VARIABILITY OF THE STREPTOZOTOCIN–NICOTINAMIDE MODEL OF EXPERIMENTAL DIABETES IN YOUNG SPRAGUE–DAWLEY RATS: A PRELIMINARY VALIDATION STUDY

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PRELIMINARY VALIDATION OF A STREPTOZOTOCIN–NICOTINAMIDE PROTOCOL FOR EXPERIMENTAL DIABETES IN YOUNG SPRAGUE–DAWLEY RATS (Abstract): The streptozotocin–nicotinamide (STZ–NIA) model is widely used to induce experimental type 2 diabetes mellitus (T2DM), but its metabolic outcome may vary according to the induction protocol. This study evaluated the response to an initial and a modified STZ–NIA protocol in young Sprague–Dawley rats, with particular emphasis on blood glucose and body weight. **Materials and methods:** Rats were allocated to control and STZ–NIA groups. The initial protocol consisted of NIA pretreatment followed by STZ administration. As persistent hyperglycemia was not achieved, the protocol was modified by reducing NIA exposure and increasing the STZ dose. Blood glucose was measured longitudinally, and body weight was monitored throughout the experimental period. Parametric and non-parametric statistical analyses were performed. **Results:** The initial protocol did not induce persistent hyperglycemia. Following protocol modification, the STZ–NIA group showed significant temporal variation in blood glucose, with the most pronounced increase occurring after the second induction phase. However, this response was transient, with glucose levels subsequently returning toward baseline. Glycemia remained comparatively stable in control animals. Body weight increased in both groups throughout the study, with no significant difference in absolute or relative weight gain between groups. **Conclusions:** The modified STZ–NIA protocol produced a transient metabolic response but did not establish a stable diabetic phenotype. These findings are consistent with an important role of the balance between STZ-induced β-cell injury and NIA-mediated protection in determining the metabolic response. Longitudinal glucose monitoring and further optimization of the induction protocol, together with direct assessment of pancreatic β-cell integrity and insulin-related parameters, are warranted to establish a reproducible model for subsequent pharmacological studies. **Keywords:** STREPTOZOTOCIN, NICOTINAMIDE, EXPERIMENTAL DIABETES, SPRAGUE–DAWLEY RATS, DIABETES MODEL VALIDATION.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major chronic metabolic disorder characterized by a complex pathophysiology involving insulin resistance, impaired pancreatic β-cell function, and, with disease progression, a decline in functional β-cell mass (1). According to the International Diabetes Federation, more than 90% of people with diabetes are affected by T2DM, underscor-

ing the need for appropriate experimental models to investigate disease mechanisms and evaluate potential therapeutic strategies (1, 2). Given the heterogeneous and multifactorial nature of human T2DM, no experimental model can fully reproduce all features of the human disease. Nevertheless, animal models, particularly rodent models, remain essential for investigating the metabolic, structural, and functional alterations associated with diabetes and for evaluating pharmacological interventions (3-5).

Experimental models of T2DM include genetic or spontaneous models, as well as models induced through pharmacological, nutritional, or surgical interventions. Pharmacologically induced models offer several practical advantages, including relatively short induction periods, low cost, and greater control over the onset and severity of metabolic disturbances (2, 3). Among these approaches, STZ, administered either alone or in combination with other interventions, is one of the most widely used methods for inducing experimental diabetes (6, 7). However, the metabolic phenotype induced by STZ is highly dependent on experimental conditions, including dose, route of administration, age and sex of the animals, and strain-specific susceptibility (6, 8, 9). Consequently, careful optimization of the induction protocol is particularly important when the resulting model is intended for subsequent pharmacological studies.

STZ is a cytotoxic compound with a pronounced tropism for pancreatic β-cells. Following cellular uptake, predominantly through the glucose transporter GLUT2, STZ induces DNA damage and promotes excessive activation of poly(ADP-ribose) polymerase-1 (PARP-1), leading to depletion of intracellular NAD+ and ATP and, ultimately, impairment of β-cell viability (10). The diabetogenic properties and relative pancreatic β-cell selectivity of STZ have provided the basis for the development of numerous experimental models of diabetes (11). Depending on the dose and experimental conditions, STZ can produce a broad spectrum of β-cell injury and metabolic disturbances, ranging from marked insulin deficiency to more moderate impairment of β-cell function.

