Journal of Developmental Origins of Health and Disease

www.cambridge.org/doh

Original Article

Cite this article: Rakhshandehroo M, Harvey L, de Bruin A, Timmer E, Lohr J, Tims S, and Schipper L. (2025) Maternal exposure to purified versus grain-based diet during early lactation in mice affects offspring growth and reduces responsivity to Western-style diet challenge in adulthood. *Journal of Developmental Origins of Health and Disease* **16**: e3, 1–12. doi: 10.1017/S2040174424000436

Received: 15 July 2024
Revised: 9 October 2024
Accepted: 15 December 2024

Keywords:
Maternal nutrition; early lactation; purified diet; grain-based diet; nutritional programming

Corresponding author:
Maryam Rakhshandehroo;
Email: maryam.rakhshandehroo@danone.com

Maternal exposure to purified versus grain-based diet during early lactation in mice affects offspring growth and reduces responsivity to Western-style diet challenge in adulthood

M. Rakhshandehroo[1], L. Harvey[1], A. de Bruin[2], E. Timmer[1], J. Lohr[1], S. Tims[1] and L. Schipper[1]

[1]Danone Research & Innovation Center, Utrecht, The Netherlands and [2]Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Abstract

The nutritional environment during fetal and early postnatal life has a long-term impact on growth, development, and metabolic health of the offspring, a process termed "nutritional programming." Rodent models studying programming effects of nutritional interventions use either purified or grain-based rodent diets as background diets. However, the impact of these diets on phenotypic outcomes in these models has not been comprehensively investigated. We used a previously validated (C57BL/6J) mouse model to investigate the effects of infant milk formula (IMF) interventions on nutritional programming. Specifically, we investigated the effects of maternal diet type (i.e., grain-based vs purified) during early lactation and prior to the intervention on offspring growth, metabolic phenotype, and gut microbiota profile. Maternal exposure to purified diet led to an increased post-weaning growth velocity in the offspring and reduced adult diet-induced obesity. Further, maternal exposure to purified diet reduced the offspring gut microbiota diversity and modified its composition post-weaning. These data not only reinforce the notion that maternal nutrition significantly influences the programming of offspring vulnerability to an obesogenic diet in adulthood but emphasizes the importance of careful selection of standard background diet type when designing any preclinical study with (early life) nutritional interventions.

Introduction

Non-communicable diseases, including obesity, diabetes, and cardiovascular disease, are a major health hazard of the modern world. While genetics and suboptimal adult environmental factors can affect an individual's propensity to develop metabolic abnormalities, early life environmental factors including (maternal) nutrition are increasingly recognized as important contributors influencing health and disease risk in later life.[1–5] Rodent models help elucidate the effects and mechanisms involved in maternal and early life dietary exposures (e.g., maternal undernutrition, high-fat diet, micronutrient exposure) and their impact on long-term health outcomes.[6–8] We previously established a mouse model of nutritional programming to determine whether dietary manipulations in early life could alter later-life health outcomes. We use this mouse model to test the effects of IMF interventions on offspring growth patterns and susceptibility to diet-induced obesity later in life. This validated model forms the basis for testing our nutritional concepts aimed at promoting healthy growth and development in children. Using this model, we have shown that lipid quality in early life diet affects offspring susceptibility to adult diet-induced obesity.[9–11]

In addition to dietary interventions, rodents in nutritional programming research are exposed to standard rodent diets prior to and/or during the experiment. These diets serve as control or base diets for a nutritional supplementation/intervention. Grain-based and purified diets are two common standard rodent diets in (metabolic) research. Both diets have long been considered nutritionally sufficient to support breeding and long lifespan. However, they are very different in nutritional composition and food matrix.[12, 13] Significant differences include the matrix (multi-nutritional ingredients versus purified, single ingredients); ingredient types (unrefined versus refined); the quantity and source of fibers (diverse range of soluble/insoluble fibers versus mainly insoluble cellulose), carbohydrates (whole grains versus combination of refined corn starch with maltodextrin and simple carbohydrates) and proteins (mainly plant based versus milk casein). Such matrix and compositional differences could lead to different effects on nutrient absorption, gut microbiota, metabolic responses, and subsequent health

outcomes. Research has shown varied effects of these diets on gut microbiota and short chain fatty acid (SCFA) profile.[14–18] In addition, rodents exposed to purified diets developed an impaired liver phenotype,[19, 20] an effect significantly reduced by addition of soluble fiber,[21] whilst higher serum cholesterol and triglycerides,[22] and slightly lower growth rate and food intake have also been observed.[23]While the impact of diet is increasingly recognized, the influence of the mother's background diet on offspring health outcomes (i.e., nutritional programming) may still be overlooked. A recent study[24] demonstrated that maternal diet lacking soluble fiber during lactation led to changes in offspring microbiota, predisposing them to obesity later in life. Building on these findings, we used our nutritional programming model to determine whether maternal exposure to a grain-based versus purified diet during early lactation could contribute to distinct programming effects on offspring growth, metabolic phenotype (focusing on body weight and composition, metabolic organs, hormones and inflammatory markers), and gut microbiota profile.

Methods

Animal procedures

The C57BL/6J mouse strain was selected as this strain is frequently used in research and is susceptible to diet-induced obesity. Mice used for this study were derived from a larger study with breeding procedures as described in detail elsewhere.[25] Briefly, breeding pairs from Charles River Laboratories (Saint-Germain-Nuelles, France) were time-mated and day of birth was recorded as postnatal day 0 (PN0). At PN2, litters were cross fostered and/or culled to six pups/dam. Each litter contained both sexes and 2 to 4 male offspring, depending on birth outcomes. Male offspring were weaned at PN21 and were pair-housed (with same-sex littermate) and followed up into adulthood. Animals were housed in IVC polycarbonate type II cages with bedding, nesting and enrichment materials as previously described.[25] All procedures took place during the light phase.

Dams were fed a grain-based diet throughout gestation. After birth, dams and litters were randomly allocated to a grain reference group [Grain-Ref] that remained on grain-based diet throughout the study, or one of four experimental groups that experienced a shift to purified diet at either PN2 [MatAIN] or PN16 [MatGrain] (Fig. 1 and Supplementary Table 1). Offspring in MatAIN and MatGrain groups were exposed to a standard AIN-93G based infant milk formula (IMF) diet containing soluble galacto-oligosaccharides and fructo-oligosaccarides (GOS/FOS) between PN16 and PN42, followed by the semisynthetic control AIN-93 M [Con] or Western-Style Diet [WSD] until PN126, following a previously described nutritional programming model.[9–11] The Grain-Ref group was included in the study as a reference to health outcomes of mice kept in same conditions as the experimental groups but without any diet interventions.

Body weight, energy intake and body composition

Body weight of dams and litters were recorded weekly and, after weaning, offspring body weight was monitored twice weekly. Body composition was measured at PN28, PN42, PN98 and PN126 by magnetic resonance imaging (EchoMRI-100™ analyzer, EchoMRI Medical Systems, Houston, TX) as previously reported.[25] Food intake was roughly monitored per cage by weighing the food on rack twice a week between PN42 and PN126.

Tissue collection

Fecal samples were collected at PN28, PN42, and PN126 and were processed for fecal DNA extraction and sequencing as previously reported.[25] At PN126 animals were deeply anesthetized (isoflurane) and sacrificed as previously reported.[25] Subcutaneous and visceral (perirenal, retroperitoneal and epididymal) white adipose tissue depots, intrascapular brown adipose tissue depots, adrenal glands, (tibialis anterior) muscle and liver were dissected and weighed. Cecum content was collected and processed for analysis of SCFAs.

Liver histology and triglycerides

For histological analysis, liver (left lobe) samples were placed in 10% formalin for approximately 48 hours followed by storage in 70% ethanol until paraffin embedded. Paraffin sections were stained with hematoxylin and eosin (H&E) for routine histological analysis.[26] Liver sections were cut to 5 µm thickness. Sections were air dried for 30 min, followed by fixation in 4% formaldehyde for 10 min. Hematoxylin nuclei staining was subsequently carried out for 5 min followed by several rinses with distilled H2O. Sections were mounted in aqueous mounting media (Imsol, Preston, UK). The H&E slides from the liver specimens were blindly evaluated by using an adapted version of the nonalcoholic steatosis scoring system for nonalcoholic fatty liver disease[27] and reviewed by two certified veterinary pathologists. This scoring system considers the presence or absence of steatosis in hepatocytes examined at low magnification, the presence of ballooning cells, and incidence of lobular inflammation.

For triglyceride analysis, part of the left lobe was snap frozen and stored at −80°C. Liver triglycerides were determined in liver homogenates prepared in buffer containing 250 mM sucrose, 1 mM EDTA, and 10 mM Tris-HCl at pH 7.5 using a commercially available kit (Instruchemie, Delfzijl, The Netherlands) according to the manufacturer's instructions.

