Bachelor's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Neuromedicine and Movement Science

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The effect of antioxidant supplementation and Nrf2 deficiency on mitochondrial function in older adults

BEV2900 - Spring 2024

Bachelor's thesis in Human Movement Science
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Abstract english:

Background: It is a well-known problem that the number of older adults in our society increases. Muscle function decreases with age and counteracting this is important so the older population could take care of themselves and not be a big load on society. A relationship has been shown between mitochondrial function and muscle function, as well as between mitochondrial function and oxidative stress. Oxidative stress is caused by free radicals and antioxidants have the ability to neutralize free radicals. Therefore, this article will look at whether supplementation of antioxidants can counteract mitochondrial dysfunction in older adults. Method: We carried out systematic searches in PubMed and Web of Science, and ended up with 8 articles that fitted our inclusion and exclusion criteria. Result: The studies showed a positive effect of antioxidant supplementation on mitochondrial function with the addition of different antioxidants. They measured mitochondrial function in different ways and some antioxidants showed more effect than others. Conclusion: Supplementation of antioxidants has an effect on mitochondrial function in older adults and several studies showed positive effects. More research is needed on humans and side effects of antioxidant supplementation to say something absolutely certain.

Keywords: aging, ATP, hydrogen peroxide, ROS, superoxide.

Abstrakt norsk:

Bakgrunn: Det er et velkjent problem at antallet eldre i samfunnet øker. Muskelfunksjonen minsker med alder, og å motvirke dette er viktig for at den eldre befolkningen skal kunne ta vare på seg selv og ikke være en stor belastning for samfunnet. Det er vist en sammenheng mellom mitokondriell funksjon og muskelfunksjon, samt mellom mitokondriell funksjon og oksidativt stress. Oksidativt stress er forårsaket av frie radikaler og antioksidanter har evnen til å nøytralisere frie radikaler. Derfor vil denne artikkelen se på om tilskudd av antioksidanter kan motvirke mitokondriell dysfunksjon hos eldre voksne. Metode: Vi gjennomførte systematiske søk i PubMed og Web of Science, og endte opp med 8 artikler som passet til våre inklusjons- og eksklusjonskriterier. Resultat: Studiene viste en positiv effekt av antioksidanttilskudd på mitokondriefunksjon ved tilskudd av ulike antioksidanter. De målte mitokondriefunksjon på forskjellige måter og noen antioksidanter viste mer effekt enn andre. Konklusjon: Tilskudd av antioksidanter har effekt på mitokondriefunksjonen hos eldre og flere studier viste positive effekter. Det er klart at det trengs mer forskning på mennesker og bivirkninger for å si noe helt sikkert.

Nøkkelord: aldring, ATP, hydrogenperoksid, ROS, superoksid.

Introduction:

It is well known that the population of older adults is increasing. An older person is known as a person that has progressive decrease in body functions that leads to low capacity to take care of themselves. The increase of older adults can therefore be a challenge for society, because a high amount of the population must be taken care of. Therefore, there is a lot focus on how to counteract age-related negative effects. One function that increases with age is muscle function, and this affects many over 60 years old (1). If we could reduce the age-related loss of muscle function, we could better the independence in older adults. This will in total lead to a positive consequence for society and the older adults.

Mitochondrial dysfunction has been suggested to play a key role in the loss of skeletal muscle function in aging. Mitochondria have an important role in a muscle cell, because the production of energy takes place here. Adenosine triphosphate (ATP) is the main source for energy in skeletal muscles and is merely produced through oxidative phosphorylation in the mitochondria. The electron transport chain (ETC) is an important part of oxidative phosphorylation and is located in the inner membrane of the mitochondria. Electron transport chain involves oxidation of molecules, electron transport and forming an electrochemical gradient (2). The oxidation of molecules and transport of electrons leads to formation of superoxide (O_2^-), and hydrogen peroxide (H_2O_2). Based on the "free radical theory", which was presented by Harman in 1956, high concentrations of superoxide and hydrogen peroxide can be linked to aging (3). In this theory they explain age-related negative effects on the body as a consequence of high concentrations of free radicals. Free radicals are partially reduced forms of oxygen, and in the body these are called reactive oxygen species (ROS) (3). Forms of ROS are superoxide and hydrogen peroxide. There are several findings that support this theory, and that these high concentrations of ROS could lead to mitochondrial dysfunction (2, 4, 5).

