

# Mismatch between obesogenic intrauterine environment and low-fat postnatal diet may confer offspring metabolic advantage

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## Abstract

**Objective:** Mismatch between a depleted intrauterine environment and a substrate-rich postnatal environment confers an increased risk of offspring obesity and metabolic syndrome. Maternal diet-induced obesity (MATOB) is associated with the same outcomes. These experiments tested the hypothesis that a mismatch between a nutrient-rich intrauterine environment and a low-fat postnatal environment would ameliorate offspring metabolic morbidity.

**Methods:** C57BL6/J female mice were fed either a 60% high-fat diet (HFD) or a 10% fat control diet (CD) for 14-week pre-breeding and during pregnancy/lactation. Offspring were weaned to CD. Weight was evaluated weekly; body composition was determined using EchoMRI. Serum fasting lipids and glucose and insulin tolerance tests were performed. Metabolic rate, locomotor, and sleep behavior were evaluated with indirect calorimetry.

**Results:** MATOB-exposed/CD-weaned offspring of both sexes had improved glucose tolerance and insulin sensitivity compared to controls. Males had improved fasting lipids. Females had significantly increased weight and body fat percentage in adulthood compared to sex-matched controls. Females also had significantly increased sleep duration and reduced locomotor activity compared to males.

**Conclusions:** Reduced-fat dietary switch following intrauterine and lactational exposure to MATOB was associated with improved glucose handling and lipid profiles in adult offspring, more pronounced in males. A mismatch between a high-fat prenatal and low-fat postnatal environment may confer a metabolic advantage. The amelioration of deleterious metabolic programming by strict offspring adherence to a low-fat diet may have translational potential.

## KEYWORDS

fetal programming, glucose tolerance, insulin resistance, maternal obesity

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## 1 | INTRODUCTION

Rates of obesity are dramatically increasing worldwide, including among pregnant women.<sup>1</sup>

One in three US women has a body mass index consistent with obesity at the time of conception.<sup>1,2</sup> Maternal obesity not only increases the risk of complications during pregnancy and at delivery, but is also associated with increased offspring risk of obesity and early-onset metabolic syndrome described in observational human studies.<sup>3</sup> Animal model studies have some advantages in studying underlying mechanisms, in that maternal and offspring diet can be precisely manipulated, isolating offspring exposure to maternal obesity and/or high-fat diet to desired developmental windows.

Animal studies have demonstrated that exposure to maternal obesity and high-fat diet is associated with an increased risk of obesity and metabolic syndrome in offspring.<sup>4-9</sup> Yet, many of these studies have been conducted by weaning the obesity-exposed offspring to the same diet as that of the dam.<sup>4-6,10</sup> This experimental paradigm makes it challenging to understand the relative contributions of the intrauterine/lactational versus the postnatal environment to offspring metabolic morbidity. In addition, many these studies have been limited by their lack of consideration of sex-specific effects.<sup>5,6,10</sup>

The literature also indicates that a mismatch between the in utero and postnatal environments is known to predispose offspring to obesity and metabolic syndrome.<sup>11-13</sup> However, most of these studies have focused on a depleted or substrate-poor intrauterine environment and a nutrient/substrate-rich postnatal environment.<sup>11,12,14</sup> Studies of pregnancy during famine have shown that a relatively nutrient-poor intrauterine environment compared to a more nutrient-rich postnatal environment predisposes offspring to obesity and cardiovascular disease.<sup>12,15,16</sup> Animal studies have also demonstrated that maternal protein malnutrition and other models of intrauterine growth restriction coupled with a nutrient-rich postnatal environment increase offspring risk for cardiometabolic morbidity, although such programming may be amenable to intervention during key windows of developmental plasticity.<sup>12,17</sup>

Few studies have evaluated offspring metabolic effects of an obese/high-fat intrauterine environment mismatched with a relative reduction in nutrient availability during the postnatal period.<sup>18-19</sup> Thus, these experiments sought to evaluate offspring metabolic programming in the setting of maternal obesity and high-fat diet, followed by a switch to 10% fat control diet immediately post-weaning and for the remainder of the lifespan. The following hypothesis was tested: a mismatch between the substrate-rich intrauterine/lactational environment of maternal obesity, and a relative reduction in nutrient availability in the post-weaning period, might ameliorate deleterious effects of offspring metabolic programming.

## 2 | METHODS

### 2.1 | Animal model and experimental design

Four-week-old C57Bl/6J mouse dams (Jackson Laboratory) were fed ad libitum either a lard-based high-fat diet (60% calories from

fat;  $N = 19$  dams, Research Diets D12492) or matched control diet (10% fat, matched for sucrose, fiber, and micronutrient content;  $N = 15$  dams, Research Diets D12450J) for 14 weeks prior to breeding, to ensure obesity in the dams fed the high-fat diet (Table 1). Maternal obesity was defined as at least a 30% increase in weight compared to age-matched controls.<sup>20</sup> Males were fed the control diet. Obese and lean dams were bred with control males. Dams remained on their assigned diet throughout pregnancy and lactation. All offspring were weaned to the control diet at P24. Figure 1 depicts the experimental timeline. Throughout the manuscript, offspring of obese dams are referred to as MATOB; offspring of control diet-fed dams are referred to as CD. Offspring were housed 2-4/cage with same-sex littermates, with ad libitum access to the control diet and water. The colony was maintained on a standard 12:12 light-dark cycle, with lights on at 07:00. The Massachusetts General Hospital Institutional Animal Care and Use Committee (IACUC) approved this protocol (#2017N00266), all guidelines for animal care and use were followed. All evaluations were performed in male and female offspring.

### 2.2 | Body composition

Adult offspring body composition was determined using nuclear magnetic resonance technology with EchoMRI™ (Echo MRI LLC). 18-24 offspring/sex/diet group were evaluated, reflecting 9 obese and 8 control litters.

