Cell Stem Cell

CellPress

Letter

Effects of Diet Choice on Stem Cell Function Necessitate Clarity in Selection and Reporting

Wenge Li,[1,4] Michele Houston,[1,4] Karina Peregrina,[1,4] Kenny Ye,[2,4] and Leonard H. Augenlicht[1,3,*]
[1]Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, USA
[2]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, USA
[3]Department of Cell Biology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, USA
[4]These authors contributed equally
*Correspondence: leonard.augenlicht@einsteinmed.org
https://doi.org/10.1016/j.stem.2020.06.014

Editor's Note: Augenlicht and colleagues raise concerns about the impact of diet on stem cell regulation and call for more careful reporting on dietary conditions used in mouse studies on this topic. We agree that specific conditions for animal housing and care should be reported, and we encourage this in our STAR Methods guidelines for all papers. However, moving forward we will require more detailed reporting on dietary information for mouse studies where the main scientific focus concerns stem cell functions, and especially metabolism. This will include vendor information, stock number, and feeding schedules within the STAR Methods section and Experimental Models section of the Key Resource Table. We hope this will provide further reproducibility, robustness, and clarity in the papers we publish.

Investigators treat "chow diet" as a singular entity and many stem cell papers do not provide any, or they provide insufficient, information to discern components of different "chows." However, chow diets are prepared from products such as corn and wheat husks, oats, alfalfa, and soybean and fish meals, inherently differing among manufacturers, varying among batches, and potentially containing mycotoxins, heavy metals, and pesticides. Their plant-based manufacture dictates that chow elevates mouse serum levels of biologically active phytoestrogens to exceed 1,000–50,000 times the level of endogenous estrogen (Brown and Setchell, 2001; Wang et al., 2005), but these are absent in mice fed purified diets and are at less than 5% of that in most humans. Thousands of publications establish that these compounds alter regulatory, signaling, oncogenic, and metabolic pathways that can affect stem cell function.

To illustrate the importance of this, *Lgr5*$^{EGFP\text{-}cre:ERT2}$ mice were fed different diets from weaning (details, Figure S1). These included a high-fat purified diet in which 60% of calories are from fat (HFD, Research Diets, 12492; Beyaz et al., 2016), a chow diet (PicoLab 5058, LabDiets) often used as a control and which was compared to the purified HFD in Beyaz et al., a purified diet that is a specific control for the purified HFD with 10% calories from fat (HFcontrol, Research Diets, 12450B), and another purified diet, AIN76A (Research Diets, D10001). The diets differed in many nutrient levels, and importantly the chow but not purified diets contained high phytoestrogen levels. After 3 months, Lgr5^{hi} cells were collected by FACS from isolated intestinal crypts, and their RNA was harvested and subjected to RNA-seq (Li et al., 2019).

Principal component analysis distinguished Lgr5^{hi} cells of chow-fed mice from Lgr5^{hi} cells of the 3 groups fed purified diets (HFcontrol, AIN76A, and HFD) (Figure S1A). Unsupervised clustering of Lgr5^{hi} cell gene expression data first separated chow-fed mice from those fed the 3 purified diets, with second-level separation of purified HFD from control purified diets, and third-level separation of the two purified control diets (Figure S1B).

As expected, genes differentially expressed between HFD mice and chow or purified HFcontrol mice decreased in number as stringency increased (P value cut-off) (Figure S1C). At each stringency, overlap of differentially expressed genes was poor using the different controls as baseline, including at highest stringency ($p = 0.0001$), where no more than one or two false positives were expected. At each stringency, there were more differentially expressed genes comparing HFD/chow than for HFD/HFcontrol, possibly due to many nutrient differences besides fat between chow control and purified HFD.

Comparing the data from HFD to HFcontrol (Figure S1D), Gene Set Enrichment analysis (GSEA) identified 33 functional groups enriched; 18 of these (55%) were not significantly enriched comparing HFD/chow, and another 6 were significantly enriched but with opposite change in direction of potential pathway function (Figure S1D), a 73% discordance with the HFcontrol comparison.

Pathways identified in comparing HFD/HFcontrol might be candidates for further investigation (e.g. upregulated DNA replication, mismatch repair, homologous recombination, or base excision repair; or downregulated toll-like receptor, B cell, T cell, and cytokine receptor signaling).

