Nuclear hormone receptors: Roles of xenobiotic detoxification and sterol homeostasis in healthy aging

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Abstract

Health during aging can be improved by genetic, dietary and pharmacological interventions. Many of these increase resistance to various stressors, including xenobiotics. Up-regulation of xenobiotic detoxification genes is a transcriptomic signature shared by long-lived nematodes, flies and mice, suggesting that protection of cells from toxicity of xenobiotics may contribute to longevity. Expression of genes involved in xenobiotic detoxification is controlled by evolutionarily conserved transcriptional regulators. Three closely related subgroups of nuclear hormone receptors (NHRs) have a major role, and these include DAF-12 and NHR-8 in C. elegans, DHR96 in Drosophila and FXR, LXR, PXR, CAR and VDR in mammals. In the invertebrates, these NHRs have been experimentally demonstrated to play a role in extension of lifespan by genetic and environmental interventions. NHRs represent critical hubs in that they regulate detoxification enzymes with broad substrate specificities, metabolizing both endo- and xeno-biotics. They also modulate homeostasis of steroid hormones and other endogenous cholesterol derivatives and lipid metabolism, and these roles, as well as xenobiotic detoxification, may contribute to the effects of NHRs on lifespan and health during aging, an issue that is being increasingly addressed in C. elegans and Drosophila. Disentangling the contribution of these processes to longevity will require more precise understanding of the molecular mechanisms by which each is effected, including identification of ligands and co-regulators of NHRs, patterns of tissue-specificity and mechanisms of interaction between tissues. The roles of vertebrate NHRs in determination of health during aging and lifespan have yet to be investigated.

Introduction

The aging process has proved to be malleable to genetic, dietary and pharmacological interventions (Fontana & Partridge, 2015; Fontana et al., 2010; Kenyon, 2010; Lamming et al., 2013). Furthermore, at least some of its mechanisms are conserved during evolution, because similar interventions, such as dietary restriction (DR), have proved capable of improving health during aging in diverse model and non-model organisms including primates (Madeo et al., 2014). However, these interventions can also have undesirable side-effects, such as impaired fecundity, immunity and wound healing (Lamming et al., 2013; Martin et al., 2008). There is therefore much interest in understanding the exact mechanisms by which different interventions improve health during aging, and in the possibility of triaging the health benefits from the side-effects.

One well-established intervention to extend lifespan, in the nematode worm Caenorhabditis elegans, the fruit fly Drosophila melanogaster, mice and, possibly, humans, is reduction of insulin/insulin-like growth factor (Igf) signaling (IIS) (Fontana et al., 2010). The IIS pathway is conserved among metazoans, and it can modulate metabolism, development, growth, body size, fecundity and resistance to different stressors including oxidative and xenobiotic stress (Broughton & Partridge, 2009). It is therefore important to establish whether any, or all, of the many pleiotropic traits associated with reduced IIS are causal in increased lifespan and health during aging.

Binding of insulin-like peptides to the insulin/Igf receptor induces a signaling cascade leading to the phosphorylation of forkhead Box O (FOXO) transcription factors by the protein kinase AKT and sequestration of FOXO in the cytoplasm. With reduced activity of the upstream pathway, FOXO translocates to the nucleus and regulates gene expression (Partridge & Bruning, 2008; Salih & Brunet, 2008; van der Horst & Burgering, 2007). In C. elegans, all phenotypes associated with reduced IIS, including xenobiotic resistance, require the gene daf-16, which encodes the single worm FOXO and, from this evidence, all of the pleiotropic traits could hence be relevant to the extension of lifespan (Honda & Honda, 1999; Riedel et al., 2013; Tissenbaum & Ruvkun, 1998). In contrast, in Drosophila, dfoxo, the daf-16 ortholog, is not required for the reduction in body size and fecundity, developmental delay or the increased oxidative.
stress resistance from reduced IIS. Only increased resistance to xenobiotics and extension of lifespan are identified FOXO-dependent effects of reduced IIS (Slack et al., 2011). It is thus possible that the increased xenobiotic resistance of insulin mutant flies is causal in their increased lifespan, while the other traits associated with reduced IIS are irrelevant.

In accordance with their increased xenobiotic resistance, the RNA transcriptomic signature of long-lived IIS mutant worms, flies and mice is enriched for xenobiotic detoxification genes (McElwee et al., 2007). Furthermore, various genetic, pharmacological and dietary interventions that promote longevity in mice also increase expression of detoxification genes. Little mice, harboring a growth hormone releasing hormone receptor knockout, and the Ames dwarf, lacking pituitary cells producing growth hormone, prolactin and thyroid stimulating hormone, have a transcriptomic signature of elevated xenobiotic detoxification genes (Amador-Noguez et al., 2004) and Little mice are resistant to hepatotoxins (Amador-Noguez et al., 2007). Other murine models of delayed aging also show increased expression of xenobiotic metabolizing enzymes (Miller et al., 2014; Steinbaugh et al., 2012). These include Snell dwarf mice, which carry mutations hindering normal pituitary development, growth hormone receptor knock out (GHRKO) mice and mice subjected to DR, reduced access to the mother during the breastfeeding period (crowded litter), or treated with rapamycin. Furthermore, DR mice show increased resistance to acetaminophen and other hepatotoxins, such as thioacetamide and bleomycin (Aido et al., 1999; Apte et al., 2003; Harper et al., 2006). Methionine-restricted mice are also less susceptible to acetaminophen (Miller et al., 2005). Taken together, these findings point to increased metabolism of endo- and xeno-biotics as a potential downstream mechanism for mediating the effects of multiple interventions promoting health during aging.

Detoxification of endo- and xeno-biotics is occurs in three phases. In phase I, cytochrome P450 enzymes (CYPs) and short chain dehydrogenases (SDRs) bioactivate the lipophilic components, providing conjugation sites for consecutive reactions. Different classes of phase II enzymes then conjugate bulky hydrophilic groups to the molecules to increase their solubility in body fluids and facilitate their excretion. Phase II enzymes include glutathione-S-transferases (GSTs), UDP-glucuronosyl-transferases (UGTs), sulfotransferases, carboxylesterases and others. The third phase is accomplished by the ABC (ATP binding cassette) transporters, which excrete the detoxified molecules (Omiecinski et al., 2011). Notably, all steps of detoxification are highly energy demanding (Gems & McElwhee, 2005).

