INTRODUCTION

The intestinal epithelium has several barriers, consisting of a mucous layer, tight junctions between cells, and a substantial set of resident immune cells, to protect the host from pathogens and toxins in the gut lumen (Peterson & Artis, 2014; Vighi et al., 2008). Disruption of the intestinal epithelial barrier permits the passage of these pathogens and toxins, which can initiate and exacerbate disease and possibly aging (Doig et al., 1998; Fink & Delude, 2005; Harris et al., 1992). Understanding and preventing the underlying causes of intestinal barrier decline could help prevent disease and possibly slow aging (Farhadi et al., 2003; Fasano & Shea-Donohue, 2005; König et al., 2016; Odenwald & Turner, 2017). Oxidative stress has been argued to be an important driver of aging and age-related pathologies, including intestinal barrier dysfunction (Hale et al., 2012; Liguori et al., 2018; Rera et al., 2012; Tian et al., 2017; Wang et al., 2014). However, the sources of oxidative stress and their relative importance in a given pathology remain unclear. In the present study, we investigated whether mitochondrial superoxide generated by complex III of the mitochondrial electron transport chain is a cause of intestinal barrier disruption, using *Drosophila* and mice fed high-nutrient diets as models of accelerated metabolic disease and aging.

In many species, restricted or *ad-libitum* feeding impacts both healthspan and lifespan. In *Drosophila*, decreasing the amount of

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**Abstract**

The underlying causes of aging remain elusive, but may include decreased intestinal homeostasis followed by disruption of the intestinal barrier, which can be mimicked by nutrient-rich diets. S3QELs are small-molecule suppressors of site III$_{Qo}$ electron leak; they suppress superoxide generation at complex III of the mitochondrial electron transport chain without inhibiting oxidative phosphorylation. Here we show that feeding different S3QELs to *Drosophila* on a high-nutrient diet protects against greater intestinal permeability, greater enterocyte apoptotic cell number, and shorter median lifespan. Hif-1α knockdown in enterocytes also protects, and blunts any further protection by S3QELs. Feeding S3QELs to mice on a high-fat diet also protects against the diet-induced increase in intestinal permeability. Our results demonstrate by inference of S3QEL use that superoxide produced by complex III in enterocytes contributes to diet-induced intestinal barrier disruption in both flies and mice.

**Keywords**

aging, complex III, diet, drosophila, intestine, intestinal permeability, leaky gut, metabolism, mitochondria, oxidative stress, superoxide
dietary protein (in the form of yeast extract, YE) below the conventional 2.5% is known to increase median lifespan and decrease intestinal permeability, whereas increasing dietary YE content decreases median lifespan and increases intestinal permeability. Work from the laboratories of Hansen (Gelino et al., 2016), Walker (Rera et al., 2012), and Patridge (Regan et al., 2016) has shown that dietary restriction can prominently modulate lifespan and intestinal barrier dysfunction in both worm and flies. We confirmed this in the two *Drosophila* strains (*w*¹¹¹⁸ and Canton S) used here (Figure S1A,B). We measured intestinal permeability of flies using the "smurf assay" which involves feeding a blue food dye that is normally not absorbed; flies with permeable intestinal epithelium become blue (Rera et al., 2012). The percentage of blue flies was higher at greater YE% in both *w*¹¹¹⁸ (Figure 1a) and Canton S flies (Figure S1C). Analysis by ANCOVA affirmed significant effects of diet and days on diet on intestinal permeability, and a significant interaction of both variables. Further analysis revealed that median lifespan was decreased in flies of either strain when the incidence of intestinal permeability was enhanced by greater dietary YE% (Figure 1d), suggesting the possibility that intestinal permeability influences lifespan.

