

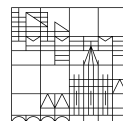
**Influence of ubiquinol/ubiquinone on energy
metabolism and DNA repair enzyme hOGG1 in
mitochondria of human skin fibroblasts**

**Dissertation zur Erlangung des
akademischen Grades eines Doktors der Naturwissenschaften
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"All our science, measured against reality, is primitive and childlike - and yet it's the most precious thing we have."

Albert Einstein

for my parents

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- Schniertshauer D, Gebhard D, Bergemann J. *A new Efficient Method for Measuring Oxygen Consumption Rate Directly ex vivo in Human Epidermal Biopsies. Bio Protoc.* 2019; DOI:10.21769/BioProtoc.3185.
- Schniertshauer D, Gebhard D, Bergemann J. *Age-Dependent Loss of Mitochondrial Function in Epithelial Tissue Can Be Reversed by Coenzyme Q₁₀. J Aging Res.* 2018; 2018:6354680.
- Schniertshauer D, Müller S, Mayr T, Sonntag T, Gebhard D, Bergemann J. *Accelerated Regeneration of ATP Level after Irradiation in Human Skin Fibroblasts by Coenzyme Q₁₀. Photochem Photobiol.* 2016; 92(3):488-94.
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Table of Contents

List of abbreviations.....	IX
Summary	XIII
Zusammenfassung	XV
1. Introduction.....	1
1.1 Anatomy and function of the human skin	1
1.1.1 Influence and protection of the skin against ultraviolet radiation	3
1.2 Mitochondrial function and genetics	4
1.2.1 Respiratory chain (oxidative phosphorylation)	6
1.2.2 Mitochondria in the process of ageing	8
1.3 Reactive oxygen species.....	9
1.3.1 Formation of ROS and oxidative stress	9
1.4 DNA damage.....	11
1.4.1 Oxidative DNA damage	12
1.4.2 7,8-dihydro-8-oxoguanine.....	13
1.5 DNA repair mechanisms.....	14
1.5.1 Direct DNA repair	15
1.5.2 Postreplicative repair (mismatch repair)	16
1.5.3 Nucleotide excision repair	16
1.5.4 Base excision repair	18
1.5.5 Mitochondrial DNA Repair	22
1.6 The human 8-oxoguanine DNA glycosylase 1.....	23
1.6.1 Gene localization and structure	24
1.6.2 Isoforms of hOGG1	25
1.6.3 DNA repair mechanism by hOGG1	26
1.7. Coenzyme Q ₁₀	29
1.7.1 Chemical composition and properties.....	29
1.7.2 Biosynthesis and intracellular transport	30
1.7.3 The role of coenzyme Q ₁₀ in the energy metabolism of eukaryotic cells ..	32
1.7.4 The role of coenzyme Q ₁₀ in the prevention of oxidative DNA damage....	33
1.8 Clinical relevance	34
1.9 Aims of this thesis	36
2. Materials	38
2.1 Antibiotics	38
2.2 Cell Culture.....	38

2.3 Cell Culture Medium	38
2.4 Chemicals and Reagents	39
2.5 Buffers and Solutions	42
2.6 Enzymes.....	45
2.7. Coenzyme Q ₁₀ formulations	45
2.8 Reporter oligonucleotides.....	45
2.9 Primer.....	46
2.10 Molecular Biological Kits.....	46
2.11 Laboratory Equipment	47
2.12 Consumables	49
2.13 Software	51
3. Methods.....	53
3.1 Cell culture	53
3.1.1 Sample collection / Ethical aspects	53
3.1.2 Isolation of skin fibroblasts	55
3.1.3 Culturing conditions	56
3.1.4 Subculturing.....	56
3.1.5 Cell number determination	57
3.1.6 Cryopreservation of Cells	57
3.1.7 Thawing of cryopreserved cells	58
3.2 Test compound supplementation	58
3.3 Irradiation of cells	59
3.4 Isolation and lysis of mitochondria.....	60
3.5 Cell viability assay	60
3.6 ATP bioluminescence assay	61
3.7 ROS- / mtROS-Assay.....	63
3.8 Mitochondrial membrane potential ($\Delta\psi_m$) measurements.....	64
3.9 Gene expression analysis	65
3.10 Mitochondrial respiration measurements - Sample preparation	66
3.10.1 Monolayer cell culture.....	66
3.10.2 Epidermis.....	66
3.11 Mitochondrial respiration measurements.....	68
3.12 Base excision repair assay.....	72
3.12.1 Reporter oligonucleotides.....	72
3.12.2 Measurement of base excision repair	72

3.13 Co-immunoprecipitation	75
3.14 Data analysis.....	75
3.15 Statistics.....	76
4. Results.....	77
4.1 Influence of ubiquinol on mitochondrial function and cellular ATP levels in human fibroblasts after UV irradiation	77
4.1.1 Key parameters of mitochondrial respiration in human fibroblasts under the influence of different concentrations of ubiquinol.....	77
4.1.2 Significance of individual coenzyme Q formulations for mitochondrial respiration parameters.....	79
4.1.3 Effect of ubiquinol on the mitochondrial respiration of fibroblasts after SSL-UVA irradiation	82
4.1.4 Effect of ubiquinol on cellular ATP level of fibroblasts after SSL-UVA irradiation.....	85
4.1.5 Influence of PARP inhibition on the ATP level of human Fibroblasts.....	86
4.1.6 Effect of ubiquinol on the mitochondrial membrane potential ($\Delta\psi_m$) of fibroblasts after SSL-UVA irradiation	88
4.2 Age-Dependent Loss of Mitochondrial Function in Epithelial Tissue Can Be Reversed by ubiquinol.....	91
4.2.1 Influence of donor age on mitochondrial parameter in epithelial tissue	91
4.2.2 Effect of ubiquinol on respiratory parameters in epithelial tissue	93
4.2.3 Influence of sampling location of biopsies on mitochondrial respiration ...	95
4.2.4 Influence of gender and smoking on the respiratory parameters in epithelial tissue	96
4.3 The activity of the DNA repair enzyme hOGG1 can be directly modulated by ubiquinol.....	98
4.3.1 Influence of ubiquinol and ubiquinone on hOGG1 enzymatic activity <i>in vitro</i>	98
4.3.2 Influence of ubiquinol on hOGG1 enzymatic activity isolated from mitochondria.....	100
4.3.3 Influence of ubiquinol on the formation of ROS and mitochondrial superoxide	101
4.3.4 Influence of redox potential on gene expression and the activity of hOGG1	103
4.3.5 Importance of calcium and magnesium for influencing the active centre of hOGG1 to increase its activity	106
4.3.6 Significance of individual structural elements of ubiquinol for hOGG1 activity	107

4.3.7 Bifunctionality of mitochondrial hOGG1 under the influence of ubiquinol	109
4.3.8 Detection of a direct hOGG1 / ubiquinol interaction by co-immunoprecipitation	111
5. Discussion	113
6. Conclusion	128
7 References	130
8. Appendix	XVII
8.1 Acknowledgement	XVII
8.2 Curriculum Vitae	XIX

List of abbreviations

-(d/dt)	first derivation of the fluorescence signal according to temperature.
$\Delta\psi_m$	mitochondrial membrane potential
5'-dRP	5'-deoxyribose phosphate
6-4PP	pyrimidine (6-4) pyrimidone photoproduct
6-FAM	6-carboxyfluorescein
8-oxoG	7, 8-dihydro-8-oxoguanine
A	adenine
acetyl-CoA	acetylated coenzyme A
ADP	adenosine diphosphate
AP	apurinic/apyrimidinic
APE1	AP endonuclease 1
ATP	adenosine triphosphate
BER	base excision repair
BHQ-1	black Hole Quencher-1
BSA	bovine serum albumin
C	cytosine
Ca ²⁺	calcium ion
CC	carrier control
cDNA	complementary deoxyribonucleic acid
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CN-8-oxoG	Chuck Norris-8-oxoguanine
CN-CTRL	Chuck Norris-control
CO ₂	carbon dioxide
CoIP	co-immunoprecipitation
CoQ	ubiquinone
CoQ ₉ (10)	coenzyme Q ₉ (10)
CoQH ₂	ubiquinol
CPD	cyclobutane pyrimidine dimer
CSA (B)	cockayne syndrome group A (B)
CTRL	control
Cu	copper
Cu ²⁺	copper ion
cyt c	cytochrome c
DDR	DNA damage response
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	1,4-dithiothreitol
ECAR	extracellular acidification rate
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid
ER	endoplasmic reticulum
ERCC1	excision repair cross complementing group 1
ETC	electron transport chain
EtOH	ethanol

F	female
FAD	flavin adenine dinucleotide
FADH ₂	hydroquinone form of the flavin adenine dinucleotide
FapyG	formamidopyrimidine-2'-deoxyguanosine
FBS	fetal bovine serum
FCCP	carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone
Fe	iron
FEN1	flap structure-specific endonuclease 1
Fe-S cluster	iron-sulfur cluster
FPP	farnesyl pyrophosphate
FRTA	free radical theory of ageing
MFRTA	mitochondrial free radical theory of ageing
G	guanine
GDH	glutamate dehydrogenase
GGR	global genome repair
GR	glutathione reductase
h	hour
H ⁺	proton
H ₂ O	water
H ₂ O ₂	hydrogen peroxide
HhH	helix-hairpin-helix
HMG-CoA reductase	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
hOGG1	human 8-oxoguanine DNA glycosylase 1
hOGG1-His	recombinant human OGG1 with a histidine-Tag
kb	kilobase
KCl	potassium chloride
kDa	kilodalton
KH ₂ PO ₄	potassium dihydrogen phosphate
LipDH	lipoamide dehydrogenase
LIG1	Ligase I
M	male
Mg ²⁺	magnesium ion
MGMT	O ⁶ -methylguanine methyltransferase
min	minute
MK-7	menaquinone-7, vitamin K2
MLH1	MutL homolog 1
mRNA	messenger ribonucleic acid
MSH2 (6)	MutS homolog 2 (6)
mtDNA	mitochondrial deoxyribonucleic acid
mtROS	mitochondrial reactive oxygen species
MTS	mitochondrial targeting sequence
mtSO	mitochondrial superoxide
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
MutL	mutator L
MutSa	mutator Sα
n	number of experiments carried out independently of each other
Na ₂ HPO ₄ ·12H ₂ O	disodium hydrogen phosphate dodecahydrate

NaCl	sodium chloride
NAD	nicotinamide adenine dinucleotide
NAD ⁺	oxidized form of nicotinamide adenine dinucleotide
NADH	1,4-dihydronicotinamide adenine dinucleotide
NaOH	sodium hydroxide
NC	negative control
NDUFB10	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10
NER	nucleotide excision repair
NLS	nuclear localisation sequence
ns	non-smoking
¹ O ₂	singlet oxygen
O ₂	oxygen
O ₂ ^{•-}	superoxide anion
OCR	oxygen consumption rate
OGG1	8-oxoguanine DNA glycosylase 1
•OH	hydroxyl radical
PAR	poly(ADP-ribose)
PARP1	poly (ADP-ribose) polymerase 1
PBS	phosphate-buffered saline
PC	positive control
PCNA	proliferating cell nuclear antigen
PGK1	phosphoglycerate kinase 1
P _i	inorganic phosphate
Pol β	polymerase β
PRR	postreplicative repair
qPCR	quantitative polymerase chain reaction
RFC	replication factor C
RNA	ribonucleic acid
RNAPII	RNA polymerase II
ROS	reactive oxygen species
RPA	replication protein A
RPL13A	ribosomal protein L13A
RPLP0	ribosomal protein, large, P0
rRNA	ribosomal ribonucleic acid
RT	room temperature
s	smoking
SDS	sodium dodecyl sulfate
SOD	superoxide dismutase
SRL	standard reaction solution
SSB	single strand breaks
SSL	simulated solar light
SSL _{6 (12)}	irradiation with 6 (12) J/cm ² UVA
T	thymine
TBHP	<i>tert</i> -Butyl hydroperoxide
TCR	transcription-coupled repair
TFIIH	transcription factor IIH
TIM	translocase of the inner membrane

TOM	translocase of the outer membrane
Tris-HCl	Tris hydrochloride
tRNA	transfer ribonucleic acid
TrxR-1	thioredoxin reductase 1
UCPs	uncoupling proteins
UV	ultraviolet
UVA (B, C)	UV-wavelength of group A (B, C)
XPA (B, C, D, F, G)	xeroderma pigmentosum complementation group A (B, C, D, F, G)
XRCC1	X-ray repair cross-complementing protein 1

Summary

Human skin is continuously exposed to a number of harmful substances, of which the ultraviolet portion of solar radiation is the most significant. UV-induced damage includes direct DNA lesions and oxidative damage to DNA, proteins and lipids caused by reactive oxygen species. Approximately 1.000 - 1.0×10^5 oxidative lesions occur daily in the cell, with one of the most common defects in this category being 7,8-dihydro-8-oxoguanine. Because of their role in oxidative phosphorylation and because mitochondria are the main site of production of reactive oxygen species in the cell, these organelles are particularly affected. Here, the above-mentioned exogenous influences can lead to an accumulation of reactive oxygen species as well as 7,8-dihydro-8-oxoguanine, which can ultimately lead to functional disorders within the electron transport chain and finally to mitochondrial dysfunctions. To counteract the accumulation of oxidative damage and the formation of reactive oxygen species, coenzyme Q₁₀ has been used for some time. Its redox-reactive character and its central role in the electron transport chain is the reason why this coenzyme is used in mitochondrial medicine for the treatment of age-related diseases. However, since the exact mechanisms of action are still unclear in many aspects, this work deals with the influence of coenzyme Q₁₀ on the energy metabolism after short-term induced damage via UV irradiation, long-term induced damage (age-related) as well as on DNA repair in human fibroblasts.

For this purpose, we analysed several mitochondrial parameters, including respiration, membrane potential and ATP levels in skin fibroblasts after UV irradiation. After treatment of the cells with coenzyme Q₁₀, an accelerated regeneration of the ATP level, a preservation of the membrane potential and thus a decrease in mitochondrial dysfunction was observed. In addition to short-term induced damage such as that caused by UV radiation, the effect of coenzyme Q₁₀ on age- and long-term induced damage was also investigated. Thus, for the first time *ex vivo* in the human epidermis, a decrease in mitochondrial respiration as well as ATP production could be shown with the age of the donor, which corresponds to the "mitochondrial theory of ageing". This decrease could be mitigated by coenzyme Q₁₀. Finally, attention was focused on human 8-oxoguanine DNA glycosylase 1, the repair enzyme for 7,8-dihydro-8-oxoguanine, under the influence of coenzyme Q₁₀. For the first time, a concentration-dependent increase in activity, a change in bifunctionality in

favor of increased N-glycosylase activity by coenzyme Q₁₀ and a direct interaction between 8-oxoguanine DNA glycosylase 1 and this coenzyme could be shown.

The results of this work suggest that the achieved effects of coenzyme Q₁₀ on mitochondrial function are mediated primarily by its antioxidant function and by increased activity of the electron transport chain. In particular, the preservation of the mitochondrial membrane potential seems to be of great importance. An increase in the activity of 8-oxoguanine DNA glycosylase 1 is probably due to the fact that coenzyme Q₁₀ contributes to the dissolution of an end product complex which is formed between the repair enzyme and the resulting unsaturated hydroxyl aldehyde at the 3' end and thus inhibits further enzymatic steps.

Based on this work, further interesting starting points for the use of coenzyme Q₁₀ as a drug for both preventive and acute treatment of numerous age-related diseases in the field of mitochondrial medicine have been identified.

Zusammenfassung

Die menschliche Haut ist ständig einer Reihe von schädlichen Stoffen ausgesetzt, von denen der ultraviolette Anteil der Sonnenstrahlung am bedeutendsten ist. Zu den UV-induzierten Schäden gehören direkte DNA-Läsionen sowie oxidative Schäden an DNA, Proteinen und Lipiden, welche durch reaktive Sauerstoffspezies verursacht werden. So kommt es täglich zu etwa. 1.000 - 100.000 oxidative Läsionen in der Zelle, wobei einer der häufigsten Defekte in dieser Kategorie das 7,8-Dihydro-8-Oxoguanin darstellt. Aufgrund ihrer Rolle bei der oxidativen Phosphorylierung und da die Mitochondrien der Hauptort der Erzeugung reaktiver Sauerstoffspezies in der Zelle darstellen, sind diese Organellen hiervon besonders betroffen. Hier kann es durch die genannten exogenen Einflüsse zu einer Akkumulation von reaktiven Sauerstoffspezies sowie 7,8-Dihydro-8-Oxoguanin kommen, was schließlich zu Funktionsstörungen innerhalb der Elektronentransportkette und letztendlich zu mitochondrialen Dysfunktionen führen kann. Um der Anhäufung von oxidativen Schäden und der Bildung von reaktiven Sauerstoffspezies entgegenzuwirken, wird seit geraumer Zeit Coenzym Q₁₀ eingesetzt. Sein redox-reaktiver Charakter und seine zentrale Rolle in der Elektronentransportkette sind der Grund, warum dieses Coenzym in der mitochondrialen Medizin zur Behandlung von altersbedingten Erkrankungen eingesetzt wird. Da die genauen Wirkmechanismen jedoch in vielen Aspekten noch nicht geklärt sind, beschäftigt sich diese Arbeit mit dem Einfluss von Coenzym Q₁₀ auf den Energiemetabolismus nach kurzzeitig induzierten Schäden über UV-Bestrahlung, Langzeit-induzierten Schäden (altersbedingt) sowie auf die DNA-Reparatur in menschlichen Fibroblasten.

Hierfür wurden mehrere mitochondriale Parameter analysiert, darunter die Respiration, das Membranpotenzial sowie ATP-Spiegel in Hautfibroblasten nach UV-Bestrahlung. Dabei konnte nach einer Behandlung der Zellen mit Coenzym Q₁₀ eine beschleunigte Regeneration des ATP-Spiegels, ein Erhalt des Membranpotenzials und dadurch eine Abnahme der mitochondrialen Dysfunktion beobachtet werden. Neben kurzzeitig induzierten Schäden, wie sie durch UV-Strahlung entstehen, wurde auch der Effekt von Coenzym Q₁₀ bei alters- und langzeitinduzierten Schäden untersucht. So konnte zum ersten Mal ex vivo in der menschlichen Epidermis eine Abnahme der mitochondrialen Atmung sowie der ATP-Produktion mit dem Alter des Spenders gezeigt werden, was der "mitochondrialen Theorie des Alterns" entspricht. Diese Abnahme konnte durch Coenzym Q₁₀ abgemildert werden. Zuletzt wurde das

Augenmerk auf die humane 8-Oxoguanin-DNA-Glykosylase 1, dem Reparaturenzym für 7,8-Dihydro-8-Oxoguanin, unter dem Einfluss von Coenzym Q₁₀ gelegt. Dabei konnte unter dem Einfluss von Coenzym Q₁₀ erstmals eine konzentrationsabhängige Aktivitätszunahme, eine Veränderung der Bifunktionalität zugunsten einer erhöhten N-Glykosylase-Aktivität sowie eine direkte Interaktion zwischen der 8-Oxoguanin-DNA-Glykosylase 1 und Coenzym Q₁₀ gezeigt werden.

Die Ergebnisse dieser Arbeit deuten darauf hin, dass die erzielten Effekte von Coenzym Q₁₀ auf die mitochondriale Funktion, primär über seine antioxidative Funktion sowie durch eine gesteigerte Aktivität der Elektronentransportkette vermittelt wird. Insbesondere ein Erhalt des mitochondrialen Membranpotenzials scheint hierbei von großer Bedeutung zu sein. Eine Aktivitätssteigerung der 8-Oxoguanin-DNA-Glykosylase 1 liegt vermutlich daran, dass Ubiquinol zur Auflösung eines Endproduktkomplexes beiträgt, welcher sich zwischen dem Reparaturenzym sowie dem resultierenden ungesättigten Hydroxyl-Aldehyd am 3'-Ende bildet und dadurch weitere enzymatische Schritte hemmt.

Auf der Grundlage dieser Arbeiten wurden weitere interessante Ansatzpunkte für den Einsatz von CoQ₁₀ als Medikament sowohl zur präventiven als auch zur akuten Behandlung zahlreicher altersbedingter Krankheiten im Bereich der mitochondrialen Medizin identifiziert.

1. Introduction

1.1 Anatomy and function of the human skin

With a total surface area of up to 2 m², the skin is the largest organ of the human body and with a weight of up to 10 kg, makes up a significant proportion of the body mass. It's extremely broad spectrum of tasks ranges primarily from protecting the human organism against external influences such as sunlight, cold and heat, but also mechanical stimuli and chemicals, to protecting against the penetration of germs and pathogens. Furthermore, it regulates the temperature and fluid balance of the body. Via sensory cells, the skin enables sensations and the feeling of touch. In addition to these tasks it is actively involved in metabolic processes such as the synthesis of vitamin D (Lehmann, 2005). The skin in general consists of three layers, the epidermis and the dermis (together called as cutis) and the hypodermis (subcutis). The following Figure 1 shows the layered structure of human skin.

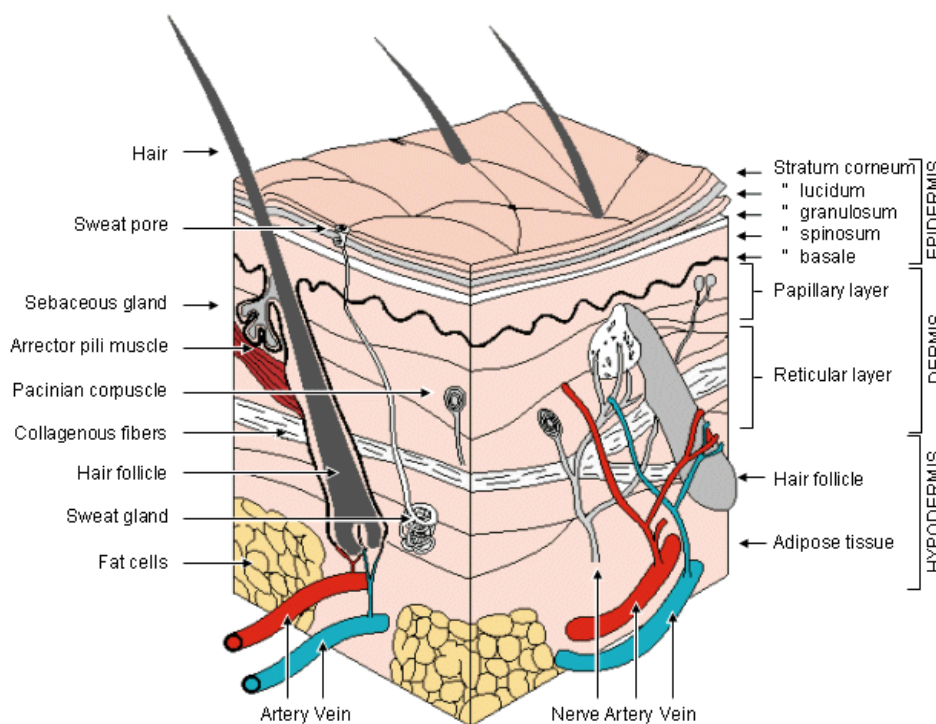


Figure 1. A cross-sectional representation of the anatomy of human skin showing the various layers. Figure from Malmivou and Plonsey, 1995.

The epidermis has no vessels and is a keratinized squamous epithelium, which can reach a thickness of 30 to 400 µm depending on body region, age and sex. The surface of the epithelium can be smooth (field skin) or develop as groin skin. It can be

divided into five further layers. Starting from the surface, these include the stratum corneum, the stratum lucidum, the stratum granulosum, the stratum spinosum and the stratum basale. Together, these layers consist of 90 % of keratinocytes and in smaller numbers, among others, of melanocytes and Merkel cells (Welsch *et al.*, 2018). The stratum basale is responsible for the continuous new production of epidermal cells and also contains the melanin-producing melanocytes known as pigment cells. These cells are characterized by the melanosomes, organelles, which synthesize and deposited the pigment melanin via the precursor tyrosine. Melanin is released to neighbouring keratinocytes in the form of vesicles, the melanosomes (Yamaguchi and Hearing, 2009). Merkel cells, slowly adapting mechanoreceptors, are also located in the stratum basale. Via desmosomes, which connect them to neighbouring keratinocytes, they are able to detect pressure in the surrounding tissue (Nakatani *et al.*, 2015). During keratinization of the epidermis, the cells formed in the stratum basale are shifted outwards in layers. In this process the cytoplasm and the cell organelles are replaced by keratin, i.e. at the beginning it is a post-mitotic keratinocyte of the epithelial base and ends with a seedless keratinized cell. The different stages of cell differentiation resulting from this process are genetically predetermined. At the top layer, the cells are rubbed off by mechanical stress (Barrandon and Green, 1987; Fuchs and Raghavan, 2002; Lavker and Sun, 1982). Below the epidermis is the dermis, which can be divided into the two layers stratum papillare and stratum reticulare. The stratum papillare is characterized by a strong interlocking with the overlying basal cell layer, also called the junction zone (Serry, 2018). The stratum papillare contains mainly fibroblasts, a population of division-active and not fully differentiated cells originating from the mesoderm, which can differentiate into a wide variety of connective tissue cells, such as chondrocytes, osteocytes or fibrocytes. This layer is also very rich in cells compared to the deeper part of the dermis. They are mainly responsible for the synthesis of collagen and elastin fibres, a basic substance that gives the dermis its stability and elasticity (Moll, 2010). The underlying layer, the reticular stratum, has fewer cells and is thicker than the papillary stratum because it's collagen fibres with a larger diameter (Meves, 2006). In addition, sweat glands and hair follicles are located here, the exits of which run through the entire remaining dermis and epidermis. The reticular stratum makes contact with the subsequent subcutis (subcutis) (Meves, 2006).

The subsequent skin layer is the subcutis, consisting of loose connective tissue. It is located above the muscle fasciae and connects them with the upper skin layers. This layer contains sections of sweat glands and hair follicles as well as larger blood vessels and nerves (Meves, 2006). Depending on body region, age, sex and physique, different numbers of fat cells are located here. Tight connective tissue runs between them. The fatty tissue serves primarily as an energy store, shock buffer and protection against cold (Welsch *et al.*, 2018).

1.1.1 Influence and protection of the skin against ultraviolet radiation

As external protection against environmental influences, the skin is confronted with ultraviolet (UV) radiation every day. The term UV radiation includes electromagnetic waves in the range of 100 nm to 400 nm. In contrast to longer-wave radiation, UV radiation is more energetic and therefore capable of damaging the skin. Basically, the longer-wave radiation is, the greater is its penetration depth. The most energy-rich part of the radiation, the short-wave UV-wavelength of group C (UVC) (100-280 nm) are absorbed by the ozone layer. Thus, with regard to skin damage, the remaining UVA and UVB components are the main factors (Jackson and Bartek, 2009). The UV radiation penetrates the skin where the energy, depending on the wavelength, is absorbed by cellular molecules. The UVB range (280-315 nm) of solar radiation causes the increased formation of the UV-absorbing skin pigment melanin in the epidermis. UVB also stimulates the formation of cholecalciferol (vitamin D3) in the skin. Besides this, UVB also has a strong erythema effect on the skin and can cause sunburn. The long-wave UVA fraction (315-380 nm) of the radiation reaches the dermis and causes direct pigmentation (conformational change of melanin) as well as damage to collagen and elastin. UVA has thereby only a small erythema effect (Berger, 2009; McDaniel *et al.*, 2018). If the personal skin type-dependent threshold dose, the so-called erythema threshold dose, is exceeded, local reddening (erythema) of the skin occurs. Even below this dose, biological interactions with UV radiation can occur within the cells localized in the skin. In addition to influencing the metabolism of collagen and elastin, which is associated with UV-induced skin ageing, there is an increased incidence of deoxyribonucleic acid (DNA) damage (Wenk *et al.*, 2001). These damages and the underlying repair mechanisms are discussed separately in this thesis (1.4 DNA damage; 1.5 DNA repair mechanism).

1.2 Mitochondrial function and genetics

Mitochondria are aerobic proteobacteria with an average size of 0.75 - 3 μm that were probably once taken up via endosymbiosis (endosymbiont theory) and are found in almost all eukaryotic cells. The number of mitochondria in a cell varies depending on the cell type and can be changed depending on the energy requirements of a cell. Due to endosymbiosis, mitochondria are surrounded by two lipid bilayers. The outer membrane, which encloses the intermembrane space, has a higher permeability than the inner membrane. It contains porins which are permeable for small molecules and ions with a size of up to 5 kilodalton (kDa) (Alberts *et al.*, 2012). The intermembrane space is a non-plasmatic phase with only a few functions. The inner membrane borders the matrix and forms cristae, the number of which varies from cell type to cell type. This leads to a significant increase in the surface area of the inner membrane, which enables increased adenosine triphosphate (ATP) production via the electron transport chain (ETC) located here as well as ATP synthetases (Madigan, 2013). There is a high metabolite exchange between cytoplasm and matrix, which is why the mitochondrial membranes have a high number of transport proteins, TIM (translocase of the inner membrane) and TOM (translocase of the outer membrane) for example. However, it is impermeable for many ions and molecules. The matrix contains ribosomes, in multiple numbers, mitochondrial DNA (mtDNA) and granules. It also houses all essential metabolic processes of the mitochondria, including genome replication, transcription and translation (Alberts *et al.*, 2012). Mitochondria have a broad spectrum of tasks and in addition to the generation of ATP they play a central role in programmed cell death (apoptosis) by the release of cytochrome C via mechanisms not yet precisely known. They are responsible for adapting the redox potential of the cell by producing reactive oxygen species (ROS) and for the release and activation of proteins (caspases) that mediate apoptosis. They also induce apoptosis by interrupting the energy metabolism through the electron transport chain (Gvozdjáková, 2008). The synthesis of iron-sulfur clusters (Fe-S cluster) is considered to be the essential function of mitochondria. Fe-S clusters are complexes of iron and sulfur, which participate as cofactors in enzyme reactions and are therefore of great importance for the survival of a cell (Lill *et al.*, 1999). The respiratory chain plays a major role in the significance of this work, why it will be discussed separately and in more detail in the following chapter.

The 16.5 kilobase (kb) mitochondrial genome is double-stranded, ring-shaped and does not have histones nor introns. In 37 genes it encodes for two ribosomal ribonucleic acids (rRNA), 22 transfer RNAs (tRNA) and 13 essential mitochondrial proteins (DiMauro, 2001; Knippers, 2006). Mitochondria are semi-autonomous organelles and therefore have a genome that encodes only a few mitochondrial proteins. The remaining proteins are encoded by nuclear DNA and must be transported from the cytoplasm into the mitochondria after their synthesis (Knippers, 2006). During cell division, the mitochondria distribute themselves to the daughter cells and reproduce themselves not by new formation, but by division. The mitochondria are inherited maternally, which prevents recombination of mtDNA, for example in meiosis (Knippers, 2006).

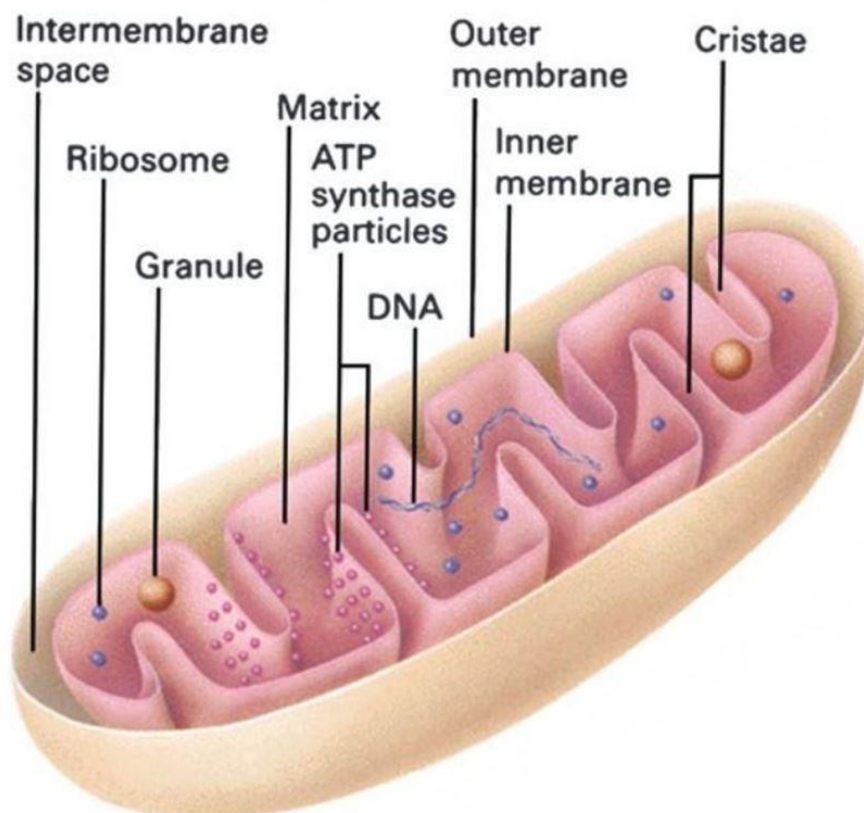


Figure 2. Schematic structure of a mitochondrion. Mitochondria are surrounded by two membranes, which define the intermembrane space and the matrix. The inner membrane is strongly folded to increase the surface area and forms cristae. Here are complexes of the electron transport chain (ETC). The matrix contains, among others, mitochondrial DNA (mtDNA), ribosomes and granula. Figure adapted from Logan, 2006.

1.2.1 Respiratory chain (oxidative phosphorylation)

The generation of ATP by oxidative phosphorylation, which takes place at the inner mitochondrial membrane, is achieved by the transport of electrons along the complexes I to IV and the mobile components ubiquinone (CoQ) and cytochrome c (cyt c) of the ETC (Figure 3). The last two serve as electron and proton carriers between these complexes.

The largest enzyme complex of the respiratory chain, complex I, the NADH-ubiquinone oxidoreductase, catalyses the transport of two electrons from the 1,4-dihydronicotinamide adenine dinucleotide (NADH) to CoQ as well as the transport of four protons (H^+) from the matrix into the intermembrane space (Q-cycle). This contributes to the formation of a proton gradient across the inner membrane. The reduced ubiquinol ($CoQH_2$) is formed, a two-electron carrier which can diffuse freely in the lipid bilayer of the inner membrane (Alberts *et al.*, 2012). At complex II (succinate dehydrogenase), also part of the citrate cycle, the oxidation from succinate to fumarate takes place and thus the electron transfer to CoQ, resulting in $CoQH_2$. In addition to three iron-sulfur clusters (Fe-S cluster), complex II has a flavin adenine dinucleotide (FAD) as a prosthetic group and a succinate binding site. The electrons migrate from the succinate via FAD and the Fe-S clusters to CoQ (Alberts *et al.*, 2012). Subsequently, at the dimeric complex III, the ubiquinone-cytochrome c oxidoreductase, a transfer of electrons from $CoQH_2$ to cytochrome c takes place with translocation of four protons from the matrix into the intermembrane space. Cytochrome c, a one-electron carrier, transports these electrons further from complex III to complex IV and releases them to the two divalent copper ions (Cu^{2+}) of cytochrome c oxidase (Cu_A), which are located in subunit II. This subunit has two heme groups (heme-a) as a prosthetic group (Alberts *et al.*, 2012). Complex IV, cytochrome c oxidase, catalyzes the electron transport from cytochrome c to oxygen (O_2), reducing it to two molecules of water (H_2O). Subunit I of complex IV has two heme groups (heme a and heme a_3) and a further Cu^{2+} ion (Cu_B). Heme a_3 and Cu_B form a further dinuclear center. Thus the electron transport of cytochrome c takes place via the Cu_A centre, the heme a and the heme a_3 - Cu_B centre on O_2 .

The energy released during the reduction of O_2 to H_2O is used to pump another four H^+ per oxygen molecule from the matrix via complex IV and the inner membrane into the intermembrane space. To prevent the formation of reactive oxygen species (ROS), it is important that the heme- a_3 - Cu_B center is first reduced with two electrons.

Only under this condition it is possible for the O_2 to bind. Thus the oxygen can be reduced directly to peroxide before it can be broken down into its individual atoms.

The proton gradient built up during this electron transport catalyses the ATP synthesis from adenosine diphosphate (ADP) and inorganic phosphate (P_i), through a proton flow from the intermembrane space back into the mitochondrial matrix using the F_0F_1 -ATP synthase (complex V). The ATP synthase in mitochondria is an F-type ATP synthase and consists of the two components F_1 and F_0 . F_1 is a peripheral membrane protein with nucleotide binding sites, F_0 is integrated into the membrane and has a proton channel (Alberts *et al.*, 2012). The F_0 subunit consists of the three main subunits, a, b, and c, and six additional subunits. F_1 is a hexamer consisting of three α - and three β -subunits. F_1 and F_0 are connected by a peripheral and a central stalk consisting of the γ -, δ - and ϵ -subunit. The γ -subunit brings the c-subunits of F_0 into contact with the δ - and ϵ -unit. According to the chemiosmotic model, the proton motor force drives the ATP synthase. The backflowing protons cause a rotational movement of the c-ring of F_0 and the γ -, δ - and ϵ -subunit of the central stalk. The resulting rotational movement is transferred to the F_1 part. This causes the ATP formation in the F_1 part via a conformational change. The peripheral stalk thereby prevents the entire F_1 subunit from rotating. Three ATP are formed during a complete rotation of the γ -subunit (Alberts *et al.*, 2012). The transfer of the electrons and the ATP synthase are coupled and do not function without the other part (Nelson and Cox, 2011). In addition to the ATP synthase, H^+ can also be pumped back into the matrix via other mitochondrial membrane proteins, so-called uncoupling proteins (UCPs). This leads to the decoupling of the electron transport chain from the ATP synthase (Nedergaard *et al.*, 2005).

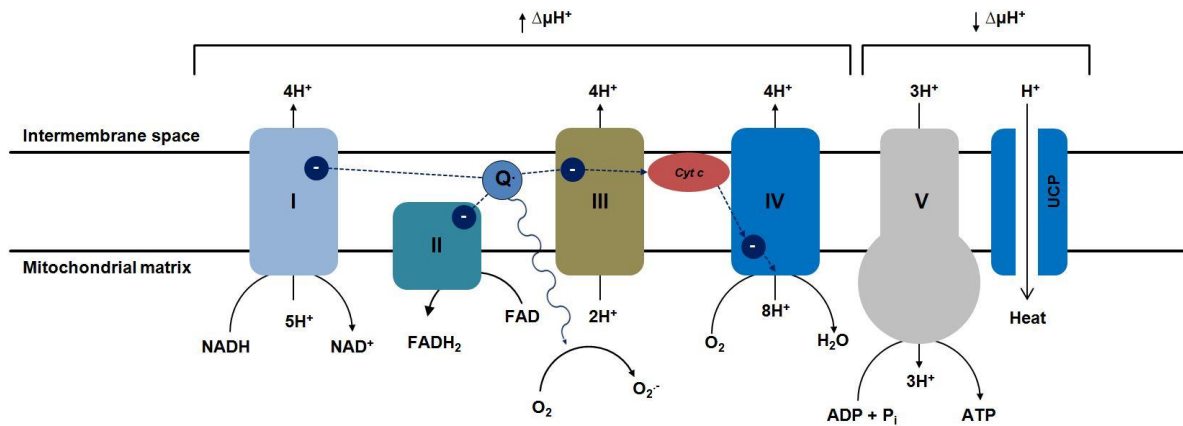


Figure 3. Simplified representation of the mitochondrial respiratory chain. The inner mitochondrial membrane and the compartments of the respiratory chain are shown. The complexes I-IV produce a proton gradient ($\Delta\mu H^+$), so that ATP can be synthesised via the complex V (ATP synthase). The electrons migrate from complex I via complex II and ubiquinol to complex III. Cytochrome c transports the electron from complex III to complex IV. Figure created after the template of Brownlee, 2001.

1.2.2 Mitochondria in the process of ageing

The ageing process is characterized by an increase in age-related disorders and serious diseases. Due to their role in oxidative phosphorylation and thus in the production of ATP, which is crucial for many cellular processes, a reason for this could be found in the mitochondria (Desler *et al.*, 2011; Wallace, 2005). Even though ageing is a multifactorial and thus much more complex process than previously thought, only the mitochondrial mechanisms that play a role here will be discussed. In the "free radical theory of ageing" (FRTA), developed by Harman in 1956, he identified free radicals as the cause of ageing processes, as they damage molecules that are important for many important cellular functions, such as DNA, RNA, but also proteins and lipids. After discovering the mitochondrial genome, he extended his theory into the "mitochondrial free radical theory of ageing" (MFRTA), which deals with the age-related loss of mitochondrial function due to an increasing accumulation of oxidative damage. This theory is also known as the "mitochondrial theory of ageing" (Harman, 1972) According to this theory, the accumulation of reactive oxygen species (ROS) that damage mitochondrial DNA and proteins can cause mitochondrial dysfunction within the electron transport chain. Due to these dysfunctions, further ROS are formed, which in turn damage the mtDNA. The

accumulation of mtDNA damage then leads to new defects in the electron transport chain, increased ROS production and higher oxidative stress. This finally leads to a vicious cycle of ROS production. In other theories, which address the effects of previous mitochondrial dysfunctions, the energy metabolism plays a central role. For example, Prinzinger's maximum slope theory deals with the correlation of lifespan with the energy production of the cell, and Prinzinger says that ageing is a consequence of the limited ability of the mitochondria to produce energy (Prinzinger, 2005). In another theory, the model of the defective powerhouse, Krutmann and his colleagues discuss the role of mtDNA mutations and dysfunctions in the respiratory chain as factors for dysfunctions in mitochondria and therefore a limited life span (Krutmann and Schroeder, 2009).

1.3 Reactive oxygen species

Reactive oxygen species are compounds formed from molecular oxygen, most of which are undesirable by-products of cellular metabolism (Yang and Lian, 2019). They are present as radicals, ions or molecules that have an unpaired electron in their outer electron shell (Krokan and Bjørås, 2013; Liou and Storz, 2010). Due to these properties, these compounds have a high reactivity and chemical aggressivity (Liou and Storz, 2010). ROS are therefore able to oxidise and thus damage cellular biopolymers - such as DNA, proteins and lipids (Birben *et al.*, 2012; Krokan and Bjørås, 2013). The group of ROS includes radical ROS such as the highly reactive hydroxyl radical ($\bullet\text{OH}$) or the superoxide anion ($\text{O}_2^{\bullet-}$), which is the precursor of almost all ROS (Lambert and Brand, 2009). Non radical ROS are represented by hydrogen peroxide (H_2O_2), hydroperoxide (ROOH) or singlet oxygen ($^1\text{O}_2$). ROS occur in all aerobic organisms and have important physiological functions, but are also involved in various diseases such as oxidative stress (Yang and Lian, 2019).

1.3.1 Formation of ROS and oxidative stress

The mitochondria are considered to be the main source of these compounds (especially mitochondrial superoxide (mtSO)). Starting from 80 % of the oxygen taken up by mammals, which is utilised in the mitochondria, 1-2 % of the oxygen taken up by these organelles is converted into ROS (Turunen *et al.*, 2004). Since ROS and superoxide are mainly unwanted by-products of the respiratory chain, the formation

of these aggressive compounds correlates with the synthesis of ATP as the last step of the respiratory chain (Turunen *et al.*, 2004). This is caused by electron leakage between complex I and III (Bottje, 2019; Hunte *et al.*, 2010; Liou and Storz, 2010). In the ubiquinol cycle two ubiquinons are formed. The release of an electron from ubiquinol to cytochrome c in the first cycle results in an unstable ubiquinone on the cytoplasmic side of the inner mitochondrial membrane. The electron of the unstable semiquinone is transferred to the ubiquinone and a stable semiquinone is formed on the matrix side, which takes up another electron from a second pass and forms ubiquinone. Due to its low stability, the unstable ubiquinone is a source of superoxide (Bottje, 2019).

After their formation, ROS can remain in the mitochondria or leave them in the direction of the cytoplasm (Liou and Storz, 2010). Due to the relatively high production of ROS in the mitochondria and the fact that mtDNA is more susceptible to external influences (such as ROS) due to the lack of protective histone proteins, the genetic information of these organelles is oxidatively damaged about ten times more frequently than nuclear DNA (Turunen *et al.*, 2004). In addition to ROS formation during mitochondrial respiration, there are oxidoreductases which contribute to the formation of reactive oxygen and nitrogen species within the framework of normal physiological processes. ROS are also used by the immune system to destroy microorganisms. Another source of ROS is the Fenton reaction, in which hydrogen peroxide and a divalent iron react to form a trivalent iron and a hydroxyl radical. This trivalent iron can be combined with other ROS to produce further divalent iron atoms, which in turn are available for the initial reaction. Also as a result of the exposure to physical influences, an increased intracellular formation of ROS can occur (Kammeyer and Luiten, 2015). For example, through the effects of solar radiation, which mainly lead to the formation of $^1\text{O}_2$ and O_2^- , the last one however to a much lesser extent (Rünger *et al.*, 1995). From these two substances, together with Cu- or Fe-ions, H_2O_2 can be formed in a Haber-Weiss reaction. H_2O_2 can also be converted into highly reactive hydroxyl radicals in a further reaction (Fenton reaction) with transition metals (iron, copper) (Halliwell and Gutteridge, 1984; Halliwell, 1996).

The presence of a high concentration of ROS or an increase in their concentration leads to a shift in the intracellular balance between oxidations and reductions towards the oxidation processes. This fact favours the formation of further ROS or

prevents a quantitatively sufficient inactivation of these oxidants by the antioxidants available to the cells, which further deflects the equilibrium (Birben *et al.*, 2012). The disturbance of the intracellular balance between the formation of ROS (oxidant) and the inactivation of these by the cellular antioxidants is called oxidative stress (Birben, *et al.*, 2012; Sies, 2015). Oxidants and antioxidants are particularly important for electron transport due to their potential to reduce or oxidize themselves. The concentration of electrons influences cell signalling systems that transfer information from the extracellular to the intracellular space through the cell membrane. The signalling processes lead to enzyme activation, activation of DNA and RNA synthesis, gene expression, cell proliferation or differentiation and apoptosis. A more reductive environment stimulates proliferation, whereas an environment shifted in favor of oxidants initiates cell differentiation (Coppotelli and Ross, 2016). However, the majority of the aggressive ROS formed in the body cells can usually be inactivated and thus rendered harmless before the onset of oxidative stress by enzymatic and non-enzymatic processes of the antioxidant system (Birben *et al.*, 2012).

1.4 DNA damage

Each of the approximately 1.0×10^{13} human body cells suffers about 1.0×10^4 to about 1.0×10^5 DNA damages per day (Jackson and Bartek, 2009; Janion, 2001). Hydrolysis, for example, results in the loss of about 1.8×10^4 purines and the deamination of thymine to uracil bases about 100 to 500 times per day and cell, and thereby violates the integrity of the genome (Bruner *et al.*, 2000; Hazra *et al.*, 2007). According to current knowledge, more than 100 different DNA base modifications have been identified (Halliwell and Aruoma, 1991; Boiteux *et al.*, 1992; Dizdaroglu, 1992). The spectrum of DNA damage ranges from direct single- or double-strand breaks caused by highly reactive ROS, through base modifications and AP (apurinic/apyrimidinic) lesions (base-free sites), to DNA-protein or DNA-DNA crosslinks (Epe, 1996). In general, the term "DNA damage" refers to molecular changes in the natural structure of the DNA or its bases. The incorporation of incorrect bases in the course of a replication process is also regarded as damage.

Possible causes of DNA damage are metabolic processes and chemical or physical influences such as UV rays. Chemical agents hydrolyse, alkylate or oxidize the DNA directly and thus cause an increased mutation rate. These substances are also

known as mutagens. However, these substances can also have an indirect effect on the DNA by inducing a higher error rate during replication or leading to the formation of ROS in the mitochondria (mtROS) (Chatterjee and Walker, 2017).

Another important role in DNA damage is played by physical factors such as solar radiation. UVB causes direct DNA damage such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) (de Gruijl *et al.*, 1993). As there are two types of pyrimidines in DNA, four different types of CPDs can be formed: T-T dimers, T-C dimers, C-T dimers and C-C dimers. The C-C dimers, which make up the smallest proportion of CPDs, have the highest mutagenicity (Douki and Cadet, 2001). Very high-energy radiation types (ionizing radiation) are also able to break up the sugar-phosphate backbone of nucleic acids and thus induce double-strand breaks (Rünger *et al.*, 1995). The UVA fraction causes, in addition to direct DNA damage, indirect damage via the generation of ROS (Cadet *et al.*, 1997; Zhang *et al.*, 1997). Some of the produced ROS are so reactive that they react immediately with substances from the environment. To cause oxidative DNA damage, ROS must therefore be located next to the DNA immediately after their formation (Yang and Lian, 2019). In contrast to contact with chemical mutagens, exposure to these physical influences cannot be completely avoided in everyday life (e.g. UV radiation from sunlight).

1.4.1 Oxidative DNA damage

With approximately 1.000 to 1.0×10^5 lesions per cell and day, oxidative damage accounts for a considerable proportion of the DNA damage that occurs daily. In total, about 100 oxidatively produced DNA damages are known. DNA protein crosslinks, base damage and oxidation of the deoxyribose units, which can even lead to strand breaks, are the main forms of oxidative damage. Since the guanine base has the lowest redox potential of all natural bases, it is the one that is most easily oxidized. Therefore, there are many different oxidized guanine bases. The other bases can also be damaged by oxidation, but this damage plays a subordinate role (Steenken *et al.*, 2001). The preferred point of attack is the 5'-guanine in a GG-rich sequence, since the redox potential of directly adjacent guanosine bases is further reduced by DNA π -base stacking. This makes guanine more sensitive to oxidative damage, because this enables charge transfer even from more distant locations. The oxidative

damage to DNA is thereby promoted from a distant location by the migration of holes through the DNA π -base stacking (Hall *et al.*, 1996).

1.4.2 7,8-dihydro-8-oxoguanine

7,8-dihydro-8-oxoguanine (8-oxoG) is one of the most common and at the same time one of the most mutagenic oxidative DNA damage induced by ROS (Hill *et al.*, 2001; Sampath *et al.*, 2012). In a healthy cell, between 1.000 and 2.000 8-oxoG damages occur per day, whereas in a tumour cell, about 1.0×10^6 damages occurs (Ravanat *et al.*, 2001). DNA damage in the form of 8-oxoG can occur in several ways. On the one hand, by direct attack of a hydroxyl radical at position C⁸ of guanine by ROS (Devasagayam *et al.*, 1991; Epe, 1996; Halliwell and Aruoma, 1991), and on the other hand, guanine already oxidized in the nucleotide pool can also be unintentionally incorporated into the DNA in the course of various cellular processes (Nakabeppu, 2015). By oxidizing guanine to 8-oxoG, this modified base in contrast to guanine is now able to hybridize not only with cytosine but also with adenine (Figure 4) (Loft and Poulsen, 1996). The pyrimidine base cytosine, which normally occurs in the replication product, is replaced by the purine base adenine. This process represents a transversion. This can lead to a complete base exchange after the second replication. The genetic information is therefore subject to a complete base pair exchange in the course of two consecutive replication processes, whereby the guanine/cytosine or 8-oxoG/cytosine base pair originally present in the DNA is replaced by thymine/adenine in the replicated daughter strand (Hill *et al.*, 2001; Sampath *et al.*, 2012).

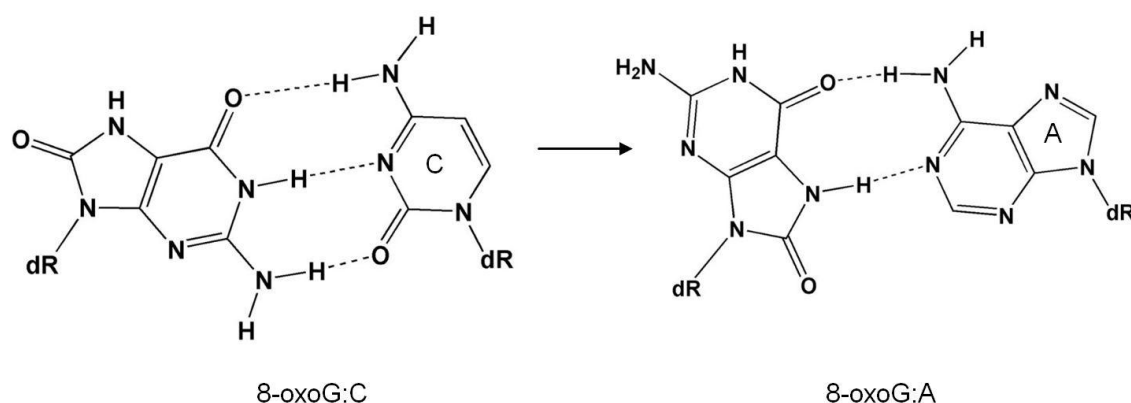


Figure 4. Possible base pairings of 8-oxoG with cytosine and adenine. On the left side the base pairing of 8-oxoG with cytosine (C) is shown. The right part of the figure shows the base pairing of 8-

oxoG with adenine (A), which is also possible due to oxidative damage. Figure created after the template of David *et al.*, 2007.

