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Contents

5

Oxidative damage to proteins and lipids during ageing
Kuka S., Tatarkova Z., Kaplan P.

12

Surfactant and its role in the upper respiratory system and Eustachian tube
Uhliarova B., Svec M., Calkovska A.

22

Nuclear magnetic resonance as a diagnostic tool in breast cancer
Hnilicova P., Dobrota D.

31

**Ovarian haemangioma with infundibulopelvic ligament torsion in
a 13-year-old girl**
Zganjer V., Zganjer M., Cigit I., Tomicic H., Cizmic L.

35

**Effect of yoga exercise on metabolic and homeostatic parameters
of insulin resistance**
Pastucha D., Filipcikova R., Cajka V., Horakova D., Radova L., Reich P., Sovova E.

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OXIDATIVE DAMAGE TO PROTEINS AND LIPIDS DURING AGEING

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Abstract

The present work is a theoretical study in the field of monitoring oxidative damage to proteins and lipids during ageing. The basic terminology and interactions are discussed as well as the sources of oxidants and their elimination through antioxidant protection. We focus on the effects of oxidative stress on the biomolecules (proteins, lipids and DNA), the role of mitochondria, antioxidants, physical activity and caloric restriction in relation to ageing. Previous research indicates the crucial role of mitochondria in the ageing process by their formation of oxidants, the accumulation of oxidative damage to mtDNA and other biomolecules leading to impairment of mitochondrial function, energy failure, apoptosis and necrosis. Although the role of oxidative stress in the ageing process is evident and well documented, the precise mechanisms of its relationships remain largely unknown. Further research is needed to clarify them and to show ways to slow down the ageing process.

Key words: ageing, oxidative damage, biomolecules, mitochondria

INTRODUCTION

Oxidative damage to biomolecules during ageing is a topic of interest of many scientists, especially for severity ageing accompanying effects. Ageing is inevitable and is characterized by a progressive deterioration in physiological functions and metabolic processes, ultimately leading to morbidity and mortality (1). There exist many theories of ageing. The *free radical theory of ageing* (2) proposes that free radicals, a by-product of normal metabolism, cause oxidative damage to macromolecules. Their accumulation causes cellular dysfunction with age and eventually cell death. Over time this theory has been further refined to reflect the fact that mitochondria are at the same time major sources and a target of free radicals and other reactive oxygen species (ROS). The main features of *mitochondrial theory of ageing* are: increased ROS production, mitochondrial DNA (mtDNA) damage accumulation and progressive respiratory chain dysfunction (3). Mitochondria ensure energetical needs of most tissues and they play a major role in cell survival. Therefore their dysfunction is causatively linked to the ageing process and a number of human degenerative diseases such as Parkinson's or Alzheimer's disease (4).

Recently, the lifespan of different organisms can be manipulated by altering ROS metabolism, especially at the level of scavenging systems, through exogenous and/or endogenous interventions. Among these, supplementation with dietary antioxidants has been one of the approaches utilized to test the free radical theory of ageing, and finally, to try to reduce the impact of age-related dysfunctions (5). Physical activity and caloric restriction represent other factors investigated in their relation to ageing.

OXIDANTS, OXIDATIVE DAMAGE TO PROTEINS AND LIPIDS, ANTIOXIDANTS

Oxidative damage to biomolecules is caused by free radicals and other (nonradical) reactive forms of oxygen and nitrogen, especially superoxide radical $O_2^{\cdot-}$, hydrogen per-

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oxide H_2O_2 , singlet oxygen $\Delta^1\text{O}_2$, hydroxyl radical $\text{OH}\cdot$ and nitric oxide $\text{NO}\cdot$. Oxidant sources can be divided into external and internal. Although internal sources (mitochondria and others) are considered as crucial, some external sources, e.g. smoking, air pollution or UV radiation, can also significantly contribute to oxidative stress.

Oxidative damage to proteins under conditions of oxidative stress involves several chemical reactions such as: amino acid side chains oxidation, fragmentation of polypeptide chains, generation of cross linkages and conformation changes. These oxidative modifications are usually irreversible and lead to serious disruption of protein function (6). The exception is the reversible oxidation of side chains of methionine and cysteine. This allows the recovery of protein property and also elimination of ROS, which is important in protecting cells from oxidative stress (7).

The oxidation of lipids is especially damaging because the formation of lipid peroxidation products leads to a spread of free radical reactions (8). Lipid peroxidation (LPO) concerns in particular polyunsaturated fatty acids (PUFA) that gradually form conjugated dienes and hydroperoxides after the radical attack. Lipid peroxidation leads also to the formation of aldehyde by-products, including malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE) and acrolein, which are often referred to as advanced lipooxidation end products (ALEs).

Antioxidant is a term widely used but rarely defined. Halliwell and Gutteridge (9) have proposed a broad definition of an antioxidant as „any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate“. Because antioxidants do not work in the body separately, more important than monitoring the activities of individual antioxidants is the determination of the total antioxidant capacity (10). Oxidative and antioxidative processes are associated with the electron transfer effect on the redox state of cells and the organisms. The changed redox state stimulates or inhibits activities of various signal proteins, resulting in a changed ability of signal pathways and the fate of the cells. Recently, the idea that oxidative stress is not always harmful has been accepted (11).

AGEING AND MITOCHONDRIA

Previous research indicates the crucial role of mitochondria in the ageing process by: their formation of oxidants; the accumulation of oxidative damage to mitochondrial DNA; and other biomolecules leading to an impairment of mitochondrial function, energy failure, apoptosis and necrosis. Interest in mitochondria relating to ageing has been apparent for a long time as shown in the article written by D. Harman in 1972 (12). Since that time, many studies have been devoted to that subject and, although the role of mitochondria and oxidative stress in the ageing process is evident and well documented, the precise mechanisms of their relationships remain largely unknown.

The mitochondria are, at the same time, major sources and a target of free radicals and other ROS (13, 14). ROS are generated in mitochondria as a by-product of energy metabolism; part of the oxygen (2 – 4%) leaks in the form of superoxide from electron transfer chain (respiratory chain) complexes (15). Although superoxide is not characterized by high reactivity to biomolecules, more dangerous products are formed from it: hydrogen peroxide, singlet oxygen and hydroxyl radical (6). Superoxide production occurs in the respiratory chain, localized to complexes I and III (16). In complex I, oxygen is likely to be reduced to superoxide at two sites: one associated with NADH oxidation in the mitochondria matrix; the second one relates to the reduction of ubiquinone in the membrane (17). Complex III leads to the formation of ROS in the binding site of antimycin A (18). Enzyme complexes oxoglutarate dehydrogenase and pyruvate dehydrogenase, in the mitochondrial matrix, are also sites of ROS production (19). Wang et al. (20) discovered a quantal mode of mitochondrial ROS production. They

showed that individual mitochondria undergo spontaneous bursts of superoxide generation, termed "superoxide flashes", indicated with the aid of a novel mitochondrial matrix-targeted superoxide indicator (circularly permuted yellow fluorescent protein cpYFP). Individual flashes are triggered by transient openings of the mitochondrial permeability transition pore (mPTP) and are fuelled by electron transfer complexes-dependent superoxide production. During cardiac hypoxia/anoxia it is decreased, whereas, during reperfusion, a flurry of superoxide flash activity occurs.

Mitochondria and other cellular compartments are a target for oxidative modifications during ageing. The results of the study from this laboratory (21) suggest a different mechanism of oxidative modification in rat heart compartments e.g. the level of lysine conjugates increased with age in homogenate while the level of modified tryptophan increased in mitochondria.

In addition to free radicals, ALEs play a significant role in oxidative damage to biomolecules during ageing: HNE creates protein adducts and induces formation of ROS in mitochondria, probably due to lipid- and protein-modification of the inner mitochondrial membrane (22). MDA shows similar adverse effects, which inhibit especially pyruvate dehydrogenase, oxoglutarate dehydrogenase, complexes I and II activities in rat liver mitochondria (23).

Many studies are focused on changes of mitochondrial respiratory chain complexes activities during ageing. The results on rats and mice mitochondria are relatively controversial: a decline in activities of the complexes I - IV with age (24, 25); decreased activities of only some complexes (others without changes), e.g. decrease of only V (26) or of I and V (27) even the contradictory activity changes: decline of complexes I and V, increase of II and III (28). The changes in the activities with age can be due to various factors: the accumulation of oxidative damaged mitochondrial proteins, as provided by the decrease of thiol groups and the increase of protein-HNE adducts and dityrosines (25). Similarly, another study (28) reports correlations between decreases in complexes I and V activities and the increase in oxidative modification of their subunits. However, activity changes may be related to oxidative damage to other components of the inner mitochondrial membrane, such as cardiolipin, supplementation of which restores age-decreased complex I activity (29). A similar effect; activity restoration of several complexes, was achieved by application of L-carnitine (24). Some studies (1, 27) suggest links between changes in respiratory chain function and oxidative damage to DNA (especially mtDNA), leading to changes in expression of genes coding respiratory chain complexes. Mitochondrial DNA is more prone to oxidative damage in comparison to nuclear DNA. Consequently, the decline in activity of complex I, composed of the largest number of subunits coded by mtDNA, has often been seen (1). Gomez et al. (30) report another mechanism of respiratory chain function deterioration with age. According to these authors, the phenomenon may arise in part from dissociation of large hypermolecular assemblies termed "supercomplexes". Mitochondrial proteins from young and old rat hearts were separated (blue native PAGE), protein bands analysed (LC-MALDI-MS/MS) and protein levels quantified by densitometry. Results showed that supercomplexes composed of various stoichiometries of complex I, III and IV were observed, and declined significantly with age, without changes in concentrations of single complexes I - IV. The deterioration in respiratory chain supercomplexes may be an important underlying factor for loss of cardiac bioenergetics with age.

AGEING AND ACCUMULATION OF OXIDATIVE DAMAGE

The possibility that ROS/RNS-mediated protein damage contributes to the ageing process is supported by results of many studies showing that ageing is associated with the accumulation of such protein damage. The accumulation of protein damage is

a complex function of a multiplicity of factors that govern the intracellular levels of ROS/RNS, on the one hand, and a multiplicity of factors that govern the degradation and/or repair of damaged proteins, on the other (31). Repair/degradation mechanism is part of the body's protective system against the negative effects of ROS/RNS. The functionality of the system is not perfect, to which also contribute ROS/RNS and secondary products of their reactions. The result is the accumulation of oxidative modified biomolecules that participate in the pathogenesis of various diseases. These include: cardiovascular diseases (6, 32); neurodegenerative diseases; cancer; many lung diseases (33, 34); different genetic diseases such as Down's syndrome (35); some eye diseases (36); digestive diseases, especially of the liver (37); rheumatoid arthritis and other chronic inflammatory diseases; diabetes; and related complications. A growing number of illnesses (schizophrenia, epilepsy, multiple sclerosis, psoriasis) with increased oxidative damage suggests its essential role in the pathogenesis of the diseases (38).