There is a critical balance between cell proliferation and apoptosis in the *Drosophila* intestine, and tipping this balance can be detrimental (Akagi et al., 2018; Liang et al., 2017). Enterocyte damage can cause apoptosis and therefore trigger proliferation of intestinal cells for repair and maintenance of tissue integrity (Amcheslavsky et al., 2009; Ohlstein & Spradling, 2006). Feeding higher YE% to *w*¹¹¹⁸ and Canton S flies increased both the number of apoptotic cells and the number of proliferating (PH3-positive) cells per intestine (Figure 1b,c; Figure S1D,E), supporting this view. Analysis by ANCOVA affirmed significant effects of diet and days on diet on intestinal apoptosis, and a significant interaction of both variables. However, only diet had a significant effect on PH3-positive intestinal cell number. Apoptotic and PH3-positive intestinal cell number

![Figure 1](image-url)
The incidence of intestinal permeability in \textit{w}^{	ext{1118}} \textit{Drosophila} fed a 5% YE diet was approximately halved by co-feeding each of the three S3QELs (Figure S2). The S3QELs also decreased the number of apoptotic cells per intestine (Figure S3a,b) and increased intestinal permeability by 10–20% (Figure S4). Interestingly, S3QELs had no significant effect on the intestinal incidence of PH3-positive cells (Figure S3C–F). Further analysis revealed that median lifespan was greater (Figure 2a–c) and apoptotic cell number was smaller (Figure 2d) when feeding S3QELs at concentrations that decreased the incidence of intestinal permeability (i.e., at concentrations greater than 0.08 \( \mu \text{M} \)). These data suggest that by inhibiting superoxide production from site IIIQo, S3QELs decrease intestinal permeability, which in turn correlates with an increase in median lifespan (Figure 2a–c). S3QELs did not significantly lower food palatability (Figure S5A) or consumption (Figure S5B), providing no evidence for the possibility that they worked by causing caloric restriction. In contrast to the effects of S3QELs, feeding of S1QEL1.1, S1QEL1.2, or S1QEL2.2 (suppressors of site I\(_2\) electron leak (Brand et al., 2016)) did not protect against induction of intestinal permeability (Figure S6). This is in contrast to our previous finding that both S1QELs and S3QELs protected against stem cell hyperplasia in flies treated with tunicamycin or genetically overexpressing the oncogene Ras\(^{V12}\) (Brand et al., 2016). Comparison of these findings illustrates that different biological perturbations can modulate different sites of mitochondrial superoxide production, and that S1QELs and S3QELs are valuable tools to address such differences (Watson et al., 2019). Note that S1QELs and S3QELs each suppress superoxide/hydrogen peroxide production from the appropriate site by mitochondria isolated from Drosophila (Brand et al., 2016). Feeding S3QELs decreased the expression of the intestinal damage and inflammatory gene markers (Figure 2e), supporting the conclusion of a decrease in intestinal permeability relative to 5% YE diet. Expression of the tight junction genes did not decrease; instead it increased (Figure 2f). Feeding S3QELs decreased the expression of the antioxidant genes (Figure 2g), consistent with a decrease in oxidative stress.

As proof of concept that S3QELs decrease mitochondrial superoxide production and can specifically work within gut enterocytes, we genetically lowered the antioxidant defenses of intestinal enterocytes. Using the NP1-GAL4 driver, we used RNAi to knock down expression of Sod1 and Sod2 specifically in intestinal enterocytes to decrease either cytosolic (Sod1) or matrix superoxide removal (Sod2) and thereby increase superoxide levels in the two compartments in intestinal enterocytes. Figure 3 shows that each knockdown significantly increased the incidence of intestinal permeability and intestinal apoptotic cell number and decreased median lifespan, showing that raised superoxide levels just in enterocytes can drive these phenotypes. During either Sod1 or Sod2 knockdown, S3QELs protected against the induced intestinal permeability (Figure 3a,f), increase in intestinal apoptotic cell number (Figure 3b,g) and decrease in median lifespan (Figure 3c,h), improving the negative correlation between intestinal permeability, intestinal apoptotic cell number, and median lifespan caused by either

correlated negatively with median lifespan (Figure 1e,f) and positively with intestinal permeability (Figure 1g,h).

We confirmed an increase in intestinal permeability using known gene expression hallmarks of a disrupted intestinal barrier (Rera et al., 2012). Antimicrobial peptides (AMPs) are expressed in response to intestinal damage and infection, and the Upd3 cytokine is released upon enterocyte damage (Lucchetta & Ohlstein, 2012). The intestinal expression of the AMP genes Dpt, Drs, and Def, and the upd3 gene increased at higher YE% on day 10 (Figure 1i) and day 30 (Figure 1f) after the diet switch. The intestinal expression of separpate junction genes (the equivalent of tight junctions in vertebrates) also increased at higher dietary YE% (Figure 1j; Figure S1G). Elevated expression of tight junction genes in flies fed a high-nutrient diet suggests a response to epithelial tight junction damage that is related to an increase in intestinal permeability.