The main transcriptional regulators of detoxifying enzymes and transporters are conserved among metazoans and include the aryl hydrocarbon receptor (AhR), the zinc-finger transcription factor Nrf-2 and members of two closely related subgroups of nuclear hormone receptors (NHRs) (Brown et al., 2005; Itoh et al., 2015; Köhle & Bock, 2009; Lindblom & Dodd, 2006; Sykiotis & Bohmann, 2008; Wang et al., 2013). NHRs are classified into groups NR1-9, and into subgroups according to their highly conserved domain structure (Nuclear Receptors Committee, 1999). The NHRs relevant for xenobiotic metabolism in invertebrates belong to the NR1J group and include DAF-12, NHR-8 and NHR-48 in C. elegans and DHR96 in Drosophila. While NHR-8 and DHR96 are demonstrated to regulate xenobiotic detoxification, DAF-12 and NHR-48 have not yet been studied regarding this possible function (King-Jones et al., 2006; Lindblom et al., 2001). The NR1J group is closely related to the NR1I group, with mammalian pregnane X receptor (PXR), constitutive androstane receptor (CAR) and vitamin D receptor (VDR) and to the NR1H group with farnesoid X receptor (FXR) and liver X receptors (LXRs; Figure 1). PXR and CAR have a well-established role in regulating xenobiotic metabolism, while VDR, FXR and LXRs, like many other mammalian NHRs, induce expression of xenobiotic detoxification genes and cross-talk with xenobiotic metabolism (Haussler et al., 2013; Wang et al., 2013; Xu et al., 2005).

Interestingly, in worms and flies, genes that regulate xenobiotic metabolism are also implicated in healthy aging. For instance, over-expression of skn-1, the Nrf2 ortholog in C. elegans, extends lifespan and its activity is required for the response of lifespan to DR (Bishop & Guarente, 2007; Tullet et al., 2008). Furthermore, over-expression of the Nrf2 ortholog cnc in Drosophila extends lifespan (Sykiotis & Bohmann, 2008), while nhr-8 is required for DR to extend lifespan in C. elegans (Chamoli et al., 2014; Thondamal et al., 2014). Although daf-12 has not been shown to be involved in xenobiotic metabolism, it is well established to promote healthy aging: daf-12 is required for increased longevity induced by loss of the germline (Hsin & Kenyon, 1999; Wollam et al., 2012). Furthermore, a gain of function mutant of daf-12 is long-lived (Fisher & Lithgow, 2006). On the other hand, although dhr96 is involved in the response to xenobiotics, it has not yet been studied regarding its involvement in healthy aging (King-Jones et al., 2006).

In addition to their possible roles in detoxification of xenobiotics, NHRs of the NR1H, NR1I and NR1J groups control homeostasis of steroid metabolites and fat metabolism. DAF-12 and NHR-8 in worms and DHR96 in flies regulate cholesterol and triacylglyceride metabolism and, furthermore all three are involved in biosynthesis and/or degradation of steroid hormones that govern developmental decisions and are involved in healthy aging (Antebi, 2013b; Bujold et al., 2009; Guitard et al., 2011; Horner et al., 2009; King-Jones et al., 2006; Magner et al., 2013; Wang et al., 2015). Mammalian LXRNS have a well-established role in controlling cholesterol homeostasis, while FXR acts as the main bile acid sensor and is also involved in triacylglyceride homeostasis (Kalanay & Mangelsdorf, 2006). Next to xenobiotics, mammalian PXR and CAR bind a wide range of endogenous ligands including bile acids, bilirubin, and steroid hormones, and are involved in control of bile acid homeostasis, lipid metabolism and gluconeogenesis, as well as regulation of steroid and thyroid hormone levels (di Massi et al., 2009; Moreau et al., 2008; Wang et al., 2013; Yang & Wang, 2014). VDR is activated by bile acids and, furthermore, polyunsaturated fatty acids are ligands for VDR, albeit with low affinities (Adachi et al., 2005; Haussler et al., 2013; Makishima et al., 2002).

The increased expression of genes involved in xenobiotic metabolism, and the xenobiotic resistance seen in animal models of healthy aging, together with the dual involvement
of transcription factors in regulating xenobiotic metabolism and healthy lifespan, all suggest that there may be a causal connection between the two traits. Since NHRs involved in detoxification of xenobiotics also regulate endogenous metabolites and hormones, this role could also contribute to healthy aging. To determine whether these associations are indeed causal will require unravelling the exact mechanisms by which NHRs modulate the different traits. In this review, we consider this issue, with particular emphasis on the invertebrate NHRs of the NR1J group, DAF-12, NHR-8 and DHR96, with discussion of their mammalian orthologs where relevant. Although NHR-48 also belongs to the NR1J group its functions are largely unknown, and it will hence not be further discussed. We focus on the role of these NHRs in worms and flies because their role in lifespan and health during aging has been much more extensively investigated than has that of vertebrate NHRs. We first provide an overview over structure and function of NHRs. We then discuss whether increased xenobiotic metabolism and/or control of sterol and triacylglyceride metabolism by NHRs contributes to health during aging.

**Nuclear hormone receptors**

Nuclear hormone receptors comprise a large superfamily of proteins that are usually ligand-dependent transcription factors. They serve a wide variety of functions, including regulation of development, mitochondria, immunity, sex determination and reproduction, as well as lipid metabolism and detoxification of endo- and xenobiotics. They are evolutionarily conserved in metazoans, with 18 members in *Drosophila*, 48 in human, 49 in mice and 284 in nematodes (Evans & Mangeldorff, 2014; Fahrbach et al., 2012). As both their DNA-binding domain (DBD) and ligand-binding domain (LBD) show high evolutionary conservation, NHRs are grouped into subfamilies and subgroups based on the phylogenetic comparison of these domains (Nuclear Receptors Committee, 1999).

The ligands of NHRs are always lipophilic but are highly variable in size and structure (Laudet et al., 2005). Examples include glucocorticoids, steroid hormones, fatty acids, phospholipids, heme, bile acids, vitamin D and xenobiotics (Huang et al., 2010). Some NHRs are orphan receptors, meaning either that they do not have ligands or that these have not yet been identified. Furthermore, some NHRs constitutively bind their ligands in the manner of a co-factor, for instance HNF-4 and fatty acids (Evans & Mangeldorff, 2014). Ligand specificity and affinity varies greatly between the family members. The human PXR, for example, binds various ligands with low affinities (in the micromolar range) whereas steroid receptors are highly specific for their cognate ligands and bind these with high affinity (0.1–1 nM) (Huang et al., 2010; Reschly & Krasowski, 2006).

