National Cholesterol Awareness Month

Interrelationships Among HDL Metabolism, Aging, and Atherosclerosis

Michael Walter

Abstract—HDL plasma concentrations decline with age in prospective studies. Decline in HDL concentration and function may occur secondary because of hormonal changes, inflammatory processes, and diabetes mellitus. Beyond these effects specific aging processes may be involved. Replicative aging, the telomere-driven loss of divisional capacity, is a species-specific aging mechanism that may decrease HDL concentration and function. Cross-sectionally, by contrast, HDL levels do not change much or even slightly increase with age, suggesting that only people with still high HDL concentrations survive. A selection bias by HDL lowering genetic variation may explain why HDL deficiency is extremely rare among centenarians. Vice versa, HDL may modulate the aging process, not only by its well-known antiatherogenic effects, eg, its ability to remove cellular lipids and by antiatherogenic pleiotropic effects on cell survival, but possibly also by direct interfering with aging signaling or survival factor KLOTHO. Most of the current findings, however, are based on cell culture and selected animal experiments and await further confirmation by appropriate in vivo models. (Arterioscler Thromb Vasc Biol. 2009;29:1244-1250.)

Key Words: atherosclerosis ■ aging ■ high density lipoproteins ■ KLOTHO 4

The influence of age on atherosclerosis is generally explained by the simple passage of time and a higher number and severity of risk factors with increasing age. However, the observation of senescence-like changes in atherosclerotic lesions,1 the severe atherosclerosis in premature aging syndromes,2 and the identification of age as by far the most predictive independent risk factor for atherosclerosis effective even in absence of other risk factors1 suggests that atherogenesis is more directly related to the aging process than previously presumed.

Aging is a complex process that (on the cellular level) includes cell cycle arrest, morphology remodeling with functional decline, chromatin silencing with profound gene expression changes, and changes in metabolism. As summarized in Figure 1, different triggers may act together in a cooperative fashion and use overlapping signaling pathways to induce and propagate the aging process.4

Reactive aging results from the progressive shortening of telomeres (composed of conserved nucleotide sequences, TTAGGG in vertebrates) attributable to incomplete end replication during cell divisions. It may protect against increased cancer risk but may also contribute to atherosclerosis in later life, particularly at “atherosclerosis-prone areas” with high replication rates.5

Next to age, a low high-density lipoprotein (HDL) cholesterol level is one of the strongest predictors of premature coronary heart disease and stroke.6 In contrast to high low-density lipoprotein (LDL) cholesterol, low HDL cholesterol remains a powerful risk predictor into old age. HDL may directly influence specific aging processes and, vice versa, aging may influence HDL concentration and function. However, it is important to bear in mind the caveats of the current studies. First, most findings are based on cell culture and animal models and have not been proven in vivo. Second, it cannot entirely be excluded that aging and decline in HDL concentration and function are parallel but not causally related processes. Third, interpretations of epidemiological studies are complicated by a selection bias against HDL lowering genetic variants.

HDL Cholesterol Levels Into Old Age

Almost all lipoprotein levels including HDL are much lower at birth than at adolescence and increase during childhood. HDL concentrations decrease in males during puberty and early adulthood, and thereafter remain lower than those in women.7 HDL cholesterol in adults was shown to decrease with age in both men and women in prospective studies.8,9 The most striking decrease, most likely attributable to hormonal changes, were observed in postmenopausal women.8 Baggio and coworkers reported that the mean HDL levels of (both female and male) centenarians are 20% lower than those of 65-year-old subjects.10 On the other hand, older people and centenarians are unlikely to have very low levels...
of HDL, and mean HDL cholesterol levels did not vary or even increase with age in cross-sectional studies.

A likely explanation for these apparent discrepancies is the existence of 2 opposite effects that determine HDL cholesterol in aged people: (1) a selection bias against HDL lowering genetic variants, and (2) a mechanism(s) intimately related to the aging process that leads(s) to a continuous reduction of HDL cholesterol levels during aging.