Next, we investigated the mechanism by which a rich diet decreased median lifespan and increased intestinal permeability. Increased oxidative stress is reported in intestines of animals fed high-nutrient diets (Feillet-Coudray et al., 2014; Lee et al., 2017; Pagliaiunga et al., 2015; Patel et al., 2007). We hypothesized that mitochondrial production of reactive oxygen species might drive increased intestinal permeability, a suggestion supported by the observation of elevated intestinal gene expression of superoxide dismutases (which dismutate superoxide to hydrogen peroxide) in the cytosol (Sod1) and mitochondria (Sod2), and of catalase (Cat), which dismutates hydrogen peroxide to water and oxygen, in flies fed higher YE% (Figure 1k).

The outer ubiquinone-binding site of complex III of the mitochondrial electron transport chain (site III\(_{Qo}\)) has the largest capacity of all mitochondrial sites to produce superoxide, which it delivers into both the mitochondrial matrix and the cytosol (Brand, 2016; St-Pierre et al., 2002). S3QELs are specific small-molecule Suppressors of site III\(_{Qo}\) Electron Leak that suppress superoxide generation at complex III of the mitochondrial electron transport chain without inhibiting normal electron flow or oxidative phosphorylation or having any other known cellular targets (Orr et al., 2015). S3QELs are a more specific way to evaluate the role of superoxide in a phenotype than conventional approaches of genetic knockdowns of superoxide-producing respiratory complexes, which (by inhibiting electron transport) have deeply confounding pleiotropic effects, or directly measuring superoxide levels in vivo, using methods that are widely recognized to be plagued by non-selectivity and/or measurement artefacts (Murphy et al., 2011).

To test whether superoxide produced by site III\(_{Qo}\) contributes to intestinal permeability, flies fed a 5% YE diet were also fed S3QELs. We tested three S3QELs: S3QEL1.2, S3QEL2.2, and S3QEL3 (Figure S10). These act as their own internal control as all three structurally different S3QELs should give the same response if their effect is on-target, but different responses if it is off-target. We previously showed that S3QELs suppress superoxide/hydrogen peroxide production from site III\(_{Qo}\) by mitochondria isolated from Drosophila (Brand et al., 2016).
Sod1 or Sod2 knockdown (Figure 3d,e,i,j). These results support the idea that S3QELs improve intestinal homeostasis and extend lifespan under a rich nutrient diet by decreasing superoxide production from complex III into both the cytosol (protection against Sod1 knockdown) and matrix (protection against Sod2 knockdown) specifically in enterocytes. Although the Sod knockdown experiments are a good way to manipulate superoxide concentrations independently in the matrix and cytosol to demonstrate their importance, we have not directly measured changes in superoxide concentration in vivo because of the unreliability of the probes that are available (Murphy et al., 2011); this is a limitation of our study.

In vitro, addition of a reductant such as succinate generates a semiquinone in complex III to drive superoxide production from site IIIQo (Brand, 2016). Previously we found that succinate concentration was elevated in flies reared on 5% YE compared to flies reared on 0.5% YE (Laye et al., 2015). Here we found that raising flies on 5% YE supplemented with 50 mM dimethyl succinate decreased median lifespan (Figure S7A) and increased intestinal permeability (Figure S7B) compared to 5% YE alone. This suggests that further elevation of succinate may exacerbate the 5% YE phenotypes by driving superoxide production from site IIIQo.

