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The role of gut microbiota alteration in rats fed a diabetogenic diet on the development of type 2 diabetes

Kuabayina, A. I.[1], Amogu, J. D.[1,2,3], Dianzuangani, D.[1], Makwela, L. N.[1], Katunda, R. M.[1], & Kabena, O. N.[1,3]

[1]*Laboratory of Applied Microbiology for Biological and Natural Resources (LaMARBN), Department of Biology, Faculty of Science, University of Kinshasa, Kinshasa XI, Democratic Republic of the Congo.*

[2]*Centers of Excellence in Nuclear, Radiological, Biological, and Chemical (CoE-CBRN), Ministry of Scientific Research and Technological Innovation, Kinshasa, Democratic Republic of the Congo.*

[3]*National Committee for Protection against Ionizing Radiation (CNPRI), Ministry of Scientific Research and Technological Innovation, Kinshasa, Democratic Republic of the Congo*

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Correspondence to:
Jean-Jacques D. Amogu
jj.amogu@unikin.ac.cd

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ABSTRACT

Introduction

Diabetes is a chronic metabolic disorder characterized by hyperglycemia, marked dyslipidemia, and pancreatic β-cell dysfunction. Despite numerous management measures for type 2 diabetes mellitus (T2DM), its incidence is not decreasing.

Objective

This study aimed to investigate the effects of gut dysbiosis induced by amoxicillin and a gut microbiota maintained by plant fiber on the prevention of T2DM development.

Methods

Sixteen Wistar rats purchased from INRB were subdivided into four groups. The intestinal microbiota of group 2 (G2) was altered by amoxicillin (AX), while that of group 4 (G4) was maintained by plant fibers. Intestinal dysbiosis was assessed using PCA culture and surface colony count. Glycemia and weight were assessed weekly, and insulin sensitivity was measured using the hyperglycemic glucose tolerance test (HGPO) after 8 weeks.

Results

AX reduced the baseline bacterial concentration by 99.99% ($p = 0.039$) after 2 weeks of treatment, but its action did not differ between males and females ($p = 0.28$). Weight gain did not differ between groups for either males ($p = 0.24$) or females ($p = 0.50$). Similarly, blood glucose levels did not differ between males ($p = 0.87$) and females ($p = 0.06$) in any of the groups. However, intestinal dysbiosis reduced the risk of diabetes in males of the G2 group but increased it in females of the same group. Conversely, the risk of T2DM was significantly reduced in both males and females in the G4 group.

Conclusion

Intestinal dysbiosis delays the onset of T2DM in males but increases the risk in females, while a well-maintained intestinal microbiota delays its onset in both sexes.

INTRODUCTION

Diabetes is a persistent metabolic condition characterised by increased blood glucose levels, pronounced dyslipidaemia, and impaired pancreatic β-cells (Guo et al., 2018; Akinlade et al., 2021). It is a major and growing public health problem worldwide. Its incidence rose from 100 million to 135 million cases in 1995 (Ji et al., 2015) to approximately 463 million cases in 2019 (International Diabetes Federation [IDF], 2019). Despite recent improvements in its management, diabetes-related mortality decreased by 18% between 2009 and 2017 (Fidila et al., 2015; IDF, 2019), but its incidence remains high (IDF, 2019). According to IDF projections, the number of cases is expected to reach 578 million in 2030 and 700 million in 2045 (IDF, 2019).

Type 2 diabetes (T2DM) accounts for approximately 90% of all diabetes cases (Ji et al., 2015; Saeedi et al., 2019; Ojo et al., 2020) and also affects young people under the age of 20 (IDF, 2019). In addition, it is associated with an increased risk of cardiovascular disease and mortality (Akinlade et al., 2021). Factors linked to T2DM currently include gender (Russo et al., 2022) and dysbiosis of the gut microbiota (Lecerf & Cani, 2022).

The work of Cani and Burcelin has demonstrated the importance of gut microbiota modulation in controlling glycaemic homeostasis. Numerous curative and preventive approaches have been developed to combat T2DM. Probiotics such as *Akkermansia muciniphila* (Knauf, 2022; Lecerf & Cani, 2022), *Lactobacillus* spp., *Bifidobacterium* spp., and *Streptococcus* spp. (Razmpoosh et al., 2019) as well as prebiotics (Khalili et al., 2019) are used in management strategies. However, probiotics have shown inconsistent effects in T2DM patients (Tao et al., 2020). In addition to probiotics, the use of prebiotics in T2DM patients has been found to improve the relative abundance of *Bifidobacterium* spp., but not necessarily the metabolic parameters of these patients (Ojo et al., 2020).