The protein maintenance system ensures repair of sulfur-containing amino acid oxidation products, either by the thioredoxin/thioredoxin reductase system or by the peptide methionine sulfoxide reductase system. Other, irreversible amino acid oxidative modifications are eliminated by the destruction of protein. Degradation of oxidized proteins in the cytosol is mainly achieved by the ubiquitin-proteasome system. Ubiquitin, small protein of 76 amino acid residues, is used for labeling of oxidized proteins that should be degraded (39). Lon protease provides degradation of the oxidized proteins in mitochondria. The lysosomal pathway has also been implicated during oxidative stress throughout a chaperone mediated autophagy. Repair/degradation systems become less efficient during ageing, leading to an increased intracellular load of damaged proteins and to the formation of protein aggregates (40). The aggregates of lipofuscin (highly oxidized, cross-linked, insoluble, not degradable, yellow-brown product) have been found in various cell types including heart, liver, kidney, neuronal tissue and dermal tissue. They are associated with the life span of a single postmitotic cell and, consequently, of the whole organism (41).

An accompanying feature of ageing cells is also the formation of the advanced glycation end products (AGEs). AGEs are a heterogeneous group of bioactive molecules that are formed by the nonenzymatic glycation of proteins, lipids and nucleic acids. Humans are exposed to AGEs produced in the body, especially in individuals with abnormal glucose metabolism, and AGEs ingested in foods. AGEs cause widespread damage to tissues through upregulation of inflammation and cross-linking of collagen and other proteins (42).

AGEING, ANTIOXIDANTS, PHYSICAL ACTIVITY AND CALORIC RESTRICTION

Ageing of humans and other organisms is accompanied by many negative phenomena (see above). There is, not surprisingly, an age-old attempt to find a way and means to eliminate these phenomena, or at least delay or mitigate. Ageing is an extremely complex process, with no factor operating in isolation. Therefore, the interpretation of results obtained *in vitro* experiments is usually of very limited validity and results obtained *in vivo* may often be different.

Ageing may cause a violation of oxidant and antioxidant balance in favor of oxidants by increasing their production and/or reducing antioxidant protection. Many experimental studies have examined the possibility of restoring the original balance by strengthening the antioxidant protection by antioxidant supplementation. Many antioxidants were tested e.g. lipoic acid alone or in combination with carnitine. Improved mitochondrial bioenergetics (43), reduced damage to mtDNA (44), decreased apoptosis (45) and increased activities of citrate cycle enzymes and respiratory chain complexes (46) in ageing rats' myocardium have been shown. Similar effects were also seen in the application of other antioxidants. Nevertheless, a clear conclusion on the role of antioxidants in ageing does not exist yet (6, 38). Based on the current data, if

oxidative stress/damage (and therefore also antioxidants) plays a role in ageing, it is much more limited than previously thought. However, oxidative stress/damage may play a role in health span, i.e., the period of life during which the animal is free of age-related pathologies, or may play a stronger role under conditions in which an animal is exposed to chronic stress over its life span, and may accelerate several features of the ageing process (47). As we have mentioned earlier, free radicals have also positive roles in the living organisms. Moreover, antioxidants may, in the non-physiological conditions, show the opposite – prooxidant effects. Example for this is the effect of ascorbic acid in the presence of excess Fe^{2+} ions. Thus, the imprudent administration of antioxidants may have a negative impact on the organism (11).

Adequate physical activity is another factor associated with ageing. Several studies indicate reduced production of H_2O_2 in myocardial mitochondria of physically active rats (48) or an increase of the life expectancy of the physically active mice from 9 to 19% (49). The mechanisms of this positive effect remain unclear. One possible mechanism is related to the change in the ratio of ROS formation and elimination. In spite of increased consumption of oxygen during physical activity, ROS production does not grow significantly because in respiratory state 3 (activity), the relative ROS production is much lower than in respiratory state 4 (resting respiration). However, physical activity and subsequent mild oxidative stress activate antioxidant protection by stimulating the expression of antioxidant enzymes (50). On the other hand, extreme physical activity leads to increased oxidative stress, probably due to muscle damage and subsequent inflammatory response (6).

Caloric restriction (CR) seems to be by far the most effective environmental manipulation that can extend maximum lifespan in many different species (51). Although the exact mechanism of action of CR is still unknown, the final effect is probably co-created by several mechanisms; not only by the reduction of energy metabolism. Li et al. (52) report that two main mechanisms leading to extended longevity during CR seem to be DNA methylation and histone modifications. Both of them are believed to dynamically influence the chromatin structure, resulting in expression changes of relevant genes. DNA methylation regulation involves DNA methyltransferase activation, resulting in silencing the expression of target genes such as *p16^{INK4a}* and *Ras* due to its hypermethylation. Histone modifications include especially deacetylation effects due to activation of histone deacetylases Sirt1 and HDAC1, leading to down-regulation of genes such as *p53*, *Foxo*, *Ku70* and *p16^{INK4a}*. Other authors (53) deny the independent action of these mechanisms and suggest that CR or CR-mimetic compounds (resveratrol) increase expression and/or activity of Sirt1, leading to increased histone deacetylation and, thereby, to increased DNA methylation. In spite of the fact that the precise mechanism is not completely known, these results suggest that epigenetic pathways play an important role in the beneficial effects of caloric restriction on lifespan.

CONCLUSIONS

Literary sources used in this review briefly document the current state of research on the problem of oxidative damage to critical biomolecules (proteins, lipids) and the role of damage in ageing. Oxidative changes are the result of an imbalance between production of free radicals and their elimination. The most important source of ROS and RNS include mitochondria that are the first targets of their harmful effects, leading to a progressive decline in mitochondrial function. Although the involvement of oxidative stress and damage to biomolecules in the ageing process is documented in many studies, the detailed mechanisms of this involvement still remain unclear. Unfortunately, many studies have published contradictory experimental results. To clarify and possibly manipulate the mechanisms acting together in the ageing process, further experiments are needed, carefully prepared, implemented and evaluated.

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SURFACTANT AND ITS ROLE IN THE UPPER RESPIRATORY SYSTEM AND EUSTACHIAN TUBE

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Abstract

Surfactant research was originally directed toward lung mechanics, however, with growing information on the biology of the surfactant system it has expanded beyond the borders of basic physiology. The research has become interdisciplinary, not only considering aspects of lungs biology relevant for breathing, but also those aspects relevant for airway defence.

Surfactant consists mainly of phospholipids that lower the alveolar surface tension to prevent lung collapse at expiration. They also support mechanical elimination of inhaled pathogens by reducing the viscosity of airway mucus. Approximately 8-10% of surfactant is made up of proteins. Among them, specific proteins SP-A and SP-D play a crucial role in the innate defence system. They belong to collectins family and serve as the first step in immune response to inhaled pathogens. In limited extent, SP-B and SP-C are also involved in immunomodulation.

Although numerous studies have focused on the physiological function of surfactant in the lower airways, relatively little is known about its role in the upper respiratory system. Identification of lamellar bodies in ciliated epithelium of the upper airways indicates that surfactant may have a role in normal sinonasal function and pathology. Decreased levels of the main component of surfactant, phospholipids, have been implicated in atrophic rhinitis and altered levels of surfactant proteins have been observed in a number of respiratory tract diseases. The pattern of inflammation in the upper respiratory tract generally appears to parallel that in the lower airways and nowadays upper respiratory disease and lower airway disease are considered as two manifestations of one pathological process. Therefore, surfactant proteins may play a significant role in the upper respiratory tract diseases.

In addition, surfactant has been identified in the Eustachian tube where it helps to lower the opening pressure between nasopharynx and middle ear. The alterations in surfactant levels may adversely affect Eustachian tube function and contribute to chronic ear infection.

The review summarizes the current knowledge on the presence and the role of surfactant in the upper respiratory system and Eustachian tube.

Key words: surfactant, specific proteins, upper airways, otitis media, Eustachian tube

INTRODUCTION

Pulmonary surfactant, a complex of lipids and proteins lining the alveolar surface, has two crucial roles in respiratory function. It reduces surface tension at the air liquid interphase, facilitating gas exchange and alveolar stability during breathing and interacts with the airway defence system (1).

In addition to the lung, surfactant-like material has been identified in many other human tissues. Lamellar bodies or phospholipids and surfactant proteins have been detected in non-pulmonary sites including upper respiratory tract, Eustachian tube, middle ear, gastrointestinal tract, salivary glands, brain, trachea, lacrimal glands, heart, kidney, pancreas, and male and female urogenital tract (2-7).

Composition of surfactant

Surfactant is composed of 85-90% lipids, about 10% proteins and 2% carbohydrates. The principal lipid constituents of surfactant are phospholipids. Phosphatidylcholine

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(PC) species comprise about 75% of surfactant phospholipids. Nearly half of the PC content is dipalmitoylphosphatidylcholine (DPPC), which is the major component of surfactant and also principal surface tension reducing compound. Up to half of the intra-alveolar content of DPPC is present in the monolayer at the air-liquid interphase. Other phospholipids include phosphatidylglycerol (~12%), phosphatidylethanolamine (~5%), phosphatidylinositol (~4%), phosphatidylserine (~1.5%), sphingomyelin (~1%), and lysophospholipid (<1%). Cholesterol is major neutral lipid, and constitutes about 6-8% of the total lipids (8).

Optimal surfactant function requires the presence of four specific proteins known as SP-A, SP-B, SP-C and SP-D. Hydrophobic surfactant proteins SP-B and SP-C facilitate the adsorption of phospholipids at the air-liquid interphase, where they reduce surface tension. Hydrophilic SP-A and SP-D play a role in the pulmonary host-defence system (9).

Metabolism of surfactant

Both surfactant lipids and proteins are produced in the alveolar type II cells. The phospholipids and three of four surfactant specific proteins, except SP-C, are also synthesized in the airways in non-ciliated epithelial Clara cells. Surfactant components were also found in secretory cells of airway submucosal glands (10).

