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Review

Biomolecular content of camel milk: A traditional superfood towards future healthcare industry

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ABSTRACT

Drinking non-bovine milk has been reported to possess bio-functionality for regular consumers. Camel milk is a traditional product that has been used for many years in arid rural communities of Asia and Africa as a biomedicine to cure several health issues such as asthma, oedema, and diabetes. The product consists of appropriate amounts of bioactive compounds. In addition, it contains low amounts of fatty acids and cholesterol, whilst it does not contain β-lactoglobulin. The latter, which is present in bovine milk, causes allergic symptoms in some people. The similarity of the formula to human milk suggests this superfood as an alternative for bovine milk with complete nutrition for infants. In this review, the biomolecules present in camel milk and their positive roles on the health of consumers are extensively discussed.

1. Introduction

Functional foods are the products which resemble traditional foods with verified physiological benefits, offering to improve public health and decreasing the risk of diseases (Al-Sheraji et al., 2013), thus the demands of consumers for these products have been increasing in recent years (Vieira da Silva, Barreira, & Oliveira, 2016). The dairy industry has the potential to become one of the main sources of these products, due to its well-adjusted composition and several biological activities (Kamal & Karoui, 2016; Shuiep, Giambra, El Zubeir, & Erhardt, 2013). Different starters of lactic acid bacteria (*LAB*) isolated from dairy products may possess properties beneficial to health (Rutella, Tagliazucchi, & Solieri, 2016).

Dairy farms have been exposed to extreme economic pressures during 2013–2015, as the prices of bovine milk (BM) have reduced by about 25% (Kersting, Hüttel, & Odening, 2016). The consumption of non-bovine milk in last fifty years has been increased to 17% of the total world milk consumption. The new sources of milk can be used as a substitute for BM to supply the required human nutrition as same quality as BM, improve the medication effects of dairy products which are daily consumed, and eliminate the allergy complications caused by BM-derived products in some people (Alhaj et al., 2013).

Camels are an ideal domestic animal, especially in hot regions, due to the remarkable capability to live in harsh conditions and with little accessibility to water (Salmen, Abu-Tarboush, Al-Saleh, & Metwalli, 2012). The presence of about 18–25 million camel heads has been reported up to now, while camel products have not been widely investigated (Ahmad et al., 2012; Al-Zoreky & Al-Otaibi, 2015; El-Fakharany, Serour, Abdelrahman, Haroun, & Redwan, 2009; Maaroufi, Rezaei, Raftaniamiri, & Mirzaei, 2015). Nevertheless, camel milk (CM) can be considered as one of the alternatives of BM with enhanced functions and better digestibility than BM in human the gastrointestinal system (Salami et al., 2009; Yaqoob & Nawaz, 2007). CM not only provides the required nutrition for local people, but also offers several therapeutic properties (Bai & Zhao, 2015). The whole production of CM was estimated to be 1.3×10^6 tons (Ziane, Couvert, Le, Moussa-boudjemaa, & Leguerinel, 2016), with the global trade of $10 billion per year. In near

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Abbreviation

α-LA	α-Lactalbumin
β-LG	β-Lactoglobulin
BM	Bovine Milk
CM	Camel Milk
CSA	Camel Serum Albumin
CN	Casein
DSC	Differential Scanning Calorimetry
FA	Fatty Acid
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LAB	Lactic Acid Bacteria
LF	Lactoferrin
LP	Lactoperoxidase
MY	Milk Yield
MW	Molecular Weight
NPN	Non-Protein Nitrogen
TG	Triglyceride
WP	Whey Protein

future however, it is expected that dairy industries produce higher amount of CM (Al-Ashqar, Al-Mohammad Salem, Al Herz, Al-Haroon, & Alluwaimi, 2015).

The lack of β-lactoglobulin (β-LG) in CM, along with larger quantities of fatty acids (FAs) and fructose, having 3–5 folds higher vitamin C in comparison with BM, high contents of antimicrobial agents such as lysozyme, lactoperoxydase, lactoferrin, immunoglobulin, and bacteriocins mean CM is considered to bea nutritious product with high stability. It is possible to store CM at 30°C for 5 days, while BM is contaminated only after 48 h (Ahamad, Raish, Ahmad, & Shakeel, 2016).

In recent years, CM as a new source for production of dairy products has been given much attention due to its therapeutic properties (Agrawal et al., 2007; Shuangquan, Tsuda & Miyamoto, 2008). Furthermore, CM has been recommended to be consumed for treatment of dropsy, jaundice, tuberculosis, asthma, leishmaniasis or kala-azar (Abdelgadir, Ahmed, & Dirar, 1998; El-agamy, Nawar, Shamsia, Awad, & Haenlein, 2009). Therefore, CM products might have a growth potential in future market of the dairy industry.

The objective of this review is to elucidate the biomolecular characterisation of CM with emphasis on the bioactivities of these molecules as well as their potential benefits for human health.