Reducing the onset of new cases of type 2 diabetes could be a key strategy for mitigating the global impact of diabetes (Piravi et al., 2018). Despite the multitude of publications, the efficacy of probiotics and prebiotics appears varied in T2DM patients. However, the preventive impact of the gut microbiota on T2DM remains underexplored. Therefore, this study aimed to investigate the effects of intestinal dysbiosis induced by amoxicillin and an intestinal microbiota maintained by plant fibres on the prevention of T2DM development.

METHODS

Selection and Preparation of the Animal Model

The animal model used in this study consisted of rats of the Wistar strain. Sixteen (16) healthy Wistar rats, weighing between 100 g and 150 g and aged 12 weeks, were purchased from the animal house of the Institut National de Recherche Biomédicale (INRB). The group included eight (8) males (123.21 g ± 13.25) and eight (8) females (114.82 g ± 8.26). The selected Wistar rats had not received antibiotic treatment for at least one month prior to the experiment. The rats were randomly divided into four groups: control group (G1), group II (G2), group III (G3), and group IV (G4), with four rats in each group (two males and two females in each group). The rats were housed in individual cages (one rat per cage) and maintained in the INRB animal house under ambient temperature and humidity conditions.

Diet

The hypercaloric (diabetogenic) diet method was used to induce insulin resistance (IR) or type 2 diabetes mellitus (T2DM) in the experimental groups, as described in the literature (Kamgang et al., 2006; Ji et al., 2015; Cao et al., 2017; Guo et al., 2018). The diets of the experimental animals consisted mainly of feed provided by INRB. Three different diets were prepared: the Normal Diet (ND), the High-Calorie Diet (HCD), and the High-Calorie Diet Rich in Plant Fibre (HCDF). The ND was a normal INRB feed modified with elements described by Kamgang et al. (2006), notably sources of plant and animal proteins. The HCD included the ND with the addition of 20% sucrose and 20% lipids of the total weight of the prepared food. The HCDF was similar to the HCD but included 20% fibre (40% wheat bran) by weight of the total mass of the prepared feed. G1 (control) received ND, while G2 and G3 received HCD. G4 received HCDF. All groups were fed ND for two weeks prior to the intervention. Animals had free access to food and water.

Disturbance of Intestinal Microbiota in G2

Of all the groups, only the microbiota of group G2 was disturbed. In this group, the bacterial community was disrupted through antibacterial treatment, as described by Yu et al. (2017) and Silva et al. (2020). Rats in G2 were

treated with the antibacterial agent amoxicillin (AX) by oral gavage. Each rat received a dose of 500 mg/kg body weight, prepared as a suspension in 2 mL of sterile distilled water. AX was administered twice daily at 6-hour intervals for two weeks. The intestinal microbiota of the animal model prior to the experiment was considered balanced.

Table 1:
Summary of the Experimental Set-up (Diets and State of Intestinal Microbiota in Each Experimental Group)

Groups	Diets	Intestinal microbiota	Abbreviations
G1 (Control)	DN	Normal	G1
G2	HCD	Disrupted (Dysbiosis)	G2
G3	HCD	Normal	G3
G4	HCDF	Normal	G4

Assessment of Intestinal Microbiota Disruption in G2

Dysbiosis in G2 was assessed based on bacterial load in rat faeces (BLF), as described by Mariani-Kurkdjian et al. (2016). The bacterial load was determined using the coproculture method after decimal dilution (down to 10^{-7}) and colony counting on PCA medium. BLF was assessed after the first week of AX treatment and again after the second week. Rat faeces were collected directly from the rats following mild stress and placed in marked sterile test tubes. The mass of faeces was determined by the difference in weight between the tube containing the faeces and the empty tube. Samples were transported to the bacteriology and research laboratory of INRB for analysis. The bacterial concentration in the faeces was determined using Equation 1, and the load in 1 g of faeces was estimated using Equation 2. When bacterial colonies could not be counted, an arbitrary value of 300 colonies was assigned.

