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PERIPUBERTAL EXPOSURE TO GLYPHOSATE-BASED HERBICIDE MODIFIES THE EFFECTS OF A HIGH-FAT DIET ON DIAPHRAGM HISTOMORPHOMETRY OF MICE

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ABSTRACT

The aim of this study was to determine whether peripubertal exposure to glyphosate-based herbicide influences the deleterious effects of obesity induced by a high-fat diet on the diaphragm muscle of adult mice. Ten male C57BL/6 mice were randomly divided into two groups (n = 5 per group): a control group that received a high-fat diet and water by gavage, and an experimental group that received a high-fat diet and glyphosate-based herbicide (50 mg Roundup®/kg per day by gavage) from 30 to 60 days of life. From 60 to 150 days, both groups received the high-fat diet. At 150 days, the animals were euthanized and weighed. The nasoanal length was measured and fats and diaphragm were collected and weighed. The muscle was stored in specific fixatives for histological analysis of muscle fibers and neuromuscular junctions (NMJs). There was no difference in macroscopic parameters between groups. Morphological analysis of muscle fibers showed degenerative changes in the diaphragm of both groups; however, these alterations were more marked in the group exposed to the glyphosate-based herbicide. Regarding the morphometric characteristics, a significant increase in the number of degenerated fibers per mm^2 was observed in the HFD-GBH group compared to the HFD-CTL group. The area and maximum diameter of NMJs were also greater in the herbicide-exposed group. In conclusion, obesity promotes degenerative changes in diaphragm muscle fibers of adult mice. Peripubertal exposure to glyphosate-based herbicide exacerbates the myotoxic effects of obesity and increases the area and maximum diameter of NMJs in obese adult mice.

KEYWORDS

Pesticides, glyphosate, obesity, skeletal muscle fibers, diaphragm

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INTRODUCTION

Developmental Origins of Health and Disease (DOHaD) is an area of science that investigates the effects caused by insults that occur at critical stages of development and that can result in physiological, morphological, and metabolic changes, consequently affecting health and disease patterns throughout life[1,2]. The different environmental insults that harm human health include the effects of pesticides[2]. Pesticides and many other industrial chemicals, plasticizers, pharmaceuticals, and metals are considered endocrine disruptors[3-6]. Ingested through contaminated food, water or air or through skin contact[6,7] these substances or exogenous mixtures can compromise normal endocrine function[3,4] and can lead to the development of a variety of reproductive, neurological or metabolic pathologies in the body[7], offspring or subpopulations[3].

Puberty is currently recognized as an important phase of life since it is characterized by a high degree of plasticity[8], and is therefore susceptible to the effects of endocrine disruptors whose deleterious effects may only manifest in adulthood[6,9,10]. Hence, endocrine changes induced by chemical compounds during this sensitive period can lead to deleterious effects in adulthood, supporting the DOHaD theory[11].

Glyphosate [N-(phosphonomethyl) glycine], an organophosphate herbicide, is the most widely used herbicide in the world and the active ingredient of all glyphosate-based herbicides[12], including Roundup®[13]. Its herbicidal activity is exacerbated when combined with other chemical products, called co-formulants[12]. Glyphosate kills weeds and grasses by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme of the shikimic acid metabolic pathway. Since this pathway does not occur in vertebrates, glyphosate is considered to be safe for mammals[14,15]. However, evidence accumulated over time has demonstrated the harmful effects of glyphosate-based herbicides on different species[16], including humans[17,18]. These herbicides are considered potential endocrine disruptors[14,19].

Recent human biomonitoring studies have demonstrated that exposure to glyphosate can occur during childhood and adolescence. In the United States, data from the National Health and Nutrition Examination Survey (NHANES) showed that glyphosate was detected in urine samples from 70% to 82% of participants, including children as young as 3 years old, indicating widespread exposure in the general population[20,21]. Similarly, Lemke et al.[22] reported detectable urinary concentrations of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in 52% of German children and adolescents aged 3 to 17 years, confirming that exposure also occurs in nonoccupational European populations. Furthermore, a longitudinal cohort study conducted in California (CHAMACOS) identified measurable urinary levels of glyphosate and AMPA from childhood through adolescence, with higher concentrations associated with proximity to agricultural applications and dietary intake of plant-based foods[23].

Humans can be exposed to glyphosate through multiple routes, primarily dietary intake, environmental contamination, and dermal or inhalation contact. Dietary exposure is considered the main pathway because glyphosate residues are frequently detected in cereals, legumes, and other plant-based foods, including imported products derived from glyphosate-resistant crops[22,23]. Additional exposure may occur through contaminated drinking water, air, or dust near agricultural areas, and through the domestic use of herbicides in lawns and gardens[20,23]. Exposure to glyphosate can cause oxidative stress, affecting the nervous system, reproductive system, and organs such as liver and kidneys[24], as well as changes in the endocrine system[13]. The high rate of morbidity observed in organophosphate poisoning is associated with the involvement of skeletal striated muscles, especially respiratory muscles[25].

Evidence indicates that exposure of humans to endocrine-disrupting chemicals at critical stages of development may also be linked to obesity[9,10,26], a disease characterized by excess body fat caused by the complex interaction between genetic and environmental factors (especially eating habits and sedentarism). Obesity has reached global epidemic proportions, a fact that renders it a human health crisis[9,10], causing or aggravating many health problems in children, adolescents, and adults[27]. The short-term effects of obesity include cardiometabolic, respiratory, musculoskeletal, endocrine and psychosocial consequences and an increase in cancer risk[9]. In addition, the disease affects and accompanies a large proportion of children and adolescents into adulthood, causing different chronic diseases[28].

