## Special | Reactive Oxygen Species

# Recent reassessment of the role of reactive oxygen species (ROS)

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## Summary

Reactive oxygen species (ROS) are closely related to pathogenic age-associated diseases, such as arteriosclerosis, diabetes and cancer. Here, important macromolecules, such as proteins, lipids or genetic information in the form of DNA, are irreversibly altered. Apart from the intensively investigated negative properties of ROS, recent studies show that these molecules have signaling functions and that a balance must be maintained between the formation of ROS and the elimination by the antioxidative enzymes and antioxidants. This implies that an excess in ROS causes damage to the organism. However, complete elimination of ROS inhibits the organism's capacity to react to external stress factors in the form of an adaptive stress response. This research is described here under the term hormesis or mitohormesis and a re-evaluation of reactive oxygen species is carried out.

**Keywords:** reactive oxygen species (ROS), antioxidants, calorie restriction, ageing, extended life span, physical exercise, mitohormesis, hormesis

## Introduction

The use of oxygen to oxidize macro-molecules as part of an aerobic metabolism is considered a basic pre-requisite for the formation of higher life forms. The transformation of nutritional energy to the available cellular energy equivalents (for example ATP) is much more efficient here than for the oxygen-free/anaerobic generation of energy. Numerous biological processes can thus be supplied with sufficient energy, supporting cellular energy homeostasis.

However, the use of oxygen leads to the formation of so-called reactive oxygen species (ROS). Due to their highly reactive nature, ROS are considered to be potentially toxic molecules capable of damaging cellular components and thus restricting their function. The discovery that cellular damage accumulates throughout life and ultimately leads to the death of the cell or organism, as well as the fact that ROS can cause damage, leads to the assumption that there is a causal relation. Accordingly, numerous studies consider ROS formation to be one possible cause for the occurrence of age-associated diseases, as well as for the ageing process per se. This research has led to the wide-spread opinion that ROS are invariably damaging and that reduced occurrence or intervention is associated with positive health effects.

Nevertheless, recent, largely controversial research in this area shows that this relationship does not al-ways apply. On the contrary, in recent years there has been a change of opinion in relation to the role of ROS under physiological conditions.
The purpose of this article is therefore to elucidate the current results in this area and to describe the physiological role of ROS in greater detail.

## What are ROS?

ROS are instable oxygen compounds characterized by high reactivity and therefore regarded as an activated form of oxygen [1]. They belong in part to the free radicals and result primarily from the transfer of an electron to molecular oxygen [2]. The resulting superoxide radical is quantitatively the most important ROS molecule [3]. Other ROS, such as hydrogen peroxide or the particularly reactive hydroxyl radical, may be formed in follow-on reactions. Furthermore, so-called singlet oxygen also belongs to the ROS group. Singlet oxygen describes a state of oxygen which is significantly more reactive than normal oxygen.
Due to their high reactivity, ROS are capable of causing damage to numerous biological molecules [4]. For example, the genetic substance DNA is susceptible to ROS related damage. Here, ROS can cause strand breakage and mutations. Lipid oxidation, i.e. the oxidative modification of lipids, can lead to extensive damage of structure generating unsaturated fatty acids in biological membranes.

## Cellular defense mechanisms

In order to combat the potential damage due to the presence of ROS, cells have manifold, highly effective defense mechanisms. These can be grouped into enzymatic and non-enzymatic mechanisms. Enzymatic detoxification includes all endogenous enzymes which eliminate ROS or play a role in eliminating the damage caused by the ROS. The most important examples are superoxide dismutase (SOD), catalase (CAT), glutathionperoxidase (GPx) and glutathione S-transferase. Non-enzymatic defense is comprised of numerous anti-oxidative molecules, such as uric acid, bilirubin, ascorbic acid (vitamin C), α tocopherol (vitamin E) and glutathione [2, 5].

## Oxidative stress

Under physiological conditions, the formation and elimination of ROS is in equilibrium. However, if there is an imbalance (that is if the equilibrium between formation and degradation shifts in the direction of the generative process resulting in alterations to redox signaling pathways and the occurrence of molecular damage) this leads to oxidative stress [6, 7]. It is generally known that long term exposure to oxidative stress has pathological consequences and thus correlates with an increased risk of disease and death [3, 8]. One example of this is the occurrence of oxidatively modified LDL molecules which are significantly involved in the occurrence of vascular diseases such as arteriosclerosis [9–11].

## Where do ROS occur?

The energy providing metabolism has been identified as one of the main sources of intracellular ROS [12]. In particular, it could be shown that in mitochondria, which are also described as the powerhouse of the cell, under physiological conditions unpaired electrons are released during the generation of cellular energy (i. e. ATP). The subsequent transfer of these electrons to oxygen leads, as already elaborated, to the formation of ROS (superoxide radical), so to say as a by-product of the aerobic mitochondrial metabolism [13].

