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Maternal treatment with oral intestinal alkaline phosphatase mitigates high fat diet-induced cognitive disorders in offspring mice

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ABSTRACT

Intestinal alkaline phosphatase (IAP) is an endogenous enzyme that promotes gastrointestinal homeostasis by detoxifying inflammatory mediators, tightening the gut barrier and promoting a healthy microbiome. Oral IAP administration was efficacious in ameliorating diabetes in a high fat diet (HFD)-induced murine model. In humans, maternal obesity and diabetes during pregnancy have been associated with an increased risk of autism spectrum disorders (ASD). In mice, HFD-induced maternal obesity leads to offspring with cognitive deficiency. Here we investigated whether IAP administration to obese dams could ameliorate autism-like disorders in mice. Using a HFD murine model, we recapitulated that maternal obesity leads to male offspring with social deficits as shown by the three chamber test and reciprocal social interaction analyses. Notably, oral delivery of IAP to dams improved those deficiencies. In addition, a jumping behavior was noted in pups from obese dams, which was rescued by maternal IAP treatment. Our findings suggest that maternal treatment with IAP can relieve some ASD-like symptoms in offspring mice.

1. Introduction

Prenatal maternal stress is a risk factor for both autism and attention deficit hyperactivity disorder [1]. In particular, obesity and diabetes during pregnancy have been associated with an increased risk of Autism Spectrum Disorders (ASD) in offspring [2]. While the mechanisms by which obesity leads to cognitive impairment are not well understood, a recent report suggested that changes to the microbiome as a consequence of high fat diet (HFD) maybe a potential cause of neurodevelopmental imbalances in offspring mice [3]. The role of the microbiome in ASD is further supported by recent evidence showing an amelioration of ASD symptoms in children treated with fecal microbiota transplantation [4]. In addition to changes in the gut microbiome, high fat diet causes local intestinal as well as systemic inflammation, which may also contribute to neurodevelopmental abnormalities [5].

IAP is an endogenous enzyme produced in the GI tract that maintains gut health by several mechanisms: 1- detoxifying inflammatory mediators, such as lipopolysaccharide (LPS), flagellin, CpG DNA and nucleotides [6]; 2- upregulation of the tight junction proteins zonulin, claudin and occludin resulting in tightening of the gut barrier [7]; and 3- favoring symbionts to pathobionts and promoting gut microbiome homeostasis [8]. The protective role of IAP in the context of obesity and diabetes has been demonstrated in humans and animal models. High fecal levels of IAP were shown to be protective in humans with type 2 diabetes (T2D) irrespective of obesity as obese individuals with high IAP levels did not develop T2D [9]. In mice, consumption of HFD reduced IAP activity, and oral IAP administration during HFD feeding prevented diabetes and metabolic syndrome [10,11]. Furthermore, subsequent studies demonstrated that IAP prevented antibiotic-associated diabetes and metabolic syndrome in mice [12]. The protective role of IAP was also corroborated in a transgenic murine model where overexpression of IAP solely in the GI tract was sufficient to attenuate HFD-induced phenotypes [13].

The ASD clinical phenotype is heterogeneous and encompasses a wide range of behaviors. Creating appropriate research animal models to study the disease is therefore challenging. However, some of the human ASD phenotypic features can be mirrored in mice [14]. Similar to humans, mice display robust and well-replicated social interactions. For example, when placed together in a confined arena, mice engage in reciprocal social interactions such as sniffing and physical play, all of which can be measured by automated software. Mouse repetitive behaviors, such as self-grooming, jumping, and circling, while dissimilar from humans, are important parameters to test, with variation from the baseline levels representing ASD-like symptoms. Anxiety, which occurs in approximately 30% of ASD individuals [15], can also be replicated and measured in mice. Furthermore, the role of maternal immune

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activation during pregnancy, which is associated with ASD in humans, is also reproduced in mice [16]. Because of these similarities, inbred mouse strains are currently considered a good research model to study idiopathic autism and are extensively used to evaluate new therapeutics [17].

Based on IAP roles in ameliorating HFD-induced diabetes and promoting a healthy microbiome, we hypothesized that IAP administration to obese dams would prevent ASD-like symptoms in offspring mice. Here, we report that indeed maternal obesity resulted in offspring with cognitive deficiency and that maternal IAP treatment improved some of their abnormal behaviors.

2. Materials and methods

2.1. Animals

The study was performed by CNS|CRO, a contract research organization based in Charlottetown, Canada, and the National Institutes of Health guide for the care and use of laboratory animals was followed. Male and female C57Bl/6J mice (Jackson Laboratories, USA) were housed alone (males) or in groups (females) at 2–3 per cage in a specific pathogen free facility and maintained on a standard 12 h day/night lighting schedule with temperature and relative humidity regulated at set points of 22 − 23 °C and 40–60%, respectively. A standard rodent chow (Lab Diet 5001) or high fat diet (Research Diets D12492) and reverse osmosis purified water were provided *ad libitum*. The study was approved by the local Animal Care Committee (Charlottetown, PEI), and all animal husbandry and experimental procedures conformed to the guidelines established by the Canadian Council for Animal Care.

2.2. Experimental design

Study design was similar to that of Buffington et al. [3]. Sixteen female C57BL/6J mice were assigned to one of three treatment groups: 1 - standard diet (SD) and vehicle; 2 - high fat diet (HFD) and vehicle; and 3 - HFD + IAP. IAP (or vehicle control) was administered in the cage drinking water from the time of diet initiation until weaning of the offspring. At 8 weeks post-initiation of feeding, females were paired with males to breed overnight. Females were returned to their home cages each morning and checked for the presence or absence of a copulatory plug. Presence of a plug indicated gestational day 0. Females with an observed plug were transferred to single housing.

