAD, Bcl-2-associated death promoter; DAG, diacylglycerol; GLUT2, glucose transporter 2; GLUT4, glucose transporter 4; GSK3, glycogen synthase kinase 3; GRB2, growth factor receptor-bound protein 2; GTT, glucose tolerance test; HFD, high-fat diet; HOMA-IR, homeostasis model assessment of insulin resistance; IP, intraperitoneally; IP3, inositol (1,4,5)-triphosphate; IRS, insulin receptor substrates; ITT, insulin tolerance test; I.V, intravenously; JNK, c-Jun N-terminal Kinase; LFD, low-fat diet; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NPD, normal-pellet diet; PC1, prohormone convertase 1; PDK1, 3-phosphoinositide-dependent protein kinase 1; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PI3K, phosphatidylinositol-3 kinase; PKC, protein kinase C; PLCγ, phospholipase Cγ; PPAR α, peroxisome proliferator-activated receptor α; QUICKI, quantitative insulin-sensitivity check index; STZ, streptozotocin; Shc, src-homology 2 and collagen homology; SOS, son-of sevenless; T2D, Type-2 diabetes; TNF-α, tumor necrosis factor-α; VHFD, very high-fat diet

* Corresponding author at: Endocrine Physiology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, No. 24, Parvaneh Street, Velenjak, P.O. Box 19395-4763, Tehran, Iran.
E-mail address: Ghasemi@endocrine.ac.ir (A. Ghasemi).

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high-fat diet (HFD) developed insulin resistance [12–16]. Therefore, a model which incorporates a HFD to induce peripheral insulin resistance, followed by low dose STZ to target the pancreatic β-cells would closely mimic not only the phenotype but also the pathogenesis of human T2D [9,17].

The aim of this review is to present features of a frequently used model of T2D in the rat, induced by HFD and low dose STZ (HFD-STZ-T2D), considering its advantages and disadvantages and to provide a practical guide for those working in this field.

2. History of HFD-STZ-T2D

Introducing a suitable animal model of T2D for research purposes can be achieved by combining a HFD which produces insulin resistance and a low dose of STZ injection that causes initial β-cell dysfunction [11]. Feeding a HFD leads to development of hyperinsulinemia, obesity, and insulin resistance but not frank hyperglycemia or diabetes [18]; therefore, to induce diabetes it would be necessary to administer low dose STZ. This model was first reported by Reed et al., who showed that animals that were fed a HFD exhibited high blood insulin levels but essentially had normal blood glucose concentrations, a model which has the hallmark characteristics of insulin resistance in human T2D. They suggested that if STZ were administered after a HFD, the functional capacity of the pancreatic β-cells would be slightly reduced without completely compromising insulin secretion. In their model, animals received STZ (50 mg/kg) via the tail vein and 3 days later, blood glucose and lipid levels were measured and their response to anti-hyperglycemic drugs was then evaluated [9]. This model was modified by Srinivasan et al. who used a lower dose of STZ (35 mg/kg) and administered it intraperitoneally (IP) [10].

3. High-fat diet for HFD-STZ-T2D

Diet is the most important factor in any experimental animal nutrition, thus the mechanisms that are relevant of specific nutrients in creating an animal model need to be fully resolved and understood [19]. A normal-pellet diet (NPD) usually contains around 26% protein, 63% carbohydrate, and 11% fat [20]; the purified diet which is made of refined ingredients including isolated proteins, refined sugars and oils, including purified sources of vitamins and minerals, is widely used in most studies [21].

Although numerous high-fat rodent diets are available, most of them differ in both the level and source of fat. Generally, if 40%-60% of calories come from fat, this can lead to metabolic disorders, hypertension, obesity, and production of pro-inflammatory cytokines [18]. Levels of fat in the diet should be taken into consideration; while there are no strict definitions, a diet with 10% calories from fat is considered a low-fat diet (LFD), while 30–50% would constitute a HFD, and > 50%, would be considered a very high-fat diet (VHFD) [22]. To induce obesity and diabetes, both the HFD and VHFD are used. Table 1 shows the composition of the HFD and the NPD.

Table 1
Composition of the normal pellet and the high fat diets in some studies.