Equation 1:
Determination of Bacterial Load in Faeces (BLF_i)

$$BLF_i = NC / (0.5 \times D)$$

Where:

- BLF_i = bacterial load in faeces of parent suspension
- NC = number of colonies counted after 24 hours of incubation
- D = seeded dilution
- 0.5 mL = inoculated volume

Equation 2:
Estimation of Bacterial Load in 1 g of Rat Faeces (BLF)

$$BLF = BLF_i / m^t$$

Where:

- BLF = bacterial load in faeces
- m^t = mass of faeces

Assessment of Blood Glucose, Weight, and Insulin Sensitivity

Rats fed a hypercaloric diet, known to be diabetogenic, were monitored for 8 weeks following the approach described by Ji et al. (2015), Cao et al. (2017), and Guo et al. (2018) to assess blood glucose levels. Blood glucose and weight were measured weekly after a 10-hour fast. Blood glucose was measured using a glucometer from blood obtained via tail vein puncture, while weight was measured using a precision balance (Sartorius). At the end of the 8-week period, insulin resistance (IR) was evidenced using an oral glucose tolerance test (OGTT). A sucrose dose, at a weight-dependent concentration of 3 g/kg, was administered to the rats after 20 hours of fasting, following the method of Kamgang et al. (2006).

Data Presentation and Statistical Analysis

Results were expressed as mean (± standard error) in graphs. Blood glucose and body weight were plotted weekly. The bacterial load in faeces (BLF) was represented as $\log_{10}$ (BLF). Data were recorded in Microsoft Excel 2013 and analysed using RStudio software (version 4.3.1, released 2023-06-16) at a significance level (α) of 0.05. The normality of data was assessed using the Shapiro-Wilk test. Friedman's ANOVA test was applied to compare the means of several dependent samples, while the Kruskal-Wallis ANOVA test was used for independent samples. The Mann-Whitney test was used to compare the means of two independent samples. Spearman's correlation coefficient (r) was calculated to assess the relationship between paired variables.

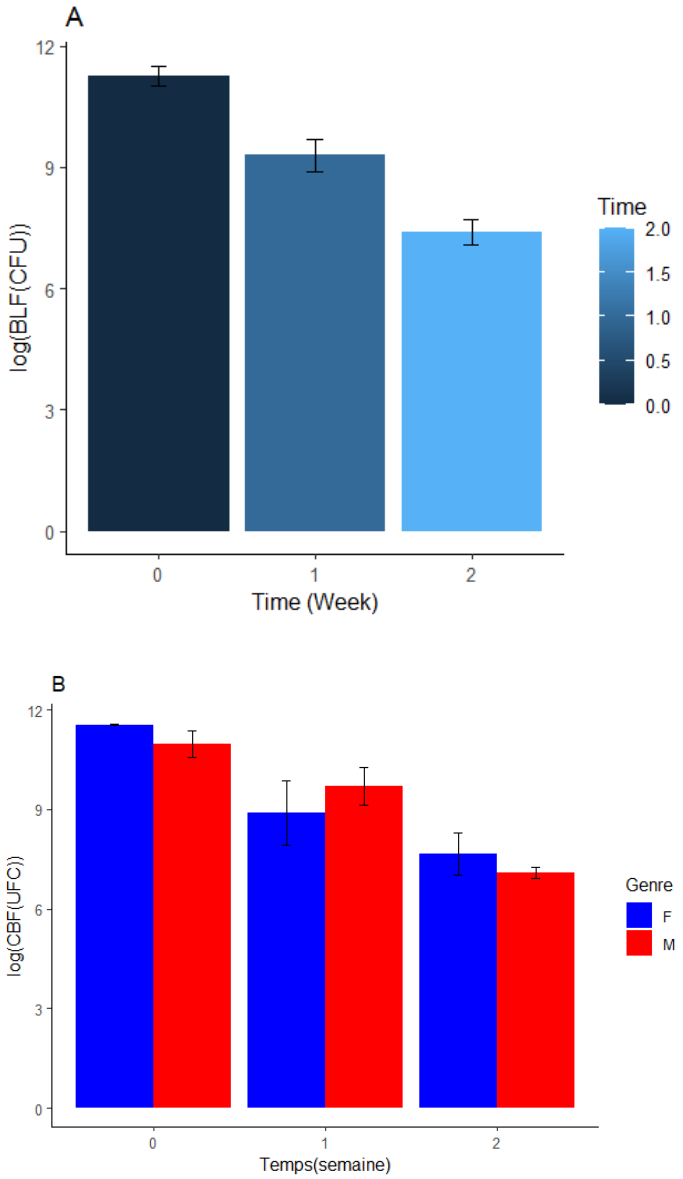
Ethical Approach

The study was approved by the ethics committee of the Biology Department, Faculty of Science, University of Kinshasa (Democratic Republic of the Congo).

