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Glucometabolic Response to Walnut (*Juglans regia* L.) Supplementation During Gestation and/or Lactation in Offspring of Sprague-Dawley Rats

[1,5]*Igbayilola Yusuff Dimeji,* [2]*Aina Olawale Samson,* [3]*Atoyebi Kayode,* [4]*Aikpitanyi Ikponwosa,* [5]*Williams Olawale Dada and* [5]*Olaoye Felix Adesina*

[1]*Department of Physiology, College of Medicine, University of Lagos, Yaba, Lagos, Nigeria*

[2]*Department of Physiology, College of Medicine, Lagos State University, Ikeja, Lagos, Nigeria*

[3]*The Arc of Washington State, 2638 State Avenue Nebraska, Olympia, Washington 98506, United States of America*

[4]*Department of Physiology, School of Basic Medical Sciences, Igbinedion University, Okada, Benin, Edo, Nigeria*

[5]*Department of Science Laboratory Technology, D.S. Adegbenro ICT Polytechnic, Eruku-Itori, Ewekoro, Ogun, Nigeria*

ABSTRACT

Background and Objective: Walnut (WN) (*Juglans regia* L.) has been reported to be the most widely planted tree nut worldwide. It is consistently referred to as the white WN, Persian WN, common WN or English WN. This study explored the impact of WN (*Juglans regia* L.) supplementation during gestation and/or lactation on glucose equilibrium in offspring of dams. **Materials and Methods:** Eighteen pregnant female and 12 male Sprague-Dawley rats were used in this study and fed either a basic diet or WN supplementation (WS). The gestating rats were exposed to WS up to parturition (gestational WN supplementation, GWS), or from parturition to day 21 after birth (lactation WN supplementation, LWS) or for a term covering both (GL WN supplementation, WN). At day 63 after birth, a blood specimen was collected for assay of fasting glucometabolic parameters. **Results:** Fasting serum glucose (FSG), fasting serum insulin (FSI) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) $p<0.05$ remarkably reduced in GWS and GLWS with a noticeable increase $p<0.05$ in LWS matched with control. Skeletal glycogen content noticeable increase of $p<0.05$ in GWS and a noticeable downturn in LWS and GLWS $p<0.05$ while hepatic glycogen content showed a noticeable downturn $p<0.05$ in GWS and LWS matched with CONT. Intestinal and pancreatic alpha-amylase and alpha-glucosidase produced a remarkably reduced $p<0.05$ in GWS, LWS and GLWS matched with CONT. **Conclusion:** The exposure to a walnut-enriched diet during pregnancy and/or lactation improved glucose metabolism, as reflected in reduced insulin resistance and down-regulation of alpha-amylase and alpha-glucosidase.

KEYWORDS

Alpha-amylase, triglyceride, intrauterine, sprague-dawley, walnut

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INTRODUCTION

Developmental planning takes place during the embryo and development foetus and it is a period in which organs and tissues are formed. Lack of sufficient nutrition during this period leads to lifelong changes in certain physiological, structural and metabolic functions of the foetus[1]. Barker, a British epidemiologist initially inveterate the postulation, referred to as the "Barker postulation," which enumerates that such programmed alterations in this time frame predispose the foetus to some postnatal disorders. The important period corresponds to the time of unstopped differentiation of cells. Importantly, planning can be referred to as the process of affecting or sustaining an insult or defacement that takes place at a pivotal juncture in its evolution[2].

It has been proven that using plant materials as remedies for illnesses dates back in time to the primitive era and restorative plants are being explored by scientists in virtually all medical fields. Indians used herbs as far back as 1900 BC describing over 600 species of plants of restorative significance. World Health Organization (WHO) reported that less than 90% of the world's population relies on traditional and herbal medicine for their primary healthcare, with the bulk of these treatments reportedly using plants or their active ingredients[3].