There was similar significant enrichment of the Ppar signaling pathway in Lgr5^{hi} cells from HFD mice compared to HFcontrol or chow (Figure S1D, black arrow). However, different genes contribute to significance in each comparison. Altered expression of genes defining the Ppar pathway (Figure S1E) was not correlated using the two different controls (Figure S1F). Expression of 17 genes contributed to Ppar pathway significance in the HFD/HFcontrol (Figure S1E), but 7 of these did not contribute in the HFD/chow comparison (41% discordance; Figure S1E). Complementarily, 29 genes contributed to significance for HFD/chow comparison, but 19 of these did not contribute for HFD/HFcontrol (66% discordance; Figure S1E).

Altered *Pparδ* expression was discordant for the two comparisons (Figure S1G). It was significantly elevated

($p = 0.006$) in Lgr^{hi} cells of mice fed HFD compared with those fed chow (Figure S1G), replicating the reported result (Beyaz et al., 2016), and contributed to the significant Ppar pathway enrichment in HFD/chow GSEA (Figure S1E, red arrow). However, higher *Pparδ* expression in the HFcontrol or AIN76A purified diets (Figure S1G) eliminated significance in comparison to HFD. Indeed, *Pparδ* was near the top of the GSEA gene list ranked by normalized enrichment score for HFD/chow while at the bottom for the HFD/HFcontrol (Figure S1E, red arrows). Thus, at every level of analysis, conclusions and impetus for follow-up of genes/pathways in $Lgr5^{hi}$ cells was dependent on the diets used.

The "right" diet depends on the question addressed, but general principles apply: diet specifics should be provided in formats journals have adopted to promote transparency necessary for data reproduction and interpretation; dietary effects can ripple through layers of biochemical, signaling, metabolic, and epigenetic regulation, and therefore purified diets should be used for experimental animals, avoiding potential complications from chow diets, as recommended (Pellizzon and Ricci, 2018; Thigpen et al., 2004; Warden and Fisler, 2008) and as more routine in other fields (e.g. chemoprevention, nutrition, and metabolism); and since results are often interpreted in the context of the etiology and mechanisms driving human disease and efficacy of preventive and therapeutic strategies, investigators should consider that human nutrient exposures differ substantially from that of rodents. This can be due to different nutritional requirements, but also due to providing optimum nutrient levels to maximize lab rodent health, longevity, and reproduction, which are not features of most human diets. Such considerations can prove insightful. For example, a purified mouse diet that provides common nutrients at the level of each equivalent to that in humans that is linked to high incidence of human colon cancer causes mouse intestinal tumors and has pronounced effects on which and how mouse intestinal stem cells function. This established that intestinal stem/progenitor-like cell function can be recruited by the nutritional environment, not only by damage or genetic manipulation of $Lgr5^{hi}$ cells (Augenlicht, 2017; Li et al., 2019, 2020; Peregrina et al., 2015). This has important implications for how intestinal homeostasis is maintained in humans where nutrient exposures vary among populations and vary for individuals daily. This highlights the importance in carefully choosing diets for mouse experiments and for clarity in reporting dietary information in stem cell-based, and other, rodent studies.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.stem.2020.06.014.

ACKNOWLEDGMENTS

This work was supported by the National Cancer Institute; National Institutes of Health grants R01CA229216, R01CA214625, and P30-13330; and the Jane A. and Myles P. Dempsey fund.

AUTHOR CONTRIBUTIONS

W.L., data analysis and manuscript revision; M.H., mouse husbandry, tissue, cell and RNA preparation, data analysis, and manuscript revision; K.P., data analysis and manuscript revision; K.Y., data analysis and manuscript revision; L.H.A., experimental conception and design, data analysis, and manuscript drafting.

REFERENCES

Augenlicht, L.H. (2017). Environmental Impact on Intestinal Stem Cell Functions in Mucosal Homeostasis and Tumorigenesis. J. Cell. Biochem. *118*, 943–952.

Beyaz, S., Mana, M.D., Roper, J., Kedrin, D., Saadatpour, A., Hong, S.J., Bauer-Rowe, K.E., Xifaras, M.E., Akkad, A., Arias, E., et al. (2016). High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. Nature *531*, 53–58.

Brown, N.M., and Setchell, K.D. (2001). Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones. Lab. Invest. *81*, 735–747.

