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ORIGINAL ARTICLE

# Short-term Treatment with Metformin During Puberty Mitigates Metabolic Dysfunctions Programmed by Neonatal Overfeeding in Male Rats

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**Abstract**

*Objective.* To address the major public health problems of increased body weight and obesity, causing conditions such as hypertension and diabetes. Early obesity in childhood and adolescence has more severe long-term health consequences. The Developmental Origins of Health and Disease (DOHaD) concept examines how early overfeeding and other factors affect health throughout life. This study hypothesized that a short-term metformin treatment during puberty could reduce metabolic dysfunction caused by neonatal overfeeding. We investigated whether the same intervention would have a similar effect on metabolic programming in adulthood.

*Methods.* Male Wistar rats raised in small (SL, three pups per mother) and normal (NL, 9 pups per mother) litters were used as models of early overfeeding. Some of the SL and NL animals received intraperitoneal injections of metformin (NL-M and SL-M), whereas the controls received saline (NL-C and SL-C) from days 35–42 (puberty) or 70–81 (adulthood).

*Results.* Two months after the intervention, at both puberty and adulthood, the SL animals exhibited metabolic dysfunction, and were significantly heavier with greater tissue fat accumulation than the NL animals ($p$ <0.0001). SL-M animals treated during puberty exhibited significant reductions in white adipose tissue and liver weight, as well as lower weight gain ($p$ <0.05). In contrast, metformin treatment in adulthood did not alter metabolism.

*Conclusion.* These findings suggest that short-term metformin treatment in rats during puberty can mitigate adult metabolic dysfunction induced by neonatal overnutrition. However, this intervention in adulthood did not result in long-term metabolic changes, which confirms the DOHaD concept. 

*Key Words:* Childhood obesity, Metabolic programming, Visceral adiposity, Adolescent intervention, Liver steatosis.

## Introduction

The World Health Organization considers obesity to be among the greatest public health risks. It is associated with cardiovascular dysfunction, glucose intolerance, insulin re-

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sistance, and dyslipidemia. Globally, approximately 390 million adolescents and 890 million adults are obese or overweight, and approximately 35.8 million will die from this disease (1,2). The causes of obesity are multifactorial. The imbalance between caloric intake and expenditure, associated with a sedentary lifestyle and emotional instability, contribute to nutritional and metabolic disorders that can lead to the development of cardiometabolic diseases (3).

The Developmental Origins of Health and Disease (DOHaD) concept posits that specific developmental phases are "plastic", meaning they can be shaped by environmental factors. These phases include preconception, gestation, lactation, and adolescence (4–6). A lactation intervention model that illustrates this concept is known as the Small Litter (SL) model. This model mimics early overfeeding and leads to obesity. In the SL model, reducing the litter size to three pups per dam results in overnutrition in these animals. Because there is no competition among the pups for food, maternal care is altered. In addition to increased triglyceride levels in the mother's milk, pup development is influenced, resulting in increased body weight, fat and altered food intake in the long term (4,7–11).

The use of interventions during plastic developmental phases and their long-term effects have been extensively studied. One such intervention is metformin (MET), a widely used drug for treating diabetes mellitus. MET has demonstrated beneficial effects on improving glycemic homeostasis and reducing body weight and appetite (12,13). When administered to Wistar rats for 12 days during the lactation period, MET improved pancreatic function and prevented long-term metabolic dysfunction in neonatally overfed rats (14).

Similar to lactation, adolescence is also being studied as a critical developmental stage. During this period, behavioral, physiological, neuroendocrine, and hormonal changes occur and interact with emotional and behavioral factors that impact overall metabolism. These changes may alter physiological pathways and program individuals toward health or disease (15,16). For example, a low-protein diet provided to animals during adolescence has been shown to impair testosterone production and energy metabolism (17). In contrast, the same diet administered during adulthood did not result in long-term metabolic consequences, suggesting that metabolic programming only occurs during the plastic phases of development (18).

Our research group previously demonstrated that short-term MET treatment during lactation prevents metabolic dysfunction in obese animals (14). According to the DOHaD concept, adolescence is the final window of opportunity for metabolic programming. Based on these initial findings, we hypothesized that short-term MET treatment during adolescence could mitigate metabolic dysfunction caused by neonatal overfeeding. In addition, we assessed whether the same intervention during adulthood would have a similar capacity for metabolic programming.

## Material and Methods

### *Ethics Approval*

This study was approved by the Animal Ethics Committee (CEUA) of the State University of Maringá, (UEM) Brazil. Experimental use of animals was performed under protocol number 3220080620. All analyses were performed according to the final protocol.

Adult Wistar rats (40 female and 20 male) were obtained from the Central Animal Facility of the UEM and housed at the DOHaD Experimental Laboratory (LEx-DOHaD-UEM). The animals were housed in polypropylene cages measuring 15 cm (height) × 30 cm (width) × 45 cm (length). The cages were lined with wood shavings that were changed three times per week. The animals were maintained under controlled environmental conditions (mean [± SD] temperature, 22 ± 2°C; 12 h light-dark cycle [from 07:00–19:00]) with *ad libitum* access to food (Nuvital, Curitiba, Brazil) and water.

After a 1 week acclimation period, the animals were housed in breeding cages at a 2:1 female-to-male ratio. Once pregnant, the females were kept in individual cages with *ad libitum* access to commercial food (Nuvilab, Curitiba, Brazil) and water, and we considered the day of birth as day zero.

On day 3 post-birth, litters were adjusted to either nine pups per dam (normal litter [NL]) or three pups per dam (small litter [SL]), prioritizing the selection of male pups. Litter reduction is essential for the induction of metabolic dysfunction during lactation, with weaning occurring at 21 days (19,20). Only male pups were used in this study due to sexual dimorphism between males and females, as well as hormonal fluctuations in females during the estrous cycle, which affect metabolism and behavior. These fluctuations could introduce bias in data interpretation. Additionally, male and female rats respond differently to neonatal overnutrition; males are more susceptible to metabolic dysfunction due to litter size reduction (21).