These are the plants that have one or more of its part contacting substances that can be explored for the care of various ailments[4]. Drugs that are obtained from plants are widely known because of their availability, safety and low cost drugs may include the whole part of a plant or are mostly prepared from leaves, bark, roots, flowers and seeds of plants. They are inhaled and administered orally or to the skin (subcutaneous)[5]. The restorative significance of these plants can be found in their bioactive and chemicals from plant origin that have a clear physiological impact on the human body.

Based on the facts above, it is important to screen plant species with the above properties to produce new drugs.

WN (*Juglans regia* L.), is the most distributed tree nut worldwide and is commonly known as the white WN, Persian WN, common WN or English WN which belongs to the species of Juglandaceae referred to by its scientific name, *Juglans regia*[6]. The WN tree species are indigenous to the ancient world and may be found in an area that stretches from the Balkans to the Himalayan chain on the west. It was first cultivated in Europe around 1000 BC. WN is currently grown commercially in all of Northern Africa, Southern Europe, Eastern Asia, Western South America and the United States. It is a dietary plant with one of the highest antioxidant levels[5] and it has been reported to have the highest level of phenolic antioxidants among all nut species[6,7]. Its extracts have been found to contain important chemicals from plant origin such as terpenoids, flavonoids, gallic acid, myricetin, caffeic acid and quercetin as well as naphthoquinones like juglone[8].

There is a dose-dependent inverse link between eating tree nuts and a lower incidence of diet-related illnesses like obesity and cardiovascular disease, according to experimental and epidemiological studies[9]. Despite the above overwhelming evidence on the restorative importance of WN, it's important to remember that its restorative full impact has not yet been realized or investigated with respect to foetal developmental planning. Precisely, there's little or no information on perinatal WN supplementation as it influences glucose homeostatic response in offspring of Sprague-Dawley rats specifically, male offspring. In this study, the role of perinatal WN supplementation was investigated in male offspring of Sprague-Dawley rats to assess its role on fasting blood glucose and fasting insulin level as well as insulin resistance and its impact on glucose storage and absorption and whether the outcome is a window of exposure dependent.

MATERIALS AND METHODS

Experimental animals: A total of 18 gesticulating Sprague-Dawley rats weighing between 130 and 160 g were used. They were housed in cages with 12 hrs light and dark cycles, free access to tap water, high-quality food and adaptation periods lasting one week at the animal house of D.S Adegbenro ICT Polytechnic in Eruku-Itori, Ogun State. The mechanisms undertaken were in line with the presentations of the Experimentation Ethics Committee on Animals Use of the College of Medicine, University of Lagos, Lagos State in accordance with the United States National Academy of Sciences Guide for the Care and Use of Laboratory animals[10]. This study was carried out between January to June, 2022.

Selection of walnut (WN): The 1 kg of WN was obtained at Gbanga market, Iyana Mortuary, Abeokuta, the seed was washed with sterilized water to remove dirt and the black outer shell was separated from the white kernel. And the white kernel was diced into smaller particles air-dried at room temperature and later ground into powdery form.

Diet, mating and grouping: The 850 g of dried WN were ground with 50 kilograms of standard rat chow to create the control (CONT) food. Another 50 kg of standard rat chow was used as a WN supplement. WN supplementation with the entire meal was available as a dried, crushed powder[10]. Female rats were kept on their individual diets throughout gestation and subjected to overnight intercourse with licensed male breeders at a ratio of one male to two females. Conception day 0 is the day that spermatozoa were discovered on a vaginal swab that had been cleaned with normal saline NaCl 0.9%. After that, rats were then assigned to one of four groups to be exposed to either a control diet or a WN supplement. Water and food were provided to all rats and grouped as follows (six animals per group):

Group i: Control-CONT (exposed to a control diet throughout the investigation)
Group ii: Gestational WN supplementation-GWS (exposed to WN supplementation only during gestation)
Group iii: Lactational WN supplementation-LWS (exposed to WN supplementation only during lactation)
Group iv: Combined WN supplementation-GLWS (exposed to WN supplementation during both windows)

By the end of the procedure, which was PND 63, all male pups were switched to regular rat chow and for coherence and uniformity in behavior, only male pups were studied for this study. This is because it has been reported that premature-life neonatal planning, which was not the focus of this study, lasts in an intimate relations-dimorphic manner[2,9]. On postnatal day 0 (birthday 0), babies were reduced to 8-10 pups. On postnatal day 21 (weaning day 21), all of them were utterly weaned and assigned to groups of between three and four male offspring per cage.