As is standard practice in the field, male pups were used to investigate the behavioral changes. There is a sex-difference in the onset and severity of ASD-like disease, which, in both humans and animals, is more pronounced in males [18]. Pups were weaned at 3 weeks of age, at which time male pups were weighed, sorted into fresh cages by treatment group, and ear notched for tracking purposes. All pups, regardless of pre-weaning treatment group, received standard rodent chow and had access to water *ad libitum*. IAP or vehicle administration was discontinued at weaning.

Following weaning of their litters, dams re-entered the breeding cycle for a maximum of 3 rounds. Stud mice were paired only with females from a single treatment group to avoid contamination through coprophagic activity.

2.3. Experimental drug

The IAP used in this study is the recombinant form of the native calf-derived IAP produced in CHO cells. IAP was delivered to mice by addition to the cage water at a final concentration of 800 U/mL in 0.4 mM Tris − HCl buffer, pH 7.5, supplemented with 20 μM $MgCl_2$ and 2 μM $ZnSO_4$. As control, the same buffer used for IAP formulation but without the drug (vehicle) was added to cage water. Cage water was replaced daily with fresh IAP (or vehicle).

2.4. Behavioral testing

All the tests were executed at CNS|CRO by experienced personnel and followed the company`s SOPs. Behavioral testing was performed on male pups between postnatal weeks 7–12, with each test presented to each animal once during this time period. All offspring underwent the tests in the same order and at approximately the same age. All tests were video recorded for scoring and documentation purposes and analyzed by experimenters blinded to treatment. Analyses were performed using a combination of manual scoring and ANY-maze™ Video Tracking System (Stoelting Co., USA) evaluation. Male offspring were evaluated in the open field, three-chamber social interaction, marble burying and reciprocal social interaction tests as described below.

2.4.1. Open field

The open field test was administered during postnatal week 7 − 9. Mice were placed in the center of a brightly lit (48 × 38 cm) arena and allowed to explore freely for 10 min. Behavioral measures included latency to reach the outer wall region, time spent in thigmotaxic behavior, number of entries into and time spent in the center region, distance travelled, average speed in each region, and comparison of activities during the first and last minute.

2.4.2. Three-chamber social interaction test

The Three-Chamber test was administered during postnatal weeks 8 − 10. This test arena consisted of three equally sized rooms (20 × 45 cm each) divided by clear Plexiglas, and with an access door between each compartment. The test occurred in three distinct stages: (1) acclimatization phase (baseline), where the test animal was placed in the center compartment and allowed to freely explore the entire empty maze for 10 min; (2) sociability phase, where an unfamiliar male mouse was contained within a wire mesh container in an outer chamber of the maze and an identical clean and empty container was placed in the chamber at the opposite side of the arena; and (3) social novelty phase, where a familiar animal was presented in one compartment while an unfamiliar mouse was presented in the other.

2.4.3. Marble burying

Marble burying was performed during postnatal weeks 9 − 11. Each mouse was placed in a 17 × 28 cm arena filled with clean bedding (5 cm depth), with 20 regularly spaced marbles in a 4 × 5 marble grid sitting on top of the bedding. After 20 min, the number of marbles that had been buried (i.e. covered to at least two thirds of its depth) were counted.

2.4.4. Reciprocal social interaction

Dyadic testing was performed during postnatal weeks 10 − 12. Mice were simultaneously placed in an open, unfamiliar arena (25 × 25 cm) with either a familiar cage-mate or an unfamiliar partner (presentation counterbalanced across groups). In all cases, pairs were from the same treatment group. Mice were allowed to interact freely for 5 min, with latency to first interaction, number of and time spent in bidirectional interactions (e.g. nose-to-nose sniffing), and total time spent interacting quantified. Interaction was defined as any of the following: bidirectional encounters, close following, touching partner, allogrooming, nose-to- anus sniffing, and crawling over/under.

2.5. Statistical analysis

Data were analyzed using one-way ANOVA with or without repeated measures, or with Independent or Paired t-tests as applicable. Correction factors were applied as required. Statistically significant main effects were further investigated using post-hoc analysis (Tukey's or other appropriate tests depending upon sample size). Data are reported as mean ± SEM. Significance level for all tests was $p \leq 0.05$.

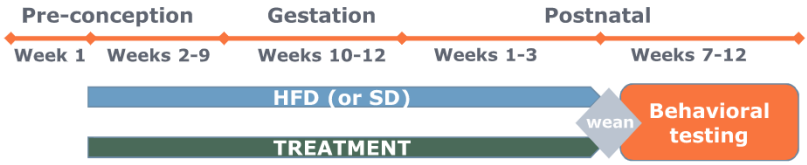

Fig. 1. Study schematic. Dams were coupled with male mice for up to three rounds of breeding, thus pre-conception period varied.

3. Results

To induce maternal obesity, female mice were fed a high fat diet (HFD) for eight weeks (Fig. 1). Standard diet (SD) was provided to a cohort of mice as normal control. During the eight weeks, one cohort of HFD mice received IAP at 800 U/mL in their drinking water, while the other two cohorts (HFD and SD) were administered vehicle. Throughout the eight weeks of HFD (or SD) treatment, both the HFD groups were significantly heavier than the control SD group ($p = 0.017$ and $p = 0.022$ for vehicle and IAP groups respectively). By week 8, dams weighed 19.1 ± 0.2, 22.9 ± 1.5, 24.7 ± 1.5, respectively for SD, HFD + vehicle and HFD + IAP. Water and food consumption amounts were the same for each group. After 8 weeks, female mice were paired with males for breeding.