We investigated how inhibiting superoxide production from site IIIQo with S3QELs ameliorates the effects of 5% YE diet other than perhaps preventing direct macromolecular damage. Superoxide generated from site IIIQo, and succinate are both known to stabilize Hif-1α (Bell et al., 2007; Brunelle et al., 2005; Guzy et al., 2005). Our previous work showed that S3QELs block the stabilization of Hif-1α under hypoxic conditions in vitro (Orr et al., 2015). Utilizing the gene switch driver 5966(GS)-GAL4 we explored whether Hif-1α contributes to the decrease in lifespan and increased intestinal permeability of flies on 5% YE compared to 0.5% YE. Specific knockdown of Hif-1α in enterocytes using RNAi resulted in an increase in lifespan (Figure S7C) and a substantial decrease in intestinal permeability (Figure S7D) in flies reared on 5% YE but not in flies reared on a 0.5% YE. Overexpression of Hif-1α in enterocytes resulted in a further decrease in lifespan and increase in intestinal permeability in flies fed 5% YE (Figure S7E,F). Overexpression also resulted in a significant decrease in lifespan and an increase in intestinal permeability in flies fed 0.5% YE. These data suggest that elevated intestinal levels of Hif-1α are detrimental and may drive some of the differences seen between 5% and 0.5 YE diet. Hif-1α is already extensively associated with intestinal barrier dysfunction making it a plausible candidate target of S3QELs (Manresa & Taylor, 2017).

To explore whether the effects of inhibition of superoxide production from site IIIQo are mediated by Hif-1α in flies reared on 5% YE, we investigated the effects of S3QELs in flies with genetically altered levels of Hif-1α in enterocytes. S3QELs protected more strongly against decreased lifespan and increased
intestinal permeability in flies overexpressing Hif-1α than in controls (Figure 4a,b). S3QEL treatment also protected against increased intestinal apoptotic cells (Figure 4c). However, in flies in which Hif-1α was knocked down, S3QEL treatment did not further increase lifespan or decrease the number of intestinal apoptotic cells any further than DMSO-treated knockdown flies (Figure 4f). S3QEL treatment had a small but significant interaction with Hif-1α knockdown (Figure 4d,e). These data suggest that the effects of S3QELs are blunted when Hif-1α is knocked down in enterocytes using RNAi, but enhanced in flies where Hif-1α is overexpressed. It is probable that superoxide from site III Qo is elevated in enterocytes in flies reared on 5% YE, which stabilizes Hif-1α, which in turn contributes to an increase in intestinal permeability and decrease in lifespan which can be ameliorated by S3QELs.

To examine whether the effects of S3QELs in Drosophila are conserved, we tested whether they protect against intestinal permeability in a mouse model. To draw parallels to Drosophila, we tested oral delivery of S3QELs in male C57BL/6J mice fed a high-fat diet (60% kcal). High-fat feeding has been shown to increase oxidative stress and induce intestinal permeability in mice (Ahmad et al., 2017; Murakami et al., 2016). We found that high-fat feeding significantly induced intestinal permeability in mice measured both by the uptake of FITC-dextran from the gut into blood plasma ($F = 48.64, p = 3.82E−09$) (Figure 5a) and by the appearance of plasma-derived albumin in feces ($F = 22.74, p = 8.75E−06$) (Figure 5b). In conjunction, there was decreased expression of tight junction (Figure 5e) and mucin genes (Figure 5f). It also induced glucose intolerance (Figure 5c; Figure S9A), increased body weight and adiposity (Figure S9B,C) and increased the expression of an ER stress gene (Figure 5d).

S3QEL1.2 and S3QEL2.2 strongly protected against the increases in intestinal permeability in mice by decreasing both plasma FITC-dextran ($F = 39.795, p = 8.78E−14$) and fecal albumin ($F = 37.913, p = 4.39E−14$) (Figure 5a,b). They protected against the decrease in tight junction and mucin gene expression in both colon and distal small intestine (Figure 5e,d; Figure S8). Expression of the goblet cell differentiation transcription factor Klf4 is known to be decreased upon high-fat feeding, and this decrease is one cause of decreased mucin expression (Gulhane et al., 2016). Klf4 expression was decreased by high-fat feeding and protected by S3QELs (Figure 5f). Together, these results strongly suggest that superoxide production from mitochondrial complex III drives intestinal permeability in mice as it does in Drosophila. This effect was probably not systemic but through direct exposure of enterocytes to S3QELs from the gut.
lumen, since unbound plasma concentrations of S3QELs measured in morning-drawn cardiac puncture blood were at least 1000× lower than their IC_{50} for suppression of superoxide production by isolated muscle mitochondria (Orr et al., 2015).

