

Quinoa Protein Supplementation as a Novel Dietotherapy for Type 2 Diabetes Mellitus

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Abstract. Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder that affects the cardiovascular and urinary systems and characterized by high blood sugar levels. Current treatments have side effects and demand strict dietary control and exercise. Moreover, complications like diabetic nephropathy remain a major concern. In this study, we sought for a new dietotherapy for T2DM. Dietotherapy, a branch of dietetics concerned with therapeutic uses of food and diet, is receiving increasing attention in recent years due to food's dual roles in health and medical care. This study explores the potential of quinoa protein as a novel food-based approach for T2DM management and prevention. *Chenopodium quinoa*, known as the "superfood" or "golden grain," contains bioactive peptides with potential health benefits for metabolic disorders. However, its role on T2DM is not fully understood. This research investigates quinoa protein supplementation's anti-diabetic potential using physiological, histological, and cytological methods with C57BL/6 male mice and db/db diabetic mutants as animal models. This study provides evidence for the significant improvements that quinoa protein supplementation has on weight and glycemic control in both diabetic and healthy mice, offering restored peripheral insulin sensitivity as a biologically plausible explanation. Quinoa protein supplementation on mice also improved their kidney architecture, increased the number of glomeruli, and enhanced renal regularity, suggesting a protection against diabetic nephropathy. In short, this study revealed the potential of quinoa protein in weight loss, glucose control, and kidney protection. Scientific evidence are provided for further development of quinoa protein, which has a practical significance.

Keywords: T2DM, quinoa, dietotherapy, weight loss, diabetic nephropathy.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by elevated blood sugar levels [1]. Epidemiological studies have implied that 537 million adults in the world have diabetes, 90% of whom have type 2 diabetes mellitus (T2DM) [2]. The consequences of diabetes can greatly reduce one's quality of life and place a financial strain on society. According to large-scale studies conducted in mainland China, more than 50% of people with T2MD have at least one chronic diabetic complication, including the highest incidence of diabetic nephropathy, leading to severe physical distress in patients and a heavy burden on the healthcare system [3].

Lifestyle intervention is a common prevention or treatment for T2DM management. Studies have shown that a 5% weight loss is beneficial for glycaemic control, and a 15% weight loss can initiate diabetes remission in most people with T2DM [4]. However, such prevention or treatment effect may be hard to achieve, since sticking to a low-carbohydrate diet and physical exercises over time is challenging and many people regain weight after a while. Human insulin and insulin analogs are the last choice for T2DM treatments, used only when β -cell functions are severely damaged [5]. Insulin therapy is comparatively costly and hard to deliver (using shoots or insulin pumps), especially for elderly patients who are vulnerable to potential infections during delivery. Given the existing prevention or treatment gap in T2DM management, we sought a new dietary prevention or treatment that can be easily applied, has minimized side effects, and is suitable for the long-term prevention or treatment of chronic disease.

The grain Quinoa (*Chenopodium quinoa*) is a gluten-free pseudocereal that originated in the South American Andes region and is mainly grown as a crop due to its immense nutritional value and potential to alleviate obesity and obesity-related illnesses. for its edible seeds [6]. Emerging studies

have highlighted the potential of quinoa to aid metabolic diseases, especially type 2 diabetes. In a 2020 study, sprouted quinoa yogurt exhibited a significant regulatory effect on hyperglycemia [7]. A 2022 study revealed that quinoa has the potential to reduce high-fat diet-induced obesity in mice [8]. The bound polyphenols in red quinoa was shown to be superior to free polyphenols in reducing postprandial blood glucose elevation [9]. Quinoa protein hydrolysates can improve insulin signaling, alleviate glucose intolerance in mouse models with Gestational Diabetes Mellitus [10]. Although a number of evidence have pointed to the immense potential of quinoa contents to alleviate T2DM conditions, the direct influence of quinoa on T2DM manifestation needs to be addressed. Furthermore, the bioactive peptides of quinoa in the form of proteins require further study to be better understood. Therefore, we designed this study to make the final step in revealing the exact influence of quinoa protein supplementation on T2DM management and prevention.

In this study, we utilized the db/db mouse as a model of T2DM conditions to study the effect of quinoa proteins on T2DM.

2. Method

2.1. Materials

Quinoa protein powder was acquired from Hanzhong Natural Gu Biology Science and Technology Co., Ltd. (Shanxi, China).

