The Weight Performance Stability of Mice on Modeling Obesity-Associated Hyperglycemia Induced by Dextrose Monohydrate

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Abstract

Previously, streptozotocin and alloxan were employed to imitate hyperglycemia in mice. High doses of sucrose were also induced as an alternative. Due to body mass index has been associated with hyperglycemia, the evidence of weight body index in various induction alternate kinds, however, have not been fully reported. Here-in, we report on the weight performance stability of mice body weight induced by dextrose, streptozotocin, and alloxan. To begin, all mice were divided into six groups of five, with one reserve in each. Following seven days of acclimatization, the mice were induced for nine days of hyperglycemia modeling; alloxan (Groups A and D), streptozotocin (Group B and E), dextrose monohydrate (Groups C and F). On preclinical research animals modelling related to obesity-associated hyperglycemia in mice, dextrose monohydrate induction was most successful than streptozocotin and alloxan induction, which performed best during the induction period (31% weight growth) and after metformin intervention (36% weight growth). Overall, dextrose monohydrate is most suitable to be used for modeling type 2 diabetes mellitus test animals rather than alloxan and streptozocotin.

Keywords: alloxan; preclinical research animals; streptozotocin; type 2 diabetes mellitus.

Abbreviations: type 1 diabetes mellitus (T1DM), type 1 diabetes mellitus (T2DM), glucose transporter 2 (GLUT2), body weight (BW)

INTRODUCTION

Diabetic mice are commonly employed in the development of anti-diabetes agent. Alloxan and streptozotocin are commonly used to induce diabetic in the mice (Al-awar et al., 2016; King & Austin, 2017; Kottaisamy et al., 2021). In a single induction, alloxan elevated the glycemic index to 127 mg/dL (Irdalisa et al., 2015), whereas three days or more raised it to 156 - 270 mg/dL (Dewi et al., 2021; Hamdani & Nurman, 2020; Lolok et al., 2019). Streptozotocin had a comparable impact, increasing glucose levels to 136 mg/dL after three days (Apriani et al., 2011) and 220-244 mg/dL after five days (Ocktarini et al., 2011; Suwanto & Rahmawati, 2019).

As an alternative to alloxan and streptozotocin, glucose induction groups have been induced. Fructose intake for 12 weeks hold glycemic levels at 81 mg/dL (Tillman et al., 2014). Dextrose outperformed fructose after seven days of induction, while by 10%-concentration raised glycemic at 148 mg/dL (Pramushinta et al., 2019) and by 40% at 154 mg/dL (Santoso & Suryanto, 2017).

Diabesity is a new term for diabetes that comes alongside obesity (Serván, 2013). Obese are roughly three times more vulnerable to diabetic (Prasetyani & Sodikin, 2017). Diabetes, most especially type 2 diabetes mellitus (T2DM), is correlated with weight gained (Droz et al., 2017) or obesity (Fang et al., 2019; Finkelstein et al., 2012). Weight gained and body mass are two factors that contribute to rising glycemic levels (Algoblan et al., 2014), so obesity and T2DM called the twin pandemic (Scheithauer et al., 2016).

Previously, streptozotocin and alloxan were employed to imitate hyperglycemia in mice. High doses of sucrose were also induced as an alternative. Due to body mass index has been associated with hyperglycemia, the evidence of weight body index in various induction alternate kinds, however, have not been fully reported. Here-in, we report on the weight performance stability of mice body weight caused by dextrose, streptozotocin, and alloxan.

MATERIALS AND METHODS

Ethical Clearence
The Medical and Health Research Ethics Committee Ethics of the Faculty of Medicine, Universitas Gadjah Mada, has accepted the whole series of research procedures under registration number KE/FK/0328/EC/2022.

Animals
Male Balb/c mice were around 2-3 months that bred by Pharmacy Laboratory of Universitas Gadjah Mada (Yogyakarta, Indonesia) and treated in accordance with current norms.