Blood and plasma measurements

Blood glucose levels were determined immediately after sacrifice using a commercial blood glucose meter and test strips (Accu-Chek Performa, Roche Diabetes Care, Inc.). Blood was collected in EDTA-coated tubes (Sarstedt, Etten-Leur), centrifuged (13,000 rpm, 15 min, 4°C), and plasma was removed and stored at –80°C until analysis. Interleukin-6 (IL-6), insulin, leptin and resistin were measured using the Mouse Metabolic Hormone Expanded Panel multiplex assay (MILLIPLEX® MAP), monocyte chemoattractant protein-1 (MCP-1) was quantified with the Mouse MCP-1 SimpleStep ELISA® Kit (Abcam) and lipopolysaccharide binding protein (LBP) was measured with the mouse LBP ELISA kit (Hycult®Biotech). All the assays were performed according to the manufacturer's instructions. Plasma analyses were performed in duplicate, and samples were excluded when duplicate measurements had coefficient of variation (CV)>20%.

SCFA analyses

Cecum content was weighed, and samples were diluted 1:10 according to weight in pre-cooled phosphate-buffered saline. Samples were vortexed 3 times for 30 s and centrifuged at 4°C for 5 min at 15 000 × g. The supernatant was collected and 200 µL was used for SCFA analysis. The following SCFAs –acetic, propionic, *n*-butyric, iso-butyric, *n*-valeric, and isovaleric acids – were quantified on a Shimadzu-GC2025 gas chromatograph with a flame ionization detector and hydrogen as mobile phase.

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press

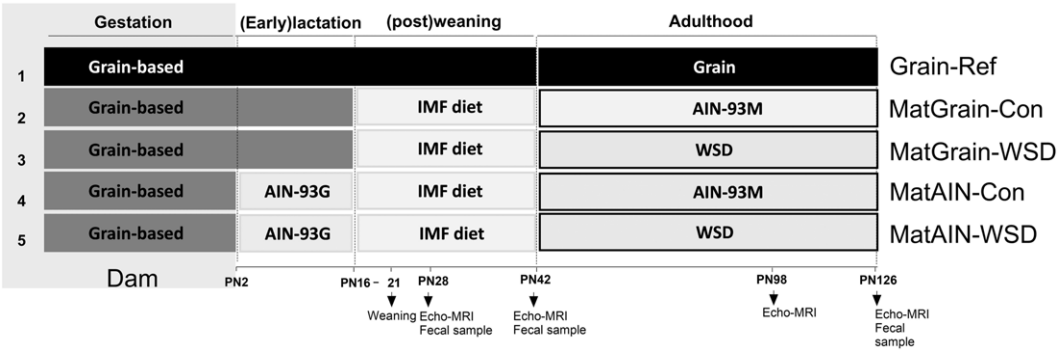

Figure 1. Experimental design. From two weeks before mating and throughout gestation dams were subjected to grain-based growth diet (Teklad 2920X-irradiated). Dams and litters in the grain reference (Grain-ref) group remained on the grain-based diet until PN42 and were switched to grain-based maintenance diet (Teklad 2916C) from PN42 to PN126. In the other four groups, from PN2 to PN16 (early lactation), dams were exposed to either the grain-based diet or purified AIN-93 growth (AIN-93-G) diet which resulted in two groups based on maternal diet type abbreviated as Mat; MatGrain or MatAIN accordingly. Between P16 and P42, dams and litters were exposed to standard infant milk formula (IMF) diet which was AIN-93G based and between P42 and P126 (adulthood), male offspring received a purified control (AIN-93-M) or Western-style diet (WSD, consisting of 20% w/w fat –17% w/w lard, 3% w/w soy, 0% w/w cholesterol). Body composition was measured by echo-MRI on PN28, PN42, PN98 and PN126. Fecal samples were collected on PN28, PN42 and PN126. The experimental groups are represented in the figure. 1) Grain-Ref ($n = 12$); 2) MatGrain-Con ($n = 12$); 3) MatGrain-WSD ($n = 12$); 4) MatAIN-Con ($n = 12$); and 5) MatAIN-WSD ($n = 12$). One mouse in the MatAIN-Con presented malocclusion, resulting in low body weight gain after PN42; data from this animal were excluded from analyses.

Quantification was performed by using 2-ethylbutyric acid as an internal standard and generating a calibration curve from the peak area after which the concentration in the samples was calculated.

Analysis of sequencing results

Analysis of fecal sequencing results was performed as extensively described previously.[25] Rarefaction was applied to the taxa by phyloseq[28] and vegan packages[29] in R v3.5.1 for α-diversity calculations using the Chao1 and Shannon index metrics. The β-diversity was computed using the Bray-Curtis distance over all samples with functions vegdist and betadisper from the vegan package in R v3.5.1. Statistical significance of differences in α-diversity were assessed with pairwise_wilcox_test function from the rstatix package in R v4.0.2[30] followed by Benjamini-Hochberg p-value adjustment per timepoint. Statistical significance of differences in β-diversity were assessed using the permutation ANOVA function adonis2 from the package vegan in R. Using Spearman, the phenotypic metadata was correlated to genera with a minimum mean relative abundance of 0.5% across all samples and tested for significance using cor.test function with default settings from the R stats package. Differential abundance was performed with generalized linear models with mixed effects on the sequencing counts using the glmmTMB package v 1.1.2.3 in R v4.0.2[31] followed by Anova.glmmTMB applying the Chi Squared test for significant differences. After adjustment, a p-value < 0.05 was considered significant for all statistical tests applied to the sequencing data.

Statistical analysis

Phenotypic data were analyzed using SPSS 20.0 (IBM software) and GraphPad Prism 8 (GraphPad software, GraphPad Holdings, LLC, La Jolla, CA, USA). Data from the Grain-Ref group were not included in the statistical analyses, but data are added to figures as a visual reference. Due to the color and texture difference between grain-based diet and purified diet, researchers were not blinded to diet type. However, ex vivo analyses and data processing was performed by researchers blinded to the groups.

Data were analyzed using linear mixed models. Effect of maternal diet type on changes in body weight and body composition over 3 weeks (PN21 – PN42) in the MatGrain (group 2 and 3 combined) versus MatAIN (group 4 and 5 combined) was analyzed by one-way repeated measures ANOVA using maternal diet type as fixed factor and time as repeated measure, excluding data at missing timepoints. Post hoc analyses were performed using Bonferroni's test. Effect of adult diet type on changes in body weight and body composition over 12 weeks (PN42-PN126) in the groups 2 – 5 was analyzed by two-way repeated measures ANOVA using maternal/adult diet types as fixed factors and time as repeated measure. Effect of diet type on organ and plasma parameters at PN126 was analyzed by two-way ANOVA using maternal and adult diet types as fixed factors. Individual animals were considered as statistical units, however, as the study included multiple batches of mice and mice were always housed two animals per cage throughout the study, all analyses included batch and cage as random factors. The relation between diet type and liver phenotype as indicated by %responder was analyzed using Chi-square test.

All data are expressed as mean ± standard error of the mean (SEM). Data were considered statistically significant when $p < 0.05$. Statistical trends were reported in case of a p-value between 0.05 and 0.06. Three-way interactions were considered statistically significant when $p < 0.1$ as a common practice in more complex models. Power calculations were based on published data from previous experiments with comparable design and based on fat accumulation in response to WSD in male adult offspring.[10] Using an error-probability of 5% and power of 80%, sample size was calculated as 12 animals per group. There was one animal in the MatAIN-Con that presented malocclusion, resulting in low body weight gain after PN42; data from this animal were excluded from all analyses.

Results

Maternal exposure to purified diet (AIN-93G) during early lactation (PN2-PN16) impacted the pattern of maternal and litter weight gain

During lactation, dams and litters in the MatGrain and MatAIN group showed a different pattern of body weight accumulation (diet*time, dams: $F_{(3,12)} = 8.51$, $p < 0.01$; litters: $F_{(3,13)} = 4.53$, $p = 0.02$) with animals in MatAIN showing lower bodyweight compared to animals in MatGrain at PN14 and PN21 (Fig. 2).

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press

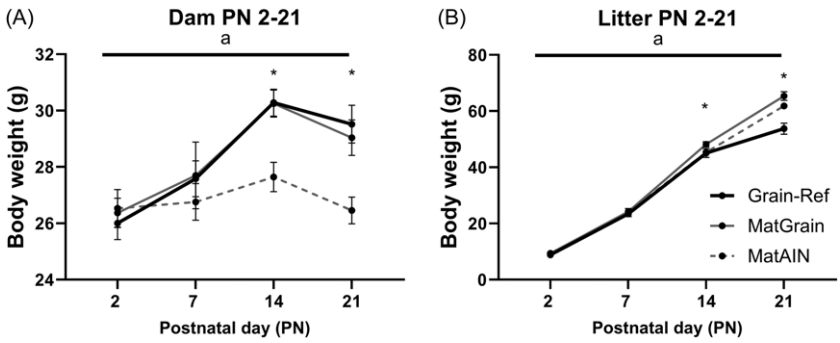

Figure 2. Dam body weight (A) and litter weight (B) in the period PN2-PN21 in the MatGrain, MatAIN and Grain-ref groups. Effect of diet type on body weight was analyzed by one-way repeated measures ANOVA using maternal diet type as fixed factor and time as repeated measure. [a]interaction effect between maternal diet and time. *MatGrain and MatAIN groups differed at depicted time points by post hoc analysis using bonferroni testing, $p < 0.05$. $n = 5$–8 (A). Grain-ref ($n = 5$ litters); MatGrain ($n = 8$ litters); and MatAIN ($n = 7$ litters). Each litter contained 6 pups in total (2–4 of which were males, depending on birth outcomes) (B). Values are given as mean ± SEM.