As illustrated in Figure 2(A), NHRs have a modular structure. The N-terminal region (A/B domain) is variable and serves in ligand-independent transactivation. The central DBD is highly conserved and has two zinc finger motifs providing DNA binding. The highly variable hinge region connects the DBD and LBD and also contains the C-terminal extensions, which bind to the DNA minor groove just C-terminal of the DBD (Huang et al., 2010; Laudet et al., 2005). The C-terminal LBD is evolutionarily conserved, but not as highly as the DBD (Bertrand et al., 2004), and contains the ligand-binding pocket. Its structure

![Figure 1. Phylogenetic relationships between NHRs implicated in xenobiotic metabolism in worms, flies and mammals.](https://www.informahealthcare.com/bmg)
allows interaction with dimerization partners as well as co-regulators and other transcription factors. The ligand-binding pocket is buried deeply in the molecule, in accordance with the lipophilic nature of the ligands. Upon ligand binding, helix 12 of the LBD is subjected to a conformational change, resulting in recruitment of co-regulators.

Classically, in the absence of the ligand, NHRs are thought to bind to co-repressors, which are exchanged for co-activators upon ligand-binding (Figure 2B) (Huang et al., 2010). However, regulation of the transactivating activity of NHRs is more complex. NHRs can also act only as activators or only as repressors (Figure 2B). Furthermore, unliganded NHRs can constitutively activate transcription, with ligands acting as inverse agonists and repressing transcription, as is the case for mammalian CAR (Figure 2B) (Evans & Mangelsdorf, 2014). Detailed models of transcriptional repression by NHRs in interaction with ligands, co-regulators and other NHRs are reviewed elsewhere (Santos et al., 2011).

Activity of NHRs is not solely regulated by the presence or absence of ligands. Transactivating activity can be regulated by phosphorylation, as in the estrogen receptor (Kato et al., 1995; White et al., 1997). Furthermore, some NHRs reside in the cytoplasm bound to chaperones in the absence of their ligands. For instance, the glucocorticoid and androgen receptors are associated with a protein complex including HSP70 and HSP90, and ligand binding induces release from the complex and translocation into the nucleus (Kawata, 2001).
The spectrum of genes regulated by a given NHR, and whether it activates or represses particular genes, can differ between tissue and cell types. This specificity probably depends upon the local availability of co-activators and corepressors, since binding of these two types of co-regulators to the surface of the NHR is mutually exclusive (Huang et al., 2010).

NHRs can bind to the DNA as monomers, homodimers or heterodimers (Figure 2C). This is reflected in the arrangement of their hexameric DNA binding motifs, with monomers binding to single hexamers, homodimers binding to inverted repeats and heterodimers binding to direct repeats of the hexameric motifs (Evans & Mangelsdorf, 2014). Many NHRs, including mammalian VDR, FXR, LXR, retinoic acid receptors (RARs), PXR and CAR, form heterodimers with retinoic X receptor (RXR). Of note, RXR is the only heterodimerization partner for all other NHRs. (Evans & Mangelsdorf, 2014). RXR heterodimers can be activated by both the RXR ligand retinoic acid and the cognate ligand of the heterodimerization partner (permissive heterodimer), or only by the ligand of the partner (non-permissive heterodimer) (Evans & Mangelsdorf, 2014). Permissive partners include peroxisome proliferator-activated receptors (PPARs), LXR, FXR, PXR and CAR, whereas thyroid receptors (TRs), VDR and RARs are non-permissive partners. In invertebrates heterodimerization of NHRs has not been described at this level of detail. However, the *Drosophila* ecypsycone receptor (EcR) heterodimerizes with USP, the RXR homolog in flies (Horner et al., 1995; Yao et al., 1993). Of note, *C. elegans* does not harbor a homolog of RXR but heterodimerization between different NHRs has been observed in worms (Li et al., 2004).

NHRs commonly have multiple isoforms that can serve different functions. Differences in the N-terminal region result in different transactivating activities, and isoforms can lack the DBD and hence sequester ligands without regulating target genes. Some isoforms act as dominant negative receptors, by binding the DNA without regulating transcription (Laudet et al., 2005). In addition, NHR signaling is autoregulated at two levels. NHRs regulate their own expression, as promoters of NHRs often hold their own binding motif (Laudet et al., 2005). NHRs can also regulate the production of their ligands by transcriptional control of enzymes necessary for their biosynthesis (Evans & Mangelsdorf, 2014).

Xenobiotic resistance: a cause for NHR dependent longevity?

The role of NHRs in longevity and healthy aging has been addressed mainly in *C. elegans* and *Drosophila*. We therefore focus on these organisms to consider the possible roles of xenobiotic, lipid and cholesterol metabolism in mediating the effects of DAF-12, NHR-8 and potentially DHR96 activity on longevity.

**DAF-12, NHR-8 and DHR96 in xenobiotic resistance and longevity**

The ligands of DAF-12 are dafachronic acids (DAs), which are synthesized by the CYP DAF-9 and other enzymes from a cholesterol precursor (Gerisch & Antebi, 2004; Gerisch et al., 2001; Mahanti et al., 2014; Rottiers et al., 2006; Schaede et al., 2012; Wollam et al., 2012). While unliganded DAF-12 is associated with the co-repressor DIN-1 and represses target gene expression (Ludewig et al., 2004), binding of DAs is thought to induce expression of target genes (Antebi, 2013b). DAF-12 signaling interacts with IIS, signals from the germline and environmental conditions to modulate healthy aging and can have opposed effects on longevity. Reduced DA/DAF-12 signaling can be achieved by mutating different components of the pathway, including the enzymes for DA biosynthesis and DAF-12 itself. The effect of daf-9 mutation on lifespan is temperature-dependent (Table 1). All four daf-9 alleles tested increase lifespan at 15°C (Gerisch et al., 2001, 2007; Jia et al., 2002). However, this effect is lost or even reversed at higher temperatures up to 25°C (Dumas et al., 2013; Gerisch et al., 2001, 2007; Jia et al., 2002; Thondamal et al., 2014). Extension of lifespan in daf-9 mutants at 15°C is completely dependent upon daf-12, indicating that unliganded DAF-12 is required for longevity at this temperature (Gerisch et al., 2001; Jia et al., 2002). Accordingly, the null allele daf-12(rh61rh411) alone either reduces (20°C) or does not change (25°C) lifespan (Dumas et al., 2013; Fisher & Lithgow, 2006) but the LBD deficient mutant daf-12(rh273) shows extended lifespan at 20°C (Fisher & Lithgow, 2006). Furthermore, the co-repressor DIN-1, which is associated with DAF-12 in the absence of ligands, is required for longevity seen in daf-9 mutants (Ludewig et al., 2004). These findings indicate that, in otherwise untreated animals, unliganded rather than liganded DAF-12 serves to extend lifespan, and DAF-12 together with DIN-1 represses genes whose functions counteract longevity.

