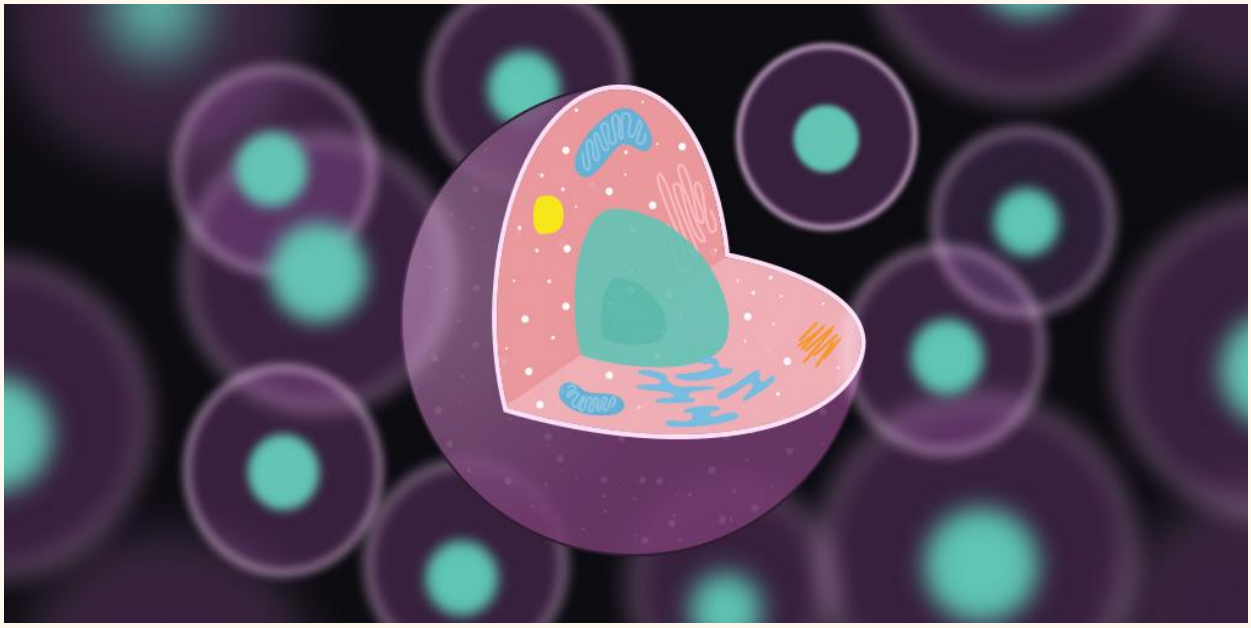
Extended Project Qualification

Antioxidants and their effect on human health: To what extent are exogenous antioxidants beneficial for health?

Xinyao Zhong



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Abstract

This project investigates the extent to which exogenous antioxidants are beneficial for human health. In vitro and in vivo studies are used to assess the effects of antioxidants on human health. Natural and supplementary antioxidants are discussed separately and their effects on different aspects of health are assessed. It is concluded that natural antioxidants are beneficial for physiological health as they are effective in preventing Cardiovascular diseases (CVD) and Type 2 Diabetes Mellitus (T2DM) and they do not produce adverse effects. In contrast, although found to be able to reduce the severity of T2DM, supplementary antioxidants are not as beneficial as they are not protective against CVD and cancer. They are also associated with pro-oxidant activities due to their high concentrations, which can lead to several adverse effects, such as higher damage to lymphocyte DNA. Both natural and supplementary antioxidants are concluded to be effective in improving mental health. However, further research, preferably randomised controlled trials, is needed to establish the causal effect of antioxidant intake on human health. Overall, fruit and vegetable consumption is recommended as they are not only a source of natural antioxidants but they also contain other substances that are good for health; dietary fibre, for instance, can prevent constipation. Antioxidant supplementation might be needed when endogenous antioxidant production declines with age, but the intake dosage should be controlled.

Introduction

It can be argued that health is the priority and foundation of everyone's life. Without a healthy body, it is impossible to carry out certain sports that one likes, to achieve one's maximum potential in their career, or to live a long happy life as a healthy person. Unfortunately, there are many diseases that prevent someone from maintaining a healthy body: some of them are mild like the common cold, while some of them can be life-threatening. According to the World Health Organisation (WHO), 7 of the top 10 causes of death in 2019 are age-related diseases such as ischaemic heart disease, stroke, lung cancer and Alzheimer's disease. Notably, around 8.9 million people died that year due to ischaemic heart disease (World Health Organisation [WHO], n.d.).[1] Therefore, it is easy to see that age-related chronic diseases are the main culprit of death and the biggest obstacle to health.

The good news is that age-related diseases can be prevented or at least mitigated through a balanced diet and healthy lifestyle. Age-related diseases are believed to be caused by the accumulation of free radicals which are produced as by-products in normal metabolic reactions. Antioxidants are substances that reduce the effect of free radicals, thus potentially preventing age-related diseases and saving millions of lives. Therefore, it is important to look at their effects on human health because they have the potential to promote cell longevity and improve life expectancy in humans.

My motivation for investigating this topic was evoked when there was a surge in the popularity of antioxidant supplements. Many people around me-my family and friends started to take antioxidant supplements even though they are totally healthy. I think the potential reason behind this is the way the supplement manufacturers portray antioxidant supplements as elixirs that can improve immunity, cure diseases and prevent ageing. I was curious to find out if they are as effective at maintaining health as they are advertised. Is it necessary to take supplements if we can obtain antioxidants from natural sources like vegetables? Are antioxidants inherently beneficial for human health or are there some adverse effects that are often neglected? Those are the questions that I would like to answer.

Therefore, I will assess the extent to which exogenous antioxidants are beneficial for human health in this dissertation and cover the questions posed. I specifically chose to talk about essential exogenous antioxidants (antioxidants that are not produced by the

body and can only be obtained from external sources) because some of their implications in human health are not fully studied and they are key in maintaining health when the internal production of endogenous antioxidants declines with age. They are also key in maintaining the balance between the amount of antioxidants and free radicals in the body, since their dosage of intake can be controlled. Furthermore, endogenous antioxidants are much more potent, which means they will always work and benefit health if their level of production is normal, so there is little point to assess their benefits to health. However, endogenous antioxidants are also very important to make up a complete antioxidant defence system, so I will briefly cover them in a small section in the background information.

Before investigating this topic in great detail, I expect to find that antioxidants are beneficial for human health, given their role in stabilising free radicals, species that are thought to cause many chronic diseases.

[1] World Health Organization. (n.d.). *Global Health Estimates: Life Expectancy and leading causes of death and disability.* World Health Organization. Retrieved December 8, 2022, from https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates

Background information

1. What are antioxidants?

According to Wikipedia, antioxidants are “compounds that inhibit oxidation, a chemical reaction that can produce free radicals” (Wikimedia, 2022).[2] Wikipedia is usually good at providing background information for a research topic; it is, however, hardly considered as scholarly as the articles on it can be edited by anyone. The definition of antioxidants from a more reliable source would be “substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures.” (Ware.M, 2018).[3] This is a more reliable source because it is medically reviewed despite the fact that it is a news article that aims to educate the general public.

2. Free Radicals

2.1 Definition of free radicals

Before going into detail about antioxidants, it is important to understand the properties and effects of free radicals, because they are the molecules that antioxidants react with and scavenge.

Free radicals are unstable (because they have one or more unpaired valence electrons), short-lived atoms or molecules.They can be made as by-products during normal metabolic processes or after exposure to external sources (eg. cigarette smoke, UV light, radon gas, ozone) (Eldridge, 2022).[4]

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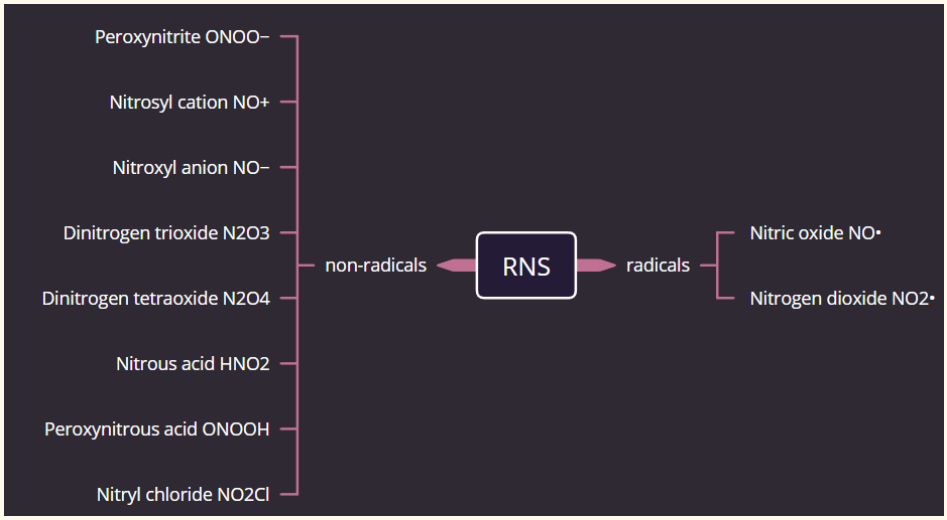
[2] Wikimedia Foundation. (2022, November 13). *Antioxidant.* Wikipedia. Retrieved November 22, 2022, from https://en.wikipedia.org/wiki/Antioxidant

[3] Ware.M (2018) *Antioxidants: Health benefits and nutritional information.* Medicalnewstoday.com. Retrieved 19 September 2022, from https://www.medicalnewstoday.com/articles/301506

[4] Eldridge,L. (2022). *What Exactly Are Free Radicals and Why Are They Important?.* Verywell Health. Retrieved 20 September 2022, from https://www.verywellhealth.com/information-about-free-radicals-2249103

