Flavin-containing monooxygenases (FMOs) are primarily studied as xenobiotic metabolizing enzymes with a prominent role in drug metabolism. In contrast, endogenous functions and substrates of FMOs are less well understood. A growing body of recent evidence, however, implicates FMOs in aging, several diseases, and metabolic pathways. The evidence suggests an important role for these well-conserved proteins in multiple processes and raises questions about the endogenous substrate(s) and regulation of FMOs. Here, we present an overview of evidence for FMOs' involvement in aging and disease, discussing the biological context and arguing for increased investigation into the function of these enzymes.

Introduction to FMOs

Evolution and classification

Flavin-containing monooxygenases (FMOs) are ancient and widely conserved enzymes, being present in all kingdoms of life (1, 2). FMOs make up a subgroup of the Group B flavin-dependent monooxygenases (EC 1.14.13.8) along with Baeyer–Villiger monooxygenases (BVMOs) and N-hydroxylating monooxygenases (NMOs) (3). FMOs, BVMOs, and NMOs are each distinguished by variations in primary structural motifs, substrate preferences, and catalytic mechanisms. FMOs are also notably similar to the Group A enzymes dihydrodipicolinate dehydrogenase (DLD), glutathione reductase (GR), and low-molecular-weight thioredoxin reductase (TRXR) (2, 4). These groups are distinguished from other flavin-dependent monooxygenases (Groups C–H) by their combined use of FAD and NAD(P)H. Any functional interplay between FMOs and Group A enzymes, perhaps as an NAD(P)H–thiol redox buffering system, is largely unexplored (5).

Catalytic cycle

The catalytic cycle of FMO enzymes is fairly well understood. FMOs utilize a tightly bound FAD prosthetic group, NAD(P)H, and molecular oxygen to monooxygenate or otherwise oxidize substrates, producing water and NAD(P)H as by-products (6) (Fig. 1). Uncoupling has been observed in both the presence and the absence of substrate in vitro, resulting in “leakage” of hydrogen peroxide, and less frequently, superoxide (7). FMOs are notable for their “cocked and loaded” mechanism where they bind NAD(P)H and reduce FAD in the absence of substrate, creating an unusually stable C4a-hydroperoxoflavin ready to oxidize any substrate that accesses the active site (6). This mechanism is in contrast to that of cytochrome P450s and the Group A enzymes discussed above, which each require the presence of substrate to begin their catalytic cycles (6). The rate-limiting step of FMOs is thought to be the release of either H2O or NADP+ (7). The significance of FMOs' reaction kinetics, reactive oxygen species leakage, and effects on cellular NAD(P)H is not known (7, 8).

Substrates

FMOs typically monooxygenate the sulfur or nitrogen atoms of small, soft nucleophiles in a charge- and stereo-selective manner, but important exceptions exist. In addition to monooxygenation, FMOs can also catalyze oxidative decarboxylation (9), oxidative demethylation (10), and disulfide bond formation (11). FMOs act on a wide variety of sulfur- and nitrogen-containing compounds, but carbon, phosphorus, selenium, and other elements are also amenable to FMO-mediated oxidation (6). Pig FMO1 has a Km from 0.3 to 10 μM in vitro for several organic selenium-containing xenobiotics (6), and both hFMO1 and hFMO3 are capable of catalyzing the formation of pyruvate from selenocysteine (12), consistent with a role in endogenous selenium metabolism. Mammalian FMO5 does not metabolize typical FMO substrates, and may act more like a BVMO (8, 13).

The depth and width of the channel leading to the active site are thought to restrict overly large substrates’ access (14), but this mechanism is not fully understood because no crystal structure has been solved for a mammalian FMO. The most evolutionarily related proteins with solved crystal structures are from bacterial trimethylamine monooxygenase (15–17) and...
hFMO1–5 show distinct developmental and tissue-specific patterns of expression (27, 28). Briefly, hFMO1 predominates in the fetal liver, whereas hFMO3 and hFMO5 are the major isoforms expressed in the postnatal liver (27, 28). In contrast, mFmo1 and mFmo5 are the major adult mouse liver FMOs, whereas mFmo3 is much more highly expressed in female mice (29). There are conflicting reports regarding conservation in humans of this sex-dependent differential expression, but it is clear that the conserved differences reported in humans are of a smaller magnitude than those in mice (30–32).

Several factors that regulate FMO transcription are known (33–37). Estrogen (29) and insulin (34) activate FMO transcription, whereas testosterone (29) and glucagon (37) are repressors of FMO transcription, but FMO5 is again an exception (38). Several hormone receptors have been placed both upstream and downstream of FMOs (39, 40). Large differences can exist between FMO mRNA abundance, FMO protein levels, and FMO functional activity (39, 41, 42), indicating multiple levels of regulation that require further study.

FMOs and disease

Although FMOs have been causally linked to only one disease, trimethylaminuria, evidence is accumulating that FMOs affect the pathology of multiple major diseases (Table 1). The nature of FMOs’ involvement in these diseases, however, remains largely undefined.

**Trimethylaminuria (TMAU)**

Much of the FMO-related literature is focused on the only disease known to be caused by altered FMO activity, TMAU (43). TMAU, or “fish odor syndrome,” is a disorder in which the volatile compound trimethylamine (TMA) cannot be converted to the soluble trimethylamine–N-oxide (TMAO), leading to excretion of TMA through the skin. TMA is a small metabolite derived from dietary intake or produced by gut bacteria that has a distinctive “fishy” smell. hFMO3 mutations are the main cause of TMAU, but other causes exist including variations in hFMO3 expression and microbiome overproduction of TMA. There is no cure for TMAU, so treatment consists of limiting dietary intake of TMA and its precursors such as choline and carnitine.

Scant epidemiological data exist on whether TMAU alters risk for other diseases. An early study observed that TMAU patients have a high incidence of hypertension (43), and there are at least two reported cases of TMAU co-presenting with neurological disorders unlikely to be related to patients’ social stress (44). These cases are interesting in relation to the other diseases recently connected to FMOs.