Genetic factors that may be responsible for higher HDL cholesterol levels and larger HDL particle size in centenarians is genetic heterogeneity in cholesteryl ester transfer protein (CETP) and in apolipoprotein C3 (apoC3). CETP genotypes that are associated with moderate inhibition of CETP activity (and modestly higher HDL cholesterol levels) show an inverse association with coronary risk and preservation of cognitive functions. Moderately reduced levels of apoC3 (an endogenous inhibitor of lipoprotein lipase [LPL]) lead to improved triglyceride clearance and a lower incidence of subclinical atherosclerosis. On the other hand, excessive CETP inhibition may impair reverse cholesterol transport (RCT), and some CETP mutants may even provoke a proatherogenic state. Therefore, the overall role of CETP mutants in longevity is still controversial, further underlined by recent disappointing clinical trials with CETP inhibitors. Also complete inhibition of apoC3 would be problematic because the enhanced formation and uptake of free fatty acids may result in obesity and insulin resistance.

Provided that centenarians and their offspring have a more favorable genetic background, a 20% lower average HDL cholesterol level in this group (compared to 65-year-old subjects) may substantially underestimate the HDL lowering effect induced by the aging process. Currently, no prospective data with centenarians are available. In the Rancho Bernardo Study, using prospective data from 50- to 93-year-old probands, it was estimated that HDL cholesterol declines by approximately 1% per year.9 The higher observed HDL level in centenarians when compared to the level predicted from this trend (20% versus 50% reduction) is likely caused by a survival bias. A decline of HDL cholesterol to such extent would be of great clinical significance because a 1% change in HDL cholesterol was estimated to change the risk of myocardial infarction or death in middle-aged persons by 2% to 3%.

### Influence of Aging on HDL Structure and Function

#### Secondary Effects of Aging on Reverse Cholesterol Transport

What could be the mechanisms for HDL decline with increasing age? A major determinant of HDL plasma levels is the RCT, which is influenced by age-sensitive factors (Table 1). Insulin resistance and impaired lipolysis are more frequent at advanced age and may impair RCT by various mechanisms. With age, there is also some change in food consumption and in activity that may have similar effects on lipolytic enzymes, HDL concentration, structure, and function. Inflammatory processes in aged people may cause low HDL cholesterol by physical replacement of apolipoprotein A-I with the acute-phase reactant serum amyloid A (SAA) and various cytokine-induced changes.

HDL metabolism is influenced by hormones that change with age. In aging men, a low testosterone level is often a component of a metabolic syndrome, which may explain the positive correlation of testosterone with HDL concentration. Testosterone decline itself may impair LPL activity and RCT. Moreover, HDL-increasing effects of estrogen on...
apoA-I production and cholesterol efflux are blunted with estrogen decline in postmenopause.18

Specific Effects of Aging on Reverse Cholesterol Transport

Influence of Cellular Senescence on Lipid Efflux

Findings from the HDL deficiency syndrome Tangier disease point to a possible involvement of replicative aging in RCT (Table 1). Defective lipid efflux in cultivated Tangier fibroblasts is associated with intracellular accumulation of lipids (including cholesterol, ceramide, cardi- olipin) and premature senescence.19–21 An inverse correlation was observed between age and HDL cholesterol levels and in vitro lipid efflux in heterozygous patients,22 suggesting that aging may impair the residual lipid efflux capacity in Tangier cells. The finding that immortalization of fibroblasts by ectopic expression of human telomerase reverse transcriptase (TERT) improves cholesterol efflux in Tangier fibroblasts in vitro may help to elucidate this inverse correlation on a molecular level.23 TERT compensates for the erosion of telomeres by synthesizing new telomeric DNA. It genetically stabilizes the cellular phenotype and counteracts the aging process (Figure 1). A possible influence of telomere attrition or DNA damage on lipid efflux is further substantiated by the finding that cholesterol efflux is also reduced in fibroblasts from patients with the genetic instability disease Werner syndrome.24