With aging an imbalance between production and detoxification of ROS could lead to higher levels of ROS (6). In a healthy human cell we have enzymatic antioxidants that regulate reactive oxygen species concentration in the mitochondria during the production of ATP. One of the enzymatic antioxidants is superoxide dismutase (SOD). SOD transforme superoxide to hydrogen peroxide and oxygen ($O_2^- + O_2^- + 2H+ \longrightarrow O_2 + H_2O_2$) (7). Then an enzyme, called catalase, transforme hydrogen peroxide to water (H_2O) and oxygen (O_2) (8). These two

reactions are important for forming harmful substances (O_2^-, H_2O_2) to less harmful substances (H_2O and O_2). (8, 9). It has been shown that the activity of SOD decreases with age and potentially contributes to the increase in superoxide levels in muscle mitochondria (10). When there are higher concentrations of reactive oxygen species in a cell we call it oxidative stress. This higher concentration is suggested to furthermore increase the ROS levels due to aging, by damaging mitochondrial functions more (6).

Mitochondrial functions are important for good muscle health, and these functions decline with age. There are several measurements that can be used as assessments for mitochondrial health. Physical tests can be used to measure mitochondrial function indirectly, and measurements on the mitochondria can be used to directly test mitochondrial functions (11, 12, 13). Analysis by using spectroscopy and microscopy is common for direct measurements (11, 1). Fluorescence is then used for measuring activity of functions (1, 14). Then the intensity of the color will say something about activity. High intensity means high activity and low intensity means low activity. Fluorescence is also used to indicate oxidative stress (4, 14). These measurements can be used for furthermore investigations of mitochondrial dysfunction.

To better muscle function in older adults it would be important to improve mitochondrial function. Increased ROS levels has been shown as a factor that leads to mitochondrial dysfunction (15). Lower SOD activity is a good reason for this. Since SOD is an enzymatic antioxidant and this decreases with age, we could discuss if antioxidants could be a solution for counteracting mitochondrial dysfunction due aging. Maybe antioxidants could regulate the imbalance between production and detoxification of ROS. We will try to investigate this more closely, and think that antioxidants could improve mitochondrial function and therefore also muscle function in older people. Therefore, the aim of this study was to investigate if supplementation of antioxidants can improve mitochondrial function in older adults.

Method:

For our bachelor thesis we used PubMed and Web of science as our databases. The search words we used on PubMed and Web of Science are present in table 1 together with the outcome of articles. Our inclusion criteria were articles in English or Norwegian, and our exclusion criteria were articles focusing on training and specifications against a particular

disease. After reading titles and abstracts, we ended up with 8 articles from PubMed and 13 articles from Web of Science following the inclusion and exclusion criteria. When we read all the 21 articles we ended up using 7 of them as primary sources. Another article from a previous search was added based on the above mentioned criteria, and therefore we ended up with 8 primary articles. As secondary literature we used articles from the reference list of our primary sources. Most of the articles that look at the influence of antioxidant supplements are done on mice, but since there are shown similarities between humans and rodents when it comes to changes that occur in muscle with aging, these studies will be relevant to look at (16).

Table 1:

Database	Search words	Articles
PubMed	(“ageing” OR “aging” OR “elderly”) AND (“mitochondrial dysfunction” OR “mitochondrial function”) AND (“antioxidants”) AND (“muscle function” or “muscular dysfunction”)	14
Web of Science	(“muscle function” OR “muscle dysfunction”) AND (“elderly” OR “aging”) AND (“oxidative stress”) AND (“mitochondrial function” OR “mitochondrial dysfunction”) AND (“antioxidants”) NOT (“sarcopenia”) NOT (“diabetes”)	219

Results:

From the 8 articles that we used, one study was done on humans and 7 were done on mice. All the studies considered a P-value < 0,05 as significant. The goal of the antioxidants that were used in the 8 articles was to protect mitochondria against oxidative stress by neutralizing free radicals. The 8 studies used 15 different antioxidants. One study used overexpression of GPx4, instead of pure supplementation. We consider this to have the same effect as supplementation. One study investigates Nuclear factor erythroid 2-related factor 2 deficiency (Nrf2 deficiency), which regulates antioxidant enzymes (17). The 8 studies did use different mechanisms and approaches. Studies were conducted in vivo (in the living), in vitro (in glass) or/and ex vivo (outside the living).