**Atherosclerosis and cardiovascular disease (CVD)**

Beginning in 2011, several studies concluded, based on both mouse and human data, that FMO3-dependent production of TMAO increases the risk for atherosclerosis and general CVD (45–47). These studies shed light on the importance of gut microbiota in determining disease risk and demonstrated a clear association between elevated TMAO and CVD. In the proposed model, gut bacteria produce TMA from dietary precursors, hepatic FMO3 converts TMA to TMAO, and TMAO...
MINIREVIEW: FMOs in aging and disease

Table 1
FMOs in human disease and disease models

| Diseases                        | Nature of association                                                                 |
|---------------------------------|---------------------------------------------------------------------------------------|
| Fmo1 Sporadic ALS               | ● 3’-UTR SNPs associated with increased disease risk (62)                            |
| Fmo-2 No data                   | No data                                                                               |
| Fmo3 TMAU                        | ● Fmo3 mutations cause TMAU (43)                                                      |
| Atherosclerosis                 | ● Increased FMO3 activity increases TMAO, in turn increasing atherosclerosis risk (45)|
| Chronic kidney disease          | ● Minor alleles at hFMO3 residue 158 were associated with increased circulating TMAO and faster epidermal growth factor receptor (eGFR) decline (95) |
| Diabetes                        | ● Fmo3 downregulated in rodent models of diabetes                                      |
| Sideroblastic anemia            | ● Single study with small sample size found Fmo3 dysfunction in cases of sideroblastic anemia (67) |
| Hemochromatosis                 | ● Most differentially expressed hepatic transcript in mouse model of hemochromatosis (68) |
| Fmo4 No data                    | No data                                                                               |
| Fmo5 Diabetes                   | ● Hepatic FMO5 expressed at ratio of 0.41 in diabetic:non-diabetic patients (55)       |
| Sporadic ALS                    | ● FMO5 SNP found associated with ALS, especially in female patients (96)               |
| Locus 1q24.3 Neurodegeneration (multiple diseases) | ● Genome-wide association study (GWAS): Fmo polymorphisms affect lentiform nucleus volume, itself associated with multiple neurodegenerative diseases (63) |
| Parkinson’s                     | ● Decreased expression of FMO1 in rotenone model of Parkinson’s in cultured primary midbrain dopaminergic neurons (64) |

increases the risk for atherosclerosis and CVD. Proposed mechanisms for TMAO increasing CVD risk include effects on cholesterol (45), a prolongation of the pressor effects of angiotensin II (48), and platelet hyperreactivity and thrombosis potential (49).

These mechanistic explanations are not fully convincing, however, and contrast with other data more consistent with TMAO production being a protective response to CVD. First, seafood rich in TMA and TMAO is widely thought to lower CVD risk (50). Second, as mentioned, an early study of TMAU patients found a high incidence of hypertension in the TMAU-afflicted group (43). The proposed mechanism was that FMO3 could metabolize tyramine, an endogenous pressor molecule, thereby reducing its pressor effects and lowering blood pressure (43). A follow-up study found that none of three examined FMO3 polymorphisms predispose to hypertension in a sample of several hundred Caucasian patients, but also noted that severe, highly penetrant loss-of-function mutations could “unmask pressor effects of variation in other drug metabolizing enzymes previously buffered by FMO3” (51). At least two more recent studies are consistent with TMAO production having a reactive, protective function in response to CVD pathology (52, 53), with one of these studies asserting that TMAO is, in fact, protective against CVD risk (53). What is clear from these publications is that TMAO is a molecule of great interest due to its diverse functions including osmolyte, chemical chaperone, reactive oxygen species scavenger, and now, potential risk factor (54).

Diabetes and metabolic disorders

FMO expression is altered in human diabetic patients and rodent models of diabetes (55, 56), and recent reports have revealed that FMOs can alter carbohydrate and lipid metabolism (40, 56–60). Two studies using streptozotocin-induced diabetes in rats show altered FMO expression in diabetic states (56). A third study comparing liver biopsy samples from Type 2 diabetes mellitus patients with samples from non-diabetic patients found hFMO5 down-regulated (55). A study examining expression of FMOs in diabetes found a trend that FMO3 is up-regulated and FMO5 is down-regulated in the disease state (61). As in CVD, it is not clear whether these FMO transcriptional changes are causative, protective, or have little effect on the disease process.

Recently, mammalian FMOs have been shown to affect intermediary carbon metabolism. Researchers who first linked FMO3 to atherosclerosis also found that mFMO3 activity correlated with hepatic and/or plasma lipids and glucose levels (40) and that FMO3 inhibition can divert cholesterol away from biliary excretion (57). The former study suggested that mFMO3’s effects were peroxisome proliferator-activated receptor α (PPARα) and KLF15-mediated, whereas the latter study concluded that mFMO3 affected cholesterol balance through TMAO production, but that mFMO3’s effects on lipids and inflammation were mediated by another substrate. Two other reports examining mFmo1 and mFmo5 knock-out mice, respectively, found that both were capable of altering metabolism and energy balance sufficiently to cause gross alterations in body size (58, 59). A recent review discusses these mFMO-related effects on carbohydrate and lipid metabolism and energy balance (60).

Neurodegeneration and neurological disease

There is substantial evidence connecting FMO expression to neurodegenerative diseases including sporadic amyotrophic lateral sclerosis (ALS), Parkinson’s disease (PD), and schizophrenia. hFMO1 expression is consistently decreased in the spinal cord of ALS patients (62), and single nucleotide polymorphisms in the hFMO1 3’-untranslated region occur more frequently in female patients with sporadic ALS (62). Additionally, mFmo1 is up-regulated in a mouse model of ALS, which, although in the opposite direction of human findings, may be explained by different stages of the disease affecting expression differently (62).
The role of FMOs in other neurodegenerative diseases is also intriguing but largely correlative. The FMO gene cluster containing FMO1–4 is associated with lentiform nucleus volume, a physiological marker associated with PD, schizophrenia, and other neurological disorders (63). Additionally, hFMO1 and Parkin are down-regulated in a rotenone model of PD carried out in cell culture (64). The same study showed up-regulation of caspase 3, and that hFMO1 inhibition was sufficient to activate caspase 3, an executor of apoptotic cell death implicated in the loss of dopaminergic neurons in PD (64). These data suggest a possible role for FMO1 in protecting against multiple neurodegenerative disorders.

