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Review

The role of peroxisomes in aging

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Abstract. Reactive oxygen species and alterations in membrane lipid homeostasis are thought to be important events in aging process and aging-related degenerative diseases. The peroxisome is a small cellular organelle involved in both oxygen and lipid metabolism, and defects in peroxisomal function are associated with major, and often fatal, changes at the neurological level during human development. Recent reports of aging-related changes in peroxisomal function raised the hypothesis that peroxisomes may also have a significant role in the aging process and aging-related degenerative diseases. This review presents the current data on changes in peroxisomal function during aging and discusses the implications of these changes for health.

Key words. Peroxisome; aging; lipid metabolism; oxidative stress.

Introduction

First characterized in 1966 [1], the peroxisome is a ubiquitous cellular organelle surrounded by a single membrane. Its size ranges from 0.2 to 1 μm diameter and so far, more than 50 biochemical pathways have been characterized within peroxisomes [2]. Among the major peroxisomal functions are peroxisomal oxidation and respiration, fatty acid β-oxidation, cholesterol synthesis, ether-lipid synthesis (plasmalogen biosynthesis), catabolism of purines (restricted to certain species) and D-amino acids, and metabolism of dolichol and glyoxylate. Peroxisomes serve either catabolic or anabolic functions that can be either exclusively peroxisomal or shared with other cell compartments (cytoplasm, endoplasmic reticulum and mitochondria). In the latter case, the peroxisomal contribution may be essential (plasmalogen synthesis) or complementary (cholesterol synthesis). While peroxisomal proteins represent only 2.5% of the total cell protein content in the liver, the existence of peroxisomal diseases (Zellweger's syndrome, adrenoleukodystrophy) which are associated with major, often fatal, neurological impairments [3, 4] and the relation between changes in peroxisomal metabolism and toxicological processes [5] have both emphasized the importance of peroxisomes in human health.

The role peroxisomes play in aging is not yet well understood due to a lack of data, in part because human peroxisomal disorders mainly affect children or young adults, and toxicological studies are usually performed on young animals. However, numerous peroxisomal metabolic pathways produce high amounts of hydrogen peroxide (H_2O_2). This release of H_2O_2 takes

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place in an organelle involved in metabolic processes of major importance for membrane lipid composition and function. The occurrence of reactive oxygen species with lipid components in the same organelle is likely to result in alterations that may modify peroxisomal function and, subsequently, the lipid composition and function of membranes. Antioxidant activities (catalase) help the peroxisome to prevent such changes by balancing the levels in reactive oxygen species. However, a number of age-related changes in peroxisomal H_2O_2-generating activities, antioxidant activities and lipid metabolism may alter the balance between pro- and antioxidants and result in major changes in the function of peroxisomes, membranes and cells. Such changes are consistent with both free-radical and membrane theories of aging [6–10]. This review presents arguments for a significant role of peroxisomes in the aging process by offering an overview of the current data available in this particular field, as well as hypotheses and future directions that can lead to a better characterization and understanding of the role of peroxisomes in aging and aging-related degenerative diseases.

Peroxisomes and the aging process

In a recent review, the role of peroxisomes in the aging and age-related degenerative diseases was mainly attributed to an age-related impairment in the oxidative stress status [11]. Peroxisomes contain multiple H_2O_2-producing oxidases as well as enzymes involved in the breakdown of free radicals (see table 1). In this context, peroxisomes are likely to participate to the aging process through the generation of reactive oxygen species, according to the free-radical theory of aging [6, 7].

However, recent data on peroxisomal function during aging suggest a more complex role of peroxisomes in the aging process. Peroxisomes play a unique role in lipid metabolism, and aging-related changes in peroxisomal fatty acid oxidation activity have been recently characterized in rodents [25, 26]. Such changes in peroxisomal lipid metabolism may alter membrane lipid composition in ways which are similar to those found in peroxisomal diseases [27–31]. Although the changes in lipid composition generated by the aging-related decrease in peroxisomal activity are likely to be milder than those observed in severe peroxisomal diseases, they may be significant enough to influence membrane function and thereby contribute to age-related declines in cell function. In this regard, the involvement of peroxisomes in the aging process may also be related to the membrane theory of aging [9, 32].

The impact of age-related decrease in peroxisomal lipid metabolism would be of significance if the class of lipids affected by such changes are critical for the organism. For example, peroxisomal β-oxidation presents a specificity for the oxidation of very long chain fatty acids (VLCFA) that are poorly oxidized by mitochondria [33] (see later). VLCFA accumulate in patients with peroxisomal disorders, and they are associated with severe and often fatal neurological impairements [28]. Peroxisomal β-oxidation is also required for the synthesis of docosahexaenoic acid (DHA or 22:6n-3) [34, 35]. DHA is a critical fatty acid for membrane structure and function especially in the brain and retina (see later), and patients with peroxisomal disorders affecting the β-oxidation system present a deficiency in DHA [31, 36].

Table 1. Enzymes involved in the production and degradation of reactive oxygen species in peroxisomes.