RESULTS

Bacterial Load in Feces of G2 Rats After Exposure to AX Antibiotic

Figure 1:
Evolution of the bacterial load in the feces (BLF) of rats in the G2 group over the exposure time of the intestinal microbiota to the antibiotic AX

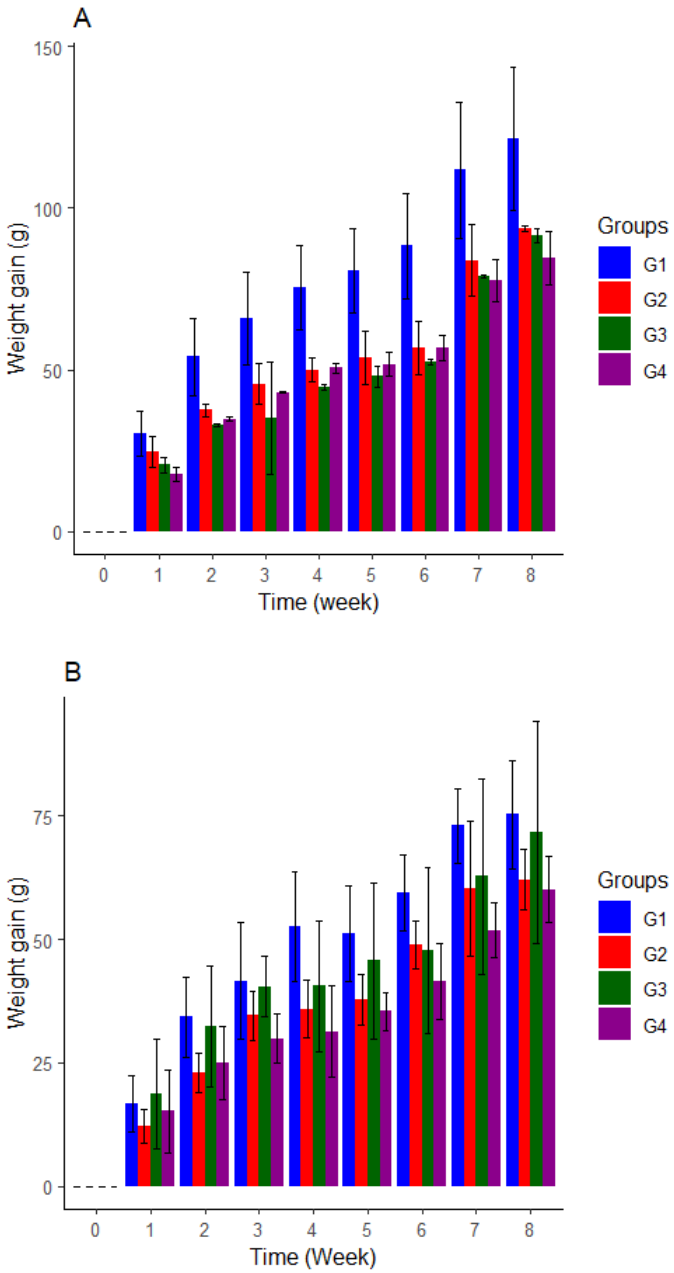


A: Evolution of the CBF of rats in group G2;
B: Evolution of the CBF according to gender.

The evolution of the bacterial load in the feces of the experimental animals highlights the disruption of the intestinal microbiota after the two-week treatment. The results obtained are shown in Figure 1, which indicates that the AX treatment given to rats in group G2 significantly reduced the rats' BLF by approximately 99.99% of the initial BLF (p = 0.039; Figure 1A). These findings suggest the state of the microbiota of rats in group G2, as the antibiotic significantly reduced bacterial populations in their gut ecosystem, promoting severe microbiota imbalance (dysbiosis). However, the effects of AX were consistent between males and females (p = 0.28; Figure 1B).