2.2. Animals and Experimental Design

Six-week-old male wild-type (WT) C57BL/6 mice and six-week-old male diabetic model (db/db) C57BL/6 mice were procured from Cyagen (Suzhou) Biotechnology Co., Ltd. (Suzhou, China). Seven days after acquisition, the WT-QPD and db/db-QPD groups were administered 400 mg/kg/day of quinoa protein via intragastric administration, while the WT-CTL and db/db-CTL groups received an equivalent dose of saline solution.

2.3. Biomedical Indexes Evaluation

Each group was measured every week. The weights of the mice were recorded bi-daily. Blood samples were collected at the onset and conclusion of the treatment to measure blood glucose, insulin, and glucagon levels. Insulin and glucagon were obtained and tested using blood samples collected on day 0, day 15, and day 29.

2.4. Renal Examination and Hematoxylin and Eosin (H&E) Staining

At the conclusion of the fourteen-day treatment period, these selected mice were humanely euthanized through cervical dislocation.

2.5. Statistical Analysis

Data are presented as mean $\pm$ SD for the experimental data in this study. One-way ANOVA, two-way ANOVA, Tukey's multiple comparisons test, multiple-effect analysis, and t-tests were performed on Prism and RStudio.

3. Results

3.1. db/db mice had a significantly higher intake of chow compared to wild-type mice.

All experimental groups were provided with an ample supply of standard chow for ad libitum consumption during the entire four-week study duration. Weekly monitoring of normal chow consumption was conducted throughout the experiment. Notably, db/db mice consumed approximately twice the quantity of standard chow in comparison to their wild-type counterparts (Fig 1A). However, it's important to note that no statistically significant differences were detected in chow

consumption between groups consisting of the same type of mice. Specifically, for the db/db mice, the p-value for chow consumption variation between the db/db-QPD and db/db-CTL groups was 0.736, while for the chow consumption variation between the WT-QPD and WT-CTL groups, the p-value was 0.678.

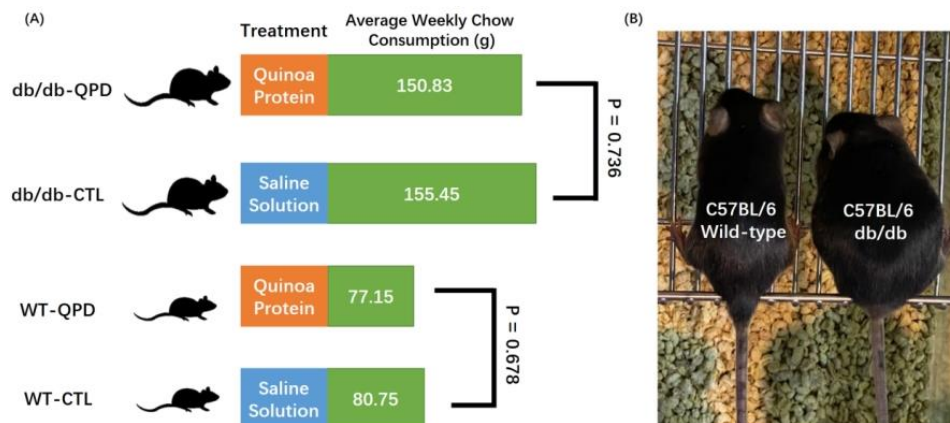


Fig. 1 Study design.

(A) The schematic diagram displays the experimental design, the groups' average weekly chow consumption over the twenty-nine-day period, and the p-values measuring the statistical significance of the difference in chow consumption between groups. (B) Picture of the six-week wild-type C57BL/6 male mouse (left) and the six-week db/db diabetic model C57BL/6 male mouse (right).

3.2. Quinoa protein temporarily inhibited weight gain in db/db diabetic mice.

Weight gain is generally regarded as a crucial physiological parameter in T2DM research. We diligently monitored and recorded the body weight of each subject every two days (Fig 2A). Prior to the four-week treatment, there was no statistically significant difference in body weight between the db/db-QPD and db/db-CTL groups ($p = 0.8988$). On average, the group-mean body weight of the db/db-QPD group was 0.7361g higher than that of the db/db-CTL group. Furthermore, the db/db-QPD group exhibited a smaller cumulative weight gain in comparison to the db/db-CTL group, with the former displaying an Area Under the Curve of 548.5 g·day and the latter 559.5 g·day (Fig 2B). However, according to the Tukey's multiple comparisons test, the variation in body weight of the two diabetic groups lacks statistical significance ($p = 0.5415$) (Fig 2A, Fig 2B).