To obtain a metabolic phenotype incorporating selected features of T2DM while limiting the severity of STZ-induced β-cell destruction, the STZ–NIA model was developed. In this approach, NIA is administered before STZ with the aim of attenuating STZ-induced β-cell toxicity (12). By reducing the extent of β-cell injury, NIA may allow the development of a more moderate metabolic phenotype than that produced by STZ alone, while retaining sufficient β-cell dysfunction to promote hyperglycemia (13). The severity and persistence of the resulting phenotype are influenced by several methodological factors, particularly the doses of NIA and STZ, the interval between their administration, the route of administration, and the characteristics of the experimental animals (8,14).

The protective effects of NIA have been associated with mechanisms including inhibition of PARP-1 activation and preservation of intracellular NAD+ availability, thereby limiting the energy depletion associated with STZ-induced DNA damage (15). Thus, the metabolic outcome of STZ–NIA administration may be regarded as the result of a balance between STZ-mediated β-cell injury and NIA-mediated cytoprotection (12). If β-cell injury is excessive, profound insulin deficiency and severe hyperglycemia may develop; conversely, if NIA-mediated protection is too pronounced, β-

cell impairment may be insufficient to produce persistent hyperglycemia (6,16). The relative contribution of these opposing effects is therefore critical for establishing a reproducible intermediate phenotype.

An important limitation of the STZ–NIA approach is the variability reported among experimental protocols. Ghasemi and Jeddi (6) emphasized that the severity of diabetes induced by STZ–NIA depends substantially on the doses of both compounds and on the experimental conditions. In addition, a methodological review identified considerable variation in published protocols, with NIA doses ranging approximately from 60 to 290 mg/kg and STZ doses from 45 to 65 mg/kg, while the interval between administration of the two compounds was commonly around 15 minutes (11). Such methodological heterogeneity may contribute to differences in the magnitude and persistence of hyperglycemia across studies. This issue is particularly relevant when the model is intended for pharmacological investigations, because an unstable metabolic phenotype may increase experimental variability and complicate the interpretation of treatment-related effects.

The characteristics of the experimental animals may further contribute to differences in response. In Sprague–Dawley rats of different ages, the response to STZ was shown to vary with age, indicating that susceptibility to both the diabetogenic and toxic effects of STZ may change during development (9,17). More broadly, reviews of experimental T2DM models have emphasized the influence of animal age, sex, and strain on susceptibility to STZ, with Sprague–Dawley and Wistar rats among the strains commonly used in this context (6, 7, 18). Therefore, the successful induction of a diabetic phenotype using a particular STZ–NIA protocol in one experimental population does not necessarily ensure comparable results in animals of a different age, sex, strain, or colony. This consideration supports the need for protocol validation under the specific experimental conditions in which the model is intended to be subsequently used.

In addition to methodological variability, the STZ–NIA model has an important conceptual limitation with regard to its representation of human T2DM. Although the model can reproduce moderate hyperglycemia and impaired β-cell function, it does not fully reproduce the insulin resistance component that characterizes human T2DM (13, 14). Accordingly, the STZ–NIA model has been described as predominantly insulinopenic rather than as a complete representation of the pathophysiology of T2DM (11). By comparison, models combining high-fat feeding with low-dose STZ administration may more closely reproduce the coexistence of insulin resistance and β-cell dysfunction observed in selected stages of human T2DM (7,12). Similarly, other studies have emphasized the value of combining a high-fat diet with STZ administration when the objective is to reproduce selected metabolic characteristics of human T2DM more closely (19). Thus, the choice of experimental model should be guided by the specific metabolic features required for the intended investigation.

Within this context, validation of the STZ–NIA protocol under the specific experimental conditions of a given study is an important methodological step before commencing subsequent pharmacological investigations. In particular, establishing persistent rather than transient hyperglycemia is essential for defining whether a

stable diabetic phenotype has been achieved. Failure to establish such a phenotype may compromise the interpretation of subsequent treatment effects, as temporal fluctuations in glucose levels may be difficult to distinguish from genuine pharmacological responses. Longitudinal assessment of blood glucose is therefore particularly valuable, as it allows the temporal profile of the metabolic response to be characterized and helps distinguish an initial, transient response from sustained hyperglycemia. Monitoring body weight in parallel provides additional information regarding the general metabolic and physiological response to the induction procedure.

The **aim** of the present study was to evaluate the reproducibility of an STZ–NIA protocol for inducing a persistent diabetic phenotype in young Sprague–Dawley rats and to characterize the metabolic response following sequential modification of the induction regimen. Specifically, blood glucose was monitored longitudinally, together with body weight, following an initial STZ–NIA protocol and its subsequent modification after persistent hyperglycemia was not achieved. The study was designed as a preliminary validation stage to determine whether the modified induction regimen could establish a stable and reproducible diabetic phenotype suitable for subsequent experimental and pharmacological investigations.