Study 1: Liu, Sophia et al. (2022): (11)

This study investigated whether supplementation of the antioxidant Urolithin A improves 6-minute walk distance, muscle endurance in hand and leg muscle, and biomarkers associated with mitochondrial health. Human population was both women and men. 66 participants were divided into either Urolithin A (1000mg per day) or placebo group (33 per), with age between 65-90 (71,7±4,9). Measures were done at baseline (before supplementation), and after 2 and 4 months.

Mitochondrial function was measured by analyses of plasma samples to detect the effect on biomarkers for mitochondrial health. At both 2 and 4 month Urolithin A supplementation shows a significant reduction in several biomarkers compared to the placebo group ($P<0,05$).

No significant effect of antioxidant supplement was found for maximal ATP production, which was measured by using magnetic resonance spectroscopy. The change after 4 months in hand muscle for Urolithin A group was 0,07±0,23 mM/s, and for placebo group it was 0,06±0,20 mM/s. The change in leg muscle for Urolithin A group was -0.03 (10) mM/s and the placebo group it was 0,03±0,10 mM/s.

Muscle endurance was measured by the ability to maintain repeated voluntary contractions (70% of max). Urolithin A significantly improved endurance after 2 months, compared to placebo ($P<0,05$). After 4 months Urolithin A had continued to improve, but a parallel improvement in the placebo group led to no significant effect.

In addition, muscle function was measured by a 6-minute walk distance. Change after 4 months was 60.8 (67,2) m. Compared to the placebo group with 42,5 (73,3)m. The increase in the Urolithon A group is found to be not significant, because both groups demonstrated significant improvements after 4 months.

No serious adverse events were reported in either of the groups. In total 33 adverse events were reported during the study, with no statistical differences between the groups.
The findings of this study were that supplementation of Urolithin A can improve mitochondrial function in older adults, but did not improve maximal ATP production, muscle endurance and function.

Study 2: Shibuya, Shuichi et al.(2022): (12)

This study investigated improvement effects of different antioxidants in the $Sod2^{-/-}$ mice population by an in vivo screening of the forced running ability. The antioxidant supplementation used was astaxanthin (500 mg/kg), genistein (10 and 50 mg/kg), MnTE-2-PyP (10 mg/kg), tempol (250 mg/kg), troglitazone (10 mg/kg), trolox (20 mg/kg), citric acid (500 mg/kg), phosphocreatine (100 mg/kg) and nicotinamide (200 mg/kg). With at least 4 mice per group. $Sod2^{-/-}$ mice have a lack of Sod2 expression in skeletal muscles and are used as an indicator for aging.

Muscle endurance was measured by mice performing a treadmill exercise by running at speed 12m/min with 0° slope, and then again 24h after getting the supplement. The combination of using $Sod2^{-/-}$ mice and measuring motor function indicate the effect on mitochondrial function in aged mice. Supplementation of astaxanthin, genistein, MnTE-2-PyP, tempol, troglitazone and trolox showed a significant increase in treadmill time ($P<0,05$). But supplementation of citric acid, phosphocreatine and nicotinamide had a significant decrease ($P<0,05$). The findings of this study were that most antioxidants did improve muscle endurance in aged mice after supplementation, but a few did not.

Study 3: Xu, Hongyang et al. (2023): (13)

This study investigated the overexpression of the antioxidant enzyme glutathione peroxidase 4 (GPx4) on mitochondrial function in 4 groups of female mice (5-11 mice per group). Group 1: wild-type mice (WT), group 2: Sod1KO mice (lack of SOD1), group 3: WT-mice with GPx4 overexpressing, and group 4: Sod1KO mice with GPx4 overexpression.

Mitochondrial function was considered by measuring mitochondrial respiration in permeabilized muscle fibers (ex vivo experiments). The results show that Sod1KO-mice had a reduction in mitochondrial respiration compared to WT-mice. Sod1KO-mice with GPx4 did not show any reduction ($P=0,0142$).

