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THE EFFECT OF DIETARY NITRATES ON THE SEVERITY OF DISEASE, THE ENDOTHELIAL FUNCTION AND INDICES OF OXIDATIVE STRESS IN PATIENTS WITH CHRONIC DISEASES

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Η επίδραση της διατροφής με νιτρικά στην βαρύτητα της νόσου, την ενδοθηλιακή λειτουργία, και δείκτες οξειδωτικού στρες ασθενών με χρόνιες παθήσεις.

Περίληψη

Οι ασθενείς με χρόνιες νόσους όπως η χρόνια αποφρακτική πνευμονοπάθεια (ΧΑΠ) και η ρευματοειδής αρθρίτιδα (ΡΑ) συχνά εμφανίζουν ενδοθηλιακή δυσλειτουργία και αγγειακά προβλήματα, τα οποία συσχετίζονται με την εμφάνιση καρδιαγγειακής νόσου. Υπάρχουν πολλοί παράγοντες που επηρεάζουν τη λειτουργία του ενδοθηλίου, ένας εκ των οποίων είναι η διατροφή. Υπάρχουν προτάσεις ότι η κατανάλωση χυμού παντζαριού (ΧΠ) μπορεί να μεταβάλλει την ενδοθηλιακή λειτουργία σε υγιή άτομα. Ωστόσο, υπάρχουν λιγοστές αναφορές σχετικά με την επίδραση του ΧΠ στην ενδοθηλιακή λειτουργία ασθενών με χρόνιες παθήσεις. Ο σκοπός της παρούσας μελέτης ήταν να διερευνηθούν οι επιδράσεις της κατανάλωσης ΧΠ για 2 εβδομάδες στη σοβαρότητα της νόσου, την ενδοθηλιακή λειτουργία και βιοχημικούς δείκτες σε ασθενείς με ΧΑΠ και ΡΑ. Δεκαοκτώ ασθενείς, 9 με ΧΑΠ (ηλικία: 60,9 ± 12,6; BMI: 26.0 ± 2.1) και 9 με ρευματοειδή αρθρίτιδα (ηλικία: 54,3 ± 11,7; BMI: 26,9 ± 3,6), και 8 υγιείς μη καπνιστές μάρτυρες (ηλικία: 55,7 ± 6,8; BMI: 27,8 ± 3,2; Ομάδα ελέγχου - ΟΕ) συμμετείχαν στη μελέτη. Οι συμμετέχοντες έλαβαν μέρος σε μια απλή τυφλή μελέτη και τυχαιοποιήθηκαν είτε στην πειραματική κατάσταση (κατανάλωση 70 ml χυμού παντζαριού ημερησίως για δύο εβδομάδες; χυμός παντζάρι - ΧΠ) ή στην κατάσταση ελέγχου (χυμός φραγκοστάφυλο; χυμός ελέγχου - ΧΕ). Υπήρχε μια περίοδος έκπλυσης δύο εβδομάδων μεταξύ των συνθηκών. Όλες οι μετρήσεις έγιναν νωρίς το πρωί, μετά από ολονύκτια νηστεία. Η ενδοθηλιακή λειτουργία βελτιώθηκε σημαντικά μετά την κατανάλωση ΧΠ τόσο στους ασθενείς με ΧΑΠ όσο και με ΡΑ, ενώ παρέμεινε σχετικά ανεπηρέαστη από την κατανάλωση του ΧΕ. Ο αιματοκρίτης μειώθηκε σημαντικά στους

ασθενείς με ΡΑ μετά την κατανάλωση του ΧΠ (39,2 ± 2,1 Vs 37,8 ± 1,8). Τα μονοκύτταρα μειώθηκαν σημαντικά στην ΟΕ μετά την κατανάλωση ΧΠ (0,52 ± 0,08 Vs 0,37 ± 0,05). Δεν παρατηρήθηκαν άλλες σημαντικές αλλαγές σε άλλες βιοχημικές και φυσιολογικές παραμέτρους σε οποιαδήποτε ομάδα μετά από την κατανάλωση ΧΠ ή ΧΕ. Η κατανάλωση ΧΠ για δύο εβδομάδες φαίνεται να είναι σε θέση να βελτιώσει τη λειτουργία του ενδοθηλίου σε ασθενείς με ΡΑ και ΧΑΠ. Ωστόσο, η μακροχρόνια κατανάλωση ΒJ θα μπορούσε επίσης να οδηγήσει σε αλλαγές σε φυσιολογικούς και βιοχημικούς δείκτες που δεν παρατηρήθηκαν μετά από δύο εβδομάδες παρέμβασης.

Λέξεις κλειδιά: διαιτητικά νιτρικά, ενδοθηλιακή λειτουργία, οξειδωτικό στρες, ρευματοειδής αρθρίτιδα, χρόνια αποφρακτική πνευμονοπάθεια

The effect of dietary nitrates in the severity of disease, the endothelial function and indices of oxidative stress in patients with chronic diseases.

Abstract

Patients with chronic diseases like obstructive pulmonary disease (COPD) and rheumatoid arthritis (RA) often present endothelial dysfunction and vascular problems, which correlate with the occurrence of cardiovascular disease (CVD). There are several factors that affect the endothelial function, one of which is nutrition. There are suggestions that beetroot juice (BJ) consumption might alter the endothelial function in healthy individuals. However, there are scarce reports on the effect of BJ on the endothelial function of patients with chronic diseases. The purpose of the present study was to investigate the effects of BJ consumption for 2 weeks on the severity of disease, endothelial function and biochemical indices in COPD and RA patients. Eighteen patients, 9 with COPD (age: 60.9 ± 12.6; BMI: 26.0 ± 2.1) and 9 with RA (age: 54.3 ± 11.7; BMI: 26.9 ± 3.6), and 8 healthy non-smoking controls (age: 55.7 ± 6.8; BMI: 27.8 ± 3.2; control group - CG) participated in the study. Participants took part in a single blinded study and they were randomly assigned to either the experimental (70 ml of beetroot juice consumption daily for two weeks; beetroot juice - BJ) or the control condition (blackcurrant juice; control juice - CJ). There was a two-week washout period between conditions. All measurements were done early in the morning, following an overnight fast. Endothelial function significantly improved following BJ consumption in both COPD and RA patients, whereas it remained relatively unaffected by the consumption of the CJ. Hematocrit significantly decreased in RA patients following BJ consumption (39.2 ± 2.1 Vs 37.8 ± 1.8). Monocytes significantly decreased in the CG following BJ consumption (0.52 ± 0.08 Vs 0.37 ± 0.05) No other significant changes

in other biochemical and physiological parameters were observed in any group following BJ or CJ consumption. A two-week consumption of BJ seems to be able to improve endothelial function among patients with RA and COPD. However, long-term BJ consumption may also result in changes in physiological and biochemical indices that were not observed after two weeks of intervention.

Key words: dietary nitrates, endothelial function, oxidative stress, rheumatoid arthritis, chronic obstructive pulmonary disease

CONTENTS

LIST OF TABLES

LIST OF GRAPHICS

LIST OF FIGURES

ABBREVIATION LIST

Activator protein1 (AP-1)

American College of Rheumatology (ACR)

Branched-chain amino acids (BCAAs)

Beetroot juice (BJ)

Blackcurrant juice; control juice (CJ)

Bone mineral density (BMD)

Cardiovascular disease (CVD)

Chronic obstructive pulmonary disease (COPD)

Complementary and alternative medicine (CAM)

Cyelooxygenase 1and 2 ($COX_{1/2}$)

Disease-modifying anti-rheumatic agent (DMARD)

Deoxyribonucleic acid (DNA)

Endothelium-derived relaxing factor (EDRF)

Fat free mass (FFM)

Fat free mass index (FFMI)

Fork head box O (FOXO)

Hydroxyl radical (OH^-)

Hypochlorus acid (HOCI)

Interleukin-6 (IL-6)

Interleukin-8 (IL-8)

Interleukin-1 beta (IL-1β)

Janus kinases (JAKs)

Hydrogen peroxide (H2O2)

Hydroxyvitamin D (25-OHD)

Lipoxygenase (LOX)

5-lipoxygenase (LOX-5)

12-lipoxygenase (LOX-12)

Mitogen-activated protein kinase (MAPK)

NG-monomethyl-L-arginine (L-NMMA)

Nitric oxide (NO)

Nitric oxide synthase (NOS)

Nitrate (NO3)

Nitrite (NO2)

Neonatal-onset multisystem inflammatory disease (NOMID)

Nonadrenergic, noncholinergic (NANC)

Nuclear factor-Kappa B (NF-κB)

Nuclear factor, k-light chain activator of B-cells (NF-kB)

Prostaglandin F2 (PGF2)

Prostaglandin E2 (PGE2)

Rheumatoid arthritis (RA)

Superoxide anion (O2)

Superoxide dismutase (SOD)

Tumor necrosis factor-alpha (TNFa)

World Health Organization (WHO)

1. INTRODUCTION

Patients with diseases like chronic obstructive pulmonary disease (COPD) and Rheumatoid arthritis (RA) often present endothelial dysfunction and vascular problems, which correlate with the occurrence of cardiovascular disease that is often present in these diseases, and the pathophysiology of each disease (Kelm & Schrader, 1990; Davignon and Ganz, 2004; Sattar et al., 2003; Moro et al 2008). Patients with COPD and RA are usually taking many medicines. Therefore, the purpose of the present study was to investigate the effects of beetroot juice (BJ) consumption for 2 weeks on the severity of disease, endothelial function and biochemical indices in RA and COPD patients.

RA is a chronic autoimmune multisystemic inflammatory disease which affects many organs but predominantly attacks the synovial tissues and joints. Onset is peaking in the 4th and 5th decades. RA has an overall prevalence of 0.5-1%, with a female predominance, with the disease being 2-3 times more common in women (Gabriel, 2001). Alternative therapies have been used in RA with promising results, such as mediterranean diet which can improve certain health indicators such as blood pressure, glucose metabolism, lipid profile, as well as reduce inflammation and markers of oxidative stress (González Cernadas et al., 2014), and others such as fish oil, olive oil, probiotics and physical activity.

COPD is characterized by persistent respiratory symptoms and airflow limitation due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases (Global Initiative for Chronic Obstructive Lung Disease, 2017). Major risk factor is cigarette smoking; other risk factor is occupational exposure to dust, chemicals and indoor air pollution from biomass fuel burning. COPD is a common disease, with a highest prevalence among those

over 60 years old, with an estimated prevalence worldwide of 10% (Halbert et al., 2006). Alternative therapies include fruits and vegetables, omega-3 fatty acids and dietary antioxidants (i.e. vitamin C).

Nitric oxide (NO) is a colorless gas that is not readily soluble in water, while it is highly reactive with other elements. NO is an important component in many physiological processes, but mostly in the function of smooth muscle surrounding arteries; muscle expansion and contraction (Vincent, 2010; Brown, 1999; Reid, 2001). The major product of NO after oxidation in aqueous solutions is nitrate (Kelm, 1999). The nitrate-nitrite-NO pathway plays an important physiological and therapeutic role in the human body (Lundberg, 2009). Dietary nitrate (i.e. inorganic nitrate anion NO3-) is found in large amounts in vegetables such as beetroot. NO may have anti-inflammatory, antiadhesive and vasodilating properties. In chronic diseases, systemic inflammation causes a significant reduction in NO bioavailability by affecting substantially the extensibility of the arteries and eventually causing severe vascular problems. Foods with high concentrations of dietary nitrates increase NO in serum. Dietary nitrates have a wide range of beneficial effects such as: blood pressure reduction, improvement of endothelial dysfunction, enhancement of performance during exercise. Both healthy people and patients with peripheral vascular disease that consume a diet rich in nitrates may have protection against ischemia, and also reduce arterial stiffness (Lidder & Webb, 2013).

Recent studies have shown that modern pharmacology has significantly improved the treatment of Rheumatoid Arthritis (RA). An increasing number of RA patients are using various complementary and alternative medicine (CAM) approaches for relief of symptoms and general well-being. CAM is the term for medical products and practices that are not part of standard care and are not generally taught in conventional medical universities. Alternative medicine is used

instead of modern pharmacology, whereas complementary medicine is used in the same time with it. CAM has been mainly used to treat back pain or other back problems, neck pain, joint pain or stiffness and anxiety or depression in RA patients (Barnes Pat al., 2002).

Alternative forms of treatment to relieve symptoms have been used also, which have been identified in part by the American College of Rheumatology (ACR), with extensive reviews which include alimentation and types of exercise and meditation. These alternative therapeutic approaches are intended to relieve back and neck pain and also to relieve anxiety and depression and for this reason the physician rheumatologist should be aware of any alternative use, preparations or treatments.

Diet and nutrition may also be important modifiable risk factors for the development, progression and management of obstructive lung diseases such as asthma and chronic obstructive pulmonary disease (COPD). Important roles for many nutrients have been suggested of which some are supported by epidemiological studies, in vivo and after animal testing. Although, very few human intervention trials are available to definitively assess the efficacy of different approaches to nutritional management of respiratory diseases diet, nutrition and exersise are increasingly becoming recognized as modifiable contributors to chronic disease development and progression. Today evidence has emerged indicating the importance of dietary intake in obstructive lung diseases such as asthma and chronic COPD in both early life and disease development (Nurmatov et al., 2011; Varraso et al., 2007) and management of disease progression (Shaheen et al., 2010, 36, 277–284; Scott et al., 2014).

Pharmacological management remains the mainstay for treatment of respiratory diseases. While treatment options are advancing, dietary intake modification could be an important adjuvant to disease management.

Pulmonary rehabilitation should be considered part of integrated patient management, and usually includes a cooperation of a wide range of healthcare professionals and a long period of time, to ensure optimum coverage of the many aspects involved (Vogiatzis et al., 2016).

2. LITERATURE REVIEW

2.1. RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune multisystemic inflammatory disease which affects many organs but predominantly attacks the synovial tissues and joints.

2.1.1. AETIOLOGY-PATHOPHYSIOLOGY

Clinical presentation. Onset may be insidious or abrupt. Early features commonly include tiredness, malaise and generalised aches. Arthritis symptoms usually first develop in the hands and wrists in a characteristic symmetric, proximal distribution. Feet and large joints may also be involved in a later stage.

Pathology. Aetiology is unknown, and probably multifactorial. It is considered that a genetic predisposition (HLA-DR B1 which is the most common allele of HLA-DR4 involved in RA) combined with an environmental trigger (EBV postulated as a possible antigen, but not proven) may lead to an autoimmune response that is directed against synovial structures and other organs.

The process of activation and accumulation of T CD4 cells in the synovium starts a cascade of inflammatory responses:

- Initially the activation of the macrophages and synovial cells that leads to the production of cytokines such as IL4 and TNF, which in turn cause proliferation of the synovial cells and increase production of destructive enzymes such as elastase and collagenase by macrophages.
- Secondly the activation of B cell lymphocytes to produce various antibodies including rheumatoid factor (RF-IgM antibodies against Fc portion of the IgG) which results in immune complexes that deposit in different tissues and contribute to further injury.
- By directly activating endothelial cells via increased production of VCAM1, there is an increase in the adhesion and further accumulation of inflammatory cells.
- Finally, by producing RANKL, which in turn activates osteoclasts causing subchondral bone destruction.

The inflammatory response leads to pannus formation, which is an oedematous thickened hyperplastic synovium infiltrated by lymphocytes T and B, plasmocytes, macrophages and osteoclasts. Pannus gradually erodes bare areas initially, continued by the articular cartilage. It causes a fibrous ankylosis which gradually ossifies (Sommer et al., 2005).

Diagnostic criteria. Diagnosis is based on a combination of clinical, radiographic and serological criteria. The 2010 ACR - EULAR classification criteria for Rheumatoid Arthritis 4 requires a score of >6/10 for a diagnosis of RA to be made:

A- Joint Involvement

0: Large Joint

1: 2-10 large joints

2: 1-3 small joints (with or without involvement of large joints)

3: 4-10 small joints (with or without involvement of large joints)

5: >10 joints (at least 1 small joint)

B- Serology

0: Negative RF and negative ACPA

2: Low-positive RF or low-positive ACPA

3: High-positive RF or high-positive ACPA

C- Acute Phase Reactants

0: Normal CRP and Normal ESR

1: Abnormal CRP and Abnormal ESR

D- Duration of Symptoms

0: <6 weeks

1: >6 weeks

Non-musculoskeletal features of RA tend to occur late in the disease and include:

A. *pulmonary involvement*,

B. *cardiovascular disease* that leads to an accelerated coronary artery and cerebrovascular atherosclerosis (Turesson et al., 1999), pericarditis and vasculitis (occurs mainly with a severe erosive disease, rheumatoid nodules and high RF titres),

C. *cutaneous involvement* consists in rheumatoid nodules formation, which is usually seen in pressure areas: elbows, occiput, lumbosacral (Robbins et al., 2010) and occurs in RF-positive patients (Ziff, 1990),

D. *ocular involvement*: keratoconjunctivitis sicca, uveitis, episcleritis.

Markers

There are various serological markers for rheumatoid arthritis:

1. The rheumatoid factor (RF) which is an IgM antibody against FC portion of the IgG antibodies, and is a traditional marker but is nonspecific that is associated with several autoimmune and chronic infectious diseases.
2. The anti-cyclic citrullinated peptide (anti-CCP) which is more than 80% sensitive and more than 95% specific.
3. Elevated erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP).

2.1.2. EPIDEMIOLOGY

RA has an overall prevalence of 0.5-1%, with a female predominance, with the disease being 2-3 times more common in women (Gabriel, 2001). Onset is peaking in the 4th and 5th decades.

2.1.3. METHODS OF TREATMENT

2.1.3.1. MEDICATIONS

Abatacept (Orencia). Abatacept is used for treatment of moderate to severe RA in adults and juvenile rheumatoid arthritis. Oftenlly prescribed after failure of a DMARD, like methotrexate but it can also be used as a first-line therapy for inflammatory joint conditions causing joint pain, swelling, redness and morning stiffness. The drug decreases the interactions of T cells and B cells to lessen joint inflammation.

Anakinra (Kineret). Anakinra (Kineret) is used to treat RA neonatal-onset multisystem inflammatory disease (NOMID), and other autoimmune diseases. It is a "biologic" medicine, which means that it is man-made through genetic-engineering techniques and closely related to a protein that occurs naturally in the body, and is used for the decrease of inflammation in arthritis.

Apremilast (Otezla). Apremilast (Otezla) is a small molecule prescription medicine that is used to psoriatic arthritis treatment and moderate to severe plaque psoriasis patients that are candidates for phototherapy (treatment of the skin with an ultraviolet light source). Apremilast slows the production of an enzyme called phosphodiesterase 4 (PDE4) and results to a reduction of inflammation.

Azathioprine (Imuran). Azathioprine *(Imuran)* is a drug used to treat dermatomyositis, systemic lupus erythematosus (lupus), inflammatory bowel disease and vasculitis (inflammation of the blood vessels), by suppressing the immune system. Azathioprine can also be used to treat RA, but it is not used as commonly as other DMARDs like methotrexate, though being an immunosuppressant also. Immunosuppressants may decrease joint damage and disability.

*Cyclophosphamide (Cytoxan).*Cyclophosphamide is reserved for severe, refractory rheumatoid arthritis or severe complications of lupus, myositis, scleroderma, or vasculitis. It belongs to a class of drugs known as alkylating agents, and have been used against cancer, been considered a potent immunosuppressant. Cyclophosphamide blocks the production of the deoxyribonucleic acid (DNA) in cells, by preventing cells from dividing, causing cell death. Such cells affected are

immune cells. They are used in autoimmune diseases such as RA, lupus, scleroderma or vasculitis.

Cyclosporine (Neoral, Sandimmune, Gengraf). Cyclosporine (Neoral, Sandimmune, Gengraf) is a potent immunosuppressant drug that is considered a DMARD, because it not only decreases the pain and swelling of arthritis, but it also may prevent joint damage and by this way reduce the risk of long term disability. It is helpfull with pain and swelling and also slows the progression of arthritis by long use. Cyclosporine is sometimes used to treatment of RA and other autoimmune diseases.

Hydroxychloroquine (Plaquenil). Hydroxychloroquine (Plaquenil) is considered DMARD, because it decreases pain and swelling of arthritis, and may prevent joint damage and reduce the risk of long-term disability.

Leflunomide (Arava). Leflunomide (*Arava*) is a drug used approved to treat moderate to severe rheumatoid arthritis in adultsand is a DMARD. This class of medicines can decrease joint damage and disability caused by rheumatoid arthritis. Leflunomide blocks the formation of DNA, and therefore suppresses the immune system resulting to reduced inflammation. However, it is not completely clear how this medication works in rheumatoid arthritis.

Methotrexate (Rheumatrex, Trexall). Methotrexate (Rheumatrex, Trexall, Otrexup, Rasuvo) is one of the most effective and commonly used immunosuppressant in the treatment of several

forms of rheumatic conditions. It is a DMARD, it decreases pain and swelling of arthritis, and also can decrease damage to joints and long-term disability.

Minocycline (Minocin). Minocycline (Minocin) is a tetracycline (antibiotic) and also belongs to DMARDs. Although RA is not thought to be caused by an infection, minocycline has shown to improve the signs and symptoms of this disease.

Mycophenolate Mofetil & Mycophenolate Sodium. Mycophenolate Mofetil (CellCept) and Mycophenolate Sodium (Myfortic) are used in the treatment of several autoimmune diseases. Mycophenolate was used originally in the management of patients with organ transplants.

Nonsteroidal anti-inflammatory drugs (NSAIDs). Traditional NSAIDs include Aspirin, ibuprofen (Advil, Motrin, etc.), naproxen (e.g., Aleve) and the newer Celecoxib (Celebrex) which belongs to a newer class of NSAIDs, the "COX-2 inhibitor" or a "COX-2 selective" NSAID, are some of the most commonly used pain medicines in adults and can decrease inflammation, such as in arthritis. NSAIDs can be very effective medications rheumatic diseases.

Rituximab (Rituxan & MabThera). Rituximab (*Rituxan* and *MabThera*) is a drug used to treat RA that has not responded to other types of medications, and also to certain forms of vasculitis. It works by turning off a part of the immune system that mulfunctions in autoimmune diseases.

Sulfasalazine (Azulfidine). Sulfasalazine (Azulfidine) belongs to a class of drugs called sulfa drugs against pain and swelling in arthritis. Resulting form a combination of salicylate (the main

ingredient in aspirin) and a sulfa antibiotic is known as a DMARD, because it not only decreases the pain and swelling of arthritis, but also prevents damage to joints and long-term disability.

TNF Inhibitors. TNF inhibitors reduce inflammation and stop disease progression and are used in inflammatory conditions such as RA, psoriatic arthritis, juvenile arthritis, inflammatory bowel disease (Crohn's and ulcerative colitis), ankylosing spondylitis and psoriasis.

*Tocilizumab (Actemra).*Tocilizumab is administered as a monthly intravenous infusion or a subcutaneous injection every 1-2 weeks. It is a biologic drug approved for treatment of adults RA, in polyarticular juvenile rheumatoid arthritis (PJIA) as well as at the systemic form of juvenile idiopathic arthritis (SJIA) in children. Biologic drugs are used to suppress the immune system.Tocilizumab is used to stop inflammation, by blocking the interleukin (IL)-6 receptor. The medicine is injected once every 1-2 weeks or infused through an IV each month.

Tofacitinib Citrate (Xeljanz). Tofacitinib citrate (Xeljanz®) is an oral drug used to treat adults with moderate-to-severe, active rheumatoid arthritis who haven't responded to methotrexate. Tofacitinib acts to block the body's production of enzymes called Janus kinases (JAKs) which have a role in joint inflammation in RA. If left untreated, RA inflammation could lead to joint erosions and organ and tissue damage. It may be used alone or in combination with methotrexate or other DMARDs. Tofacitinib is currently studied for use in other autoimmune diseases, including psoriasis, psoriatic arthritis, ulcerative colitis, Crohn's disease and ankylosing spondylitis (American College of Rheumatology, 2017).

Treatment and prognosis.

Current treatment of RA consists in improving the symptoms and slowing disease progression. Therapy conbines corticosteroids, NSAIDs, DMARDs and TNF antagonists.

RA carries a significant burden of disability and a reduction in life expectancy. Excess mortality is usually related to its non-articular manifestations (Chehata et al., 2001; Young et al., 2007).

2.1.3.2. DIET - OTHER THERAPEUTIC APPROACHES

Diet

It has been shown that the Mediterranean diet can improve certain health indicators such as blood pressure, glucose metabolism, lipid profile, as well as reduce inflammation and markers of oxidative stress (González Cernadas et al., 2014).