**Quantitatively speaking, approximately 0.2–2 % of the overall oxygen consumed is transformed into ROS [14], corresponding to roughly 90 % of the overall amount of intracellular ROS [15].**

Furthermore, granulocytes (white blood cells) form a targeted ROS defense against bacteria [2] by means of NADPH oxidase. The release of superoxide radicals resulting from an infection is considered, among others, to be an atherogenic risk factor. In addition to the endogenous energy metabolism, ROS also result as a by-product of enzymatic reactions (for example in the microsomal ethanol-oxidizing system [16]) and through environmental influences, such as environmental toxins, cigarette smoke, UV light, X-rays and Gamma rays [17].

## The significance of ROS in the ageing process

### Mitochondrial free-radical theory of aging (FRTA/MFRTA)

Ageing/senescence (lat. *senescere* 'ageing') of an organism is understood as a progressive process leading to a general restriction or reduction of cellular and systemic function [18].

One of the most important theories about the process of ageing is the free radical theory of ageing presented by Denham HARMAN in the 1950s and 1970s (FRTA), and the mitochondrial free radical theory of ageing (MFRTA) [19, 20]. According to this, the ROS continuously produced in metabolic processes cause permanent cellular damage, which manifests itself particularly in the form of modified proteins, lipids and nucleic acids. Over time, this damage accumulates and leads to the loss of cellular and systemic function and ultimately to the demise of the organism. In addition to this – according to HARMAN – the main source of ROS, the mitochondria, at the same time represent the main point of attack. In this vicious circle, oxidative damage to mitochondria not only reduces the capacity to generate energy, but also causes additionally rising ROS levels, in turn leading to further damage.

The discovery of the SOD enzyme in 1969 by J. M. McCORD and I. FRIDOVICH, which serves only as a defense mechanism against ROS, lends considerable credibility to HARMAN's theory [21]. In addition, the theory is based on experiments which show that the formation of ROS and the resulting damage increase with age [14]. Furthermore, numerous studies based on different models lead to the belief that there is a negative correlation between ROS formation and life span [22–29].

## Modification of the aerobic metabolism, calorie restriction and influence on ROS formation

Considering the assumption that ROS represent by-products of the aerobic metabolism, the reduction of the mitochondrial metabolism must consequently be accompanied by a decrease in overall ROS levels, and therefore have a positive effect on life span. This belief is based on the observation of Max RUBNER who postulated an inverse connection between the metabolic rate and life span [30].

The realization that energetic metabolism determines the life span was taken up again in the rate of living hypothesis [31]. In combination with FRTA, this hypothesis supplied a plausible explanation for observations in mice over the following years which showed that a reduction in calorie intake, that is the metabolizable macronutrients for the production of energy, increases longevity [32].

> **Calorie restriction (CR) refers to a reduction of the *ad libitum* calorie intake by 10–50 % without the occurrence of deficiency symptoms, such as a lack of vitamin supply or important trace elements. Up until now, CR is the only convincing intervention in relation to reducing age-associated diseases as well as increasing life span.**

Consequently, effects which extend life span have already been observed in simple organisms such as yeast fungus, worms, flies, mice and primates [33, 34].

It is not known whether CR can also extend the life span of humans. However, we do know that CR can positively influence risk factors for age-associated diseases [35–37]. Nevertheless, the hypothesis that reduced metabolic rate paired with reduced ROS formation is responsible for the positive characteristics of CR could not be confirmed in recent research [38, 39]. On the contrary, this indicated that CR increases the metabolic rate, observed in the form of increased mitochondrial cellular respiration and biogenesis [40–42]. Increased ROS formation could therefore also be measured on the basis of CR [43–45].

Furthermore, other indications contradict the hypothesis of a simple relationship between ROS formation and ageing. One example of this is the naked mole rat, which has a life expectancy of around 10 times longer than that of laboratory mice. Interestingly, it was determined that this long-living rodent demonstrated higher ROS formation as well as an increased level of oxidatively modified macromolecules [46, 47]. In addition, current studies on model organisms have shown that targeted mild ROS induction can extend life span [48–50].

Along with this, numerous – in part large-scale – human intervention studies have been unable to corroborate the expected positive influence of supplementation with antioxidants (i.e. natural or synthetic substances capable of eliminating ROS), as summarized elsewhere [51, 52]. Some of these studies even came to the conclusion that supplementation with antioxidants has a negative influence on health [53] and life span [54].

Overall, this new knowledge indicates that ROS, apart from their undisputed potentially damaging properties, also fulfill important physiological functions. On the one hand, this clearly demonstrates that increased ROS formation can have positive effects. On the other hand, there is a danger of damage to health through the reduction of ROS levels using antioxidant supplementation.

