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Effect of barley β-glucan on weight gain prevention and angiotensin-converting enzyme 2 (ACE2) activity in Wistar rats

Kawthar Belkaaloul[1,*], Kaoutar Bouaziz[1], Youcef Bouferkas[1,2], Leila Amier[1], Thomas Haertlé[3], and Samia Addou[1]

[1]*Laboratory of Physiology of Nutrition and Food Safety, Department of Biology, Faculty of Natural and Life Sciences, University of Oran 1*, Ahmed Ben Bella, 31000, Oran, Algeria

[2]*Laboratory of Bioeconomy, Food Safety and Health, Food Sciences Department, Faculty of Natural and Life Sciences, Abdelhamid Ibn Badis University of Mostaganem*, 27000, Mostaganem, Algeria

[3]*Institut National de la Recherche Agronomique et Environnementale*, Nantes, France

***Corresponding author**: belkaaloul.kawthar@univ-oran1.dz

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Abstract: Obesity, dyslipidemia, and hypertension are key risk factors for cardiovascular disease. This study investigated the preventive effects of barley β-glucan (BG) supplementation on morphometric parameters, body weight, and the renin-angiotensin system in a high-fat diet (HFD)-induced obesity model. Male Wistar rats were randomly assigned to four groups for 12 weeks. The control group (CT) received a standard diet (SD), while the CT+BG group was fed the same diet enriched with 5% BG. The HFD group received an HFD, and the HFD+BG group was given the HFD supplemented with 5% BG. Morphometric analysis showed that the HFD+BG group exhibited significantly reduced thoracic and abdominal circumferences compared to the HFD group, while the nose-to-anus length remained unchanged. Weight gain was significantly lower in the HFD+BG group compared to the HFD group, with a similar trend observed between the CT+BG and CT groups. ACE2 enzymatic activity revealed a notable increase in renal ACE2 in the CT+BG group vs the CT. Conversely, ACE2 activity was significantly higher in the HFD group compared to the HFD+BG group. Cardiac ACE2 activity was elevated in the CT+BG group relative to the HFD+BG group. Regarding pulmonary ACE2, significant differences were observed between the HFD+BG and CT+BG groups, and between the HFD and CT+BG groups. These findings highlight the beneficial role of BG supplementation, supporting its potential as a dietary strategy to prevent metabolic and cardiovascular disorders.

Keywords: β-glucan, high-fat diet, obesity, weight gain, angiotensin, anti-hypertensive.

INTRODUCTION

Obesity is increasing across all regions of the world and has become a global epidemic, responsible for 2.8 million deaths annually [1]. Obesity is one of the main risk factors for high blood pressure. The connection between weight loss and a reduction in blood pressure has been established in several studies [2], with abdominal fat mass often linked to high blood pressure [3]. It remains the leading cause of cardiovascular diseases worldwide, despite various preventive strategies, and affects one in three adults globally. Over 10 million Algerians suffer from this condition [4]. Overweight leads to alterations in blood distribution and cardiac morphology. Indeed, the prevalence of hypertension is about threefold higher in individuals with obesity compared to those of normal weight [5]. Additionally, certain cytokines secreted by adipocytes play a significant role in increasing blood pressure. Research reports a positive correlation between body mass index (BMI), angiotensinogen, and leptin [6-7].

Obesity arises from a disruption in energy balance, caused by an imbalance between energy intake and expenditure. This leads to dysregulated carbohydrate and lipid metabolism, ultimately resulting in excessive fat accumulation. Obesity is considered the most critical risk factor for metabolic diseases [8]. From a physiological standpoint, eating activates dual pathways: the anabolic parasympathetic nervous system, which

enhances insulin secretion, intensifies peristalsis, and boosts gastrointestinal secretions, and the catabolic sympathetic nervous system, which promotes thermogenesis, diminishes gastrointestinal motility and secretions, and supports the maintenance of postprandial blood pressure. This latter effect is achieved through peripheral vasoconstriction, which counterbalances the splanchnic vasodilation occurring after a meal [9]. The ingestion of food not only augments blood flow to the splanchnic vessels but also increases the release of vasoactive peptides and triggers gastric distension. These physiological changes, in conjunction with their influence on the autonomic nervous system and baroreflex mechanisms, can result in a relative or absolute decrease in blood pressure, a condition known as postprandial hypotension. Additionally, this process is often accompanied by an increase in heart rate, which is commonly detected by wearable heart rate monitors following meals and may sometimes lead to sensations of lightheadedness [10].