Pups were weaned at 3 weeks of age and weights were recorded weekly. At the time of weaning, male pups from maternal HFD (MHFD) + vehicle and MHFD + IAP were heavier than pups from maternal SD (MSD) pups with weights of 9.9 ± 0.3, 10.4 ± 0.2, and 8.2 ± 0.4, respectively ($p = 0.011$ and $p = 0.021$ respectively; Tukey's post hoc) (Fig. 2A). By week 4, however, pups in all groups were similar in weight. Interestingly, when analyzed by percentage of weight gain, the difference in weight reached statistical significance with both MHFD groups not gaining as well post-weaning as MSD pups ($p \leq 0.001$; Fig. 2B). These results are similar to what is seen in other mouse models of ASD [19,20].

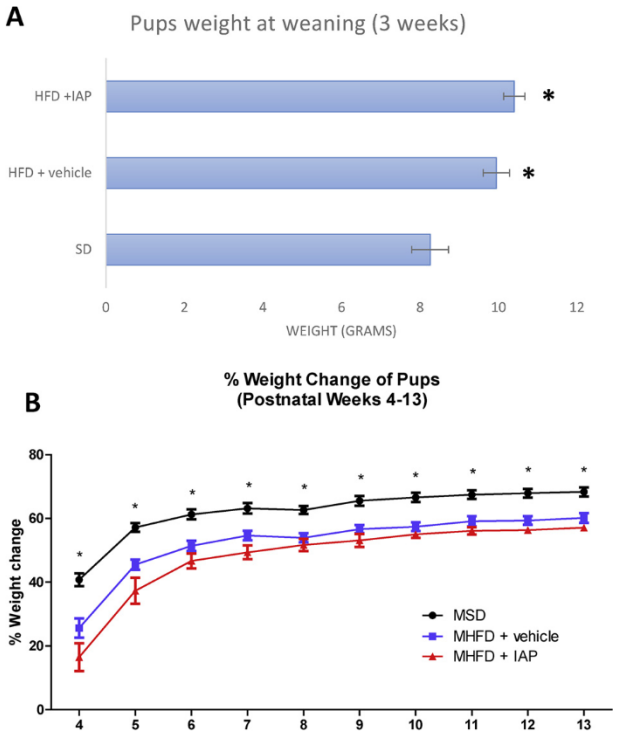

Fig. 2. Maternal obesity produced heavier offspring that did not gain as much weight over time compared to controls.
A) Weight of pups at weaning (3 weeks old) showing that MHFD pups are significantly heavier than MSD pups. *$p = 0.011$ and $p = 0.021$ for vehicle and IAP, respectively; Tukey's post hoc; mean ± SEM; MSD n = 14; MHFD + vehicle n = 14; MHFD + IAP n = 5.
B) Weight gain in offspring over time. An evaluation of weight gained post-weaning compared to weaning weight revealed that both MHFD groups did not gain as well as MSD offspring. Asterisks indicate a statistically significant difference between MHFD groups and MSD mice ($p \leq 0.001$; Tukey's post hoc); mean SEM ± ; MSD n = 14; MHFD + vehicle n = 14; MHFD + IAP n = 5.

Male pups were assessed for neurobehavioral deficiencies. Offspring were first tested in an open field paradigm, in which no differences between any of the groups were noted, as was also shown in Buffington et al. [3]. Similarly, the marble burying test resulted in no differences among groups.

Offspring were evaluated in the three-chamber social interaction test. One of the features of ASD patients is being less "sociable", a feature that can be assessed in this set of experiments. An analysis of baseline exploration revealed no bias for left or right chambers with any treatment group. During the sociability phase, there were no between-group differences in time spent or number of entries into any of the 3 chambers (stranger mouse, novel object, or center; $p > 0.05$). However, there was a trend for MHFD + vehicle offspring to spend less time interacting, hence mimicking one of the human ASD features. This behavior was rescued by maternal IAP treatment (Fig. 3) with offspring showing an improvement in their ability to socialize with other mice. Overall, the three chamber tests suggest that treatment with IAP may be ameliorating some of the ASD-like social anomalies in this model.

Alterations in social behavior is a key component of ASD and the reciprocal social interaction testing provides a more complex evaluation of social activities in rodents. When tested with a familiar cage mate, offspring showed no differences in any measure of social interaction behavior for any group. However, when tested with an unfamiliar mouse, MHFD + vehicle pups showed different behavior compared to the MSD and MHFD + IAP groups (Fig. 4). In particular, there was a significant increase in the number of head-to-head (reciprocal) interactions for the MHFD + vehicle pups as compared to both the MSD ($p = 0.022$, Tukey's post hoc) and MHFD + IAP ($p = 0.046$, Tukey's post hoc) groups. Similarly, the number of nose-to-tail