Li, W., Zimmerman, S.E., Peregrina, K., Houston, M., Mayoral, J., Zhang, J., Maqbool, S., Zhang, Z., Cai, Y., Ye, K., and Augenlicht, L.H. (2019b). The nutritional environment determines which and how intestinal stem cells contribute to homeostasis and tumorigenesis. Carcinogenesis *40*, 937–946.

Li, W., Peregrina, K., Houston, M., and Augenlicht, L.H. (2020). Vitamin D and the nutritional environment in functions of intestinal stem cells: Implications for tumorigenesis and prevention. J. Steroid Biochem. Mol. Biol. *198*, 105556.

Pellizzon, M.A., and Ricci, M.R. (2018). The common use of improper control diets in diet-induced metabolic disease research confounds data interpretation: the fiber factor. Nutr. Metab. (Lond.) *15*, 3.

Peregrina, K., Houston, M., Daroqui, C., Dhima, E., Sellers, R.S., and Augenlicht, L.H. (2015). Vitamin D is a determinant of mouse intestinal Lgr5 stem cell functions. Carcinogenesis *36*, 25–31.

Thigpen, J.E., Setchell, K.D., Saunders, H.E., Haseman, J.K., Grant, M.G., and Forsythe, D.B. (2004). Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. ILAR J. *45*, 401–416.

Wang, H., Tranguch, S., Xie, H., Hanley, G., Das, S.K., and Dey, S.K. (2005). Variation in commercial rodent diets induces disparate molecular and physiological changes in the mouse uterus. Proc. Natl. Acad. Sci. USA *102*, 9960–9965.

Warden, C.H., and Fisler, J.S. (2008). Comparisons of diets used in animal models of high-fat feeding. Cell Metab. *7*, 277.