2.2 Classification



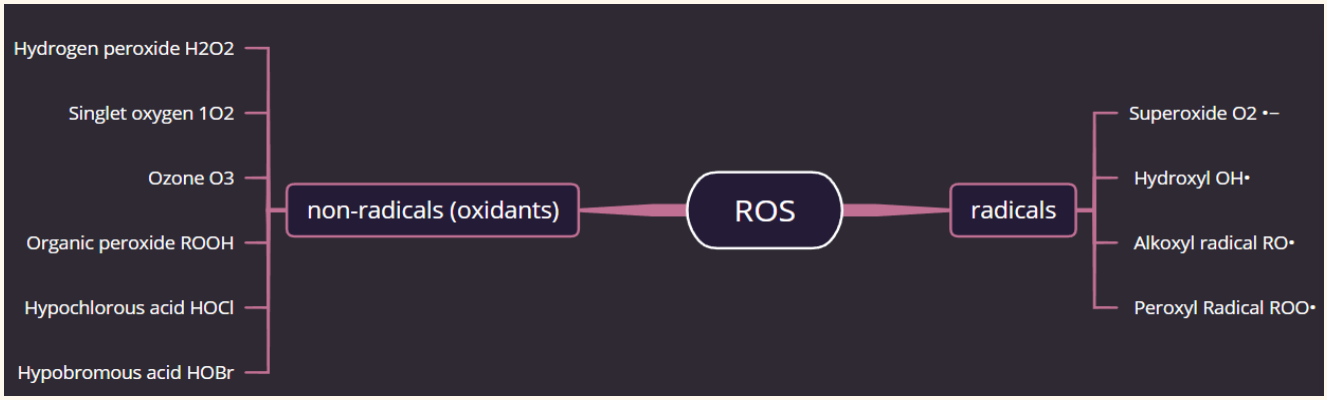


Figure 1 & 2. Schematic showing the classification of ROS and RNS. (own images)

Free radicals are generally divided into 2 groups (see Figure 2): Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (see Figure 1). They collectively make up all the radicals and non-radicals (also known as oxidants). Radicals are more reactive and less stable than non-radicals because of their unpaired electrons. However, non-radicals can be easily converted to free radicals or trigger various free radical reactions in living organisms (Ahmad, 2018).[5]

[5] Ahmad, R. (2018). Introductory Chapter: Basics of Free Radicals and antioxidants. *Free Radicals, Antioxidants and Diseases*. https://doi.org/10.5772/intechopen.76689

2.3 Effects of free radicals

Since free radicals have an uneven number of electrons in their outer shell and are unstable, they seek stability by stealing electrons from other molecules they collide with (oxidising other molecules). The affected molecule loses electrons and turns into a free radical itself, thus starting the chain reaction and increasing the concentration of free radicals (Phaniendra et al., 2015).[6]

At high concentrations, ROS and RNS can cause damage to various biomolecules including DNA, membrane lipids and proteins. They can even cause lysis of endothelial cells and fibroblasts (Pham-Huy, 2008).[7]

Continuous increase in accumulation of ROS and RNS can also lead to an imbalance between free radicals/oxidants and antioxidants in the body, which is known as oxidative/nitrosative stress. Oxidative/nitrosative stress contributes to many diseases including neurodegenerative diseases, rheumatoid arthritis, cardiovascular diseases and the ageing process (Phaniendra et al., 2015; Pham-Huy, 2008).[6,7]

However, at low or moderate concentrations, free radicals are beneficial for human health as they help phagocytes to fight off pathogens and are involved in many other physiological processes (Phaniendra et al., 2015; Pham-Huy, 2008).[6,7]

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[6] Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian journal of clinical biochemistry : IJCB*, *30*(1), 11–26. https://doi.org/10.1007/s12291-014-0446-0

[7] Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International journal of biomedical science : IJBS*, *4*(2), 89–96.

2.4 Examples of oxidative damage to macromolecules

1) Lipid peroxidation

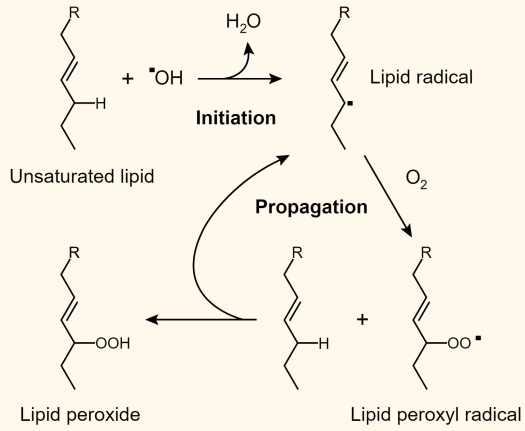

Figure 3. Schematic showing the process of lipid peroxidation (Source: Abylinn, n.d.)[8]

Stage 1-initiation:

FR hydroxyl attacks a polyunsaturated lipid to form a water molecule and a lipid radical (Hussain, 2018).[10]

Stage 2-propagation:

One oxygen molecule reacts with the lipid radical to produce the lipid peroxyl radical, which in turn reacts with another polyunsaturated lipid. This generates lipid peroxide and another lipid radical which in turn reacts with an oxygen molecule. Therefore, the process is repeated hence it becomes a chain reaction (Santos-Sánchez et al., 2019; Hussain, 2018).[9,10]

Stage 3-termination:

This is when two lipid radicals react to produce peroxide and oxygen, thus ending the production of more radicals (Santos-Sánchez et al., 2019; Hussain, 2018).[9,10]

Polyunsaturated lipids are an important component of cell membranes. This process damages cell membranes. Therefore, it can lead to loss of cell fluidity and even cell lysis. The products of this process can lead to age-related diseases such as atherosclerosis and cancer (Hussain, 2018).[10]

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[8] Abylinn. (n.d.). *English: Mechanism of lipid peroxidation*. Retrieved from https://en.wikipedia.org/wiki/File:Lipid_Peroxidation_Pathway.svg.

[9] Santos-Sánchez, N.F., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019). Antioxidant Compounds and Their Antioxidant Mechanism. *Antioxidants.* doi: 10.5772/intechopen.85270

[10] Hussain. [Hussain Biology]. (2018) *Mechanism of Lipid Peroxidation* [Video] Youtube. Retrieved 25 September 2022, from https://www.youtube.com/watch?v=Ut4Gri_dpxk.

2) DNA oxidation:

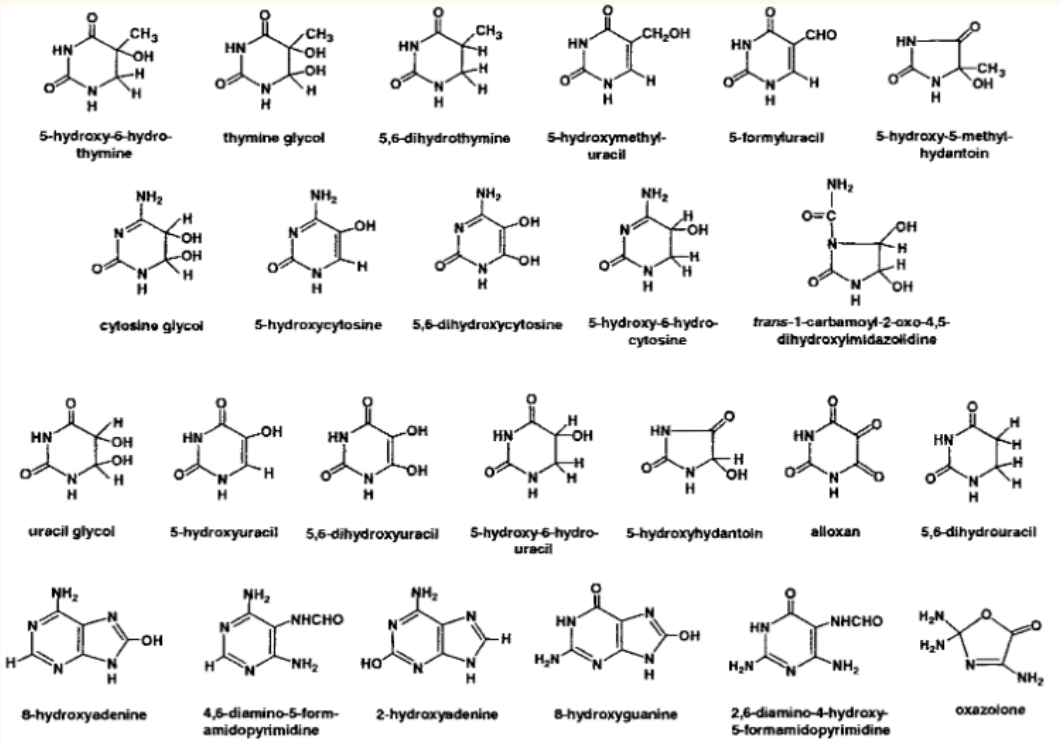

Figure 4. showing possible DNA base products after their reaction with ROS and free radicals. (Source: Cooke et al., 2003, p1196)[12]

DNA contains 4 types of bases (adenine, thymine, guanine and cytosine) and once those bases are oxidised, DNA is said to be damaged (Hussain, 2017).[11] As shown in Figure 4, reactions between different DNA bases and different free radicals can produce different products. Those product DNA bases are called base lesions (Cooke et al., 2003).[12] All bases have the possibility to be oxidised but guanine reacts with free radicals and ROS more than other bases because it has a high oxidation potential (Hussain, 2017).[11] Figure 5 shows the oxidation reaction of guanine to form 8-oxoguanine (Santos-Sánchez et al., 2019; Hussain, 2017).[9,11]

[11] Hussain. [Hussain Biology]. (2017) *DNA Oxidation* [Video] Youtube.Retrieved 25 September 2022, from https://www.youtube.com/watch?v=7_RbLIN-WY4.