### 2.3 | Glucose and insulin tolerance tests

Glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs) were performed on adult offspring (12-16 weeks old,  $N = 25-35$  offspring/sex/diet group, 19 obese and 15 control litters). For the GTT, after an overnight fast, offspring were injected intraperitoneally with D-glucose (50% dextrose; Hospira, NDC) at 2 mg/g body weight, a standard dose and mode of delivery for rodent GTTs.<sup>21</sup> For the ITTs, Novolin-R human insulin (Novo Nordisk; 0.4 mU/g body weight) was administered intraperitoneally after a 4-h fast.<sup>22</sup> For both tests, 1  $\mu$ l blood was collected in conscious animals via tail prick immediately before glucose or insulin injection (time = 0) and at 15, 30, 60, and 120 min after injection. Glucose levels were determined using a glucometer (Abbott, AlphaTrak2).

### 2.4 | Fasting cholesterol and triglyceride levels

Cholesterol and triglyceride levels were evaluated in adult offspring (16-20 weeks old,  $N = 14-18$  offspring/sex/diet group, 9 obese and 9 control litters). After an overnight fast, facial vein draws were performed at 9 AM. Serum total triglyceride and cholesterol levels were determined using a Dri-Chem 7000 instrument (HESKA).

## 2.5 | Metabolic cage analyses

Metabolic rate, locomotor activity, and sleep behavior were measured by indirect calorimetry in metabolic cages using a computer-controlled system (Promethion Metabolic Screening, Sable Systems International) in adult offspring (16–20 weeks,  $N = 12$ –21/sex/diet group, 12 obese and 11 control litters). Animals were individually housed and habituated to the apparatus for 48 h, followed by a 72-h data acquisition period. A standard 12:12 light–dark cycle, with lights on at 07:00 was utilized. All mice had ad libitum access to usual diet and water. Energy expenditure was calculated from  $O_2$  and  $CO_2$  continuous sampling. Body weight as a covariate on energy expenditure was analyzed by ANCOVA ( $\alpha = 0.05$ ). Physical activity was detected with XYZ beam arrays. Data acquisition and system control was coordinated using MetaScreen v. 2.2.8, and raw data were processed using ExpeData v. 1.8.2 (Sable Systems International).

## 2.6 | Statistical analyses

Statistical analyses were performed using GraphPad Prism (v8, GraphPad Software). The Shapiro–Wilk and D'Agostino–Pearson

TABLE 1 Maternal diet composition

Contents	High-fat diet D12492		Control diet D12450J	
	g (%)	kcal (%)	g (%)	kcal (%)
Protein	26.2	20	19.2	20
Carbohydrate	26.3	20	67.3	70
Fat	34.9	60	4.3	10
Kcal/gram	5.24		3.85	
	g	Kcal	g	Kcal
Sucrose	68.8	275.2	68.8	275.2
Casein (30 mesh)	200	800	200	800
Lard	245	2205	20	180

tests were used to evaluate normal distribution of data. Comparisons between groups were made using two-tailed student's  $t$  test for normally distributed data, or Mann–Whitney test for non-normally distributed data. Male and female offspring data were analyzed in a pre-planned, sex-stratified fashion. Statistical significance was defined as  $p < 0.05$ . Only 1–2 offspring/sex/litter were analyzed, to avoid litter effects.

## 3 | RESULTS

### 3.1 | Offspring weight gain and adiposity

There were no significant differences between litters from obese and lean dams with respect to litter size or sex distribution by litter (Table 2). Male and female MATOB-exposed post-weaning juveniles had significantly higher body weight compared to sex-matched controls; however, only the obesity-exposed females weighed significantly more as adults, as depicted in growth trajectories by sex (Figure 2A,B). Female MATOB-exposed adult offspring had significantly increased body fat percentage compared to their sex-matched controls, while MATOB-exposed male differences did not achieve statistical significance (MATOB F vs. CD F,  $p < 0.01$ ; MATOB M vs. CD M  $p = 0.06$ ; Figure 2C). MATOB-exposed male offspring had significantly lower percent lean body mass compared to their sex-matched controls (MATOB F vs. CD F,  $p = 0.50$ ; MATOB M vs. CD M  $p = 0.04$ ; Figure 2D).

### 3.2 | Glucose and insulin tolerance tests

MATOB-exposed adult offspring demonstrated significantly improved glucose tolerance compared to their sex-matched controls. Both males and females demonstrated significantly lower blood glucose 30 and 60 min after IP glucose injection (Figure 3A). The glucose AUC over 120 min after glucose IP injection was significantly reduced in MATOB-exposed offspring of both sexes (Figure 3B). MATOB-exposed adult offspring of both sexes also

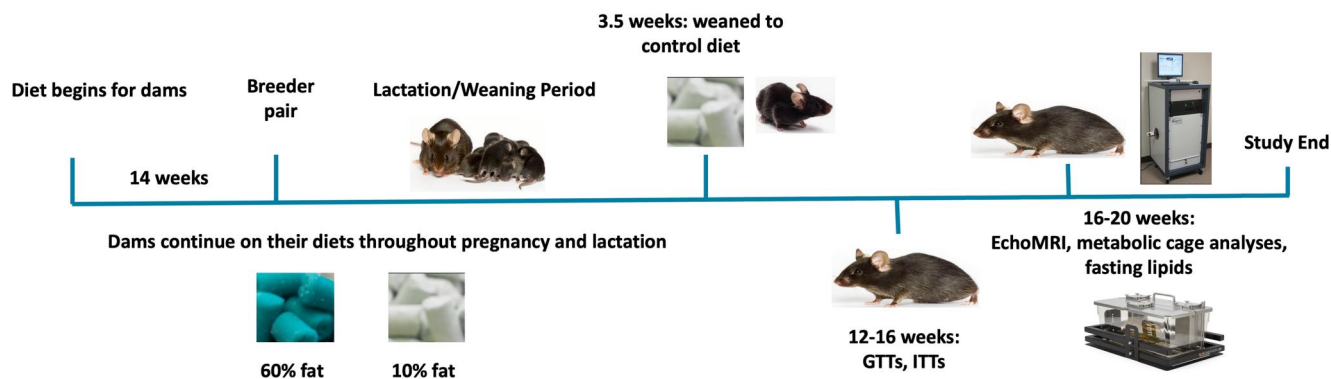


FIGURE 1 Experimental timeline. GTT, glucose tolerance test; ITT, insulin tolerance test