Analyses of proximate, vitamins and mineral compositions: Prior to pelletizing the animal feeds, specimens of dried WN were determined for protein, moisture, fat, fibre, ash nitrogen-free extract and mineral and vitamin composition by the methods of the Association of Official Analytical Chemists and as presented in the previous investigation[9].

Assessment of fasting blood glucose: The fasting blood glucose (FBG) level after overnight fasting was determined with an Accu-Check Glucose Meter[10].

Blood specimen: A cardiac puncture was employed to retrieve four (4 mL) specimens of blood, each of which was subsequently left to clot for an hour at 4°C. The serum was carefully collected with a rubber pipette into a clean Eppendorf flask and kept at -20°C until analysis after blood that had clotted had been spun at a speed of 3,000 rpm for 15 min[11].

Skeletal and hepatic glycogen: This was assessed using homogenate samples of skeletal and hepatic tissue[12].

Fasting serum insulin: This was assayed by enzyme-linked immunosorbent assay test as described by Igbayilola *et al.*[10].

Insulin resistance: This was determined using this formula[13]:

$$\text{HOMA} - \text{IR} = \frac{\text{FSG} \times \text{FSI}}{22.5 \times 18}$$

Organ extraction: The rats were put to sleep on day 63 by cervical dislocation following regional anesthesia. The skeletal, liver, pancreatic and intestines of the rats were extracted and cleansed in chilled water, after which they were washed with 1.15% KCl. These organs were immediately blotted and weighed.

Assay of pancreatic and intestinal amylase: Pancreatic amylase and intestinal tissue were evaluated using an approach presented by Igbayilola *et al.*[10].

Assay of pancreatic and intestinal α-glucosidase: According to the procedure given by Igbayilola *et al.*[10], pancreatic glucosidase and intestinal tissue were evaluated.

Statistical analysis: The mean as well as the standard error of the mean (SEM) for the results are reported. GraphPad Prism 5 software (GraphPad, Inc., in La Jolla, California, USA) was used for the statistical computation. A one-way investigation of variance utilizing *post hoc* with a 0.05 adjusted level of significance, Tukey's numerous comparison tests were conducted.

Ethical consideration: Ethical approval was sought and given by the Institutional Animal Care and Use Research Ethics Committee (ACUREC) to conduct the study.

RESULTS

Outcome of perinatal WN supplementation on glucose parameters: The FBG level in GWS and GLWS reduced remarkably by $p<0.05$ and increased noticeably by $p<0.05$ in LWS matched with CONT. However, perinatal WN supplementation produced a noticeable increase and reduced $p<0.05$, respectively in LWS and GWS matched with GLWS and LWS, respectively (Table 1).

The FSI level in GWS and GLWS reduced by $p<0.05$ and increased by $p<0.05$ in LWS matched with CONT. However, perinatal WN supplementation produced a noticeable increase and reduction of FSI $p<0.05$, respectively in LWS and GWS matched with GLWS and LWS, respectively as shown in Table 1.

The HOMA-IR was remarkably $p<0.05$ reduced in GWS and GLWS matched with CONT and remarkably heightened in LWS matched with GLWS (Table 1).

Outcome of perinatal WN supplementation on skeletal and hepatic glycogen contents: The result from the skeletal glycogen assay exhibited an apparent rise. The $p<0.05$ in GWS and a noticeable downturn $p<0.05$ in GLWS matched with CONT. However, perinatal WN supplementation produced a noticeable downturn $p<0.05$ in LWS and GLWS matched with GWS (Fig. 1a).

