RESEARCH DIETS FOCUS

Open Standard Diets Improve Metabolic and Gut Health Compared to Traditional AIN Diets in Mice

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Diet is one of many environmental variables which affects the phenotype of an animal and is one that can be easily controlled. Laboratory rodent diets are classified into two main types: grain-based diets (GBDs) or purified diets (PDs). GBDs (also referred to as chow diets) are typically closed formulas and made with grain-based ingredients and animal byproducts [1]. While they provide nutrition for growth and overall health, they contain non-nutritive ingredients such as phytochemicals and potential toxins such as endotoxins, mycotoxins and heavy metals from several ingredients, which may vary from batch-to-batch and potentially influence phenotype [2-5]. In contrast, PDs are 'open' formulas made with defined concentrations of highly refined purified ingredients, each providing one main nutrient (i.e., sucrose is mainly carbohydrate, corn oil is mainly fat and casein is mainly protein). This minimizes non-nutrient contamination and allows for greater control relative to grain-based diets (GBDs) [3, 6].

Product #	D10001 76A		D10012G 93G		D11112201 OSD		D11112202 OSD-F	
Macronutrient	**gm%**	***kcal%***	**gm%**	***kcal%***	**gm%**	***kcal%***	**gm%**	***kcal%***
Protein	20	*21*	20	*20*	19	*20*	17	*20*
Carbohydrate	66	*68*	64	*64*	61	*65*	53	*65*
Fat	5	*12*	7	*16*	7	*15*	6	*15*
Fiber	5		5		9		20	
Soluble	0		0		2		2	
Insoluble	5		5		7		18	
Total		***100***		***100***		***100***		***100***
kcal/gm	3.90		4.00		3.81		3.34	
Ingredient	**gm**	***kcal***	**gm**	***kcal***	**gm**	***kcal***	**gm**	***kcal***
Protein (Mainly casein)	203	*812*	203	*812*	203	*812*	203	*812*
Carbohydrate	650	*2600*	629.5	*2518*	641	*2564*	641	*2564*
Sucrose	50%		10%		< 1%		< 1%	
Fiber								
Soluble (Inulin)	0	*0*	0	*0*	25	***38***	25	***38***
Insoluble (Cellulose)	50	*0*	50	*0*	75	*0*	225	*0*
Fat	50	*450*	70	*630*	70	*630*	70	*630*
Mineral Mix	35	*0*	35	*0*	45	*0*	45	*0*
Vitamin Mix	10	*40*	10	*40*	10	*40*	10	*40*
Choline Bitartrate	2	*0*	2.5	*0*	2	*0*	2	*0*
Total	**1000**	***3902***	**1000**	***4000***	**1071**	***4084***	**1221**	***4084***

Table 1: Composition of the diets used in Study 1. The diet formulation table was modified from Griffin et al, Curr Dev Nutr 2022;6:nzac105.

The American Institute of Nutrition (AIN) formulated the AIN-76A PD over 4 decades ago and around 30 years ago, another AIN committee formulated the AIN-93 series PDs. Since the inception of these AIN diets, we have learned more about how different nutrients alter the metabolic health of mice and rats and this knowledge can be applied to improve the formulation of these diets for future studies. The AIN diets are the most commonly used control PDs, which can provide adequate growth and health of rodents [7]; however, there have been reports of mild metabolic dysfunction (e.g. increased body weight, body fat, mild insulin resistance, hyperlipidemia, etc.) in animals consuming these diets [8, 9], relative to GBD fed animals. While several differences exist between these two types of diets, these perturbations may be in part due to certain ingredients in these diets, including their higher sucrose content (10% and 50% w/w in AIN-93G and AIN-76A, respectively) and a low amount of total and mostly non-fermentable fiber (5% cellulose) [10]. This is in stark contrast to the presence of minimal amounts of sucrose and relatively higher amounts (15-25% w/w) of fiber in GBDs. In addition, GBDs also contain diverse sources of fiber including soluble (beta-glucan, pectin), partially soluble (hemicellulose) and insoluble fibers (cellulose, lignin) [1, 3].

We conducted a study to determine if PDs modified to contain lower sucrose and increased fiber (soluble and insoluble) improve metabolic and gut health in mice compared to traditional AIN diets [11]. Modifications included replacement of sucrose with sources such as corn starch and dextrose to minimize fructose, an initiator of metabolic disease, including insulin resistance, glucose intolerance, and hyperlipidemia [12, 13]. Additionally, we also increased the total fiber content and included inulin, a refined soluble fiber, which may promote metabolic health via gut microbiome alterations [14-19]. These Open Standard Diets (OSDs) contained only trace levels of sucrose (in the vitamin and mineral mixes), providing around 1% of total calories. The OSD D11112201 contained 100 gm of added fiber per 4084 kcals in a 3:1 ratio of cellulose to inulin (9.3% fiber w/w). The OSD-F diet D11112202 (20.5% total fiber w/w) contained three times as much cellulose (225 g), but the same amount of inulin (25 g), to be more in line with GBDs which contain higher amounts of fiber as insoluble fiber with some soluble fiber. The nutritional profiles of these diets are presented in Table 1. In addition to the AIN diets, we compared these OSDs to the GBD, LabDiet 5002 (insoluble fiber 18.6% and soluble fiber 5.3%). The study was done in male C57Bl/6N mice (N=15/treatment) and the mice were fed these diets for 88 days [11].