Food supplements

Fish oil. Fish oil is rich in omega-3 polyunsaturated fatty acids (PUFAs), which are associated with a reduction in inflammatory markers such as TNF-a and interleukin-1 (Calder, 2008; Proudman et al., 2008). These characteristics are similar to anti-inflammatory and anti-TNF blockers used in the treatment of RA.

Fish oil is recommended by the American Heart Association for reducing events in patients with coronary heart disease (CHD), and therefore the RA patients can take advantage of the additional fish oil intake, by both preventing elevated rates of CHD and reducing inflammation (Kris-Etherton et al., 2002).

Indeed, a meta-analysis has shown that cosuming fish oil in an amount greater than 2.7 gr/day for more than 3 months may result in reducing the amount of non-steroidal antiinflammatory drugs (NSAIDs) required (Lee et al., 2012).

Interestingly, a systematic review that studied the effect of omega-3 fatty acids in patients with PA, demonstrated a moderate beneficial effect on joint swelling, pain, duration of morning stiffness, the generalized estimation of pain and disease activity, and lower doses of NSAIDs (Miles & Calder, 2012).

Olive oil. Virgin olive oil contains several substances with excellent anti-inflammatory and antioxidant effects (Lucas et al., 2011). The main components are oleic acid, linolenic acid, alpha linolenic acid, and phenolic compounds (Waterman & Lockwood, 2007; Wardhana et al., 2011).

A study on supplementation with olive oil in patients with RA who were already taking fish oil, observed that the combination of both oils resulted in more early and intense clinical and laboratory improvement of participants (Berbert et al., 2005).

Vitamin D. Vitamin D is involved in bone metabolism, and its deficiency is associated with osteopenia, a disorder that often coexists in the PA. Furthermore, the effects of vitamin D are extending to the regulation of immune system and interfere with both the innate and the acquired immune systems (Wen & Baker, 2011).

In vitro studies demonstrate the inhibition of activation of interleukins IL-2, IL-12 and IL-6, and interferon-gamma (IF-c) and TNF. Instead, vitamin D promotes the differentiation of monocytes into macrophages, and further displays anti-inflammatory characteristics, through the ability of prostaglandins output adjustment (Kriegel et al., 2011).

Generally multi research analysis suggests that vitamin D deficiency is associated with increased risk of developing RA, while vitamin D supplements could have a role in the treatment of RA (Song et al., 2012). Also, a team of researchers has demonstrated improved disease course after increased intakes of calciferol for a year (Brohult & Jonson, 1973).

In another study, it appeared that the administration of vitamin D, as additional of treatment with DMARDs, is associated with decreased pain. It is important that no side effects were observed during administration of vitamin D (Andjelkovic et al., 1999).

Probiotics. Probiotics are live microorganisms which when administered in adequate amounts are beneficial for host's health (FAO / WHO, 2006). The current understanding is that probiotics can be used in the treatment of chronic diseases such as flegmonondon PA (Yeoh et al., 2013). It has been shown that the Lactobacillus casei induces improvement of histopathological changes and intra-articular lymphocytic infiltrates, likewise those of the administration of methotrexate (MTX) (So et al., 2008).

Today there are signs that probiotics has beneficial effects on pain sensitivity and articular edema, and have also shown a decrease in various inflammation markers (Alipour et al., 2014). Most importantly, no side effects were observed during administration of probiotics.

Herbal Medicine. Some herbs have interaction with mediators of inflammation and therefore may be used in the treatment of rheumatoid arthritis (Yang et al., 2013; Cameron et al., 2009). They can act as antagonists of free radicals, but in other ways, such as capsaicin, which acts competitively high of TRPV1 receptors resulting in the local call of substance P, which is believed to interfere with the pain relieving device (Cameron et al., 2011).

Extract of the herb Tripterygium Wilfordii (TWE; Vine of the god of thunder) was used in eleven randomized controlled trials of 940 people with unsafe results (Liu et al., 2013). The TWE is incorporated in Chinese medicine with major components of triptolide (triptolide) and celastrol (quinone triterpene). Triptolide exhibits anti-inflammatory, antiproliferative and proapoptotic effects and diversifies the immune system (Liu, 2011). Moreover, TWE may show protective cartilage actions (Bao & Dai, 2011; Moudgil et al., 2011).

Other therapeutic approaches

Physical activity. Lack of physical activity is the 4th pre-disposing factor of mortality mortality (World Health Organization, 2009). Exercise leads to smaller percentages of vascular heart disease, hypertension, cerebral ischemic attacks, metabolic syndrome, bowel and breast cancer, depression, and death from all causes (US Department of Health and Human Services, 2008), while decreases the likelihood of fracture and bone density reduction, and increases muscle mass, strength and intrinsic neuromuscular activity (US Department of Health and Human Services, 2008; Tremblay et al., 2011). Although the PA has been shown that physical activity increases the stress on the joints, pain, disease activity and joint destruction, Hurkmans al. (2009) suggested the combination of aerobic exercise and resistance training in the daily practice of patients with RA. Therefore, exercise may be recommended in patients with RA, while any physical activity should have a low joint impact to avoid pain and musculoskeletal injury (US Department of Health and Human Services, 2008).

Yoga. In modern literature, it has been suggested that mind-body interventions, such as Yoga which combines the management of stress with physical activity, may be beneficial. Yoga regulates sweating during dynamic exercise and improves the strength of the respiratory muscles, the strength of the hand and the grip, and elasticity (Oken et al., 2004; Madanmohan Mahadevan et al., 2008; Hart & Tracy, 2008). Scientific studies have shown improvement of the questionnaire results DAS28 (Badsha et al., 2009; Haaz & Bartlett, 2011), and side effects have not been reported from the use of yoga (Uhlig, 2012). It has been demonstrated that it could also be used in younger patients with RA (Evans et al., 2013).

Tai Chi. Tai Chi is a martial Chinese art which combines meditation with slow soft and graceful movements with deep breaths and relaxing (Wang, 2011) and could be used in older age patients with RA that have reduced strength, mobility and morbidities (increased cardiovascular risk, osteoporosis and depression) (Wang, 2011). No side effects have been reported from the use of Tai Chi (Wang, 2011).

Meditation. Meditation incorporates multiple techniques in order to focus attention, empathy and inner peace. It can relieve psychosomatic and psychiatric disorders, and reduce cardiovascular risk (Grossman et al., 2004), pain (Kabat-Zinn et al., 1985), stress (Miller et al., 1995) and depression in patients with RA (Dickens et al., 2003; Ma & Teasdale, 2004) and fibromyalgia (Sephton et al., 2007).

Acupuncture. Methods such as acupuncture (Ernst & Lee, 2010), the electroacupuncture (David et al., 1999) and acupuncture with bee venom (Lee et al., 2014) have been used in studies

without sufficient data for reliable conclusions. However, there are positive signs in reducing pain.

Homeopathy. There are very limited clinical trials (Chatfield et al., 2011; Vithoulkas, 2011), while no contraindications have been reported.

Hydrotherapy. Balneotherapy. Prolonged use of heat, pressure exerted by the water that transfers the vibrations from the skin deeper into the body cause mechanical irritation of the immune system, reduction of pain and inhibition of the sympathetic system (Gabrielsen et al., 2000). Most studies show positive results, but with low methodological quality (Kamioka et al., 2010).

Joint Injection/Aspiration. Joint injections or aspirations (taking fluid out of a joint) are performed in an office or hospital setting, often with a cold spray or other local anesthesia. After the skin surface is thoroughly cleaned, the joint is entered with a needle attached to a syringe. At this point, either joint fluid can be obtained (aspirated) and used for appropriate laboratory testing or medications can be injected into the joint space. This technique also applies to injections into a bursa or tendon sheath to treat bursitis and tendonitis, respectively. Commonly injected joints include the knee, shoulder, ankle, elbow, wrist, base of the thumb and small joints of the hands and feet. Hip joint injection may require the aid of an ultrasound or X-ray called fluoroscopy for guidance. Some small joints may also be more easily aspirated or injected with aid of ultrasound (American College of Reumatology, 1027).

Joint Surgery. Joint replacement surgery is typically recommended to patients who have tried non-surgical treatment but still have joint pain. While this is an extremely effective surgical

treatment, total joint replacement should be considered as the last, rather than the first, treatment option for patients with advanced arthritis of the hip, knee or shoulder. Modern joint replacement surgery involves removal of the worn cartilage from both sides of the joint, followed by resurfacing of the joint with a metal and plastic replacement implant that looks and functions much like your normal joint. Although nearly every joint in the body can be replaced, most replacement surgeries involve the hip or knee. Over the last 30 years, improved surgical techniques and new implant materials have been developed, making total joint replacement one of the most reliable and durable procedures in any area of medicine (American College of Reumatology, 1027).

Clinical Research Trials. Participation in a clinical trial is entirely voluntary, and depends on an understanding of the possible benefits and risks associated with participating in a particular trial and happens after written approval of the participant. A variety of different medications have been used to treat patients who have arthritis and other rheumatic diseases. Before any of these medicines can be prescribed, the Federal Food and Drug Administration (FDA) has required strict testing for the safety and effectiveness. Testing and evaluation process is mainly done through clinical trials. Such trials, lasting from a few weeks to many years, determine how safe and effective new treatments are.

2.2. CHRONIC OBSTRUCTIVE PULMONARY DISEASE

2.2.1. AETIOLOGY-PATHOPHYSIOLOGY

Definition: Chronic obstructive pulmonary disease (COPD) is a common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases (Global Initiative for Chronic Obstructive Lung Disease, 2017).

Terminology. The current definition of COPD does not include the terms chronic bronchitis or emphysema. As *chronic bronchitis* we define the presence of productive cough for three months in each of two successive years in a patient in whom other causes of chronic cough (e.g. bronchiectasis) have been excluded. We don't consider patients with chronic bronchitis as COPD, unless they have airflow obstruction. As *emphysema* we define the pathology of the destruction of the alveoli walls. This is one of the structural abnormalities that can be present in patients with COPD but can also be found in persons with normal lung function (Global Initiative for Chronic Obstructive Lung Disease, 2017).

Etiology, pathobiology and pathology of COPD leading to airflow limitation and clinical manifestations.

Pathology

COPD is characterised by poorly reversible airflow obstruction and an abnormal inflammatory response in the lungs. The latter represents the innate and adaptive immune responses to long

term exposure to noxious particles and gases, particularly cigarette smoke. All cigarette smokers have some inflammation in their lungs, but not all of them develop COPD, patients having an enhanced or abnormal response to inhaling toxic agents. This amplified response may result in mucous hypersecretion (chronic bronchitis), tissue destruction (emphysema) and/or disruption of normal repair and defence mechanisms causing small airway inflammation and fibrosis (bronchiolitis). These pathological changes result in increased resistance to airflow in the small conducting airways, increased compliance of the lungs, air trapping and progressive airflow obstruction, resulting COPD. The cellular and molecular mechanisms underlying the pathological changes found in COPD, are largely understood. Pathology, pathogenesis, and pathophysiology William MacNee

Chronic irritants cause an inflammatory response in the respiratory tract which is amplified in COPD-prone patients. Other environmental exposures such as bioamass fuel exposure and air pollution as well as genetic abnormalities, abnormal lung development and accelerated aging may contribute. Chronic inflammation leads to structural changes in the peripheral airways but also in the large airways, lung parenchyma and pulmonary vasculature (Hogg & Timens, 2009). The pattern of change varies between patients and can include narrowing or reduction in number of the small airways, destruction of the lung parenchyma or emphysema, enlarged mucous glands with mucus hypersecretion and hyperplasia of the wall of the small pulmonary arteries Global Initiative for Chronic Obstructive Lung Disease, 2017).

Pathogenesis

Inflammation is present in the lungs particularly the small airways of all people who smoke.This normal protective response to the inhaled toxins is amplified in COPD, leading to tissue

destruction and impairment of the defence mechanisms that limit such destruction and disruption of the repair mechanisms. These changes in the airways increase with disease severity and persist even after smoking cessation. Besides inflammation, there two other processes that are involved in the pathogenesis of COPD - an imbalance between proteases and antiproteases and an imbalance between oxidants and antioxidants (oxidative stress) in the lungs. Inflammatory cells that are present are neutrophils, macrophages and T lymphocytes (CD8 more than CD4) in the lungs. The extent of the inflammation is related to the degree of the airflow obstruction.These inflammatory cells release a variety of cytokines and mediators that participate in the disease process. This inflammatory pattern is markedly different from that of patients with asthma. Pathology, pathogenesis, and pathophysiology William MacNee

Inflammatory mediators

Many inflammatory mediators are increased in COPD, including Leucotriene B4, a neutrophil and T cell chemoattractant which is produced by macrophages, neutrophils and epithelial cells, chemotactic factors such as the CXC chemokines, interleukin 8 and growth related oncogene, which are produced by macrophages and epithelial cells.

These attract cells from the circulation and amplifypro-inflammatory responses. Pro-inflammatory cytokines such as tumour necrosis factor and interleukins 1 and 6, Growth factors such as transforming growth factor, which will facilitate fibrosis in the airways either directly or through release of another cytokine, connective tissue growth factor. Pathology, pathogenesis, and pathophysiology William MacNee

Protease and antiprotease imbalance

Increased production of proteases and inactivation of antiproteases results in imbalance. Cigarette smoke and inflammation itself produce oxidative stress which primes several inflammatory cells to release a combination of proteases and inactivates several antiproteases by oxidation. The main proteases involved are those produced by neutrophils (including the serine proteases elastase, cathepsin G and protease 3) and macrophages (cysteine proteases and cathepsins E, A, L and S and various matrix metalloproteases (MMP-8, MMP-9 and MMP-12). The main antiproteases involved in the pathogenesis of emphysema are 1 antitrypsin, secretory leucoprotease inhibitor and tissue inhibitors of metalloproteases. Pathology, pathogenesis, and pathophysiology William MacNee

Oxidative stress.

The oxidative burden is increased in COPD. Sources of oxidants include cigarette smoke and reactive oxygen and nitrogen species released from the attracted inflammatory cells. This creates an imbalance in oxidants and antioxidants resulting oxidative stress. Many markers of oxidative stress are increased in stable COPD and are further increased in exacerbations of the disease. Oxidative stress may lead to inactivation of antiproteases or stimulation of mucous production. It may also increase inflammation by enhancing transcription factor activation (such as nuclear factor B) and hence gene expression of pro-inflammatory mediators. Pathology, pathogenesis, and pathophysiology William MacNee

Pathophysiology

The above pathogenic mechanisms result in the pathological changes of the disease. These in turn activate physiological abnormalities—mucous hypersecretion and ciliary dysfunction, airflow obstruction and hyperinflation, gas exchange abnormalities, pulmonary hypertension and systemic effects.

Mucous hypersecretion results in a chronic productive cough. This is a characteristic of chronic bronchitis and thought not necessarily associated with airflow obstruction, not all COPD patients have symptomatic mucous hypersecretion. Hypersecretion happens due to squamous metaplasia, increased numbers of goblet cells and increased size of bronchial submucosal glands in response to chronic irritation by noxious particles and gases.

Ciliary dysfunction is due to squamous metaplasia of epithelial cells and comes as a result in abnormal mucociliary escalator or difficulty in expectorating. Pathology, pathogenesis, and pathophysiology William MacNee

Clinical features

Presentation. The main symptoms of COPD are dyspnoea (exertional dyspnoea is the most common early symptom), chronic cough and sputum production. These symptom may be under-reported by the patients. Less often symptoms are wheezing and chest tightness. Symptoms usually start in mid-life and are slowly progressive.

Patients with COPD can present with chronic daily respiratory symptoms, recurrent acute exacerbations. They may present with relatively little respiratory complaints but with an extremely sedentary lifestyle due to the exertional dyspnea (Global Initiative for Chronic Obstructive Lung Disease, 2017). Chronic inflammation causes structural changes, narrowing of

the small airways and destruction of the lung parenchyma that leads to the loss of alveolar attachments to the small airways and decreases lung elastic recoil.

Diagnosis. Spirometry is required to establish the diagnosis of COPD. A post-bronchidialtor FEV1/FVC ratio less than 0.7 confirms the presence of persistent airflow limitation. In the absence of an alternative explanation for the symptoms and airflow limitation, the diagnosis is COPD (Global Initiative for Chronic Obstructive Lung Disease, 2017).

2.2.2. EPIDEMIOLOGY

COPD is a common disease, with a highest prevalence among those over 60 years old, with an estimated prevalence worldwide of 10% (Halbert et al., 2006). An increased prevalence is estimated amongst men, although the number of women who have COPD has been rising over recent years. A severe genetic form is seen in younger females.

COPD is currently the 4th leading cause of death worldwide (Lozano et al., 2012) but is projected to be the 3rd by 2020 (Mathers & Loncar, 2006).

Aetiology. The most commonly encountered risk factor for COPD is cigarette smoking. Other types of tobacco and marijuana are also encountered risk factors. Occupational exposure to dust and chemicals and indoor air pollution from biomass fuel burning have been seen to increase risk of developing COPD.

The best known genetic risk factor is hereditary deficiency of alpha-1 antitriprysin. These patients typically develop emphysema earlier; other genetic risk factors are also thought to contribute to COPD (Global Initiative for Chronic Obstructive Lung Disease, 2017).

2.2.3. METHODS OF TREATMENT

2.2.3.1. MEDICATIONS

Smoking cessation has the greatest impact on the evolution of the disease. The pharmacologic treatment of stable symptomatic COPD includes bronchodilators (beta agonists, anticholinergics, teophylline) alone or in combination with inhaled glucocorticoids +/- phosphosterase 4 inhibitors.

Pulmonary rehabilitation and oxygen therapy in patients with chronic hypoxemia at rest is also important. In selected patients with severe emphysema lung volume reduction, surgery (removing an area of emphysematous lung to allow the rest of the lung to expand better when breathing) is proposed.

Oxygen therapy, short-acting bronchodilators and systemic corticosteroids are the main treatments for exacerbations. In specific situations antibiotics are used (Global Initiative for Chronic Obstructive Lung Disease, 2017; Celli et al., 2004).

The natural history and prognosis of COPD are variable but there is usually a slow gradual decline with increasing symptoms. The reduction in life expectancy depends on the severity of the disease (Shavelle et al., 2010).

Differential diagnosis. The finding of irreversible airflow limitation on spirometry in a patient with risk factors (smoking history) and clinical symptoms of COPD is diagnostic. A chest radiograph can help to exclude other possible diagnosis as heart failure or interstitial lung disease.

Asthma, bronchiectasis and scarring from previous tuberculosis need to be considered in the differential diagnosis. To be taken into account is the fact that these can also occur concomitant with COPD (Global Initiative for Chronic Obstructive Lung Disease, 2017; Halbert et al., 2006).

2.2.3.2. DIET - OTHER THERAPEUTIC APPROACHES

There is evidence to suggest that a "western" style dietary pattern increases the risk of asthma in children, has worse outcomes for adults with asthma, and is related to COPD risk.

Fruit and Vegetables. Fruit and vegetable intake has been investigated for potential benefits in association with respiratory conditions due to their nutrient profile consisting of antioxidants, vitamins, minerals, fibre and phytochemicals. In adults, Grieger et al. (2013) discussed the heterogeneous nature of the data describing fruit and vegetable intake and lung function, with one study showing no effect on lung function of higher fruit and vegetable intake over 10 years (Butland et al., 2000), yet in another study, increased fruit intake over 2 years was associated with increased FEV1 (Butland et al., 2000), while another study showed that a large decrease in fruit intake over 7 years was associated with decreased FEV1 (Carey et al., 1998). Increased fruit and vegetable intake may be protective against COPD development, with consumption of a "prudent" diet, including increased fruit and vegetables, being protective against lung function decline (Shaheen et al., 2010). Two randomized controlled trials (RCT's) manipulating fruit and vegetable intake have been conducted in COPD. A 12 week study showed no effect of a high fruit and vegetable intake on FEV1, systemic inflammation or airway oxidative stress (Baldricket al., 2012). However, a 3-year study in 120 COPD patients revealed an improvement in lung function in the high fruit and vegetable group compared to the control group (Keranis et al.,

2010), suggesting that longer term intervention is needed to provide a therapeutic effect. There is considerable evidence to suggest that a high intake of fruit and vegetables is favorable for all life stages of asthma and evidence is emerging which suggests the same in COPD.

Omega-3 Fatty Acids and Fish. Omega-3 polyunsaturated fatty acids (PUFA) from marine sources and supplements have been shown to be anti-inflammatory through several cellular mechanisms including their incorporation into cellular membranes and resulting altered synthesis of eicosanoids (Thies et al., 2001).

Omega-3 PUFA may have positive effects in COPD, as higher levels of DHA in serum were found to decrease the risk of developing COPD (Shahar et al., 1999). Experimental studies in humans with COPD, including supplementation with omega-3, found lower levels of TNF-α (De Batlle et al., 2012) and improved rehabilitation outcomes (Broekhuizenet al., 2005), though no improvements were seen in FEV1.

Antioxidants. Dietary antioxidants are an important dietary factor in protecting against the damaging effects of oxidative stress in the airways, a characteristic of respiratory diseases (Wood et al., 2003). Oxidative stress caused by reactive oxygen species (ROS) is generated in the lungs due to various exposures, such as air pollution, airborne irritants and typical airway inflammatory cell responses (Kelly, 2005). Also, increased levels of ROS generate further inflammation in the airways via activation of NF-κB and gene expression of pro-inflammatory mediators (Rahman, 2003). Antioxidants including vitamin C, vitamin E, flavonoids and carotenoids are abundantly present in fruits and vegetables, as well as nuts, vegetable oils, cocoa, red wine and green tea. Dietary antioxidants may have beneficial effects on respiratory health,

from influences of the maternal diet on the fetus, and intake in children through to adults and pregnant women with asthma and adults with COPD. Finally, α-tocopherol (a form of vitamin E) helps cells maintain integrity of membrane fatty acids, by inhibiting lipid peroxidation (Grieger et al., 2013).

Metabolic phenotypes and nutritional risk profile in COPD

Large population studies have shown that the age-standardised rate of death from any cause was lowest among participants with a BMI of 22.5–24.9 kg/m^2 and of 20–25 kg/m^2 in analyses made restricted to nonsmokers (Berrington de Gonzalez et al., 2010; Whitlock et al., 2009). In patients with moderate to severe airflow obstruction, a BMI <25 kg/m^2 was associated with constant high mortality risk relative to overweight or obese patients (landbo et al., 1999; Schols et al., 1998; Lainscak et al., 2011). This ‘‘obesity paradox’’of increased BMI in COPD, could be related to the direct effect of adipose tissue on lung mechanics (Ora et al., 2011). It might also be an epiphenomenon of other, yet unknown diseases that lead to both a reduced mortality risk and preserved fat mass and/or fat free mass (FFM). It is not yet clear whether it is excessive fat or preserved FFM that contributes to the survival advantage in COPD, as it is proven that a low fat free mass index (FFMI) (<10th percentile), independent of BMI and fat mass, is a strong predictor of mortality (Schols et al., 2005). Prevalence of underweight in COPD increases with disease severity (Schols et al., 2005) and is directly associated with the emphysema (Engelen et al., 1999). Normal to overweight patients, that present a low FFMI have a proportionally high fat mass index. Furthermore, a redistribution of fat mass from subcutaneous to visceral adipose tissue has been associated with increased cardiovascular risk in mild to moderate COPD (van den Borst et al., 2012). Underweight patients or with low FFM are more prone to loss of bone

mineral density (BMD) than overweight patients (Bolton et al., 2004). DEXA is used for combined screening of osteoporosis, FFM and fat mass. Distinction between abdominal visceral and subcutaneous fat mass requires advanced imaging technologies (e.g. computed tomography and magnetic resonance imaging), but a clinically useful estimate can be derived by DEXA.

Pathophysiology of abnormal body composition and targets for nutritional intervention

Understanding the pathophysiology of muscle loss and adiposity in COPD is important for the development of targeted nutritional interventions to address specific metabolic phenotypes. We present a summary relevant to alterations in nutritional status and possible nutritional intervention. For a detailed account of skeletal muscle wasting in COPD, the reader is directed to the recently updated American Thoracic Society/ERS statement on lower limb muscle dysfunction in COPD (Maltais et al., 2014).

TABLE 1. Metabolic phenotypes in COPD patients (Schols et al., 2014).