Iron dyshomeostasis

Studies by a single research group support FMOs acting in iron homeostasis. Building on a known FMO–calreticulin complex (65), a ferrireductase role for FMO in an iron import complex termed “paraferritin” was described (66). The complex was further described as including FMO, calreticulin, DMT1, and other proteins (66). Its suggested roles include serving as an alternative, non-transferrin mechanism for cellular iron uptake and as a means to deliver iron to mitochondrial ferrochelatase for incorporation into heme. While acknowledging that larger studies would be necessary to determine prevalence, this group also suggested diminished FMO activity as a risk factor for sideroblastic anemia based on four cases (67).

Mouse models of hereditary hemochromatosis, a disease characterized by excessive intestinal iron absorption and subsequent iron overload throughout bodily tissues, alter hepatic mFmo transcription. Mutation of the hemochromatosis gene Hfe (high iron Fe) and high dietary iron both cause liver iron loading, but they do so via secondary and primary iron overload, respectively. Hepatic mFmo3 transcription was highly up-regulated by genetic hereditary hemochromatosis, but counter-intuitively, heavily down-regulated in mice fed high dietary iron (68). By magnitude, mFmo3 was the most altered transcript across both conditions. In a separate study, mFmo3 was up-regulated in Hfe-deficient D2 mice, and although the data were not shown, mFmo3 was described not to change in WT mice fed a high-iron diet (69). These studies, in addition to those describing paraferritin, suggest that FMOs act in iron homeostatic pathways in the liver or elsewhere, and that this novel role for FMOs requires further exploration.

FMOs and aging

Published data from the past decade provide increasing evidence that FMOs play an important role during aging. Specific Fmo genes are transcriptionally activated in numerous mouse longevity models including dietary restriction (DR), growth hormone/insulin-like growth factor 1 (GH/IGF1) signaling disruption, and rapamycin treatment (70–73). These are among the most robustly conserved longevity–promoting interventions (74). The correlation between increased mFmo expression and longevity suggests that FMOs could play a causal role in promoting longevity. Further supporting this, Caenorhabditis elegans (nematode worm) fmo-2 is up-regulated by DR and is necessary for lifespan extension from solid DR, a form of DR (75). Nematode FMO-2 is also sufficient to extend lifespan and improve healthspan and stress resistance when ubiquitously overexpressed (75).

FMOs and mammalian aging

Multiple gene expression analyses from mouse models of delayed aging show that mFmo gene expression is often increased in long-lived mice (Table 2). For example, a 2007 meta-analysis of published liver microarray data found that mFmo3 is consistently up-regulated in a variety of long-lived knock-out models and in response to longevity-promoting interventions (73). Similarly, a 2008 study of XME gene expression in liver found mFmo3 and mFmo4 to be up-regulated in long-lived male mice following DR, GH/IGF1 mutation, or rapamycin treatment (71). Of particular interest, an independent study showed that growth hormone receptor mutant mice have increased levels of both mFmo3 and TMAO, further correlating mFmo3 expression and activity with longevity (76).

Dietary restriction and treatment with the drug rapamycin are the two best-documented and most effective interventions for delaying diseases of aging and increasing lifespan in mice (77), and several studies, including those listed above, have observed increased FMO gene expression in animals subjected to both interventions (Table 2). For example, a comparison of mouse DR and gene expression over time found multiple FMOs up-regulated by DR (70), with mFmo3 and mFmo5 among the most significantly up-regulated liver transcripts, along with heart mFmo3. Overall, mFmo1 and mFmo2 were among the most significantly elevated genes when all 17 tested tissues were considered as a group. Interestingly, the same study found that mFmo1 was significantly down-regulated with age in animals fed a normal diet, suggesting that reduced FMO1 expression could be a biomarker of normative aging or even causally involved in the aging process. The most extensive analysis to date of gene expression changes associated with rapamycin treatment in mice found that hepatic mFMO levels are consistently elevated by both DR and rapamycin in both male and female mice (72).

Crowded litter mice represent an alternative model of DR where animals experience nutrient restriction only during the first 3 weeks of life (78). Interestingly, even this early life restriction is sufficient to cause persistent induction of mFmo3 in liver up to 12 months later. This could suggest that epigenetic changes associated with DR, and perhaps other longevity interventions, induce persistent changes in FMO expression that contribute to healthy aging even after the intervention is discontinued.

In contrast to data supporting a role for FMOs in promoting longevity, loss of mFmo5 results in a blood profile of cholesterol, glucose/insulin, and other biomarkers that resembles a more youthful state (59). The authors suggest that this could mean that FMO5 itself promotes metabolic aging. However, because this study did not test the long-term health effects of the metabolic profile, it is unclear whether these results are representative of aging or metabolic reprogramming (59). FMO5 also has a unique substrate profile among mammalian FMOs (8), so it may not be representative of the majority of FMOs. Given their wide taxonomic distribution and numerous evolutionary modifications, there are likely to be exceptions to any broad claims about FMOs as a group.