Table 1. Effects of Aging on Reverse Cholesterol Transport

<table>
<thead>
<tr>
<th>Age-Dependent Trigger</th>
<th>Affected Molecules and Possible Mechanisms</th>
<th>Effect on HDL</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin resistance</td>
<td>Expression of apoA-I, ABCA1 and ABCG1 ↓; VLDL production ↑; LPL/HL ratio ↓</td>
<td>HDL formation (liver and plasma) ↓; lipid efflux ↓; HDL remodeling</td>
<td>15 (C,A,H)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Expression/activity of apo A-I ABCA1, ABCG1, PON1, LCAT, CETP, HL, PLTP, apoE ↓; expression/activity of PPARζ, SAA, EL, sPLA2, PAF-AH ↑</td>
<td>HDL formation (liver and plasma) ↓</td>
<td>16 (C,A,H)</td>
</tr>
<tr>
<td>Testosterone decline</td>
<td>Consequence of insulin resistance; direct effect: expression of SR-B1, LPL and HL ↓</td>
<td>HDL formation ↓; HDL remodeling (see also: insulin resistance)</td>
<td>17 (C,A,H)</td>
</tr>
<tr>
<td>Estrogen decline</td>
<td>Expression of apoA-I and nCEH (in cultivated macrophages) ↓</td>
<td>HDL formation (liver) ↓; lipid efflux ↓</td>
<td>18 (C,A,H)</td>
</tr>
<tr>
<td>Cellular senescence</td>
<td>Impaired lipid trafficking; loss of signaling competent caveolae; Cdc42, PKC/PLD, PKA, nCEH ↓; ACAT ↑</td>
<td>Lipid efflux ↓ and lipid storage ↑</td>
<td>23–26 (C,A)</td>
</tr>
<tr>
<td>HDL “particle aging”</td>
<td>LCAT activity and formation of large HDL ↓</td>
<td>Lipid efflux ↓</td>
<td>13,14,27 (C,A,H)</td>
</tr>
</tbody>
</table>

Deduced from findings in cell culture (C), animals (A), or humans (H); further references are available upon request.

ABCA1/ABCG1 indicates ATP-binding cassette transporter A1/G1; ACAT, acyl-CoA: cholesterol acyltransferase; apoA-I, apolipoprotein A-I; apoE, apolipoprotein E; EL, endothelial lipase; HL, hepatic lipase; LCAT, lecithin: cholesterol acyltransferase; LPL, lipoprotein lipase; nCEH, neutral cholesteryl ester hydrolase; PAF-AH, platelet-activating factor acetylhydrolase; PKA, protein kinase A; PKC, protein kinase C; PLD, phospholipase D; PLTP, phospholipid transfer protein; PON1, paraoxonase 1; PPAR, peroxisome proliferator-activated receptor; SAA, serum amyloid A; sPLA2, serum phospholipase A2; SR-B1, scavenger receptor B1; VLDL, very low-density lipoproteins.

Influence of Age on HDL Particle

HDL isolated from older subjects present a reduced capacity to promote cholesterol efflux.27 Age-dependent reductions in LPL or LCAT activity and HDL particle size are possible reasons. These alterations, however, greatly vary and seem to have a strong genetic background. For example, individuals with exceptional longevity and their offspring have larger HDL₂-like particles and a lower prevalence of cardiovascular disease.13 In very old people age is positively correlated with HDL₃ and negatively correlated with HDL₂,28 possibly because of selective mortality. This does not necessarily mean, however, that HDL₃ is not protective because selection and age may influence HDL subfractions in a different manner. Moreover, it cannot entirely be excluded that lipoprotein particle size is only a marker of the longevity phenotype. For example, in offspring of centenarians lipoprotein particle size can distinguish those with hypertension and insulin resistance and those without these conditions,13 pointing to a common physiological background or unidentified pleiotropic vascular effects of larger HDL.

Effects of Aging on Pleiotropic effects of HDL

The pleiotropic antiatherogenic effects of HDL may decline parallel to decline of HDL plasma levels with advanced age (Table 2). Even more important, however, might be changes in the functionality of HDL induced by age-dependent modifications of the HDL particle or the effector cells.24–27,29–34

Age-Sensitive Factors of HDL Metabolism

ATP-Binding Cassette Transporters

There are no specific reports about age-dependent changes of ABC transporter activities in cholesterol efflux. However, many members of the family of ABC transporters, including ABCA1, were shown to be sensitive to aging phenomena or
to be involved in age-related diseases including Alzheimer’s disease and diabetes mellitus.\textsuperscript{25}

Mechanisms that may account for age-dependent ABC transporter dysfunction include alterations in gene expression, protein synthesis, catabolism, or cellular localization, but also functional changes, triggered by age-dependent decreases in the level of cellular ATP or by altered interaction with molecules involved in stress and senescence signaling. Such age-dependent changes may be exacerbated or mimicked by functional polymorphisms.