H_2O_2-generating enzymes	Antioxidant enzymes
Acyl-CoA oxidase [12]	catalase [1]
D-Amino acid oxidase [1]	superoxide dismutase [23]
Cytochrome b_5 reductase [13]	glutathione peroxidase [24]
Cytochrome P450 [14]	
Glutaryl-CoA oxidase [15]	
L-α-Hydroxyacid oxidase A and B [1]	
Oxalate oxidase [16]	
Pipecolic acid oxidase [17, 18]	
Polyamine oxidase [16, 19]	
Pristanoyl-CoA oxidase [20]	
Trihydroxycholestanoyl-CoA oxidase [21]	
Urate oxidase [1]	
Xanthine oxidase [22]	

Current data

Peroxisomal oxidases, antioxidant enzymes and aging

Peroxisomes were named after their ability to produce H_2O_2 by the action of several enzymes termed oxidases [37]. A list of these oxidases can be found in table 1. These oxidases have the ability to use molecular oxygen directly for the transformation of their substrates, with a concomitant release of H_2O_2. H_2O_2 is toxic for cells and can be subsequently converted to the hydroxyl radical ($\cdot OH^-$), the most potent reactive oxygen species.

To prevent cell damage from the production of H_2O_2 and/or its reactive oxygen derivatives, peroxisomes contain large amounts of the enzyme catalase [1]. Catalase, an enzymatic marker of peroxisomes, decomposes hydrogen peroxide into water and oxygen. The combination of H_2O_2 production and decomposition is termed peroxisomal respiration. Peroxisomes are estimated to contribute up to 20% of total liver respiration [1]. Therefore, an increase in the ratio between peroxisomal

Table 2. Changes in peroxisomal enzyme activities and functions during aging (?, unknown; *, unpublished results).

Enzyme/Function	Tissue	Species	Strain	Gender	Age-related changes
Catalase	liver	mouse	OF1	F	decreased [25, 26, 47]
			CSWW	F	decreased [25]
		rat	Wistar	M	decreased [46]
			Wistar	?	decreased [54]
			Sprague-Dawley	M	decreased*
Urate oxidase	liver	mouse	OF1	F	decreased [26,47]
		rat	Wistar	M	increased [46]
D-Amino acid oxidase	liver	rat	Wistar	?	increased [54]
Acyl-CoA oxidase	liver	mouse	OF1	F	decreased [26, 47]
		rat	Wistar	M	decreased [46]
			F344	M	unchanged [55]
			Sprague-Dawley	M	decreased*
Bi(tri)functional enzyme	liver	rat	Wistar	M	increased [46
Thiolase	liver	rat	Wistar	M	increased [46]
β-Oxidation	liver	mouse	OF1	F	decreased [25, 26]
			CSWV	F	decreased [25]
	brain	rat	Wistar	M	decreased [56]

free-radical-generating and free-radical-degrading enzymes is likely to result in an increase in cellular damage caused by an increased leakage of reactive oxygen species.

When rodents are exposed to peroxisome proliferators (PP), a class of structurally diverse chemicals which trigger a pleiotropic response in specific tissues, a tremendous alteration in lipid homoeostasis occurs, and several organelles and lipid-related metabolic pathways are affected. The peroxisome is the most sensitive organelle to PP, and peroxisomal metabolic pathways are affected differently. The peroxisomal β-oxidation system can be induced 10-fold, whereas catalase activity and dihydroxyacetone-phosphate acyltransferase (DHAP-AT, the key enzyme in plasmalogen synthesis) activity undergo a 2-fold increase [38]. Additionally, the response to PP is tissue- as well as species-specific. The liver is the most responsive tissue, whereas the brain is unresponsive. Rats and mice are generally highly sensitive to PP, whereas guinea pigs, monkeys and humans are not (or almost not) responsive. The induction of peroxisomal metabolism by PP has clear implications for oxidative stress, since the key enzyme in peroxisomal β-oxidation, acyl-coenzyme A (CoA) oxidase, belongs to the group of H_2O_2-generating enzymes. The differential induction of H_2O_2-generating and H_2O_2-degrading enzymes by PPs eventually led to the proposition that carcinogenicity associated with long-term treatment with PPs results from an increased release of H_2O_2 [5]. Additionally, treatments with PP were associated with an increase in lipoperoxidation and lipofuscin, an end product of free-radical alteration [39–41].

Lipofuscin accumulation is considered to be a marker of aging [42]. Since alterations in pro- and antioxidant peroxisomal enzyme activities influence the rate of lipofuscin formation, it seems likely that age-related changes in peroxisomal metabolism would influence the aging process. However, the relationship between peroxisomal function and lipofuscin production under normal conditions (without PP treatment) and its significance for the aging process is not yet established. Nevertheless, the occurrence of metabolic pathways for lipids sensitive to free radicals (polyunsaturated fatty acids (PUFA) and plasmalogens) and H_2O_2-generating metabolic processes in the same organelle is likely to result in lipoperoxidation and lipofuscin production. This point remains to be clarified, but it is likely that its impact on human aging is quite different from the PP-treated animal situation (human peroxisomes do not respond to PP). Additionally, as described below, the changes in peroxisomal function occurring during aging have nothing in common with those in PP-treated animals.

