INTRODUCTION

Normal aging

Aging is the process of becoming older, a process that is genetically determined and environmentally modulated. Aging is characterized by progressive and broadly predictable changes that are associated with increased susceptibility to many diseases. Aging is not a homogenous process. Rather, organs in the same person age at different rates influenced by multiple factors, including; genetic, lifestyle choices, and environmental exposures [(1)].

Demographics indicate that the world's population aged 60 years and over will be more than triple within 50 years from 600 million in the year 2000, to more than 2 billion by 2050. As a result of this the fastest growing sub-population of society, in the developed world, is adults aged ≥ 80 [(2)]. Aging is accompanied by numerous increasingly prevalent clinical conditions ,which can lessen the quality of life, and reduce independence such as, rheumatoid and osteoarthritis, vascular disease, type 2 diabetes, and osteoporosis that require extensive health care resources [(3)] .

Age-associated physiologic changes

- **Physiologic rhythms**: The organization of rhythmic physiologic processes is altered by aging. Age impacts the circadian pattern of body temperature, plasma cortisol, and sleep, and can cause desynchronization or "internal phase drift". Phase advances can lead to the occurrence of some rhythmic functions (eg, the 24-hour body temperature trough and sleep onset) one to two hours earlier in older adults. The pulsatile secretion of gonadotropins, growth hormone, thyrotropin, melatonin, and adrenocorticotropic hormone (ACTH) are attenuated with age [(4)].

- **Loss of complexity**: Loss of complexity, a concept derived from the field of non linear dynamics, may be a general principle of all aging systems. This loss of complexity may result in decreased heart rate variability, blood pressure variability, electroencephalographic frequencies, response to auditory frequencies, and response to stress. Age-related loss of complexity may not be immutable, however; as an example, senior athletes show greater heart rate variability than sedentary age matched controls[(4)].

- **Homeostenosis:** Homeostenosis refers to the concept that, from maturity to senescence, diminishing physiologic reserves are available to meet challenges to homeostasis. This concept was first recognized by Walter Cannon in the 1940s. Homeostenosis leads to the increased vulnerability to disease that occurs with aging. ***Figure(1)*** graphically displays the traditional thinking about homeostenosis. The endpoint of this process is frailty, where even the smallest challenge overwhelms the available reserves and results in disaster. The “precipice” may be variably defined: death, cardiac arrest, hospital admission, or onset of a symptom such as confusion or incontinence. Aging itself brings the individual closer to the precipice by the loss of physiologic reserves. With aging, the area in which the older person can bring themselves back to homeostasis narrows or becomes stenotic[(4)].

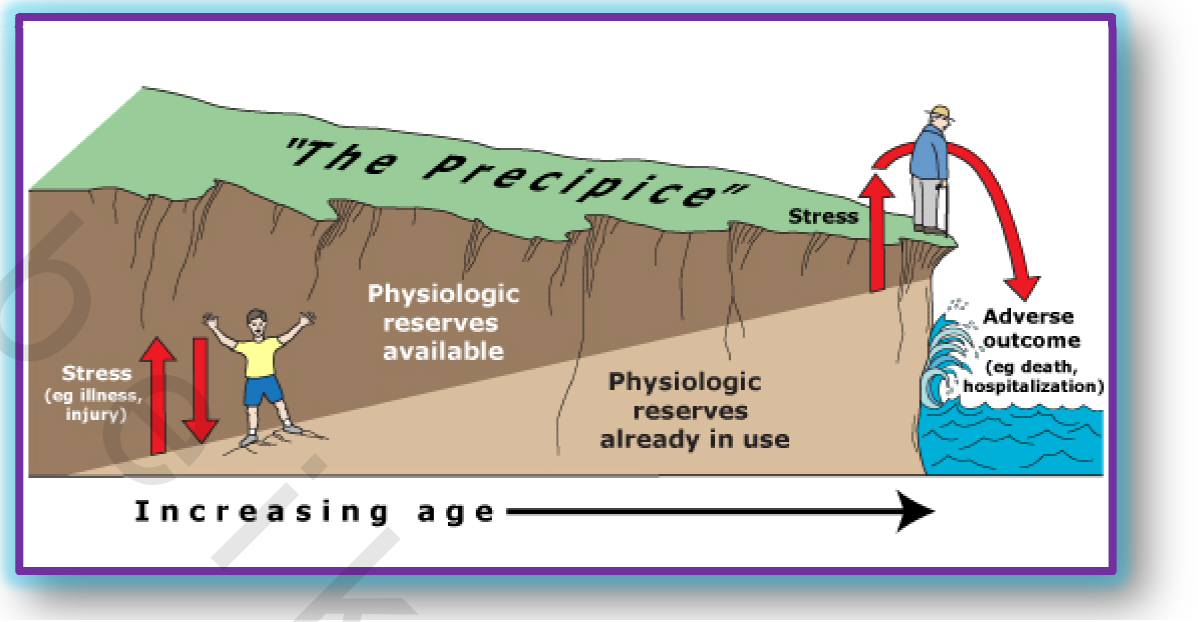

Figure 1: Traditional thinking about homeostenosis [4].