Figure 1b showed a downturn $p<0.05$ in hepatic glycogen in GWS and LWS matched with CONT while GLWS remarkably increased $p<0.05$ matched with GWS and LWS.

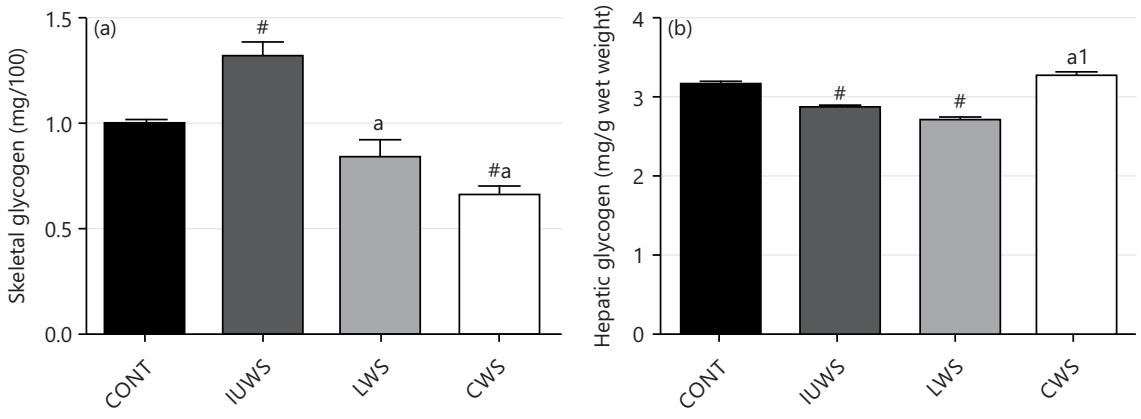

Fig. 1(a-b): Outcome of WN supplementation on (a) Skeletal glycogen and (b) Hepatic glycogen in CONT and treated rats

Represent value Mean±SEM, n = 6, noticeable levels ($^{\#}p<0.05$ vs CONT, $^{a}p<0.05$ vs 0GWS and $^{1}p<0.05$ vs LWS), #, a and 1: Represent significant levels when compared with CONT, GWS and LWS, respectively, CONT: Control, GWS: Gestational walnut supplementation, LWS: Lactational walnut supplementation and GLWS: Gestation and lactation walnut supplementation

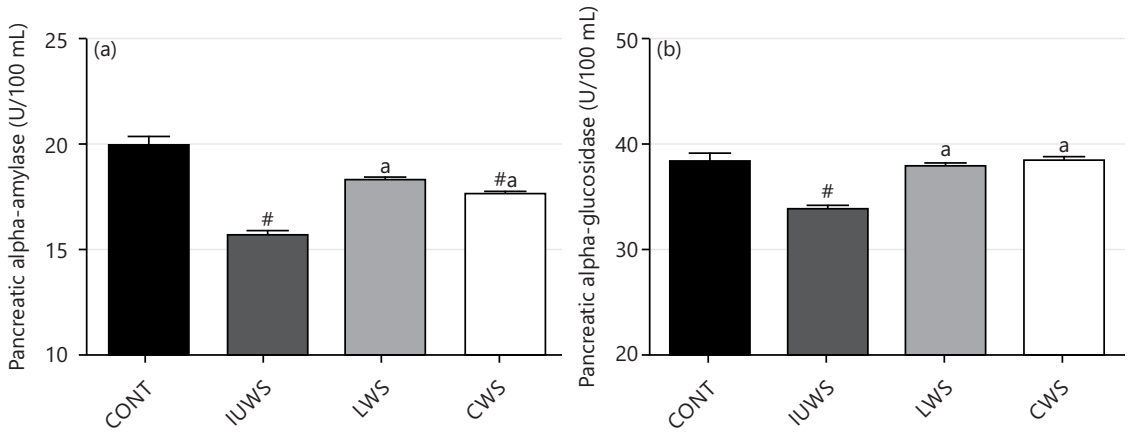

Fig. 2(a-b): Outcome of WN supplementation on (a) Pancreatic alpha amylase and (b) Pancreatic alpha glucosidase in CONT and treated rats