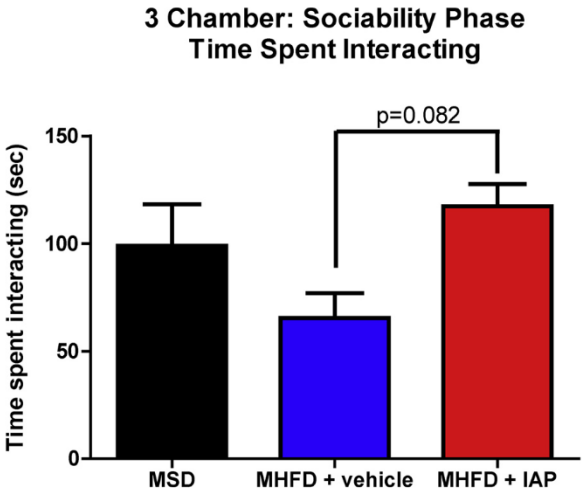

Fig. 3. Decreased overall interaction time in offspring from MHFD. MSD and MHFD + IAP offspring spent more time interacting compared to MHFD + vehicle during the sociability phase (novel mouse plus novel object) of the three chamber test. Mean ± SEM. MSD n = 10; MHFD + vehicle n = 14; MHFD + IAP n = 5.

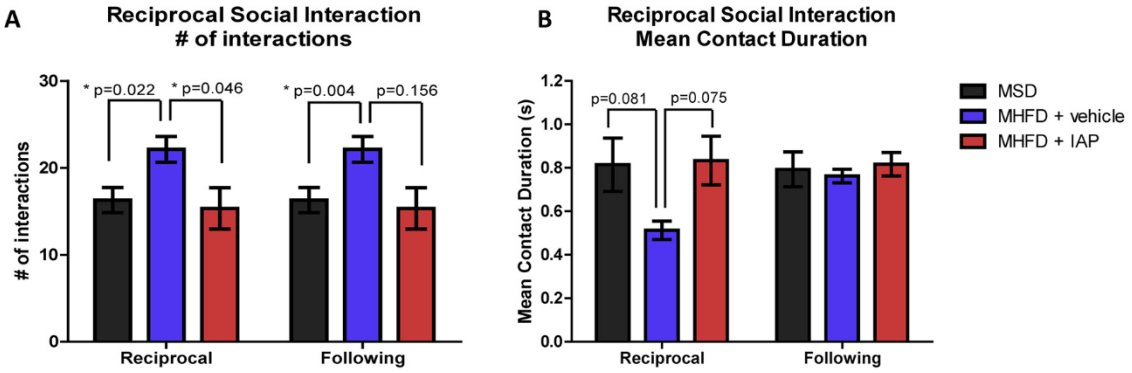

Fig. 4. Increased number of interactions and decreased contact time in MHFD pups. When tested with a stranger mouse, MHFD + vehicle offspring performed an increased number of reciprocal social interactions but showed a trend toward decreased reciprocal contact time compared to both MSD and MHFD + IAP mice. Mean ± SEM. MSD n = 14; MHFD + vehicle n = 16; MHFD + IAP n = 6.

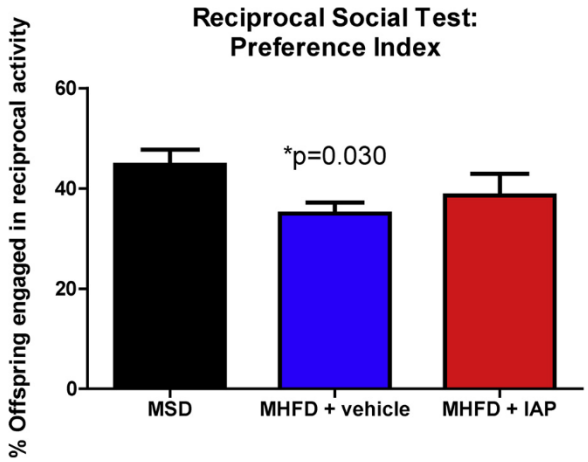

Fig. 5. Decreased contact duration time in MHFD pups. MFHD + vehicle animals demonstrated a preference to engage in individual (i.e. following) rather than reciprocal interactions during dyadic social interaction testing which was not seen in MSD pups and was rescued by IAP administration. Mean ± SEM. MSD n = 14; MHFD + vehicle n = 16; MHFD + IAP n = 6. Asterisk indicates a significant difference between the MSD and MHFD + vehicle groups (p = 0.030; Tukey's post hoc).

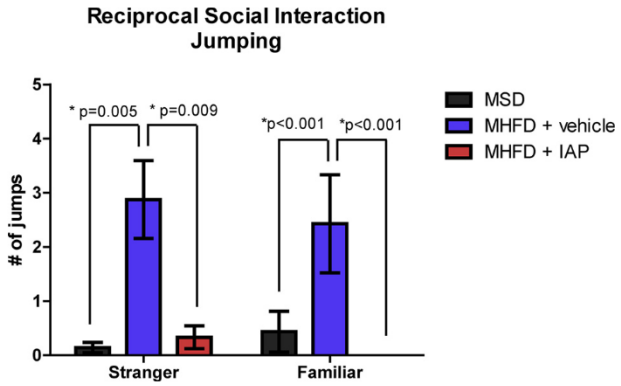

Fig. 6. MHFD alone resulted in offspring displaying a jumping behavior. Quantification of jumping behavior during dyadic testing revealed that MHFD + vehicle mice jumped more than either MDS and MHFD + IAP groups. This effect persisted regardless of whether pairing occurred with a familiar or a stranger mouse. Error bars represent SEM. Mean ± SEM. MSD n = 14; MHFD + vehicle n = 16; MHFD + IAP n = 6.