[12] Cooke, M. S., Evans, M. D., Dizdaroglu, M., & Lunec, J. (2003). Oxidative DNA damage: Mechanisms, mutation, and disease. *The FASEB Journal*, 17(10), 1195–1214. https://doi.org/10.1096/fj.02-0752rev

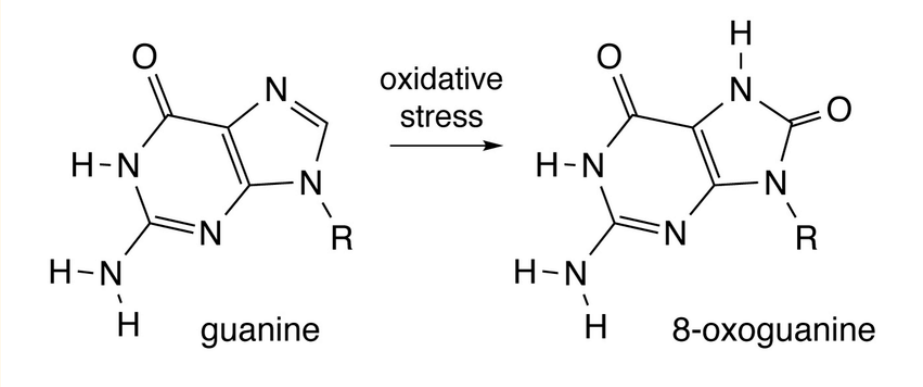

Figure 5. showing oxidation of the DNA base guanine, forming 8-oxoguanine as a product (Source: Lanier, 2017)[13]

There are a number of detrimental biological consequences as a result of DNA oxidation. Firstly, DNA oxidation is mutagenic, which means it can cause mutations as it changes the structure of DNA bases. This can lead to base mispairing, misincorporation and deletion. As a result, stages of protein synthesis (transcription and translation) can also be affected. DNA oxidation is also carcinogenic because it plays a role in the "initiation, promotion, and malignant conversion", which are stages that lead to cancer (Pham-Huy, 2008; Hussain, 2017).[7,11] This process also contributes to other gene-related diseases such as leukaemia, cystic fibrosis and dementia (Hussain, 2017).[11]

3) Inactivation and denaturation of proteins:

As mentioned in the previous section, oxidation to DNA can impact protein synthesis, resulting in an abnormal or non-functional protein. This is an indirect effect of oxidation on proteins. Free radicals and ROS can also react directly with amino acid residues on the proteins, leading to the formation of "protein aggregates by cross-linking". This means the proteins produced lose their specific shapes and therefore become inactive (Santos-Sánchez et al., 2019; Anguilar et al., 2016).[9,14]

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[13] Lanier, K. (2017). *The Origin of Life: Models and Data*. ResearchGate. Retrieved February 26, 2023, from https://www.researchgate.net/figure/The-oxidation-of-guanine-to-form-8-oxoguanine-Guanine-spontaneously-degrades-in-the_fig4_314088680.

[14] Aguilar, T. A., Navarro, B. C., & Pérez, J. A. (2016). Endogenous antioxidants: A review of their role in oxidative stress. *A Master Regulator of Oxidative Stress - The Transcription Factor Nrf2*. https://doi.org/10.5772/65715

3. Antioxidants

3.1 Classification of antioxidants

Antioxidant is a term that refers to any compound with antioxidant properties (ability to scavenge free radicals) (Wikimedia, 2022).[2] There are numerous antioxidants and they can be classified in many ways. As shown in figures 6, 7 and 8, they can be categorised according to their nature, source and mechanism (Jamshidi-kia et al., 2020).[15]

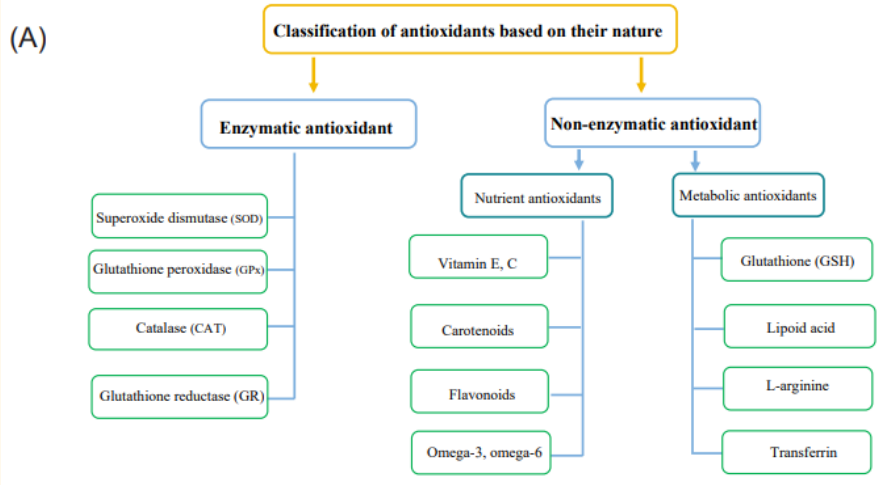


Figure 6 shows the classification of antioxidants based on their nature (Source: Jamshidi-kia et al., 2020)

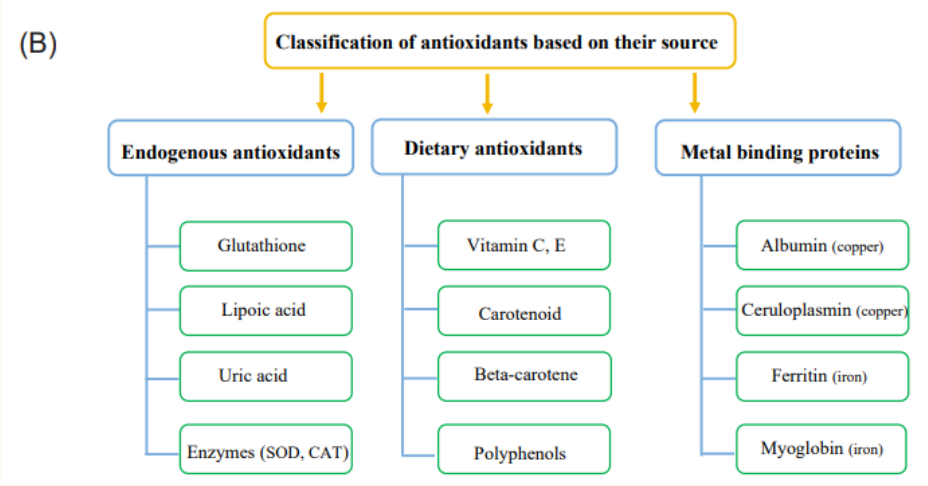


Figure 7 shows the classification of antioxidants based on their source (Source: Jamshidi-kia et al., 2020)

[15] Jamshidi-kia, F., Wibowo, J. P., Elachouri, M., Masumi, R., Salehifard-Jouneghani, A., Abolhasanzadeh, Z., & Lorigooini, Z. (2020). Battle between plants as antioxidants with free radicals in human body. *Journal of Herbmed Pharmacology*, *9*(3), 191–199. https://doi.org/10.34172/jhp.2020.25

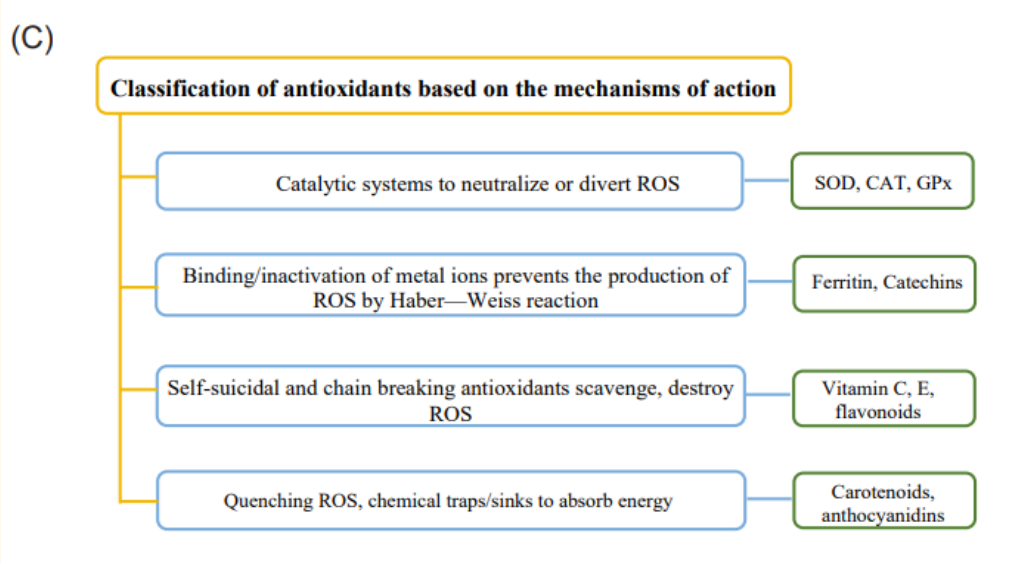


Figure 8 shows the classification of antioxidants based on their mechanism (Source: Jamshidi-kia et al., 2020)

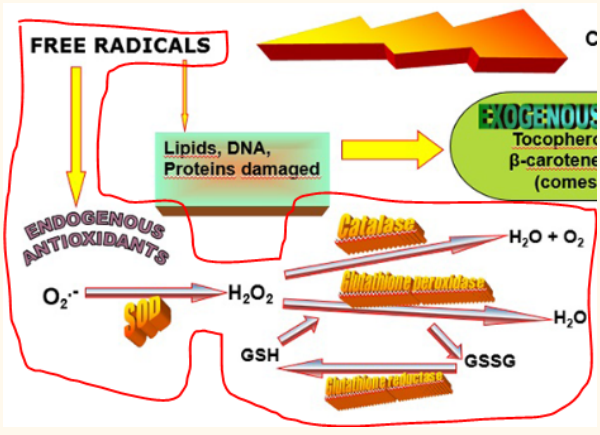
In this dissertation, I will classify antioxidants according to their sources-endogenous and exogenous. Endogenous antioxidants are the ones that are produced inside our bodies. Exogenous antioxidants are antioxidants obtained through diet (either through natural sources or through dietary supplements).

3.2 The Role of Endogenous antioxidants

Endogenous antioxidants are antioxidants that are made by our bodies. They can be further divided into enzymatic and non-enzymatic antioxidants.

1) Primary enzymatic system: (region circled by red line in Figure 9)

Component enzymes (biological catalysts that speed up chemical reactions):



superoxide dismutase (SOD)
catalase (CAT)
Glutathione peroxidase (GPx)
Glutathione reductase

Figure 9 showing the series of reactions between the enzymatic antioxidant system and ROS. (Source: Angular et al., 2016)

Firstly, superoxide ($O_2^{\bullet-}$) is converted to hydrogen peroxide in the presence of superoxide dismutase (SOD). This reaction is known as dismutation. Then, catalase (CAT) catalyses the reaction in which hydrogen peroxide is converted to water and molecular oxygen.