### *Experiments*

*Experiment 1 (E1): Intervention at Puberty:* Analyses began during puberty. The treatment protocol was initiated on postnatal day (PND) 34, and the groups were divided as follows: NL-C and SL-C (control groups receiving saline); and NL-M and SL-M (treatment groups receiving MET). Treatment was administered from PND 34 to PND 45 via intraperitoneal injection at a dose of 100 mg/kg (14). The animals were monitored until PND 105, at which point they were euthanized following the completion of the experimental procedures. The decision to wait two months

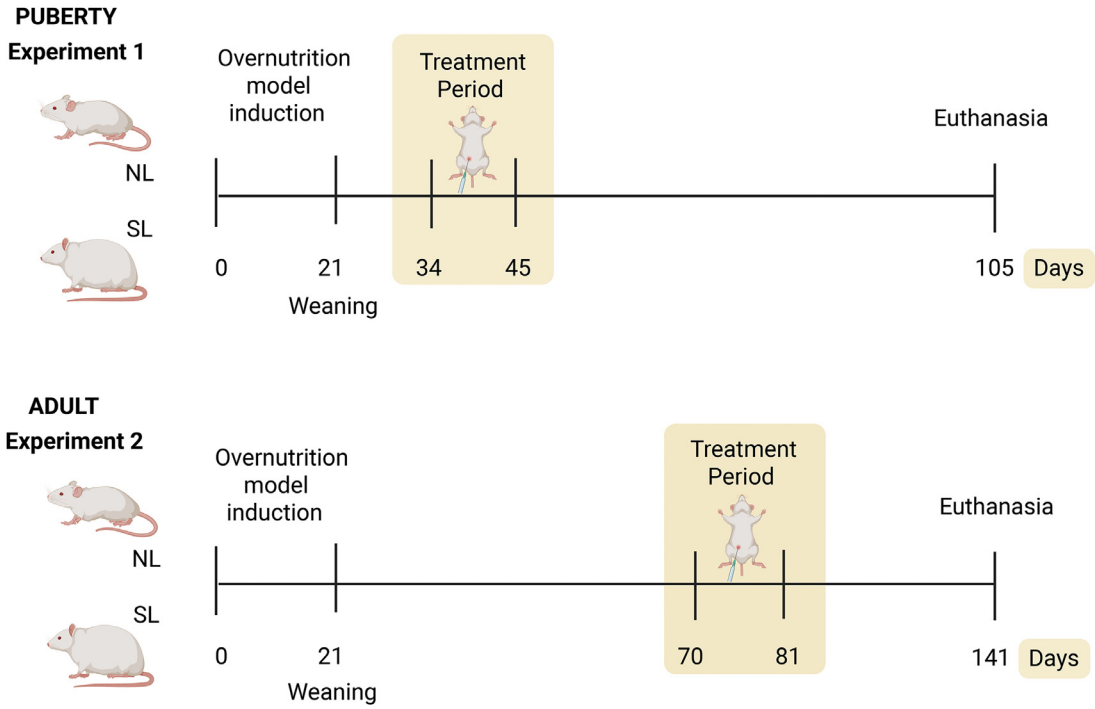


**Figure 1.** Experimental protocol – Small litter (SL) and Normal litter (NL).

after treatment termination was based on the established literature demonstrating that this timeframe is sufficient to assess long-term metabolic programming effects (6,17,22). The objective was to evaluate the effects of a short-term intervention; thus, a 12 days treatment period was chosen, as previously described by Previate C, et al. (14). The PND 34–45 timeframe was selected because previous studies have indicated that puberty is a critical developmental window characterized by hormonal changes that prepare the organism for reproductive capability. This period is a critical stage for long-term metabolic programming, supporting the hypothesis (Figure 1) (23–27).

*Experiment 2 (E2): Intervention in Adulthood:* To investigate programming capacity during adulthood, the intervention began at PND 70. For 12 consecutive days, the NL and SL groups received either intraperitoneal saline injections (NL-C and SL-C groups) or MET at a dose of 100 mg/kg (NL-M and SL-M groups). Treatment was discontinued on PND 81 and the animals were maintained until PND 141, when the experimental procedures were completed, followed by euthanasia (Figure 1).

### *Biometric and Biochemical Parameters*

The body weight and food intake of the animals were assessed weekly from weaning (21 days) until euthanasia at 105 or 141 days. Laparotomy was performed to collect blood, and tissues from the liver, perigonadal fat, mesenteric fat, and retroperitoneal fat. The collected tissues were weighed and stored at –80°C. The collected blood was heparinized to prevent coagulation, then centrifuged at 10,000 rpm for 5 min to obtain plasma and stored at –20°C for subsequent analysis of total cholesterol, triglyceride and fasting glucose levels.

Absolute food intake was calculated as the difference between the total amount of food provided one week earlier (initial amount) and the remaining amount of food (final amount), divided by the number of days and the number of rats per cage: (food intake [in grams] = [initial − final]/[7 × 3]).

### *Oral Glucose Tolerance Test*

*Experiment 1 (E1):* At 105 days of age, the rats underwent a 12 h fasting period. Subsequently, all groups received an oral glucose solution via gavage (2 g/kg body weight). An oral glucose tolerance test (OGTT) was performed by collecting a blood sample from the animals' tails. Samples were collected at 0, 15, 30, 45, 60, and 120 min. Whole blood was collected, and plasma was separated for biochemical analysis.

*Experiment 2 (E2):* At 141 days of age, the animals were anesthetized with a mixture of ketamine (75 mg/kg body weight) and xylazine (15 mg/kg body weight). A silicone cannula was implanted into the right jugular vein via an incision in the anterior cervical region, followed by tissue dissection until the vein was exposed. A silicone cannula was then inserted into the vein and secured to the pectoralis major muscle with a simple suture. The cannula

was filled with a 10% heparin solution (Liquemine, Roche, Brazil) diluted in 0.9% physiological saline (0.9% NaCl) to prevent blood entry and subsequent clot formation. After surgery, the animals received a subcutaneous injection of an analgesic (acetylsalicylic acid [aspirin], 20 mg/kg body weight, twice daily): one dose immediately after surgery and another dose 8 h later. The animals were housed in individual cages for 24 h following this procedure. After a 1 day recovery period, the animals underwent an overnight 12 h fasting period. Then, a blood sample (400 $\mu$L) was collected directly from the jugular vein via the cannula to determine fasting blood glucose levels (time 0′). During the test, glucose solution (1 g/kg body weight) was administered. Blood samples (400 $\mu$L) were collected at 5, 15, 30, 45, and 60 min after glucose administration. The corresponding volume was replaced with a 0.9% NaCl solution. Blood samples were collected using heparinized syringes and transferred to tubes that were placed in ice baths. The samples were subsequently centrifuged at 4,500 rpm for 5 min, and the plasma was used to determine glucose levels.