2. Camel milk composition

2.1. General composition

Until the fifth parity, female-camels can produce large amounts of milk during whole the year, even in dry seasons, due to exceptional adaptation of camel to poor quality and quantity of feed in harsh conditions (Konuspayeva et al., 2010). Milk yield (MY) in camels usually ranges from 3.5 kg/day in a hot summer and under intensive management to 20.0 kg/day in a rainy season and under more favorable conditions (Ahmad et al., 2012). Nevertheless, contribution of CM in household food baskets of the inhabitants of dry regions in dry seasons is higher than any other times (Elhadi, Nyariki, & Wasonga, 2015). MY and milk compositions are influenced by several factors such as age, parity, seasonal variations, ecological conditions, geographical origins, feeding strategy and stage of lactation, and individual variation (Abdalla et al., 2015; Al-Masri et al., 2014; Nagy & Juhasz, 2016). Also, time and number of milkings significantly influence CM composition. It has been shown that with 4 L/day as a result of 6 milkings/day, the MY is reduced to 2.5 L/day if milking once a day (Abdalla et al., 2015). Machine milking of dromedaries is now under design to improve milk production (Nagy & Juhasz, 2016). Nevertheless, camels have not yet been well appreciated for milk production. Accordingly, genetic improvement of camels in order to increase the MY has been rarely considered (Almutairi, Boujenane, Musaad, & Awad-Acharari, 2010; Jans et al., 2013). The only published example in this case has reported a yearly genetic development equal to 50 g MY (Nagy, Skidmore, & Juhasz, 2013).

Either in raw or fermented form, CM is a crucial product which provides nutrition and energy requirements of the minor population of rural communities in dry regions of Africa and Middle East (Benkerroum, Boughdadi, Bennani, & Hidane, 2003; Shori, 2012, 2015). It covers all essential biomaterials including proteins, carbohydrates, fats, high amounts of vitamins and minerals, and possesses great biological value due to its significant content of heat resistant antimicrobials (Ahamad et al., 2016; Felfoul, Lopez, Gaucheron, & Attia, 2015; Rahman, Al-hakmani, Al-alawi, & Al-marhubi, 2012).

Camel's access to water is an important factor which affects the concentration of the various CM components. Milk from camels is more diluted than BM, sometimes reaching up to 91% water, which in desert areas is desirable for the calf (Zhao, Bai, & Niu, 2015). The composition of CM is different from that of milk from other ruminants. It contains lower amounts of fats, proteins and carbohydrates, but higher amounts of vitamins and minerals (Arab et al., 2014) (Konuspayeva, Faye, Pauw, & Focant, 2011). Two important camel types are the Dromedary (one-humped camel) living in the deserts (90% of total camels) and Bactrian (two-humped camel) living in cooler areas (10% of total camels) (Salmen et al., 2012), the Bactrian camel is a low dairy product producer (Konuspayeva et al., 2010). The variation in milk components from these, listed in Table 1, is due to physicochemical parameters rather than seasonal or regional variables (Faye, Konuspayeva, Messad, & Loiseau, 2008).

There are no reports we are aware of concerning allergy indicators possessed by CM. Thus, CM can be considered as a safe product for consumption by those with weak immune-systems. The lactose in CM is metabolized by lactose intolerant people with no trouble, probably because this lactose is more exposed to the action of the lactase (Shori, 2015).

CM consists of high amount of vitamins, especially thiamine (B1), riboflavin (B2) and ascorbic acid (C) (Ereifej, Alu'datt, Alkhalidy, Alli, & Rababah, 2011; Mohamed, Mousa, & Beynen, 2005). The availability of large amount of vitamin C in CM (24–52 mg/kg), i.e. three to five times higher than BM and 1.5 times higher than human milk, is of importance in arid areas where green foods are not easily accessed (Kamal & Karoui, 2016; Zhao et al., 2015; Ziane et al., 2016).

Non-protein nitrogen (NPN) is the second source of nitrogen in milk after proteins. In BM, NPN (including 50% urea) consists less than 6% of the nitrogen content, though in CM, this value is under

Table 1
The components of Bactrian and Dromedary camel milk (Faye et al., 2008).

Component	Bactrian camel milk	Dromedary camel milk
Protein content (%)	5.23 ± 1.17	4.76 ± 1.13
Lactose content (%)	2.77 ± 0.96	3.12 ± 0.92
Fat content (%)	6.67 ± 2.93	5.94 ± 2.26
Ash content (%)	1.0	1.0
Skimmed dry matter (%)	10.64 ± 3.11	10.87 ± 3.19

2% (i.e. in the range of 0–300 mg/L), governed by the seasonal conditions and directly correlated to total protein content (Faye, Konuspayeva, & Loiseau, 2010). This low value may be due to the adaptation of camels to harsh environmental conditions.

Minerals make up less than 1% of the CM including K > Cl > Ca > P > Na > Mg, Cu, Fe, and Zn (Yaqoob & Nawaz, 2007). The iron content of CM is about ten times higher than in BM (Ziane et al., 2016). Also the amounts of K and Cu are higher in CM than BM (Zhao et al., 2015). The heavy metals are in the range of harmless limits concerning the maximum daily intake of these elements (Ahamad et al., 2016; Nagy & Juhasz, 2016). The mineral content in CM is very similar to human milk. Thus, it is well possible to provide the nutritional mineral requirements of human by CM.

A collection of 130 volatile components, mostly from the groups of alcohol, acids and esters, have also been detected in CM using GC/MS (Li et al., 2011). Some examples are: 2-Methyl-1-propanol, 3-Methyl-1-butanol, 2-Hydroxy-propanoic acid ethyl ester, Octanoic acid ethyl ester, Acetic acid, 1-Heptanol, 1,2-Dichloro-benzene, 1-Octanol, Propanoic acid, 1-Nonanol, 3-(Methylthio)-1-propanol, Acetic acid 2-phenylethyl ester, 2-Decen-1-ol, Hexanoic acid, Phenylethyl alcohol, Heptanoic acid, Octanoic acid, etc.