We observed that the OSDs only marginally influenced body weight and adiposity. At the end of the study, body weights were similar across the five groups and all the individual fat pad (mesenteric, gonadal, retroperitoneal and inguinal) weights were generally similar among the groups. Although all groups had similar 6-hr fasting blood glucose levels, glucose tolerance was significantly reduced in mice consuming the AIN diets compared to both the GBD and the OSD diet D11112201. We also measured other markers including serum cholesterol, triglycerides, leptin, liver triglycerides and they were generally similar across all the groups with some exceptions. Our data suggested that the addition of inulin was key to maintaining glucose tolerance in the OSD groups relative to animals on GBD. In addition, replacement of sucrose with glucose-derived carbohydrates may also have benefited these mice on OSDs as sucrose at doses similar to the AIN-76A diet induce metabolic disease in rats and mice [17, 20]. While sucrose levels were reduced to 10% in the AIN-93G diet relative to 50% in the AIN-76A diet [7], even relatively low levels of sucrose may elicit changes in glucose tolerance over a chronic feeding period, which is due to the fructose component of this carbohydrate [21].

"If you want additional details on these diets, please refer to our recently published paper."

Addition of Soluble Fiber in Low-Fat Purified Diets Maintains Cecal and Colonic Morphology, Modulates Bacterial Populations and Predicted Functions, and Improves Glucose Tolerance Compared with Traditional AIN Diets in Male Mice

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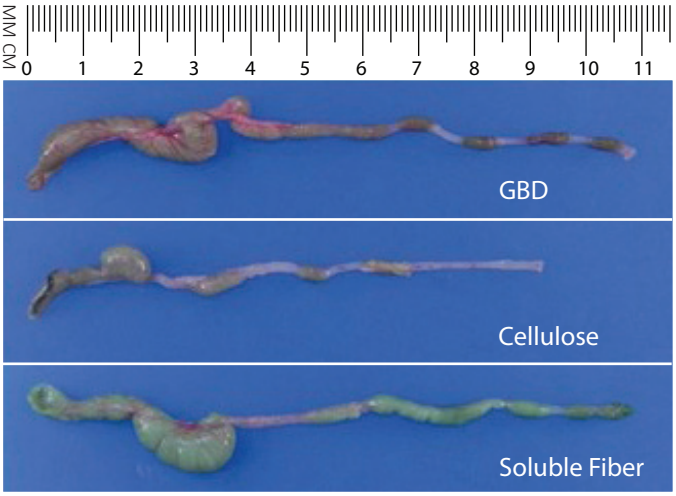

Figure 1. A representative figure to show how the soluble fiber type affects the phenotype in C57Bl/6N mice. This figure was adapted from Griffin et al, Curr Dev Nutr 2022;6:nzac105.

The current recommendation for fiber in the AIN diets at 5% cellulose, an insoluble fiber, provides little fermentable dietary substrate accessible to the intestinal microbiota. To further understand the role of fiber type and concentration in PDs and how they compare to GBDs, we used 6 additional versions of the OSD with either 100 or 200 g of cellulose, inulin or fructo-oligosaccharides (FOS, ~9 or 18% total fiber). The six experimental OSDs were compared to two GBDs: LabDiet 5002, same as in the previous study, and LabDiet 5001, in male C57Bl/6N mice (n = 6/treatment) maintained on these diets for 14 days. Despite minimal differences in weight in the two-week period, we observed rapid changes to the lower intestinal morphology. Representative pictures of cecums and colons (Figure 1) from each group indicated that a replacement of cellulose with soluble fiber-based OSDs prevented the rapid cecum and colonic weight loss associated with traditional cellulose-based diets. We have observed this previously in the context of a high-fat diet, where inulin helped to maintain cecum and colon weight changes similar to GBDs. This effect was microbiome-linked as inulin's ability to promote colon and cecum health was abrogated in germ-free mice [19].

16S rRNA sequencing of the cecum and colonic contents showed that soluble fiber groups (i.e. inulin or FOS) had similar beta diversity (Figure 2). However, the soluble fibers lowered the alpha diversity relative to GBDs and cellulose fed OSD groups. The GBDs were able to support the greatest number of species in both tissue contents regardless of the diversity metric used and in most cases, both the cellulose based OSDs were also able to maintain a statistically similar number of species as GBDs [11]. This could be partially because the fiber types present in GBDs are predominantly insoluble types, like cellulose [3]. Previous data suggest that cellulose is important for age-related diversification of the intestinal microbiota [22] and thus our results indicate that it may be better to continue having cellulose in future designs of OSDs. The soluble fiber OSDs significantly reduced the Firmicutes/Bacteroidota ratio in the cecum samples (relative to the GBDs), and increased certain genera associated with improved gut and metabolic health such as *Bifidobacteria and Akkermansia* [11]. Together, these observations could partially explain why adding soluble fiber such as inulin to diets is associated with improved metabolic health compared to the insoluble fiber (cellulose) based low-fiber, high-sucrose AIN diets as seen from results of our first study.