## ROS as important signaling molecules

An increasing number of studies in this field prove that ROS function as important signaling molecules which play an important role in the regulation of cellular processes [55]. They have typical properties which predestine them as signaling molecules. For example, certain ROS are capable of permeating biological membranes. Their intracellular concentration can be regulated (at the synthesis and elimination level) or depending on external stimulants. Furthermore, there are numerous functional cell components, known as target molecules, which they can activate or deactivate [3, 55]. Accordingly, it could be demonstrated that ROS are involved, for example, in the regulation of processes such as cellular growth (proliferation), inflammatory processes, glucose homeostasis, programmed cell death and the induction of defense mechanisms [43, 44, 56–60].

As shown in ◆Figure 1, ROS carry out different functions depending on their concentration. Under normal physiological conditions, that is in the absence of cellular stress, they serve to maintain cellular homeostasis. With increased concentration, however, for example in the event of a moderately stressful state, they play a decisive role in what is called the cellular stress response or adaptive response. Here, ROS function as stress signals, which trigger signal cascades through the activation of

numerous stress-activated protein kinases. This signal transmission ultimately terminates in the cell nucleus, where so-called transcription factors (for example, NRF–2, FoxO transcription factors, HSF1), bring about the expression of numerous genes associated with defense mechanisms [3, 61]. This high regulation of cellular defense systems, which not only incorporates the antioxidative protection systems, serves on the one hand to defend against initial stress and on the other increases the general stress resistance. This circumstance makes the cell more resistant against a number of external influences and also possibly against the ageing process.

The transcription factor NRF–2 is worthy of mention in this context. It is part of a conserved oxidative stress response, which means that it can also be found in other species [62]. NRF–2 is coupled with a repressor protein (KEAP-1) in the cytosol when in a state of rest, that is, outside the cell nucleus, whereas under stress this transcription factor is directly activated by the previously mentioned stress-activated protein kinases or by ROS. The bond to the repressor is triggered and NRF–2 moves into the nucleus. Here, it binds to the DNA and initiates the expression of numerous genes asso-

ciated with defense mechanisms, for example, antioxidative enzymes (SOD, catalase, GPx), redox regulators (thioredoxine, glutathion) or phase 2 detoxification enzymes (glutathion S-transferases [GSt], UDP glucuronosyl transferases [UDPGT]) [63–67].

The fact that ROS signaling molecules are capable of inducing stress resistance as a result of a complex adaptive response leads to the assumption that they could also influence life span. This assumption is well supported by extensive research which shows a correlation between longevity and increased stress resistance [8, 68–73].

Under very high conditions of stress, that is circumstances which are unfavorable for the maintenance of cellular functions, the result is an induction of substantial amounts of ROS. This results in massive damage to the cellular components and the demise of the cells, for example through apoptosis (programmed cell death).

## Hormesis/Mitohormesis

The idea that a potentially damaging substance can have a positive effect in low concentrations was first described by two German researchers in relation to the effect of medicines.
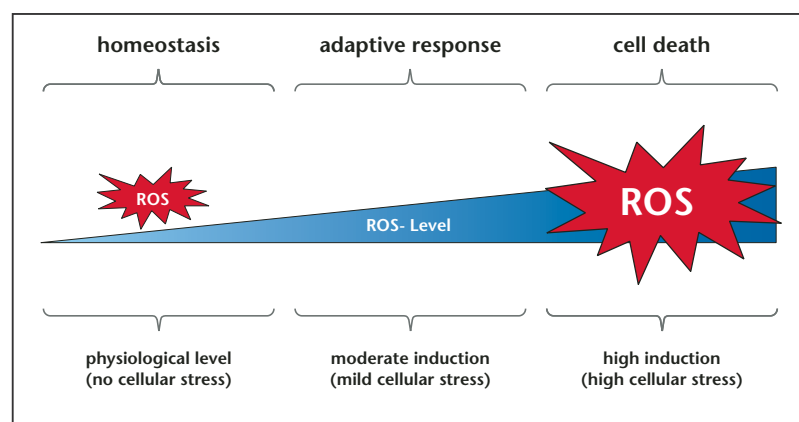


Fig. 1: **Different ROS functions as a function of concentration**
(modified after [55])
ROS = reactive oxygen species

The ARNDT-SCHULZ law that they postulated describes the possibility of a non-linear dose-effect relationship with pharmaceutical substances [74]. This implies that the effect of the substance in low concentrations is different from that in higher doses. The term hormesis was introduced to describe this non-linear dose-effect relationship [75].