Angiotensinogen, primarily produced by the liver, increases in concentration with obesity [11]. It is converted into angiotensin I through the action of renin released by the kidneys. Angiotensin I is further converted into angiotensin II by the angiotensin-converting enzyme (ACE). Angiotensin II contributes to oxidative stress and sympathetic activation [12], and functions as a vasoconstrictor, increasing peripheral vascular resistance and elevating blood pressure. The increase in vascular vasoconstriction, coupled with an increase in water reabsorption, raises blood pressure [13].

Dietary fibers are undigested carbohydrate fractions that reach the upper part of the digestive tract. Dietary fibers are found in vegetables, fruits, and cereals [14]. The beneficial effects of dietary fibers are diverse, and studies have shown that fibers can improve the inflammatory state of the digestive tract and reduce associated symptoms. They also possess a satiety-inducing effect, thereby promoting weight loss and preventing obesity [15]. Dietary fibers are characterized as a group of polysaccharide components that exert a significant impact on the intestine, nutritional mechanisms, and most human diseases. BG is a key component of soluble fiber that has the potential to decrease the risk of hypoglycemia, hypercholesterolemia, and lower the risk of colon cancer in experimental animals [16].

Barley and oats exhibit the highest BG contents, ranging from 2% to 16%, in comparison to other grains [17]. Pectin, BG, guar gum, and psyllium are more commonly associated with reducing appetite and enhancing feelings of satiation compared to foods with little to no fiber [16]. A dietary plan incorporating fiber has numerous health advantages, such as slowing down gastrointestinal transit time, increasing bulk, extending nutrient absorption, and promoting feelings of satiety [18].

Epidemiological studies have shown that the consumption of food rich in whole grains is inversely associated with weight gain in adulthood [19] and body mass index [20]. Long-term high-fat diets are one of the major factors contributing to lipid metabolism disorders. Disruption of lipid metabolism has been identified as the main cause of numerous conditions, including obesity, insulin resistance, and hypertension [21]. The objective of this research is to investigate the impacts of BG supplementation derived from barley in an overweight animal model. The investigation focuses on evaluating anthropometric parameters, weight gain, and ACE2 activity.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Algerian Institutional Ethical Committee for Animal Research (approval no. 45/DGLPAG/DVA. SDA.14). Every possible measure was taken to minimize stress on the animals.

Diet ingredients

Components of the various diets utilized in this study are given in Supplementary Table S1. Barley (*Hordeum vulgare*) grains were harvested in August 2021 in Tiaret, Algeria. They were washed in the Laboratory of Physiology of Nutrition and Food Safety, Faculty of Natural and Life Sciences, Oran 1 University Ahmed Ben Bella to remove inedible parts; they were then air-dried, shredded in a high-speed electric mill (A-V100 Grain Mill Grinder), and passed through a 20-mm-mesh sieve for bread preparation. β-glucan was extracted from the barley grains using the protocols of Mohamed et al. [22] and Bouaziz et al. [23]. The

percentage of BG recovered was 81.12±1.38%. Sucrose (Sigma-Aldrich), an important source of glucose, was used. The standard diet (SD), composed of grass-based compounds formulated for rodents, served as the staple food for the rats.