Variable	Research	Clinical practice
Fat-free mass/fat mass	Deuterium dilution	DEXA, single-frequency BIA Anthropometry (sum of four skin folds)
Intracellular mass	Deuterium dilution combined with bromide dilution	Multifrequency BIA
Muscle mass	CT MRI Biomarkers (*i.e.* D_3-creatine dilution)	DEXA Ultrasonography Biomarkers (*i.e.* creatine height index) Anthropometry (mid-arm muscle circumference)
Abdominal fat	CT	DEXA
Abdominal visceral fat	MRI Biomarkers (*i.e.* PAI-1)	Anthropometry (*i.e.* sagittal diameter and/or waist/hip circumference) Ultrasonography
Bone mass and density	DEXA	DEXA HRCT
Muscle strength and related physical performance	Isokinetic quadriceps strength (Repetitive) magnetic stimulation Timed up-and-go test Stair-climb power test Cycle ergometry	One-repetition maximum Handgrip strength Timed up-and-go test Stair-climb power test

DEXA: dual-energy X-ray absorptiometry; BIA: bioelectrical impedance; CT: computed tomography; MRI: magnetic resonance imaging; PAI: plasminogen-activator inhibitor; HRCT: high-resolution computed tomography.

Fat loss

Loss of body weight and fat mass happens when energy expenditure in grater than energy intake. Eating per se, can adversely affect haemoglobin saturation and increase dyspnoea in patients with severe COPD (Schols et al., 1991a). Ageing is a contributing factor to reduced dietary intake in COPD due to loss of taste, poor dentition, dysphagia, poor chewing and swallowing ability, poor appetite, or food aversion, as well as the presensce of social problems (e.g. living or eating alone, or poverty) and inability to self-feed (Gronberg et al., 2005). Anorexia is not the primary trigger of energy unbalance in clinically stable disease, as generally, a normal appetite to increased dietary intake is seen in underweight patients (Goris et al., 2003; Schols et al., 1991b). While the normal response to semi-starvation is a reduced metabolic rate and depressed protein turnover, weight-losing in COPD patients may display elevated resting energy expenditure and generally increased whole-body protein turnover (Kao et al., 2011). In addition to an increased cost of ventilation due to abnormal pulmonary mechanics, a higher energy cost is required for muscular contraction (Layec et al., 2011), fact that may contribute to decreased mechanical efficiency of lower limb exercise (Baarends et al., 1997a) and elevate daily energy requirements in certain COPD patients (Baarends et al., 1997b). Furtheremore, weight gain after lung volume reduction surgery was proved as a result of better lung function and reduced work of breathing (Kim et al., 2012). Collectively, this causes a hypermetabolic state that may contribute to weight loss. After hyperalimentation adverse effects were observed in carbohydrate supplementation in COPD due to increased carbon dioxide production, resulting from carbohydrate oxidation loading ventilation but have not been substantiated in more recent studies (Efthimiou et al., 1992); this is, in practice, unlikely to happen with oral nutrition and can easily be avoided by smaller meal portions well spread over the day.

Muscle loss

Muscle mass is determined by the net balance of muscle protein synthesis and protein breakdown. An increased muscle protein degradation rate was present in cachectic COPD patients characterised by low BMI and low FFMI (Rutten et al., 2006). After analyses of the effector pathways of protein degradation a consistent elevation of components of the ubiquitin 26S proteasome system (Langen et al., 2013) and enhanced autophagy (Guo et al., 2013) were observed.

Furtheremore, distal protein synthesis signalling cues (insulin-like growth factor I and phospho-Akt expression levels) are mainly stable (Langen et al., 2013). In cachectic patients, stimulating protein synthesis more proximally with nutritional intervention to balance elevated proteolysis may contribute to muscle mass maintenance in the presence of increased protein turnover, fact that is taking for granded that there is no impairment in protein synthesis signalling (i.e. its responsiveness to catabolic triggers), and requires further research (Jonker et al., 2014). Presuming a positive energy balance, nutritional intervention should target at provision of sufficient amino acids to support protein synthesis signalling and could evoke a compensatory response to increases in proteolysis cues (Jonker et al., 2014). Stimulation of protein synthesis depends on the availability of amino acids in the circulation. COPD patients with low FFM have low plasma levels of branched-chain amino acids (BCAAs) (Engelen et al., 2000). BCAAs, particularly leucine, are stimulators of muscle protein synthesis. The extraction of amino acids by the intestine has a critical influence in availability to peripheral tissues. Lower splanchnic extraction associated with an enhanced anabolic response to a protein meal (Engelen et al., 2012) was observed in sarcopenic patients with COPD, which probably is related to compromised intestinal function (Rutten et al., 2013). Supplementation of soy protein with BCAAs improoved

inter-organ metabolism in the muscle compartment in COPD (Engelen et al., 2007). The emphysematous phenotype exhibited a blunted whole-body protein turnover after acute exercise and so, further research is required to investigate if the anabolic potential of high-quality protein is less in chronic respiratory failure or in the cachexia-susceptible emphysematous phenotype (Engelen et al., 2003). Increased levels of oxidative stress have been consistently reported in the skeletal muscle of COPD patients. Muscle biopsy analyses have prooved activation of FOXO (fork head box O), MAPK (mitogen-activated protein kinase) and NF-kB (nuclear factor, k-light chain activator of B-cells) in signalling pathways sensitive to oxidative stress and involved in muscle mass regulation. MAPK and NF-kB signalling are also introdused by inflammation and increased inflammatory cell infiltration, with a result of pro-inflammatory cytokine expression. These catabolic pathways or upstream triggers such as oxidative stress and inflammation may be suitable targets for nutritional modulation (Langen et al., 2013).

Bone mineral density loss

Osteoporosis is a skeletal disease characterised by loss of bone mass and microarchitectural deterioration resulting in bone fragility and high susceptibility to fracture (Sambrook & Cooper, 2006). Hip fractures are directly related to falls, while vertebral fractures often occur silently and are the result from routine activities such as bending or lifting causing hospitalisation and excess mortality. In COPD patients vertebral and rib cage fractures may lead to increased kyphosis and reduced rib cage mobility resulting further reduction in pulmonary function. Prevalence data of coincidence of COPD and osteoporosis vary from 5% to 60% depending on the diagnostic methods used (Lehouck et al., 2011). A reason for this association is the presence of common risk factors such as ageing, smoking, underweight, sarcopenia and physical or functional

limitation. Furtheremore, systemic inflammation, use of systemic corticosteroids and the high prevalence of vitamin D deficiency, are frequently observed in more severe stages of COPD, and contribute to a further loss of bone and muscle mass (Lehouck et al., 2011; Graat-Verboom et al., 2009).

Studies also suggest that emphysema represents a particular phenotype as a result of musculoskeletal impairment but the underlying mechanisms remaine unclear (Bon et al., 2011; Makita et al., 2007; Ohara et al., 2008). Bone tissue is continuously renewed throughout life, with a reaching peak bone mass at the age of 25 to 30 years and an expected bone formation balance back to resorption with an annual loss of 0.5–1%. On the cellular level, remodelling of bone consists in an interaction between osteoblasts, cells producing osteoid protein matrix that subsequently mineralises, and osteoclasts, which absorb bone and release calcium back from its stores, which is regulated by NF-kB and its ligand (receptor activator of NF-kB (RANK)/RANK ligand (RANKL) system) expressed on the surfaces of both cell types. On this pathway Vitamin D plays a key role in the regulation of calcium and bone homeostasis with other factors, although several proinflammatory cytokines also present a regulating role. Production of parathyroid hormone is triggered by low 25-hydroxyvitamin D (25-OHD) levels, which, through the activation of the RANK/RANKL system, activates osteoclasts into bone resorption, calcium release and subsequent stabilisation of blood calcium levels (Lips, 2001). Associations between low 25-OHD levels and BMD have been shown in different populations, as well as in COPD patients (Graat-Verboom et al., 2009; Franco et al., 2009). Sufficient intake of vitamin D and calcium, as well as lifestyle modifications (increased physical activity, spending more time outside, smoking cessation and limited alcohol use), composes the basis of all prevention and

treatment strategies of osteoporosis, as low 25-OHD levels are also associated with muscle weakness and increased risk of falls (Rachner et al., 2011).

Adiposity

In patients with advanced disease and sarcopenia and cachexia as risk factors, respiratory failure is the most common cause of death. COPD patients with mild-to-moderate disease in which adiposity is a lifestyle-induced risk factor, the primary cause of death is ischaemic cardiovascular disease (McGarvey et al., 2007). There is strong evidence that abundance of adipose tissue in COPD patients is a significant contributor to the systemic inflammatory load (van den Borst et al., 2011). Abdominal visceral fat is more strongly associated with cardiovascular risk, while subcutaneous fat could be related to a higher inflammatory capacity. In mild to moderate, nonobese COPD patients compared with controls, a fat redistribution was shown towards more abdominal visceral fat, despite measured comparable total fat mass (van den Borst et al., 2012). To what extent this redistribution reflects unhealthy lifestyle or is disease-induced is yet unclear and remains for further research whether the two act synergistically (van den Borst et al., 2013). The fact that obese COPD patients have increased dyspnoea at rest and poorer health status related to normal-weight patients, while static lung hyperinflation is reduced, seems to be unrelated to the severity of disease (Ora et al., 2011). The coexistance of obesity and COPD has an effect on exercise tolerance that seems to depend on the type of exercise (weight-bearing versus non-weight-bearing). While peak cycling capacity is preserved in obese COPD patients compared with nonobese patients and the dyspnoea ratings are systematically lower during cycling in obese patients, the 6-min walk distance (6MWD) is lower and the degree of fatigue is higher in obese patients (Bautista et al., 2011). There are no systemical studies in the

investigation of the effects of weight loss interventions on adiposity, functionality and systemic inflammatory profile in COPD patients. Weight maintenance after a small weight loss period is reported as a challenge in all risk populations, while even modest reductions in weight may reduce the cardiovascular disease risk through improvements in body fat distribution (Chaston et al., 2008). Aerobic exercise training is known to improve insulin sensitivity, induce mitochondrial biogenesis in skeletal muscle and induce loss of visceral fat mass. A dietary intervention combined with aerobic exercise may have best results (van den Borst et al., 2013). Efficacy of this approach may be limited in advanced COPD because of ventilatory restraints on exercise intensity.

As an adjunct, administation of bioactive nutrients (e.g. polyphenols, polyunsaturated fatty acids (PUFAs) and vitamin B_3) could be helpfull to boost muscle mitochondrial metabolism and limit visceral fat accumulation (Schols et al., 2013), but this requires similar clinical trials in COPD in the future.

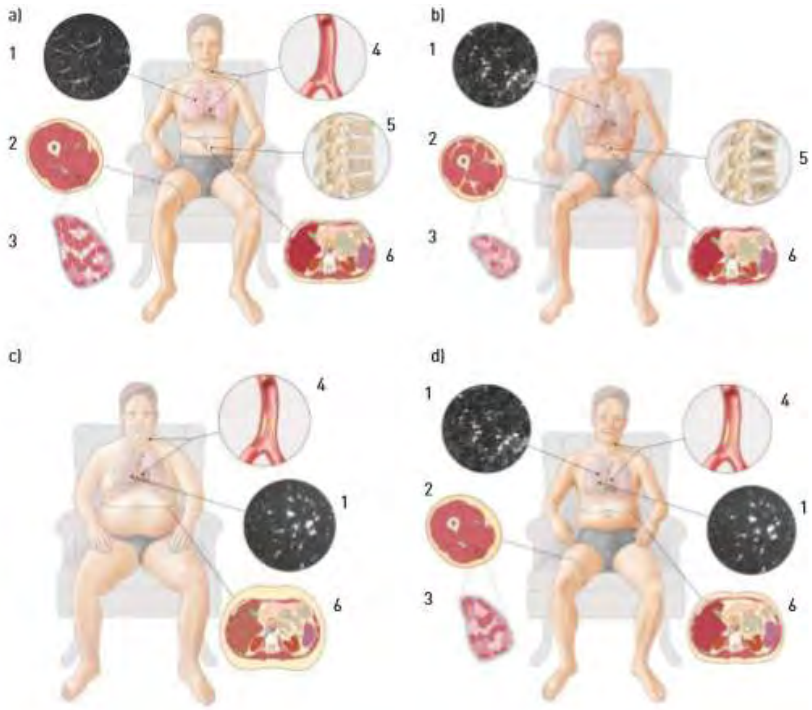

Figure 1. Abnormal metabolic phenotypes and related nutritional risk in chronic obstructive pulmonary disease. a) healthy (reference) with: 1) normal-high rsolution computer tomography of lung tissue; 2) graphic presentation of magnetic resonance imaging (MRI) with quadriceps muscle (red) and adipose tissue (yellow;) 3) normal quadriceps muscle cross section area and fibre type distribution (red: type I; pink: type IIA; white: type IIX); 4) healthy arterial blood vessel; 5) Normal bone tissue; and 6) graphic representation of MRI image of abdomen showing visceral and subcutaneous adipose tissue (yellow). b) Cachexia is often linked to 1) emphysema and hyperinflation, with 2) loss of skeletal muscle mass combined with 3) muscle fidbre atrophy, and a type I to II shift leading to decreased skeletal muscle function, 5) osteoporosis and 6) wasting of fat mass. c) Obesity is often linked to 1) chronic bronchitis with 6) increased subcutaneous and visceral adipose tissue, and 4) arterial stiffness and increased cardiovascular risk. d) Sarcopenia and hidden obesity is not clearly linked to a specific pulmonary phenotype, but is characterized by 2) loss of skeletal muscle mass combined with 3) muscle fibre atrophy and type I or II shift leading to decreased muscle function, preservation of fat mass but redistribution of adipose tissue towards increased 6) visceral adipose tissue, 4) arterial stiffness and increased cardiovascular risk.

Acute exacerbations

Weight loss and wasting of muscle and bone tissue may happen during severe acute exacerbations requiring hospitalisation, due to conguity of catabolic stimuli including malnutrition (Varmeeren et al., 1997), physical inactivity (Ehsan et al., 2013), hypoxia, inflammation (Creutzberg et al., 2000) and systemic glucocorticoids (Saudny-Unterberger et al., 1997).

Practical difficulties due to breathlessness or treatments such as ventilation may reduse energy intake. Moreover, impaired responsiveness to signalling cues of muscle regeneration and protein synthesis may delay recovery and diminiss possibility of readmission (Pouw et al., 2000). In acute respiratory exacerbations phase, the expected loss of appetite results in elevated systemic levels of the appetiteregulating hormone leptin and pro-inflammatory cytokines (Vermeeren et al., 1997; Creutzberg et al., 2000). In cases of hospitalisations an additional opportunity for detailed nutritional assessment could be considered as well as an oppostunity of longer term nutritional management, as they represent a period of heightened ‘‘nutritional risk’’ that may require intensive nutritional therapy (Lainscak et al., 2013). This is to be established with similar clinical trials in COPD in the future.

Dietary management and nutritional supplementation

Nutrient or micronutrient randomised clinical trials face specific obstacles, some of which are difficult to resolve, such as having a placebo or blinding of food. Nutrients, due to their multiplemetabolic impact, present difficulties in choosing a primary outcome and a determination of sample. Research on single foods is complex because it exploits a big number of bioactive compounds acting on a very lerge network of interacting processes.

Treatment of weight loss in COPD

A COPD patient in a negative energy balance and losing weight will require increased energy intake, as an additional reduction in energy expenditure is undesirable. Suitable energy- and proteinenriched diet can be achieved by many small portions throughout the whole day (Broekhuizen et al., 2005). The energy and protein-enriched diet should have a higher fat content

(45% of total energy) in healthy individuals. Due to high proportion of fat requirements, a consideration is raised about the quality of the fat. It is recommended currently by guidelines that protein should provide 20% of the total energy intake.

Many products can be used to increase energy and protein content in the different small meals (Weekes et al., 2009). A dietician is able to create energy- and protein-enriched diet taking into account each subject's eating habits. At low energy intakes, it is hard to fulfil the expectd needs for vitamins, minerals or other elements. If this is the case, oral nutritional supplements (as powders, puddings or liquids) can be used. In weight-losing and underweight COPD patients the rationale is to support nutrition status and to maintain or increase energy availability and muscle protein synthesis. Randomised clinical trials investigating the clinical efficacy of which are generally small and initial meta-analyses revealed small estimates of effect only. The Cochrane review by FERREIRA et al. (Ferreira et al., 2012) was recently updated and now includes 17 trials (632 participants) of o2 weeks of nutritional support. The post-treatment values were pooled for all outcomes and the changes from baseline scores (change scores) were pooled for primary outcomes. The updated review incorporated the Grading of Recommendations Assessment, Development and Evaluation (Atkins et al., 2004) approach aswell, to determine the quality of the evidence (i.e. risk of bias of included studies, inconsistency of results, indirectness of the evidence, imprecision of the data and possible publication bias). The increased body of evidence gives a clear picture of the overall effects of nutritional supplementation and the impact in each specific COPD subgroup. Moderate-quality evidence (due to mixed risks of bias) suggests that nutritional supplementation promotes weight gain among patients with COPD, especially if cahectic. There was important improvement in anthropometric measures (FFM, mid-arm muscle circumference and triceps skin folds) (fig. 3), 6MWD, respiratory muscle

strength (maximal inspiratory and expiratory pressures) and overall health-related quality of life as valued by the St George's Respiratory Questionnaire in undernourished patients with COPD. The increase in 6MWD reached the minimal clinically important difference in severe COPD (Puhan et al., 2011; Polkey et al., 2013). The results of recent systematic reviews and meta-analyses now suggest that nutritional supplementation should be incorporated to the management of undernourished patients with COPD. Five out of 17 trials included in the updated meta-analysis (Ferreira et al., 2012), had nutritional supplementation combined with exercise. It is likely that the benefits of supplementation will be maximised if combined with exercise, although based on the current literature, the effects of nutrition and exercise cannot clearly be distinguished, which is a subject for future research.

Nutrition as ergogenic aid

Nutrition is important for the enhancement of performance and training and has long been recognised in the fields of sports and athletics. The benefits of ensuring adequate carbohydrate and protein intake (depending on the athletic discipline) in optimising performance are today known (van Loon, 2014) and there is evidence that some specific nutrients (e.g. creatine, PUFAs and nitrate) may enhance physical performance (Devries & Phillips, 2014; van de Bool et al., 2012; Mickleborough, 2013; Cermak et al., 2012). Enhancing physical performance is a key therapeutic goal in COPD. Therefore, there are reasons for hypothesising that nutritional intervention might improve performance in this population or enhance the outcome of exercise training, something that is of proven clinical and physiological benefit in COPD.

Aerobic exercise training is of established efficacy in COPD thus it remains uncertain whether the benefit is comparable to similar aged healthy subjects. Moreover, lower limb muscles in

COPD are characterised by a decreased proportion of type I muscle fibres associated with decreased levels of muscle oxidative metabolic markers and nutrient sensing regulators of cellular energy state (e.g. peroxisome proliferator-activated receptor (PPAR)-c coactivator 1, PPARs, AMP-activated kinase and sirtuins) (Schols, 2013).

Task Force members (van de Bool et al., 2012) have recentnly reviewed exercise training with nutritional therapies in COPD. A variety of interventions including carbohydrate and fat-rich supplements (Steiner et al., 2003), essential amino acids (Baldi et al., 2010), whey protein (rich in BCAAs) (Sugawara et al., 2011), creatine (Deacon et al., 2008; Fuld et al., 2005; Faager et al., 2006) and PUFAs (natural ligands of PPARs) was reviewed (Broekhuizen et al., 2005). In the literature involved there is no homogenicity is considering the nature of the intervention, the populations enrolled and the exercise outcomes that were studied. The studies were underpowered or single-centre investigations. The first investigations involved fat-rich supplements but did not suggest a performance advantage in the intervention groups, while later studies using a carbohydrate-rich supplement and PUFAs suggested the outcome or exercise training might be enhanced in selected patients (Steiner et al., 2003; Broekhuizen et al., 2005).

Potential benefits of whey protein and carnitine have been studied, but with no insufficient statistical power for wider conclusions to be drawn. A systematic review and meta-analysis of three trials that tested the effect of creatine supplementation during exercise training in COPD found no consistent positive effect (Al-Ghimlas & Todd, 2010). In a group of COPD patients, with overall non-wasted protein and carbohydrate supplementation after resistance exercise they did not augment functional or molecular exercise responses (Constantin et al., 2013). Whether nutritional support can help the performance outcomes of exercise training and pulmonary rehabilitation remains largely unanswered.

Cost-effectiveness issues

In other countries nutritional counselling and oral nutritional supplements compete with other treatments for a part of the publicly funded healthcare budget; something that is not the case in our country. It is thought important to assess their cost-effectiveness. There are no data on the economic implications of these interventions in COPD. Numerous studies, however, reported on the association between nutritional status and healthcare utilisation, focusing on hospitalised patients. They showed that being undernourished in COPD is likely to be associated with longer hospitalization (Giron et al., 2009l Gupta et al., 2010), a higher probability of being readmitted (Pouw et al., 2000; Hallin et al., 2006) and an increase in healthcare utilization (odencrants et al., 2008) in comparison with normally nourished patients. Three randomised controlled trials in COPD investigated the effects of nutritional supplementation on healthcare utilisation and their costs (Weekes et al., 2009; van Wetering et al., 2010; Edington et al., 2004). Two studies did not find a difference in hospital admissions. It is very probable that in these studies, the duration of follow-up of 6 months was too short to detect an effect on healthcare utilisation. The only full economic evaluation, which was a pre-specified subgroup analysis of the 24-month Interdisciplinary Community-Based COPD Management Program trial, comparing usual care in COPD with nutritional rehabilitation in patients with low muscle mass, found a significant reduction in hospital costs (van Wetering et al., 2010). The mean total COPD and non- COPD related costs per patient after 2 years were J12 830 for the intervention group and J14 025 for the usual care group, resulting in net savings of J1195 (95% CI -7905–5759). Compared with the usual care group, the intervention group had a significant decrease in hospitalisation costs J-4724 (95% CI -7704– -1734). Because of these net cost savings, no cost-effectiveness ratio was calculated. There is a need for more cost-effectiveness studies of nutritional counselling and

supplementation to support decision making about reimbursement of these additional interventions in COPD. There are several possibilities. There is the conventional approach of designing randomised clinical trials in which the additional costs and benefits of adding a nutritional intervention to usual care are investigated. Because usual care is a multimodal pulmonary rehabilitation programme or disease management programme that already includes nutritional counselling, the newly designed trials should focus on assessing the added value of the oral supplements and/or the long-term nutritional counselling. Because of the current lack of any cost-effectiveness data, these trials could use patients from different target groups including end-stage COPD patients with both muscle loss and weight loss (cachexia) as well as weight-stable COPD patients with muscle wasting (sarcopenia). More risk factors should be investigated as weight change, BMI and FFMI, and the risk of COPD exacerbations and hospitalizations, in observational studies. Such data could be used in cost-effectiveness modelling studies to simulate potential long-term effects of changes in weight, BMI and FFM on health status, healthcare utilisation and costs. Cost should be determined as it seems that the costs of nutritional intervention in sarcopenic COPD patients are likely to precede the benefits by far. Vitamin D deficiency and insufficient intake of vitamins with antioxidant capacity (vitamins A, C and E) have been reported in COPD. Vitamin D has an important role in bone and calcium homeostasis but effects are also anti-inflammatory, anti-infectious and anti-tumoural, as well as neuromuscular improvements, have been attributed to vitamin D [112]. Vitamin D status is assessed by the measurement of serum levels of 25-OHD, a precursor of the active hormone., vitamin D status is an independent predictor of all-cause mortality, upper airway respiratory infections and pulmonary function, in the general population. For COPD, evidence is conflicting on whether 25-OHD levels correlate with lung function decline, infectious exacerbations and

muscular function [113–116]. Vitamin D level is determined by the synthesis capacity of the skin, the hours of sun (ultraviolet) exposure, possible genetic variation in key enzymes of the involved pathway and supplemental intake in food. In COPD, vitamin D deficiency possibly occurs because of smoke-induced skin ageing, reduced outdoor activity and/or low-quality dietary intake. It is internationally accepted that vitamin D deficiency (25-OHD levels ,20 ng?mL-1) is highly prevalent in COPD and increases with disease severity. Recent prospective epidemiological evidence associates vitamin D deficiency with an increased incidence of COPD and a more rapid decline of pulmonary function in subjects with COPD [117]. The higher prevalence in more advanced COPD and in nutritionally depleted states suggests that screening for vitamin D deficiency may be of value in these populations. Screening for vitamin D deficiency may restrict to vitamin D deficient patients, in whom the beneficial effects on the bones and fall prevention, especially if combined with calcium intake, are proven. Daily intakes in addition to a minimal amount of ultraviolet radiation exposure vary with age but a dose of 800 IU with 1 g calcium is considered to be largely sufficient. The possibility of highdosage supplementation to obtain other than calcaemic effects, including lung function decline and COPD exacerbations, needs further exploration [118].