### *Insulin Tolerance Test*

At 105 and/or 141 days of age, the animals underwent a 6 h fasting period, followed by an intraperitoneal injection of insulin (1 U/kg body weight). Blood was collected from the tail vein at 0 (baseline), 15, 30, 45, and 60 min after insulin injection, and the glucose disappearance rate constant (Kitt) was then calculated.

### *Biochemical Analyses*

Baseline plasma levels of glucose, total cholesterol, and triglycerides were quantified in plasma samples obtained from whole blood via intravenous glucose tolerance test (ivGTT) and/or OGTT (time 0). Blood glucose levels were quantified using the glucose oxidase method by spectrophotometry with a semi-automatic biochemical analyzer (BIO 200FL, Bio Plus, São Paulo/SP, Brazil), and a commercially available kit (Gold Analisa, Belo Horizonte/MG, Brazil).

### *Liver Histology*

Liver tissue was fixed in saline formalin (formaldehyde at 40% in phosphate-buffered saline [PBS], pH ± 6.8), embedded in paraffin, and sectioned into 5 $\mu$m thick serial slices using a microtome. Three non-serial sections from three animals per experimental group were mounted on glass slides and stained with hematoxylin and eosin. The analysis was performed using randomly acquired digital images (TIFF format, 36 bit color, 1360 × 1024 pixels) taken with a 12.0 megapixel camera and microscope (28,29).

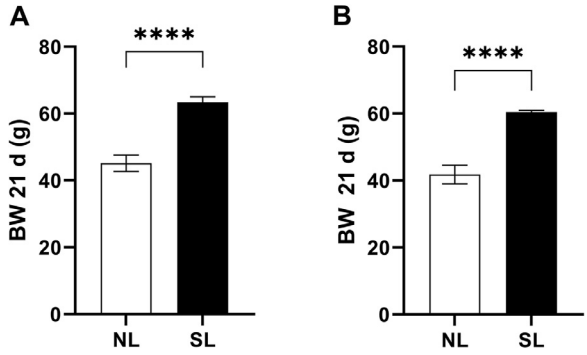


**Figure 2.** A, and B show the final body weight at 21 days in E1 and E2, respectively. Data are expressed as the mean ± SEM of $n = \pm 12$ rats from $n = 5$ different litters. Statistical analysis was performed using an unpaired *t*-test (****$p < 0.0001$). NL: Normal litter; SL: Small litter. E1: Experiment 1 (puberty); E2: Experiment 2 (adulthood).

## Statistical Analyses

Statistical analyses were performed using Prism version 9.00 (GraphPad Inc., San Diego, CA, USA) for Windows (Microsoft Corp., Redmond, WA, USA). Results are expressed as the mean ± standard error of the mean (SEM). Data were compared using a Student's *t*-test or two-way analysis of variance (ANOVA), and a Tukey's post-hoc test. Differences with $p < 0.05$ were considered statistically significant.

## Results

### *Biometric Parameters*

*Body Weight (21 days):* Final body weights of the animals were analyzed immediately after weaning. The results of Experiment 1 (i.e., "E1") demonstrated the effect of the SL model on body weight gain on day 21. SL animals were significantly heavier than NL animals (SL-C, 63.3 ± 4.7 g vs. NL-C, 45.1 ± 5.9 g; $p < 0.0001$). The same trend was observed in Experiment 2 ("E2") (SL, 66.23 ± 1.72 g vs. NL, 44.27 ± 1.41 g; $p < 0.0001$) (Figures 2A and 2B).

*Body Weight Evolution:* Figure 3 shows the progression of body weight and the area under the curve (AUC) from 21 days (weaning) to 105 days (E1, Figure 3A) and from 21 to 141 days (E2, Figure 3B) across all groups. Throughout the experimental period, SL-C animals remained significantly heavier than NL-C animals (E1: SL-C, 22.979.0 ± 251.2 vs. NL-C, 21.229.0 ± 332.3; $p < 0.0001$; E2: SL-C, 42,366.1 ± 558.2 vs. NL-C, 34.,870.7 ± 473.2; $p < 0.001$). A two-way ANOVA revealed a treatment effect on body weight reduction in both experimental groups; however, this effect was only significant when the treatment was administered during adolescence (E1) ($p < 0.05$).

*Food Intake:* Food intake was assessed in all groups from day 30 until the end of the experimental protocol. No significant differences were observed in E1 (Figure 4A) for any of the parameters. In contrast, a significant effect of