2.2. Fat content of camel milk

CM has a very low fat content, including 96% triglycerides (TGs) (Ereifej et al., 2011) and quite a low amount of cholesterol, i.e. 30 mg/100 g dry matter (Salwa & Lina, 2010) (Ali M S Gorban & Izzeldin, 1999). The main part of fats is long chain FAs (FAs) (92–99%), though CM is poor source of short chain FAs (Ereifej et al., 2011). About 50–65% of total FAs are saturated, predominantly composed of C16:0 (35%), C14:0 (15%), and C18:0 (10%) (Gorban & Izzeldin, 2001). Around 35–50% of FA are polyunsaturated (C18:1 - C18:3) (Nagy et al., 2013), higher than that of in other milk sources (Ereifej et al., 2011). The homogenous form of FAs and low amount of carotenes are probably the reason of the smooth white colour of CM (Ibrahem & Zubeir, 2016).

The composition of FAs obtained from CM has been found to be different from country to country, and correlated to the environmental and farming conditions (Konuspayeva, Lemarie, Faye, Loiseau, & Montet, 2008). There is also a difference between the concentration of cholesterol in serum from cow and camel, suggesting a difference in lipid metabolism. The time of milk drawing, environmental factors (e.g. temperature and relative humidity), and physiological factors (e.g. diet, stage of lactation which normally takes 270–540 days (Abdurahman, 1996), and genetic differences within the species) govern the milk fat composition and cholesterol concentration. Addition of crude olive cake to the diet of settled lactating camels has been reported to have no effect on the fat content of the milk, but can modulate the FA composition by increasing linolenic and palmitic acid (Faye et al., 2013).

Among different tested milk samples (from buffalo, cow, goat and camel), the smallest fat globules have been found to correspond to camel (Fig. 1). It is maybe the reason that the digestibility of CM is the highest (Meena, Rajput, & Sharma, 2014). On the other hand, TGs' arrangement results in the existence of several crystalline forms, accordingly, the melting behaviour of the fat in CM is very complicated (Haddad, Mozzon, Strabbioli, & Frega, 2011). The size of fat globules and melting behaviour cause troubles in technological applications.

According to several studies, the FA composition of CM in comparison with BM contains a lower percentage of short-chain saturated FAs (C4-C12) and higher concentration of long-chain FAs such as stearic and palmitic acids (Farah, Streiff, & Bachmann, 1989).

2.3. Proteins and bioactive peptides

CM is an important source of proteins for a broad range of the population (Al-Ashqar et al., 2015). The total amount of protein is not similar in all CMs, even for the same breeds, but it is in the range of 2.5–5.5% according to season conditions (Zhao et al., 2015). The effect of lactation stage on protein content has been reported to be negligible (Konuspayeva et al., 2010).

Proteins of CM are split into caseins (CN) and whey proteins (WP) (Shuiep et al., 2013). CN involves 80% of BM proteins, though the amount of this protein in CM is in the range of 50–80%,

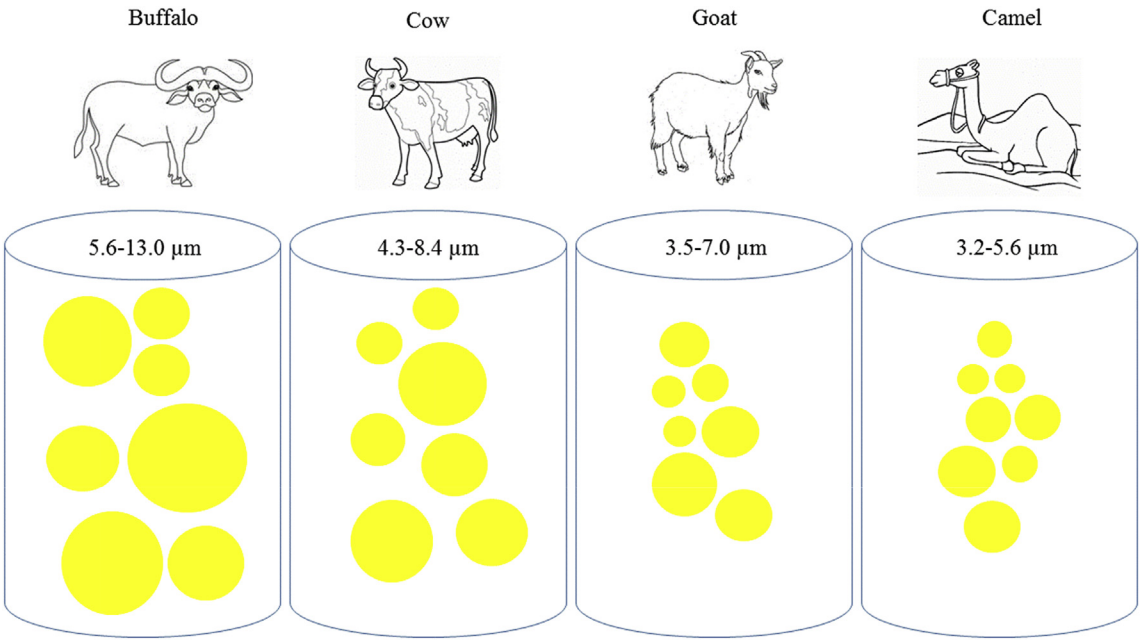

Fig. 1. Size distribution of fat particles in the milk from buffalo, cow, goat and camel (Meena et al., 2014).

Despite having 85% similarity in sequences, camel chymosin has different characteristics than bovine chymosin and shows seven-fold higher ratio of clotting to normal proteolytic action (Langholm Jensen et al., 2013). The enzyme from CM is slightly more thermostable than bovine chymosin. CM is not sensitive to coagulation after exposure to bovine chymosin. Given this information, performing the same procedure as used in the production of bovine cheese to produce camel cheese results in a product with an undesirable taste and not acceptable for local consumers. Fermentation by using camel chymosin has partially solved this problem by unique specificity towards camel κ-CN, thus providing a way to produce high quality camel cheese (Kappeler et al., 2006).