MINIREVIEW: FMOs in aging and disease
**MINIREVIEW: FMOs in aging and disease**

### Table 2

**FMOs and aging in model systems.**

| Species                  | FMO genes            | Lifespan/healthspan effects                                      | Expression changes in longevity interventions |
|--------------------------|----------------------|-----------------------------------------------------------------|-----------------------------------------------|
| *Mus musculus* (mouse)  | Fmo1                 | No data                                                         | Caloric restriction ↑ (70)                    |
|                          | Fmo2                 | Regulates whole-body cholesterol balance in mice (57)          | Caloric restriction ↑ (70, 71)                |
|                          | Fmo3                 | • Necessary for solid DR longevity (75)                        | Hypoxic response ↑ (79, 80)                  |
|                          |                      | • Necessary for hypoxic response longevity (75)                | Mitochondrial disruption ↑ (81)               |
|                          | Fmo4                 | No data                                                        | Mitochondrial disruption ↑ (81)               |
|                          |                      | • Dietary restriction ↑ (75, 82)                                | Long-telomere worms ↑ (79)                    |
|                          | Fmo5                 | Suggested to be a metabolic regulator of aging (59)            | Caloric restriction ↑ (70)                    |
| *D. melanogaster* (fly) | Fmo1                 | RNAi shortens adult lifespan (86)                              |                                               |
|                          | Fmo2                 | No data                                                        |                                               |
| *C. elegans* (worm)     | fmo-1                | No data                                                        |                                               |
|                          | fmo-2                | • Overexpression sufficient for LS extension (75, 97)          | Dietary restriction ↑ (82, 83)                |
|                          |                      | • Necessity for solid DR longevity (75)                        | Dietary restriction ↑ (75, 82)                |
|                          | fmo-3                | No data                                                        | Hypoxic response ↑ (79, 80)                  |
|                          | fmo-4                | No data                                                        | Micthondrial disruption ↑ (81)                |
|                          | fmo-5                | No data                                                        | Mitochondrial disruption ↑ (81)               |
|                          | C46H11.2             | No data                                                        |                                               |
|                          | C01H6.4              | No data                                                        |                                               |
| *S. cerevisiae* (yeast) | fmo1                 | No data                                                        | Responds to altered sulfur availability (possibly related to methionine restriction) (98, 99) |
| *A. thaliana* (plant)   |                      | OE sufficient for longevity and stress resistance (87)        |                                               |
|                         | YUC6                 | No data                                                        |                                               |

**FMOs and aging in non-mammalian species**

Extensive data linking FMO function to aging have come from studies performed in the nematode *C. elegans*. Several independent studies show that *fmo-2* is up-regulated by both genetic and environmental models of increased lifespan in this organism, including DR, hypoxia, mutation of the Von Hippel Lindau tumor suppressor, stabilization of the hypoxic response transcription factor, and developmental electron transport chain inhibition (79–83). Recently, a direct, causal role for FMO-2 as a longevity-promoting factor was uncovered by work showing that either ubiquitous or intestine-specific overexpression of FMO-2 in otherwise wild-type animals is sufficient to extend lifespan in worms (75). Consistent with this, deletion of *fmo-2* prevented full lifespan extension following activation of the hypoxic response or DR, further supporting a model that activation of *fmo-2* contributes to lifespan extension under these conditions (75).

Additional worm *fmo* genes are up-regulated by these same longevity interventions, but they have not yet been studied for their roles in aging. Of note, *fmo-1* is up-regulated in a more lasting manner than *fmo-2* by fasting (83). Also, *fmo-4* is induced similarly to *fmo-2* by hypoxia (79). Worm *fmo-4* is expressed in the hypodermis, whereas *fmo-2* is expressed in the intestine, pharynx, and excretory cells (84). Worm *fmo-4* also displays a hypoosmotic sensitivity phenotype unique among worm *fmo* genes (85). Taken together, this evidence suggests that further valuable details can be learned about worm FMOs’ roles in healthy aging, stress resistance, and normal physiological processes.

Evidence from flies and plants further suggests that FMOs promote longevity. RNAi knockdown of *Drosophila melanogaster* (fly) Fmo2 shortens adult lifespan (86). As with worm and mammalian FMOs, fly Fmo2 is not directly orthologous to worm *fmo-2* or mammalian FMO2. In fact, both fly Fmo genes are more similar to the ancestral yeast Fmo genes (19). Plants have evolved a distinct group of FMOs termed “YUCCAs.” *Arabidopsis thaliana* YUC6 overexpression in potato plants results in increased height, erect stature, and longevity due to YUC6’s role in auxin production (87). These phenotypes may be plant-specific, or there may be overlap with FMO functions conserved in animal species. YUCCAs also promote drought resistance, and some FMOs are involved in osmoregulation in worms (85) and fish (88), in addition to producing the osmolyte TMAO in humans (43). Interestingly, there is overlap between the DR and osmotic stress pathways (89, 90), and it is plausible that FMOs act at this intersection.

**Concluding remarks**

**FMOs and disease: Unifying mechanisms?**

Our understanding of FMO–disease relationships is nascent, but the data already suggest two common mechanisms. Altered sulfur amino acid (SAA) metabolism affects CVD (92),...
metabolic disease (92), and neurodegenerative disease pathology (93). Again, several SAA pathway metabolites that are credibly FMO substrates remain untested as such. Iron metabolism is a second pathway that links FMOs, SAAs, CVD, metabolic disease, and neurological disease.

**FMOs and aging: Evolutionary considerations**

Data from non-mammalian model systems demonstrate that FMOs can play a direct, causal role in promoting longevity. In worms, fmo-2 is sufficient to extend lifespan and is required for lifespan extension from numerous interventions. FMO induction in response to multiple longevity-enhancing interventions in mice supports a conserved role, as do the primary structural similarities shared by worm and mammalian FMOs.

If FMOs promote longevity in a conserved manner, then the conditions shaping both the evolution of FMOs and aging will merit attention. Induction of FMOs in response to both DR and osmotic stress, for example, suggests that a “harsh times” survival strategy can underlie longevity. Model systems will continue to be of great utility in such investigations.

**Future directions**

FMOs are emerging as enzymes of considerable interest, with clear experimental and theoretical research directions identifiable. Two lines of experimentation will greatly solidify our foundational knowledge of FMOs. First, solving the crystal structures of hFMOs would conclusively answer structural questions and directly inform functional ones. Second, thorough testing for endogenous substrates, aging, disease, and basic cellular function would solidify the conserved endogenous role of these proteins. There are several candidate endogenous substrates of major biological interest that have not been tested in vitro or otherwise. Metabolites in the sulfur amino acid metabolism pathway, including S-adenosyl methionine, homocysteine (91), and homocysteine adducts, are potential targets for prioritization.

Evidence points to FMO involvement in multiple major diseases and the aging process (Fig. 2). There is an ongoing debate whether aging should be reclassified as a disease, but it is clearly established that aging is the biggest risk factor for the major causes of death including CVD, cancer, and neurodegeneration (94). The risk for each of these increases exponentially with age, independent of other risk factors. Taking everything into consideration, FMOs are an exciting, undercharacterized subgroup of well-conserved enzymes that may play central roles in basic biological processes affecting human health, and there are clear first steps to characterizing them more fully.