TABLE 2 Litter size and sex distribution by litter

Characteristic	Sex	Diet group		p-Value
		MATOB	CD	
Litter size, n (mean ± SEM)	Combined	6.1 ± 0.28	6.68 ± 0.25	0.43
Offspring per sex/litter/group, n (mean ± SEM)	Male	3.25 ± 0.37	3.15 ± 0.36	0.87
	Female	2.85 ± 0.39	3.52 ± 0.36	0.21

Abbreviations: CD: control diet; MATOB: maternal diet-induced obesity.

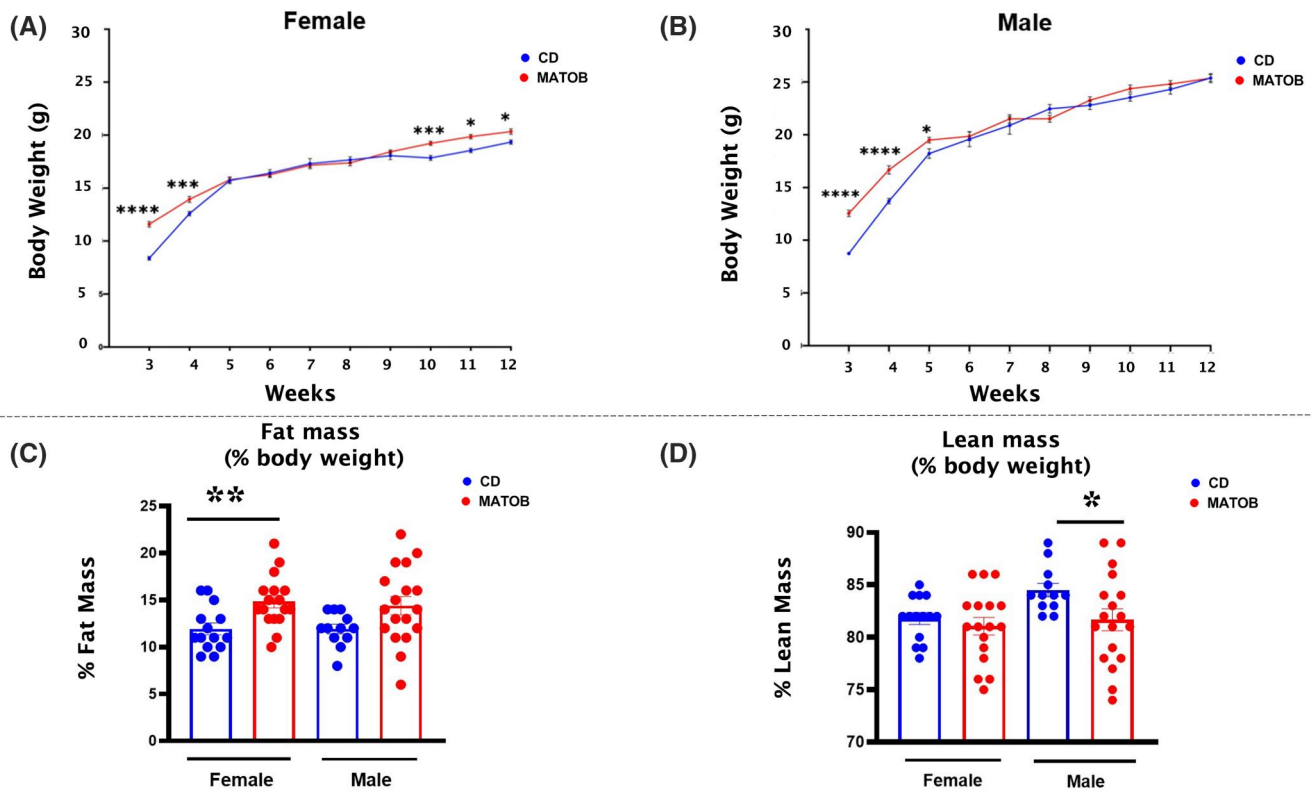


FIGURE 2 Offspring growth trajectories and body composition by sex. (A) Female offspring weight trajectory. (B) Male offspring weight trajectory. (C) Adult offspring percent fat mass by sex. (D) Adult offspring percent lean mass by sex. MATOB-exposed female offspring weighed significantly more and had significantly increased adiposity compared to sex-matched controls as adults. These changes were not observed in male offspring. CD, control diet; MATOB; maternal diet-induced obesity. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ . All data are depicted as mean ± SEM

demonstrated significantly increased insulin sensitivity compared to sex-matched controls. Both males and females had significantly reduced blood glucose levels at 15 and 30 min after IP insulin injection (Figure 4A). The glucose AUC in response to insulin was significantly reduced in MATOB-exposed offspring of both sexes (Figure 4B).