Oxidative stress was measured by using F_2-isoprostanes as a marker for oxidative stress. Results show higher levels of oxidative stress in muscle from Sod1KO-mice compared to

WT-mice (P=0,0002). Overexpression of GPx4 did reduce oxidative stress compared to WT-mice and Sod1KO-mice (P=0,0274).

In addition, muscle function was measured by measuring maximum force in extensor digitorum. Maximum force was 30% lower in Sod1KO mice than WT mice, and there was an increase in maximum force in the group with overexpression of GPx4 in the Sod1KO mice (P=0.0401). The same was observed in specific muscle force in a single fiber (P<0.001).

The findings of this study were that overexpression of GPx4 can improve mitochondrial respiration and muscle function (both single muscle fiber and whole muscle), and reduce oxidative stress in Sod1KO mice.

Study 4: Fernandes-Ortiz, Marisol et al. (2020): (18)

This study investigated if antioxidants can improve or restore mitochondrial function in WT-mice. The antioxidant supplementation used was melatonin (10mg/kg/day) given orally for 2 months. WT-mice were divided into 5 groups, Group 1: young (3 months old), group 2: early-aged (12 months old), group 3: early-aged with melatonin, group 4: old-aged (24 months old) and group 5: old-aged with melatonin, with 7 mice per group.

Mitochondrial function was measured in vitro by looking at levels of protein involved in mitochondrial dynamics. Levels did decrease in early-age mice and old-aged mice (P<0,05), and results showed an increase in protein levels by supplementation of melatonin in both groups. Mitochondrial function was also measured by using transmission electron microscopy on the cardiac muscle. There were observed mitochondrial damage in early-age and old-aged mice, but highest in old-age mice. With melatonin supplementation the two groups did maintain normal mitochondria function.

Numbers of mitochondria were measured by using morphometric analysis of cardiac mitochondria. Melatonin did increase the numbers of mitochondria (P<0,05) in early-aged and old-aged mice.

Nrf2 (a gen regulator for antioxidants) was measured by using markers for Nrf2 protein. Early-aged and old-aged mice show a decrease in protein levels of Nrf2 (P<0,001), but protein levels did recover in early-age and old-aged mice with melatonin.

The findings of this study were that melatonin can increase mitochondrial function in aged mice and recover the lack of Nrf2 proteins.

Study 5: Kitaoka, Yu et al. (2019): (15)

This study investigated whether the lack of Nrf2 (a gen regulator for antioxidants) promotes age-related mitochondrial oxidative stress in skeletal muscles in WT-mice. 3 groups were used, group 1: aged Nrf2 KO-mice (22 months), group 2: young WT-mice (4 months) and group 3: aged WT-mice (22 months), with 6-7 mice per group.

Mitochondrial function was assessed by measuring mitochondrial respiration and ROS production by using fluorescence. ROS production was significantly higher for Nrf2 KO-mice than both young and aged WT-mice (P<0,05). While mitochondrial respiration was lower in both aged groups compared to the young WT-mice group (P<0,05).

Oxidative damage was measured ex vivo by looking at levels of 4-HNE and protein carbonyl which are indicators for oxidative stress. Nrf2 KO-mice shows an increase in 4-HNE and protein carbonyl compared to the other groups (P<0,05). Catalase activity was lower in Nrf2 KO-mice compared to young WT-mice (P<0,05).This indicates less detoxification of ROS.

The findings of this study shows that lack of Nrf2 did decrease mitochondrial function and increase oxidative damage and stress.

Study 6: Zhu, Anni et al. (2023): (1)

This study investigated whether supplementation of the antioxidants ginsenoside Rh4 can regulate mitochondrial homeostasis to delay skeletal muscle aging in mice. D-gal is used to increase oxidative stress for simulating aging. For this study, 1-6 weeks old mice with severely inhibited immune response due to the absence of the thymus were used. These mice were randomly divided into 6 groups, 12 per group. Group 1: control group (no substance), group 2-6: got D-gal injections (200mg/kg/day) for 10 weeks, group 3-5: also got low

(50mg/kg), medium (100mg/kg) or high (200mg/kg) dose of Rh4 orally for 6 weeks, and group 6: received nicotinamide mononucleotide (NMN, 200 mg/kg BW) for 6 weeks.