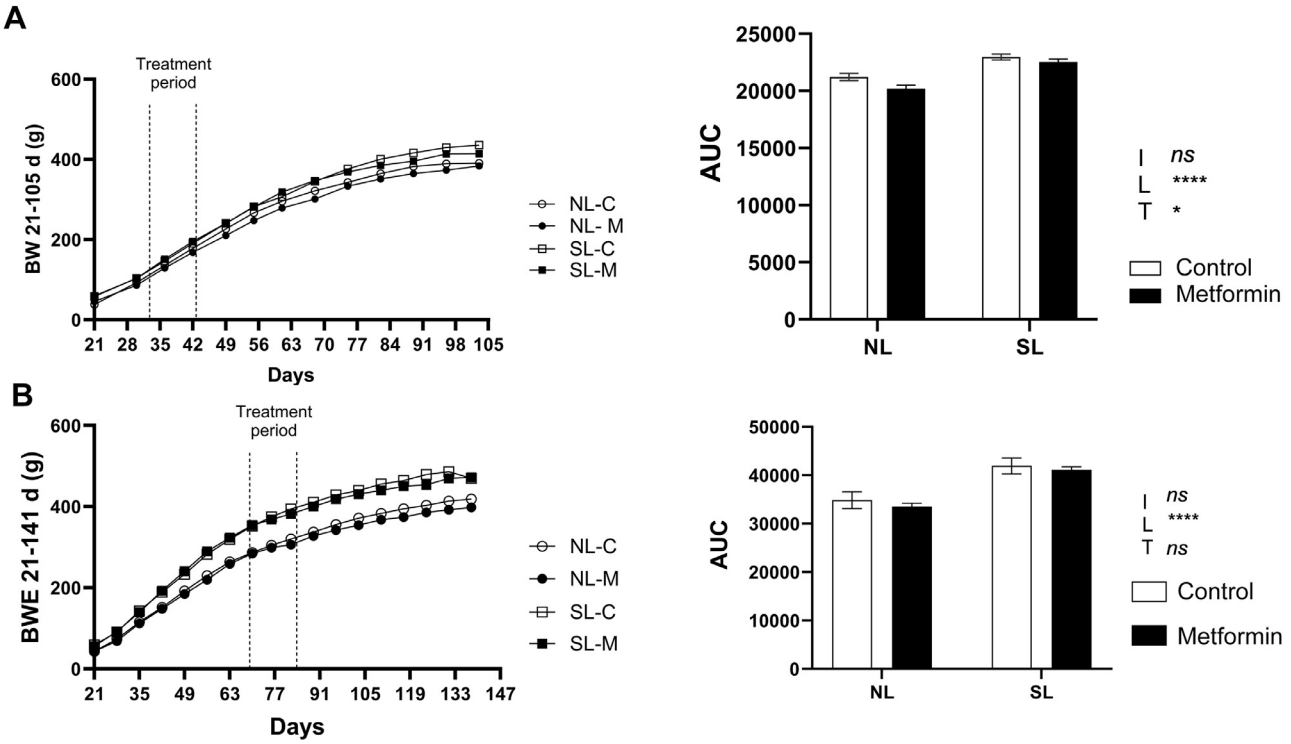


**Figure 3.** A, and B depict body weight evolution from day 21–104 or 141 days for E1 and E2, respectively. Statistical analysis was performed using a two-way ANOVA. Data are expressed as the mean ± SEM of $n = \pm 12$ rats from $n = 5$ different litters ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$; *ns*: not significant). Treatment period: E1 (34–45 d); E2 (70–81 d). E1: Experiment 1 (puberty); E2: Experiment 2 (adulthood).

**Table 1.** Puberty.

| **Puberty** | **Groups** | | | | **Factors** | | |
|---|---|---|---|---|---|---|---|
| | **NL-C** | **NL-M** | **SL-C** | **SL-M** | **I** | **L** | **T** |
| Retroperitoneal fat weight (g/100bw) | 1.2 ± 0.06 | 1.3 ± 0.04 | 1.8 ± 0.06 | 1.81 ± 0.1 | ** | *** | * |
| Perigonadal fat weight (g/100bw) | 1.2 ± 0.04 | 1.1 ± 0.05 | 1.5 ± 0.1 | 1.2 ± 0.06 | *ns* | ** | * |
| Mesenteric fat weight (g/100bw) | 0.6 ± 0.01 | 0.6 ± 0.02 | 0.8 ± 0.03 | 0.7 ± 0.03 | * | **** | * |
| Triglycerides (mg/dL) | 50.9 ± 7.5 | 50.3 ± 10.5 | 81.5 ± 24.3 | 50.4 ± 19.4 | * | * | * |
| Total cholesterol (mg/dL) | 51.4 ± 9.7 | 50.9 ± 9 | 79.2 ± 31.5 | 43.2 ± 15.3 | ** | *ns* | ** |
| Fasting glucose (mg/dL) | 85.4 ± 3.9 | 79.6 ± 5.8 | 86.5 ± 3.3 | 79.1 ± 4.6 | *ns* | *ns* | *ns* |

NL: Normal litter; SL: Small litter; C: Control; M: Metformin; I: Interaction; L: Litter; T: Treatment. Data are expressed as the mean ± SEM of $n = \pm 10$ rats from $n = 4$ different litters. A two-way ANOVA test was used to compare the experimental groups. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$, *ns*: Not significant.

litter size was observed in adulthood (E2, Figure 4B) ($p < 0.0001$).

*Liver Weight:* Relative liver weights are presented in Figures 5A and 5B. In both E1 and E2, a litter size effect was observed, demonstrating that SL animals had significantly heavier livers than NL animals ($p < 0.0001$). In E1, MET treatment attenuated liver weight in treated animals ($p < 0.05$), whereas in E2, an interaction effect was detected ($p < 0.05$).

### *Lipid and Glycemic Profiles*

*Puberty:* A significant effect of litter size on retroperitoneal ($p < 0.001$), perigonadal ($p < 0.01$), and mesenteric ($p < 0.0001$) fat was reported in Table 1A. MET treatment effectively reduced the weight of all analyzed fat deposits ($p < 0.05$). Plasma triglyceride levels were significantly higher in SL animals than NL animals ($p < 0.0001$), and treatment significantly reduced this parameter ($p < 0.05$). MET also significantly lowered total cholesterol levels in the treated groups, with an observed interaction effect ($p < 0.01$). However, no significant differences were found in fasting glucose levels.