Whether DA signaling affects xenobiotic resistance has not yet been assessed, but reduced DA signaling increases resistance to other stressors. Both long-lived daf-9 and LBD deficient daf-12(rh273) mutants exhibit resistance to thermal and oxidative stress (Fisher & Lithgow, 2006; Gerisch et al., 2007). In daf-9 mutants, resistance is abrogated when daf-9 expression is restored or when they are treated with a DAF-12 agonist (Gerisch et al., 2007). Furthermore, increased resistance of daf-9 mutants depends upon daf-12 and its co-repressor din-1, indicating that unliganded DAF-12 acts to increase stress resistance (Gerisch et al., 2007). It has been proposed that resistance against a broad range of stressors is a longevity-assurance mechanism (Gems & McElwee, 2005; Shore & Ruvkun, 2013), which could suggest that long-lived mutants that are resistant to thermal and oxidative stress would also be more resistant to xenobiotic stress. The hypothesis further implies that interventions that abrogate increased stress resistance should also abrogate longevity of these mutants. However, the increased stress tolerance of daf-9 mutants is partially dependent on daf-16 while their longevity is not abrogated by daf-16 mutation (Gerisch et al., 2001, 2007; Jia et al., 2002), indicating that increased resistance against these two stressors can be at least partially uncoupled from longevity.

Further evidence arguing against xenobiotic detoxification as a cause for longevity in mutants with reduced DA signaling comes from comparison of genes differentially regulated between long-lived daf12(rh273) and short-lived daf-12(rh61rh411) mutants. Genes involved in phase I and II
Table 1. DA/DAF-12 signaling affects longevity in a context-dependent manner.

| DA/DAF-12 signaling mutant/ intervention | Temperature (°C) | daf-2 (class 1) | daf-2 (class 2) | daf-2 RNAi | daf-16 | Germs abl. | References |
|----------------------------------------|-----------------|----------------|----------------|------------|--------|-----------|------------|
| daf-9 (e1406)                          | 15              | +              |               |            |        |           | Jia et al. (2002) |
| daf-9 (e1406)                          | 15              | +              |               |            |        |           | Gerisch et al. (2001) |
| daf-9 (db8)                            | 15              | +              |               |            |        |           | Gerisch et al. (2001) |
| daf-9 (db6)                            | 15              | +              |               |            |        |           | Gerisch et al. (2007) |
| daf-9 (e1406)                          | 15              | +              |               |            |        |           | Gerisch et al. (2007) |
| daf-9 (rh50)                           | 15              |               |               |            |        |           | Gerisch et al. (2001) |
| daf-9 (e1406)                          | 15              |               |               |            |        |           | Jia et al. (2002) |
| daf-9 (rh50)                           | 15              |               |               |            |        |           | Gerisch et al. (2001) |
| daf-9 (e1406)                          | 20              | =             |               |            |        |           | Jia et al. (2002) |
| daf-9 (rh50)                           | 20              |               |               |            |        |           | Thondamal et al. (2014) |
| daf-9 (e1406)                          | 22.5            |               |               |            |        |           | Gerisch et al. (2001) |
| daf-9 (k182)                           | 25              | =             |               |            |        |           | Jia et al. (2002) |
| daf-9 (k182)                           | 25              |               |               |            |        |           | Dumas et al. (2013) |
| daf-9 (rh50) + Δ7-DA                    | 20              | =             |               |            |        |           | Dumas et al. (2013) |
| Δ4-DA                                  | 22.5            | +             |               |            |        |           | Thondamal et al. (2014) |
| daf-12 (m20)*                          | 15              |               |               |            |        |           | Gems et al. (1998) |
| daf-12 (rh61rh411)**                   | 20              | =             |               |            |        |           | Dumas et al. (2013) |
| daf-12 (rh61rh411)**                   | 20              |              |               | Fisher & Lithgow (2006) |        |           | Dumas et al. (2013) |
| daf-12 (rh273)**                       | 22.5            |               |               |            |        |           | Gems et al. (1998) |
| daf-12 (m20)*                          | 22.5            | =             |               |            |        |           | Dumas et al. (2013) |
| daf-12 (m20)*                          | 22.5            | =             |               |            |        |           | Dumas et al. (2013) |
| daf-12 (rh61rh411)**                   | 25              | =             |               |            |        |           | Dumas et al. (2013) |
| daf-12 (m20)*                          | 25              | =             |               |            |        |           | Larsen et al. (1995) |
| daf-9 (rh50)                           | 20              | =             |               |            |        |           | Gerisch et al. (2001) |
| daf-9 (db6)                            | 20              | =             |               |            |        |           | Gerisch et al. (2001) |
| daf-12 (m20)*                          | 20              | =             |               |            |        |           | Hsin & Kenyon (1999) |
| daf-12 (rh61rh411)**                   | 20              | =             |               |            |        |           | Yamawaki et al. (2010) |

*Non-null, only DAF-12A is affected, DAF-12B is transcribed normally; ** null; ***LBD deficient; + DA/DAF-12 mutant/intervention increased longevity compared to background; = no change compared to background; − DA/DAF-12 mutant/intervention decreased longevity compared to background.

detoxification, including CYPs, GSTs and glucuronosyltransferases, are down-regulated in long-lived daf-12(rh273) mutants (Fisher & Lithgow, 2006) while, in contrast, xenobiotic detoxification genes are up-regulated in long-lived worms, flies and mice (McElwee et al., 2007). Xenobiotic resistance is thus unlikely to be causal for healthy aging of mutants with reduced DA/DAF-12 signaling. However, due to the correlative nature of these findings this conclusion remains speculative. Future work should assess the xenobiotic resistance of long-lived DA/DAF-12 mutants and any possible role in determination of lifespan.