Mitochondrial function and respiration was measured by using COX protein as a marker. COX 4 is an important protein for mitochondrial respiration chain assembly and function. Compared to the control group, the D-gal group had a significantly lower content of COX 4. After supplementation of Rh4 the content of COX 4 did increase significantly ($P<0,05$).

Mitochondrial membrane potential (MMP) was measured by using a fluorescent probe. MMP is important in production of ATP, because this affects the transport of substances through the membrane in the electron transport chain. The D-gal group had a decrease in mitochondrial membrane potential, but with Rh4 there was an increase in MMP. They also found a significant partially restored ATPase activity in mitochondria after Rh4 supplementation, which is an important enzyme in the production of ATP (7), compared to the control group ($P<0,001$).

The morphological changes of mitochondria in cells were measured using transmission electron microscopy (TEM). The group with only D-gal had significant morphological changes, leading to the disappearance of mitochondrial cristae. But in the ginsenoside Rh4 groups structurally cristae was still intact.

The findings of this study were that ginsenoside Rh4 improves mitochondrial function by increasing mitochondrial membrane potential, mitochondrial respiration and counteract damaging mitochondrial structure.

Study 7: Dong, Wenixet al. (2022): (4)

This study investigated the effect of antioxidant ginsenoside Rb1 supplementation on improving muscle stem cells function and mitochondrial function in old mice, by reducing oxidative stress levels. Muscle stem cells (myoblast) from mice were cultured for 24h, and substantially treated with Rb1 20μM and 40μM then with 1000μM hydrogen peroxide for 6h (in vitro). Hydrogen peroxide was used to indicate aging. They also used young (6 month old) and old mice (20 month old) (minimum 3 per group), where old mice were injected once a day for 4 weeks with ginsenoside Rb1 (20 mg/kg BW). They had two control groups, older

mice and younger mice that got normal saline injections once a day for 4 weeks (in vivo). 24h after the last injection, ex vivo fluorescence was used to detect muscle cell damage and mitochondrial hydrogen peroxide.

Mitochondrial membrane potential and total ATP content was measured by using a probe. Hydrogen peroxide did reduce the MMP of myoblasts, and did improve with Rb1 treatment ($P<0,05$). There were also observed a loss of total ATP and a significantly restored ATP content with Rb1.

Oxidative stress was measured by using fluorescence to detect mitochondrial ROS levels. Cells treated with hydrogen peroxide have significant enhancement in mitochondrial ROS level, but cell's pretreatment with Rh1 did reduce those levels.

Mitochondrial content, which is the quantity or density of mitochondria within a cell, was measured using fluorescence. Compared to the other groups myoblasts exposed with hydrogen peroxide showed the lowest fluorescence intensity. Treatment of Rb1 did reverse the hydrogen peroxide effect. Also several protein markers for mitochondrial biogenesis were measured as an indicator for mitochondrial number and size. Rb1 induced mice showed a significant increase on several protein markers.

In addition, mitochondrial damage was measured by using ultrastructural analysis by TEM. Which showed damaged mitochondria and a significantly higher percentage of abnormal mitochondria in the hydrogen peroxide group compared to the control group. Rb1 treatment did decrease mitochondrial damage caused by hydrogen peroxide.

The findings of this study were that ginsenoside Rb1 can decrease damage to mitochondrial function and oxidative stress caused by hydrogen peroxide.

Study 8: Feng, Wenjing et al. (2021): (14)

This study investigated whether the antioxidant alginate oligosaccharide (AOS) could be used as an anti-ageing drug to alleviate cardiac aging in mice. AOS is a natural substance that is extracted from algae. 8 week old mice were divided into 5 groups, 8 mice per group. Group 1: control with no supplementation, group 2: D-gal + low-dose AOS (50mg/BW), group 3:

D-gal + middle-dose AOS (100mg/BW), group 4: D-gal + high-dose AOS (150mg/BW) and group 5: control with just D-gal. D-gal was injected for 8 weeks, then AOS was injected for 4 weeks. Analysis was done ex vivo of the heart.

Mitochondrial membrane potential was measured by using fluorescence. Results showed that MMP was much lower in the D-gal group than in the control group, and AOS significantly helped protect against the D-gal effect on MMP ($P<0.05$).

