MAIZE ANTHOCYANIN IMPROVES HEALTH PARAMETERS IN OBESE RATS

Pedro REVILLA[1*]; María DE LA FUENTE[1]; Marcos COUSELO CAREIRA [2,3]; Rosa Ana MALVAR[1]

[1] Misión Biológica de Galicia (CSIC). Pontevedra, Spain.
[2] Laboratory of Molecular Endocrinology, Instituto de Investigación Sanitaria (IDIS), Complejo Hospitalario Universitario de Santiago (CHUS/SERGAS). Santiago de Compostela, Spain.
[3] CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Complejo Hospitalario Universitario de Santiago (CHUS/SERGAS). Santiago de Compostela, Spain.

* previlla@mbg.csic.es

Received: September-12, 2023
Acepted: October-28, 2023
Published on-line: December-29, 2023

Citation:
Revilla P, De la Fuente M, Couselo Careira M, Malvar RA. 2023. Maize anthocyanin improves health parameters in obese rats. Mol 23: 4.

Abstract

Antioxidant activity of maize (*Zea mays* L.) anthocyanin has potential health benefits. The objective of this work was to evaluate the effects of anthocyanin-rich black maize on health parameters of obese rats. Rats were fed with high-fat diets while a control group was fed with balanced diet (CD). Then, the high-fat diet group was split in three treatments high-fat diet (HF), white maize flour (WM), and black maize flour (BM), during four weeks. Body weight decreased for BM and increased for HF and CD. Visceral and subcutaneous adipose tissue was lower in BM than in WM and HF. Glucose was higher in BM than in WM; urea was lower in both maize diets than in HF; creatinine was lower in BM than in WM and in both maize diets than in HF; proteins were higher in both maize diets than in HF; albumin was higher in BM than in WM and HF; BM had lower triglycerides content than HF; maize diets had higher HDL and non-HDL, and lower LDL cholesterol than HF. Therefore, maize reduced obesity and help to reach healthy parameters in a short time; furthermore, anthocyanin could explain the superior health effects of black maize compared to white maize.

Resumen

La actividad antioxidante de la antocianina del maíz (*Zea mays* L.) tiene posibles beneficios para la salud. El objetivo de este trabajo fue evaluar los efectos del maíz negro rico en antocianinas sobre parámetros de salud de ratas obesas. Las ratas fueron alimentadas con dietas ricas en grasas, mientras que un grupo control fue alimentado con una dieta equilibrada (CD). Luego, el grupo de dieta alta en grasas se dividió en tres tratamientos: dieta alta en grasas (HF), harina de maíz blanco (WM) y harina de maíz negro (BM), durante cuatro semanas. El peso corporal disminuyó para BM y aumentó para HF y CD. El tejido adiposo visceral y subcutáneo fue menor en BM que en WM y HF. La glucosa fue mayor en BM que en WM; la urea fue menor en ambas dietas de maíz que en HF; la creatinina fue menor en BM que en WM y en ambas dietas de maíz que en HF; las proteínas fueron mayores en ambas dietas de maíz que en HF; la albúmina fue mayor en BM que en WM y HF; BM tuvo menor contenido de triglicéridos que HF. Las dietas de maíz tenían niveles más altos de HDL y no HDL, y niveles más bajos de colesterol LDL que los HF. Por tanto, el maíz reduce la obesidad y ayuda a alcanzar parámetros saludables en poco tiempo; Además, las antocianinas podrían explicar los efectos superiores del maíz negro sobre la salud en comparación con el maíz blanco.

Key words: maize, whole grain; black maize; anthocyanins; obesity.

Abbreviations: CD: Control diet, HF: High fat diet, WM: White maize diet, BM: Black maize diet.

Introduction

Obesity has nearly tripled since 1975 worldwide, with more than 1.9 billion adults having overweight and, among these; over 650 million were obese (World Health Organization 2019). Diabetes, musculoskeletal disorders, and some cancers (Williams et al. 2015), together with High Body Mass Index are major risk factors for cardiovascular diseases, particularly heart disease and stroke, which were the main causes of death in 2012. Within the global action plan for the prevention and control of non-inheritable diseases 2013-2020, the diseases related to overweight and obesity are a priority (World Health Organization 2019). Recent reports support health measures to reduce obesity in order to decrease premature causes of mortality (Ma et al. 2017).