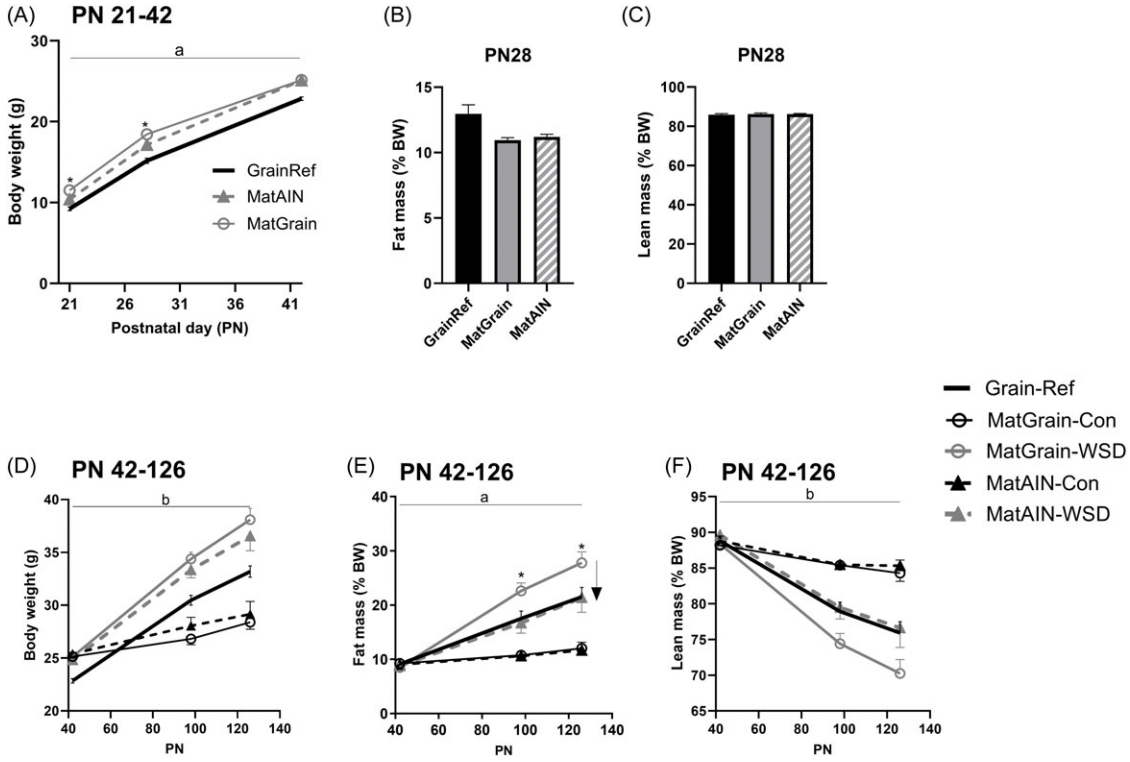

Figure 3. Longitudinal body weight (BW) in the post-weaning period PN21-PN42 (A), in the groups MatGrain, MatAIN and Grain-Ref. Average fat mass (% BW) at PN28 (B) and lean mass (% BW) at PN28 (C), longitudinal BW (D), fat mass (% BW) (E) and lean mass (% BW) (F) in the groups MatGrain-Con, MatGrain-WSD, MatAIN-Con, MatAIN-WSD and Grain-Ref. Maternal diet (Grain versus AIN-93G), adult diet (WSD versus AIN-93M), time, and diet-by-time interaction effects were determined by repeated measures one-way ANOVA for the period (PN21-PN42) and repeated measures two-way ANOVA for the period (PN42-PN126). [a] interaction effect between maternal diet and time (A) and interaction between maternal diet, adult diet and time (E), [b] interaction effect between adult diet and time (D–F), $p < 0.05$. *MatAIN and MatGrain groups in panel a and MatGrain-WSD and MatAIN-WSD groups in panel E differed at depicted time points by post hoc analysis using Bonferroni testing, $p < 0.05$. $n = 11$**–12. **MatAIN-Con group in panel A-D. Values are given as mean ± SEM.

Maternal exposure to purified diet during early lactation increased offspring growth velocity after weaning and decreased offspring response to WSD challenge in adulthood

In the offspring, there was a significant increase in body weight over time in both MatGrain and MatAIN groups (time: $F_{(2,69)} = 5586.97$, $p < 0.001$) during the post-weaning period (PN21 – PN42), as well as an interaction between maternal diet and time on offspring body weight (maternal diet*time: $F_{(2,69)} = 9.26$, $p < 0.001$) during the same period. Post hoc testing indicated that offspring from MatAIN mice had lower body weight compared to offspring from MatGrain mice at PN21 ($p < 0.001$) and PN28 ($p < 0.001$) and had similar body weight at PN42 ($p = 0.97$) (Fig. 3A). There was no difference in fat mass and lean body mass at PN28 and PN42 (Fig. 3).

During the adult phase (PN42 – PN126) there was an interaction effect between adult diet and time on offspring body weight ($F_{(2,79)} = 81.10$, $p < 0.001$) and relative lean body mass ($F_{(2,88)} = 40.56$, $p < 0.001$)(Fig. 3D and 3F). Post hoc analysis indicated that body weight was significantly higher and relative lean body mass was significantly lower at PN98 and PN126 due to adult WSD exposure.

There was an interaction between maternal diet, adult diet and time on offspring fat mass ($F_{(2,86)} = 3.95$, $p = 0.02$). Post hoc analysis indicated that relative fat mass was significantly lower in the offspring from MatAIN compared to MatGrain at PN98 ($p < 0.01$) and PN126 ($p = 0.02$) only following WSD challenge in adulthood (Fig. 3E). There was no statistically significant effect, though visually there seemed to be an interaction between maternal diet, adult diet and time on offspring relative lean body mass when exposed to WSD challenge; lean body mass seemed to be higher in the offspring from MatAIN compared to MatGrain at

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press

Table 1. Average weight of fat depots and organs at PN126

	Grain-Ref	MatGrain-Con	MatGrain-WSD	MatAIN-Con	MatAIN-WSD	Mat diet *p*-value	Adult diet *p*-value
Muscle (% BW) (tibialis anterior)	0.15 ± 0.01	0.17 ± 0.00	0.14 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.05	0.00
Total fat (% BW)	7.17 ± 0.62	3.47 ± 0.41	9.73 ± 0.81	3.30 ± 0.34	7.27 ± 1.05	0.15	0.00
Visceral fat (% BW)	4.97 ± 0.43	2.15 ± 0.28	6.84 ± 0.55	2.07 ± 0.24	5.14 ± 0.75	0.18	0.00
Subcutaneous fat (% BW)	1.51 ± 0.20	0.88 ± 0.10	2.13 ± 0.25	0.82 ± 0.08	1.54 ± 0.21	0.11	0.00
Brown fat (% BW)	0.69 ± 0.06	0.44 ± 0.05	0.76 ± 0.06	0.76 ± 0.06	0.59 ± 0.11	0.22	0.00
Cecum content	1 ± 0.1	0.8 ± 0.07	0.9 ± 0.3	1 ± 0.2	0.5 ± 0.06	0.29	0.37
Adrenal gland	0.03 ± 0.00	0.02 ± 0.00	0.027 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.99	0.41

Values are mean ± SEM, $n = 11$–12. Statistical analyses were performed using two-way ANOVA, no interaction effects.