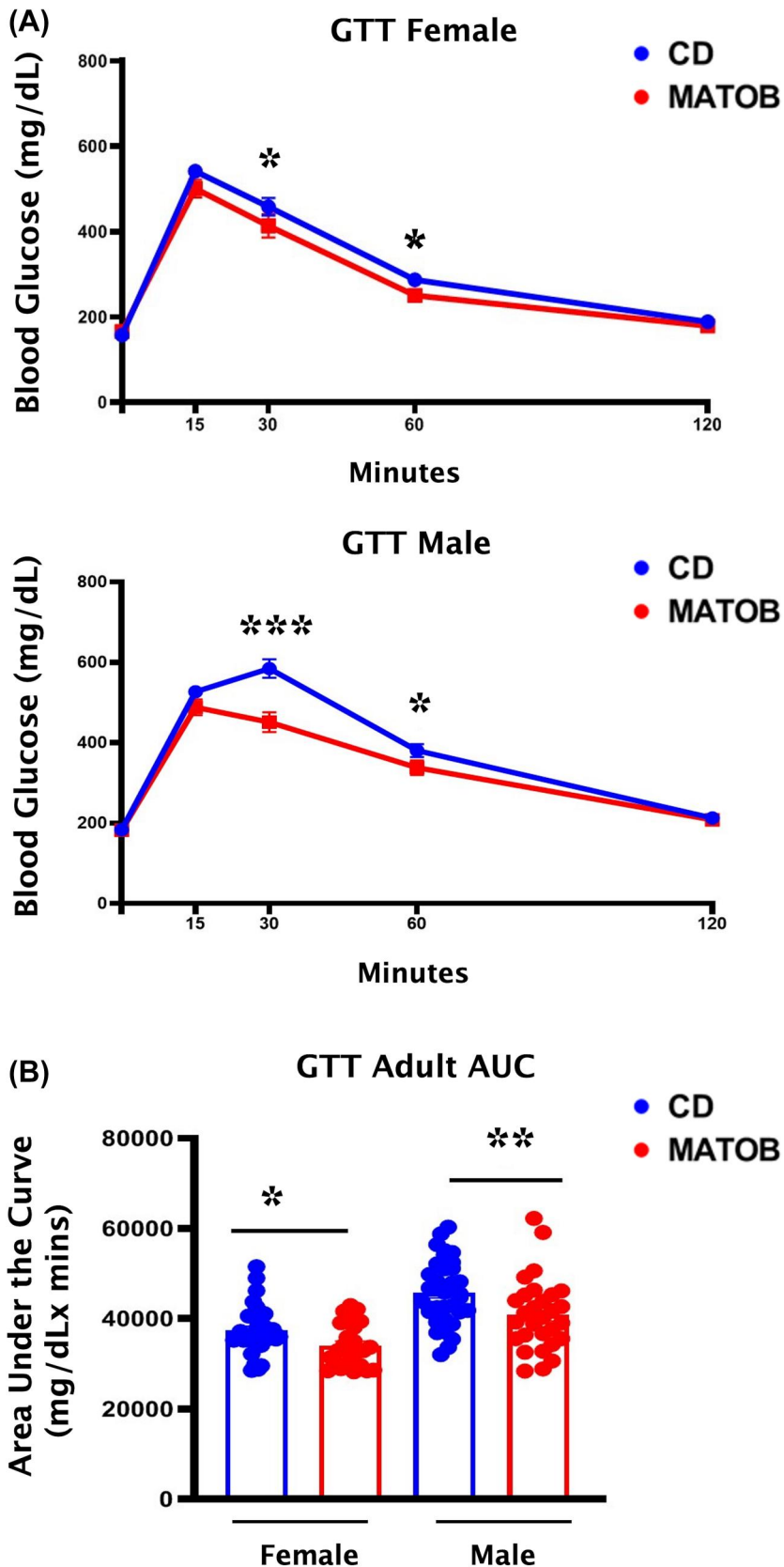
### 3.3 | Cholesterol and triglyceride levels

MATOB-exposed adult males had significantly lower fasting cholesterol than sex matched controls (MATOB M  $120.5 \pm 22.50$  mg/dl vs.

CD M  $143 \pm 10.22$  mg/dl,  $p = 0.04$ ; Figure 5) and reduced triglycerides compared to sex-matched controls, although this finding did not achieve statistical significance (MATOB M  $132.6 \pm 29.32$  mg/dl vs. CD M  $161.9 \pm 16.11$  mg/dl,  $p = 0.08$ ; Figure 5). In female offspring, there were no significant differences in lipids between MATOB-exposed and lean-exposed groups (Figure 5).