High intake of phenolic compounds present in whole-grain cereals could be used for reducing obesity (Castro-Barquero et al. 2018). Phenolic compounds are metabolites with antioxidant activity considered the basis of the health effects attributed to the Mediterranean diet. However, the beneficial effects of phenolic compounds are not always consistently demonstrated; for example, Bart et al. (2012) reported that polyphenol-enriched foods, such as apple juice, showed a significant reduction in body fat mass but not in body weight, body mass index, or waist circumference, while a clinical trial with obese patients found that a polyphenol supplement significantly reduced body weight, body mass index and waist and hip circumference (Cases et al. 2015). Evidence for polyphenols' effects on human obesity is probably inconsistent due to the complexity of the projects involving humans, as well as to the heterogeneity among the characteristics of the diverse studies, such as diet composition and source of polyphenols (Castro-Barquero et al. 2018). The potential benefits of functional nutrients have been checked mainly by using extracts added to a diet, instead of using real food supplies. Tsuda et al. (2003) found that extracts of cyaniding 3-glucosiderich purple maize color ameliorated insulin resistance caused by high fat diet-induced in mice contributing to the suppression of mRNA levels of enzymes and regulatory elements involved in sterol, fatty acid and tryacylglycerol, results that support the benefits of anthocyanins for the prevention of obesity and diabetes. Results from experiments with isolated compounds differ from experiments using real food (Jang et al. 2016) and, indeed, evaluations of foods are more realistic than evaluations of extracts. A more precise and reliable approach is studying the effects of each functional nutrient by using simple natural food models. A simple diet with a single nutrient is not possible with humans, but animal models can provide valuable information about the effects of isolated foods. Nevertheless, few studies have been made with this kind of simple diets, for example involving cereal whole-grains. In a previous study, Revilla et al. (2018) studied the effects of black maize flour on larvae development of the insect *Sesamia nonagrioides*, and concluded that antioxidant activity has insecticide effects during the first stages of larval development, while antioxidant activity favors larvae growth in larger larvae. Toufektsian et al. (2008) reported that maize anthocyanins increased cardio-protection against heart attack in rats by including black and white maize in the diet; later, Toufektsian et al. (2011) demonstrated that maize anthocyanins increase poly unsaturated fatty acids in plasma or rats; however, these authors did not analyze the specific health parameters affected by anthocyanins. In the current study, we used white and black maize whole-grain, differing only in anthocyanin content, for feeding obese rats. The objective of this work was to evaluate the effects of anthocyanin-rich whole-grain black maize on health parameters of obese rats.

Materials and methods

We used the multicolor maize synthetic population EPS4 made in 1981 by mixing 100 kernels from the open-pollinated populations (Salcedo, Taboadelo and Cambados) from northwestern Spain with diverse kernel colors, followed by 10 generations of recombination, using each plant solely either as male or female (Rodríguez et al. 2013). Two samples of white and black kernels were separated from the population EPS4. These two samples were grinded in a laboratory grinding mill. The flour was screened with a sieve of one millimeter in diameter. Anthocyanin content of black maize was 5.38±0.018 μmol/g fresh weight while white maize had only 0.28±0.018 μmol/g (Rodríguez et al. 2013). The average nutritive composition of this maize variety was around 11% of total protein, 5.5% of total fat, 1% of brute fiber, and 58% starch, without significant differences among kernel colors.