*Adulthood:* In adulthood, significant differences were only observed in litter size ($^{****}p < 0.0001$), suggesting that the SL animals developed long-term metabolic dysfunction (Table 1B). Short-term treatment with MET in

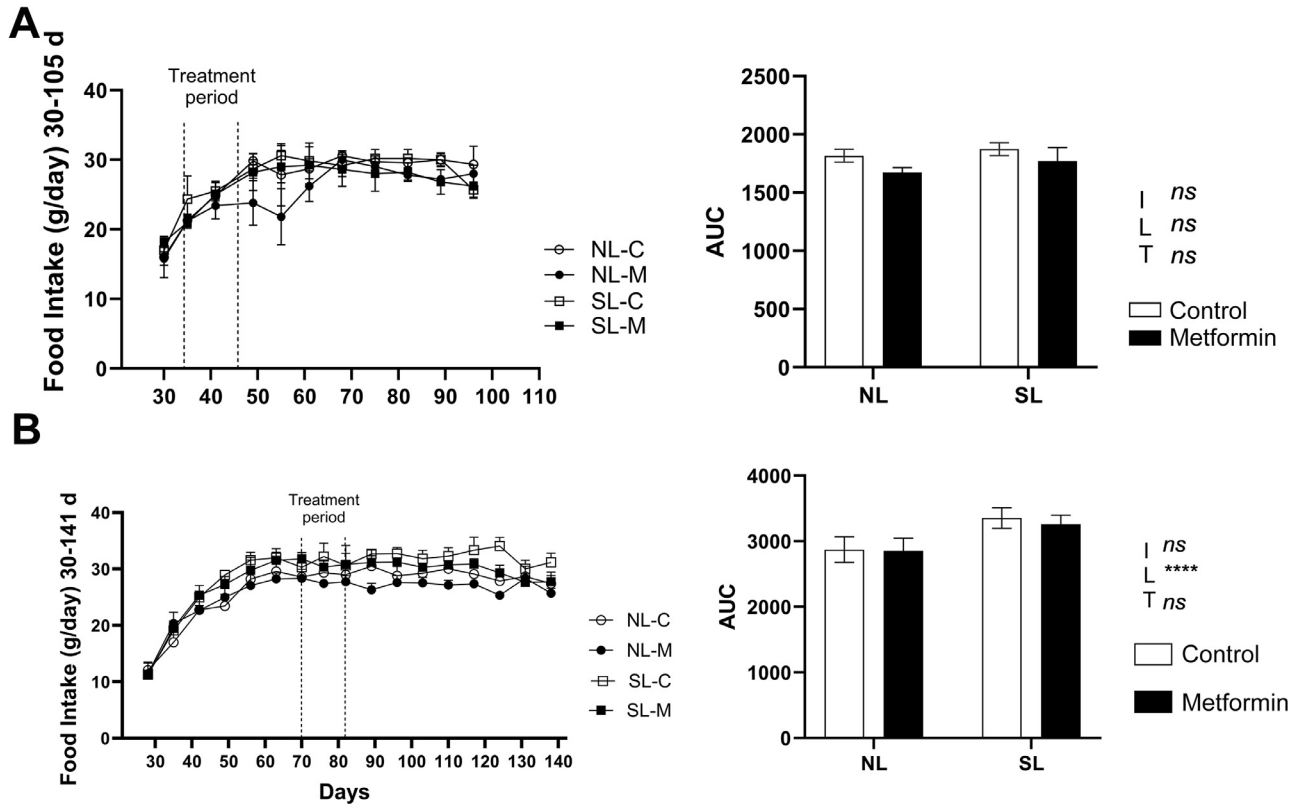


**Figure 4.** A, and B illustrate food intake from day 30 until the end of the experimental period for E1 and E2, respectively. Data are expressed as the mean ± SEM of $n = \pm 12$ rats from $n = 5$ different litters. Statistical analysis was performed using two-way ANOVA (****$p$ <0.0001). Treatment period: E1 (34–45 days); E2 (70–81 days). E1: Experiment 1 (puberty); E2: Experiment 2 (adulthood).

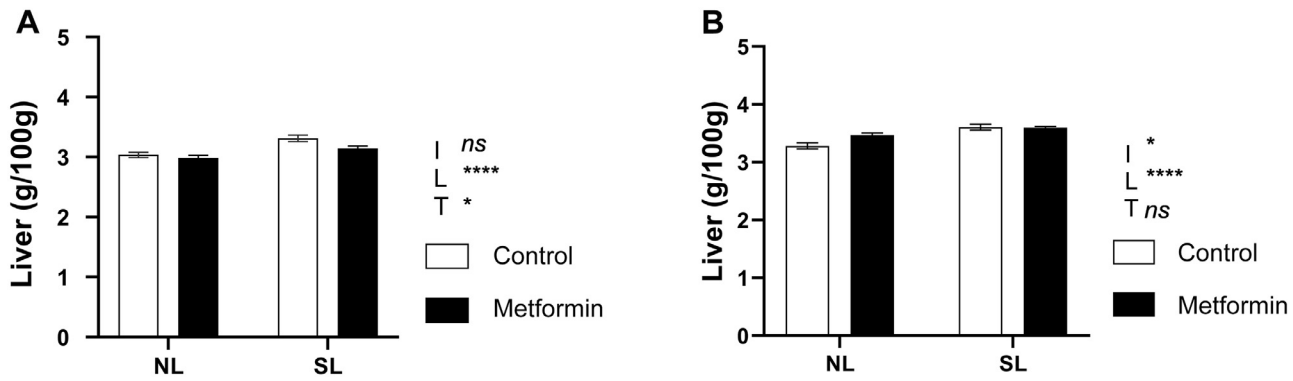


**Figure 5.** A (E1), and B (E2) illustrate the relative liver weight at the end of the experimental protocol (E1: 105 days; E2: 141 days). Data are expressed as the mean ± SEM of $n = \pm 12$ rats from $n = 5$ different litters. Statistical analysis was performed using a two-way ANOVA (*$p$ <0.05; ****$p$ <0.0001). E1: Experiment 1 (puberty); E2: Experiment 2 (adulthood).

adulthood was insufficient to mitigate the effects of any of the evaluated parameters.

*OGTT*

In E1, the OGTT revealed no significant differences in any evaluated parameter (Figure 6A). In E2, the ivGTT demonstrated a significant effect on litter size, with SL animals exhibiting markedly impaired glucose tolerance compared with NL animals ($p$ <0.0001) (Figure 6B).