(following) interactions was also increased in the MHFD + vehicle group compared to the MSD and MHFD + IAP (Fig. 4A). The increase in the number of contacts did not translate to an increase in social behavior, however, as analysis of the mean contact duration showed a different behavior in MHFD + vehicle, with pups engaging in a shorter head to head contact duration time compared to MSD and MHFD + IAP (Fig. 4B). No differences were observed between groups for mean contact duration in head-to-tail interactions. When examined in the context of preference for individual interactions versus preference for social contact, the MHFD + vehicle group was found to have a significant decrease in social interaction overall during dyadic testing compared to MSD controls (p = 0.030; Tukey's post hoc), a difference not noted in MHFD + IAP mice (Fig. 5). Overall, this set of results showed that MHFD + vehicle offspring preferred to spend more time in individual interactions, a feature that is common in ASD patients, whereas MSD and MHFD + IAP offspring did not present this bias. This social interaction test further confirms the trend noted in the three chamber test, and provides additional support for the association of maternal obesity with offspring dysfunction while demonstrating that maternal treatment with IAP can ameliorate these social deficits.

In addition to social measures during reciprocal social interaction testing, pups from MHFD + vehicle exhibited an unusual jumping behavior. This activity may be used by ASD-like mice to reduce anxiety levels and may model behavioral patterns commonly seen in humans with ASD (Fig. 6). MHFD + vehicle pups exhibited a significant number of jumps during testing when encountering stranger and familiar mice. This behavior was completely reversed by oral administration of IAP, suggesting reduced anxiety levels in those mice.

4. Discussion

In this paper, we described the amelioration of maternal HFD-induced ASD-like syndrome in offspring by oral treatment of obese dams with IAP.

ASD is a heterogeneous group of neurodevelopmental disorders. While the exact etiology and pathogenesis are not well defined, increasing evidence supports an important role of the immune system in the pathogenesis of ASD. The increased incidence of ASD in the human population has been associated, in part, with environmental factors. In particular, maternal prenatal stress is a nonspecific risk factor for a wide array of neurodevelopmental outcomes in offspring. Stress has been associated with alterations of the immune response and hormone production in both humans and animal models [21]. Among stressors, obesity and diabetes during pregnancy are associated with increased risk for ASD in offspring [22]. While the mechanisms are incompletely understood, obesity/diabetes creates an inflammatory response that can lead to immune system dysfunction. This in turn creates the neuroinflammation and neuro-immune abnormalities that have now been established as key factors in ASD development and maintenance [23]. Moreover, inflammation is associated with changes to the gut

microbiome. Many studies have now shown alterations in the composition of the fecal microbiota and metabolic products of the gut microbiome in patients with ASD [24,25]. With the gut microbiome harboring and directing the immune response, changes to the resident bacteria may indeed not only result in local GI insult, which are common in ASD patients, but also systemic effects, such as neurodevelopmental deficiencies.

The role of obesity as a stressor in ASD pathoetiology has been modeled in mice [3]. In this model, maternal obesity during pregnancy led to offspring with social behavior impairments as shown by reciprocal social interaction and three-chamber test abnormalities. The observed neurobehavioral changes were associated with alteration of the gut microbiome. Intervention aimed at improving the gut microbiome was successful in ameliorating the disease severity.

It is interesting to note that in humans an enriched-fat diet causes metabolic endotoxemia, which is associated with obesity, diabetes and insulin resistance [26]. HFD is also associated with a reduction in intestinal bacterial diversity, increased gut barrier permeability, acute systemic inflammation and increased lipopolysaccharide (LPS) translocation [27]. In particular, LPS plays a central role in the pathogenesis of metabolic endotoxemia. In humans, administration of LPS leads to weight gain, acute inflammation and ultimately insulin resistance [28]. Similar effects are seen in animal models either fed a HFD or dosed with LPS. Importantly, maternal inflammation induced by LPS at late gestation led to offspring with increased anxiety and reduced social activity due to a pro-inflammatory reaction in the fetal brain [29]. Therefore, while the predictive value of animal models of ASD is incompletely clear, the important role of HFD and LPS in the pathogenesis of ASD seems to be consistent among humans and animals.

Here, we treated obese dams with IAP, an agent that functions to preserve intestinal homeostasis and diminish inflammation [30]. IAP administration had been shown to treat metabolic syndrome in animal models [11]. It is anti-inflammatory, prevents gut leakage and also promotes a healthy microbiome [31]. Therefore, here we employed IAP with the intent of ameliorating ASD caused by HFD-related stressors. In this first pilot study, we focused on analyzing behavioral ASD-like phenotypes in male offspring. We did not focus on measuring some other key parameters such as gut barrier permeability at both maternal and offspring levels, which will be evaluated in follow-up studies.

The offspring from MHFD groups were heavier at weaning compared to MSD pups. This is in agreement with the increased weight of children from diabetic mothers [32]. However, by the second post-weaning week, this increased weight was no longer evident, and offspring from HFD dams did not gain as much weight as the control animals for the remainder of the study. These results are similar to observations with other mouse models of ASD where prenatal zinc deficiency and genetic deletion resulted in offspring with reduced weight gain compared to controls [19,20]. Weight is also an issue for children with ASD [33,34]; however, medications taken by ASD patients can confound the role of weight in the disease. The difference in weight gain in the current study appears to be related to the HFD and not to the IAP treatment. One explanation for this phenomenon might be that pups from HFD dams had access only to HFD during the first few weeks of life and thus acquired a “taste” for the softer, more easily chewed pellets; alternatively, the microbiome of animals fed HFD early in life could become used to digesting more calories, and when transferred to lean, standard-diet fed, these animals maintained this higher metabolic rate, resulting in less weight gain.