Normal pellet diet			High fat diet			Ref.
Carbohydrate	Fat	Protein	Carbohydrate	Fat	Protein	
70.0	10.0	20.0	35.0	45.0	20.0	[27]
60.0	12.0	28.0	42.0	38.0	20.0	[71]
65.8	10.3	24.2	59.8	20.1	20.1	[24]
60.0	10.0	22.0	50.0	37.0	13.0	[74]
62.0	12.0	26.0	42.0	40.0	18.0	[33]
70.8	5.9	23.3	17.0	58.0	25.0	[13]
66.0	12.0	22.0	5.0	60.0	35.0	[26]
60.0	12.0	28.0	41.0	40.0	18.0	[9]
72.1	5.7	22.0	27.5	58.0	14.5	[4]

It should be noted that different types of fats have different effects on glucose homeostasis and insulin sensitivity [23]. Animal fat [14,24], coconut oil [25,26], soybean oil [27], and other vegetable oils can be used for preparing a HFD. Whereas diets that contain high saturated fat such as lard, beef tallow, or coconut oil are more likely to induce obesity and diabetes in susceptible strains [28]; diets that contain polyunsaturated fatty acids such as fish oil and oils derived from variety of seeds have beneficial effects on insulin's action and overall body composition [29,30]. However, the role of monounsaturated fatty acids, such as olive oil remains to be determined [28]. It has been shown that animals fed diets with low saturated fat (less than 15% of the calories coming from saturated fat) such as omega-3 fatty acids, do not gain as much weight as those fed diets with high saturated fat [31] and are more insulin sensitive [28]. Although linoleic and linolenic are dietary essential fatty acids; other oils such as soybean [21,32], corn [33,34], sunflower [34], or safflower [35] maybe added to the fat source, as they can also provide the basic requirements for linoleic and linolenic fatty acids (Table 2).

While most obesity/diabetes researches use purified ingredient diets, some studies add the fat to a chow [36]. This obviously leads to an unbalanced diet composition and dilutes other nutrients like protein, vitamins, minerals, and fiber, which can lead to nutritional inadequacies [22,37]. In order to avoid reductions in the amount of protein, one can add casein, milk powder, and soybean to the normal diet [38]. Of these, casein can be a good option because its amino acid composition is sufficient, it is readily accessible, and its cost is lower compared to other sources [21]. One limitation is the low sulfur amino acids, particularly cystin/cysteine; to overcome this problem, DL-methionine can be used [4,21] which in vivo can be converted to cysteine thus preventing any potential deficiency of this amino acid [4,19,21]. Cornstarch and sucrose are the most important sources of carbohydrates, and amongst them, cornstarch is even better than sucrose [19,21], as a sucrose-rich diet can cause hyperlipidemia, hepatic lesions [39], and nephrocalcinosis [40].

4. Preparation of a HFD

The normal-pellet diet (Pars animal feed) that we used in our animal facilities contained 2% fat, 57% carbohydrate, 17.5% protein, 4.9% vitamin and mineral mix, 6.6% fiber, and 12% water, with a total caloric value of ~3160 kcal/kg (percent by weight). Since protein and carbohydrate both contain 4 kcal/g, and fat contains 9 kcal/g, the 57 grams of carbohydrate in our NPD provided 228 kcal, the 17.5 g of protein provided 70 kcal, and the 2 grams of fat provided 18 kcal. Hence each 100 grams of NPD produced 316 kcal (228 + 70 + 18), with 72.1% calories of which were derived from carbohydrates, 22% from protein, and 5.7% from fat (percent by calorie).

Preparations of HFD in the literature are quite variable. For example, ranges between 15 and 60% of calories from fat [38], 3–59.8% from carbohydrate [24,38], and 13–74% from protein [38] have been reported. For making a HFD which incorporated 58% of calories from fat, 27.5% of calories from carbohydrate, and 14.5% of calories from protein with a total caloric value of ~4900 kcal/kg, we used butter and chose casein as our source of protein [4,19]. To overcome the limitation of casein and preventing vitamin and mineral dilution; DL-methionine, vitamin (100×), and mineral (10×) mix were added (Tables 3 and 4). For this purpose, powdered NPD (1000 g), butter (531 g), casein (125 g), DL-methionine (3 g), vitamin mix (7 g), and mineral mix (42 g) were thoroughly mixed to produce 1708 g of HFD. Since each 1000 g of NPD contained 570 g of carbohydrate, 175 g of protein, and 20 g of fat, by adding the above compounds to this base, the final amounts of fat, protein, and carbohydrate reached 551 g, 300 g, and 570 g, respectively. To acquire the weight percent, if 1708 g of our HFD contain 551 g of fat, 300 g of protein, and 570 g of carbohydrate, then 100 g of this HFD would hence have 32.3 g of fat, 17.5 g of protein, and 33.3 g of carbohydrate. The total caloric value can be obtained by multiplying