**Other Cell and Plasma Proteins Involved in Cholesterol Homeostasis**

In rats aging is associated with a significant increase in intracellular cholesterol esters, increased ACAT activities, and reduced expression of neutral cholesterol ester hydrolase and caveolin-1, proteins involved in cholesterol ester hydrolysis and export. Overexpression of caveolin leads to premature senescence, whereas loss of function of caveolae occurs in fully senescent human fibroblasts.\textsuperscript{26} The activity of enzymes involved in HDL formation and conversion of HDL subclasses may change as persons age.\textsuperscript{27,28} Moreover, most of the HDL signaling pathways\textsuperscript{40–50} are potentially compromised in aged cells.\textsuperscript{24–26} For example, cdc42 activity is reduced in senescent cells and may contribute to impairment of lipid transport in Werner syndrome.\textsuperscript{24}

**Influence of HDL on the Aging Process**

**Lipid Efflux and Pleiotropic Antiatherogenic Effects of HDL**

It is obvious that HDL-inducible lipid efflux and pleiotropic HDL effects may influence vascular aging. HDL-dependent removal of lipid deposits is accompanied by the reduction of cytotoxic processes and may prevent lipoproteinosis in the arterial wall. Oxidative stress is a key factor promoting aging. HDL reduces the oxidative burst in both the plasma and cellular compartment. Moreover, HDL counteract the mitochondrial pathway of apoptosis and stimulate the proliferation of cultivated cells in vitro.\textsuperscript{29}

The physiological in vivo significance of these in vitro effects is difficult to predict. For example, mitogensics may contribute to senescence by inducing mitogenic stress signals but may also orchestrate a survival pathway. A complex interplay of signaling molecules and the duration and intensity of insults determines if activation leads to transient cell cycle arrest (with repair), to senescence, or to apoptosis. Moreover senescence and apoptosis of single cells can be beneficial for the whole organism under certain circumstances.\textsuperscript{4} It remains remarkable, however, that HDL-inducible signaling pathways intersect with key stress response and survival pathways (Figures 1 and 2). It is thus possible (but not yet proven) that HDL signaling
Figure 2. HDL-inducible signaling pathways and potential functions. HDL-associated apolipoprotein A-I may trigger mobilization of cellular lipids by binding to ABCA1 and subsequent activation of Cdc42 and its downstream target JNK. Other signaling pathways involved in lipid efflux in cultured fibroblasts and macrophages are phosphatidylinositol-specific phospholipases (PC-PLC, PC-PLD) and protein kinase C (PKC), which may cooperate with an adenylate cyclase (AC) and protein kinase A (PKA)-dependent mechanism to trigger ABCA1 phosphorylation and apoA-I lipida
tion. The small G proteins Rac1 and RhqA (not shown) may additionally stimulate and inhibit cholesterol efflux, respectively. Based on experiments with cultivated endothelial cells, it is thought that HDL brings together sphingosine 1 phosphate receptor Edg-3 (S1P3) with different binding partners: scavenger receptor class B1 (SR-B1), heterotrimeric G protein, and possibly tyrosine kinase Src to induce NO release, anti-apoptosis, and cell migration; SR-B1, heterotrimeric G protein, and small G protein Ras to induce proliferation and angiogenesis. Both HDL-associated apolipoproteins and phospholipids seem to be required for these pleiotropic HDL effects.