The lower percentage of muscle mass in obese individuals is associated with diseases such as hypertension and diabetes mellitus[29]. Obesity may also impair muscle architecture and function due to systemic inflammatory processes affecting myofiber morphology and extracellular matrix composition[30]. The disease can also affect respiratory function, including the diaphragm muscle, as a result of greater respiratory effort and impaired gas exchange. In addition, abdominal muscle hypertonia caused by obesity can compromise respiratory function dependent on diaphragmatic action as a result of reduced muscle performance and chest expansion[31]. A recent study has demonstrated an inverse association between obesity and cardiorespiratory and muscle performance in adolescents. Overweight was also inversely correlated with cardiorespiratory performance during the 4-year follow-up period[28].

Given that peripubertal exposure to glyphosate-based herbicide (GBH) alone has already been associated with histological and functional impairment in the diaphragm muscle[32], it is crucial to investigate whether this exposure

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potentiates additional metabolic insults, such as those induced by a high-fat diet. The aim of this study was to determine whether peripubertal exposure to a GBH influences the deleterious effects of obesity induced by a high-fat diet (HFD) on the diaphragm muscle of adult mice.

METHODS

Ethical aspects

The Ethics Committee on Animal Use of Universidade Estadual do Oeste do Paraná (UNIOESTE) approved all experimental procedures on May 11, 2018 (Protocol nº 08/18). The institutional guidelines for the use of animals in research were followed for maintenance and care of animals.

Animals

Ten male C57BL/6 mice, 21 days old and mean body weight of 20 g, were used. The animals were obtained from the central animal house of UNIOESTE and were kept in the sectorial animal house of the Laboratory of Endocrine Physiology and Metabolism (LAFEM) under controlled conditions of temperature (28 ± 2ºC) and lighting (12-h light/dark) in polypropylene cages.

Experimental groups

At 30 days of age, the animals were weaned and randomly divided into two groups. Only two offspring of the same dam were allocated to the same group: control group (CTL, n = 5) and glyphosate-based herbicide group (GBH, n = 5). From 30 to 60 days of age, the CTL group received water and the GBH group received 50 mg/kg body weight[33] of Roundup® Original DI (Monsanto, Brazil), which contained 445 g/l of N-(phosphonomethyl) glycine diammonium salt, corresponding to 370 g/l or 37.0% (w/v) of the active component glyphosate. According to the European Food Safety Authority (EFSA), this concentration corresponds to the no-observed-adverse-effect level (NOAEL) for glyphosate, representing a sub-toxicological dose commonly employed in experimental studies to investigate mechanistic and developmental effects[34]. Both groups were fed daily the established solution by gavage, always at the same time. The pH of the GBH solution and water was corrected to 5-5.5 with hydrochloric acid. All mice had ad libitum access to water and were fed a standard rodent diet (Nuvital, Brazil) during this period.

Induction of obesity

Once the animals had completed 60 days of life, the CTL and GBH groups were exposed to the HFD as follows: high-fat diet control (HFD-CTL group, n = 5) –animals received water by gavage and were fed the standard diet from 30 to 60 days and the HFD from 60 to 150 days for the induction of obesity; high-fat diet + glyphosate-based herbicide (HFD-GBH group, n = 5)– GBH was administered by gavage and the animals were fed the standard diet from 30 to 60 days and the HFD from 60 to 150 days.

The standard diet for laboratory rodents consisted of 70% carbohydrates, 20% proteins and 10% fats, containing 3.8 kcal/g. The diet was obtained from the central animal house of UNIOESTE (Nuvital, Brazil). The HFD was prepared with 50% triturated standard ration, 31.2% pork fat, 14.8% casein, and 4% soybean oil, containing 6.2 kcal/g.

Euthanasia and tissue collection

At 150 days of age, the animals were weighed and the nasoanal length was measured. These measurements were used for calculation of the Lee index [body weight $(g)^{1/3}$ / nasoanal length (cm) × 1000][35]. The animals were anesthetized with a mixture of xylazine hydrochloride (9 mg/kg; Anasedan®, Vetbrands, Brazil) and ketamine hydrochloride (90 mg/kg; Dopalen®, Vetbrands, Brazil). Laparotomy was then performed. The retroperitoneal and perigonadal fat were collected and weighed. For collection of the diaphragm muscle, the animals were placed in a ventral position and a midline incision was made just below the thorax, followed by elevation of the skin and muscles.

Macroscopic parameters	HFD-CTL	HFD-GBH	p value
Body weight (g)	39.73 ± 4.02	38.06 ± 4.73	0.5274
Nasoanal length (cm)	9.28 ± 0.22	9.54 ± 0.26	0.1216
Lee index	367.46 ± 11.44	352.16 ±1 4.52	0.2351
Retroperitoneal fat (g)	0.69 ± 0.07	0.67 ± 0.12	0.7768
Perigonadal fat (g)	2.13 ± 0.38	1.83 ± 0.49	0.4439
Diaphragm muscle weight (g)	0.05 ± 0	0.04 ± 0	0.2875

Table 1. *Macroscopic parameters of C57BL/6 mice at 150 days of age in the high-fat diet control group and high-fat diet + glyphosate-based herbicide group.*

Legend: Values are expressed as the mean ± standard deviation. Kolmogorov-Smirnov test. Student t- test ($p < 0.05$). Abbreviations: HFD-CTL, high-fat diet control, HFD-GBH, high-fat diet + glyphosate-based herbicide.

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Only the costal part of the diaphragm was removed and divided into right and left antimeres. Each antimere was cut into two fragments. The upper and lower fragments of the right antimere were processed and embedded in paraffin for the histological study of muscle fibers and the upper and lower fragments of the left antimere were submitted to histochemical staining for the study of neuromuscular junctions (NMJs).