Inside the cells, the surfactant components are stored in dense, multilayered membrane structures – the lamellar bodies. Lamellar bodies are excreted into the alveoli and converted into a lattice-like structure of tubular lipid double-layers, called tubular myelin, from which the monolayer at the air-liquid interphase is formed. Formation of these structures and their transformation is facilitated by surfactant proteins (10).

A major clearance pathway for surfactant is an uptake and reutilization by the type II cells. A significant fraction of surfactant is degraded by alveolar macrophages, with minor amounts moving up to the airways and across the epithelial-endothelial barrier into the blood stream (11).

The immunological role of surfactant

The main function of the **phospholipids**, primarily DPPC, is to lower the surface tension at the air-liquid interphase, however, they also possess immunomodulating properties. Surfactant lipids have been shown to suppress the release of inflammatory cytokines and prostanoids by monocytes and to inhibit activation of both T and B lymphocytes. They are able to quench surface free radical activity of mineral dust particles and reduce their toxicity in vitro (12). Moreover, the surfactant phospholipids coat the gel layer to reduce the surface tension, decrease mucus viscosity and enhance the elimination of inhaled pathogens (13).

The hydrophobic proteins **SP-B and SP-C** have been characterized extensively for their ability to affect lamellar body formation, secretion and creation of the surfactant monolayer that is critical to the lowering surface tension at the air-liquid interphase. Their role is crucial as children born with inherited SP-B and/or SP-C deficiency usually develop acute respiratory failure non-responding to exogenous surfactant replacement (14).

Increasing evidence indicates that SP-B and SP-C are also involved in immunomodulation that is critical for the host defence of the airways. For example, SP-B reduces inflammatory response in the lungs to bacterial lipopolysaccharide (15). In transgenic mice reduction in SP-B expression and the associated abnormalities in reducing surface tension evoked an inflammatory response in alveolar macrophages and alveolar type II cells (16). SP-C-deficient mice are susceptible to bacterial and viral infections and they suffer from excessive inflammation (17). Moreover, surfactant in combination with hydrophobic SP-B and SP-C possesses viscoelastic and rheologic properties that enhance mucociliary clearance (18). It accelerates ciliary beat frequency, decreases mucus viscosity and improves particle clearance from the lungs (19).

SP-A and SP-D are members of collectins family that belong to C-type lectins named for their aminoterminal collagen-like region and carboxyterminal or carbohydrate recognition domain (CRD). The collectins participate in innate immunity in the period before induction of an antibody-mediated response. These proteins selectively recognize and bind via their CRD to carbohydrate, but also lipopolysaccharide and protein moieties on the surface of bacteria, fungi, viruses and allergens. This binding is followed by interactions with dendritic cells or macrophages and leads to opsonization and pathogen clearance, complement activation, and modulation of leukocyte function including chemotaxis and subsequent cytokine and/or chemokine responses (20, 21).

Taken together, SP-A and SP-D are critical in the initial interaction, recognition, processing and subsequent adaptive immune response for a wide number of inhaled pathogens. In addition, SP-A and SP-D promote apoptotic cell uptake by innate immune cells and regulate cytokine and free radical production (9).

Several studies have shown that SP-A and SP-D bind to and inactivate wide range of pathogens, such as *Staphylococcus aureus*, *Haemophilus influenzae* type A, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and a group A streptococci, *Klebsiella pneumoniae*, *Aspergillus*, *Salmonella minnesota*, *E. coli*, herpes simplex virus, influenza virus, *M. tuberculosis*, *Pneumocystis carinii*, *Mycoplasma pneumoniae* (22, 23).

Beside binding to pathogens and affecting antigen processing, SP-A and SP-D affect immunoglobulin E binding to the allergen and cause a shift in the polarization of T-lymphocytes subpopulations Th1 and Th2 (13). In addition, topical application of SP-A and SP-D have been shown to decrease immunoglobulin E levels and reduce eosinophilia in mouse model of allergic bronchopulmonary aspergillosis and to cause a marked shift from a pathogenic Th2 profile to a protective Th1 cytokine pattern (22).

While SP-D not being associated with surfactant lipids is solely included in immune processes, surfactant protein A cooperates with SP-B in formation of the tubular myelin and the surface film (24). It does not have an essential role in reduction of surface tension, since mice deficient in SP-A will survive and have normal compliance and lung volumes (25). Moreover, SP-A increases resistance of surfactant to some inhibitors (26).

SURFACTANT IN THE UPPER RESPIRATORY SYSTEM

The nose and sinuses play an important role in the first line-defence of the respiratory tract. By warming up, humidifying and filtering incoming air the nose and sinuses are essential in the protection and homeostasis of lower airways (27). As in lower airways, surfactant in upper respiratory system may also be impaired by inhaled noxious agents (28).

Despite their differences, both the upper and the lower airways are crucial in the body's defences against inhaled pathogens, and the pattern of inflammation in the upper respiratory tract generally appear to parallel that in the lower airways (29). Recently, the concept of "united airway disease" or "one linked airway disease" has been proposed (30). In this concept, upper airway disease and lower airway disease are considered as two manifestations of one pathological process.

The close relationship between asthma, allergic and nonallergic rhinosinusitis and nasal polyps has been acknowledged for many years. It has been estimated that approximately 90% of allergic asthmatics suffer from rhinitis, and around 30% of rhinitis patients suffer from asthma (31). Individuals with asthma sensitive to the ingestion of aspirin may suffer from nasal polyps as part of the disease process (32). Allergic fungal rhinosinusitis is considered to be the upper airway correlate to allergic bronchopulmonary aspergillosis (33). Patients with cystic fibrosis invariably develop chron-

ic rhinosinusitis (CRS) in addition to their pulmonary disease. This is through the similar mechanism of inspissated mucus, impaired mucociliary clearance, and persistent bacterial infections and inflammation (34). Several studies report the prevalence of chronic nasal symptoms in patients with chronic obstructive pulmonary disease as 40-70% (35). On the other hand, there is 40% prevalence of lower airway disease in patients with CRS (36) and, interestingly, 70% of those patients were first diagnosed as having lower airway disease. Thus, the lungs and the paranasal sinuses share contact with inhaled pathogens and include many of the same morphological and functional properties.

Surfactant phospholipids in the upper airways

Although numerous studies have focused on the nature and defensive role of surfactant in the lower airways, relatively little is known about its role in the upper respiratory system.

Identification of lamellar bodies in ciliated pseudostratified epithelium of the upper airways (5, 6) indicates that surfactant may have a role in normal sinonasal function and pathology. These lipid storage and secretory organelles possibly undergo exocytosis and organize to form surfactant in the lumen of the sinonasal cavity in a fashion similar to that in the lower airways. Biochemical analysis of the nasal aspirate in healthy individuals revealed the presence of phospholipids constituting surfactant as phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and other phospholipids (37). It was observed that phosphatidylcholine constituted ~75% phospholipids of the nasal aspirate, while phosphatidylethanolamine constituted ~15 %, sphingomyelin ~5% and other phospholipids 4% (Fig.1).

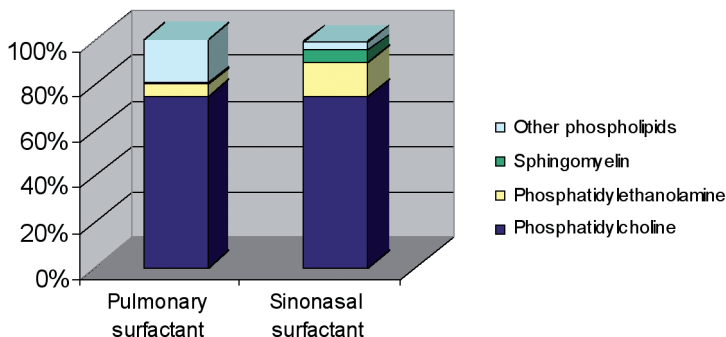


Fig 1. Differences in phospholipid content of pulmonary and sinonasal surfactants

This phospholipids profile corresponds with that observed in the lung wash (38) and in the Eustachian tube and nose (39) in healthy humans.

In patients with primary atrophic rhinitis compared to the healthy people total phospholipids content decreases and its profile changes (37). The changes are characterized by significant decrease in phosphatidylcholine and increase in phosphatidylethanolamine and sphingomyelin. This is in agreement with the study done by Gunther et al. (40) who demonstrated reduced phospholipids concentrations in the bronchoalveolar lavage fluid in all patients with inflammatory lung injury. The compositional changes in the phospholipids profile are similar to that observed in premature infants with neonatal respiratory distress syndrome and in acute pulmonary inflammation in adult respiratory distress syndrome and/or pneumonia (40, 41).

Surfactant proteins in the upper airways

Recent studies identified surfactant proteins and their messenger RNA (mRNA) in normal and diseased sinonasal tissue (42, 43). Immunolocalization of surfactant specific proteins demonstrates their presence in pseudostratified ciliated epithelium and submucosal secretory ducts of sinonasal mucosa (44, 45).

The location of SP-A and D is consistent with the role of these proteins in the innate defence against pathogens at sites of potential invasion of microorganisms. The discovery of surfactant production and secretion by sinonasal mucosa indicates that initial contact and interaction between pathogens and surfactant proteins occurs relatively early after inhalation and deposition into the mucus of the upper respiratory tract.

In sinus mucosal biopsies from patients with cystic fibrosis hydrophilic SP-A and SP-D (42), as well SP-B mRNA (46), were up-regulated when compared with healthy controls. The upregulation is likely due to the substantial bacterial infections that accompany this form of chronic rhinosinusitis (CRS), although undetermined genetic factors and immunologic dysfunction could also play a role. *Pseudomonas aeruginosa* invariably colonizes and infects the sinuses of patients with CF and has been shown to degrade surfactant components including surfactant proteins (47). This may result in a compensatory response at the cellular level to increase expression of SP mRNA and surfactant production. However, content of SP-A and D in bronchoalveolar lavage fluid was reduced in CF patients and it was even lower during an active infection (48). In these studies, protein levels only and not the cellular mRNA were measured. It is possible, that in CF patients there is an upregulation of SP-A and D gene expression and subsequent protein production, but these are rapidly degraded in the presence of bacteria.