Table 2 summarizes the known age-related changes in different peroxisomal enzymes and functions reported so far. Each change will be addressed, and the significance of such changes for the aging process discussed.

Catalase activity has been shown to decrease during aging [43–45]. This raises the possibility of increased levels of free radicals in peroxisomes and, since H_2O_2 freely diffuses across the peroxisomal membrane, within the whole cell as well. Such changes in catalase support the free-radical theory of aging. However, some studies have shown that both acyl-CoA oxidase and catalase present a similar pattern of activity throughout life, including the aging period, in the liver [25]. In fact, the calculated catalase-to-acyl-CoA oxidase ratio remains unchanged from adulthood throughout aging, suggesting no impairment in the balance between these two activities during aging and, therefore, no increased levels in the peroxisomal H_2O_2 content. A subsequent study showed that, in addition to the age-related de-

crease in acyl-CoA oxidase, urate oxidase activity was found to be decreased as well [26]. The age-related decrease in peroxisomal catalase, acyl-CoA oxidase, β-oxidation and urate oxidase activity partly confirms a previous study describing the enzyme contents in isolated peroxisomes from young and old male Wistar rat liver [46]. Quantitative SDS-polyacrylamide gel electrophoresis (PAGE) immunoblot analysis revealed that compared with young animals, peroxisomes from old animals presented a decreased content of the enzyme catalase and the three components of acyl-CoA oxidase. These results are arguments for preservation of the balance between peroxisomal free-radical-generating and free-radical-scavenging enzymes in aging. The same study also found that the protein content for both the bi(tri)functional enzyme and the thiolase (component of the peroxisomal β-oxidation) is decreased in isolated peroxisomes from old animals compared with young. However, urate oxidase content was found to be increased in liver peroxisomes isolated from old rats [46]. The apparent discrepancy between the reduction in urate oxidase activity in mouse liver homogenate [26, 47] and the increase in urate oxidase protein in isolated peroxisomes [46] could be explained if equal amounts of protein from the young and old isolated peroxisome fraction were loaded in the SDS-PAGE gel prior to immunoanalysis [46]. As long as there is a decrease in catalase and acyl-CoA oxidase protein content in isolated peroxisomes, then loading equal amounts of protein in the gel will generate an apparent increase in the protein content of other enzymes (urate oxidase). Additionally, heterogeneity is a characteristic of peroxisomes [48, 49], and densitometric methods used so far to isolate peroxisomes fail to recover the full range of peroxisomes because of contamination by other organelles with peroxisome-like density and sedimentation coefficients. The technique used by Beier and co-workers in 1993 for the preparation of isolated peroxisomes from young and old rat livers allows the recovery of a narrow range of peroxisomes with a density of 1.245 g/cm^3 [46]. Later, the same group demonstrated the existence of two peroxisome subpopulations using a modification of their original technique [49]. The first subpopulation, termed 'heavy' peroxisomes, bands in metrizamide gradient at a density of 1.24 g/cm^3, and the second subpopulation, termed 'light' peroxisomes, bands at a density of 1.20 g/cm^3. Therefore, isolated peroxisomes obtained from young and old rat livers belong mainly to the heavy fraction. Differences in the protein content between heavy and light peroxisome populations have been demonstrated, the former being more enriched in urate oxidase. This heterogeneity is likely to influence the results when studying peroxisomes that are isolated using a densitometric method. New peroxisome purification techniques such as the recently developed immunomagnetic isolation of peroxisomes may help to clarify the problem with urate oxidase, since this technique will allow investigators to recover a broader range of peroxisomes without contaminating organelles [50]. Finally, species differences may explain the discrepancy observed for urate oxidase activity as a function of aging in rat [46] and mouse [26, 47].

The significance of changes in uric acid oxidase in the aging process is dual, as this enzyme produces H_2O_2 and since its substrate (uric acid) has been characterized as an antioxidant. The antioxidant property of uric acid is thought to be a major factor in decreasing radical-induced aging and cancer [51]. An age-related decrease in urate oxidase activity should lead to an increase in uric acid levels and a lower production of H_2O_2. As a result, less damage related to urate oxidase mediated-H_2O_2 generation would be expected, with a concomitant increased antioxidant status. On the other hand, an increase in urate oxidase activity would lead to an increase in damage caused by H_2O_2 with a concomitant lower antioxidant status. One report described an increase in uric acid levels in striatal synaptosomes in aged rats compared with young [52], suggesting a lower urate oxidase activity. However, the same study demonstrated that total striatal uric acid levels are decreased in these same aged rats. The way urate oxidase activity is affected by aging remains to be clarified, as contradictory data exist on this point. However, whether urate oxidase is decreased or increased during aging represents a minor point in the field of human aging, since urate oxidase is not expressed in humans [53].

D-Amino acid oxidase activity was reported to be increased in the liver of 26-month-old Wistar rats as compared with 14-month-old animals [54]. This supports the hypothesis of an aging-related increase in the levels of free radicals generated within the peroxisome. It also emphasizes that additional studies on all the peroxisomal H_2O_2-producing enzymes are required, to clearly determine if there is any change in the peroxisomal free-radical status, and to give arguments to the oxidative stress hypothesis proposed by Masters and Crane [11].