**References**

1. Mascotti, M. L., Lapadula, W. J., and Juri Ayub, M. (2015) The origin and evolution of Baeyer–Villiger monooxygenases (BVMOs): an ancestral family of flavin monooxygenases. *PLoS ONE* 10, e0132689

2. Mascotti, M. L., Juri Ayub, M., Furnham, N., Thornton, J. M., and Lasowski, R. A. (2016) Chopping and changing: the evolution of the flavin-dependent monooxygenases. *J. Mol. Biol.* 428, 3131–3146

3. Huijbers, M. M. E., Montersino, S., Westphal, A. H., Tischler, D., and Van Berkel, W. J. H. (2014) Flavin-dependent monooxygenases. *Arch. Biochem. Biophys.* 544, 2–17

4. Ojha, S., Meng, E. C., and Babbitt, P. C. (2007) Evolution of function in the “two dinucleotide binding domains” flavoproteins. *PLoS Comput. Biol.* 3, e121

5. Ziegler, D. M., Duffel, M. W., and Poulsen, L. L. (1979) Studies on the nature and regulation of the cellular thioldisulphide potential. *Ciba Found. Symp.* 191–204

6. Krueger, S. K., and Williams, D. E. (2005) Mammalian flavin-containing monooxygenases: structure/function, genetic polymorphisms and role in drug metabolism. *Pharmacol. Ther.* 106, 357–387

7. Siddens, L. K., Krueger, S. K., Henderson, M. C., and Williams, D. E. (2014) Mammalian flavin-containing monooxygenase (FMO) as a source of hydrogen peroxide. *Biochem. Pharmacol.* 89, 141–147

8. Fiorentini, F., Geier, M., Binda, C., Winkler, M., Faber, K., Hall, M., and Mattevi, A. (2016) Biochemical characterization of human FMO5: unearthing Baeyer–Villiger reactions in humans. *ACS Chem. Biol.* 11, 1039–1048

9. Hashiguchi, K., Tanaka, K., Sakai, T., Sugawara, S., Kawaide, H., Natsume, M., Hanada, A., Yano, T., Shirasu, K., Yao, H., McSteen, P., Zhao, Y., Hayashi K., Kamiya, Y., and Kasahara, H. (2011) The main auxin biosynthesis pathway in *Arabidopsis*. *Proc. Natl. Acad. Sci.* 108, 18512–18517

10. Gut, I., and Conney, A. H. (1993) Trimethylamine N-oxidation and N-demethylation in rat liver microsomes. *Biochem. Pharmacol.* 46, 239–244

11. Suh, J. K., Poulsen, L. L., Ziegler, D. M., and Robertus, J. D. (1999) Yeast flavin-containing monooxygenase generates oxidizing equivalents that control protein folding in the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* 96, 2687–2691

12. Rooseboom, M., Commandeur, J. N. M., Floor, G. C., Rettie, A. E., and Vermeiden, N. P. E. (2001) Selenoxidation by flavin-containing monooxygenases as a novel pathway for β-elimination of selenocysteine Se-conjugates. *Chem. Res. Toxicol.* 14, 127–134

13. Neuhoff, C., Gunawan, A., Farrow, M. O., Cinar, M. U., Große-Brinkhaus, C., Sahadevan, S., Frieden, L., Tesfaye, D., Tholen, E., Looft, C., Schelander, K., and Uddin, M. J. (2015) Preliminary study of FMO1, FMO5, CYP21, ESR1, PLIN2 and SULT2A1 as candidate gene for compounds related to boar taint. *Meat Sci.* 108, 67–73

14. Ziegler, D. M. (2002) An overview of the mechanism, substrate specificities, and structure of FMOs. *Drug Metab. Rev.* 34, 503–511

15. Alfieri, A., Malito, E., Orru, R., Fraiaje, M. W., and Mattevi, A. (2008) Revealing the moonlighting role of NADP in the structure of a flavin-containing monooxygenase. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6572–6577

16. Cho, H. J., Cho, H. Y., Kim, K. J., Kim, M. H., Kim, S. W., and Kang, B. S. (2011) Structural and functional analysis of bacterial flavin-containing monooxygenase reveals its ping-pong-type reaction mechanism. *J. Struct. Biol.* 175, 39–48

17. Li, C. Y., Chen, X. L., Zhang, D., Wang, P., Sheng, Q., Peng, M., Xie, B. B., Qin, Q. L., Li, P. Y., Zhang, X. Y., Su, H. N., Song, X. Y., Shi, M., Zhou, B. C.,...
MINIREVIEW: FMOs in aging and disease

Xun, L. Y., et al. (2017) Structural mechanism for bacterial oxidation of oceanic trimethylamine into trimethylamine N-oxide. *Mol. Microbiol.* **103**, 992–1003

18. Esawaramoothy, S., Bonanno, J. B., Burley, S. K., and Swamianathan, S. (2006) Mechanism of action of a flavin-containing monooxygenase. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 9832–9837

19. UniProt Consortium (2015) UniProt: A hub for protein information. *Nucleic Acids Res.* **43**, D204–D212

20. Suh, J. K., Poulsen, L. L., Ziegler, D. M., and Robertus, J. D. (1996) Molecular cloning and kinetic characterization of a flavin-containing monooxygenase from *Saccharomyces cerevisiae*. *Arch. Biochem. Biophys.* **336**, 268–274

21. Henderson, M. C., Krueger, S. K., Stevens, J. F., and Williams, D. E. (2002) Alternative processing of the human FMO6 gene renders transcripts incapable of encoding a functional flavin-containing monooxygenase. *Mol. Pharmacol.* **62**, 320–325

22. Tattersall, V., Zhang, J., and Cashman, J. R. (2004) Alternative processing events in human FMO genes. *Mol. Pharmacol.* **65**, 1517–1525