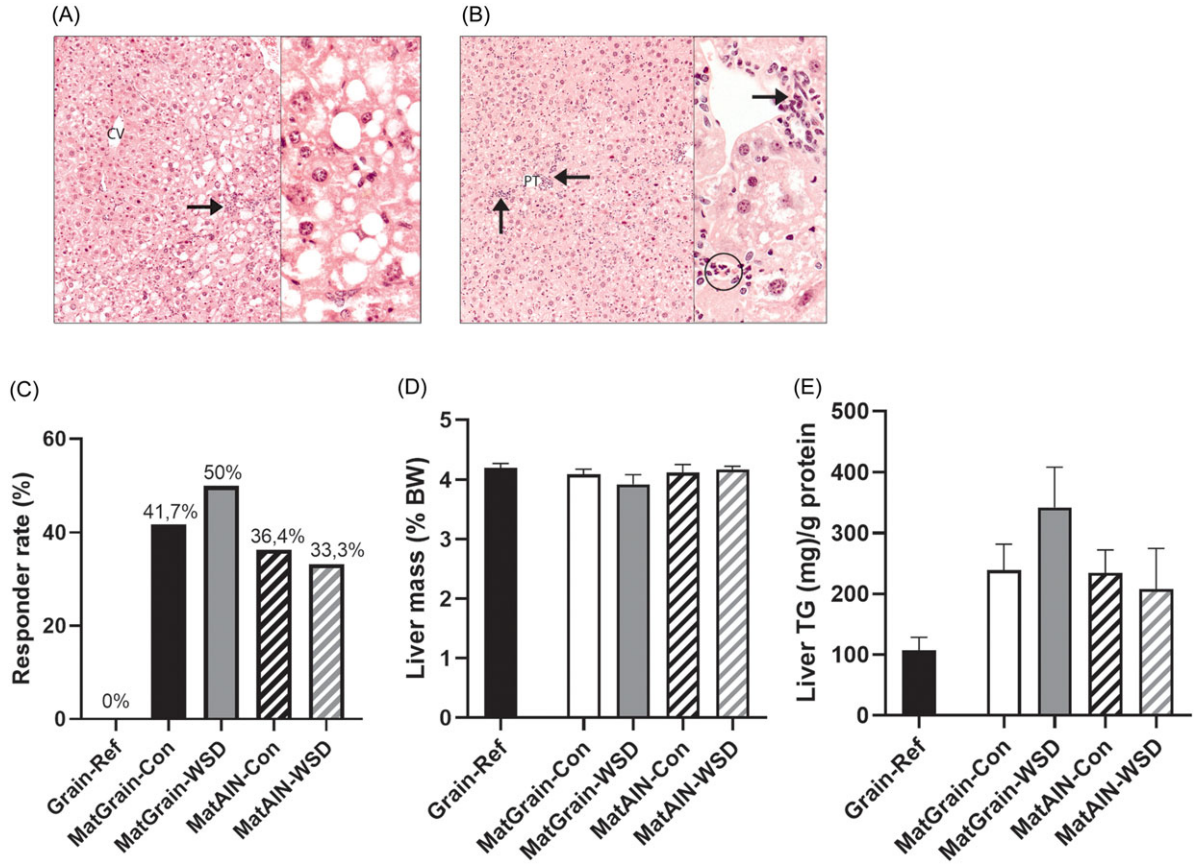

Figure 4. Hematoxylin and eosin (H&E) staining of representative liver sections of the mice scored positive for steatosis (A) and inflammation (B) in the study groups with a switch to AIN-93 diet. % responder rate (defined by the outcome of H&E staining and based on the presence of steatosis and/or inflammation) (C), liver mass (% BW) (D), liver triglyceride (TG) content (mg/g protein) (E). The relation between experimental diet group and liver phenotype as indicated by %responder was analyzed using chi-square test. Values are given as mean ± SEM. $n = 11$*-12 mice per group (C–E). *MatAIN-Con. central vein (CV), portal tract (PT).

PN98 and PN126 when exposed to WSD (Fig. 3F). Maternal diet had no effect on offspring energy intake from PN42 to PN126 whereas, WSD exposure increased caloric intake ($F_{(1,20)} = 66.72$, $p < 0.001$) (Supplementary Fig. 1).

The Grain-Ref group showed similar patterns of weight gain to both MatGrain and MatAIN groups in the post-weaning period (PN21-PN42) (Fig. 3A). There was a difference between the Grain-Ref, MatGrain-Con and MatAIN-Con groups in terms of body weight and body composition development in adulthood period (PN42-PN126); numerically the Grain-Ref group had higher body weight and relative fat mass and lower relative lean mass compared to the other groups (Fig. 3D and 3E and 3F).

Maternal brief exposure to purified diet during early lactation did not have a significant effect on organ weights

At PN126, WSD resulted in a decrease in relative tibialis anterior muscle mass and an increase in relative total fat, visceral fat,

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press

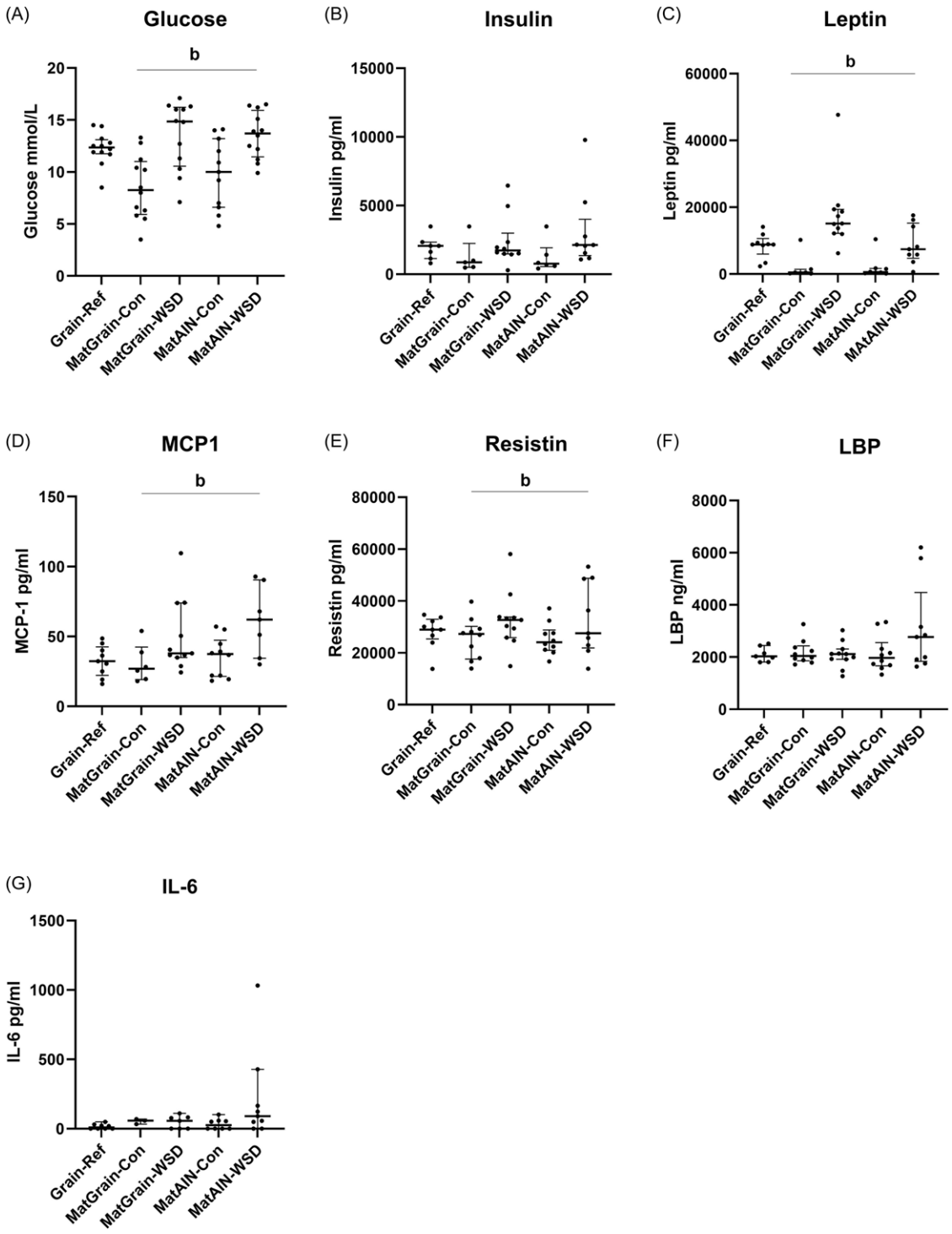

Figure 5. Plasma glucose is expressed as mmol/L ($n = 11$–12). Plasma markers (leptin ($n = 7$–11), monocyte protein-1 (MCP-1, $n = 6$–11), resistin ($n = 9$–11) and interleukin-6 (IL-6, $n = 3$–9)) and insulin ($n = 5$–10) are expressed as pg/ml and lipopolysaccharide binding protein (LBP) as ng/ml ($n = 7$–11). Volume of plasma collected was not insufficient for all analyses resulting in lower n/group. Effect of diet type on plasma measures was analyzed by two-way ANOVA using maternal and adult diet types as fixed factors. Data presented as mean ± SEM. [b] significant effect of adult diet, $p < 0.05$.

subcutaneous fat and brown fat mass. Relative tibialis anterior muscle mass was higher in offspring from MatAIN compared to MatGrain ($p = 0.05$). In line with effect of maternal diet type on changes in body composition observed during adulthood, the weight of the adipose tissue depots in the groups exposed to WSD appeared to be lower in MatAIN compared to MatGrain at dissection, however this effect was not significant in the statistical model used (Table 1). Moreover, neither maternal nor adult diet affected cecum content weight and adrenal gland weights. At PN126, cecum short chain fatty acid profile was analyzed which indicated no effect of maternal diet nor an interaction between maternal and adult diet on cecum content weight, total amount of