Animals and diets

This study was conducted on 24 male Wistar rats, aged 5 to 7 weeks and weighing approximately 142.63±5 g, obtained from the Pasteur Institute in Algiers, Algeria. The rats were accommodated in polystyrene cages, designed according to best practices, and were provided *ad libitum* access to food and water. The controlled environmental conditions included a stable temperature of 22±3°C and a 12-h light-dark cycle. The animals were divided into 4 homogeneous groups (n=6 rats per group) as follows: Group CT served as the control, and received a standard diet (SD); group CT+BG was fed SD supplemented with 5% BG; group HFD was fed a high-fat diet (HFD); group HFD + BG was fed HFD with a 5% BG addition. Over the 12-week study period, rats were monitored for dietary intake, weight progression, and anthropometric changes (Supplementary Table S2). At the end of the experiment, the rats were ethically killed, and their major organs, including lungs, heart, and kidneys, were harvested for detailed analysis of angiotensin-converting enzyme II (ACE2) activity.

Body weight gain and morphometric measurements

At the end of the experiment, the rats underwent the following measurements: abdominal circumference, thoracic circumference, body length (nose-anus), and body weight gain.

Rat euthanasia and organ collection

At the end of the experimental period, all 24 rats fasted overnight and were anesthetized with an intraperitoneal injection of sodium pentothal (50 mg kg^{-1}). The heart, kidneys, and lungs were carefully removed, rinsed with an ice-cold 0.9% NaCl solution, then stored at -80°C for analysis of ACE2 levels.

The animals' health and behavior were monitored daily through systematic observation of physical activity, behavioral responses, and cardiorespiratory parameters. Any signs of distress, abnormality, or mortality were promptly recorded.

ACE2 activity

ACE2 activity was measured using the ACE2 Activity Assay Kit (Fluorometric) (Cat. No. MAK377; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). This method utilizes ACE2 capacity to hydrolyze a specialized synthetic peptide substrate containing 7-methoxycoumarin-4-acetic acid (MCA). The enzymatic action releases the MCA fluorophore, the intensity of which can be precisely measured using a fluorescence microplate reader (Biotek FLx800 TBI, Instruments Inc., Highland Park, USA). To ensure the specificity of ACE2 activity measurement, the assay kit includes a selective ACE2 inhibitor that effectively differentiates ACE2 activity from that of other proteases.

Sample preparation

For sample preparation, 100 mg of each organ (heart, kidneys, and lungs) was homogenized in 400 µL ACE2 lysis buffer. The mixture was then incubated on ice and vortexed for 10 s, followed by an additional 5-min ice incubation. Following centrifugation at 16,000 x g for 10 min at 4˚C, the supernatant was collected in pre-cooled tubes and kept on ice. In the dosing procedure, 4 µL of this prepared sample was mixed with 46 µL of ACE2 assay buffer in a well of a white, flat-bottomed 96-well plate. After mixing thoroughly, the solution was incubated for 15 min at room temperature. Subsequently, 50 µL of the ACE2 substrate was added to each well. Fluorescence was measured in kinetic mode for 30 min to 2 h at room temperature. The activity of ACE2 was calculated according to the following formula:

$$(ACE2)\ (pmol/min/mg) = BxD/\Delta TxP$$

where B is the MCA released into the sample based on the slope of the standard curve (pmol), ΔT is the reaction time (T2-T1 in min), P is the sample used (in mg), and D is the sample dilution factor.

Table 1. Assessment of the morphometric dimensions across different rat groups

Groups	CT	CT+BG	HFD	HFD+BG
Nose-anus length (cm)	22.13±0.08	22.13 ±3.87 *	22.78 ±0.07 **	22.36 ±0.09
Thoracic Circumference (cm)	14.32±0.08	14.78 ±0.09 **	16.85 ±0.09 ***	15.52 ±0.14 $^{**, \$\$}$
Abdominal circumference (cm)	14.7±0.15	14.71 ±0.14 $^{\$, £££}$	18.38 ±0.09 ***	16.97 ±0.11 $^{** \$}$