Oxidative stress was measured by using fluorescence to measure levels of ROS production. There was an increase in levels of ROS in D-gal induced mice and AOS significantly decreased these levels ($P<0.05$). The higher dose of AOS in mice, the lower levels of ROS were observed.

In addition, mitochondrial biogenesis was measured by analyzing protein expressions. They observed a decrease in protein expression in D-gal-induced mice, and with AOS there was a significant increase in protein expression ($P<0.01$). The higher the dose of AOS the higher decrease was observed. The same was observed in other mitochondrial biogenesis factors with a significant increase.

The findings of this study were that alginate oligosaccharide increases mitochondrial function and biogenesis, and reduces oxidative stress in D-gal induced mice.

Discussion:

In this bachelor thesis we have investigated whether antioxidants can have a positive effect on mitochondrial function with aging. The main findings were that most of the studies showed a positive effect on antioxidants supplementation, but some showed no effect or a combination of both. Mitochondrial function was measured in various ways. Most of them have tested directly up against mitochondria but two of the studies also did indirect measurements of mitochondrial function. This was done with physical tests and investigations on muscle tissues. There are some similarities between the studies, but also many differences. None of the studies in mice did compare or look at supplementation over different periods of time. They only compare different doses of antioxidants, and therefore we can't say anything about long-term effects and benefits.

Oxidative stress and less antioxidant defense

Studies have shown that low activity of antioxidant defense leads to higher levels of ROS, and that this increases with age (17, 12). All articles that measured ROS in older cells, showed an increased ROS level with aging and linked this to mitochondrial dysfunction. Seven studies looked directly at the effect of antioxidant supplementation, but we also choose to include a study that looked at the lack of antioxidant defense. Which is Nrf2-deficiency. That study showed a decrease in mitochondrial content in muscle tissue, lower mitochondrial respiration and lower catalase activity in the relation of higher ROS levels (17). These results support the theory of a relation between reduction in antioxidant defense and high levels of ROS (oxidative stress) in the mitochondria that leads to mitochondrial dysfunction.

Supplementation of the antioxidants GPx4, AOS and Ginsenoside Rb1 showed a reduced level of ROS. These supplements did also improve mitochondrial content in muscle tissue, mitochondrial respiration and mitochondrial membrane potential (8, 18, 4). This further indicates that lower levels of ROS increase mitochondrial function, and that antioxidants could lower these levels.

One study measured the effect of Melatonin on Nrf2 deficiency in aged mice, and this showed that the supplement did recover the Nrf2 deficiency. This showed that antioxidants can counteract reduction in antioxidant defense (16). This is interesting since we thought that antioxidants did remove the ROS itself, but maybe some of the antioxidants had a positive effect on mitochondrial function by counteracting the reduced antioxidative defense.

Direct measurements of mitochondrial function

Supplementation of GPx4 and Ginsenoside Rh4 showed significant improvement in mitochondrial respiration of aged muscle tissue (13, 19). Mitochondrial respiration is important in the production of ATP, and is therefore a good indicator for mitochondrial function. It also says something about formation of oxidative stress. When there is observed a decrease in mitochondrial respiration this leads to higher levels of oxidative stress, because there will be higher production and less detoxification of ROS (2). Therefore better respiration could improve mitochondrial function, by improving production of ATP and decreasing oxidative stress.

There were also observed improvements in mitochondrial membrane potential with supplementation of the antioxidants AOS, Ginsenoside Rh4 and Ginsenoside Rb1 (20, 19, 21). Mitochondrial membrane potential is important in production of ATP, because this affects the transport of electrons (e-) and protons (H+) through the membrane. Transport of protons (H+) through the membrane is important for making an electrochemical gradient and also a source for potential energy in the production of ATP at the end of the electron transport chain (2, 8). Therefore, a low membrane potential across the mitochondrial membrane could lead to low potential energy available, which then leads to low production of ATP. These observations show us that supplementations of antioxidants could improve mitochondrial membrane potential, and therefore mitochondrial function.

Mitochondrial biogenesis is the ability of the mitochondria to divide and reproduce. These functions are important for the size and number of mitochondria. Studies showed an increase in different factors of mitochondria biogenesis by supplementations of antioxidants (18, 22, 4). Based on this result, we expect that supplementation of antioxidants can increase the number of mitochondria in muscle cells and therefore improve mitochondrial function.