Insufficient dietary habits of intake of fresh fruits and vegetables may result in deficiency of vitamins with antioxidant capacity. On the other hand, long-term supplementation with vitamin E has been shown to reduce the risk of COPD [119] although there is no evidence on the positive effects of additional vitamin intake on clinical outcome in a COPD population. Smoking and lung inflammation in COPD are known to cause significant oxidative stress, so the reduction of the antioxidative capacity may have negative effects on the course of COPD.

Large, population-based epidemiological studies have shown that a poper diet is associated with better pulmonary function, less lung function decline and reduced risk of COPD [120–122]. Interestingly, intake of dietary fibre has been largely associated with reduced COPD risk, better lung function and reduced respiratory symptoms [123]. Three studies have reported hight prevalence of developing COPD when frequent or high consumption of cured meats [120, 124, 125]. A study has extended this association to include the evolution of the disease, revealing that high cured meat consumption is linked to a higher risk of rehospitalization in COPD [126]. Finally, even rarely assessed in clinical practice, iron deficiency occurs often in COPD patients, caused by several factors including systemic inflammation, malabsorption of iron from the gut, renal failure (as a consequence of concomitant chronic kidney disease or diabetes mellitus), and medications such as angiotensin-converting enzyme inhibitors and corticosteroids [127]. The above evidence indicates that a well-balanced diet with sufficient intake of fresh fruits and vegetables is beneficial to COPD patients, not only for its potential benefits on the lung, but also for its proven benefits on metabolic and cardiovascular risk.

Interventional therapy in stable COPD

Lung volume reduction surgery improves survival in severe emphysema patients with an upper-lobe emphysema and low post-rehabilitation exercise capacity.

Bullectomy: In selected patiens bullectomy is associated with decreasd dyspnea, improved lung function and exercise tolerance.

Transplantation in appropriately selected patients with very severe COPD, lung transplantation has been shown to improve quality of life and functional capacity.

Bronchoscopic interventions in selected patinents with advanced emphysema reduses end-expiratory lung volume and improves exercise tolerancw, health status and lung function at 6-12 months following treatment (endobronchial valves and lung coils).

2.3. NITRATES

The discovery of nitric oxide and its role in vascular biology *S. Moncada & E.A. Higgs

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Nitric oxide (NO) is a relative newcomer to pharmacology, as the first paper was published only 25 years ago. Today its impact is such that to date more than 31,000 papers have been published with NO in the title and more than 65,000 refer to it in some way. NO pathway is widespread and plays a variety of physiological roles, as proved after the identification of NO with endothelium-derived relaxing factor and the discovery of its synthesis from L-arginine. These physiological roles include the maintenance of vascular tone, neurotransmitter function in both the central and peripheral nervous systems, and mediation of cellular defence. In addition, NO interacts with mitochondrial systems to regulate cell respiration and to augment the generation of reactive oxygen species, thus triggering mechanisms of cell survival or death. NO inhibits platelet aggregation and adhesion and modulates smooth muscle cell proliferation. It has been implicated in a number of cardiovascular diseases and every risk factor for these appears to be associated with a reduction in endothelial generation of NO. Reduced basal NO synthesis or

action leads to vasoconstriction, resulting elevated blood pressure and progressively thrombus formation. Adversilly overproduction of NO leads to vasodilatation and resulting hypotension, vascular leakage and disruption of cell metabolism. Appropriate pharmacological or molecular biological manipulation of the generation of NO will doubtless prove beneficial in such conditions. British Journal of Pharmacology (2006) 147, S193–S201. doi:10.1038/sj.bjp.0706458

Nitric oxide (NO) is a colorless gas that is not readily soluble in water, while it is highly reactive with other elements. NO reacts with oxygen and it may also react by winning an electron and forming NO- or losing an electron and forming NO+. The major product of NO after oxidation in aqueous solutions is nitrate (Kelm, 1999).

Nitrate salts are inorganic compounds which can be both natural and synthetic. They are synthesized from a nitrogen atom (N) and three oxygen atoms (O) and their chemical symbol is NO_3.

Nitrite salts (NO_2) are formed from nitrates by reduction (McCasland et al., 1985).

The inorganic nitrate and nitrite salts are used by humans as preservatives in foods for about 5000 years. However, a significant public health issue concerning nitrites was raised in the '70s, as there was evidence that nitrates and nitrites could be converted in the human body in endogenous N-nitrosamines, which are associated with cancer (Bryan, 2006).

In the early 80s, it was discovered that nitrates and nitrites are also composed endogenously in human body via the path of l-arginine-NO. Thus, although researchers used to consider nitrates and nitrites as hazardous, it is now emerged that the nitrate-nitrite-NO pathway plays an important physiological and therapeutic role in the human body (Lundberg, 2009) (Figure 2).

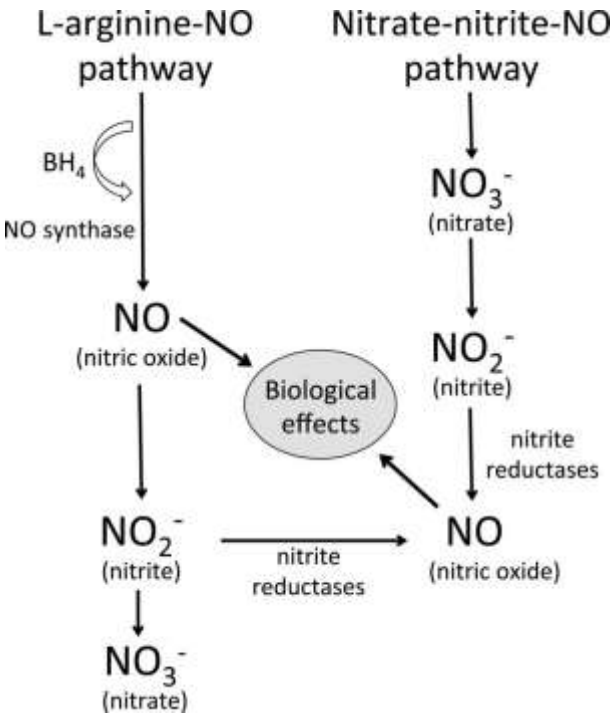

Figure 2. Two main nitric oxide production pathways; L-Arginine-NO pathway and nitrate-nitrite-NO pathway.

Nitric oxide (NO) is an important component in many physiological processes in the transmission of nerve impulses, the mitochondrial respiration, the muscle contraction, but mostly in the function of smooth muscle surrounding arteries. NO is responsible for muscle expansion and contraction (Vincent, 2010; Brown, 1999; Reid, 2001).

The bioavailability of NO can affect all the aforementioned functions, especially the endothelial function. In chronic diseases, systemic inflammation causes a significant reduction in NO bioavailability by affecting substantially the extensibility of the arteries and eventually causing severe vascular problems. Patients with diseases like RA and COPD, often present endothelial dysfunction and vascular problems, which correlate with the occurrence of cardiovascular

disease that is often present in these diseases, and the pathophysiology of each disease (Kelm & Schrader, 1990; Davignon and Ganz, 2004; Sattar et al., 2003; Moro et al 2008).

Foods with high concentrations of dietary nitrates increase NO in serum. It seems that NO has anti-inflammatory, antiadhesive and vasodilating properties. In other words, reduced NO bioavailability can lead to cardiovascular disease, including atherosclerosis and hypertension (Ignarro, 2002).

Moreover, it has been shown that dietary nitrates have a wide range of beneficial effects such as blood pressure reduction, improvent of endothelial dysfunction, and enhancement of performance during exercise. Both healthy people and patients with peripheral vascular disease that consume a diet rich in nitates may have protection against ischemia, and also reduce arterial stiffness (Lidder & Webb, 2013).

All these data have caused a gradually increase in the reputation of dietary nitrates as a dietary supplement during exercise (Jones, 2014) while there is also evidence that they may protect against diseases such as gastroenteritis (Powlson et al, 2008).

2.1.2. Metabolism of nitrates

Dietary nitrate (i.e. inorganic nitrate anion NO3-) is rapidly absorbed in the upper gastrointestinal tract and distributed through the bloodstream. The biological availability of the cooked spinach, raw cabbage and cooked beetroot is almost 100% and reaches the highest percentage in blood plasma one hour after consumption. Plasma nitrate levels remain high for long enough, as the half-life in plasma is 6.5 hours. Instead, the biological availability of nitrite reaches about 95-98% after consuming large quantities (Lidder & Webb, 2013). Most of the

ingested dietary nitrate is found in blood plasma, while about 25% of it is absorbed by the salivary glands and concentrated in saliva (Jones, 2014) (Figure 3).

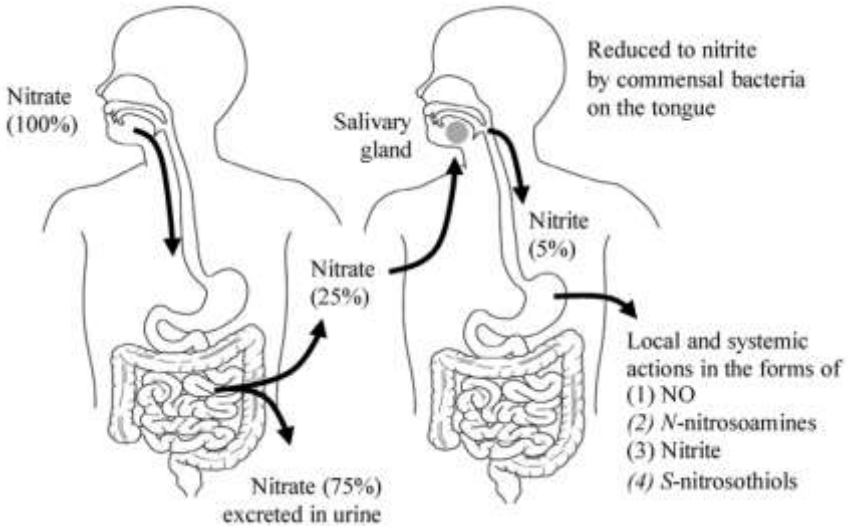

Figure 3. Nitrate and nitrite metabolism in the human body (retrieved from Kobayashi et al., 2015).

More than 25% of the ingested NO3- enters the entero-salivary circulation, it is then recycled in the blood, collected in the salivary glands and secreted in saliva, where bacteria of the oral cavity convert NO3- to NO2-. A part of the NO2- is swallowed and converted in NO within the acid environment of the stomach. The remaining NO2- is absorbed in order to increase the NO2- concentration in the blood plasma (McKnight et al, 1999; Kelly et al, 2013) (Figure 4).

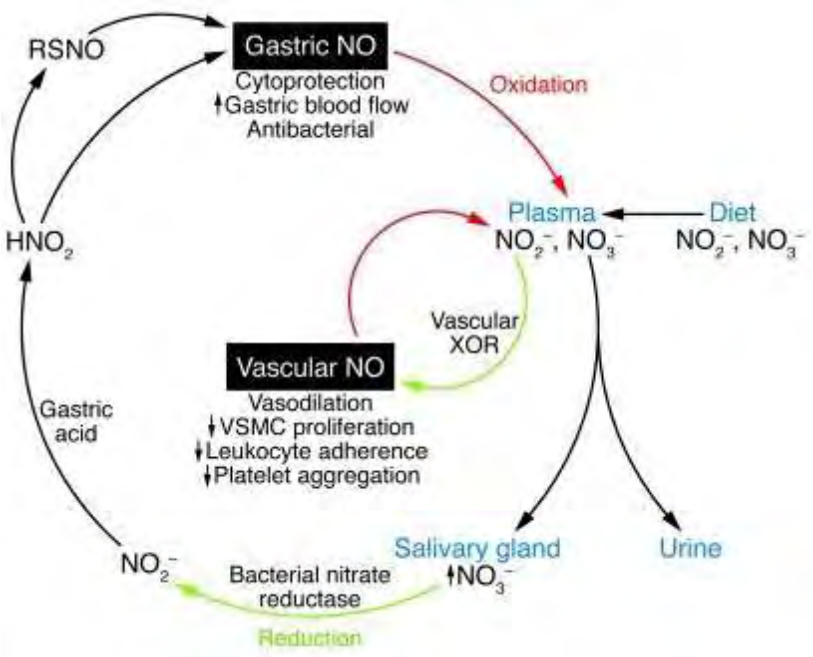

Figure 4. Absorption of dietary nitrate in the gastrointestinal tract.

The metabolism of nitrate was first investigated in animals. Fritsch et al. (1976) showed that a specific amount of nitrates are reduced to nitrites by the ileo-cecal microflora in rats. This procedure can also be done by the action a nitrate reductase, which can appear in the digestive mucosa. Moreover, it has been indicated that there is a possible transition of nitrates in the intestinal tract. In 1985, Schultz et al. investigated the transport of nitrates from blood flow in the lumen of the colon and their metabolism by intestinal bacteria. They concluded that the colon may be responsible for the removal of approximately half the percentage of nitrates that are not removed from the body throught the kidneys.

The endogenous production of nitrates and nitrites in the human body greatly increases their total concentration in blood and tissues. NO is endogenously produced by the reduction of L-arginine to L-citrulline through three isoforms of NO synthase (NOS) (Ferguson et al, 2013). Nitrites are endogenously produced by the oxidation of NO and the reduction of nitrate salts induced by

commensal bacteria in the oral cavity and in the gastrointestinal system (Hord et al, 2009).

NO can also be produced in the absence of NOS. NOS enzymes are using L-arginine and cellular oxygen to produce the free radical NO, which is a key regulator of vascular homeostasis, neurotransmission and immune defense. Therefore, NO is a autocrine and paracrine signaling molecule (Lundberg et al, 2008). Nevertheless, NO is stabilized in blood and tissues when it is oxidized to nitrate and nitrite. Nitrate and nitrite can be considered endocrine molecules which are transported in the blood, they are installed in the tissues and can be converted again to NO under normal or pathological conditions (Lundberg et al, 2008).

Studies have shown that there is a variety of enzymes and proteins that can be a catalyst for the reduction of nitrates to NO in the blood and tissues. These substances include blood proteins, xanthine oxidoreductase, some components of the mitochondrial respiratory chain and other reducing agents such as vitamin C and polyphenols that can significantly accelerate the reduction of nitrate (Lundberg, 2009).

As previously mentioned, a significant amount of nitrites are concentrated in saliva. One parameter that increases their concentration in saliva is that nitrates are metabolized into nitrites and by bacterial microflora located in the posterior surface of the tongue (Pannala et al, 2003). Thus, the nitrates are initially reduced to nitrites under the influence of oral bacteria and enzymes, and then they can enter the bloodstream. These symbiotic bacteria are necessary for the reduction of nitrates in humans, as mammalian cells can not effectively metabolize them.

2.2 CONTENT OF NITRATE IN VARIOUS FOODS

The endogenous synthesis of nitrate includes oxidizing endogenously existing NO and reducing the nitrates by symbiotic bacteria in the saliva in the mouth, and gastrointestinal tract. However, nitates are also found in foods such as meat and vegetables, and water. The average quantity of nitrite that is consumed from diet ranges from 0-20 mg, while the amount of nitrate ranges from 53-300 mg per day (Machha & Schechter, 2011). Most of the nitrates consumed (approximately 85%) are derived from vegetables and very little from fruits. Concentration of nitrates in the drinking water is <10mg/L in the absence of bacterial contamination, although it can differ widely depending on the country and the area of the country of origin (Machha & Schechter, 2011; Lidder & Webb, 2013).

Dietary nitrite is primarily derived from the nitrites added to foods as preservatives, especially from processed meat products (~ 39%) and toasted food and cereals (~ 34%) (Machha & Schechter, 2011; Lidder & Webb, 2013). Dietary nitrites are added in meats to stabilize the characteristic red color of these meat products, to give them the characteristic flavor and as a preservative to prevent the spores of bacterium Clostridium Botulinum from producing toxin that cause fatal illness known as «botulism» (Lidder & Webb, 2013).

The nitrate content of various vegetables is affected by environmental and genetic factors, and cultivation techniques. The main environmental factors include temperature, humidity, sunlight and nitrate content of irrigation water (Lidder & Webb, 2013). The nitrate content is also influenced by the genotype of the plant, the soil composition, conditions of maturation as well as the storage and transport conditions (Hord et al, 2009).

2.3 NITRATES AND CHRONIC DISEASES

There is a growing research interest for the potential benefits of the dietary nitrates on human health. Several studies have shown their benefitial effects on the cardiovascular system; they improve the condition of the endothelium and protect it from injury after ischemia-reperfusion, and they also reduce blood pressure in healthy populations.

Nitrates and cardiovascular health

Endothelium plays an important role in maintaining vascular homeostasis, with the release of a variety of vascular local mediators, such as endothelium-derived NO. NO is essential for maintaining vascular homeostasis because of its actions on both smooth muscle cells and circulating blood cells (platelets, monocytes, etc.). Changes in endothelial NO bioavailability can lead to endothelial dysfunction, leading to impaired vascular homeostasis and thus in the pathogenesis and the clinical expression of a variety of cardiovascular diseases.

Over the last decades, research has indicated that cardiovascular risk factors (such as hypertension, diabetes, menopausal status, aging, lack of physical exercise, smoking, inflammation, and dyslipidemia) are associated with endothelial dysfunction due to reduced bioavailability of NO in the vascular system. Additionally, endothelial dysfunction causes a reduction in vasodilation, which favors the adhesion of platelets and activation of coagulation, which could act as a risk factor for cardiovascular events. Thus, restoring or improving the bioavailability of NO in the vascular system is of great importance in the treatment of cardiovascular diseases.

There is evidence indicating that dietary nitrates can improve the bioavailability of NO in the vascular system, exert vasodilation, inhibit platelet aggregation, and improve cardiovascular

health (Moncada S et al., 1991; Cines DB et al.,1998; Quyyumi AA, 1998; Naseem KM. et al., 2005; Hadi HAR et al., 2005; Ajay M et al.,2006; Hirata Y et al., 2010).

Atherosclerosis is a diffuse process of the arterial tree that starts in childhood and early adult life [1 Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993; 362: 801±809.]. Its `preclinical' stage may last for decades, during which time athero-sclerotic changes progress slowly, eventually causing lumenal stenosis and/or disruptive lesions leading to clinical symptoms. Endothelial dysfunction, especially reduction in the bioavailability of endothelium-derived nitric oxide, is a key early event in atherogenesis, appearing long before the formation of structural athero-sclerotic changes [2 Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992; 340: 1111±1115, 3 Reddy KG, Nair NR, Sheehan HM, Hodgson JM. Evidence that selective endothelial dysfunction may occur in the absence of angiographic or ultrasound atherosclerosis in patients with risk factors for atherosclerosis. J Am Coll Cardiol 1994;23: 833±843]. Nitric oxide is released from the endothelial cells in response to increased shear stress and certain pharmacologic stimuli [Celermajer DS. Endothelial dysfunction: Does it matter? Is it reversible? J Am Coll Cardiol 1997; 30: 325±333]. Nitric oxide may function as an endogenous antiatherogenic molecule by maintaining low arterial tone at rest, inhibiting leucocyte ± endothelial interactions, attenuating platelet aggregationand inhibiting smooth muscle cell proliferation [4 Celermajer DS. Endothelial dysfunction: Does it matter? Is it reversible? J Am Coll Cardiol 1997; 30: 325±333]. Therefore the testing of endothelial nitric oxide release in humans allows investigation of one particularly important aspect of normal arterial physiology.

Cardiovascular disease

Cardiovascular disease (CVD) refers to any disease affecting the cardiovascular system, including heart diseases, vascular diseases, diseases of the brain and kidney and peripheral arterial disease (Bridget, 2010). There are various risk factors for CVD, such as atherosclerosis, hypertension and aging (Dantas et al., 2012).

According to the World Health Organization (WHO), CVD is the leading cause of death. It is estimated that 30% of the total worldwide mortality in 2008 was due to CVD. It is also estimated that by 2030, deaths from CVD will reach 23 million each year. Thus changes in lifestyle and eating habits that contribute to CVD are of great importance. For example, a study by Webb et al. (2008) showed that ingestion of 200 mg dietary nitrate reduced blood pressure, inhibited platelet aggregation and improved ischemia-induced endothelial dysfunction in healthy individuals. Moreover, apples that are rich in flavonoids and spinach that is rich in nitrates, can increase levels of nitric oxide, enhance endothelial function, improve blood pressure, and indirectly benefit cardiovascular health (Bondonno et al., 2011). Small changes in diet, such as adding foods rich in nitrates, may benefit and protect those at risk for myocardial infarction (Kleinbongard et al, 2006). All these results are in accordance with epidemiological evidence which indicates that consumption of fruits and vegetables can reduce the risk of CVD.

Diabetes mellitus

Diabetes mellitus (DM) is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body can not effectively use the insulin that produces. Moreover, patients with DM have increased risk of heart disease and stroke, while 50% of them die of CVD (especially myocardial infarction and stroke) (Morrish et al., 2001). For that reason,

reseach should focus on the prevention and treatment of both microangiopathy and macroangiopathy, which are two common long-term complications of DM.

In a recent study by Gilchrist et al. (2013), supplementation with 7.5 mmol of nitrate per day for 2 weeks did not result in changes in microvascular or macrovascular endothelial function and insulin sensitivity. Although an increase in nitrite and nitrate concentrations in plasma was observed, there was no change in blood pressure, endothelial function or insulin sensitivity in individuals with type 2 DM. However, in a study by Anderson et al. (2005), platelet response to sodium nitroprusside (SNP) and glyceryl trinitrate (GTN) was tested in patients with type 2 DM and compared to healthy individuals, with an increase in induced by adenosine diphosphate (ADP) platelet aggregation. Platelet aggregation in the fasting state was increased in DM patients compared to healthy individuals, while SNP and GTN inhibited ADP aggregation in healthy individuals compared to DM patients. In another study, the levels of cyclic guanosine monophosphate (cGMP) were found to be significantly lower in DM patients compared to healthy individuals. cGMP is responsible for the relaxation of vascular smooth muscle that leads to vasodilation and increased blood flow. Moreover, DM patients without late complications had significantly higher levels of nitrite/nitrate in comparison to DM patients with complications (Farkas et al., 2004).

Reumatoid arthritis

Rheumatoid arthritis (RA) is a chronic systemic disease that affects the joints, the connective tissues, muscles, tendons and fibrous tissue. Its onset is mainly during the most productive years of adulthood, between the ages of 20 and 40. RA is a chronic disabling condition often causing pain and deformity. The prevalence of RA is between 0.3% and 1%, and is more common in

women, with higher prevalence of disease in developed countries. Only 50% of patients with RA in developed countries are able to hold a full time job within the first 10 years of the onset of disease (Woolf & Pfleger, 2003).

There is evidence suggesting that RA patients have a high risk of developing coronary heart disease (Van Doornum et al., 2002). Indeed, studies confirm the presence of significant endothelial dysfunction in RA patients correlated with markers of systemic inflammation. In addition to that, some studies indicate that inflammation may be a mediator of endothelial dysfunction in those patients (Hurlimann et al., 2002). Inflammation could mediate endothelial dysfunction by reduced expression of eNOS (Vallance et al., 1997). NO as mentioned before is of great importance for endothelial function.

Chronic Obstructive Pulmonary Disease

Individuals with COPD have daily minimal excersise as it is limited by multiple factors which can result in hypoxemia. Some of these factors include loss of normal lung architecture, impaired cardiac function [American ST. ATS statement: guidelines for the six-minute walk test. American Journal of Respiratory and Critical Care Medicine. 2002; 166:111–117. [PubMed: 12091180], abnormal pulmonary blood flow distribution [Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP, Benjamin N, Jones AM. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. Journal of Applied Physiology. 2010; 109:135–148. [PubMed: 20466802] and peripheral muscle de-conditioning [Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the

O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. Journal of Applied Physiology. 2009; 107:1144–1155. [PubMed: 19661447].

