showed that the production of free radicals was not substantially higher than in age-matched controls, despite accumulated mitochondrial mutations.[93] Although the damage to mtDNA does not appear to be decisive, senescence-associated rises in free radical formation are observed, which should be rather attributed to a higher frequency of partial ETC blockades.

Several actions of melatonin seem to reduce the aging-related increases in mitochondrial free radical generation. Melatonin is capable of efficiently scavenging free radicals of higher reactivity, in particular, •OH[94–96] and CO_3•$^-$,[97] but poorly interacts directly, in the absence of catalysts, with O_2•$^-$.[98] The real contribution of direct scavenging remains, however, to be convincingly determined with regard to physiologically available melatonin levels, even though melatonin attains higher levels in mitochondria than in the circulation.[5] The reduction of electron leakage by melatonin and, thus, of free radical formation does not require high concentrations and may be more important than direct scavenging.[77] A high-affinity binding site for melatonin located in the amphipathic ramp of Complex I has been assumed to modulate electron flux and to reduce electron backflow.[77] The avoidance of secondary bottlenecks at other respirasomes such as Complexes III and IV, which can be caused by protein nitrosation, nitration and oxidation, is thought to be another means of protecting mitochondria. Notably, the kynuric melatonin metabolites N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) and N^1-acetyl-5-methoxykynuramine (AMK) (Figure 19.1) may contribute to the anti-oxidative and anti-nitrosative protection. AFMK and, even more potently, AMK act as scavengers of oxygen free-radicals.[99,100] AMK is also an efficient scavenger of •NO and its congeners as well as of •NO_2, and forms a stable nitrosation product, 3-acetamidomethyl-6-methoxycinnolinone.[101–103] Moreover, it is a potent inhibitor of neuronal nitric oxide synthase (nNOS) and down-regulator of inducible NOS (iNOS), including its mitochondrially located subform.[104–106] The formation of these metabolites, which are poorly apparent in the circulation, is nevertheless relevant to mitochondria, in which melatonin is metabolized to these kynuramines.[107] However, most of the published evidence on respirasomal protection has been obtained under conditions of high-grade inflammation and oxidative stress, but only rarely in the context of aging.[77] On the other hand, the fact that only nanomolar concentrations of melatonin or AMK were needed to enhance activities of Complexes I and IV as well as ATP synthesis[108,109] speaks for a physiological role of melatonin that can be expected to decline during its senescence-associated decrease.

Other actions of melatonin connect mitochondrial protection to prevention of apoptosis. The frequently observed up-regulation of anti-apoptotic factors such as Bcl-2 or Bcl-xL and down-regulation of pro-apoptotic factors such as Bax and Bad[110] represent changes that may halt the progression towards apoptosome formation and activation of caspases. Moreover, melatonin is capable of counter-acting early causes of apoptosis initiation as well. A direct inhibition of the mtPTP opening was described, with an IC_{50} of 0.8 μM.[111] Meanwhile, another potentially important finding has been obtained concerning the duration of the mtPTP opening. So-called "superoxide