Although it is possible to make butter from CM, it takes a long time to churn the milk (Berhe, Seifu, & Kurtu, 2013). Pastoralists claim the difficulty of churning CM into butter because of its low tendency to cream due to the lack of a protein, namely agglutinin. The fat in CM is distributed as micelle-like globules and it is strictly bound to the protein. Moreover, the fat globule membrane of CM is thick (Haddad et al., 2011). The butter from CM has been used for clinical purposes or as hair pomade (Kappeler et al., 2006).

5. Camel milk as healthcare product

CM and its fermented products have been traditionally used for many years due to the belief that those promote bone formation in infants, and possess healing properties against many internal diseases (Lü, Hu, Dang, & Liu, 2014). Nowadays, it has been verified that these products include valuable nutrition, in addition to possessing exceptional functions such as antigenotoxic, anticytotoxic, antimicrobial, antioxidative, antithrombotic, antihypertensive, anthelmintic activity, immuno-modulatory, anti-inflammatory, hypoallergenic, hypoglycemic, and anti-hypertensive (Abdalla et al., 2015; Alhaider et al., 2014; Alimi et al., 2016; Arab et al., 2014; Nagy et al., 2013; Osman et al., 2014; Salwa & Lina, 2010). CM and its derived products have been therapeutically used to treat jaundice, lung and spleen-related ailments, asthma, anemia, autism, oedema, piles, milk allergies, hepatitis C virus, diarrhea-causing viruses tuberculosis, gastrointestinal ulcers, dermatological autoimmune diseases and more importantly, diabetes (Arab et al., 2014; Gorban & Izzeldin, 1999; Konuspayeva et al., 2011; Osman et al., 2014; Salwa & Lina, 2010). In addition, the cholesterol-lowering activity of CM by an undefined mechanism has been reported in rats (Al haj & Al Kanhal, 2010; Al-Numair, 2010). CM is digested in the stomach with no trouble and no allergic reactions. It also positively protects consumers against heavy metal toxicity and potential infections (Ahamad et al., 2016).

Yogurt from CM can be used as a probiotic with promising dose-dependent therapeutic properties (Elayan, Sulieman, & Salah, 2010). The minimum viable numbers of probiotics in the final fermented CM is considered to be around 10^6–10^7 CFU/g. Recently, a novel bacteriocin with special heat and pH stability produced by *Lactobacillus casei* isolated from fermented CM has been purified and characterized (Lü et al., 2014).

Moreover, there are several promising applications for daily consumption of CM for human health. For instance, CM has been proposed as a potential candidate for suppression of both alcoholic and non-alcoholic fatty liver disease (Althnaian, Albokhadaim, & El-Bahr, 2013). The alleviating effect of CM as complementary approach for the management of inflammatory bowel diseases has been also suggested (Arab et al., 2014).

As stated earlier, LF from CM induces oxidant stability and inhibits DNA damage through binding catalytic iron, and exhibits antibacterial and antiviral activities. Consequently, it can be influential to control diseases such as cancer, Alzheimer's, Hepatitis C, HIV and Tuberculosis. LF prevents the growth of colon tumour cells as well. Also, having high content of iron promoted using of CM to control hypochromic anemia (Ebaid et al., 2015; Habib, Ibrahim, Schneider-Stock, & Hassan, 2013).

It is common for inhabitants of camel rich regions to consume CM in its fresh or sour state with the purpose of diabetes treatment and improvement of wound healing (Althnaian et al., 2013). The latter is the consequence of interaction between inflammatory cells and biochemical mediators (Althnaian et al., 2013). CM has been reported to exhibit protective properties against diabetes, including type I and II, by reducing demand for insulin in patients and improving residual β-cell function in the pancreas, considering its immunomodulatory influence. The anti-inflammatory effect and high concentration of antioxidants probably possess positive roles in curing diabetes too (Limon et al., 2014). CM with high amount of insulin can resist against coagulum formation in the stomach, and therefore becomes available for absorption in the small intestine (Korish, Abdel Gader, & Alhaider, 2015). Interestingly, it has been reported that camel breeders in India, who regularly drink CM, have shown no diabetes mellitus, though in places which CM is not consumed, 5.5% have shown diabetes mellitus (Korish et al., 2015).

Additionally, regularly drinking of 500 mL CM over a period of a few months by type I diabetes mellitus patients has resulted in 30–35% reduction in the daily insulin requirements, with a significant drop in blood glucose (R P Agrawal et al., 2003; Agrawal, Jain, Shah, Chopra, & Agarwal, 2011; Agrawal et al., 2009). A similar study on type II diabetes patients has been carried out, showing increasing of insulin concentration from 64.59 to 84.03 pmol/L in only two months, probably due to glycemic control (Ejtahed et al., 2015). This time-, quantity-dependent effect has been previously reported in both humans and animals such as dogs and rats (Ebaid, 2014; Sboui et al., 2010). Also, the capability of insulin-like small molecules in CM to mimic insulin interaction with its receptor, transformation of insulin to indigestible nanoparticles and carry on this hormone into the bloodstream have been reported as the possible mechanisms to reduce the blood glucose (Malik, Al-Senaidy, Skrzypczak-Jankun, & Jankun, 2012). The above mentioned results support the idea of the beneficial effect of CM in the management of diabetes. Nevertheless, upscaling the laboratory experiments with larger human groups are required to endorse the curative results obtained from CM.