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press

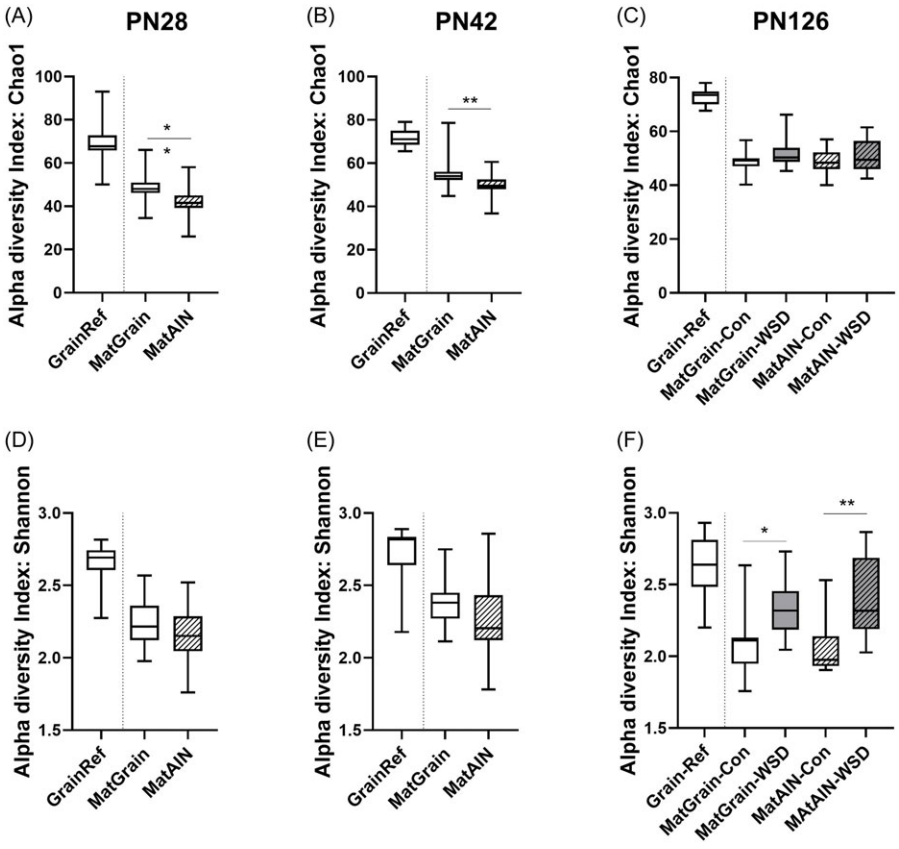

Figure 6. Alpha diversity assessed by Chao1 index at PN28 (A), PN42 (B) and PN126 (C) and Shannon index at PN28 (D), PN42 (E) and PN126 (F). Statistical significance of differences in alpha diversity were assessed with pairwise_wilcox_test followed by Benjamini-Hochberg *p*-value adjustment per timepoint. Data presented as median ± interquartile range. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ $n = 23$*-24 mice per group (panel A, *B*, D, E) * MatAIN. $n = 11$*-12 mice per group (panel C and F) * MatAIN-con.

SCFAs and the relative levels of individual SCFAs (data not shown).

Histological analysis indicated presence of liver steatosis and inflammation in all the experimental groups that switched to purified diet at either PN2 or PN16

Liver sections obtained at PN126 were stained and scored for anomalies/pathologies. While there was no fat accumulation in the liver of the Grain-Ref mice, a marked heterogeneity in fat accumulation and histology was observed in the four experimental groups. A few animals in all experimental groups developed liver steatosis (Fig. 4A) or inflammation (Fig. 4B) while there was no evidence of nonalcoholic fatty liver disease (Supplementary Table 2). We measured the response rate to purified diet based on the presence of steatosis and/or inflammation, which indicated, surprisingly, that 30%–50% mice per experimental group were responders yet there was no significant correlation between experimental diet groups and response rate (Fig. 4C). Liver weight was not modulated by maternal nor adult diet. Quantitation of hepatic triglycerides confirmed liver fat accumulation in all the experimental groups. Hepatic triglycerides seemed to be lower following WSD challenge in the offspring from MatAIN compared to MatGrain group, however, this effect was not statistically significant (Fig. 4E).

Maternal brief exposure to purified diet during early lactation seemed to decrease plasma leptin levels following WSD challenge

The WSD challenge resulted in an overall increase in blood glucose and plasma insulin levels at PN126 (glucose: adult diet, $F_{(1,21)} = 20.79$, $p < 0.001$; insulin: adult diet, $F_{(1,15)} = 3.18$, $p = 0.09$), however, maternal diet type had no effect (Fig. 5A and 5B). The WSD challenge also increased plasma leptin, MCP-1 and resistin, supporting a WSD induced obesogenic phenotype, but did not modulate IL-6 and LBP levels (leptin: $F\,(1,31) = 22,12$, $p < 0.001$; MCP-1: $F_{(1,19)} = 7.60$, $p = 0.01$; resistin: $F_{(1,37)} = 5.51$, $p = 0.02$, Fig. 5C–G). Plasma leptin levels seemed to be lower in MatAIN versus MatGrain group following WSD challenge, although the interaction effect did not reach significance ($F_{(1,31)} = 3.55$, $p = 0.07$, Fig. 5C). The Grain-Ref group had a visually higher leptin level compared to MatGrain-Con and MatAIN-Con groups.

Maternal diet type during early lactation affected the offspring gut microbiota diversity and composition in the post-weaning period

Analysis of alpha diversity showed lower species richness, measured by Chao1 index, in the offspring from MatAIN compared to MatGrain group at early time points PN28 (Fig. 6A) and PN42 (Fig. 6B) which was not present at later time point PN126 (Fig. 6C). Shannon index indicated no differences at PN28 and PN42 and a significantly increased diversity by WSD challenge at PN126 (Fig. 6D–F). Grain-Ref group visually had a higher alpha diversity by both indices at all the time points (Fig. 6).

Analysis of beta diversity, quantifying (dis-)similarities in microbiota composition between samples, showed differences in microbiota composition due to maternal diet at PN28, PN42 and PN126 as well as main effect of adult diet type at PN126 (Fig. 7). The Grain-Ref group was clearly separated from all the other groups at early and later time points (Supplementary Fig. 2).

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press

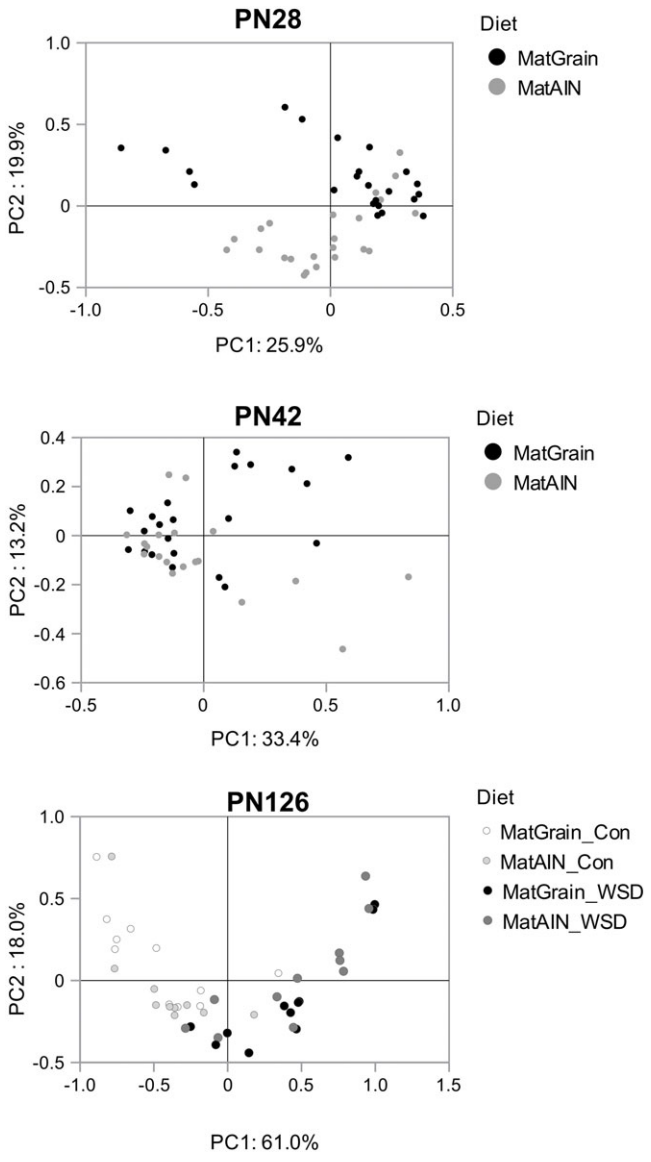

Figure 7. Beta diversity computed with functions vegdist and betadisper from the vegan package in R v3.5.1. Statistical significance of differences in the beta diversity were assessed using the permutation ANOVA function adonis2 from the package vegan in R. PN28; MatDiet: $F = 3.62$; $p = 0.00$. PN42; MatDiet: $F = 2.54$; $p = 0.00$. PN126; MatDiet: $F = 2.64$; $p = 0.04$; AdultDiet: $F = 17.85$; $p = 0.00$; MatDiet:AdultDiet: $F = 1.16$; $p = 0.26$.