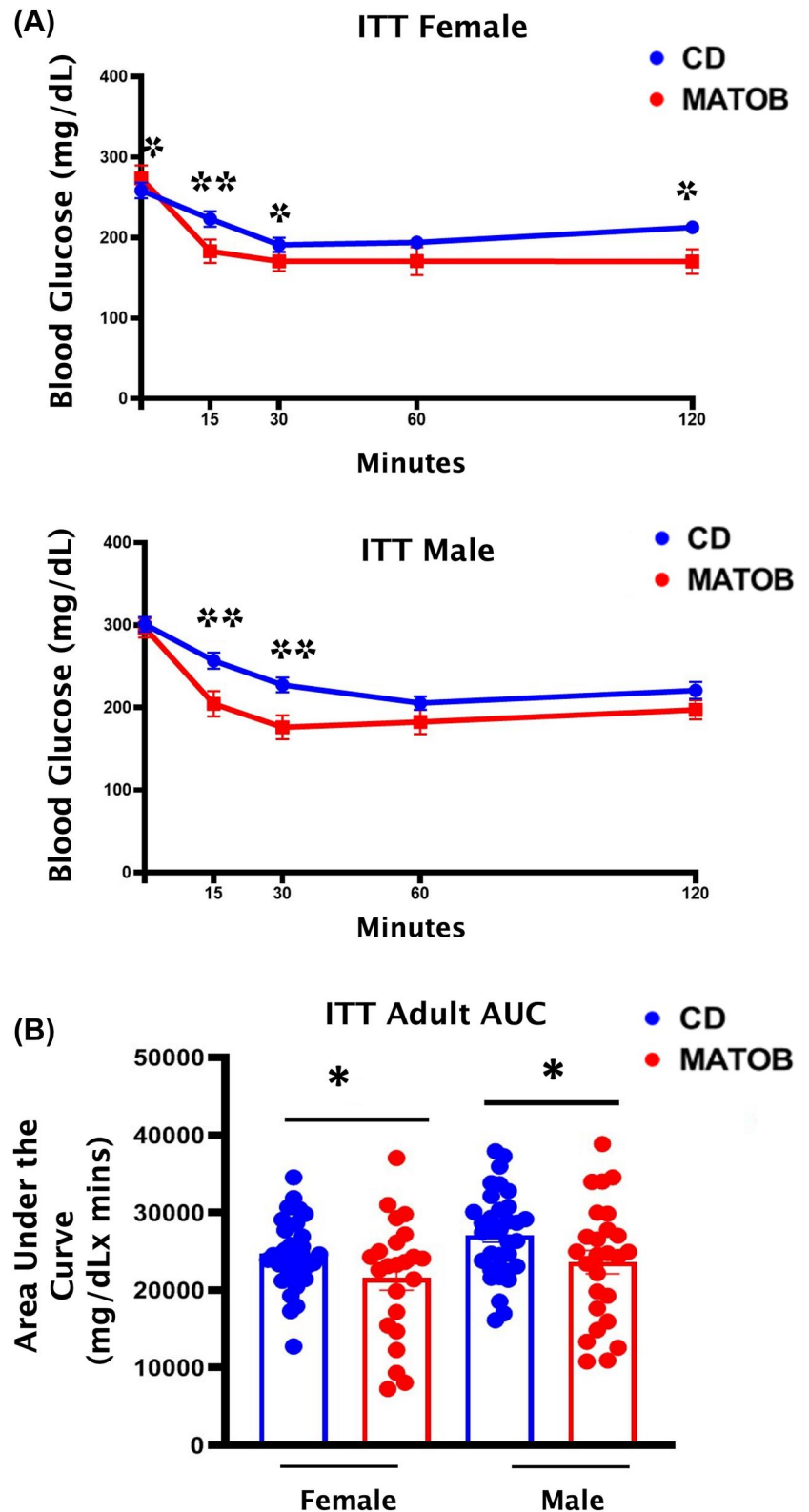
### 3.4 | Indirect calorimetry analyses

There were no significant differences in metabolic rate between the MATOB-exposed and lean-exposed offspring of either sex



**FIGURE 3** Obesity-exposed offspring weaned to control diet have improved glucose tolerance. Male and female offspring exposed to MATOB followed by 10% CD demonstrated improved glucose tolerance compared to their sex-matched controls (evidenced by reduced blood glucose after 2 mg/g IP glucose injection). This was true for both analyses by time (3A) and AUC analyses (3B). AUC, area under the curve; CD, control diet; GTT, glucose tolerance test; IP, intraperitoneal injection; MATOB, maternal diet-induced obesity. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . All data are depicted as mean  $\pm$  SEM

**FIGURE 4** Obesity-exposed offspring weaned to control diet have improved insulin sensitivity. Male and female offspring exposed to MATOB followed by 10% CD showed improved insulin sensitivity compared to their sex-matched controls (evidenced by reduced blood glucose after 0.4 mu/g IP injection). This was true for both analyses by time (4A) and AUC analyses (4B). AUC, area under the curve; CD, control diet; ITT, insulin tolerance test (glucose is the measured value); IP, intraperitoneal injection; MATOB, maternal diet-induced obesity. \* $p < 0.05$ ; \*\* $p < 0.01$ . All data are depicted as mean  $\pm$  SEM



during adulthood (Figure 6A). MATOB-exposed females demonstrated significantly increased sleep duration during the dark cycle, the usual active period, compared to sex-matched controls ( $p = 0.02$ , Figure 6B). MATOB-exposed females also had

significantly reduced locomotor activity in the dark cycle compared to sex-matched controls, with significantly reduced locomotor speed ( $p = 0.04$ ) and total distance traveled ( $p < 0.01$ ) (Figure 6C).



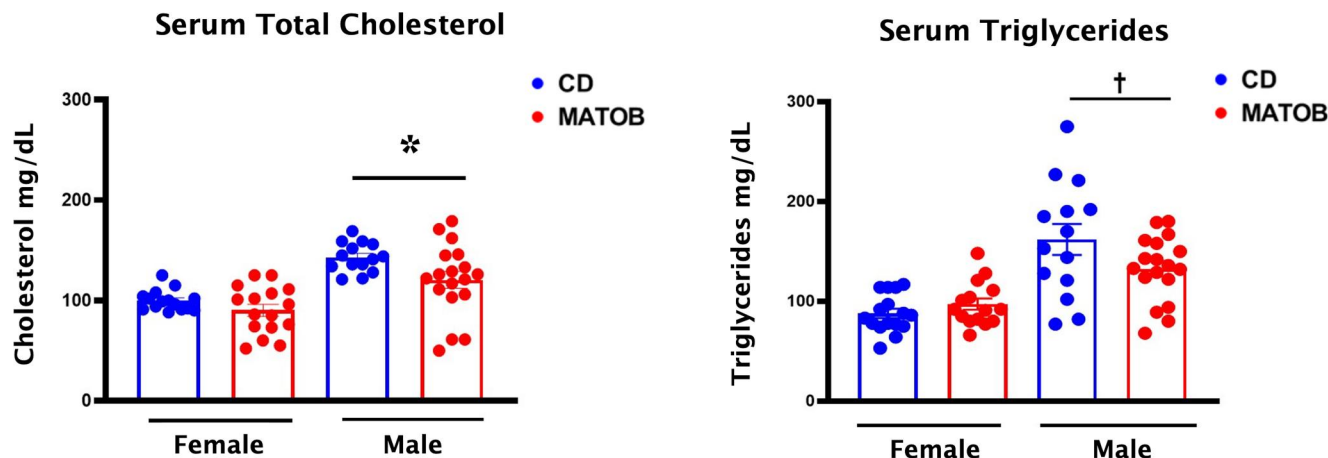


FIGURE 5 Obesity-exposed adult males have improved fasting lipids. Obesity-exposed adult males had significantly lower fasting cholesterol and trended toward reduced triglycerides compared to sex-matched controls. No significant difference was observed in female offspring. CD, control diet; MATOB, maternal diet-induced obesity. \* $p < 0.05$ ; † $p = 0.08$ . All data depicted are mean  $\pm$  SEM