The CT (control) group received a standard diet; the CT+BG group received a standard diet + 5% BG; the HFD group received a high-fat diet, and the HFD+BG group received a high-fat diet + 5% BG. Results are expressed as the mean±SD. The comparison of the means between the four groups of rats under diet (CT, CT+BG, HFD, HFD+BG) was performed using one-way ANOVA with Tukey's post hoc test. Differences are considered significant at P<0.05, *P<0.05, **P<0.01, ***P<0.001 (CT vs. HFD, CT vs. CT+BG, CT vs. HFD+BG);$^{\$}$ P<0.05, $^{\$\$}$ P<0.01, $^{\$\$\$}$ P<0.001 (HFD vs. HFD + BG, HFD vs. CT + BG). $^{£}$P<0.05, $^{££}$P<0.01, $^{£££}$P<0.001 (HFD+BG vs. CT+BG).

Statistical analysis

All *in vitro* experimental results are presented as the mean±standard error of the mean (SEM) from three independent parallel experiments. Data among groups were analyzed using one-way ANOVA, followed by Tukey's honestly significant differences post-hoc test. P<0.05 was considered statistically significant. Results were processed using GraphPad PRISM v5.0 (GraphPad Software, Inc.).

RESULTS

Morphometric measurements of rats

The morphometric characteristics of the rats are presented in Table 1. Significant differences emerged, particularly in thoracic and abdominal circumferences. A significant difference in thoracic circumference was revealed between the CT and HFD groups (P<0.001), between the CT and HFD+BG groups (P<0.01), between the CT and CT+BG groups (P<0.01), and between the HFD and HFD+BG groups (P<0.01). A significant difference in abdominal circumference was observed between the CT and HFD groups (P<0.001), between the CT and HFD+BG groups (P<0.01), between the HFD and HFD+BG groups (P<0.05), the HFD and CT+BG groups (P<0.05), and between the HFD+BG and CT+BG groups (P<0.001). A significant difference in nose-anus length was observed between the CT and HFD groups (P<0.01), and between the CT and CT+BG groups (P<0.05). The morphometric findings suggest that HFD consumption leads to significant alterations in body composition, characterized by increased thoracic and abdominal circumferences and a reduction in nose-to-anus length, indicative of enhanced adiposity and potential growth impairment. The attenuation of these alterations in the BG-supplemented groups underscores the beneficial effect of barley BG in mitigating HFD-induced morphometric disruptions. In particular, the reduction in body circumferences may reflect improved fat distribution and a decrease in visceral adipose tissue. These results support the potential of BG as a functional dietary component capable of counteracting the deleterious effects of excessive fat intake on body morphology.

Body weight gain

Over 12 weeks, the four groups of Wistar rats were fed different diets: A normal diet (CT group), a normal diet supplemented with 5% BG (CT+BG group), a high-fat diet (HFD group), and an HFD with 5% BG (HFD+BG) group. Rats on the HFD exhibited a significant increase in body weight from the 4th week (P<0.05) compared with those on the normal diet, and this trend continued until the end of the 12th week. At the end of the experiment, the CT group exhibited a significant weight gain (P<0.001) compared to the HFD, HFD+BG, and CT+BG groups. Compared to the CT+BG group, the HFD group showed a significantly lower body weight (Fig. 1A), suggesting that the addition of barley β-glucan may have mitigated the weight gain usually induced by a high-fat diet.

ACE2 activity assessment

The present study revealed that the highest level of ACE2 was recorded in the lungs for all groups. Comparison between the HFD and CT groups showed that overfeeding leads to a significant decrease (57.36%) in ACE2 expression in the lungs (P<0.01), without affecting the heart and kidneys. Comparison between