Materials
Alloxan monohydrate (Sigma-Aldrich), streptozotocin (Sigma-Aldrich), and dextrose monohydrate 40% (Otsuka) were used to induce mice. Metformin (Dexa Medica) and Natrium Carboxy Methylcellulose (Dai-ichi Kogyo Seiyaku) were prepared to assess the stability of hyperglycemia treatment. Citrate buffer pH 4.5 is solved for intraperitoneal preparation. Supplies for glycemic testing, such as strip and glucometer (easytouch). Among the other consumables are a handscoon, syringe for 1mL and 5 mL, blood lancet, and aquadest. As support equipment, analytical scales, beakers, mouse cages, markers, mortars and stampers, and oral probes were used.

Procedures
All mice were divided into six groups of five, with one reserve in each. Following seven days of acclimatization, the mice were induced for nine days of hyperglycemia modeling and five days of metformin intervention.

▪ *Induction Period*
To get baseline data, we assessed the weight of the mice following the acclimatization. For 9 days, Groups A and D were induced alloxan 0.12 mg/gram body weight (BW) intraperitoneally, Group B and E were induced streptozotocin 0.05 mg/gram BW intraperitoneally, and Groups C and F were induced dextrose monohydrate 6 mg/gram BW orally. At the end of the induction, we weighed the mice again.
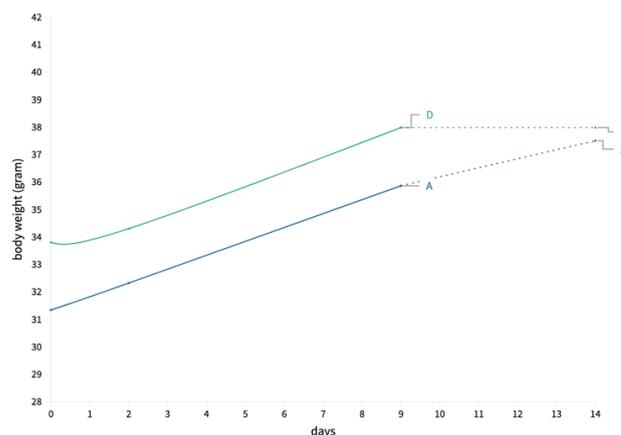
▪ *Intervention Period*
Following the induction period, groups A, B, and C were treated metformin orally, whereas groups D, E, and F as negative controls and were treated Na CMC orally. Dextrose monohydrate continued to activate Groups C and F throughout the intervention. On day 14, the body weight was measured.

RESULTS AND DISCUSSION

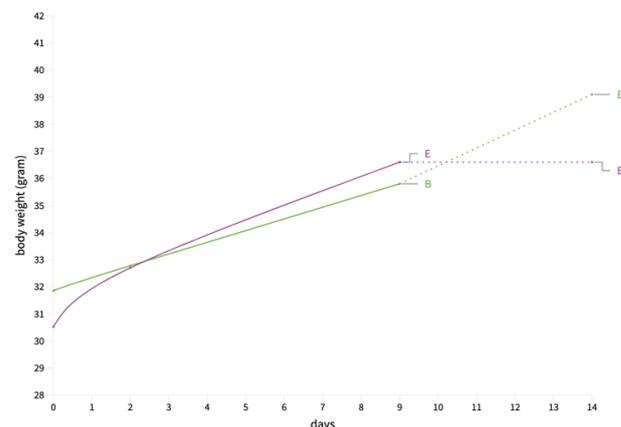### Performance of Alloxan Induction
Mice in groups A and D gained 35.86 grams and 37.98 grams of body weight after nine days of alloxan induction, respectively. Mice on Group A grew to 37.50 grams after a five-day metformin intervention, whereas control mice (Group D) stayed constant at 37.98 grams. (Figure 1).

Figure 1. The body weight gain due to alloxan induction in the intervention group (A) and the control group (D). The solid line represents the induction period, the dashed line represents the intervention period.