Based on available data, it is difficult to provide a definitive assessment of whether the aging process affects the peroxisomal free-radical status, since many peroxisomal oxidases remain to be studied as a function of aging. It is recommended that future work on the peroxisomal free-radical status during aging include data on both the free-radical generating and degrading pathways [25].

Peroxisomal β-oxidation, fatty acids, membrane composition and aging

In addition to oxidases and free-radical scavenging enzymes, peroxisomes have unique biochemical pathways

involved in various lipid metabolic processes. An aging-related decrease in peroxisomal lipid metabolism is likely to affect the peroxisomal β-oxidation pathway [38], specific for VLCFA that are poorly oxidized by mitochondria [33], DHA synthesis [35] and plasmalogen biosynthesis [57]. The following paragraphs describe these metabolic pathways, the importance of these lipids in health, the current data on aging-related changes in these peroxisomal metabolic pathways and their relevance for the aging process.

Peroxisomal β-oxidation. One of the main physiological roles of peroxisomal β-oxidation is to prevent the accumulation of VLCFA within the organism. These fatty acids are toxic for the cell and for the organism. VLCFA accumulation is the hallmark of all peroxisomal disorders affecting the β-oxidation pathway and is associated with severe neurological disorders [27, 28, 64, 65].

The oxidation of fatty acids takes place in both mitochondria and peroxisomes. Though each organelle oxidizes fatty acids through a similar β-oxidation system, the peroxisomal β-oxidation pathway (fig. 1) differs at many points [58]. Peroxisomal β-oxidation shows a specificity for the VLCFA (chain length of 20 and more carbon atoms). These fatty acids are preferentially, if not exclusively, oxidized within the peroxisomes. Several factors explain this difference, the main one being a lack of acyl-CoA synthetase specific for VLCFA in mitochondria [59]. Unable to activate the VLCFA (see fig. 1), the mitochondria are unable to metabolize them.

The unsaturation index influences the rate of fatty acid oxidation by peroxisomes, polyunsaturated fatty acids being oxidized faster than the corresponding saturated fatty acids [25, 26, 60, 61]. Additionally, the position of the first carbon-carbon double bond from the carboxylic function of the fatty acid influences the oxidation rate [61]. The combination of these two properties (chain length and unsaturation specificity) gives peroxisomes a wider range of action regarding fatty acid oxidation than mitochondria.

Peroxisomal β-oxidation, however, is not as efficient as its mitochondrial counterpart for fatty acid oxidation and is usually referred to as not going to completion. Whereas the mitochondrial β-oxidation is a complete oxidative pathway, degrading a given fatty acid into its corresponding amount of acetyl-CoA units, the peroxisomal β-oxidation is usually considered a chain-shortening pathway, permitting only a few cycles of β-oxidation for a given fatty acid. The exact number of cycles completed by the peroxisomal β-oxidation still remains to be clarified [62, 63]. It is likely that many factors such as chain length, unsaturation index, current metabolic state of the cell and competition between different fatty acids all influence the number of β-oxidation cycles in vivo.

The chain-shortening property of peroxisomal β-oxidation unexpectedly results in an anabolic process: the terminal step in DHA biosynthesis [35]. This fatty acid is of major importance for the structure and function of the retina and brain membranes. Data from studies of developing rats given a diet restricted in n-3 fatty acids show DHA-deficient membranes as well as alterations in biochemical, toxicological and neurological parameters [66]. Retinal response to light has been reported to be 10 times lower in n-3 deficient rats [66] and fish [67]. Sensitivity to neurotoxins was also increased, scores in learning task tests were dramatically decreased, and Na^+, K^+-adenosine triphosphatase (ATPase) activity was also altered in n-3 deficient rats [68].

The exact biosynthetic pathway of DHA was until recently a question of debate. The biosynthesis of all PUFA occurs mainly in the endoplasmic reticulum, where two different sets of enzymes, desaturases and elongases, metabolize PUFA precursors, linoleic acid and α-linolenic acid, to eventually produce PUFA with longer chain lengths such as arachidonic (ARA or 20:4n-6) acid and eicosapentaenoic acid (EPA or 20:5n-3). Desaturases add a carbon-carbon double bond to the fatty acid carbon backbone, resulting in an increase in the unsaturation index, whereas elongases add an acetyl residue to the fatty acid carbon backbone, resulting in an increase in the fatty acid chain length. Although it was initially believed that DHA synthesis occurs from EPA by elongation and Δ4-desaturation, it has recently been shown that the final step is peroxisomal and proceeds through retroconversion of 24:6n-3. This latter fatty acid results from the elongation of EPA to 24:5n-3 followed by Δ6-desaturation [34]. This finding explains why a severe deficiency in DHA occurs in patients suffering from some peroxisomal disorders [31]. These recent data on DHA synthesis [35] have led to a complete reevaluation of the pathway for PUFA synthesis [69], and they have established the existence of a close collaboration between the endoplasmic reticulum and peroxisomes in the determination and maintenance of membrane lipid composition [70].

