

# Development of Type 1 and Type 2 Diabetes in Rats Using Streptozotocin and Nicotinamide/Streptozotocin

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## Abstract

Diabetes mellitus is a rapidly growing disease worldwide. Experimental animal models for diabetes mellitus are beneficial for understanding the complex nature of the disease. The purpose of this study was to develop type 1 and type 2 diabetes mellitus in rats using streptozotocin and nicotinamide/streptozotocin, respectively. Results of this study showed that type 1 diabetes was developed using streptozotocin, and type 2 diabetes using nicotinamide/ streptozotocin. These methods used for the development of type 1 and type 2 diabetes can be easily used in the diabetes-related studies.

**Keywords:** Type 1 diabetes, Type 2 diabetes, streptozotocin, nicotinamide, fasting blood glucose

## Özet

# Streptozotosin ve Nikotinamid/Streptozotosin Kullanılarak Sıçanlarda Tip 1 ve Tip 2 Diyabet Geliştirilmesi

Diyabet dünya çapında hızla büyüyen bir hastalıktır. Diyabet için geliştirilen deneysel hayvan modelleri hastalığın karmaşık yapısını anlamak için faydalıdır. Bu çalışmanın amacı, sırasıyla streptozotosin ve nikotinamid / streptozotosin kullanarak sıçanlarda tip 1 ve tip 2 diyabet geliştirmektir. Bu çalışmanın sonuçları streptozotosin kullanılarak tip 1 diyabet, ve nikotinamid / streptozotosin kullanarak tip 2 diyabet geliştirildiğini göstermiştir. Tip 1 ve tip 2 diyabet geliştirmek için kullanılan bu yöntemler diyabet ile ilgili çalışmalar da kolaylıkla kullanılabilir.

**Anahtar kelimeler:** Tip 1 diyabet, Tip 2 diyabet, streptozotosin, nikotinamid, açlık kan şekeri

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## 1. Introduction

Diabetes (Diabetes Mellitus) is a rapidly growing disease in the world due to increase in the world population, aging, urbanization and increase in the frequency of obesity and physical inactivity [1]. All over the world, there were 387 million diabetic patients in 2014, and this number is expected to reach 592 million in 2035. In Turkey, prevalence of diabetes was reported as 7.2 % (8.0% in women and 6.2% in men) in 2002 [2]. According to Social Security Administration data, number of diabetic patients in Turkey was about 2.5 million in 2013. Type 2 diabetes was responsible for more than 4.9 million deaths in 2014, and this number is increasing steadily [3]. Diabetes ranks fifth among the diseases causing death in the world. The money spent for the diagnosis, treatment and complications of diabetes is also a big burden for the world economy. 3-12% of total health care spending constitutes diabetes expenditures in several countries [4]. Because of all these, the studies on diabetes have been increased dramatically all over the world.

Diabetes is a chronic complex metabolic disease, which is associated with high blood glucose levels resulting from defects in the body's ability to produce and/or use insulin [5], and it is characterized by failure in carbohydrate, fat and protein metabolism. Weight loss, fatigue, polyuria, polydipsia, polyphagia, ketoacidosis, pruritus are the typical symptoms of diabetes [6]. Acute (e.g. hypoglycemia, hyperglycemia, lactic acidosis, bacterial and fungal infections) and chronic (e.g. retinopathy, neuropathy, nephropathy, cardiovascular complications and chronic ulcers) complications are also seen as a result of decreased insulin secretion and metabolic failure. Diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves. So, it may adversely affect the quality of life. Fasting plasma glucose level is usually used for diagnosis of diabetes. In addition, oral glucose tolerance test, glycosylated hemoglobin test (hemoglobin A1c), glucose urine test, breath acetone measurements might also be used for diagnosis of diabetes [7].