Besides 8-oxoG, there are other important oxidation products of guanine, including formamidopyrimidine-2'-deoxyguanosine (FapyG) (Figure 5). Various mechanisms within the cell produce hydroxyl radicals ($\cdot\text{OH}$), which then attack guanosine and thus form FapyG and its derivatives (Snyder *et al.*, 2005). As hydroxyl radicals attack mainly guanine and its oxidation products, their importance for oxidation is considerable. Within a cycloaddition, a cyclic endoperoxide is initially formed, which reacts in a reaction cascade to two further important oxidation products, guanidinohydantoin and spiroiminohydantoin (Nyaga *et al.*, 2007).

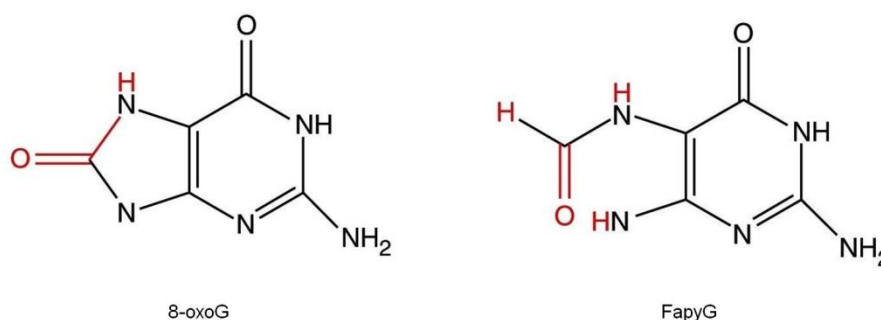


Figure 5. Chemical structure of 8-oxoG and FapyG. Besides 8-oxo-7,8-dihydroguanine (8-oxoG) the formamidopyrimidine FapyG is one of the most common oxidative purine modifications. Figure adapted from Krokan and Bjørås, 2013.

1.5 DNA repair mechanisms

As already described, there are a number of damaging influences on DNA, lipids and proteins, the effects of which can have fatal consequences for the affected organism over time. Especially in an oxygen-transforming organ, the cells are constantly exposed to reactive oxygen species, which is mainly associated with an oxidation of nucleic acids. Without effective repair, this damage would sooner or later lead to mutations, a loss of function at the protein level and finally to cell death. Due to the high susceptibility of DNA to damage, it is one of the few biomolecules that possesses a large number of specific and highly conserved defence and repair mechanisms to ensure, if possible, an error-free and complete repair of the occurring damage. Thus, a complex network of DNA damage response (DDR) mechanisms has developed, including a number of DNA repair pathways, damage tolerance

processes and cell cycle control pathways. Since DNA damage can occur at any time at any genomic site, DDR is a process that requires special spatio-temporal orchestration (Giglia-Marie *et al.*, 2011). Each mechanism of damage response depends on the type and location of the lesion in the genome (Essers *et al.*, 2006). The importance of maintaining genomic integrity is reflected in the presence of more than 150 different DNA repair genes (Wood *et al.*, 2005). In the following, some of the most important DNA repair mechanisms for direct damage (e.g. CPDs, 6-4PPs) as caused by irradiation with UVB light as well as indirect damage, e.g. 8-oxoG, caused by ROS, induced by UVA irradiation, will be explained in detail.

1.5.1 Direct DNA repair

In the best case, damage to the DNA can be restored to its original form by a simple enzymatic process. However, a direct reversion of DNA damage is relatively rare according to current knowledge, since the great chemical diversity of damage has been supplemented by other more complex systems in the course of evolution. Photolyases are the best known representatives of direct repair. Photolyases use the blue part of visible light to repair UV-induced DNA damage such as CPDs or 6-4PPs. When the photolyase encounters a lesion in the genome, the enzyme opens and bends the DNA strand by 50° (CPDs) and 180° (6-4PPs), respectively, so that the dimer enters the active site of the photolyase and is split into two separate bases again (Müller and Carell, 2009; Maul *et al.*, 2008; Kim and Sancar, 1993; Mitani and Shima, 1995; van de Poll *et al.*, 2002).

In addition, four types of alkylated DNA have been known for some time (1-methyladenes, methyl-fapyG, 3-methyladenine and O⁶-methylguanine), which can also be directly repaired by alkyltransferases such as the O⁶-methylguanine methyltransferase (MGMT) (Sedgwick, 2004; Kurowski *et al.*, 2003). In this process, the methyl residue (O⁶-methylguanine) covalently bound to the DNA base is removed and transferred to enzyme-own cysteine residues (Pegg, 2000; Wibley *et al.*, 2000; Xu-Welliver *et al.*, 2000). However, it is also possible to load several alkyl groups onto the enzyme, although the effectiveness decreases with increasing length of the alkyl residue (Sedgwick, 2004; Kurowski *et al.*, 2003). In this process, the enzyme is deactivated, since the formed S-methylcysteine is chemically very stable and the reaction is therefore irreversible (suicidal enzyme).

1.5.2 Postreplicative repair (mismatch repair)

To remove replication errors, eukaryotic cells use a number of mechanisms of postreplicative repair (PRR). Normally, replication errors are repaired in a kind of direct DNA Repair mechanism by the proofreading activity of DNA polymerases. Each nucleotide incorporated into the newly synthesized strand during DNA replication is checked immediately after incorporation for proper base pairing with the corresponding base in the strand to be replicated and, if necessary, removed if mismatched. If this is not the case, another mechanism of PRR is used, the mismatch repair. A mismatch is detected by binding Mutator α (MutS α), a heterodimer consisting of the subunits MutS homolog 2 (MSH2) and 6 (MSH6), to the mismatched base. Subsequently, the proliferating cell nuclear antigen (PCNA), which is recruited to the lesion via an open 3' end, interacts with MutS. MutL, another heterodimer consisting of MutL homolog 1 (MLH1) and PMS2, also reaches the lesion site and is activated by MutS and PCNA. This results in a 3'-directional incision next to the mismatched base by the PMS2 subunit of MutL (Kolodner and Marsischky, 1999; Kolodner *et al.*, 1999; Yang, 2007). Mismatch repair can also be used to remove insertion loops with a length of up to 16 nucleotides, which are very common in the genome of repetitive microsatellites, for example (Marti *et al.*, 2002; Peltomaki, 2003; Renkonen *et al.*, 2003).

1.5.3 Nucleotide excision repair

Another essential DNA repair process is the nucleotide excision repair (NER). Here, the DNA is first scanned for structural distortions by NER enzymes. If a structural anomaly is identified (e.g. based on dimerized nucleobases or intercalants), a section of approximately 24 to 32 nucleotides long is cut out enzymatically from the DNA single strand that shows the damage (Ura and Hayes, 2002). Since both the chemical modification and the steric distortion of the DNA helix (bulky adducts) must be present for damage detection, this is referred to as two-part substrate detection. These so-called "bulky lesions", cross-linkings within the DNA strand include UV-induced CPDs, 6-4PPs and various alkylation products on the DNA, which are able to block replication or transcription (Ura and Hayes, 2002). The cutting of the damaged area is done within five steps according to the so-called "cut and patch" mechanism. After the detection of the damage and opening of the helix at the position of the damage, it is removed. The resulting single-stranded area within the DNA is then

reconstructed into an intact double strand by a DNA polymerase and a DNA ligase using the undamaged strand as a template with the correct nucleotide sequence (Rademakers *et al.*, 2003; Prakash and Prakash, 2000).

Eukaryotic NER can take two different pathways, the global genome repair (GGR) and transcription-coupled repair (TCR). Global genome repair takes place throughout the genome, both in transcribed and non-transcribed regions. Its efficiency depends on the substrate, e.g. 6-4PP lesions are repaired faster than CPDs. Initially, damage is detected by the xeroderma pigmentosum complementation group C (XPC)-hHR23 complex with its ubiquitin-like domain, which triggers a conformational change in the DNA, enabling XPA and replication protein A (RPA) to bind to the damage site. Subsequently, the transcription factor IIH (TFIIH) (Svejstrup *et al.*, 1995; Bhatia *et al.*, 1996), XPB and XPD, two DNA helicases and the two endonucleases XPG and excision repair cross complementing group 1 (ERCC1) -XPF are bound. During the subsequent incision of the DNA, an oligonucleotide of 24 - 32 bases is cut out (Klungland *et al.*, 1999). The new synthesis is carried out complementary to the counter strand by the DNA polymerase δ/ϵ -RPA-replication factor C (RFC) complex. In the final step, the remaining gap is closed with the help of ligase I.

Transcription-coupled repair, in contrast, removes damage that blocks RNA polymerase II (RNAPII) from reading activated genes in transcribed regions. In contrast to GGR, different substrates are removed with equal efficiency. The fact that "bulky adducts" blocks the polymerase during elongation initiates the TCR mechanism and the (Cockayne syndrome group B) CSB protein is recruited to the damage site (Friedberg, 1996). Damage recognition therefore takes place by blocking or slowing down the RNA polymerase (Friedberg, 1996; Tornaletti *et al.*, 1999). Under ATP hydrolysis a stable CSB-RNAPII-DNA complex is formed. If the lesion is small, the polymerase is able to overcome the damage (Tantin *et al.*, 1997; Citterio *et al.*, 2000). However, if the damage is UV-induced, cisplatin or greater, the polymerase cannot read over the damage and recruitment of further proteins such as Cockayne syndrome group A (CSA), TFIIH and XPG occurs. Subsequently, as in GGR, the double helix is wrested and the oligofragment is cut out (Kamiuchi *et al.*, 2002; Tantin, 1998; Iyer *et al.*, 1996).

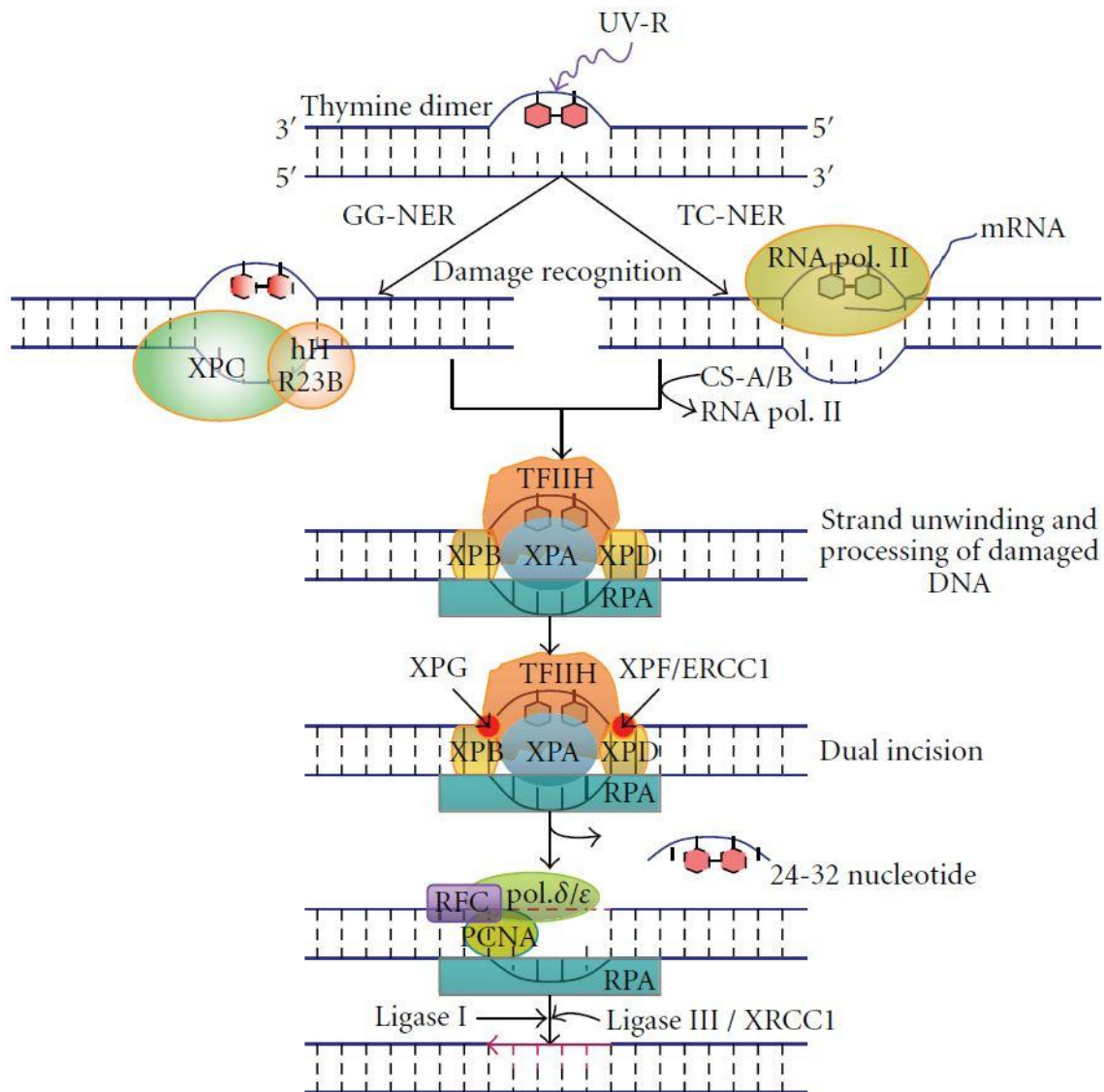


Figure 6. Schematic overview of the two different NER pathways, the global genome repair (GGR) and transcription-coupled repair (TCR). Figure from Rastogi *et al.*, 2010.

1.5.4 Base excision repair

Base excision repair (BER) is used to remove or replace deaminated, alkylated, fragmented or ROS-oxidized DNA bases as well as induced single-strand breaks and is similar to the damage response by NER. In both cases, it consists of detecting the DNA modification, its removal, the subsequent re-synthesis using a matrix strand and the ligation of the resulting gap (Krokan *et al.*, 1997; Lindahl and Wood, 1999; Gros *et al.*, 2002). The identification and removal of damaged bases is done by specialized enzymes called DNA glycosylases. These are responsible for the initiation of BER (Gorbunova, *et al.*, 2007). DNA glycosylases are repair proteins, of which eleven different ones are known to date in humans, which are characterized by their high

damage specificity and whose substrate specificity partly overlaps (Jacobs and Schär, 2012; Zharkov, 2008). In addition, a distinction is made between monofunctional and bifunctional glycosylases. For example, a monofunctional glycosylase recognizes uracil produced by deamination, a bifunctional glycosylase recognizes 8-oxoG induced by oxidation processes (Lee *et al.*, 2015; Ba and Boldogh, 2018). Glycosylases migrate along DNA molecules and check them for the presence of damaged bases (Zharkov *et al.*, 2010). If they encounter a specific damage, the repair process is initiated.

Monofunctional glycosylase

In the case of a monofunctional glycosylase, the base recognized as damaged is removed enzymatically by folding it out of the DNA double helix (base flipping) and then cleaving the N-glycosidic bond between the modified base and the C1 atom of the deoxyribose of the sugar phosphate backbone of the DNA by the glycosylase (Gorbunova, *et al.*, 2007). After base removal by an AP lyase, an apurinic/apyrimidinic (AP) site remains in the DNA, depending on the type of base removed by the glycosylase (Gorbunova, *et al.*, 2007). This AP site is then further processed by the AP endonuclease 1 (APE1) by incising it at its 5' end. APE1 removes the α , β -unsaturated aldehyde and in this process, the AP site is transformed into a single strand break (SSB) with a 3'-OH group and a 5'-deoxyribose phosphate (5'-dRP) residue (Barzilay *et al.*, 1995; Masuda *et al.*, 1998; Dianov *et al.*, 2003). The resulting gap within the polynucleotide is then reconstructed enzymatically by DNA polymerases (DNA polymerase β (Pol β)) and ligases using the undamaged, complementary DNA strand as a template to form an intact, continuous strand (Klungland and Lindahl, 1997; Gorbunova, *et al.*, 2007). The cooperation between Pol β and the last enzyme of the cascade, ligase III, is mediated by the X-ray repair cross-complementation protein 1 (XRCC1), which also gives the activating impulse to APE1 (Nash *et al.*, 1997; Vidal *et al.*, 2001; Petermann *et al.*, 2006; Nazarkina *et al.*, 2007).

Bifunctional glycosylase

In addition to monofunctional DNA glycosylases, there are also bifunctional DNA glycosylases such as 8-oxoguanine DNA glycosylase 1 (OGG1). This glycosylase catalyzes the cleavage of the modified DNA base as well as the subsequent reaction

resulting in a single strand break (Fromme *et al.*, 2003; Hazra *et al.*, 2002;). This reaction is a β elimination, which leads to the incision of the affected DNA strand at the AP lesion. At the end of the β elimination, an unsaturated hydroxyl aldehyde (3-trans-4-hydroxy-2-pentenal-5-phosphate) remains at the 3' end and a phosphate group at the 5' end (Svilar *et al.*, 2011). The removal of the 3'-aldehyde and the associated generation of an OH-group is achieved by the 3'-phosphodiesterase activity of APE1 (Almeida and Sobol, 2007; Wallace, 1998). The resulting DNA single-strand break is then further processed as described above.

Short-patch / long-patch

BER can be classified into two subtypes. In a short-patch BER, as described above, the nucleotide present at the AP site is removed enzymatically from the DNA. In contrast, the so-called long-Patch-BER is more similar to a NER. In this case, a 2 - 13 nucleotide long section, starting from the resulting single-strand break, is inserted by the DNA polymerases β , δ or ϵ , displacing the original strand. This displaced strand is removed by the flap structure-specific endonuclease 1 (FEN1). The gap is then closed by the Ligase I (LIG1) (Gary *et al.*, 1999; Pascucci *et al.*, 1999). Due to the topic of this thesis, the procedure of the bifunctional glycosylase hOGG1 is described in more detail under 1.6.3 *DNA repair mechanism by hOGG1*. Figure 7 shows the molecular mechanism of the BER.

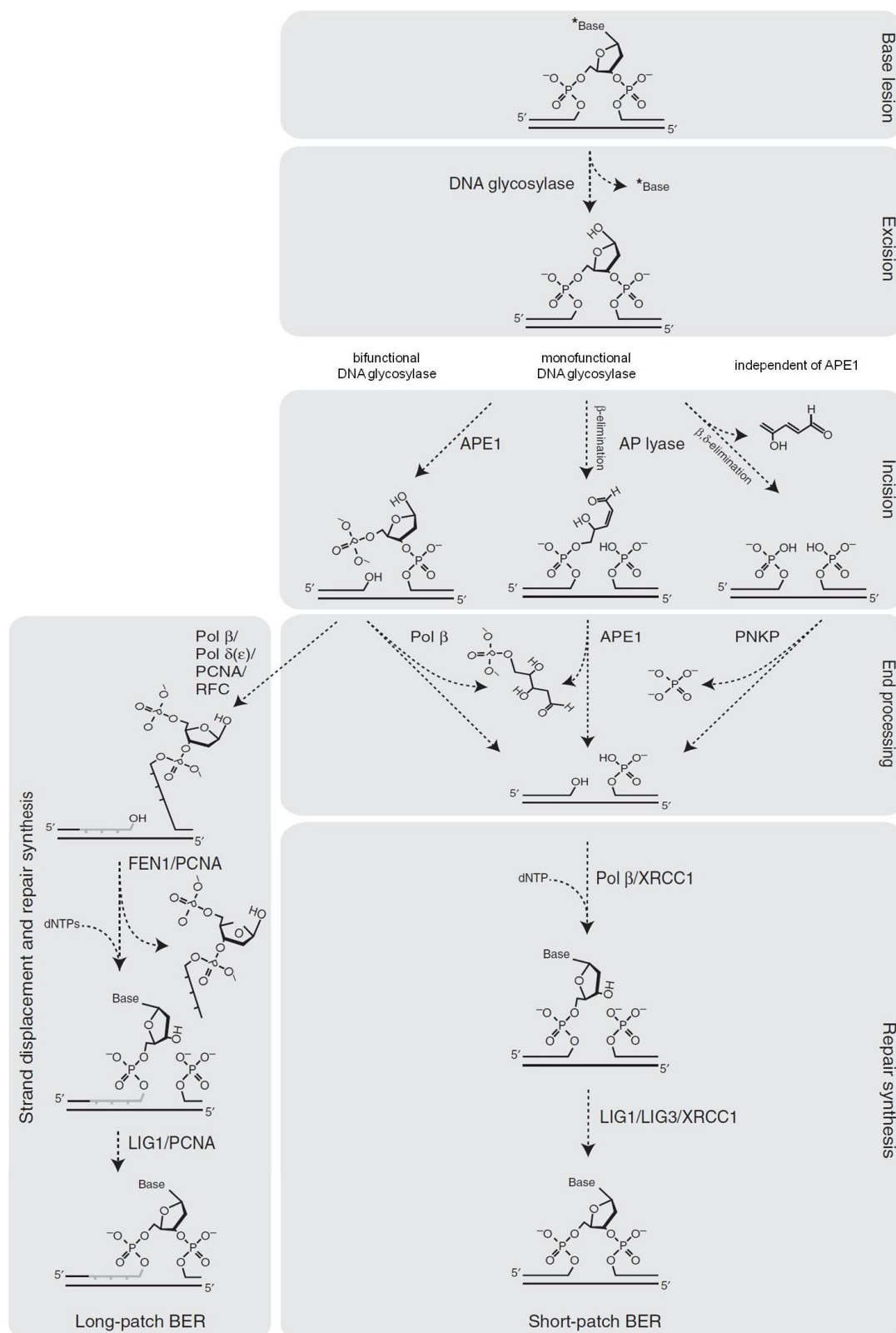


Figure 7. Schematic overview of different BER pathways. In BER, a distinction is made between short-patch repair and long-patch repair. Furthermore, the subpathways for monofunctional and bifunctional enzymes are different. Figure adapted from Krokan and Bjørås, 2013.

Single strand break repair

There are several possibilities for the formation of single strand breaks. Firstly, UV radiation, which can induce SSBs in addition to DNA damage such as CPDs, 6-4PPs or 8-oxoG, and secondly, temporary single-strand breaks occur during BER and NER as repair intermediates (Greinert *et al.*, 2012). However, SSBs can also be formed by spontaneous depurination with subsequent hydrolysis of the phosphodiester bond. SSBs can be divided into four categories. In the first category are strand breaks, which are caused by an incision in the phosphodiester bond without modification of sugar or base. A strand break due to the absence of a phosphate group without modification of sugars or bases marks the second category. Strand breaks due to modification of sugars or bases with or without the absence of the phosphate group as well as strand breaks induced by the loss of a nucleotide are found in categories three and four (Obe *et al.*, 1992). SSBs do not pose a particular danger to the cell, as they are easy to repair (Ward *et al.*, 1985). Direct SSBs, such as those caused by ROS, can be repaired in two different ways - the shortpatch or the long-patch pathway. In both cases, occurring SSBs activate the enzyme poly (ADP-ribose) polymerase 1 (PARP1), which binds to the damage. PARP is a key enzyme in the regulation of many cellular processes and can be activated by DNA damage, for example. The strongest stimulation of PARP1 activity is mediated by its binding to DNA strand breaks (Mangerich and Bürkle, 2012). PARP catalyses the synthesis of negatively charged poly(ADP-ribose) molecules (PARs). In the next step, XRCC1 uses this as a signal to diffuse to the damage site and serve as a platform protein for the BER enzymes present (Ba and Garg, 2011, Oliver *et al.*, 1999). The rest of the repair of SSBs is carried out as already described under 1.5.4 *Base excision repair*.

1.5.5 Mitochondrial DNA Repair

Due to the absence of a repair of photoproducts in mitochondria, it has long been assumed that mitochondria have little or no mtDNA repair and thus explain the high mutation rate in mitochondria, which is about ten times higher than in the nucleus (Clayton *et al.*, 1974). This view has changed completely in recent years and it has been shown that most DNA repair pathways in mitochondria are fully functional. The only DNA repair pathway described in the nucleus, which is certainly not present in the mitochondria, is the NER (Liu and Demple, 2010). However, to compensate for a missing NER, mitochondria have found their own ways to deal with NER-specific

DNA damage. For example, close links between mitochondrial function and NER have been demonstrated, and it now seems evident that NER proteins, in addition to their roles in DNA repair, are also involved in redox homeostasis and energy metabolism. Furthermore, the DNA polymerase in mitochondria, the DNA polymerase γ (Poly) has a largely error-free trans-lesion replication, it reads over these errors. Otherwise, replication is completely blocked at these defects. The repair of oxidative damage in the mitochondria seems to be a priority compared to the repair of photoproducts. The fact that even primary NER proteins are recruited for this purpose alone underlines this (Hosseini *et al.*, 2015).

However, if the mitochondria are too badly damaged, they are degraded by a specific quality control mechanism called mitophagy. These organelles are disposed of via an autophagic-lysosomal degradation pathway dependent on PTEN-induced kinase 1 (PINK1) and Parkin (Dombi *et al.*, 2018).

1.6 The human 8-oxoguanine DNA glycosylase 1

Oxidative damage to DNA such as 8-oxoG, as mentioned above, is mainly repaired by BER processes (Hill *et al.*, 2001; Sampath *et al.*, 2012). The enzyme responsible for the recognition and removal of 8-oxoG is the human 8-oxoguanine DNA glycosylase 1 (hOGG1). It is found in the cytoplasm, in the cell nucleus as well as in the mitochondria (Fauchner *et al.*, 2012; Sampath *et al.*, 2012) and is the main antagonist against oxidative damage to DNA. It is a bifunctional glycosylase, has both N-glycosylase and AP-lyase activity (Hill *et al.*, 2001) and uses FapyG, 8-oxoG as substrate (Klungland and Lindahl, 1997). It removes 8-oxoG via the BER, replaces it with a guanine base and thus prevents the formation of mutations (Lindahl and Nyberg, 1972; Sancar, 2008). Due to its bifunctionality, hOGG1 is able to induce single-strand breaks by cutting DNA phosphodiester bonds without the involvement of AP endonucleases (Lu and Liu, 2010). Although hOGG1 is classified as a bifunctional enzyme, several recent experiments suggest that the *in vivo* relevant mechanism of hOGG1 is a monofunctional mode in which only the 8-oxoG nucleobase is removed (Müller and Carell, 2009).

1.6.1 Gene localization and structure

The gene OGG1 which codes for the enzyme hOGG1 is located on the third chromosome at position p25.3 and consists of a total of eight exons (Shinmura and Yokota, 2001). The structure of hOGG1 results from a single amino acid chain and is characterized by its domain folding with a DNA binding helix-hairpin-helix (HhH) motif and a glycine-proline-rich stretch with an aspartate . This allows hOGG1 to interact with the DNA to catalyze substrate recognition and the subsequent reaction. In addition, the enzyme has further functional domains, mainly binding domains, most of which are important for the binding of DNA and 8-oxoG (Table 1). The enzyme is additionally associated with two calcium ions to stabilize the deformed phosphate backbone of the DNA during damage response and subsequently return it to its native structure (Boiteux and Radicella, 2000).

Table 1. Overview of the functional domains of hOGG1 as well as the corresponding position in the amino acid sequence and their description.

Functional domains of hOGG1		
Feature key	Position	Description
Binding site	149	DNA
Binding site	154	DNA
Binding site	204	DNA
Active site	249	Schiff's-base intermediate with DNA
Binding site	266	8-oxoguanine; via carbonyl oxygen
Binding site	268	8-oxoguanine
Binding site	270	DNA
Binding site	287	DNA
Binding site	315	8-oxoguanine
Binding site	319	8-oxoguanine

1.6.2 Isoforms of hOGG1

Isoenzymes are several forms of an enzyme which catalyse the same processes, but differ both in their structure and in their amino acid sequence. They occur between individuals of a species and in different organs of a person or even in a single cell. The reason for the development of isoforms is the need to be able to work optimally with different activity under different environmental conditions or in the presence of different substrates. A change in the temperature or the pH-value for example can cause an enzyme to deviate from its optimum activity and thus ensure that certain reactions are either insufficient or no longer possible. Because of this, isoenzymes, which catalyse the same reaction but have different activity optimums, are indispensable for the internal processes of the cells. Eight active isoforms of this enzyme are registered on NCBI, which are composed of a combination of eight exons by alternating splicing of the messenger RNAs (mRNAs) of OGG1 (Ogawa *et al.*, 2015). However, not all exons are required per isoform. The different isoforms are divided into two main groups (type 1 and type 2) depending on the last exon of their mRNA sequence. Type 1a (targeted to the nucleus) and type 2a (targeted to mitochondria) have been identified as main isoforms in different human tissues, including lung, kidney, brain, and fetal brain. It has also been identified in peripheral blood lymphocytes, fibroblasts and many other cells (Aburatani *et al.*, 1997; Nishioka *et al.*, 1999). However, the exact characteristics of most of these isoforms have not yet been fully clarified. Since, in addition to the cell nucleus, the mitochondria also contain their own DNA and this mtDNA is particularly susceptible to oxidative stress due to the cell respiration that takes place there, it is not surprising that seven of the eight isoforms previously known to be active are located in the mitochondria (Furihata, 2015). Although the isoform hOGG1-1a, like all other isoforms contains a mitochondrial targeting sequence (MTS), it is localised in the cell nucleus, since it carries a additionally nuclear localisation sequence (NLS) at the C-terminal end (Boiteux and Radicella, 1999). Comparative studies between the isoforms -1a and -1b have shown that both use the same repair mechanism. It can therefore be assumed that DNA repair with regard to hOGG1 takes place in the nucleus in the same way as in the mitochondria (Furihata, 2015). The following figure shows the composition of the individual isoforms from the different exons.

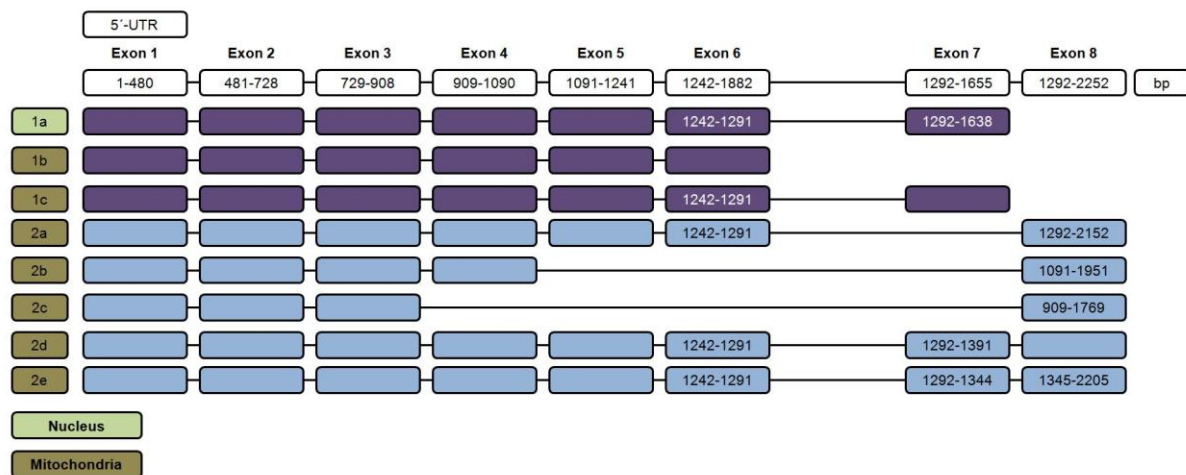


Figure 8. Simplified illustration of the different isoforms of hOGG1. Shown is the composition of the individual isoforms from the different exons and the nucleotide length. Data from Furihata, 2015.

1.6.3 DNA repair mechanism by hOGG1

In a somatic human cell there are about 5.0×10^4 hOGG1 molecules in a diploid chromosome set with about 6.4×10^9 base pairs. Moreover, since guanine and 8-oxoG differ in their structure only by two oxygen atoms (O^8 and N^7), it is obvious that hOGG1 has a very specific repair mechanism for damage search and damage detection (Cappelli *et al.*, 2001).

Initiation of the BER of 8-oxoG

The enzyme migrates along the DNA and scans it for the presence of oxidative damage. If this enzyme encounters an 8-oxoG paired with cytosine, it initiates the BER process (in the case of 8-oxoG repair, a short patch BER is performed). In hOGG1 the damage is detected by an amino acid residue of the active centre of the enzyme. With this residue the DNA strand is checked for its formability and stability, as stability decreases and formability increases in the case of a lesion. A change in these parameters may indicate a possible 8-oxoG mismatch (Bruner *et al.*, 2000). The enzyme differentiates between guanine and 8-oxoG by a single hydrogen bond, the bond between an oxygen atom located on a G42 carbonyl of the main chain of hOGG1 and the protonated N^7 atom of 8-oxoG. An amino acid residue of the hOGG1 enzyme thereby stabilizes the DNA strand, while the base on the exo side of the enzyme is controlled for an 8-oxoG specific binding (Dalhus *et al.*, 2011; Lingaraju *et al.*, 2005). If this is the case, after hOGG1 has bound to the site of DNA damage, the base identified as damaged is folded out of the DNA. In this process, known as base

flipping, the base carrying the damage is extruded from the helix so that it faces outwards away from the double helix in order to better reach the active centre of the enzyme (Banerjee *et al.*, 2005). The movement is called "pinch-push-plug-pull". First, the glycase induces bending and deformation of the DNA double helix (pinch). Then an amino acid side chain of the enzyme intercalates into the double helix to push the target base out of the helix (push). The same or a different intercalated amino acid side chain (not yet known) now acts as a "plug" to fill the gap created by the extruded base and thereby support the DNA (plug). Using side chains of the active site that specifically bind the damaged base now brings the base finally into the active center of the glycosylase (Pull) (Stivers, 2004).

A particularity arises when a mismatch of 8-oxoG with adenine occurs. Here the DNA-glycosylase MUTYH (human MutY Homolog) plays an important role. This enzyme has a adenine glycosylase activity, which enables it to remove the incorrectly incorporated adenine instead of the modified base in this type of mismatch (Michaels and Miller, 1992). The removal is carried out by a repair mechanism similar to the Long-Patch-BER (Parlanti *et al.*, 2002). This mechanism also leads to the subsequent generation of an 8-oxoG:C base pair. The excision of 8-oxoG is then performed by hOGG1, as described below (Lu *et al.*, 2001).

Reaction mechanism in the active centre of hOGG1

The structure of hOGG1 is similar to an endonuclease III and a 3-methyladenine DNA glycosylase II and is characterized by a helix-hairpin-helix DNA binding motif and a glycine-proline-rich loop terminated by an invariant catalytic aspartate residue. All members of this BER family with high lyase activity have a lysine residue with high lyase activity (Lys249 in hOGG1) within the helix-hairpin-helix DNA binding motif (van der Kemp *et al.*, 2004). Dalhus and his colleagues have now disproved that the lyase activity of hOGG1 is based on this amino acid, as previously suspected. They could show that aspartic acid (Asp268) plays a greater role here. His270, which is also located in the active centre, serves as a proton donor for the oxygen atom in the deoxyribose ring (Sebera *et al.*, 2017; Dalhus *et al.*, 2011). The enzymatic removal of 8-oxoG takes place via the N-glycosylase activity of hOGG1. This is based on an activated, nucleophilic aspartic acid. This acid attacks the N-glycosidic bond between 8-oxoG and the deoxyribose of the nucleotide and cleaves it enzymatically (Hill *et al.*, 2001). After removal of the damaged guanine, the amino group at the C ϵ atom of the

hOGG1 lysine residue reacts with the C1' carbon of the aldehyde group of the deoxyribose present in open-chain form (as aldehyde) due to base cleavage, which leads to the formation of a Schiff's base (an imine) intermediate and a covalent bond between hOGG1 and the DNA (Hill *et al.*, 2001; Müller and Carell, 2009). Schiff's bases are essential for the combination of lyase and glycosylase activity of a bifunctional enzyme. The resulting imine is subsequently cleaved either hydrolytically or in the course of β elimination as described (1.5.4 Base excision repair). In case of hydrolytic cleavage via an activated water molecule, hOGG1 is released immediately, leaving an intact AP site in the DNA double strand (Dalhus *et al.*, 2011). The enzyme can then bind back to this site and continue with the repair (removal of the AP nucleotide by the APE1 is also possible (Vidal *et al.*, 2001). In case of β elimination, hOGG1 is released during the elimination process. For further steps of the hOGG1-initiated BER of 8-oxoG, the already described short patch path is the preferred mechanism. This has already been demonstrated by Pascucci *et al.*, 2002 in in vitro experiments. They were able to show that only one nucleotide is incorporated during the repair process (Pascucci *et al.*, 2002). Figure 9 below shows the simplified reaction mechanism of hOGG1.

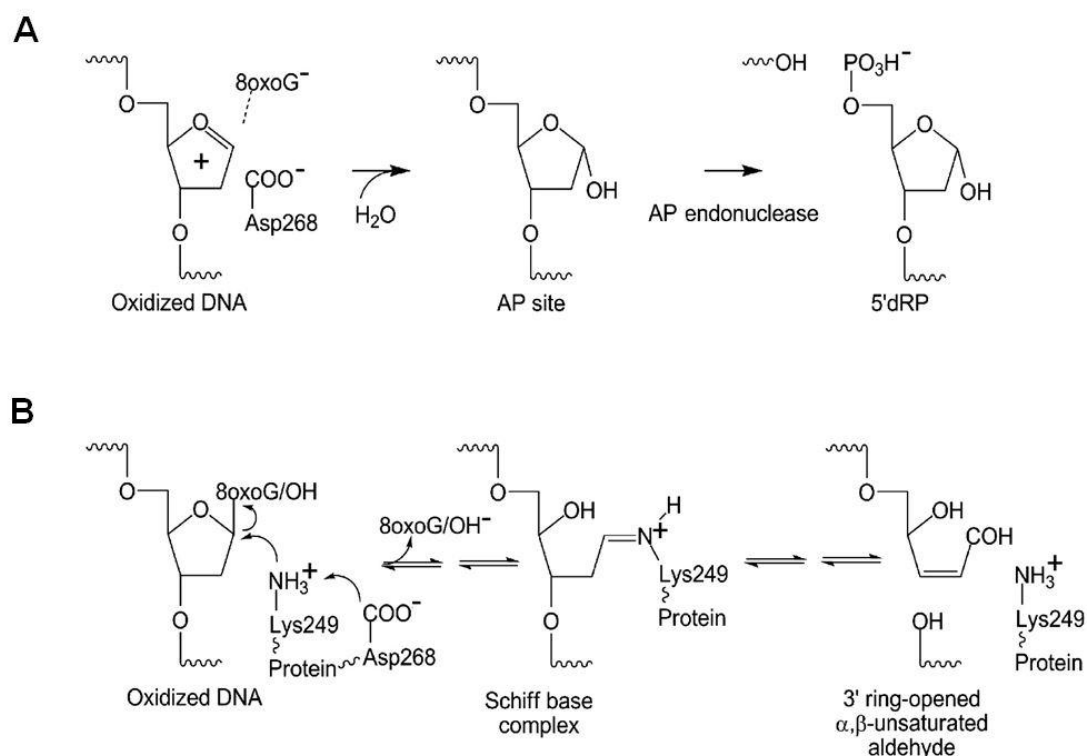


Figure 9. Simplified description of the bifunctional and monofunctional reaction mechanism of hOGG1. [A] During the bifunctional mechanism of hOGG1, Lys249 forms a covalent Schiff's base

intermediate, wherein Asp268 plays a role in the activation of the Lys249 nucleophile. The intermediate Schiff's base protein-DNA complex is dissolved by hydrolysis with water, resulting in β elimination and 3'-strand insertion. **[B]** In monofunctional mode, the reaction proceeds through an oxocarbenium ion, which is stabilized by the preserved Asp268 and then reacts with a water nucleophile to form an AP site. The AP site containing the DNA is incised by an AP endonuclease. The dotted line shows the lysis of the N-glycosidic bond. Figure adapted from Dalhus *et al.*, 2011.

1.7. Coenzyme Q₁₀

Coenzyme Q₁₀ (CoQ₁₀) is an ubiquitous endogenous quinone derivative found in the biological membranes of the majority of all cells in the body. It is also present as an anti-oxidative component in circulating lipoproteins (Bentinger *et al.*, 2010; Turunen *et al.*, 2004).

1.7.1 Chemical composition and properties

Structurally, CoQ₁₀ consists of a redox-active 2,3-dimethoxy-5-methylbenzoquinone ring and an isoprenoid side chain coupled to it at position 6. Depending on the sequence of functional groups attached to the quinone ring, physiologically irrelevant conformation (cis-conformation) can be distinguished from effective isomers (trans-conformation) (Crane, 2001). The number of linked dihydroisoprene units differs in different organisms and varies from six to ten dihydroisoprene units (Bentinger *et al.*, 2010). In humans, the chain is predominantly made up of ten monomers, which is why the lipid is accordingly referred to as CoQ₁₀. 7-9 % of the coenzyme is present in the human organism in the form of CoQ₉ (Turunen *et al.*, 2004). The 1,4-benzoquinone ring of ubiquinone and the 1,4-benzohydroquinone of ubiquinol represents the active functional group of the lipid. Via this redox-active structural element, electrons are taken up and released as a result of reduction and oxidation processes. Besides its antioxidative properties, the lipophilic isoprenoid side chain serves to anchor the coenzyme in biological membranes, thereby increasing the fluidity and permeability of the membrane (Turunen *et al.*, 2004). In its function as antioxidant, the predominantly present reduced form of the coenzyme Q₁₀, ubiquinol (CoQH₂), prevents the oxidation of DNA, lipids and proteins (Tomasetti *et al.*, 2001). CoQ₁₀, which is oxidized to ubiquinone (CoQ) is continuously converted back to its reduced form CoQH₂ by enzymatic processes in order to maintain its antioxidative properties (Bentinger *et al.*, 2010). This ensures that the CoQ₁₀ contained in blood

plasma usually consists of about 95 % CoQH₂ and about 5 % CoQ (Evans *et al.*, 2009).

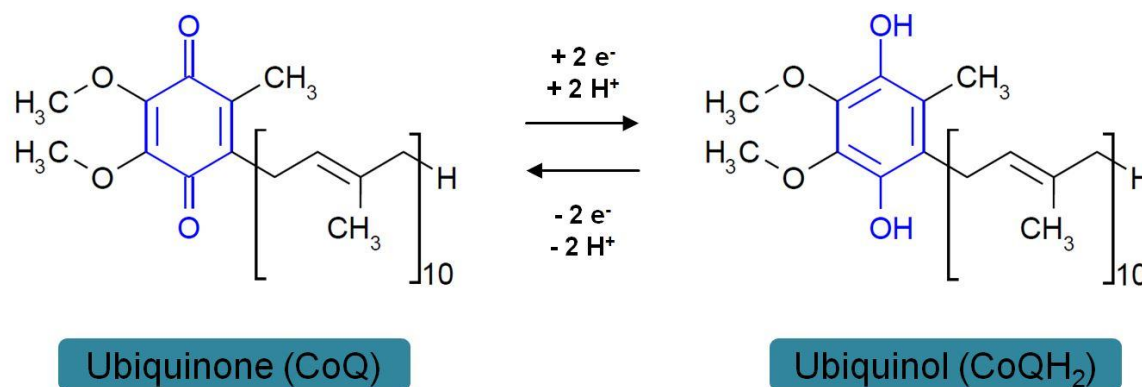


Figure 10. Chemical structures of the oxidized and reduced form of CoQ₁₀. Shown are the oxidized (CoQ) and reduced form (CoQH₂) of the coenzyme and the corresponding redox reactions. The quinone part of CoQ (1,4-benzoquinone) and the hydroquinone part of COQH₂ (1,4-benzohydroquinone) are marked in blue. Figure created after the template of Karlson *et al.*, 2005.

1.7.2 Biosynthesis and intracellular transport

Under normal physiological conditions, CoQ₁₀ can be synthesized in sufficient quantities by all cells of the human organism (Raizner, 2019). However, the exact process of biosynthesis in humans has not yet been fully clarified. Current knowledge and assumptions are mainly based on the results of extensive studies of synthesis in bacteria, yeasts and to a certain extent in animal cells (Bentinger *et al.*, 2010; Turunen *et al.*, 2004).

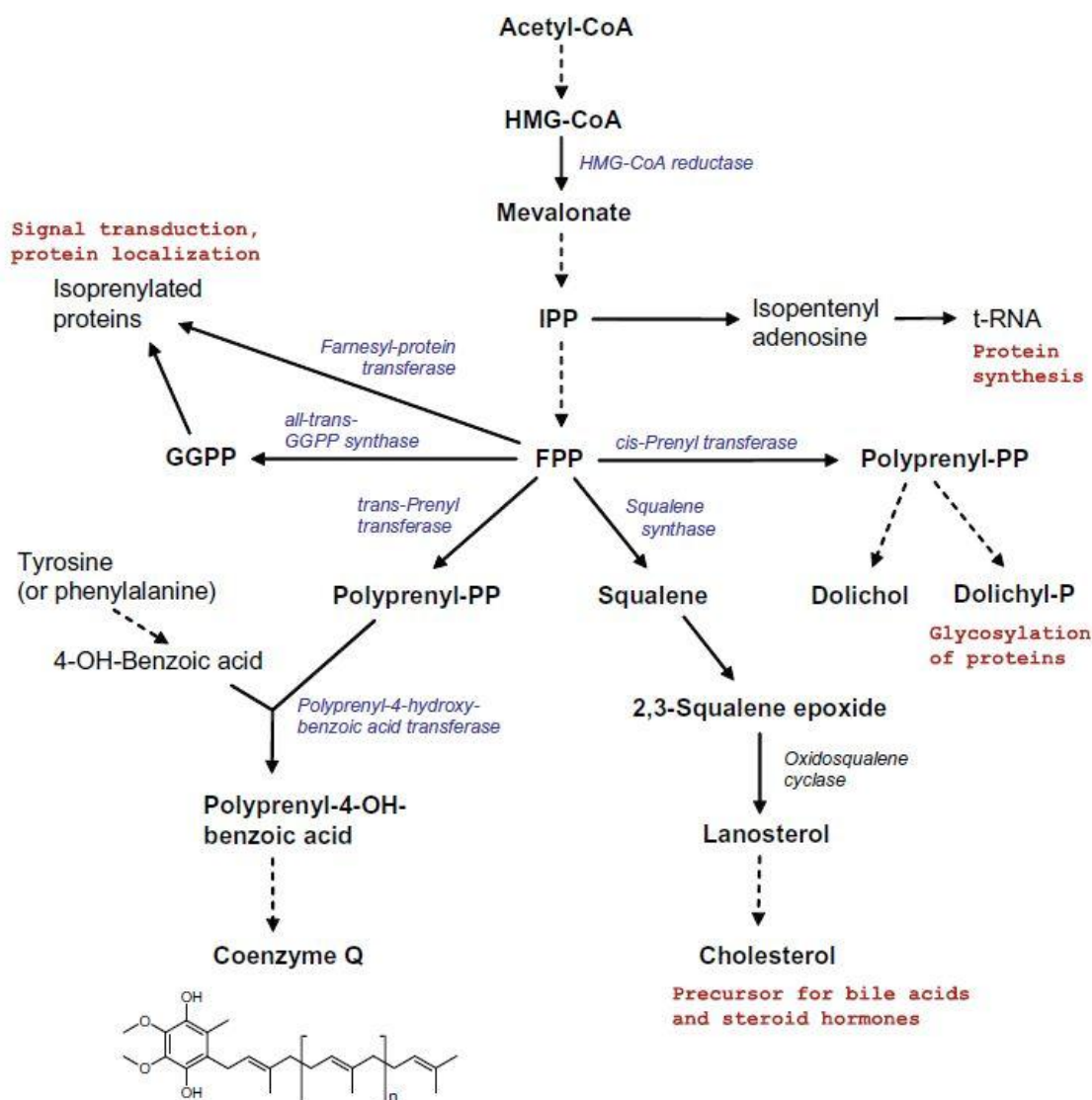


Figure 11. Schematic procedure of the coenzyme Q₁₀ biosynthesis. Shown is the biosynthesis of CoQ₁₀ via the mevalonate pathway, starting from acetylated coenzyme A (acetyl-CoA). The names of the enzymes responsible for the catalysis of the individual reactions are shown in blue. The primary functions of individual components are shown in red. Figure from Bentinger *et al.*, 2010.

The biosynthesis of CoQ₁₀ represents a complex process that can be implemented in different cell compartments and is described below. Starting from acetylated coenzyme A (acetyl-CoA), the formation of the CoQ₁₀ precursor farnesyl pyrophosphate (FPP) initially takes place in the cytoplasm and peroxisomes (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) is localized in peroxisomes) (Turunen *et al.*, 2004) via the reactions of the mevalonate metabolic pathway (Bentinger *et al.*, 2010; Turunen *et al.*, 2004). After uptake into the mitochondria, peroxisomes or into the endoplasmic reticulum (ER) Golgi system, the FPP is linked to isopentenyl pyrophosphate

molecules to form the isoprenoid side chain (Bentinger *et al.*, 2010; Turunen *et al.*, 2004). In the following step, this side chain reacts by splitting off water with 4-hydroxybenzoate, which is formed from tyrosine in advance and is usually present in excess. This condensation reaction is catalyzed by the polyprenyl 4-hydroxybenzoate transferase (Bentinger *et al.*, 2010; Turunen *et al.*, 2004). The resulting polyprenyl 4-hydroxybenzoate then undergoes a series of different modification reactions. Carbon hydroxylation, decarboxylation, oxygen methylation, and final carbon methylation take place (Bentinger *et al.*, 2010). At the end of this reaction sequence, the final CoQ₁₀ is obtained. Subsequently, the synthesized coenzymes are distributed or transported intracellularly to the respective target sites. CoQ₁₀ that has been finally synthesised in the mitochondrial matrix, remains in the mitochondria. It is probable that the entire newly formed CoQ₁₀ is integrated into the inner mitochondrial membrane (Turunen *et al.*, 2004). CoQ₁₀ synthesised in the ER-Golgi system, on the other hand, is incorporated as an antioxidative component into lipoproteins also formed there and leaves the cells in the form of these into the bloodstream (Turunen *et al.*, 2004). The transport of CoQ₁₀, which was formed in the ER-Golgi system, to other cell compartments or the associated membranes has not yet been clearly proven. However, based on the results of previous investigations, it is assumed that the transport takes place via vesicles or the Golgi apparatus (Turunen *et al.*, 2004).

1.7.3 The role of coenzyme Q₁₀ in the energy metabolism of eukaryotic cells

The chemical properties of CoQ₁₀ enable this molecule to perform numerous functions in the human organism. Due to its redox-active character, the ability to transport protons as well as electrons by redox reactions, CoQ₁₀ plays a central role in the course of the mitochondrial respiratory chain. The lipid located in the inner mitochondrial membrane acts as a transport system, which transports electrons from complexes I and II through the hydrophobic membrane interior to complex III of the respiratory chain (Bentinger *et al.*, 2010). CoQ is reduced to ubiquinol CoQH₂ by the uptake of two electrons and two protons. In the opposite direction, CoQH₂ can be oxidized to CoQ by the release of two electrons and protons (Figure 10). This redox reaction pair, consisting of 2,3-dimethoxy-5-methyl-1,4-benzoquinone and 2,3-dimethoxy-5-methyl-1,4-benzohydroquinone, represents the Q cycle and reflects the role of Q₁₀ as electron carrier. Extramitochondrial electron transport (e.g. in lysosomes) also occurs via this coenzyme (Turunen *et al.*, 2004). First, the electrons

originating from the cellular redox equivalents NADH and the hydroquinone form of the flavin adenine dinucleotides (FADH₂) are transferred to CoQ by complex I or complex II. CoQH₂ then transfers the electrons to cytochrome c via the enzyme ubiquinone-cytochrome c oxidoreductase (complex III). Thus, the electron flow is used to pump protons from the mitochondrial matrix into the intermembrane space to create an electrochemical proton-gradient across the inner mitochondrial membrane. This gradient is used to generate ATP using complex V (Figure 3) (Mitchel, 1975; Mitchel, 1991). The diffusion of CoQ₁₀ is significantly faster than its conversion at the respiratory complexes, which is why CoQ₁₀ behaves like a mobile diffusing CoQ₁₀ pool within the inner mitochondrial membrane (Lenaz and Genova, 2009). In addition to its role in energy metabolism, CoQ₁₀ is also thought to be involved in the inhibition of apoptosis, the formation of thiol groups and hydrogen peroxide, and the control of membrane channels (Crane, 2001).