Analysis of microbial taxa relative abundance at phylum level both at PN28 and at PN42 showed a slightly higher relative abundance of Verrucomicrobiota and Actinobacteria and a lower relative abundance of Firmicutes and Bacteroidota in MatAIN compared to MatGrain group. Analysis at PN126 showed an increase in the relative abundance of the phylum Firmicutes, particularly in the WSD-challenged groups, compared to the levels observed at PN42. No differences between MatGrain and MatAIN groups were observed at P126. In the Grain-Ref group, the microbiota profile was dominated by the phyla Firmicutes and Bacteroidota (Supplementary Fig. 3).

Analysis at genus level showed that at PN28, the offspring from MatAIN compared to MatGrain group, showed a significantly higher relative abundance of *Bacteroides* and lower relative abundances of *Faecalibaculum*, an unknown genus of Muribaculaceae and *Parasutterella* (Fig. 8A). At PN42 only the abundance of the genera *Alistipes* and the *Lachnospiraceae NK4A136* group were lower in the MatAIN compared to the MatGrain group (Fig. 8B). At PN126, offspring exposed to WSD compared to AIN control diets showed significant difference in the relative abundance of many bacterial genera, among which a few belonging to the Firmicutes phylum, such as *Colidextribacter and Lactobacillus,* were higher and *Akkermansia* and *Parasutterella* were lower in the groups exposed to WSD (Supplementary Fig. 4). There was no significant maternal diet effect nor an interaction effect between maternal and adult diet on relative abundance of microbial taxa at PN126.

Next, we examined the cross-sectional correlations between relative abundance of bacterial groups at genus level and measured metabolic outcomes, i.e., body weight, relative fat mass and relative lean body mass, at PN28 and PN42. At PN28, a few bacterial taxa from Bacteroidetes phylum correlated ($\rho > 0.5$ or $\rho < -0.5$) with body weight. There was a negative correlation between body weight and the *Bacteroides* genus, and positive correlations between body weight and the following genera: *Alistipes*, an unknown genus of Muribaulaceae, Rikenellaceae RC9 gut group, and an unknown genus of Tannerellaceae. At PN42, relative lean body mass correlated positively with the *Akkermansia* genus ($\rho = 0.48$, data not shown).

Discussion

We have previously shown effects of early life nutrition on adult (metabolic) health outcomes using a nutritional programming model.[10,11,32–34] In this study, we describe the persistent programming effects of maternal exposure to standard purified diet versus grain-based diet during early lactation on offspring's response to WSD in adulthood. Offspring of dams exposed to a purified compared to grain-based diet exhibited reduced body weight at weaning, increased growth velocity in the post-weaning period and a lower fat accumulation (% total weight) in response to adult WSD challenge. These effects were in parallel with an adolescent microbiota profile characterized by reduced alpha diversity and a distinct composition depending on maternal diet type.

Considering the nutritional differences and the less favorable attributes of a purified diet on health outcomes in metabolic research,[14–23] one might speculate that early life exposure to such a diet, compared to a grain-based diet, could increase susceptibility to adult diet-induced obesity. We observed decreased fat mass accumulation in response to WSD challenge due to maternal exposure to a purified diet during early lactation. This effect was seen in WSD-challenged offspring but not in non-WSD groups, suggesting it is not due to a general alteration in body fat accumulation. The response to a high-fat diet varies across studies and even among mice within the same study group,[35] often attributed to gene-environment-microbiome interactions[36–38] and sexual dimorphism.[39] However, early life nutrition is an often-overlooked determinant of this variability.

Emerging evidence suggests that early nutritional experiences significantly influence metabolic responses to dietary challenges later in life. While we cannot conclusively determine whether a maternal grain-based diet promotes fat mass accumulation in response to WSD or if a purified diet impedes it, we can assert that fat mass accumulation triggered by WSD is substantially influenced by the maternal diet during early lactation. Contrary to our findings, a recent study[24] observed that feeding dams a standard purified diet during lactation resulted in offspring with

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press

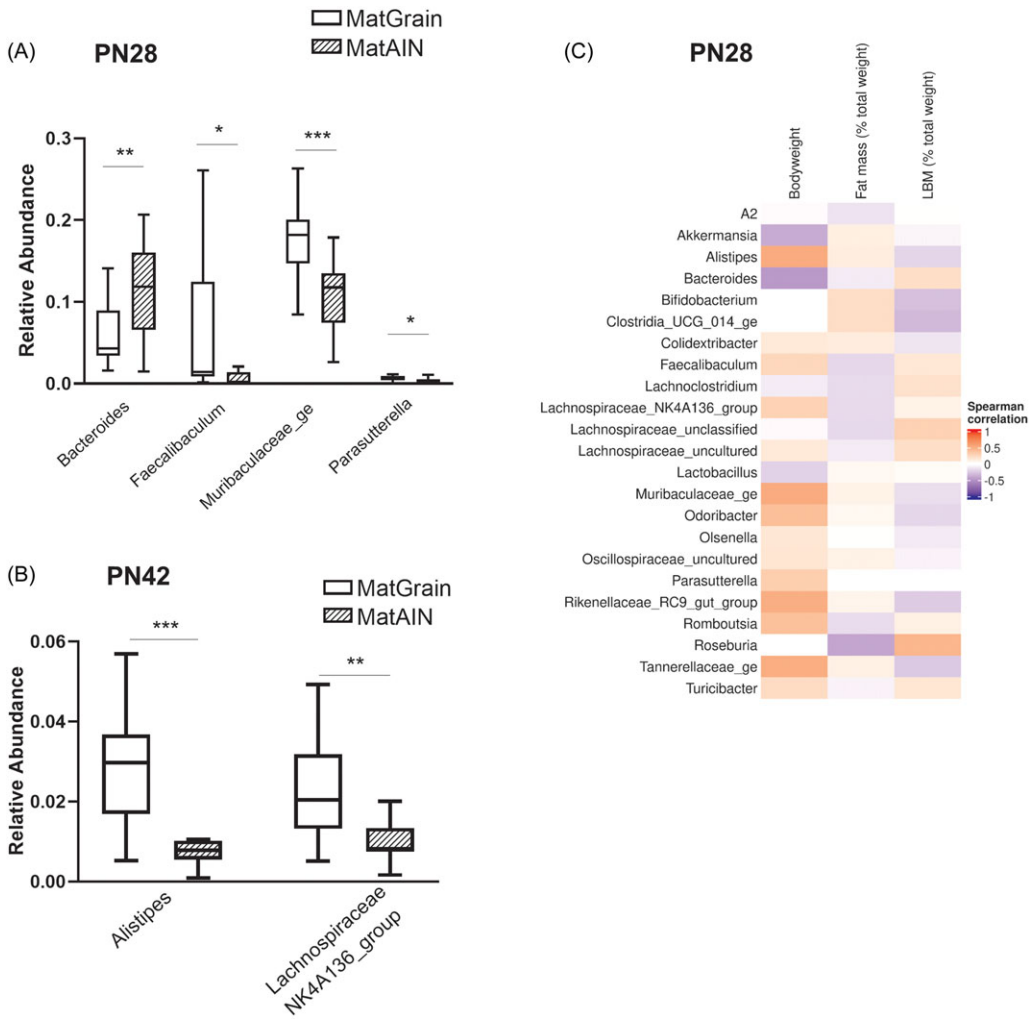

Figure 8. Relative abundance at genus level for PN28 (A) and PN42(B) performed with generalized linear models. Cross-sectional correlations between bacterial taxa at PN28 (groups 2, 3, 4 and 5 combined) and body weight, fat mass (% body weight) and lean mass (% body weight) using Spearman correlation analysis (C). Statistical significance of the relative abundance data was assessed using Chi Squared test. The resulting p-values were corrected using Benjamini-Hochberg. Data presented as median ± interquartile range. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.00$. $n = 23$*-24 mice per group. * MatAIN.

higher body weight and adiposity at weaning, and increased sensitivity to diet-induced obesity later in life. These discrepancies may be due to differences in study design, particularly the nutritional environments between PN16 and PN21. In the previous study,[24] pups were exposed to a fiber-rich grain-based diet starting at PN21, whereas in our study, exposure to a diet devoid of soluble fiber was limited to early lactation and ended at PN16. At PN16, pups were transitioned to an AIN-93G-based IMF diet with GOS/FOS as a source of soluble fiber. This critical phase for organ development and programming, including adipose tissue development,[40] may account for the different outcomes observed.

Given the critical role of gut microbiota in nutrient digestion, energy harvest and production of bioactive metabolites,[41–44] we assessed gut microbiota profiles. We identified an (adolescent) microbiota profile characterized by reduced alpha diversity and a distinct composition at PN28 and PN42 in MatAIN versus MatGrain groups. This distinct composition was further characterized by a decrease in the Bacteroidota phylum and an increase in the genera *Bacteroides* in the offspring from dams exposed to a purified compared to a grain-based diet specifically at PN28. *Bacteroides* species are well-known for their ability to utilize various carbohydrate structures. The higher abundance of *Bacteroides* in MatAIN versus MatGrain may imply that more carbohydrates are reaching and/or being released in the colon when exposed to purified versus grain-based diets and that, in addition to (soluble) fiber, digestible carbohydrate composition of these diets could contribute to the observed effects.