Regarding NO, the best known is the classical L-arginine nitric oxide synthase (NOS) pathway which is oxygen dependent [Jones AM, Vanhatalo A, Bailey SJ. Influence of dietary nitrate supplementation on exercise tolerance and performance. Nestle Nutrition Institute Workshop Series. 2013; 75:27–40. [PubMed: 23765348]]. The second is the entero-salivary pathway and is oxygen independent. Briefly, nitrate from the diet is rapidly and extensively absorbed in the stomach and proximal small intestine with bioavailability approaching 100% [Kelly J, Fulford J, Vanhatalo A, Blackwell JR, French O, Bailey SJ, Gilchrist M, Winyard PG, Jones AM. Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle and cognitive function in older adults. American Journal of Physiology Regulatory, Integrative and Comparative Physiology. 2013; 304:R73–83.]. Nitrate is then concentrated in the salivary glands, with concentrations 10 fold greater in saliva than in plasma. Nitrate secreted in saliva is reduced to nitrite by facultative anaerobic bacteria on the dorsum of the tongue [Kenjale AA, Ham KL, Stabler T, Robbins JL, Johnson JL, Vanbruggen M, Privette G, Yim E, Kraus WE, Allen JD. Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. Journal of Applied Physiology. 2011; 110:1582–1591. [PubMed: 21454745]]. On swallowing, the acidic environment of the stomach results in NO formation with important local effects on gastric function and host defence [Berry MJ, Rejeski WJ, Miller ME, Adair NE, Lang W, Foy CG, Katula JA. A lifestyle activity intervention in patients with chronic obstructive pulmonary disease. Respiratory Medicine. 2010; 104:829–839. [PubMed: 20347286], Lansley KE, Winyard PG, Fulford J, Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Gilchrist M, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the O2 cost of walking and

running: a placebo-controlled study. Journal of Applied Physiology. 2011; 110:591–600. [PubMed: 21071588]]. Some nitrite enters the circulation where it acts as a storage pool for subsequent NO production [Gosker HR, Zeegers MP, Wouters EF, Schols AM. Muscle fibre type shifting in the vastus lateralis of patients with COPD is associated with disease severity: a systematic review and meta-analysis. Thorax. 2007; 62:944–949. [PubMed: 17526675]]. The conversion of nitrite to NO is expedited in conditions of acidosis [Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. The New England Journal of Medicine. 2006; 355:2792–2793. [PubMed: 17192551]] or hypoxemia [Gosker HR, Zeegers MP, Wouters EF, Schols AM. Muscle fibre type shifting in the vastus lateralis of patients with COPD is associated with disease severity: a systematic review and meta-analysis. Thorax. 2007; 62:944–949. [PubMed: 17526675]] which often occur in the exercising muscle of individuals with COPD [Larsen FJ, Schiffer TA, Borniquel S, Sahlin K, Ekblom B, Lundberg JO, Weitzberg E. Dietary inorganic nitrate improves mitochondrial efficiency in humans. Cell Metabolism. 2011; 13:149– 159. [PubMed: 21284982]].

It is well established that dietary nitrate consumption increases plasma nitrate and nitrite levels in COPD patients, as well as that dietary nitrate consumption increases submaximal exercise capacity in COPD patients.

Dietary nitrate consumption also decreases resting systolic blood pressure in COPD patients.

NO is a signalling molecule with multiple functions including regulation of vascular tone, mitochondrial respiration and skeletal muscle function [14. Gosker HR, Zeegers MP, Wouters EF, Schols AM. Muscle fibre type shifting in the vastus lateralis of patients with COPD is associated with disease severity: a systematic review and meta-analysis. Thorax. 2007; 62:944–949. [PubMed: 17526675] 15. Hernandez A, Schiffer TA, Ivarsson N, Cheng AJ, Bruton JD,

Lundberg JO, Weitzberg E, Westerblad H. Dietary nitrate increases tetanic [Ca2+]i and contractile force in mouse fast-twitch muscle. The Journal of Physiology. 2012; 590:3575–3583. [PubMed: 22687611] 16. Ingram TE, Pinder AG, Bailey DM, Fraser AG, James PE. Low-dose sodium nitrite vasodilates hypoxic human pulmonary vasculature by a means that is not dependent on a simultaneous elevation in plasma nitrite. American Journal of Physiology Heart and Circulatory Physiology. 2010; 298:H331–339. [PubMed: 19940079]].

NO is produced in two distinct ways in man. In many individuals with COPD, functional capacity is reduced to a level where activities of daily living may impose a challenge due to an energy requirement representing a high fraction of their maximal oxygen uptake. Whilst a number of cardiovascular and physiological benefits have been shown as a result of dietary nitrate supplementation in healthy populations, it tle is known about possible effects in clinical populations.

These abnormalities result in feelings of breathlessness and fatigue [Berry MJ, Rejeski WJ, Adair NE, Zaccaro D. Exercise rehabilitation and chronic obstructive pulmonary disease stage. American Journal of Respiratory and Critical Care Medicine. 1999; 160:1248–1253. [PubMed: 10508815]], with individuals often finding that activities of daily living are physically challenging. The beneficial effects of a diet rich in vegetables upon cardiovascular health [Berry MJ, Rejeski WJ, Miller ME, Adair NE, Lang W, Foy CG, Katula JA. A lifestyle activity intervention in patients with chronic obstructive pulmonary disease. Respiratory Medicine. 2010; 104:829–839. [PubMed: 20347286]], risk of morbidity and mortality [Bescos R, Ferrer-Roca V, Galilea PA, Roig A, Drobnic F, Sureda A, Martorell M, Cordova A, Tur JA, Pons A. Sodium nitrate supplementation does not enhance performance of endurance athletes. Medicine and Science in Sports and Exercise. 2012; 44:2400–2409. [PubMed: 22811030]], and COPD

development [8. Borg GA. Psychophysical bases of perceived exertion. Medicine Science in Sports and Exercise. 1982; 14:377–381. 9. Breese BC, McNarry MA, Marwood S, Blackwell JR, Bailey SJ, Jones AM. Beetroot juice supplementation speeds O2 uptake kinetics and improves exercise tolerance during severe-intensity exercise initiated from an elevated metabolic rate. American Journal of Physiology Regulatory, Integrative and Comparative Physiology. 2013; 305:R1441–1450.] have been well described. These positive effects have, in part, been attributed to inorganic nitrate which is found in particularly high quantities in leafy green vegetables and some root vegetables such as beetroot [Butler J, Chomsky DB, Wilson JR. Pulmonary hypertension and exercise intolerance in patients with heart failure. Journal of the American College of Cardiology. 1999; 34:1802–1806. [PubMed: 10577573] Berry]. Nitrate supplementation in the form of sodium nitrate or nitrate-rich beetroot juice has been shown to have remarkable effects in healthy young individuals and athletes, including reductions in the oxygen cost of exercise [Cermak NM, Res P, Stinkens R, Lundberg JO, Gibala MJ, van Loon LJ. No improvement in endurance performance after a single dose of beetroot juice. International Journal of Sport Nutrition and Exercise Metabolism. 2012; 22:470–478.], enhanced exercise tolerance/performance and reduced blood pressure (BP) [Cermak NM, Res P, Stinkens R, Lundberg JO, Gibala MJ, van Loon LJ. No improvement in endurance performance after a single dose of beetroot juice. International Journal of Sport Nutrition and Exercise Metabolism. 2012; 22:470–478., Christensen PM, Nyberg M, Bangsbo J. Influence of nitrate supplementation on VO(2) kinetics and endurance of elite cyclists. Scandinavian Journal of Medicine & Science in Sports. 2013; 23:e21–31. [PubMed: 23020760]]. Assesed effects have subsequently been exercise and restored exercise tolerance and oxidative function to values observed in normoxia (Vanhatalo A, Fulford J, Bailey SJ, Blackwell JR, Winyard PG, Jones AM. Dietary nitrate

reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. The Journal of Physiology. 2011; 589:5517–5528. [PubMed: 21911616]). These results suggest that stimulating the NO3−→NO2−-→NO pathway via ingestion of dietary NO3− may have important therapeutic applications in improving muscle energetics and functional capacity during hypoxic conditions such as mild exercise in patients with cardiovascular, pulmonary and/or sleep disorders.

Kelly et al. hypothesized that NO3− supplementation may also provide beneficial effects for older adults because of the age associated decline in NO signaling (Kelly J, Fulford J, Vanhatalo A, Blackwell JR, French O, Bailey SJ, Gilchrist M, Winyard PG, Jones AM. Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle and cognitive function in older adults. American Journal of Physiology Regulatory, Integrative and Comparative Physiology. 2013; 304:R73–83.). NO defect is a result of reduced availability of L-arginine or the cofactor tetrahydrobiopterin, reduced endothelial NOS activity and/or increased superoxide production (Reckelhoff JF, Kellum JA, Blanchard EJ, Bacon EE, Wesley AJ, Kruckeberg WC. Changes in nitric oxide precursor, L-arginine, and metabolites, nitrate and nitrite, with aging. Life Sciences. 1994; 55:1895–1902. [PubMed: 7990649], Tiefenbacher CP. Tetrahydrobiopterin: a critical cofactor for eNOS and a strategy in the treatment of endothelial dysfunction? American Journal of Physiology Heart and Circulatory Physiology. 2001; 280:H2484–2488. [PubMed: 11356602]). Kelly et al. reported that dietary supplementation of NO3− over a 2.5 day period significantly increased plasma NO2− levels and decreased resting blood pressure and the mean response time of VO2 in healthy older adults (Kelly J, Fulford J, Vanhatalo A, Blackwell JR, French O, Bailey SJ, Gilchrist M, Winyard PG, Jones AM. Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle

and cognitive function in older adults. American Journal of Physiology Regulatory, Integrative and Comparative Physiology. 2013; 304:R73–83.). These results suggest that dietary NO3− intake has the potential to improve the exercise capacity and general health of older adults.

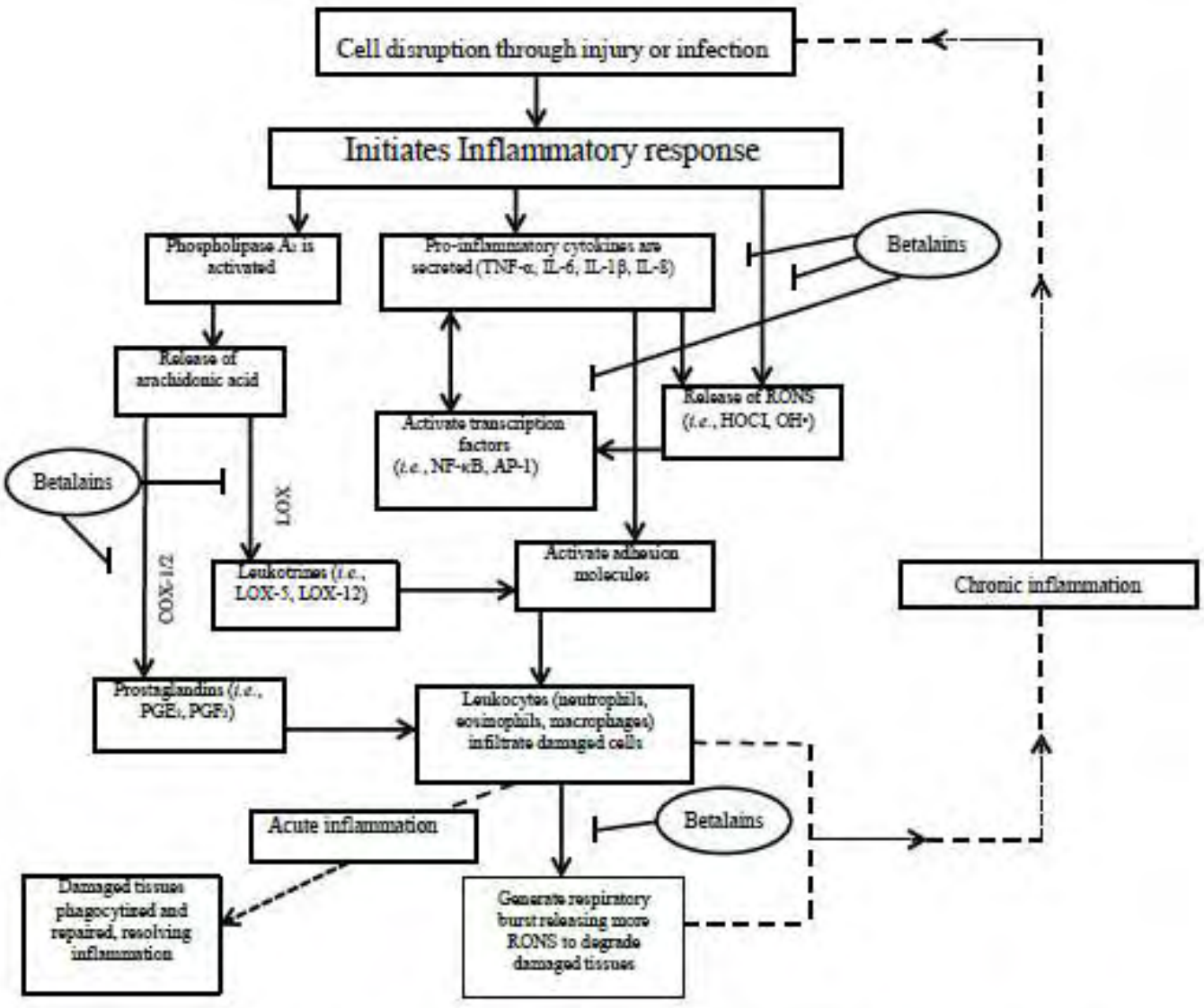

Figure 5. Illustation of the inflammatory cascade in response to cellular attack and possible pathways where betalains may exhibit inhibitory effects. PGF2, Prostaglandin F2; PGE2, Prostaglandin E2; $COX_{1/2}$, Cyelooxygenase 1and 2; LOX, lipoxygenase; LOX-5, 5-lipoxygenase; LOX-12, 12-lipoxygenase, HOCI, Hypochlorus acid; OH^-, Hydroxyl radical; NF-κB, Nuclear factor-Kappa B; AP-1, Activator protein1: IL-6, Interleukin-6; IL-8, Interleukin-8; IL-1β, Interleukin-1 beta; TNFa, tumor necrosis factor-alpha.

Beetroot

The well-documented health benefits of a diet high in fruit and vegetables has led to a growing interest in so-called "functional foods" and their application in health and disease. In recent years, the root vegetable Beta vulgaris rubra, otherwise known as red beetroot (herein referred to as beetroot) has attracted much attention as a health promoting functional food. Reports of its use as a natural medicine dates back to Roman times [Ninfali, P.; Angelino, D. Nutritional and functional potential of Beta vulgaris cicla and rubra. Fitoterapia 2013, 89, 188–199.]. Curently beetroot is agricultured in many countries worldwide, it is regularly consumed as part of the normal diet and is commonly used in manufacturing as a food colouring agent known as E162 [2. Georgiev, V.G.; Weber, J.; Kneschke, E.M.; Denev, P.N.; Bley, T.; Pavlov, A.I. Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot Beta vulgaris cv. Detroit dark red. Plant Foods Hum. Nutr. 2010, 65, 105–111. 3. Zielińska-Przyjemska, M.; Olejnik, A.; Dobrowolska-Zachwieja, A.; Grajek, W. In vitro effects

of beetroot juice and chips on oxidative metabolism and apoptosis in neutrophils from obese individuals. Phytophera Res. 2009, 23, 49–55.]. Recent interest in beetroot has been primarily driven by the discovery that sources of dietary nitrate may have important implications for managing cardiovascular health [Lundberg, J.O.; Weitzberg, E.; Gladwin, M.T. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat. Rev. 2008, 7, 156–167.]. However, beetroot is rich in several other bioactive compounds that may provide health benefits, particularly for disorders characterised by chronic inflammation. Consequently, the potential role for beetroot as an adjunct treatment in several clinical conditions that will be presented; Specifically, part of the aims of this review are: (1) to highlight evidence from recent studies showing the physiological and biological actions of beetroot; and (2) to evaluate its use as a nutritional intervention in health and disease, with a special emphasis on experimental studies relating to oxidative stress, inflammation, endothelial function and cognition. Recent studies have provided compelling evidence that beetroot ingestion offers beneficial physiological effects that may translate to improved clinical outcomes for several pathologies, such as hypertension, atherosclerosis, type 2 diabetes and dementia [1. Ninfali, P.; Angelino, D. Nutritional and functional potential of Beta vulgaris cicla and rubra. Fitoterapia 2013, 89, 188–199, 5–8 5. Gilchrist, M.; Winyard, P.G.; Fulford, J.; Anning, C.; Shore, A.C.; Benjamin, N. Dietary nitrate supplementation improves reaction time in type 2 diabetes: Development and application of a novel nitrate-depleted beetroot juice placebo. Nitric Oxide 2014, 40, 67–74. 6. Presley, T.D.; Morgan, A.R.; Bechtold, E.; Clodfelter, W.; Dove, R.W.; Jennings, J.M., Kraft, R.A.; King, R.A.; Laurienti, P.J.; Rejeskib, J.; et al. Acute effect of a high nitrate diet on brain perfusion in older adults. Nitric Oxide 2011, 24, 34–42. 7. Vanhatalo, A.; Bailey, S.J.; Blackwell, J.R.; di Menna, F.J.; Pavey, T.G.; Wilkerson, D.P.; Benjamin, N.; Winyard, P,G.; Jones, A.M. Acute and

chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. Am. J. Physiol. -Reg. I 2010, 299, 1121–1131. 8. Wootton-Beard, P.C.; Brandt, K.; Fell, D.; Warner, S.; Ryan, L. Effects of a beetroot juice with high neobetanin content on the early-phase insulin response in healthy volunteers. J. Nutr. Sci. 2011, 3, 1–9.]. Hypertension in particular has been the target of many therapeutic interventions and there are numerous studies that show beetroot, delivered acutely as a juice supplement [9. Bailey, S.J.; Winyard, P.; Vanhatalo, A.; Blackwell, J.R.; Dimenna, F.J.; Wilkerson, D.P.; Tarr, J.; Benjamin, N.; Jones, A.M. Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. J. Appl. Physiol. 2009, 107, 1144–1155. 10. Webb, A.J.; Patel, N.; Loukogeorgakis, S.; Okorie, M.; Aboud, Z.; Misra, S.; Rashid, R.; Miall, P.; Deanfield, J.; Benjamin, N.; et al. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. Hypertension 2008, 51, 784–790. 11. Jajja, A.; Sutyarjoko, A.; Lara, J.; Rennie, K.; Brandt, K.; Qadir, O.; Siervo, M. Beetroot supplementation lowers daily systolic blood pressure in older, overweight subjects. Nutr. Res. 2014, 34, 1–8.] or in bread [12. Hobbs, D.A.; Goulding, M.G.; Nguyen, A.; Malaver, T.; Walker, C.F., George, T.W.; Lovegrove, J.A. Acute ingestion of beetroot bread increases endothelium-independent vasodilation and lowers diastolic blood pressure in healthy men: A randomized controlled trial. J. Nutr. 2013, 143, 1399–1405. 13. Hobbs, D.A.; Kaffa, N.; George, T.W.; Methven, L.; Lovegrove, J.A. Blood pressure-lowering effects of beetroot juice and novel beetroot-enriched bread products in normotensive male subjects. Brit. J. Nutr. 2012, 108, 2066–2074.] significantly reduce systolic and diastolic blood pressure. Further discussion of beetroot's anti-hypertensive potential is summarised in several reviews: [14. Hobbs, D.A.; George, T.W.; Lovegrove, J.A. The effects of dietary nitrate on blood

pressure and endothelial function: A review of human intervention studies. Nutr. Res. Rev. 2013, 26, 210–222. 15. Kapil, V.; Weitzberg, E.; Lundberg, J.O.; Ahluwalia, A. Clinical evidence demonstrating the utility of inorganic nitrate in cardiovascular health. Nitric Oxide 2014, 38, 45–57. 16. Lidder, S.; Webb, A.J. Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. Br. J. Clin. Pharmacol. 2013, 75, 677–696.]. Beetroot's effect on the vasculature is largely attributed to its high inorganic nitrate content (250 mg·kg−1 of fresh weight; [Ormsbee, M.J.; Lox, J.; Arciero, P.J. Beetroot juice and exercise performance. J. Int. Soc. Sports Nutr. 2013, 5, 27–35.]). Nitrate itself is not considered to mediate any specific physiological function; rather, nitrates beneficial effects are attributed to its in vivo reduction to nitric oxide (NO), a multifarious messenger molecule with important vascular and metabolic functions [14. Hobbs, D.A.; George, T.W.; Lovegrove, J.A. The effects of dietary nitrate on blood pressure and endothelial function: A review of human intervention studies. Nutr. Res. Rev. 2013, 26, 210–222. 15. Kapil, V.; Weitzberg, E.; Lundberg, J.O.; Ahluwalia, A. Clinical evidence demonstrating the utility of inorganic nitrate in cardiovascular health. Nitric Oxide 2014, 38, 45–57. 16. Lidder, S.; Webb, A.J. Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. Br. J. Clin. Pharmacol. 2013, 75, 677–696. Ormsbee, M.J.; Lox, J.; Arciero, P.J. Beetroot juice and exercise performance. J. Int. Soc. Sports Nutr. 2013, 5, 27–35. 18. Machha, A.; Schechter, A.N. Dietary nitrite and nitrate: A review of potential mechanisms of cardiovascular benefits. Eur. J. Nutr. 2011, 50, 293–303.]. The generation of NO via nitrate involves a series of sequential steps that have been well described in the literature [4. Lundberg, J.O.; Weitzberg, E.; Gladwin, M.T. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat. Rev. 2008, 7, 156–167., 19. Lundberg, J.O.; Carlström, M.; Larsen, F.J.;

Weitzberg, E. Roles of dietary inorganic nitrate in cardiovascular health and disease. Cardiovas Res. 2011, 89, 525–532]. Briefly, ingested nitrate is first absorbed through the upper part of the small intestine into the systemic circulation [4. Lundberg, J.O.; Weitzberg, E.; Gladwin, M.T. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat. Rev. 2008, 7, 156–167.,, 15. Kapil, V.; Weitzberg, E.; Lundberg, J.O.; Ahluwalia, A. Clinical evidence demonstrating the utility of inorganic nitrate in cardiovascular health. Nitric Oxide 2014, 38, 45–57.]. It is then estimated that 25% of the circulating nitrate enters the entero-salivary cycle where bacterial species located at the posterior aspect of the tongue bioactivate or reduce salivary nitrate to nitrite [16. Lidder, S.; Webb, A.J. Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. Br. J. Clin. Pharmacol. 2013, 75, 677–696., Lundberg, J.O.; Carlström, M.; Larsen, F.J.; Weitzberg, E. Roles of dietary inorganic nitrate in cardiovascular health and disease. Cardiovas Res. 2011, 89, 525–532.]. Because salivary bacteria facilitate the reduction reaction that converts nitrate to nitrite, spitting out saliva or taking oral anti-bacterial treatments, like dental mouthwash for example, has been shown to diminish nitrate-nitrite conversion [Webb, A.J.; Patel, N.; Loukogeorgakis, S.; Okorie, M.; Aboud, Z.; Misra, S.; Rashid, R.; Miall, P.; Deanfield, J.; Benjamin, N.; et al. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. Hypertension 2008, 51, 784–790.,18 Machha, A.; Schechter, A.N. Dietary nitrite and nitrate: A review of potential mechanisms of cardiovascular benefits. Eur. J. Nutr. 2011, 50, 293–303.]. Under normal circumstances, however, salivary nitrite is re-absorbed into the circulation via the stomach where it is metabolised to NO and other nitrogen oxides by a variety of reductase enzymes [Lundberg, J.O.; Weitzberg, E.; Gladwin, M.T. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat. Rev. 2008, 7, 156–167.,10. Webb, A.J.;

Patel, N.; Loukogeorgakis, S.; Okorie, M.; Aboud, Z.; Misra, S.; Rashid, R.; Miall, P.; Deanfield, J.; Benjamin, N.; et al. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. Hypertension 2008, 51, 784–790., 13. Hobbs, D.A.; Kaffa, N.; George, T.W.; Methven, L.; Lovegrove, J.A. Blood pressure-lowering effects of beetroot juice and novel beetroot-enriched bread products in normotensive male subjects. Brit. J. Nutr. 2012, 108, 2066–2074.]. However, as previously mentioned, nitrate is not the only constituent of beetroot proposed to have beneficial effects in health and disease. Beetroot is a rich source of phytochemical compounds (Figure 1), that includes ascorbic acid, carotenoids, phenolic acids and flavonoids [Georgiev, V.G.; Weber, J.; Kneschke, E.M.; Denev, P.N.; Bley, T.; Pavlov, A.I. Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot Beta vulgaris cv. Detroit dark red. Plant Foods Hum. Nutr. 2010, 65, 105–111., 20. Kujala, T.S.; Vienola, M.S.; Klika, K.D.; Loponen, J.M.; Pihlaja, K. Betalain and phenolic compositions of four beetroot (Beta vulgaris) cultivars. Eur. Food. Res. Technol. 2002, 214, 505–510. 21. Wootton-Beard, P.C.; Ryan, L. A beetroot juice shot is a significant and convenient source of bioaccessible antioxidants. J. Funct. Foods 2011, 3, 329–3 34.].