1.7.4 The role of coenzyme Q₁₀ in the prevention of oxidative DNA damage

CoQ₁₀ plays a separate role in the antioxidant system of the human body alongside carotenoids, estrogens and tocopherols, the naturally occurring antioxidants, as it is the only fat-soluble antioxidant that can be synthesized endogenously (Bentinger *et al.*, 2007; Bentinger *et al.*, 2010; Turunen *et al.*, 2004). As an antioxidant, the reduced form CoQH₂ effectively prevents oxidation of DNA, lipids and proteins (Tomasetti *et al.*, 2001). The extraordinary efficiency as an antioxidant is due to the numerous cellular mechanisms for the regeneration of CoQH₂ and the localization of CoQ₁₀ (Bentinger *et al.*, 2010). Since cell-damaging ROS are mainly formed in the inner membranes of the mitochondria (site of the respiratory chain), the CoQH₂ localised there can capture the oxidants formed directly at the site of their formation, neutralise them as a result of reduction processes and thus protect cellular structures as well as proteins and DNA from oxidative damage (Bentinger *et al.*, 2010; Turunen *et al.*, 2004). Furthermore, its antioxidant property is associated with the ability to interfere with the initiation and proliferation of lipid peroxidation (a radical oxidation of unsaturated fatty acids) (Bentinger *et al.*, 2007). The regeneration of the CoQH₂ oxidised in these reactions takes place outside the inner mitochondrial membrane with the enzymes lipoamide dehydrogenase (LipDH), glutathione reductase (GR) and thioredoxin reductase 1 (TrxR-1) through zinc- and selenium-dependent reactions (Nordman *et al.*, 2003).

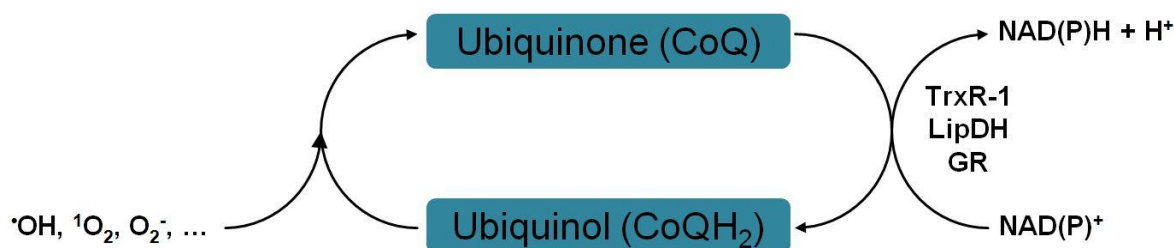


Figure 12. Schematic illustration of the regeneration of the antioxidant ubiquinol. The regeneration of ubiquinol (CoQH₂) after oxidation by reactive oxygen species (ROS) through the enzymes lipoamide dehydrogenase (LipDH), glutathione reductase (GR) and thioredoxin reductase 1 (TrxR-1) through zinc- and selenium-dependent reactions. Figure created after the template of Nordman *et al.*, 2003.

1.8 Clinical relevance

Diseases of the mitochondria, also known as mitochondriopathies, are diseases in which there is a defect in the mitochondria for which the genetic cause is often found in the mtDNA. Due to its structure and localisation directly next to the respiratory chain, mtDNA is particularly susceptible to oxidative stress. As a result, the rate of mutation in the mitochondrial genome is ten times higher than in the nuclear DNA (Wirth and Zichner, 2003). Without external influences, the rate of mutation in eukaryotes is about one nucleotide exchange per 1.0×10^9 base pairs in each replication round (Knippers, 2006). In addition, oxidative mtDNA damage in the respiratory chain leads to an increase in electron leakage and the number of radicals that contribute to further damage to the mtDNA. This creates a "vicious circle" of ROS production in the cells (Furda *et al.*, 2012; Yoshida *et al.*, 2012; Chinnery *et al.*, 2012; DeBalsi *et al.*, 2017). According to the "mitochondrial theory of ageing", these mtROS together with other oxidative mtDNA damage play a very important role in ageing processes and age-associated diseases. However, the details, for example the exact origin of mtROS and its exact amount, remain controversial (Lagouge and Larsson, 2013; Grivennikova and Vinogradov, 2013), although it was already shown in the 1980s and 1990s that mtDNA is severely damaged by ROS (Richter *et al.*, 1988; Yakes and Van Houten, 1997). The balance between oxidants and antioxidants plays an important role in this topic. For example, many anti-cancer agents use their molecular properties to generate high levels of reactive radical

species capable of directly or indirectly inducing lethal oxidative stress in cancer cells by destroying mitochondria (Coppotelli and Ross, 2016). At the same time as the production of ROS increases with age, the activity of glycosylases and thus also the activity of hOGG1 decreases with increasing age - the amount of 8-oxoG increases (Blasi *et al.*, 2001; Chen *et al.*, 1997). An increased 8-oxoG concentration in the organism has been demonstrated in many different clinical pictures. These include diabetes, cardiovascular diseases, cancer and age-related neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, as mtDNA damage mainly affects cells with high energy requirements and consequently an increased number of mitochondria, such as brain, heart and muscle cells (Krutmann and Schroeder, 2009; Prinzinger, 2005; Bentinger *et al.*, 2010). 8-oxoG as well as its removal therefore show great pathophysiological relevance and hOGG1 therefore plays an important role in preventing pathological processes such as skin ageing and carcinogenesis. It is suspected that the amount of the human 8-oxoguanine DNA glycosylase 1 at the level of gene expression and thus its protein expression can be actively controlled by the redox-active properties of ubiquinol (Tomasetti *et al.*, 2001). To restore the balance between oxidants and antioxidants, the use of CoQ₁₀ as a therapeutic agent has been proposed for some time and has been implemented in many areas of mitochondrial medicine. Mitochondrial medicine tries to address exactly this point. It is a new form of therapy in human medicine. It is intended to have a greater influence on the defective metabolism in the cells of the patient. Today it is known that many diseases are caused by errors in the metabolism of cells and especially of the mitochondria. The thousands of copies of mtDNA in a cell are normally identical at birth. In contrast, patients with mitochondrial dysfunction have a mixture of mutant and wild type mtDNA in each cell (Hol *et al.*, 1988; Hol *et al.*, 1990). Furthermore, the level of mutated DNA varies within the organs and tissues of the same individual (Macmillan *et al.*, 1993). This is one explanation for the high diversity of phenotypes of patients with dysfunction caused by mutations of mtDNA (Koopman *et al.*, 2012). So far, only the inherited mitochondriopathies were known, but for some time now the focus has been on many different environmental influences, such as UV rays, which damage the mitochondria. Besides genetic causes, drugs are another effector. Mainly antibiotics and chemotherapeutics, but also methylphenidate, which for example blocks complex I of the mitochondrial respiratory chain and thus increases superoxide production. In long

term, inflammations can cause damage in the mitochondria, as this is always accompanied by the release of ROS. Likewise, mental suffering in the body leads to the formation of ROS and thus to neurostress, which can cause damage to the mitochondria (Mutschler, 2012).

Due to its antioxidant property and membrane activity, CoQ₁₀ represents a promising possibility to influence the formation of ROS. For example, treatment with CoQ₁₀ induces increased activity of other antioxidant enzymes such as superoxide dismutase and glutathione peroxidase through increased gene expression of these enzymes (Blatt and Littarru, 2011). As an obligatory cofactor for mitochondrial uncoupling proteins, CoQ₁₀ is also involved in the prevention of ROS formation in mitochondria (Echtay *et al.*, 2000; Echtay *et al.*, 2001). If these uncoupling proteins are activated, this leads to a decoupling of oxidative phosphorylation, thereby lowering the proton gradient across the inner mitochondrial membrane and thus to a reduction in mitochondrial ROS formation. Given the multiple effects of CoQ₁₀ on cell metabolism, it is not surprising that CoQ₁₀ has been shown to be a useful therapeutic agent in various age-related and degenerative diseases such as neurodegeneration, mitochondrial myopathies, age-related macular degeneration and cardiovascular diseases (Beal, 2004; Blasi *et al.*, 2001; Chen *et al.*, 1997; Damian *et al.*, 2004). In all these clinical conditions, low levels of endogenous CoQ₁₀ were reported in comparison to healthy subjects. It has also been shown that ageing is associated with reduced CoQ₁₀ levels in the body (Bentinger *et al.*, 2010).

1.9 Aims of this thesis

Oxidative damage to mtDNA plays a major role in the daily damage to the DNA of human cells, since mitochondria are the main site of ROS formation and therefore their genome is particularly affected by oxidative stress. Lesions on the mtDNA and the resulting mitochondrial dysfunction can have negative effects on many essential processes such as cellular energy metabolism and are therefore considered to be triggers for a wide range of age-associated diseases such as neurodegenerative diseases like Parkinson's disease, but also diseases such as cancer or diseases of the cardiovascular system. According to the "mitochondrial theory of ageing", oxidative mtDNA damage in the respiratory chain leads to increased electron leakage and ROS, which in turn contributes to further damage of the mtDNA. This leads to a cycle of cellular ROS production and as a consequence, to an age-related increase

in mitochondrial dysfunctions. To counteract this process, ubiquinol is used in mitochondrial medicine for the treatment of age-associated diseases due to its antioxidant properties. However, the exact mechanisms of action in many aspects have not yet been clarified.

As UV radiation is one of the most harmful external influences on the skin, the first part of this work should examine the effect of UV-induced damage on the mitochondrial energy metabolism. This included cellular ATP levels, the mitochondrial respiratory profile and the mitochondrial membrane potential. In addition, the possibility of regeneration of these parameters after UV irradiation with ubiquinol should be investigated. Due to their role in the ageing process, the second part of this work should then analyse to what extent the age-related accumulation of mtDNA damage is reflected in the mitochondrial respiratory profile and how this "long-term induced" damage can be attenuated by the use of ubiquinol. Also associated with ageing processes and degenerative diseases, 8-oxoG is one of the most common oxidative damages of mtDNA. Since ubiquinol is suspected of influencing the repair enzyme hOGG1, which is responsible for this damage, the third and final part of the project focused on investigating the influence of ubiquinol on hOGG1 and the underlying mechanisms.

In times of demographic change, the average age of the population is constantly increasing, which is why the number of people with age-associated diseases is also rising. The results of this work should not only provide a better understanding of the influence of short- and long-term induced damage on the mitochondrial respiratory profile, but also help to identify further interesting targets for the use of ubiquinol as a drug for preventive as well as acute treatment in the field of mitochondrial medicine.

2. Materials

2.1 Antibiotics

Designation	Manufacturer
Gentamycin (10 mg/ml)	Gibco® by Life Technologies, Carlsbad, USA

2.2 Cell Culture

Cell type	Source
Human primary fibroblasts isolated from skin biopsies	District hospital Sigmaringen, general surgery unit, Sigmaringen, Germany District hospital Sigmaringen, center for urology, Pfullendorf, Germany Aesthetic Perfection Lake Constance, plastic surgery unit, Constance, Germany Joint surgical practice, Dr. Fuhrer, H. Nonnenmacher, Dr. Astfalk und Dr. Fauser, Reutlingen, Germany

2.3 Cell Culture Medium

Designation	Supplements	Manufacturer
Dulbecco's Modified Eagle's Medium (DMEM)-high glucose		Merck KGaA, Darmstadt, Germany
DMEM (1x)	10 % FBS, Gentamycin (50 µg/ml)	Gibco® by Life Technologies™, Carlsbad, USA
KBM-Gold™	KGM-Gold™ BulletKit™	Lonza Group AG,

Keratinocyte Basal
Medium

Basel, Switzerland

2.4 Chemicals and Reagents

Designation	Manufacturer
1,4-Dithiothreitol (DTT)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany and Life Technologies GmbH, Darmstadt, Germany
2-Mercaptoethanol ($\geq 99\%$)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl- 2H-tetrazolium bromide (MTT)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
3-[(3-Cholamidopropyl)dimethylammonio]-1- propanesulfonate (CHAPS, $\geq 98\%$, PUFFERAN [®])	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
ABT-888 (Veriparib, 1 mM)	AbbVie Deutschland GmbH & Co. KG, Wiesbaden, Germany
Acetic acid (100 %, ROTIPURAN [®])	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Adenosine 5'-triphosphate (ATP, 5 mM)	Life Technologies GmbH, Darmstadt, Germany
Antimycin A from <i>Streptomyces</i> sp. ($\geq 95\%$)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Benzamidine hydrochloride hydrate ($\geq 98\%$)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Bovine serum albumin (BSA, 20 $\mu\text{g}/\mu\text{l}$)	New England BioLabs [®] GmbH, Frankfurt am Main, Germany
Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP)	Cayman Chemical Company, Ann Arbor, USA
Carbonyl cyanide-4-	Sigma-Aldrich Chemie GmbH,

(trifluoromethoxy)phenylhydrazone (FCCP, ≥ 98 %)	Steinheim, Germany
CellROX [®] Green Reagent	Life Technologies GmbH, Darmstadt, Germany
cComplete [™] Lysis-M	Roche Diagnostics International AG, Rotkreuz, Switzerland
cComplete [™] Mini Protease Inhibitor Cocktail	Roche Diagnostics International AG, Rotkreuz, Switzerland
Dimethyl sulfoxide (DMSO, $\geq 99,5$ %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, ≥ 98 %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
D-Luciferin	Life Technologies GmbH, Darmstadt, Germany
Ethanol (EtOH, $\geq 99,8$ %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Ethylenediaminetetraacetic acid (EDTA, ≥ 99 %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Ethylene glycol-bis(2-aminoethylether)- <i>N,N,N',N'</i> -tetraacetic acid (EGTA, $\geq$ 99 %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
FACS Clean	BD Biosciences, Heidelberg, Germany
FACS Sheat solution	BD Biosciences, Heidelberg, Germany
Fetal bovine serum (FBS)	Gibco [®] by Life Technologies [™] , Carlsbad, USA
Glycerol ($\geq 99,5$ %, ROTIPURAN [®])	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
His Mag Sepharose [™] Ni	GE Healthcare Life Sciences, Chicago, USA
Hydrochloric acid (HCl, 37 %, ROTIPURAN [®])	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Isopropyl alcohol ($\geq 99,5$ %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany

JC-1	Cayman Chemical Company, Ann Arbor, USA
LightCycler [®] 480 Probes Master	Roche Diagnostics International AG, Rotkreuz, Switzerland
Luciferase, firefly recombinant (5 mg/ml)	Life Technologies GmbH, Darmstadt, Germany
Magnesium chloride (MgCl ₂ , ≥ 98,5 %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Menaquinone-7 (Vitamin K ₂ , MK-7)	Merck KGaA, Darmstadt, Germany
MitoSOX [®] Red	Life Technologies GmbH, Darmstadt, Germany
NEBuffer [™] 2 (10x)	New England BioLabs [®] GmbH, Frankfurt am Main, Germany
Oligomycin from <i>Streptomyces diastatochromogenes</i> (≥ 90 %)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Potassium chloride (KCl, ≥ 99 %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Potassium dihydrogen phosphate (KH ₂ PO ₄ , ≥ 99 %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Reaction buffer 20x	Life Technologies GmbH, Darmstadt, Germany
Rotenone (≥ 95 %)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Sodium chloride (NaCl, ≥ 99,9 %, CELLPURE [®])	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Sodium dodecyl sulfate (SDS, ≥ 99 %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Sodium hydroxide (NaOH, ≥ 98 %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
<i>tert</i> -Butyl hydroperoxide solution (TBHP, 70 %, in H ₂ O)	Life Technologies GmbH, Darmstadt, Germany
Tri-sodium citrate dihydrate (≥ 99 %)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Tris hydrochloride (Tris-HCl, ≥ 99 %,	Carl Roth GmbH & Co. KG, Karlsruhe,

PUFFERAN [®])	Germany
Triton [™] X100	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Ultrapure Water (ddH ₂ O) - PureLab flex - water preparation unit	Veolia Water Technologies Deutschland GmbH, Celle, Germany
XF24 Calibrant Solution (pH 7.4)	Agilent Technologies, Santa Clara, USA

2.5 Buffers and Solutions

Designation	Composition
10x PBS	80.0 g NaCl 2.0 g KCl 28.8 g Na ₂ HPO ₄ ·12H ₂ O 12.4 g KH ₂ PO ₄ <i>ad</i> 1000 ml ddH ₂ O, pH 7.4
1x PBS	50 ml 10x PBS <i>ad</i> 500 ml ddH ₂ O
Antimycin A (2.5 mM)	1.35 mg Antimycin A <i>ad</i> 1000 µl DMSO
BSA solution (2 µg/µl)	130 µl BSA (20 µg/µl) <i>ad</i> 1300 µl ddH ₂ O
Cell lysis buffer	5 ml Triton [™] X100 4.38 g NaCl 3.03 g Tris-HCl 0.73 g EDTA <i>ad</i> 500 ml ddH ₂ O, sterile filtration (0.2 µm), pH 8.0
CellROX solution (0.25 mM)	10 µl CellROX [®] Green Reagent <i>ad</i> 100 µl 1x PBS
CHAPS lysis buffer	1.25 ml Glycerol (80 %) 1 ml CHAPS (5 %) 100 µl Tris-HCl (1 M) 100 µl EGTA (0.1 M)

	10 µl MgCl ₂ (1 M)
	5 µl Benzamidine (0.2 M)
	3.52 µl 2-Mercaptoethanol (14.19 M)
	<i>ad</i> 10 ml ddH ₂ O, one tablet cOMplete™
	Mini Protease Inhibitor Cocktail
Coenzyme Q ₁ stock solution (10 mM)	2 mg Q ₁ dissolved in 800 µl DMSO
Coenzyme Q ₁₀ stock solution (10 mM)	10 mg Q ₁₀ dissolved in 1159 µl DMSO
DTT stock solution (2.5 M)	193 mg DTT
	<i>ad</i> 500 µl ddH ₂ O, sterile filtration
	(0.2 µm)
Enzymatic digestion solution	8 ml KBM-Gold™ Keratinocyte Basal Medium
	4 ml Dispase II (final concentration
	2.4 U/ml)
FCCP (2.5 mM)	635.4 µg FCCP
	<i>ad</i> 1000 µl DMSO
hOGG1 assay buffer (base excision repair assay)	4872 µl ddH ₂ O
	2564 µl DMSO
	1282 µl BSA solution (2 µg/µl)
	1282 µl NEBuffer™ 2 (10x)
Menaquinone-7 solution (2.5 mM)	24.3 mg Menaquinone-7
	<i>ad</i> 15 ml EtOH, sterile filtration (0.2 µm)
MitoQ® stock solution (10 mM)	10 mg MitoQ® dissolved in 1.473 ml DMSO
MitoSOX solution (5 mM)	50 µg MitoSOX™ Red
	<i>ad</i> 16 µl DMSO
MTT solution I	5 mg 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
	<i>ad</i> 1 ml 1x PBS, sterile filtration (0.2 µm)
MTT solution II	99.4 ml DMSO
	10 g SDS
	0.6 ml Acetic acid
NaOH (10 M)	40 g NaOH
	<i>ad</i> 100 ml ddH ₂ O

NEBuffer™ 2 (1x)	50 mM NaCl 10 mM Tris-HCl 10 mM MgCl ₂ 1 mM DTT pH 7.9
Oligomycin (2.5 mM)	5 mg Oligomycin dissolved in 2528 µl DMSO
QuinoMit® Q ₁₀ -Fuid stock solution (10 mM)	0.904 g QuinoMit® Q ₁₀ -Fuid ad 5 ml ddh ₂ O, sterile filtration (0.2 µm)
QuinoMit® - carrier control stock solution (10 mM)	0.904 g QuinoMit® Q ₁₀ -Fuid ad 5 ml ddh ₂ O, sterile filtration (0.2 µm)
Rotenone (2.5 mM)	986.1 µg Rotenone ad 1000 µl DMSO
SiaMit® Q ₁₀ stock solution (2.5 mM)	0.108 g SiaMit® Q ₁₀ ad 5 ml ddh ₂ O, sterile filtration (0.2 µm)
Sodium citrate solution (2 M)	5.882 g Tri-sodium citrate dihydrate ad 10 ml ddH ₂ O
Standard reaction solution (SRL)	4810 µl ddH ₂ O 270 µl D-Luciferin 270 µl Reaction buffer 20x 54 µl DTT 1.35 µl Luciferase
Stop solution	50 ml FBS ad 500 ml 1x PBS
TBHP solution (50 mM)	3.22 µl TBHP ad 500 µl 1x PBS
XF assay medium	0.675 g Dulbecco's Modified Eagle's Medium-high glucose ad 50 ml ddH ₂ O, sterile filtration (0.2 µm), pH 7.4

2.6 Enzymes

Designation	Manufacturer
Recombinant human OGG1 (1600 Unit/ml)	New England BioLabs® GmbH, Frankfurt am Main, Germany
Recombinant human OGG1 (0.50mg/ml)	Abcam plc, Cambridge, UK
0.5 % Trypsin/EDTA (10x)	Gibco® by Life Technologies™, Carlsbad, USA
Dispase II (neutral protease, grade II)	Roche Diagnostics International AG, Rotkreuz, Switzerland

2.7. Coenzyme Q₁₀ formulations

Designation	Manufacturer
Coenzyme Q ₁	Merck KGaA, Darmstadt, Germany
Coenzyme Q ₁₀	Merck KGaA, Darmstadt, Germany
Mitoquinone (mesylate) (MitoQ®)	MedChemTronica AB, Sollentuna, Sweden
QuinoMit® Q ₁₀ -Fuid	mse Pharmazeutika GmbH, Bad Homburg, Germany
QuinoMit® - carrier control	mse Pharmazeutika GmbH, Bad Homburg, Germany
SiaMit® Q ₁₀	mse Pharmazeutika GmbH, Bad Homburg, Germany

2.8 Reporter oligonucleotides

Description
CN-8-oxoG: [6FAM]CCATAATAATAAAC[8-oxo-dG]CAATAATAATAATACC[6FAM]
CN-CTRL: [6FAM]CCATAATAATAAACGCAATAATAATAATACC[6FAM]

BHQ-1-tagged complementary strand:

[BHQ1]GGTATTATTATTATTGCGTTATTATTATTATGG[BHQ1]

Manufacturer

All oligonucleotides were ordered from Sigma-Aldrich Chemie GmbH, Steinheim, Germany

2.9 Primer**Description**

Target	Assay ID, Primer sequence, UPL no.
OGG1	Acc:8125]",111525,CTGCATCCTGCCTGGAGT, CCTGGGGCTTGTCTAGGG,83
RPL13A	Acc:10159]",102119,CTGGACCGTCTCAAGGTGTT GCCCCAGATAGGCAAACCTT,157
RPLP0	Acc:10371]",101144,TCGACAATGGCAGCATCTAC GCCAATCTGCAGACAGACAC,6
PGK1	Acc:8896]",102083,GGAGAACCTCCGCTTTCAT GCTGGCTCGGCTTTAACC,69

Manufacturer

Gene expression was performed with the qPCR, RealTime ready Custom Panels (Roche Diagnostics International AG, Rotkreuz, Switzerland). In this system, primer and probe are already lyophilized in the LC480 plates.

2.10 Molecular Biological Kits

Designation	Manufacturer
ATP Determination Kit	Life Technologies GmbH, Darmstadt, Germany
Cell Titer-Glo [®] Luminescent Cell Viability Assay	Promega Corporation, Fitchburg, USA

High Pure RNA Isolation Kit	Roche Diagnostics International AG, Rotkreuz, Switzerland
His Buffer Kit	GE Healthcare Life Sciences, Chicago, USA
KGM-Gold™ BulletKit™	Lonza Group AG, Basel, Switzerland
Seahorse XF Cell Mito Stress Test Kit	Agilent Technologies, Santa Clara, USA
Transcriptor First Strand cDNA Synthesis Kit	Roche Diagnostics International AG, Rotkreuz, Switzerland

2.11 Laboratory Equipment

Description	Manufacturer
BD FACSCalibur™ System	BD Biosciences, Heidelberg, Germany
Beakers	Schott Duran® Group, Mainz, Germany
Centrifuge Eppendorf 5417 R	Eppendorf AG, Hamburg, Germany
Centrifuge Eppendorf 5804 R	Eppendorf AG, Hamburg, Germany
Centrifuge Mikro 24-48	Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany
Erlenmeyer flasks	Schott Duran® Group, Mainz, Germany
Freezer (- 80 °C) - Thermo Fisher HFU 600TV	Thermo Fisher Scientific GmbH, Dreieich, Germany
Freezer (- 20 °C) - Liebherr Comfort	Liebherr-International Deutschland GmbH, Ochsenhausen, Germany
Glass bottles	Schott Duran® Group, Mainz, Germany
HERA Cell 240 CO ₂ -Incubator	Heraeus Holding GmbH, Hanau, Germany
Incubator 4010	GFL - Gesellschaft für Labortechnik mbH, Burgwedel, Germany
Laminar flow cabinet class II	BDK Luft- und Reinraumtechnik GmbH, Sonnenbühl-Genkingen, Germany
LightCycler® 480	Roche Diagnostics International AG, Rotkreuz, Switzerland
Magnetic Separator MagRack6	GE Healthcare Life Sciences, Chicago, USA
Magnetic Stirrer IKA-Combimag RCT	Janke & Kunkel GmbH & Co. KG, Staufen,

Measuring cylinders	Germany Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Micro balance Mettler AE 160 (Max. 30 g, d = 0.01 mg)	Mettler-Toledo GmbH, Gießen, Germany
Micro balance Mettler AE 200 (Max. 200 g, d = 0.1 mg)	Mettler-Toledo GmbH, Gießen, Germany
Microplate Reader Victor ³ 1420- fluorometer	PerkinElmer [®] , Waltham, USA
Microscope Axiovert 40 CFL	Carl Zeiss Microscopy GmbH, Jena, Germany
Microscope Axiovert Primovert	Carl Zeiss Microscopy GmbH, Jena, Germany
Multi-channel pipettes Transferpette [®] - 8 5-50/20-200 µl	Brand GmbH & Co. Kg, Wertheim, Germany
Nalgene [®] Cyro Freezer Container	Life Technologies GmbH, Darmstadt, Germany
NanoDrop One (spectralphotometer)	Thermo Fisher Scientific GmbH, Dreieich Germany
Neubauer improved counting chamber (0.1 mm depth/ 0.0025 mm ²)	Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Germany
PCR-Cooler	Eppendorf AG, Hamburg, Germany
pH Meter FiveEasy [™] F20	Mettler-Toledo GmbH, Gießen, Germany
Pipetboy acu	INTEGRA Biosciences Deutschland GmbH, Biebertal, Germany
Pipettes Reference [®] 0.1-2.5/0.5-10/2-20/20-200/100-1000 µl	Eppendorf AG, Hamburg, Germany
Pipettes Research [®] plus 0.5-10 /2-20/10-100/20-200/100-1000 µl	Eppendorf AG, Hamburg, Germany
Precision balance PCB 250-3 (Max. 250 g, d = 1 mg)	KERN & SOHN GmbH, Balingen- Frommern, Germany
PureLab flex - water preparation unit	Veolia Water Technologies Deutschland GmbH, Celle, Germany
Refrigerator (4 °C)	Gorenje Vertriebs GmbH, München, Germany

Refrigerator (4 °C) Exquisit	GGV Handelsgesellschaft mbH & Co. KG, Kaarst, Germany
Rotilabo® Tabletop centrifuge	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Scissor, stainless stell	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Seahorse Bioscience XF24 Extracellular Flux Analyzer	Agilent Technologies, Santa Clara, USA
Seahorse Capture Screen Insert Tool	Agilent Technologies, Santa Clara, USA
Solar simulator 400F/5C	Dr. Hönle AG, UV-Technologie, Gräfelfing, Germany
Sonoplus Ultraschall-Homogenisator UW 3100	Bandelin electronic GmbH & Co. KG, Berlin, Germany
Systec VX-75 with exhaust air filtration	Systec GmbH, Linden, Germany
Thermomixer MKR 13 HLC	DITABIS Digital Biomedical Imaging Systems AG, Pforzheim, Germany
Tweezers, stainless steel	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
UV meter µC, HighEnd	Dr. Hönle AG, UV-Technologie, Gräfelfing, Germany
VACUSAFE Aspiration System	INTEGRA Biosciences Deutschland GmbH, Biebertal, Germany
ViCell™ XR cell viability analyzer	Beckman Coulter GmbH, Krefeld, Germany
Vortex-Genie™ 2 G-560E	Scientific Industries Inc., Bohemia, USA
Water bath	GFL - Gesellschaft für Labortechnik mbH, Burgwedel, Germany

2.12 Consumables

Consumables	Manufacturer
Cell scraper	Sarstedt AG & Co. KG, Nümbrecht, Germany
Cell Strainer	Corning GmbH, Kaiserslautern, Germany
Centrifugation Tubes (15/50 ml)	Sarstedt AG & Co. KG, Nümbrecht,

Cover slip 24x24 mm	Germany Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Cryovials 2 ml	Sarstedt AG & Co. KG, Nümbrecht, Germany
Disposal bags	Sarstedt AG & Co. KG, Nümbrecht, Germany
FACS Tubes 0,5/5 ml)	Sarstedt AG & Co. KG, Nümbrecht, Germany
Injection cannula (20 G x 1 ½ ")	B. Braun Melsungen AG, Melsungen, Germany
LightCycler® 480 Multiwell Plate 96 (white)	Roche Diagnostics International AG, Rotkreuz, Switzerland
LightCycler® 480 Sealing Foil	Roche Diagnostics International AG, Rotkreuz, Switzerland
Microscope slides (frosted one end) 25 x 75 mm	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Multi-channel reservoir	neoLab® MIGGE GmbH, Heidelberg, Germany
Parafilm M	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Pasteur pipettes (glass)	Poulten & Graf GmbH, Wertheim, Germany
PCR plates Multiply	Sarstedt AG & Co. KG, Nümbrecht, Germany
Petri dish 10 cm Cell+	Sarstedt AG & Co. KG, Nümbrecht, Germany
Pipette filter tips Biosphere® (various)	Sarstedt AG & Co. KG, Nümbrecht, Germany
Pipette tips (various)	Sarstedt AG & Co. KG, Nümbrecht, Germany
qPCR, RealTime ready Custom Panels 96 - 16	Roche Diagnostics International AG, Rotkreuz, Switzerland
Reaction tubes 0.2/1.5/2 ml	Sarstedt AG & Co. KG, Nümbrecht,

Serological pipettes (various)	Germany Sarstedt AG & Co. KG, Nümbrecht, Germany
Syringe 5, 10, 20 ml Norm Ject®	Henke Sass Wolf GmbH, Tuttlingen, Germany
Syringe filter Filtropur S 0,2 (0,2 µm)	Sarstedt AG & Co. KG, Nümbrecht, Germany
Tissue culture plates Cell+ 6-, 12-, 24-, 96-well	Sarstedt AG & Co. KG, Nümbrecht, Germany
Tissue culture flask Cell+ T25/75/175	Sarstedt AG & Co. KG, Nümbrecht, Germany
Vasco® Nitril long (gloves)	B. Braun Melsungen AG, Melsungen, Germany
ViCell™ 4 ml Sample Vials	Beckman Coulter GmbH, Krefeld, Germany
XF24 cell culture microplates	Agilent Technologies, Santa Clara, USA
XF24 Flux-Plates (sensor cartridge)	Agilent Technologies, Santa Clara, USA
XF24 islet capture microplates	Agilent Technologies, Santa Clara, USA

2.13 Software

Software	Source
CELLQuestPro software, Version 5.1	BD Biosciences, Heidelberg, Germany
Citavi 4, Version 4.5.0.11	Swiss Academic Software GmbH, Wädenswil, Switzerland
GraphPad Prism, Version 5.03, 7.04, 8.2.1	GraphPad Software®, San Diego, USA
LightCycler® 480 Software (instrument software), Version 1.5.0.39	Roche Diagnostics International AG, Rotkreuz, Switzerland
Microsoft Office Standard Edition 2010	Microsoft, Redmond, USA
Seahorse XF24 Flux Analyzer Software (instrument software), Version 1.8.1.1	Agilent Technologies, Santa Clara, USA

Vi-CELL™XR Cell Viability Analyzer
(instrument software), Version 2.03

Wallac victor³ 1420-fluorometer
(instrument software), Version 3.00

Revision 5

Wave analysis software, Version 2.6

Beckman Coulter GmbH, Krefeld,
Germany

PerkinElmer®, Waltham, USA

Agilent Technologies, Santa Clara, USA

3. Methods

3.1 Cell culture

3.1.1 Sample collection / Ethical aspects

Human fibroblasts were isolated from skin biopsies which were received from the district hospital Sigmaringen, department of general surgery (Sigmaringen, Germany) and from the center for urology, Pfullendorf, Germany, from Aesthetic Perfection Lake Constance, department of plastic surgery (Constance, Germany); or from the Joint surgical practice Dr. Fuhrer, H. Nonnenmacher, Dr. Astfalk und Dr. Fauser (Reutlingen, Germany). Experiments were carried out with skin biopsies from donors of different ages and fibroblasts isolated therefrom. The sampling sites of the biopsies ranged from abdomen, femoral, foreskin to upper eyelids (Figure 13). The profiles of all donors are summarized in Table 2. After surgery, the biopsies were stored in 1x phosphate-buffered saline (PBS) at 4 °C for a maximum of 36 hours (h). During this period it was important to isolate the cells as well as to perform further measurements directly on the biopsies, as the mitochondrial functions and thus the quality of the cells decrease significantly for further experiments after this period. All experiments were conducted in accordance with the Declaration of Helsinki and approved by the Ethics Commission of the State Medical Association of Baden-Württemberg, Germany (187-03). Patients were informed in advance and gave their written consent to the use of their samples.

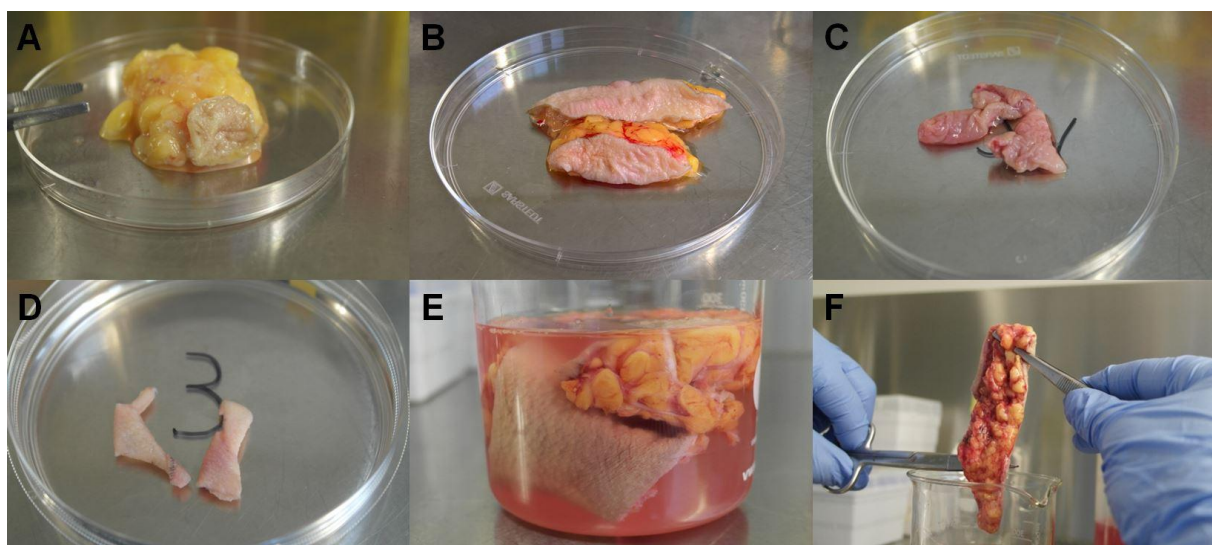


Figure 13. Variety of biopsies obtained. **A/B.** Abdominal specimens - wound margins, artificial intestinal outlets. **C.** Foreskin samples - circumcision for medical and religious reasons. **D.** Upper eyelids - plastic surgery (Eyelid Lifting). **E/F.** Femoral - plastic surgery (thigh lift).

Table 2. Summary of all donors. Biopsies were used either for cell isolation or for direct measurement of OCR. (F - female, M - male, ns - non-smoking, s - smoking).

Donor no.	Age [y]	Sex	Smoking/ non smoking	Body site
1	6	M	ns	Foreskin
2	8	M	ns	Foreskin
3	9	M	ns	Foreskin
4	14	M	ns	Foreskin
5	29	F	s	Femoral
6	35	F	ns	upper eyelids
7	41	F	ns	upper eyelids
8	45	M	ns	Foreskin
9	46	M	ns	Abdomen
10	46	F	ns	upper eyelids
11	50	M	ns	upper eyelids
12	53	M	ns	Foreskin
13	54	F	ns	upper eyelids
14	55	F	ns	upper eyelids
15	57	F	ns	Abdomen
16	57	M	ns	upper eyelids
17	60	M	ns	Foreskin
18	60	M	ns	Abdomen
19	61	M	ns	Foreskin
20	62	F	ns	Abdomen
21	63	F	s	Abdomen
22	63	M	ns	Abdomen
23	64	M	s	Abdomen
24	64	M	s	Abdomen
25	65	F	s	Abdomen
26	65	F	ns	Abdomen
27	68	F	ns	Abdomen
28	68	M	ns	Abdomen
29	69	F	ns	Abdomen
30	70	F	s	Abdomen
31	70	M	ns	Abdomen
32	71	M	ns	Abdomen
33	72	F	ns	Abdomen
34	73	F	ns	Abdomen
35	74	F	s	Abdomen
36	75	F	s	Abdomen
37	75	M	ns	Abdomen
38	76	M	ns	Abdomen
39	77	M	s	Abdomen
40	77	M	ns	upper eyelids
41	77	M	s	Abdomen
42	78	M	ns	Abdomen
43	79	M	ns	Abdomen
44	80	F	ns	Abdomen
45	82	F	ns	Abdomen
46	82	M	ns	Abdomen
47	83	M	ns	Abdomen
48	83	F	ns	Abdomen
49	84	M	ns	Abdomen
50	86	M	ns	Abdomen

3.1.2 Isolation of skin fibroblasts

For the isolation of fibroblasts from the biopsies, they first had to be purified of blood and other impurities. For this purpose, the samples were transferred to a reaction tube with 30 ml of 70 % isopropanol and immersed five times using sterile tweezers. The samples were then transferred to another reaction tube with 30 ml of 1x PBS and also immersed 5 times. The biopsies were then transferred with into a petri dish with 15 ml of 1x PBS using another pair of sterile tweezers (Figure 14A) to remove fat and connective tissue adherent to the dermis with a sterile scissor. The remaining skin (Figure 14B) was then cut into small pieces (0.4 x 0.4 cm approximately) and placed in enzymatic digestion solution - 8 ml keratinocyte growth medium (KBMTM Gold, Lonza) plus 4 ml Dispase II (Roche Diagnostics) (final concentration 2.4 U/ml) (Figure 14C). The samples were then incubated either at 4 °C overnight (16 - 18 h) in the refrigerator or for 6 h at 37 °C in the incubator (HERA Cell 240) under non-stirring conditions. The following day, the epidermis was separated from the dermis with fine tweezers. The dermis was placed into a T25 flask with 4 ml of fibroblast medium (DMEM, Gibco) and incubated at 37 °C, 5 % carbon dioxide (CO₂) and 90 % humidity in the incubator. Dermis should not be covered completely (Figure 14D). After 3 days, the first media change took place. The old medium was removed without touching or moving the dermis and replaced by 4 ml fresh fibroblast medium. The separated epidermis was used in the course of this work for the measurement of the oxygen consumption rate (OCR) directly in epidermis (3.10 Mitochondrial respiration measurement - Sample preparation).

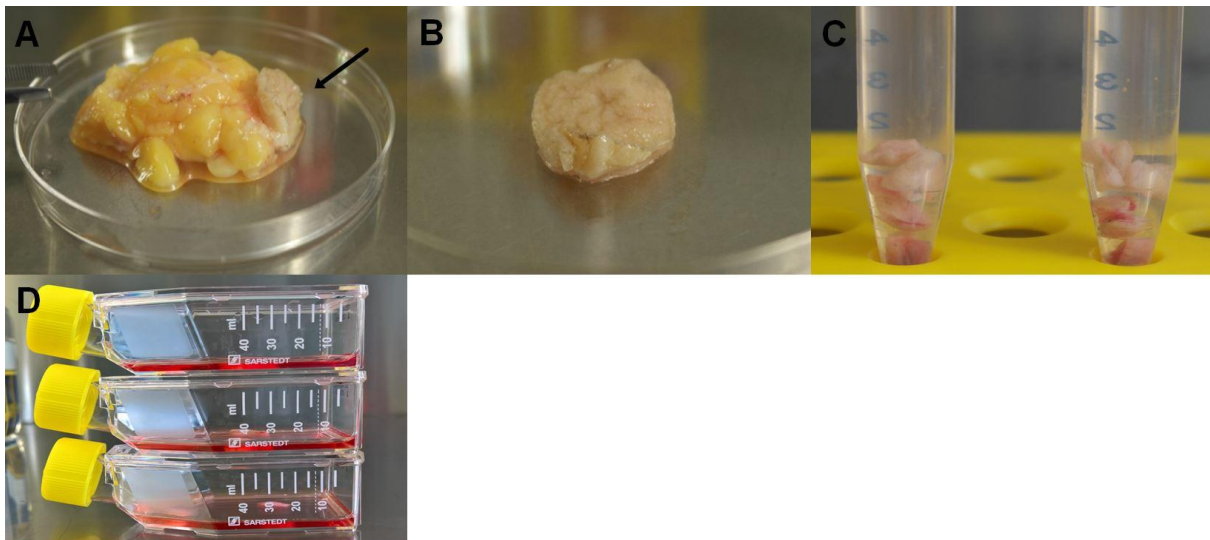


Figure 14. Isolation steps of the biopsy. **A.** Fat and connective tissue adherent to the dermis/epidermis (arrow). **B.** Dermis/Epidermis after first purification step. **C.** Divided biopsy in solution for enzymatic digestion. **D.** Dermis after separation from epidermis in a T25 flask with fibroblast medium.

3.1.3 Culturing conditions

The human primary fibroblasts used in the subsequent experiments were maintained in culture flasks in cell-specific medium at 37 °C, a CO₂ content of 5 % and a relative humidity of 90 % (standard conditions). All fibroblasts were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum and 50 µg/ml gentamycin (all from Gibco).

3.1.4 Subculturing

In order for subculturing the cells, the old growth medium was removed and the cells washed twice with prewarmed 1x PBS (37 °C) after a check for sufficient confluent growth (80 - 90 %). The cells were then overlayed with 0.5 % Trypsin/EDTA (Gibco), which caused the cells to detach from the cell culture flask during an incubation period of approximately 5 minutes (min) at 37 °C in the incubator. After the cells had completely dissolved, the reaction was stopped with 10 % fetal bovine serum (PAA Laboratories) in 1x PBS. Thereafter cell suspension was transferred to a 50 ml centrifugation tube and centrifuged for 5 min at 300 g. Then the supernatant was removed and fresh cell culture medium was added. The cells were transferred to new cell culture bottles without calculation of the cell count every 3 - 4 days. Due to a lack

of immortality of primary fibroblasts, the cells were subcultured up to a maximum of 12 passages. In order to seed the cells in multi-well plates, the cells were counted (3.1.5 *Cell number determination*) and the required dilution for the desired cell density calculated.

3.1.5 Cell number determination

The cell count was determined using a hemocytometer, the Neubauer counting chamber. Therefore 10 µl of the cell suspension was pipetted underneath the coverslip and the outer four large squares (A, B, C, D) were counted under a microscope. For the determination of the viable cells, 80 µl of the cell suspension were stained with 20 µl trypan blue (Carl Roth) prior to application into the counting chamber and only the number of unstained cells were counted. In order to achieve sufficient accuracy of the results, the cell count per square should be between 20 and 100 cells. Otherwise, the cell suspension must be diluted in cell culture medium or concentrated by centrifugation before counting. Cells lying on the upper and left boundary lines of the large squares were counted; cells on the lower and right boundary lines were not counted. The calculation of the number of cells per volume (cells / ml) was then carried out using the following formula: Number of cells / ml = average cell count $\times 10^4 \times$ dilution factor. As an alternative to the Neubauer counting chamber and especially for many cell counts, the cell number were determined with a ViCell™ XR cell viability analyzer (Beckman Coulter). For this purpose, 500 µl of the cell suspension were pipetted into a reaction tube and added to the device. The determination was carried out analogously to the method described above. Depending on the experiment, the required volume of cell suspension with the appropriate amount of cells was diluted using cell culture medium and seeded into the cell culture vessels required for the respective experiment.

3.1.6 Cryopreservation of Cells

In order to keep the cells isolated and cultured from biopsies in low passages they were cryopreserved in liquid nitrogen for longer periods of time. The cells were cultivated under standard conditions up to a confluence of 80 - 90 % and detached from the cell culture flask as described (3.1.4 *Subculturing*). After a subsequent cell count, the volume, corresponding to 1.5×10^6 - 3.0×10^6 cells, was first centrifuged for

5 min at 300 g, the resulting cell pellet was carefully resuspended in 1 ml of freezing medium (fetal bovine serum (FBS) + 10 % dimethyl sulfoxide (DMSO)) and transferred into a cryovial. These aliquots were initially frozen at -80 °C for 24 h using freezing containers (Life Technologies). The temperature drop rate was approx. 1 °C per minute. The next day, the aliquots were converted into liquid nitrogen for long-term storage.

3.1.7 Thawing of cryopreserved cells

For thawing human fibroblasts stored at -80 °C or in liquid nitrogen at -196 °C, the cryovials were first thawed in a water bath at 37 °C until only a small amount of ice remained. The cell suspension was then transferred to a 50 ml centrifuge tube containing 19 ml prewarmed (37 °C) cell culture medium. In order to prevent a cytotoxic effect of the DMSO contained in the freezing medium, the cells were pelleted by centrifugation for 5 min at 300 g, resuspended in 20 ml fresh prewarmed cell culture medium and transferred to a new T175 cell culture flask. The next day there was another media change. Further cultivation was carried out according to 3.1.4 Subculturing.

3.2 Test compound supplementation

We used both ubiquinol and ubiquinone formulations (QuinoMit® Q₁₀-Fluid, SiaMit® Q₁₀, mse Pharmazeutika) to test the effect of Coenzyme Q₁₀ on the energy metabolism and mtDNA repair in human skin fibroblasts. Both formulations were always freshly weighed and diluted with ultrapure water (PureLab). The carrier control (CC) was administrated exactly as described for QuinoMit® Q₁₀-Fluid. QuinoMit® Q₁₀-Fluid, SiaMit® Q₁₀ and carrier control are emulsions of phospholipid nanoparticles (30 - 90 nm). The exact composition of these test compounds can be found in Table 3 below. In further experiments also CoQ₁₀, CoQ₁ (Merck KGaA) and Mitoquinone (mesylate) (MitoQ®) (MedChemTronica) was used. All these substances were dissolved in DMSO. 1,4-dithiothreitol (DTT) (Carl Roth) stock solution was also diluted in ultrapure water and then sterile filtered. Menaquinone-7 (MK-7) (Merck) was dissolved in ethanol (Carl Roth) (96%, pure) at 37 °C and 500 rpm and then also sterile filtered. For the inhibition of PARP, the PARP inhibitor Veliparib (ABT-888) (AbbVie) was diluted with DMSO. Magnesium (Mg²⁺) and calcium (Ca²⁺) (both from

Sigma Aldrich) were weighed and dissolved in ultrapure water (PureLab) to make a stock solution. The test-specific addition of the active substances was done either directly into the cell culture medium or immediately before the measurement. In this case, the substances were diluted in the appropriate reaction buffer.

Table 3. Exact composition of QuinoMit® Q₁₀-Fluid, SiaMit® Q₁₀ and carrier control

CoQ ₁₀ formulations and carrier control			
	QuinoMit® Q ₁₀ -Fluid ubiquinol (red.)	SiaMit® Q ₁₀ ubiquinone (ox.)	carrier control
Glycerin	+	+	+
Ubiquinol	+	-	-
Ubiquinone	-	+	-
Ethanol	-	-	-
Deionized water	+	+	+
Soy lecithin	+	+	+
Niacinamide	+	+	+

3.3 Irradiation of cells

The cells were irradiated with simulated solar light (SSL) and were performed using a 400F/5C solar simulator (Hönle). The cells were either irradiated in 6-well plates (Sarstedt) or in XF cell culture plates. When irradiated in 6-well plates, the cells were first washed with 3 ml of 1x PBS and then irradiated without lid in the presence of 3 ml of 1x PBS in order to avoid phototoxic effects by the cell culture medium. The UVA component of SSL was measured with a handheld UV meter and a sensor for UVA manufactured by Hönle. Cells were irradiated with 6 or 12 J/cm² SSL-UVA depending on the experiment. After irradiation, 1x PBS was immediately exchanged with fresh cell culture medium supplemented with or without test compounds (3.2 Test compound supplementation). Irradiation with 12 J/cm² SSL-UVA was accomplished directly prior to the subsequent experiment, whereas the irradiations with 6 J/cm² SSL-UVA were conducted 16 h prior to the experiment and the cells were kept in test compound-supplemented medium afterward. To ensure

comparability, the cells in the unirradiated control samples were also first washed with 1x PBS, stored in 1x PBS for the duration of irradiation and then supplied with fresh cell culture medium. Irradiation for subsequent measurement of mitochondrial respiration parameters was performed as described above, but the wells were washed with 150 µl 1x PBS and irradiated in the presence of 150 µl 1x PBS respectively.

3.4 Isolation and lysis of mitochondria

For the isolation of mitochondria 3.0×10^6 fibroblasts were first centrifuged for 5 min at 500 g and 4 °C. After supernatant was discarded, the resulting cell pellet was resuspended in 200 µl CHAPS lysis buffer supplemented with 3 µl of tri-sodium citrate dihydrate (Carl Roth) and then incubated on ice for 45 min. Thereafter the samples were homogenized with a syringe and a cannula (diameter = 0,9 mm) by pipetting up and down on ice 10 times. To completely separate the mitochondria from the remaining cellular structures, samples were again centrifuged for 10 min at 1200 g and 4 °C. The supernatants with the mitochondria were transferred into new reaction tubes. The lysis of the isolated mitochondria was carried out in a further lysis step on ice with pulsed ultrasound (Bandelin electronic GmbH & Co. KG) at 20 watts for one minute (0.5 s pulse on, 1.0 s pulse off). Finally, another centrifugation step followed for 45 min at 21.000 g and 4 °C. The supernatants were then kept on ice until further use.

3.5 Cell viability assay

To obtain a first indication of the viability of the cells under ubiquinol, a cell viability assay was performed. This assay was performed according to the manufacturer's instructions (Cell Titer-Glo[®] Luminescent Cell Viability Assay; Promega). Cell Titer Glo[®] is based on a quantification of the existing ATP, which signals the presence of metabolically active cells. In a mono-oxygenation reaction, luciferin is catalyzed in the presence of luciferase, ATP, Mg^{2+} and molecular oxygen. The amount of ATP is directly proportional to the number of viable cells. 3.600 cells per well were seeded into 96-well plates and incubated under standard conditions at 37 °C in 100 µl cell culture medium per well with or without test compounds until the measurement at different time points took place. Control wells containing medium without cells were

prepared to obtain values for the background luminescence. For each treatment, three wells were analyzed in parallel. After completion of the incubation 100 µl Cell Titer-Glo® substrate was added to the cells and mixed for 2 min on an orbital shaker to induce cell lysis. Luminescence was measured after a further incubation for 10 min at room temperature (RT). At the beginning of the measurement, the 96-well plates were shaken by the fluorometer for 2 s, and then the samples were measured for 1 s at an emission maximum of 560 nm at RT. The values obtained were normalized to the untreated control.

Because this test is based on the production of ATP and the substances to be tested partly influence the ATP production of mitochondria, a further cell viability assay was performed which is based on the measurement of both mitochondrial and cytosolic dehydrogenase activity. This results in a reduction of the tetrazolium ring of the yellow 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) in living cells, primarily by the cytosolic succinate-tetrazolium reductase system. The result is the dark blue formazan, which is insoluble in water and must be triggered by a solubilization solution. This test was performed as described above, but after incubation under standard conditions 20 µl per well of MTT solution I (formazan) were added to the cells and incubated for further 2 h at 37 °C and discarded afterwards. The formazan was then triggered through 100 µl of solubilization solution (MTT solution II) during a 5 min incubation on a orbital shaker at RT and its intensity measured photometrically at 570 nm and 630 nm in a Wallac victor³ 1420-fluorometer (PerkinElmer) at 37 °C.