There were no differences noted in Open Field testing for any locomotor measures. This result is similar to what was reported for this model in Buffington et al. [3].

The 3 chamber test allows evaluation of both sociability and social novelty measures. In the current study, no statistically significant difference was noted between groups for any basic measures during sociability testing, although trends toward differences were seen that indicated a tendency for the offspring of MHFD + vehicle mice to spend less time interacting with the novel mouse compared to offspring of MHFD + IAP. When interaction time was evaluated as a whole (time interacting with novel mouse + time interacting with novel object), a trend toward an overall decrease in interaction time was noted for MHFD + vehicle offspring, particularly when compared to the MHFD + IAP group (Fig. 3). This result is in agreement with data from Buffington et al. [3] and suggests that dysfunctional social behaviors in MHFD offspring are attenuated by maternal IAP treatment.

Reciprocal Social Interaction testing provides a more complex evaluation of social activities in rodents compared to the three chamber paradigm, allowing assessment of dyadic interactions with familiar and unfamiliar conspecifics. During dyadic testing with familiar mice, there were no differences between groups for basic measures. With unfamiliar mice, however, MHFD + vehicle pups exhibited an increased number of overall interactions, both reciprocal and individual(i.e. following), compared to both MSD and MHFD + IAP groups. Interestingly, contact duration time for reciprocal interactions, but not individual interactions, was decreased for the MHFD + vehicle offspring. To further elucidate these data, a preference index was performed to compare overall reciprocal and individual activities for the groups. Results showed MHFD + vehicle mice preferred to spend more time in individual interactions, whereas MSD and MHFD + IAP animals did not have this bias. Alterations in social behavior are a key component of ASD-like models. Here, MHFD + vehicle offspring displayed altered social function compared to both MSD controls and MHFD + IAP mice by preferring head to tail rather than head to head contacts. These results provide further support of improved outcome in MHFD + IAP animals.

A final measure evaluated during dyadic testing was jumping behavior, as it was noted that some mice were persistently engaging in this activity. It was discovered that MHFD + vehicle mice were almost exclusively exhibiting such jumping behavior, while MSD and MHFD + IAP mice rarely jumped during testing. The jumping was observed with either familiar or stranger animals. Jumping was not, however, seen in the home cage, a phenomenon that might be due to the more confined conditions (i.e. from the cage lid) or that the mice experienced increased agitation from a novel environment combined with direct social stimulation. Since mice did not jump when cage lids were removed during normal husbandry, and this behavior was also not evident during open field testing, it is more likely the latter. Persistent jumping has been reported in other mouse models of ASD [35,36]. Jumping is a typical repetitive behavior seen in murine models, associated with genetic modifications leading to different phenotypic functions in the brain. The jumping activity has been proposed to be indicative of stereotypes commonly seen in ASD, and it has been suggested that this type of repetitive behavior might be used by ASD-like mice to help reduce elevated anxiety levels that result from a primary deficit in the ability to understand social situations. This possibility is of particular interest for the current study, as higher corticosterone levels have also been previously reported in offspring of HFD-fed mice with neurobehavioral disorders [37]. Indeed, HFD during hippocampal development was associated with complex patterns of gene expression leading to autism-like behaviors [37]. In a future study, it would be interesting to assess whether IAP administration has any effect on gene expression and therefore phenotypic changes in the brain. In addition, measurements of pro-inflammatory cytokines in both dams and offspring, levels of hormones such as corticosterone, and epigenetic assessments could provide important information to further understand the disease model and the effects of IAP. Once again results from reciprocal social interaction testing provide compelling evidence that IAP treatment normalizes social behavior in the HFD model.

Taken together, the current study shows that, while IAP treatment administered daily to dams fed HFD does not ameliorate all issues in offspring that are associated with an ASD-like phenotype, it does appear to rescue many of the social deficits inherent in the model. In future studies, it would be interesting to determine if IAP administration to the

offspring alone would mitigate the disease severity. Finally, it would also be interesting to measure IAP levels in stools from ASD patients as well as their mothers.

Authors' contributions

CFF and MK were responsible for study conceptualization, study design, data curation and interpretation. CFF was responsible for writing the manuscript original draft. AM and DG assisted with study design and data interpretation, and were responsible for study methodology, formal analysis, project administration, and coordinated and oversaw the study activities. CFF, AM, DG, and MK reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Declaration of Competing Interest

The authors declare the following potential conflicts of interest with respect to the research, authorship and/or publication of this article: CFF and MK are employees of Synthetic Biologics, Inc. AM and CG are employees of CNS |CRO, a fee-for-service provider engaged by Synthetic Biologics, Inc.