6. Conclusion

Dairy industries have produced bovine milk and bovine milk-derived products at different scales for many years. The benefits of dairy products to human health have been extensively studied and repeatedly emphasised. Although the recent development of new products from milk have been advanced slowly, the industry still survived, due to the importance of health-required components present in the milk. Nevertheless, the future industries tend to develop new products for consumers and finding new sources rather than cow. In this case, camel is probably a good candidate. The exceptional properties of camel milk encourage food technologists in the regions where there exist large camel populations, to produce and process camel milk. Towards bringing the benefits of camel milk in human diet, we recommend establishing a camel milk industry-traditional system.

In the current study, several recent advances concerning camel milk as a superfood and its applications with an emphasis on healthcare properties were described. The highlights of using camel milk are given in brief as below:

- CM is accessible in dry and semi-dry area.
- The nutritional value of CM is higher than BM.

- CM contains higher amounts of proteins with positive roles in immunology systems than BM.
- CM possesses little or no allergy effects, due to the lack of β-LG.
- The lactose from CM is metabolized by lactose intolerant people with no difficulty.
- CM exhibits great biological values due to its significant amount of bioactive peptides.
- CM's digestibility is greater than BM's.
- CM has been reported to possess alleviative roles against diabetes.

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CHAPTER 20

20. Free Radicals, Relation to Diseases and Protection against Them

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20.1. What Is Oxidative Stress?

Oxidative stress is a mechanism of damage to the body which co-participates in the pathogenesis of many diseases and their complications, such as atherosclerosis, diabetes mellitus, renal disease, ischaemia-reperfusion injury, neurodegenerative diseases, carcinogenesis and inflammatory diseases.

Oxidative stress is defined as an imbalance between the increased production of oxidants and insufficient antioxidant defence mechanisms, which results in damage to tissues.

20.2. Oxidants – Free Radicals and Reactive Forms of Oxygen and Nitrogen

Oxidants are free radicals and other reactive forms of oxygen and nitrogen which are closely related to radical reactions. Free radicals are atoms, molecules or ions containing one or more unpaired electrons in the bonding orbital. They are unstable, highly reactive and tend to make chain reactions. Examples of important oxidants include the hydroxyl radical $OH^{\cdot}$, superoxide $O_2^{\cdot -}$, singlet oxygen $1O_2$, hydrogen peroxide H_2O_2, nitric oxide $NO^{\cdot}$, hypochlorous acid HClO, and the peroxyl and alkoxyl radicals $ROO^{\cdot}$ and $RO^{\cdot}$. Figure 1 shows the reactions leading to oxidant generation.