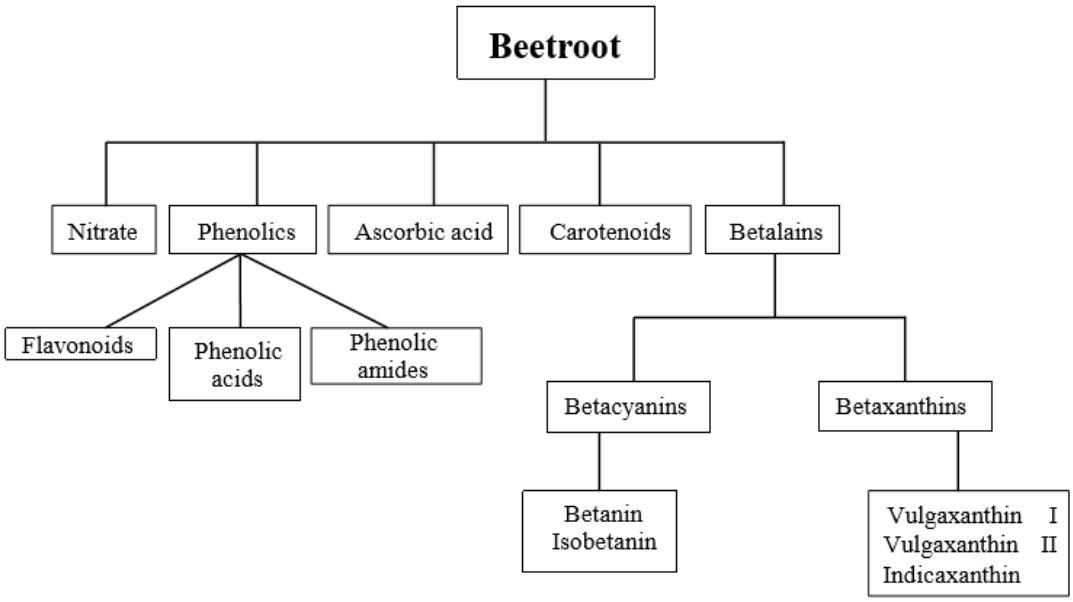

Figure 6. Overview of potentially bioactive compounds in beetroot (based on data from [1. Ninfali, P.; Angelino, D. Nutritional and functional potential of Beta vulgaris cicla and rubra. Fitoterapia 2013, 89, 188–199. 2. Georgiev, V.G.; Weber, J.; Kneschke, E.M.; Denev, P.N.; Bley, T.; Pavlov, A.I. Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot Beta vulgaris cv. Detroit dark red. Plant Foods Hum. Nutr. 2010, 65, 105–111. 20. Kujala, T.S.; Vienola, M.S.; Klika, K.D.; Loponen, J.M.; Pihlaja, K. Betalain and phenolic compositions of four beetroot (Beta vulgaris) cultivars. Eur. Food. Res. Technol. 2002, 214, 505–510.]).

Beetroot is also one of the few vegetables that contain a group of highly bioactive pigments known as betalains [22. Lee, C.H.; Wettasinghe, M.; Bolling, B.W.; Ji, L.L.; Parkin, K.L. Betalains, phase II enzyme-inducing components from red beetroot (Beta vulgaris L.) extracts. Nutr. Cancer 2005, 53, 91–103, 23. Vulić, J.J.; Ćebović, T.N.; Čanadanović-Brunet, J.M.; Ćetković, G.S.; Čanadanović, V.M.; Djilas, S.M.; Tumbas Šaponjac, V.T. In vivo and in vitro

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P.; Tresher, W.; Keller, R.; Jiminez, R.; Michalowski, T.; Wybraniec, S. Influence of betalin-rich extracts on reduction of discomfort associated with osteoarthritis. New. Med. 2010, 1, 12–17.] and even cancer [28. Das, S.; Williams, D.S.; Das, A.; Kukreja, R.C. Beet root juice promotes apoptosis in oncogenic MDA-MB-231 cells while protecting cardiomyocytes under doxorubicin treatment. J. Exp. Second. Sci. 2013, 2, 1–6. 29. Kapadia, G.J.; Azuine, M.A.; Rao, G.S.; Arai, T.; Iida, A.; Tokuda, H. Cytotoxic effect of the red beetroot (Beta vulgaris L.) extract compared to doxorubicin (Adriamycin) in the human prostate (PC-3) and breast (MCF-7) cancer cell lines. Anti -Cancer Agent Med. Chem. 2011, 11, 280–284. 30. Kapadia, G.J.; Azuine, M.A.; Sridhar, R.; Okuda, Y.; Tsuruta, A.; Ichiishi, E.; Mukainakec, T.; Takasakid, M.; Konoshimad, T.; Nishinoc, H.; et al. Chemoprevention of DMBA-induced UV-B promoted, NOR-1-induced TPA promoted skin carcinogenesis, and DEN-induced phenobarbital promoted liver tumors in mice by extract of beetroot. Pharmacol. Res. 2003, 47, 141–148. 31. Kapadia, G.J.; Rao, G.S.; Ramachandran, C.; Iida, A.; Suzuki, N.; Tokuda, H. Synergistic cytotoxicity of red beetroot (Beta vulgaris L.) extract with doxorubicin in human pancreatic, breast and prostate cancer cell lines. J. Complement. Med. 2013, 10, 113–122.].

Knowing all these benefits of dietary nitrate on cardiovascular function and more, we decided to investigate the effect of this juice in patients with RA and COPD.

2.4. CHRONIC INFLAMMATION AND OXIDATIVE STRESS

Chronic inflammation is a pathological condition characterized by continued active inflammation response and tissue destruction. Many of the immune cells including macrophages, neutrophils and eosinophils are involved directly or by production of inflammatory cytokine production in pathology of chronic inflammation. From literatures, it appeares that there is a general concept that chronic inflammation can be a major cause of cancers and express aging processes. Moreover, many studies suggest that chronic inflammation could have serious role in wide variety of age-related diseases including diabetes, cardiovascular and autoimmune diseases. Inflammatory process induces oxidative stress and reduces cellular antioxidant capacity. Overproduced free radicals react with cell membrane fatty acids and proteins impairing their function permanently. In addition, free radicals can lead to mutation and DNA damage that can be a predisposing factor for cancer and age-related disorders. (Recent Pat Inflamm Allergy Drug Discov. 2009 Jan;3(1):73-80.

2.4. ENDOTHELIAL FUNCTION, CHRONIC INFLAMMATION AND OXIDATIVE STRESS

Chronic obstructive pulmonary disease (COPD) is associated with increased cardiovascular mortality. Endothelial dysfunction supports this association. In cross-sectional study the impact of airflow obstruction, systemic inflammation, oxidative stress, sympathetic activation, hypoxaemia and physical activity on endothelial function in COPD was determined. The findings of this study demonstrate that the severity of airflow obstruction is a significant determinant of endothelial function in patients with COPD and that the level of physical activity seems to have a favourable effect on this association. Determinants of endothelial function in

patients with COPD Christian F. Clarenbach1, Oliver Senn2, Noriane A. Sievi1, Giovanni Camen1, Arnoldus J.R. van Gestel1, Valentina A. Rossi1, Milo A. Puhan3, Robert Thurnheer4, Erich W. Russi1,5 and Malcolm Kohler1,

Moreover, chronic inflammation is a pathological condition characterized by continued active inflammation response and tissue destruction. The majority of the immune cells including macrophages, neutrophils and eosinophils are involved directly or by production of inflammatory cytokine production in pathology of chronic inflammation. From literatures, it appeares that there is a general concept that chronic inflammation can be a major cause of cancers and express aging processes. Nevertheless, many studies suggest that chronic inflammation could have serious role in wide variety of age-related diseases including diabetes, cardiovascular and autoimmune diseases. Inflammatory process induces oxidative stress and reduces cellular antioxidant capacity. The overproduced free radicals react with cell membrane fatty acids and proteins impairing their function permanently. In addition, free radicals can lead to mutation and DNA damage that can be a predisposing factor for cancer and age-related disorders. (Recent Pat Inflamm Allergy Drug Discov. 2009 Jan;3(1):73-80. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Khansari N1, Shakiba Y, Mahmoudi M.)

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants.

Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies. Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA. However, oxidative stress also has a useful role in

physiologic adaptation and in the regulation of intracellular signal transduction. Therefore, a more useful definition of oxidative stress may be "*a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them*."

(What Is Oxidative Stress? JMAJ 45(7): 271–276, 2002 Toshikazu YOSHIKAWA* and Yuji NAITO** Professor* and Associate Professor**, First Department of Medicine, Kyoto Prefectural University of Medicine)

3. METHODOLOGY

Taken into consideration all the aforementioned possible benefits of dietary nitrate on cardiovascular function and other health aspects, we decided to investigate the effects of BJ, which is rich in nitrates, in chronic patients that are susceptible to CVD.

The purpose of the present study was to investigate the effects of beetroot juice (BJ) consumption for 2 weeks on the severity of disease, endothelial function and biochemical indices in RA and COPD patients.

3.1. PARTICIPANTS

Eighteen patients, 9 with COPD (6 men, 3 women; age: 60.9 ± 12.6; BMI: 26.0 ± 2.1) and 9 with RA (2 men, 7 women; age: 54.3 ± 11.7; BMI: 26.9 ± 3.6), and 8 healthy controls (3 men, 5 women; age: 55.7 ± 6.8; BMI: 27.8 ± 3.2; control group - CG) participated in the study. RA patients met the diagnostic criteria of RA (Arnett et al., 1988). COPD patients were of all stages of disease as defined by the GOLD 2011 (Han et al., 2013).

Inclusion/exclusion criteria

RA: PA patients with a diagnosis of RA according to the criteria of the American company rheumatology for less than 5 years, stable status of disease for four weeks before the study, constant medication for four weeks before the study, without known CVD or hypertension.

COPD: COPD patients with stabilized disease status for four weeks without response to bronchodilator, full capacity cooperation in spirometry, COPD of any stage if the patient is able to walk, patients not receiving oxygen therapy at house.

CG: Non-smoking individuals with non-declared pathology that do not receive medication, exercising less than 3 times per week.

3.2. EXPERIMENTAL DESIGN

Participants were screened by their doctor and were informed about the study protocol, the associated risks and benefits and they signed an informed consent form. The procedures of the study were in accordance with the 1975 Declaration of Helsinki. Ethics approval was received from the University of Thessaly review board.

Participants were randomly assigned to either the experimental (beetroot juice - BJ) or the control leg (blackcurrant juice; control juice - CJ). They were then asked to report at the testing venue (Ultrasonography Laboratory, General Hospital of Trikala) early in the morning, following an overnight fast. Demographic and disease characteristics were recorded and somatometric characteristics (i.e. height, weight and waist/hip) were assessed. Endothelial function the ability to exercise and other physiological parameters were also measured. Moreover, a blood sample was obtained in order to later determined indices of redox status and complete blood count. Participants consumed 70ml of BJ daily for two weeks. After that, they reported to the testing venue where they were re-evaluated. They crossed over to the other leg of the study after a two-week washout period. A 2-day diet recall was also obtained at the first evaluation, and then participants were adviced to follow the same diet for 2 days before each next evaluation.

3.3. BLOOD COLLECTION AND HANDLING

Blood samples were drawn from a forearm vein. A small portion of blood was collected into ethylenediamine tetra acetic acid (EDTA) tubes and shaken thoroughly for the determination of complete blood count (CBC). For plasma preparation, a portion of blood was collected in vacutainer tubes containing EDTA and shaken thoroughly. Plasma was separated by centrifugation at 1370 x g for 10 min at 4^{o}C. The supernatant was transferred into Eppendorf tubes® and was immediately stored at 80^{o}C for later determination of total antioxidant capacity (TAC). For red blood cell lysate preparation, packed erythrocytes were diluted with distilled water (1:1 v/v), vortexed vigorously, and centrifuged at 4000 x g for 15 min at 4^{o}C. The supernatant was transferred into Eppendorf tubes® and stored at -80^{o}C for later determination of catalase activity. For serum separation, another portion of blood was collected in tubes containing clot activator, left at room temperature for 20 min to clot, and centrifuged at 1370 x g for 10 min at 4^{o}C. The supernatant was transferred into Eppendorf tubes® and was immediately stored at 80^{o}C for later determination of uric acid (UA) and bilirubin.

3.4. METHODS

Anthropometric and physiological measurements

Body weight was measured to the nearest 0.1 kg (Tanita Body Fat Monitor/Scale TBF-521; Tanita, Inc., IL, USA), with participants lightly dressed and barefoot; standing height was measured to the nearest 0.1 cm (Stadiometer 208; Seca, Birmingham, UK). Blood pressure (BP) was measured with a manual sphygmomanometer (FC-101 Aneroid Sphygmomanometer; Focal

Corporation, Japan). The ability to exercise using the 6-min walk test (Balke, 1963) was also evaluated.

Endothelial function

Endothelial function was estimated using the technique of flow-mediated vasodilatation, which assesses the diameter of the lumen of the bracial artery pre and post a 5-minute occlusion to the blood flow of the forearm. According to Corretti et al. (2002), endothelial function was examined by high-resolution ultrasound in the brachial artery after blocking blood flow in the forearm for 5 minutes. The diameter of the artery before blocking (reference diameter) was compared to the maximum diameter of the artery after the restoration of blood flow, and the vasodilation rate was estimated.

Severity of disease

MRC Dyspnoea Scale (Fletcher et al., 1959) and COPD Assessment Test (COPD Assessment Test website, 2017) were used for COPD patients. DAS-28 (DAS-score website, 2017) and the Health assessment questionnaire (HAQ-DI) (Fries et al., 1980) for RA patients.

Blood samples analyses

Assays were performed in duplicate.

Assays in whole blood: CBC [white blood cell (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRA), lymphocyte percentage (LYM%), monocyte percentage (MON%), granulocyte percentage (GRA%), red blood cell count (RBCc), hemoglobin (HGB), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin

concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV), platelecrit (PCT), platelet distribution width (PDW)] was measured with a Mythic 18 (Orphee S.A., Geneva, Switzerland) autoanalyser.

Assays in plasma: TAC was determined by a method based on the scavenging of 1,1-diphenyl-2-picrylhydrazyl, according to Janaszewska and Bartosz (2002).

Assays in serum: UA and bilirubin were measured in a Clinical Chemistry Analyzer Z 1145 (Zafiropoulos Diagnostica, Athens, Greece) with commercially available kits (Zafiropoulos, Athens, Greece).

Assays in red blood cell lysate: Catalase activity was determined according to a method by Aebi (1984).

Test juice

The BJ used in this study was Beet It Sport shots (James White Drinks Ltd, U.K.), which is a 100% natural source of nitrate. It constitutes of concentrated beetroot juice (98%) and lemon juice (2%). It contains at least 400 mg of natural nitrate, and no preservatives, additives or artificial flavours. It is suitable for vegetarians and vegans, and it is GMO free, gluten and dairy free.

Ingredients. Concentrated beetroot juice (98%), lemon juice (2%); made from concentrates.

Table 1. Nutritional info of the test juice (per 100g).

Energy	**414kJ / 97kcal**
Fat	0.2g
of which saturates	<0.1g

Carbohydrates	20g
of which sugars	13g
Protein	4g
Salt	0.3g

Control juice

The control juice (CJ) was a product of blackcurrant juice (Ribena, Lucozade Ribena Suntory Ltd, U.K.)

Ingredients. Water, Sugar, Blackcurrant Juice from Concentrate (23%), Acid (Citric Acid), Vitamin C, Preservatives (Potassium Sorbate, Sodium Bisulphite), Colour (Anthocyanins).

Table 2. Nutritional info of the control juice (per 100g).

Energy	**181kJ / 43kcal**
Fat	0g
of which saturates	0g
Carbohydrates	10.5g
of which sugars	10.5g
Protein	0g
Salt	0g

3.5. STATISTICAL ANALYSIS

Two-way repeated measures ANOVA was performed to analyze the data. Moreover, pairwise comparisons were performed through simple contrasts and simple main effects analysis using the Bonferroni test method.

The level of statistical significance was set at $p<0.05$. The statistical programme used was SPSS version 15.0 (SPSS Inc., USA).

4. RESULTS

Antropometric and physiological characteristics

Table 3. Antropometric and physiological characteristics of COPD patients following BJ and CJ consumption (Mean ± SD).

Index	Pre BJ	Post BJ	Pre CJ	Post CJ
BMI **(kg/m^2)**	26.0 ± 2.1	26.1 ± 2.1	26.9 ± 2.1	26.3 ± 2.2
Waist **(cm)**	94.0 ± 6.8	93.9 ± 6.7	94.1 ± 6.6	94.8 ± 6.1
Hip **(cm)**	92.4 ± 5.8	92.0 ± 5.7	92.9 ± 5.9	93.7 ± 5.6
WHR	1.078 ± 0.17	1.081 ± 0.17	1.076 ± 0.17	1.076 ± 0.17
Resting Heart Rate	79.5 ± 4.5	80.8 ± 4.4	76.8 ± 3.4	83.7 ± 3.3[#]
Systolic Blood Pressure	131.4 ± 13.4	128.3 ± 13.8	127.3 ± 12.2	126.3 ± 12.2
Diastolic Blood Pressure	83.1 ± 3.5	77.4 ± 4.1	79.7 ± 2.9	78 ± 2.9
6-min Walk Test (m)	379.2 ± 30.3	432.3 ± 39.5	418.9 ± 37.1	382.3 ± 26.1

[#]Significant difference ($p<0.05$) from BJ consumption at the same time point.

Table 4. Antropometric and physiological characteristics of RA patients following BJ and CJ consumption (Mean ± SD).

Index	Pre BJ	Post BJ	Pre CJ	Post CJ
BMI **(kg/m^2)**	26.9 ± 3.6	27.1 ± 3.8	27.2 ± 3.8	27.4 ± 3.7
Waist **(cm)**	89.3 ± 4.8	88.8 ± 5.3	89.1 ± 5.3	89.3 ± 5.2
Hip **(cm)**	85.0 ± 7.2	83.0 ± 7.1	83.0 ± 7.1	83.5 ± 7.3
WHR	1.138 ± 0.14	1.162 ± 0.15	1.172 ± 0.16	1.172 ± 0.16
Resting Heart Rate	69.0 ± 4.1	80.5 ± 4.4	71.5 ± 2.3	76.5 ± 3.4
Systolic Blood Pressure	122.9 ± 3.3	116.8 ± 2.5	117.2 ± 3.7[#]	119.7 ± 3.9
Diastolic Blood Pressure	77.1 ± 2.5	73.2 ± 1.6	73.0 ± 2.1[#]	74.0 ± 2.0

6-min Walk Test **(m)**	456.6 ± 29.8	456.1 ± 26.5	460.0 ± 24.1	458.6 ± 26.1

[#]Significant difference ($p<0.05$) from BJ consumption at the same time point.

Table 5. Antropometric and physiological characteristics of the control group following BJ and CJ consumption (Mean ± SD).

Index	Pre BJ	Post BJ	Pre CJ	Post CJ
BMI **(kg/m^2)**	27.8 ± 1.1	27.8 ± 1.2	27.6 ± 1.2	27.6 ± 1.1
Waist **(cm)**	96.0 ± 4.8	95.0 ± 4.8	93.8 ± 5.1	92.7 ± 4.8[#]
Hip **(cm)**	89.7 ± 7.7	89.5 ± 7.6	88.3 ± 7.2	89.4 ± 7.5
WHR	1.149 ± 0.15	1.143 ± 0.15	1.139 ± 0.14	1.115 ± 0.14*
Resting Heart Rate	78.8 ± 6.3	74.6 ± 4.9	71.4 ± 4.9	77.8 ± 4.8*
Systolic Blood Pressure	115.8 ± 8.6	112.0 ± 6.6	100.8 ± 8.8	113.6 ± 9.3
Diastolic Blood Pressure	66.0 ± 2.4	65.2 ± 2.2	62.0 ± 3.7	66.8 ± 6.7
6-min Walk Test **(m)**	486.3 ± 24.1	530.9 ± 19.7	527.1 ± 21.3[#]	486.3 ± 42.9

*Significant difference ($p<0.05$) from pre-intervention at the same condition.

[#]Significant difference ($p<0.05$) from BJ consumption at the same time point.

Endothelial function

No difference in pre-occlusion diameter between any of the time-points was observed. Endothelial function significantly improved following BJ consumption in both experimental groups (RA: pre: 2.6% [0.9 – 6.2], post: 10.7% [6.2 – 11.7]; p=0.013) (COPD: pre: 3.4% [1.2 – 4.8], post: 7.8% [3.6 – 10.2]; p=0.034). However, endothelial function remained relatively unaffected by the consumption of the control juice (Overall: pre: 3.9% [0 – 5.1], post: 4.2% [2.3 – 6.1]; p=0.26).

Complete blood count

Table 6. Complete blood count indices after PJ and CJ consumption in COPD patients.

Index	Pre BJ	Post BJ	Pre CJ	Post CJ	Normal rage
WBC (**10^3/μl)**	6.82 ± 0.7	8.68 ± 1.9	6.60 ± 0.7	8.68 ± 2.4	4.0-12.0
LYM (**10^3/μl)**	1.88 ± 0.3	2.20 ± 0.3	2.20 ± 0.3	2.14 ± 0.4	1.0-5.0
MON (**10^3/μl)**	0.52 ± 0.08	0.62 ± 0.14	0.44 ± 0.06	0.58 ± 0.16	0.1-1.0
GRA (**10^3/μl)**	4.42 ± 0.7	5.90 ± 1.8	3.94 ± 0.4	5.94 ± 2.3	2.0-8.0
LYM **%**	28.5 ± 5.0	29.1 ± 6.3	33.1 ± 2.0	29.8 ± 5.6	25.0-50.0
MON **%**	7.46 ± 0.7	6.78 ± 0.4	6.82 ± 0.8	6.82 ± 0.6	2.0-10.0
GRA **%**	64.0 ± 5.2	64.1 ± 6.2	60.0 ± 2.4	63.4 ± 5.8	50.0-80.0
RBCc (**10^6/μl)**	4.96 ± 0.3	5.10 ± 0.3	4.77 ± 0.3	4.88 ± 0.2	4.00-6.20
HGB **(g/dl)**	13.3 ± 0.3	13.5 ± 0.2	12.3 ± 0.5$^{\#}$	12.8 ± 0.2$^{\#}$	11.0-18.0
HCT **%**	44.2 ± 0.7	45.0 ± 0.5	41.6 ± 1.3$^{\#}$	43.6 ± 0.3	35.0-55.0
MCV **(μm^3)**	90.3 ± 4.6	89.1 ± 4.1	88.2 ± 4.1	89.6 ± 4.7	80.0-100.0
MCH **(pg)**	27.1 ± 1.3	26.8 ± 1.4	26.0 ± 1.1	26.5 ± 1.3	26.0-34.0
MCHC **(g/dl)**	30.1 ± 0.3	30.0 ± 0.4	29.5 ± 0.5	29.6 ± 0.2	31.0-35.5
RDW **%**	14.1 ± 0.5	13.7 ± 0.4	14.0 ± 0.6	14.2 ± 0.5	10.0-16.0
PLT (**10^3/μl)**	264.3 ± 25.5	256.8 ± 27.1	247.3 ± 16.2	253.0 ± 10.9	150-400
MPV (**μm^3)**	7.88 ± 0.3	7.96 ± 0.4	8.02 ± 0.3	7.88 ± 0.4	7.0-11.0
PCT **%**	0.208 ± 0.02	0.204 ± 0.01	0.196 ± 0.01	0.200 ± 0.01	0.200-0.500
PDW **%**	14.6 ± 0.7	14.3 ± 1.0	14.4 ± 1.2	14.7 ± 0.4	10.0-18.0
ESR **(mm/h)**	32.5 ± 13.5	31.0 ± 12.9	31.8 ± 14.1	33.0 ± 10.4	<20

$^{\#}$Significant difference ($p<0.05$) from BJ consumption at the same time point.