MATERIALS AND METHODS

All experimental procedures were conducted at the CEMEX – Ostin C. Mungiu Advanced Research and Development Center for Experimental Medicine, within the Grigore T. Popa University of Medicine and Pharmacy, Iași, Romania. The animals were maintained under standardized laboratory conditions, with environmental parameters and husbandry practices controlled throughout the experimental period.

Animals

Male Sprague–Dawley rats weighing 220–260g were included in the study. The animals were healthy, genetically unmodified, and obtained from the Cantacuzino National Military-Medical Institute for Research and Development, Băneasa Facility, Bucharest, Romania.

Reagents and substances

The chemicals used in the experimental procedures were obtained from Sigma-Aldrich (Steinheim, Germany), including streptozotocin (STZ; approximately 75% β-anomer, ≥98% purity by HPLC, molecular weight 265.22; cat. no. S0130), citrate buffer (0.09 M; cat. no. C2488), and distilled water (molecular weight 18.02; cat. no. 07-6061). Niacinamide was obtained from DSM Nutritional Products Ltd. (Sisseln, Switzerland).

Experimental protocol

The study included two experimental groups, each comprising six animals: Group 1 (Control), consisting of non-diabetic animals receiving physiological saline, and Group 2 (STZ–NIA), consisting of animals subjected to pharmacological induction with NIA followed by STZ. The STZ–NIA induction procedure was performed sequentially in two phases using the same animals. The initial induction protocol was administered first. Following assessment of the glycemic response, the induction regimen was subsequently modified and reapplied on day 7 after the first STZ administration.

First induction phase. Following a 12-hour fasting period, animals in the STZ–

NIA group received NIA at a dose of 120 mg/kg by intraperitoneal (i.p.) administration. Fifteen minutes later, STZ was administered i.p. at a dose of 50 mg/kg. Control animals underwent the corresponding experimental procedures and received physiological saline instead of NIA and STZ. Blood glucose concentrations were assessed at 72 hours and 7 days after the first STZ administration. Thus, the first induction regimen consisted of: 12-hour fasting → NIA 120 mg/kg i.p. → 15-minute interval → STZ 50 mg/kg i.p. → glucose assessment at 72 hours and day 7.

Second induction phase was performed in the same animals immediately after the blood glucose assessment on day 7 following the first STZ administration. The induction regimen was modified by reducing the NIA dose and increasing the STZ dose. Following a 12-hour fasting period, NIA was administered i.p. at a dose of 100 mg/kg, followed 15 minutes later by STZ at a dose of 55 mg/kg i.p. Blood glucose concentrations were assessed at 72 hours and 7 days after the second STZ administration. Thus, the modified induction regimen consisted of: day 7 after the first STZ administration → 12-hour fasting → NIA 100 mg/kg i.p. → 15-minute interval → STZ 55 mg/kg i.p. → glucose assessment at 72 hours and day 7 after the second STZ administration. The modification of the induction regimen was intended to alter the balance between NIA-mediated β-cell protection and STZ-induced β-cell injury. Specifically, the NIA dose was reduced from 120 to 100 mg/kg, whereas the STZ dose was increased from 50 to 55 mg/kg.

Blood glucose concentrations were determined from capillary blood obtained by puncture of the distal portion of the tail after a 12-hour fasting period, using an Accu-Chek Active glucometer (Roche, Basel, Belgium). Measurements were obtained at baseline and at predetermined post-induction time points. Following the first induction, glucose concentrations were assessed at 72 hours and 7 days after STZ administration. Following the second induction, glucose concentrations were assessed at 72 hours and 7 days after the second STZ administration. For experimental model validation, a blood glucose concentration of 180–250 mg/dL was defined as the target range for the intended diabetic phenotype. However, a single elevated glucose measurement was not considered sufficient to confirm successful induction. Establishment of a stable diabetic phenotype required persistence of hyperglycemia during follow-up.

Body weight was recorded at baseline, before initiation of the experimental procedures, and at the end of the experimental period. Absolute and relative changes in body weight were calculated to assess changes in body mass during the study.

Statistical analysis

Data were processed and statistically analyzed using IBM SPSS Statistics for Windows, version 24. Continuous variables were expressed as mean ± standard deviation (SD). Statistical tests were selected according to the experimental design and distributional characteristics of the data.