Glutathione peroxidase (GPx) catalyses the decomposition of hydrogen peroxide (H_2O_2) or organic peroxide (ROOH) to water or alcohol. Glutathione (GSH) is also involved in this process and is oxidised to Glutathione disulfide (GSSG). GSSG can be converted back to GSH by the enzyme glutathione reductase. This reaction is very important in protecting the polyunsaturated lipids in the cell membrane (Anguilar et al., 2016; Moussa et al., 2020).[14,16]

2) Non-enzymatic system:

A main component of the non-enzymatic endogenous antioxidant system is glutathione (GSH), also known as the “master” antioxidant due to its presence in all plant and animal cells. It has 3 amino acids: glycine, cysteine, glutamic acid and is mainly synthesised in the liver. It has several redox forms (reduced glutathione-GSH, oxidised glutathione-GSSG). GSH is the active form while GSSG is the inactive form. As GSH is involved in prevention of oxidative stress, it becomes oxidised and turns into GSSG. However, GSSG can be converted to GSH by the glutathione reductase enzyme, as mentioned in the last section. Its functions include boosting the utilisation and regeneration of other antioxidants eg. vitamins C and E, alpha-lipoic acid and coenzyme 10, repairing lipids after lipid peroxidation and acting as a sacrificial reductant during catalytic reduction of peroxides by glutathione peroxidase (GSHPx) because it gets oxidised in this process. Some other non-enzymatic endogenous enzymes include uric acid, bilirubin, polyamines, alpha-lipoic acid, coenzyme Q and ferritin. (Anguilar et al., 2016; Moussa et al., 2020).[14,16]

[16] Moussa, Z., M.A. Judeh, Z., & A. Ahmed, S. (2020). Nonenzymatic exogenous and endogenous antioxidants. *Free Radical Medicine and Biology*. https://doi.org/10.5772/intechopen.87778

3.3 Exogenous antioxidants

Exogenous antioxidants are antioxidants that are obtained through diet.

3.31 Vitamin C

Vitamin C, also known as ascorbate or ascorbic acid, is a water-soluble vitamin. Its sources include fruits (especially citrus fruits) and vegetables. It has to be obtained through diet as humans do not have the enzyme, l-gulono-γ-lactone to synthesise it. It is necessary to prevent scurvy (characterised by bleeding gums). More importantly, it also functions as an antioxidant (Goszcz et al., 2015).[18]

Vitamin C scavenges ROS (superoxide, hydroxyl radicals) and non-radicals (hydrochlorous acid and nitrosating agent) by donating two electrons from a double bond between carbon 2 and 3 in the ring (see Figure 10) to help free radicals to gain stability. This helps to reduce free radical concentration, thus protecting lipids, DNA and proteins from oxidative stress. After the electron donation, the compound itself becomes oxidised to inactive dehydroascorbic acid/dehydroascorbate. Therefore, it is known as a "sacrificial" antioxidant. Nevertheless, dehydroascorbic acid can be reduced back to ascorbic acid by dehydroascorbate reductase in the presence of GSH (See Figure 10) (Goszcz et al., 2015; Pehlivan, 2017).[18,19]

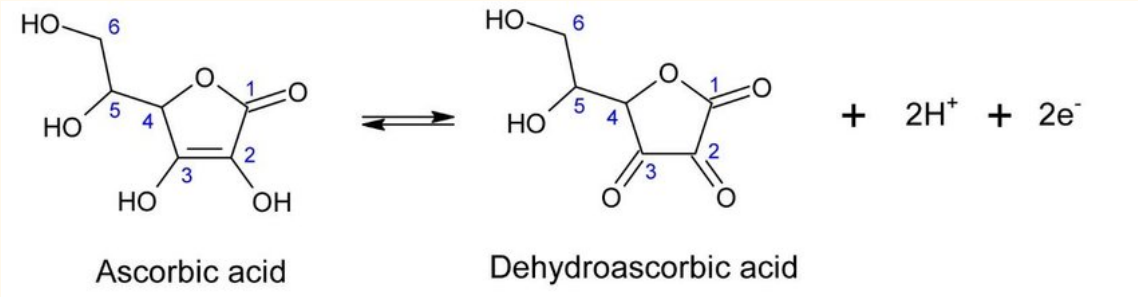


Figure 10 showing conversion from ascorbic acid (reduced form) to dehydroascorbic acid (oxidised form) and vice versa. (Source: Šafranko, 2020)[17]

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[17] Šafranko, S. (2020). *Electrochemical Detection of Vitamin C in Real Samples*. ResearchGate. Retrieved February 26, 2023, from https://www.researchgate.net/figure/Oxidation-mechanism-of-vitamin-C-L-ascorbic-acid_fig2_342918066.

[18] Goszcz, K., Deakin, S. J., Duthie, G. G., Stewart, D., Leslie, S. J., & Megson, I. L. (2015). Antioxidants in cardiovascular therapy: Panacea or false hope? *Frontiers in Cardiovascular Medicine*, *2*. https://doi.org/10.3389/fcvm.2015.00029

[19] Pehlivan, F. E. (2017). Vitamin C: An Antioxidant agent. *Vitamin C*. https://doi.org/10.5772/intechopen.69660

Vitamin C converts lipid peroxidation products into unreactive C-LPO products. This prevents lipid peroxyl radicals from interacting with macromolecules as DNA and proteins (Pehlivan, 2017).[19] Vitamin C also helps to regenerate Vitamin E by donating electrons to tocopheryl radical (vitamin-E–O•) thus reducing it to tocopheryl (Vitamin E) (Moussa et al., 2020; Goszcz et al., 2015; Pehlivan, 2017).[16,18,19]

Vitamin C can also act as a pro-oxidant which encourages free radical generation in the presence of metal ions, as observed in in vitro studies. Vitamin C can reduce Fe^{3+} ion to Fe^{2+} ion according to this equation: $2Fe^{3+}+ascorbate \rightarrow 2Fe^{2+}+dehydroascorbate$.

The Fe^{2+} ion then reacts with hydrogen peroxide to produce hydroxyl radical, which is a ROS by the equation: $2Fe^{2+}+2H2O2 \rightarrow 2Fe^{3+}+2OH\bullet+2OH^{-}$ This process is known as the Fenton reaction. However, pro-oxidant activity has only been observed in in-vitro studies, where the experiment environment is highly controlled and thus may not represent conditions in living organisms. Therefore, pro-oxidant activity is likely to be irrelevant in organisms (in vivo conditions) (Pehlivan, 2017).[19] The difference between in vitro and in vivo studies will be discussed in section 4.

Vitamin C is found to inhibit oxidation to LDL (low-density lipid) in vitro and subsequently the uptake of ox-LDL by white blood cells, which lead to atherosclerosis (a major cause of CVD) so it is likely to prevent atherosclerosis. There is also in vivo evidence in animal studies as mice that lacked l-gulono-γ-lactone oxidase (Gulo−/−), the enzyme for vitamin C synthesis and were not given vitamin C in diet, showed more vascular damage (Goszcz et al., 2015).[18]

3.32 Vitamin E

Vitamin E is a fat-soluble vitamin found in cooking oils, egg yolks, butter and green-leafy vegetables. It has eight isomers (natural chemical forms): alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol. The major isomers in the human diet are α- and γ-tocopherol (Goszcz et al., 2015; U.S. Department of Health and Human Services, n.d.).[20] Vitamin E is present in human cell membranes and they help to reduce or prevent lipid peroxidation of the cell membranes by preferentially reacting with free radicals. They stabilise free radicals by transferring/accepting electrons (Tokoph, 2017).[21]

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[20] U.S. Department of Health and Human Services. (n.d.). *Office of dietary supplements - vitamin E.* NIH Office of Dietary Supplements. Retrieved November 24, 2022, from https://ods.od.nih.gov/factsheets/VitaminE-HealthProfessional/

[21] Tokoph, K. [Catalyst University]. (2017) *Vitamin E: Anti-oxidant Function* [Video] Youtube. Retrieved 23 November 2022, from https://www.youtube.com/watch?v=sD1dlplmxEg

3.33 Phenolic compounds

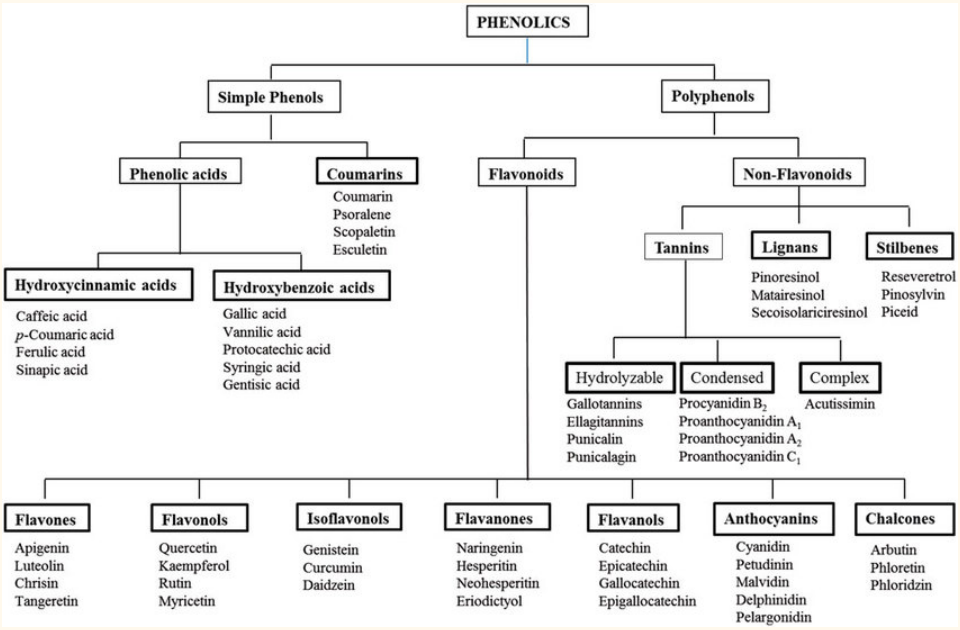

Figure 11 showing classification of phenolic compounds (Source: Vardhan, 2017)[22]

Phenolic compounds are defined as “secondary metabolites in plants with a common aromatic ring bearing one or more hydroxyl groups” (Anantharaju et al., 2016).[23] They are a large group of more than 8000 chemical substances and can be subdivided into groups like flavonoids, tannins and stilbenes (as shown in Figure 10) (Santos-Sánchez et al., 2019; Goszcz et al., 2015).[9,18]

Dietary sources include cereals, legumes and soybeans (Anantharaju et al., 2016).[23]

Phenolic compounds act as antioxidants by three main mechanisms. Firstly, they neutralise free radicals and oxidants by donating hydrogen atoms from their hydroxyl groups. They also act as antioxidants by chelating (binding tightly to) metal ions like iron and copper. Lastly, they are able to block the action of some enzymes involved in the production of superoxide radicals, such as protein kinase C (Santos-Sánchez et al., 2019; Goszcz et al., 2015; Anantharaju et al., 2016).[9,18,23]

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[22] Vardhan, V. (2017). *Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production*. ResearchGate. Retrieved February 26, 2023, from https://www.researchgate.net/figure/Schematic-representation-of-classification-of-phenolic-compounds_fig1_318476026.