Table 7. Complete blood count indices after PJ and CJ consumption in RA patients.

Index	Pre BJ	Post BJ	Pre CJ	Post CJ	Normal rage
WBC (**10^3/μl)**	5.89 ± 0.3	6.66 ± 0.7	5.80 ± 0.4	6.34 ± 0.5	4.0-12.0
LYM (**10^3/μl)**	1.88 ± 0.22	2.14 ± 0.16	2.01 ± 0.14	2.00 ± 0.18	1.0-5.0
MON (**10^3/μl)**	0.44 ± 0.06	0.44 ± 0.05	0.44 ± 0.05	0.43 ± 0.04	0.1-1.0
GRA (**10^3/μl)**	3.56 ± 0.2	4.09 ± 0.6	3.36 ± 0.3	3.93 ± 0.5	2.0-8.0
LYM **%**	31.66 ± 2.7	33.04 ± 2.3	35.07 ± 1.8	32.13 ± 3.1	25.0-50.0
MON **%**	7.37 ± 0.9	6.69 ± 0.8	7.36 ± 0.7	7.00 ± 0.9	2.0-10.0
GRA **%**	60.96 ± 3.3	60.11 ± 3.1	57.57 ± 2.1	60.87 ± 3.8	50.0-80.0
RBCc (**10^6/μl)**	4.62 ± 0.31	4.50 ± 0.32	4.56 ± 0.30	4.42 ± 0.29	4.00-6.20
HGB **(g/dl)**	12.4 ± 0.7	12.0 ± 0.6	11.9 ± 0.7	11.6 ± 0.4	11.0-18.0
HCT **%**	39.2 ± 2.1	37.8 ± 1.8*	38.2 ± 2.1	37.2 ± 1.4	35.0-55.0
MCV **(μm^3)**	86.1 ± 4.9	85.9 ± 5.9	85.06 ± 5.1	85.8 ± 5.2	80.0-100.0
MCH **(pg)**	27.2 ± 2.0	27.3 ± 2.1	26.6 ± 1.8	26.7 ± 1.8	26.0-34.0
MCHC **(g/dl)**	31.6 ± 1.0	31.8 ± 1.0	31.2 ± 0.7	31.1 ± 0.5	31.0-35.5
RDW **%**	14.8 ± 0.6	14.5 ± 0.5	14.5 ± 0.5	15.0 ± 0.6$^{\#}$	10.0-16.0
PLT (**10^3/μl)**	218.2 ± 11.7	230.0 ± 12.9	235.3 ± 9.4	227.3 ± 7.4	150-400
MPV (**μm^3)**	8.20 ± 0.1	8.40 ± 0.3	8.53 ± 0.1$^{\#}$	8.32 ± 0.2	7.0-11.0
PCT **%**	0.177 ± 0.01	0.193 ± 0.01	0.201 ± 0.01	0.188 ± 0.01	0.200-0.500
PDW **%**	14.97 ± 0.6	15.10 ± 0.5	14.02 ± 0.3	14.62 ± 0.6	10.0-18.0
ESR **(mm/h)**	30.38 ± 9.6	30.63 ± 10.0	41.50 ± 11.4	36.5 ± 11.2	<20

*Significant difference ($p<0.05$) from pre-intervention at the same condition.

$^{\#}$Significant difference ($p<0.05$) from BJ consumption at the same time point.

Table 8. Complete blood count indices after PJ and CJ consumption in control group.

Index	Pre BJ	Post BJ	Pre CJ	Post CJ	Normal rage
WBC (**10^3/μl)**	6.57 ± 0.8	5.85 ± 0.4	5.70 ± 0.3	5.53 ± 0.4	4.0-12.0
LYM (**10^3/μl)**	2.58 ± 0.5	2.28 ± 0.3	2.18 ± 0.2	1.98 ± 0.2	1.0-5.0
MON (**10^3/μl)**	0.52 ± 0.08	0.37 ± 0.05*	0.42 ± 0.03	0.37 ± 0.05	0.1-1.0
GRA (**10^3/μl)**	3.47 ± 0.3	3.22 ± 0.2	3.13 ± 0.2	3.20 ± 0.3	2.0-8.0
LYM **%**	38.2 ± 3.1	38.3 ± 1.7	38.1 ± 1.3	35.9 ± 1.6	25.0-50.0
MON **%**	7.60 ± 0.6	6.45 ± 0.5	7.00 ± 0.5	6.48 ± 0.4	2.0-10.0
GRA **%**	54.3 ± 2.9	55.5 ± 2.0	54.9 ± 1.5	57.6 ± 1.3	50.0-80.0
RBCc (**10^6/μl)**	5.04 ± 0.4	5.02 ± 0.3	5.03 ± 0.4	4.80 ± 0.4	4.00-6.20
HGB **(g/dl)**	13.2 ± 0.5	13.1 ± 0.6	12.9 ± 0.5	13.6 ± 0.3	11.0-18.0
HCT **%**	40.0 ± 1.0	39.7 ± 1.2	39.6 ± 1.2	40.9 ± 1.1	35.0-55.0
MCV **(μm^3)**	81.4 ± 6.1	81.0 ± 6.2	80.7 ± 5.9	87.3 ± 6.0	80.0-100.0
MCH **(pg)**	26.9 ± 2.2	26.8 ± 2.4	26.5 ± 2.3	29.2 ± 2.1	26.0-34.0
MCHC **(g/dl)**	33.0 ± 0.6	32.9 ± 0.6	32.7 ± 0.6	33.3 ± 0.4	31.0-35.5
RDW **%**	14.1 ± 0.5	14.0 ± 0.6	14.1 ± 0.7	13.7 ± 0.5	10.0-16.0
PLT (**10^3/μl)**	263.7 ± 31.1	262.3 ± 18.5	256.2 ± 16.4	247.5 ± 20.1	150-400
MPV (**μm^3)**	8.24 ± 0.4	8.02 ± 0.4	7.94 ± 0.4	8.20 ± 0.5	7.0-11.0
PCT **%**	0.212 ± 0.03	0.216 ± 0.02	0.210 ± 0.02	0.203 ± 0.02	0.200-0.500
PDW **%**	15.1 ± 0.3	14.5 ± 0.6	15.0 ± 0.5	14.8 ± 0.5	10.0-18.0
ESR **(mm/h)**	19.0 ± 7.5	19.0 ± 6.1	18.6 ± 7.4	20.8 ± 5.6	<20

*Significant difference ($p<0.05$) from pre-intervention at the same condition.

Table 9. Indices of oxidative stress after PJ and CJ consumption in COPD patients.

Index	Pre BJ	Post BJ	Pre CJ	Post CJ
TAC **(mmol DPPH/L)**	1.15 ± 0.06	1.20 ± 0.05	1.17 ± 0.06	1.17 ± 0.05
UA **(mg/dl)**	7.83 ± 0.6	7.93 ± 0.5	8.08 ± 0.4	7.95 ± 0.8
Bilirubin **(mg/dl)**	0.66 ± 0.09	0.67 ± 0.10	0.65 ± 0.09	0.69 ± 0.09
Catalase **(U/g Hb)**	344.0 ± 21.1	334.3 ± 18.0	322.1 ± 16.1	343.6 ± 17.6*

*Significant difference ($p<0.05$) from pre-intervention at the same condition.

Table 10. Indices of oxidative stress after PJ and CJ consumption in RA patients.

Index	Pre BJ	Post BJ	Pre CJ	Post CJ
TAC **(mmol DPPH/L)**	0.80 ± 0.03	0.82 ± 0.03	0.79 ± 0.03	0.80 ± 0.03
UA **(mg/dl)**	5.51 ± 0.9	5.13 ± 0.8	5.79 ± 0.9	5.43 ± 0.8
Bilirubin **(mg/dl)**	0.43 ± 0.04	0.45 ± 0.05	0.44 ± 0.05	0.45 ± 0.04
Catalase **(U/g Hb)**	348.0 ± 23.3	346.2 ± 24.3	348.6 ± 23.6	346.9 ± 17.7

Table 11. Indices of oxidative stress after PJ and CJ consumption in control group.

Index	Pre BJ	Post BJ	Pre CJ	Post CJ
TAC **(mmol DPPH/L)**	0.94 ± 0.04	0.98 ± 0.03	0.96 ± 0.04#	0.96 ± 0.04
UA **(mg/dl)**	5.40 ± 0.7	5.32 ± 0.5	5.54 ± 0.5	5.67 ± 0.3
Bilirubin **(mg/dl)**	0.90 ± 0.4	0.93 ± 0.3	0.96 ± 0.3	0.92 ± 0.3
Catalase **(U/g Hb)**	328.3 ± 31.7	324.6 ± 25.1	330.9 ± 22.2	334.9 ± 27.9

Conclusions

Based on the available data, beetroot appears to be a powerful dietary source of health promoting agents that holds potential as therapeutic treatment for several pathological disorders. The powerful antioxidant, anti-inflammatory and vascular-protective effects offered by beetroot and its constituents have been clearly demonstrated by several in vitro and in vivo human and animal studies; hence its increasing popularity as a nutritional approach to help manage cardiovascular disease and cancer. In the human studies to date, beetroot supplementation has been reported to reduce blood pressure, attenuate inflammation, avert oxidative stress, preserve endothelial function and restore cerebrovascular haemodynamics. Furthermore, although beyond the scope of this review, several studies have now established beetroot supplementation as an effective means of enhancing athletic performance [96,97].

The present work reveals that two weeks of BJ consumption may improve endothelial function in patients with RA and COPD. Since these patients are prone to CVD, this effect of BJ could elicit significant health benefits. Further research is needed to investigate the benefits of longer-term effects of BJ consumption on endothelial function and related cardiovascular health, as well as disease symptoms, and quality of life should be conducted.

5. DISCUSSION

Dietary nitrate supplementation in COPD: An acute, double-blind, randomized, placebo-controlled, crossover trial ☆ Conor P. Kerley a,b,*, Kathleen Cahill a, Kenneth Bolger a, Aisling McGowan a, Conor Burke a, John Faul a, Liam Cormican a a Respiratory and Sleep Diagnostics Department, Connolly Hospital, Blanchardstown, Dublin 15, Ireland b School of Medicine and Medical Sciences, University College Dublin, Belfield, Dublin 4, Ireland

To our knowledge, this is the first trial of dietary nitrate in patientswithCOPD.Inthiscrossoverstudy,wedemonstratethatacute consumptionof BRJsignificantlyincreasedserumnitrate/nitrite,increased exercise capacity and decreased systemic arterial blood pressureinselectedpatientswithCOPD.Thesefindingssupportprevious findings of improved exercise capacity among healthy volunteers [10,22], athletes [11] and subjects with PVD [12] after nitrate consumption. Weobservedasignificantincreaseinserumnitrate >9fold(55– 508uM)andnitrite >4fold(139–612nM)3hafteringestionof BRJ compared with 3 h after PL (Fig.2). These values are similar to previous studies involving PVD subjects [12]. There was marked variation in the elevation of serum nitrate (375–1767%) and nitrite (−16% to +1662%) in response to BRJ. BRJ increased ISWT distance by 11% (−6% to +28%), while PL resulted in a decrease of 7.6% (−33% to 0%) (Fig. 3). ISWT responses varied (−10 to +80 m). Our data suggest that dietary nitrate supplementation appears to abrogate

exercise related fatigue in some patients with COPD, but the effect is unpredictable. Wecannotfullyexplainthevariationinresponseofserumnitrate/ nitrite responses and exercise capacity to BRJ and PL. Advanced age [23], specific medications [24], altered oral bacteria [25] and stomach acidity [24] may partially account for the variation in serum nitrite in response to dietary nitrate provision. It is noteworthy that there was a strong correlation between the elevations in serum nitrate and serum nitrite ($r = 0.83$, $p = 0.0015$) (data not shown) suggesting that the elevation in serum nitrite may depend on the elevation in serum nitrate. Potential contributors to the varied exercise response include: differences in habitual physical activities, COPD severity and nitrate/nitrite conversion. Some studies of nitrate supplementation and exercise performance have shown varied beneficial changes [10–12,22,26,27] but othersshownoeffect [28].Manyof thesestudies,similartoourobservations,reportedinter-individualvariabilitybothinbiochemical and physiological responses to dietary nitrate. A recent report has suggestedthatageneticinfluenceispossible[29].Wedidnotassess genotype here. It is difficult to compare our results with other studies, as the protocols differ in terms of nitrate dosing, exercise assessment and population studied. Our trial involved COPD subjects performing a progressive and maximal exercise test of short duration (0.33– 7.6min). Previous reports of nitrate provision before short, intense

exercisehavenotedimprovements [11].Inaddition,nitratesupplementation has previously been demonstrated to improve exercise tolerance in hypoxic conditions. In one trial, 9.3mmol nitrate increased time to exhaustion by 15% compared with placebo during a 5–10m constant-load maximal knee-extension test [22]. Similarly, 0.07mmol nitrate/kg body wt resulted in 36% greater time to exhaustion during a maximal incremental cycle test [27]. Finally, our results parallel those from a trial of inorganic nitrate supplementation in a PVD population – a disease characterized by peripheralischemiauponexertion.12.1mmoldietarynitrateacutely increasedexercisetoleranceby17%[12].Alikelyexplanationisthat thesetrialshavebeenperformedinstatesassociatedwithlowtissue oxygentension,suchashypoxia,ischemia,and,acidosis.Itisknown that during these conditions the nitrate–nitrite–NO pathway is upregulated [6]. In contrast to previous reports [10,12], there was no correlation between absolute change in exercise performance/MAP and percent change in serum nitrite (Fig.3a and b). A potential explanation for this finding is that we included heterogeneous COPD subjects, differing by COPD severity, age, BMI and smoking history. Additionally,themajorityofparticipantshadmultipleco-morbidities and hence complex medication regimens. Nineofelevensubjects(82%)hadincreasedexercisecapacityafter BRJcomparedwithPL.Onesubjectwalkedidenticaldistancesbefore

andafterbothBRJandPL(100m).Thissubjecthadthemostsevere (GOLD stage IV; FEV1% = 15) and was the only current-smoker. Smoking may interfere with nitrate/nitrite metabolism [30]. The secondnon-responderwalked10mlessontheISWTafterBRJ(150 to 140m) compared with PL (140m before and after PL), and was the only subject who had a decrease in serum nitrite following BRJ. This subject was taking a proton pump inhibitor (Lansoprazole). It is known that the acidic conditions of the stomach upregulate the reductionof nitritetoNO [24].Itispossiblethatincreasedstomach pH may account for the decreased serum nitrite and lack of exercise benefit due to nitrate supplementation in this subject. We also observed significant decreases in resting systolic BP (−12.8 mmHg), diastolic BP (−3.2mmHg), and MAP (−6.2mmHg) (Table3). This is noteworthy as hypertension is a frequently occurringcomorbidityinCOPD [31].Indeed,6(55%)of ourstudysample were established on anti-hypertensive therapy. This observation is consistent with some previous reports of BP reductions following

dietary nitrate supplementation [10,12,32]. There was no significant difference between the two beverages regarding %SpO2, HR or dyspnoea either before- or after-ISWT (Table3). Considering that mostsubjectswalkedfurtherwithBRJ,changesin%SpO2,HRordyspnoea would have been unlikely. Even though exercise testing is frequently used in the clinical

evaluation of patients with COPD and to evaluate the functional impactof atreatment[33]quantifyingimprovementinexercisetolerance is difficult. Hence, there is no consensus regarding which exercise testing protocol should be used to assess COPD symptomsandtreatmentimpact.ISWTperformancemorestronglyreflects theabilitytoundertakeeverydayphysicalactivitiesthanlaboratorybasedtesting[34].Alt houghISWTisavalidatedmeasureof exercise capacityinCOPD[17,35]andhasexcellenttest–retestreliability[35], its use does not elucidate the mechanism(s) involved.

Potential mechanisms

Several mechanisms may have contributed to our observed results and suggested mechanisms by which dietary nitrate improved exercise capacity have recently been reviewed [36] and include reduced O2 cost of exercise [10], increased mitochondrial eficiency [36], as well as increased oxygenation status [12,27] and blood volume in active skeletal muscle [10,12]. Further mechanisms may include an improvement in dynamic hyperinflation and a pulmonary vasodilatory action, leading to abrogation of ventilation mismatch during exercise. However in this pilot study, we did not explore mechanisms.

Limitations

Our study has several important limitations. We did not use a true placebo but rather manufactured a similar appearing and tasting no-nitrate, placebo juice. However,aresearchernotinvolvedintheassessmentsblindedthebeveragesandtherefore study subjects and assessors were blinded throughout. Nevertheless, BRJ contains multiple, non-nitrate components that may influence

exerciseperformancee.g.betaine,potassium,carbohydrateenergy(as sugar),antioxidants,andpolyphenols.Wefeelitisunlikelythatany of these food components account for the acute improvement in exercise capacity we observed. For example, the BRJ contained 146kcal from carbohydrates compared with 26kcal in the PL. It is unlikely that the energy content of the BRJ accounts for our observations. Vermeeren et al. (2001) [37] demonstrated no difference inexercisecapacity(submaximalcycleenduranceexercisetest)followingacuteconsumptionof a250kcalcarbohydratedrinkamong COPD subjects. Multiple investigations have noted superiority of nitrate rich BRJ compared with a recently developed, nitrate

Furthermore, previous trials utilizing BRJ vs.anon-BRJ,nitrate-freejuicehaveshownthatBRJresultedinbeneficial exercise/BP response compared with PL [12,26]. Therefore, we feel it is likely that the inorganic nitrate was responsible for the observed effects. While a limitation of this study is the heterogeneous nature of thesamplestudied,thisisrepresentativeof theCOPDspectrumobservedclinically [31].Whetherourresultscanbeappliedtoalarger group of COPD subjects in the chronic setting requires further research. Another limitation is the absence of the ISWT practice walk as recommended[16].Theprotocolwasidenticalonbothvisitsexcept for the beverages. Seven (63%) of our subjects were randomly

assignedtoBRJfollowedbyPL.Meanbaseline(i.e.pre-beverage)ISWT scores were found to be similar between weeks 1 and 2 (197.6 vs. 195 m) (Fig. 3). Therefore, it is unlikely the increased exercise capacity we observed with BRJ can be attributed to a learning effect. Although NO may be important in COPD, therapy with inhaled NO has yielded disappointing results in COPD and can worsen ventilation perfusion matching [13]. Indeed, inhaled NO is contraindicatedinCOPDasperthe2013GlobalStrategyfortheDiagnosis, Management and Prevention of COPD (2014) [38] guidelines. No recommendation exists about the benefits or risks of supplementaldietarynitrate.However,directconsumptionof nitrite(typically in the form of processed meat) has been associated with COPD incidence and severity [39]. Nitrite formed endogenously from dietary nitrate appears beneficial by forming NO endogenously, whereas directly consumed nitrite has been associated with detrimental effects, potentially by forming carcinogenic nitrosamines.

4.3. Clinical relevance

The same group who developed the ISWT recently recommended the minimum clinically important improvement for the ISWT to 47.5m ([40]). According to this criteria, 3 of our 12 subjects (25%) improved to a clinically significant degree (+ 50, 70, 80m respectively). Ever decreasing exercise tolerance is central to the progression of the downward spiral of COPD. Augmenting chronic dietary nitrate

intake through nutritional strategies may represent a possible opportunity to impact on exercise tolerance in COPD; however we did not assess chronic effects in this study.

5. Conclusions

Acutesupplementationofdietarynitrateincreasedserumnitrate/nitritelevels.Thiscorrespondedtoameanincreaseof11%indistance walked on ISWT and a 5.4% decrease in MAP. However, our preliminary trial has important limitations, namely the inclusion of a small, heterogeneous sample, short trial period (3h) and the lack of a true placebo. Since COPD is often associated with decreased exercise capacity, nitrate-rich vegetables may represent an inexpensive, acceptable, novel, adjunct therapeutic option. Our preliminaryacuteresultsrequireconfirmationwithalargersample, a true placebo and a longer intervention period.

6. CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

Nutritional status is an important determinant of outcome of COPD and RA and may be assessed by longitudinal measurement of body weight and body composition.

The prevalence of vitamin D nutrient deficiency is high in COPD and could be incorporated into nutritional risk screening.

The nutritional risk profiles associated with different metabolic phenotypes of COPD patients could be useful in patient counselling.

Nutritional intervention is likely to be effective in undernourished patients (based on the Cochrane review [66]) and is probably most effective if combined with an exercise programme.

Providing evidence of the cost-effectiveness of nutritional intervention is required to support reimbursement of, and thus increase access to, nutritional intervention.

Overall, the evidence indicates that a well-balanced diet with sufficient intake of fresh fruits and vegetables is beneficial to COPD patients, not only for its potential benefits on the lung but also for its proven benefits on metabolic and cardiovascular risk.

Future research priorities

Nutritional assessment

Validate the criteria for risk stratification phenotypes of COPD as set out in figure 1

Investigate whether these phenotypes are characterised by specific mechanisms/pathophysiology

Standardise protocols for lifestyle determinants (diet, smoking and physical activity level) and for metabolic phenotyping to facilitate betweencentre comparisons and multicentre studies

Pathophysiology of abnormal body composition

Explore the role of systemic inflammation and of inflammatory genotypes on body composition changes.

Explore the role of adipose tissue macrophages in the systemic inflammatory response and related extra pulmonary pathology, consider sex differences in adipose tissue metabolism and inflammation, and investigate effects of COPD exacerbations on adipose tissue inflammation and metabolism.

Investigate the aetiology of muscle wasting on a cellular basis by analysing the regulatory and effector pathways of muscle protein and myonuclear turnover in muscle biopsies of in well-deep phenotyped COPD patients and by longitudinal data collection.

Investigate the added value of pharmacological modulation of regulatory pathways of proteolysis, including NF-kB, FOXO, MAPK, or their triggers oxidative stress and inflammation on the outcome of anabolic nutritional and multimodal interventions.

Analyse the impaired response to anabolic stimuli after acute nutritional, pharmacological or exercise challenges in analogy to the glucose tolerance test.

Investigate the putative influence of abnormal microbiota shifts in the lung or intestine on abnormal metabolic phenotypes.

Determine whether targeting exacerbations with intensive nutritional therapy (perhaps combined with exercise and anabolic drugs) would improve outcome.

Determine the effectiveness and safety of weight reduction programmes in obese patients with COPD and RA.

Outcome analysis

It is likely that the benefits of supplementation will be maximised if combined with exercise, although based on the current literature, the effects of nutrition and exercise cannot clearly be distinguished, which is a subject for future research.

Cost-effectiveness

Assess the added value of oral nutritional supplements and long-term nutritional counselling in terms of costs and effects in different phenotypes in RCTs

Use of real-life data from continuous patient registries in weight-losing patients for cost-effectiveness analysis of dietary counselling and nutritional supplements

Use longitudinal real-life data from patient registries to study the association between change in body composition and disease progression risk, functional impairment, hospitalisations and mortality; this information can be used to perform a model-based analysis of long-term cost-effectiveness of nutritional interventions

REFERENCES

APPENDIX I

Έγκριση επιτροπής βιοηθικής

APPENDIX II

Τ.Ε.Φ.Α.Α., ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ

ΣΤΟΙΧΕΙΑ ΣΥΜΜΕΤΕΧΟΝΤΟΣ

ΟΝΟΜΑΤΕΠΩΝΥΜΟ:

ΗΜΕΡΟΜΗΝΙΑ ΓΕΝΝΗΣΗΣ:

ΗΜΕΡΟΜΗΝΙΑ:

ΚΩΔΙΚΟΣ ΣΥΜΜΕΤΕΧΟΝΤΑ:

ΑΝΘΡΩΠΟΜΕΤΡΙΚΑ ΧΑΡΑΚΤΗΡΙΣΤΙΚΑ

Μέτρηση	1η	2η	3η	4η
Βάρος (kg)				
Ύψος από όρθια θέση (cm)				
Ποσοστό σωματικού λίπους				
Ποσοστό ενυδάτωσης				
Λόγος περιμέτρου μέσης /γοφών (WHR)				

ΚΥΚΛΟΦΟΡΙΚΟ – ΚΑΡΔΙΑ

Μέτρηση	1η	2η	3η	4η
Αρτηριακή πίεση ηρεμίας				
Καρδιακή συχνότητα ηρεμίας				

Παρατηρήσεις

Μέτρηση	1η	2η	3η	4η
6 min walk test				
Σπιρομέτρηση				

Ο ΥΠΕΥΘΥΝΟΣ ΤΟΥ ΕΡΓΑΣΤΗΡΙΟΥ

(Υπογραφή)

APPENDIX III

Ερωτηματολόγιο υγείας

Όνομα.....