Diabetes was classified into four classes (type 1, type 2, other specific types and gestational diabetes mellitus) by American Diabetes Association in 1997, and is still in use [8]. In type 1 diabetes (insulin dependent diabetes or juvenile diabetes), the body does not produce enough insulin due to failure of pancreas. Approximately 5-10% of all individuals with diabetes are type 1 diabetic patients and the disease starts before the age of 30 in 75% of type 1 diabetic patients [9]. Type 2 diabetes (non-insulin dependent diabetes or adult diabetes), is characterized by a high glucose level in the context of insulin resistance and relative lack of insulin [10]. It is usually seen after the age of 40, and approximately 90-95% of diabetic patients have type 2 diabetes. Gestational diabetes develops in pregnancy and indicates similar symptoms with type 2 diabetes [11]. After delivery, blood glucose usually return to normal levels. However, these patients are high risk group for diabetes [12]. Other specific types of diabetes are rare and include genetic defects of  $\beta$ -cell function, genetic defects in insulin action, diseases of the exocrine pancreas, endocrinopathies, drug or chemical induced infections, uncommon forms of immune-mediated diabetes, other genetic syndromes (e.g. Down syndrome, Klinefelter syndrome, Turner syndrome, Wolfram's syndrome) sometimes associated with diabetes [13].

Different approaches such as chemical [14], surgical [15], spontaneous [16], viral [17], transgenic [18] models are used to develop experimental diabetes in animals. Although diabetic animal models may not display the full symptoms of diabetic patients, these are the utility models

for identification of the development, progression and pathogenesis of diabetes and characterize to antidiabetic agents.

The purpose of this study was to develop experimental diabetes mellitus in male Sprague Dawley rats using streptozotocin and nicotinamide/streptozotocin. Variation in body weights, fasting glucose levels were used for diagnosis of diabetes, and histopathological examination was performed to determine the type of diabetes developed.

## **2. Material and Methods**

### **Materials**

Nicotinamide and streptozotocin were purchased from Sigma-Aldrich (Germany). Bayer Contour TS Blood Glucose Meter (Germany) was used for measurement of blood glucose levels.

### **Methods**

All animal experiments were conducted using protocols approved by the Animal Experimentations Local Ethics Board of Hacettepe University, Turkey (ethical committee approval number :2011/33-4). Sprague Dawley male rats (275-365 g) were maintained in a conventional housing with unrestricted access to food and water. However, rats were fasted the night before the measurement of blood glucose level with free access to water. Nine Sprague-Dawley male rats were randomly divided into three groups namely control (C), streptozotocin (S) and nicotinamide/streptozotocin (N/S) groups. Only sterile 0.1 M cold citrate buffer was injected to the control group intraperitoneally. Streptozotocin dissolved in 0.1 M cold citrate buffer (sterile, 65 mg/kg) was injected to rats intraperitoneally for streptozotocin group. For nicotinamide group, nicotinamide dissolved in the physiological saline (sterile) was injected to rats intraperitoneally at a dose of 110 mg/kg, and 15 minutes later, streptozotocin dissolved in 0.1 M cold citrate buffer (sterile, 65 mg/kg) was administered to rats. All solutions, which were administered to rats, were sterilized by filtration through filters with a diameter of 0.22  $\mu\text{m}$  in a laminar flow cabinet. Six hours after injection, 10 % glucose solution was given to all groups for the next 24 hours to prevent hypoglycemia. Three and 7 days after the application of chemical agents, blood samples were taken from tail vein of the rats, and blood glucose levels were measured by a glucometer (BayerContour TS, Germany). To determine the type of diabetes, animals were euthanized one week after the injection of chemical agents, their pancreases were removed, and then fixed in 10 % buffered formaldehyde solution (4 g monobasic sodium phosphate, 6.5 g dibasic sodium phosphate, 100 ml formaldehyde, 900 ml distilled water) before histopathological examination. Hematoxylin and eosin stained slides were examined for islet number and morphology. In addition, the body weights of rats were measured before and one week after the injections.