In most of the studies they measured mitochondrial structure and different factors that indicate mitochondrial function in aged muscle mitochondria. Antioxidant supplementations like Urolithin A, Ginsenoside Rh4 and Melatonin showed increase in mitochondrial structure that are damaged due aging. Damage on the structure in mitochondria could be an explanation why there are observed decreases in mitochondrial functions. In most of the studies they found a positive increase in mitochondrial function by supplementation of antioxidants. Production of ATP is important for muscle function, and since there was observed an increase in mitochondrial functions that are important in the production of ATP, we can expect that ATP production increases. Out of this we can expect improvement in muscle function with aging by antioxidant supplementation.

Indirect measurements of mitochondrial function

Physical tests were done in two of the studies. These results are interesting because they are more directly related to muscle function and out of this can consider if antioxidant supplementation improves mitochondrial function with aging. These studies did a 6-minute

walk test and a treadmill time test (11, 12). The first study was done on humans and the second study on mice. When a person gets older they often walk slower and a 6-minute walk test would be a good measure of this. On the 6 minute walk they didn't see any significant effects of the supplement of antioxidants, because both groups (placebo and Urolithin A) increased walk length (11). This was the only study that didn't find any effect of the supplement. The reason for an increase in both groups can be that the participants wanted to see an effect and therefore pushed themself harder at 2 and 4 months than at baseline. This indicates that it wasn't the antioxidants that caused the increase in walking length, but an inside motivation.

In the study that used older mice they tested out how long they could run on a treadmill with a specific speed (12). This is interesting when we discuss physical endurance to an older person. They observed a positive increase in treadmill time with supplementation of some antioxidants, but not in all of them (12). The test was just done after 24 hours, and therefore indicated that supplementation of some antioxidants could acutely improve physical endurance and therefore also mitochondrial function. The study in older humans also measured muscle endurance. Supplementation of Urolithin A showed a significant positive effect after 2 months in muscle endurance, but did not have a further significant positive effect after 4 months (11). This investigation was done over a longer time than the study in older mice. Since there weren't any specific effects over time, this could indicate that supplementations of antioxidants have the biggest impact on muscle endurance at the beginning.

Contractile function was measured in one of the studies and showed that overexpression of GPx4 improved mitochondrial contractile function on Sod1 KO mice (13). Since we consider overexpression of antioxidants as the same as supplementation, this could have an impact on the results. Based on the results it indicates better muscle function with supplementation of antioxidants. ATP content is important in the relation of contractile function, because a muscle needs energy to contract. Studies that measured ATP content got different results. Supplementation of Ginsenoside Rb1 restored the loss of total ATP, but supplementation of Urolithin A didn't find any significant effect on ATP production.

We only found one study in older humans, and they didn't find a significant effect of antioxidant supplementation (11). This indicates that the improvement in mitochondrial function from antioxidants may not be big enough to affect muscle function in older adults. The lack of research in humans makes it hard to know exactly how antioxidants will affect older adults' muscle function. Since there are observed similarities between humans and rodents in age-related effects on muscles (16), it would be relevant to use the results from studies done in mice to make a conclusion. Studies in old mice showed increased mitochondrial function with antioxidants supplementation and can expect that this increases muscle function. Even though there are similarities between aging induced mice and natural aging in humans, we don't know if the different antioxidants are absorbed the same way in mice as in humans. To say something more about this we need more studies in humans to compare the results to.

All the studies used different antioxidants and this can have an impact on the results. Antioxidants are a big selecting name for many different substances that could have an effect on different places in mitochondria. The antioxidants have the ability to neutralize free radicals, but we didn't find any other direct information about where they specifically neutralize. If they have an effect in different phases, maybe some antioxidants could use their ability to neutralize and decrease oxidative stress and some don't. To say something about reasons for different results we need more research on different functions of antioxidants.

Side effects

Supplementation of Urolithin A in humans was well tolerated and safe (11). Side effects were measured by asking participants and taking blood samples, which is easier in humans than mice. That might be the reason for lack of measures on side effects in mice. However, this means that we can't say anything specific about the safety of antioxidant supplementation, or its side effects on other mechanisms.