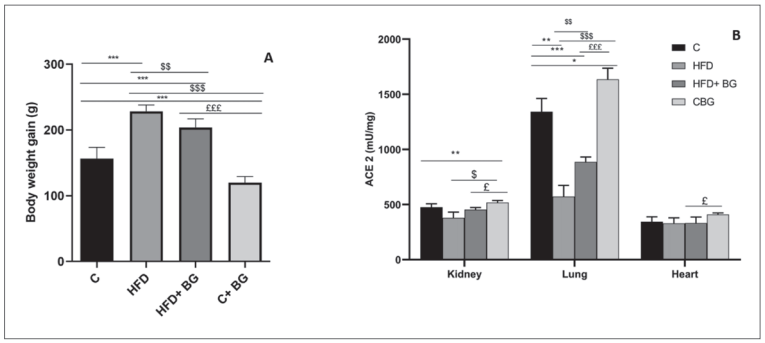

Fig. 1. Body weight gain (**A**) and ACE2 activity (mU/mg) in different rat tissue samples (kidneys, lungs, and heart) (**B**) at the end of the experiment. The CT group received a SD; the CT+BG group received SD+5% BG; the HFD group received an HFD; the HFD+BG group received an HFD + 5% BG. Results are expressed as the mean±standard error of the mean. The comparison of the means among the four groups of rats under diet conditions (CT, CT+BG, HFD, HFD+BG) was carried out using one-way ANOVA followed by Tukey's post hoc test. P<0.05 was considered statistically significant. *P<0.05, **P<0.01, ***P<0.001 (CT vs HFD, CT vs HFD+BG, CT vs CT+BG). $P<0.05, $$P<0.01, $$$P<0.001 (HFD vs HFD+BG, HFD vs CT+BG). £P<0.05, ££P<0.01, £££P<0.001 (HFD+BG vs BG). SD, standard diet. CT, control; BG, β-glucan; HFD, high-fat diet.

DISCUSSION

This study investigated the effects of barley β-glucan (BG) supplementation on morphometric alterations, body weight gain, and angiotensin-converting enzyme 2 (ACE2) expression in rats subjected to a high-fat diet (HFD). The primary objective was to assess whether this soluble dietary fiber could mitigate the adverse effects associated with excessive fat intake, particularly regarding body composition and metabolic regulation. Morphometric analysis revealed significant differences, particularly in thoracic and abdominal circumferences. These parameters were markedly increased in rats fed the HFD compared to the control group, indicating excessive fat accumulation. BG supplementation caused a significant reduction in circumferences, suggesting a protective effect on fat distribution. A reduction in nose-to-anus length was also observed in the HFD group, pointing to an impact of high-fat feeding on linear growth. These findings are consistent with those reported by Mutiso et al. [24], who described anthropometric alterations such as increased body weight and changes in body proportions in animals exposed to high-calorie diets. Furthermore, the improvements observed in the HFD+BG group align with the work of Mateos et al. [25], who demonstrated that BG supplementation in obese individuals improved anthropometric parameters. In terms of body weight, a significant weight increase was observed from the 4th week in HFD-fed rats. By the end of the study, the control group exhibited higher final body weight than the HFD group and both BG-supplemented groups. This finding may reflect appetite suppression or impaired energy metabolism in HFD-fed rats. BG supplementation appeared to modulate weight gain, even in the context of high-fat feeding, suggesting mechanisms beyond caloric intake. These findings support those of Obadi et al. [14], who confirmed that excessive energy intake leads to weight gain, although soluble fibers such as BG may attenuate this effect. Similarly, Lee et al. [26] reported that BG supplementation reduces weight gain in standard and high-fat diet models. These beneficial effects may be attributed to the lower energy density and higher satiety potential of high-fiber foods, as

the HFD and HFD+BG groups revealed that treating overfed rats with BG induces a significant increase (35.42%) in ACE2 in the lungs (P<0.001), without causing significant differences in either the kidneys or heart. These results suggest that overfeeding decreases ACE2 expression in the lungs, while supplementation with barley BG increases this expression. BG supplementation in overfed rats did not affect ACE2 activity in the kidneys and heart. At the renal level, no significant difference was observed between the HFD and CT groups, or between the HFD and HFD+BG groups. However, a significant increase (P<0.001) was noted in the CT group compared with the CT+BG group (Fig. 1B). As regards the heart, a significant increase was observed only in the CT+BG group compared with the HFD+BG group (P<0.05). In the pulmonary context, supplementing the SD with 5% BG for 3 months resulted in a significant increase in the ACE2 level in the CT group compared with the CT+BG group (P<0.05). Significant differences (P<0.001) were observed between the HFD+BG and CT+BG groups, and between the HFD and CT+BG groups. The CT+BG group exhibited the highest levels of ACE2 in the heart, lungs, and kidneys, demonstrating that supplementation of the SD with 5% BG affects ACE2 activity. Overfeeding influenced ACE2 activity in the lungs, while the consumption of barley BG regulated ACE2 activity in the lungs and kidneys.