The studies were performed in 30 eight-week old male Sprague Dawley rats purchased from the central animal facilities of the Universidad de Santiago de Compostela Rats were housed in air-conditioned rooms (22°C –24°C) under a controlled (12:12-h) light-dark cycle. They were allowed to acclimatize for 1 week on arrival. After the acclimatization period, the animals were randomized into four weight-matched groups (n=7/group). One group had *ad libitum* access to a standard diet (SAFE- Panlab, Spain), with 5.5% lipid, 23% protein, and 70% carbohydrate content. The other three groups were fed with high fat diet (HFD, Open Source Diets, Research Diets; Brogaarden, Denmark, Reference D 12492) with 60% lipid, 20% protein, and 20% carbohydrate for 16 weeks. Then, obese rats were divided into three experimental groups: a group that remained with ad libitum access to high dietary fat (Ob control n=7) and the other two groups changed their feed to maize, one with white grain maize (Ob WC, n=7) and the other with black grain maize (Ob BC, n=7) for 4 weeks. Body weight, food, and water intake were measured during the experimental period. All animal experiments and procedures involved in this study were approved by the Animal Care Committee of University of Santiago de Compostela in accordance with our institutional guidelines and the European Union standards for the care and use of experimental animals.

At the end of the experiment, animals were euthanized and decapitated and the visceral and subcutaneous adipose tissue were obtained, weighted and immediately frozen on dry ice, and kept at -80 °C until analysis. Blood was collected from the tail vein with a cannula. Serum from the blood was collected by centrifugation at 4,000 × g for 30 min and then stored at -80 °C.

Serum tests for total proteins, albumin, urea, creatinine, cholesterol, triglycerides, HDL and LDL were performed using an automated chemistry analyzer (ADVIA 2400 Chemistry System, Siemens Medical Solutions Inc.; USA). All biochemical parameters were measured at the end of the experimental period prior to the slaughter of the animals.

Analysis of variance were made for each trait by using the using the GLM procedure of SAS (SAS® 9.2 Enhanced Logging Facilities. 2018. SAS Inst.Inc.; Cary, NC, USA), considering rats as random effects and treatment as fixed effects. Comparisons of means among treatments were calculated for each trait with the Fisher's protected LSD.

Results and discussion

As the CD group had not been previously fed with fattening diet as the maize group did; therefore, we consider CD values as a reference for healthy levels. CD had lower values than both maize diet groups for visceral and subcutaneous adipose tissue, as well as for triglycerides and cholesterol, while CD values were the highest for glucose and creatinine (table 1). The BM diet was closer to CD than the WM for visceral and subcutaneous adipose tissue, glucose, creatinine, and triglycerides. Body weight significantly decreased for BM, while the weight loss was not significant for WM, being the reduction more evident during the first three weeks, and increased for normal diet and fattening diet (figure 1).

The analyses of variance showed significant differences among treatments for all traits (table 1). The values of both maize diets (WM and BM) were lower than those of HF for visceral and subcutaneous adipose tissue, urea, triglycerides, and the three types of cholesterol, and were not significant different from those of the control group for proteins, non-HDL and LDL cholesterol. BM had lower values than WM for visceral and subcutaneous adipose tissue, and the opposite was true for glucose, creatinine and albumin. Urea levels were lower in BM and WM than in CD. Even though BM caused the lowest urea content, this effect was not significantly different from WM; therefore, the reduction of urea caused by whole grain maize could not be explained solely by polyphenols.

The maize diets reduced visceral and subcutaneous adipose tissue, triglycerides, cholesterol and urea, and produced normal levels of proteins, non-HDL and LDL cholesterol. Furthermore, the effect of BM diet was even more important than that of WM for adipose tissue, glucose, creatinine, albumin, and triglycerides. Body weight significantly decreased for BM, in agreement with the reduction of body weight associated to consumption of whole grains reported for other cereals, which was probably due to the synergistic action of nutrients provided by whole grains (Castro-Barquero et al. 2018; Campbell and Fleenor 2018). Conversely, previous studies with whole grains from other cereals have not reported a significant reduction of urea (Behall et al. 2016). Even though BM caused the lowest urea content, this effect was not significantly different from WM; therefore, the reduction of urea caused by whole grain maize could not be explained solely by polyphenols, which have a demonstrated a clear effect in urea in vinegar and garlic (Ali et al. 2018). Nevertheless, Wang et al. (2017) detected that polyphenols from a *Solidago virgaurea* var. *gigantea* extract significantly reduced creatinine and urea in fat mice. Since the only difference between BM and WM was polyphenol content, the higher body weight reduction in BM was likely caused by polyphenols, which agrees with previous reports that associated the beneficial effects of whole grain on body weight to phenolic content (Castro-Barquero et al. 2018).