23. Hao, D. C., Chen, S. L., Mu, J., and Xiao, P. G. (2009) Molecular phylogeny, long-term evolution, and functional divergence of flavin-containing monooxygenases. *Genetica* **137**, 173–187

24. Koukouritaki, S. B., Simpson, P., Yeung, C. K., Rettie, A. E., and Hines, R. N. (2001) Regulation of flavin-containing monooxygenase mRNAs in mouse liver. *Drug Metab. Dispos.* **29**, 17–26

25. Zhang, J., Chaluvadi, M. R., Reddy, R., Motika, M. S., Richardson, T. A., Cashman, J. R., and Morgan, E. T. (2009) Hepatic flavin-containing monooxygenase gene regulation in different mouse inflammation models. *Drug Metab. Dispos.* **37**, 462–468

26. Zhang, J., Jung, A. V., Manthana, P. V., Gearing, M. E., Graham, M. I., Crooke, R. M., Croce, K. J., Esquejo, R. M., Clish, C. B., Morbid Obesity Study Group, Vicent, D., and Biddinger, S. B. (2015) Flavin-containing monooxygenase 3 as a potential player in diabetes-associated atherosclerosis. *Nat. Commun.* **6**, 6498

27. Celli, T., Roblin, S., Harper, P. A., Matthews, J., Boutros, P. C., Pohjanvirta, R., and Okey, A. B. (2008) Aryl hydrocarbon receptor-dependent induction of flavin-containing monooxygenase mRNAs in mouse liver. *Drug Metab. Dispos.* **36**, 2499–2505

28. Celius, T., Parsyan, A., Matthews, J., Okey, A. B., Henderson, M. C., Krueger, S. K., and Williams, D. E. (2010) Flavin-containing monooxygenase-3: induction by 3-methylcholanthrene and complex regulation by xenobiotic chemicals in hepatoma cells and mouse liver. *Toxicol. Appl. Pharmacol.* **247**, 60–69

29. Shih, D. M., Wang, Z., Lee, R., Meng, Y., Che, N., Charugundla, S., Qi, H., Wu, J., Pan, C., Brown, J. M., Vallim, T., Bennett, B. J., Graham, M., Hazen, S. L., and Luis, A. J. (2015) Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. *J. Lipid Res.* **56**, 22–37

30. Hines, R. N., Hopp, K. A., Franco, J., Saeian, K., and Begun, F. P. (2002) Induction of flavin-containing monooxygenase regulatory elements in human liver. *Hum. Mol. Genet.* **11**, 1421–1430

31. Sadeque, A. J., Thummel, K. E., and Rettie, A. E. (1993) Purification of human liver flavin-containing monooxygenase and pseudogene clusters. *Pharmacogenetics* **14**, 117–130

32. Phillips, I. R., Palm, C., Hadley, M. R., Hutt, A. I., McCombie, J. R. R., Smith, R. L., and Shephard, E. A. (1995) The molecular biology of the flavin-containing monooxygenases of man. *Chem. Biol. Interact.* **96**, 17–32

33. Luo, Z., and Hines, R. N. (2001) Regulation of flavin-containing monooxygenase and cytochrome P450 activities in experimental diabetes. *Drug Metab. Dispos.* **29**, 11138–11146

34. Tomova, L., Konopelski, P., and Ufnal, M. (2016) Gut bacteria and host factors influence FMO3 expression: sulfenic acid formation from thioureas and oxidation of glutathione. *Chem. Res. Toxicol.* **17**, 653–660

35. Klick, D. E., Shadley, J. D., and Hines, R. N. (2008) Differential regulation of the flavin-containing monooxygenases of man. *Biochem. Pharmacol.* **76**, 524–531

36. Bennett, B. J., de Aguiar Vallim, T. Q., Wang, Z., Shih, D. M., Meng, Y., Che, N., Charugundla, S., Qi, H., Wu, J., Pan, C., Brown, J. M., Vallim, T., Bennett, B. J., Graham, M., Hazen, S. L., and Luis, A. J. (2015) Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. *J. Lipid Res.* **56**, 22–37

37. Celius, T., Parsyan, A., Matthews, J., Okey, A. B., Henderson, M. C., Krueger, S. K., and Williams, D. E. (2010) Flavin-containing monooxygenase-3: induction by 3-methylcholanthrene and complex regulation by xenobiotic chemicals in hepatoma cells and mouse liver. *Toxicol. Appl. Pharmacol.* **247**, 60–69

38. Novick, R. M., Vezina, C. M., and Elfarra, A. A. (2010) Isoform distinct time-, dose-, and castration-dependent alterations in flavin-containing monooxygenase expression in mouse liver after 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment. *Biochem. Pharmacol.* **79**, 1345–1351

39. Treacy, E. P., Akerman, B. R., Chow, L. M. L., Youl, R., Bibeau, C., Lin, J., Bruce, A. G. Knight, M., Danks, D. M., Cashman, J. R., and Forrest, S. M. (1998) Mutations of the flavin-containing monooxygenase gene (FMO3) cause trimethylaminuria, a defect in detoxication. *Hum. Mol. Genet.* **7**, 839–845

40. McConnell, H. W., Mitchell, S. C., Smith, R. L., and Brewster, M. (1997) Trimethylaminuria associated with seizures and behavioural disturbance: a case report. *Seizure* **6**, 317–321

41. Wang, Z., Klipfell, E., Bennett, B. J., Koeh, R., Levison, B. S., Dugar, B., Feldstein, A. E., Britt, E. B., Fu, X., Chung, Y.-M., Wu, Y., Schauer, P., Smith, J. D., Allayee, H., Tang, W. H. W., et al. (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57–63

42. Tang, W. H. W., Wang, Z., Levison, B. S., Koeh R. A., Britt, E. B., Fu, X., Wu, Y., and Hazen, S. L. (2013) Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N. Engl. J. Med.* **368**, 1575–1584

43. Koeh R. A., Wang, Z., Levison, B. S., Buffa J. A., Org., E., Sheehy, B. T., Britt, E. B., Fu, X., Wu, Y., Lee, L., Smith, J. D., DiDonato J. A., Chen, J., Li, H., Wu, G. D., et al. (2013) Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature Med.* **19**, 576–585