3.6 ATP bioluminescence assay

In order to measure the content of ATP in human fibroblasts, 3.0×10^5 cells per well were seeded into 6-well plates and incubated under standard conditions at 37 °C in cell culture medium with or without test compounds until the measurement at different time points (6, 16, 24, 32 h) took place. As a control PARP was added to some approaches. Control wells containing medium without cells were prepared to obtain values for the background luminescence. In addition to each measurement, an ATP dilution series from 50 pM to 1.28 pM was prepared based on a 5 mM ATP solution (Life Technologies) (Figure 15). The dilution of the ATP stock solution was prepared using ultrapure water (Purelab). For each treatment, three wells were analyzed in parallel. In case of irradiation of the cells, this took place as previously described (3.3

Irradiation of cells) and the incubation for 6, 16, 24, 32 h respectively took place directly after irradiation. Subsequently the cells were detached from the culture dishes with 0.5 % Trypsin/EDTA (Gibco). The reaction was stopped with 10 % fetal bovine serum (Gibco) in 1x PBS and the viable cell numbers were determined. After this, 3.5×10^4 cells were collected by centrifugation for 6 min at 500 g, washed with 100 μ l 1x PBS, centrifuged again and resuspend in 100 μ l ultrapure water (PureLab). The lysis of the cells was carried out for 5 min at 95 °C in a thermocycler (HLC BioTech). Then, the samples were centrifuged again for 6 min at 500 g to remove all cell fragments. The supernatants were stored until measurement at -20°C. The bioluminescence measurements were carried out with a Wallac victor3 1420-fluorometer (Perkin Elmer) as a luciferin-luciferase reaction ($\text{Luciferin} + \text{O}_2 + \text{ATP} \rightarrow \text{Oxyluciferin} + h\nu + \text{AMP} + \text{PP}_i + \text{CO}_2$) in a 96-well plate. The ATP-Assay was performed according to the manufacturer's instruction (ATP Determination Kit; Life Technologies GmbH, Darmstadt, Germany). First, 200 μ l per well of the standard reaction solution (SRL) (included in the ATP Determination Kit) were measured in the fluorometer after incubation for 1 min, followed by the addition of 10 μ l of the ATP-samples. Afterwards, the 96-well plate was incubated in the fluorometer prior to the start of measurement for another minute. The measurements were conducted at 28 °C. At the beginning of the measurement, the 96-well plates were shaken by the fluorometer for 2 s, and then the samples were measured for 1 s at an emission maximum of 560 nm. The values obtained were normalized to the untreated control.

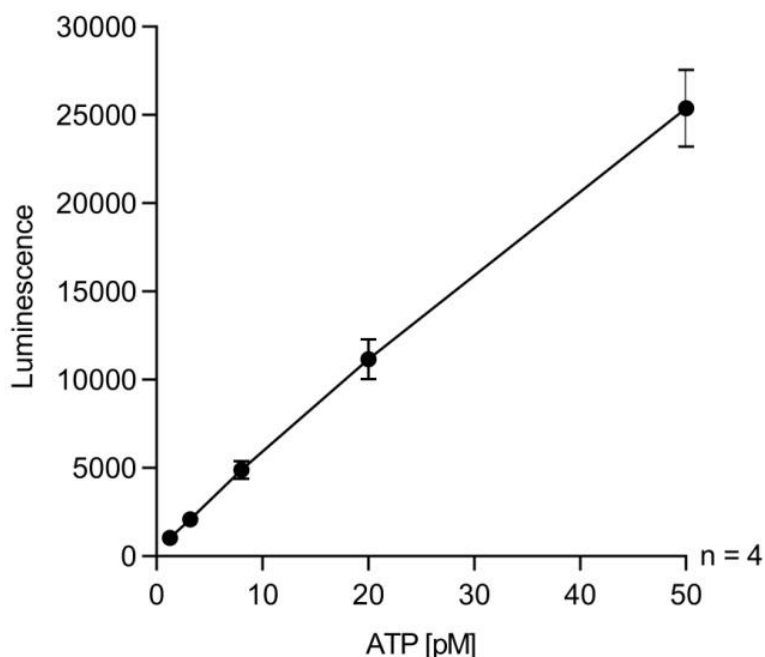


Figure 15. ATP dilution series. Shown is the measured luminescence depending on the amount of ATP used (1,28 - 50 pM) ($n = 4$, linear reg. with 95 % confidence interval, $R^2 = 0.9537$ / $p = <0.0001$, deviation from zero = significant).

3.7 ROS- / mtROS-Assay

For the measurements of ROS and mtROS 1.0×10^4 fibroblasts per well were seeded in a 96-well plate and then incubated in cell culture medium with or without test compounds for 16 h in the incubator at 37 °C. After this, 400 μ M *tert*-Butyl hydroperoxide (TBHP) was added to some approaches as a positive control and incubated for another hour at 37 °C. For each treatment, eight wells were analyzed in parallel. The fluorescence measurements were carried out with a Wallac victor³ 1420-fluorometer (Perkin Elmer). Both assays were performed according to the manufacturer's instructions (CellROX[®] Green Reagent (C10444), MitoSOX[™] Red (M36008); Life Technologies GmbH). CellROX[®] Green Reagent is a cell-permeable and reduced leuco-dye that fluoresces by oxidation with ROS at an absorption/emission maximum of 480/520 nm. After oxidation, the dye binds to the DNA, which increases the fluorescence in the nucleus and in the mitochondria. However, it is not possible to distinguish between signal from general and from mitochondrial ROS. For a distinction between ROS and mtROS, we used therefore MitoSOX[™] Red a mtSO indicator for the detection of superoxide in the mitochondria.

In the oxidized state, the dye fluoresces at an absorption/emission maximum of 510/580 nm and has a fluorescence excitation which can only be produced by the oxidation with superoxide. Thus mtROS can be indirectly detected as mtSO. Cells were stained with 5 μ M CellROX® Green Reagent or 5 μ M MitoSOX™ Red by adding it to the media and another incubation for 1 h at 37 °C or 30 min at the same temperature using MitoSOX™. Prior to the measurement, cells were washed twice with prewarmed 1x PBS (37 °C). The measurements were carried out at 37 °C in the presence of 150 μ l 1x PBS. At the beginning of the measurement, the 96-well plates were shaken by the fluorometer for 2 s, and then the samples were measured for 1 s. The values obtained were normalized to the untreated control.

3.8 Mitochondrial membrane potential ($\Delta\psi$ m) measurements

To detect the mitochondrial membrane potential ($\Delta\psi$ m) in human fibroblasts a total of 1.0×10^5 cells per well were seeded in 6-well plates. In each run, three controls and two treated samples were analyzed in parallel (Table 4). The SSL-UVA irradiation (as described above) with a dose of 12 J/cm^2 (SSL₁₂) took place 7 h or 24 h after seeding, respectively. Following the irradiations, the cells were incubated in cell culture medium with or without test compounds. If no supplementation with active ingredient was administered, the appropriate amount of ultrapure water (PureLab) was added to the cell culture plates. Prior to measurement, the cells were washed with 3 ml of prewarmed (37 °C) 1x PBS and stained with 1 ml JC-1 (Cayman Chemical Company) (3.9 μ M in cell culture medium) for 30 min at 37 °C. Then the cells were detached from the culture dishes with 0.5 % Trypsin/EDTA (Gibco). After 5 min, the reaction was stopped with 10 % fetal bovine serum in 1x PBS. As a control, 2.5 μ M FCCP (Cayman Chemical Company) was added to the stop solution of the negative control samples. Immediately after cell detachment, the mitochondrial membrane potential was analyzed. JC-1 was measured using a blue laser (488 nm) and two band pass filters (585 nm; JC-1-aggregates (red) and 530 nm; JC-1-monomers (green)) from the Fluorescence Activated Cell Sorter FACSCalibur (BD Biosciences). Data were analyzed with CELLQuestPro software (BD Biosciences). To calculate a measure for the $\Delta\psi$ m, the median of the red fluorescence intensity (medianFI_{red}) was divided by the median of the green fluorescence intensity (medianFI_{green}). To calculate the normalized ratio, the ratio of the sample (ratio_{sample}) was divided by the ratio of the control (ratio_{CTRL}).

Table 4. Controls and samples to evaluate the effect of ubiquinol and SSL-UVA irradiation on $\Delta\psi_m$ of human fibroblasts.

Controls/Samples		Treatment			
		JC-1	FCCP	Ubiquinol	SSL ₁₂
Controls	Untreated	-	-	-	-
	Untreated + stained	+	-	-	-
	FCCP	+	+	-	-
Samples	UVA	+	-	-	+
	Ubiquinol + SSL ₁₂	+	-	+	+

3.9 Gene expression analysis

1.0x10⁵ fibroblasts were plated into 10 cm petri dishes. 3 h after seeding, cells were treated with 100 μ M ubiquinol and incubated for the appropriate time in the incubator at 37 °C. Sampling took place 4, 12, 24, 48 h after treatment and harvested cells were kept at -80 °C until RNA extraction. For gene expression analysis via quantitative polymerase chain reaction (qPCR), RealTime ready Custom Panels 96 - 16 (Roche Diagnostics) with ready-made primers and probes for the selected gene of interest (hOGG1) and reference genes (RPL13A, ribosomal protein L13A; RPLP0, ribosomal protein, large, P0; PGK1, phosphoglycerate kinase 1) were used. Reference genes were experimentally determined and calculated with the software GenEx (functions 'GeNorm' and 'Normfinder', MultiD Analyses AB). Total RNA of all samples was extracted with a High Pure RNA Isolation Kit (Roche Diagnostics) and purity and concentration of RNA was determined using an ExperionTM system with a RNA Sens Analysis Kit (Bio-Rad). RNA isolation was followed by synthesis of complementary DNA (cDNA) with a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics) and cDNA precipitation. It was proceeded according to the manufacturer's instructions. cDNA was resuspended in 200 μ l ultrapure water and stored at -80 °C until qPCR. Reactions were carried out in a volume of 20 μ L with 10 μ L LightCycler R 480 Probes Master, and 0.625 μ M of each primers and probe. Amplifications were conducted as follows: 10 min of initial denaturation at 95 °C, followed by 45 cycles of 10 s denaturation at 95 °C, 30 s primer annealing and

elongation at 60 °C, and 1 s fluorescence acquisition at 72 °C. Relative expression levels were calculated with the $2^{-\Delta\Delta C_p}$ method and then normalized to all reference genes.

3.10 Mitochondrial respiration measurements - Sample preparation

To get a mitochondrial respiration profile, samples were measured with a Seahorse Bioscience XF24 Extracellular Flux Analyzer (Agilent Technologies). XF24 Flux-Plates (sensor cartridge) were incubated overnight in a non-CO₂ incubator at 37 °C in 1 ml of XF24 calibrant solution (Agilent Technologies) per well.

3.10.1 Monolayer cell culture

One day prior to measurement, a total of 5.0×10^4 fibroblasts per well were seeded into XF24 cell culture microplates. For each treatment, five wells were analyzed in parallel. 5 h after seeding, cells were treated with test compounds and incubated under standard culture conditions according to the experiment. For measurement, cells were washed with 450 µl of prewarmed (37 °C) assay medium (D7777, pH 7.4; Sigma-Aldrich) and incubated in 450 µl of the same medium per well in a non-CO₂ incubator for 60 min at 37 °C.

3.10.2 Epidermis

16 - 18 h prior to measurement, biopsies were treated with or without ubiquinol or carrier control and incubated at 4 °C. For each treatment, a minimum of three wells were analyzed in parallel. The change of OCR (pmol/min)) and extracellular acidification rate (ECAR (mpH/min)) in medium surrounding the epidermis was measured using XF24 islet capture microplates (Agilent Technologies). In these plates, small capture screens can be fixed to the plate bottom. To measure the oxygen consumption of the vital layer of the epidermis (stratum basale), epidermis and dermis were enzymatically separated (3.1.2 *Isolation of skin fibroblasts*) and the epidermis placed with the vital - basal - side on capture screen. To accomplish this, the epidermis had to be carefully picked up from the petri dish with 1x PBS (Figure 17A) with a microscopic slide and wiped it off on a second slide (epidermis had to be rotated once). This orientation was essential for the measurement as the side facing the capture screen gets detected by the sensor (Figure 16). Thereafter, with

the second slide the epidermis was placed on the capture screen, pulled smooth with a sterile tweezer and the capture screen was then inserted into the islet capture microplate by using the capture screen insert tool (Figure 17B). Since the measurement of respiration parameters was followed by normalization of the data obtained over the area of the epidermis, it was necessary to ensure that the epidermis covered the entire surface within the inner ring of the capture screen (Figure 17C-D). There should be also no air bubbles (Figure 17E) under the epidermis, as they would interfere with the measurement. The excess overhanging skin in the well of the islet capture microplate was then removed with tweezers and 450 μ l of prewarmed (37 °C) unbuffered assay medium (D7777, pH 7.4; Sigma-Aldrich) per well were added. After incubation in a non-CO₂ incubator for 45 min at 37 °C, the mitochondrial respiration was measured (3.10. *Mitochondrial Respiration Measurements*).

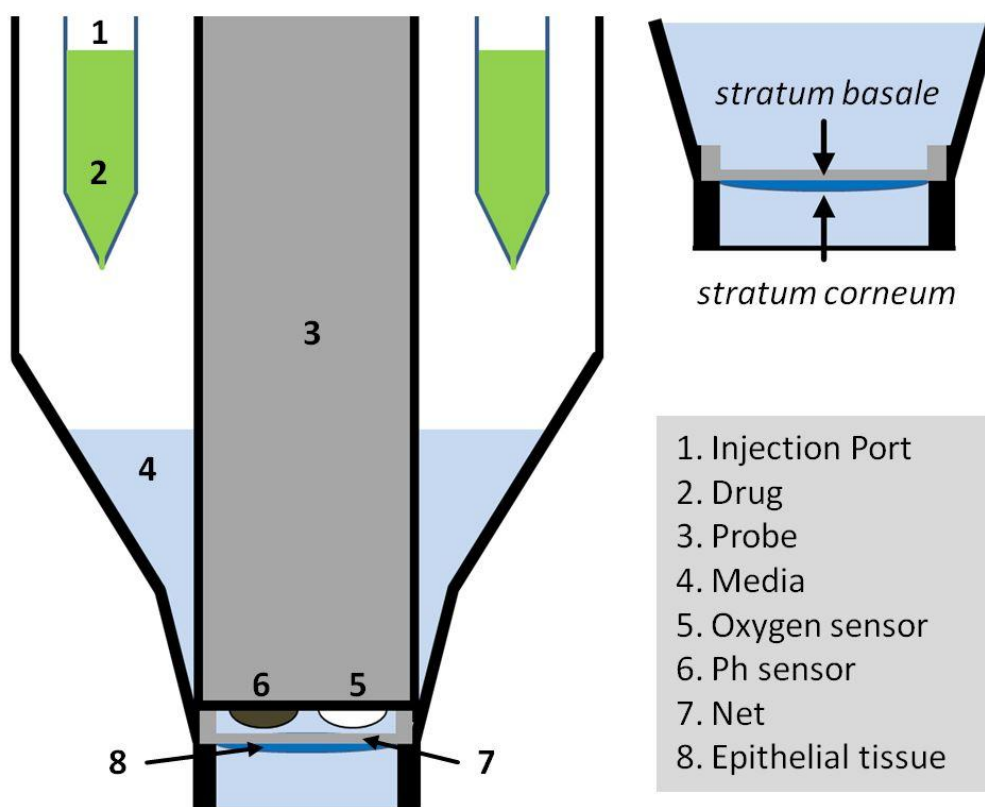


Figure 16. Schematic setup of the XF24 measuring system with an islet capture microplate.

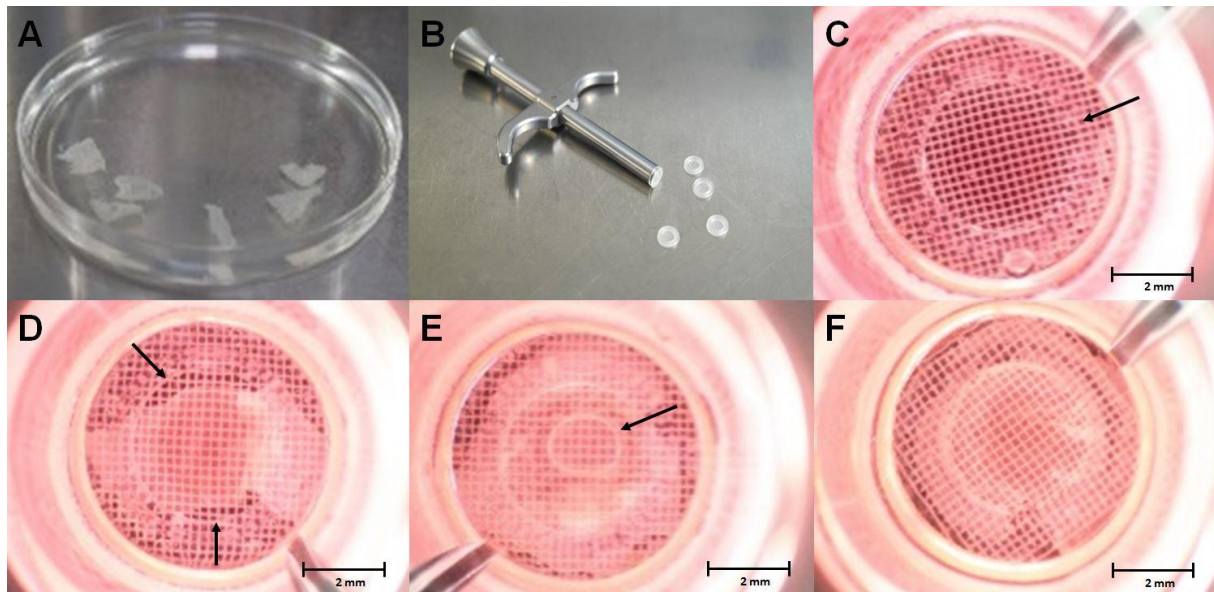


Figure 17. Microscopic image of epidermis in the XF24 islet capture microplate. [A] Separated epidermis sheets after incubation with Dispase II. [B] Capture screen insert tool with Islet capture screens (Agilent Technologies). [C] The measurement of oxygen consumption takes place over the surface within the ring marked with the arrow. [D/E] Within the ring in the center of the net, there should be no holes or air bubbles. [F] Flat-mounted epidermis, without air bubbles, which covers the entire surface and with the right orientation. Scale bars corresponds to 2 mm.

3.11 Mitochondrial respiration measurements

During the preincubation phase in a non-CO₂ incubator for 45 or 60 min respectively the sensor cartridge was loaded with the stress reagents (XF Cell Mito Stress Test Kit, Agilent Technologies) and the instrument calibration were started. To measure changes in the parameters of mitochondrial respiration, oligomycin, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) and rotenone combined with antimycin A were all prepared to result in a 1 μ M final concentration after injection for respiration measurement on cell cultures. The concentration of oligomycin, FCCP and rotenone / antimycin A was determined in preliminary experiments for the respective experimental conditions, as they may differ depending on the sample to be measured. For measurement on epidermis for example, a higher concentration of the stress reagents was required (4 μ M final concentration) because epidermis separated from a biopsy is a cell complex in which the substances simply take longer to penetrate through the entire sample. In preliminary studies, the concentration of 4 μ M was determined. Lower concentrations showed little or no effect in contrast to

monolayer cell cultures. After the insertion of the cell culture microplates or the islet capture microplate in case of measuring epidermis, a 15 - 35 min equilibration phase was implemented before measurements. First, the initial respiration (basal response) was measured. Then, the active ingredients in the three ports were injected consecutively to determine the effect of each compound. This makes it possible to determine the efficiency of the individual complexes within the respiratory chain. Oligomycin blocks the F_0 subunit of the ATP synthase. The decrease in respiration can thus be regarded as a measure of the ATP production of the mitochondria. The decoupling of the respiratory chain by the protonophore FCCP allows the maximal respiration (ETC accelerator response) to be determined independently of the proton gradient. Spare respiratory capacity was calculated from the division of the ETC accelerator response by the basal response. Finally, complexes I and II are blocked by the addition of rotenone and antimycin A and thus the difference between basal response (oxygen consumption of the third cycle) and the lowest oxygen consumption after the injection of rotenone and antimycin A gave information about the mitochondrial respiration. The remaining oxygen consumption is thus attributable to the non-mitochondrial respiration. Proton leak was then calculated by the subtraction of the lowest OCR signal after the injection of rotenone and antimycin A from the OCR signal after the addition of oligomycin (Figure 18 and Figure 19). OCR was reported in the unit of pmol/min and the extracellular acidification rate (ECAR) in the unit of mpH/min.

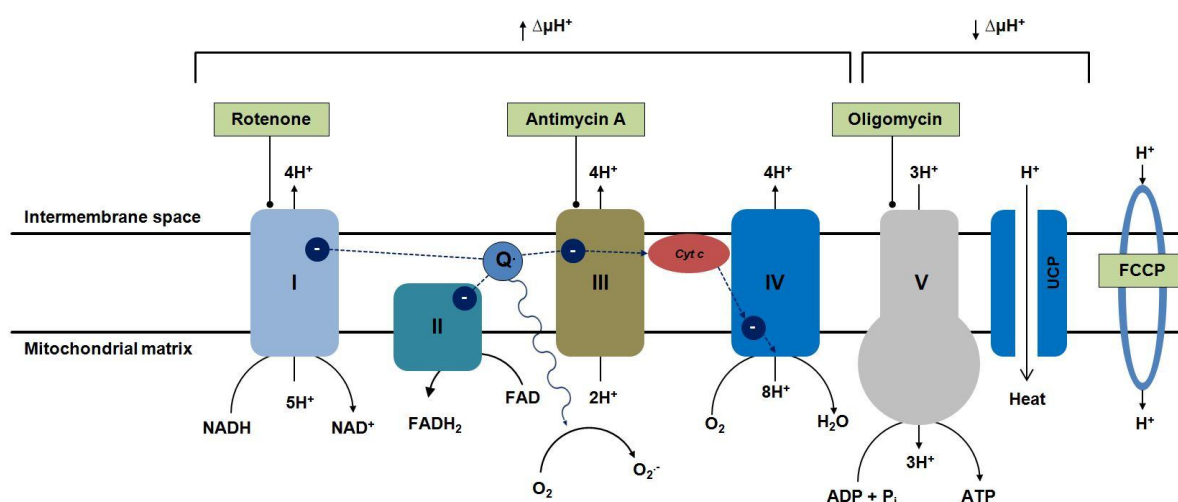


Figure 18. Electron transport chain (ETC) with the points of attack of the stress reagents (XF Cell Mito Stress Test Kit, Agilent Technologies). Oligomycin blocks the F_0 subunit of the ATP synthase. The protonophore FCCP decouples the ETC from the ATP synthesis. Complexes I and II are blocked by rotenone and antimycin A. Figure created after the template of Brownlee, 2001.

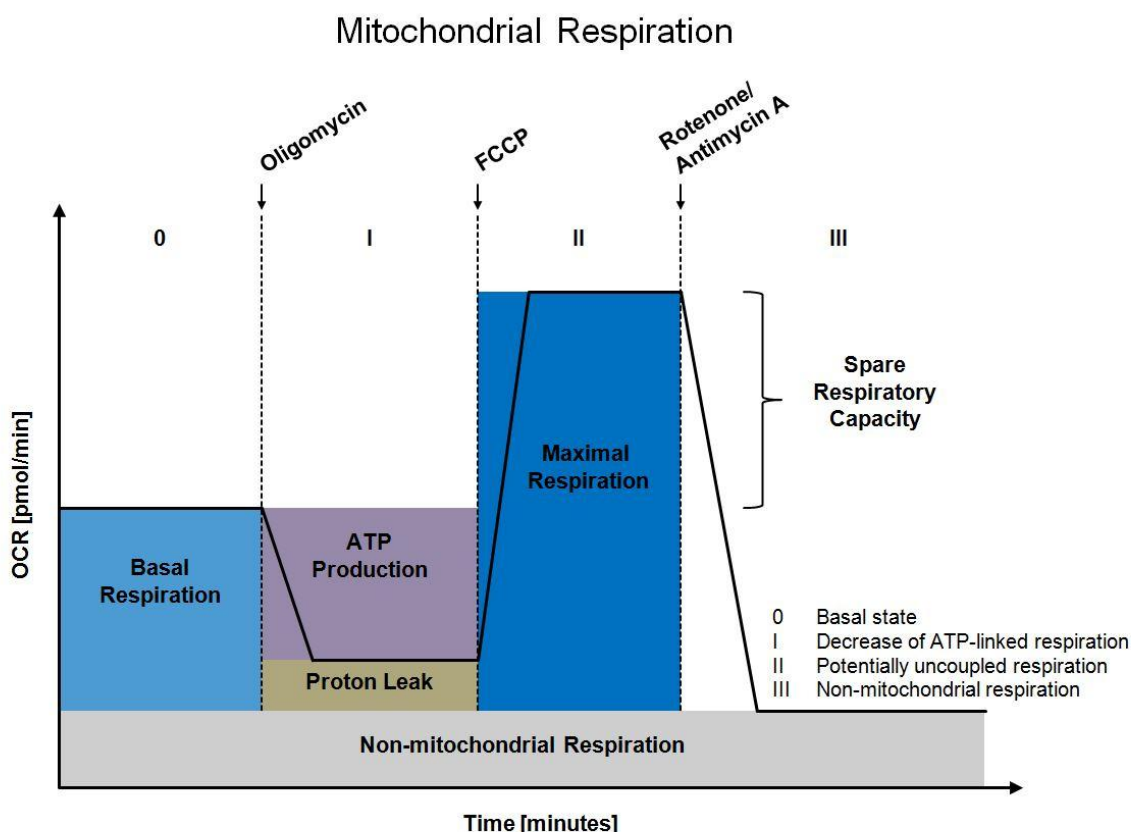


Figure 19. Exemplary XF24 measurement using the XF Cell Mito Stress Test Kit (Agilent Technologies). Shown is the oxygen consumption rate (OCR; pmoles/min) as a function of time (minutes). Scheme depicting the main stages after injecting the four active ingredients in three injecting steps. This makes it possible to determine the efficiency of the individual complexes within the respiratory chain. First, the basal respiration is recorded. The addition of oligomycin determines proton leakage and ATP-linked respiration. FCCP is used to measure maximal respiration. Antimycin A and rotenone block respiration of the mitochondria, to determine non-mitochondrial respiration. Figure created after the template of Agilent Technologies (Agilent Technologies. Retrieved October 15, 2019, from <https://www.agilent.com/en/products/cell-analysis/seahorse-xf-consumables/kits-reagents-media/seahorse-xf-cell-mito-stress-test-kit#additionalinformation>).

All measurements were done in three cycles. Each cycle involved 4 min mixing, 2 min waiting, and 3 min OCR and ECAR measurement. When measuring epidermis, the measurement of the initial phase and after addition of FCCP was also done in three, all other phases in six cycles (Figure 20). Following the measurement, the data obtained had to be normalized. For this, the cells were dissolved with 50 μ l 0.5 % Trypsin/EDTA (Gibco) per well as described under 3.1.4 *Subculturing* and the reaction was stopped with 450 μ l 10 % fetal bovine serum (PAA Laboratories) in 1x PBS. and the number of viable cells were measured with a ViCellTM XR cell viability

analyzer (Beckman Coulter). The measured values (OCR, ECAR) were normalized to the number of living cells. The normalization of the epidermis values was omitted since this already took place above the surface in the inner ring of the capture screen (Figure 17C-D).

Workflow of the XF controller

1. Calibrate Probes

2. Time of Delay: 15 - 35 min

3. First Loop (Basal state)

- 3 times for cell culture and epidermis

- a. Mixing: 4 min
- b. Time of Delay: 2 min
- c. Measurement: 3 min

4. Injection of Port A (50 μ l Oligomycin)

- 1 μ M final concentration for cell culture - 4 μ M for epidermis

5. Second Loop (decrease of ATP-linked respiration)

- 3 times for cell culture, 6 times for epidermis

- a. Mixing: 4 min
- b. Time of Delay: 2 min
- c. Measurement: 3 min

6. Injection of Port B (55 μ l FCCP)

- 1 μ M final concentration for cell culture, 4 μ M for epidermis

7. Third Loop (potentially uncoupled respiration)

- 3 times for cell culture and epidermis

- a. Mixing: 4 min
- b. Time of Delay: 2 min
- c. Measurement: 3 min

8. Injection of Port C (60 μ l Rotenone/Antimycin A)

- 1 μ M final concentration for cell culture - 4 μ M for epidermis

9. Fourth Loop (non-mitochondrial respiration)

- 3 times for cell culture, 6 times for epidermis

- a. Mixing: 4 min
- b. Time of Delay: 2 min
- c. Measurement: 3 min

10. End of Program

Figure 20. Workflow of the XF controller. All measurements were done in three cycles. Each cycle involved 4 min mixing, 2 min waiting, and 3 min OCR and ECAR measurement. When measuring epidermis, the measurement of the initial phase and after addition of FCCP was also done in three, all other phases in six cycles

3.12 Base excision repair assay

For the measurement of the hOGG1 activity we developed an assay that exploits the principle of enzymatic removal of 8-oxoG from DNA as well as the cut into its sugar-phosphate backbone by hOGG1 as a part of the BER.

3.12.1 Reporter oligonucleotides

Commercially purchased recombinant human OGG1 (Abcam plc) and the lysates of isolated mitochondria from human fibroblasts were incubated with specifically designed reporter oligonucleotides for this assay. Each of the two sequence-identical double-stranded constructs consisted of 33 nucleotides. Both carried a 6-carboxyfluorescein molecule (6-FAM) at the 3'-end of the reporter strand and a black hole quencher (BHQ-1) at the 5'-end of the complementary strand (Figure 21). For the preparation of the reporter oligonucleotides Chuck Norris-8-oxoguanine (CN-8-oxoG) ([6FAM]CCATAATAATAAAC[8-oxo-dG]CAATAATAATAATACC[6FAM]) and Chuck Norris-control (CN-CTRL) ([6FAM]CCATAATAATAAACGCAATAATAATAATACC[6FAM]), 5 µl of each of the single-stranded CN oligonucleotides CN-8-oxoG and CN-CTRL (100 µM each) were mixed together with 10 µl of the BHQ-1-tagged complementary strands ([BHQ1]GGTATTATTATTATTGCGTTATTATTATTATGG[BHQ-1]) (100 µM) (all Sigma Aldrich) and 35 µl NEBuffer™ 2 (NEB). This was followed by an incubation for 15 min at 95 °C for hybridization of the CN-(6-FAM) oligonucleotides and complementary strands in the thermocycler (Digital Biomedical Imaging Systems AG). The reporter oligonucleotide batches were then centrifuged briefly. The cooling and subsequent storage of the finished constructs took place at -20 °C.

3.12.2 Measurement of base excision repair

The measurement was carried out in a Light Cycler 480 (Roche Diagnostics), which performed a real-time fluorescence measurement at 37 °C for 80 cycles of 1 min each and a subsequent melting curve analysis. The reaction buffer for this assay was prepared as follows: 20 % DMSO; 1x NEBuffer™ 2; 10 % BSA; 10 pmol reporter constructs; recombinant human OGG1 for control (0.5 µg/µl = 0.8 U/µl). The test-specific addition of the active substances CoQ₁₀, DTT or MK-7 as well as the CoQ₁₀-treated mitochondrial lysates was done immediately before the measurement. The

DNA glycosylase present in the lysates from mitochondria recognizes the damage on the reporter oligonucleotide (CN-8-oxoG) and cuts out the oxidized base first followed by a cut in the phosphate backbone. As a result, the incised DNA strand dissociates from the opposite strand at the incubation temperature of 37 °C and a fluorescence signal can be measured. The strength of the signal correlates with the repair activity or the AP lyase activity of the hOGG1 enzyme. The use of an undamaged reporter oligonucleotide (CN-CTRL) served as a control in order to exclude nonspecific digestion of the oligonucleotide constructs (Figure 22A). For a determination of the resulting oligonucleotide fragments a melting curve analysis at a temperature of 95 °C to 20 °C was performed after completion of the real-time activity measurement. For this purpose, the first derivative of the trend of the fluorescence signal according to temperature was recorded $-(d/dT)$. The resulting minima represent the melting temperatures of the different fragments (Figure 22B).

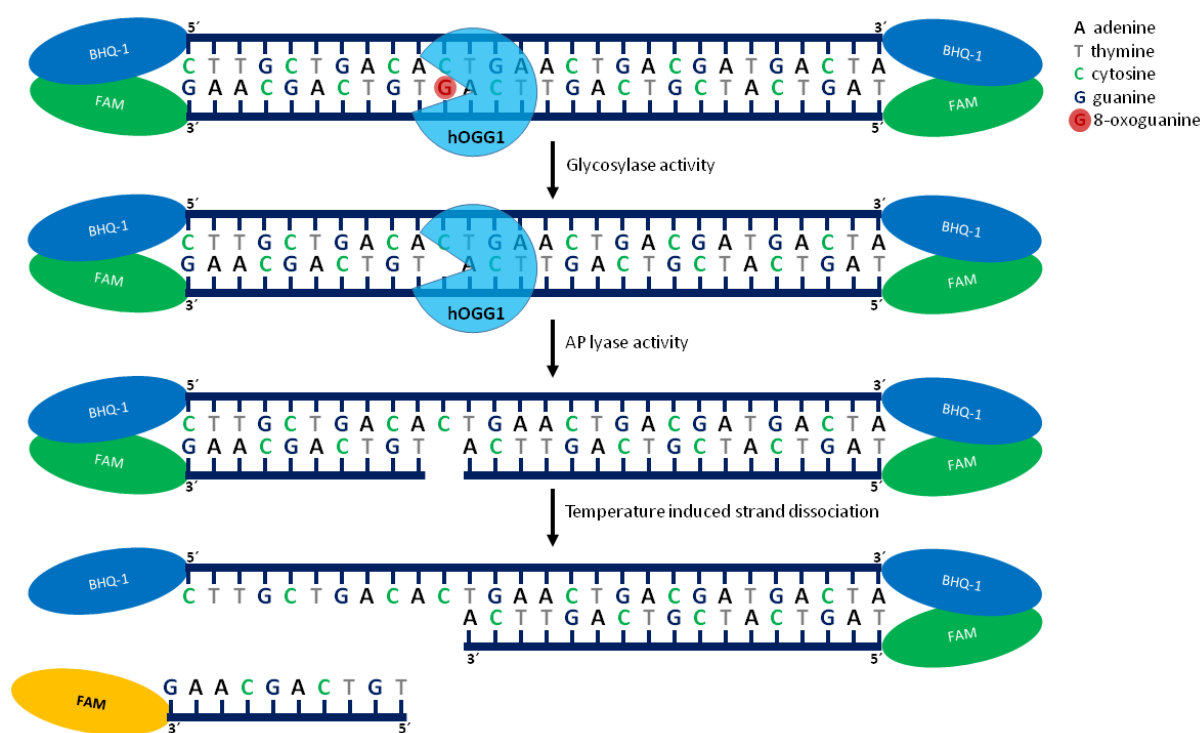


Figure 21. Basic principle of the assay. A double-stranded nucleotide strand carrying an 8-oxoG base (shown in red) is used to detect the activity of hOGG1. The strand with the damage carries at both ends a 6-FAM molecule as a fluorescence reporter and on the opposing quencher are added at both ends. In the uncut reporter-oligonucleotide both strands are hybridized with each other whereby the fluorescence signal of the 6-FAM molecules is intercepted by the quenchers. If the strand with the

damage is cut, the two nucleotide strands dissolve from each other and the fluorescence signal can be measured.

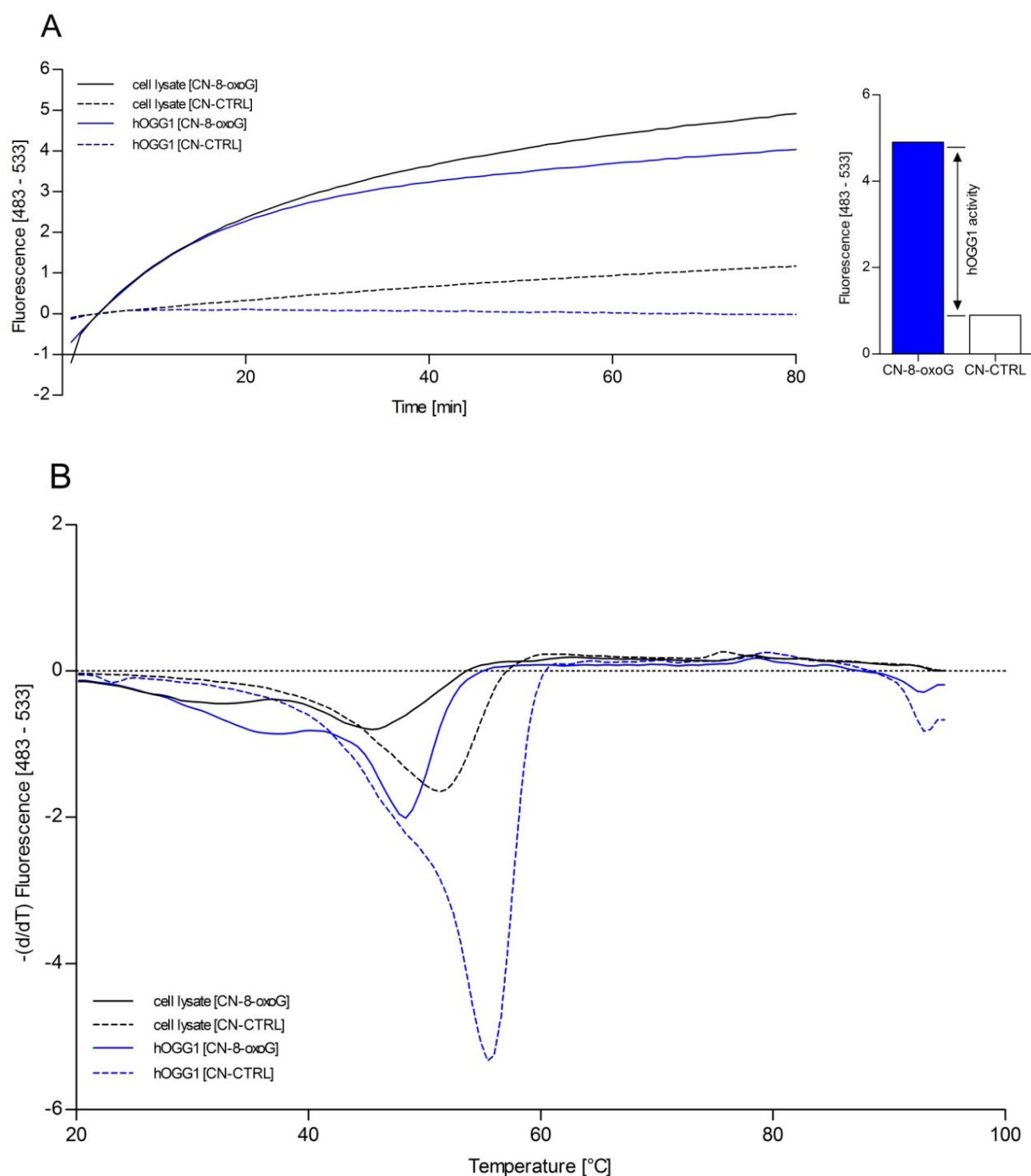


Figure 22. Schematic representation of a determination of the hOGG1 activity by fluorescence and melting curve analysis. **[A]** Fluorescence curves of the reporter-oligonucleotides CN-8-oxoG (with damage) and CN-CTRL (without damage) after digestion with 1.6 units of commercially purchased hOGG1 as well as hOGG1 obtained from cell lysates over a period of 80 cycles of 1 min each at 37 °C. The hOGG1 activity can be determined by means of the end point (maximum fluorescence) of the fluorescence curve of the defective reporter-oligonucleotide (CN-8-oxoG) minus the nonspecific digestion (CN-CTRL). **[B]** Melting curves of the reporter-oligonucleotides CN-8-oxoG

and CN-CTRL after digestion with 1,6 units of commercially purchased hOGG1 and hOGG1 obtained from cell lysates., a fragment analysis can be carried out after completion of the real-time activity measurement using the function of the recorded melting curves. For this, the first derivative of the trend of the fluorescence signal is recorded at a temperature change from 95 °C to 20 °C $-(d/dT)$. The resulting minima represent the melting temperatures of the different fragments.

3.13 Co-immunoprecipitation

For the detection of a direct interaction between hOGG1 and ubiquinol, a co-immunoprecipitation (CoIP) was performed. For this, 200 µl of magnetic beads (His Mag Sepharose™ Ni, GE Healthcare Life Sciences) were prepared per approach. The storage solution was removed and replaced with equilibration buffer containing 5 mM imidazole (His Buffer Kit, GE Healthcare Life Sciences). This step was repeated once. Following the preparation of the beads, the samples were added. Depending on the approach, commercially purchased recombinant human OGG1 with a histidine-Tag (hOGG1-His) (Abcam plc) and ubiquinol were added to the beads. For the binding of ubiquinol to hOGG1, all the mixtures were incubated for 30 min at 10 °C. in the thermocycler (Digital Biomedical Imaging Systems AG). Beads incubated only with ubiquinol served as a control in order to exclude binding of ubiquinol to the beads. After incubation of the beads with and without ubiquinol and hOGG1, the supernatant of the beads was removed (for binding efficiency testing) and the beads were washed three times with wash buffer with 5 mM imidazole (His Buffer Kit, GE Healthcare Life Sciences). To remove the enzymes from the beads, 100 µl of elution buffer containing 500 mM imidazole (His Buffer Kit, GE Healthcare Life Sciences) were added to the samples and after one minute of incubation, the samples were transferred to a new reaction tube. The eluted samples were then used directly in the previously described base excision repair assay.

3.14 Data analysis

In addition to Microsoft Excel 2007 and 2010 (Microsoft Corporation) and GraphPad Prism 5.03, 7.04 or 8.2.1 software (GraphPad Software, Inc.), the analysis of the collected data was also carried out with the device-specific evaluation programs (2.13 Software)

3.15 Statistics

Statistical analysis was performed using GraphPad Prism 5.03, 7.04 or 8.2.1 software (GraphPad Software Inc.). Values are presented as mean $\pm$ SEM or individual values. Comparison between multiple groups was done by one-way ANOVA with Dunnett's multiple comparison test or Bonferroni's multiple comparison test. Statistical significance was defined as $p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$. n = number of experiments carried out independently of each other.

4. Results

PART I

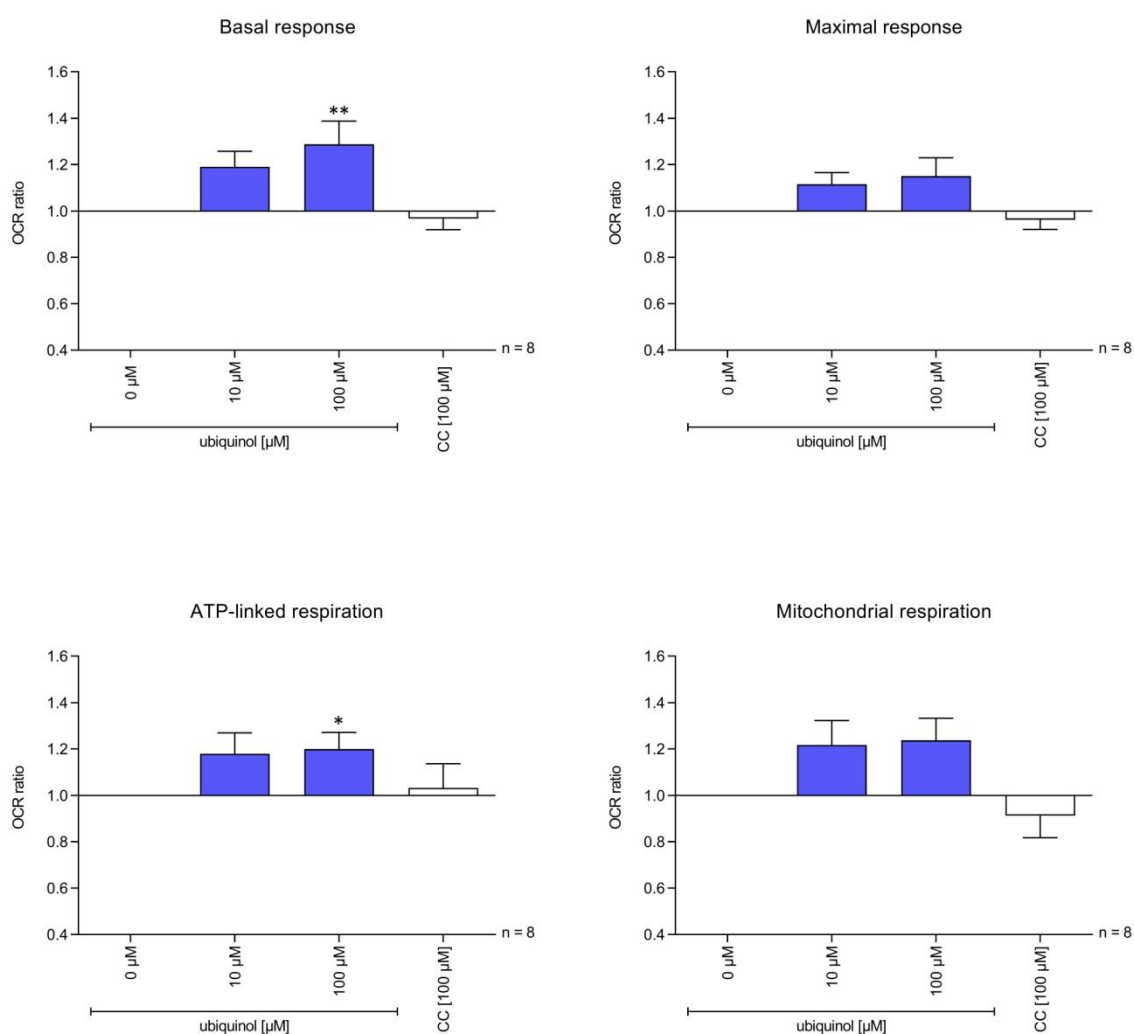
4.1 Influence of ubiquinol on mitochondrial function and cellular ATP levels in human fibroblasts after UV irradiation

The most common UV-induced damage includes both, direct DNA lesions as well as oxidative damage to DNA, proteins and lipids caused by ROS. If these damages are not getting repaired, mutations occur that play a special role in mitochondrial DNA, because mutations in genes coding for proteins of the respiratory chain contribute to an increased production of mtROS. This mtROS in turn accelerates the damage and mutation of the mtDNA and thus leads to a renewed production of mtROS. This leads to a kind of vicious circle. To prevent this dysfunction, the body tries to counteract mtROS with natural substances such as ubiquinol. The resulting mitochondrial dysfunctions can have a negative effect on many essential cellular processes and thus contribute to the development of age-associated diseases. In order to prevent this dysfunction, the body tries to counteract mtROS with natural substances such as ubiquinol. The first part of this work was to investigate the influence of ubiquinol on mitochondrial function and cellular ATP levels in human fibroblasts after UV irradiation with physiologically relevant doses of simulated solar light. Furthermore, the effect of different CoQ₁₀ formulations on mitochondrial respiration parameters was investigated.

4.1.1 Key parameters of mitochondrial respiration in human fibroblasts under the influence of different concentrations of ubiquinol

First, it should be investigated to what extent the administration of different concentrations of ubiquinol has an influence on the key parameters of mitochondrial respiration in human fibroblasts. For this, on the day before the measurement took place 5.0×10^4 fibroblasts per well were seeded into XF24 cell culture microplates in cell specific growth medium and incubated with or without 10 and 100 μM of ubiquinol (QuinoMit[®] Q₁₀-Fluid) for 16 h at 37 °C in an incubator under standard conditions. As a control, one approach was treated with 100 μM carrier control (CC [100 μM]). The measurement of the mitochondrial respiration profile was carried out as previously described in 3.10.1 *Monolayer cell culture* and 3.11 *Mitochondrial*

respiration measurements using the Seahorse Bioscience XF24 Extracellular Flux Analyzer. As can be seen in the following figure, the treatment of human fibroblasts with ubiquinol resulted in a concentration-dependent increase in the key parameters of mitochondrial respiration. Especially the basal response, the ATP-linked respiration and the proton leak were significantly increased. The maximal response and mitochondrial respiration were increased in both cases and did not differ between the two concentrations. Only the spare respiratory capacity was reduced. The carrier control showed no significant increase in all mitochondrial parameters.



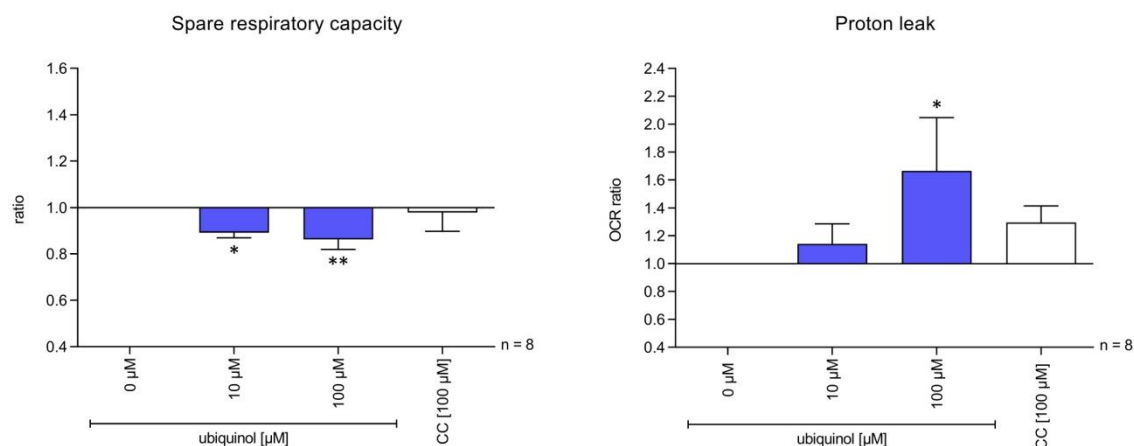
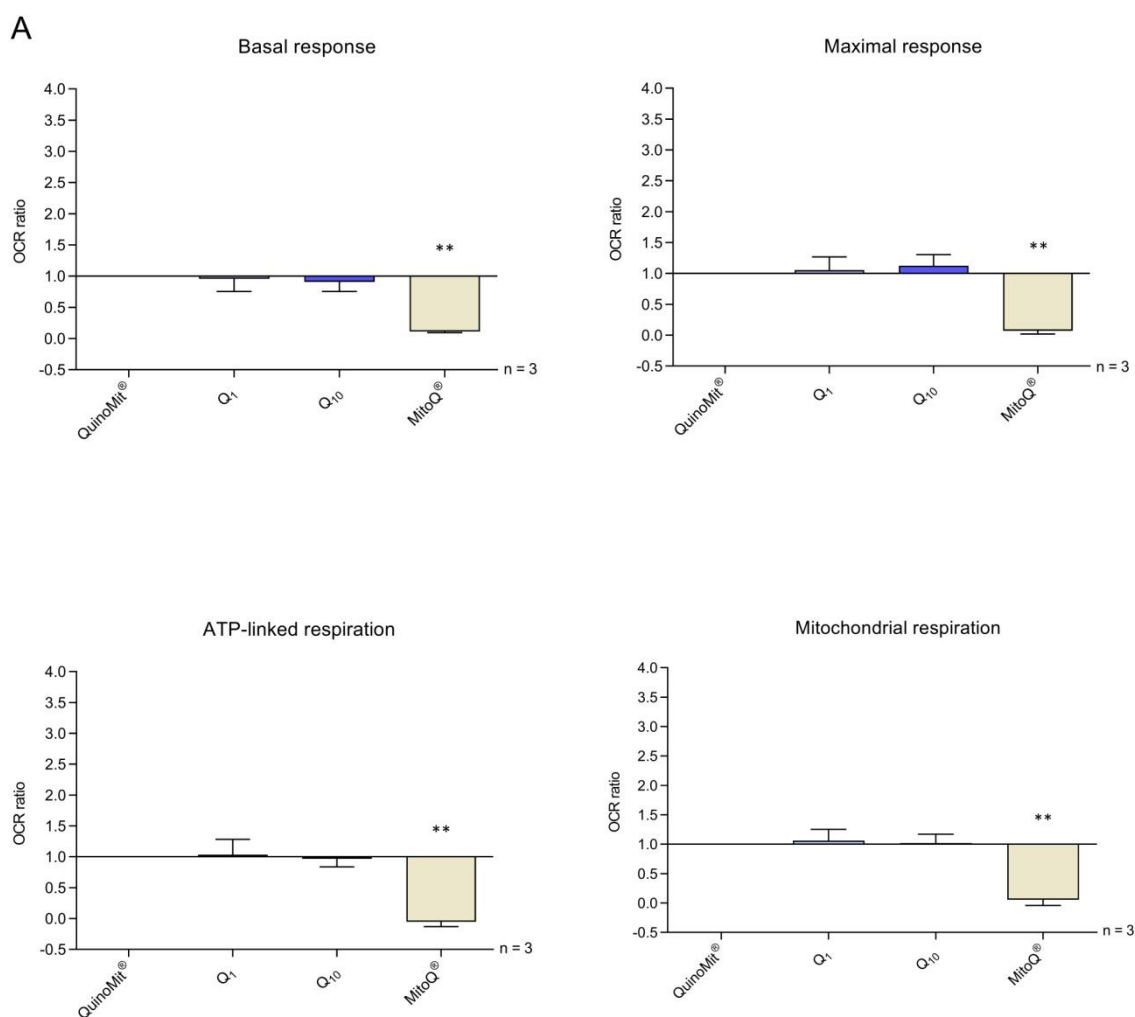


Figure 23. Key parameters of mitochondrial respiration in human fibroblasts under the influence of different concentrations of ubiquinol. 5.0×10^4 fibroblasts per well were incubated with or without 10 or 100 μM ubiquinol (QuinoMit[®] Q₁₀-Fluid) for 16 h at 37 °C in an incubator under standard conditions. As a control, one approach was treated with 100 μM carrier control (CC [100 μM]). After incubation, the mitochondrial respiration was analyzed with a Seahorse Bioscience XF24 Extracellular Flux Analyzer (Agilent Technologies) (n = 8, mean $\pm$ SEM, one-way ANOVA with Dunnett's multiple comparison test versus control, *p < 0.05; **p < 0.01). Control cells (0 μM - without ubiquinol or carrier control) correspond to 1.0. OCR = oxygen consumption rate. Data partly received from Tanja Sonntag - Master Thesis.