Figure 3. KLOTHO survival function and possible relationships to HDL. KLOTHO functions as a circulating hormone that represses intracellular signals of insulin and IGF1. The extracellular domain(s) of KLOTHO circulate in the blood stream and signal suppression of tyrosine phosphorylation of insulin/IGF-1 receptors (IR/GFR), insulin receptor substrate 1 (IRS1), and downstream signals (shown in dark boxes). Inhibition of insulin/IGF-1 signaling delays aging by allowing activation of forkhead transcription factors of the forkhead box gene group O (FOXO): when insulin levels fall (or insulin action is inhibited by KLOTHO) FOXO is dephosphorylated and translocated into the nucleus, where it allows DNA repair and increases stress resistance, by upregulating a series of key target genes. By contrast, the sequestration of FOXO into the cytoplasm (in the presence of insulin) inhibits FOXO-dependent transcription and allows cell proliferation, accompanied by higher stress sensitivity. Hypothetical mechanisms as to how HDL may compensate for KLOTHO functional decline are numbered in circles: (1) HDL may induce lipolipidosis by promoting cholesterol efflux; (2) HDL and KLOTHO may have characteristics of partial agonists and may compromise insulin signaling by redirecting the signaling molecules to other pathways (PI-3-K/Akt for both and JAK2 for HDL only); (3) HDL may influence insulin signaling (and compensate for KLOTHO dysfunction) indirectly by modulating cholesterol content of lipid rafts, which serve as scaffolds for various signaling intermediates; (4) HDL may directly mimic KLOTHO effects by inhibition of IRS1 phosphorylation; (5) Apolipoprotein-inducible activation of JNK, which is a KLOTHO-independent activator of FOXO, is another possible mechanism by which HDL may override KLOTHO dysfunction; or (6) HDL may compensate for insulin-independent KLOTHO functions such as (NO/Akt-mediated) vasorelaxation or (ERK-mediated) growth factor effects. Possible HDL receptors are described in Figure 2. The transmembrane KLOTHO protein is an obligatory coreceptor for FGF23. The postulated cell surface receptors for secreted KLOTHO protein, however, are unknown. An intrinsic sialidase activity may be involved in KLOTHO cell surface interactions.

Influence of HDL on KLOTHO Function

The KLOTHO gene, identified by insertional mutagenesis in mice, is a suppressor of the expression of multiple aging phenotypes, including atherosclerosis. Antiaging properties of the shed transmembrane form of KLOTHO protein are mostly explained by its hormonal effect on insulin signaling: KLOTHO moderately inhibits insulin/IGF1 signaling, which is the only proven evolutionary conserved mechanism for life span extension in men. However, other potentially atheroprotective effects of KLOTHO on vasotonus and calcium metabolism can also not be dismissed as possible explanations for severe atherosclerosis in KLOTHO deficient mice (Figure 3).

HDL cholesterol levels are inversely associated with the detrimental effect of a dysfunctional KLOTHO protein in humans. HDL cholesterol levels in the high normal range seem to be completely protective against KLOTHO dysfunction. The underlying mechanism of this relationship is unknown. It is obvious, however, that HDL and KLOTHO modulate similar signaling pathways. Both molecules induce NO synthesis, counteract apoptosis, induce angiogenesis, and counteract insulin signaling in cell culture models.

Several mechanisms may be involved to explain a functional interrelationship between KLOTHO and HDL. HDL may also modulate the aging process in other organ systems. A candidate target is c-Jun N-terminal kinase (JNK), which is typically activated by a variety of insults (but also by HDL) and serves a strong protective function in flies.
Edg proteins are therefore candidates where the various KLOTHO/HDL regulated pathways may intersect.

**Perspectives**

Future studies are required to find strategies to mimic the positive (antiaging) effects of HDL and to prevent the negative effects of aging on HDL. The following questions have to be addressed: (1) To what extent does HDL induce survival mechanisms in vivo and which molecules are involved? (2) By which mechanisms does (replicative) aging influence HDL metabolism? (3) Where do HDL and KLOTHO metabolism intersect, and what is the basis for multifunctionality of these proteins? (4) Is telomere attrition a primary abnormality that renders the organism more susceptible to cardiovascular risk or is reduced telomere length in patients with cardiovascular diseases mere consequence of increased cell turnover induced by the chronic inflammatory response? (5) The physiological role of senescence has to be clarified. Senescence per se is not necessarily negative as it keeps the cells in a stable viable condition. The role of such aging processes can be addressed using premature aging syndromes, centenarians, or primate models. Moreover, long-term prospective studies might be helpful to separate age-dependent from selection bias effects.

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**Disclosures**

None.

**References**


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