NHR-8 is required for normal levels of xenobiotic resistance, because both nhr-8(ok186) mutants and worms subjected to nhr-8 RNAi treatment are sensitive to xenobiotics (Lindblom et al., 2001). However, nhr-8(ok186) mutants do not exhibit reduced resistance against phenazine, indicating that NHR-8 confers resistance against a specific subset of xenobiotics (Lindblom et al., 2001). Furthermore, NHR-8 appears to have redundant functions with other transcriptional regulators in regulating xenobiotic detoxification genes, because NHR-8 RNAi treatment does not abrogate expression of selected phase I and II enzymes (Chamoli et al., 2014; Jones et al., 2013). Interestingly, NHR-8 is necessary for the response of lifespan to DR in C. elegans (Chamoli et al., 2014; Thondamal et al., 2014). It remains to be tested whether xenobiotic resistance is increased by DR and contributes to longevity of DR worms. The finding that skn-1, the Nrf-2 ortholog in C. elegans, is also necessary for DR in C. elegans supports this idea (Bishop & Guarente, 2007).

Analogous to nhr-8 mutants, dhr961 loss of function mutants are sensitive to xenobioc, including phenobarbital, DDT and permethrin (Beaver et al., 2010; King-Jones et al., 2006). Over-expression of dhr96 in L3 larvae induces expression of xenobiotic detoxification genes independently of treatment with xenobiotics and, in addition DHR96 is required for induction of xenobiotic detoxification genes by phenobarbital treatment, including Turandot genes, acyl-CoA synthetases, CYPs, GstD7 and juvenile hormone binding proteins (JHBPs), which serve as lipid carriers in the hemolymph (King-Jones et al., 2006). However, the responses to phenobarbital of some xenobiotic detoxification genes, including Cyp6a8 and GstD2, are unaffected by loss of dhr96 (King-Jones et al., 2006). These findings suggest that DHR96 is not solely responsible for the gene expression changes in response to phenobarbital and other transcriptional regulators. For instance, the Nrf2 ortholog CNC or spineless (ss), the ortholog of the AhR, could also be involved in xenobiotic metabolism because their mammalian counterparts regulate xenobiotic detoxification genes (Köhle & Bock, 2009; Okawa et al., 2006).
Increased xenobiotic resistance of long-lived IIS mutants – mediated by NHRs?

In flies, long-lived IIS mutants are resistant to xenobiotics and, furthermore, xenobiotic detoxification genes are up-regulated in long-lived mutants with reduced IIS in worms, flies, and mice (Gronke et al., 2010; McElwee et al., 2007; Slack et al., 2011). DHR96 is likely to act downstream of reduced IIS in flies because it is a direct target gene of FOXO and xenobiotic resistance is a FOXO-dependent trait of IIS mutants (Alic et al., 2011; King-Jones et al., 2006; Slack et al., 2011). These findings suggest that DHR96 might contribute to increased xenobiotic resistance and longevity of IIS mutants.

In *C. elegans*, long-lived *daf-2* mutants share transcriptomic signatures of increased detoxification genes with fly IIS mutants (McElwee et al., 2004, 2007). Whether long-lived *daf-2* mutants are actually resistant to xenobiotics has not been assessed but further evidence suggests that xenobiotic detoxification and longevity are coupled in worms (Shore et al., 2012): many mutations that disrupt cytoprotective mechanisms including resistance to different xenobiotics also abrogate longevity of *daf-2* mutants (Shore et al., 2012). Necessity of *nhr-8* or *daf-12* for *daf-2*-dependent longevity would provide an indirect hint for the putative involvement of these NHRs in the regulation of xenobiotic detoxification downstream of IIS. *nhr-8* is not necessary for longevity from reduced IIS, indicating that it does not contribute to xenobiotic resistance and longevity from reduced IIS (Thondamal et al., 2014). In contrast, DA/DAF-12 signaling interacts with IIS in a context- and temperature-dependent manner to modulate lifespan (Table 1). Longevity induced by both weak *daf-2(e1368)* and strong *daf-2(e1370)* alleles is abrogated by *daf-9* mutation at 15°C, but at higher temperatures *daf-9* mutation shortens lifespan of *daf-2(e1368)* and further extends lifespan of *daf-2(e1370)* (Dumas et al., 2013; Gerisch et al., 2001). Correspondingly, Δ^4-DA supplementation further extends *daf-2(e1368)* lifespan but has no effect on *daf-2(e1370)* longevity (Gerisch et al., 2007). Furthermore, different *daf-12* mutations affecting different DAF-12 isoforms influence longevity of *daf-2* mutants in opposing directions (Dumas et al., 2013; Gems et al., 1998; Larsen et al., 1995; McCulloch & Gems, 2007). These complex phenotypes suggest intense cross-talk between DA/DAF-12 and IIS pathways. However, since combination of different *daf-2* mutations with mutants impaired in DA/DAF-12 signaling have different effects on longevity, assessing their xenobiotic resistance in parallel to their lifespan phenotypes could prove useful to test whether xenobiotic resistance and longevity are coupled in these long-lived mutants.

Xenobiotic resistance as a cause for longevity – open questions

The experimental evidence is not yet sufficient to determine whether xenobiotic resistance per se improves health during aging. In *C. elegans*, a causal connection between xenobiotic detoxification and longevity could be implied by the common role of *skn-1*, reduced activity of which interferes with both xenobiotic resistance and longevity (Shore et al., 2012; Tullet et al., 2008). However, it remains to be assessed whether xenobiocic resistance is increased by interventions that extend lifespan in *C. elegans*. Many DR-like interventions increase xenobiotic resistance in rodents. Therefore, it would be interesting to investigate whether DR worms are resistant to xenobiotics as well and, if so, to assess the role of *skn-1* and *nhr-8* as candidates for mediating the effect, since both regulate expression of xenobiotic detoxification genes and are necessary for longevity of DR worms (Bishop & Guarente, 2007; Chamoli et al., 2014; Lindblom et al., 2001; Thondamal et al., 2014).

In flies and rodents, a correlation between increased xenobiotic resistance and longevity has been observed. However, it is unclear whether increased resistance is causal for healthy aging. In flies, longevity and increased xenobiotic resistance are phenotypes resulting from reduced IIS in a FOXO-dependent manner (Slack et al., 2011). As *dhr96* is a target gene of FOXO (Alic et al., 2011), DHR96 is a promising candidate to mediate the beneficial effect of reduced IIS on xenobiotic resistance and longevity. To confirm this hypothesis it needs to be tested whether interventions that increase the activity of DHR96 extend lifespan. Furthermore, if IIS mutants are long-lived because of their increased xenobiotic resistance, then longevity of these mutants should be abrogated in a DHR96 null background. However, since DHR96 is probably not the only transcriptional regulator of xenobiotic metabolism in flies, other relevant regulators of xenobiotic detoxification genes, namely CNC and SS, should also be examined for a possible role downstream of interventions that improve health during aging.