Weight gain (Figure S9B), and food consumption (Figure S9C) of mice fed the high-fat diet were not significantly altered by S3QEL feeding. Treatment with S3QELs did not improve glucose tolerance in mice fed the high-fat diet (Figure 5c; Figure S9A). Hyperglycemia induced by a high-fat diet has been proposed to drive intestinal permeability (Thaiss et al., 2018). The lack of significant effect of S3QELs on glucose tolerance despite the improvement in intestinal permeability suggests that hyperglycemia is upstream of intestinal permeability and supports this model (Thaiss et al., 2018) over the alternative model that increased gut permeability drives glucose intolerance. We propose that high-fat diet and the resulting hyperglycemia increase superoxide production from site III_{Qo} of the mitochondrial electron transport chain in gut epithelial cells, which in turn drives ER stress, increased intestinal permeability, and associated sequelae. This hypothesis explains why treatment with S3QELs protects against ER stress (Figure 5d) and intestinal permeability (Figure 5a,b), but not against impaired glucose tolerance (Figure 5c; Figure S9A).
DISCUSSION

There is an emerging realization that intestinal barrier dysfunction contributes to almost every major disease, including aging, and it has become critically important to understand the mechanisms that drive this dysfunction (Farhadi et al., 2003; Fasano & Shea-Donohue, 2005; König et al., 2016; Odenwald & Turner, 2017). Oxidative stress has consistently been argued to be an important driver of aging and age-related pathologies, including intestinal barrier dysfunction (Hale et al., 2012; Liguori et al., 2018; Rera et al., 2012; Tian et al., 2017; Wang et al., 2014). However, the sources of oxidative stress are often not addressed and their relative importance in a given pathology remains unclear. There are few examples connecting mitochondrial superoxide/hydrogen peroxide with intestinal barrier dysfunction. Prior studies report that mitochondrially targeted general antioxidants such as MitoQ protect against intestinal barrier disruption (Hale et al., 2012; Wang et al., 2014). However, they used a dextran sulfate sodium-induced colitis mouse model (DSS model), which is an unphysiological “sledge-hammer” approach. We utilized a diet-induced barrier disruption model, which is more physiologically relevant to human aging and disease, and we used S3QELs and S3QELs to identify the source of the superoxide that causes intestinal barrier disruption.

It is becoming increasingly apparent that the site of superoxide/hydrogen peroxide production is important when understanding and treating pathology given that general antioxidants often have no benefit or have detrimental side effects (Bjelakovic et al., 2007). General antioxidants act as a sponge “mopping” up ROS in a non-specific
manner, which interferes with potentially important oxidative signaling necessary to normal physiology. The identification of S1QELs, which are specific small-molecule Suppressors of site I_Q Electron Leak, and S3QELs, which are specific small-molecule Suppressors of site III_Qo Electron Leak, offers precise tools to identify and prevent superoxide/hydrogen peroxide production by complex I or III and its downstream effects without interfering with other sites (Brand et al., 2016; Orr et al., 2015). Studies using these compounds have established that sites I_Q and III_Qo not only have the highest capacity of all mitochondrial sites to produce superoxide/hydrogen peroxide in vitro, but also that they are the main contributors of superoxide in the mitochondrial matrix in several cell lines (Brand, 2016; Fang et al., 2020; Wong et al., 2019). These tools enable investigation of the contributions and importance of superoxide/hydrogen peroxide production by mitochondrial sites I_Q and III_Qo in pathologies and physiology (Brand, 2020; Watson et al., 2019).

Understanding the mechanisms that drive intestinal barrier dysfunction is crucial to understanding the impact and connection of this barrier to both healthspan and lifespan. Three interrelated candidate mechanisms are ER stress (Gulhan et al., 2016) and hyperglycemia (Thaiss et al., 2018), both known to induce mitochondrial ROS production, and stabilization of Hif-1α in response to superoxide/hydrogen peroxide produced from site III_Qo (Bell et al., 2007; Brunelle et al., 2005; Guzy et al., 2005). Our work builds mechanistically upon these earlier studies to show that superoxide produced specifically by site III_Qo of mitochondrial complex III is a crucial cause of the downstream damage and signaling caused by hyperglycemia and ER stress that leads to pathology.