4.1.2 Significance of individual coenzyme Q formulations for mitochondrial respiration parameters

In this experiment, the efficacy of different coenzyme Q formulations on the key parameters of mitochondrial respiration compared to ubiquinol in the QuinoMit[®] Q₁₀ fluid formulation was investigated. For this, on the day before the experiment, 5.0×10^4 cells were seeded in an XF24 cell culture microplate in cell specific growth medium and treated with either 10 μM of QuinoMit[®] Q₁₀ fluid, coenzyme Q₁, coenzyme Q₁₀ or mitoquinone (mesylate, MitoQ[®]). MitoQ[®] has a positively charged triphenylphosphonium group on its isoprenoid side chain. This allows an increased attachment of MitoQ[®] into the mitochondria. Following a 16 h incubation at 37 °C in an incubator under standard conditions, the mitochondrial respiration profile was measured as described in 3.10.1 *Monolayer cell culture* and 3.11 *Mitochondrial respiration measurements* using the Seahorse Bioscience XF24 Extracellular Flux Analyzer. Figure 24A shows that the key parameters of mitochondrial respiration do not differ significantly between QuinoMit[®], Q₁ and Q₁₀. In the presence of MitoQ[®],

these parameters decrease significantly compared to QuinoMit[®]. Only the proton leak was not significantly affected. In addition, the relationship between OCR and ECAR was also considered (Figure 24B, 24C). This ratio indicates to what extent the cells switch their metabolism from energetically more favorable oxidative phosphorylation to glycolysis. This leads to an increased ECAR when energy is generated via glycolysis. It can be seen that the ratio of OCR to ECAR under MitoQ[®] shifts in favor of glycolysis. An increasing OCR/ECAR ratio also suggests increased oxidative phosphorylation, starting with QuinoMit[®] and ending with Q₁₀.



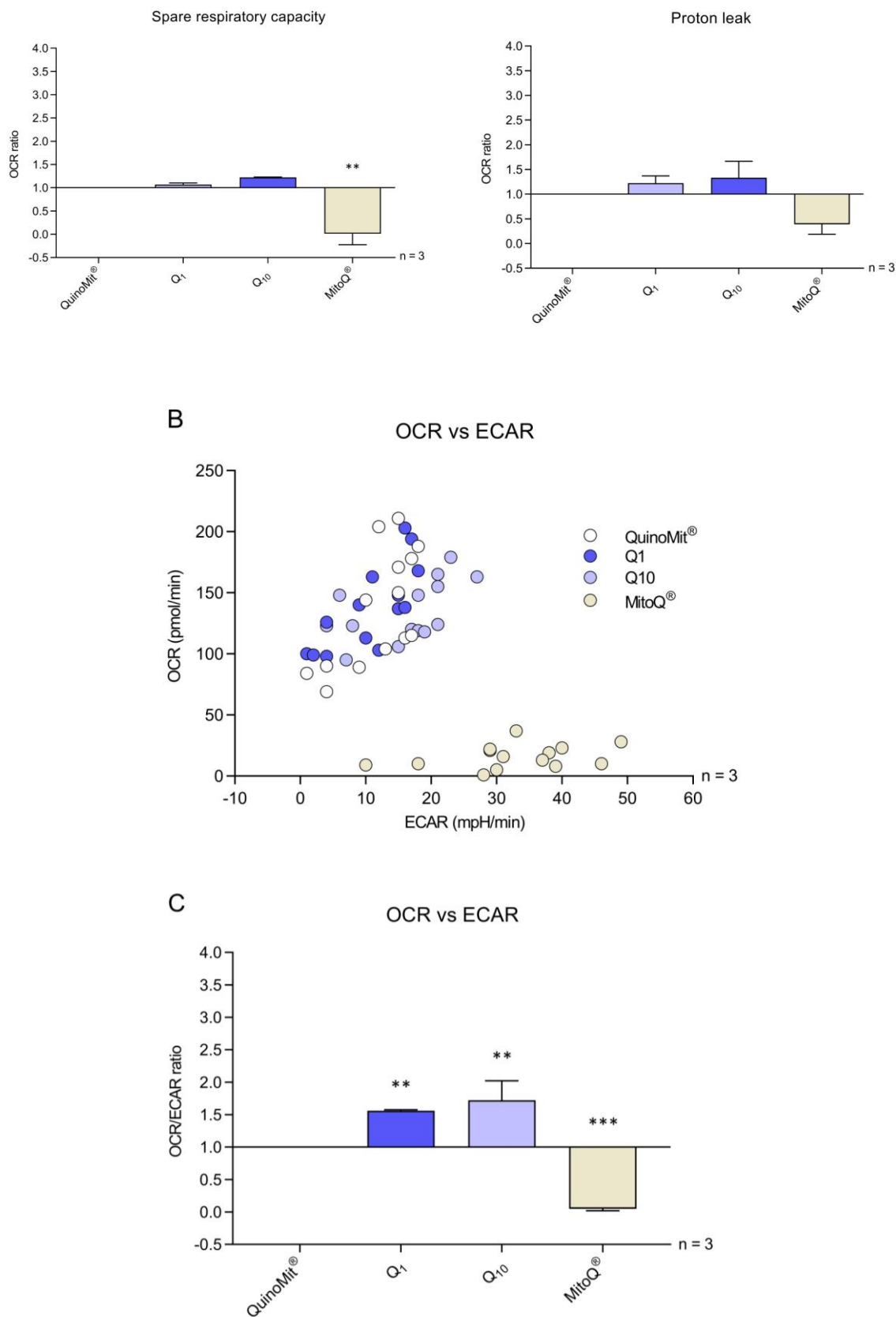
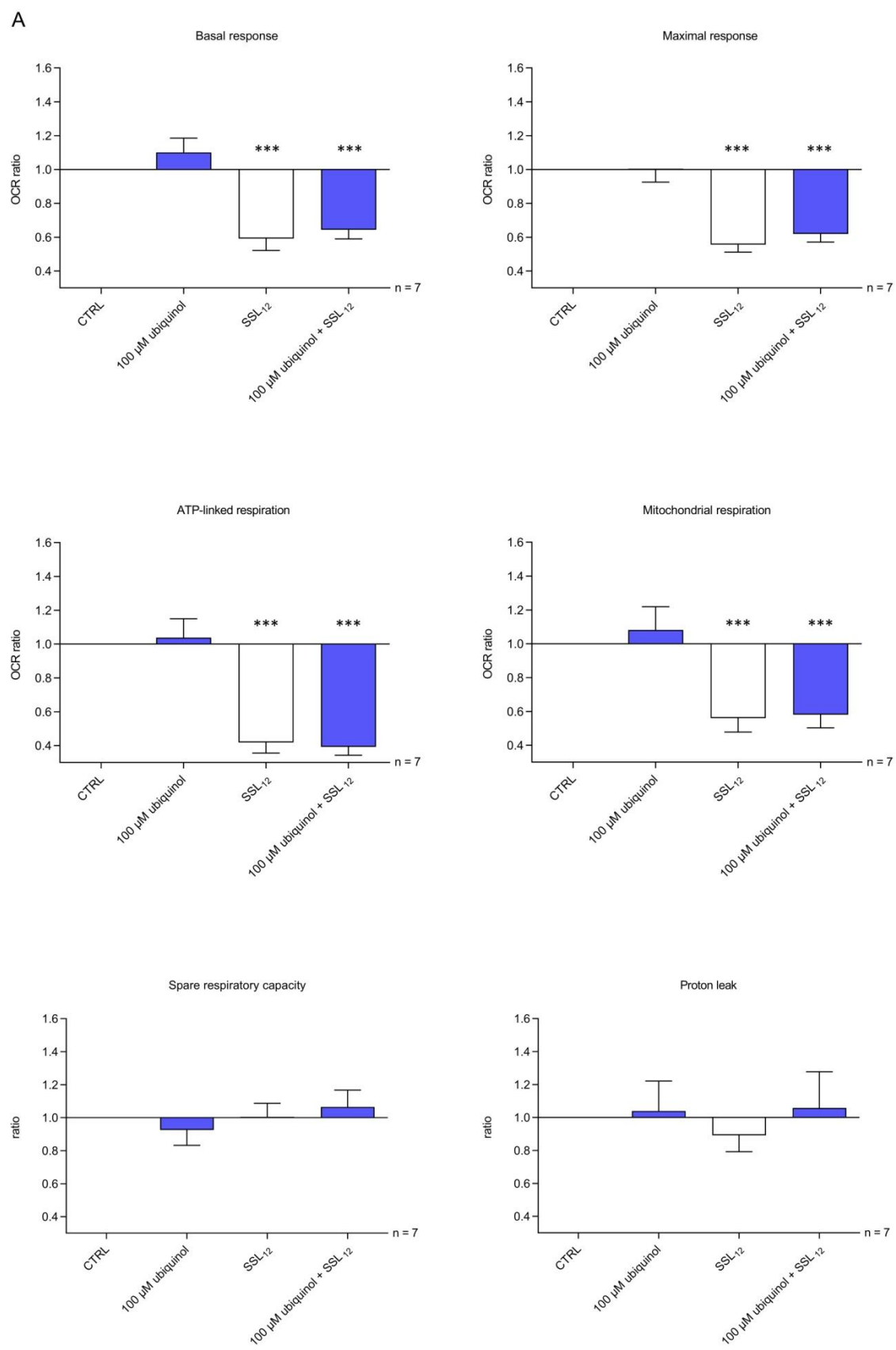


Figure 24. Significance of individual coenzyme Q formulations for mitochondrial respiration parameters. 5.0×10^4 fibroblasts per well were incubated with either 10 μ M of QuinoMit[®], coenzyme

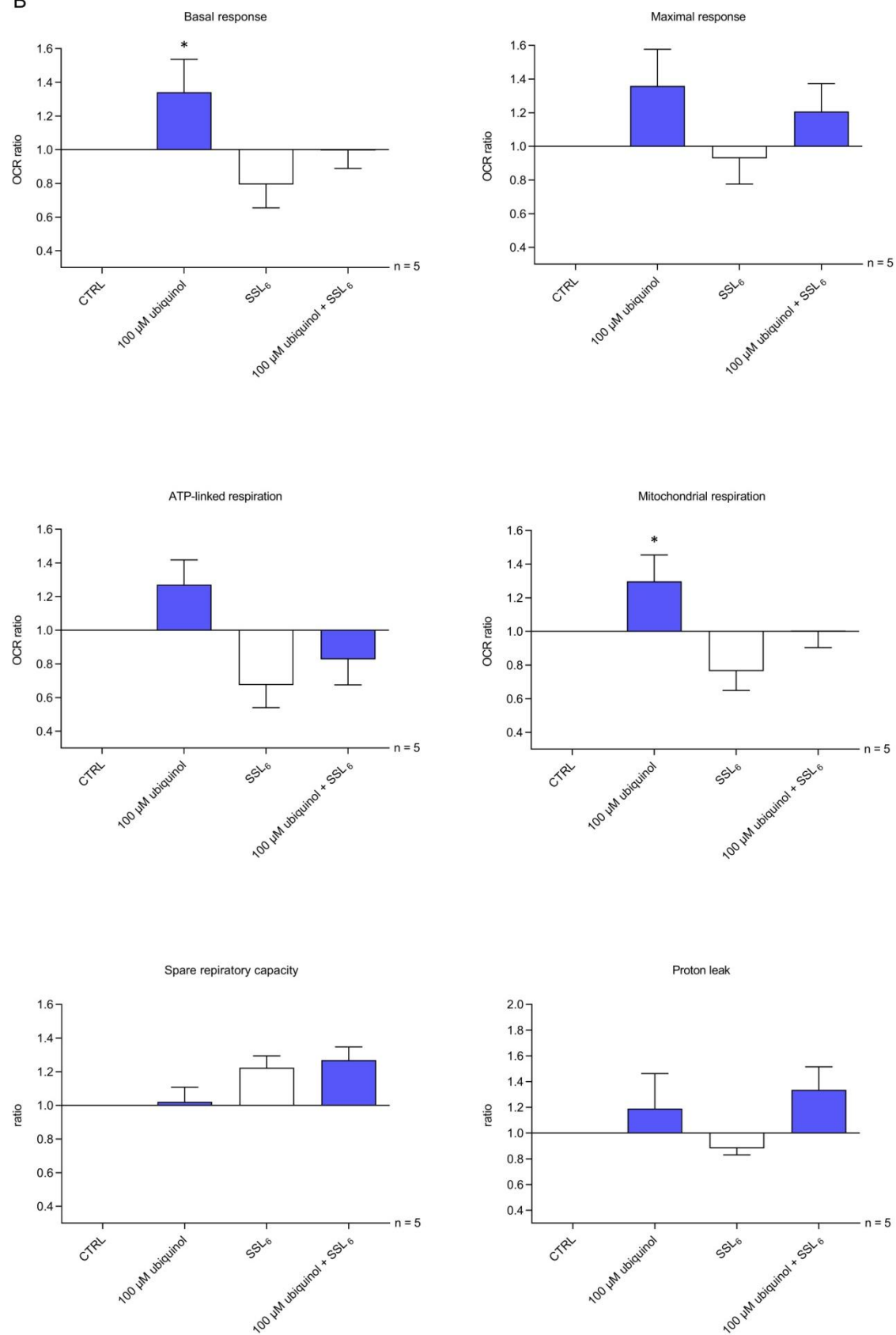
Q₁, coenzyme Q₁₀ or MitoQ[®] for 16 h at 37 °C in an incubator under standard conditions. After incubation, the mitochondrial respiration was analyzed with a Seahorse Bioscience XF24 Extracellular Flux Analyzer (Agilent Technologies) (n = 3, mean +/- SEM, one-way ANOVA with Dunnett's multiple comparison test versus QuinoMit[®], **p < 0.01; ***p < 0.001). QuinoMit[®] correspond to 1.0. OCR = oxygen consumption rate.

4.1.3 Effect of ubiquinol on the mitochondrial respiration of fibroblasts after SSL-UVA irradiation

Since ubiquinol has already shown a positive effect on the key parameters of mitochondrial respiration in untreated cells, the following experiment should investigate the influence of ubiquinol on human fibroblasts after irradiation with SSL. 5.0x10⁴ fibroblasts per well were seeded into XF24 cell culture microplates one day prior to measurement and incubated with or without 100 µM of ubiquinol (QuinoMit[®] Q₁₀-Fluid) in cell specific growth medium at 37 °C in an incubator under standard conditions. Because in the previous experiment an ubiquinol concentration of 100 µM showed the highest potency, only this concentration was used here. If the cells were irradiated with 12 J/cm² SSL-UVA (SSL₁₂) (3.3 *Irradiation of cells*), the irradiation took place immediately before the measurement of the respiration parameters, while the irradiation with 6 J/cm² SSL-UVA (SSL₆) was performed 16 h before. In this case, the cells were treated again with or without 100 µM ubiquinol after irradiation. The measurement of the mitochondrial respiration profile was carried out as described in 3.10.1 *Monolayer cell culture* and 3.11 *Mitochondrial respiration measurements* using the Seahorse Bioscience XF24 Extracellular Flux Analyzer. Irradiation of the cells with SSL₁₂ leads to a decrease in all key parameters of respiration. The spare respiratory capacity did not change due to irradiation. The proton leak decreased slightly under SSL₁₂ and increased again under 100 µM ubiquinol, but not significantly in both cases. Treatment of the cells with ubiquinol prior to irradiation showed no effect on the remaining parameters (Figure 25A). Irradiation with SSL₆ (Figure 25B) resulted in a significantly higher basal response and increased mitochondrial respiration in untreated and unirradiated cells. Maximum response and ATP-linked respiration were increased but not significant. If the cells were irradiated, an increase of the spare respiratory capacity was observed, all other mitochondrial parameters showed a decrease of the values. This decrease could be counteracted by treating the cells with 100 µM ubiquinol after irradiation.



B



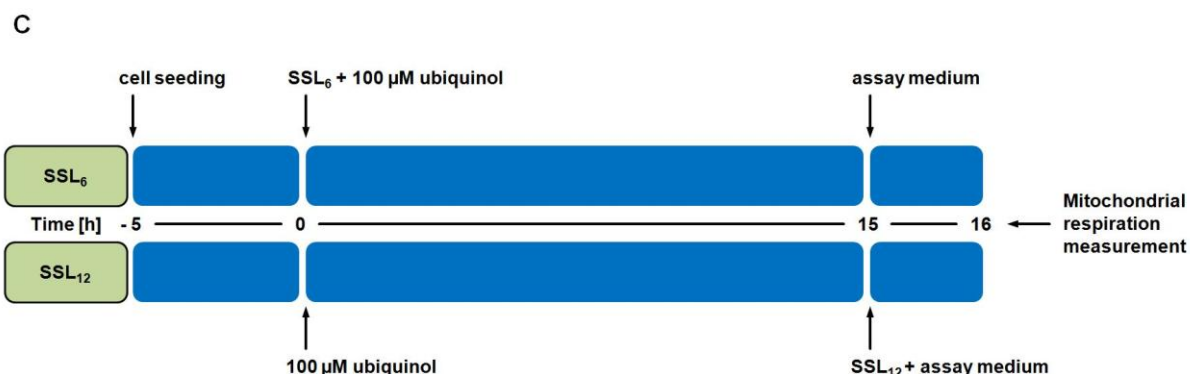


Figure 25. Effect of ubiquinol on the mitochondrial respiration of fibroblasts after irradiation with simulated solar light. 5.0×10^4 fibroblasts per well were seeded into XF24 cell culture microplates one day prior to measurement and incubated with or without 100 μ M of ubiquinol (QuinoMit[®] Q₁₀-Fluid) at 37 °C in an incubator under standard conditions. **[A]** 16 h later, cells were irradiated with 12 J/cm² (SSL₁₂) and then the mitochondrial respiration profile was analyzed. using the Seahorse Bioscience XF24 Extracellular Flux Analyzer (n = 7, mean $\pm$ SEM, one-way ANOVA with Dunnett's multiple comparison test versus control, *p < 0.05; ***p < 0.001). **[B]** Cells were incubated for 1 h in cell specific growth medium with or without 100 μ M ubiquinol and then irradiated with 6 J/cm² (SSL₆) 16 h prior to the measurement of the mitochondrial respiration profile. After irradiation cells were treated again with or without 100 μ M ubiquinol (n = 5, mean $\pm$ SEM, one-way ANOVA with Dunnett's multiple comparison test versus control, *p < 0.05). Control cells correspond to 1.0. OCR = oxygen consumption rate. **[C]** Flow chart for the measurement of the mitochondrial respiration parameters in UV-irradiated cells. Data partly received from Tanja Sonntag - Master Thesis.

4.1.4 Effect of ubiquinol on cellular ATP level of fibroblasts after SSL-UVA irradiation

Based on the previous experiments, in which mitochondrial respiration parameters, including ATP-linked respiration, were impaired after irradiation with simulated solar light, it was now necessary to investigate how SSL irradiation and combined treatment of the cells with ubiquinol over a longer period of time affects cellular ATP levels. Therefore, 3.0×10^5 fibroblasts per well were seeded into 6-well plates in cell specific growth medium and incubated with or without 100 μ M of ubiquinol (QuinoMit[®] Q₁₀-Fluid) for 16 h at 37 °C in an incubator under standard conditions followed by irradiation with SSL₁₂ (3.3 Irradiation of cells). Measurement of ATP levels were carried out with 3.5×10^4 lysed cells in a Wallac victor³ 1420-fluorometer as a luciferin-luciferase reaction as previously described (3.6 ATP bioluminescence assay) 6, 16, 24, 32 h after irradiation, respectively. The results showed that the ATP levels of the cells treated with ubiquinol but not irradiated were almost identical to the

ATP levels of the untreated control cells. After irradiation with SSL₁₂ the ATP level in the irradiated control cells, as well as in the cells treated with ubiquinol, decreased strongly to about 60 % compared to the non-irradiated control cells. After irradiation, the ATP level of the irradiated and additionally ubiquinol-treated cells regenerated and were near to the control levels 24 h after irradiation. Even 32 h after irradiation there was no significant difference between these two approaches. However, even after 32 h the ATP values of the untreated and irradiated cells were strongly reduced (Figure 26).

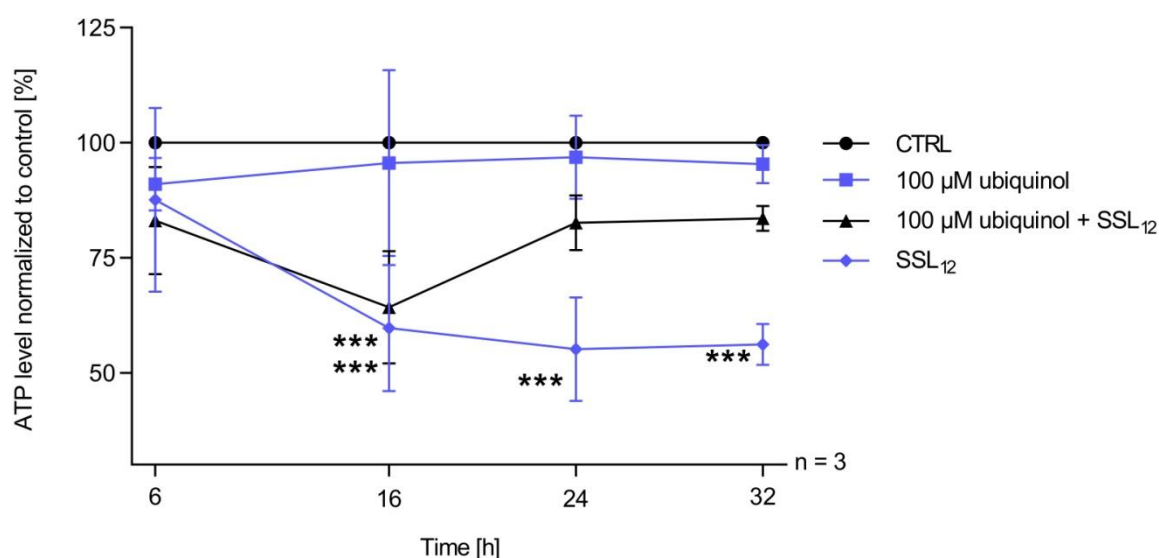


Figure 26. Effect of ubiquinol on cellular ATP level of fibroblasts after SSL (simulated solar light)-UVA irradiation. 3.0×10^5 fibroblasts per well were incubated with or without 100 µM ubiquinol (QuinoMit® Q₁₀-Fluid) for 16 h at 37 °C in an incubator under standard conditions followed by irradiation with or without 12 J/cm² SSL-UVA. After irradiation, there was a further incubation of the cells for 6, 16, 24, 32 h, respectively. Following incubation, cells were harvested and 3.5×10^4 cells were used for the determination of the ATP level by a luminescent reaction (n = 3, mean +/- SEM, two-way ANOVA, ***p < 0.001). Control cells (CTRL) correspond to 100 % at each time point. Data partly received from Tobias Mayr - Bachelor Thesis.

4.1.5 Influence of PARP inhibition on the ATP level of human Fibroblasts

The aim of this experiment was to find out whether a decrease in cellular ATP levels after UV irradiation is related to increased demand of DNA repair. To find this out, the enzyme PARP, a key enzyme in DNA repair, was inhibited. For this, 3.0×10^6 fibroblasts per well were seeded into 6-well plates in cell specific growth medium and incubated with or without 100 µM of ubiquinol (QuinoMit® Q₁₀-Fluid) as well as with or

without 10 μ M of the PARP inhibitor Veliparib (ABT-888) (AbbVie) for 16 h at 37 °C in an incubator under standard conditions followed by irradiation with SSL₁₂ (3.3 *Irradiation of cells*). Measurement of ATP levels after PARP inhibition were carried out with 3.5×10^4 lysed cells in a Wallac victor3 1420-fluorometer as a luciferin-luciferase reaction as previously described (3.6 *ATP bioluminescence assay*) 24 h after irradiation, because at this time the strongest reduction of the ATP level took place. As already shown, cells irradiated with SSL₁₂ had a significantly lower ATP level compared to unirradiated cells (Figure 27). If unirradiated cells were treated with ubiquinol, a slightly but not significantly increased content of cellular ATP could be measured after 24 h compared to control. A decrease of the ATP level after irradiation could be counteracted by the addition of ubiquinol. Inhibition of the PARP enzyme with ABT-888 in unirradiated cells led to an increase in ATP levels. This could also be observed in unirradiated cells treated with ubiquinol and ABT-888. Here, the cellular ATP content was even higher than that of cells that were not treated with ubiquinol. It was also found that irradiated cells treated with ubiquinol and ABT-888 showed only slightly elevated ATP levels compared to irradiated cells. In general, however, no large effect of PARP inhibition on the total amount of ATP in the cell after irradiation can be observed. This would suggest that the reduced ATP levels are only due to mitochondrial dysfunction and not to increased repair of DNA damage.

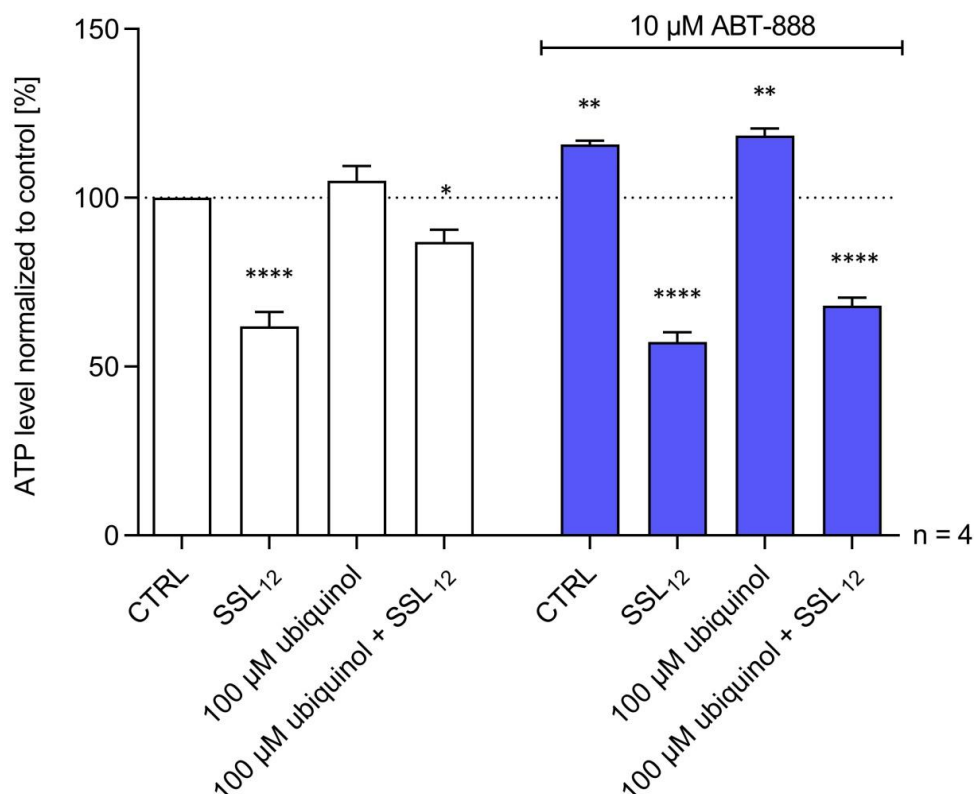
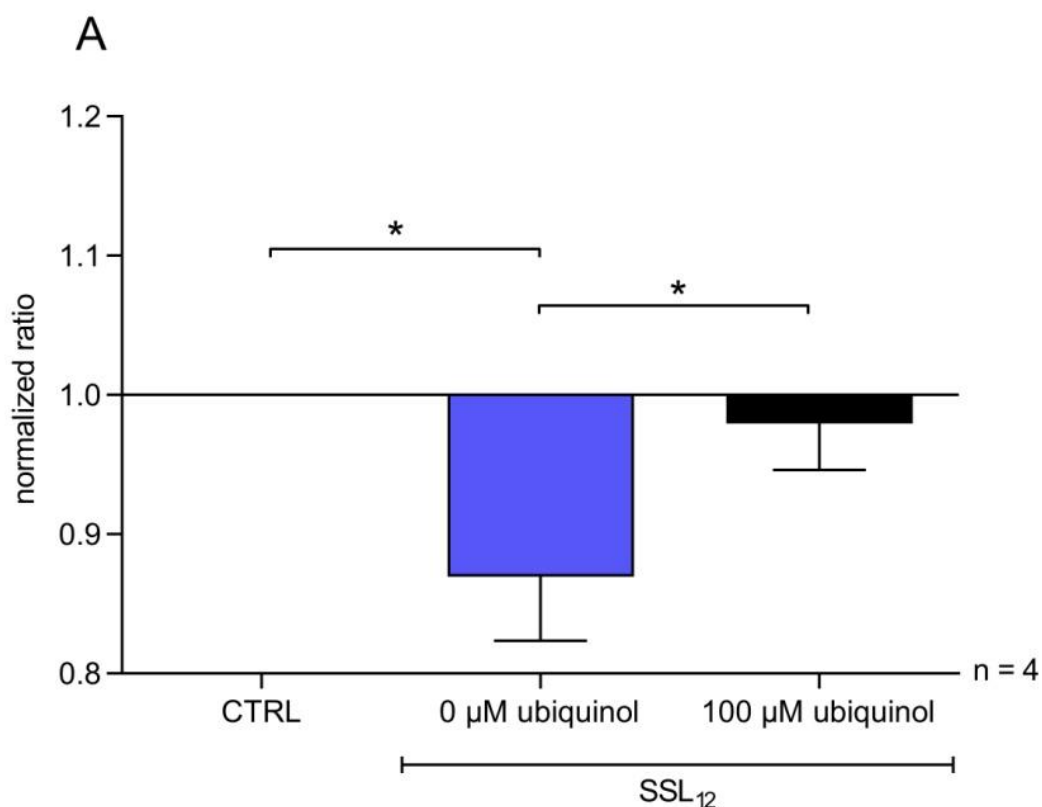


Figure 27. Influence of PARP inhibition on the ATP level of human Fibroblasts. 3.0×10^6 fibroblasts per well were incubated with or without 100 μM ubiquinol (QuinoMit[®] Q₁₀-Fluid) or 10 μM ABT-888 for 16 h at 37 °C in an incubator under standard conditions followed by irradiation with or without 12 J/cm² SSL-UVA. After irradiation, there was a further incubation of the cells for 24 h. Following incubation, cells were harvested and 35.000 cells then were used for the determination of the ATP level by a luminescent reaction (n = 4, mean $\pm$ SEM, one-way ANOVA with Dunnett's multiple comparison test versus control, *p < 0.05; **p < 0.01; ****p < 0.0001). Control cells (CTRL) correspond to 100 %.

4.1.6 Effect of ubiquinol on the mitochondrial membrane potential ($\Delta\psi\text{m}$) of fibroblasts after SSL-UVA irradiation

In order to find out whether an accelerated regeneration of the ATP level is based on the preservation of the mitochondria, in the following experiment the influence of irradiation and treatment of the cells with ubiquinol on the mitochondrial membrane potential was investigated. A total of 1.0×10^5 cells per well were seeded in 6-well plates. 7 h or 24 h after seeding the SSL-UVA irradiation with a dose of 12 J/cm² took place (3.3 Irradiation of cells). If the cells were irradiated 7 h after seeding, following the irradiation there was a further incubation of the cells in cell specific growth medium with or without 100 μM ubiquinol. Prior to measurement, the cells were

stained with JC-1 (Cayman Chemical Company) for 30 min at 37 °C and subsequently detached. As a negative control FCCP (Cayman Chemical Company) was added to the stop solution of one approach. The measurement of the mitochondrial membrane potential was analyzed using the Fluorescence Activated Cell Sorter FACSCalibur (3.8 *Mitochondrial membrane potential ($\Delta\psi_m$) measurements*). It was shown that the irradiation of the cells with SSL₁₂ led to an impairment of the mitochondrial membrane potential. Both directly (Figure 28A) and 17 h (Figure 28B) after irradiation, depolarization of the mitochondrial membrane occurred. However, if the cells were treated with ubiquinol before and immediately after irradiation, there was no significant change in membrane potential compared to the untreated and non-irradiated control cells. Treatment with ubiquinol had a protective effect on $\Delta\psi_m$ when the cells were irradiated with SSL₁₂.



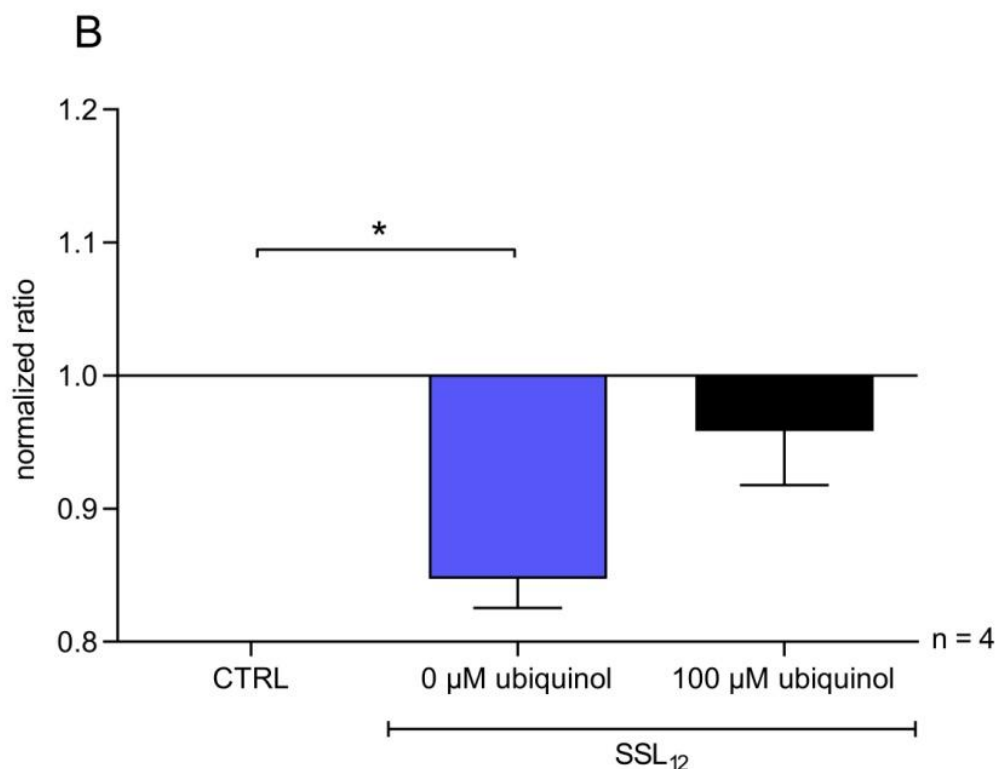


Figure 28. Effects of ubiquinol on the mitochondrial membrane potential ($\Delta\psi_m$) of fibroblasts after SSL-UVA irradiation. 1.0×10^5 fibroblasts per well were incubated with or without 100 μ M ubiquinol (QuinoMit® Q₁₀-Fluid), irradiated with 12 J/cm² SSL-UVA 24 h **[A]** or 7 h **[B]** after seeding, respectively. If the cells were irradiated 7 h after seeding, following the irradiation there was a further incubation of the cells in cell specific growth medium with or without 100 μ M ubiquinol. 24 h after seeding, cells were stained with JC-1 for 30 min at 37 °C. After reaching the incubation time, the cells were detached and the mitochondrial membrane potential were analyzed with the Fluorescence Activated Cell Sorter FACSCalibur. $\Delta\psi_m$ was calculated as follows $[(\text{medianFI}_{\text{red}})/(\text{medianFI}_{\text{green}})]$. Data are shown as normalized ratio $[(\text{ratio}_{\text{sample}})/(\text{ratio}_{\text{CTRL}})]$ (n = 4, mean $\pm$ SEM, one-way ANOVA with Tukey's post-tests, *p < 0.05). Control cells (CTRL) correspond to 1.0. Data partly received from Sonja Müller - internal research.

PART II

4.2 Age-Dependent Loss of Mitochondrial Function in Epithelial Tissue Can Be Reversed by ubiquinol

Dysfunctions of the mitochondria and their effects on oxidative phosphorylation and thus ATP production are regarded as the main reason for the increase in age-related and severe diseases during the ageing process. ROS-damaged mtDNA is at the beginning of a process which, according to the "mitochondrial theory of ageing", is a major cause of cell ageing, tissue dysfunction and degeneration. According to this theory, understanding the effects of harmful influences on mitochondrial function is therefore essential for a better understanding of the molecular mechanisms of ageing. After showing in cell culture that fibroblasts treated with ubiquinol improve the key parameters of mitochondrial respiration and cellular ATP levels after irradiation with SSL, these findings should be confirmed in the following section directly on human epithelial tissue. The question was whether ubiquinol can attenuate the mitochondrial dysfunctions caused by short-term induced damage, e.g. UV irradiation, as well as age-related and long-term induced damage. Because measurement of mitochondrial respiration in isolated primary fibroblasts cannot reflect the actual situation of the skin, the second part of this work was about to measure mitochondrial parameters directly in epithelial tissue.

4.2.1 Influence of donor age on mitochondrial parameter in epithelial tissue

In this first study, we analyzed the mitochondrial respiration and ATP-linked respiration as a function of age to find out whether, according to the "mitochondrial theory of ageing", the mitochondrial function decreases with age. To do so, 16 h prior to measurement purified biopsies were stored overnight at 4 °C in Dispase II to enzymatically separate the epidermis from the dermis (*3.1.2 Isolation of skin fibroblasts*). The epidermis were then placed on a capture screen (*3.10.2 Epidermis*) and the mitochondrial respiration was measured with a Seahorse Bioscience XF24 Extracellular Flux Analyzer (*3.11 Mitochondrial respiration measurements*). As shown in the figures below, both the mitochondrial respiration (Figure 29A) and ATP-linked respiration (Figure 29B) in the measured donor collective (Table 2) decrease drastically with increasing age. This results in a reduction of mitochondrial respiration by an average of approximately 10 % per decade.

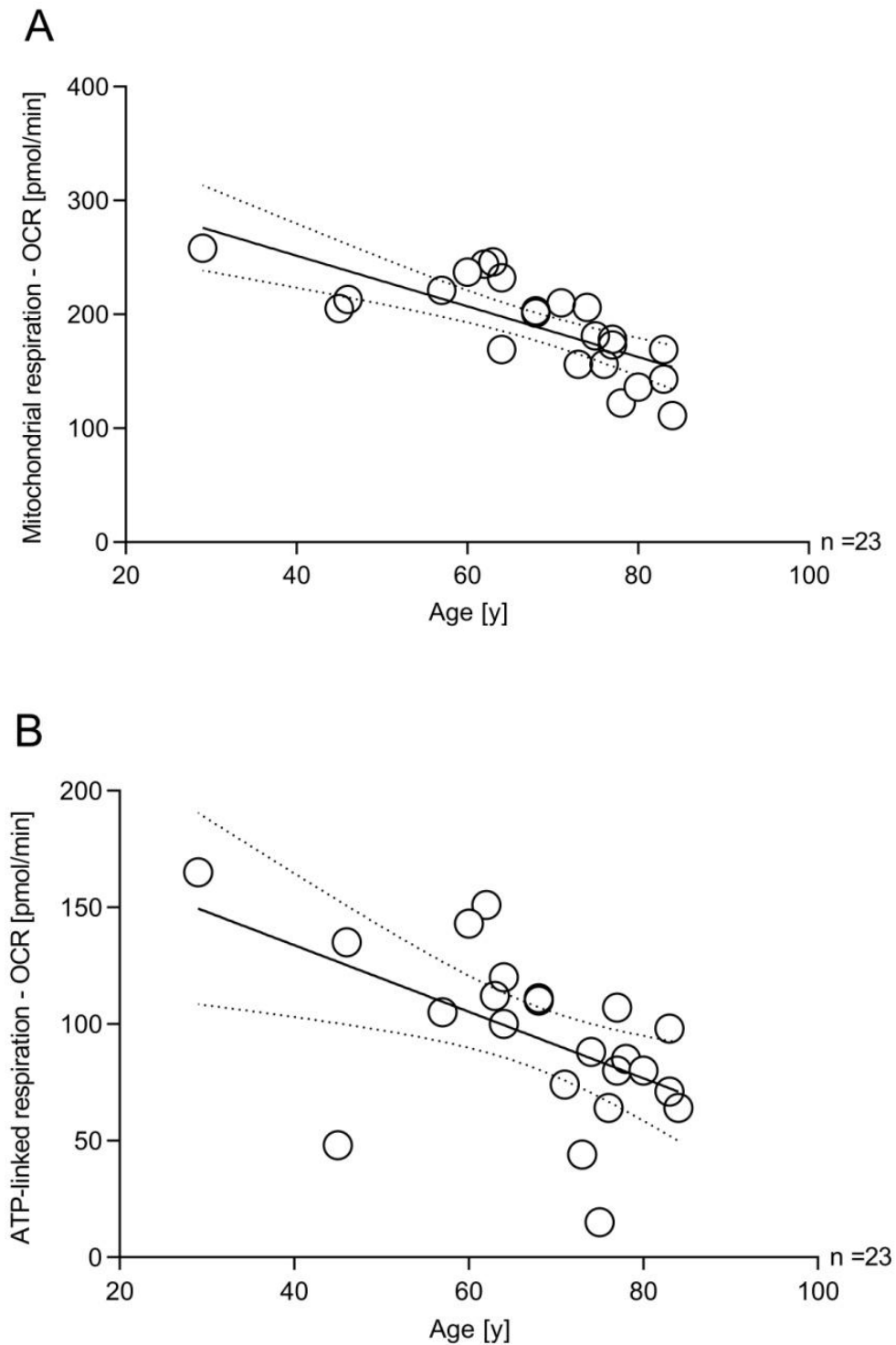
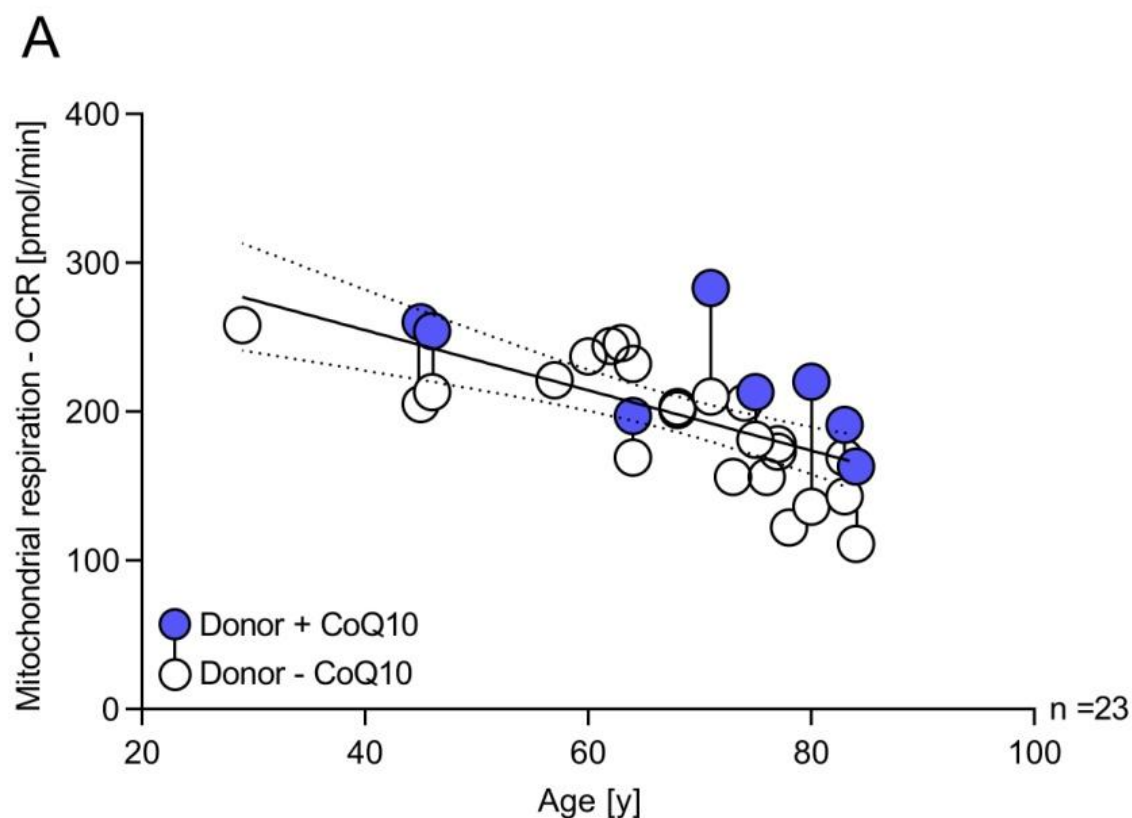


Figure 29. Influence of donor age on mitochondrial parameter in epithelial tissue. 16 h prior to measurement purified biopsies were stored overnight at 4 °C in Dispase II. On the next day the mitochondrial respiration **[A]** and ATP-linked respiration **[B]** of the separated epidermis was analyzed with a Seahorse Bioscience XF24 Extracellular Flux Analyzer (n = 23, linear reg. with 95 % confidence interval, R^2 mitochondrial respiration = 0.5487 / $p = < 0.0001$, ATP-linked respiration = 0.2945 / $p = < 0.01$, deviation from zero = significant). OCR = oxygen consumption rate. Data partly received from Daniel Gebhard - internal research.

4.2.2 Effect of ubiquinol on respiratory parameters in epithelial tissue

In the next experiment, the impact of ubiquinol and carrier control treatment on respiratory parameters was examined to see if ubiquinol can attenuate age-related mitochondrial dysfunctions, how they were observed in the previous study. Prior to measurement, biopsies were treated with or without 100 μ M ubiquinol or carrier control (CC) and incubated at 4 °C for 16 h. The test compounds were added directly to the enzymatic digestion solution. The following day the dermis was separated from the epidermis (3.1.2 *Isolation of skin fibroblasts*) and mitochondrial parameters were measured as described under 3.10.2 *Epidermis* and 3.11 *Mitochondrial respiration measurements*. Due to the small size of many biopsies, treatment with ubiquinol and carrier control could not be performed on all samples listed in Table 2. As can be seen from Figure 30A, treatment of the epidermis with ubiquinol increases mitochondrial respiration and almost all important respiratory parameters (Figure 30B). Thus, in addition to a significant increase in mitochondrial respiration, a significant increase in basal response, maximal response and proton leak could be observed. ATP-linked respiration was also increased, but not significant. The carrier control, however, did not lead to a significant effect (Figure 30B).



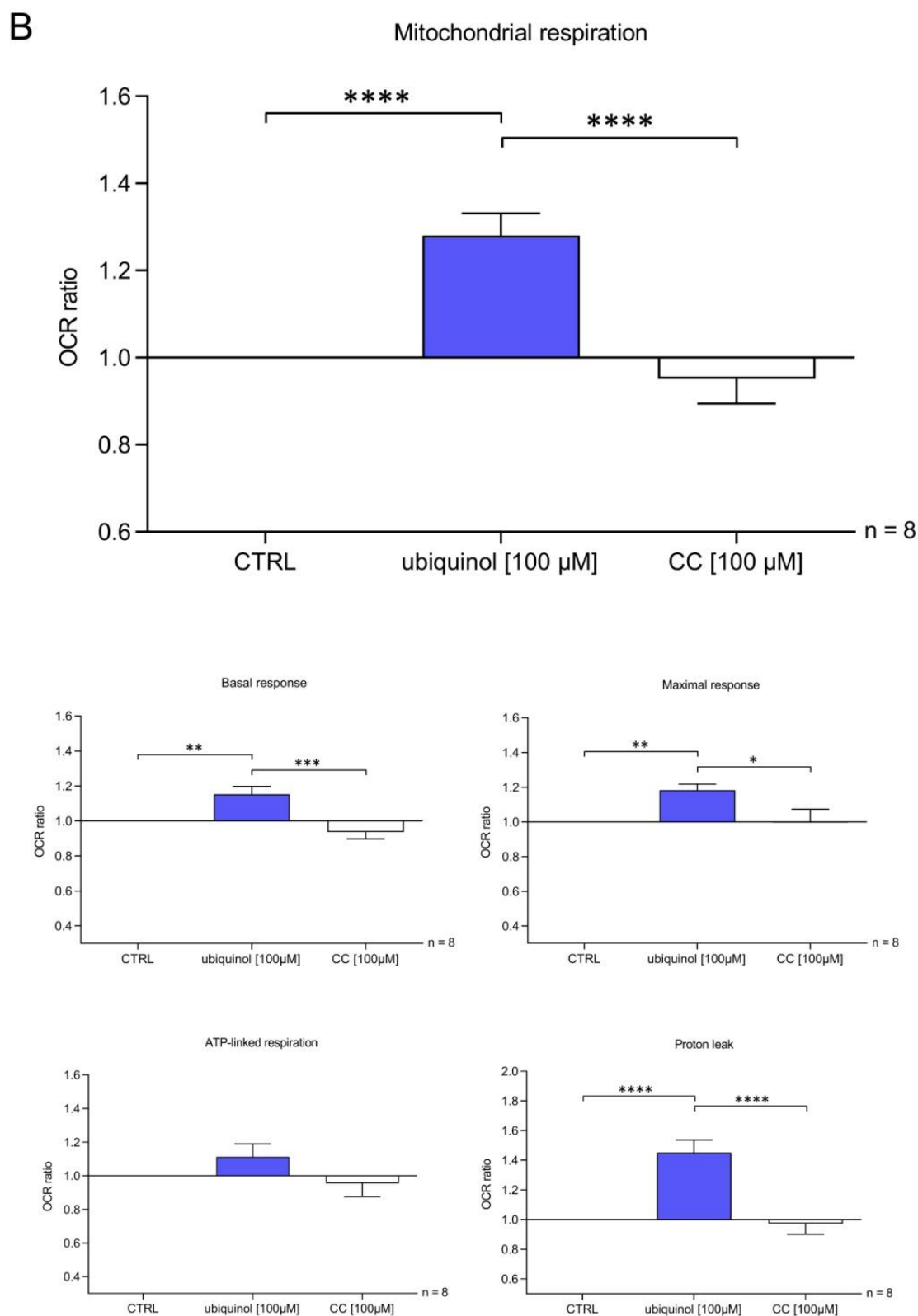


Figure 30. Effect of ubiquinol on respiratory parameters in epithelial tissue. 16 h prior to measurement, purified biopsies were stored overnight at 4 °C in Dispase II. During incubation with Dispase II the samples were treated with or without 100 μM ubiquinol or carrier control (CC [100 μM]). The following day the respiration profile of the separated epidermis was analyzed with a Seahorse

Bioscience XF24 Extracellular Flux Analyzer. **[A]** White circles represent untreated samples. Samples treated with ubiquinol are shown in blue. Connected circles represent samples from the same donor. ($n = 23$, linear reg. with 95 % confidence interval, $R^2 = 0.4354$ / $p = < 0.0001$, deviation from zero = significant). **[B]** Influence of carrier control (CC [100 μ M]) on mitochondrial respiratory parameters ($n = 8$, mean $\pm$ SEM, one-way ANOVA with Dunnett's multiple comparison test versus control and CoQ₁₀ carrier control, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). Control cells (CTRL) correspond to 1.0. OCR = oxygen consumption rate.