Histological study of muscle fibers

The fragments of the right antimere of the diaphragm were fixed in methacarn, cut transversely, and stained with hematoxylin-eosin[36]. Ten microscopic fields per animal were analyzed at 40× objective and used for the quantification of muscle fibers, peripheral and central nuclei, capillaries, nuclei/fiber ratio, capillary/fiber ratio, and muscle fiber degeneration. The field area at this objective was 0.147 mm^2, resulting in a total analyzed area of 1.472 mm^2 per animal. All measurements, including the identification and counting of degenerated fibers, were performed using Image Pro Plus 6.0® (Media Cybernetics, Maryland, USA). For most variables, the mean value from the ten fields was considered representative for each animal, while the degenerated fibers were expressed as the number of fibers per mm^2, calculated by dividing the total number of degenerated fibers counted in the ten microscopic fields by the total analyzed area (1.472 mm^2 per animal). The mean value obtained for each animal was used for statistical analysis.

Morphological and morphometric study of NMJs

The fragments of the left antimere of the diaphragm were fixed in Karnovsky solution[37] and cut longitudinally into several thin sections with a stainless-steel blade. The sections were submitted to the nonspecific esterase reaction[38]. Morphometric analysis of NMJs consisted of the measurement of the area and maximum and minimum diameters of 100 NMJs per animal on microscopic images (20× objective).

Statistical analysis

The data were analyzed statistically using the GraphPad Prism® program (La Jolla, USA). Normality was evaluated by the Kolmogorov-Smirnov test. The Student t-test was applied and a p value <0.05 was considered to be statistically significant.

RESULTS

Macroscopic parameters

There was no significant difference in macroscopic parameters between the HFD-GBH and HFD-CTL groups *(Table 1)*.

Morphological and morphometric analysis of muscle fibers

The standard morphological structure was preserved in most muscle fibers of the diaphragm in both groups, i.e., polygonal multinucleate cells with peripheral nuclei that were arranged in fascicles *(Fig. 1A and 1B)*. However, some areas in the muscle contained fibers with large nuclei exhibiting a basophilic halo *(Fig. 1C and 1D)* and central nuclei *(Fig. 1E and 1F)*. Many rounded and hypereosinophilic muscle fibers were also observed in the

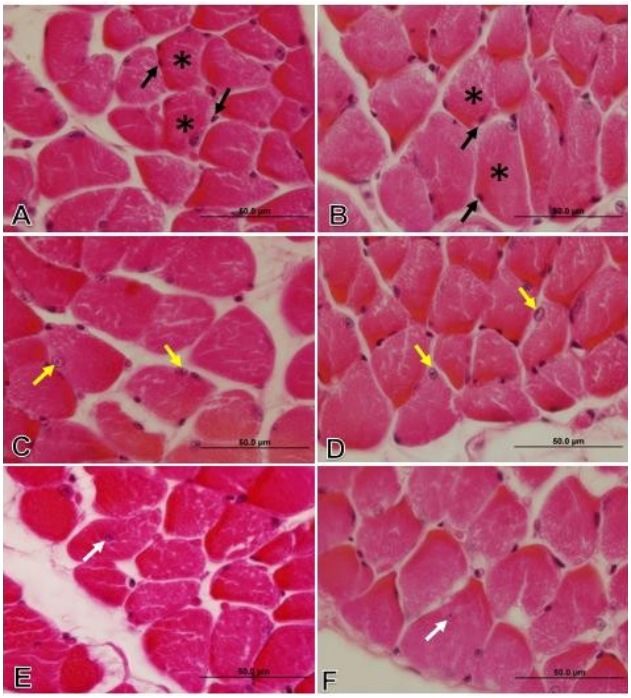


Figure 1. *Photomicrographs of the diaphragm muscle of C57BL/6 mice at 150 days of age. Cross-section, hematoxylin-eosin staining. High-fat diet control group (A, C, and E). High-fat diet + glyphosate-based herbicide group (B, D, and F). A and B) polygonal muscle fibers (*) with peripheral nuclei (black arrow). C and D) large nuclei with a basophilic halo (yellow arrow). E and F) central nuclei (white arrow).*

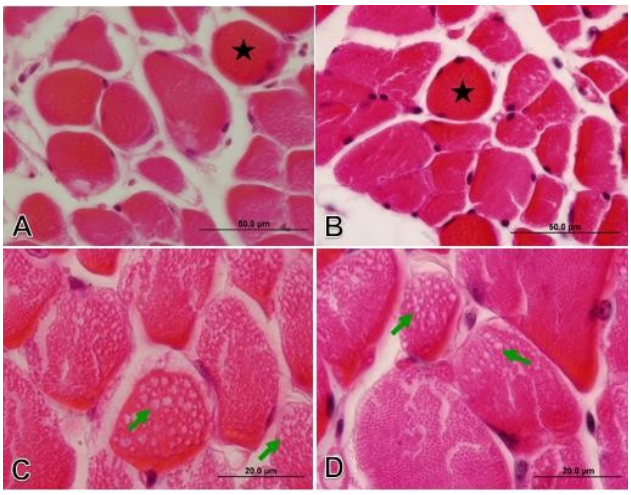


Figure 2. *Photomicrographs of the diaphragm muscle of C57BL/6 mice at 150 days of age. Cross-section, hematoxylin-eosin staining. High-fat diet control group (A and C). High-fat diet + glyphosate-based herbicide group (B and D). A and B) rounded and hypereosinophilic muscle fibers (star). C and D) muscle fibers with vacuolized cytoplasm (green arrow).*