*Insulin Tolerance Test*

*E1 (Figure 7A):* The insulin sensitivity index (Kitt) inferred from the insulin tolerance test (ITT) indicated that SL animals had significantly higher insulin resistance compared

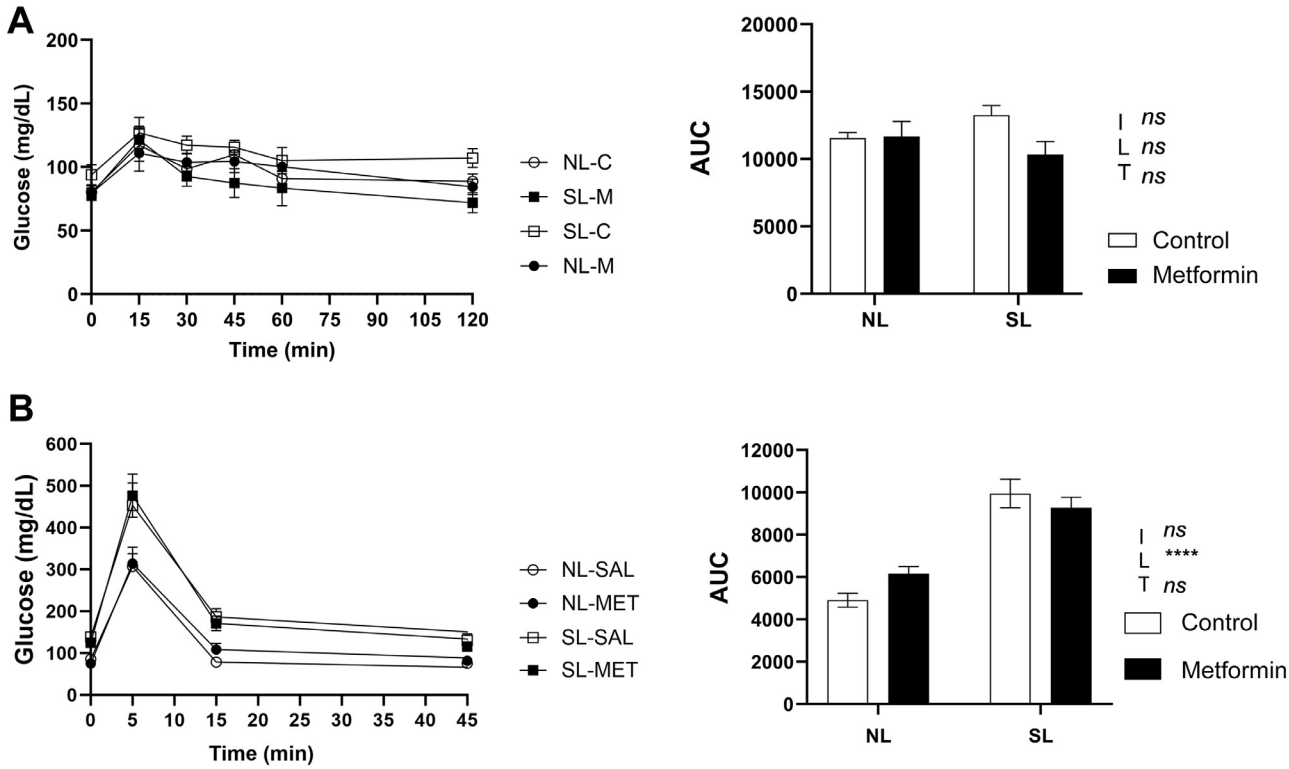


**Figure 6.** A (E1), and B (E2) display glucose levels during the test and their corresponding AUC. Data are expressed as the mean ± SEM of $n = \pm 12$ rats from $n = 5$ different litters. Statistical analysis was performed using a two-way ANOVA (****$p < 0.0001$). E1: Experiment 1 (puberty); E2: Experiment 2 (adulthood).

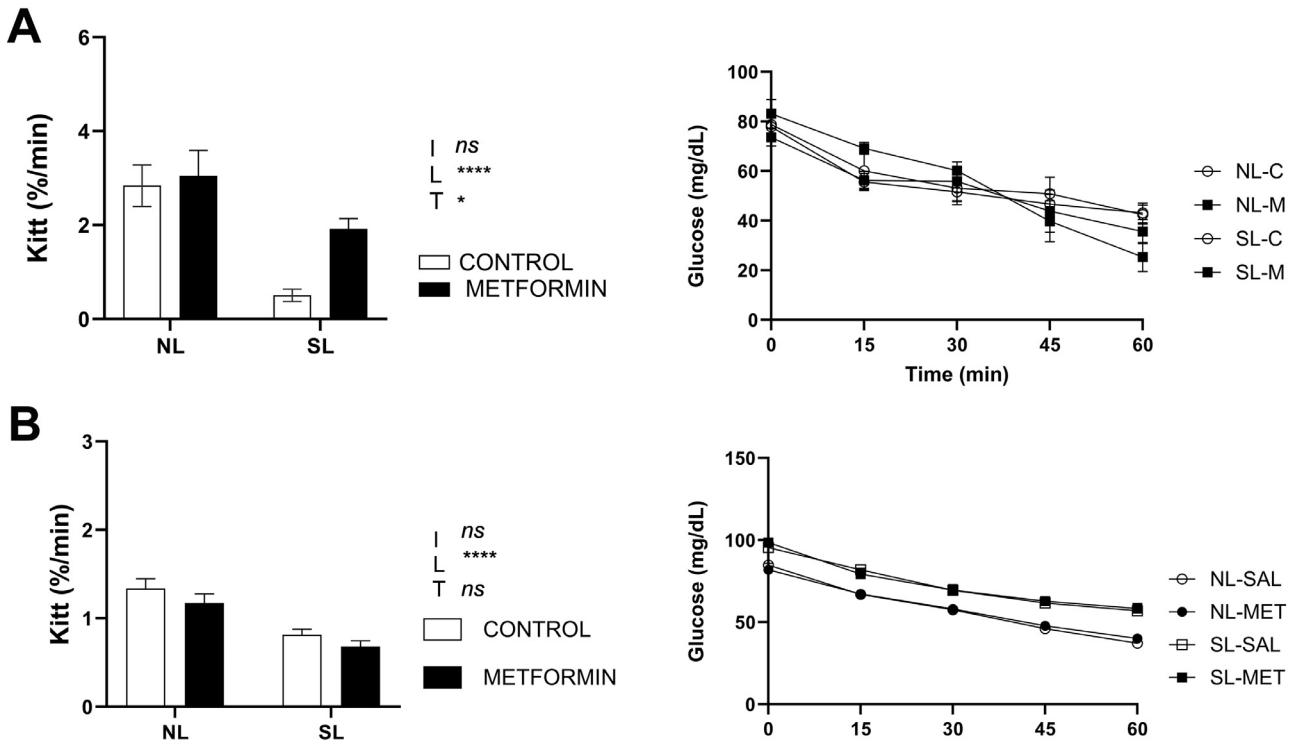


**Figure 7.** A (E1), and B (E2) show glucose levels during an insulin tolerance test. The bar graphs at the top of the figure represent the Kitt. Data are expressed as the mean ± SEM of $n = \pm 12$ rats from $n = 5$ different litters. Statistical analysis was performed using a two-way ANOVA (*$p < 0.05$, ****$p < 0.0001$). E1: Experiment 1 (puberty); E2: Experiment 2 (adulthood).