[23] Anantharaju, P. G., Gowda, P. C., Vimalambike, M. G., & Madhunapantula, S. R. V. (2016). An overview on the role of dietary phenolics for the treatment of cancers. *Nutrition Journal*, *15*(1). https://doi.org/10.1186/s12937-016-0217-2

3.34 Carotenoids

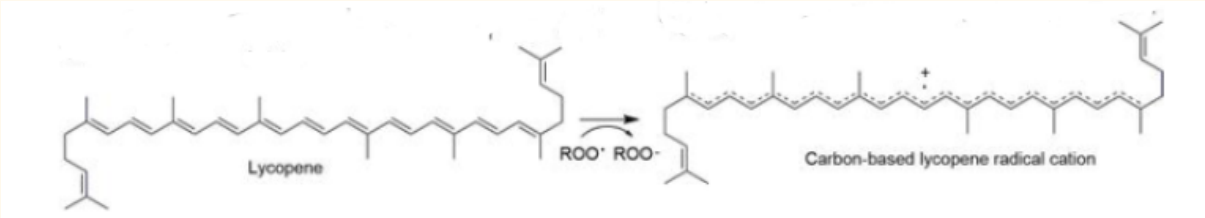

Figure 12 Free radical addition reaction of lycopene (Source: Moussa et al., 2020)[16]

Carotenoids are a group of lipid soluble phytonutrients found in almost all plants and algae (Santos-Sánchez et al., 2019; Moussa et al., 2019; Goszcz et al., 2015).[9,16,18] They have a long unsaturated hydrocarbon alkyl chain that makes them soluble in lipids (Moussa et al., 2019).[16] Carotenoids have a characteristic symmetrical tetraterpene skeleton that contains 40 carbons and can undergo various changes in structure, thus allowing three mechanisms of scavenging free radicals (Santos-Sánchez et al., 2019).[9] Around 700 of them are identified and characterised. They are primarily classified into 2 groups: xanthophylls that contain oxygen atoms and carotenes that are hydrocarbons (Santos-Sánchez et al., 2019; Moussa et al., 2019).[9,16]
There are three antioxidant mechanisms used by carotenoids:

1. Single electron transfer- carotenoids transfer one electron to free radicals in order to stabilise them. For example, lycopene, the strongest antioxidant among all carotenoids, reduces peroxyl radicals through electron transfer.
2. Hydrogen atom transfer- stabilising free radicals by transferring hydrogen atoms to them
3. Free radical addition reaction- combining with radicals to form a stabilised carbon-based radical (see Figure 12 lycopene reaction)

Both hydrogen atom transfer and free radical addition reaction are due to the strongly-reducing extended conjugated system of carotenoids, which eases those two mechanisms (Santos-Sánchez et al., 2019; Moussa et al., 2019; Goszcz et al., 2015).[9,16,18]

4. In vitro vs In vivo studies

The adjective “In vitro” has a Latin origin which means “in glass” and it describes medical experiments and tests that are conducted in a controlled environment such as test tubes or petri dishes. In vitro studies usually involve “studying microorganisms, human or animal cells in culture”. They are useful for researchers to observe and

assess certain biological phenomena without confounding variables. However, the results obtained from in vitro studies may not be applicable to the actual living organisms where conditions are not controlled.

On the other hand, “In vivo” which means “within the living” in Latin, refers to tests or clinical trials that are conducted in living organisms. The results represent how things work in a real living organism, which makes this method useful (Santos-Sánchez et al., 2019; Eske, 2020).[9,24]

5. Dosage of exogenous antioxidants

Dosage of antioxidants is a factor that affects plasma antioxidant concentration. Antioxidants are effective to delay or prevent oxidative damage caused by ROS to cells and tissues when present at low concentrations. According to in vitro studies, when at high concentrations, exogenous antioxidants can display pro-oxidant activity, which encourages free radical generation (Bouayed & Bohn, 2010).[25] For example, at low concentrations flavonoids protect rat H4IIE cells against H2O2-induced cytotoxicity and DNA strands break whereas at high concentrations they cause cytotoxicity and DNA damage. However, pro-oxidant activities are not inherently harmful, as stated by Bouayed and Bohn (2010) “beneficial or harmful effects of natural compounds may also occur independently from their (anti-) oxidative properties”. For instance, the pro-oxidant activity of some polyphenols can cause mitochondrial dysfunction thus leading to cell death. This could actually be a possible mechanism to kill cancer cells.

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[25] Bouayed, J., & Bohn, T. (2010). Exogenous antioxidants--Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative medicine and cellular longevity*, *3*(4), 228–237. https://doi.org/10.4161/oxim.3.4.12858

5.1 Natural vs Supplementary

Natural antioxidants are found in many foods (vegetables, fruits and nuts) and we can obtain them through diet by consuming foods rich in antioxidants. Antioxidants supplements are concentrated forms of natural antioxidants. The harm of taking antioxidant supplements may outweigh the benefit (Walle, 2018).[26] As mentioned above, when present at high concentrations, antioxidants can become pro-oxidants and increase ROS production, which contributes to oxidative-induced diseases (Bouayed & Bohn, 2010).[25] Taking large doses of antioxidant supplements can increase the risk of many types of cancers, decrease exercise performance and add to the risk of birth defects. Therefore, it is recommended to obtain exogenous antioxidants from foods rather than from supplements (Walle, 2018).[26]

5.2 Age and Recommended Dosage

Endogenous antioxidant and ROS productions are balanced in young people. However, as age increases, endogenous antioxidant production declines and concentrations of ROS increase, leading to oxidative damage. Therefore, oxidative-induced age-related diseases appear in the elderly. As a result, older people should take larger doses of exogenous antioxidants and antioxidant supplements when appropriate to prevent oxidative damage (Poljsak & Milisav, 2013).[27]

[26] Walle, G. V. D. (2018, November 13). *Should you take antioxidant supplements?* Healthline. Retrieved December 9, 2022, from https://www.healthline.com/nutrition/antioxidant-supplements

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Discussion

1. Defining and measuring health

The aim of this project is to find out the extent to which exogenous antioxidants are beneficial for health. According to the World Health Organisation (WHO), health is “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.” (WHO, n.d.).[28] Therefore, I will assess the effects of exogenous antioxidants on physiological and mental health. For the purpose of this discussion, I will leave out the social aspect of health because I do not see how they exert an effect directly on someone’s social well-being as they do not change the relationships between people.

1.1 Physiological Health

1.1.1 Disease prevention

One measurement of physiological health is the absence of diseases. Within the confines of this project, I will only assess the role of exogenous antioxidants in the prevention and treatment of free radical-induced diseases, namely age-related or chronic diseases such as cardiovascular disease, cancers and type 2 diabetes.

1.1.11 Cardiovascular disease (CVD)

Cardiovascular disease (CVD) is the “collective term named for disorders afflicting the blood vessels and heart”. Inflammation and severe oxidative stress caused by free radicals are fundamental risk factors for CVD (Mimiran et al., 2022).[29] Therefore, Mimiran et al. (2022)[29] stated that “a sufficient intake of antioxidants is suggested to beneficially interfere with CVD by quenching ROS”, so they can delay and prevent CVD.

In vitro evidence

In vitro evidence shows that antioxidants are effective in preventing CVD. For instance, vitamin C prevents lipid peroxidation, thus inhibiting oxidation to LDL (low-density lipoprotein) and the subsequent formation of ox-LDL (oxidised LDL), which are events occurring in atherosclerosis. Phenolic compounds are also found to prevent atherosclerosis by inhibiting lipid oxidation and the uptake of ox-LDL by macrophages in vitro. High levels of cholesterol can cause fat deposits in blood vessels, which lead to

CVD-like atherosclerosis, thrombosis and heart attack. β-carotene and lycopene are able to reduce plasma cholesterol levels by inhibiting the action of HMG-CoA reductase (an enzyme involved in cholesterol synthesis) in vitro, highlighting their protective role in the circulatory system (Goszcz et al., 2015).[18] However, in vitro studies are inadequate, because the results are obtained in a highly controlled lab environment and the complexity of living organisms fails to be acknowledged, so they may be inapplicable to the actual living entities (Eske, 2020).[24] This means that antioxidants that are found to be capable of preventing CVD might not be effective in real life. For example, bioavailability of polyphenols is very low; less than 1% of ingested polyphenols are absorbed into plasma and they are also rapidly excreted. Maximum concentrations of blood polyphenols after consuming polyphenol-rich foods tend to be 0.1–1 μM, which is much lower than that used in vitro (Goszcz et al., 2015).[18] Therefore, although in vitro evidence unanimously supports the role of antioxidants in preventing CVD, it is inadequate to draw a conclusion.

In vivo evidence-natural antioxidants

In order to assess whether natural antioxidants are effective to prevent CVD, in vivo evidence has to be considered. It mostly supports the protective role of natural antioxidants against CVD.

Mirmiran et al. (2022) conducted a cohort study on 15,005 individuals that were selected from the Tehran lipid and glucose study (TLGS), of which, 5120 subjects remained and entered analysis after a follow-up of 15 years (1999-2014). This was a large sample size, which means any statistical correlation found is unlikely to be due to chance. Potential confounding factors like physical activity and blood pressure were all taken into consideration, which improved the validity of the study. Although they found that the associations between CVD risk and vitamin A and C intake are statistically insignificant, there was an inverse association between vitamin E intake and CVD risk, suggesting its protective role in the prevention of CVD. Antioxidants used in this study all came from natural sources (fruits and vegetables). The dietary intake of each participant was recorded in detail using a 168-item semi-quantitative food frequency questionnaire (FFQ), which included the daily, weekly and monthly intake of each food. After a series of computations, dietary intakes of common antioxidants (vitamin C, A, E) were calculated and considered in grams per week (Mirmiran et al., 2022).[29] Although FFQ is claimed to be reliable and valid; dietary intake, however, is not an accurate indication of antioxidant levels in vivo, because it is possible that some people absorb

particular antioxidants better than others. Therefore, studies that measure antioxidant levels using plasma/serum concentration are more accurate.