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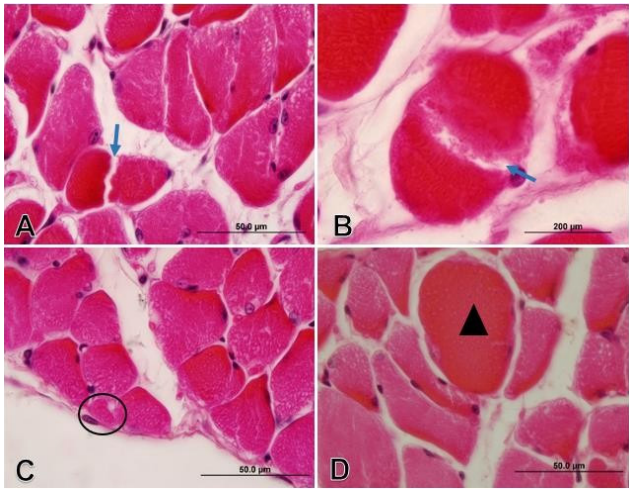


Figure 3. *Photomicrographs of the diaphragm muscle of C57BL/6 mice at 150 days of age. Cross-section, hematoxylin-eosin staining. High-fat diet + glyphosate-based herbicide group. A and B) muscle fiber splitting (blue arrow). C) atrophied muscle fiber (ellipse). D) hypertrophic and hypereosinophilic fiber (triangle).*

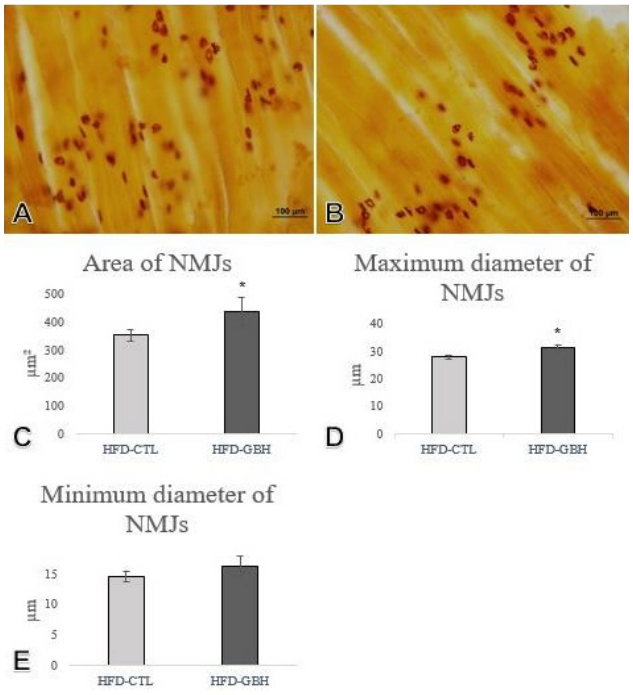


Figure 4. *Photomicrographs of neuromuscular junctions (NMJs) in the diaphragm muscle of C57BL/6 mice at 150 days of age. Longitudinal section, nonspecific esterase reaction. High-fat diet control group (HFD-CTL) (A). High-fat diet + glyphosate-based herbicide group (HFD-GBH) (B). A and B) polymorphic NMJs showing no morphological changes. C, D and E) show the area, maximum diameter and minimum diameter of NMJs, respectively. The asterisk indicates a significant difference ($p < 0.05$). Kolmogorov-Smirnov test. Student t-test.*

two groups studied *(Fig. 2A and 2B)*, as well as fibers with destructured myofibrils in the cytoplasm (vacuolization) *(Fig. 2C and 2D)*.

Muscle fiber splitting (longitudinal division of the muscle fiber) *(Fig. 3A and 3B)*, degenerating muscle fibers, some atrophic fibers *(Fig. 3C)*, and other hypertrophic and hypereosinophilic fibers with homogeneous cytoplasm without striations (hyalinization) *(Fig. 3D)* were observed exclusively in the HFD-GBH group.

Regarding the morphometric characteristics of diaphragm muscle fibers, a significant increase in the number of degenerated fibers per mm^2 was observed in the HFD-GBH group compared to the HFD-CTL group *(Table 2)*.

Morphological and morphometric analysis of NMJs

The NMJs found in the diaphragm of the animals studied were polymorphic, exhibiting an oval, round or elliptic shape. No morphological changes were observed *(Fig. 4A and 4B)*.

Morphometric analysis showed an increase in the area and maximum diameter of NMJs in the HFD-GBH group compared to the HFD-CTL group *(Fig. 4C and 4D)*. There was no significant difference in minimum diameter between groups *(Fig. 4E)*.

DISCUSSION

According to the literature, individual insults do not necessarily promote significant changes in adulthood but the combination of two insults at different stages may explain the emergence of some pathologies in the future. In other words, genetic susceptibility combined with an insult occurring at some stage of development (first-hit) can lead to the reorganization of different organ systems, which alone may be insufficient to alter the adult phenotype. However, a “second-hit” caused by new insults at a later stage in life can result in endocrine alterations that are sufficient to reveal unseen damage or even to amplify it, resulting in pathologies[39]. Hence, in the present study, the exposure to glyphosate during the peripubertal period was considered the first-hit and the induction of obesity by the HFD in adulthood the second-hit.

Peripubertal exposure to GBH from 30 to 60 days of age did not exacerbate the body weight of male adult mice with HFD-induced obesity. Romano et al. (2008)[33] also found no body weight changes in male rats receiving doses of Roundup Transorb of 5, 50 and 250 mg/kg live weight by gavage from days 23 to 53. According to Zhao et al. (2021) [40], oral treatment with glyphosate in water for 4 weeks also did not alter the body weight of male mice. The present results agree with the literature showing that glyphosate was unable to alter the body weight of male mice.