Represent value Mean±SEM, n = 6, noticeable levels ($^{\#}p<0.05$ vs CONT, $^{a}p<0.05$ vs GWS and $^{1}p<0.05$ vs LWS), #, a and 1: Represent significant levels when compared with CONT, GWS and LWS, respectively, CONT: Control, GWS: Gestational walnut supplementation, LWS: Lactational walnut supplementation, GLWS: Gestation and lactation walnut supplementation

Table 1: Outcome of perinatal WN supplementation FBG, FSI and HOMA-IR in CONT and treated rats

Parameter	CONT	GWS	LWS	GLWS
FBG (mg dL^{-1})	53.60±1.21	43.00±0.95$^{\#}$	57.40±0.51$^{\#a}$	41.80±0.37$^{\#1}$
FSI (ng mL^{-1})	8.30±0.17	7.33±0.16$^{\#}$	8.97±0.15$^{\#a}$	7.28±0.14$^{\#1}$
HOMA-IR (AU)	2.48±0.86	1.77±0.08$^{\#}$	2.46±0.14^{a}	1.78±0.06$^{\#a}$

Mean±SEM, n = 6, noticeable levels: ($^{\#}p<0.05$ vs CONT, $^{a}p<0.05$ vs GWS and $^{1}p<0.05$ vs LWS), #^{a} and 1: Represent significant levels when compared with CONT, GWS and LWS, respectively, CONT: Control, GWS: Gestational walnut supplementation, LWS: Lactational walnut supplementation and GLWS: Gestation and lactation walnut supplementation

Outcome of perinatal WN supplementation on pancreatic alpha-amylase and alpha-glucosidase: Results from pancreatic alpha-amylase assay showed a noticeable downturn $p<0.05$ in GWS and GLWS matched with CONT. However, perinatal WN supplementation produced a noticeable increase of $p<0.05$ in LWS and GLWS matched with GWS Fig. 2a.

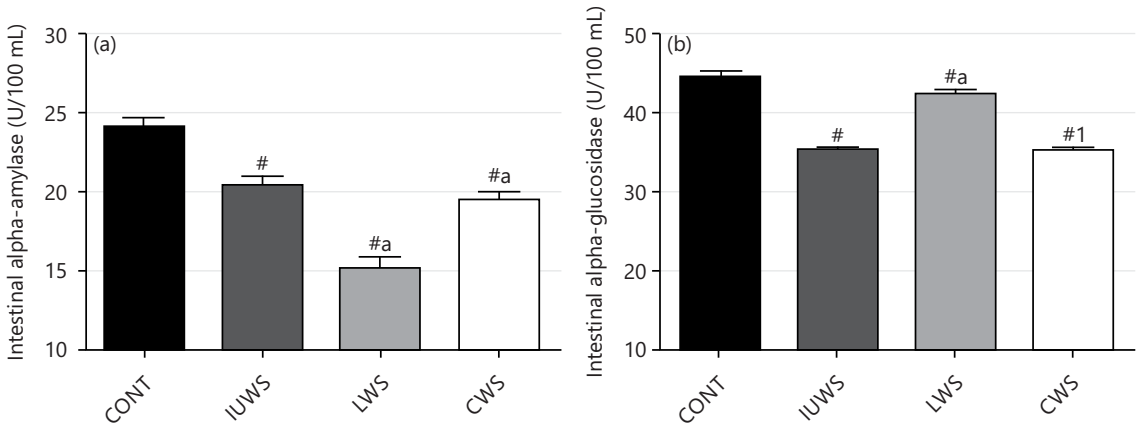

Fig. 3(a-b): Outcome of WN supplementation on (a) Intestinal alpha-amylase and (b) Intestinal alpha-glucosidase in CONT and treated rats