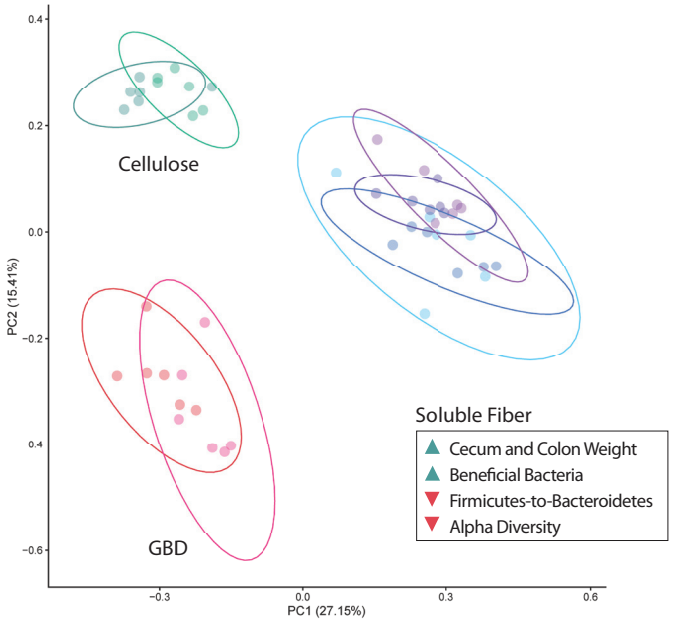

Figure 2. Beta diversity (Bray-Curtis Dissimilarity plots) of colonic microbiome in C57Bl/6N mice (N=6) consuming either GBDs or OSDs containing either cellulose, or soluble fibers (inulin/FOS). The impacts of soluble fiber on gut morphology and microbes are also detailed. Figure adopted from Griffin et al, Curr Dev Nutr 2022;6:nzac105.

In conclusion, it is clear that these changes to the formulation of traditional AIN PDs (increased fiber and addition of soluble fiber, replacement of sucrose with glucose derived carbohydrate sources) provide improvements to metabolic and gut health. This may have been in part due to changes in the gut microbiota profile. However, to more closely mimic gut microbiota in mice fed GBDs, the addition of multiple, diverse fiber sources will likely be required [3, 11]. Future efforts should be directed towards determining the optimal ratios of soluble and insoluble fibers in PDs, as well as exploring how these changes to the gut microbiome may influence the animal's metabolic health throughout the life cycle including during gestation/rapid growth phases and later in life. Ultimately a metabolically healthy control PD would greatly help the research community to decipher nutrient related phenotypic differences in a wide range of scientific domains.

References:

1. Pellizzon, M., Lab Anim (NY), 2016. 45(6): p. 238-9.
2. Kozul, C.D., et al., Chem Biol Interact, 2008. 173(2): p. 129-40.
3. Pellizzon, M.A., et al., Curr Protoc Toxicol, 2018: p. e55.
4. Radhakrishnan, S., et al., Society for Toxicology Annual Meeting, 2019. 2676.
5. Waldemarson, A.H., et al., Lab Anim, 2005. 39(2): p. 230-5.
6. Pellizzon, M.A., et al., Curr Dev Nutr, 2020. 4(4): p. nzaa031.
7. Reeves, P.G., J Nutr, 1997. 127(5 Suppl): p. 838S-841S.
8. Klurfeld, D.M., et al., J Nutr, 2021. 151(6): p. 1380-1382.
9. Syed, R., et al., Metab Syndr Relat Disord, 2016. 14(4): p. 202-9.
10. Gonzalez-Blazquez, R., et al., Sci Rep, 2020. 10(1): p. 2902.
11. Griffin, L.E., et al., Curr Dev Nutr, 2022. 6(10): p. nzac105.
12. Sleder, J., et al., Metabolism, 1980. 29(4): p. 303-5.
13. Thresher, J.S., et al., Am J Physiol Regul Integr Comp Physiol, 2000. 279(4): p. R1334-40.
14. Brooks, L., et al., Mol Metab, 2017. 6(1): p. 48-60.
15. Campbell, J.M., et al., J Nutr, 1997. 127(1): p. 130-6.
16. Levrat, M.A., et al., J Nutr, 1991. 121(11): p. 1730-7.
17. O'Brien, P., et al., Int J Mol Sci, 2020. 22(1).
18. Chassaing, B., et al., Am J Physiol Gastrointest Liver Physiol, 2015. 309(7): p. G528-41.
19. Zou, J., et al., Cell Host Microbe, 2018. 23(1): p. 41-53 e4.
20. Sumiyoshi, M., et al., J Nutr, 2006. 136(3): p. 582-7.
21. Baena, M., et al., Sci Rep, 2016. 6: p. 26149.
22. Fischer, F., et al., Gut Microbes, 2020. 12(1): p. 1-17.

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