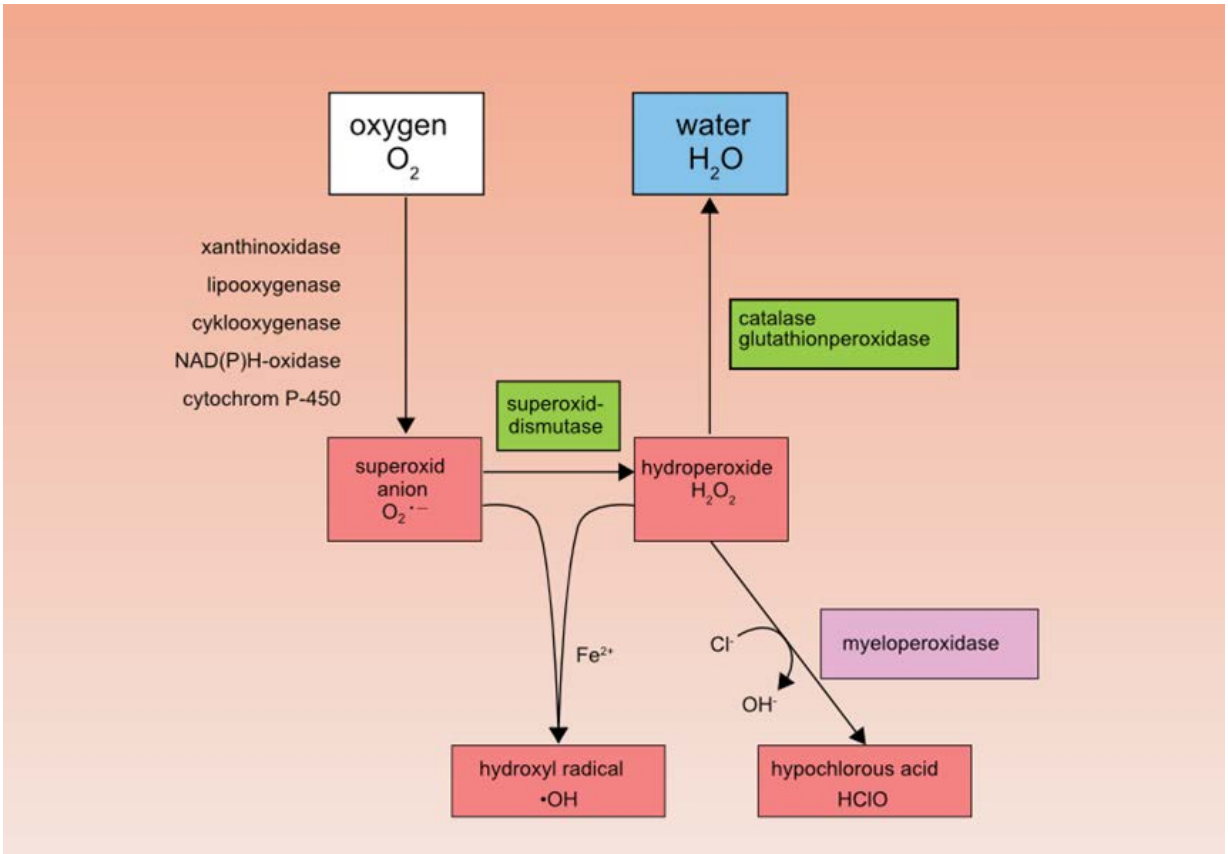

Figure 20.1. Oxidant generation and oxidant inter-reactions

The most important source of free radicals in the body is the mitochondrial respiratory chain. Oxygen commonly accepts 4 electrons and is transformed into water. This reaction, however, takes place in successive steps: oxygen → superoxide → hydrogen peroxide → hydroxyl radical → water. Another important producer of oxidants is phagocytes (neutrophils and monocytes-macrophages), specifically their NADPH oxidase and myeloperoxidase. In the respiratory burst (Figure 21.2), following activation by inflammatory stimuli, NADPH oxidase reduces molecular oxygen to superoxide, which is subsequently converted to hydrogen peroxide. These substances can be another source of oxidants – nitric oxide, peroxynitrite and hydroxyl radical. In the presence of chloride ions, hydrogen peroxide is also metabolized by myeloperoxidase to hypochlorous acid, which may subsequently react with endogenous amines to form chloramines. Free radicals are also generated during xenobiotic detoxification and in many other chemical reactions, such as reactions catalyzed by cytochrome P450, xanthine oxidase, lipoperoxidase or cyclooxygenase. Their generation is accelerated by transition metals - iron, copper – Fenton's reaction:

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\cdot} + OH^{-} + Fe^{3+}$$

Nitric oxide ($NO^{\cdot}$) is generated from L-arginine by the action of NO synthase (NOS). Constitutive NOS is found primarily in the endothelium and neurons, and inducible NOS primarily in macrophages.

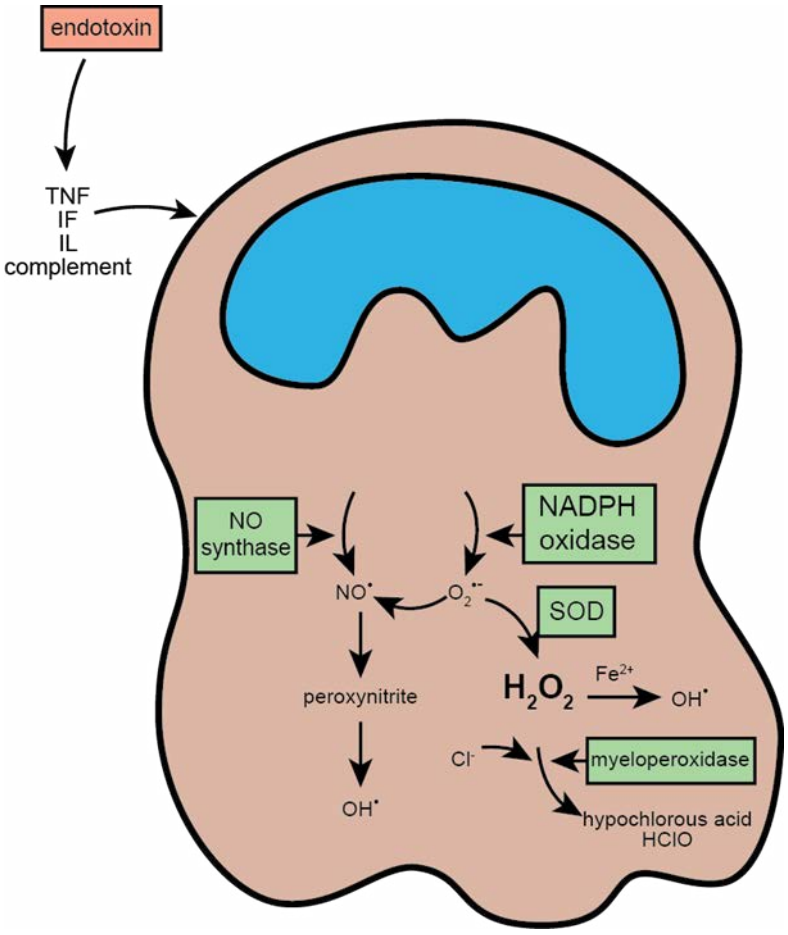

Figure 20.2. Respiratory burst of neutrophils

20.3. Antioxidants – Substances Acting against Oxidants

Antioxidants are substances working against the generation and effect of free radicals – as scavengers (interceptors) of free radicals, as free radical generation inhibitors through the bond to transition metals, for example, or else they eliminate hydroperoxides and repair damage. Antioxidants in the human body can be divided into two groups:

Enzymes and enzyme cofactors = trace elements (superoxide dismutase – copper, zinc, manganese; glutathione peroxidase – selenium; catalase – iron; cytochrome P450, lactoperoxidase;

Substrates – lipophilic (tocopherols and carotenoids) and hydrophilic (ascorbic acid, glutathione, thiols, caerulo-

plasmin, transferrin, ferritin, albumin, bilirubin, uric acid and others).

Antioxidant properties can also be found in many synthetic compounds such as the iron and copper chelates deferroxamine and penicillamine, the xanthine oxidase inhibitors allopurinol and oxypurinol, probucol, lazaroids, angiotensin-converting-enzyme inhibitors and statins.