Still regarding body weight, Kubsad et al. (2019)[41] observed important generational toxicology when pregnant rats were exposed to glyphosate (25 mg/kg) for 14 days during gestation. The results showed body weight loss at weaning in the F1 generation in both females and males, while there were no body weight changes in the F2 generation. In the F3 generation, only male rats exposed to glyphosate exhibited body weight gain. In addition, an increase in the frequency of obesity was observed in the

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Morphometric parameters	HFD-CTL	HFD-GBH	p value
Fiber area (µm²)	885.15 ± 123.82	770.02 ± 186.06	0.3411
Maximum diameter (µm)	41.99 ± 3.38	38.20 ± 5.17	0.2692
Minimum diameter (µm)	25.04 ± 1.73	23.28 ± 2.58	0.2747
Number of fibers	953.20 ± 153.02	937.60 ± 207.81	0.9106
Peripheral nuclei	1376 ± 188.71	1516.80 ± 202.94	0.2824
Central nuclei	43.20 ± 13.48	40.20 ± 4.71	0.6004
Number of capillaries	541 ± 99.33	591.20 ± 70.74	0.4870
Nuclei/fiber ratio	1.45 ± 0.17	1.64 ± 0.17	0.2148
Capillary/fiber ratio	0.57 ± 0.08	0.64 ± 0.08	0.1494
Number of degenerated fibers (per mm²)	11.3 ± 3.03	18.6 ± 6.02[A]	0.0411

Table 2. *Morphometric analysis of diaphragm muscle fibers of C57BL/6 mice at 150 days of age in the high-fat diet control group and high-fat diet + glyphosate-based herbicide group.*

Legend: Values are expressed as the mean ± standard deviation. Kolmogorov-Smirnov test. Student t- test (p <0.05). HFD-CTL, high-fat diet control; HFD-GBH, high-fat diet + glyphosate-based herbicide; A, statistical difference versus control HFD-CTL (p <0.05).

F2 and F3 generations. The authors concluded that direct exposure has low toxicity, and that glyphosate promotes transgenerational epigenetic inheritance[41]. Within this context, the present results confirm that direct exposure to GBH during the peripubertal period does not cause body weight changes in adulthood. However, the literature shows that the endocrine disruption potential of glyphosate can modify metabolism and thus interfere with body weight and predisposition to obesity in future generations[41].

Yan et al. (2021)[42] observed gastrocnemius muscle atrophy in obese mice induced by feeding the animals a HFD for 3 months. Another recent study on obese male rats induced by a hypercaloric diet for 16 weeks showed muscle fiber degeneration, increased fat accumulation, and recruitment of macrophages in the biceps femoris muscle. Small and rounded fibers and hypertrophic fibers were also found. According to the authors, these fibers are the result of repeated cycles of muscle degeneration and regeneration[43]. The findings of the present study corroborate literature data showing that obesity induced by a HFD can cause degenerative lesions in diaphragm muscle fibers. Furthermore, the diaphragm is considered to be the main respiratory muscle[25] and obesity is known to cause chronic respiratory overload[44]. Thus, the choice of the studied muscle (diaphragm) may have also influenced the myopathic changes found in obese mice.

Areas containing muscle fibers with altered morphology were detected in the diaphragms of the HFD-GBH and HFD-CTL groups. The nuclei had a larger size. An increased number of sarcolemmal nuclei is indicative of an early regenerative response[45], increasing the protein synthesis rate[46]. Thus, the enlarged volume of myonuclei, together with the other results of this study, demonstrates that the muscle fibers underwent a regeneration process in order to minimize the damage caused by fiber degeneration induced by obesity alone and by obesity associated with GBH.

In addition to enlarged nuclei, mice of the two groups exhibited many rounded and hypereosinophilic fibers as well as vacuolization of the cytoplasm. Rounded muscle fibers suggest myopathy[47], as do vacuolization and fiber division. Such alterations also occurred in skeletal muscle after systemic administration of agents with direct myotoxic effects[48].

Taken together, the results suggest that obesity induced by a HFD alone promoted toxic effects on skeletal muscle. When the HFD was combined with GBH (HFD-GBH group), some myopathic features were only found in this group, which can be explained by the myotoxicity of pesticides, as demonstrated by Cavaliere et al. (1996)[25] who reported muscle fiber necrosis in the diaphragm of rats poisoned with sublethal doses of the organophosphate paraoxon. Bright et al. (1991)[49] administered a sublethal dose of the organophosphate sarin to mice and observed muscle fiber degeneration and mononuclear infiltration, preceded by calcium accumulation.

Obese adult mice exposed to GBH from 30 to 60 days of life exhibited fibers with a longitudinal division of the cytoplasm (splitting). This morphophysiological adaptation of segmentation (internal or lateral) of the fiber is aimed at favoring metabolic exchanges and at preventing injuries by reducing the distance between the capillary network and cytoplasm[50]. Studies have shown that these alterations can be triggered by different processes such

as stress resulting from exercise, metabolic stress, and microcirculatory changes[50]. We therefore believe that this division of the muscle fiber is the result of attempted cell recovery after damage caused by the stress arising from the combination of GBH exposure at a critical stage of development and obesity in adulthood.

Atrophic, hypertrophic and hypereosinophilic fibers were found in the HFD-GBH group. The enlargement of some muscle fibers observed in this study can be explained by compensatory hypertrophy, which is characterized by concomitant atrophic changes in the muscle[47], i.e., in response to the atrophy of some fibers, there was an overload of other fibers, inducing fiber hypertrophy. Atrophic fibers also indicate a degenerative process. Atrophy resulting from non-neurogenic causes, such as the administration of myotoxic chemicals, is generally characterized by the presence of myopathic changes such as rounded fibers, cells with central nuclei, and fibers undergoing splitting[47].