Represent value Mean±SEM, n = 6, noticeable levels ($^{\#}p<0.05$ vs CONT, $^{a}p<0.05$ vs GWS and $^{1}p<0.05$ vs LWS), #, a and 1: Represent significant levels when compared with CONT, GWS and LWS, respectively, CONT: Control, GWS: Gestational walnut supplementation, LWS: Lactational walnut supplementation and GLWS: Gestation and lactation walnut supplementation

Figure 2b showed a noticeable downturn $p<0.05$ in pancreatic alpha glucosidase matched with CONT. The result was not noticeable ($p>0.05$) in LWS and GLWS matched with CONT. However, a noticeable downturn $p<0.05$ was observed in LWS and GLWS matched with GWS.

Outcome of perinatal WN supplementation on intestinal alpha-amylase and alpha-glucosidase: Results from intestinal alpha amylase assay showed a noticeable downturn $p<0.05$ in GWS, LWS and GLWS matched with CONT. However, perinatal WN supplementation produced a noticeable downturn $p<0.05$ in LWS and GLWS matched with GWS (Fig. 3a).

Figure 3b exhibited a noticeable downturn $p<0.05$ in intestinal alpha glucosidase in GWS, LWS and GLWS matched with CONT. The result was remarkably increased $p<0.05$ in LWS matched with GWS and GLWS.

DISCUSSION

WN has generally been used as a supplement for human consumption since ancient times. The high oil and protein values of the *Juglans regia* L., kernels make them essential for human use. As such, it is classified as a vital agent for the human diet and is added to the FAO list of plants with high advantage[14]. There was a noticeable reduction in fasting blood glucose in GWS and LWS which is suggestive of the hypoglycemic outcome of the perinatal WN supplementation. The blood sugar-lowering outcome of WN leaves has been reported by previous studies[14]. Intake of WN leaf pellets in diabetic rats has also been reported to reduce fasting blood sugar *J. regia* and leaves methanolic extract was equally reported to downturn the postprandial plasma glucose in short and long-term models[15].

Perinatal WN supplementation produced a downturn in fasting serum insulin levels in GWS and LWS offspring which is suggestive of hypoinsulinaemia. A previous study reported that there was no noticeable difference in the level of insulin of leaf and peel extract as well as insulin groups in juxtaposition with control and treated groups and the glucose-lowering outcome of these plants may be a result of stimulation of β-cells of the pancreas to produce more insulin increasing glucose equilibrium improving insulin action and binding carbohydrate with high fibre level[16]. Lower HOMA-IR is in tandem with a measure of insulin resistance where a lower number indicates that the tissues are less resistant to insulin.

Lower FBG and FSI in the progeny of GWS and GLWS exposed to perinatal WN supplementation to reduce insulin resistance. It is therefore evident that perinatal WN supplementation in this study positively influenced glucose equilibrium which may be connected to the role of WN as a hypoglycemic plant.

The synthesis of glucose (via glycogenolysis and gluconeogenesis) and its release into the circulation are two of the many functions of the liver. The liver is the second largest organ and is largely a metabolic organ[17]. It is a crucial organ that controls blood sugar, as an imbalance between the liver's release of glucose and its uptake by peripheral tissues can cause blood sugar to rise steadily, which is a major risk factor for developing diabetes[11]. Increased insulin response in peripheral tissues resulted in increased glucose uptake, utilization and absorption and glycogen release in the liver and skeletal muscle of GLWS and GWS offspring, respectively.