In rodents, the causal connection between increased xenobiotic resistance and longevity also requires testing. However, this will not be a trivial undertaking, because many transcriptional regulators, including PXR, CAR, VDR, FXR, Nrf2 and AhR, regulate detoxification enzymes, and it is likely that they can compensate for each other (Haussler et al., 2013; Ito et al., 2015; Köhle & Bock, 2009; Wang et al., 2013). One possibility would be to test whether longevity of Little mice is abrogated in a FXR null background. These mice have elevated levels of xenobiotic detoxification genes, which is lost in an FXR null background, while xenobiotic gene expression is unaffected in Little mice in a PXR/CAR double mutant background (Amador-Noguez et al., 2007). If longevity of Little mice is also abrogated in a FXR null background then this would suggest that increased xenobiotic resistance could be causal for longevity. However, FXR has a well-established role in regulation of bile acid levels by regulating detoxification enzymes, and this function of FXR could also contribute to longevity of Little mice.

Xenobiotic resistance of long-lived models – a bystander effect?

Deregulated expression of xenobiotic detoxification genes in long-lived mutants could also point to changes in levels of steroid hormones or other lipophilic metabolites. In mammals, homeostasis of lipophilic signaling molecules and metabolites such as steroid hormones, bile acids or bilirubin, is controlled by the enzyme classes that act in xenobiotic detoxification (di Masi et al., 2009; Gibson & Skett, 1986). This implies that, for example, steroid hormone homeostasis and xenobiotic
detoxification are intimately connected. It remains to be studied whether these processes are interrelated also in the long-lived invertebrate models, although it seems very likely given the structural similarities of, for example, DAs with bile acids (Mahanti et al., 2014). Therefore, the unexpected down-regulation of detoxification genes in long-lived LBD deficient daf-12(rh273) mutants in *C. elegans* might reflect changes in DA homeostasis. Bile acids are degraded by CYPs (di Masi et al., 2009) and the same is likely to apply to degradation of DAs. Furthermore, the production of DAF-12 ligands is achieved by CYPs and short chain dehydrogenases and other enzymes that are yet to be identified (Mahanti et al., 2014). Given the broad substrate specificities of CYPs (Gibson & Skett, 1986), some of the enzymes that have, as of yet, been described only as detoxification enzymes might also be involved in DA biosynthesis. In further support of this idea, both DAF-12 and DHR96 regulate CYPs involved in steroid hormone homeostasis. DAF-12 regulates expression of DAF-9, required for biosynthesis of DAs (Gerisch & Antebi, 2004; Mak & Ruvkun, 2004) and DHR96 regulates CYP18a1, which is involved in catabolism of 20 hydroxy ecodycene (20E), a major steroid hormone in *Drosophila* (Guittard et al., 2011; King-Jones et al., 2006). In summary, differential expression of xenobiotic detoxification genes in long-lived animal models suggests that, as well as the potential to reduce toxic endo- and xenobiotic molecules, levels of steroid hormones and their metabolites are changed. This implies that physiological functions that are under control of these hormones may be causal for healthy aging of these animals. Accordingly, steroid hormones like DAs in worms and 20 E in flies modulate lifespan (Gerisch et al., 2007; Simon et al., 2003; Tricoire et al., 2009). The roles of steroid signaling in healthy aging are reviewed in detail elsewhere (Galikova et al., 2011; Toivonen & Partridge, 2009). Comprehensive analyses of the substrate specificities of CYPs, SDRs and other enzymes involved in detoxification and steroid hormone biosynthesis could prove very useful to disentangle cause from effect.

Apart from endo- and xenobiotic metabolism, members of the NR1J group control lipid metabolism and reproduction. These functions, which we shall consider in the next section, are likely to mediate healthy aging and, furthermore, their effects on longevity might be interrelated.

**NHRs, lipid metabolism, germline and aging**

Deregulated fat and cholesterol homeostasis has a major impact on health during aging resulting in type II diabetes and cardiovascular disease (Barzilai et al., 2012). In addition, lipid metabolism and reproduction mutually influence each other and both affect the aging process (Hansen et al., 2013). However, the connection of the three processes is as of yet unresolved. Interestingly, DAF-12, NHR-8 and DHR96 all either cross-talk with signals from the germline to modulate healthy aging or control reproduction in response to nutrient availability.

Dafachronic acid (DA)/DAF-12 signaling is required for longevity of worms lacking a germline, a phenomenon referred to as germline longevity or gonadal longevity (Table 1) (Gerisch et al., 2001; Hsin & Kenyon, 1999; Yamawaki et al., 2010). The primordial germline of *C. elegans* comprises four stem cells, with two somatic and two germline stem cells. When the germline stem cells are ablated by laser microsurgery, worms are sterile and live 60% longer than intact animals (Hsin & Kenyon, 1999). However, if the gonadal stem cells are ablated as well, animals are not long-lived (Arantes-Oliveira et al., 2002; Hsin & Kenyon, 1999). Importantly, modulation of adult lifespan by the germline is conserved in flies and mice (Cargill et al., 2003; Flatt et al., 2008; Mason et al., 2009). Much effort has been made to understand how signals from the germline affect aging reviewed in detail by Antebi (2013a). One candidate mechanism by which germline signals may modulate healthy aging is through regulation of fat metabolism. This idea is supported by the finding that the triacylglycerol lipases *lips-17* and *lipl-4* and the fatty acyl-CoA reductase *fard-1* are necessary for germline longevity, and that overexpression of *lipl-4* is sufficient to extend lifespan in *C. elegans* (McCormick et al., 2012; Wang et al., 2008). Interestingly, *fard-1* is a target gene of DAF-12, suggesting that control of lipid metabolism is a mechanism by which DAF-12 modulates healthy aging. In further support of this idea, DA/DAF-12 signaling induces mobilization of fat stores and fatty acid oxidation (Wang et al., 2015).