Conclusion:

Based on our findings we concluded that supplementation of antioxidants can improve mitochondrial function in older adults because almost every study showed an increase in mitochondrial function. The studies were conducted within a short period of time, with the longest duration of 4 months. This makes it difficult to say something about long-term and

side effects. We can only say that a short time of antioxidant supplementation can improve mitochondrial function. Our findings indicate that supplementations improve muscle function indirectly, but we need more research to say something about the direct effect on muscle function in humans.

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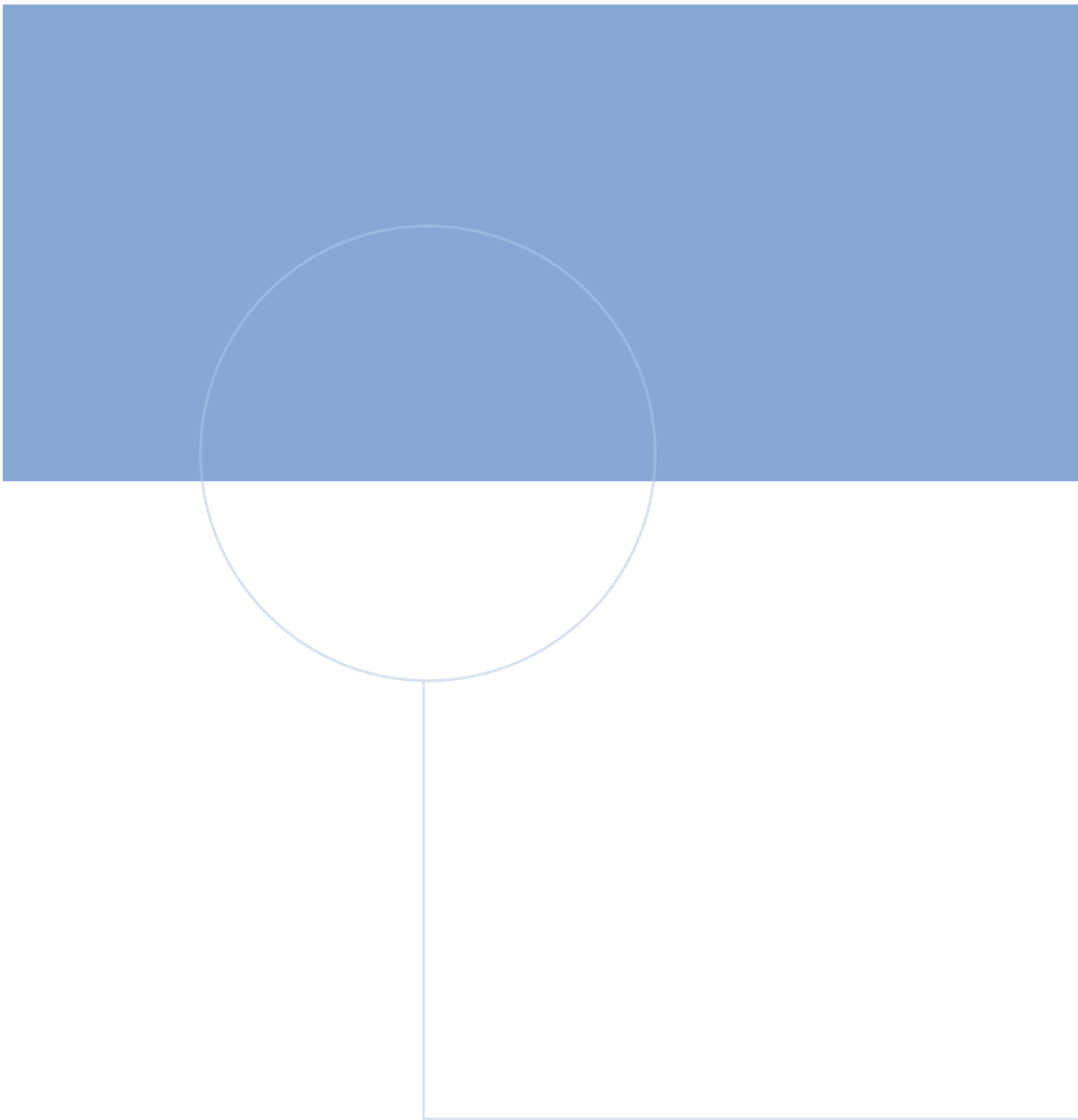

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