20.4. Compounds Generated Due to Oxidative Stress – Radical Reaction Products and Their Importance in Tissue Damage

Oxidative stress **modifies biologically important compounds**. It changes their structure by fragmentation, aggregation, cross-linking; and, it changes their properties – hydrophobicity, proneness to proteolysis and function, as well as their immunological properties. These changes depend on the chemical nature of the agent and the intensity of attack. The damage affects lipids, proteins, carbohydrates and nucleic acids. Fatty acids with multiple double bonds – polyunsaturated fatty acids – are most prone to radical reactions in lipids, and the process is referred to as **lipid peroxidation**. Their structure is gradually rearranged; conjugated dienes, peroxyl and alkoxyl radicals and hydroperoxides are generated, and the modified molecule may also cleave into shorter products to form malondialdehyde or 4-hydroxynonenal. Membrane lipids as well as lipoproteins may become damaged. The amino acids tyrosine, methionine, cysteine and tryptophane are subject to damage in **proteins** by oxidation, nitration, chlorination and dimer formation. Proteins may then aggregate and cross-link to generate advanced oxidation protein products (AOPP). **Advanced glycation end products** (AGEs) are generated with the help of carbohydrates and also due to carbonyl stress. Proteins can also be modified through the action of lipid peroxidation products (malondialdehyde lysine, hydroxynonenal and acrolein-protein adduct) to form advanced lipid peroxidation end products (ALEs). **DNA damage** affects deoxyribose and bases, which results in DNA chain breaks and chain cross-linking. *Figure 3* shows damage to biological structures and its effects.

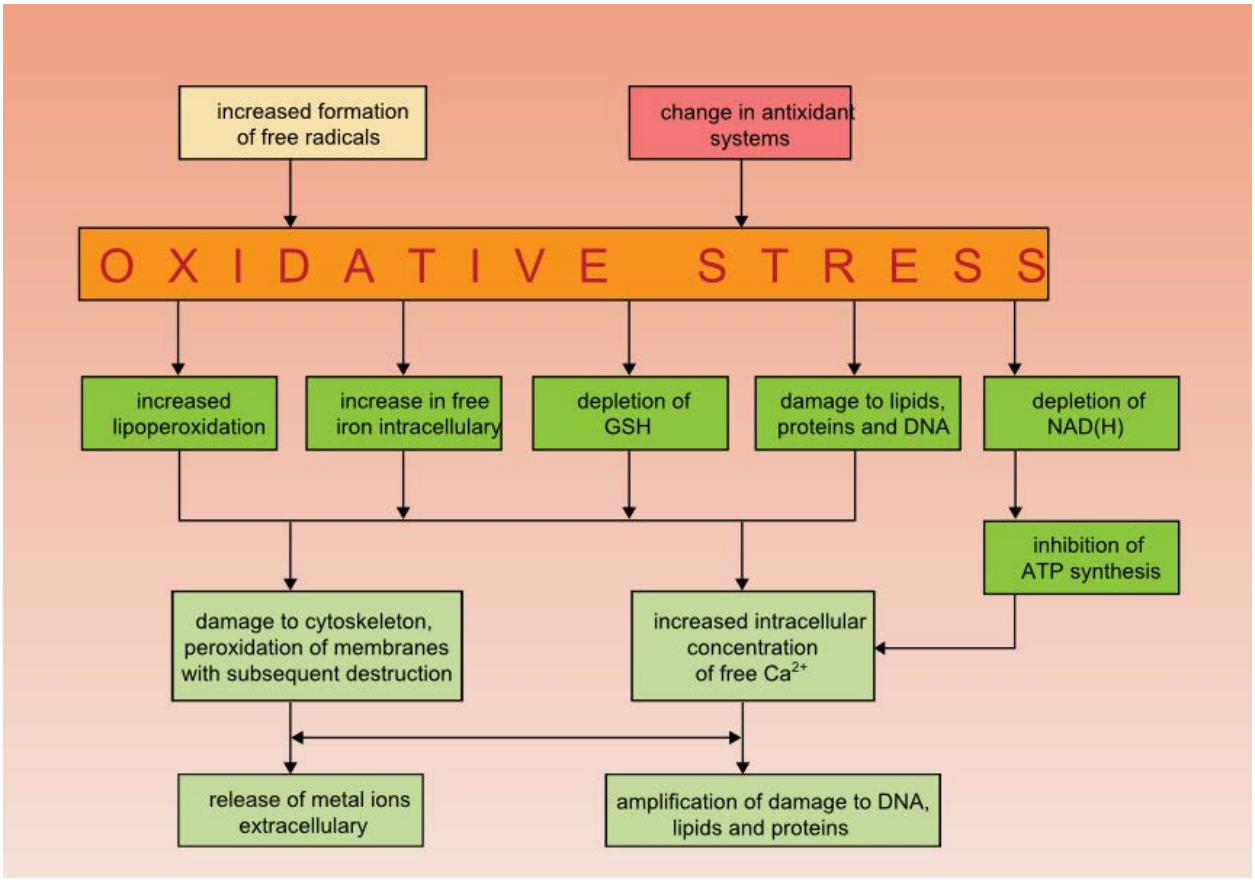

Figure 20.3. Effects of oxidative stress

Oxidative stress is closely related to **carbonyl stress**, which is characterized as an increase in reactive carbonyl compounds caused by their reduced production and/or reduced elimination and excretion. These are compounds containing a carbonyl group such as glyoxal and methylglyoxal, or products of lipid peroxidation, malondialdehyde and 4-hydroxynonenal. Reactive carbonyl compounds may also be generated from carbohydrates, lipids and amino acids in oxidative and non-oxidative reactions, and may give rise to ALEs and AGEs. AGEs, a heterogeneous group of compounds such as carboxymethyllysine, pentosidine, glyoxal-lysine dimer and methylglyoxal-lysine dimer, have been