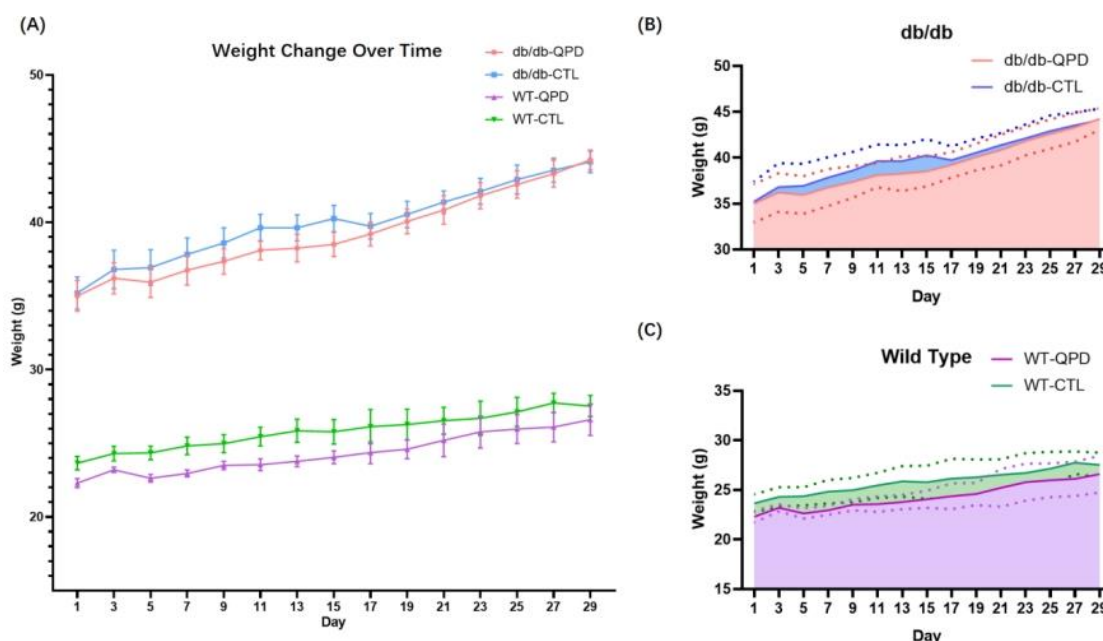


Fig. 2 The suppressing effect of quinoa protein on weight gain.

(A) The bi-daily-measured average body weight (g) of all mice in every treatment group. The x-axis represents the days from the beginning of the treatment, and the y-axis represents the group's average body weight. (B) The bi-daily-measured average body weight (g) of all db/db diabetic model mice. (C) The bi-daily-measured average body weight (g) of all wild-type mice. Data are presented as mean $\pm$ Standard Deviation (SD) (n=4). $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***; $p < 0.001$, ****.

3.3. Quinoa protein consistently maintained lower body weight in wild-type mice.

In this study, wild-type C57BL/6 mice served as healthy controls compared to db/db C57BL/6 mice. Four wild-type mice received quinoa protein solution treatment (WT-QPD group), while another four received an equivalent volume of saline solution (WT-CTL group). Throughout the four-week experimental duration, the group-mean body weight of quinoa-treated mice was consistently 1.511g lower on average compared to the control group, equivalent to approximately 6% of the weight of a typical male wild-type C57BL/6 mouse. Additionally, the cumulative weight gain (Area Under Curve) for the quinoa-treated group was 340.1 g·day, whereas the control group exhibited a cumulative weight gain of 361.6 g·day (Fig 2C). However, an initial statistically significant difference ($p = 0.0486$, *) was noted in the individual initial body weights between the WT-QPD and WT-CTL groups, with the quinoa-treated group starting at a lower group-mean body weight, which could account for the aforementioned pattern. Nonetheless, the disparity in weight gain between the two groups intensified over the four weeks, ultimately resulting in a more substantial difference as confirmed by the Tukey's multiple comparisons test comparing the four-week data of the WT-QPD and WT-CTL groups ($p < 0.0001$, ****).