Jayedi et al. (2019) conducted a meta-analysis on 15 prospective cohort studies and 3 prospective evaluations within interventional studies (clinical trials). The sources of antioxidants were limited to fruits and vegetables. They concluded that a higher dietary intake and a higher circulating concentration of vitamin C are associated with a lower CVD mortality risk. Higher circulating concentrations of vitamin E and β-carotene displayed a significantly inverse association with mortality of CVD while their dietary intakes did not show this association (Jayedi et al., 2019).[30] Both dietary intake and blood concentration of antioxidants were taken into consideration, so this piece of evidence provides more confidence about the protective role of natural antioxidants against CVD. One might believe that a higher intake of antioxidants would result in a higher plasma concentration of antioxidants. However, it is not the case, because not all antioxidants ingested are absorbed into the bloodstream since some antioxidants with low bioavailability might be egested straightaway. Therefore, a point could be made is that antioxidants with higher bioavailability are more likely to be utilised in the body to stabilise ROS and prevent oxidative stress, thus lowering the risk of CVD.

In vivo evidence-supplementary antioxidants

Most studies that use supplements as a source of antioxidants found that antioxidant supplements are not effective in preventing CVD.

Myung et al. (2013) conducted a meta-analysis on 50 randomised control studies in order to find out the efficacy of antioxidant supplements in prevention of CVD. They found that supplementation with antioxidants was not associated with reduced risk of CVD and some were even associated with increased risk of angina pectoris. Although some beneficial effects were seen in some randomised control trials such that vitamin E supplementation was associated with lowered risk of myocardial infarction, these trials used supplements supplied by the pharmaceutical industry, so they had a vested interest in speaking in favour of supplementation. As a result, the final conclusion of this study is that antioxidant supplements are not effective in preventing CVD (Myung et al., 2013).[31] This might be due to the fact that supplementary antioxidants are at high concentrations, so they may display pro-oxidant activities, as mentioned in section 5.1. Pro-oxidant activities promote the generation of ROS, adding to oxidative stress and inflammation of the arterial lining. Another reason might be that people usually inevitably ingest different natural antioxidants together, since there are

many different types of antioxidants present in a particular vegetable or fruit. This might mean that taking antioxidants in combinations might be more beneficial than taking a single type of extracted antioxidant, making natural antioxidants more effective in preventing CVD than supplementary antioxidants.

Sub-conclusion

To conclude this section, evidence shows that some natural antioxidants (especially Vitamin C and E) are able to reduce the risk of CVD while supplementary antioxidants do not. However, more studies are needed for a solid conclusion.

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[29] Mirmiran, P., Hosseini-Esfahani, F., Esfandiar, Z., Hosseinpour-Niazi, S., & Azizi, F. (2022). Associations between dietary antioxidant intakes and cardiovascular disease. *Scientific Reports,* 12(1). https://doi.org/10.1038/s41598-022-05632-x

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1.1.12 Cancers

Cancer happens when abnormal cells divide uncontrollably and spread to other parts of the body. Normally, damaged cells undergo apoptosis (programmed cell death) controlled by tumour suppressor proteins coded by tumour suppressor genes. However, high concentrations of free radicals cause damage to DNA. When the tumour suppressor genes in the DNA are mutated, the tumour suppressor proteins coded can become non-functional and as a result, cells proliferate and cancer develops. Antioxidants have the ability to scavenge free radicals and consequently reduce or prevent ROS-induced damage to DNA. Therefore they can potentially prevent cancers (National Cancer Institute, n.d.).[32] Apart from that, some phenolic compounds are also found to inhibit cell proliferation and cell migration by interfering with epithelial-to-mesenchymal transition (EMT), cell invasion and extravasation in vitro (Anantharaju et al., 2016).[23]

In vivo evidence-natural antioxidants

Observational studies on natural antioxidants and the risk of cancers produced inconsistent results. Most of them do not support the protective role of natural antioxidants against cancer.

The Rotterdam Study is a population-based prospective cohort study on 7983 Dutch women aged 55 and above with no history of breast cancer. It aimed to find the association between total dietary antioxidant capacity, individual antioxidant intake and breast cancer risk. They found that a high total dietary antioxidant capacity was associated with lower postmenopausal breast cancer risk. Although this shows that natural antioxidants are overall effective at preventing breast cancer, that might not be the case, because supplement intake, which could have affected the total antioxidant capacity, was not taken into consideration. The total antioxidant capacity was assessed by dietary ferric reducing antioxidant potential (FRAP), which measures the ability of antioxidants in food items to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). FRAP scores and antioxidant intake were assessed by a semiquantitative food-frequency questionnaire (SFFQ) (Pantavos et al., 2014).[33] The ability of antioxidants to reduce ferric ions measured in vitro may not reflect its effectiveness in vivo owing to factors like bioavailability. Nevertheless, it was a useful method to estimate and quantify the effectiveness of each antioxidant in terms of reducing and stabilising free radicals. Even though no overall association was found between individual antioxidant intake and risk of breast cancer, low intake of flavonoids was associated with higher breast cancer risk

among women aged 70 and above. This means that flavonoids may be effective at preventing cancer. The study provides some confidence for the protective role of natural antioxidants against breast cancer, since there were adjustments for confounding variables like hormone use, reproductive history and dietary fat intake and the median follow-up was 17 years, which was appropriate to show the long-term effect of natural antioxidant intake on the risk of breast cancer.

Feskanich et al. (2000)[34] conducted a prospective cohort study on 77283 women and 47778 men and found that high fruit and vegetable consumption was associated with modestly lower lung cancer risk among non-smokers and women who smoke (Feskanich et al., 2000).[34] In contrast, Michels et al. (2000), another prospective cohort study on 88764 women and 47325 men, found little association between colon and rectal cancer incidence and fruit and vegetable consumption. Therefore, it was concluded that frequent intake of fruits and vegetables does not appear to provide protection against colon and rectal cancers (Michels et al., 2000).[35] Both studies investigated the relationship between cancer and fruit and vegetable consumption rather than antioxidant intake. Despite that, fruits and vegetables are natural sources of antioxidants, so it is appropriate to use such studies.

According to evidence presented above, natural antioxidants generally have little or no association with the risk of developing cancer. Although some show an inverse relationship between them, this is usually limited to sub-groups within the studies (eg. women over 70, non-smokers), which means a number of factors, both genetic and environmental, contribute to the development of cancer. Unlike in the case of CVD, oxidative stress and free radical-induced damage may play a little role in the development of cancer, making the effect of natural antioxidants on cancer ambiguous.

In vivo evidence-supplementary antioxidants

Evidence of antioxidant supplements is also highly variable.

A randomised controlled trial was conducted in Linxian, China and all participants were given daily supplements (a combination of selenium, vitamin E and β-carotene). Ten years after the cessation of supplementation, they found that participants who took the supplements had a reduction of 11% in gastric cancer mortality than those who did not receive the supplements, and this effect was more evident in younger participants (aged 55 and below). Esophageal cancer mortality decreased by 17% in participants aged below 55 and increased by 14% in those older than 55 years (Qiao et al., 2009).[36] This suggests that antioxidant supplements are more effective at reducing cancer death

rates in younger populations compared to older ones. As discussed before, antioxidants are able to inhibit cell proliferation and cell migration by interfering with epithelial-to-mesenchymal transition (EMT) in vitro. As a result, they are able to slow down the growth of cancerous cells, which could be the reason why they reduced cancer mortality rate. The double-blind technique means that the decrease in mortality rate was unlikely to be the result of participants detecting the incidence of cancer and receiving treatment at an early stage of cancer development, because they were unaware of the aim of the study and that the study was about cancer. This gives more confidence that the reduction in the cancer mortality rate was due to antioxidant supplementation, rather than other factors. However, the fact that the Linxian population was undernourished might limit the generalisability of the results to well-nourished populations.

A randomised controlled trial conducted on 29133 male smokers from southwestern Finland found that long term supplementation of alpha-tocopherol and beta-carotene had no protective effect against urothelial cancer (Vitamo et al., 2000).[37] Similarly, according to Rautalahti et al. (1999), alpha-tocopherol and beta-carotene had no statistically significant preventive effect against the rate of incidence of pancreatic carcinoma. Supplementation with alpha-tocopherol even increased the rate of incidence of pancreatic carcinoma by 34% relative to those who did not take supplements (Rautalahti et al., 1999).[38] This paradoxical role of supplementary antioxidants in cancer could be explained by their pro-oxidant activities and the fact they suppress normal functions of ROS, which will be discussed in more detail in the section of adverse effects.

Sub-conclusion

The inconsistency in the findings could be explained by the difference in sample, dose used and the duration of the studies. Some studies used are population-based (Pantavos et al., 2014; Qiao et al., 2009; Vitamo et al., 2000; Rautalahti et al., 1999),[34,36,37,38] which means the results might only be applicable to the particular population upon which the study was conducted. Some studies recruited participants who are at high risk of developing cancers, for example, smokers (Vitamo et al., 2000)[37] while some did not.

Furthermore, cancer is a multifactorial disease. This means that it is developed due to both genetic and environmental factors, and different types of cancers may be influenced by different factors. When cancer develops due to genetic predisposition, then antioxidants are unlikely to prevent its occurrence. On the other hand, when one

type of cancer (for example, gastric and colorectal cancer) is more influenced by environmental factors like diet, then the intake of antioxidants is more likely to have an effect on it. Despite that, as discussed earlier, little association was found between fruit and vegetable consumption and colon and rectal cancer incidence, adding to the ambiguity of the effect of antioxidants on cancer.

In conclusion, the effect of both natural and supplementary antioxidants on cancer is not clear. This is because some cancers such as gastric and colorectal cancers are more related to diet and environmental factors than others, so the role of antioxidants may vary in different types of cancers.

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[38] Rautalahti, M. T., Virtamo, J. R., Taylor, P. R., Heinonen, O. P., Albanes, D., Haukka, J. K., Edwards, B. K., Kärkkäinen, P. A., Stolzenberg-Solomon, R. Z., & Huttunen, J. (1999). The effects of supplementation with alpha-tocopherol and beta-carotene on the incidence and mortality of carcinoma of the pancreas in a randomized, controlled trial. *Cancer*, 86(1), 37–42.

1.1.13 Type 2 Diabetes Mellitus (T2DM)

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterised by elevated blood glucose levels. Although obesity and physical inactivity are known to be major risk factors for type 2 diabetes (T2DM), recent evidence suggests that oxidative stress may contribute to the pathogenesis of T2DM by increasing insulin resistance or impairing insulin secretion. Insulin is a hormone secreted by pancreatic beta cells to stimulate the uptake of blood glucose by liver and muscle cells (Bajaj & Khan, 2012).[39] When insulin secretion is impaired, blood glucose level would become too high, leading to T2DM. Antioxidants prevent oxidative stress by scavenging free radicals and ROS, so they have the potential to prevent T2DM.