NHR-8 also regulates lipid metabolism. RNAi against *nhr-8* increases fat content and, furthermore, NHR-8 controls cholesterol homeostasis in worms, which are cholesterol auxotrophs (Ashrafi et al., 2003; Magner et al., 2013). By regulation of apolipoproteins, NHR-8 regulates distribution of cholesterol throughout the body and, specifically, its transport into eggs (Magner et al., 2013). Evidently, this function has implications for the control of reproduction in the adult worm, because NHR-8 is necessary for decreased proliferation of germline stem cells under nutrient deprivation (Thondamal et al., 2014). This opens up the possibility that NHR-8 mediates the beneficial effects of DR by the reduction of germ cell signals that shorten lifespan. In addition, by controlling cholesterol uptake, NHR-8 also regulates availability of precursors for biosynthesis of steroid hormones such as DAs, reflected in the developmental phenotypes of *nhr-8* mutants, which resemble those of DA-deficient animals (Magner et al., 2013). This suggests that NHR-8 signaling might also interfere with effects of DA/DAF-12 signaling on health during aging.

DHR96 performs very similar functions in flies to those of NHR-8 in worms. Flies are also cholesterol auxotrophs, and DHR96 regulates cholesterol metabolism to varying levels of cholesterol in the food (Bujold et al., 2009; Horner et al., 2009). As implied by the parallel regulation of DA levels by NHR-8 in worms, DHR96 may also control availability of precursors for steroid hormones that modulate healthy aging. Furthermore, DHR96 also regulates reproduction in response to nutrient availability. In response to starvation, germline stem cells, as well as follicular stem cells, which give rise to the somatic gonad in flies, cease to proliferate until nutrients are available again (LaFever & Drummond-Barbosa, 2005). DHR96 is necessary for proliferation of follicular stem cells when flies are re-fed after a starvation period (Hartman et al., 2013). The response to re-feeding is further dependent on the cholesterol content of the food, indicating that the cholesterol-sensing function of DHR96 is responsible for the control of the response. Although it has not yet been addressed whether DHR96 also controls germline stem cell proliferation, by
inference from the role of NHR-8 in adjusting germline stem cell proliferation to cholesterol availability, DHR96 could play a role in germline longevity in flies.

Finally, DHR96 regulates many genes involved in fat metabolism including magro, a gastric triacylglycerol lipase and cholesterol esterase (Sieber & Thummel, 2009). In addition to its function in cholesterol homeostasis, magro facilitates uptake of lipids from the food by liberating fatty acids from triacylglycerides (Bujold et al., 2009; Horner et al., 2009; Sieber & Thummel, 2009, 2012). Since magro is an ortholog of lipl-4 in C. elegans, it would be interesting to investigate whether over-expression of magro also improves health during aging in flies.

**Molecular functions of NR1J group members**

While invertebrate animal models have proven very useful to gain insights into the biological mechanisms of the aging process and have helped to identify members of the NR1J group as important mediators of interventions that promote health during aging, we lack comprehensive knowledge of their molecular functions. To better understand the proximal mechanisms by which these NHRs might confer longevity, it will be necessary to decipher how they function molecularly to control the correlated phenotypes, i.e. xenobiotic resistance, steroid/lipid homeostasis and lifespan.

In C. elegans DAF-12 has three isoforms, with DAF-12A1 and DAF-12A3 being very similar and DAF-12B lacking the DBD (Antebi et al., 2000). Interestingly, the daf-12(m20) mutation, which affects only the DAF-12A isoforms, has different effects on longevity from those of the daf-12(rh61rh411) null mutation, which deletes all isoforms. Strikingly, these differences are apparent in two different contexts. On one hand, at higher temperatures longevity of IIS mutants is further increased by the daf-12(m20) mutation while the daf-12(rh61rh411) null allele does not change their lifespan (Table 1) (Dumas et al., 2013; Gems et al., 1998; Larsen et al., 1995; McCulloch & Gems, 2007). On the other hand, the different daf-12 mutations have different effects on germline longevity (Table 1). The original experiments showing that germline longevity is dependent on daf-12, with deletion of the somatic gonad having no effect, were done with the daf-12(m20) allele, which leaves the DAF-12B isoform unaffected (Antebi et al., 2000; Hsin & Kenyon, 1999). However, in daf-12(rh61rh411) null mutants, ablation of germline stem cells and somatic gonad increases lifespan slightly (Yamawaki et al., 2010). These findings imply that different DAF-12 isoforms exhibit important differences in their impact on longevity. DAF-12B lacks the DBD, but the functional relevance of this isoform is unknown. It is thought to sequester DAs without affecting transcription of DAF-12 targets and/or to heterodimerize with other NHRs and to modulate their transactivating activity (Figure 3) (Antebi et al., 2000; Gissendanner et al., 2004). If DAF-12B acts solely as a ligand scavenger, this finding would imply that DAs have targets other than DAF-12. Since the fully functional DAF-12 protein is not present in the daf-12(m20) mutants, scavenging of DAs can only be effective if there is another receptor that can respond to their absence or presence. If DAF-12B acts as a heterodimerization partner for other NHRs, its presence can modulate the activity of DAF-12.

![Figure 3](https://www.informahealthcare.com/bmg/fig3.png)

**Figure 3.** Different molecular functions of DAF-12 isoforms. DAF-12 A isoforms comprise both LBD and DBD while DAF-12B lacks the DBD. In the absence of DAs, DAF-12A is bound to the co-repressor DIN-1 and represses transcription of target genes while transcription is activated when DA is bound. DAF-12B might either sequester DAs and thereby repress transcription from DAF-12A (A) or heterodimerize with another unknown NHR and regulate its transactivating activity dependent or independent of DA availability (B). (see colour version of this figure at www.informahealthcare.com/bmg.)
Other NHRs, then their interaction has considerable relevance for the modulation of healthy aging. In either case, the functional differences of DAF-12 isoforms and their impact on healthy aging could provide detailed molecular evidence on the possible roles of both xenobiotic and lipid metabolism in healthy aging from DAF-12.