> **Accordingly, hormesis is defined as a biphasic dose effect, with a potentially dangerous, non-lethal stressor in low concentrations resulting in a positive or stimulating effect, whereas with high concentrations inhibitory or toxic effects are observed.**

Here, the stimulating effect which occurs with low doses refers to the capability of cells in the organism to react to external signals. This enables their activation as a result of an adaptive response mechanism which renders them capable of surviving more severe stress influences [76, 77]. The assumption that mitochondrial ROS formation also involves a mild stressor serves as the basis for the formulation of the concept of mitochondrial hormesis, or *mitohormesis* [78]. In relation to the regulation of life span, the theory states that a slight increase in the mitochondrial ROS production (for example due to increased physical activity) may significantly extend life span through a secondary induction of defense mechanisms, whereas high mitochondrial ROS levels reduce life span [44, 51, 60]. As already described, the oxidative, mitochondrial metabolism permanently produces ROS. Here, increased mitochondrial activity is associated with increased ROS formation [43, 79–81]. Accordingly, mitohormetic mechanisms were observed, in particular, in life-prolonging interventions which accompany an increase in mitochondrial respiration, for example CR (see above) or moderate physical activity (see below) [53, 82].

## Nutrition and mitohormesis

In this respect, calorie restriction (CR) is a well described example. As already mentioned above, mitochondrial biogenesis is activated by CR, followed by an increase in mitochondrial respiration [38, 40–43, 83]. Furthermore, it was demonstrated long ago that CR contributes to increased stress defense in organisms [84–93]. Accordingly, CR reduces the risk factors for stress-associated diseases, such as diabetes mellitus type 2 and cardiovascular diseases [35–37]. Very recent investigations have now proven that increased mitochondrial ROS formation is responsible for increased stress resistance and thus also for the life-prolonging potential of CR [44, 45, 92, 93]. Consequently, it can be assumed that CR also represents an optimum intervention in humans for healthy ageing. However, as CR is tantamount to a permanent state of hunger, this intervention is not an option for the majority of the population.

Initial indications from work with model organisms allow the conclusion that similar effects could also possibly be achieved by varying the combination of macro-nutritional components (that is, the fractions of carbohydrates, proteins and lipids in the overall nutritional intake). Here it has been shown that glucose restriction extends the life span of model organisms through mitohormetic processes [40, 43, 94]. Conversely, an excess of glucose intake in worms led to decreased life span [43, 95, 96]. Furthermore, there are indications that diets low in carbohydrates decrease the risk of cardiovascular diseases in humans [97].

On the other hand, an increased supply of branched amino acids leads to mitochondrial biogenesis and increased longevity in mice [98].

However, no clear nutritional recommendations can yet be derived on the basis of this knowledge from work with model organisms. On the contrary, further studies on humans must define the role that of our nutritional composition and the optimal amount of, for example, carbohydrate intake.

A further, possibly nutritionally relevant aspect results from the observation that the phytochemicals found in fruit, vegetables and spices are capable of inducing cellular defense mechanisms via the activation of the NRF-2 path. Classic antioxidants are found under the compounds described as *hormetics or hormetines*, such as resveratol, curcumin and also isothiocyanate [99]. In pharmacological doses, some of these compounds offer life-prolonging potential, at least in model organisms [100, 101].

## Physical exercise and mitohormesis

Moderate physical activity is a generally recognized, health-promoting intervention known to reduce the risk of illness and death [102–107]. Here, regular physical activity leads to increased mitochondrial metabolics due to the high energy requirements and therefore to increased ROS formation and enhanced defense capacity [82, 102, 108–110]. Furthermore, it has been reported that the intake of antioxidative supplements parallel to physical exercise completely inhibits the health-promoting aspects [53, 111]. It therefore seems plausible that mitohormetic mechanisms mediate the positive effect of physical activity.

On the other hand, it is known that excessive training causes massive oxidative stress which may exceed the capacity of a positive hormetic response and is therefore associated with health problems [112].

# Conclusion

The function of reactive oxygen molecules in complex organisms is vastly more complex than previously realized. If the balance between ROS and the associated defense mechanisms is disturbed, classic age and nutrition influenced symptoms (diabetes mellitus, arteriosclerosis, cancer etc.) can result. However, in addition to the damaging effects of ROS, recent research has revealed properties indicating the importance of ROS as signal molecules, which are now in the focus of ROS research. An adaptive response to exogenous stress can only be imparted by the stress molecules themselves. Therefore, the objective of future research will be to identify and characterize every factor which induces an adaptive stress response. Candidates for this are physical activity, calorie restriction (for example specific dietary energy suppliers) and other food constituents, especially secondary plant substances.

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**Conflict of Interests**

The authors declare no conflict of interests according to the guidelines of the International Committee of Medical Journal Editors.

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