Lee et al. (49) demonstrated upregulation of SP-A mRNA and more intense expression of SP-A protein in paranasal sinus mucosa of patients with chronic rhinosinusitis than in healthy control. It indicates that the SP-A gene in paranasal sinus mucosa is not only constitutively expressed, but it is also upregulated during inflammation. The upregulation of SP-A mRNA and SP-A protein in the paranasal sinus mucosa in patients with CRS suggests its role in the local defence mechanism of the paranasal sinus mucosa. These findings are similar to that in patients with chronic allergic rhinosinusitis (50). Moreover, the degree of SP-A mRNA expression correlated with severity of disease measured by Rhinitis Symptom Utility Index in patients with allergic rhinitis symptoms. Linking SP-A expression to the severity of nasal symptoms, sneezing and running nose suggests that SP-A may be an important molecule in local nasal inflammation as well, but additional work is necessary to determine whether SP-A elevation is a reaction to local allergy or a mediator of it. In addition, the expression of SP-A and SP-D is positively influenced by the degree of cell differentiation into mucociliary epithelium (45).

EFFECT OF SURFACTANT ON EUSTACHIAN TUBE

Eustachian tube (ET) connects the middle ear with the nasal cavity and it is important for ventilation, protection and clearance of the middle ear (ME). The structure of the ET is similar to other respiratory airways in which the lumen of the tube is bound by a thin fluid layer at the mucosal surface and is surrounded by cartilaginous and muscular elements. Under physiological conditions ET exists in a "collapsed" configuration that protects the middle ear from nasopharyngeal secretions. However, ET is also responsible for maintaining ambient middle ear pressures and clearing the ME fluid into the nasopharynx. The pressure-regulating and clearance functions require an

open ET in which the resistance to air and fluid flow is minimal. Opening pressure is assumed to reflect the pressure that is needed to overcome all closing forces, including the solid-to-solid adhesion of the ET walls (luminal forces) and pressure of the cartilage and other surrounding tissues (extraluminal forces). The closing pressure is believed to reflect the extraluminal forces, so it has passive ventilatory function (51). Thus, ET dysfunction and the resulting disease complications can develop when the tube is excessively patent or cannot be readily open.

Reduced tubal patency is regarded as possible factor in the development of middle ear disease. Otitis media (OM) is a common childhood disease that includes inflammation of the middle ear mucosa and an accumulation of fluid within the ME. By age of 3 years, a significant number of children (33%) experience more than three episodes of OM (52). The persistence of OM often results in hearing loss, with possible effects on language acquisition, speech production, and social and educational development (53). Although bacterial or viral infections and nasal allergies contribute to the onset of OM, the development of persistent OM is associated with a functional impairment of the Eustachian tube (51).

The role of surfactant in ET function has been questioned for several years. As early as in 1963, Flisberg et al. (54) suggested that the surface activity of the mucous lining of the tubal lumen may be important in tubal opening (Fig.2).

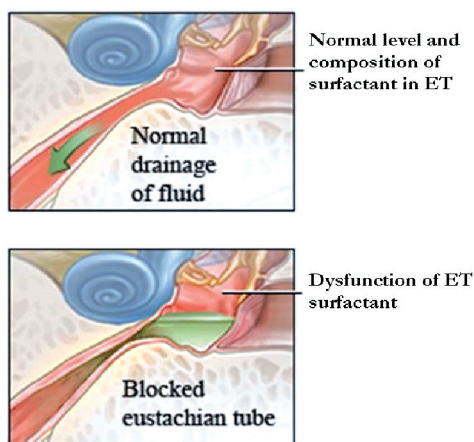


Fig. 2 The role of surface-active material in Eustachian tube (modified according to <http://ptolentinobioresearch.wikispaces.com>). ET – Eustachian tube

Since then, surface tension-lowering factors (surfactants) have been identified in both the middle ear and ET, both in animals (55) and humans (56). Surfactant-producing cells morphologically similar to the alveolar type II cells in the lungs were found in the dorsal part of the ET (57).

ET surfactant is composed of specific surfactant proteins (58) and a mixture of predominantly phospholipids, especially phosphatidylcholines and sphingomyelins (59). Phospholipids are known to reduce the surface tension at an air-aqueous interphase. However, because the ET is normally closed, the ET surfactant covering the epithelium is supposed to act as a release agent by preventing solid-to-solid adhesion (59). Grace et al. (56) compared the phospholipids content of middle ear effusions resulting from ET obstruction in adult patients with that of children with secretoric otitis media. In both groups, surface tension-lowering substances were isolated but the composition

was different from adults having a higher amount of sphingomyelin. In other study, a higher sphingomyelin/phosphatidylcholine ratio was present in children with secretory otitis media than in those without OM (60) indicating a lower degree of surface-lowering properties.

Several recent studies have investigated the effect of exogenous surfactant on ET mechanics. Exogenous surfactant reduces opening pressure of the ET (61), also under pathological conditions in patients with otitis media (62, 63). It also seems to improve clearance function of the ET as a significant enhancement of mucociliary transport has been observed after the application of surfactant, both in vitro and in vivo (64). Theoretically, enrichment of administered surfactant by substances increasing its resistance (65) or having antimicrobial effect (66) could improve efficacy of such therapy.

Thus, the presence of a sufficient quantity and quality of ET surfactant may be an important determinant of ET functions and mechanics. Dysfunction of ET surfactant could be a possible factor in the development of serous otitis media.

CONCLUSION

Although numerous studies have focused on the nature and defensive role of surfactant in the lower airways, relatively little is known about its role in the upper respiratory system.

The lamellar body arrangement of phospholipids has now been demonstrated in the both normal and diseased sinus tissue, resulting in the implication that these structures may play a crucial role in the regulation of mucus viscosity and in mucociliary clearance against inhaled pathogens as well. Decreased levels of phospholipids have been found in atrophic rhinitis. Surfactant proteins make up a relatively small portion of surfactant, but appear to have an important role especially in innate immunity. They are crucial in the initial interaction, recognition, processing, and subsequent adaptive immune responses for a wide variety of inhaled pathogens and allergens. Presence of surfactant proteins in a variety of normal and diseased sinonasal tissue indicates that these proteins may play a significant role in physiology and pathophysiology of sinonasal diseases. Understanding the role of surfactant proteins in diseased and healthy states may help to develop novel treatments for sinonasal pathologies.

In addition, surfactant has been identified in the Eustachian tube where it helps to lower the opening pressure between the nasopharynx and middle ear. Therefore, alteration in surfactant levels may adversely affect Eustachian tube function and contribute to chronic ear infection.

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NUCLEAR MAGNETIC RESONANCE AS A DIAGNOSTIC TOOL IN BREAST CANCER

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Abstract

The early detection and treatment of breast cancer is of direct benefit to patients. Magnetic resonance imaging (MRI) is a promising modality for detection, diagnosis, and staging of breast cancer. MRI enables two methods: the diffusion-weighted MRI (DW MRI) and the dynamic contrast enhanced MRI (DCE MRI). DW MRI reflects the diffusion of water molecules in the extracellular fluid space and allows the estimation of cellularity and tissue structure. The value of the diffusion of water in tissue is called the apparent diffusion coefficient (ADC). ADC values in malignant lesions are smaller than in benign tissue. DCE MRI yields appropriate pharmacokinetic data of physiological parameters that relate to tissue perfusion, microvascular vessel wall permeability and extracellular volume fraction. Gadolinium based contrast agent is usually used in breast DCE MRI diagnostics. Changes in the post-contrast signal intensity help to distinguish lesions according to characteristically enhanced accumulation of contrast agent. Malignant lesions are characterized by a faster and stronger signal enhancement than benign lesions which relate to their neoangiogenesis. Over the last few years, there has been appreciable interest in the use of magnetic resonance spectroscopy (MRS) for the non-invasive analysis of breast tissue metabolites. One of the spectroscopic hallmarks of the neoplastic process appears to be the presence of total choline signal in the *in vivo* spectrum. Despite the fact that MRI and MRS achieve excellent results, they are still not so frequently used in comparison to mammography and breast ultrasound.

Key words: magnetic resonance imaging, diffusion weighted MRI, dynamic contrast enhanced MRI, magnetic resonance spectroscopy, breast cancer.

INTRODUCTION

Breast cancer is a major cause of cancer-related deaths among women in most western countries (1 - 4). According to recent statistics, the mortality and morbidity rates of breast cancer are the highest of all cancers in women in the Slovak Republic (4, 5). Therefore, early detection and treatment of breast cancer is necessary to patient survival.

MAGNETIC RESONANCE IMAGING (MRI)

The use of MRI as a diagnostic tool for detecting breast cancer began in the 1970s (2, 6). With the introduction of contrast agents, advances in surface coil technology, and development of new imaging protocols, MRI has emerged as a promising modality for detection, diagnosis, and staging of breast cancer (1, 6 - 9). MRI enables three investigative methods: the conventional MRI, the diffusion-weighted MRI (DW MRI), which aims at a meticulous analysis of the lesion's structure, and the dynamic contrast enhanced MRI (DCE MRI), which is based on tissue pharmacokinetic investigations (8, 10 - 12).

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DIFFUSION-WEIGHTED MRI (DW MRI)

First proposed for the purposes of breast cancer detection in 1997, DW MRI has been suggested as a potential adjunct to the existing MR protocol in order to improve the overall specificity of breast MRI. DW MRI provides information on microstructure which has been shown to be an important index of tumor grade, and local tissue architecture, which is a sensitive early indicator of abnormality (51, 52).

DW MRI reflects the diffusion of water molecules in the extracellular fluid space and allows estimation of cellularity and tissue structure (48 - 55). DW MRI is based on random Brownian motion of molecules during the interval of excitation and signal measurement which reduces the amplitude of the resulting signal. The application of an appropriate pulse allows the measurement of the signal cancellation due to diffusion in a given direction. While normal tissue exhibits gross signal loss, areas with restricted motion of molecules like densely packed tumor cells show less signal loss and become bright in diffusion-weighted images (Figure 1).