Analysis of NMJs showed an increase in area and maximum diameter in the HFD-GBH group when compared to the HFD-CTL group. Acetylcholinesterase plays a key role in terminating cholinergic signaling at the NMJ[51]. However, since acetylcholinesterase activity was not evaluated in the present study, the mechanisms underlying the observed morphological changes remain unclear and cannot be directly attributed to alterations in cholinergic transmission.

The enlargement of the NMJs observed in the HFD-GBH group may reflect an adaptive or compensatory remodeling in response to synaptic stress induced by peripubertal exposure to GBH. According to Mantilla and Sieck (2009)[52], inactivity or altered synaptic input in the diaphragm promotes morphological adaptations such as increased junctional area and terminal branching, which represent attempts to maintain neurotransmission under stressful or unbalanced conditions rather than indicators of enhanced function. Similarly, Campanari et al. (2016)[53] reported that structural remodeling of the NMJ—characterized by altered acetylcholine receptor (AChR) distribution and disorganization of the postsynaptic membrane—is often associated with impaired cholinergic transmission efficiency. Given that the diaphragm requires continuous and finely regulated activation to sustain effective ventilation, such remodeling may compromise excitation–contraction coupling, leading to subtle but functionally relevant impairments in respiratory performance[52].

It is important to note that the present study compared two groups exposed to a high-fat diet in adulthood, differing only in the peripubertal exposure to GBH. As such, the strong metabolic impact of the high-fat diet may have masked some of the specific effects attributable to GBH exposure. Although the inclusion of groups fed a standard diet (with or without peripubertal GBH exposure) would allow a clearer isolation of these effects, this limitation was addressed in a previous study by Zazula et al. (2024)[32], which was conducted using the same experimental cohort and exposure protocol. That study demonstrated that peripubertal GBH exposure alone induces histopathological changes in the diaphragm muscle of adult mice. Taken together, these findings indicate that GBH exposure can cause myotoxic effects even under normal dietary conditions and may exacerbate the damage induced by obesity-related metabolic stress.

CONCLUSION

The results of the present study show that HFD-induced obesity promotes degenerative changes in the diaphragm muscle fibers of adult mice. Peripubertal exposure to GBH from 30 to 60 days of age exacerbates the myotoxic effects of obesity.

REFERENCES

1. Gluckman PD, Hanson MA, Mitchell MD. Developmental origins of health and disease: reducing the burden of chronic disease in the next generation. Genome Med. 2010;2(2):14. doi: 10.1186/gm135.
2. Suzuki K. The developing world of DOHaD. J Dev Orig Health Dis. 2018;9(3):266-269. doi: 10.1017/S2040174417000691.
3. Bergman A, Heindel JJ, Jobling S, Kidd AK, Zoeller RT; World Health Organization, United Nations Environment Programme. State of the science of endocrine-disrupting chemicals - 2012. Geneva: WHO; 2013. 296 p.
4. Lucaccioni L, Trevisani V, Marrozzini L, Bertoncelli N, Predieri B, Lugli L, et al. Endocrine-Disrupting Chemicals and Their Effects during Female Puberty: A Review of Current Evidence. Int J Mol Sci. 2020;21(6):2078. doi: 10.3390/ijms21062078.
5. Yilmaz B, Terekeci H, Sandal S, Kelestimur F. Endocrine disrupting chemicals: exposure, effects on human health, mechanism of action, models for testing and strategies for prevention. Rev Endocr Metab Disord. 2020;21(1):127-147. doi: 10.1007/s11154-019-09521-z.
6. Kalofiri P, Balias G, Tekos F. The EU endocrine disruptors' regulation and the glyphosate controversy. Toxicol Rep. 2021;8:1193-1199. doi: 10.1016/j.toxrep.2021.05.013.
7. Schneider M, Pons JL, Labesse G, Bourguet W. In Silico Predictions of Endocrine Disruptors Properties. Endocrinology. 2019;160(11):2709-2716. doi: 10.1210/en.2019-00382.
8. Silveira PP, Portella AK, Goldani MZ, Barbieri MA. Origens desenvolvimentistas da saúde e da doença (DOHaD). J Pediatr (Rio J). 2007;83(6):494-504. doi: 10.2223/JPED.1728.
9. Newbold RR, Padilla-Banks E, Jefferson WN, Heindel JJ. Effects of endocrine disruptors on obesity. Int J Androl. 2008;31(2):201-8. doi: 10.1111/j.1365-2605.2007.00858.x.
10. Boudalia S, Bousbia A, Boumaaza B, Oudir M, Canivenc Lavier MC. Relationship between endocrine disruptors and obesity with a focus on bisphenol A: a narrative review. Bioimpacts. 2021;11(4):289-300. doi: 10.34172/bi.2021.33.
11. Ivanski F, de Oliveira VM, de Oliveira IM, de Araújo Ramos AT, de Oliveira Tonete ST, de Oliveira Hykavei G, et al. Prepubertal acrylamide exposure causes dose-response decreases in spermatic production and functionality with modulation of genes involved in the spermatogenesis in rats. Toxicology. 2020;436:152428. doi: 10.1016/j.tox.2020.152428.
12. Milesi MM, Lorenz V, Durando M, Rossetti MF, Varayoud J. Glyphosate Herbicide: Reproductive Outcomes and Multigenerational Effects. Front Endocrinol (Lausanne). 2021;12:672532. doi: 10.3389/fendo.2021.672532.
13. Owagboriaye F, Dedeke G, Ademolu K, Olujimi O, Aladesida A, Adeleke M. Comparative studies on endogenic stress hormones, antioxidant, biochemical and hematological status of metabolic disturbance in albino rat exposed to roundup herbicide and its active ingredient glyphosate. Environ Sci Pollut Res Int. 2019;26(14):14502-14512. doi: 10.1007/s11356-019-04759-1.
14. Romano RM, Romano MA, Oliveira CA. Glifosato como desregulador endócrino químico. Ambiência. 2009;5(2):359-72.
15. Ingaramo P, Alarcón R, Muñoz-de-Toro M, Luque EH. Are glyphosate