Cell Stem Cell, Volume *27*

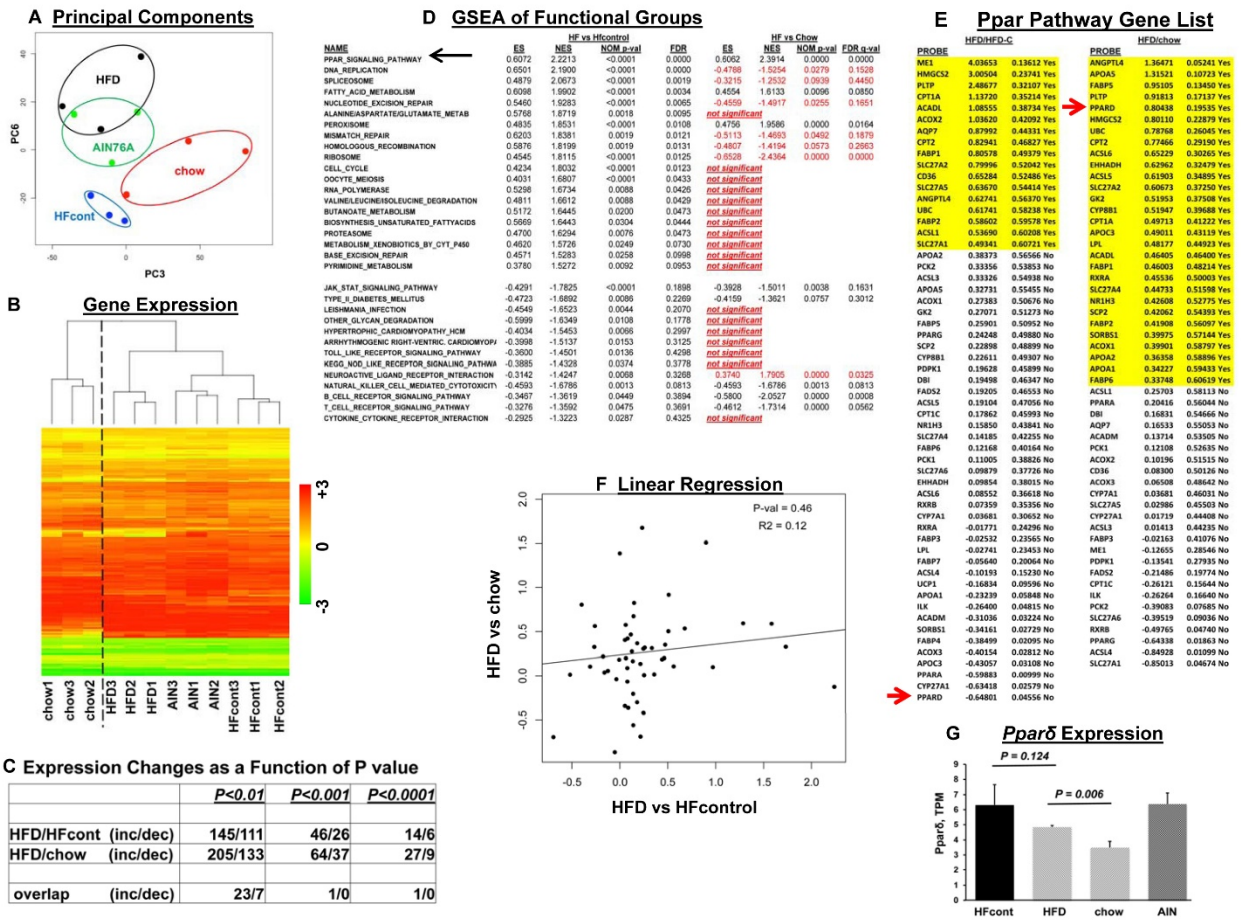
Supplemental Information

Effects of Diet Choice on Stem Cell Function

Necessitate Clarity in Selection and Reporting

Wenge Li, Michele Houston, Karina Peregrina, Kenny Ye, and Leonard H. Augenlicht

Supplemental Figure 1



C Expression Changes as a Function of P value		P<0.01	P<0.001	P<0.0001
HFD/HFcont	(inc/dec)	145/111	46/26	14/6
HFD/chow	(inc/dec)	205/133	64/37	27/9
overlap	(inc/dec)	23/7	1/0	1/0

Figure S1: Gene expression analysis of Lgr5^{hi} intestinal cells as a function of diet fed to the mice. Lgr5tm1(cre/ERT2)Cle/J mice (Jax #008875) were fed different diets *ad lib* from weaning (N=3/diet group; the chow cohort consisted of male mice, the purified control diet cohorts were mixed groups of male and female mice, and the HFD group were female mice). The diets were: the high fat purified diet (HFD) used in ((Beyaz et al., 2016), 60% calories from fat, Research Diets, 12492); a purified control (HFcontrol, 10% fat, Research Diets 12450B). This is 1 of 4 purified diets marketed by Research diets as a control for the HFD, all of which are 10% fat, 20% protein, and 70% carbohydrate, with the carbohydrate raised in different ways to balance the higher fat in HFD; another purified control diet, AIN76A (Research Diets, D10001); a chow diet (PicoLab 5058, LabDiets). Mice were sacrificed at 3 months, the small intestine dissected, Lgr5^{hi} cells from purified crypts isolated by FACS as the highest 2-3% of the green fluorescent cells, and RNAseq data generated with RNA prepared from each cell preparation (Li et al., 2019b; Peregrina et al., 2015). **A)** Principal Component Analysis as a function of diet; **B)** One-way ANOVA identified

162 genes that best distinguish the 4 groups, but without regard to diet identity. These were then used for unsupervised clustering in the heat map. **C)** Number of expressed genes up or down regulated in the Lgr5hi cells from the HFD group compared to either the HFcontrol or the chow group as a function of stringency of cut-off, and the overlap of the differentially expressed genes determined at each stringency. At $P<0.0001$, it is expected that only 1-2 false positives may be detected. **D)** The RNAseq data for the $Lgr5^{hi}$ cell populations were analyzed by GSEA (Li et al., 2019b). Tabulation of the statistically enriched functional groups in comparing the HFD to HFDcontrol mice, and for each of these its corresponding GSEA analysis when the data from HFD fed mice were compared to that from chow fed mice (black arrow, PPAR pathway). **E)** Of the 57 genes that define the Ppar Kegg pathway, those that contributed to the significant GSEA enrichment are highlighted in yellow for the HFD mice compared to either the HFcontrol or chow diet mice. In each comparison, genes are ranked from highest to lowest NES (normalized enrichment score); Red arrows indicate the Pparδ gene for each comparison. **F)** Linear regression of the ratio of gene expression for each gene of the Ppar pathway when the $Lgr5^{hi}$ cell data from mice fed HFD were compared to the data from mice fed either HFcontrol or chow diet. **G)** Pparδ expression (TPM=transcripts per million) in $Lgr5^{hi}$ cells as a function of the diet fed the mice. RNAseq data is deposited in Geo, accession number GSE151498.