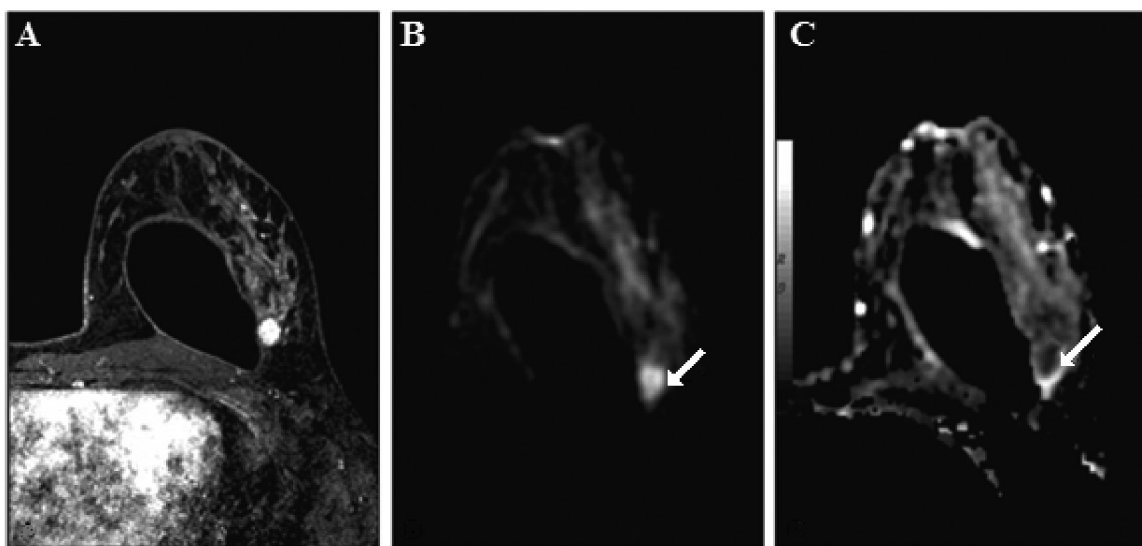


Fig. 1 Female, 48-year-old patient presenting infiltrating ductal carcinoma in the left breast. Axial, 3D gradient T_1 -weighted sequence with fat-suppression at early postcontrast phase (A), diffusion-weighted sequence ($b = 500 \text{ s} / \text{mm}^2$) in the axial plane (B), and apparent diffusion coefficient (ADC) black / white map in the axial plane (C) show microlobulated nodule with suspicious contrast-enhancement. Note that the nodule presents high signal intensity on the diffusion (arrow) and signal loss on the ADC map (arrow), suggesting restricted diffusion of water molecules (55)

The value of the diffusion of water in tissue is called the apparent diffusion coefficient (ADC). Based on the diffusion-weighted images an ADC map can be calculated which shows the ADC value of each voxel in every slice. Restricted water movement in tumors with high cellularity leads to smaller ADC values than in benign tissue. This is likely to be caused by increased cellularity, larger nuclei, and less extracellular space. Furthermore, in malignant tumors, a higher percentage of microvessels is present than in benign tissue. Perfusion may, therefore, artificially increase ADC in malignant lesions and hinder differentiation (44, 46 - 48, 51, 53, 54, 55).

However, in tumor clusters the diffusion is vastly restricted in every direction and therefore it is sufficient to measure DWI of the breast in just one orientation. In most

applications the diffusion gradients are integrated in echo planar imaging (EPI) sequences which exhibit high signal intensity in areas with restricted diffusion as well as in fatty tissue. Furthermore, the fat signal is displaced in the direction of the chemical shift as compared to the water signal. This makes fat saturation techniques necessary to identify the lesions in the diffusion-weighted images (44, 47 - 49).

Image interpretation starts like conventional breast MRI. If a lesion is visualized in the dynamic scan it has to be identified in the corresponding slice of the diffusion-weighted images. As a second step, a region-of-interest (ROI) is drawn in the centre of the lesion and copied to the ADC map. The scanner software provides the mean value within the ROI which equals the ADC value. The ADC in each voxel is a sum of the true ADC and noise. To estimate the true ADC, the noise must be minimized. This is especially important for small lesions, where the accurate determination of the mean ADC relies on only a few voxels. The precision of ADC determination reaches a maximum at a b value, which is a scan parameter. The b factor depends on target organs, lesions, and equipment. The optimum b factor should sufficiently suppress the background signal of the mammary gland and provide a cancer signal that is strong enough to allow image interpretation. Previous studies have suggested that the optimal b value for breast lesion classification with DW MRI is between 700 and 1000. However, it is also well known that higher b values result in reduced signal to noise ratio (SNR) and therefore lower image quality. If the lesion is not visible in the b 800 images, the location of the lesion can be identified at least b in the 50 image, otherwise the ADC value can not be evaluated (44, 47, 50, 51, 53, 55). Lesions smaller than 5 mm and lesions with central necrosis and an enhancing rim smaller than 5 mm are often harder to delineate in the DWI or lead to wrong ADC values (44, 47). Application of DW MRI to the breast has been limited by movement artifacts such as breathing and heartbeats (49, 52, 55).

DYNAMIC CONTRAST ENHANCED MRI (DCE MRI)

DCE MRI yields appropriate pharmacokinetic data of physiological parameters that relate to tissue perfusion, microvascular vessel wall permeability and extracellular volume fraction (9, 12 - 15). DCE MRI, on the other hand, is a technological process in which serial T_1 - weighted 3D MR images of both breasts are acquired before and repetitively after the administration of the contrast agent. This agent, usually gadolinium based, is a paramagnetic substance which generates its own magnetic field. This magnetic field decreases relaxation times (T_1 , T_2) and so enables differences between tissues according to the volume of contrast agent (9, 10, 11, 14, 16). Gadolinium - diethylenetriamine pentaacetic acid (Gd - DTPA) contrast agent is commonly used in breast DCE MRI (10, 11). The first to apply Gd - DTPA as a contrast agent in breast MRI was Heywang et al. in 1985. Following that in 1989 Kaiser et al. first reported dynamic breast MRI, as a modality designed to track rapid changes in signal intensity (7, 14, 24). Changes in the post-contrast signal intensity helped to distinguish lesions according to characteristic enhanced accumulation of contrast agent could then be related to higher tissue microvasculature (1, 7, 12, 13, 15, 17, 18). Tumor growth is dependent on angiogenesis, which provides the tumor with nutrients. Therefore, the microvessel density is much higher in cancers than in healthy tissue (9, 14 - 16, 19). Malignant lesions are characterized by a faster and stronger signal enhancement than benign lesions which relate to their neoangiogenesis. However, benign lesions may also show rapid enhancement during strong hormonal stimulation (e.g., pregnancy, oral contraception, fibroadenomas) (6, 9, 10, 14, 15, 17 - 20).

Subsequently, a small ROI is drawn over the region that appears to be the most enhancing in the lesion and the enhancement values at different pre-and post-contrast time points are calculated over this to form the kinetic curve (Figure

2) (7, 8, 10, 11, 20, 21). With the use of dynamic data sets, two different criteria may ensue to describe lesion enhancement kinetics:

1. The first is to analyse the behaviour of signal intensity in the early phase after the administration of contrast material. It is evaluated by means of the steepness of the postcontrast signal intensity curve. Several descriptors are applied to this criterion: curve slope, early-phase enhancement rate, enhancement velocity, or percentage of increase in signal intensity. The largest increase in relative signal intensity which usually occurs in the first postcontrast minute is referred to as the enhancement or wash-in rate. The malignant lesions tend to have higher enhancement velocities than benign ones (1, 7, 11).
2. A more common practice, as a modality for any cancer detection is to observe the signal intensity behaviour in intermediate and late post-contrast phases of the DCE MRI curves (7, 8, 9, 22). The late post-contrast phases of the DCE MRI curves depend on their shapes and are categorized into three types as shown below in Figure 3 (7, 8, 10, 11, 13, 21):
 - ❖ Type I, a persistent curve with continuous increase in enhancement for benign lesions,
 - ❖ Type II, a plateau curve that reaches a maximum signal intensity approximately 2 to 3 min after contrast agent injection. The signal intensity remains constant after reaching the maximum because of saturation effects. A plateau curve can be seen with both benign and malignant lesions.
 - ❖ Type III, a washout curve with decreasing signal intensity after peak enhancement for malignant lesion;

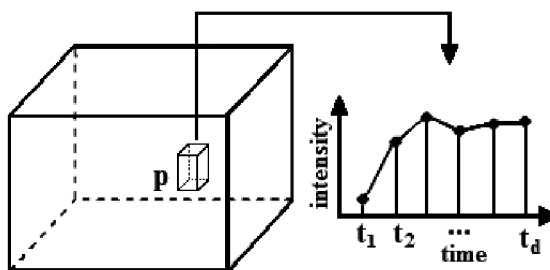


Fig. 2 The principle of creating kinetic DCE MRI curves (21)

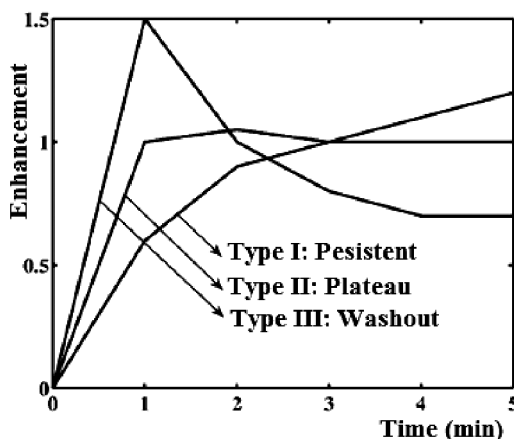


Fig. 3 The three types of kinetic DCE MRI curves; type I: a persistent curve for benign lesions, type II: a plateau curve for both types of lesions, type III: a wash out curve for malignant lesions (10)

A breast tumor leads to angiogenesis, i.e. the formation of new vessels and/or the sprouting of existing capillaries. These newly formed vessels yield an early contrast agent enhancement, and therefore, a strong contrast agent washin. Furthermore, the vessels, as a result of the angionetic activity of the tumor, become highly permeable and a rapid contrast agent washout would then be expected in tumorous tissue (2, 9, 14 - 16, 19). In many cases, the targeted tumor is very heterogeneous and the most appropriate part of it then is not so easily determined. To avoid the distortion of the average time-intensity curve, the ROI should be very small and must not include dead tumor cells (necrosis) or surrounding tissue. The heterogeneity of tumor vascularization, the proximity of necrotic and vital tumor tissue, and the subjectiveness of ROI placement complicate the interpretation of the kinetics and may even result in unrevealed malignant tissue. This could happen, if an ROI covers malignant and benign tissue, with an average curve shape indicating benignity (2, 7, 8, 10). Whereas it is still not standardized the method for generating kinetic curves is accepted as both inter-and intraobserver variability (7, 8, 10, 11, 20, 21). In general, though, DCE MRI has excellent sensitivity despite its variable specificity for breast cancer detection (10, 14, 15, 22, 24).