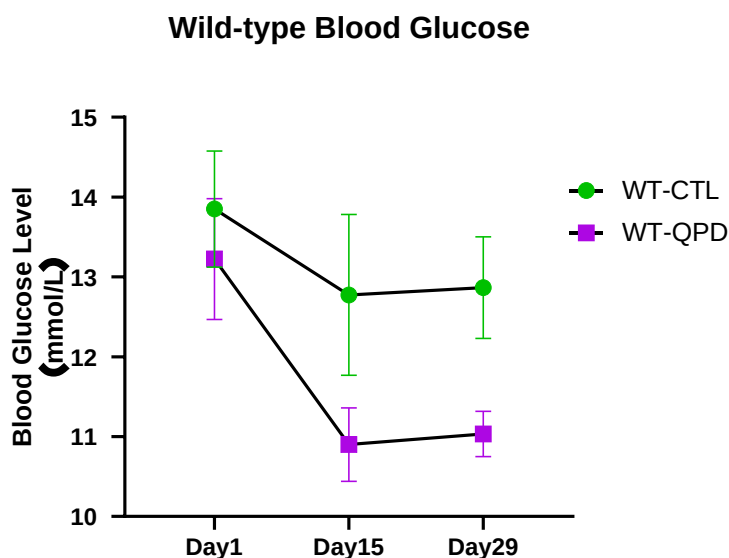


Fig. 3 Random blood glucose (RBG) testing results of wild-type mice over the four weeks.

The line graph displaying the average random blood glucose of the WT-CTL group and the WT-QPD group on Day 1, Day 15, and Day 29, with ANOVA-analyzed statistical significance shown. The x-axis indicates the days from the beginning of the treatment while the y-axis represents the blood glucose level (mmol/L). Data are presented as mean $\pm$ Standard Deviation (SD) (n=4). $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***; $p < 0.001$, ****.

3.4. Blood tests hint at quinoa protein's potential to reduce blood sugar levels in wild-type mice.

Random blood glucose (RBG) levels play a pivotal role in T2DM screening and diagnosis[11]. From our RBG data, initially, no statistically significant difference was evident between the WT-QPD and WT-CTL groups ($p = 0.5730$). However, differences became apparent as the experiment progressed. Mixed-effects analysis conducted over the four weeks suggested a noticeable disparity in

RBG data between the two groups ($p = 0.0104$, *), with the predicted mean RBG level in the quinoa-treated group at 11.60 mmol/L and the control group at 13.01 mmol/L (Fig 3A). Further analysis within the WT-QPD group revealed a notable decrease in RBG from day 1 to day 15 ($p = 0.0393$, *) (Fig 3B). It is noteworthy that both groups exhibited an uptick in RBG levels on day 29 compared to day 15, although a statistical significance was not observed ($p = 0.8313$ for WT-QPD, $p = 0.5915$ for WT-CTL) (Fig 3A).

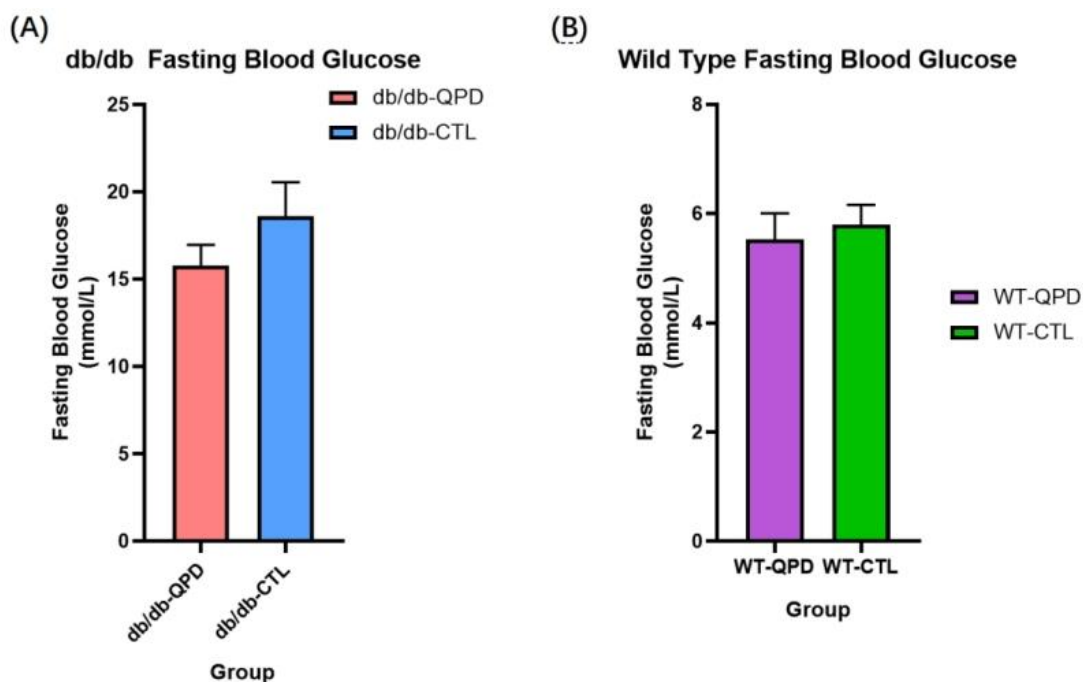


Fig. 4 Fasting blood glucose (FBG) testing results of db/db and wild-type mice.