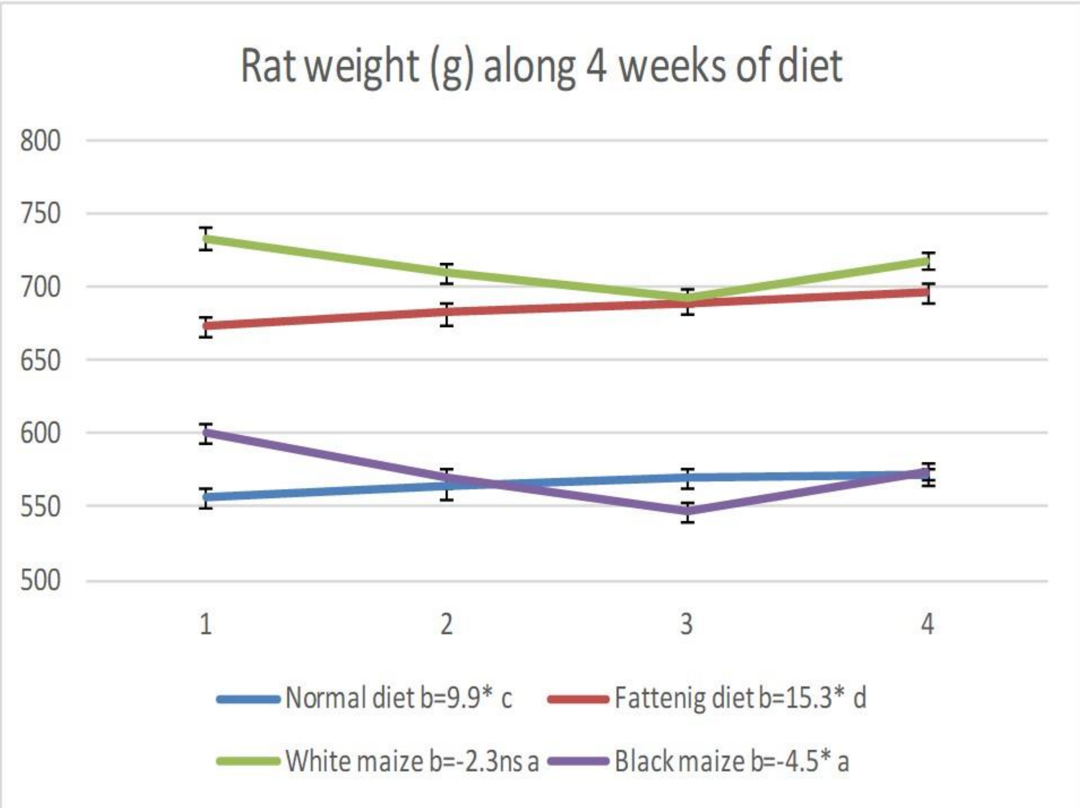

Figure 1. Evolution of rats' weight (means and LSD at p = 0.05) along four weeks under normal diet, fattening diet, white maize diet and black maize diet after four months with fattening diet (except the set of normal diet). *, ns Significant at P = 0.05, and non-significant, respectively. Coefficients of regression followed by the same letter do not differ significantly at P = 0.05.

The beneficial effects of whole grains and polyphenols are supported by studies made with humans and complex diets and also by our study with whole grain diets and obese rats (Lutsey et al. 2007). These results agree with the postulated effects of polyphenols in weight loss by inducing satiety and thermogenesis in brown adipose tissue, and by inhibiting adipocyte differentiation and promoting adipocyte apoptosis, modulating lipolysis, and activating oxidation (Castro-Barquero et al. 2018; Wang et al. 2017; Rupasinghe et al. 2018). The effects of BM on glucose are consistent with the results of Tenore et al. (2013), who found that polyphenols of tea improved glucose metabolism mediated by glucose transporters and had potential hypocholesterolemic effect by stimulating LDL receptor binding activity in vitro.