Weight Change During Diabetogenic Feeding

Figure 2:
Weight gain of rats fed a high-calorie diet from different groups over the 8 weeks of experimentation according to gender



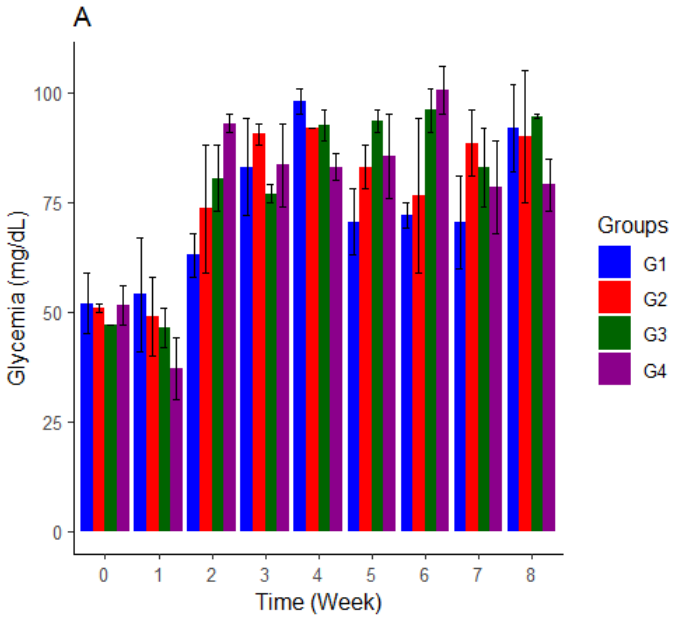
A: Weight gain evolution in males;
B: Weight gain evolution in females.

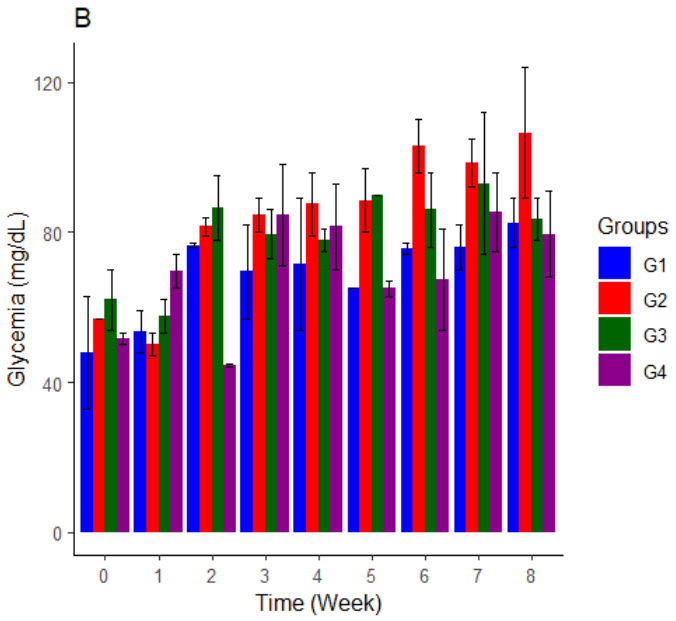
The evolution of weight gain for all rats according to gender is presented in Figure 2. Both male ($p < 0.0001$) and female ($p < 0.0001$) weights increased significantly during the feeding period. Males gained weight faster than females in each group ($p = 0.0001$). In the G4 group, weight gain was more pronounced in females (Figure 2B) than in males (Figure 2A), although it increased more slowly than in the G2 and G3 groups. However, differences between the test groups were not significant in males ($p = 0.24$) and females ($p = 0.50$; Figure 2). Overall, the test groups in both genders gained weight less rapidly than the control group. Despite these observations, statistical analysis indicated that the differences were not significant for males ($p = 0.06$) or females ($p = 0.29$).

Changes in Blood Glucose Levels and Insulin Sensitivity

a. Changes in Blood Glucose Levels in Rats Fed a Diabetogenic Diet

Figure 3:
Evolution of blood glucose levels in rats over the 8 weeks of feeding according to gender.





A: Evolution of blood glucose levels in males;
B: Evolution of blood glucose levels in females.