## **3. Results and Discussion**

Various methods are available in the literature for development of experimental diabetes. These methods include surgical, transgenic, spontaneous, viral, hormone induced, insulin deficiency due to insulin antibodies, diet induced metabolic dysregulation and chemical methods. In

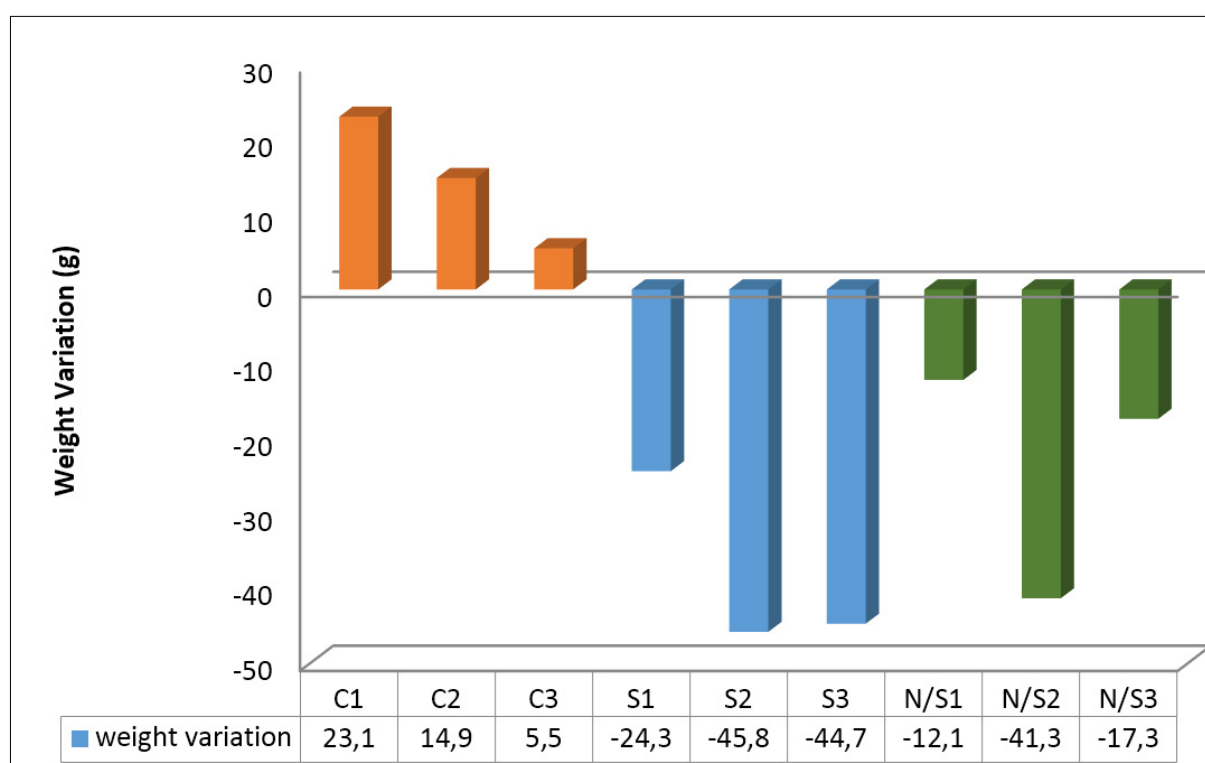
surgical diabetes, partial or full pancreatectomy is performed. Mortality rate is higher than the other experimental diabetes methods, therefore, surgical method was not preferred in our study [19]. Genetically modified transgenic animals have hyperglycemia due to expressing transgenes. Therefore, they are very expensive for regular experiments for diabetes [20]. On the other hand, development of spontaneous diabetes using Bio Breeding rats, obese diabetic mice, non-obese diabetic mice takes about three months as the animal (rats or mice) grows diabetes develops [21]. Viruses (e.g. coxsackie B virus, rubella virus, mumps virus, cytomegalovirus, Epstein-Barr virus and Varicella Zoster virus) can affect beta cells or stimulate to autoimmune destruction of pancreatic beta cells [22]. Viral diabetes development method is not used due to the complexity of the method [19]. Hormone induced diabetes is developed by treating the animals with corticosteroid or growth hormone [23]. This method is also expensive than the chemically induced method, and may affect the other organs. Anti-insulin serum can cause a diabetic syndrome due to neutralization of endogenous insulin by insulin antibodies resulting insulin deficiency [23]. In diet induced metabolic dysregulation method, animals are induced with high sugar, high fat diet after 12 hour fasting. After 8 weeks, body fat weight, percentage of HbA1c and triglyceride concentration are increased [24]. Chemically induced diabetes methods have many advantages such as easy implementation, low cost, a short time such as one week for development diabetes in animals. Therefore, in this study, streptozotocin and nicotinamide/streptozotocin methods were preferred.