4.2.3 Influence of sampling location of biopsies on mitochondrial respiration

In order to exclude a photoageing effect on the values obtained in the previous experiments, the mitochondrial respiration of biopsies from body regions with a high UV exposure (HE) (e.g., eyelids) and biopsies from low UV-exposed body regions (LE) (e.g., abdomen) were compared. Only donor pairs of the same age were compared in order to exclude an age-induced effect (Figure 31). The processing of the samples as well as the measurement of the mitochondrial respiration was proceeded as in the previous experiments (3.1.2 *Isolation of skin fibroblasts*, 3.10.2 *Epidermis*, 3.11 *Mitochondrial respiration measurements*). As can be seen below, samples with a high UV exposure showed a significantly lower mitochondrial respiration on average than samples with low UV exposure. As a result, samples with high UV exposure were not included in the evaluation of age dependence as shown in Figure 29A.

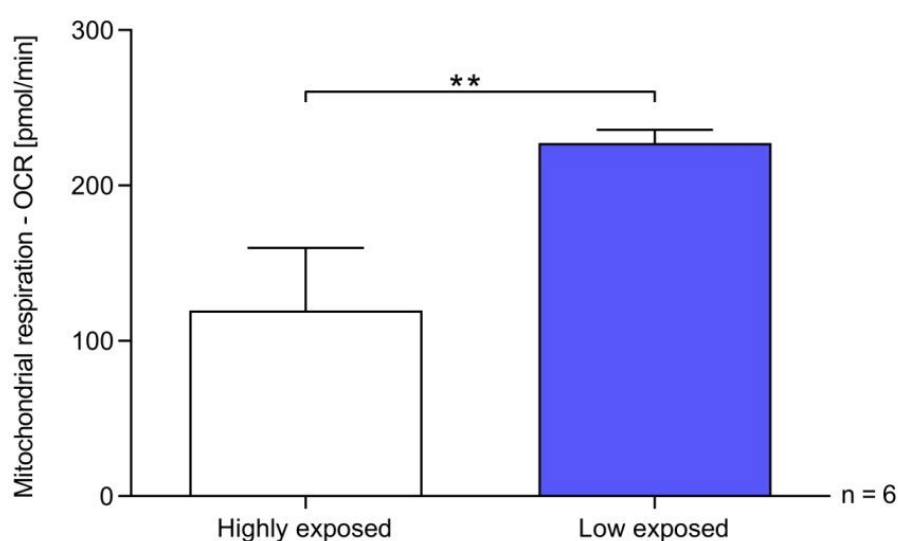


Figure 31. Influence of sampling location of biopsies on mitochondrial respiration. One day before measurement, purified biopsies were stored overnight at 4 °C in Dispase II for 16 h. The

following day the respiration profile of the separated epidermis was analyzed with a Seahorse Bioscience XF24 Extracellular Flux Analyzer. The range of age in both groups (HE & LE) was between 29 and 77 years ($n = 6$ highly exposed, $n = 6$ low exposed, linear reg., mean $\pm$ SEM, t-test, $**p < 0.05$).

4.2.4 Influence of gender and smoking on the respiratory parameters in epithelial tissue

In addition to the influence of the sampling site of the biopsies, it was also important to find out whether the gender, the smoking or non-smoking of the volunteers had an influence on respiratory parameters. It should also be investigated to what extent the age dependence of mitochondrial respiration depends on the sex of the donors. For this the measured collective of 20 donors was divided into male and female donors. The processing of the samples as well as the measurement of the mitochondrial respiration was proceeded as described under *3.1.2 Isolation of skin fibroblasts*, *3.10.2 Epidermis* and *3.11 Mitochondrial respiration measurements*. Figure 32A shows that the gender of the donors and the aspect of smoking have no significant influence on respiratory parameters in epithelial tissue. When investigating the age dependence of mitochondrial respiration on sex, however, it was noticeable, that there was a difference in the decrease of mitochondrial respiration with age between male and female donors (Figure 32B). The data show that there is a higher decrease of mitochondrial respiration with increasing age in male subjects.

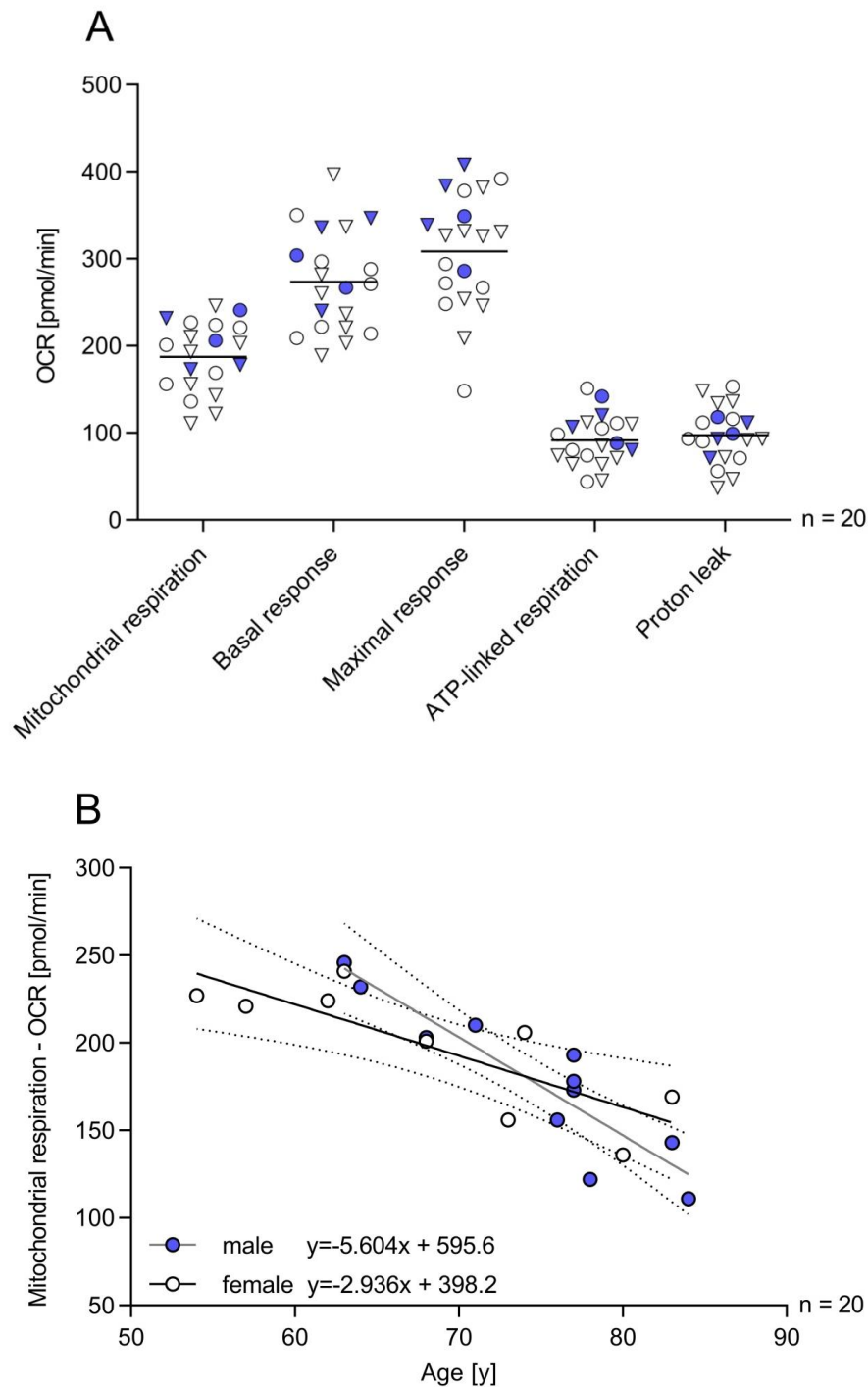


Figure 32. Influence of gender and smoking on respiratory parameters in epithelial tissue. 16 h prior to measurement, purified biopsies were stored overnight at 4 °C in Dispase II. On the next day the respiration profile of the separated dermis was analyzed with a Seahorse Bioscience XF24 Extracellular Flux Analyzer (female n = 9, male n = 11). **[A]** circles = female, triangles = male, blue = smoker, white = non-smoker. **[B]** Donors were separated into female and male, linear reg. with 95 % confidence interval, R^2 female = 0.6681, male = 0.8243, deviation from zero = significant). Data partly received from Daniel Gebhard - internal research.

PART III

4.3 The activity of the DNA repair enzyme hOGG1 can be directly modulated by ubiquinol

The DNA of human cells suffers about 1.000 to 1.0×10^5 oxidative lesions per day. One of the most common defects in this category is represented by 7,8-dihydro-8-oxoguanine. There are numerous exogenous effects on the DNA that induce the intracellular generation of 7,8-dihydro-8-oxoguanine. In this case, the solar radiation should be particularly emphasized. Therefore, a quantitatively sufficient repair of all occurring oxidative damaged guanine bases is often only partially feasible, especially in advanced age. Inadequate removal of these damages can subsequently lead to mutations and thus to serious diseases. All these aspects represent a dangerous situation for an organism. However, it is suspected that the amount of the 8-oxoguanine DNA glycosylase 1 can be actively regulated on the level of gene expression by the redox-active properties of ubiquinol and thus its protein expression can be controlled. The aim of this part of the work was to find out to what extent ubiquinol influences the activity of the human DNA repair enzyme 8-oxoguanine DNA glycosylase 1.

4.3.1 Influence of ubiquinol and ubiquinone on hOGG1 enzymatic activity *in vitro*

In order to analyze a potential, activity-enhancing effect of CoQ₁₀ on hOGG1 *in vitro*, test solutions in concentrations of 12.5 to 100 µM were initially prepared from both CoQ₁₀ formulations, the reduced and the oxidized form (ubiquinol and ubiquinone). These test solutions were added in a base excision repair assay (3.12.2 *Measurement of base excision repair*) to the reporter oligonucleotides CN-8-oxoG and CN-CTRL (3.12.1 *Reporter oligonucleotides*). Together with commercially purchased and purified hOGG1, the enzymatic digestion of the reporter oligonucleotides was then analyzed in the presence of different CoQ₁₀-concentrations of both formulations because *in vivo* mainly the reduced form ubiquinol is present and is continuously converted into ubiquinone. As a positive control (PC) approaches were included with all assay components but without test solution. This was used for the detection of the hOGG1 basic activity without CoQ₁₀. In addition, two negative controls (NC - 100 µM test solution) were used. NC1 without reporter

oligonucleotides and hOGG1, for the detection of a possible autofluorescence of the test solutions. NC2 with reporter oligonucleotides but without hOGG1 in order to investigate a spontaneous decay of reporter oligonucleotides in the presence of the test solution. Because the CoQ₁₀ formulations we used was a nanostructured lipid formulation, a carrier control (CC) was included as an additional control in order to exclude possible effects of the ingredients on the activity of hOGG1. As can be seen in Figure 33, both ubiquinone and ubiquinol show a significant almost linear increase in hOGG1 enzymatic activity at concentrations of 50 - 100 μ M of CoQ₁₀. There was no significant increase in the presence of carrier control. An autofluorescence of the test solution as well as a self-cutting of the reporter oligonucleotide could be excluded.

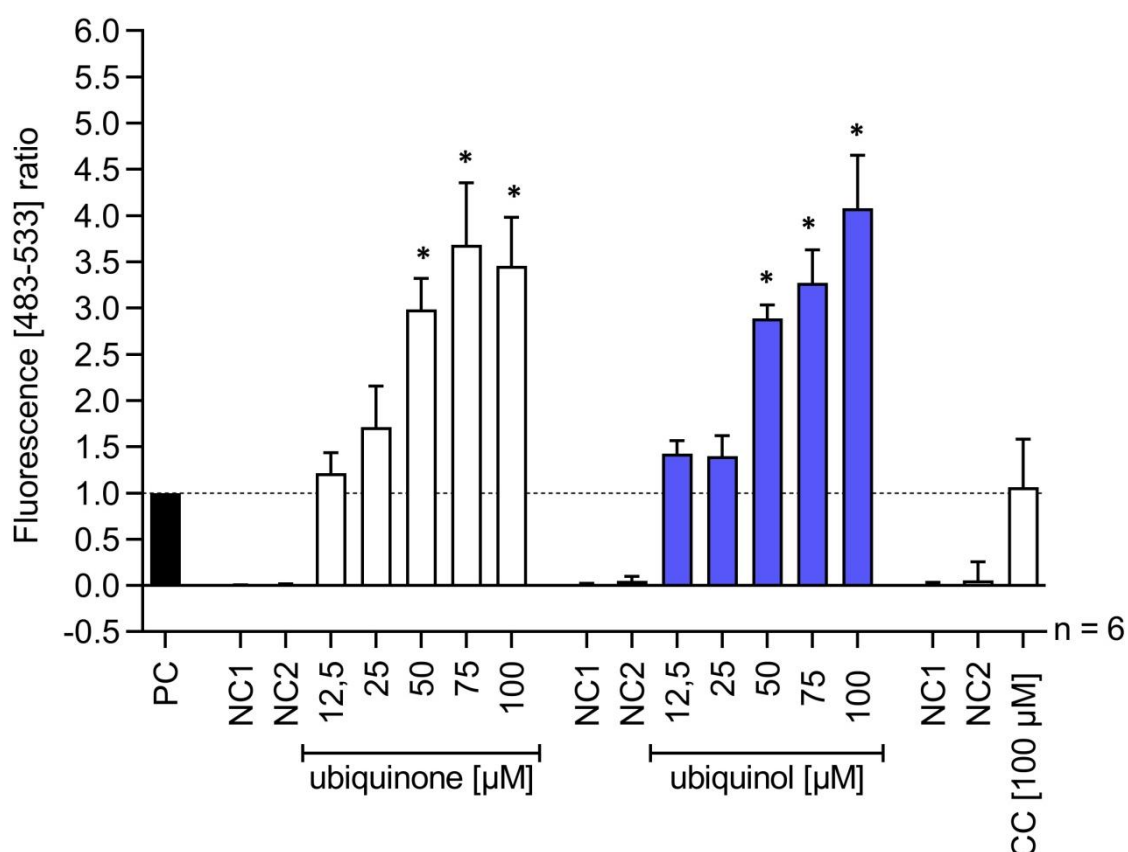


Figure 33. Influence of ubiquinol and ubiquinone on hOGG1 enzymatic activity *in vitro*. Addition of different concentrations (12.5 - 100 μ M) of ubiquinone, ubiquinol and carrier control to commercially purchased hOGG1 and reporter oligonucleotides. The fluorescence signals were recorded with a LightCycler® 480 in a wavelength range between 483 and 533 nm. The fluorescence signals of the digested reporter oligonucleotides based on the hOGG1 activity are shown (n = 6, mean $\pm$ SEM, one-way ANOVA with Bonferroni's multiple comparison test, *p < 0.05). NC1 = test solution without reporter oligonucleotides and hOGG1. NC2 = test solution with reporter oligonucleotides but without

hOGG1. Values normalized to the positive control (PC = all assay components without test solution). PC correspond to 1.0. Data partly received from Heiko van Beek - Bachelor Thesis.

4.3.2 Influence of ubiquinol on hOGG1 enzymatic activity isolated from mitochondria

Following the experiments with commercially purchased and purified hOGG1, the next step was to determine whether an increase in the activity of this enzyme also takes place on mitochondrial hOGG1. For the recovery of mitochondrial hOGG1, mitochondria from 3.0×10^6 human fibroblasts were first isolated over several centrifugation steps and then lysed via an ultrasonic disintegrator (*3.4 Isolation and lysis of mitochondria*). In order to investigate an activity-increasing effect of ubiquinol on mitochondrial hOGG1, only ubiquinol (10 μ M - 1000 μ M) was used because it showed the best results and an almost linear increase in hOGG1 activity with increasing ubiquinol concentration (50 – 100 μ M) in the *in vitro* experiments. In a subsequent base excision repair assay (*3.12.2 Measurement of base excision repair*) ubiquinol was given to the reporter oligonucleotides CN-8-oxoG and CN-CTRL as in the previous experiment. In addition, instead of purchased and purified hOGG1, the isolated mitochondrial hOGG1 was added. As a positive control an approach was made with all assay components but no ubiquinol. A negative control with test solution but without hOGG1 and a carrier control were also performed. An increase in hOGG1 activity on mitochondrial hOGG1 could be detected with increasing ubiquinol concentration. While low ubiquinol concentrations (10, 50, and 100 μ M) resulted in little stimulation of hOGG1 activity, higher concentrations (500 and 1000 μ M) induced a statistically significant increase in enzymatic activity.

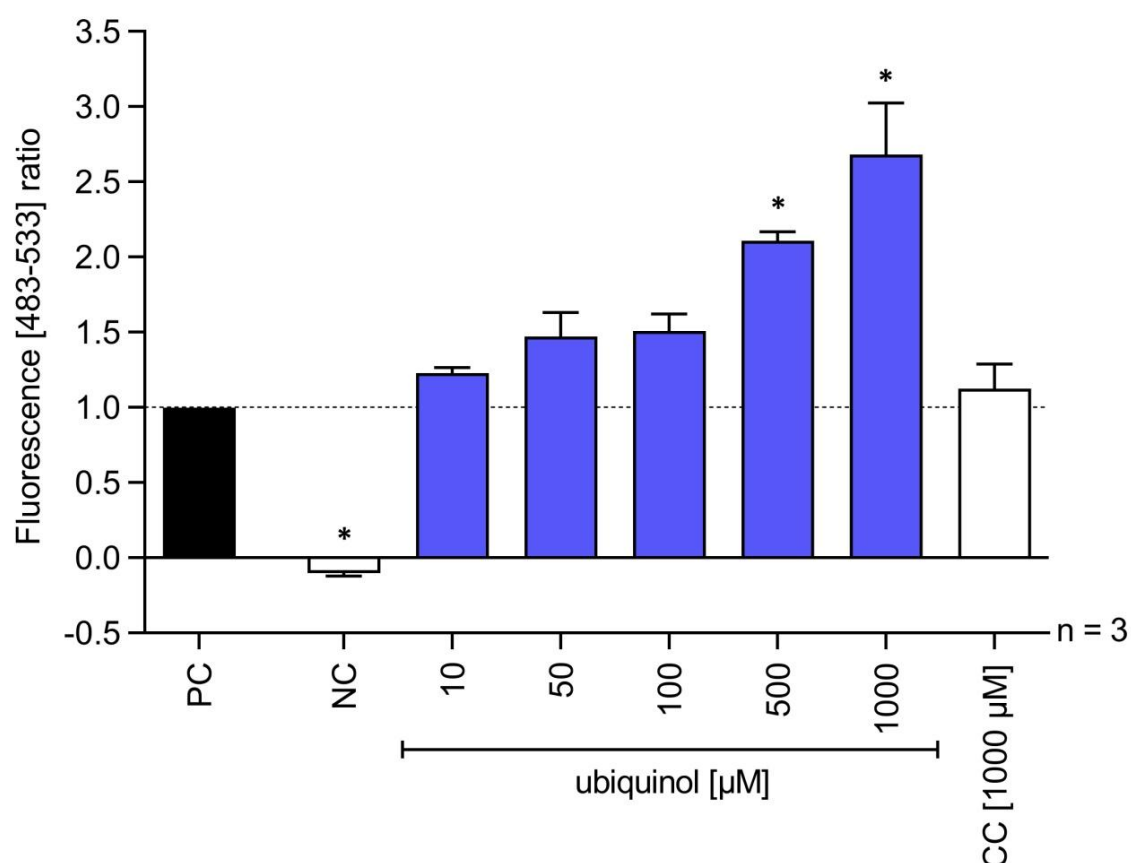
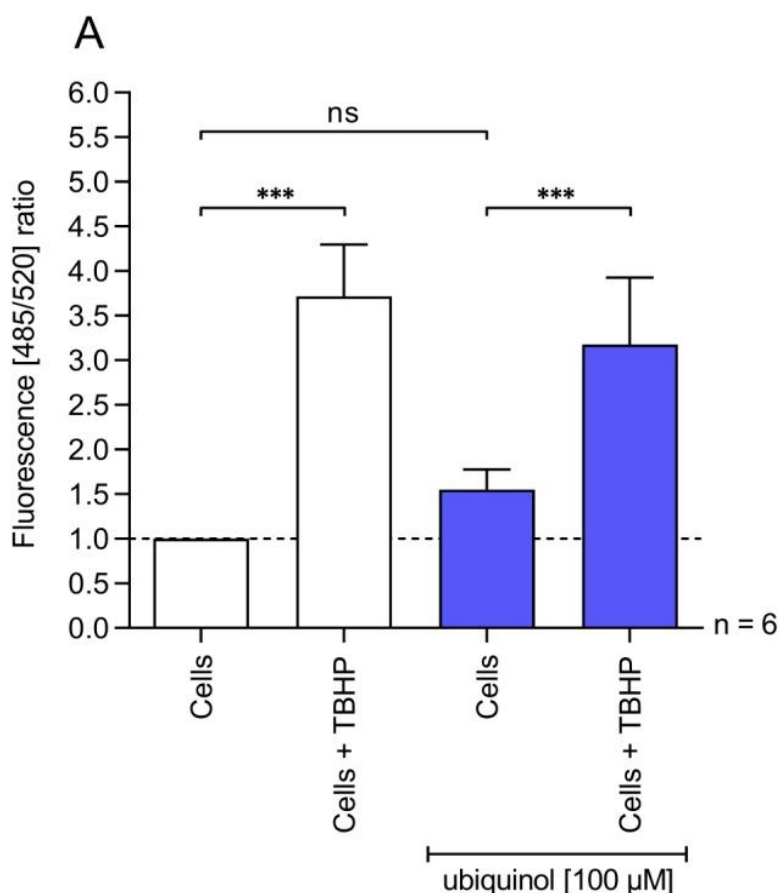


Figure 34. Influence of ubiquinol on hOGG1 enzymatic activity isolated from mitochondria. Addition of different concentrations (10 - 1000 μM) of ubiquinone and carrier control to reporter oligonucleotides and hOGG1 from mitochondrial lysates, isolated from 3.0×10^6 human fibroblasts. As a negative control one approach with test solution and reporter oligonucleotides but without hOGG1 were included. The fluorescence signals were recorded with a LightCycler® 480 in a wavelength range between 483 and 533 nm. The fluorescence signals of the digested reporter oligonucleotides based on the hOGG1 activity are shown ($n = 3$, mean $\pm$ SEM, one-way ANOVA with Bonferroni's multiple comparison test, $*p < 0.05$). Values normalized to the positive control (PC = all assay components without test solution). PC correspond to 1.0. Data partly received from Julia Schon and Vivien Nöth - Bachelor Thesis.

4.3.3 Influence of ubiquinol on the formation of ROS and mitochondrial superoxide

After an increase in the activity of mitochondrial hOGG1 by ubiquinol was shown, it should now be investigated whether the addition of ubiquinol also leads to an increased formation of ROS in the mitochondria and thus to a possible increase in 8-oxoG damage. This was of particular interest since the addition of ubiquinol leads to an already known increase in the key parameters of mitochondrial respiration, such

as ATP-linked respiration or basal reaction (Schniertshauer *et al.*, 2016). 1.0×10^4 human fibroblasts per well were seeded on a 96-well plate and incubated for 16 h at 37 °C with and without ubiquinol (100 μ M). After incubation of the cells, TBHP (400 μ M) was added to the respective wells to generate oxidative stress and then incubated again at 37 °C for 1 h. After this time 5 μ M CellROX[®] Green reagent were added for 1 h at 37 °C or MitoSOX[®] Red for 30 min at 37 °C. Subsequently the fluorescence measurement was carried out at an absorption/emission maximum of 485 nm / 520 nm for the detection of CellROX[®] and 510 nm / 580 nm for the detection of MitoSOX[®] (3.7 ROS- / mtROS-Assay). As seen below, the ubiquinol-treated cells showed a slight increase in the amount of ROS compared to the untreated cells. However, this increase was not significant. The addition of TBHP resulted in a significant increase in ROS compared to untreated cells. On the other hand, ubiquinol significantly reduced the amount of ROS compared to the control with TBHP (Figure 35A). When monitoring the amount of mitochondrial superoxide, there was a large increase in the amount of superoxide on addition of TBHP. However, in the ubiquinol approach a significant mitochondrial superoxide mitigation can be seen compared to the control (Figure 35B).



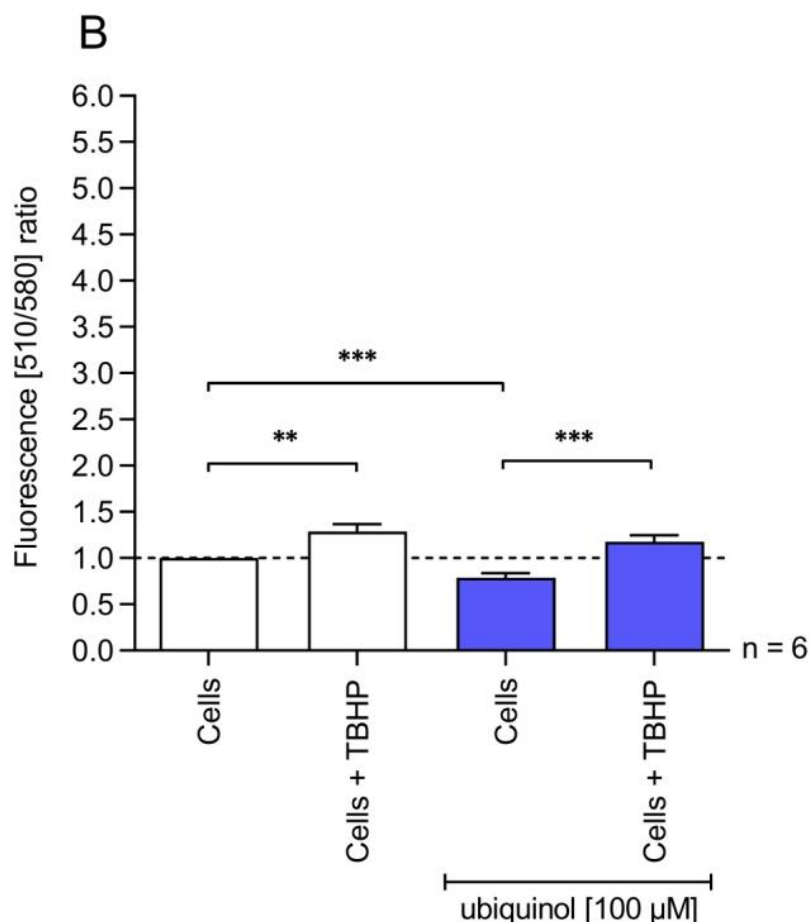


Figure 35. Influence of ubiquinol on the formation of ROS [A] and mitochondrial superoxide [B]. To study the effect of ubiquinol on the amount of ROS and mitochondrial superoxide, 1.0×10^4 human fibroblasts per well were seeded in a 96-well plate and incubated in the presence of ubiquinol (100 μ M) for 16 h at 37 °C. After adding ROS-inducing *tert*-Butyl hydroperoxide (TBHP) to the respective wells, incubation was continued for 1 h at 37 °C. After this time, 5 μ M CellROX[®] Green reagent or MitoSOX[®] Red were added, 1 h at 37 °C for CellROX[®] and 30 min at 37 °C for MitoSOX[®]. After this, the fluorescence measurement was done in a Wallac victor3 1420-fluorometer at an absorption/emission maximum of 480 nm / 520 nm for the detection of CellROX[®] and 510 nm / 580 nm to detect MitoSOX[®] (n = 6, mean $\pm$ SEM, one-way ANOVA with Bonferroni's multiple comparison test, **p < 0.01; ***p < 0.001). Values normalized to the untreated cells. This cells corresponds to 1.0. Data partly received from Julia Schon - Bachelor Thesis.

4.3.4 Influence of redox potential on gene expression and the activity of hOGG1

In this experiment it is no longer about the effect of ubiquinol on hOGG1, but about the identification of the underlying mechanisms. We start with the influence of the redox potential on hOGG1 activity. Based on the previous work done by Tomassetti and his Colleagues (Tomassetti *et al.*, 2001), CoQ₁₀ is supposed to change the redox

potential within the cell and thus the gene expression of hOGG1. Because in the previous studies an increase in activity of hOGG1 by ubiquinol could be detected, the underlying cause of the activity-increasing effect should now be determined. First, we investigated whether the stimulation of hOGG1 activity in the presence of ubiquinol was based on a change in the redox status of the reaction solution. Therefore we used different concentrations (1 – 100 mM) of 1,4-dithiothreitol (DTT), a strong redox status changing substance to see if this has an influence on the activity of hOGG1. As a positive control one approaches was made with all assay components but no ubiquinol or DDT. Two negative controls (NC - 100 μ M ubiquinol / 100 mM DDT) were used. NC1 without reporter oligonucleotides and hOGG1, for the detection of a possible autofluorescence of the test solutions. NC2 with reporter oligonucleotides but without hOGG1 in order to investigate a spontaneous decay of reporter oligonucleotides in the presence of the test solution (3.12.2 *Measurement of base excision repair*). As shown below in Figure 36A, no significant increase in hOGG1 activity was observed compared to the samples with ubiquinol. In order to exclude altered gene expression after ubiquinol treatment a gene expression analysis was performed with 1.0×10^5 fibroblasts. Cells were treated with 100 μ M ubiquinol and incubated for 4, 12, 24 or 48 h in the incubator at 37 °C. For gene expression analysis (Figure 36B) via qPCR, RealTime ready Custom Panels 96 - 16 (Roche Diagnostics) with ready-made primers and probes for the selected gene of interest (hOGG1) and reference genes (RPL13A, RPLP0, PGK1) were used (3.9 *Gene expression analysis*). Relative expression levels were calculated with the $2^{-\Delta\Delta C_p}$ method and then normalized to all reference genes. As shown in Figure 36B, ubiquinol treatment did not have significant impact on gene expression over a period of 48 h.

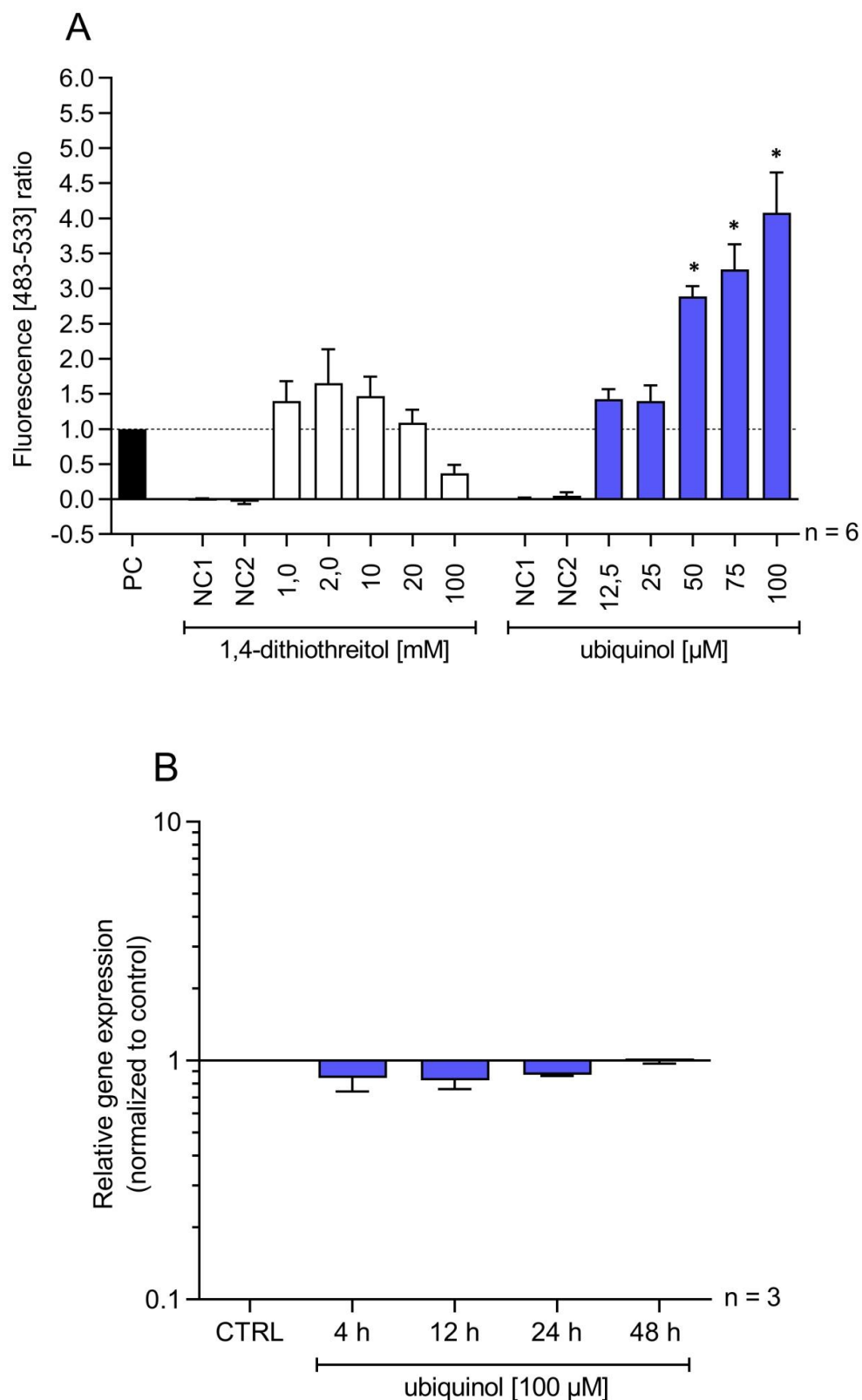


Figure 36. Influence of redox potential on gene expression and the activity of hOGG1. [A] To study the effect of redox potential on hOGG1 activity, 1,4-dithiothreitol at concentrations of 1-100 mM was added in a base excision repair assay to the reporter oligonucleotides CN-8-oxoG and CN-CTRL. NC1 = test solution without reporter oligonucleotides and hOGG1. NC2 = test solution with reporter

oligonucleotides but without hOGG1. The fluorescence signals were recorded with a LightCycler® 480 in a wavelength range between 483 and 533 nm. The fluorescence signals of the digested reporter oligonucleotides based on the hOGG1 activity are shown. For comparative purposes, the results obtained in the previous study regarding the effect of ubiquinol on hOGG1 activity were also presented ($n = 6$, mean $\pm$ SEM, one-way ANOVA with Bonferroni's multiple comparison test, $* p < 0,05$). Values normalized to the positive control (PC = all assay components without test solution). PC correspond to 1.0. Data partly received from Heiko van Beek - Bachelor thesis. **[B]** Gene expression analysis of hOGG1 was performed with 1.0×10^5 fibroblasts via qPCR, RealTime ready Custom Panels 96 - 16 (Roche Diagnostics) with ready-made primers and probes for the selected gene of interest (hOGG1) and reference genes (RPL13A, RPLP0, PGK1). Cells were treated with 100 μ M ubiquinol or without (CTRL) and incubated for 4/12/24/48 h in the incubator at 37 °C. Relative expression levels were calculated with the $2^{-\Delta\Delta C_p}$ method and then normalized to all reference genes ($n = 4$, mean $\pm$ SEM, one-way ANOVA with Dunnett's multiple comparison test, $*p < 0.05$). Data partly received from Tanja Sonntag - Master Thesis.

4.3.5 Importance of calcium and magnesium for influencing the active centre of hOGG1 to increase its activity

Because the active centre of hOGG1 has two Ca^{2+} ions, which help to stabilize the deformed DNA backbone at the site of the extruded lesion, it should be investigated to what extent the activity of hOGG1 changes with increasing concentrations of calcium. The NEB2 buffer used for the preparation of the assay buffer also contained a bivalent cation in the form of MgCl_2 [10 mM], so the influence of magnesium on the enzyme activity was also investigated. CaCl_2 (0.125, 0.50, 1,0 mM) and MgCl_2 (10, 20, 40 mM) were added in different concentrations in a base excision repair assay (3.12.2 *Measurement of base excision repair*) to the reporter oligonucleotides CN-8-oxoG, CN-CTRL (3.12.1 *Reporter oligonucleotides*) and commercially purchased and purified hOGG1. As a control (CTRL) ultrapure water were included with all assay components but without Ca^{2+} or Mg^{2+} . As can be seen below (Figure 37), the addition of magnesium results in a concentration-dependent and significant reduction of hOGG1 activity with increasing magnesium concentrations. By adding calcium, however, the activity of this enzyme increased, also depending on the concentration and in a significant way.

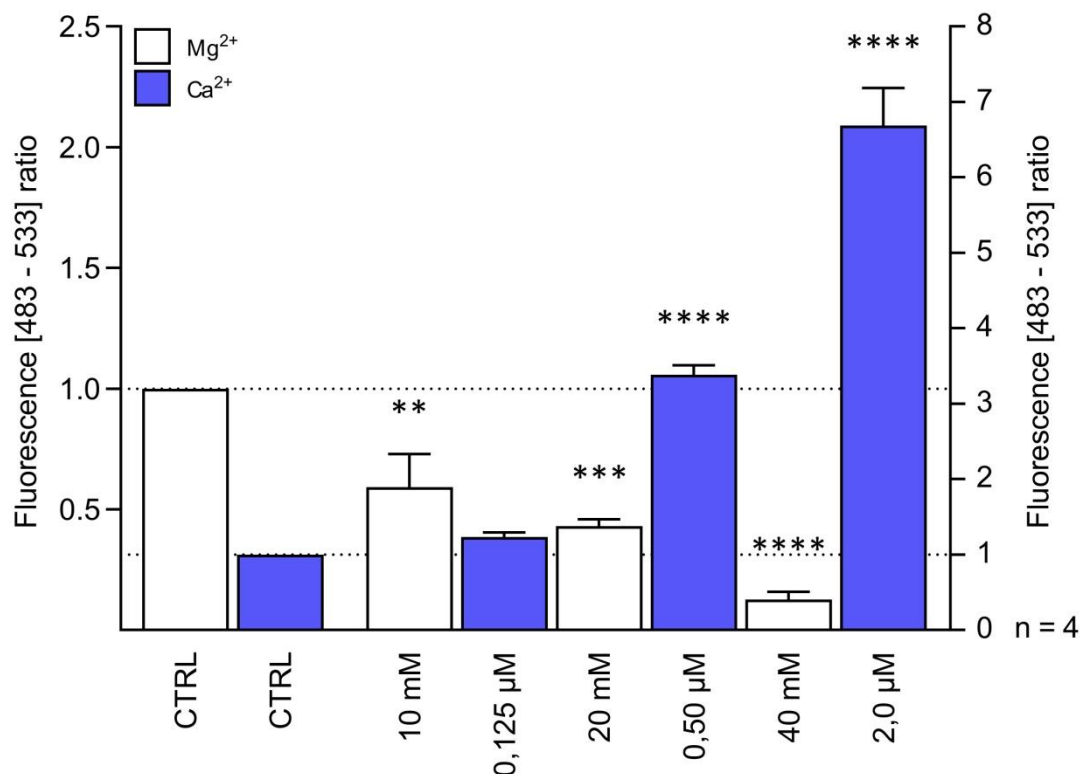
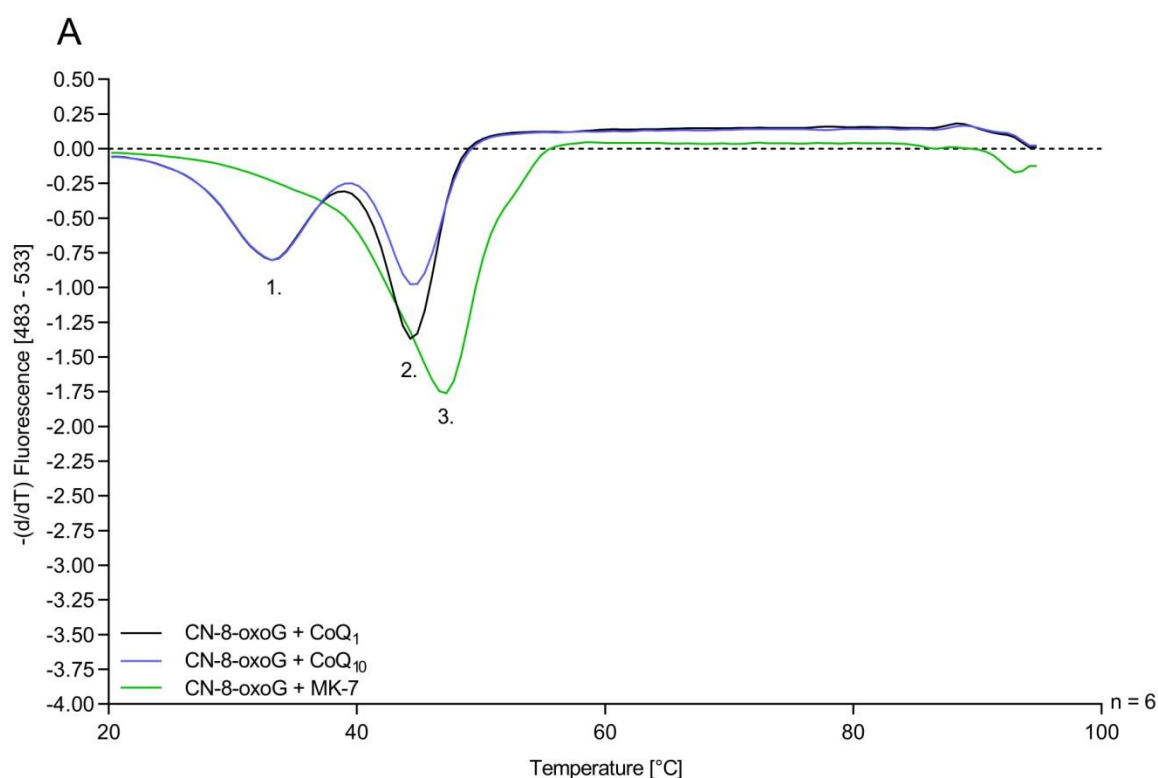


Figure 37. Importance of calcium and magnesium for influencing the active centre of hOGG1 to increase its activity. Addition of different concentrations of Ca²⁺ (CaCl₂) (0.125, 0.50, 1.0 mM) and Mg²⁺ (MgCl₂) (10, 20, 40 mM) to commercially purchased hOGG1 and reporter oligonucleotides. The fluorescence signals were recorded with a LightCycler® 480 in a wavelength range between 483 and 533 nm. The fluorescence signals of the digested reporter oligonucleotides based on the hOGG1 activity are shown (n = 4, mean +/- SEM, one-way ANOVA with Dunnett's multiple comparison test, **p < 0.01; ***p < 0.001; ****p < 0.0001). Values normalized to the control (CTRL). CTRL correspond to 1.0.

4.3.6 Significance of individual structural elements of ubiquinol for hOGG1 activity

In the previous experiment, the change in redox potential by ubiquinol as the cause of an increase in the enzymatic activity of hOGG1 could be excluded. In the next experiment, it was investigated whether the activity-enhancing effect is based on the structure of the coenzyme and a possibly resulting behavior as an effector molecule. For this purpose 100 µM CoQ₁ was used which has in contrast to CoQ₁₀ no lipophilic isoprenoid side chain. Thus, it should be determined whether this structural element is crucial for an increase in activity of hOGG1. In a LightCycler® 480 a melting point analysis from 95 °C to 20 °C was carried out after completion of the real-time activity measurement, to be able to determine the activity of each individual enzymatic step

(N-glycosylase and AP lyase activity) of this bifunctional enzyme (3.12.2 *Measurement of base excision repair*) (Figure 38B). As can be seen in Figure 38A, the two melting curves of CoQ₁ and CoQ₁₀ do not differ concerning the amount of completely cut substrate only CoQ₁ has a higher N-glycosylase activity compared to CoQ₁₀. In both cases, there was no longer any uncut reporter oligonucleotide. After an involvement of the isoprenoid side chain in an activity-enhancing effect of ubiquinol on hOGG1 could be excluded, the importance of the head group was analyzed. For this, we used 100 μ M of menaquinone-7 (MK-7), a structurally related molecule to CoQ₁₀. This molecule differs in the head group (quinone group). The analysis of the influence on the hOGG1 activity was done analogous to the previous experiments with CoQ₁ and CoQ₁₀. It can be seen that MK-7 leads to an inhibition of the enzymatic activity of hOGG1 and therefore no digestion of the reporter oligonucleotides occurs.



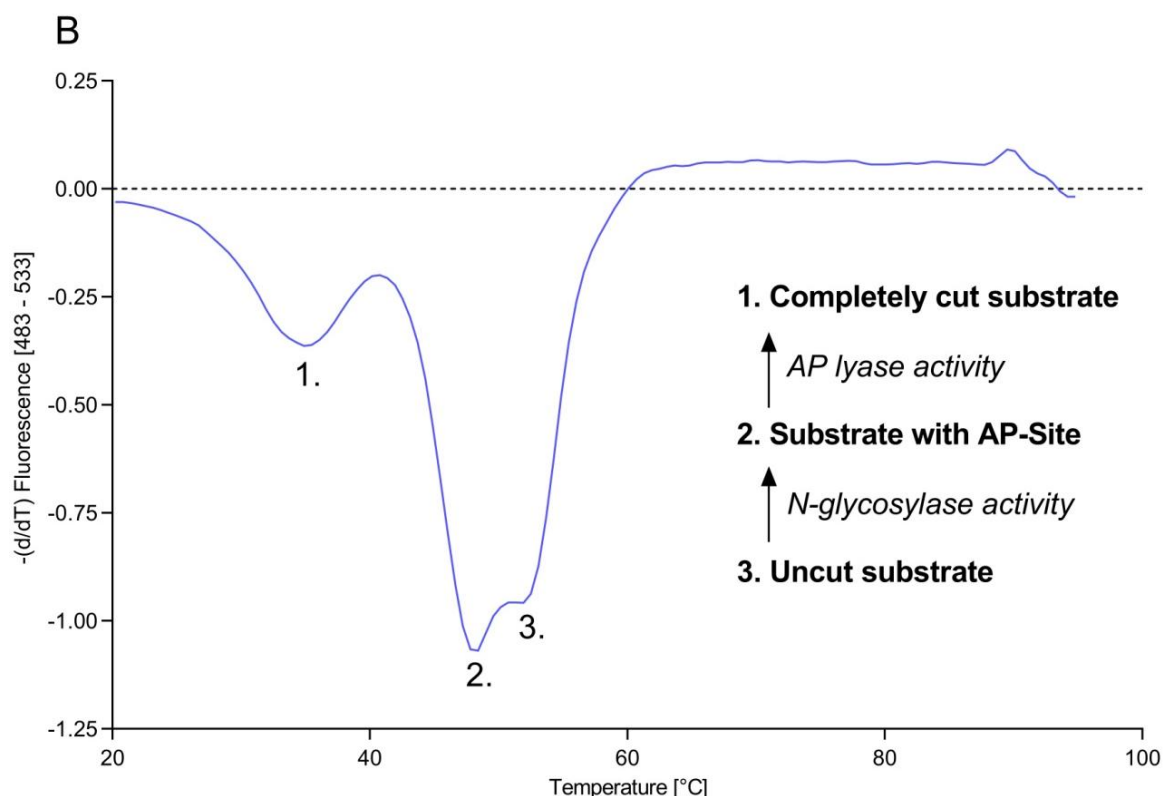
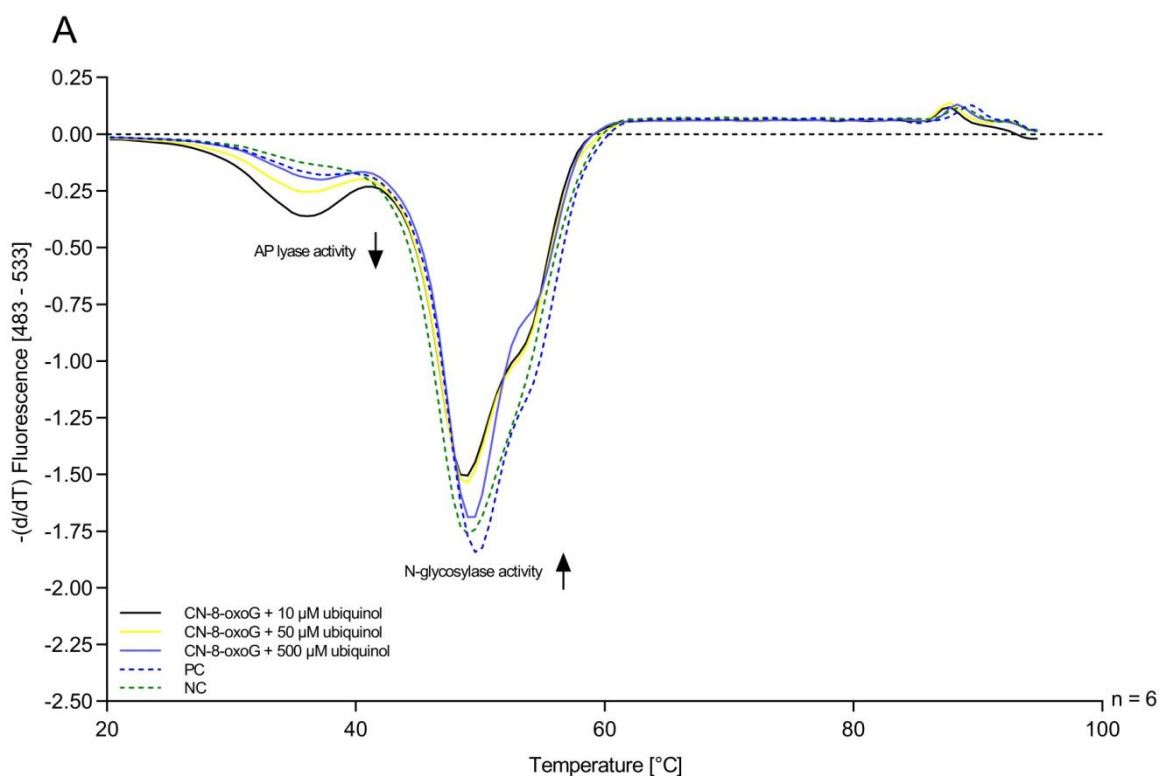


Figure 38. Significance of individual structural elements of ubiquinol for hOGG1 activity. [A] Melting curves of the reporter oligonucleotides CN-8-oxoG under the presence of CoQ₁, CoQ₁₀ and menaquinone-7 (MK-7) (100 μ M each) after digestion with commercially purchased hOGG1. Fragment analysis was done using the function of the recorded melting curves. For fragment analysis, the first derivative of the trend of the fluorescence signal was recorded at a temperature from 95 $^{\circ}$ C to 20 $^{\circ}$ C ($n = 6$). **[B]** Schematic representation of the recorded melting curve for fragment analysis. The peak at position 1 represents a complete cut substrate of hOGG1 activity. In this substrate, both the modified base (8-oxoG) was excised from the DNA and the sugar-phosphate backbone of the DNA was cut. The peak labeled at position 2 represents substrate with an AP site in which the N-glycosylase function of the enzyme was already active but the AP lyase was not. At position 3 there is uncut substrate, in which the glycosylase nor the ligase function became neither active. Depending on where the larger peak is located, statements can be made about the activity of the respective enzymatic steps of hOGG1.

4.3.7 Bifunctionality of mitochondrial hOGG1 under the influence of ubiquinol

After an increase in the activity of hOGG1 in the presence of ubiquinol could be shown, the next step is to investigate the extent to which the kinetics of hOGG1 change under the influence of increasing ubiquinol concentrations. For this, mitochondrial hOGG1 was obtained from the isolated mitochondria of 3.0×10^6 human fibroblasts as already described under 3.4 *Isolation and lysis of mitochondria*, mixed

with ubiquinol (10 - 500 μM) and added into the base excision repair assay (3.12.2 *Measurement of base excision repair*) to the reporter oligonucleotides CN-8-oxoG (3.12.1 *Reporter oligonucleotides*). As a control one approaches was made with all assay components but no ubiquinol (PC) and for the negative control (NC) with 500 μM ubiquinol but without hOGG1. As in the previous experiment, a melting point analysis from 95 $^{\circ}\text{C}$ to 20 $^{\circ}\text{C}$ was carried out after completion of the real-time activity measurement. It can be seen from Figures 37A and 37B that there is an increase in the amount of reporter oligonucleotide with an AP site with increasing concentrations of ubiquinol (increase in N-glycosylase activity), whereas the proportion of completely cut reporter oligonucleotides decreased significantly with increasing amount of ubiquinol (reduction in AP lyase activity). An increase in the total activity of hOGG1 can be seen in Figure 34 (4.3.2 *Influence of ubiquinol on hOGG1 enzymatic activity isolated from mitochondria*).