44. Ufnal, M., Jazwicz, R., Dadlez, M., Drapala, A., Sikora, M., and Skrzypecki, J. (2014) Trimethylamine–N-oxide: a carnitine-derived metabolite that prolongs the hypertensive effect of angiotensin II in rats. *Can. J. Cardiol.* **30**, 1700–1705

45. Zhu, W., Gregory, J. C., Org., E., Buffa J. A., Gupta, N., Wang, Z., Li, L., Fu, X., Wu, Y., Mehrabian, M., Sartor, R. B., McIntyre, T. M., Silverstein, L. R., Tang, W. H., DiDonato, J. A., et al. (2016) Gut microbial metabolite TMAO enhances platelet article gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell* **165**, 111–124

46. Tomosa, L., Konopelski, P., and Ufnal, M. (2016) Gut bacteria and hydrogen sulfide: the new old players in circulatory system homeostasis. *Molecules* **21**, E1558

47. Dolan, C., Shields, D. C., Stanton, A., O’Brien, E., Lambert, D. M., O’Brien, J. K., and Treacy, E. P. (2005) Polymorphisms of the flavin containing monooxygenase 3 (FMO3) gene do not predispose to essential hypertension in Caucasians. *BMC Med. Genet.* **6**, 41
duces markers of vascular injury in hemodialysis patients. *J. Cardiovasc. Pharmacol.* **56**, 289–295

53. Collins, H. L., Drazul-Schrader, D., Sulphioz, A. C., Koster, P. D., Williams-Young, S., Adelman, S. J., Owen, K., Sanli, T., and Bellamine, A. (2016) \( {\delta } \)-Carotene intake and high trimethylamine \( {\mathbf{N}} \)-oxide plasma levels correlate with low aortic lesions in ApoE\( ^{-/-} \) transgenic mice expressing CETP. *Atherosclerosis* **244**, 29–37

54. Ufnal, M., Zadlo, A., and Ostaszewski, R. (2015) TMAO: a small molecule with great expectations. *Nutrition* **31**, 1317–1323

55. Takamura, T., Sakurai, M., Ota, T., Ando, H., Honda, M., and Kaneko, S. (2004) Genes for systemic vascular complications are differentially expressed in the livers of Type 2 diabetic patients. *Diabetologia* **47**, 638–647

56. Vahabzadeh, M., and Mohammadmour, A.-H. (2015) Effect of diabetes mellitus on the metabolism of drugs and toxins. *J. Clin. Toxicol.* **5**, 233

57. Warren, M., Shih, D. M., Burrows, A. C., Ferguson, D., Gromovsky, A. D., Brown, A., Marshall, S., McDaniel, A., Schugar, C. R., Wang, Z., Sacks, I., Rong, X., Vullim, T., Chou, J., Iovanov, P. T., et al. (2015) The TMAO-generating enzyme flavin monoxygenase 3 is a central regulator of cholesterol balance. *Cell Rep.* **10**, 326–338

58. Veeravalli, S., Omar B. A., Houseman, L., Hancock, M., Gonzalez Malagon, S. G., Scott, F., Jamnomed, A., Phillips, I. R., and Shephard, E. A. (2014) The phenotype of a flavin-containing monoxygenase knock-out mouse implicates the drug-metabolizing enzyme FMO1 as a novel regulator of energy balance. *Biochem. Pharmacol.* **90**, 88–95

59. Gonzalez Malagon, S. G., Melidoni, A. N., Hernandez, D., Omar B. A., Houseman, L., Veeravalli, S., Scott, F., Varshavi, D., Everett, J., Tsuchiya, Y., Timms, J. F., Phillips, I. R., and Shephard, E. A. (2015) The phenotype of a knockout mouse identifies flavin-containing monoxygenase 5 (FMO5) as a regulator of metabolic ageing. *Biochem. Pharmacol.* **96**, 267–277

60. Petriello, M. C., Hoffman, J. B., Morris, A. J., and Hennig, B. (2017) Emerging roles of xenobiotic detoxification enzymes in metabolic diseases. *Rev. Environ. Health*. **32**, 105–110

61. Motika, M. S., Zhang, J., and Cashman, J. R. (2007) Flavin-containing monoxygenase 3 and human disease. *Expert Opin. Drug Metab. Toxicol.* **3**, 831–845

62. Bozzone, V., Pansarasa, O., Dimanti, L., Nosari, G., Cereda, C., and Ceroni, M. (2016) Amyotrophic lateral sclerosis and environmental factors. *Funct. Neurol.* **31**, 7–19

63. Hibar, D. P., Stein, J. L., Ryles, A. B., Kohannim, O., Jahanshad, N., Medline, G. W., Martin, N. G., Wright, M. J., Saykin, A. J., Jack, C. R., Jr., Weiner, M. W., et al. (2013) Genome-wide association identifies genes variants associated with lentiform nucleus volume in AD patients. *Brain Imaging Behav.* **7**, 1345–1356

64. Miller, R. A., Harrison, D. E., Aistle, C. M., Fernandez, E., Flurkey, K., Han, M., Javors, M. A., Li, X., Nadon, N. L., Nelson, J. F., Fletcher, S., Salmon, A. B., Sharp, Z. D., Van Roekel, S., Winkelman, L., and Strong, R. (2014) Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell* **13**, 468–477

65. Swindell, W. R. (2007) Gene expression profiling of long-lived dwarf mice: longevity-associated genes and relationships with diet, gender and aging. *BMC Genomics* **8**, 353

66. Pitt, J. N., and Kaebelerlein, M. (2015) Why is aging conserved and what can we do about it? *PLOS Biol.* **13**, e1002131; Correction (2015) *PLOS Biol.* **13**, e1002176

67. Leiser, S. F., Miller, H., Rossner, R., Fletcher, M., Leonard, A., Primitivo, M., Rintala, N., Ramos, F. J., Miller, D. L., and Kaebelerlein, M. (2015) Cell nonautonomous activation of flavin-containing monoxygenase promotes longevity and health span. *Science* **350**, 1375–1378