Figure 3 shows the evolution of fasting blood glucose levels in the different groups, according to gender. Fasting blood glucose levels in males ($p < 0.0001$) and females ($p = 0.0051$) varied significantly in all groups over the feeding period. In males, blood glucose levels in group G3 appeared to increase progressively over time compared to those in groups G2 and G4 (Figure 3A), but the difference was not statistically significant ($p = 0.87$). Conversely, in females, blood glucose levels in the G2 group increased over time compared to the other groups (G3 and G4; Figure 3B), though these differences were not significant ($p = 0.06$). Compared to the G1 control group, variations in blood glucose levels in the test groups were not significantly different for males ($p = 0.74$; Figure 3A) or females ($p = 0.06$; Figure 3B).

b. Insulin Sensitivity After 8 Weeks of High-Calorie Diet

The main effect of the high-calorie diet on blood glucose levels was to alter the insulin sensitivity of certain target tissues. Insulin sensitivity results are presented in Figure 4, which shows that the blood glucose levels of males in groups G2 and G3 increased to 126 mg/dL or higher after 3 hours of observation. In group G2, blood glucose levels varied minimally during the three hours following gavage ($p = 0.83$) compared with group G3, whose blood glucose levels dropped by 30.71% over the same period ($p < 0.0001$). In contrast, in group G4, blood glucose levels fell

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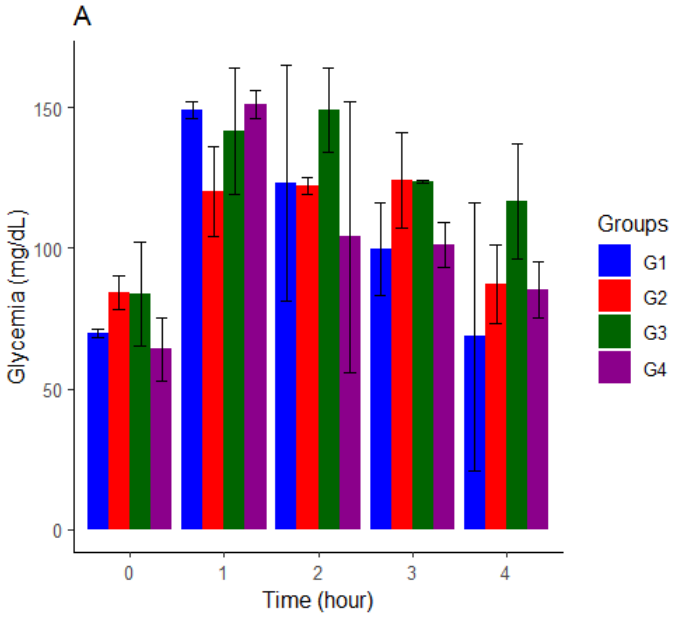
below 126 mg/dL two hours after gavage in males (p < 0.0001; Figure 4A).

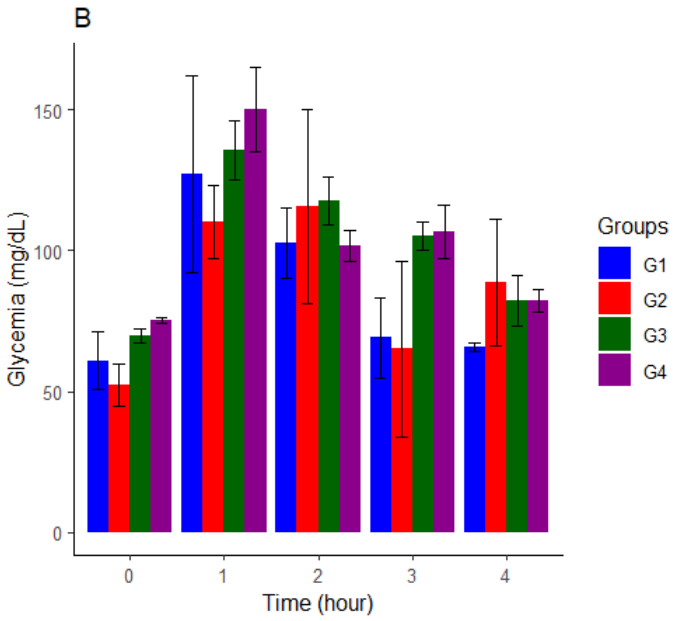
In females, blood glucose levels in groups G3 and G4 exceeded 126 mg/dL after 3 hours. In group G3, the mean blood glucose level remained above 126 mg/dL but decreased significantly (p < 0.0001). In group G4, blood glucose levels dropped below 126 mg/dL two hours after gavage (p < 0.0001). In group G2, blood glucose levels reached 126 mg/dL after 2 hours but fell sharply after 3 hours (p < 0.0001; Figure 4B).