Chemical diabetes is the most preferred method among all these methods. Alloxan (40-80 mg/kg) and streptozotocin (25-80 mg/kg) are the most used chemicals to develop diabetic animal (e.g. rats, mice, hamsters, dogs, sheep and monkeys) models [20, 24]. Streptozotocin is a narrow-spectrum antibiotic with diabetogenic features and has toxic effects on beta cells of the islets of Langerhans in the pancreas. It has also genotoxic effect [25]. Usually, single dose of streptozotocin (40-65 mg/kg, intraperitoneal) is used to develop diabetes. This dose can be increased or can be applied several times at lower doses. However, it is not effective below 40 mg/kg. It was reported that after administration of streptozotocin, structural changes occur in the pancreatic beta cells within 48 hours, and continue up to 4 months. Experimental diabetes develops in several stages after administration of streptozotocin: hyperglycemia occurs at the first stage due to release of excess glucose because of sudden destruction of glycogen from the liver. At the next stage, hypoglycemia occurs. Hypoglycemia is thought to be caused by suppression of glucose release from tissues into the blood, increased use of glucose in tissues, and insulin release especially during the destruction of beta cells. Finally, permanent hyperglycemic phase occurs. At this stage, insulin level is reduced proportionally to the dose of the chemical agent and increased blood sugar. Conflicting results are available in the literature regarding the type of diabetes developed using streptozotocin. Type 1 diabetes was reported in some studies [26], whereas type 2 diabetes was reported in others [27-30]. Cytotoxic effect of streptozotocin in the pancreatic beta cells can be prevented by nicotinamide [31].

Although the body weights of rats were increased in the control group, there was a decrease in the body weights both in the streptozotocin and nicotinamide/ streptozotocin groups (Table 1 and Figure 1). Akbarzadeh et al. induced diabetes by streptozotocin in rats. They compared average of body weight of normal and diabetic adult rats, and found that body weight of diabetic rats decreased while normal rats increased [32]. Similarly, in another studies, a significant weight loss was observed at diabetic rats which were induced by streptozotocin or streptozotocin and nicotinamide [33].

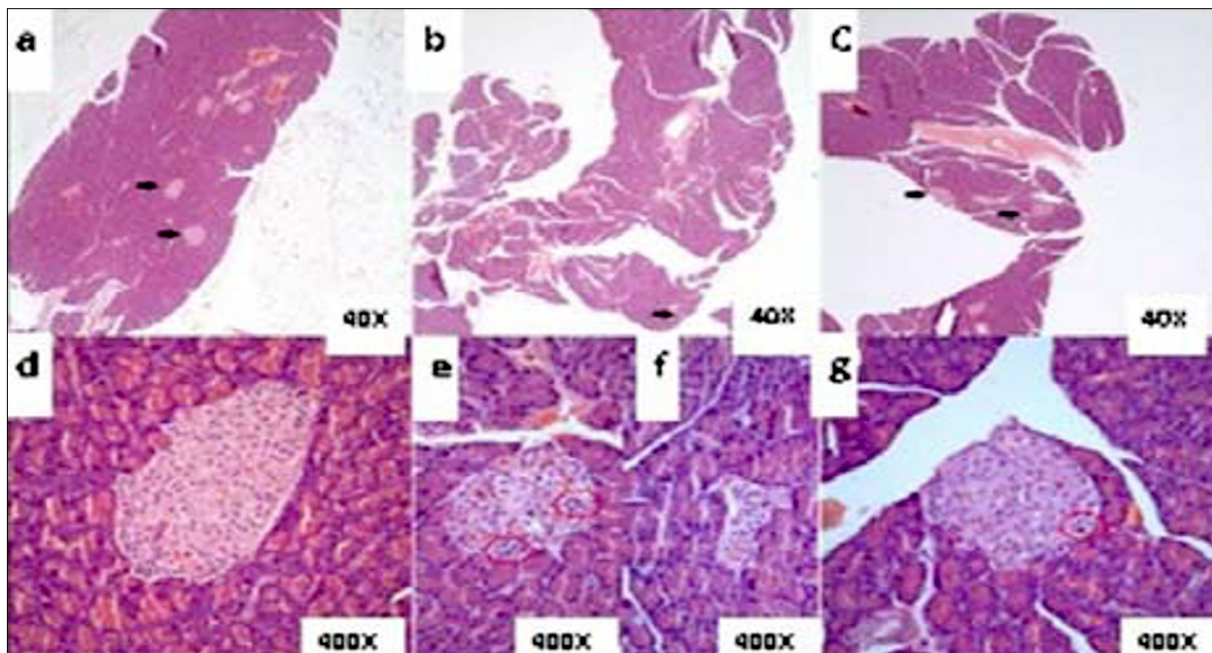
**Table 1.** Body weights and weight variation of rats in control and diabetic groups.