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References

[1] A. Ronald, C.E. Pennell, A.J. Whitehouse, Prenatal maternal stress associated with ADHD and autistic traits in early childhood, Front. Psychol. 1 (2010) 223.
[2] M. Li, M.D. Fallin, A. Riley, R. Landa, S.O. Walker, M. Silverstein, D. Caruso, C. Pearson, S. Kiang, J.L. Dahm, X. Hong, G. Wang, M.C. Wang, B. Zuckerman, X. Wang, The association of maternal obesity and diabetes with autism and other developmental disabilities, Pediatrics 137 (2) (2016) e20152206.
[3] S.A. Buffington, G.V. Di Prisco, T.A. Auchtung, N.J. Ajami, J.F. Petrosino, M. Costa-Mattioli, Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring, Cell 165 (7) (2016) 1762–1775.
[4] D.W. Kang, J.B. Adams, D.M. Coleman, E.L. Pollard, J. Maldonado, S. McDonough-Means, J.G. Caporaso, R. Krajmalnik-Brown, Long-term benefit of microbiota transfer therapy on autism symptoms and gut microbiota, Sci. Rep. 9 (1) (2019) 5821.
[5] A. Agusti, M.P. Garcia-Pardo, I. Lopez-Almela, I. Campillo, M. Maes, M. Romani-Perez, Y. Sanz, Interplay between the gut-brain Axis, obesity and cognitive function, Front. Neurosci. 12 (2018) 155.
[6] K.T. Chen, M.S. Malo, A.K. Moss, S. Zeller, P. Johnson, F. Ebrahimi, G. Mostafa, S.N. Alam, S. Ramasamy, H.S. Warren, E.L. Hohmann, R.A. Hodin, Identification of specific targets for the gut mucosal defense factor intestinal alkaline phosphatase, Am. J. Physiol. Gastrointest. Liver Physiol. 299 (2) (2010) G467–75.
[7] W. Liu, D. Hu, H. Huo, W. Zhang, F. Adiliaghdam, S. Morrison, J.M. Ramirez, S.S. Gul, S.R. Hamarneh, R.A. Hodin, Intestinal alkaline phosphatase regulates tight junction protein levels, J. Am. Coll. Surg. 222 (6) (2016) 1009–1017.
[8] M.S. Malo, S.N. Alam, G. Mostafa, S.J. Zeller, P.V. Johnson, N. Mohammad, K.T. Chen, A.K. Moss, S. Ramasamy, A. Faruqui, S. Hodin, P.S. Malo, F. Ebrahimi, B. Biswas, S. Narisawa, J.L. Millan, H.S. Warren, J.B. Kaplan, C.L. Kitts, E.L. Hohmann, R.A. Hodin, Intestinal alkaline phosphatase preserves the normal homeostasis of gut microbiota, Gut 59 (11) (2010) 1476–1484.
[9] M.S. Malo, A high level of intestinal alkaline phosphatase is protective against type 2 diabetes mellitus irrespective of obesity, EBioMedicine 2 (12) (2015) 2016–2023.
[10] S.S. Ghosh, J. Bie, J. Wang, S. Ghosh, Oral supplementation with non-absorbable antibiotics or curcumin attenuates western diet-induced atherosclerosis and glucose intolerance in LDLR-/- mice–role of intestinal permeability and macrophage activation, PLoS One 9 (9) (2014) e108577.
[11] K. Kaliannan, S.R. Hamarneh, K.P. Economopoulos, S. Nasrin Alam, O. Moaven, P. Patel, N.S. Malo, M. Ray, S.M. Abtahi, N. Muhammad, A. Raychowdhury, A. Teshager, M.M. Mohamed, A.K. Moss, R. Ahmed, S. Hakimian, S. Narisawa, J.L. Millan, E. Hohmann, H.S. Warren, A.K. Bhan, M.S. Malo, R.A. Hodin, Intestinal alkaline phosphatase prevents metabolic syndrome in mice, Proc. Natl. Acad. Sci. U.S.A. 110 (17) (2013) 7003–7008.
[12] K.P. Economopoulos, N.L. Ward, C.D. Phillips, A. Teshager, P. Patel, M.M. Mohamed, S. Hakimian, S.B. Cox, R. Ahmed, O. Moaven, K. Kaliannan, S.N. Alam, J.F. Haller, A.M. Goldstein, A.K. Bhan, M.S. Malo, R.A. Hodin, Prevention of antibiotic-associated metabolic syndrome in mice by intestinal alkaline phosphatase, Diabetes Obes. Metab. 18 (5) (2016) 519–527.
[13] S.S. Ghosh, H. He, J. Wang, W. Korzun, P.J. Yannie, S. Ghosh, Intestine-specific expression of human chimeric intestinal alkaline phosphatase attenuates Western diet-induced barrier dysfunction and glucose intolerance, Physiol. Rep. 6 (14) (2018) e13790.
[14] J. Ellegood, J.N. Crawley, Behavioral and neuroanatomical phenotypes in mouse models of autism, Neurotherapeutics 12 (3) (2015) 521–533.
[15] F.J. van Steensel, S.M. Bogels, S. Perrin, Anxiety disorders in children and adolescents with autistic spectrum disorders: a meta-analysis, Clin. Child Fam. Psychol. Rev. 14 (3) (2011) 302–317.
[16] K.C. Kim, E.L. Gonzales, M.T. Lazaro, C.S. Choi, G.H. Bahn, H.J. Yoo, C.Y. Shin, Clinical and neurobiological relevance of current animal models of autism Spectrum disorders, Biomol. Ther. (Seoul) 24 (3) (2016) 207–243.
[17] T.M. Kazdoba, P.T. Leach, M. Yang, J.L. Silverman, M. Solomon, J.N. Crawley, Translational mouse models of autism: advancing toward pharmacological therapeutics, Curr. Top. Behav. Neurosci. 28 (2016) 1–52.