(A) FBG of the two diabetic groups upon completion of the four-week period. The x-axis indicates the group while the y-axis represents the blood glucose level (mmol/L). (B) FBG of the two wild-type groups upon completion of the four-week period. The x-axis indicates the group while the y-axis represents the blood glucose level (mmol/L). Data are presented as mean $\pm$ Standard Deviation (SD) ($n=4$).

3.5. Quinoa protein supplementation exhibited a suppressing effect on the fasting blood glucose of diabetic mice.

Fasting blood glucose was tested for each subject immediately upon completion of the four-week period. According to our testing results, an observable differentiation was demonstrated between the two diabetic groups (Fig A). The group-mean FBG of the quinoa-treated group was 15.76 mmol/L, while the group mean of the control group was 18.60 mmol/L. A total difference of 2.833 ± 1.321 mmol/L existed between the two groups, with the control group being approximately $18 \pm 8.381\%$ higher in FGB compared to the quinoa-treated group.

However, the difference between FBG levels of the two wild-type groups was arguably less significant. The difference between the two group-mean FBG levels was calculated to be 0.2667 ± 0.3432 mmol/L, which indicated that the sugar-lowering effect of quinoa protein on wild-type mice reduced after fasting.

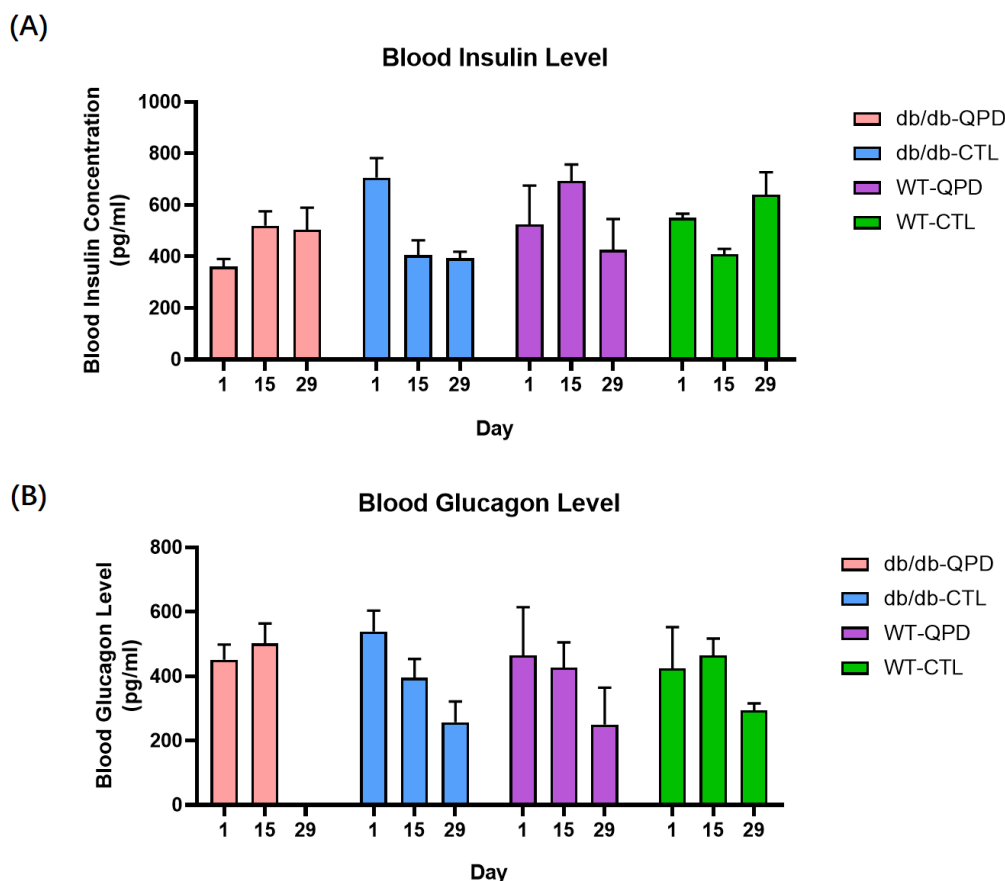


Fig. 5 Blood insulin level and blood glucagon level examination.