MAGNETIC RESONANCE SPECTROSCOPY (MRS)

Over the last few years, there has been appreciable interest in the use of proton magnetic resonance spectroscopy for the non-invasive diagnosis of breast lesions. The normal breast consists of glandular and adipose tissue, which in the proton spectrum exhibit large signals from water (4.7 ppm) and lipid resonances (multiple lipid resonances: at 0.9 and 1.3 ppm, at 2.2 and 2.5 ppm, at 5.2 ppm). When water and lipid suppression pulses are turned on, it becomes possible to observe much smaller metabolite signals: various compounds including choline (Cho, 3.2 ppm), creatine (Cr, 3.0 and 3.9 ppm), taurine (Tau, 3.3 ppm), glycine (Gly, 3.5 ppm), and alanine (Ala, 3.8 ppm), amongst others (27, 28, 38, 39, 43). It should be noted that these signals are generally only clearly observed using high-strength magnetic fields (at least 4 T) and state-of-the-art methodology. Using lower field strength (1.5 T) systems, the only observable signals, in the normal breast tissue, may be residual water and lipids. One

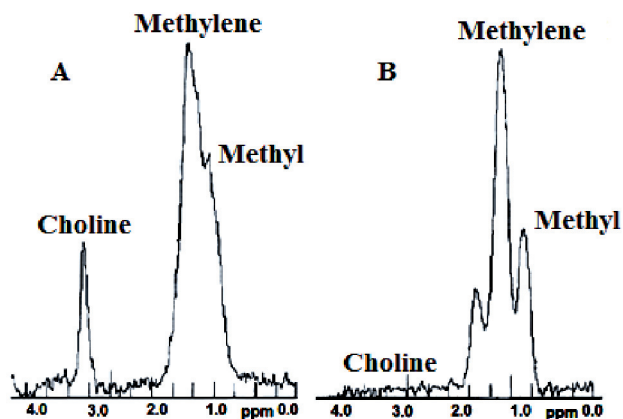


Fig. 4 Localized proton spectra from the human breast recorded using the PRESS pulse sequence (1.5 T, TR 2 sec, TE 350 msec, 256 averages, 3.8 cm³ voxel size).

(A) Infiltrating ductal carcinoma, (B) fibroadenoma. Lipid methyl and methylene signals are observed in both spectra, while an elevated choline peak at 3.2 ppm is only detected in the carcinoma (A) (27)

of the spectroscopic hallmarks of the neoplastic process appears to be the presence of a choline signal in the *in vivo* spectrum, predominantly consisting of phosphocholine (PC), glycerophosphocholine (GPC), and some free choline itself usually in higher concentrations than in normal tissue or in non-malignant lesions (Figure 4). However, the precise mechanisms that produce an elevated total choline (tCho) concentration have not yet been fully identified. A working hypothesis is that elevated tCho is an indicator of increased cellular proliferation. The largest component contributing to the tCho peak from neoplastic tissue is phosphocholine, a known precursor of membranes. Thus, the increased tCho in neoplastic tissues may be a reflection of increased membrane turnover by replicating cells (27, 29, 30, 37 - 39, 43). Normal tissue, and many fibroadenomas, should not exceed a Cho concentration of 1 mmol.kg⁻¹, so a concentration higher than this value is suspicious for malignancy. However, this may not be the case under all circumstances; for instance, it has been reported that Cho and lactose signals (~3.8 ppm) can be observed in women who are lactating (27, 37, 39).

MRS may also be used to determine if a tumor is responding to a particular chemotherapy regime. It has been hypothesized that biochemical measurements of tumor Cho will decrease as cells are killed, prior to reduction in tumor size. An earlier indicator of tumor response could in principle be used to alter chemotherapeutic agent or dosage, rather than performing several weeks of ineffective therapy (27, 38 - 40).

Various factors, as listed below, should be kept in mind when evaluating spectra from breast lesions (27, 31, 32, 37, 39):

- ❖ Lesion size: Since the signal-to-noise ratio (SNR) of a spectrum depends linearly on the voxel size (amongst other factors), smaller lesions will be less likely to show detectable tCho signal than larger ones. Limitation of lesions is 2 cm³ or larger at 1.5 T.
- ❖ SNR: It should also be kept in mind that, under high SNR conditions, it is possible to detect tCho signal from normal fibroglandular tissue. Spectral resolution and SNR are critically dependent on magnetic field homogeneity, which can be challenging to optimize in the breast, even at field strengths of 1.5 T.
- ❖ Field homogeneity: For better homogeneity sensitive phased-array receiver coils should be used. Breast and respiratory motions are undesirable under these circumstances.
- ❖ Suppression of water and lipids: It has been shown that small magnetic field instabilities can modulate the large lipid signals typically found in the breast to produce discrete sidebands, which can overlap with or mimic small Cho signals.

CONCLUSION

After establishing the existence of a lesion in breast, it is critical to determine whether this lesion is benign or malignant. The sensitivity of breast cancer detection by mammography is 69 % – 90 %. It has been estimated that between 5 % and 15 % of tumors are missed. Sonographic classification of benign and malignant tumors is of a low specificity as well—approximately 30 % (6, 27, 37, 45 - 49).

MRI has been the most commonly used modality due to its wide availability and lack of ionizing radiation (20). Due to increased vascularity and capillary permeability of tumors, DCE MRI shows better distinction between lesions and normal tissue than conventional MRI alone (1). Despite the fact that MRI and MRS achieve excellent results, they are still not so frequently used in comparison to mammography and breast ultrasound. From current consensus, NMR is particularly suited for specific cases, such as patients who have undergone breast-conserving therapy, patients who have high risk of developing breast cancer, patients with implants, plus, postoperative scars, or clinical evidence of breast cancer that can not be detected by other diagnostic me-

thods (1, 6, 10 - 12, 15, 22, 24). Breast MR imaging may improve detection of localized breast cancer by revealing multifocal growth in patients scheduled for conservative breast surgery (9, 11, 12, 22). In general, the sensitivity reported for diagnosis of breast cancer using MRI is larger than 90 %, but the reported specificity varies considerably and may be substantially lower (1). The sensitivity of breast DCE MRI has been reported to range from 90 % to 100 % for the detection of breast cancer, whereas the specificity has been reported to be between 20 % and 90 %, which is likely to be lower than the sensitivity (3, 6, 8, 26, 27, 52). DW MRI is the only method to measure molecular diffusion in vivo. Furthermore, it provides extensive information without contrast medium. An ADC threshold level between malignant and benign lesions with a diagnostic accuracy is around 95 % (49, 52, 53).

MRI's main disadvantages are: it has a long scan time and it is a relatively expensive examination. The use of contrast agent is contraindicated in patients with a high risk of nephrogenic systemic fibrosis. The Committee for Medicinal Products for Human Use (CHMP) does not recommend their use in the following cases (10, 15, 25):

- ❖ in patients with kidney problems and patients receiving a liver transplant,
- ❖ in neonates and infants,
- ❖ in the elderly,
- ❖ in pregnant or breastfeeding women.

It is still valuable to add additional modalities which could increase diagnostic confidence. Magnetic resonance spectroscopy has the potential to fulfil this role. Furthermore, MRS is low-risk in that it is non-invasive, and does not involve radiation. It can also be cost-effective and useful in radiographically dense breasts. ¹H MRS of the breast has a sensitivity of 70 % - 96 % and a specificity of 83 % - 93 % for the detection of breast cancer (37, 38, 41 - 43). Nevertheless, at present it largely remains mainly an experimental procedure (27, 33 - 37).

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OVARIAN HAEMANGIOMA WITH INFUNDIBULOPELVIC LIGAMENT TORSION IN A 13-YEAR-OLD GIRL

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Abstract

Background. Ovarian haemangioma are very rare type of tumors, although the ovary itself is a highly vascularised organ. Such benign tumors of the blood vessels are rare in ovaries during childhood.

Clinical case. We described a 13-year-old girl with an ovarian haemangioma presented clinically with acute abdomen symptoms - abdominal and pelvic pain and vomiting. The pelvic mass was noted and sonography revealed a huge cystic tumor with probably ovarian torsion. A left salpingo-oophorectomy and appendectomy were performed with an uneventful postoperative course. Pathology examination revealed a cavernous haemangioma of the ovary.

Conclusion. Haemangiomas of the ovary, especially in children, are very rare type of the benign tumor. Surgical extirpation of the involved areas is the treatment of the choice.

Key words: haemangioma, ovary, ovarian torsion, children

INTRODUCTION

Infantile haemangiomas are described as a benign (non-cancerous) condition affecting cutaneous blood vessels. It is also known as proliferative haemangioma because it is due to proliferating endothelial cells. Haemangiomas usually develop shortly after birth and they are distinct from vascular malformations, which are usually present at birth and are less common. Over 80% of infantile haemangiomas occur on the head and neck area. They grow to 80% of maximum size in the first three months and most stop growing at about 5 months. However, they may keep growing for up to 18 months (1, 2). Although ovaries have a very rich vascular blood supply, haemangiomas of the ovary are extremely rare especially in ovaries during childhood. The number of well-documented cases of ovarian haemangioma, in both adult and children, does not exceed 100 patients.

Haemangioma of the ovary was first described by Payne in 1869 (cited by Talerman) (3, 4). Mostly they are the small tumors discovered incidentally. In low percentage of cases ovarian haemangiomas presented clinically with an acute abdomen. The presenting symptoms of acute abdomen and the ultrasonographic study in most cases led to the preoperative diagnosis of torsion of an ovarian tumor. When we found a tumor on the ovaries, in the most cases ovarian cysts are present. Ovarian cysts are most commonly in children undergoing the conservative treatment, but larger cyst or ovarian torsion need surgical treatment. The purpose of this presentation is how to deal with changes in the ovaries. Only cysts of smaller dimensions could be treated conservatively. Ovarian cysts larger than 5 cm and tumors should be treated surgically due to the possibility of malignant alteration and / or ovarian torsion (5).