Performance of Streptozotocin Induction
Mice in groups B and E gained 35.80 grams and 36.60 grams of body weight after nine days of streptozotocin induction, respectively. Mice on Group B grew to 39.10 grams after a five-day metformin intervention, whereas control mice (Group D) stayed constant at 36.60 grams. (Figure 2).
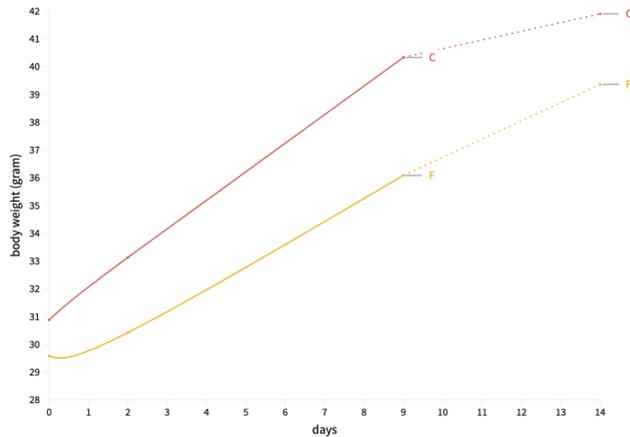

Figure 2. The body weight gain due to streptozotocin induction in the intervention group (B) and the control group (E). The solid line represents the induction period, the dashed line represents the intervention period.

Performance of Dextrose Monohydrate Induction
Mice in groups C and F gained 40.33 grams and 36.08 grams of body weight after nine days of dextrose monohydrate induction, respectively. Both groups thereafter continued to rise to 41.90 grams and 39.36

grams respectively, after receiving metformin intervention for five days (Figure 3).
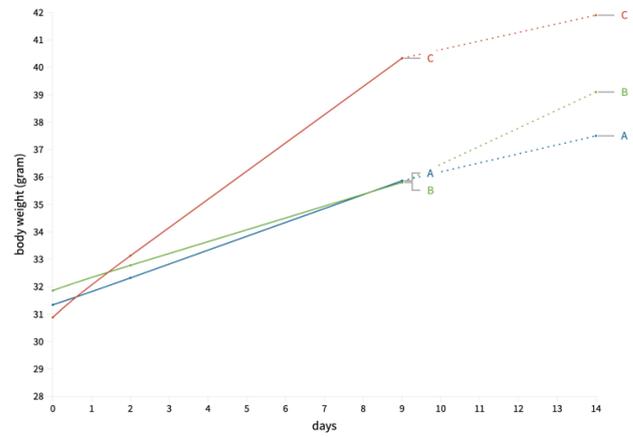
streptozotocin-induced (39.10 grams) and alloxan-induced (37,50 gram)

Figure 3. The body weight gain due to dextrose monohydrate induction in the intervention group (C) and the control group (F). The solid line represents the induction period, the dashed line represents the intervention period.

Figure 4. The body weight gain in mice following 9 days of induction (solid line) to alloxan (A), streptozotocin (B), and dextrose monohydrate (C), and persistence rate following 5 days of metformin intervention (dash line) after obesity (day 14th).

Induction variant performance in mice treated with metformin was compared.

After the 9-day induction period, dextrose-induced mice gained the most weight (40.33 grams), followed by alloxan-induced mice (35.86 grams), and streptozotocin-induced mice (35.80 grams). After 5 days of metformin treatment, dextrose-induced mice had the best body weight performance (41.90 grams), followed by

Dextrose monohydrate induction in mice was most successful than streptozocotin and alloxan induction, which performed best during the induction period (31% weight growth) and after metformin intervention (36% weight growth).

Table 1. Percentage of body weight gain in mice following 9 days of induction to alloxan (A), streptozotocin (B), and dextrose monohydrate (C), and persistence rate following 5 days of metformin intervention after obesity (day 14th).