[18] S.J. Jeon, E.L. Gonzales, D.F.N. Mabunga, S.T. Valencia, D.G. Kim, Y. Kim, K.J.L. Adil, D. Shin, D. Park, C.Y. Shin, Sex-specific behavioral features of rodent models of autism Spectrum disorder, Exp. Neurobiol. 27 (5) (2018) 321–343.
[19] S. Grabrucker, T.M. Boeckers, A.M. Grabrucker, Gender dependent evaluation of autism like behavior in mice exposed to prenatal zinc deficiency, Front. Behav. Neurosci. 10 (2016) 37.
[20] M. Shibutani, T. Horii, H. Shoji, S. Morita, M. Kimura, N. Terawaki, T. Miyakawa, I. Hatada, Arid1b haploinsufficiency causes abnormal brain gene expression and autism-related behaviors in mice, Int. J. Mol. Sci. 18 (9) (2017).
[21] T.W. Klein, Stress and infections, J. Fla. Med. Assoc. 80 (6) (1993) 409–411.
[22] K.J. Varcin, J.P. Newnham, A.J.O. Whitehouse, Maternal pre-pregnancy weight and autistic-like traits among offspring in the general population, Autism Res. 12 (1) (2019) 80–88.
[23] D. Siniscalco, S. Schultz, A.L. Brigida, N. Antonucci, Inflammation and neuro-immune dysregulations in autism spectrum disorders, Pharmaceuticals (Basel) 11 (2) (2018).
[24] A. Fattorusso, L. Di Genova, G.B. Dell'Isola, E. Mencaroni, S. Esposito, Autism Spectrum disorders and the gut microbiota, Nutrients 11 (3) (2019).
[25] F. Liu, J. Li, F. Wu, H. Zheng, Q. Peng, H. Zhou, Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review, Transl. Psychiatry 9 (1) (2019) 43.
[26] P.D. Cani, J. Amar, M.A. Iglesias, M. Poggi, C. Knauf, D. Bastelica, A.M. Neyrinck, F. Fava, K.M. Tuohy, C. Chabo, A. Waget, E. Delmee, B. Cousin, T. Sulpice, B. Chamontin, J. Ferrieres, J.F. Tanti, G.R. Gibson, L. Casteilla, N.M. Delzenne, M.C. Alessi, R. Burcelin, Metabolic endotoxemia initiates obesity and insulin resistance, Diabetes 56 (7) (2007) 1761–1772.
[27] T.L. Netto Candido, J. Bressan, R.C.G. Alfenas, Dysbiosis and metabolic endotoxemia induced by high-fat diet, Nutr. Hosp. 35 (6) (2018) 1432–1440.
[28] N.N. Mehta, F.C. McGillicuddy, P.D. Anderson, C.C. Hinkle, R. Shah, L. Pruscino, J. Tabita-Martinez, K.F. Sellers, M.R. Rickels, M.P. Reilly, Experimental endotoxemia induces adipose inflammation and insulin resistance in humans, Diabetes 59 (1) (2010) 172–181.
[29] G. Hava, L. Vered, M. Yael, H. Mordechai, H. Mahoud, Alterations in behavior in adult offspring mice following maternal inflammation during pregnancy, Dev. Psychobiol. 48 (2) (2006) 162–168.
[30] F. Kuhn, F. Adiliaghdam, P.M. Cavallaro, S.R. Hamarneh, A. Tsurumi, R.S. Hoda, A.R. Munoz, Y. Dhole, J.M. Ramirez, E. Liu, R. Vasan, Y. Liu, E. Samarbafzadeh, R.A. Nunez, M.Z. Farber, V. Chopra, M.S. Malo, L.G. Rahme, R.A. Hodin, Intestinal alkaline phosphatase targets the gut barrier to prevent aging, JCI Insight 5 (6) (2020).
[31] M. Estaki, D. DeCoffe, D.L. Gibson, Interplay between intestinal alkaline phosphatase, diet, gut microbes and immunity, World J. Gastroenterol. 20 (42) (2014) 15650–15656.
[32] L.L. Liu, J.M. Lawrence, C. Davis, A.D. Liese, D.J. Pettitt, C. Pihoker, D. Dabelea, R. Hamman, B. Waitzfelder, H.S. Kahn, S.F.D.I.Y.S. Group, Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for diabetes in youth study, Pediatr. Diabetes 11 (1) (2010) 4–11.
[33] K.K. Dhaliwal, C.E. Orsso, C. Richard, A.M. Haqq, L. Zwaigenbaum, Risk factors for unhealthy weight gain and obesity among children with autism spectrum disorder, Int. J. Mol. Sci. 20 (13) (2019).
[34] S. Healy, C.R. Pacanowski, E. Williams, Weight management interventions for youth with autism spectrum disorder: a systematic review, Int. J. Obes. (Lond) 43 (1) (2019) 1–12.
[35] H. Won, H.R. Lee, H.Y. Gee, W. Mah, J.I. Kim, J. Lee, S. Ha, C. Chung, E.S. Jung, Y.S. Cho, S.G. Park, J.S. Lee, K. Lee, D. Kim, Y.C. Bae, B.K. Kaang, M.G. Lee, E. Kim, Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function, Nature 486 (7402) (2012) 261–265.
[36] H. Kim, C.S. Lim, B.K. Kaang, Neuronal mechanisms and circuits underlying repetitive behaviors in mouse models of autism spectrum disorder, Behav. Brain Funct. 12 (1) (2016) 3.
[37] K.C. Page, E.K. Jones, E.K. Anday, Maternal and postweaning high-fat diets disturb hippocampal gene expression, learning, and memory function, Am. J. Physiol. Regul. Integr. Comp. Physiol. 306 (8) (2014) R527–37.