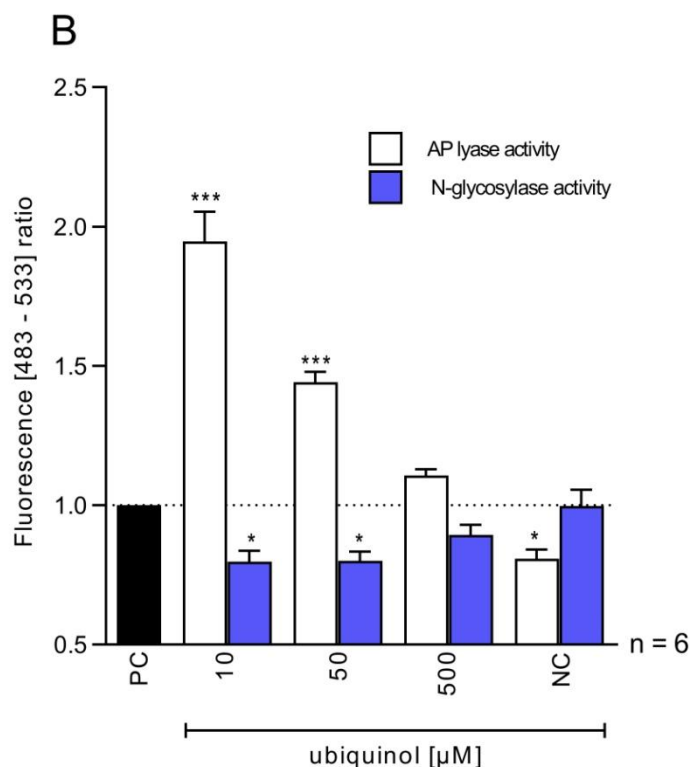


Figure 39. Bifunctionality of hOGG1 under the influence of ubiquinol [A]. Melting curves of the reporter oligonucleotides CN-8-oxoG under the presence of ubiquinol (10 - 500 μ M) after digestion with mitochondrial hOGG1 from 3.0×10^6 human fibroblasts. NC = test solution without reporter oligonucleotides and hOGG1. PC = all assay components without test solution. Fragment analysis was done using the function of the recorded melting curves. For fragment analysis, the first derivative of the trend of the fluorescence signal was recorded at a temperature from 95 $^{\circ}$ C to 20 $^{\circ}$ C ($n = 6$). **[B]** Fragment analysis of the individual enzymatic steps of mitochondrial hOGG1 from human fibroblasts under the influence of increasing concentration of ubiquinol. The fluorescence signals are shown at 36 $^{\circ}$ C (AP lyase activity and at 49 $^{\circ}$ C (N-glycosylase activity) ($n = 6$, mean $\pm$ SEM, one-way ANOVA with Dunnett's multiple comparison test, * $p < 0.05$; *** $p < 0.001$). Values normalized to the positive control (PC = all assay components without test solution). PC correspond to 1.0.

4.3.8 Detection of a direct hOGG1 / ubiquinol interaction by co-immunoprecipitation

Co-immunoprecipitation (3.13 *Co-immunoprecipitation*) was performed to demonstrate a direct interaction between ubiquinol and hOGG1. A histidine tag-coupled hOGG1 and ubiquinol (100 μ M) were incubated together with CN-8-oxoG and His Mag magnetic beads for 30 min at 10 $^{\circ}$ C. After three washing steps hOGG1-His, which had been bound to the beads, was eluted from this again. Upon binding of ubiquinol to hOGG1-His, this is eluted together with the beads and subsequently generates an increase in activity of the repair enzyme in the base excision repair

assay at 37 °C. In addition to a positive control, to check the components of the base excision repair assay and a carrier control to exclude the influence of the phospholipid nanoparticles on the binding of ubiquinol to hOGG1 also an approach without hOGG1-His were used to check the possible binding of ubiquinol to the beads. This was done to exclude the carryover of ubiquinol in the subsequent base excision repair assay. In this approach, hOGG1-His was directly added to the base excision repair assay (3.12.2 *Measurement of base excision repair*). As shown in Figure 40, the addition of ubiquinol to the approach of the CoIP with hOGG1-His results in an increased hOGG1 activity in the subsequent base excision repair assay compared to the CoIP approach without ubiquinol. Based on the control without hOGG1-His in the approach of the CoIP, binding of ubiquinol to the beads can be excluded. This indicates a direct binding of ubiquinol to hOGG1-His.

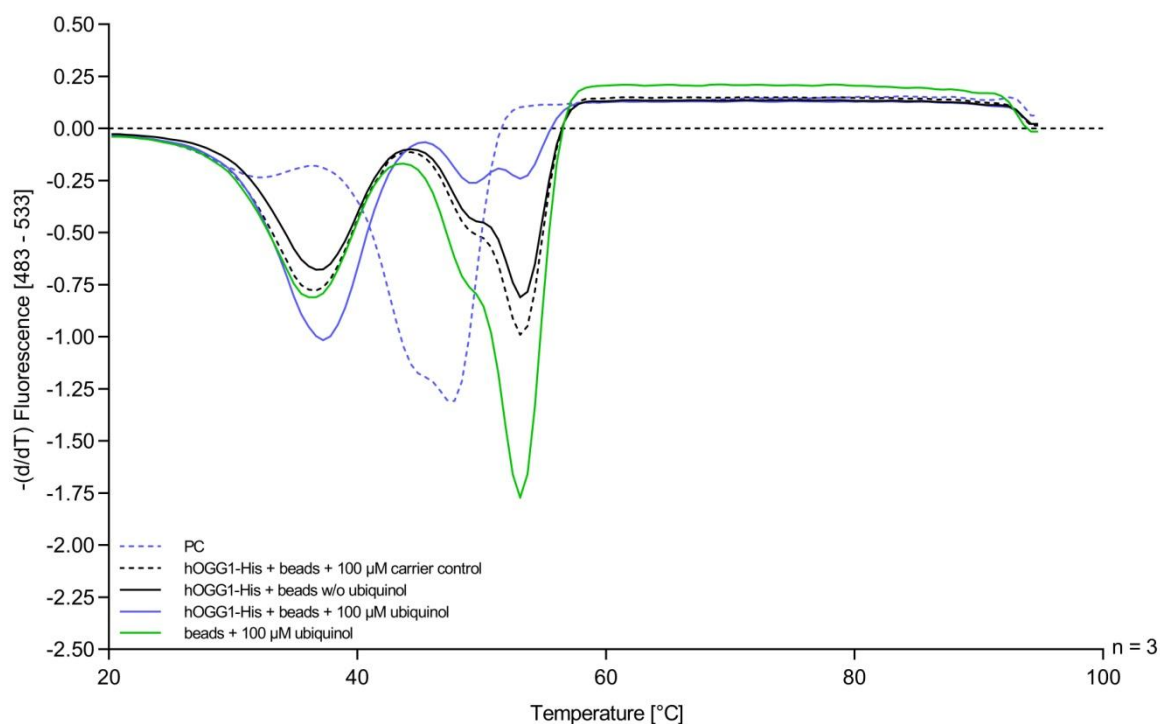


Figure 40. Detection of a direct hOGG1 / ubiquinol interaction by co-immunoprecipitation.

Melting curves of the reporter oligonucleotides CN-8-oxoG under the presence with or without ubiquinol (100 µM) or carrier control (100 µM) after co-immunoprecipitation with hOGG1-His and magnetic beads. PC = all assay components without test solution and without beads). Fragment analysis was done using the function of the recorded melting curves. For fragment analysis, the first derivative of the trend of the fluorescence signal was recorded at a temperature from 95 °C to 20 °C (n = 3).

5. Discussion

Every day, human skin is exposed to a number of harmful substances, of which the UV component of solar radiation is the most significant. Among the most common UV-induced damages are direct DNA lesions and up to 1.0×10^5 oxidative damages to DNA, proteins and lipids caused by ROS. One of the most common oxidative defects is 7,8-Dihydro-8-Oxoguanine. Since the mitochondria are the main site of ROS generation in the cell, they are particularly affected by this so-called oxidative stress caused by UV radiation. Due to their role in oxidative phosphorylation and thus the production of ATP, the resulting mitochondrial dysfunctions may have negative effects on many essential cellular processes, including the aging process. This process is characterised by an increase in age-related disorders and severe diseases. To counteract the accumulation of oxidative damage and the formation of ROS, CoQ₁₀ is used as a powerful therapeutic agent in a number of diseases.

PART I

Influence of ubiquinol on mitochondrial function and cellular ATP levels in human fibroblasts after UV irradiation

To understand the effects of ubiquinol on the cells of human fibroblasts in this context, the influence of different ubiquinol concentrations on the mitochondrial respiratory profile was first investigated. Subsequently, the mitochondrial respiratory profile, mitochondrial membrane potential and cellular ATP levels in human skin fibroblasts after SSL irradiation were analysed again. In addition, the effect of different CoQ₁₀ formulations on mitochondrial respiratory parameters was investigated. In addition to an increase in all mitochondrial respiratory parameters, an accelerated regeneration of the cellular ATP level, a decrease in mitochondrial dysfunction and a maintenance of the mitochondrial membrane potential after SSL irradiation in human skin fibroblasts after treatment with ubiquinol were observed.

In the first instance, the testing of different ubiquinol concentrations in human fibroblasts led to a concentration-dependent increase in the key parameters of mitochondrial respiration. In particular, basal response, ATP-linked respiration and proton leak were significantly increased at a concentration of 100 μ M ubiquinol. The maximum response and mitochondrial respiration were increased in both cases (10

and 100 μM) and did not differ between the two concentrations. Only the spare respiratory capacity was reduced. Since the carrier control did not show a significant increase in all mitochondrial parameters due to the substances contained in the ubiquinol formulation, such as glycerol, soy lecithin or nicotinamide, all results were related to the administration of ubiquinol. In previous internal experiments, no toxic effects could be detected at ubiquinol concentrations of up to 1000 μM , as used in the investigation of ubiquinol on the hOGG1 activity in isolated mitochondria. In addition, the concentrations used here and their safety when used in humans are well documented in the literature (Hoppe *et al.*, 1999; Ikematsu *et al.*, 2006; Raizner, 2019). An increase in respiratory parameters can be attributed to an increased ETC. Estornell *et al.*, 1992 reported a correlation between CoQ₁₀ concentration and respiratory rate that led to the assumption that physiological CoQ₁₀ concentrations do not saturate the respiratory chain (Estornell *et al.*, 1992; Littarru and Tiano, 2007). Thus, the administration of CoQ₁₀ was expected to induce an increase in mitochondrial respiration. These assumptions were confirmed. It is assumed that the increase in respiratory parameters is caused, among other things, by an incorporation of ubiquinol in the mitochondrial membrane. Such an intercalation of ubiquinol in mitochondria in general was proven by the experiments of Tatsuta *et al.* in mice, since ubiquinol as well as ubiquinone accumulated in their membranes especially in mouse brain regions with a high number of mitochondria. The concentration of ubiquinol in the mitochondria increased with the concentration of exogenous ubiquinol (Tatsuta *et al.*, 2017). However, the increase in respiratory parameters was not dose-dependent in these studies, which could be due to the saturation state of ubiquinol in the mitochondrial membrane (Takahashi and Takahashi, 2013). In the membrane, ubiquinol could function as an additional charge carrier between the complexes or have a membrane-stabilising effect. The former would mean that respiration at complex I and III would be increased, as protons are transported between these membrane proteins by CoQ₁₀. This assumption can be confirmed by previous work, which demonstrated increased respiration due to exogenous ubiquinone at complex I and III in mitochondria in slaughter pigs (Fišar *et al.*, 2016). Another effect of ubiquinol could be the stimulation of just these complexes. This has already been suspected in another experiment with aged mice, since the exogenous addition of ubiquinol to the mitochondria of aged mice increased their mitochondrial respiration, although it was already assumed that the activity of

complex III (ubiquinone-cytochrome c oxidoreductase) in these mice decreased with age (Takahashi and Takahashi, 2013). This assumption is derived from the study, in which the total CoQ₁₀ (ubiquinol and ubiquinone) content was the same in young and aged mice despite a decrease in respiratory activity (Takahashi and Takahashi, 2013). Only the amount of ubiquinol was increased in the aged mice, which is due to complex III, since this complex forms ubiquinol from ubiquinone (Takahashi and Takahashi, 2013). Ubiquinol may also increase the amount of complexes in the membrane, thereby stimulating increased respiratory activity. It has been shown that administration of CoQ₁₀ increases the NADH-ubiquinone oxidoreductase (complex I) concentration while succinate dehydrogenase (complex II) concentration does not change (Gvozdjáková, 2008). In addition, it has been demonstrated in bovine cardiac mitochondria that the concentration of CoQ₁₀ is approximately equal to the amount of NADH dehydrogenase (Gvozdjáková, 2008). With this background and our assumption that exogenously added CoQ₁₀ acts as an "electron clamp" and thus increases the electron transport between complexes I/II and III in the mitochondrial respiratory chain, an increase in the proton leak may initially appear to be incorrect, but the administration of CoQ₁₀ leads to an activation of UCPs which transport protons across the inner mitochondrial membrane independently of the complexes in the respiratory chain (Nedergaard *et al.*, 2005; Turunen *et al.*, 2004). Thus, they cause a change in membrane potential, cause an increase in proton leak and consequently lead to an activation of the respiratory chain, since the proton gradient for ATP synthase decreases (Echtay *et al.*, 2001; Nedergaard *et al.*, 2005). Ubiquinol is an obligatory cofactor for UCP function, since protons are difficult to transport in the absence of CoQ₁₀ (Turunen *et al.*, 2004). CoQ₁₀ stimulates H⁺ transport by interacting with the UCPs in the hydrophobic bilayer. Although only ubiquinol has been investigated for UCP so far, this could be a reason for the increased proton leak. A decrease in spare respiratory capacity can be explained by the fact that the cell is generally unable to provide higher performance (maximum response) with increasing ubiquinol concentrations. This can be confirmed by the fact that ubiquinol does not have a performance-enhancing effect on athletes for example (Orlando *et al.*, 2018). If basal respiration increases when using ubiquinol and approaches to the maximum response, the spare respiratory capacity decreases. The spare respiratory capacity indicates the extent to which the cell can respond to an increased energy

demand by increasing respiratory capacity in extreme situations such as UV exposure.

After these initial experiments, which were carried out only with the QuinoMit[®] fluid formulation, further coenzyme Q formulations and their influence on the key parameters of mitochondrial respiration were then tested in comparison. It was found that the key parameters of mitochondrial respiration did not differ significantly between QuinoMit[®], Q₁ and Q₁₀. However, in the presence of MitoQ[®] these parameters decrease drastically, in most cases significantly, compared to QuinoMit[®] fluid. When investigating the relationship between OCR and ECAR, which is an indication of the extent to which cells switch their metabolism from energetically more favourable oxidative phosphorylation to glycolysis, it can be seen that the ratio of OCR to ECAR shifts in favour of glycolysis under MitoQ[®]. Taken together, these results indicate that the cells are in poor condition after treatment with MitoQ[®]. It has been demonstrated that the use of MitoQ[®] leads to swelling of mitochondria in renal cells and loss of membrane potential (Gottwald *et al.*, 2018). This side effect can be attributed to the alkyl chain and is not related to the antioxidative function of the molecule. It is assumed that the swelling is not caused by the opening of pores in the mitochondrial membrane but by direct influences of the alkyl chain. A reduction in mitochondrial respiratory parameters could be explained by the fact that MitoQ[®] blocks respiration and inhibits energy metabolism by inducing a pseudo-membrane potential (Sun *et al.*, 2017). In this process, MitoQ[®] anchors itself in the inner mitochondrial membrane, but the cationic part of the molecule remains in the intermembrane area. Here the membrane potential is maintained for the time being, since MitoQ[®] inhibits the complexes I, III and IV of the respiratory chain (main producers of protons). The positive charge from MitoQ[®] replaces protons of the electrochemical gradient, which, however, cannot be pumped back and thus reduce the ATP synthesis. Although the results shown here indicate that mitochondrial respiration is strongly impaired after treatment with MitoQ[®], this has not been shown in previous studies. This discrepancy can be explained by the different incubation periods. While Gottwald *et al.*, 2018 examined the cells shortly after treatment with MitoQ[®] and was able to detect a swelling of the mitochondria within minutes, the cells in this study were incubated with MitoQ[®] for 16 h for better comparability with the other CoQ₁₀ formulations. An inhibitory effect is therefore unlikely to occur immediately. The fact that there is no significant difference in the results between Q₁

and Q_{10} suggests that the activity-increasing effect of ubiquinol can be attributed to its quinone group. The importance of the head group for the function of ubiquinol has already been underlined in earlier studies (Ernster and Dallner, 1995; Turunen *et al.*, 2004). Based on the results and experience, only the QuinoMit[®] fluid formulation was used in the subsequent studies of the mitochondrial respiratory parameters.

What effect does ubiquinol have on mitochondrial respiration in human fibroblasts after irradiation with SSL-UVA? Irradiation of cells with SSL₁₂ led to a decrease in all key respiratory parameters. The spare respiratory capacity was not changed by irradiation. The proton leak decreased slightly under SSL₁₂ and increased again under 100 μ M ubiquinol, but not significantly in both case. Treatment of the cells with 100 μ M ubiquinol prior to irradiation with SSL₁₂ showed no effect on the other parameters compared to the samples without ubiquinol. It is concluded that the damage induced by the higher dose of irradiation may have been too strong and the CoQ₁₀ incubation time too short to observe a CoQ₁₀-induced effect on fibroblast respiration. In contrast, if the cells were irradiated with SSL₆ only, this led to a significantly higher basal response and increased mitochondrial respiration in untreated and unirradiated cells. The maximum response and ATP-linked respiration were increased, but not significantly. If the cells were only irradiated, an increase in spare respiratory capacity was observed, while all other mitochondrial parameters showed a decrease. This decrease could be counteracted by treating the cells with 100 μ M ubiquinol after irradiation. The time of CoQ₁₀ addition before or after irradiation seems to play an important role. Looking at the results of the respiration measurement after irradiation with SSL₁₂, it seems that CoQ₁₀ does not provide protection for the cells in case of pretreatment. In contrast, an increase in ATP levels in irradiated and CoQ₁₀-treated cells shows that a certain incubation of the cells after irradiation (post-treatment) leads to a significant improvement in mitochondrial functions. It can be assumed that the effect of CoQ₁₀ could be time-dependent and that posttreatment with CoQ₁₀ is more important than pretreatment.

Based on these results, the question then was, how irradiation with SSL₁₂ affects ATP production and thus the cellular ATP content. Since a time dependence of CoQ₁₀ was previously suspected, the cells irradiated with SSL₁₂ were subsequently treated again with 100 μ M ubiquinol and incubated for different periods of time. It was observed that treatment with CoQ₁₀ leads to an accelerated regeneration of the

cellular ATP level after irradiation. Thus, the ATP level in the CoQ₁₀-treated cells regenerated and was close to the control values after 24 h.

The observed drastic drop in the ATP level after irradiation could be explained by two points. First, an increased mitochondrial dysfunction due to UVA-induced ROS. This assumption is confirmed by the measurement of mitochondrial respiration, since a significant reduction in respiration was previously observed in untreated cells after irradiation with SSL₁₂. According to the mitochondrial theory of ageing, mitochondrial dysfunction promotes the formation of ROS. This then leads to increased damage to mitochondrial components, which further promotes mitochondrial dysfunction (Van Remmen and Jones, 2009). This vicious cycle could explain the reduction in ATP levels in the irradiated cells. A second explanation could be that irradiation induces an increased ATP requirement, which could explain the first effect of reducing ATP levels. UV radiation causes DNA damage such as CPDs, 8-OxoG and also DNA single-strand breaks (SSBs) (Greinert *et al.*, 2012). These SSBs activate the enzyme poly (ADP-ribose) polymerase (PARP). PARP then catalyses the synthesis of highly negatively charged poly(ADP-ribose) molecules (PARs). PARP is a key enzyme in the regulation of many cellular processes and can be activated by DNA damage, for example. The PARP gene family consists of 17 homologues, with PARP 1 being the founding member and the strongest stimulation of PARP1 activity is mediated by its binding to DNA strand breaks (Mangerich and Bürkle, 2012). For the synthesis of PAR, which consists of up to 200 ADP-ribose subunits, PARP requires NAD⁺ molecules (oxidized form of nicotinamide adenine dinucleotide (NAD)) as substrate (Mangerich and Bürkle, 2012). These PARs bind various proteins (histones and factors of DNA repair mechanisms) and thereby initiate processes of DNA repair (Ba and Garg, 2011; Oliver *et al.*, 1999). An increased production of PARs by PARP leads to an increased consumption of NAD⁺ molecules within the cell. ATP molecules are required for the resynthesis of NAD⁺, which leads to a decrease in cellular ATP levels (Buonvicino *et al.*, 2013). In addition to mitochondrial dysfunction, this mechanism could be another reason for the drastic reduction in ATP levels.

Inhibition of the PARP enzyme with ABT-888 in non-irradiated cells led to an increase in ATP levels. This was also observed in non-irradiated cells treated with ubiquinol and ABT-888. Here the cellular ATP level was even higher than in cells not treated with ubiquinol. It was also found that irradiated cells treated with ubiquinol and ABT-888 showed only slightly increased ATP levels compared to irradiated cells. In

general, however, no major effect of PARP inhibition on the total amount of ATP in the cell after irradiation can be observed. This would indicate that the reduced ATP levels are only due to mitochondrial dysfunction and not to increased repair of DNA damage.

This assumption that the reduced ATP levels are due to mitochondrial dysfunction is supported by the fact that the normalised ratio of the mitochondrial membrane potential in SSL-irradiated cells decreases significantly as a result of depolarisation. This again shows the harmful effects of radiation on the mitochondria. In contrast, there were no differences in mitochondrial membrane potential between the unirradiated, untreated control cells and the irradiated cells treated with CoQ₁₀. Papucci *et al.* have already shown in corneal keratocytes of rabbits that CoQ₁₀ drastically reduces apoptotic cell death and prevents a reduction in ATP levels corresponding to apoptotic stimuli by inhibiting mitochondrial depolarisation (Papucci *et al.*, 2003). This stabilisation of the membrane potential is essential for ATP synthesis. From our analyses of respiratory parameters and measurements of mitochondrial membrane potential, we assume that the faster regeneration of ATP-linked respiration has been achieved due to the addition of CoQ₁₀. It can be said that CoQ₁₀ is able to maintain the mitochondrial membrane potential after SSL-UVA irradiation and thus reduce the level of mitochondrial dysfunction. This ultimately leads to a faster regeneration of cellular ATP levels. An explanation for the fact that unirradiated and ubiquinol-treated cells do not show any increase in cellular ATP levels compared to untreated cells can be found in the phosphocreatine-creatine system. This system serves as an energy buffer for the cells by keeping the ATP/ADP ratio in balance. This mechanism enables the cell to react to an excess or deficiency of ATP and thus keep ATP levels at a constant level (Saks *et al.*, 2006, Wallimann *et al.*, 2011).

Part II

Age-Dependent Loss of Mitochondrial Function in Epithelial Tissue Can Be Reversed by ubiquinol

In addition to short-term induced damage caused by UV radiation, the second part of the study examined the effect of ubiquinol on age- and long-term induced damage. However, since the measurement of mitochondrial respiration in isolated primary fibroblasts cannot completely reflect the actual situation of the skin, the mitochondrial

respiration parameters were measured directly in biopsies of human epidermis. A reduction in mitochondrial respiration of about ten percent per decade and a decrease in ATP-linked respiration with increasing donor age were observed, which corresponds to the "mitochondrial theory of ageing". However, when the reduced form of CoQ₁₀, ubiquinol, was added to the cells, there was a regeneration of mitochondrial respiration and almost all important respiratory parameters, including basal response, maximum response and proton leak. ATP-linked respiration was also increased, but not significantly. On closer examination of the data, it was also observed that samples with high UV exposure, such as eyelids, showed significantly lower mitochondrial respiration on average than samples with low UV exposure. The aspect of smoking, on the other hand, showed no significant influence on the mitochondrial respiration parameters in epithelial tissue. When investigating the age dependency of mitochondrial respiration on sex, it was found that mitochondrial respiration decreases more strongly with increasing age in male donors.

A decrease in mitochondrial respiration and ATP production as a function of donor age is consistent with the "mitochondrial theory of ageing" established by Harman in 1972. This theory deals with the possibility of a vicious cycle, caused by an impairment of DNA repair and mitochondrial function, which in turn leads to increased ROS formation. This contributes to a decrease in the mtDNA repair capacity and mitochondrial function with increasing age of the subjects, as observed here (Damian *et al.*, 2004; Harman, 1972; Hazane *et al.*, 2006; Prah *et al.*, 2008; Sauvaigo *et al.*, 2007; Wei *et al.*, 2009). With increasing age, a reduced DNA repair capacity leads to an accumulation of DNA damage in the mitochondria, which, if insufficiently repaired, leads to mutations and thus to age-associated diseases, or at least to a loss of capacity in the ETC (Prinzinger *et al.*, 2005). The age-related decrease in mitochondrial respiration and ATP-linked respiration observed here correlates with Prinzinger's "Maximum Metabolic Slope Theory", which suggests a connection between lifespan and energy production (Prinzinger *et al.*, 2005). According to this theory, ageing is a consequence of the fact that the mitochondria are no longer able to supply sufficient energy.

To compensate for this, cells were treated with CoQ₁₀, as in the experiments mentioned above, which led to a significant increase in most mitochondrial respiratory parameters in the epithelial tissue. An increase can be attributed to an increased ETC. It is assumed that the addition of CoQ₁₀ leads to improved electron

transport and thus to an increase in respiratory parameters. A correlation between CoQ₁₀ concentration and respiratory rate has already been reported in the literature, which led to the assumption that physiological CoQ₁₀ concentrations alone are not able to saturate the respiratory chain (Estornell *et al.*, 1992; Littarru *et al.*, 2007). It was therefore expected that administration of additional CoQ₁₀ would induce an increase in mitochondrial respiration, which was confirmed here. One aspect of the mitochondrial theory of ageing, deals with a possible "leakage" of the electron transport chain, which causes more ROS to be released (Samper *et al.*, 2003). A connection between increased ROS release and the development of mitochondrial dysfunction in age-related diseases, as well as the accumulation of oxidative damage during the ageing process, has already been demonstrated (Harman, 1972; Hudson *et al.*, 1998; Lagouge *et al.*, 2013; Marzetti *et al.*, 2013). The results suggest the possibility that exogenously added CoQ₁₀ acts as an "electron clamp" and thus increases the electron transport between complexes I/II and III in the mitochondrial respiratory chain, which has become "leaky" over the years.

In order to be able to exclude an effect of the sampling location of the biopsies on mitochondrial respiration (high exposure or low exposure to UV radiation), a possible photo-aging effect was investigated on the samples measured. A lower mitochondrial respiration in samples with high UV exposure compared to samples with low UV exposure indicates a photoaging effect.

Besides the changes in gene expression pathways already shown in the literature, which are related to collagen degradation in the skin, ROS play an important role in photoaging. UV-generated ROS have been suggested as a reason for photoaging. This leads increasingly to mutations of the mitochondrial genome and via the disruption of oxidative phosphorylation, to a decrease in ATP levels and to an increased exposure to ROS (Scharffetter-Kochanek *et al.*, 2000). Thus, repeated UV exposure to physiological UVA radiation led to an approximately 40 % increase in common deletions (CD) of a 4977 base pair deletion of mtDNA (Berneburg *et al.*, 2004). A disturbance of oxidative phosphorylation as well as a decrease of ATP levels after UVA irradiation could be confirmed in this study. The results also show that repeated UV exposure leads to long-term dysfunctions of mitochondrial respiration, independent of age. This correlates with the results of Birch-Machin *et al.*, 1998, which show that photoaging does not correlate with chronological age (Birch-Machin *et al.*, 1998).

In addition to the influence of the sampling location of the biopsies, gender obviously plays a role in the age-dependent decrease in mitochondrial respiration. Thus, for the first time, gender-dependent decrease in mitochondrial respiration could be shown in human epithelial tissue. A more rapid decrease in mitochondrial respiration was observed in male donors than in female donors. In experiments with mice, women showed greater resistance of mitochondrial function (oxygen consumption and ATP production) to ROS exposure and higher antioxidant enzyme activities compared to men (Farhat *et al.*, 2017). Another study in the hearts and brains of mice showed increased superoxide dismutase activity in the female brain and improved mitochondrial respiratory function in the mitochondria of the female brain. However, increased rates of H₂O₂ formation in mitochondria from the male brain were observed in male mice (Khalifa *et al.*, 2017). An influence of smoking on respiratory parameters could not be detected, although studies using the comet assay have already shown that smoking can lead to a decrease in DNA repair capacities (Sauvaigo *et al.*, 2007). In order to obtain statistically significant results, the group of test persons would have to be enlarged.

Part III

The activity of the DNA repair enzyme hOGG1 can be directly modulated by ubiquinol

In the third and last part of this work, attention was paid to the formation of ROS, as well as the repair of the resulting damage 8-oxoG by hOGG1 under the influence of ubiquinol. The aim was not, as in the previous parts, to mitigate already existing mitochondrial dysfunctions, but to prevent their formation. For the first time, a concentration-dependent increase in the activity of hOGG1 under the influence of CoQ₁₀ was observed. This could be shown both on purified hOGG1 and on enzyme isolated from the mitochondria of human fibroblasts. A change in bifunctionality in favour of increased N-glycosylase activity and a direct interaction between ubiquinol and 8-oxoguanine DNA glycosylase 1 was also demonstrated. In addition, the addition of CoQ₁₀ resulted in a reduction of ROS and mitochondrial superoxide under induced oxidative stress.

The use of both ubiquinone and ubiquinol resulted in an almost linear increase in hOGG1 enzyme activity. Since there are no cellular components in the used assay system using commercially purchased recombinant human hOGG1, a first

assumption is the allosteric effect of CoQ₁₀ on hOGG1 when the coenzyme is added directly. Experiments already carried out indicate exactly this. For example, it has been shown that a mutant form of hOGG1 (replacement of the serine at position 326 of the amino acid sequence by a cysteine) still showed low enzymatic activity in a dimer formation with another mutant hOGG1 (Hill and Evans, 2006). This would indicate an allosteric influence caused by the second hOGG1 enzyme.

Because 8-oxoG lesions mainly occur in the mtDNA, in addition to commercially purchased and purified hOGG1, an ubiquinol-induced effect on mitochondrial hOGG1 was investigated, which was isolated from the mitochondria of human fibroblasts. Again, an increase of the enzymatic hOGG1 activity could be demonstrated with increasing ubiquinol concentrations. Previous studies have already reported on the stimulation of hOGG1 activity by oxidants and repair enzymes of BER. Among others, hOGG1 could be stimulated by APE1 and XRCC1 (Bravard *et al.*, 2006; Hill *et al.*, 2001; Marsin *et al.*, 2003). By stabilising AP sites and transferring intermediates during the repair process, these enzymes increase the repair capacity (Marsin *et al.*, 2003).

With regard to the hOGG1 enzyme, they prevent unwanted rearrangement reactions of the DNA and a reassociation of hOGG1 and DNA after N-glycosylase activity (Hill *et al.*, 2001; Maroz *et al.*, 2009). In addition, after cutting into the sugar-phosphate backbone of the DNA, hOGG1 forms a complex with the resulting unsaturated hydroxyl aldehydes (3-trans-4-hydroxy-2-pentenal-5-phosphates) at the 3' end, which has a half-life of about 2 h and inhibits the process (Hill *et al.*, 2001). The data shown here suggest that CoQ₁₀ contributes to the dissolution of this complex and thus accelerates the dissociation process. It can also confirm the hypothesis from previous work that CoQ₁₀ mainly stimulates the activity of the hOGG1 glycosylase function, as has already been suspected for oxidants and APE1 (Bravard *et al.*, 2006; Hill *et al.*, 2001; Vidal *et al.*, 2001). This was shown by the fact that in the investigation of bifunctionality, an increase in the amount of reporter oligonucleotide with an AP site (increase in N-glycosylase activity) occurred with increasing ubiquinol concentration, whereas the proportion of completely cleaved reporter oligonucleotides decreased significantly with increasing ubiquinol amount (decrease in AP lyase activity).

In order to exclude the possibility that the addition of ubiquinol leads to an increased formation of ROS and thus to an increased damage by 8-oxoG, the amounts of cellular ROS as well as of mtSO were measured. A significant increase in cellular

ROS could be excluded in ubiquinol-treated cells. A slight increase in ROS values is due to a general increase in mitochondrial respiratory parameters caused by ubiquinol, which was already demonstrated in previous experiments in human fibroblasts and epithelial tissue. The recording of mtSO in ubiquinol-treated cells showed a significant reduction compared to the control. The expression of wild-type hOGG1 also showed a reduction (Baptiste *et al.*, 2018). These experiments showed that the addition of ubiquinol did not lead to an additional significant increase in ROS or mtSO. It can therefore be assumed that an activity-increasing effect is not due to higher ROS and mtSO values. The reduction of oxygen radicals is initially attributed to the antioxidative function of ubiquinol. CoQ₁₀ used in this experiment is present in the reduced form (ubiquinol) and thus represents a respiratory-active form, which in case of ROS formation immediately leads to an antioxidative effect. In addition, it is assumed that stimulation of some enzymes which contribute to neutralizing ROS, including superoxide dismutase (SOD) and glutamate dehydrogenase (GDH). These enzymes, which are particularly present in the mitochondria, require antioxidants as cofactors to neutralize ROS (Edeas, 2011). Superoxide is the precursor of all ROS and thus an important primary trigger of oxidative stress. A decrease in mtSO after ubiquinol addition could be explained by stimulation of SOD. An increase of these enzymes when using CoQ₁₀ was shown in cancer cells of rats during tamoxifen therapy (Perumal *et al.*, 2005). A ubiquinol-dependent increase in the enzyme activity of SOD and GDH has also been demonstrated (Papucci *et al.*, 2003; Perumal *et al.*, 2005). Another possibility of ubiquinol-induced effects on ROS formation after oxidative stress could be a stabilization of the respiratory chain complexes. This is important in this context, since ROS are formed between complex I and III and especially since the Q cycle is located at complex III (Villamena, 2013). The electron transfer from ubiquinol to ubiquinone, which takes place here, is a suitable point of attack in the case of unstable electron transport in the respiratory chain due to the reactive ubiquinone, which is formed as an intermediate product and can form superoxide (Chen *et al.*, 2008). Furthermore, CoQ₁₀ is involved as a cofactor for mitochondrial UCPs in the prevention of excessive mtROS production (Echtay *et al.*, 2000; Echtay *et al.*, 2001). In particular, UCP2 and UCP3 are responsible for maintaining a low ROS concentration, which, due to the reduction of the proton motive force, reduces ROS formation and thus minimises the probability of electron interaction with oxygen (Nedergaard *et al.*, 2005). UCPs are stimulated by activators

that consist mainly of ROS itself (Turunen *et al.*, 2004). This negative feedback mechanism represents a regulatory mechanism and counteracts an overproduction of oxygen radicals (Nedergaard *et al.*, 2005). Besides a reduction of ROS, this could, as already mentioned, be a reason for the increased proton leak in previous experiments on fibroblasts and epithelial tissue. Based on their redox-active properties, it has so far been assumed that antioxidants such as CoQ₁₀ can actively intervene in the regulation of numerous transcription factors that are dependent on the redox status and thus also in the expression of cellular proteins (Müller *et al.*, 1997; Tomasetti *et al.*, 2001). For hOGG1, a redox-induced intracellular increase in hOGG1 gene expression by CoQ₁₀ was assumed on the basis of previous studies, which show that hOGG1 can be influenced by various factors such as a change in the redox status of the cell, and thus accompanied by an indirect increase in hOGG1 activity in the form of higher enzyme levels (Bravard *et al.*, 2006; Pool-Zobel *et al.*, 1998; Tomasetti *et al.*, 2001). Neither a direct increase in hOGG1 enzyme activity when the redox status is changed by DTT, nor an increased gene expression during treatment of the cells with CoQ₁₀ over a period of 48 h could be observed in this study. Experiments with cells treated with cadmium show that a change in the redox status can lead to a change in the activity of hOGG1 (Youn *et al.*, 2005). Presumably, strongly oxidizing conditions lead to a change in the eight cysteine residues of the enzyme and thus to a reversible loss of activity. This could be a regulatory possibility, since these cysteine residues are surrounded by positively charged amino acids and are therefore susceptible to oxidation. Accordingly, CoQ₁₀ would be able to protect hOGG1 from the effects of oxidation or reactivate it after a reaction with oxidants (Bravard *et al.*, 2006). A change in the redox status of hOGG1 or the redox potential of the assay buffer by the addition of CoQ₁₀ and thus a change in the enzymatic hOGG1 activity could not be detected in this work. Therefore, CoQ₁₀ mediated redox processes are most likely not the cause of an increase in enzyme activity. An induction of gene expression of hOGG1 by CoQ₁₀ and thus higher intracellular hOGG1 levels can also be excluded, because the fact that CoQ₁₀ causes an increase in hOGG1-activity direct after the addition without incubation time in a cell-free assay contradicts the assumptions in which an increased number of cellular hOGG1 was suspected due to an increase in gene expression (Tomasetti *et al.*, 2001). These results support the assumption that CoQ₁₀ can directly stimulate hOGG1 activity. In addition, a stimulation of hOGG1 activity by changes in pH when CoQ₁₀ is added can

be excluded, as this has already been investigated in preliminary experiments. An increase in hOGG1 activity through the formation of lipid compartments in aqueous solution when using the CoQ₁₀ formulation can also be largely excluded, as the observed effects also occurred when CoQ₁₀ without lipid formulation was used. In addition, the water-insoluble CoQ₁₀ becomes water-soluble in nanoparticles by the formulation, which prevents the formation of lipid compartments.

Because the active center of hOGG1 has two Ca²⁺ ions which are important for the stabilization of the deformed DNA backbone at the site of the extruded lesion and thus to the activity of the enzyme, it was assumed that another possibility of modulating hOGG1 activity lies in the concentration and type of the ion in the active center of the enzyme. Therefore, the hOGG1 activity was tested under the influence of different calcium and magnesium concentrations, which led to a concentration-dependent and significant reduction of the hOGG1 activity when magnesium was added. However, the addition of calcium also resulted in a concentration-dependent and significant increase in the activity of this enzyme. When magnesium is added, it is assumed that the Ca²⁺ ion in the active centre is probably displaced by Mg²⁺, which leads to a steric impairment of the enzyme activity. It has already been shown that magnesium significantly reduces not only the efficiency of base excision and strand incision but also AP lyase activity in hOGG1 repair (Morland *et al.*, 2005).

After a change in the redox potential by CoQ₁₀ as well as a change in the magnesium and calcium concentration of the assay buffer used can also be excluded as a cause for an increase in the enzymatic activity of hOGG1, it was investigated whether the activity-increasing effect is based on the structure of the coenzyme and thus a possibly resulting behaviour as an effector molecule. When investigating the individual structural elements of CoQ₁₀, it was found that CoQ₁, which in contrast to CoQ₁₀ does not have a lipophilic isoprenoid side chain, does not differ from CoQ₁₀ in the amount of completely cut substrate. Although CoQ₁ has a slightly higher N-glycosylase activity, in both cases no uncut reporter oligonucleotide was present. This indicates that an involvement of the lipophilic isoprenoid side chain in increasing the activity of hOGG1 can be largely excluded. However, the side chain is an important structural element for the anchoring of CoQ₁₀ in the mitochondrial membrane, which increases its fluidity and permeability (Turunen *et al.*, 2004). Since participation of the side chain in an increase in activity of hOGG1 can be largely excluded, the head group MK-7, a molecule structurally related to CoQ₁₀, which

differs only in the head group, was used to investigate the significance of the head group MK-7. This led to a complete inhibition of hOGG1 activity and therefore suggests that the activity-increasing effect of CoQ₁₀ can be attributed to the benzoquinone ring, whose function as a redox-active part of the enzyme further underlines its importance and significance (Ernster and Dallner, 1995; Turunen *et al.*, 2004). These are all indications that a direct interaction between hOGG1 and coenzyme Q₁₀ leads to an increase in activity of the repair enzyme. This could be confirmed by extended coimmunoprecipitation. Interactions between hOGG1 and other proteins have already been shown. For example, β -hOGG1 in complex I of the respiratory chain interacts with the mitochondrial protein NDUFB10 (NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10) (Su *et al.*, 2013).

6. Conclusion

Low levels of endogenous CoQ₁₀ were considered in the clinical pattern of various age-related and chronic diseases. Often mitochondrial dysfunction and/or low antioxidant activity play an important role. This work addresses the properties of CoQ₁₀ in the development of mitochondrial dysfunction by UV radiation and in the process of aging. The influence of ubiquinol on the energy metabolism as well as the repair of oxidative damage by human 8-oxoguanine DNA glycosylase 1 is addressed.

Based on the results generated during this work, it can be said that CoQ₁₀ is able to maintain mitochondrial membrane potential in the context of short-term induced damage after UVA irradiation, reduce the level of mitochondrial dysfunction and thus lead to a faster regeneration of energy metabolism in human fibroblasts. Furthermore, the results suggest that the time of CoQ₁₀ addition before or after irradiation seems to play an important role. Looking at the mitochondrial respiratory parameters after irradiation, it appears that CoQ₁₀ does not provide protection for the cells in the case of pre-treatment because the short-term exposure is too high. In contrast, an increase in ATP levels in irradiated and CoQ₁₀-treated cells shows that a specific incubation of the cells after irradiation leads to a significant improvement in mitochondrial functions. It can be assumed that the effect of CoQ₁₀ could be time-dependent and that, in the case of short-term induced damage, post-treatment with CoQ₁₀ is more important than pretreatment. The study of mitochondrial respiratory parameters in human epithelial tissue confirmed an age-related decrease in mitochondrial respiration and ATP production in accordance with the mitochondrial theory of ageing, which could be mitigated by the administration of CoQ₁₀. We hypothesize that exogenously added CoQ₁₀ acts as an "electron clamp" and thus improves electron transport between complexes I/II and III in the mitochondrial respiratory chain, which has become "leaky" over the years. An increase in proton leak by ROS, which may initially appear to be incorrect, indicates the activation of UCPs by CoQ₁₀. Increased levels of ROS can increase the proton leak by activating UCPs, and an increased proton leak in turn reduces ROS generation. This indicates the existence of a protective feedback loop that helps to mitigate the negative effects of ROS on mitochondria. An increase in the activity of 8-oxoguanine DNA glycosylase 1 may be due to the fact that ubiquinol contributes to the dissolution of

an end product complex during direct interaction with this repair enzyme. After incision into the sugar-phosphate backbone of the DNA, this complex is formed between the repair enzyme and the resulting unsaturated hydroxyl aldehyde at the 3' end, thereby inhibiting further enzymatic steps. In addition to the effects of CoQ₁₀, e.g. an increase in mitochondrial respiratory parameters and simultaneous actor as antioxidant, it could be speculated that direct binding of CoQ₁₀ to the 8-oxoguanine DNA glycosylase 1 could mean an additional mechanism by which the repair capacity of oxidative damage in extreme situations (increased UV exposure) can be increased within a very short time without having to increase the expression of this DNA repair enzyme.

Due to the multiple properties of CoQ₁₀, it is difficult to attribute only one effect to the results presented here. However, the ability of CoQ₁₀ to improve the electron transport chain and its antioxidant potential seem to play a major role. The possibility of influencing the activity of 8-oxoguanine DNA glycosylase 1 could also be an important indication. All these properties make CoQ₁₀ an important therapeutic tool in mitochondrial medicine for both preventive and acute treatment of numerous age-related diseases. As an endogenous substance, CoQ₁₀ also has fewer or no side effects compared to conventional xenobiotics. In times of demographic change, the average age of the population is constantly increasing and with it the number of people with age-related diseases. In this context, CoQ₁₀ is a promising candidate to mitigate the harmful effects of both short- and long-term induced damage on the mitochondrial respiratory profile. Based on this work, further interesting starting points for the use of CoQ₁₀ as a drug in the field of mitochondrial medicine have been identified.

7 References

Aburatani H, Hippo Y, Ishida T, Takashima R, Matsuba C, Kodama T, Takao M, Yasui A, Yamamoto K, Asano M. Cloning and characterization of mammalian 8-hydroxyguanine-specific DNA glycosylase/apurinic, apyrimidinic lyase, a functional mutM homologue. *Cancer Res.* 1997 Jun 1;**57(11)**:2151-6.

Alberts B. *Lehrbuch der Molekularen Zellbiologie*. Wiley-VCH. 2015. ISBN 978-3-527-32384-5.

Almeida KH, Sobol RW. A unified view of base excision repair: lesion-dependent protein complexes regulated by post-translational modification. *DNA Repair (Amst)*. 2007 Jun 1;**6(6)**:695-711. Epub 2007 Mar 6.

Banerjee A, Yang W, Karplus M, Verdine GL. Structure of a repair enzyme interrogating undamaged DNA elucidates recognition of damaged DNA. *Nature*. 2005 Mar 31;**434(7033)**:612-8.

Baptiste BA, Katchur SR, Fivenson EM, Croteau DL, Rumsey WL, Bohr VA. Enhanced mitochondrial DNA repair of the common disease-associated variant, Ser326Cys, of hOGG1 through small molecule intervention. *Free Radic Biol Med*. 2018 Aug 20;**124**:149-162. doi: 10.1016/j.freeradbiomed.2018.05.094. Epub 2018 Jun 5.

Barrandon Y, Green H. Three clonal types of keratinocyte with different capacities for multiplication. *Proc Natl Acad Sci U S A*. 1987 Apr;**84(8)**:2302-6.

Barzilay G, Walker LJ, Robson CN, Hickson ID. Site-directed mutagenesis of the human DNA repair enzyme HAP1: identification of residues important for AP endonuclease and RNase H activity. *Nucleic Acids Res*. 1995 May 11;**23(9)**:1544-50.

Ba X, Boldogh I. 8-Oxoguanine DNA glycosylase 1: Beyond repair of the oxidatively modified base lesions. *Redox Biol*. 2018 Apr;**14**:669-678. doi: 10.1016/j.redox.2017.11.008. Epub 2017 Nov 10.

Ba X, Garg NJ. Signaling mechanism of poly(ADP-ribose) polymerase-1 (PARP-1) in inflammatory diseases. *Am J Pathol*. 2011 Mar;**178(3)**:946-55. doi: 10.1016/j.ajpath.2010.12.004.

Beal MF. Mitochondrial dysfunction and oxidative damage in Alzheimer's and Parkinson's diseases and coenzyme Q10 as a potential treatment. *J Bioenerg Biomembr*. 2004 Aug;**36(4)**:381-6.

Bentinger M, Brismar K, Dallner G. The antioxidant role of coenzyme Q. Mitochondrion. 2007 Jun;**7 Suppl**:S41-50. Epub 2007 Mar 16.

Bentinger M, Tekle M, Dallner G. Coenzyme Q--biosynthesis and functions. Biochem Biophys Res Commun. 2010 May 21;**396(1)**:74-9. doi: 10.1016/j.bbrc.2010.02.147.

Berger U. Diagnose Hautkrebs - Die Krankheit, ihre Ursachen und Behandlungsmethoden. Baltic Sea Press. 2009. ISBN 978-3-942129-02-2.

Berneburg M, Plettenberg H, Medve-König K, Pfahlberg A, Gers-Barlag H, Gefeller O, Krutmann J. Induction of the photoaging-associated mitochondrial common deletion in vivo in normal human skin. J Invest Dermatol. 2004 May;**122(5)**:1277-83.

Bhatia PK, Wang Z, Friedberg EC. DNA repair and transcription. Curr Opin Genet Dev. 1996 Apr;**6(2)**:146-50.

Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012 Jan;**5(1)**:9-19. doi: 10.1097/WOX.0b013e3182439613. Epub 2012 Jan 13.

Birch-Machin MA, Tindall M, Turner R, Haldane F, Rees JL. Mitochondrial DNA deletions in human skin reflect photo- rather than chronologic aging. J Invest Dermatol. 1998 Feb;**110(2)**:149-52.

Blasi MA, Bovina C, Carella G, Genova ML, Jansen AM, Lenaz G, Brancato R. Does coenzyme Q10 play a role in opposing oxidative stress in patients with age-related macular degeneration? Ophthalmologica. 2001 Jan-Feb;**215(1)**:51-4.

Blatt T, Littarru GP. Biochemical rationale and experimental data on the antiaging properties of CoQ(10) at skin level. Biofactors. 2011 Sep-Oct;**37(5)**:381-5. doi: 10.1002/biof.169. Epub 2011 Oct 11.

Boiteux S, Gajewski E, Laval J, Dizdaroglu M. Substrate specificity of the Escherichia coli Fpg protein (formamidopyrimidine-DNA glycosylase): excision of purine lesions in DNA produced by ionizing radiation or photosensitization. Biochemistry. 1992 Jan 14;**31(1)**:106-10.

Boiteux S, Radicella JP. Base excision repair of 8-hydroxyguanine protects DNA from endogenous oxidative stress. Biochimie. 1999 Jan-Feb;**81(1-2)**:59-67.

Boiteux S, Radicella JP. The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. Arch Biochem Biophys. 2000 May 1;**377(1)**:1-8.

- Bottje WG. Oxidative metabolism and efficiency: the delicate balancing act of mitochondria. *Poult Sci*. 2019 Oct 1;**98(10)**:4223-4230. doi: 10.3382/ps/pey405.
- Bravard A, Vacher M, Gouget B, Coutant A, de Boisferon FH, Marsin S, Chevillard S, Radicella JP. Redox regulation of human OGG1 activity in response to cellular oxidative stress. *Mol Cell Biol*. 2006 Oct;**26(20)**:7430-6. Epub 2006 Aug 21.
- Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001 Dec 13;**414(6865)**:813-20.
- Bruner SD, Norman DP, Verdine GL. Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature*. 2000 Feb 24;**403(6772)**:859-66.
- Buonvicino D, Formentini L, Cipriani G, Chiarugi A. Glucose deprivation converts poly(ADP-ribose) polymerase-1 hyperactivation into a transient energy-producing process. *J Biol Chem*. 2013 Dec 20;**288(51)**:36530-7. doi: 10.1074/jbc.M113.506378. Epub 2013 Nov 5.
- Cadet J, Berger M, Douki T, Morin B, Raoul S, Ravanat JL, Spinelli S. Effects of UV and visible radiation on DNA-final base damage. *Biol Chem*. 1997 Nov;**378(11)**:1275-86.
- Cappelli E, Hazra T, Hill JW, Slupphaug G, Bogliolo M, Frosina G. Rates of base excision repair are not solely dependent on levels of initiating enzymes. *Carcinogenesis*. 2001 Mar;**22(3)**:387-93.
- Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. *Environ Mol Mutagen*. 2017 Jun;**58(5)**:235-263. doi: 10.1002/em.22087. Epub 2017 May 9.
- Chen Q, Moghaddas S, Hoppel CL, Lesnefsky EJ. Ischemic defects in the electron transport chain increase the production of reactive oxygen species from isolated rat heart mitochondria. *Am J Physiol Cell Physiol*. 2008 Feb;**294(2)**:C460-6. Epub 2007 Dec 12.
- Chen RS, Huang CC, Chu NS. Coenzyme Q10 treatment in mitochondrial encephalomyopathies. Short-term double-blind, crossover study. *Eur Neurol*. 1997;**37(4)**:212-8.
- Chinnery PF, Elliott HR, Hudson G, Samuels DC, Relton CL. Epigenetics, epidemiology and mitochondrial DNA diseases. *Int J Epidemiol*. 2012 Feb;**41(1)**:177-87. doi: 10.1093/ije/dyr232. Epub 2012 Jan 28.