<i>Codes</i>	<i>Body weights (g)</i>	<i>Body weights 1 week after (g)</i>	<i>Weight variation (g)</i>
C1	279.9	303.0	+23.10
C2	333.1	348.0	+14.90
C3	365.5	371.0	+5.50
S1	335.4	311.1	-24.30
S2	314.3	268.5	-45.80
S3	320.2	275.5	-44.70
N/S1	354.9	342.8	-12.10
N/S2	335.1	293.8	-41.30
N/S3	347.7	330.4	-17.30

**Figure 1.** Weight variation of control (C), streptozotocin (S) and nicotinamide/streptozotocin (N/S) administered rats.

In general, rats are accepted to be hyperglycemic and diabetic when fasting glucose levels are more than 200 mg/dL [34]. Although the blood glucose level was less than 200 mg/dL in control group, it was higher than 200 mg/dL in both diabetic groups, indicating that experimental diabetes was developed by injecting nicotinamide/streptozotocin and streptozotocin. These values were consistent with the literature [34-37]. However, measurement blood glucose levels are not enough to determine the type of diabetes. Histopathological examination of pancreas demonstrated that distribution of the islets of Langerhans cells in normal pancreas tissue is 7-8 islets per pancreatic lobule. Compared to control group, the number of islets was reduced significantly in the streptozotocin group (0-1 islets/lobule). However, 4- 5 islets per pancreatic lobule were observed in the nicotinamide/streptozotocin group (Figure 2) [38]. These results indicate

that islets are missing in streptozotocin group which type 1 diabetes develops whereas islets are preserved in nicotinamide/streptozotocin group which type 2 diabetes develops. Detailed examination of the neuroendocrine cells within Langerhans islets demonstrated that there was a decrease in the size of islets because of both shrinkage and diminished number of islet cells in streptozotocin group compared to control group. Morphological changes of injury, such as apparent nucleoli, hyperchromasia and pyknosis were observed in streptozotocin group. In the case of nicotinamide/streptozotocin group, shrinkage of cytoplasm was minimal indicating that islet volume was protected, although there were apparent nucleoli. Pyknotic cells were also observed in nicotinamide/streptozotocin group but number of these cells was quite few compared to streptozotocin group [39]. Results of the histopathological examination clearly indicated that type 1 diabetes was developed in the streptozotocin group, and type 2 diabetes in the nicotinamide/streptozotocin group.



**Figure 2.** Microscopy of Langerhans islets in pancreatic tissue of control group (a, 40x), streptozotocin group (b, 40x), nicotinamide/streptozotocin group (c, 40x), control group (d, 400x), streptozotocin group (e-cytoplasm shrinkage, nuclear hyperchromasia and pyknosis (ellipse), prominence of nucleoli), f (decreasing volume of islets), 400x), nicotinamide/ streptozotocin group (g, 400x) rats.

#### 4. Conclusions

Results of the histopathological examination and other results were clearly indicated that type 1 diabetes was developed in the streptozotocin group, and type 2 diabetes in nicotinamide/streptozotocin group. Although streptozotocin causes irreversible loss of beta cells of the pancreas, nicotinamide prevents death of the beta cells. The methods used for the development of type 1 (streptozotocin) and type 2 (nicotinamide/ streptozotocin) diabetes are simple and not-time consuming; therefore, they can be easily used in the diabetes-related studies.

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