We conclude that superoxide production from site III_Qo infered from use of S3QELs contributes to the development of diet-induced intestinal barrier dysfunction in flies and mice. In flies, raising superoxide levels by Sod knockdown in enterocytes is sufficient to cause intestinal barrier dysfunction. Increased diet-induced intestinal permeability tightly correlates with decreased lifespan, and feeding S3QELs extends diet-compromised lifespan in flies. This is important, as our study makes the first robust link from superoxide produced by mitochondrial complex III to the intestinal pathology that impacts lifespan, with important ramifications for mechanisms of metabolic disease and aging. Suppressing superoxide production by site III_Qo in complex III of the mitochondrial electron transport chain using S3QELs has potential therapeutic value in intestinal disorders and premature aging caused by overnutrition.

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CONFLICT OF INTERESTS
The authors declare no competing interests.

AUTHOR CONTRIBUTIONS
M.A.W., P.K and M.D.B. designed the experiments, M.A.W. performed the experiments and wrote the manuscript; P.K and M.D.B. edited the manuscript. B.P. helped perform succinate feeding and Hif-1α fly experiments. T.A.U.H. helped with statistical analysis, and J. L.-D. gavaged the mice.

DATA AVAILABILITY STATEMENT
All data is available in the manuscript or the supplementary materials.

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REFERENCES
Ahmad, R., Rah, B., Bastola, D., Dhawan, P., & Singh, A. B. (2017). Obesity- induces organ and tissue specific tight junction restructuring and barrier deregulation by claudin switching. Scientific Reports, 7, 5125.
Akagi, K., Wilson, K., Katewa, S., Ortega, M., Simons, J., Hilsabeck, T., Kapuria, S., Sharma, A., Jasper, H., & Kapahi, P. (2018). Dietary restriction improves intestinal cellular fitness to enhance gut barrier function and lifespan in D. melanogaster. PLoS Genetics, 14, e1007777.
Amchelrlsky, A., Jiang, J., & Ip, Y. T. (2009). Tissue damage-induced intestinal stem cell division in Drosophila. Cell Stem Cell, 4, 49–61. https://doi.org/10.1016/j.stem.2008.10.016.
Bell, E., Klimova, T., Eisenbarth, J., Moraes, C., Murphy, M., Budinger, G., & Chandel, N. (2007). The Q_o site of the mitochondrial complex III is required for the transduction of hypoxic signaling via reactive oxygen species production. Journal of Cell Biology, 177, 1029–1036.
Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., & Gluud, C. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: Systematic review and meta-analysis. JAMA, 297, 842–857. https://doi.org/10.1001/jama.297.8.842.
Brand, M. D. (2016). Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. Free Radical Biology and Medicine, 100, 14–31. https://doi.org/10.1016/j.freeradbiomed.2016.04.001.
Brand, M. D. (2020). Riding the tiger - physiological and pathological effects of superoxide and hydrogen peroxide generated in the mitochondrial matrix. Critical Reviews in Biochemistry and Molecular Biology, 55, 592–661. https://doi.org/10.1080/10409238.2020.1828258.
Brand, M., Goncalves, R., Orr, A., Vargas, L., Jensen, M., Wang, Y., Melov, S., Turk, C., Matzen, J., Dardov, V., Petrasii, M., Meeusen, S., Perevoschikova, I., Jasper, H., Brookes, P., & Ainscow, E. (2016). Suppressors of superoxide-H2O2 production at site I_Q of mitochondrial complex I protect against stem cell hyperplasia and ischemia-reperfusion injury. Cell Metabolism, 24, 582–592.
Brunelle, J. K., Bell, E. L., Quesada, N. M., Vercauteren, K., Tiranti, V., Zeviani, M., Scarpulla, R. C., & Chandel, N. S. (2005). Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. Cell Metabolism, 1, 409–414.
Doig, C. J., Sutherland, L. R., Sandham, J. D., Fick, G. H., Verhoef, M., & Meddings, J. B. (1998). Increased intestinal permeability is associated with the development of multiple organ dysfunction syndrome in critically ill ICU patients. American Journal of Respiratory and Critical Care Medicine, 158, 444–451.
Fang, J., Wong, H.-S., & Brand, M. D. (2020). Production of superoxide and hydrogen peroxide in the mitochondrial matrix is dominated by site IQ of complex I in diverse cell lines. Redox Biology, 37, 101722.
Farhadi, A., Banan, A., Fields, J., & Keshavarzian, A. (2003). Intestinal barrier: An interface between health and disease. Journal of Gastroenterology and Hepatology, 18, 479–497.