- Citterio E, Van Den Boom V, Schnitzler G, Kanaar R, Bonte E, Kingston RE, Hoeijmakers JH, Vermeulen W. ATP-dependent chromatin remodeling by the Cockayne syndrome B DNA repair-transcription-coupling factor. *Mol Cell Biol*. 2000 Oct;**20(20)**:7643-53.
- Clayton DA, Doda JN, Friedberg EC. The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. *Proc Natl Acad Sci U S A*. 1974 Jul;**71(7)**:2777-81.
- Coppotelli G, Ross JM. Mitochondria in Ageing and Diseases: The Super Trouper of the Cell. *Int J Mol Sci*. 2016 May 11;**17(5)**. pii: E711. doi: 10.3390/ijms17050711.
- Crane FL. Biochemical functions of coenzyme Q10. *J Am Coll Nutr*. 2001 Dec;**20(6)**:591-8.
- Dalhus B, Forsbring M, Helle IH, Vik ES, Forstrøm RJ, Backe PH, Alseth I, Bjørås M. Separation-of-function mutants unravel the dual-reaction mode of human 8-oxoguanine DNA glycosylase. *Structure*. 2011 Jan 12;**19(1)**:117-27. doi: 10.1016/j.str.2010.09.023.
- Damian MS, Ellenberg D, Gildemeister R, Lauermann J, Simonis G, Sauter W, Georgi C. Coenzyme Q10 combined with mild hypothermia after cardiac arrest: a preliminary study. *Circulation*. 2004 Nov 9;**110(19)**:3011-6. Epub 2004 Nov 1.
- David SS, O'Shea VL, Kundu S. Base-excision repair of oxidative DNA damage. *Nature*. 2007 Jun 21;**447(7147)**:941-50.
- DeBalsi KL, Hoff KE, Copeland WC². Role of the mitochondrial DNA replication machinery in mitochondrial DNA mutagenesis, aging and age-related diseases. *Ageing Res Rev*. 2017 Jan;**33**:89-104. doi: 10.1016/j.arr.2016.04.006. Epub 2016 Apr 30.
- de Gruijl FR, Sterenborg HJ, Forbes PD, Davies RE, Cole C, Kelfkens G, van Weelden H, Slaper H, van der Leun JC. Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. *Cancer Res*. 1993 Jan 1;**53(1)**:53-60.
- Desler C, Marcker ML, Singh KK, Rasmussen LJ. The importance of mitochondrial DNA in aging and cancer. *J Aging Res*. 2011 Mar 30;**2011**:407536. doi: 10.4061/2011/407536.
- Dianov GL, Sleeth KM, Dianova II, Allinson SL. Repair of abasic sites in DNA. *Mutat Res*. 2003 Oct 29;**531(1-2)**:157-63.

- DiMauro S, Schon EA. Mitochondrial DNA mutations in human disease. *Am J Med Genet.* 2001 Spring;**106(1)**:18-26.
- Dizdaroglu M. Oxidative damage to DNA in mammalian chromatin. *Mutat Res.* 1992 Sep;**275(3-6)**:331-42.
- Dombi E, Mortiboys H, Poulton J. Modulating Mitophagy in Mitochondrial Disease. *Curr Med Chem.* 2018;**25(40)**:5597-5612.
- Douki T, Cadet J. Individual determination of the yield of the main UV-induced dimeric pyrimidine photoproducts in DNA suggests a high mutagenicity of CC photolesions. *Biochemistry.* 2001 Feb 27;**40(8)**:2495-501.
- Echtay KS, Winkler E, Klingenberg M. Coenzyme Q is an obligatory cofactor for uncoupling protein function. *Nature.* 2000 Nov 30;**408(6812)**:609-13.
- Echtay KS, Winkler E, Frischmuth K, Klingenberg M. Uncoupling proteins 2 and 3 are highly active H(+) transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquinone). *Proc Natl Acad Sci U S A.* 2001 Feb 13;**98(4)**:1416-21.
- Edeas M. Strategies to target mitochondria and oxidative stress by antioxidants: key points and perspectives. *Pharm Res.* 2011 Nov;**28(11)**:2771-9. doi: 10.1007/s11095-011-0587-2. Epub 2011 Sep 15.
- Epe B. DNA damage profiles induced by oxidizing agents. *Rev Physiol Biochem Pharmacol.* 1996;**127**:223-49.
- Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta.* 1995 May 24;**1271(1)**:195-204.
- Essers J, Vermeulen W, Houtsmuller AB. DNA damage repair: anytime, anywhere? *Curr Opin Cell Biol.* 2006 Jun;**18(3)**:240-6. Epub 2006 May 2.
- Estornell E, Fato R, Castelluccio C, Cavazzoni M, Parenti Castelli G, Lenaz G. Saturation kinetics of coenzyme Q in NADH and succinate oxidation in beef heart mitochondria. *FEBS Lett.* 1992 Oct 19;**311(2)**:107-9.
- Evans M, Baisley J, Barss S, Guthrie N. A randomized, double-blind trial on the bioavailability of two CoQ10 formulations. *J of Functional Foods.* 2009:65-73.
- Farhat F, Amérand A, Simon B, Guegueniat N, Moisan C. Gender-dependent differences of mitochondrial function and oxidative stress in rat skeletal muscle at rest and after exercise training. *Redox Rep.* 2017 Nov;**22(6)**:508-514. doi: 10.1080/13510002.2017.1296637. Epub 2017 Mar 1.

Faucher F, Doublé S, Jia Z. 8-oxoguanine DNA glycosylases: one lesion, three subfamilies. *Int J Mol Sci*. 2012;**13(6)**:6711-29. doi: 10.3390/ijms13066711. Epub 2012 Jun 1.

Fišar Z, Hroudová J, Singh N, Kopřivová A, Macečková D. Effect of Simvastatin, Coenzyme Q10, Resveratrol, Acetylcysteine and Acetylcarnitine on Mitochondrial Respiration. *Folia Biol (Praha)*. 2016;**62(2)**:53-66.

Friedberg EC. Relationships between DNA repair and transcription. *Annu Rev Biochem*. 1996;**65**:15-42.

Fromme JC, Bruner SD, Yang W, Karplus M, Verdine GL. Product-assisted catalysis in base-excision DNA repair. *Nat Struct Biol*. 2003 Mar;**10(3)**:204-11.

Fuchs E, Raghavan S. Getting under the skin of epidermal morphogenesis. *Nat Rev Genet*. 2002 Mar;**3(3)**:199-209.

Furda AM, Marrangoni AM, Lokshin A, Van Houten B. OXIDANTS AND NOT ALKYLATING AGENTS INDUCE RAPID MTDNA LOSS AND MITOCHONDRIAL DYSFUNCTION. *DNA Repair (Amst)*. 2012 Aug 1;**11(8)**:684-92. doi: 10.1016/j.dnarep.2012.06.002. Epub 2012 Jul 4.

Furihata C. An active alternative splicing isoform of human mitochondrial 8-oxoguanine DNA glycosylase (OGG1). *Genes Environ*. 2015 Oct 1;**37**:21. doi: 10.1186/s41021-015-0021-9. eCollection 2015.

Gary R, Kim K, Cornelius HL, Park MS, Matsumoto Y. Proliferating cell nuclear antigen facilitates excision in long-patch base excision repair. *J Biol Chem*. 1999 Feb 12;**274(7)**:4354-63.

Giglia-Mari G, Zotter A, Vermeulen W. DNA damage response. *Cold Spring Harb Perspect Biol*. 2011 Jan 1;**3(1)**:a000745. doi: 10.1101/cshperspect.a000745.

Gorbunova V, Seluanov A, Mao Z, Hine C. Changes in DNA repair during aging. *Nucleic Acids Res*. 2007;**35(22)**:7466-74. Epub 2007 Oct 2.

Gottwald EM, Duss M, Bugarski M, Haenni D, Schuh CD, Landau EM, Hall AM. The targeted anti-oxidant MitoQ causes mitochondrial swelling and depolarization in kidney tissue. *Physiol Rep*. 2018 Apr;**6(7)**:e13667. doi: 10.14814/phy2.13667.

Greinert R, Volkmer B, Henning S, Breitbart EW, Greulich KO, Cardoso MC, Rapp A. UVA-induced DNA double-strand breaks result from the repair of clustered oxidative

DNA damages. *Nucleic Acids Res.* 2012 Nov 1;**40(20)**:10263-73. doi: 10.1093/nar/gks824. Epub 2012 Aug 30.

Grivennikova VG, Vinogradov AD. Mitochondrial production of reactive oxygen species. *Biochemistry (Mosc).* 2013 Dec;**78(13)**:1490-511. doi: 10.1134/S0006297913130087.

Gros L, Sapparbaev MK, Laval J. Enzymology of the repair of free radicals-induced DNA damage. *Oncogene.* 2002 Dec 16;**21(58)**:8905-25.

Gvozdjaková A. *Mitochondrial Medicine: Mitochondrial Metabolism, Diseases, Diagnosis and Therapy.* Springer. 2008. ISBN 978-1402067136.

HaTull DB, Holmlin RE, Barton JK. Oxidative DNA damage through long-range electron transfer. *Nature.* 1996 Aug 22;**382(6593)**:731-5.

Halliwell B. Mechanisms involved in the generation of free radicals. *Pathol Biol (Paris).* 1996 Jan;**44(1)**:6-13.

Halliwell B, Aruoma OI. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett.* 1991 Apr 9;**281(1-2)**:9-19.

Halliwell B, Gutteridge JM. Role of iron in oxygen radical reactions. *Methods Enzymol.* 1984;**105**:47-56.

Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc.* 1972 Apr;**20(4)**:145-7.

Hazane F, Sauvaigo S, Douki T, Favier A, Beani JC. Age-dependent DNA repair and cell cycle distribution of human skin fibroblasts in response to UVA irradiation. *J Photochem Photobiol B.* 2006 Mar 1;**82(3)**:214-23. Epub 2006 Feb 7.

Hazra TK, Izumi T, Boldogh I, Imhoff B, Kow YW, Jaruga P, Dizdaroglu M, Mitra S. Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. *Proc Natl Acad Sci U S A.* 2002 Mar 19;**99(6)**:3523-8.

Hazra TK, Das A, Das S, Choudhury S, Kow YW, Roy R. Oxidative DNA damage repair in mammalian cells: a new perspective. *DNA Repair (Amst).* 2007 Apr 1;**6(4)**:470-80. Epub 2006 Nov 20.

Hill JW, Evans MK. Dimerization and opposite base-dependent catalytic impairment of polymorphic S326C OGG1 glycosylase. *Nucleic Acids Res.* 2006 Mar 20;**34(5)**:1620-32. Print 2006.

Hill JW, Hazra TK, Izumi T, Mitra S. Stimulation of human 8-oxoguanine-DNA glycosylase by AP-endonuclease: potential coordination of the initial steps in base excision repair. *Nucleic Acids Res.* 2001 Jan 15;**29(2)**:430-8.

Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature.* 1988 Feb 25;**331(6158)**:717-9.

Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet.* 1990 Mar;**46(3)**:428-33.

Hoppe U, Bergemann J, Diembeck W, Ennen J, Gohla S, Harris I, Jacob J, Kielholz J, Mei W, Pollet D, Schachtschabel D, Sauermann G, Schreiner V, Stäb F, Steckel F. Coenzyme Q10, a cutaneous antioxidant and energizer. *Biofactors.* 1999;**9(2-4)**:371-8.

Hosseini M, Ezzedine K, Taieb A, Rezvani HR. Oxidative and energy metabolism as potential clues for clinical heterogeneity in nucleotide excision repair disorders. *J Invest Dermatol.* 2015 Feb;**135(2)**:341-351. doi: 10.1038/jid.2014.365. Epub 2014 Oct 9.

Hudson EK, Hogue BA, Souza-Pinto NC, Croteau DL, Anson RM, Bohr VA, Hansford RG. Age-associated change in mitochondrial DNA damage. *Free Radic Res.* 1998 Dec;**29(6)**:573-9.

Hunte C, Zickermann V, Brandt U. Functional modules and structural basis of conformational coupling in mitochondrial complex I. *Science.* 2010 Jul 23;**329(5990)**:448-51. doi: 10.1126/science.1191046. Epub 2010 Jul 1.

Ikematsu H, Nakamura K, Harashima S, Fujii K, Fukutomi N. Safety assessment of coenzyme Q10 (Kaneka Q10) in healthy subjects: a double-blind, randomized, placebo-controlled trial. *Regul Toxicol Pharmacol.* 2006 Apr;**44(3)**:212-8. Epub 2006 Jan 23.

Iyer N, Reagan MS, Wu KJ, Canagarajah B, Friedberg EC. Interactions involving the human RNA polymerase II transcription/nucleotide excision repair complex TFIIH, the nucleotide excision repair protein XPG, and Cockayne syndrome group B (CSB) protein. *Biochemistry.* 1996 Feb 20;**35(7)**:2157-67.

Jackson SP, Bartek J. THE DNA-DAMAGE RESPONSE IN HUMAN BIOLOGY AND DISEASE. *Nature.* 2009 Oct 22;**461(7267)**:1071-8. doi: 10.1038/nature08467.

Jacobs AL, Schär P. DNA glycosylases: in DNA repair and beyond. *Chromosoma*. 2012 Feb;**121**(1):1-20. doi: 10.1007/s00412-011-0347-4. Epub 2011 Nov 3.

Janion C. Some Provocative Thoughts on Damage and Repair of DNA. *J Biomed Biotechnol*. 2001;**1**(2):50-51.

Kamiuchi S, Saijo M, Citterio E, de Jager M, Hoeijmakers JH, Tanaka K. Translocation of Cockayne syndrome group A protein to the nuclear matrix: possible relevance to transcription-coupled DNA repair. *Proc Natl Acad Sci U S A*. 2002 Jan 8;**99**(1):201-6.

Kammeyer A, Luiten RM. Oxidation events and skin aging. *Ageing Res Rev*. 2015 May;**21**:16-29. doi: 10.1016/j.arr.2015.01.001. Epub 2015 Jan 31.

Karlson P, Doenecke D, Koolman J. *Biochemie und Pathobiochemie*. Georg Thieme Verlag. 2005. ISBN 978-3133578158

Khalifa AR, Abdel-Rahman EA, Mahmoud AM, Ali MH, Noureldin M, Saber SH, Mohsen M, Ali SS. Sex-specific differences in mitochondria biogenesis, morphology, respiratory function, and ROS homeostasis in young mouse heart and brain. *Physiol Rep*. 2017 Mar;**5**(6). pii: e13125. doi: 10.14814/phy2.13125.

Kim ST, Sancar A. PHOTOCHEMISTRY, PHOTOPHYSICS, AND MECHANISM OF PYRIMIDINE DIMER REPAIR BY DNA PHOTOLYASE. *Photochem Photobiol*. 1993 May;**57**(5):895-904.

Klungland A, Höss M, Gunz D, Constantinou A, Clarkson SG, Doetsch PW, Bolton PH, Wood RD, Lindahl T. Base excision repair of oxidative DNA damage activated by XPG protein. *Mol Cell*. 1999 Jan;**3**(1):33-42.

Klungland A, Lindahl T. Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). *EMBO J*. 1997 Jun 2;**16**(11):3341-8.

Knippers R. *Molekulare Genetik*. Georg Thieme Verlag. 2006. ISBN 978-3134770094.

Kolodner RD, Marsischky GT. Eukaryotic DNA mismatch repair. *Curr Opin Genet Dev*. 1999 Feb;**9**(1):89-96.

Kolodner RD, Tytell JD, Schmeits JL, Kane MF, Gupta RD, Weger J, Wahlberg S, Fox EA, Peel D, Ziogas A, Garber JE, Syngal S, Anton-Culver H, Li FP. Germ-line msh6 mutations in colorectal cancer families. *Cancer Res*. 1999 Oct 15;**59**(20):5068-74.

- Koopman WJ, Willems PH, Smeitink JA. Monogenic mitochondrial disorders. *N Engl J Med*. 2012 Mar 22;**366**(12):1132-41. doi: 10.1056/NEJMra1012478.
- Krokan HE, Bjørås M. BASE EXCISION REPAIR. *Cold Spring Harb Perspect Biol*. 2013 Apr 1;**5**(4):a012583. doi: 10.1101/cshperspect.a012583.
- Krutmann J, Schroeder P. Role of mitochondria in photoaging of human skin: the defective powerhouse model. *J Investig Dermatol Symp Proc*. 2009 Aug;**14**(1):44-9. doi: 10.1038/jidsymp.2009.1.
- Kurowski MA, Bhagwat AS, Papaj G, Bujnicki JM. Phylogenomic identification of five new human homologs of the DNA repair enzyme AlkB. *BMC Genomics*. 2003 Dec 10;**4**(1):48.
- Lagouge M, Larsson NG. The role of mitochondrial DNA mutations and free radicals in disease and ageing. *J Intern Med*. 2013 Jun;**273**(6):529-43. doi: 10.1111/joim.12055. Epub 2013 Mar 7.
- Lambert AJ, Brand MD. Reactive oxygen species production by mitochondria. *Methods Mol Biol*. 2009;**554**:165-81. doi: 10.1007/978-1-59745-521-3_11.
- Lavker RM, Sun TT. Heterogeneity in epidermal basal keratinocytes: morphological and functional correlations. *Science*. 1982 Mar 5;**215**(4537):1239-41.
- Lee DH, Liu Y, Lee HW, Xia B, Brice AR, Park SH, Balduf H, Dominy BN, Cao W. A structural determinant in the uracil DNA glycosylase superfamily for the removal of uracil from adenine/uracil base pairs. *Nucleic Acids Res*. 2015 Jan;**43**(2):1081-9. doi: 10.1093/nar/gku1332. Epub 2014 Dec 30.
- Lehmann B. The vitamin D3 pathway in human skin and its role for regulation of biological processes. *Photochem Photobiol*. 2005 Nov-Dec;**81**(6):1246-51.
- Lenaz G, Genova ML. Mobility and function of coenzyme Q (ubiquinone) in the mitochondrial respiratory chain. *Biochim Biophys Acta*. 2009 Jun;**1787**(6):563-73. doi: 10.1016/j.bbabi.2009.02.019. Epub 2009 Mar 4.
- Lill R, Diekert K, Kaut A, Lange H, Pelzer W, Prohl C, Kispal G. The essential role of mitochondria in the biogenesis of cellular iron-sulfur proteins. *Biol Chem*. 1999 Oct;**380**(10):1157-66.
- Lindahl T, Nyberg B. Rate of depurination of native deoxyribonucleic acid. *Biochemistry*. 1972 Sep 12;**11**(19):3610-8.

Lindahl T, Wood RD. Quality control by DNA repair. *Science*. 1999 Dec 3;**286**(5446):1897-905.

Lingaraju GM, Sartori AA, Kostrewa D, Prota AE, Jiricny J, Winkler FK. A DNA glycosylase from *Pyrobaculum aerophilum* with an 8-oxoguanine binding mode and a noncanonical helix-hairpin-helix structure. *Structure*. 2005 Jan;**13**(1):87-98.

Liou GY, Storz P. Reactive oxygen species in cancer. *Free Radic Res*. 2010 May;**44**(5):479-96. doi: 10.3109/10715761003667554.

Littarru GP, Tiano L. Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. *Mol Biotechnol*. 2007 Sep;**37**(1):31-7.

Liu P, Demple B. DNA repair in mammalian mitochondria: Much more than we thought? *Environ Mol Mutagen*. 2010 Jun;**51**(5):417-26. doi: 10.1002/em.20576.

Logan DC. The mitochondrial compartment. *J Exp Bot*. 2006;**57**(6):1225-43. Epub 2006 Mar 23.

Lu AL, Li X, Gu Y, Wright PM, Chang DY. Repair of oxidative DNA damage: mechanisms and functions. *Cell Biochem Biophys*. 2001;**35**(2):141-70.

Lu J, Liu Y. Deletion of Ogg1 DNA glycosylase results in telomere base damage and length alteration in yeast. *EMBO J*. 2010 Jan 20;**29**(2):398-409. doi: 10.1038/emboj.2009.355. Epub 2009 Nov 26.

Macmillan C, Lach B, Shoubridge EA. Variable distribution of mutant mitochondrial DNAs (tRNA(Leu[3243])) in tissues of symptomatic relatives with MELAS: the role of mitotic segregation. *Neurology*. 1993 Aug;**43**(8):1586-90.

Madigan MT. *Brock Mikrobiologie*. Pearson. 2013. ISBN 978-3868941449.

Malmivou J, Plonsey R. *Bioelectromagnetism - Principles and Applications of Bioelectric and Biomagnetic Fields*. Oxford University Press. 1995. ISBN 978-0195058239

Mangerich A, Bürkle A. Pleiotropic cellular functions of PARP1 in longevity and aging: genome maintenance meets inflammation. *Oxid Med Cell Longev*. 2012;**2012**:321653. doi: 10.1155/2012/321653. Epub 2012 Sep 19.

Maroz A, Anderson RF, Smith RA, Murphy MP. Reactivity of ubiquinone and ubiquinol with superoxide and the hydroperoxyl radical: implications for in vivo antioxidant activity. *Free Radic Biol Med*. 2009 Jan 1;**46**(1):105-9. doi: 10.1016/j.freeradbiomed.2008.09.033. Epub 2008 Oct 14.

Marsin S, Vidal AE, Sossou M, Ménissier-de Murcia J, Le Page F, Boiteux S, de Murcia G, Radicella JP. Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1. *J Biol Chem*. 2003 Nov 7;**278(45)**:44068-74. Epub 2003 Aug 21.

Marti TM, Kunz C, Fleck O. DNA mismatch repair and mutation avoidance pathways. *J Cell Physiol*. 2002 Apr;**191(1)**:28-41.

Marzetti E, Csiszar A, Dutta D, Balagopal G, Calvani R, Leeuwenburgh C. Role of mitochondrial dysfunction and altered autophagy in cardiovascular aging and disease: from mechanisms to therapeutics. *Am J Physiol Heart Circ Physiol*. 2013 Aug 15;**305(4)**:H459-76. doi: 10.1152/ajpheart.00936.2012. Epub 2013 Jun 7.

Masuda Y, Bennett RA, Demple B. Dynamics of the interaction of human apurinic endonuclease (Ape1) with its substrate and product. *J Biol Chem*. 1998 Nov 13;**273(46)**:30352-9.

Maul MJ, Barends TR, Glas AF, Cryle MJ, Domratcheva T, Schneider S, Schlichting I, Carell T. Crystal structure and mechanism of a DNA (6-4) photolyase. *Angew Chem Int Ed Engl*. 2008;**47(52)**:10076-80. doi: 10.1002/anie.200804268.

Mayr T. Beschleunigte Regenerierung der ATP-Levels in solar-bestrahlten humanen Fibroblasten durch Coenzyme Q₁₀. Bachelor Thesis, 2015. University of Applied Sciences Albstadt-Sigmaringen.

McDaniel D, Farris P, Valacchi G. Atmospheric skin aging-Contributors and inhibitors. *J Cosmet Dermatol*. 2018 Apr;**17(2)**:124-137. doi: 10.1111/jocd.12518.

Meves A. Intensivkurs Dermatologie. Urban & Fischer / Elsevier. 2006. ISBN 978-3-437-41162-5.

Michaels ML, Miller JH. The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). *J Bacteriol*. 1992 Oct;**174(20)**:6321-5.

Mitani H, Shima A. INDUCTION OF CYCLOBUTANE PYRIMIDINE DIMER PHOTOLYASE IN CULTURED FISH CELLS BY FLUORESCENT LIGHT AND OXYGEN STRESS. *Photochem Photobiol*. 1995 Apr;**61(4)**:373-7.

Mitchell P. Protonmotive redox mechanism of the cytochrome b-c1 complex in the respiratory chain: protonmotive ubiquinone cycle. *FEBS Lett*. 1975 Aug 1;**56(1)**:1-6.

Mitchell PD. Foundations of vectorial metabolism and osmochemistry. Biosci Rep. 2004 Aug-Oct;**24(4-5)**:386-434; discussion 434-5.

Moll I. Duale Reihe Dermatologie. GeorgThieme Verlag. 2010. ISBN 978-3-13-126687-3.

Morland I, Luna L, Gustad E, Seeberg E, Bjørås M. Product inhibition and magnesium modulate the dual reaction mode of hOgg1. DNA Repair (Amst). 2005 Mar 2;**4(3)**:381-7.

Mutschler R. Regenerative Mitochondrien-Medizin - von der Forschung in die Praxis. Orthomol. Med. 2012 Mar;**3**:20-22

Müller JM, Cahill MA, Rupec RA, Baeuerle PA, Nordheim A. Antioxidants as well as oxidants activate c-fos via Ras-dependent activation of extracellular-signal-regulated kinase 2 and Elk-1. Eur J Biochem. 1997 Feb 15;**244(1)**:45-52.

Müller M, Carell T. Structural biology of DNA photolyases and cryptochromes. Curr Opin Struct Biol. 2009 Jun;**19(3)**:277-85. doi: 10.1016/j.sbi.2009.05.003. Epub 2009 May 30.

Nakabeppu Y. Cellular levels of 8-oxoguanine in either DNA or the nucleotide pool play pivotal roles in carcinogenesis and survival of cancer cells. Int J Mol Sci. 2014 Jul 15;**15(7)**:12543-57. doi: 10.3390/ijms150712543.

Nakatani M, Maksimovic S, Baba Y, Lumpkin EA. Mechanotransduction in epidermal Merkel cells. Pflugers Arch. 2015 Jan;**467(1)**:101-8. doi: 10.1007/s00424-014-1569-0. Epub 2014 Jul 23.

Nash RA, Caldecott KW, Barnes DE, Lindahl T. XRCC1 protein interacts with one of two distinct forms of DNA ligase III. Biochemistry. 1997 Apr 29;**36(17)**:5207-11.

Nazarkina ZK, Khodyreva SN, Marsin S, Lavrik OI, Radicella JP. XRCC1 interactions with base excision repair DNA intermediates. DNA Repair (Amst). 2007 Feb 4;**6(2)**:254-64. Epub 2006 Nov 21.

Nedergaard J, Ricquier D, Kozak LP. Uncoupling proteins: current status and therapeutic prospects. EMBO Rep. 2005 Oct;**6(10)**:917-21.

Nelson D, Cox M. Lehninger Biochemie. Springer. 2011. ISBN 978-3540686378

Nishioka K, Ohtsubo T, Oda H, Fujiwara T, Kang D, Sugimachi K, Nakabeppu Y. Expression and differential intracellular localization of two major forms of human 8-

oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. Mol Biol Cell. 1999 May;**10(5)**:1637-52.

Nöth V. Einfluss von Coenzyme Q₁₀ auf die *in vivo*-Aktivität der humanen 8-Oxoguanin-DNA-Glykosylase 1 (hOGG1). Bachelor Thesis, 2018. University of Applied Sciences Albstadt-Sigmaringen.

Nordman T, Xia L, Björkhem-Bergman L, Damdimopoulos A, Nalvarte I, Arnér ES, Spyrou G, Eriksson LC, Björnstedt M, Olsson JM. Regeneration of the antioxidant ubiquinol by lipoamide dehydrogenase, thioredoxin reductase and glutathione reductase. Biofactors. 2003;**18(1-4)**:45-50.

Nyaga SG, Jaruga P, Lohani A, Dizdaroglu M, Evans MK. Accumulation of oxidatively induced DNA damage in human breast cancer cell lines following treatment with hydrogen peroxide. Cell Cycle. 2007 Jun 15;**6(12)**:1472-8. Epub 2007 Apr 13.

Obe G, Johannes C, Schulte-Frohlinde D. DNA double-strand breaks induced by sparsely ionizing radiation and endonucleases as critical lesions for cell death, chromosomal aberrations, mutations and oncogenic transformation. Mutagenesis. 1992 Jan;**7(1)**:3-12.

Ogawa A, Watanabe T, Shoji S, Furihata C. Enzyme kinetics of an alternative splicing isoform of mitochondrial 8-oxoguanine DNA glycosylase, ogg1-1b, and compared with the nuclear ogg1-1a. J Biochem Mol Toxicol. 2015 Feb;**29(2)**:49-56. doi: 10.1002/jbt.21605. Epub 2014 Sep 4.

Oliver FJ, Menissier-de Murcia J, de Murcia G. Poly(ADP-ribose) polymerase in the cellular response to DNA damage, apoptosis, and disease. Am J Hum Genet. 1999 May;**64(5)**:1282-8.

Orlando P, Silvestri S, Galeazzi R, Antonicelli R, Marcheggiani F, Cirilli I, Bacchetti T, Tiano L. Effect of ubiquinol supplementation on biochemical and oxidative stress indexes after intense exercise in young athletes. Redox Rep. 2018 Dec;**23(1)**:136-145. doi: 10.1080/13510002.2018.1472924.

Papucci L, Schiavone N, Witort E, Donnini M, Lapucci A, Tempestini A, Formigli L, Zecchi-Orlandini S, Orlandini G, Carella G, Brancato R, Capaccioli S. Coenzyme q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property. J Biol Chem. 2003 Jul 25;**278(30)**:28220-8. Epub 2003 May 7.

- Parlanti E, Fortini P, Macpherson P, Laval J, Dogliotti E. Base excision repair of adenine/8-oxoguanine mispairs by an aphidicolin-sensitive DNA polymerase in human cell extracts. *Oncogene*. 2002 Aug 8;**21(34)**:5204-12.
- Pascucci B, Stucki M, Jónsson ZO, Dogliotti E, Hübscher U. Long patch base excision repair with purified human proteins. DNA ligase I as patch size mediator for DNA polymerases delta and epsilon. *J Biol Chem*. 1999 Nov 19;**274(47)**:33696-702.
- Pegg AE. Repair of O(6)-alkylguanine by alkyltransferases. *Mutat Res*. 2000 Apr;**462(2-3)**:83-100.
- Peltomäki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. *J Clin Oncol*. 2003 Mar 15;**21(6)**:1174-9.
- Perumal SS, Shanthi P, Sachdanandam P. Augmented efficacy of tamoxifen in rat breast tumorigenesis when gavaged along with riboflavin, niacin, and CoQ10: effects on lipid peroxidation and antioxidants in mitochondria. *Chem Biol Interact*. 2005 Feb 28;**152(1)**:49-58.
- Petermann E, Keil C, Oei SL. Roles of DNA ligase III and XRCC1 in regulating the switch between short patch and long patch BER. *DNA Repair (Amst)*. 2006 May 10;**5(5)**:544-55. Epub 2006 Jan 26.
- Pool-Zobel BL, Bub A, Liegibel UM, Treptow-van Lishaut S, Rechkemmer G. Mechanisms by which vegetable consumption reduces genetic damage in humans. *Cancer Epidemiol Biomarkers Prev*. 1998 Oct;**7(10)**:891-9.
- Prahl S, Kueper T, Biernoth T, Wöhrmann Y, Münster A, Fürstenau M, Schmidt M, Schulze C, Wittern KP, Wenck H, Muhr GM, Blatt T. Aging skin is functionally anaerobic: importance of coenzyme Q10 for anti aging skin care. *Biofactors*. 2008;**32(1-4)**:245-55.
- Prakash S, Prakash L. Nucleotide excision repair in yeast. *Mutat Res*. 2000 Jun 30;**451(1-2)**:13-24.
- Prinzinger R. Programmed ageing: the theory of maximal metabolic scope. How does the biological clock tick? *EMBO Rep*. 2005 Jul;**6 Spec No**:S14-9.
- Rademakers S, Volker M, Hoogstraten D, Nigg AL, Moné MJ, Van Zeeland AA, Hoeijmakers JH, Houtsmuller AB, Vermeulen W. Xeroderma pigmentosum group A protein loads as a separate factor onto DNA lesions. *Mol Cell Biol*. 2003 Aug;**23(16)**:5755-67.

Raizner AE. Coenzyme Q₁₀. *Methodist Debaque Cardiovasc J*. 2019 Jul-Sep;**15(3)**:185-191. doi: 10.14797/mdcj-15-3-185.

Rastogi RP, Richa, Kumar A, Tyagi MB, Sinha RP. Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *J Nucleic Acids*. 2010 Dec 16;**2010**:592980. doi: 10.4061/2010/592980.

Ravanat JL, Di Mascio P, Martinez GR, Medeiros MH. Singlet oxygen induces oxidation of cellular DNA. *J Biol Chem*. 2001 Feb 23;**276(8)**:40601-4.

Renkonen E, Zhang Y, Lohi H, Salovaara R, Abdel-Rahman WM, Nilbert M, Aittomaki K, Jarvinen HJ, Mecklin JP, Lindblom A, Peltomaki P. Altered expression of MLH1, MSH2, and MSH6 in predisposition to hereditary nonpolyposis colorectal cancer. *J Clin Oncol*. 2003 Oct 1;**21(19)**:3629-37.

Richter C, Park JW, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci U S A*. 1988 Sep;**85(17)**:6465-7.

Rünger TM, Epe B, Möller K. Processing of directly and indirectly ultraviolet-induced DNA damage in human cells. *Recent Results Cancer Res*. 1995;**139**:31-42.

Saks V, Dzeja P, Schlattner U, Vendelin M, Terzic A, Wallimann T. Cardiac system bioenergetics: metabolic basis of the Frank-Starling law. *J Physiol*. 2006 Mar 1;**571(Pt 2)**:253-73. Epub 2006 Jan 12.

Sampath H, Vartanian V, Rollins MR, Sakumi K, Nakabeppu Y, Lloyd RS. 8-Oxoguanine DNA glycosylase (OGG1) deficiency increases susceptibility to obesity and metabolic dysfunction. *PLoS One*. 2012;**7(12)**:e51697. doi: 10.1371/journal.pone.0051697. Epub 2012 Dec 17.

Samper E, Nicholls DG, Melov S. Mitochondrial oxidative stress causes chromosomal instability of mouse embryonic fibroblasts. *Aging Cell*. 2003 Oct;**2(5)**:277-85.

Sancar A. Structure and function of photolyase and in vivo enzymology: 50th anniversary. *J Biol Chem*. 2008 Nov 21;**283(47)**:32153-7. doi: 10.1074/jbc.R800052200. Epub 2008 Aug 4.

Sauvaigo S, Bonnet-Duquennoy M, Odin F, Hazane-Puch F, Lachmann N, Bonté F, Kurfürst R, Favier A. DNA repair capacities of cutaneous fibroblasts: effect of sun exposure, age and smoking on response to an acute oxidative stress. *Br J Dermatol*. 2007 Jul;**157(1)**:26-32.

Scharffetter-Kochanek K, Brenneisen P, Wenk J, Herrmann G, Ma W, Kuhr L, Meewes C, Wlaschek M. Photoaging of the skin from phenotype to mechanisms. *Exp Gerontol.* 2000 May;**35(3)**:307-16.

Schon J. Einfluss von Ubiquinol auf die *in vivo*-Aktivität der humanen 8-Oxoguanin-DNA-Glykosylase 1 sowie die Bildung von reaktiven Sauerstoffspezies in den Mitochondrien. Bachelor Thesis, 2019. University of Applied Sciences Albstadt-Sigmaringen.

Šebera J, Hattori Y, Sato D, Reha D, Nencka R, Kohno T, Kojima C, Tanaka Y, Sychrovský V. THE MECHANISM OF THE GLYCOSYLASE REACTION WITH HOGG1 BASE-EXCISION REPAIR ENZYME: CONCERTED EFFECT OF LYS249 AND ASP268 DURING EXCISION OF 8-OXOGUANINE. *Nucleic Acids Res.* 2017 May 19;**45(9)**:5231-5242. doi: 10.1093/nar/gkx157.

Sedgwick B. Repairing DNA-methylation damage. *Nat Rev Mol Cell Biol.* 2004 Feb;**5(2)**:148-57.

Shinmura K, Yokota J. The OGG1 gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis. *Antioxid Redox Signal.* 2001 Aug;**3(4)**:597-609.

Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol.* 2015;**4**:180-3. doi: 10.1016/j.redox.2015.01.002. Epub 2015 Jan 3.

Snyder JW, Skovsen E, Lambert JD, Ogilby PR. Subcellular, time-resolved studies of singlet oxygen in single cells. *J Am Chem Soc.* 2005 Oct 26;**127(42)**:14558-9.

Sonntag T. Evaluation of coenzyme Q₁₀ for the prevention of UV-induced damages in skin cells. Master Thesis, 2014. University of Konstanz.

Steenken S, Jovanovic SV, Candeias LP, Reynisson J. Is "frank" DNA-strand breakage via the guanine radical thermodynamically and sterically possible? *Chemistry.* 2001 Jul 2;**7(13)**:2829-33.

Sterry W. Kurzlehrbuch Dermatologie. Georg Thieme Verlag. 2018. ISBN 978-3-13-146272-5.

Stivers JT. Site-specific DNA damage recognition by enzyme-induced base flipping. *Prog Nucleic Acid Res Mol Biol.* 2004;**77**:37-65.

Su YH, Lee YL, Chen SF, Lee YP, Hsieh YH, Tsai JH, Hsu JL, Tian WT, Huang W. Essential role of β -human 8-oxoguanine DNA glycosylase 1 in mitochondrial

oxidative DNA repair. *Environ Mol Mutagen.* 2013 Jan;**54(1)**:54-64. doi: 10.1002/em.21742. Epub 2012 Oct 11.

Sun C, Liu X, Di C, Wang Z, Mi X, Liu Y, Zhao Q, Mao A, Chen W, Gan L, Zhang H. MitoQ regulates autophagy by inducing a pseudo-mitochondrial membrane potential. *Autophagy.* 2017 Apr 3;**13(4)**:730-738. doi: 10.1080/15548627.2017.1280219. Epub 2017 Jan 25.

Svejstrup JQ, Wang Z, Feaver WJ, Wu X, Bushnell DA, Donahue TF, Friedberg EC, Kornberg RD. Different forms of TFIIH for transcription and DNA repair: holo-TFIIH and a nucleotide excision repairosome. *Cell.* 1995 Jan 13;**80(1)**:21-8.

Svilar D, Goellner EM, Almeida KH, Sobol RW. Base excision repair and lesion-dependent subpathways for repair of oxidative DNA damage. *Antioxid Redox Signal.* 2011 Jun 15;**14(12)**:2491-507. doi: 10.1089/ars.2010.3466. Epub 2010 Oct 28.

Takahashi K, Takahashi M. Exogenous administration of coenzyme Q10 restores mitochondrial oxygen consumption in the aged mouse brain. *Mech Ageing Dev.* 2013 Nov-Dec;**134(11-12)**:580-6. doi: 10.1016/j.mad.2013.11.010. Epub 2013 Dec 11.

Tantin D, Kansal A, Carey M. Recruitment of the putative transcription-repair coupling factor CSB/ERCC6 to RNA polymerase II elongation complexes. *Mol Cell Biol.* 1997 Dec;**17(12)**:6803-14

Tantin D. RNA polymerase II elongation complexes containing the Cockayne syndrome group B protein interact with a molecular complex containing the transcription factor IIH components xeroderma pigmentosum B and p62. *J Biol Chem.* 1998 Oct 23;**273(43)**:27794-9.

Tatsuta Y, Kasai K, Maruyama C, Hamano Y, Matsuo K, Katano H, Taira S. Imaging mass spectrometry analysis of ubiquinol localization in the mouse brain following short-term administration. *Sci Rep.* 2017 Oct 11;**7(1)**:12990. doi: 10.1038/s41598-017-13257-8.

Tomasetti M, Alleva R, Borghi B, Collins AR. In vivo supplementation with coenzyme Q10 enhances the recovery of human lymphocytes from oxidative DNA damage. *FASEB J.* 2001 Jun;**15(8)**:1425-7.

Tornaletti S, Reines D, Hanawalt PC. Structural characterization of RNA polymerase II complexes arrested by a cyclobutane pyrimidine dimer in the transcribed strand of template DNA. *J Biol Chem.* 1999 Aug 20;**274(34)**:24124-30.

Turunen M, Olsson J, Dallner G. Metabolism and function of coenzyme Q. *Biochim Biophys Acta.* 2004 Jan 28;**1660(1-2)**:171-99.

- Ura K, Hayes JJ. Nucleotide excision repair and chromatin remodeling. *Eur J Biochem.* 2002 May;**269(9)**:2288-93.
- van Beek H. Einfluss verschiedener Coenzym Q₁₀-Präparate auf die Aktivität der humanen 8-Oxoguanin-DNA-Glykosylase 1 (hOGG1). Bachelor Thesis, 2017. University of Applied Sciences Albstadt-Sigmaringen.
- van de Poll WH, Hanelt D, Hoyer K, Buma AG, Breeman AM. Ultraviolet-B-induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. *Photochem Photobiol.* 2002 Nov;**76(5)**:493-500.
- van der Kemp PA, Charbonnier JB, Audebert M, Boiteux S. Catalytic and DNA-binding properties of the human Ogg1 DNA N-glycosylase/AP lyase: biochemical exploration of H270, Q315 and F319, three amino acids of the 8-oxoguanine-binding pocket. *Nucleic Acids Res.* 2004 Jan 29;**32(2)**:570-8. Print 2004.
- Van Remmen H, Jones DP. Current thoughts on the role of mitochondria and free radicals in the biology of aging. *J Gerontol A Biol Sci Med Sci.* 2009 Feb;**64(2)**:171-4. doi: 10.1093/gerona/gln058. Epub 2009 Jan 30.
- Vidal AE, Boiteux S, Hickson ID, Radicella JP. XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. *EMBO J.* 2001 Nov 15;**20(22)**:6530-9.
- Vidal AE, Hickson ID, Boiteux S, Radicella JP. Mechanism of stimulation of the DNA glycosylase activity of hOGG1 by the major human AP endonuclease: bypass of the AP lyase activity step. *Nucleic Acids Res.* 2001 Mar 15;**29(6)**:1285-92.
- Villamena FA. Molecular Basis of Oxidative Stress: Chemistry, Mechanisms, and Disease Pathogenesis. John Wiley & Sons. 2013. ISBN 978-1-118-35588-6
- Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet.* 2005;**39**:359-407.
- Wallace SS. Enzymatic processing of radiation-induced free radical damage in DNA. *Radiat Res.* 1998 Nov;**150(5 Suppl)**:S60-79.
- Wallimann T, Tokarska-Schlattner M, Schlattner U. The creatine kinase system and pleiotropic effects of creatine. *Amino Acids.* 2011 May;**40(5)**:1271-96. doi: 10.1007/s00726-011-0877-3. Epub 2011 Mar 30.

- Ward JF, Blakely WF, Joner EI. Mammalian cells are not killed by DNA single-strand breaks caused by hydroxyl radicals from hydrogen peroxide. *Radiat Res.* 1985 Sep;**103(3)**:383-92.
- Wei YH, Wu SB, Ma YS, Lee HC. Respiratory function decline and DNA mutation in mitochondria, oxidative stress and altered gene expression during aging. *Chang Gung Med J.* 2009 Mar-Apr;**32(2)**:113-32.
- Welsch U, Kummer W, Deller T. *Histologie - Das Lehrbuch.* Urban & Fischer / Elsevier. 2018. ISBN 978-3-437-44434-0.
- Wenk J, Brenneisen P, Meewes C, Wlaschek M, Peters T, Blaudschun R, Ma W, Kuhr L, Schneider L, Scharffetter-Kochanek K. UV-induced oxidative stress and photoaging. *Curr Probl Dermatol.* 2001;**29**:83-94.
- Wibley JE, Pegg AE, Moody PC. Crystal structure of the human O(6)-alkylguanine-DNA alkyltransferase. *Nucleic Acids Res.* 2000 Jan 15;**28(2)**:393-401.
- Wirth CJ, Zichner L, Knippers R. *Orthopädie und orthopädische Chirurgie: Stoffwechsel- und Systemerkrankungen.* Georg Thieme Verlag. 2003. ISBN 9783131269317.
- Wood RD, Mitchell M, Lindahl T. Human DNA repair genes, 2005. *Mutat Res.* 2005 Sep 4;**577(1-2)**:275-83.
- Xu-Welliver M, Kanugula S, Loktionova NA, Crone TM, Pegg AE. Conserved residue lysine165 is essential for the ability of O6-alkylguanine-DNA alkyltransferase to react with O6-benzylguanine. *Biochem J.* 2000 Apr 15;**347(Pt 2)**:527-34.
- Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci U S A.* 1997 Jan 21;**94(2)**:514-9.
- Yamaguchi Y, Hearing VJ. Physiological factors that regulate skin pigmentation. *Biofactors.* 2009 Mar-Apr;**35(2)**:193-9. doi: 10.1002/biof.29.
- Yang S, Lian G. ROS and diseases: role in metabolism and energy supply. *Mol Cell Biochem.* 2019 Dec 7. doi: 10.1007/s11010-019-03667-9. [Epub ahead of print]
- Yang W. Human MutLalpha: the jack of all trades in MMR is also an endonuclease. *DNA Repair (Amst).* 2007 Jan 4;**6(1)**:135-9. Epub 2006 Dec 4.
- Yoshida T, Goto S, Kawakatsu M, Urata Y, Li TS. Mitochondrial dysfunction, a probable cause of persistent oxidative stress after exposure to ionizing radiation.

Free Radic Res. 2012 Feb;**46(2)**:147-53. doi: 10.3109/10715762.2011.645207. Epub 2012 Jan 12.

Youn CK, Kim SH, Lee DY, Song SH, Chang IY, Hyun JW, Chung MH, You HJ. Cadmium down-regulates human OGG1 through suppression of Sp1 activity. J Biol Chem. 2005 Jul 1;**280(26)**:25185-95. Epub 2005 Mar 9.

Zhang X, Rosenstein BS, Wang Y, Lebwohl M, Wei H. Identification of possible reactive oxygen species involved in ultraviolet radiation-induced oxidative DNA damage. Free Radic Biol Med. 1997;**23(7)**:980-5.

Zharkov DO. Base excision DNA repair. Cell Mol Life Sci. 2008 May;**65(10)**:1544-65. doi: 10.1007/s00018-008-7543-2.

Zharkov DO, Mechetin GV, Nevinsky GA. Uracil-DNA glycosylase: Structural, thermodynamic and kinetic aspects of lesion search and recognition. Mutat Res. 2010 Mar 1;**685(1-2)**:11-20. doi: 10.1016/j.mrfmmm.2009.10.017. Epub 2009 Nov 10.

8. Appendix

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8.2 Curriculum Vitae

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Education

2015-Present PhD (Dept. of Biology)

University of Konstanz, Germany - Molecular Toxicology Group, Bürkle lab

University of Applied Sciences Albstadt-Sigmaringen, Germany - Biomedical Sciences, Bergemann lab

Graduate School Biological Sciences

Graduate School InViTe (In Vitro Technologies)

2013-2015 M.Sc. (Biomedical Sciences)

University of Applied Sciences Albstadt-Sigmaringen, Germany

Thesis title: **Importance of palmitoylation of viral proteins in the infection cycle of the human cytomegalovirus**

2009-2013 B.Sc. (Pharmaceutical Biotechnology)

University of Applied Sciences Biberach, Germany

Thesis title: **Characterization and significance of posttranslational lipid modifications of the viral tegument protein pUL71 of the human cytomegalovirus**

2002-2004 Education as a state-certified environmental technical assistant

Employment

01/2015-09/2019

Project Leader / Scientific Assistant - University of Applied Sciences Albstadt-Sigmaringen, Sigmaringen, Germany

03/2014-09/2014

Scientific Assistant - Department of Virology, University Medical Center Ulm, Germany

05/2011-06/2011

Tutor - Institute of Pharmaceutical Biotechnology, University of Applied Sciences Biberach, Germany

Teaching Experience**Co-supervision of Bachelor degree students**

1. Jannika Scholle (B.Sc. August 2019)
2. Julia Schon (B.Sc. May 2019)
3. Vivien Mockenhaupt (B.Sc. July 2018)
4. Heiko van Beek (B.Sc. May 2018)
5. Jessica Cerne (B.Sc. January 2018)
6. Florian Mutschler (B.Sc. October 2017)
7. Claudia Beerweiler from the University of Konstanz (B.Sc. August 2016)
8. Thomas May (B.Sc. July 2016)
9. Gerhard-Robert Hohadi (B.Sc. September 2015)

Co-Supervision of interns from the University of Konstanz

1. Julian Hogg, internship - September 2018

Co-supervision in practical courses**1. Immunology and cell biology - Bachelor degree students - 6th semester**

Summer 2019	44 hours
Winter 2018	22 hours
Summer 2018	22 hours
Winter 2017	22 hours
Summer 2017	44 hours
Winter 2016	44 hours

2. Stem cell technologies - Master degree students - 2th semester

Summer 2016	40 hours
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3. Molecular biology - Bachelor degree students - 4th semester

Summer 2015	40 hours
Winter 2015	40 hours

4. Virology - undergraduate medical / molecular medicine students at the University Medical Center Ulm

Summer 2014	6 hours
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Lectures for Master degree students / 2th semester Biomedical Sciences

Winter 2018 Microbiology / virology

- Topic: herpesviruses - an underrated virus

Winter 2018 Pathophysiology of the Cell

- Topic: eukaryotic model organisms

Summer 2018 Microbiology / virology

- Topic: herpesviruses - an underrated virus

Awards

1. Junior IMMA Award - International Mitochondrial Medicine Association (IMMA) 2017
2. Award for Poster Presentation – 7th World Congress on Targeting Mitochondria 2016
3. Award for excellent technology transfer - Chamber of Commerce and Industry 2016
4. Seahorse Poster Award – 6th World Congress on Targeting Mitochondria 2015

Funding

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Publications

1. **Schniertshauer D, Gebhard D, van Beek H, Nöth Vivien, Schon J, Bergemann J.** The activity of the DNA repair enzyme hOGG1 can be directly modulated by ubiquinol. *DNA Repair*. 2020; DOI:10.1016/j.dnarep.2019.102784.
2. **Schöller-Mann A, Matt K, Schniertshauer D, Hochecker B, Bergemann J.** 12 Days of *in vivo* caloric reduction can improve important parameters of aging in humans. *Mech Ageing Dev*. 2020; DOI:10.1016/j.mad.2020.111238.
3. **Schniertshauer D, Gebhard D, Bergemann J.** A new Efficient Method for Measuring Oxygen Consumption Rate Directly *ex vivo* in Human Epidermal Biopsies. *Bio Protoc*. 2019; DOI:10.21769/BioProtoc.3185.
4. **Schniertshauer D, Gebhard D, Bergemann J.** Age-Dependent Loss of Mitochondrial Function in Epithelial Tissue Can Be Reversed by Coenzyme Q₁₀. *J Aging Res*. 2018; 2018:6354680.
5. **Schniertshauer D, Gebhard D, Bergemann J.** Einfluss von solarer Strahlung auf die Mitochondrien. *OM & Ernährung*. 2018; SH08: 50-57.
6. **Schniertshauer D, Müller S, Mayr T, Sonntag T, Gebhard D, Bergemann J.** Accelerated Regeneration of ATP Level after Irradiation in Human Skin Fibroblasts by Coenzyme Q₁₀. *Photochem Photobiol*. 2016; 92(3):488-94.
7. **Gebhard D, Kirschbaum K, Matt K, Müller S, Schniertshauer D, Bergemann J.** Biologie von Alterungsvorgängen - Rolle der mitochondrialen Funktion am Modellsystem Haut. FHprofUnt2012 "MitoFunk". Abschlussbericht 2015; DOI:10.2314/GBV:867965967.

Poster / Oral Presentations

Poster Presentation

1. **Schniertshauer D, Gebhard D, Bergemann J.** Age-Dependent Loss of Mitochondrial Function in Epithelial Tissue Can Be Reversed by Coenzyme Q₁₀. *Poster presentation*, EBEC, Budapest, **2018**
2. **Schöller-Mann A, Matt K, Schniertshauer D, Bergemann J.** Caloric Reduction affects DNA Repair Capacity and Mitochondrial Function. *Poster presentation*, 9th World Congress on Targeting Mitochondria, Berlin, **2018**
3. **Schniertshauer D, Gebhard D, Bergemann J.** Development of a real-time assay to measure the activity of the human 8-oxoguanine-DNA glycosylase 1 and its splice variants. *Poster presentation*, EUROMIT, Köln, **2017**
4. **Schöller-Mann A, Matt K, Schniertshauer D, Bergemann J.** Influence of Caloric Reduction on Mitochondrial Function. *Poster presentation*, 8th World Congress on Targeting Mitochondria, Berlin, **2017**
5. **Schniertshauer D, Müller S, Mayr T, Sonntag T, Gebhard D, Bergemann J.** Accelerated Regeneration of ATP Level after Irradiation in Human Skin Fibroblasts by Coenzyme Q₁₀. *Poster presentation*, 7th World Congress on Targeting Mitochondria, Berlin, **2016** → **Award for Poster Presentation**
6. **Schniertshauer D, Gebhard D, Bifl M, Bergemann J.** Establishment of an assay for the measurement of the mitochondrial respiration in epidermis. *Poster presentation*, 6th World Congress on Targeting Mitochondria, Berlin, **2015** → **Seahorse (Agilent Technologies) Poster Award**

Oral Presentation

1. **Schniertshauer D.** *Ex vivo* measurement of respiratory parameters in epidermis, *Oral presentation*, Seahorse Workshop - Measuring Metabolic Engines and Fuels with the Seahorse XF Analyzer, **2016**