**Table 2.** Adult.

| Adult | Groups | | | | Factors | | |
|---|---|---|---|---|---|---|---|
| | NL-C | NL-M | SL-C | SL-M | I | L | T |
| Retroperitoneal fat weight (g/100bw) | 0.8 ± 0.05 | 0.8 ± 0.08 | 1.05 ± 0.09 | 0.9 ± 0.05 | *ns* | **** | *ns* |
| Perigonadal fat weight (g/100bw) | 1.1 ± 0.05 | 1.1 ± 0.04 | 1.6 ± 0.07 | 1.4 ± 0.05 | *ns* | **** | *ns* |
| Mesenteric fat weight (g/100bw) | 0.7 ± 0.03 | 0.7 ± 0.06 | 0.9 ± 0.07 | 1.04 ± 0.04 | *ns* | **** | *ns* |
| Triglycerides (mg/dL) | 75.6 ± 4.7 | 68.4 ± 4.3 | 143.3 ± 7.5 | 128.2 ± 6.8 | *ns* | **** | *ns* |
| Total cholesterol (mg/dL) | 70.4 ± 2.4 | 64.8 ± 2.1 | 88.4 ± 5.1 | 87.3 ± 5.1 | *ns* | **** | *ns* |
| Fasting glucose (mg/dL) | 85.7 ± 4.03 | 75.3 ± 6.3 | 138.8 ± 11.3 | 125.07 ± 9.5 | *ns* | **** | *ns* |

NL: Normal litter; SL: Small litter; C: Control; M: Metformin; I: Interaction; L: Litter; T: Treatment. Data are expressed as the mean ± SEM of $n = \pm 10$ rats from $n = 4$ different litters. To compare the experimental groups a two-way ANOVA test was used. ****$p < 0.0001$; *ns*: Not significant.

with NL animals ($p < 0.001$). Short-term MET treatment during puberty improved this parameter ($p < 0.05$).

*E2 (Figure 7B):* The same test performed in adulthood demonstrated that SL animals remained insulin resistant ($p < 0.0001$), with no observed treatment effect.

### *Liver Histology*

In Figures 8A–8D, the arrows indicate the areas of hepatic microsteatosis. SL-C animals exhibited a significantly greater number of lipid droplets than NL-C animals (SL-C, 0.30 ± 0.07 vs. NL-C, 0.18 ± 0.08; $p < 0.05$). Furthermore, SL-C animals had significantly higher hepatic fat content than SL-M animals (SL-C, 0.30 ± 0.07 vs. SL-M, 0.16 ± 0.03; $p < 0.01$). An interaction effect between the factors was also observed ($p < 0.001$) (Figure 8E).

## Discussion

The results of the present study demonstrate that even a short-term intervention with MET during puberty can mitigate metabolic dysfunction induced during lactation, but not during adulthood. To the best of our knowledge, this is the first study to investigate short-term MET interventions during puberty and compare them to interventions in adulthood. This study provides scientific corroboration of the DOHaD concept, which proposes that puberty represents a "plastic" developmental window, whereas adulthood does not.

SL model rodents are widely used to mimic obesity because they develop metabolic dysfunctions throughout life. One of the most prominent characteristics is increased body weight associated with high food intake, as observed in our animals (14,30,31).

Previous research from our laboratory has demonstrated that intraperitoneal administration of MET during lactation protected SL animals from metabolic dysfunction (14). These results align with our current findings, demonstrating that a similar 12 days intervention during puberty can mitigate body weight gain and body fat in MET-treated animals. However, these effects were not observed in adulthood which reinforces the idea that adulthood is not a "plastic" window. Furthermore, our findings provide crucial insight into the conditional nature of MET's efficacy. Notably, MET treatment in the control animals did not induce any significant metabolic alterations. This absence of effect implies that MET cannot alter an already normal physiological state. The data imply that a pre-existing metabolic dysfunction, as programmed by the early-life SL model, acts as a necessary trigger for MET's mitigating effects to be observed. It appears that a pathological deviation from homeostasis is required for the drug to exert its corrective influence, as if a "broken" metabolic substrate is necessary for MET's repair mechanisms to be activated.

The positive effects observed when MET is administered during puberty can be explained by physiological mechanisms specific to this period. Puberty is characterized by intense metabolic and endocrine remodeling, changes in insulin sensitivity and adipogenesis, and interactions with sex hormones. These changes can make tissues, such as the liver and adipose tissue, more responsive or "reprogrammable" to interventions (32). Thus, the activation of pathways such as AMPK by MET can produce lasting effects if activated while metabolic circuits are still undergoing dynamic maturation. This interpretation is consistent with reviews that highlight adolescence as a critical period for interventions to reduce cardiometabolic risk (33).