(A) Group-mean blood insulin levels in all four groups on Day 1, Day 15, and Day 29, with statistical significance yielded from the t-test shown. The x-axis indicates the days from the beginning of the treatment while the y-axis represents the blood insulin level (pg/ml). (B) Group-mean blood glucagon levels of all four groups on Day 1, Day 15, and Day 29, with statistical significance yielded from the t-test shown [12].

3.6. Quinoa treatment had no significant impact on insulin and glucagon levels.

We sought to measure the blood insulin level of subjects at the study's outset, midpoint, and conclusion. There wasn't any statistically significant difference in blood insulin levels between diabetic mice and healthy controls over the four-week period ($p = 0.5838$). For the wild-type mice, no statistically significant variation in initial blood insulin level was observed between the two groups as well ($p = 0.7312$). Nonetheless, the group-mean blood insulin level of the WT-QPD group became significantly higher than that of the WT-CTL group on day 15 ($p = 0.0400$, *) (Fig 5A).

Glucagon is one of significant hormone in glucose regulation that opposes insulin secretion by enhancing hepatic glucose production [13]. Blood test results did not reveal significant alterations in glucagon levels within groups over the four-week period except for group db/db-CTL. However, a peculiar constant decrease in blood glucagon level was observed in group db/db-CTL on day 15 and day 29 compared to day 1 and day 15 respectively (Fig 5B).

3.7. Quinoa protein improved glomerular hypertrophy, and mesangial matrix expansion in kidney by H&E staining.

We used hematoxylin and eosin (H&E) staining to scrutinize alterations in the morphological characteristics of renal tissue across experimental groups [14]. Our data revealed that saline-treated wild-type mice displayed a well-ordered configuration of renal tubules surrounding glomeruli (Fig 6A and 6B). In contrast, db/db-CTL mice unequivocally displayed a conspicuous reduction in the number of glomeruli. This reduction was indicative of glomerular hypertrophy, mesangial matrix

expansion, and a degree of tubular atrophy (Fig 6E and 6F). Conversely, mice treated with quinoa protein exhibited a discernible enhancement in kidney architecture, characterized by an increased number of glomeruli and an improved structural regularity. These enhancements apply to both quinoa-treated WT mice and diabetic mice when compared to their corresponding control groups (Fig 6 C-H).