#### 4 | DISCUSSION

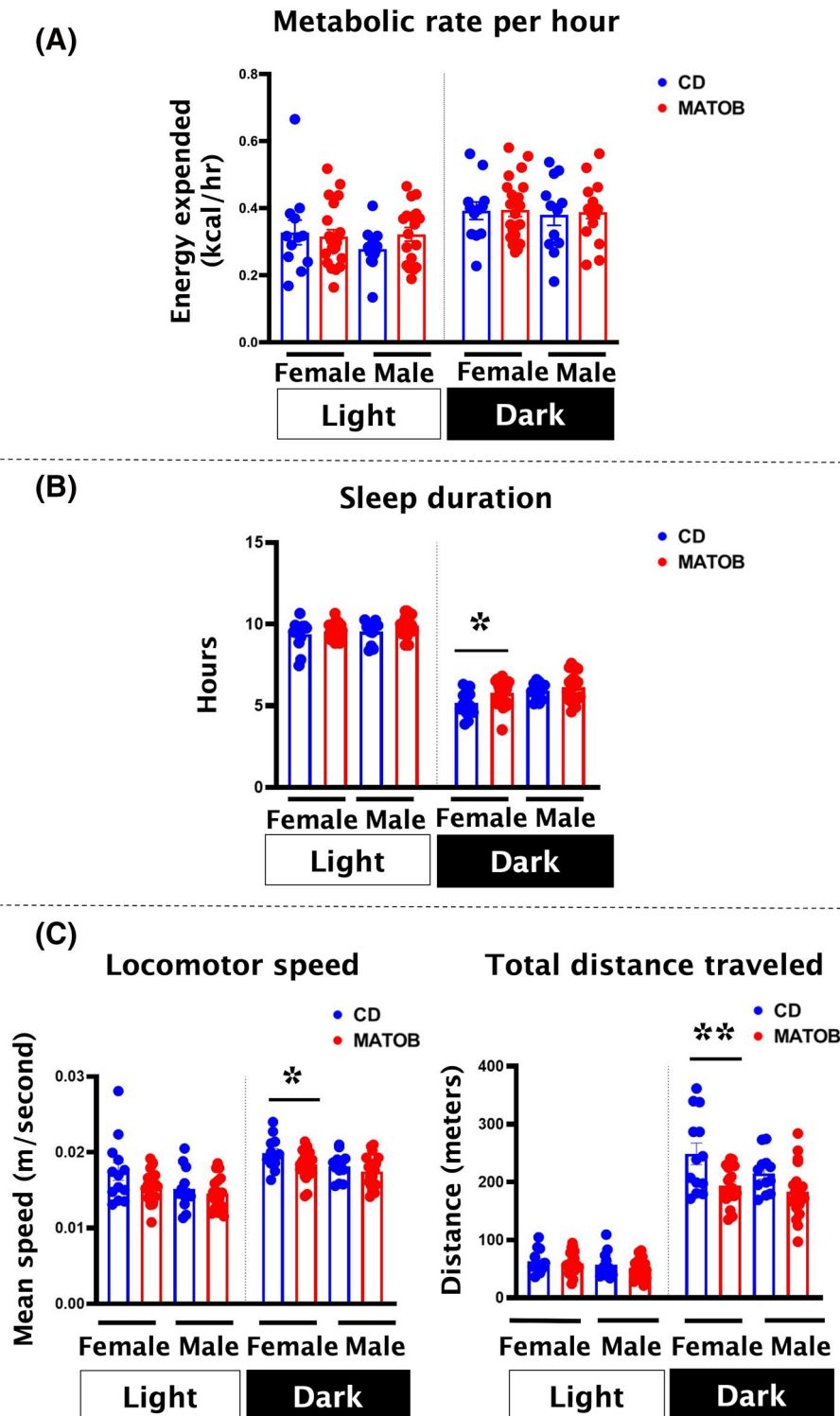
Adult offspring exposed to maternal obesity and a high-fat diet in utero and during lactation, followed by switch to a 10% fat control diet in the immediate post-weaning period and continued across the lifespan, have improved metabolic parameters compared to lean-exposed offspring that remained on the control diet postnatally. These include improved glucose tolerance and insulin sensitivity in offspring of both sexes, and improved fasting cholesterol in male offspring. Female obesity-exposed offspring gained more weight and had increased adiposity in adult life compared to sex-matched controls, and demonstrated reduced locomotor activity and increased sleep duration compared to sex-matched controls. Maternal obesity was not associated with significant differences in adult offspring metabolic rate in either sex.

To place these results in the context of the existing literature, exposure to maternal obesity and high-fat diet has been demonstrated to result in metabolic dysfunction in offspring,<sup>6,9,23,24</sup> including glucose intolerance,<sup>9,23,25</sup> insulin insensitivity,<sup>25,26</sup> hyperlipidemia,<sup>6,10,24</sup> fatty liver disease,<sup>24</sup> and increased body weight and adiposity.<sup>9</sup> The results presented here demonstrate improved glucose tolerance, insulin sensitivity, and fasting lipids robust across multiple litters. The results thus differ from some of the aforementioned studies, with several possible explanations for the differences. First, many prior studies are limited by their design of weaning offspring to the mother's diet.<sup>4-6,10</sup> In this study, offspring were weaned to the 10% fat control diet for the remainder of the lifespan. As mentioned, many of the above studies examined male offspring only,<sup>4,5,10,27,28</sup> and some studies differed from the present study in the route of administration of the glucose tolerance test, using oral administration.<sup>5</sup> Although oral gavage is also a common technique for GTT in rodents, it is known to increase animal stress, raising the potential for confounding.<sup>29</sup>