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CASE REPORT

A 13-years-old girl was admitted to the surgical clinic with severe pain in the abdomen which lasted for 24 hours. Prior hospitalization she was vomiting 6 times. After being admitted to the hospital, we started with the treatment. Inflammatory parameters were elevated, Sedimentation rate was 65 mm/h (normal 0-15 mm/h), leukocyte count was 18.9 (normal $4-12 \times 10^9/L$) and C-reactive protein was 82mg/L (normal <5mg/L). Patient was immediately subjected to abdominal ultrasound (Figure 1). In ultrasonographic evaluation of our patient a pelvic mass was found located at the left ovary. Ovary size was 10 x 6.4 cm, which had no blood flow. Ultrasound showed a large ovarian torsion. Then she was examined by a gynaecologist. Rectal and abdominal ultrasound transducer probe found solid pelvic mass with impaired flow through it. We suspected ovarian torsion and immediately surgery was induced. We have not even attempted laparoscopic operative treatment. The pain lasted a long time, an ultrasound showed torsion of the ovary and we were not sure they could perform surgery with laparoscopic management of the mass and untwisting the ovary and the tube. A Pfannenstiel incision was done and displayed left ovarian tumor the size of 10 x 7 x 4 cm. Ovary with the tumor and the fallopian tube was turned 5 x 180 degrees. Fallopian tube and ovary with the tumor were necrotic and the only treatment option was the removal of the tumor, ovaries and fallopian tubes (Figure 2). The right ovary was adequate layout and size for gestational age girls and grossly was unremarkable. During surgery we made removal of the appendix. We always removed appendix when surgery in the abdomen is necessary for other reasons. It is known that after abdominal surgery adhesions are present, with appendectomy we prevent re-surgery which may require an appendicitis. Postoperative course was uneventful, the wound healed properly. Tumor markers including CA125 (cancer antigen 125), and CEA (carcinoembryonic antigen) were within normal limits /normal values: CA125 < 35 mU/L; CEA < 3.4 g/L /.

Removed parts were sent for histopathological diagnosis. Fallopian tube was 5 cm long filled with blood. In addition to the Fallopian tube was an oval lesion size

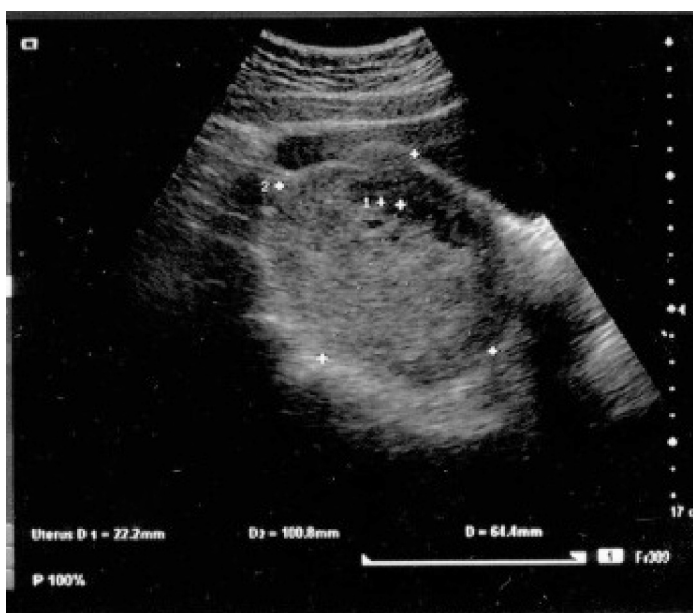


Fig. 1. Abdominal ultrasound with ovarian torsion

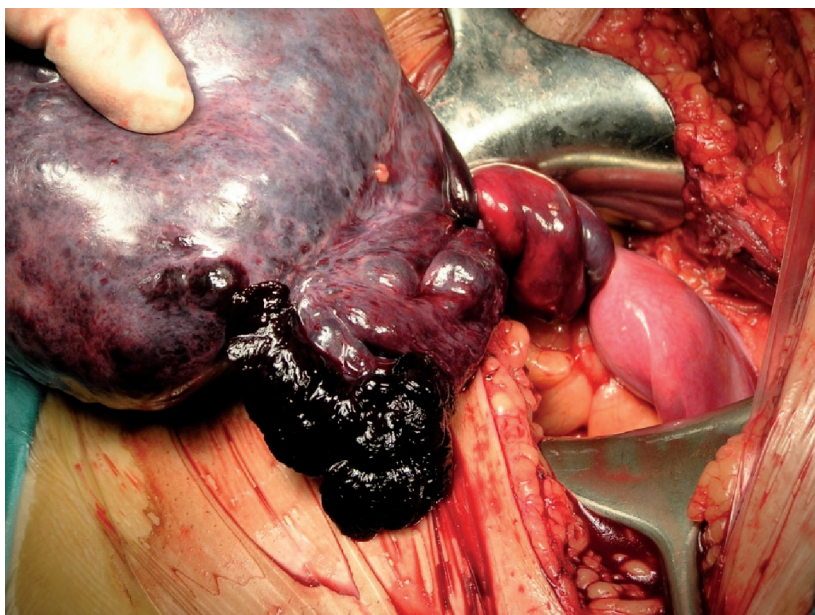


Fig. 2. Haemangioma of the ovary with torsion and necrosis

10x7x4 cm. The formation of the cut was fully encapsulated and haemorrhagic. Macroscopically, the tissue of the ovary was not found. Microscopically examinations was made of 23 slices and find different wide vascular spaces, in some places, spaces were dilated with a thick or thin-walled space. Described properties were lined with flattened endothelium and filled with erythrocytes. Histologically, there were parts as ovarian and fallopian tube and tumor formation are fully imbued with bleeding, ovarian structure was lost to necrosis. Histological picture, histochemistry (Mallory) and immunohistochemical (CD31, D2-40) analysis of the received and reviewed materials corresponds to haemangioma of the ovary with torsion of the ovary.

DISCUSSION

Haemangiomas are benign lesions arising from a failure in vascular formation forming abnormal vascular channels and are of two types- capillary and cavernous. The blood vessels in uppermost layers of the skin are dilated and called capillary haemangioma. Deep infantile haemangiomas are also called cavernous haemangiomas and they appear as a bluish soft to firm swelling. The difference between them relates to the size of the blood vessels formed. They may be localised (confined to a small area) or segmental (involving a larger neuroectodermal unit). The superficial infantile haemangioma is also called capillary haemangioma, capillary naevus, strawberry haemangioma, strawberry naevus, and haemangioma simplex. Capillary haemangiomas are made of the blood vessels of the caliber of normal capillaries, and cavernous types consist of larger channels which are more deeply set in the dermis and subcutis. Both types of haemangioma may occur together in mixed angiomatous naevi(1,5). Vascular tumors of the female genital tract, especially those arising in the ovary in childhood, are very rare although the ovary has rich blood supply (6). The most cases of the ovarian haemangioma are small and incidental findings at operation. Large lesion tends to present clinically with symptoms typical for presence of the mass or such as abdomi-

nal pain due to torsion. In our case haemangioma was large and symptomatic such as acute abdomen due to torsion (7-9). Cavernous haemangioma of the ovary is more prevalent than other histological types. The etiology of the ovarian haemangioma is unknown. Some authors think that the hemangiomas are hamartomas, true tumors, stimulated the blood vessels or congenital malformations (10, 11).

Our surgical approach is debatable because some would strongly advocate for laparoscopic management of the mass, and untwisting the ovary and tube; the literature seems to support this approach. In tumors changes in the ovary, where there is no torsion or torsion of the ovary takes up to 6 hours we always do laparoscopic surgery. In ovarian torsion lasting as long in our patient, always choose the classic surgery. In torsion of the ovary that last longer than 24 hours, the success of laparoscopic surgery was low in our hospital. On several occasions we tried to do laparoscopic untwisting the ovary and tube and in most cases the surgery could not be done for strong edema. A Pfannenstiel incision is often used in preference to other incision types for the sake of aesthetics, because the scar will be hidden by the pubic hair. The incision does not distort the belly button and heals faster than the traditional vertical incision. A Pfannenstiel incision is a type of surgical incision that allows access to the abdomen. Because the exposure is limited this incision should be used only when surgery is planned on the pelvic organs. The Pfannenstiel incision offers large view of central pelvis but limits exposure to the lateral pelvis and upper abdomen, factors that limit the usefulness of this incision for gynecologic cancer surgery.

Haemangioma of the ovary, especially in children, is very rare neoplasm. Surgical extirpation, laparoscopic or open method, of the involved areas is the treatment of the choice. After histopatological examination and correct diagnosis, definitive surgical treatment was done. In our patient we had only one method of treatment because it was haemangioma with an ovarian torsion and both of them were necrotic.

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EFFECT OF YOGA EXERCISE ON METABOLIC AND HOMEOSTATIC PARAMETERS OF INSULIN RESISTANCE

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Abstract

The aim of this study is to assess the effect of yoga exercise on the anthropometric parameters and metabolic parameters in individuals regularly exercising yoga as well as to assess the possible impact on cardiovascular risk prevention. The study sample composed of two groups: A (57 healthy control) and B (61 subjects exercised yoga). For each group were measured: blood pressure, body mass index (BMI) and laboratory values of total cholesterol, triglycerides, high density lipoproteins (HDL) and low density lipoproteins (LDL) cholesterol, glycaemia and insulinemia, homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index (QUICKI). BMI was significantly lower ($p = 0.005923$) in the group B (yoga). We found an impact of yoga on the homeostatic indices HOMA-IR and QUICKI and it means significantly lower value of systolic blood pressure ($p = 0.043543$) and LDL cholesterol levels ($p = 0.008714$).

Based on these outcomes, we consider yoga to be a suitable physical activity to prevent cardiovascular diseases.

Key words: yoga, insuline resistance, cardiovascular diseases, HOMA-IR, QUICKI.

INTRODUCTION

Owing to the intensive interdisciplinary co-operation between the respective medical fields the mortality rate from cardiovascular diseases has been significantly reduced. However, cardiovascular diseases remain the most frequent cause of death in adults, account for most sick leaves, incur significant financial burden from treatment and prevention, and continue to represent a major health and social-economic problem not only for the developed countries (1).

For a very long time the causality between some nosological features, such as hypertension, obesity, diabetes mellitus (DM), dyslipidemia and some others, and the development of cardiovascular diseases has been known. Vascular disease is here the main cause of morbidity and mortality, and accelerated atherosclerosis is responsible for about 80% of mortality and for about 75% of hospitalizations (2). The cost of cardiovascular diseases in European Union is estimated to be as high as 169 billion Euro early (3). There is a need for efficient primary prevention that specifically focuses on the risk factors (RF) influencing the onset of such diseases. The most significant role here

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is assigned to the risk factors linked with the metabolic syndrome (MS). The impact of changes in diet and, in particular, in the physical activities has been demonstrated by a number of studies. For example, Ivy correlated the relationships between hypokinesia under genetic pre-disposition with the development of insulin resistance (4).