68. Schirra, H. J., Anderson, C. G., Wilson, W. J., Kerr, L., Craik, D. J., Waters, M. L., and Lichanska, A. M. (2008) Altered metabolism of growth hormone receptor mutant mice: a combined NMR metabolomics and microarray study. *PLOS ONE* **3**, e2764

69. Kaebelerlein, M., Rabinovitch, P. S., and Martin, G. M. (2015) Healthy aging: the ultimate preventative medicine. *Science* **350**, 1191–1193

70. Sun, L., Sadigah Akha, A. A., Miller, R. A., and Harper, J. M. (2009) Lifespan extension in mice by preweaning food restriction and by methionine restriction in middle age. *J. Gerontol. A. Biol. Sci. Med. Sci.* **64**, 711–722

71. Shen, C., Nettleton, D., Jiang, M., Kim, S. K., and Powell-Coffman, J. A. (2005) Roles of the HIF-1 hypoxia-inducible factor during hypoxia response in *Caenorhabditis elegans*. *J. Biol. Chem.* **280**, 20580–20588

72. Bishop, T., Lau, K. W., Epstein, A. C. R., Kim, S. K., Jiang, M., O’Rourke, D., Pugh, C. W., Gleadie, J. M., Taylor, M. S., Hodgkin, J., and Ratcliffe, P. J. (2004) Genetic analysis of pathways regulated by the von Hippel-Lindau tumor suppressor in *Caenorhabditis elegans*. *PLOS Biol.* **2**, e289

73. Lee, S.-J., Hwang, A. B., and Kenyon, C. (2010) Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity. *Curr. Biol.* **20**, 2131–2136

74. uno, M., Honjoh, S., Matsuda, M., Hoshikawa, H., Kishimoto, S., Yamamoto, T., Ebisuya, M., Yamamoto, T., Matsumoto, K., and Nishida, E. (2013) A fasting-responsive signaling pathway that extends life span in *C. elegans*. *Cell Rep.* **3**, 79–91

75. Petalcorin, M. I. R., Joshua, G. W., Agapow, P. M., and Dolfin, C. T. (2005) The *fmo* genes of *Caenorhabditis elegans* and *C. briggsae*: characterization, gene expression and comparative genomic analysis. *Gene* **346**, 83–96

76. Hirani, N., Westenberg, M., Seed, P. T., Petalcorin, M. I., and Dolfin, C. T. (2016) *C. elegans* flavin-containing monoxygenase-4 is essential for oospermulation in hypotonic stress. *Biochim. Biophys. Acta* **1758**, 537–549

77. Attrill, H., Falls, K., Goodman, J. L., Millburn, G. H., Antonazzo, G., Rey, A. J., and Marygold, S. J., FlyBase Consortium (2016) FlyBase: establishing a Gene Group resource for *Drosophila melanogaster*. *Nucleic Acids Res.* **44**, D786–D792

78. Cheol Park, H., Cha, J.-Y., and Yun, D.-J. (2013) Roles of YUCCAs in auxin biosynthesis and drought stress responses in plants. *Plant Signal. Behav.* **8**, e24995

79. Shenkel, D. (1993) A comparison of endogenous and exogenous substrates of the flavin-containing monoxygenases in aquatic organisms. *Aquat. Toxicol.* **26**, 157–162

80. Matzkin, L. M., and Markow, T. A. (2009) Transcriptional regulation of metabolism associated with the increased desiccation resistance of the cactophilic *Drosophila mojavensis*. *Genetics* **182**, 1279–1288

81. Chandler-Brown, D., Choi, H., Park, S., Ocampo, B. R., Chen, S., Le, A., Sutphin, G. L., Shamie, L. S., Smith, E. D., and Kaebelerlein, M. (2015)
Sorbitol treatment extends lifespan and induces the osmotic stress re-
response in *Caenorhabditis elegans*. Front. Genet. 6, 316
91. McCully, K. S. (2015) Homocysteine metabolism, atherosclerosis, and dis-
eases of aging. Compr. Physiol. 6, 471–505
92. Poloni, S., Blom, H. J., and Schwartz, I. V. (2015) Stearoyl-CoA desatu-
rase-1: Is it the link between sulfur amino acids and lipid metabolism? Biology (Basel) 4, 383–396
93. Gibrat, C., and Cicchetti, F. (2011) Potential of cystamine and cysteamine in the treatment of neurodegenerative diseases. Prog Neuropsychophar-
macol. Biol. Psychiatry 35, 380–389
94. Kaeberlein, M. (2013) Longevity and aging. F1000Prime Rep. 5, 5
95. Robinson-Cohen, C., Newitt, R., Shen, D. D., Rettie, A. E., Kestenbaum, B. R., Himmelfarb, J., and Yeung, C. K. (2016) Association of FMO3 vari-
ants and trimethylamine N-oxide concentration, disease progression, and mortality in CKD patients. PLoS ONE 11, e0161074
96. Gagliardi, S., Gallo, A., Policicchio, S., La Salvia, S., Diamanti, L., Bernuzzi, S., Pansarasa, O., and Cereda, C. (2016) Environmental and genetic factors in ALS: positive correlation of Snps in flavin-containing monooxygenase 5 gene. Ann. Neurodegener. Disord. 1, 1014
97. Park, M. C., Park, D., Lee, E. K., Park, T., and Lee, J. (2010) Genomic analysis of the telomeric length effect on organismic lifespan in *Caenorh-
abditis elegans*. Biochem. Biophys. Res. Commun. 396, 382–387
98. Brauer, M. J., Huttenhower, C., Airoldi, E. M., Rosenstein, R., Matses, J. C., Gresham, D., Boer, V. M., Troyanskaya, O. G., and Botstein, D. (2008) Coordination of growth rate, cell cycle, stress response, and metabolic activity in yeast. Mol. Biol. Cell 19, 352–367
99. Kumar, A., John, L., Maity, S., Manchanda, M., Sharma, A., Saini, N., Chakraborty, K., and Sengupta, S. (2011) Converging evidence of mito-
chondrial dysfunction in a yeast model of homocysteine metabolism im-
balance. J. Biol. Chem. 286, 21779–21795