Compared to the control group, blood glucose levels in the test groups declined more slowly over time. However, in the G4 group, blood glucose levels fell more rapidly than in the G2 and G3 groups. Males experienced a slower decline in blood glucose levels than females, indicating a rapid loss of insulin sensitivity in males compared to females. Individuals in the G2 and G3 groups developed insulin resistance (IR) more progressively and rapidly than those in the G4 group.

Spearman's correlation coefficient showed a positive, moderate, and significant correlation between feeding time and blood glucose in the G2 (r = 0.68; p < 0.0001) and G3 (r = 0.65; p < 0.0001) groups. In the G4 group, the correlation was positive but weak (r = 0.43; p = 0.008).

Figure 4:
Insulin sensitivity of rats after 8 weeks of feeding with a hypercaloric diet according to gender





A: Insulin sensitivity in males;
B: Insulin sensitivity in females.

When considering gender, Spearman's correlation coefficient revealed a positive, strong, and significant correlation in the G3 group (r = 0.75; p = 0.0003) in males, and in the G2 group (r = 0.82; p < 0.0001) in females. The correlation was also positive and moderate in males and females in the G2 (r = 0.54; p = 0.02) and G3 (r = 0.54; p = 0.02) groups. Conversely, in the G4 group, it was positive but weak and non-significant in males (r = 0.38; p = 0.12) and females (r = 0.39; p = 0.11). These findings align with insulin sensitivity results, indicating a more rapid loss of insulin sensitivity in the G2 and G3 groups. The risk of diabetes was more pronounced in the G3 and G2 groups, especially among males and females, respectively.

DISCUSSION

According to recent studies, the gut microbiota significantly influences the host's metabolism by regulating and maintaining its balance (Milani et al., 2017; Monnier & Schlienger, 2018). Disruption of this balance leads to multiple health issues, including metabolic disturbances and increased inflammation (Silva et al., 2020). Additionally, nutrition plays a key role in modulating blood sugar levels and diabetes, partly by affecting the gut microbiota (Rossmeisl et al., 2003). This study examined the effects of amoxicillin (AX)-induced intestinal dysbiosis and the maintenance of gut microbiota

through plant fibre consumption on the prevention of type 2 diabetes (T2D) development.

Recent research indicates that antibiotics cause gut microbiota dysbiosis by significantly reducing its taxonomic diversity (Ge et al., 2017; Yu et al., 2017; Silva et al., 2020; Fu et al., 2023). This finding aligns with our observations, as AX induced a significant reduction in the bacterial population. When antibiotics are administered orally, their action directly impacts the composition and taxonomic organisation of the gut microbiota (Ghoshal & Ghoshal, 2017). However, the effects of antibiotics may differ between males and females. For instance, a 2023 study reported that the Gram-positive/Gram-negative (Gram+/Gram-) bacterial ratio varied between male and female mice after treatment with the broad-spectrum antibiotic kanamycin (Fu et al., 2023). This is contrary to our findings, as we did not examine the Gram+ and Gram- bacterial groups separately.

Unlike our study, numerous reports have demonstrated that high-energy diets play a role in the pathogenesis of metabolic diseases, particularly obesity. Studies by Melo et al. (2019), Akinlade et al. (2021), and Zhang et al. (2022) reported faster weight gain in groups subjected to hypercaloric diets compared to control groups. We believe that the unchanged initial diet of the control group allowed the rats to adapt more quickly than those in the test groups, whose diets were significantly altered. Additionally, the high nutrient content of the control group's diet could explain the observed differences in weight gain. However, unlike our results, Yu et al. (2017) found that intestinal dysbiosis induced weight loss. This discrepancy may be due to the combined impact of intestinal dysbiosis and the hypercaloric diet used in our study.