Group Label	Induction Treatment	Body Weight (Percentage Increase)		
		Baseline	9th day treatment	14th day treatment
A	Alloxan	31.34±1.27 (-)	35.86±3.58 (14%)	37.50±4.54 (20%)
B	Streptozotocin	31.86±2.69 (-)	35.80±3.58 (12%)	39.10±3.01 (23%)
C	Dekstrose Monohydrate	30.88±2.35 (-)	40.33±2.66 (31%)	41.90±3.28 (36%)

Discussion

On preclinical research animals, we simulate obesity-associated hyperglycemia in mice using dextrose monohydrate induction. The bulk previous studies employed streptozotocin and alloxan to imitate hyperglycemia. However, as one of the important criteria in hyperglycemia etiology, the investigation of body weight issues was disregarded. Due to the importance of publishing our findings, we would show the evidence of steady rates of dextrose monohydrate-induced body weight during the experiment.

According to the availability of test animals in preclinical research on the developing of anti-diabetic agents, there are two options: genetic modification, or chemical induction (Kottaisamy et al., 2021). In the unavailability of genetically diabetic test animals, alloxan and streptozotocin are widely used to cause

diabetes in mice (Al-awar et al., 2016; Kottaisamy et al., 2021).

Streptozotocin and alloxan, on the other hand, act on beta cells via the glucose transporter 2 (GLUT2) and cause complete ablation of beta cells inside the islets, leading in severe insulin insufficiency, hyperglycemia, and weight loss, they are more suited for modeling Type 1 diabetes (King & Austin, 2017). Mice given alloxan or streptozotocin lost weight because they were unable to use the available glucose for energy. To meet the body's energy requirements, excessive catabolism of protein and fat in muscle and adipose tissue was performed (Malik et al., 2015; Sinata & Arifin, 2016; Suwanto & Rahmawati, 2019).

Since the great majority of T2DM are overweight (Finkelstein et al., 2012; Wilding, 2014), body mass index has long been linked to hyperglycemia and T2DM

(Algoblan et al., 2014; Jung & Choi, 2014), with the potential of obesity to promote insulin resistance being the primary explanation (Jung & Choi, 2014). Obesity was proclaimed in mice as test animals when they weighed more than 20% of their age-matched mates. (Patonah et al., 2018).

Common sugar exacerbates obesity and increases adiposity in mice (Kleinert et al., 2018). Adipocyte hypertrophy and hyperplasia lead to excessive lipid accumulation (lipotoxicity), which activate insulin resistance (Lee et al., 2020; Longo et al., 2019). Indeed, the diets manipulated of mice with high sucrose feeding generate a compensatory response from some beta cells, (King & Austin, 2017). Additionally, the weight gain was also linked to the disfunction of leptin (anorexigenic hormone) due to the circulating of glucose which led the test animals to consume more feed (Tillman et al., 2014).

Throughout the investigation, the induction performance of dextrose monohydrate has been shown to increase glycemia. Dextrose monohydrate is more suitable to be used for modeling type 2 diabetes mellitus test animals due to the aspect of increasing body weight, rather than the two toxic compounds that actually suppress body weight, and they are more suitable for modeling mice with diabetes mellitus type 1 as well, aside from having a higher level of glucose elevation than alloxan and streptozotocin.

CONCLUSIONS

On preclinical research animals modelling related to obesity-associated hyperglycemia in mice, dextrose monohydrate induction was most successful than streptozotocin and alloxan induction, which performed best during the induction period (31% weight growth) and after metformin intervention (36% weight growth). Throughout the investigation, dextrose monohydrate is most suitable to be used for modeling type 2 diabetes mellitus test animals rather than alloxan and streptozotocin.

Authors' Contributions: Conceptualization, SBS; Methodology, SBS and HL; Data Collection, DYSP; Writing-Original Draft Preparation, SBS and DYSP Writing-Review & Editing, DYSP, SBS. and HL. All authors read and approved the final version of the manuscript.

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