and glyphosate-based herbicides endocrine disruptors that alter female fertility? Mol Cell Endocrinol. 2020;518:110934. doi: 10.1016/j.mce.2020.110934.
16. Walsh LP, McCormick C, Martin C, Stocco DM. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environ Health Perspect. 2000;108(8):769-76. doi: 10.1289/ehp.00108769.
17. Bai SH, Ogbourne SM. Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. Environ Sci Pollut Res Int. 2016;23(19):18988-9001. doi: 10.1007/s11356-016-7425-3.
18. Thakur DS, Khot R, Joshi PP, Pandharipande M, Nagpure K. Glyphosate poisoning with acute pulmonary edema. Toxicol Int. 2014;21(3):328-30. doi: 10.4103/0971-6580.155389.
19. Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE. Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ Health Perspect. 2005;113(6):716-20. doi: 10.1289/ehp.7728.
20. Ospina M, Schütze A, Morales-Agudelo P, Vidal M, Wong LY, Calafat AM. Exposure to glyphosate in the United States: Data from the 2013-2014 National Health and Nutrition Examination Survey. Environ Int. 2022;170:107620. doi: 10.1016/j.envint.2022.107620.
21. Ospina M, Schütze A, Morales-Agudelo P, Vidal M, Wong LY, Calafat AM. Temporal trends of exposure to the herbicide glyphosate in the United States (2013-2018): Data from the National Health and Nutrition Examination Survey. Chemosphere. 2024;364:142966. doi: 10.1016/j.chemosphere.2024.142966.
22. Lemke N, Murawski A, Schmied-Tobies MIH, Rucic E, Hoppe HW, Conrad A, Kolossa-Gehring M. Glyphosate and aminomethylphosphonic acid (AMPA) in urine of children and adolescents in Germany - Human biomonitoring results of the German Environmental Survey 2014-2017 (GerES V). Environ Int. 2021;156:106769. doi: 10.1016/j.envint.2021.106769.
23. Eskenazi B, Gunier RB, Rauch S, Kogut K, Perito ER, Mendez X, Limbach C, et al. Association of Lifetime Exposure to Glyphosate and Aminomethylphosphonic Acid (AMPA) with Liver Inflammation and Metabolic Syndrome at Young Adulthood: Findings from the CHAMACOS Study. Environ Health Perspect. 2023;131(3):37001. doi: 10.1289/EHP11721.
24. Soudani N, Chaâbane M, Ghorbel I, Elwej A, Boudawara T, Zeghal N. Glyphosate disrupts redox status and up-regulates metallothionein I and II genes expression in the liver of adult rats. Alleviation by quercetin. Gen Physiol Biophys. 2019;38(2):123-134. doi: 10.4149/gpb_2018043.
25. Cavaliere MJ, Calore EE, Perez NM, Puga FR. Miotoxicidade por organofosforados. Rev Saude Publica. 1996;30(3):267-72. doi: 10.1590/S0034-89101996000300010.
26. Baillie-Hamilton PF. Chemical toxins: a hypothesis to explain the global obesity epidemic. J Altern Complement Med. 2002;8(2):185-92. doi: 10.1089/107555302317371479.
27. Li G, Liu JY, Zhang HX, Li Q, Zhang SW. Exercise training attenuates sympathetic activation and oxidative stress in diet-induced obesity. Physiol Res. 2015;64(3):355-67. doi: 10.33549/physiolres.932851.
28. Petrovics P, Sandor B, Palfi A, Szekeres Z, Atlasz T, Toth K, et al. Association between Obesity and Overweight and Cardiorespiratory and Muscle Performance in Adolescents. Int J Environ Res Public Health. 2020;18(1):134. doi: 10.3390/ijerph18010134.
29. Sizoo D, de Heide LJM, Emous M, van Zutphen T, Navis G, van Beek AP. Measuring Muscle Mass and Strength in Obesity: a Review of Various Methods. Obes Surg. 2021;31(1):384-393. doi: 10.1007/s11695-020-05082-2.
30. Lopes Cappellaro G, Petry de Paula TN, Gerson Kuhn M, Lima da Silva RC, Nassar CA, Meire Costa R, et al. The Influence of Fetal Programming due to Maternal Obesity and Periodontitis on the Extensor Digitorum Longus Muscle of Wistar Rats. Muscles, Ligaments and Tendons Journal. 2025;15(2):162–173. doi: 10.32098/mltj.02.2025.06.
31. Rasslan Z, Stirbulov R, Lima CA da C, Júnior RS. Função pulmonar e obesidade. Rev Bras Clin Med. 2009;7(1):36-9.
32. Zazula MF, Marca AP, de Campos Oliveira M, Barbosa A, Bonfleur ML, de Fátima Chasko Ribeiro L, et al. Peripubertal Exposure to Glyphosate-Based Herbicides Promotes Histopathological Impairment in the Structure of the Diaphragm Muscle of C57BL/6 Mice. Muscles, Ligaments and Tendons Journal. 2024;14(1):12–21. doi: 10.32098/mltj.01.2024.02.
33. Romano RM, Romano MA, Moura MO, Oliveira CA. A exposição ao glifosato- Roundup causa atraso no início da puberdade em ratos machos. Braz. J. vet. Res. anim. Sci. 2008;45:481-487. doi: 10.11606/issn.1678-4456.bjvras.2008.26672.