In humans, all fatty acids can be synthesized de novo except fatty acids from the n-6 and n-3 series. These n-6 and n-3 unsaturated fatty acids are called essential fatty acids because they are absolutely required for the biosynthesis of longer n-6 and n-3 fatty acids. Both 18:2n-6 and 18:3n-3 must be provided by the diet in order to avoid any deficiencies in ARA, EPA or DHA. As in rodents and fish, in humans a deficiency in n-3 fatty acids precursors results in retinal and neurological impairments [66–68, 71, 72]. Interestingly, each fatty acid family is metabolically independent, so that n-6 fatty acids cannot be used to generate n-3 fatty acids.

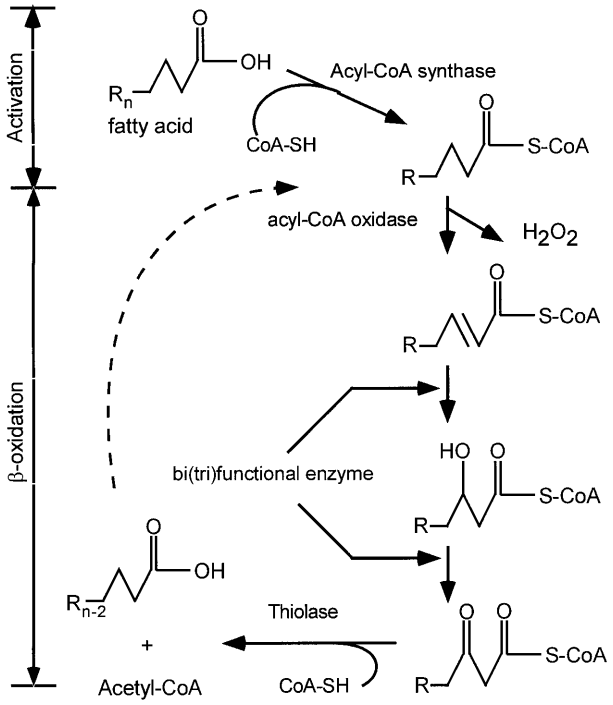

Figure 1. The oxidation of fatty acids by peroxisomes: activation step and subsequent β-oxidation cycle.

The key enzyme in n-6 and n-3 fatty acid synthesis is the Δ6-desaturase. There is evidence of an age-related decrease in Δ6-desaturase activity in rodent liver [73, 74]. This finding led to the proposal that a decrease in Δ6-desaturase activity might produce an impairment in PUFA synthesis and subsequently modify cell membrane PUFA composition.

Another report that examined Δ6-desaturase activity in mouse liver throughout the entire life span [75] showed that Δ6-desaturase displays distinct patterns of activity during development, adulthood and aging. Adult animals showed no change in activity until they were 300 days old. Thereafter, Δ6-desaturase activity declined, suggesting that the aging-related decrease in Δ6-desaturase activity starts later but proceeds faster than previously expected [73, 74].

This detailed report of changes in Δ6-desaturase activity as a function of age [75] was followed by a similar study of catalase and peroxisomal β-oxidation activity for various 18-carbon fatty acids in mouse liver [25]. This showed that liver peroxisomal activities present a pattern of activity similar to that of Δ6-desaturase activity. This reinforced the proposition of a tight collaboration between peroxisomes and endoplasmic reticulum in lipid metabolism [70]. Morphological data which always show colocalization of peroxisomes and endoplasmic reticulum support this concept [76]. The spatial association of peroxisomes with the endoplasmic reticulum led to the proposition that peroxisomes are of microsomal origin [77], but later studies ruled out this hypothesis [78–80].

Peroxisomal β-oxidation, membrane composition and aging. Since both peroxisomal β-oxidation [25, 47] and endoplasmic reticulum Δ6-desaturase [73–75] activity decline as a function of aging, the resultant balance between PUFA synthesis and degradation was hypothesized to be qualitatively but not quantitatively preserved during aging [25]. However, supporting data for this hypothesis come from separate experiments carried out either in rats [73, 74] or mice [25, 26, 47, 75]; a study measuring both parameters as a function of age in the same animal model may help to confirm this hypothesis.

Currently available data have led to the proposition that aging affects endoplasmic reticulum PUFA synthesis and peroxisomal β-oxidation in quantitative rather than qualitative terms. The earlier hypothesis that the age-related decrease in Δ6-desaturase activity causes unbalanced PUFA homeostasis [74] is less likely since there are now data showing a similar aging-related decrease in peroxisomal activity [25, 47]. On the other hand, since both endoplasmic reticulum and peroxisome metabolism are less able to metabolize a given amount of fatty acid, dietary fat intake may overwhelm or bypass the endoplasmic reticulum and peroxisome system more easily and lead to changes in membrane lipid composition. This suggests that dietary intake of fat should be adjusted so as to remain within the metabolic capacity of the endoplasmic reticulum-peroxisomal system metabolic capability [25]. Some of the beneficial effects of dietary restriction may be explained by such a mechanism [81–85]. It has been demonstrated that dietary restriction helps prevent age-related membrane peroxidative deterioration as well as age-related changes in membrane fatty acid composition [81–83]. Figure 2 illustrates the effects of an age-related decline in peroxisomal activity on membrane lipid composition and function. In young animals peroxisomal function is optimum and results in an efficient degradation of toxic VLCFA (large gray arrow) and the biosynthesis of sufficient amounts of DHA and plasmalogens (large white arrow). This permits cells to create and maintain optimum membrane lipid composition. Subsequently, membrane-associated functions, like membrane receptor signal transduction activity, ion-channel function and enzymatic activities are optimum. An aging-related decrease in peroxisomal activity (fig. 2, ①) will affect the ability of the cell to eliminate VLCFA and will allow these VLCFA to accumulate in membranes. This, in addition to the decrease in endoplasmic reticulum function, will lead to insufficient synthesis of DHA and