Several studies have demonstrated results comparable to this study's, where a maternal or offspring dietary or nutritional

intervention resulted in an improvement in offspring developmental programming (both neurodevelopmental and cardiometabolic) in the setting of maternal obesity.<sup>30-34</sup> In a murine model of maternal diet-induced obesity, social deficits and neuroinflammation in offspring were improved by a maternal lactational dietary switch, with female offspring demonstrating more plasticity than males in this regard.<sup>30</sup> Adverse neurodevelopmental programming in male offspring of obese ewes was improved by maternal pre-gestational, gestational and lactational dietary interventions.<sup>31</sup> Amelioration of MATOB-associated offspring metabolic dysfunction by maternal or offspring dietary interventions has also been described.<sup>33,34</sup> Adverse metabolic phenotype in male offspring of obese rats was partially reversed by switching obese dams from a high-fat diet to normal chow one month prior to mating.<sup>34</sup> Studies have also demonstrated improved offspring metabolic profile in the setting of relative undernutrition/reduced substrate availability during lactation, including improved insulin sensitivity, reduced body fat and perigonadal adipocyte size, and reduced total body weight.<sup>32,33</sup>

Few studies have directly examined the impact of maternal high-fat diet feeding and/or maternal obesity followed by weaning to a control diet on metabolic programming of both male and female offspring.<sup>18,19,23,24,35,36</sup> These studies have had conflicting results, with one demonstrating no significant impact of maternal diet-induced obesity and high-fat diet during lactation on offspring metabolic phenotype;<sup>35</sup> two demonstrating protective effect of maternal HFD on offspring weight gain and metabolic profile when re-challenged with either a high-fat or palatable diet;<sup>18,19</sup> two demonstrating increased adiposity and body weight and adverse impact on blood pressure, cholesterol or glucose homeostasis among female but not male offspring exposed to high-fat or high-fat/high-sugar diet in pregnancy and lactation, even when weaned to a chow diet;<sup>23,24</sup> and one demonstrating increased fat preference and food intake in offspring exposed to a junk food diet in utero and during lactation, with hyperphagia corrected by weaning



**FIGURE 6** Obesity-exposed females have increased sleep duration and reduced locomotor activity. (A) There was no significant difference in metabolic rate between the MATOB-exposed and control offspring of either sex. (B) MATOB-exposed female offspring spent significantly more time sleeping during the dark cycle, the usual active period. (C) MATOB-exposed female offspring had significantly decreased locomotor activity (both speed and total distance traveled). CD, control diet; MATOB, maternal diet-induced obesity. \* $p < 0.05$ ; \*\* $p < 0.01$ . Data are depicted as mean  $\pm$  SEM

offspring to standard chow.<sup>23,24,36</sup> The aforementioned studies differed from this study with respect to species or strain of rodent used, macro- and micronutrient content of diets utilized, duration of

maternal high-fat diet feeding/establishment of maternal obesity, duration of offspring follow-up, and key metabolic programming endpoints examined.



There are several biologically-plausible explanations for the improved metabolic profile of obesity-exposed offspring reported here. First, just as malnutrition in utero followed by abundance of postnatal nutrition resources has been associated with increased risk of cardiometabolic disease in human epidemiologic studies<sup>14,37</sup> and animal models,<sup>38,39</sup> the opposite type of mismatch (between an obese intrauterine/lactational environment and a postnatal diet with relatively reduced fat availability) may confer a metabolic advantage. Which factors might mediate this advantage is an important question for future studies. Body fat distribution is one candidate mediator of improved offspring glucose handling and insulin sensitivity. It is known that increased subcutaneous fat depots can act in an insulin-sensitizing capacity.<sup>40</sup> While the results demonstrated that obesity-exposed female offspring had increased body fat mass compared to control offspring, EchoMRI does not determine the distribution of body fat. Future experiments will therefore utilize DEXA to determine visceral versus subcutaneous deposition of adipose in offspring. Similarly, quantifying liver triglycerides in obese- and lean-exposed offspring is an important future direction, given that increased liver triglyceride deposition is associated with insulin resistance.<sup>41</sup> If liver triglyceride deposition is reduced in obesity-exposed offspring, this could suggest an important mechanism underlying increased insulin sensitivity and improved glucose tolerance.

The results demonstrate that after weaning, male and female obesity-exposed juveniles had increased body weight compared to their sex-matched controls; however, only obesity-exposed females weighed significantly more in adulthood. The literature is mixed regarding which sex is more vulnerable to increased weight gain and adiposity in the setting of maternal obesity. While some studies have suggested increased female vulnerability,<sup>23,24,26</sup> others have shown greater impact on male offspring.<sup>42,43</sup> In addition, it is unclear which period—pregnancy, lactation, or weaning—is most critical to programming weight gain, adiposity, and hyperphagia.<sup>23,26,36</sup>

With regard to the metabolic cage results, some studies suggest that maternal obesity is associated with increased locomotor activity in offspring, although data are conflicting regarding which sex is more affected.<sup>30,44</sup> In contrast, this study found increased sedentary behavior in obesity-exposed female offspring in the metabolic cage assessment, without a change in metabolic rate. The reduced locomotor activity and increased sleep duration in female adult offspring suggest that increased obesity liability in obesity-exposed female offspring may be mediated in part by increased sedentary behavior. This will be an important direction for future research.

A key strength of the study is the separation of maternal pregnancy and lactational diet from the offspring post-weaning diet, given that many studies in the literature continued the offspring on the dam's diet, failing to isolate perigestational exposure to maternal obesity and high-fat diet as the causative variable.<sup>4-6,10</sup> The novel finding that maternal obesity-exposed offspring have improved metabolic parameters after post-weaning dietary switch suggests that the deleterious effects of in utero and lactational exposure to maternal obesity and high-fat diet may be amenable to intervention, with male offspring demonstrating the most benefit. Another key

strength of the study is the examination of offspring of both sexes, given that many studies in the literature have focused on male offspring only.<sup>4,5,10,27,28</sup> A more mechanistic understanding of how sex modifies the effects of maternal obesity on offspring metabolic programming is critical to designing effective and targeted interventions. The examination of maternal obesity is a strength, given that many studies in the literature place dams on a high-fat diet for 4-6 weeks pre-breeding, failing to achieve maternal obesity.<sup>5,19,45</sup> This study's focus on obese dams provides more translational potential to human maternal obesity.