Damage to biological structures	Lipid peroxidation
	Glycoxidation
	Protein modification
	DNA modification
Role in the pathogenesis of many diseases and their complications	Atherosclerosis
	Diabetes mellitus
	Renal diseases
	Tumour diseases
	Inflammatory diseases
	Neurodegenerative diseases

Table 20.2. Pathological roles of free radicals

20.6. Laboratory Diagnostics

The determination of free radicals in body fluids is problematic due to their short biological half-life. It is therefore more appropriate to measure levels of compounds generated due to oxidative stress or antioxidation protection. Assays can be made on various biological materials such as serum, plasma, urine, whole blood, blood elements or exhaled air. Laboratory diagnosis must always be comprehensive and the results of regular tests may often point to the probable presence of oxidative stress. Laboratory results must be evaluated on an individual basis in relation to the patient's clinical condition. Laboratory assays for oxidative stress and antioxidant parameters are used mainly to study the cause and course of a disease; they are not used in normal clinical practice.

Direct measurements include assays for generating radicals and the reactive forms of oxygen and nitrogen using methods such as pulse radiolysis, electron spin resonance and chemiluminescence. These methods are used especially in basic research and are not commonly available.

Indirect measurements determine substances generated in radical reactions, antioxidants and autoantibodies. It is also possible to determine degradation products in NO generation (nitrites, nitrates) or use an immunochemical assay (ELISA) for enzymes generating radicals – myeloperoxidases or xanthine oxidase.

It is possible to determine many **substances generated in radical reactions (classic oxidative stress markers)**; for example, lipid peroxidation is characterized by malondialdehyde, oxidized LDLs or 8-isoprostane as an arachidonic acid metabolite. Protein impairment can be mapped by measuring modified amino acids or as AOPP; AGEs or sRAGE (soluble receptor for AGEs) are typical for the effects of carbohydrates. 8-hydroxy-2´-deoxyguanosine is a marker of DNA damage. ELISA, high-performance liquid chromatography (HPLC) and gas chromatography with mass spectrometry (GC/MS) are the most commonly applied methods.

Assays for selected **antioxidants** are more available: vitamins A and E (HPLC), trace elements, zinc and selenium in particular (atomic absorption spectrometry); it is also possible to determine the activity of antioxidant enzymes or total antioxidant capacity using spectrophotometry.

Another possibility, again more used for research, is the **assay for autoantibodies** generated in reaction to the modification of biological structures. The oxidative stress process involves the generation of compounds that are immunogenic and the body produces antibodies against them. Another system (immune system) thereby becomes involved and may be impaired by oxidative stress; an example would be antibodies against oxidized LDL determined by ELISA.

Molecular biology techniques are able to determine a genetic predisposition to tissue damage (e.g. polymorphism of genes for antioxidant enzymes or enzymes degrading reactive carbonyl compounds, or genes for receptors mediating tissue damage such as RAGE – receptor for AGEs).

Basic laboratory assays can point to various pathologies and signal the probable presence of oxidative stress. The

function of kidneys is examined routinely, and it is known that oxidative stress is present in patients with impaired renal function. Oxidative stress also has an important connection with inflammatory reaction (indicators such as C-reactive protein, orosomucoid) and the atherogenesis process (modified LDL particles, myeloperoxidase). An elevated glucose or lipid level means more substrates for the production of reactive carbonyl compounds. On the other hand, albumin and bilirubin (higher levels without hepatic involvement, e.g. Gilbert's syndrome) and also glucose have antioxidant effects. Erythrocytes contain another important antioxidant, glutathione, and so a drop in total glutathione is expected in anaemia.

Table 21.3 summarizes selected assays for detecting oxidative stress. The methods listed below describe the reactions and processes that take place in the human body during oxidative stress. Individual analytes should be considered in context of their mutual relations before evaluating the degree of damage to the body by oxidative stress.

Group of Assays	Parameters
Direct measurement – determination of generated radicals	Pulse radiolysis, electron spin resonance, chemiluminescence
Oxidative stress damage markers	Lipid peroxidation – malondialdehyde, conjugated dienes, oxLDL, ALEs, 8-isoprostane
	Amino acid and protein oxidation – modified amino acids (3-chlorotyrosine, 3-nitrotyrosine, dichlorotyrosine), AOPP
	Glycoxidation – AGEs, sRAGE
	Oxidative DNA damage – 8-hydroxy 2´deoxyguanosine
	Degradation products in NO generation (nitrites, nitrates)
	Radical-generating enzymes – xanthine oxidase, myeloperoxidase
Antioxidants	Vitamins A and E
	Trace elements – Se, Zn
	Albumin, bilirubin
	Glucose, uric acid
	Antioxidant enzymes – superoxide dismutase, glutathione peroxidase, catalase
	Total antioxidant capacity
Autoantibodies	Antibodies against oxidized LDL
Molecular biology techniques	Polymorphism of genes for antioxidant enzymes and enzymes degrading reactive carbonyl compounds, or genes for receptors mediating tissue damage such as RAGE – receptor for AGEs
Basic laboratory assays	Renal function – estimated glomerular filtration, serum creatinine
	C-reactive protein, albumin, glucose level, HbA1c, microalbuminuria, lipids, bilirubin, blood count

Table 20.3. Selected assays for detecting oxidative stress

20.7. Possible Therapies

There are no standard recommendations for oxidative stress therapy at present. However, many studies have