Therefore, maize, and particularly high-anthocyanin maize, had healthier effects in obese rats for body weight, adipose tissue, glucose, urea, creatinine, total proteins, triglycerides, and cholesterol. Our results agree with Wang et al. (2017), who detected that polyphenols significantly reduced triglycerides and cholesterol in fat mice. Similarly, Toufektsian et al. (2008) demonstrated that maize anthocyanin protects rats against heart attack and that reduce poly unsaturated fatty acids. Similarly, Lutsey et al. (2007) studied the relationship between whole grain diet and obesity, insulin resistance, inflammation, diabetes and subclinical cardiovascular disease and found favorable effects of whole grains in urine albumin excretion, obesity, insulin resistance, inflammation, and glucose levels.

Conclusions

As conclusion, whole grain maize reduces obesity and improves some health parameters in obese rats, and those effects are even more important for black maize than for white maize; therefore, anthocyanin could explain the superior health effects of black maize compared to white maize. In addition, the greatest weight loss occurs in the first three weeks of the experiment, which can be a positive point to promote weight loss diets with anthocyanins, since quick weight loss can have a positive impact on the psychology of the patient, which increase the hope of success.

Acknowledgements

Grant PID2019-108127RB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and by “ERDF A way of making Europe”. The authors thank Amando Ordás for providing the plant material (the maize synthetic EPS4) used for this experiment.

Table 1. Final mean[1] value after four weeks under four diets of biological traits measured in rats feed during four weeks with normal diet, fattening diet, white maize diet and black maize diet, after four months with fattening diet (except the set of normal diet).

Diet	Adipose tissue (g)		Glucose	Urea	Creatinine	Proteins	Albumin	Triglycerides	Cholesterol (mg/dl)		
	Visceral	Subcutaneous	(mg/dl)	(mg/dl)	(mg/dl)	(µg/ml)	(g/dl)	(mg/dl)	HDL	Non-HDL	LDL
HF[2]	16.38 a	7.42 a	158.9 d	34.4 a	0.224 d	5.31 b	3.47 b	115 a	19.7 a	67.4 a	9.4 a
WM	13.17 b	8.09 a	170.4 c	21.6 c	0.297 c	6.06 a	3.70 b	100 ab	18.1 b	49.7 b	4.7 b
BM	6.48 c	3.90 b	180.0 b	20.4 c	0.328 b	5.87 a	5.87 a	79 bc	18.1 b	44.4 b	4.1 b
CD	4.27 d	2.97 c	193.9 a	25.1 b	0.370 a	5.97 a	3.70 b	61 c	14.9 c	49.7 b	5.0 b
LSD	1.98	0.85	8.09	2.1	0.026	0.30	0.25	26	1.5	7.5	1.3

[1] Means followed by the same letter do not differ significantly, according to the Fisher's protected LSD at P = 0.05

[2] HF = high fat diet, WM = white maize diet, BM = black maize diet, and CD = control group with balanced diet