previously demonstrated in human studies [27] and supported by the findings of Burton-Freeman [28] and Rolls [29], who emphasized the role of dietary fiber in increasing fullness and reducing energy intake.

This study found that a high-fat diet led to a marked decrease in ACE2 levels in lung tissue, while no significant changes were observed in the heart or kidneys. This pulmonary downregulation suggests a tissue-specific sensitivity to dietary fat, which may have implications for inflammation and cardiovascular risk. BG supplementation significantly restored ACE2 expression in the lungs, indicating a possible protective role against diet-induced downregulation. In normal metabolic conditions, BG supplementation resulted in the highest ACE2 expression across all studied organs, particularly in the lungs, suggesting a beneficial regulatory effect even in the absence of metabolic stress. A decrease in renal ACE2 expression was observed in the CT+BG group compared to the CT group, highlighting a differential response that may depend on baseline metabolic status. These observations are consistent with the findings of Lin et al. [30], who reported the involvement of ACE2 in blood pressure regulation and its role in obesity-related disorders such as diabetes and hypertension. The ACE/ACE2 balance modulates inflammation and fibrosis, and its expression can be affected by dietary factors, such as fat, sodium, and sugar intake [31]. Our findings agree with Snelson et al. [32], who reported that dietary fiber, particularly BG, may enhance ACE2 expression in renal tissue. Weight reduction itself has been associated with increased ACE2 expression [33], suggesting that the anti-obesity properties of soluble fiber may contribute to this regulation. Although the exact mechanisms by which BG affects ACE2 expression remain unclear, it is suggested that improved insulin sensitivity and enhanced energy metabolism may play a role [34,35]. Gut microbiota may play a key mediating role, and studies by Guimarães et al. [36] and Marques et al. [37] suggest that short-chain fatty acids produced through fiber fermentation could regulate ACE2 and contribute to the attenuation of hypertension.

CONCLUSION

This study demonstrates that barley BG supplementation exerts beneficial effects on morphometric parameters, body weight control, and ACE2 activity under high-fat dietary conditions. These findings highlight the potential of BG as a functional dietary component to mitigate the adverse metabolic effects of obesity and promote cardiopulmonary health by modulating ACE2 activity.

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Author contribution: KB (KBelkaaloul) designed the experimental protocol, supervised the work, coordinated the project, and finalized the manuscript. SA oversaw the project. KB (KBouaziz) performed the experiments and prepared the results for analysis and presentation. YB contributed to the realization of the results and the finalization of the manuscript. LA assisted in implementing experimental protocols and contributed to the preparation of reagents and materials used in the study. TH contributed by revising the manuscript and improving the scientific writing and English language.

Conflict of interest disclosure: The authors declare that they have no competing interests.

Data availability: The raw data underlying this article is available as an online supplementary research dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Belkaaloul%20et%20al_Dataset.pdf

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Components of the diets used in this study according to [27].

Ingredients	CT	CT+BG	HFD	HFD+BG
Standard diet (%)	100	95	50	50
Bread (%)	-	-	30	30
Sucrose (%)	-	-	10	5
B-glucan (%)	-	5	-	5
Red palm oil (%)	-	-	10	10
Total (%)	100	100	100	100
Caloric value (kcal/g)	3.49	3.31	4.24	4.04

ONLINE SUPPLEMENTARY RESEARCH DATASET

The raw data underlying this article is available as an online supplementary research dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Belkaaloul%20et%20al_Dataset.pdf

Supplementary Table S2. Animal experimentation protocol [22].