Changes in body weight from baseline to the end of the experimental period were assessed using the paired *Student's t*-test. Comparisons between the control and STZ–NIA groups were performed using the independent-samples *Student's t*-test when the assumptions for parametric analysis were satisfied. Non-parametric alternatives were used when these assumptions were

not fulfilled.

Longitudinal changes in blood glucose concentrations were assessed using repeated -measures analysis. Repeated-measures ANOVA was used for parametric analysis, while the Friedman test was used as a non-parametric alternative. Between-group differences at individual experimental time points were assessed using the independent-samples *Student's t*-test. The overall longitudinal effects of time and experimental group, as well as their interaction, were evaluated using two-way repeated-measures analysis. All statistical tests were two-sided, and a p-value<0.05 was considered statistically significant.

Ethical aspects

The experimental procedures received prior approval from the University Ethics Committee (Ethical Approval No. 675/12.11.2025) and were conducted under the authorization issued by the Veterinary Sanitary Authority (Authorization No. 83/16.12.2025). All procedures involving laboratory animals were performed in accordance with applicable ethical principles and regulations governing the use of animals for scientific research. The study was designed and conducted in compliance with relevant national and international requirements, including Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Rationale for the sequential modification of the induction protocol

The induction regimen was modified because the initial NIA–STZ protocol did not result in persistent hyperglycemia. Importantly, the second induction protocol was applied to the same animals rather than to an independent experimental group. This sequential design allowed the glycemic response of the same animals to be evaluated following two different combinations of NIA and STZ doses.

The initial protocol consisted of NIA at 120 mg/kg followed by STZ at 50 mg/kg, whereas the modified protocol consisted of NIA at 100 mg/kg followed by STZ at 55 mg/kg. The modification was based on the hypothesis that the extent of β-cell injury and the resulting glycemic response depend, at least in part, on the balance between STZ-induced cytotoxicity and NIA-mediated β-cell protection. Accordingly, reducing the NIA dose was intended to decrease the protective effect on β-cells, while increasing the STZ dose was intended to enhance the diabetogenic stimulus.

Definition of experimental model establishment

Successful establishment of the experimental diabetic phenotype was defined a priori by the presence of hyperglycemia within the predefined target range of 180–250 mg/dL together with persistence of the elevated blood glucose concentration during follow-up. Accordingly, blood glucose measurements obtained at 72 hours were interpreted in conjunction with measurements obtained 7 days after STZ administration. An isolated elevation in blood glucose at a single time point was not considered sufficient to establish a stable experimental diabetic phenotype. Persistent elevation during the subsequent follow-up period was considered necessary for classification of the induction protocol as successful.

The predefined criterion was applied to both the initial and modified induction protocols. The glycemic response following each induction phase was subsequently

evaluated according to the magnitude and temporal persistence of the observed changes.

RESULTS

Body weight increased progressively from baseline to the end of the experimental period in both groups. In the control group, mean body weight increased from 237.0 ± 6.81 g to 297.5 ± 11.73 g, corresponding to an absolute increase of 60.50 ± 8.24 g (25.53 ± 3.36%) (fig. 1).

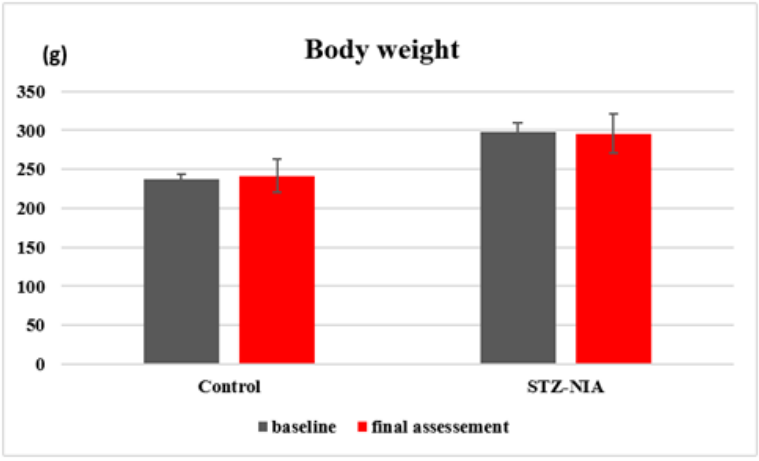

Fig. 1. Variation in the body weight during the experiment. The values are presented as mean± S.D. (n=6).