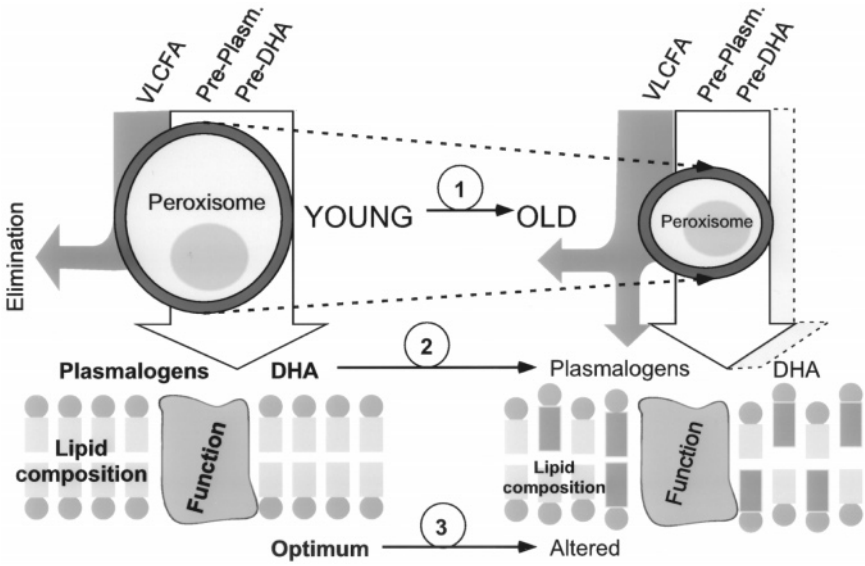

Figure 2. Effect of aging on peroxisomal activity and its impact on membrane lipid composition and function.

plasmalogens in old animals (fig. 2, ②). This is likely to be detrimental for both membrane lipid composition and function (fig. 2, ③). It was previously proposed that changes in membrane lipid composition may be responsible for the changes in membrane-associated receptor activity occurring in aging and in aging-related degenerative diseases such as Alzheimer's disease [86]. Beneficial effects may result by adjusting the diet with respect to the capacity of the endoplasmic reticulum-peroxisome system, which means lower VLCFA intake and increased DHA and plasmalogen intake. In this regard, dietary restriction would help prevent the accumulation of VLCFA (as well as the increase in membrane cholesterol). It is also possible that dietary restriction may help to maintain peroxisomal function. This point remains to be addressed, since there are currently no data available on the effect of dietary restriction on peroxisomal activities.

The following paragraphs describe the importance of VLCFA, DHA and plasmalogens for membrane lipid composition and function and thus emphasize the critical role of peroxisomes in cell function and the impact of an aging-related decrease in peroxisomal function on health.

VLCFA, membrane composition and function. One unique role of peroxisomes is to oxidize toxic saturated and monounsaturated VLCFA. These fatty acids are provided to the organism by diet and endogenous synthesis in the endoplasmic reticulum. As previously emphasized, mitochondria are unable to activate VLCFA due to a lack of acyl-CoA synthetase specific for VLCFA. In contrast, both endoplasmic reticulum and peroxisomes possess such an enzyme [87].

The decrease in peroxisomal acyl-CoA oxidase activity and by extension in peroxisomal β-oxidation activity [26, 46, 47] is expected to lead to a decrease in VLCFA breakdown and consequently an accumulation of these fatty acids in the body. This is important, since hexacosanoic acid (the typical VLCFA) has been shown to increase membrane disorders [88] and to remain tightly associated with membranes: its rate of release from model membranes has been calculated to be 10^5–10^6 times slower than that of palmitic acid [88, 89]. Accumulation of hexacosanoic acid has also been associated with increased membrane microviscosity [90] and a decrease in basal and adrenocorticotropic hormone (ACTH)-stimulated cortisol-release in human adrenocortical cells [91].

There are reports of age-related increases in membrane cholesterol content and changes in membrane unsaturation [32, 92]. The aging-related decrease in peroxisomal activity may alter the unsaturation index of membranes. Since in peroxisomal diseases VLCFA have been shown to accumulate in the cholesterol-ester fraction [27], an aging-related accumulation of VLCFA resulting from the aging-related decrease in peroxisomal β-oxidation is also likely to occur in the cholesterol-ester fraction.