In vivo evidence-natural antioxidants

Past observational studies found an inverse association between natural antioxidant intake and the risk of T2DM.
Montonen et al. (2004) was a cohort study involving following up “a cohort of 2,285 men and 2,019 women 40–69 years of age and free of diabetes” for 23 years. They found that intakes of vitamin E, α-tocopherol, γ-tocopherol, δ-tocopherol, and β-tocotrienol were inversely related to a risk of T2DM. Covariates like smoking status, blood pressure, body weight and height were adjusted, which ensured the validity of the results. (Montonen et al., 2004).[40] Another cohort study conducted on 338 men found also found an inverse relationship between vitamin C intake from fruits and vegetables and glucose intolerance, which suggests the protective role of vitamin C against T2DM (Feskens et al., 1995).[41] Plasma glucose level was accurately measured 2 hours after the glucose load of 75g and there were adjustments for age, past body mass index, and past energy intake, making the study convincing. It is clear that a variety of natural antioxidants are effective at preventing the incidence of T2DM. Particularly, vitamin E is suggested to protect the cell plasma membranes by inhibiting the chain reaction of lipid peroxidation, which prevents damage to pancreatic beta cells and ensures normal production of insulin.

In vivo evidence-supplementary antioxidants

Evidence of antioxidant supplements showed their protective role against T2DM. Paolisso et al.(1993) conducted a randomised controlled study by randomly assigning type 2 diabetic patients to the group that received vitamin E supplementation or the

group that received placebo. They found that daily vitamin E supplements seem to produce an improvement in the metabolic control in type II diabetic patients (Paolisso et al., 1993).[42] Its double-blind technique reduced bias, thus increasing the validity and reliability of the results. However, the sample size was very small (25 patients) and this means the results are less likely to be generalisable to a wider population. Furthermore, the follow-up period was 3 months so it is inadequate to draw conclusions about the effect of long-term administration of vitamin E. Nevertheless, the long-term benefit of vitamin E supplementation can be backed up by the meta-analysis conducted by Akbar et al. (2011), which concluded that a higher dose and longer duration studies with vitamin E were associated with a reduction in the early glycation end product, HbA1C (a biomarker of type 2 diabetes), after assessing 14 relevant studies (Akbar et al., 2011).[43] However, antioxidant supplementation does not affect plasma glucose or insulin levels, suggesting that they do not impact the pathogenesis of insulin resistance (Akbar et al., 2011).[43] This means that supplementary antioxidants are unlikely to prevent the occurrence of type 2 diabetes and they could only protect type II diabetic patients from severe symptoms and complications.

Sub-conclusion

According to past observational studies, it can be concluded that natural antioxidants are effective at preventing healthy people from type 2 diabetes. Antioxidant supplementation, especially vitamin E, is effective at reducing the severity of the disease in patients with type 2 diabetes.

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1.1.2 Adverse effects

In vitro evidence

In vitro evidence shows that antioxidants can display pro-oxidant activities when at high concentrations, under certain pHs and in the presence of metal ions. Pro-oxidants are substances that encourage the generation of free radicals and contribute to oxidative stress. For example, quercetin, when at high concentrations, can promote superoxide radical (O2•-) generation within isolated mitochondria and cultured cells. Phenolic compounds show pro-oxidant activities in the presence of copper ions (Bouayed & Bohn, 2010).[25]

In vivo evidence

In vivo evidence also demonstrates the adverse effects of high doses of antioxidant intakes. Podmore et al. (2010) found that there was an increase of oxidative damage in the DNA of lymphocytes in patients who were supplemented with high doses of vitamin C (500 mg/d). This shows the damage caused by the pro-oxidative effects of antioxidants when at high concentrations (Podmore et al., 2010).[44] Another meta-analysis by Bjelakovic et al. (2012) concluded that high doses of vitamin beta-carotene, vitamin E and vitamin A supplementation seem to increase overall mortality in both healthy people and patients with various diseases (Bjelakovic et al., 2012).[45] They found that the group taking antioxidant supplements were 1.03 times more likely to die, compared to the control group that received placebo or no intervention. Firstly, an increase of 3% in mortality rate is not very significant and it is unclear if antioxidant supplements are responsible for this increase, because people die due to various reasons and there might be other unconsidered reasons that have caused this. Therefore, I would argue that the overall mortality rate would be too broad as a measurement for the adverse effects of supplementary antioxidants. Also, this meta-analysis may be biassed in study selection because it included quasi-randomised trials for the assessment of the danger of antioxidants but not for the assessment of the benefit of antioxidants. Quasi-randomized trials are less reliable than randomised trials because participants are not assigned to the groups completely randomly, so there is selection bias. The criteria for selecting studies used in both assessments should be standardised. Therefore, I believe that this meta-analysis is not convincing enough to demonstrate the adverse effects of antioxidants.

A better demonstration of the adverse effect of antioxidants in vivo would be the fact that high doses of antioxidant supplementation increased esophageal cancer mortality by 14% in those who aged above 55 years in the Linxian population and increased pancreatic carcinoma by 34% in male smokers (Qiao et al., 2009; Rautalahti et al., 1999).[36,38] These figures are more significant and the sample sizes of those two studies (29,584 and 29,133 respectively) are large enough to prove that the results were not found by chance. These results could be explained by the pro-oxidant activities discussed earlier and also by the suppression of normal functions of ROS, which will be discussed later.

It is worth noticing that pro-oxidant effects of antioxidants are not inherently detrimental to health. For example, polyphenols exhibit antidepressant activities by modulating neurotransmitters (serotonin and noradrenaline) when present at high concentrations (Bouayed & Bohn, 2010).[25] As mentioned in section 5, the pro-oxidant activity of some polyphenols can cause mitochondrial dysfunction, thus leading to cell death. This can potentially be a mechanism to kill cancer cells. Despite that, in most cases, pro-oxidant activities are undesirable as they add to oxidative stress by encouraging the production of free radicals.

As mentioned in section 2.3 Effects of free radicals, at low concentrations, ROS are beneficial for human health. They play an important role in normal physiological functions such as cellular signalling, gene expression, the regulation of immune responses and fostering antioxidative defence mechanisms. (Bouayed & Bohn, 2010; Martin & Barrett, 2002).[25,46] Antioxidants protect the cells from the toxic effects of ROS but they also suppress the normal physiological functions of ROS, thus disrupting normal metabolic processes. For instance, ROS could induce apoptosis of genetically damaged and preneoplastic cells. As mentioned before, cells attacked by ROS can die. There are two types of death - necrosis and apoptosis. Necrosis is when severely injured cells die from extensive plasma membrane damage and loss of cellular homeostasis. Necrosis of normal working cells is always unwanted. In contrast, apoptosis is the self-destruction of genetically damaged and preneoplastic cells without eliciting an immune response, which is beneficial (Martin & Barrett, 2002).[46] Antioxidants suppress both types of cell death. When apoptosis is prevented, cancerous cells do not die and proliferate, thus leading to the development of cancer. This could explain the increase in cancer incidence and mortality rate after antioxidant supplementation and also the paradoxical effect of antioxidant supplementation on cancer.

Therefore, to conclude this section, antioxidants are needed to prevent the concentration of ROS from getting too high, thus protecting cells from oxidative damage. However, the dose of antioxidant intake should be controlled, because at high doses, antioxidants exhibit pro-oxidant activities and suppress the normal physiological functions of ROS. The balance between the concentration of antioxidants and the concentration of free radicals/ROS is important.

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1.2 Mental Health

Mental disorders are the disorders that can affect one's mood, thinking and behaviour. Some common mental disorders are depression and anxiety. Depression is characterised by low mood, lack of interest, a feeling of low self-worth and disturbed sleep and appetite. Anxiety is a "a psychological state in which the feeling of fear is disproportionate to the nature of the threat" (Bouayed, 2010).[51]

Excessive ROS causes oxidative stress and damages the cells. Brain cells are particularly vulnerable to oxidative stress because of their higher oxygen consumption, higher lipid content and weaker antioxidative defence (Bhatt et al., 2020).[47] Therefore, oxidative stress is involved in the pathogenesis of many mental health disorders, including depression and anxiety.

In vivo evidence-natural antioxidants

Observational studies found that natural antioxidants are beneficial for mental health as they are able to reduce the risk of common mental disorders and improve symptoms. Fruits and vegetables contain natural antioxidants. A cross-sectional study conducted on 60404 adults in Australia found that increasing fruit and vegetable consumption may help reduce psychological distress in middle-aged and older adults (Nguyen et al., 2017).[48] Similarly, another study based on the National Population Health Survey in Canada also found an inverse relationship between fruit and vegetable intake and the risk of depression. (Kingsbury et al., 2015).[49]

Data including fruit and vegetable consumption in these two studies, are self-reported by participants through questionnaires, so they might be unreliable due to inaccurate memory. However, the large sample gathered and the adjustments for covariates like sociodemographic characteristics and lifestyle risk factors mean that the results should be representative and valid. Although behaviours like smoking and physical exercise may have a more important impact on depression than fruit and vegetable intake, these two pieces of evidence still provide confidence in the protective role of natural antioxidants against mood disorders, thus benefiting mental health.

In vivo evidence-supplementary antioxidants

According to evidence, supplementary antioxidants are also found to be beneficial for mental health. Gautam et al. (2012) found that patients with GAD and depression had significantly lower levels of vitamin C, A and E plasma concentrations compared to the

healthy controls. They also found that there was a reduction in depression and anxiety both in all of the groups (both the experimental group consisted of depression and generalised anxiety disorder (GAD) patients and the control group consisted of mentally healthy participants) who received antioxidant supplementation for 6 weeks. This suggests that supplementary antioxidants are effective in reducing symptoms of GAD and depression, thus improving mental health. One strength of this study is that all patients of the groups were matched on sociodemographic data and they had similar diets during the period of the study, so the results are comparable. However, this study had a small sample size of 100 people, therefore the results may not be generalisable to a wider population. Furthermore, the GAD and depression scores were obtained from the Max Hamilton Anxiety Rating Scale (HAM-A) and Max Hamilton Depression Rating Scale (HAM-D), respectively. These two rating scales depend on the clinician's interpretation of the subjects' response during the interview, so there might be interviewer biases (Gautam et al., 2012).[50] However, it could be argued that the use of an interview is still better than the use of a questionnaire, which entirely relies on self-report and each participant's subjective feelings. Therefore, this study indeed provides some confidence that supplementary antioxidants are effective at reducing symptoms of mood disorders.