Our findings on the evolution of blood glucose levels are consistent with those of Kamgang et al. (2006), Melo et al. (2019), and Zhang et al. (2022), who observed that a diabetogenic diet led to increased fasting blood glucose levels in test groups. The concordance between our results and theirs can be attributed to the fact that energy-dense foods significantly alter the mechanisms of blood glucose regulation. However, compared to our study, the test groups in the studies by Kamgang et al. (2006) and Zhang et al. (2022) had higher blood glucose levels than the control groups. This difference may be attributed to variations in the feeding duration and the composition of the normal diet. For example, Kamgang et al. (2006) fed rats for 16 weeks and supplemented the diet with approximately 40% of energy components (carbohydrates and lipids), while Zhang et al. (2022) extended the feeding period to 24 weeks.

The development of insulin resistance (IR) and hyperglycaemia occurs more slowly in females than in males (Kamgang et al., 2006; Ji et al., 2015; Cao et al., 2017; Guo et al., 2018; Melo et al., 2019; Akinlade et al., 2021; Zhang et al., 2022). Evidence suggests that females develop IR and hyperglycaemia after 16–24 weeks of a diabetogenic diet (Kamgang et al., 2006; Zhang et al., 2022), whereas males develop these conditions after only 8 weeks (Ji et al., 2015; Cao et al., 2017; Guo et al., 2018; Melo et al., 2019; Akinlade et al., 2021). Hyperglycaemic diets disrupt blood glucose regulation over time (Burchfield et al., 2018) by damaging the normal structure of pancreatic tissues (Zhan-Zho et al., 2015). The risk of developing diabetes also varies with gender, as females have better blood glucose regulation than males. This difference is attributed to variations in energy source utilisation, with males primarily metabolising sugars and females metabolising fats (Brockman & Yardley, 2018).

High-energy-density diets significantly alter the gut microbiota, increasing the abundance of harmful bacteria (Yang et al., 2022). Notably, these diets are linked to an increase in Prevotella spp. and certain bacteria from the phylum Bacteroidetes, which are associated with type 1 diabetes (T1D) and T2D (Schlienger, 2019). Furthermore, the intake of plant fibres provides a substrate for the gut microbiota, supporting the production of short-chain fatty acids (SCFAs), which are essential for maintaining glycaemic homeostasis (Makki et al., 2018). The role of plant fibres in preserving gut health underscores the potential of dietary interventions in managing diabetes risk.

Limitations

The limitations of our study arise from the small sample size, the low number of rats per group, and the short duration of the feeding period. These factors could

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potentially introduce biases in our conclusions. To improve the reliability of the findings, it may be necessary to conduct a similar study with an increased sample size and a longer experimental duration, such as 24 weeks or more, for more comprehensive observations.

CONCLUSION

This study aimed to investigate the effects of amoxicillin (AX)-induced intestinal dysbiosis and a gut microbiota maintained by plant fibres on the development of type 2 diabetes (T2D). The objective was achieved by assessing the combined effects of a high-calorie diet and the state of the microbiota on weight and blood glucose levels, as well as by demonstrating the preventive role of the intestinal microbiome in the development of T2D.

In conclusion, AX-induced dysbiosis and the intestinal microbiota did not have a significant impact on the weight and blood glucose levels of the rats. However, the microbiota maintained by plant fibres slowed the deterioration of glycaemic regulation parameters in both male and female rats. In contrast, while intestinal dysbiosis slowed the deterioration of glycaemic parameters in males, it promoted glycaemic deterioration in females.

For future research, it would be important to investigate the interactions between different taxonomic groups and their role in the regulation and onset of T2D. Additionally, studies should explore the specific influence of amoxicillin on the composition of the gut microbiota in males and females, focusing on both taxonomic and metabolomic perspectives. Finally, further research is recommended to examine the role of AX-induced intestinal dysbiosis in males in relation to variations in T2D risk.

Authors' Contributions

- Conceptualisation: O. N. K. and A. I. K.;
- Formal analysis: J. J. D. A.;
- Validation: O. N. K. and D. L. D.;
- Drafting and preparation of the original version: A. I. K., R. M. K., and L. N. M.

All authors have read and approved the final version of the manuscript.

Ethics Approval: The study was approved by the ethics committee of the Biology Department, Faculty of Science, University of Kinshasa (Democratic Republic of the Congo).

Conflicts of Interest: None declared.

ORCID iDs:

Kuabayina, A. I.[1]: Nil identified
Amogu, J. D.[1,2,3]: Nil identified
Dianzuangani, D.[1]: Nil identified
Makwela, L. N.[1]: Nil identified
Katunda, R. M.[1]: Nil identified
Kabena, O. N.[1,3]: Nil identified

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