Accumulation of VLCFA in peroxisomal diseases leads to an increase in the cholesterol content [27], and it has been shown that there is an increase in the cholesterol-to-phospholipid ratio during aging [32]. However, to the extent of our knowledge, there are no available data on brain lipid composition for the cholesterol-ester fraction or VLCFA as a function of aging. If such a process does occur in the aging brain, alterations in myelin structure and/or a demyelination process might also

occur. Alterations in myelin structure [93] along with myelin degeneration [94] have been observed in the brain of old monkeys. The relevance of these changes in age-related degenerative diseases is speculative, but Svennerholm and Gottfries studied membrane lipids in the brain of Alzheimer's disease patients and their data suggest a significant loss of myelin lipids is a primary event in the late-onset form [95].

PUFA, membrane composition and function. The aging-related decrease in acyl-CoA oxidase activity has been correlated with a decrease in peroxisomal degradation of both ARA and DHA, a finding which reinforces the key role of acyl-CoA oxidase in the peroxisomal β-oxidation pathway [26]. It has been hypothesized that the aging-related decrease in membrane unsaturation is due to the different effects of aging on acyl-CoA oxidase (decreased) and trifunctional enzyme (increased) [46]. Since the latter enzyme possesses an isomerase activity (Δ3,Δ2-enoyl-CoA isomerase) required for the degradation of some unsaturated fatty acids, the age-related increase in this enzyme would suggest that there might be an increase in degradation of unsaturated fatty acids [46]. However, the extent of the aging-related decrease in the β-oxidation of various 18-carbon fatty acids (stearic, oleic, linoleic and α-linolenic) appears to be similar in mice [25]. The technique used to measure the β-oxidation activity in this study was designed to estimate the rate of [1-^{14}C]-acetate release by peroxisomal β-oxidation using specific [1-^{14}C]-fatty acids as substrate. It did not permit the estimation of the rate of β-oxidation after removal of the first acetyl group. Therefore, the estimated degradation rate obtained by this technique provided no information on the Δ3,Δ2-enoyl-CoA isomerase function of the trifunctional enzyme. This leaves open the possibility that the age-related increase in trifunctional enzyme leads to an increase in PUFA breakdown [46]. However, peroxisomal β-oxidation of ARA and DHA was also studied as a function of aging [26], and whereas ARA does not require the isomerase activity for the removal of its first double bond, DHA does. The release of the first acetyl-CoA residue from this fatty acid involves acyl-CoA oxidase and yields 2,4-docososahexadienoyl-CoA. This is metabolized by a 2,4-dienoyl-CoA reductase to give 3-docosahexaenoyl-CoA, which is further metabolized to 2-docosahexaenoyl-CoA by Δ3,Δ2-enoyl-CoA isomerase activity. 2-Docosahexaenoyl-CoA then undergoes a complete cycle of peroxisomal β-oxidation, resulting in the release of one [1-^{14}C]-acetyl-CoA residue. Despite this difference between ARA and DHA, β-oxidation of these two fatty acids shows a similar decrease during aging. This suggests that acyl-CoA oxidase activity is the limiting step in the degradation of unsaturated fatty acids and that the relative increase in trifunctional enzyme in peroxisomes from old rat liver [46] has no or little effect on the rate of PUFA oxidation.

Since the production of 22:6n-3 has been demonstrated to be dependent on peroxisomal β-oxidation [26], the observed decrease in both peroxisomal fatty acid degradation and acyl-CoA oxidase activity may contribute to an aging-related decrease in 22:6n-3 synthesis and consequently to an aging-related decrease in membrane DHA content. A major component of retina and brain membranes [96, 97], DHA is of major importance for retina function [98, 66]. It has been hypothesized that DHA-containing phospholipids create a specific microenvironment required for rhodopsin function [99]. In the developing animal, 22:6n-3 is supplied to the retina by the liver [100]. However, synthesis of n-3 fatty acids like 24:5n-3, an intermediate in DHA synthesis, has also been demonstrated in retina [101]. There is a decrease in DHA content in retinal membranes during aging [102], and this decrease is not due to an impairment in phospholipid turnover or a decrease in the rate of incorporation of DHA into membranes. This parameter, in fact, has been shown to be increased during aging [102]. Taken together, these results suggest that the age-related decrease in retinal n-3 PUFA content may be due to a decrease in DHA synthesis. An aging-related defect in Δ4-desaturase activity [102] was first hypothesized. Because it is now well established that the production of DHA requires peroxisomes [35], retinal tissue is likely to exhibit aging-related changes generated by a defective peroxisomal β-oxidation.