The Bacteroidota phylum has been associated with the modulation of body weight, and we also found moderate, but significant, correlations between the PN28 levels of bacterial genera belonging to the phylum Bacteroidota and body weight. We acknowledge that these observed changes might function more as markers than direct causative factors. For instance, cultured isolates from the *Alistipes* genus have demonstrated bile resistance.[45] Therefore, variations in *Alistipes* abundance could potentially serve as a marker of alterations in the host's fat metabolism rather than a direct cause. However, it is particularly intriguing that these correlations, along with significant changes in the relative abundance of certain genera, are evident at the time point when differences in body weight are observed, specifically at PN28.

There were two interesting additional observations in the microbiota data. The MatGrain group, despite the exposure to (AIN-based) IMF diet containing soluble fibers (GOS/FOS), had a very distinct microbiota composition compared with the Grain-Ref group at PN42 (Supplementary Fig. 2). It is noteworthy to mention that the (AIN-based) IMF diet contains less fiber (AIN-based IMF: 3% GOS/FOS and 3% cellulose) than a grain-based diet (15%–25% mostly soluble fiber) which could have played a role. In addition, after the WSD challenge at PN126, we observed a notable

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press

stimulatory impact of the WSD on gut microbiota diversity and certain bacterial genera, consistent with previous findings.[46–48] Although we detected a statistically significant effect of the maternal diet type on Beta diversity at PN126, this effect was not attributable to consistent taxonomic changes and was not as pronounced as the impact of WSD at this timepoint.

Previously, it has been noted that mice fed AIN-93G diet have higher TG accumulation in the liver[20,49] and C57BL/6J inbred mice showed heterogeneity in liver response to WSD challenge.[35, 50, 51] Our findings indicate a clear development of liver steatosis and/or inflammation in 30%–50% of the offspring across all groups that transitioned to a purified diet, a phenomenon not observed in the Grain-Ref group. Importantly, the liver phenotype was not influenced by the maternal diet type. Mechanistically, the process by which a purified diet induces the development of liver steatosis is not well understood. However, the lower quality and quantity of fiber in a purified diet compared to a grain-based diet[19–21, 49] suggests a significant role for microbial involvement. Notably, the observation of liver steatosis and/or inflammation across all study groups after transitioning to a purified diet (compared to the Grain-Ref group), even in the absence of a WSD challenge later in life, raises legitimate concerns about the long-term effect of purified AIN diet on liver health.

This study has some limitations. First, we studied the effects of maternal dietary exposure on offspring health outcomes in male mice only. While exclusion of female offspring reduced the total number of animals needed for this study, we acknowledge that this choice contributes to the sex bias prevailing in preclinical research.[52] Next, we indicated an accelerated growth rate in the MatAIN group compared to the MatGrain group during the post-weaning period. Unfortunately, individual-level caloric intake during in this period could not be determined due to pair housing. While we didn't expect variations in food intake, we cannot eliminate the possibility. Moreover, we investigated the weight of both dams and litters during the pre-weaning period (PN2-PN21). Notably, differences in weight accumulation were already evident by PN7, as indicated by lower weight in dams and litters exposed to the purified diet. However, the underlying mechanisms responsible for this apparent disparity in offspring weight – whether related to altered energy transfer from mother to pup (such as variations in dam milk availability or composition) or other contributing factors – remain to be elucidated. In addition, we identified a different microbiota profile in MatAIN compared to MatGrain group at PN28 and PN42. Whilst we acknowledge the role of SCFAs in host energy metabolism[53, 54] we could not measure ceacal SCFAs at these earlier time points. Finally, we compared different diets, rather than focusing on single nutrient variations, this approach restricted our ability to draw definitive conclusions regarding the underlying nutrients behind the observed effects. While we elaborated on potential effects of soluble fiber exposure, the differences in fat, protein and carbohydrate profiles between purified and grain-based diets could have also contributed to the observed phenotype in our study.[55–58]

Our findings not only reconfirm the role of maternal diet on offspring growth, development, and programming response to an obesogenic environment in later life, but also strongly highlight the critical impact of standard background diet choice in any study with (early life) nutritional interventions. In line with this, others have previously reported that the prevalent practice of using inappropriate control diets, like employing grain-based diets as controls for refined high-fat diets,[18, 59] introduces considerable challenges in isolating the effects that are solely attributable to the dietary intervention from those created by the background diet/s. Further research is crucial to safeguard the quality of preclinical animal research by unraveling the mechanisms that drive the impact of maternal purified diets during early lactation on offspring growth velocity and responsivity to a high-fat diet in adulthood.

Supplementary material. For supplementary material accompanying this paper visit https://doi.org/10.1017/S2040174424000436

Acknowledgments. The authors would like to acknowledge the many people who contributed to this study either in the design phase, the execution or in the analysis: Cleo Arkenaar, Martin Balvers, Eline van der Beek, Martijn Breeuwsma, Nicole Buurman, Francina Dijk, Miriam van Dijk, Jessica Freesse, Johanneke van der Harst, Andrea Kodde, Stephan Pouw, Hanil Quirindongo, Noela Schaap, Sudarshan Shetty, Heleen de Weerd, Tjalling Wehkamp, Rachel Thomas and Simon De Neck.

Financial support. This work was supported by Danone Research & Innovation, Utrecht, The Netherlands.

Competing interests. Rakhshandehroo M, Harvey L, Lohr J, Tims S, Schipper L are employees of Danone Research & Innovation, Utrecht, The Netherlands. De Bruin A and Timmer E declare no conflict of interest.

Ethical standard. This study was conducted under an ethical license of the national competent authority (CCD, Centrale Commissie Dierproeven) following a positive advice from an external, independent Animal Ethics Committee (St. DEC consult, Soest, the Netherlands), and all animal procedures were captured in a detailed protocol approved by the Animal Welfare Body – by this process securing full compliance the European Directive 2010/63/EU for the use of animals for scientific purposes.

References

1. Koletzko B, Godfrey KM, Poston L, et al. Nutrition during pregnancy, lactation and early childhood and its implications for maternal and long-term child health: the early nutrition project recommendations. *Ann Nutr Metab*. 2019; 74, 93–106.
2. Koletzko B, Brands B, Poston L, Godfrey K, Demmelmair H, Early Nutrition Project. Early nutrition programming of long-term health. *Proc Nutr Soc*. 2012; 71, 371–378.
3. Gawlinska K, Gawlinski D, Filip M, Przegalinski E. Relationship of maternal high-fat diet during pregnancy and lactation to offspring health. *Nutr Rev*. 2021; 79, 709–725.
4. North S, Crofts C, Thoma C, Zinn C. The role of maternal diet on offspring hyperinsulinaemia and adiposity after birth: a systematic review of randomised controlled trials. *J Dev Orig Health Dis*. 2022; 13, 527–540. DOI: 10.1017/S2040174421000623.
5. Koletzko B. Early nutrition and its later consequences: new opportunities. *Adv Exp Med Biol*. 2005; 569, 1–12.
6. Fernandez-Twinn DS, Ozanne SE. Early life nutrition and metabolic programming. *Ann N Y Acad Sci*. 2010; 1212, 78–96.
7. Fernandez-Twinn DS, Hjort L, Novakovic B, Ozanne SE, Saffery R. Intrauterine programming of obesity and type 2 diabetes. *Diabetologia*. 2019; 62, 1789–1801.
8. Bianco-Miotto T, Craig JM, Gasser YP, van Dijk SJ, Ozanne SE. Epigenetics and DOHaD: from basics to birth and beyond. *J Dev Orig Health Dis*. 2017; 8, 513–519.
9. Oosting A, Kegler D, Boehm G, Jansen HT, van de Heijning BJ, van der Beek EM. N-3 long-chain polyunsaturated fatty acids prevent excessive fat deposition in adulthood in a mouse model of postnatal nutritional programming. *Pediatr Res*. 2010; 68, 494–499.
10. Oosting A, Kegler D, Wopereis HJ, et al. Size and phospholipid coating of lipid droplets in the diet of young mice modify body fat accumulation in adulthood. *Pediatr Res*. 2012; 72, 362–369.