Although antioxidants are shown to be effective in improving symptoms of mood disorders, one might question why we use them, since we already have effective treatments such as antidepressants. The first reason is that it has been found that dietary polyphenols display antidepressant activity at relatively lower doses, therefore they can be a new approach to treat depression (Bouayed, 2010).[51] Furthermore, many medications aimed to treat mood disorders have severe adverse effects like dependency. In contrast, polyphenols are found to have similar anxiolytic effects as benzodiazepines without side effects like dependency, so administration of polyphenols can potentially be an effective alternative to benzodiazepines to treat anxiety (Bouayed, 2010).[51] It is interesting that the anxiolytic effect of polyphenols does not come from their antioxidant properties as one might have expected. It is due to their ability to act as a benzodiazepine receptor agonist, which means that they combine with benzodiazepine receptors on the neurons to induce an anti-anxiety effect. Therefore, although antioxidants are found to be beneficial for mental health, it is worth investigating whether the mechanism behind this is due to their antioxidant properties (ie. their ability to prevent oxidative stress) or their other properties. Although it is clear that antioxidants have the potential to treat mental disorders, more clinical trials need to be conducted to

determine the effectiveness, potential side effects and cost-efficacy before prescribing them to patients.

Sub-conclusion

Overall, research showed a positive effect of both natural and supplementary antioxidants in terms of improving symptoms and reducing risks of mental health disorders. However, there are limited studies conducted on this topic and most of them are observational studies that do not establish a causal relationship, so more randomised controlled trials are needed for a definite conclusion.

[47] Bhatt, S., Nagappa, A. N., & Patil, C. R. (2020). Role of oxidative stress in depression. *Drug discovery today*, *25*(7), 1270–1276. https://doi.org/10.1016/j.drudis.2020.05.001

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2. Types of in vivo studies

There are many types of in vivo studies and they all have associated advantages and disadvantages. This section will discuss the nature of the studies used and how they impact the strength of the conclusions.

The first type is observational studies, which are “a type of study in which individuals are observed or certain outcomes are measured. No attempt is made to affect the outcome.” (National Cancer Institute, n.d.).[32] Observational studies include case-control and cohort studies. Case-control studies are studies that compare two groups of people - one group consisted of participants with the disease (case) while the other consisted of participants without the disease (control). The researchers study the medical and lifestyle histories of the participants and try to find out what factors might be associated with the development of the disease. Cohort studies are longitudinal studies that are used to determine the incidence or risk of a condition by following a group of participants for a long period of time. They allow the researchers to quantify the strength of association between exposure and the risk of developing a condition. However, observational studies are inadequate to establish causal relationships because there is a risk of selection and observation biases. Also, there are confounding variables which are difficult to account for (National Cancer Institute, n.d.; “Randomised controlled trials”, n.d.).[32, 52]

Another type is randomised controlled trials (RCTs). They involve randomly assigning a group of similar participants into two groups (experimental/treatment and control/placebo). The results obtained from the two groups are obtained. This type of study is more reliable than observational studies and is able to establish a causal relationship between the independent and dependent variables. This is because experimental techniques are used to reduce biases. For example, confounding variables, whether measured or unmeasured, can be accounted for by the technique of randomisation. Furthermore, blinding is used to reduce observation bias (“Randomised controlled trials”, n.d.).[52]

The in vivo evidence used to evaluate the effectiveness of supplementary antioxidants is mostly RCTs, which are very reliable. In contrast, the in vivo evidence used for natural antioxidants (obtained from fruits and vegetables) are mostly cohort studies. This can lower the quality of the conclusion made. However, it is very difficult to conduct RCTs in this case since it is arguably unethical to limit or eliminate participants’ fruit and

vegetable consumption for the sake of the study. Therefore, observational studies where no intervention is involved are more appropriate.

[52] *Randomized controlled trials - the gold standard research design for causal effects*. Accredited Professional Statistician For Hire. (n.d.). Retrieved January 17, 2023, from https://www.scalestatistics.com/randomized-controlled-trial.html

Conclusion

The aim of this project is to assess the extent to which exogenous antioxidants are beneficial for health. Exogenous antioxidants can be further categorised into natural and supplementary, and health can be separated into physiological and mental health. Physiological health is further measured by the ability to prevent or treat age-related diseases (CVD, cancers and T2DM) and adverse effects.

Regarding disease prevention, all in vitro evidence included suggests that antioxidants offer protection against age-related diseases. In vivo evidence suggests that some types of natural antioxidants from fruits and vegetables (Vitamin C and E) are effective for protecting from CVD. In contrast, supplementary antioxidants are found ineffective in protecting against CVD. Although some studies found that vitamin E supplements are associated with reduced risk of CVD, these studies were mostly funded by pharmaceutical companies so the results can be biassed.
The studies show controversial results on the role of both natural and supplementary antioxidants in cancers, since there are many different types of cancers and their pathogenesis can be different. Therefore, the effect of antioxidants on cancers is unclear.
In contrast, both natural and supplementary antioxidants are able to offer protection against type II diabetes, according to in vivo evidence. This is probably because type II diabetes is more related to diet, so antioxidant intakes can have a greater impact on improving the symptoms of this disease.
Overall, it can be concluded that natural antioxidants are effective in preventing age-related diseases, whilst the effect of supplementary antioxidants needs to be assessed by more RCTs to see if long-term supplementation is appropriate.

Long-term supplementation increases the concentration of antioxidants in the body very rapidly and this is also linked to pro-oxidant activities, which happen when antioxidants promote the generation of free radicals rather than stabilising them. Pro-oxidant activities are ambivalent but in most cases undesirable, so they are considered as an adverse effect of antioxidants. Furthermore, high concentrations of antioxidants also prevent ROS from performing their normal physiological functions, thus affecting health negatively. Despite that, I believe that the benefits of antioxidants still outweigh their adverse effects. However, it is important to keep a balance between the concentration

of antioxidants and free radicals, so the dose of antioxidant intake needs to be monitored.

According to evidence, both natural and supplementary antioxidants improve mental health as they are effective in reducing symptoms of depression and anxiety in patients. However, there are limited studies available on this topic and the sample sizes investigated in those studies are quite small. Therefore, more studies are needed before making a definite conclusion.

In summary, to a larger extent, exogenous antioxidants are beneficial for health. They generally play a preventive role in age-related diseases and mood disorders. Although there can be some undesirable side effects when the intake dose is large, they are still essential for maintaining health both physiologically and mentally, as long as the dose is moderate.

Evaluation

This project aims to explore the extent to which exogenous antioxidants are beneficial for human health and I believe this aim has been achieved by looking at the mechanisms of antioxidants and their effects on physiological and mental health. However, there are a few limitations of this project.

Limitations

Some studies used to draw the conclusion are of low quality. Firstly, some of them have flaws in methods of data collection, like the use of questionnaires, which can lead to unreliable results. Secondly, some are conducted on a particular population, so the results may not be generalisable to the whole human population. Furthermore, case-control studies which may involve selection bias are used. These are all factors that can decrease the validity of my conclusion.
Another issue is that the studies I used to assess the effect of natural antioxidants on human health are all observational studies because I did not find any relevant randomised controlled trials (RCTs), which are better to establish a causal relationship. This can lower the validity of my conclusion about natural antioxidants.
A reason for the lack of RCTs could be that it is unethical to control fruit and vegetable consumption of participants as it is apparent that a reduction in them would harm participants' health, so it would be difficult to conduct RCTs involving natural antioxidants. However, it might also mean that my research was not thorough enough as I obtained evidence mainly from online sources. To improve this, I will also include printed sources such as books. Despite that, a lot of the time I did not have open access to some high-quality studies so I would have to compromise by citing only the abstract or give up the whole study altogether if there was no useful information in the abstract. This limited the quality of the evidence used for conclusion in this project.

Extension & Further work

The project can be further expanded in several ways. Due to the time limit, I omitted the section discussing the relationship between social well-being and antioxidants. Although antioxidant intakes do not directly affect someone's social status and relationships, as I mentioned in section 1 of the discussion, they can indirectly affect them. As antioxidants are concluded to be beneficial for mental health since they alleviate mood disorders,

this can make the person more willing to socialise with others, thus potentially improving their social well-being.
Furthermore, people's socioeconomic status can affect their availability of antioxidants. For example, the more affluent may be more educated about the benefits of antioxidants and more likely to be able to afford antioxidant supplements than others. This can bring the topic to a wider context as some social and economic aspects of antioxidants, for example, their cost-efficacy, can also be involved.
Another potential topic that I can include is antioxidants as food additives. This is because many countries use antioxidants to preserve food. The implications on health of those antioxidant preservatives may be worth investigating.
Cultural differences in antioxidant intake can also lead to an interesting discussion. For instance, people in Japan usually have a diet rich in antioxidants and Japan has been ranked top for highest life expectancy. Therefore, more research could be conducted to investigate the level of antioxidant intakes and health status between different cultures and populations.
For this project, I extensively used studies conducted by others in this project without collecting my own data. Therefore, for further work, I could gather my own data by interviewing an expert in the field and use it in my discussion.

Problems encountered

I encountered many problems on the way. I found it challenging to understand many journal articles written by professionals, so I had to search for the meaning of difficult terms and ask my tutor. Another problem is that during initial research, there was too much information on antioxidants and free radicals, since this was a broad topic and I was tempted to include everything. Then, I realised that much of the information was repetitive, so I learned to be more selective and included only the necessary ones.
Furthermore, I also had problems structuring my project in a reasonable way because I found that much information in the literature review would be repeated in the discussion. Then, I realised that I did not need to follow a conventional structure, so I arranged it in a thematic way, which allows the reader to acquire relevant information first before reading my discussion. Overall, I managed to stick to my original plan despite the procrastination. Of course, there were some slight changes such as delaying the presentation from February to March.

What I learned

The process of completing this project was hard but rewarding as I have learned a lot. I have gained more knowledge about the topic of antioxidants after doing extensive research and this can be useful for my future studies as I want to study biology at university. I have also developed several skills. Evaluating the sources improved my critical thinking skill as I learned to look at both sides of the sources rather than trusting what is given straight away. Furthermore, my time management skill has been enhanced as I have learned to divide my time between EPQ and school work.

Final Summary & Suggestions for future projects

Overall, this project has been a very valuable experience for me as I am now more confident in doing academic writing and undertaking other projects in the future. If I were to undertake another project, I would plan more realistically by giving myself more time for initial research and final review. This is because I realised that I underestimated the time I needed for proofreading and reviewing. More importantly, I would break the project down into smaller, manageable tasks from the beginning to reduce my procrastination.

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