Ημερομηνία.....

Παρακαλώ συμπληρώστε

1. Έχετε κάποιο πρόβλημα υγείας για το οποίο
   - Α) είστε υπό φαρμακευτική αγωγή ναι [ ] όχι [ ]
   - Β) είστε υπό ιατρική παρακολούθηση ναι [ ] όχι [ ]

2. Τα τελευταία 2 χρόνια, εξαιτίας κάποιας ασθένειας
   - Α) επισκεφτήκατε το γιατρό σας ναι [ ] όχι [ ]
   - Β) επισκεφτήκατε εξωτερικά ιατρεία ναι [ ] όχι [ ]
   - Γ) μείνατε στο νοσοκομείο ναι [ ] όχι [ ]

3. Είχατε ποτέ κάποια από τις παρακάτω καταστάσεις;
   - Α) Επιληψία ναι [ ] όχι [ ]
   - Β) Έκζεμα ναι [ ] όχι [ ]
   - Γ) Διαβήτης ναι [ ] όχι [ ]
   - Δ) Άσθμα ναι [ ] όχι [ ]
   - Ε) Καρδιαγγειακά νοσήματα ναι [ ] όχι [ ]
   - Ζ) Πεπτικά προβλήματα ναι [ ] όχι [ ]
   - Η) Προβλήματα γυναικολογικά ναι [ ] όχι [ ]
   - Θ) Προβλήματα οστών και αρθρώσεων ναι [ ] όχι [ ]
   - Ι) Προβλήματα ισορροπίας και συναρμογής ναι [ ] όχι [ ]
   - Κ) Προβλήματα όρασης/ ακοής ναι [ ] όχι [ ]
   - Λ) Προβλήματα θυρεοειδούς ναι [ ] όχι [ ]
   - Μ) Ορμονικά προβλήματα ναι [ ] όχι [ ]

Ν) Προβλήματα ήπατος και νεφρών ναι [ ] όχι [ ]

Ξ) Μούδιασμα άκρων ναι [ ] όχι [ ]

Ο) Άλλα προβλήματα ναι [ ] όχι [ ]

4. Αν είστε γυναίκα

Α) σχεδιάζετε να μείνετε έγκυος ναι [ ] όχι [ ]

Β) είστε η νομίζετε ότι είστε έγκυος ναι [ ] όχι [ ]

Γ) παίρνετε χάπια αντισύλληψης ή ορμόνες ναι [ ] όχι [ ]

Δ) είστε στην εμμηνόπαυση ναι [ ] όχι [ ]

5. Κάποιος από τους συγγενείς πρώτου βαθμού είχε

Α) Καρδιαγγειακά προβλήματα ναι [ ] όχι [ ]

Β) Διαβήτη ναι [ ] όχι [ ]

Γ) Εγκεφαλικό ναι [ ] όχι [ ]

Δ) Κάποια άλλη ασθένεια ναι [ ] όχι [ ]

6. Καπνίζετε αυτή την περίοδο ναι [ ] όχι [ ]

Έχετε καπνίσει ποτέ ναι [ ] όχι [ ]

Αν ναι, για πόσο καιρό και πότε το κόψατε.....

Πόσες μονάδες αλκοόλ πίνετε σε μια εβδομάδα.....

7. Γυμνάζεστε ναι [ ] όχι [ ]

Πόσες φορές την εβδομάδα.....

Αναφέρετε το είδος γυμναστικής.....

Αν απαντήσατε ναι σε κάποια από τις ερωτήσεις, παρακαλώ περιγράψτε εν συντομία

.....

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.....

☐ Δηλώνω ότι είμαι σωματικά υγιής και δεν πάσχω από κάποια πάθηση, βλάβη, ασθένεια ή αναπηρία που θα μπορούσε να εμποδίσει τη συμμετοχή μου στην πειραματική διαδικασία.

☐ Αναγνωρίζω ότι έχω εξετασθεί και ο γιατρός μου έχει δώσει την άδεια να συμμετάσχω, ή έχω αποφασίσει να συμμετάσχω στην πειραματική διαδικασία χωρίς την έγκριση του γιατρού μου. Αναλαμβάνω κάθε ευθύνη για την συμμετοχή μου.

Ημ/νία

Υπογραφή ερευνητή Υπογραφή συμμετέχοντα

APPENDIX IV

ΔΙΕΘΝΕΣ ΕΡΩΤΗΜΑΤΟΛΟΓΙΟ ΦΥΣΙΚΗΣ ΔΡΑΣΤΗΡΙΟΤΗΤΑΣ (IPAQ)

Όνομα/ Κωδικός:

Ημερομηνία:

Ενδιαφερόμαστε να μάθουμε για τις σωματικές δραστηριότητες που κάνουν οι άνθρωποι σαν κομμάτι της καθημερινής τους ζωής. Οι ερωτήσεις αναφέρονται σε δραστηριότητες που κάνατε τις **τελευταίες 7 ημέρες.** Παρακαλούμε απαντήστε σε όλες τις ερωτήσεις ακόμα και αν δεν θεωρείτε τον εαυτό σας δραστήριο άτομο. Παρακαλούμε σκεφτείτε τις δραστηριότητες που κάνετε στην εργασίας σας, σαν μέρος της δουλειάς που κάνετε στο σπίτι και στην αυλή, για να μετακινηθείτε από ένα μέρος σε άλλο και στον ελεύθερο χρόνο σας για αναψυχή, εξάσκηση ή για σπορ.

Σκεφτείτε όλες τις έντονες δραστηριότητες κάνατε τις **τελευταίες 7 ημέρες**. Οι έντονες σωματικές δραστηριότητες χρειάζονται προσπάθεια και μπορεί να σας κάνουν να αναπνέετε πιο δύσκολα από το κανονικό. Σκεφτείτε μόνο εκείνες τις σωματικές δραστηριότητες που κάνατε για τουλάχιστον 10 λεπτά συνεχόμενα.

1. Κατά ην διάρκεια των **τελευταίων 7 ημερών**, πόσες μέρες κάνατε **έντονες** σωματικές δραστηριότητες όπως το να σηκώσετε βαριά αντικείμενα, σκάψιμο, aerobics ή γρήγορο ποδήλατο;

____ Ημέρες ανά εβδομάδα.

Καμιά έντονη σωματική δραστηριότητα => περάστε στην ερώτηση 3.

2. Πόσο χρόνο καταναλώσατε σε **έντονες** σωματικές δραστηριότητες την κάθε μία από αυτές τις ημέρες;

____ Ώρες ανά ημέρα

____ Λεπτά ανά ημέρα

Δεν ξέρω/ δεν είμαι σίγουρος

Σκεφτείτε όλες τις δραστηριότητες που χρειάζονται μέτρια σωματική προσπάθεια και τις οποίες κάνατε τις **τελευταίες 7 ημέρες**. Οι μέτριες σε ένταση δραστηριότητες μπορεί να σας κάνουν να αναπνέετε πιο λίγο πιο δύσκολα από το κανονικό. Σκεφτείτε μόνο εκείνες τις σωματικές δραστηριότητες για τις οποίες διαθέσατε τουλάχιστον 10 λεπτά συνεχόμενα.

3. Κατά την διάρκεια των **τελευταίων 7 ημερών**, πόσες μέρες κάνατε **μέτριες** σωματικές δραστηριότητες όπως τη μεταφορά ελαφριών φορτίων, ποδήλατο σε κανονικό ρυθμό, ή τένις. Μην συμπεριλάβετε το περπάτημα.

____ Ημέρες ανά εβδομάδα.

Καμιά μέτριας έντασης σωματική δραστηριότητα => περάστε στην ερώτηση 5

4. Πόσο χρόνο καταναλώσατε συνήθως σε **μέτριες** σωματικές δραστηριότητες την κάθε ημέρα;

____ Ώρες ανά ημέρα

____ Λεπτά ανά ημέρα

Δεν ξέρω/ δεν είμαι σίγουρος

Σκεφτείτε το χρόνο που διαθέσατε στο **περπάτημα** τις **τελευταίες 7 ημέρες**. Αυτό περιλαμβάνει και το περπάτημα στο σπίτι και στη δουλειά, την μετακίνηση από το ένα μέρος στο άλλο και οτιδήποτε περπάτημα κάνετε απλά στον ελεύθερο σας χρόνο για άθληση, εξάσκηση η αναψυχή.

5. Κατά τις **τελευταίες 7 ημέρες** πόσες μέρες περπατήσατε για τουλάχιστον 10 λεπτά συνεχόμενα;

____ Ημέρες ανά εβδομάδα.

Καθόλου περπάτημα = περάστε στην ερώτηση 7

6. Πόσο χρόνο συνήθως περάσατε περπατώντας μία από αυτές τις ημέρες;

____ Ώρες ανά ημέρα

____ Λεπτά ανά ημέρα

Δεν ξέρω/ δεν είμαι σίγουρος

Σκεφτείτε το χρόνο που περάσετε **καθισμένοι κ**ατά τις εργάσιμες ημέρες στο διάστημα των **τελευταίων 7 ημερών.** Συμπεριλάβετε τον χρόνο στη δουλειά, στο σπίτι, στη διάρκεια μαθημάτων ή της ξεκούρασης. Αυτό μπορεί να περιλαμβάνει τον χρόνο που περάσατε καθισμένοι σε ένα θρανίο, επισκεπτόμενοι φίλους, διαβάζοντας ή τον χρόνο που περάσατε καθισμένοι ή ξαπλωμένοι βλέποντας τηλεόραση.

7. Κατά τη διάρκεια των **τελευταίων 7 ημερών**, πόση ώρα περάσατε καθισμένοι σε μία από τις εργάσιμες ημέρες;

____ Ώρες ανά ημέρα

____ Λεπτά ανά ημέρα

Δεν ξέρω/ δεν είμαι σίγουρος

Αυτό είναι το τέλος του ερωτηματολογίου, ευχαριστούμε για την συμμετοχή σας

APPENDIX V

ΔΙΑΤΡΟΦΙΚΗ ΑΝΑΚΛΙΣΗ 24ΩΡΟΥ (24h recall)

Όνομα/ Κωδικός:

Ημερ/νία:

ΒΗΜΑ 1: Λίστα τροφών που καταναλώθηκαν εχθές/ μια τυπική ημέρα

1. **ΠΡΩΙΝΟ**

2. **ΠΡΟΓΕΥΜΑ**

3. **ΜΕΣΗΜΕΡΙ**

4. **ΑΠΟΓΕΥΜΑ**

5. **ΒΡΑΔΥ**

6. **ΑΛΛΟ**

ΒΗΜΑ 2: Αναλυτική περιγραφή τροφίμων

Ερωτήσεις περιγραφής

- Θυμάσαι κάτι άλλο που κατανάλωσες με αυτό το φαγητό?
- Τι άλλο έφαγες σε αυτό το γεύμα?
- Σε αυτό έβαλες κάτι από πάνω?

Ερωτήσεις τύπου τροφίμου

- Τύπος φαγητού, μορφή, μέθοδος παρασκευής, εταιρεία παρασκευής, ποσότητα και μέρη, υλικά, επιπρόσθετα

Ερωτήσεις ωραρίου

- Ποιό ήταν το πρώτο πράγμα που έφαγες όταν ξύπνησες το πρωί?
- Έφαγες κάτι μετά τα μεσάνυχτα?
- Έφαγες ή ήπιες κάτι όταν βγήκες έξω?

Ερωτήσεις δραστηριότητας

- Καθώς δούλευες (εντός ή εκτός σπιτιού) έκανες κάποιο διάλειμμα να φας ή να πιείς κάτι?
- Είδες τηλεόραση εχθές? Καθώς έβλεπες τηλεόραση έτρωγες κάτι παράλληλα?
- Σταμάτησες στο δρόμο από το σπίτι στη δουλειά να πάρεις κάποιο φαγητό ή ποτό?

Ομάδες τροφίμων/ Προτιμήσεις

Δημητριακά	**(ΝΑΙ/ ΟΧΙ, περιγραφή)**
ψωμί	
ρύζι	
δημητριακά	
πατάτες	
ζυμαρικά	
Φρούτα	
εσπεριδοειδή	
χυμοί	
άλλα φρούτα	
ξερά φρούτα	
Λαχανικά	
Κρέας	
ψάρι	
θαλασσινά	
αυγά	
όσπρια	
ξηροί καρποί	
Γαλακτοκομικά προϊόντα (γάλα, γιαούρτι, τυρί κτλ)	
Λίπη, Έλαια, Γλυκά (ελιές, ελαιόλαδο, σπορέλαιο, μαγιονέζα, μαργαρίνη, βούτυρο, σάλτσες, σως, ζάχαρη)	
Νερό	
Αλάτι	
Αλκοόλ	
Αναψυκτικά	
Άλλο, σούπες, snacks, τσάι, καφές	

APPENDIX VI

COPD - Κλίμακα δύσπνοιας MRC

Ημερομηνία:

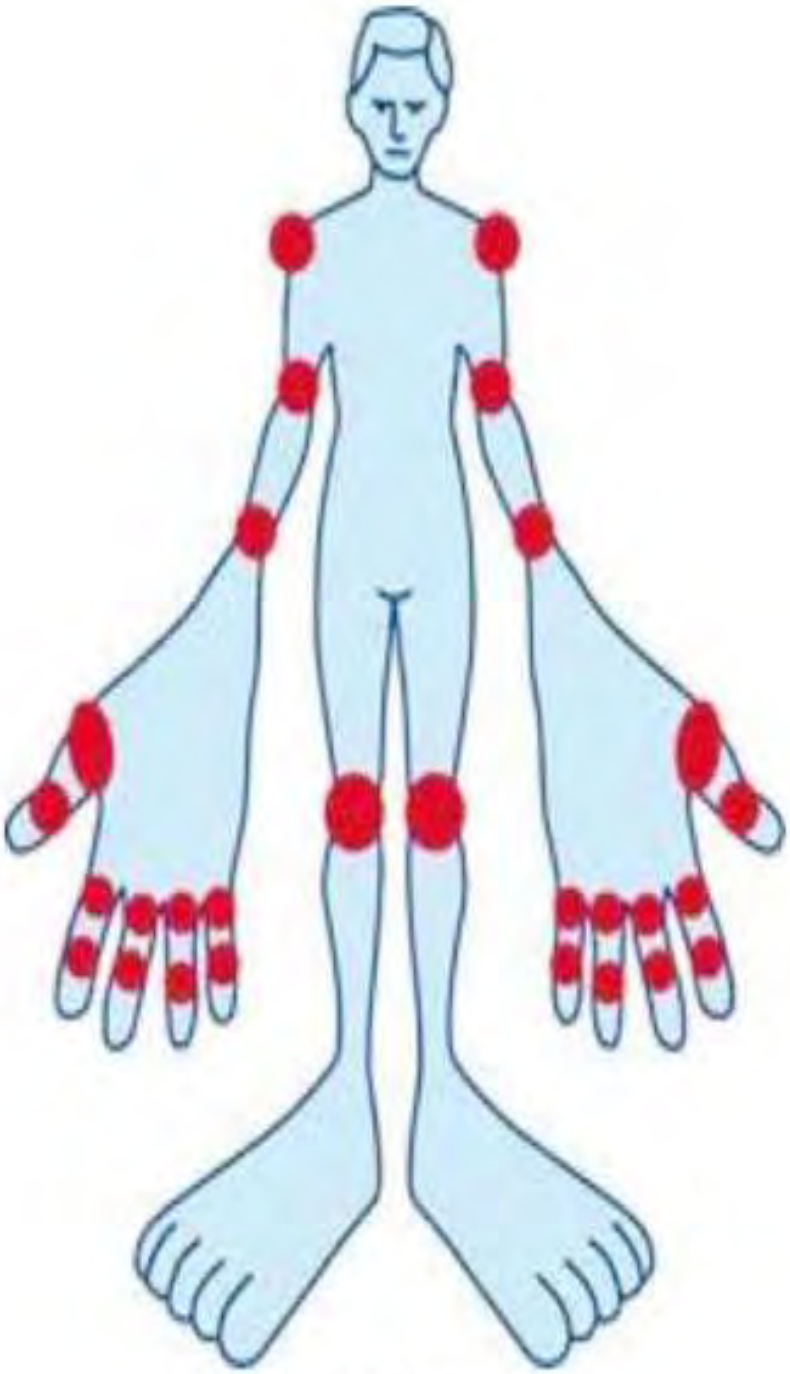
Κωδικός:

Με την παρακάτω κλίμακα, ο εξεταζόμενος βαθμολογείται από 0-4, ανάλογα με την ικανότητά του στην κίνηση. Με την κλίμακα MRC δεν αποτιμάται η ένταση της δύσπνοιας, αυτής καθαυτής, όπως με τις αναλογικές ή κατηγορικές διαβαθμίσεις, αλλά μόνο η σχέση της με τροποποιημένη δραστηριότητα (Προσαρμογή από Fletcher et al., 1959).

0	Απουσία δύσπνοιας εκτός από την περίπτωση έντονης σωματικής άσκησης
1	Εμφάνιση δύσπνοιας όταν βιάζεται στο ίσιωμα ή όταν ανεβαίνει σε μικρή ανηφόρα
2	Βαδίζει βραδύτερα από ανθρώπους της ίδιας ηλικίας στο ίσιωμα ή σταματά όταν βαδίζει με το δικό του βήμα στο ίσιωμα
3	Σταματά για να ανασάνει όταν βαδίζει 100 μ. ή μετά από λίγο στο ίσιωμα
4	Πολύ μεγάλη δύσπνοια για να μπορέσει να βγει από το σπίτι ή όταν ντύνεται ή ξεντύνεται

APPENDIX VII

RA - DAS28



DAS28

APPENDIX VIII

Όνομα:

Ημερομηνία:

CAT
COPD Assessment Test

Πώς είναι η κατάσταση της υγείας σας λόγω της ΧΑΠ; Συμπληρώστε το Τεστ Αξιολόγησης για την ΧΑΠ (COPD Assessment Test™, CAT)

Αυτό το ερωτηματολόγιο θα βοηθήσει εσάς και το γιατρό σας να εκτιμήσετε την επίδραση που έχει η ΧΑΠ (Χρόνια Αποφρακτική Πνευμονοπάθεια) στην ευεξία και στην καθημερινή σας ζωή. Οι απαντήσεις σας, καθώς και η βαθμολογία από το τεστ μπορούν να χρησιμοποιηθούν από εσάς και το γιατρό σας ως βοήθημα στη διαχείριση και βελτίωση της ΧΑΠ σας, ώστε να λάβετε τα μέγιστα δυνατά οφέλη από τη θεραπεία.

Για κάθε μια από τις παρακάτω δηλώσεις σημειώστε ένα X στο τετραγωνάκι που περιγράφει με τη μεγαλύτερη ακρίβεια την τρέχουσα κατάστασή σας. Βεβαιωθείτε ότι έχετε επιλέξει μία μόνο απάντηση για κάθε ερώτηση.

Παράδειγμα: Είμαι πολύ ευχαριστημένος/η	0 X 2 3 4 5	Είμαι πολύ στενοχωρημένος/η	ΒΑΘΜΟΛΟΓΙΑ
Δεν βήχω ποτέ	0 1 2 3 4 5	**Βήχω συνέχεια**	
Δεν έχω καθόλου φλέγμα (βλέννα) στο στήθος	0 1 2 3 4 5	**Το στήθος μου είναι εντελώς γεμάτο με φλέγμα (βλέννα)**	
Δεν αισθάνομαι καθόλου σφίξιμο στο στήθος	0 1 2 3 4 5	**Αισθάνομαι έντονο σφίξιμο στο στήθος**	
Δεν λαχανιάζω όταν περπατάω σε ανηφόρα ή όταν ανεβαίνω τις σκάλες ενός ορόφου	0 1 2 3 4 5	**Λαχανιάζω πολύ όταν περπατάω σε ανηφόρα ή όταν ανεβαίνω τις σκάλες ενός ορόφου**	
Δεν έχω κανένα περιορισμό όταν πραγματοποιώ οποιαδήποτε δραστηριότητα στο σπίτι	0 1 2 3 4 5	**Περιορίζομαι πολύ όταν πραγματοποιώ οποιαδήποτε δραστηριότητα στο σπίτι**	
Νιώθω αυτοπεποίθηση όταν βγαίνω από το σπίτι παρά την πνευμονική πάθησή μου	0 1 2 3 4 5	**Δεν νιώθω καθόλου αυτοπεποίθηση όταν βγαίνω από το σπίτι λόγω της πνευμονικής πάθησής μου**	
Κοιμάμαι ήρεμα	0 1 2 3 4 5	**Δεν κοιμάμαι ήρεμα λόγω της πνευμονικής πάθησής μου**	
Έχω πολλή ενέργεια	0 1 2 3 4 5	**Δεν έχω καθόλου ενέργεια**	

APPENDIX IX

HEALTH ASSESSMENT QUESTIONNAIRE (HAQ-DI)©

Name: _____ Date: _____

Please place an "x" in the box which best describes your abilities OVER THE PAST WEEK:

	WITHOUT ANY DIFFICULTY	WITH SOME DIFFICULTY	WITH MUCH DIFFICULTY	UNABLE TO DO
DRESSING & GROOMING				
Are you able to:				
Dress yourself, including shoelaces and buttons?	☐	☐	☐	☐
Shampoo your hair?	☐	☐	☐	☐
ARISING				
Are you able to:				
Stand up from a straight chair?	☐	☐	☐	☐
Get in and out of bed?	☐	☐	☐	☐
EATING				
Are you able to:				
Cut your own meat?	☐	☐	☐	☐
Lift a full cup or glass to your mouth?	☐	☐	☐	☐
Open a new milk carton?	☐	☐	☐	☐
WALKING				
Are you able to:				
Walk outdoors on flat ground?	☐	☐	☐	☐
Climb up five steps?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

☐ Devices used for Dressing (button hook, zipper pull, etc.)
☐ Built up or special utensils
☐ Crutches
☐ Cane
☐ Wheelchair
☐ Special or built up chair
☐ Walker

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

☐ Dressing and grooming ☐ Arising ☐ Eating ☐ Walking

Please place an "x" in the box which best describes your abilities OVER THE PAST WEEK:

	WITHOUT ANY DIFFICULTY	WITH SOME DIFFICULTY	WITH MUCH DIFFICULTY	UNABLE TO DO
HYGIENE				
Are you able to:				
Wash and dry your body?	☐	☐	☐	☐
Take a tub bath?	☐	☐	☐	☐
Get on and off the toilet?	☐	☐	☐	☐
REACH				
Are you able to:				
Reach and get down a 5 pound object (such as a bag of sugar) from above your head?	☐	☐	☐	☐
Bend down to pick up clothing from the floor?	☐	☐	☐	☐
GRIP				
Are you able to:				
Open car doors?	☐	☐	☐	☐
Open previously opened jars?	☐	☐	☐	☐
Turn faucets on and off?	☐	☐	☐	☐
ACTIVITIES				
Are you able to:				
Run errands and shop?	☐	☐	☐	☐
Get in and out of a car?	☐	☐	☐	☐
Do chores such as vacuuming or yard work?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

☐ Raised toilet seat ☐ Bathtub bar ☐ Long-handled appliances for reach

☐ Bathtub seat ☐ Long-handled appliances in bathroom ☐ Jar opener (for jars previously opened)

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

☐ Hygiene ☐ Reach ☐ Gripping and opening things ☐ Errands and chores

Your ACTIVITIES: To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?

COMPLETELY	MOSTLY	MODERATELY	A LITTLE	NOT AT ALL
☐	☐	☐	☐	☐

Your PAIN: How much pain have you had IN THE PAST WEEK?
On a scale of 0 to 100 (where zero represents "no pain" and 100 represents "severe pain"), please record the number below.

☐☐☐

Your HEALTH: Please rate how well you are doing on a scale of 0 to 100 (0 represents "very well" and 100 represents "very poor" health), please record the number below.

☐☐☐

Your ACTIVITIES: To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?

COMPLETELY	MOSTLY	MODERATELY	A LITTLE	NOT AT ALL
☐	☐	☐	☐	☐

Your PAIN: How much pain have you had IN THE PAST WEEK?
On a scale of 0 to 100 (where zero represents "no pain" and 100 represents "severe pain"), please record the number below.

☐☐☐

Your HEALTH: Please rate how well you are doing on a scale of 0 to 100 (0 represents "very well" and 100 represents "very poor" health), please record the number below.

☐☐☐