11. Baars A, Oosting A, Engels E, et al. Milk fat globule membrane coating of large lipid droplets in the diet of young mice prevents body fat accumulation in adulthood. *Br J Nutr*. 2016; 115, 1930–1937.
12. Ricci M, Ulman EJALN. Laboratory animal diets: a critical part of your in vivo research. *Res Diets*. 2005; 4, 1–6.
13. Pellizzon MA, Ricci MR. Choice of laboratory rodent diet may confound data interpretation and reproducibility. *Curr Dev Nutr*. 2020; 4, nzaa031.
14. Toyoda A, Shimonishi H, Sato M, Usuda K, Ohsawa N, Nagaoka K. Effects of non-purified and semi-purified commercial diets on behaviors, plasma corticosterone levels, and cecum microbiome in C57BL/6J mice. *Neurosci Lett*. 2018; 670, 36–40.
15. Kuo SM. The interplay between fiber and the intestinal microbiome in the inflammatory response. *Adv Nutr*. 2013; 4, 16–28.
16. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes*. 2017; 8, 172–184.
17. Makki K, Deehan EC, Walter J, Backhed F. The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host Microbe*. 2018; 23, 705–715.
18. Pellizzon MA, Ricci MR. The common use of improper control diets in diet-induced metabolic disease research confounds data interpretation: the fiber factor. *Nutr Metab (Lond)*. 2018; 15, 3.
19. Daubioul C, Rousseau N, Demeure R, et al. Dietary fructans, but not cellulose, decrease triglyceride accumulation in the liver of obese Zucker fa/fa rats. *J Nutr*. 2002; 132, 967–973.
20. Ronda O, van de Heijning BJM, de Bruin A, et al. Spontaneous liver disease in wild-type C57BL/6JOlaHsd mice fed semisynthetic diet. *PLoS One*. 2020; 15, e0232069.
21. Pontifex MG, Mushtaq A, Le Gall G, et al. Differential influence of soluble dietary fibres on intestinal and hepatic carbohydrate response. *Nutrients*. 2021; 13, 4278.
22. Lien EL, Boyle FG, Wrenn JM, Perry RW, Thompson CA, Borzelleca JF. Comparison of AIN-76A and AIN-93G diets: a 13-week study in rats. *Food Chem Toxicol*. 2001; 39, 385–392.
23. Rutten AA, de Groot AP. Comparison of cereal-based diet with purified diet by short-term feeding studies in rats, mice and hamsters, with emphasis on toxicity characteristics. *Food Chem Toxicol*. 1992; 30, 601–610.
24. Zou J, Ngo VL, Wang Y, Wang Y, Gewirtz AT. Maternal fiber deprivation alters microbiota in offspring, resulting in low-grade inflammation and predisposition to obesity. *Cell Host Microbe*. 2023; 31, 45–57 e47.
25. Schipper L, Tims S, Timmer E, Lohr J, Rakhshandehroo M, Harvey L. Grain versus AIN: common rodent diets differentially affect health outcomes in adult C57BL/6j mice. *PLoS One*. 2024; 19, e0293487.
26. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. In *Histopathology*, 2014; pp. 31–43. Springer.
27. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005; 41, 1313–1321.
28. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. 2013; 8, e61217.
29. Jari Oksanen FGB, Friendly M, Kindt R, et al. Vegan Community Ecology Package. 2020. Available from: =https://CRAN.R-project.org/package=vegan
30. Rstatix AK. Pipe-friendly framework for basic statistical tests 2021. Available from: https://cran.r-project.org/
31. Brooks ME. GlmmTMBed balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*. 2017; 9, 378–400.
32. Oosting A, van Vlies N, Kegler D, et al. Effect of dietary lipid structure in early postnatal life on mouse adipose tissue development and function in adulthood. *Br J Nutr*. 2014; 111, 215–226.
33. Ronda O, van de Heijning BJM, Martini IA, et al. An early-life diet containing large phospholipid-coated lipid globules programmes later-life postabsorptive lipid trafficking in high-fat diet- but not in low-fat diet-fed mice. *Br J Nutr*. 2021; 125, 961–971.
34. Kodde A, van der Beek EM, Phielix E, Engels E, Schipper L, Oosting A. Supramolecular structure of dietary fat in early life modulates expression of markers for mitochondrial content and capacity in adipose tissue of adult mice. *Nutr Metab (Lond)*. 2017; 14, 37.
35. Duval C, Thissen U, Keshtkar S, et al. Adipose tissue dysfunction signals progression of hepatic steatosis towards nonalcoholic steatohepatitis in C57BL/6 mice. *Diabetes*. 2010; 59, 3181–3191.
36. Siersbaek MS, Ditzel N, Hejbol AK, et al. C57BL/6J substrain differences in response to high-fat diet intervention. *Sci Rep*. 2020; 10, 14052.
37. Dumas ME, Rothwell AR, Hoyles L, et al. Microbial-host co-metabolites are prodromal markers predicting phenotypic heterogeneity in behavior, obesity, and impaired glucose tolerance. *Cell Rep*. 2017; 20, 136–148.
38. Yang Y, Smith DL Jr., Keating KD, Allison DB, Nagy TR. Variations in body weight, food intake and body composition after long-term high-fat diet feeding in C57BL/6J mice. *Obesity (Silver Spring)*. 2014; 22, 2147–2155.
39. Casimiro I, Stull ND, Tersey SA, Mirmira RG. Phenotypic sexual dimorphism in response to dietary fat manipulation in C57BL/6J mice. *J Diabetes Complications*. 2021; 35, 107795.
40. Kodde A, Engels E, Oosting A, van Limpt K, van der Beek EM, Keijer J. Maturation of white adipose tissue function in C57BL/6j mice from weaning to young adulthood. *Front Physiol*. 2019; 10, 836.
41. Wopereis H, Oozeer R, Knipping K, Belzer C, Knol J. The first thousand days - intestinal microbiology of early life: establishing a symbiosis. *Pediatr Allergy Immunol*. 2014; 25, 428–438.
42. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. *Nat Med*. 2016; 22, 713–722.
43. Dabke K, Hendrick G, Devkota S. The gut microbiome and metabolic syndrome. *J Clin Invest*. 2019; 129, 4050–4057.
44. Blanton LV, Charbonneau MR, Salih T, et al. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. *Science*. 2016; 351, aad3311.
45. Parker BJ, Wearsch PA, Veloo ACM, Rodriguez-Palacios A. The genus alistipes: gut bacteria with emerging implications to inflammation, cancer, and mental health. *Front Immunol*. 2020; 11, 906.
46. Malesza IJ, Malesza M, Walkowiak J, et al. High-fat, Western-style diet, systemic inflammation, and gut microbiota: A Narrative Review. *Cells*. 2021; 10, 3164.
47. Wang B, Kong Q, Li X, et al. A high-fat diet increases gut microbiota biodiversity and energy expenditure due to nutrient difference. *Nutrients*. 2020; 12, 3197.
48. Jo JK, Seo SH, Park SE, et al. Gut microbiome and metabolome profiles associated with high-fat diet in mice. *Metabolites*. 2021; 11, 482.
49. Aguiar LM, Moura CS, Ballard CR, et al. Metabolic dysfunctions promoted by AIN-93G standard diet compared with three obesity-inducing diets in C57BL/6J mice. *Curr Res Physiol*. 2022; 5, 436–444.
50. Koza RA, Nikonova L, Hogan J, et al. Changes in gene expression foreshadow diet-induced obesity in genetically identical mice. *PLoS Genet*. 2006; 2, e81.
51. Burcelin R, Crivelli V, Dacosta A, Roy-Tirelli A, Thorens B. Heterogeneous metabolic adaptation of C57BL/6J mice to high-fat diet. *Am J Physiol Endocrinol Metab*. 2002; 282, E834–E842.
52. Karp NA, Reavey N. Sex bias in preclinical research and an exploration of how to change the status quo. *Br J Pharmacol*. 2019; 176, 4107–4118.
53. LeBlanc JG, Chain F, Martin R, Bermudez-Humaran LG, Courau S, Langella P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb Cell Fact*. 2017; 16, 79.
54. Portincasa P, Bonfrate L, Vacca M, et al. Gut microbiota and short chain fatty acids: implications in glucose homeostasis. *Int J Mol Sci*. 2022; 23, 1105.
55. EMvdBaA Oosting. Nutritional programming in early life: the role of dietary lipid quality for future health. *Int Congr Ser*. 2020; 27, 12–24.
56. Abrahamse-Berkeveld M, Jespers SN, Khoo PC, et al. Infant milk formula with large, milk phospholipid-coated lipid droplets enriched in dairy lipids affects body mass index trajectories and blood pressure at school age: follow-up of a randomized controlled trial. *Am J Clin Nutr*. 2024; 119, 87–99.
57. Desclee de Maredsous C, Oozeer R, Barbillon P, et al. High-protein exposure during gestation or lactation or after weaning has a

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press

period-specific signature on rat pup weight, adiposity, food intake, and glucose homeostasis up to 6 Weeks of age. *J Nutr*. 2016; 146, 21–29.
58. Bouwman LMS, Fernandez-Calleja JMS, van der Stelt I, Oosting A, Keijer J, van Schothorst EM. Replacing part of glucose with galactose in the postweaning diet protects female but not male mice from high-fat diet-induced adiposity in later life. *J Nutr*. 2019; 149, 1140–1148.
59. Pellizzon MA, Ricci MR. Effects of rodent diet choice and fiber type on data interpretation of gut microbiome and metabolic disease research. *Curr Protoc Toxicol*. 2018; 77, e55.
60. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr*. 1993; 123, 1939–1951.

https://doi.org/10.1017/S2040174424000436 Published online by Cambridge University Press