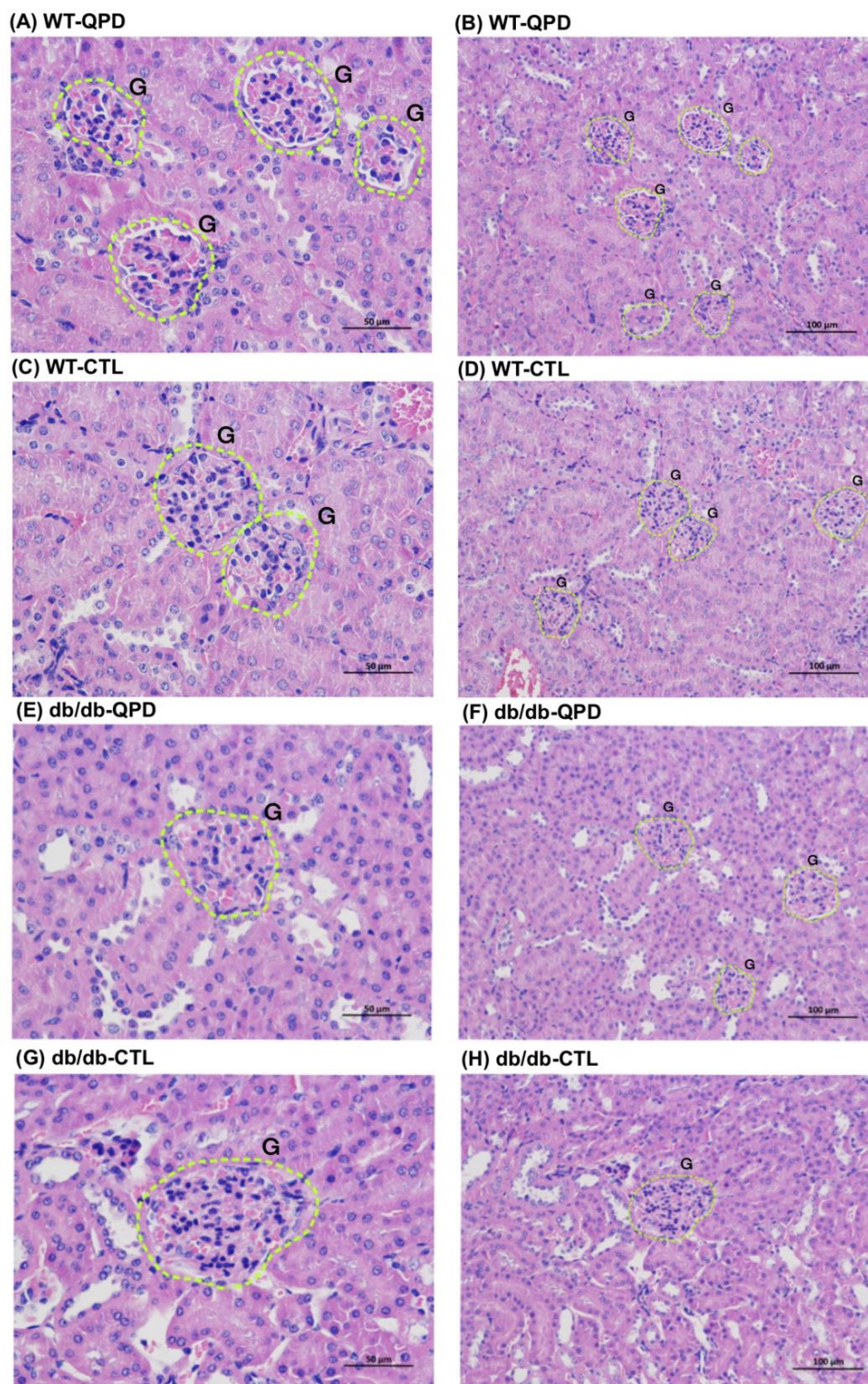


Fig. 6 Histological examination of H&E-stained kidney tissue slices.

(A)&(B) WT-CTL group H&E-stained kidney tissue slices. (C)&(D) WT-QPD group H&E-stained kidney tissue slices. (E)&(F) db/db-QPD group H&E-stained kidney tissue slices. (G)&(H) db/db-QPD group H&E-stained kidney tissue slices. Glomeruli are circled out and marked with “G”.

4. Discussion

Quinoa protein as novel treatments and preventions for T2DM improves glycemic control. FBG testing demonstrated a considerably lower FBG level in the quinoa-treated db/db-QPD group compared to that of the control group (Fig 4A). Specifically, the average FBG level of the db/db-CTL group was 18.0 % higher than that of the db/db-QPD group (SEM = ± 1.321 mmol/L). Although our results did indicate a possible sugar-lowering effect of quinoa protein supplementation, further investigation is necessary for drawing a conclusion since we were unable to measure the random blood glucose of db/db mice due to limitations in available measuring techniques. Moreover, the quinoa protein extraction procedure adopted in this study does not guarantee a purity greater than 60%. We believe that future studies should employ more advanced extraction methods to better exclude the potential influence of other quinoa contents, such as minerals and vitamins [15].

Diabetic nephropathy represents a serious complication of diabetes, associated with elevated mortality rates. Our histological analysis using H&E staining reveals substantial structural alterations within the kidneys of db/db mice, findings consistent with those reported by Fengjuan et al. Their investigation demonstrated pronounced glomerular hypertrophy, mesangial matrix expansion, and partial tubular atrophy in db/db mice compared to the control group, as evidenced in H&E staining [16]. Moreover, Our discovery of quinoa protein's kidney-protective potential in db/db mice opens doors for studying the molecular mechanisms it influences in the kidney.

Michael et al. embarked on the identification of prospective biomarker genes for diabetic nephropathy through an extensive transcriptomic analysis of 8-week and 16-week-old db/db mice kidneys. Their findings unveiled significant upregulation of fibrinogen gamma chain, endothelin 3, and bone morphogenetic protein and, at the same time a notable downregulation of cholecystokinin[17]. In future research, we plan to use fluorescent qPCR to further explore the molecular mechanisms behind quinoa protein's kidney protection and its ability to delay kidney damage.

5. Conclusion

This study revealed the potential of quinoa protein in weight loss, glucose control, and kidney protection. Scientific evidence are provided for further development of quinoa protein, which has a practical significance.