Other strengths of the study include: (1) the use of a control diet matched to the high-fat diet, permitting the isolation of maternal obesity and dietary fat content as the key variables. Recent work has demonstrated the importance of selecting the correct control diet in studies examining offspring developmental programming.<sup>46</sup> (2) The large number of offspring tested and significant litter diversity, with metabolic results robust across numerous test cohorts. (3) All rodent testing was performed by the same two experienced individuals, minimizing animal stress that could confound the results.

Obesity-exposed offspring were not weaned to a high-fat diet in this study as these experiments have already been performed elegantly by other groups.<sup>8,10,19,35,47,48</sup> While two studies by the same research group demonstrated an unexpected protective benefit of maternal high-fat diet on rat offspring later challenged with a palatable high-sucrose or high-fat diet,<sup>18,19</sup> the majority of groups have demonstrated a deleterious effect of post-weaning high-fat diet on offspring glucose tolerance, insulin sensitivity, hyperlipidemia and weight gain.<sup>8,10,36,47,48</sup> Male offspring demonstrated increased vulnerability to worsened metabolic parameters in the setting of maternal obesity and weaning to a postnatal high-fat diet.<sup>35,47</sup> These findings, taken together with the data presented here that male offspring demonstrated the most metabolic benefit from a post-weaning dietary switch, suggests that male offspring may have both increased vulnerability to adverse metabolic programming in the setting of maternal obesity, and increased capacity for metabolic improvement in the setting of intervention. To better understand the sex-specific differences in outcomes found in this study, future research can evaluate possible underlying mechanisms including malprogramming of hypothalamic satiety setpoints, central reward circuitry, and hyperphagia, as well as pro-inflammatory malprogramming of skeletal muscle, liver, and pancreas of offspring of both sexes.

As the fat, protein, lactose, fatty acid and insulin composition of human milk may impact infant growth and fat deposition in the first 12 months of age,<sup>49-51</sup> the lactational period is also a critical period to consider in initiation and maintenance of maternal dietary changes. Evaluating the efficacy of lactational interventions in altering metabolic programming of obesity-exposed offspring poses a challenge in rodent models, however. Cross-fostering experiments have been reported to increase pup stress, anxiety, and risk for weight gain in adulthood.<sup>52</sup> Such a design would raise the potential for confounding given this study's outcomes of interest. Other studies have examined lactational nutritional switches,

demonstrating improved insulin sensitivity when male offspring of obese dams are cross-fostered to lean dams, and increased obesity and insulin resistance in pups of lean dams exposed to high-energy diet.<sup>53,54</sup>

In addition to the question of lactational versus post-weaning dietary change, there are other relevant considerations relating to breastmilk and offspring metabolic morbidity in the setting of maternal obesity. Maternal breast milk reflects in part the maternal diet, and maternal obesity and the Western diet may contribute to higher overall fat content and increased long-chain polyunsaturated fatty acids (LC-PUFAS) that may be obesity-promoting in infants.<sup>49,55</sup> Published data are conflicting regarding the impact of maternal obesity on the omega-6:omega-3 (N6:N3) fatty acid composition of human milk, with some studies reporting significantly increased total saturated fatty acids with increased N6:N3 LC-PUFA ratio, and others finding no impact of maternal pre-pregnancy weight on the N6:N3 LC-PUFA ratio.<sup>56,57</sup> In addition, both animal and human studies have suggested that maternal obesity is linked to impaired lactogenesis.<sup>58–61</sup> The impact of maternal obesity on the hormonal and macronutrient composition of human milk has also been examined, with higher insulin, leptin, adiponectin, ghrelin, IL-6, and TNF-alpha noted in the milk of women with obesity.<sup>49,62,63</sup>

Previous work has demonstrated that maternal diet-induced obesity is associated with deficits in central catecholamine neurotransmitter synthesis and reduced mesolimbic dopamine release, with female offspring more vulnerable across the lifespan.<sup>64</sup> The experiments described here clearly demonstrate that peripheral metabolic programming is not a substantial driver of offspring obesity risk in this model. The predisposition to weight gain observed among female offspring in the model might therefore be driven by central malprogramming, such as increased propensity for reward-driven eating, in combination with the increased sedentary behavior and sleep noted in females during the usual active period. Thus, future experiments will evaluate central mesolimbic dopamine malprogramming as a targetable mechanism driving sex-specific offspring vulnerability to weight gain in the setting of maternal obesity.

These data are presented to spur the investigation of underlying mechanisms prior to translating this study's findings to humans. A more mechanistic understanding of the sex differences demonstrated here will be of importance in designing effective and targeted interventions. Ultimately, careful dietary regulation in offspring of women with obesity might hold promise as an intervention to attenuate deleterious obesity-associated metabolic programming.

## 5 | CONCLUSION

A low-fat dietary switch following intrauterine and lactational exposure to maternal diet-induced obesity and high-fat diet was associated with improved glucose handling, insulin sensitivity, and fasting lipids in adult offspring. These findings suggest that a mismatch between the intrauterine and postnatal environments,

from increased to reduced substrate availability, may confer a metabolic advantage. Male offspring may be more sensitive to improvement of metabolic parameters in this setting. Reversal of deleterious metabolic programming by maternal diet-induced obesity may be possible with strict offspring adherence to a low-fat diet.

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## AUTHOR CONTRIBUTIONS

Study design, funding acquisition, data collection, data analysis, data interpretation, literature search, and writing of the manuscript: Andrea G. Edlow. Data collection, data analysis, data interpretation, literature search, and writing of the manuscript: Sezen Kislal. Data collection, Writing—review and editing: William Jin. Data collection and writing—review and editing: Claire Maesner.

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