DHA has been shown to be involved in cholinergic signal transduction at the synaptic level [103]. Therefore, any change in DHA content in the synaptic membrane would be likely to influence the efficiency of cholinergic neurotransmission, which is involved in learning and in particular in memory processes [104]. As noted previously, evidence for this essential role of DHA comes from studies of dietary n-3 fatty acid deficiencies in animals [66] and humans [71, 72] as well as in peroxisomal diseases [31]. Moreover, DHA supplementation has been found to reverse some of the symptoms associated with Zellweger syndrome [36]. The primary improvement resulting from the DHA therapy was in visual function. Some of the DHA-treated patients have also shown improved muscular tone and reduced hepatomegaly. The aging-related decrease in peroxisomal activity is likely to result in a decrease in the DHA content in excitable membranes, such as in retina [102], and therefore a decrease in membrane-receptor signal transduction activity. This supports the hypothesis of membrane alterations as causes of impaired signal transduction in aging and in aging-related diseases [86], but it also supports the idea that aging-related decreases in peroxisomal lipid metabolism are likely to alter mem-

brane lipid composition and function, and have a significant impact on the aging process [25].

At the biochemical level, the strict monitoring of PUFA intake in these patients leads to a decrease in plasma VLCFA and an increase in plasma DHA content. DHA therapy of Zellweger patients also led to an increase in erythrocyte plasmalogen levels in these patients. This highlights the interrelationship between the different products of peroxisomal function. Indeed, some plasmalogens are particularly enriched in DHA [105], and it has been shown that ether phospholipids are selectively acylated with DHA [106].

Plasmalogen synthesis, membrane composition and aging. Plasmalogens are ether phospholipids. The etherlipid synthesis pathway involves both the endoplasmic reticulum and peroxisomes, but plasmalogen synthesis is dependent on peroxisomal function [57]. The finding of plasmalogen deficiency in different peroxisomal diseases argues for the essential role of peroxisomes in plasmalogen synthesis [4, 29]. The exclusive localization of some enzymes involved in plasmalogen synthesis (including the key enzyme DHAP-AT) within the peroxisome provides definitive evidence [107]. However, the exact function of plasmalogens is still unknown. It appears that they are important for excitable membranes, since they are present in large amounts in both synapses and myelin. It has been proposed that plasmalogens may have an antioxidant role, since they have been shown to protect lipoproteins from free radicals [108]. Another report has suggested that plasmalogens facilitate membrane fusion [109]. This later point is of particular interest, since plasmalogens are a major constituent of synaptic membranes. Plasmalogens also interact closely with DHA, as demonstrated by both the beneficial effect of DHA therapy on plasmalogen levels in Zellweger patients [36] and the preferential acylation of ether lipids by DHA [106]. As mentionned previously, DHA is involved in cholinergic signal transduction [103], and the relationship of plasmalogens with DHA is likely to reflect an involvement of plasmalogens in signal transduction as well. This is not surprising, since it has been shown that hydrolysis of plasmalogens is involved in the synthesis of platelet-activating factor [110]. The role of plasmalogens in signal transduction was recently reviewed by Farooqui and co-workers. [111]. An aging-related decrease in peroxisomal function would be expected to impair the homeostasis of both DHA and plasmalogens and signal transduction pathways associated with these lipids, particularly muscarinic cholinergic signal transduction. It is interesting to note that a specific decrease in plasmalogens was characterized in patients with Alzheimer's disease [112]. However, no biochemical data exist on plasmalogen biosynthesis activity during aging to support the decrease in plasmalogen content reported in Alzheimer's patients. These crucial data are still required.

Conclusions and perspectives

A growing body of evidence from animal and human studies suggests that an aging-related decrease in peroxisomal function may play a role in mammalian aging processes. It is likely that the role of peroxisomes in aging is a result of complex interactions between changes in lipid metabolism and oxidative stress. One can speculate that aging-related changes in peroxisomal activity could influence the organism in a way similar to peroxisomal diseases, though with milder functional consequences.

The conceptual similarity between the impact of peroxisomal changes on aging and peroxisomal diseases can be demonstrated by morphological observations. It has been shown that in patients with pseudo-neonatal adrenoleukodystrophy (ps-NALD), a peroxisomal disease characterized by a specific defect for acyl-CoA oxidase, peroxisomes are larger than from healthy individuals [113]. In peroxisomes from old rats, Fahimi and co-workers made the double observation of a decrease in acyl-CoA oxidase and an increase in the size of peroxisomes [46].

Additional points support a significant role of peroxisomes in the aging process. The membrane cholesterol content has been shown to be increased with age [32], and peroxisomes are involved in cholesterol synthesis [114]. Unfortunately, no data are available on peroxisomal 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity during aging, but one study using a Chinese hamster ovary (CHO) cell line deficient in peroxisomes revealed increased rates of cholesterol synthesis [115]. Thus, aging-related changes in the peroxisomal function may contribute to increased cholesterol content in membranes. In this regard results obtained by Theda and co-workers have shown that the incorporation of VLCFA in the cholesterol-ester fraction occurring in peroxisomal disease may represent a protective process for the cell [116]. As a result, membrane cholesterol content is increased, and it has been shown that an increase in membrane cholesterol affects muscarinic cholinergic signal transduction in rat brain synaptosomes [117].

In conclusion, the impact of peroxisomes in the aging process requires further study. Taken together, the data presented in this review suggest that peroxisomal function may have a significant role in aging and aging-related degenerative diseases. It is our opinion that the impact of peroxisomes on aging involves a complex interaction between changes in oxidative stress and lipid metabolism.

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