Fasano, A., & Shea-Donohue, T. (2005). Mechanisms of disease: The role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. Nature Clinical Practice. Gastroenterology & Hepatology, 2, 416–422.

Feillet-Coudray, C., Fourtet, G., Ebabe Elle, R., Rieusset, J., Bonafos, B., Chabi, B., Crouzier, D., Zarkovic, K., Zarkovic, N., Ramos, J., Badia, E., Murphy, M. P., Cristol, J. P., & Coudray, C. (2014). The mitochondrial-targeted antioxidant MitoQ ameliorates metabolic syndrome features in obeseogenic diet-fed rats better than Apocynin or Allopurinol. Free Radical Research, 48, 1232–1246.

Fink, M. P., & Delude, R. L. (2005). Epithelial barrier dysfunction: A unifying theme to explain the pathogenesis of multiple organ dysfunction at the cellular level. Critical Care Clinics, 21, 177–196.

Gelino, S., Chang, J. T., Kumsta, C., She, X., Davis, A., Nguyen, C., Panowski, S., & Hansen, M. (2016). Intestinal autophagy improves healthspan and longevity in C. elegans during dietary restriction. PLoS Genetics, 12, e1006135.

Gulhane, M., Murray, L., Lourie, R., Tong, H., Sheng, Y. H., Wang, R., Kang, A., Schreiber, V., Wong, K. Y., Magor, G., Denman, S., Begun, J., Florin, T. H., Perkins, A., Cuiv, P. Ō., McGuckin, M. A., & Hasnain, S. Z. (2016). High-fat diets induce colonic epithelial cell stress and inflammation that is reversed by IL-22. Science Translational Medicine, 8, 347ra132.

Hale, L. P. & Greer, P. K. (2012). A novel murine model of inflammatory bowel disease and inflammation-associated colon cancer with ulcerative colitis-like features. PLoS One, 7, e41797. https://doi.org/10.1371/journal.pone.0041797.

Harris, C. E., Griffiths, R. D., Freestone, N., Billington, D., Atherton, S. T., & Macmillan, R. R. (1992). Intestinal permeability in the critically ill. Intensive Care Medicine, 18, 38–41. https://doi.org/10.1007/BF01764242.

König, J., Wells, J., Cani, P. D., García-Ródenas, C. L., MacDonald, T., Mercenier, A., Whyte, J., Troost, F., & Brummer, R. J. (2016). Human intestinal barrier function in health and disease. Clinical and Translational Gastroenterology, 7, e196.

Laye, M. J., Tran, V., Jones, D. P., Kapahi, P., & Promislow, D. E. L. (2015). The effects of age and dietary restriction on the tissue-specific metabolome of Drosophila. Aging Cell, 14, 797–808.

Lee, H.-Y., Lee, J. S., Alves, T., Ladiges, W., Rabinovitch, P. S., Jurczak, M. J., Choi, C. S., Shulman, G. I., & Samuel, V. T. (2017). Mitochondrial-targeted catalase protects against high-fat diet-induced muscular insulin resistance by decreasing intramuscular lipid accumulation. Diabetes, 66, 2072–2081. https://doi.org/10.2337/db16-1334.

Liang, J., Balachandra, S., Ngo, S., & O’Brien, L. E. (2017). Feedback regulation of steady-state epithelial turnover and organ size. Nature, 548, 588–591. https://doi.org/10.1038/nature23678.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. Clinical Interventions in Aging, 13, 757–772.

Lucchetta, E. M., & Ohsitain, B. (2012). The Drosophila midgut: A model for stem cell driven tissue regeneration. Wiley Interdisciplinary Reviews-Developmental Biology, 1, 781–788.

Manresa, M. C., & Taylor, C. T. (2017). Hypoxia inducible factor (HIF) hydroxylases as regulators of intestinal epithelial barrier function. Cellular and Molecular Gastroenterology and Hepatology, 3, 303–315.