The physiological insulin resistance that occurs during puberty is primarily driven by growth hormone, generating a state of compensatory hyperinsulinemia to maintain normoglycemia (30). However, in obese adolescents, this natural resistance may be severe enough to overwhelm pancreatic beta cells, increasing the risk of progression to metabolic syndrome (31). In this clinical context, MET serves as a therapeutic agent to "neutralize" this metabolic tension. MET primarily suppresses hepatic gluconeogenesis, thereby reducing liver glucose production and decreasing the body's demand for high insulin levels. It also increases peripheral glucose uptake and improves muscle insulin sensitivity (34,35). Therefore, by targeting both liver glucose production and tissue glucose disposal, MET may mitigate the consequences of insulin resistance during

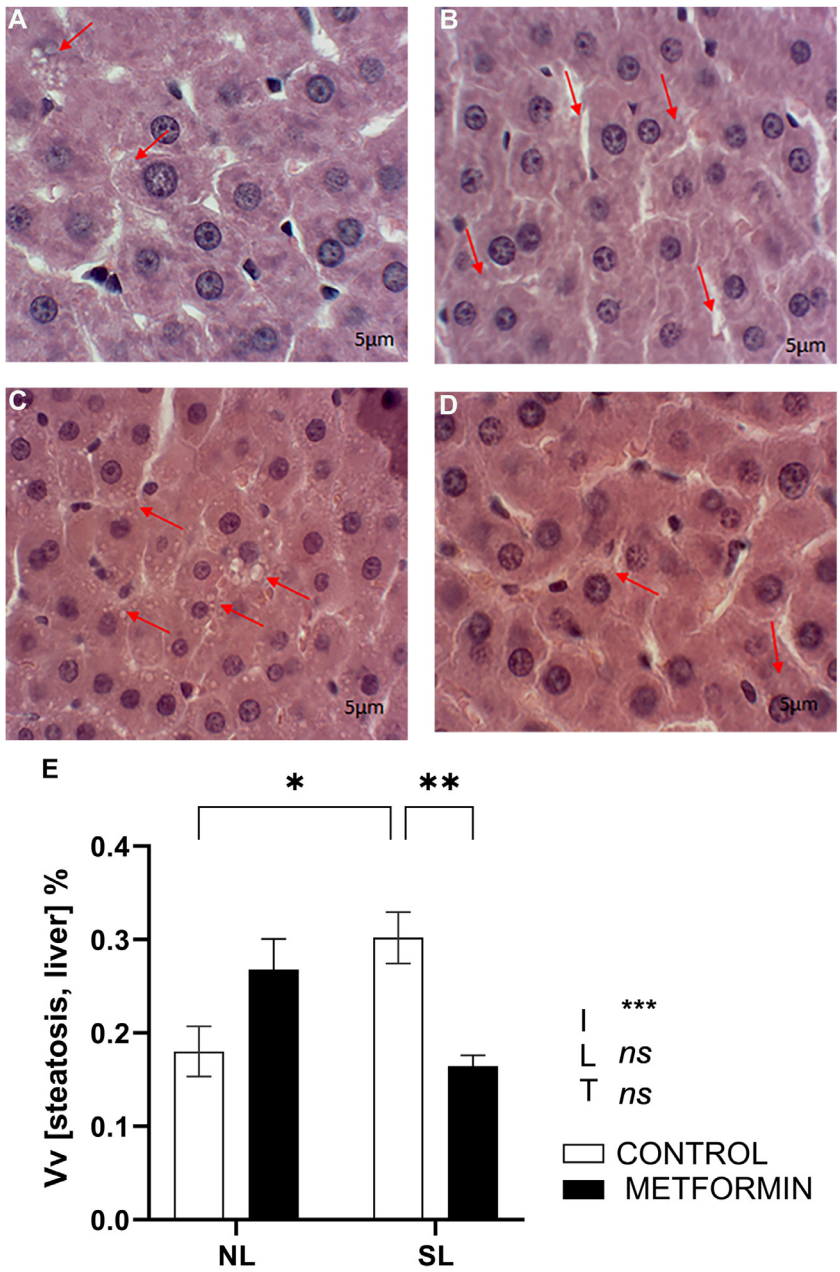


**Figure 8.** Represents histological sections with hematoxylin/eosin (HE) staining. Percentage area of hepatic steatosis. Data are expressed as the mean ± SEM of $n = \pm 12$ rats from $n = 5$ different litters. Statistical analysis was performed using a two-way ANOVA (*$p$ <0.05, **$p$ <0.01, ****$p$ <0.0001). E1: Experiment 1 (puberty); E2: Experiment 2 (adulthood). A. NL-C; B. NL-M; C. SL-C; D. SL-M.

puberty and help restore metabolic balance, as observed in our findings.

Studies show that SL animals present hepatic steatosis due to fat accumulation, which contributes to poor organ function. MET has demonstrated the potential to improve hepatic steatosis (36–38). In the present study, a similar improvement was observed after MET treatment during puberty. Despite the short treatment duration, MET reduced liver weight and fat accumulation, as confirmed by histological analyses. At the molecular and tissue level, another plausible explanation for the reduction in hepatic lipid deposition and decreased liver weight in animals treated during puberty is the action of MET on the AMPK pathway in the liver. This action reduces lipogenesis, increases fatty acid oxidation, and decreases local inflammation. These mechanisms have already been observed in experimental models of steatosis and in clinical meta-analyses of non-alcoholic fatty liver disease (NAFLD) (39,40). The greater efficacy observed during puberty may reflect greater hepatic sensitivity to AMPK activation during this period or a

greater capacity for adipose and liver tissue remodeling to reestablish energy homeostasis. From a translational perspective, the data suggest that pharmacological or behavioral interventions, when administered during adolescence, may have greater potential to reverse metabolic risks programmed early in life than interventions initiated only in adulthood. Therefore, our results reinforce the idea that puberty is a promising window for intervention.

We acknowledge that our work is limited by the absence of in-depth molecular assessments, such as those of AMPK activity, insulin signaling, and lipogenic profile, which would allow for a more direct link between the observed phenotypic effects and the proposed mechanisms. Furthermore, the study was conducted on a single sex, which precludes conclusions about sex differences in response to the intervention; therefore, future investigations should analyze both sexes.

## Conclusion

In conclusion, we present the first evidence that short-term MET treatment during puberty can improve metabolic parameters induced by overnutrition during lactation. The same treatment in adulthood does not yield similar benefits. However, the precise mechanism through which MET acts within this specific timeframe to induce long-term metabolic programming remains unclear. Importantly, our findings scientifically confirm that a short-term intervention during adulthood is insufficient to mitigate metabolic dysfunction in obese animals, reinforcing the importance of the DOHaD concept.

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## Conflict-of-Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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