34. Panzacchi S, Tibaldi E, De Angelis L, Falcioni L, Giovannini R, Gnudi F, et al. Carcinogenic effects of long-term exposure from prenatal life to glyphosate and glyphosate-based herbicides in Sprague-Dawley rats. Environ Health. 2025;24(1):36. doi: 10.1186/s12940-025-01187-2.
35. Bernardis LL, Patterson BD. Correlation between 'Lee index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. J Endocrinol. 1968;40(4):527-8. doi: 10.1677/joe.0.0400527.
36. Junqueira LC, Carneiro J. Histologia Básica, 12th ed. Guanabara Koogan, Rio de Janeiro; 2013.
37. Karnovsky MJ. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell. Biol. 1965;27:1A-149A.
38. Lehrer GM, Ornstein L. A diazo coupling method for the electron microscopic localization of cholinesterase. J. Biophys. Biochem. Cytol. 1959;6:399-419. doi: 10.1083/jcb.6.3.399.
39. Padmanabhan V, Cardoso RC, Puttabyatappa M. Developmental Programming, a Pathway to Disease. Endocrinology. 2016;157(4):1328-40. doi: 10.1210/en.2016-1003.
40. Zhao L, Zhang J, Yang L, Zhang H, Zhang Y, Gao D, et al. Glyphosate exposure attenuates testosterone synthesis via NR1D1 inhibition of StAR expression in mouse Leydig cells. Sci Total Environ. 2021;785:147323. doi: 10.1016/j.scitotenv.2021.147323.
41. Kubsad D, Nilsson EE, King SE, Sadler-riggleman I, Beck D, Skinner MK. Assessment of Glyphosate Induced epigenetic transgenerational Inheritance of pathologies and sperm epimutations: Generational toxicology. Sci. Rep. 2019;9:6372. doi: 10.1038/s41598-019-42860-0.
42. Yan X, Niu Q, Gao X, Shen S, He N, Wang H, et al. Differential Protein Metabolism and Regeneration in Gastrocnemius Muscles in High-fat Diet Fed Mice and Pre-hibernation Daurian Ground Squirrels: A Comparison between Pathological and Healthy Obesity. Zool Stud. 2021;60:e6. doi: 10.6620/ZS.2021.60-06.
43. Asghar A, Akhtar T, Batool T, Khawar MB, Nadeem S, Mehmood R, Sheikh N. High-fat diet-induced splenic, hepatic, and skeletal muscle architecture damage: cellular and molecular players. Mol Cell Biochem. 2021;476(10):3671-3679. doi: 10.1007/s11010-021-04190-6.
44. Espino-Gonzalez E, Tickle PG, Benson AP, Kissane RWP, Askew GN, Egginton S, et al. Abnormal skeletal muscle blood flow, contractile mechanics and fibre morphology in a rat model of obese-HFpEF. J Physiol. 2021;599(3):981-1001. doi: 10.1113/JP280899.
45. Crabbs TA. NTP nonneoplastic lesion atlas: skeletal muscle - degeneration [Internet]. 2021. Available from: https://ntp.niehs.nih.gov/nnl/musculoskeletal/skel_musc/degen/skeletal-muscle degeneration_508.pdf. Accessed 2024 Apr 12.
46. Santos VC, Vechetti Júnior IJ, Sant'Ana DMG, Almeida FLA. Vias de sinalização celular envolvidas no crescimento e manutenção do músculo estriado esquelético. Arq Mudi. 2019;23(3):220-48. doi: 10.4025/arqmudi.v23i3.51539.
47. Camargo Filho JCS, Vanderlei LCM, Camargo RCT, Oliveira DAR, Oliveira Júnior SA, Dal Pai V, Belangero WD. Análise histológica, histoquímica e morfométrica do músculo sóleo de ratos submetidos a treinamento físico em esteira rolante. Arq Cienc Saude. 2006;12(3):196-9.
48. Greaves P, Chouinard L, Ernst H, Mecklenburg L, Pruimboom-Brees IM, Rinke M, et al. Proliferative and non-proliferative lesions of the rat and mouse soft tissue, skeletal muscle and mesothelium. J Toxicol Pathol. 2013;26(3 Suppl):1S-26S. doi: 10.1293/tox.26.1S.
49. Bright JE, Inns RH, Tuckwell NJ, Griffiths GD, Marrs TC. A histochemical study of changes observed in the mouse diaphragm after organophosphate poisoning. Hum Exp Toxicol. 1991;10(1):9-14. doi: 10.1177/096032719101000102.
50. Ciabattari O, Dal Pai A, Dal Pai V. Efeito da natação associado a diferentes dietas sobre o músculo tibial anterior do rato: estudo morfológico e histoquímico. Rev Bras Med Esporte. 2005;11(2):113-8. doi: 10.1590/S1517-86922005000200005.
51. Araújo CRM, Santos VLA, Gonsalves AA. Acetilcolinesterase - AChE: uma enzima de interesse farmacológico. Rev Virtual Quim. 2016;8(4):1818-34. doi: 10.21577/1984-6835.20160122.
52. Mantilla CB, Sieck GC. Neuromuscular adaptations to respiratory muscle inactivity. Respir Physiol Neurobiol. 2009;169(2):133-40. doi: 10.1016/j.resp.2009.09.002.
53. Campanari ML, García-Ayllón MS, Ciura S, Sáez-Valero J, Kabashi E. Neuromuscular Junction Impairment in Amyotrophic Lateral Sclerosis: Reassessing the Role of Acetylcholinesterase. Front Mol Neurosci. 2016;9:160. doi: 10.3389/fnmol.2016.00160.