References

World Health Organization. 2019. Available on line: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight) (accessed 7 February 2019).
Williams EP, Mesidor M, Winters K, Dubbert PM, Wyatt SB. 2015. Overweight and obesity: prevalence, consequences and causes of a growing public health problem. Current Obesity Reports 4: 363–370.
Ma C, Avenell A, Bolland M, Hudson J, Stewart F, Robertson C, MacLennan G. 2017. Effects of weight loss interventions for adults who are obese on mortality, cardiovascular disease, and cancer: systematic review and meta-analysis. BMJ 359: j4849.
Castro-Barquero S, Lamuela-Raventos RM, Domenech M, Estruch R. 2018. Relationship between Mediterranean dietary polyphenol intake and obesity. Nutrients 10: 1523. DOI: 10.3390/nu10101523
Barth SW, Koch TCL, Watzl B, Dietrich H, Will F, Bub A. 2012. Moderate effects of apple juice consumption on obesity-related markers in obese men: Impact of diet-gene interaction on body fat content. European Journal of Nutrition 51: 841–850.
Cases J, Romain C, Dallas C, Gerbi A, Cloarec M. 2015. Regular consumption of Fiit-ns, a polyphenol extract from fruit and vegetables frequently consumed within the Mediterranean diet, improves metabolic ageing of obese volunteers: A. randomized, double-blind, parallel trial. International Journal of Food Science and Nutrition 66: 120–125.
Tsuda T, Horio F, Uchida K, Aoki H, Osawa T. 2003. Dietary cyanidin 3-O-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. The Journal of Nutrition 133(7): 2125-2130.
Jang YS, Wang Z, Lee JM, Lee JY, Lim SS. 2016. Screening of Korean natural products for anti-adipogenesis properties and isolation of kaempferol-3-O-rutinoside as a potent anti-adipogenetic compound from *Solidago virgaurea*. Molecules 21: 226.
Revilla P, Soengas P, Malvar RA. 2018. Effects of Antioxidant Activity of black maize in corn borer larval survival and growth. Spanish Journal of Agricultural Research 16 (1) # e1004.
Toufektsian MC, de Lorgeril M, Nagy N, Salen P, Donati MB, Giordano L, Mock HP, Peterek S, Matros A, Petroni K, Pilu R, Rotilio D, Tonelli C, de Leiris J, Boucher F, Martin C. 2008. Chronic dietary intake of plant-derived anthocyanins protects the rat heart against ischemia-reperfusion injury. Journal of Nutrition 138: 747–752.
Toufektsian MC, Salen P, Laporte F, Tonelli C, de Lorgeril M. 2011. Dietary flavonoids increase plasma very long-chain (n-3) fatty acids in rats. Journal of Nutrition 141: 37-41.
Rodríguez VM, Soengas P, Landa A, Ordás A, Revilla P. 2013. Effects of selection for color intensity on antioxidant capacity in maize (*Zea mays* L.). Euphytica 193: 339-345.
Campbell MS, Fleenor BS. 2018. Whole grain consumption is negatively correlated with obesity-associated aortic stiffness: A hypothesis. Nutrition 45: 32–36.
Behall KM, Scholfield DJ, Hallfrisch J. 2016. Whole-grain diets reduce blood pressure in mildly hypercholesterolemic men and women. Journal of the American Dietary Association 106: 1445-1449.
Ali Z, Ma H, Rashid MT, Ayim I, Wali A. 2018. Reduction of body weight, body fat mass, and serum leptin levels by addition of new beverage in normal diet of obese subjects. Journal of Food Biochemistry 42, N. 5 Ms e12554.
Wang Z, Kim JH, Jang YS, Kim CH, Lee JY, Lim SS. 2017. Anti-obesity effect of *Solidago virgaurea* var. *gigantea* extract through regulation of adipogenesis and lipogenesis pathways in high-fat diet-induced obese mice (C57BL/6N). Food & Nutrition Research 61(1): 1273479.
Lutsey PL, Jacobs DR, Kori S, Mayer-Davis EJ, Shea S, Steffen LM, Szklo M, Tracy R. 2007.

Whole grain intake and its cross-sectional association with obesity, insulin resistance, inflammation, diabetes and subclinical CVD: The MESA Study. British Journal of Nutrition 982: 397-405.

Rupasinghe HPV, Sekhon-Loodu S, Mantso T, Panayiotidis MI. 2016. Phytochemicals in regulating fatty acids-oxidation: Potential underlying mechanisms and their involvement in obesity and weight loss. Pharmacology & Therapeutics 165: 153–163.

Tenore GC, Stiuso P, Campiglia P, Novellino E. 2013. In vitro hypoglycaemic and hypolipidemic potential of white tea polyphenols. Food Chemistry 141: 2379–2384.