A similar increase was observed in the STZ–NIA group, from 238.50 ± 21.53 g to 295.83 ± 25.45 g, corresponding to an absolute increase of 57.33 ± 16.48 g (24.25 ± 7.50%). The increase in body weight from baseline was statistically significant in both groups (paired *t*-test, $p < 0.001$), and all animals showed an increase in body weight during the study (fig. 1).

Baseline body weight was comparable between the control and STZ–NIA groups (237.0 ± 6.81 vs. 238.50 ± 21.53 g, respectively; $p = 0.876$). No significant between-group difference was observed at the end of the experimental period (297.50 ± 11.73 vs. 295.83 ± 25.45 g; $p = 0.888$). Similarly, neither absolute weight gain (60.50 ± 8.24 vs. 57.33 ± 16.48 g; $p = 0.686$) nor relative weight gain (25.53 ± 3.36 vs. 24.25 ± 7.50%; $p = 0.716$) differed significantly between the control and STZ–NIA groups (fig. 1). Both groups exhibited a comparable increase in body weight throughout the experimental period, with no evidence of impaired weight gain in the STZ–NIA group compared with controls (fig. 1).

Blood glucose concentrations were assessed at five experimental time points in the control and STZ–NIA groups (n = 6 per group). Glucose levels remained relatively stable over time in the control group, with no significant temporal variation (repeated-measures ANOVA, $F(4,20) = 1.512$, $p = 0.237$) (fig. 2).

In contrast, the STZ–NIA group showed a significant temporal variation in blood glucose concentrations (repeated-measures ANOVA, $F(4,20) = 4.274$, $p = 0.012$), which was also supported by the Friedman test ($\chi^2(4) = 11.664$, $p = 0.020$). The most

pronounced increase was observed 72 hours after the second induction phase, when blood glucose was significantly higher in the STZ–NIA group than in the control group (109.8 ± 8.1 vs. 97.8 ± 9.8 mg/dL, respectively; $p = 0.049$). However, this difference was not maintained at the subsequent assessment, with glucose concentrations returning toward baseline values in the STZ–NIA group.

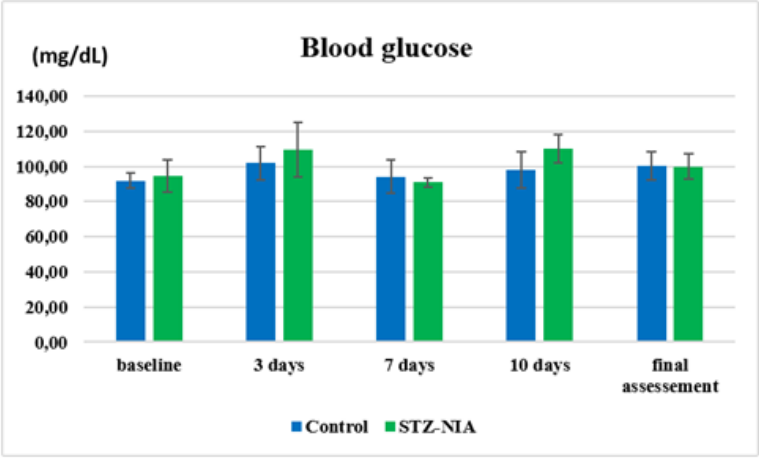

Fig. 2. Variation in blood glucose level during the experiment.
The values are presented as mean ± standard deviation (SD) of mean (n=6).

Across the remaining experimental time points, no significant between-group differences were observed. The longitudinal analysis revealed a significant effect of time ($F(4,40) = 5.011$, $p = 0.002$), whereas the main effect of group ($F(1,10) = 3.261$, $p = 0.101$) and the interaction effect ($F(4,40) = 1.279$, $p = 0.294$) did not reach statistical significance. The modified STZ–NIA protocol produced a significant but transient alteration in blood glucose, without establishing sustained hyperglycemia or a stable diabetic phenotype.

DISCUSSION

The present findings highlight the sensitivity of the STZ–NIA model to the specific conditions used for diabetes induction. The initial protocol did not produce persistent hyperglycemia, suggesting that the degree of β-cell impairment achieved under these conditions was insufficient to induce sustained disruption of glucose homeostasis. This finding may be related to the protective effect of NIA, which represents an important component of the STZ–NIA model and is intended to attenuate STZ-induced β-cell injury. Masiello *et al.* demonstrated that the metabolic phenotype produced by STZ–NIA depends strongly on the balance between STZ-induced β-cell damage and NIA-mediated protection (20). Similarly, Szkudelski emphasized that variations in the doses of both compounds can substantially influence the severity of the resulting diabetic phenotype (21).