Another important step to understand the role of NR1J group members in healthy aging will be to identify their cognate ligands and the enzymes involved in their biosynthesis and degradation. NHR-8 is an orphan. However, given its modular structure and its close homology to DAF-12, it is highly probable that it has a ligand. Extrapolation from the DAF-12 ligands is unlikely to be informative because worm NHRs show low sequence identity within the LBD (<30%) (Gissendanner et al., 2004). In an attempt to identify NHR-8 ligands, the xenobiotics chloroquine and colchicine, as well as several sterol derivatives known to be bound by mammalian LXR, were tested in a ligand-sensor screen, but they did not transactivate NHR-8 (Magner et al., 2013). Several attempts have been made to identify DHR96 ligands. A promising candidate is cholesterol or a closely related derivative, because cholesterol co-purifies with the DHR96 LBD (Horner et al., 2009). However, neither supplementation of cholesterol nor reduction of cholesterol availability by different means changed DHR96 activity (Horner et al., 2009). To identify and/or confirm the ligands of NHR-8 and DHR96 it will be preferable to use in vivo rather than in vitro assays, because their transactivating activity is dependent on heterodimerization partners and co-regulators which may not be present in cell culture systems.

Apart from the known interaction of DAF-12 with the corepressor DIN-1, it is unclear which dimerization partners and/or co-regulators are required for DAF-12, NHR-8 and DHR96 to exert their functions, and whether availability of these proteins differs at different life history stages and in different tissues. DAF-12 contains a homodimerization domain in its LBD (Antebi et al., 2000) and its response elements commonly contain inverted or direct repeats, indicating that it binds to the DNA as a dimer (Shostak et al., 2004). However, the formation of homo- or heterodimers by DAF-12 has not yet been reported. One interesting candidate for a DHR96 dimerization partner is Ultraspiracle.
can maintain a healthy lipid profile. Steroid hormones, bile acids and other sterol metabolites and endo- and xeno-biotic molecules and promote somatic main-xenobiotic detoxification, NHRs can decrease the load of toxic hand, by regulating enzymes of phase I, II and III of endo-and metabolism and xenobiotic resistance (Figure 4). On the one hand, by regulating enzymes of phase I, II and III of endo-and xenobiotic detoxification, NHRs can decrease the load of toxic endo- and xeno-biotic molecules and promote somatic main-xenobiotic detoxification, NHRs can decrease the load of toxic hand, by regulating enzymes of phase I, II and III of endo-and metabolism and xenobiotic resistance (Figure 4). On the one

Increased xenobiotic resistance is a commonly observed phenotype in many long-lived worms, flies and rodents. Members of the NR1J group are important modulators of health during aging. These NHRs and their close mammalian homologs control xenobiotic detoxification but also sterol and triglyceride metabolism. Evidence is not yet sufficient to assess xenobiotic resistance as a cause for healthy aging, because most of the data are correlative in nature, and they are also largely confined to C. elegans and Drosophila. Furthermore, due to the overlapping functions of xenobiotic detoxification genes with sterol metabolism, xenobiotic resistance is tightly linked with the biosynthesis and degradation of steroid hormones and bile acids. It is therefore possible that the increased expression of xenobiotic detoxification genes in long-lived models reflects changes in levels of bile acids or steroid hormones. In invertebrates, steroid hormones modulate health during aging and in mice bile acids elicit expression profiles of detoxification genes similar to the ones found in long-lived mice. Finally, it is likely that NHRs of the NR1J group contribute to health during aging by controlling lipid metabolism because they regulate lipid-modifying enzymes that are necessary and/or sufficient to extend lifespan in C. elegans. NHRs of the NR1J group and their mammalian homologs may therefore contribute to health during aging by balancing sterol and lipid metabolism and xenobiotic resistance (Figure 4). On the one hand, by regulating enzymes of phase I, II and III of endo-and xenobiotic detoxification, NHRs can decrease the load of toxic endo- and xeno-biotic molecules and promote somatic main-xenobiotic detoxification, NHRs can decrease the load of toxic hand, by regulating enzymes of phase I, II and III of endo-and metabolism and xenobiotic resistance (Figure 4). On the one

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Brown RP, McDonnell CM, Berenbaum MR, Schuler MA. (2005). Nuclear receptor DHR96 signaling might cross-talk in modulating healthy aging, possibly by sharing the heterodimerization partner USP, the ortholog of RXR in flies. USP forms the receptor for 20E together with ecdysone receptor (EcR), which has been implicated in modulating healthy aging (Horner et al., 1995; Maletta et al., 2014; Simon et al., 2003; Tricmoire et al., 2009; Yao et al., 1993). Interestingly, DHR96 binds the hsp27 ecdysone response element, suggesting a possible heterodimerization between DHR96 and USP (Fisk & Thummel, 1995). Notably, in mammals, RXR is the only heterodimerization partner for all other NHRs and USP may have a similar function in flies. However, it is also possible that DHR96 competes with EcR/USP for binding sites and shares target genes with the 20E receptor. Given that DHR96 also regulates catabolism of 20E by regulating expression of CYP18a1 (Guitard et al., 2011; King-Jones et al., 2006), EcR and DHR96 signaling might cross-talk in modulating healthy aging, possibly by sharing the heterodimerization partner USP. Studying the interaction of DHR96 and USP could thus be revealing of molecular mechanisms.

Conclusion and outlook

Increased xenobiotic resistance is a commonly observed phenotype in many long-lived worms, flies and rodents. Members of the NR1J group are important modulators of health during aging. These NHRs and their close mammalian homologs control xenobiotic detoxification but also sterol and triglyceride metabolism. Evidence is not yet sufficient to assess xenobiotic resistance as a cause for healthy aging, because most of the data are correlative in nature, and they are also largely confined to C. elegans and Drosophila. Furthermore, due to the overlapping functions of xenobiotic detoxification genes with sterol metabolism, xenobiotic resistance is tightly linked with the biosynthesis and degradation of steroid hormones and bile acids. It is therefore possible that the increased expression of xenobiotic detoxification genes in long-lived models reflects changes in levels of bile acids or steroid hormones. In invertebrates, steroid hormones modulate health during aging and in mice bile acids elicit expression profiles of detoxification genes similar to the ones found in long-lived mice. Finally, it is likely that NHRs of the NR1J group contribute to health during aging by controlling lipid metabolism because they regulate lipid-modifying enzymes that are necessary and/or sufficient to extend lifespan in C. elegans. NHRs of the NR1J group and their mammalian homologs may therefore contribute to health during aging by balancing sterol and lipid metabolism and xenobiotic resistance (Figure 4). On the one hand, by regulating enzymes of phase I, II and III of endo-and xenobiotic detoxification, NHRs can decrease the load of toxic endo- and xeno-biotic molecules and promote somatic maintenance. On the other hand, they provide for homeostasis of steroid hormones, bile acids and other sterol metabolites and can maintain a healthy lipid profile.

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