An important enzyme called pancreatic amylase converts large, soluble starch molecules into smaller, soluble ones that can then be absorbed and converted into maltose[9]. It is reported that *in vitro*, alpha-glucosidase activity for the sucrase and maltase enzymes was substantially reversed by WN plant extract.[8], whereas insulin and Glut-4 gene expression remained unaffected. According to the author, the caffeoylquinic acid and gallic acid in the leaves are what provide the plant extract with their inhibitory effects[18]. The small intestine's lining is home to alpha-glucosidase, which catalyzes the last phase of the digestion of the many starches and disaccharides found in the typical human diet. For diabetics, these enzyme inhibitors lower post-meal blood glucose levels by delaying the digestion of carbohydrates. Alpha-glucosidase GWS, LWS and GLWS offspring decreased as a result of this study indicative of hypoamylasemia. A previous research project by Fukuda *et al*.[18] concluded that WN polyphenols along with other polyphenolic components including tellimagradin I, tellimagradin II and casuarictin act on many enzymes including amylase, glycosidase, maltase and sucrose. Researchers also found that the polyphenol-rich component of WN has triglyceride and urinary peroxide-lowering effects in type II diabetes mellitus, which is caused by hereditary inheritance. The results of this study showed that glucosidase activity was reduced and probably inhibited in GWS, LWS and GLWS offspring, suggesting that there was less alpha-glucosidase transit in the blood.

The implication of this study is that understanding the metabolic effects of studying the glucometabolic response to a walnut-supplemented diet in rats can provide valuable insights into how specific dietary components may influence blood glucose levels and metabolic processes in animals and the potential health benefits suggest potential health benefits for humans with similar metabolic conditions, such as diabetes or impaired glucose tolerance. The study's outcomes contributed to dietary recommendations for individuals at risk of or already experiencing metabolic disorders.

Findings from this study can be a basis of application for further investigation in human clinical trials, where the effects of Walnut consumption on glucometabolic parameters can be evaluated in greater detail and this study could be incorporated into dietary guidelines or recommendations for individuals and/or pregnant mothers with metabolic concerns.

Recommendations include controlled experiments: The study design should include appropriate controls to isolate the effects of the walnut diet on glucometabolic responses.

Sample size and randomization: An adequate sample size and randomization of the animals into experimental groups to reduce bias and increase statistical power.

Monitoring and measurements: Regularly monitor the rats' glucose levels and other relevant metabolic markers throughout the study.

Standardized diets: Maintenance of consistent and standardized diets for all experimental groups, except for the walnut intervention, to control for confounding factors.

Limitations of this study include animal-to-human extrapolation: Findings from this study may not directly translate to human responses due to differences in metabolism, physiology and diet.

Study duration: This study is a short-term and may not capture the long-term effects of a walnut diet on glucometabolic responses.

Specificity of results: The effects observed may be specific to rats or this particular group of rats, making it essential to replicate the study in different animal models or human subjects for validation.

CONCLUSION

In conclusion, this study suggests that exposure to a walnut-enriched diet during pregnancy and/or lactation improved glucose metabolism, as reflected in reduced insulin resistance and down-regulation of the enzymes alpha-amylase and alpha-glucosidase. In clinical studies, regular consumption of nuts has a dose-dependent sugar-lowering effect, so small amounts have only a moderate effect. Because of their high activity, walnut supplementation can be used in lower doses than when using whole walnuts to provide a glucose effect. However, it is worth noting that the outcomes were dependent on the window of exposure.

SIGNIFICANCE STATEMENT

The long-term goal of this research is to develop a formalized nutritional awareness with respect to Walnut consumption. The current study sought to fill a gap in the literature on the role of Walnut consumption in the offspring of mothers of child-bearing age and the potential impact on glucose homeostasis. Findings from this study will help to define the role of a perinatal Walnut (*Juglans regia* L.) supplementation on glucose homeostasis. The current study will also yield information on the impact of a perinatal Walnut (*Juglans regia* L.) supplementation during pregnancy and/or lactation in influencing the growth and development of the offspring. Furthermore, the study will serve as a tool to elucidate other metabolic programming that may lead to a decreased risk of chronic diseases in later life.

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