Regarding the strong influence of psychosocial factors on the development of MS and cardiovascular diseases (CVD), on the key role of activation of sympathetics in the pathogenesis of insulin-resistant conditions, on the ability of regeneration and restoration of changes resulting from MS, the mind-body therapy represents a considerable potential in the prevention and treatment of cardiovascular diseases (5,6,7). In this respect, yoga enjoys a special status. It is the oldest known system of personal development covering the body, mind and spirit. There are only a few studies in international literature documenting the positive impact of regular yoga exercises on the CVD RF. The studies demonstrate that regular yoga exercises regulates arterial hypertension and improves cardiac performance, positively affects insulin resistance, abdominal obesity and improves type 2 diabetes mellitus, normalizes lipid profile, modifies coagulopathy and oxidation stress through balancing the sympathetic and parasympathetic tonus, positively affects certain anthropometric parameters and improves many other clinical parameters (8, 9, 11). Also yoga has a potential impact on posture and stability control which is a process of maintaining balance and position of the body and its parts in a constantly changing environment. It is an important regulatory mechanism of the body, because it precedes movement, is upon the completion of which this system tries to maintain the body position (10).

The objective of this study is to assess the effect of yoga exercise on the anthropometric parameters : body mass index (BMI), metabolic parameters (glycaemia, insulinemia, LDL and HDL cholesterol,) and the homeostatic indices homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index (QUICKI) in individuals regularly exercising yoga for more than 1 year and to compare these parameters with the standard population without any apparent signs of insulin resistance.

SUBJECTS AND METHODS

In present study the subjects were divided in two independent groups. All subjects signed an informed consent prior to their entry into the study.

Group A contained 57 biological sample of the population subjects (29 women, 18 men, average age 50.93 SD 9.58) without clinical or metabolic signs of insulin resistance and the subjects were selected randomly out of a larger set of patients monitored by practitioners to ensure the study groups are consistent in terms of age and sex. This group included subjects who have not been treated yet for any chronic disease; however, their lab results subsequently showed deviations from physiological values.

Group B comprised 61 subjects (43 women and 18 men, average age 48.6 SD 11.6) who regularly, twice a week, exercised yoga for longer than 1 year.

The subjects in both groups were not on any special diet and observed their regular eating habits.

For each subject, the following parameters were measured: blood pressure, BMI and laboratory values of total cholesterol, triglycerides, HDL and LDL cholesterol, glycaemia and insulinemia. The values of glucose and insulin concentration were used to calculate HOMA-IR and QUICKI.

The HOMA- IR was calculated according to the homeostatic mode as (12, 13, 14):

$$HOMA\ IR = insulin\ fasting\ (IU/ml) \times glycaemia\ fasting\ (mmol/l) / 22.5.$$

QUICKI was calculated according to formula:
 $QUICKI = 1 / [\log \text{ insulin fasting (IU/ml) } + \log \text{ glycaemia fasting (mmol/l) } \times 18.182]$

Statistical evaluation was done using the programme Statistics version 6.0 for analysis of the means, medians and standard deviations. T-test was calculated to determine statistical significance ($p < 0.05$) in clinical and biochemical characteristics.

RESULTS

We have demonstrated statistically lower values of glycaemia ($p = 0.017164$) and insulinemia ($p<10^{-6}$) in the group of individuals exercising yoga for more than 1 year. We have also demonstrated a statistically significant difference in values of homeostatic indices HOMA-IR (decrease) and QUICKI (elevation) in a group of individuals exercising yoga ($p<10^{-6}$). (Table 1,2).

Table 1 Results of examination in the groups A (controls) and B (yoga)

		N	Average	95% Confidence limit		Median	Min	Max	SD
Yoga (B)	SYSTOL BP	61	118.68	113.92	123.45	120.00	90.00	160.00	18.61
	DIASTOL BP	61	74.75	71.83	77.67	80.00	60.00	120.00	11.4
	GLY (mmol/l)	61	5.02	4.86	5.19	4.90	4.10	8.80	0.65
	HDL (mmol/l)	61	1.60	1.50	1.70	1.52	0.82	2.95	0.39
	LDL (mmol/l)	61	3.12	2.90	3.33	3.07	1.79	5.87	0.83
	BMI (kg/m²)	61	23.11	22.32	23.90	22.70	17.40	30.90	3.08
	INZULIN (mIU/l)	61	4.53	3.66	5.40	3.20	2.00	19.80	3.39
	Quicki	61	0.40	0.38	0.41	0.39	0.30	0.46	0.04
	HOMA_IR	61	1.03	0.81	1.24	0.78	0.36	4.92	0.84
	Age at examination	61	48.62	45.65	51.59	48.00	26.00	68.00	11.60
Control group (A)	SYSTOL	57	125.08	121.28	128.88	125.00	95.00	170.00	14.31
	DIASTOL	57	76.31	73.66	78.96	75.00	60.00	110.00	9.97
	GLY (mmol/l)	57	5.18	5.06	5.31	5.10	4.30	6.40	0.45
	HDL (mmol/l)	57	1.68	1.59	1.77	1.70	1.08	2.47	0.34
	LDL (mmol/l)	57	3.23	3.07	3.38	3.30	1.30	4.26	0.58
	BMI (kg/m2)	57	24.42	23.75	25.09	24.44	19.03	29.32	2.52
	INZULIN (mIU/l)	57	8.57	7.34	9.80	7.70	2.20	22.10	4.59
	Quicki	57	0.35	0.34	0.36	0.34	0.29	0.44	0.03
	HOMA_IR	57	2.01	1.69	2.33	1.81	0.43	5.83	1.19
	Age at examination	57	50.92	48.38	53.47	54.00	22.00	63.00	9.58

SYSTOL BP- systolic blood pressure, DIASTOL BP- diastolic blood pressure, GLY- glycaemia, HDL- high density lipoprotein, LDL low density lipoprotein, BMI –Body Mass Index

Table 2 Statistical significance of individual parameters in A and B

	N (A)	N (B)	p-value (Mann-Whitney)
SYSTOL BP	57	61	0.029212
DIASTOL BP	57	61	0.411358
GLY	57	61	0.017164
HDL	57	61	0.182376
LDL	57	61	0.146395
BMI	57	61	0.005923
INZULIN	57	61	p<10⁻⁶
Quicki	57	61	p<10⁻⁶
HOMA_IR	57	61	p<10⁻⁶
AGE	57	61	0.252377

SYSTOL BP- systolic blood pressure, DIASTOL BP- diastolic blood pressure, GLY- glycaemia, HDL- high density lipoprotend, LDL low density lipoprotein, BMI –Body Mass Index

Group B (yoga) demonstrated statistically significantly lower value of systolic blood pressure, value of serum insulin, both homeostatic indices HOMA-IR and QUICKI, BMI value and serum glycaemia.

Comparing the individual parameters in both groups using sex as a parameter of comparison, in men, group B- yoga subjects, was significantly lower BMI, systolic blood pressure, insulinemia, LDL cholesterol and both homeostatic indices HOMA-IR and QUICKI.

Comparing women from both groups A and B we found in group B only a statistically significantly lower insulinemia and significant difference in both homeostatic indices HOMA-IR and QUICKI. We have not found significant difference in systolic blood pressure, LDL cholesterol and BMI as in the group of men (Table 3).

Table 3 Comparison of statistical significance of individual parameters in A and B; gender is the parameter for comparison

	Men			Women		
	N (A)	N (B)	p-value (Mann-Whitney)	N (A)	N (B)	p-value (Mann-Whitney)
SYSTOL BP	18	18	0.043543	39	43	0.218130
DIASTOL BP	18	18	0.742798	39	43	0.287289
GLY	18	18	0.050859	39	43	0.121413
HDL	18	18	0.481230	39	43	0.517727
LDL	18	18	0.008714	39	43	0.904451
BMI	18	18	0.007105	39	43	0.068615
INZULIN	18	18	p<10⁻⁶	39	43	0.000300
Quicki	18	18	p<10⁻⁶	39	43	0.000336
HOMA_IR	18	18	p<10⁻⁶	39	43	0.000336

SYSTOL BP- systolic blood pressure, DIASTOL BP- diastolic blood pressure, GLY- glycaemia, HDL- high density lipoprotein, LDL low density lipoprotein, BMI –Body Mass Index

DISCUSSION

There is rather limited number of studies analysing the impact of yoga exercise on CVD RF. Most studies were carried out in Asia.

The results of our study confirmed the positive effect of regular exercise not only on the anthropometric parameters, where in the group of yoga men the BMI was significantly lower ($p=0.005923$), but also on the homeostatic indices HOMA-IR and QUICKI demonstrated a decrease in risk factors for CVD in patients who exercised yoga through reduction of glycaemia, cholesterol, triglycerides, improvement of subjective condition and the quality of life and reduction in blood pressure. They also demonstrated reduction in catecholamine levels, reduced sympathetic tonus and oxidation stress (15). Our study also demonstrates significantly lower value of systolic blood pressure in men ($p=0.043543$). Therefore, it is possible to agree with the studies carried out by other authors that yoga exercise may be recommended as an efficient, non-pharmacological, safe and not so difficult way to significantly reduce hypertension, primarily in men (9,16).

Simple fasting methods to measure insulin resistance, such as the homeostasis model assessment (HOMA-IR), and quantitative insulin sensitivity check index (QUICKI)

methods, have been widely promoted for studies, because of the possibility of evaluating insulin sensitivity from the levels of glycaemia and insulinaemia with the help of the homeostatic indices HOMA-IR and QUICKI which is for use in general practice (17,18). The significant difference in the glycaemia level ($p=0.017164$), insulinemia ($p<10^{-6}$) and homeostatic indices calculated based on these values HOMA-IR ($p<10^{-6}$) and QUICKI ($p<10^{-6}$) may be seen very positively as they indicate the protective effect of yoga exercise against the development of insulin resistance or metabolic syndrome. These results are in compliance with a similar monitoring by Bijani (2005), which demonstrated a decrease in glycaemia and adjustment of total cholesterol, LDL cholesterol and HDL cholesterol already after a 9-week yoga course (10).

Our study demonstrated a significant impact on LDL cholesterol levels ($p=0.008714$) and BMI ($p=0.007105$) for men, in women the difference was not statistically significant. The possible explanation is, that men exercised more frequently and for more years than women.

We have demonstrated statistically lower values of glycaemia and insulinemia in the group of individuals exercising yoga for more than 1 year. We have also demonstrated a statistically significant difference in values of homeostatic indices HOMA-IR and QUICKI. Based on these outcomes we consider yoga could be a suitable physical activity and may be recommended as an efficient, non-pharmacological, safe and not so difficult ways to prevent cardiovascular diseases. On the other hand, the authors are aware of the fact that the positive effect of regular yoga exercise may also be influenced by a different diet and lifestyle of individuals regularly exercising yoga and they recommend to focus on further studies in this area.

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