Murakami, Y., Tanabe, S., & Suzuki, T. (2016). High-fat diet-induced intestinal hyperpermeability is associated with increased bile acids in the large intestine of mice. Journal of Food Science, 81, H216–H222. https://doi.org/10.1111/1750-3841.13166.

Murphy, M. P., Holmgren, A., Larsson, N. G., Halliwell, B., Chang, C. J., Kalyanaraman, B., Rhee, S. G., Thornalley, P. J., Partridge, L., Gems, D., Nystroém, T., Belousov, V., Schumaker, P. T., & Winterbourn, C. C. (2011). Unraveling the biological roles of reactive oxygen species. Cell Metabolism, 13, 361–366.

Odenwald, M. A., & Turner, J. R. (2017). The intestinal epithelial barrier: A therapeutic target? Nature Reviews Gastroenterology & Hepatology, 14, 9–21.

Olive, B., & Spradling, A. (2006). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature, 439, 470–474. https://doi.org/10.1038/nature04333.

Orr, A. L., Vargas, L., Turk, C. N., Baaten, J. E., Matzen, J. T., Dardov, V. J., Attle, S. J., Li, J., Quackenbush, D. C., Goncalves, R. L. S., Perevoschikova, I. V., Petrassi, H. M., Meeusen, S. L., Ainscow, E. K., & Brand, M. D. (2015). Suppressors of superoxide production from mitochondrial complex III. Nature Chemical Biology, 11(11), 834–846. https://doi.org/10.1038/nchembio.1910.

Pagialunga, S., Ludzki, A., Root-McCaig, J., & Holloway, G. P. (2015). In adipose tissue, increased mitochondrial emission of reactive oxygen species is important for short-term high-fat diet-induced insulin resistance in mice. Diabetologia, 58, 1071–1080. https://doi.org/10.1007/s00125-015-3531-x.

Patel, C., Ghanim, H., Ravishankar, S., Sia, C. L., Viswanathan, P., Mohanty, P., & Dandona, P. (2007). Prolonged reactive oxygen species generation and nuclear factor-κB activation after a high-fat, high-carbohydrate meal in the obese. Journal of Clinical Endocrinology and Metabolism, 92, 4476–4479.

Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. Nature Reviews Immunology, 14, 141–153.

Regan, J. C., Khericha, M., Dobson, A. J., Bolukbasi, E., Rattanavirotkul, N., & Partridge, L. (2016). Sex difference in pathology of the aging gut mediates the greater response of female lifespan to dietary restriction. Elife, 5, e10956. https://doi.org/10.7554/elife.10956.

Rera, M., Clark, R. I., & Walker, D. W. (2012). Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. Proceedings of the National Academy of Sciences of the United States of America, 109, 21528–21533.

St-Pierre, J., Buckingham, J. A., Roebuck, S. J., & Brand, M. D. (2002). Topology of superoxide production from different sites in the mitochondrial electron transport chain. Journal of Biological Chemistry, 277, 44784–44790.

Thaiss, C. A., Levy, M., Grosheva, I., Zheng, D., Soffer, E., Blacher, E., Braverman, S., Tengeler, A. C., Barak, O., Elazar, M., Ben-Zeev, R., Lehavi-Regev, D., Katz, M. N., Pevsner-Fischer, M., Gertler, A., Halpern, Z., Harmelin, A., Aamar, S., Serradas, P., & Elinav, E. (2018). Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. Science, 359, 1376–1383. https://doi.org/10.1126/science.aar3318.

Tian, T., Wang, Z., & Zhang, J. (2017). Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. Oxidative Medicine and Cellular Longevity, 2017, 18. https://doi.org/10.1155/2017/4535194.

Vighi, G., Marcucci, F., Sensi, L., Di Cara, G., & Frati, F. (2008). Allergy and the gastrointestinal system. Clinical and Experimental Immunology, 153(Suppl 1), 3–6.

Wang, A., Keita, Å. V., Phan, V., McKay, C. M., Schoutz, I., Lee, J., Murphy, M. P., Fernando, M., Ronaghan, N., Balce, D., Yates, R., Dicay, M., Beck, P. L., MacNaughton, W. K., Söderholm, J. D., & McKay, D. M. (2014). Targeting mitochondria-derived reactive oxygen species to reduce epithelial barrier dysfunction and colitis. American Journal of Pathology, 184, 2516–2527.
SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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