References

- [1] E. Ahmad, S. Lim, R. Lamptey, D. R. Webb, and M. J. Davies, "Type 2 diabetes," (in eng), *Lancet*, vol. 400, no. 10365, pp. 1803-1820, Nov 19 2022.
- [2] (2023, September 14). *Diabetes*. Available: <https://www.who.int/news-room/fact-sheets/detail/diabetes>
- [3] Z. Liu, C. Fu, W. Wang, and B. Xu, "Prevalence of chronic complications of type 2 diabetes mellitus in outpatients - a cross-sectional hospital based survey in urban China," *Health Qual Life Outcomes*, vol. 8, p. 62, Jun 26 2010.
- [4] M. E. Lean *et al.*, "Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial," *Lancet*, vol. 391, no. 10120, pp. 541-551, Feb 10 2018.
- [5] J. Upadhyay *et al.*, "Pharmacotherapy of type 2 diabetes: An update," *Metabolism*, vol. 78, pp. 13-42, Jan 2018.
- [6] E. A. Martínez, F. F. Fuentes, and D. Bazile, "History of Quinoa: Its Origin, Domestication, Diversification, and Cultivation with Particular Reference to the Chilean Context," *Quinoa: Improvement and Sustainable Production*, vol. 19, no. 24, 2015.
- [7] J. U. Obaroakpo *et al.*, "The hyperglycemic regulatory effect of sprouted quinoa yoghurt in high-fat-diet and streptozotocin-induced type 2 diabetic mice via glucose and lipid homeostasis," (in eng), *Food Funct*, vol. 11, no. 9, pp. 8354-8368, Sep 23 2020.

- [8] T. Y. Wang *et al.*, "Quinoa Reduces High-Fat Diet-Induced Obesity in Mice via Potential Microbiota-Gut-Brain-Liver Interaction Mechanisms," *Microbiol Spectr*, vol. 10, no. 3, p. e0032922, Jun 29 2022.
- [9] Y. Zhang, B. Bai, Y. Yan, J. Liang, and X. Guan, "Bound Polyphenols from Red Quinoa Prevailed over Free Polyphenols in Reducing Postprandial Blood Glucose Rises by Inhibiting α -Glucosidase Activity and Starch Digestion," (in eng), *Nutrients*, vol. 14, no. 4, Feb 9 2022.
- [10] D. Busso *et al.*, "A Quinoa Protein Hydrolysate Fractionated by Electrodialysis with Ultrafiltration Membranes Improves Maternal and Fetal Outcomes in a Mouse Model of Gestational Diabetes Mellitus," (in eng), *Mol Nutr Food Res*, p. e2300047, Sep 4 2023.
- [11] M. E. Bowen, L. Xuan, I. Lingvay, and E. A. Halm, "Random blood glucose: a robust risk factor for type 2 diabetes," (in eng), *J Clin Endocrinol Metab*, vol. 100, no. 4, pp. 1503-10, Apr 2015.
- [12] Data is missing for average blood glucagon level of db/db-QPD as the remaining sample was insufficient for measurement
- [13] I. Caruso *et al.*, "Glucagon in type 2 diabetes: Friend or foe?," (in eng), *Diabetes Metab Res Rev*, vol. 39, no. 3, p. e3609, Mar 2023.
- [14] A. Al Drees, M. Salah Khalil, and M. Soliman, "Histological and Immunohistochemical Basis of the Effect of Aminoguanidine on Renal Changes Associated with Hemorrhagic Shock in a Rat Model," (in eng), *Acta Histochem Cytochem*, vol. 50, no. 1, pp. 11-19, Feb 28 2017.
- [15] S. Dakhili, L. Abdolalizadeh, S. M. Hosseini, S. Shojaee-Aliabadi, and L. Mirmoghtadaie, "Quinoa protein: Composition, structure and functional properties," (in eng), *Food Chem*, vol. 299, p. 125161, Nov 30 2019.
- [16] F. Tang, Y. Hao, X. Zhang, and J. Qin, "Effect of echinacoside on kidney fibrosis by inhibition of TGF- β 1/Smads signaling pathway in the db/db mice model of diabetic nephropathy," (in eng), *Drug Des Devel Ther*, vol. 11, pp. 2813-2826, 2017.
- [17] J. Guan, M. Wang, M. Zhao, W. Ni, and M. Zhang, "Discovery of Fibrinogen γ -chain as a potential urinary biomarker for renal interstitial fibrosis in IgA nephropathy," (in eng), *BMC Nephrol*, vol. 24, no. 1, p. 60, Mar 20 2023.
- [18] Data is missing for average blood glucagon level of db/db-QPD as the remaining sample was insufficient for measurement