Following modification of the protocol by reducing the NIA dose and increasing the STZ dose, a transient elevation in blood glucose was observed after the second induction phase. This response indicates that the modified regimen produced a

greater metabolic effect than the initial protocol, although the response was insufficient to establish sustained hyperglycemia. The subsequent decrease in glucose concentrations suggests that the induced metabolic disturbance was transient rather than progressive. Importantly, because the NIA and STZ doses were modified simultaneously, the present study cannot determine whether the observed response was predominantly related to the increased STZ dose, the reduced NIA dose, or the combined effect of both modifications.

The temporal variation observed in the STZ–NIA group, together with the relative stability of glucose concentrations in control animals, indicates that the induction procedure elicited a measurable metabolic response. However, the absence of a significant group × time interaction indicates that a consistent difference in the longitudinal glycemic profile between groups was not detected. These findings emphasize the importance of longitudinal glucose assessment during model validation, as an early increase in blood glucose does not necessarily indicate the establishment of a stable diabetic phenotype. Ghasemi and Jeddi similarly highlighted the substantial influence of STZ dose, administration route, animal characteristics, and other methodological factors on the reproducibility of STZ-induced diabetes models (6).

The preservation of body weight in both groups provides additional evidence that the induction protocol was not associated with marked deterioration of general metabolic status. Both groups showed comparable weight gain throughout the experimental period, with no significant difference in absolute or relative weight gain. The absence of weight loss in the STZ–NIA group is consistent with the lack of a severe insulin-deficient phenotype. Nevertheless, body weight alone cannot be used to determine the severity or nature of the diabetic phenotype and should therefore be interpreted together with glycemic, insulin-related, and pancreatic parameters.

Animal-related characteristics may also have contributed to the observed variability in the response to STZ–NIA induction. Age, sex, strain, body weight, and experimental conditions are recognized determinants of susceptibility to STZ. Previous studies have reported differences in STZ sensitivity according to age and sex, while commonly used rat strains may also differ in their metabolic response (6, 12, 18). The relatively young age of the animals and the use of Sprague–Dawley rats may therefore have contributed to the response observed in the present study. In addition, differences in administration route, the interval between NIA and STZ administration, and other protocol-specific factors may influence the resulting metabolic phenotype (8, 16, 21, 22).

From a methodological perspective, these findings emphasize the importance of validating the STZ–NIA model before initiating pharmacological experiments. A transient metabolic response may be insufficient for evaluating antidiabetic interventions because variability in the induction of the diabetic phenotype could confound the interpretation of subsequent treatment effects. Further optimization should therefore consider systematic variation of STZ and NIA doses, together with longitudinal glucose monitoring and direct assessment of pancreatic β-cell integrity. Histological and immunohistochemical evaluation of insulin-positive β-cells, as well as assessment of insulin secretion or pancreatic insulin content, would provide additional infor-

mation regarding the extent of β-cell injury and could help clarify the mechanisms underlying the transient hyperglycemic response observed in the present study.

The modified STZ–NIA protocol produced a measurable but transient metabolic disturbance rather than a stable diabetic phenotype. The findings support the concept that the severity and persistence of the response depend on the balance between STZ-induced β-cell injury and NIA-mediated protection and are influenced by experimental and animal-related factors. These observations provide a basis for further optimization of the induction protocol and underscore the need to establish a reproducible metabolic phenotype before subsequent pharmacological investigations.

CONCLUSIONS

The present study shows that the metabolic response to the STZ–NIA induction protocol was influenced by modifications of the NIA and STZ dosing regimen. The initial protocol did not produce persistent hyperglycemia, whereas the modified protocol resulted in a transient increase in blood glucose, particularly after the second induction phase. This response indicates that the modified regimen produced a measurable metabolic disturbance, but was insufficient to establish a stable diabetic phenotype. Body weight increased in all animals, with no significant differences in weight gain between groups, and no evident weight loss was observed following induction.

The findings support the concept that the balance between STZ-induced β-cell injury and NIA-mediated protection contributes to the severity and persistence of the metabolic response in this model. These results emphasize the importance of preliminary validation and longitudinal glucose monitoring before the STZ–NIA model is used for pharmacological studies. Further optimization of the induction regimen, including systematic evaluation of STZ and NIA doses and direct assessment of pancreatic β-cell integrity and insulin-related parameters, is warranted to establish a reproducible and stable diabetic phenotype suitable for subsequent therapeutic investigations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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