There are a great overlap between the physiologic changes that are attributable to disuse and those that typically have been observed in aging populations.These effects span a wide range of organ systems and functional capacities potentially relevant to health status in older adults. In most physiologic systems, the normal aging processes do not result in significant impairment or dysfunction in the absence of pathologic conditions or under resting conditions. In response to a stress or significant disuse, however, the age-related reduction in physiologic reserves causes a loss of homeostatic balance or an inability to complete a task requiring near maximal effort [5].

Aging theories

The traditional aging theories hold that aging is not an adaptation or genetically programmed. Modern biological theories of aging in humans fall into two main categories:

Programmed and damage or error theories.

The **damage or error theories:** emphasize environmental assaults to living organisms that induce cumulative damage at various levels as the cause of aging, including;

1. **Wear and Tear Theory:** The very general idea that changes associated with aging are the result of chance damage that accumulates over time.
2. **Rate of Living Theory:** The greater an organism's rate of oxygen basal metabolism, the shorter its life span [6].
3. **Cross-Linking Theory:** According to this theory, an accumulation of cross-linked proteins damages cells and tissues, slowing down bodily processes resulting in aging.
4. **Free Radicals Theory:** This theory proposes that superoxide and other free radicals cause damage to the macromolecular components of the cell, giving rise to accumulated damage causing cells, and eventually organs, to stop functioning [7].

5. **Somatic DNA Damage Theory:** DNA damages occur continuously in cells of living organisms. Therefore, aging results from damage to the genetic integrity of the body's cells.

The programmed theories: imply that aging follows a biological time table, perhaps a continuation of the one that regulates childhood growth and development. This regulation would depend on changes in gene expression that affect the systems responsible for maintenance, repair and defense responses. The programmed theory has three sub-categories:

1. **Programmed Longevity**: Aging is the result of a sequential switching on and off of certain genes [(8)].
2. **Endocrine Theory:** Biological clocks act through hormones to control the pace of aging [(9)].
3. **Immunological Theory:** The immune system is programmed to decline over time, which leads to an increased vulnerability to infectious disease and thus aging and death[(10)].

The redox stress hypothesis of aging is based on age-related alterations in cellular redox balance [(11)] accompanied by age-related dysregulation of the immune system **immunosenescence**[(12)] .Other processes such as **endocrinosenescence** and declining levels of sex hormones also likely contribute to elevated inflammation in older age[(13)]. In addition, a number of diseases, especially age-related diseases such as atherosclerosis and dementia, have strong inflammatory components[(14).]

Redox stress:

Endogenous reactive oxygen species (ROS) are hypothesized to play a key role in molecular, cellular, and structural damage over time .Under normal physiological conditions, reactive oxygen species like hydrogen peroxide (H_2O_2) have vital roles in signal transduction cascades and function . However, age-associated increases in reactive oxygen species leads to over-oxidation and irreversible changes in protein structure and function [(11)].The biological process to remove these accumulated damaged proteins stimulates inflammatory responses leading to a chronic inflammatory state [(15)].

Mitochondrial damage:

Mitochondria, the primary sites for chemical energy (ATP) production, are essential for normal cell function and maintenance of redox homeostasis as well as regulating programmed cell death . The **mitochondrial free radical theory of aging** is based on oxidative damage to mitochondrial DNA (mtDNA) due to overproduction of reactive oxygen species. This damage results in dysregulation of cell and organ function leading to the overall system decline recognized as aging [(16)]. mtDNA mutations have been reported to accumulate with age [(17)]; the ensuing loss of energy production likely underlies sarcopenia (age-related muscle loss) [(18)]. In addition, *apoptosis*, or *programmed cell death*, is a highly regulated process that leads to cell death without stimulation of the inflammation response nor damage to surrounding tissue [(19)].

Immunosenescence:

Oxidative stress and energy dysregulation are also hypothesized to play a key role in ***immunosenescence***; which is the gradual decline of the immune system with age. Immunosenescence results from the accumulation of molecular and cellular defects due to oxidative damage and thymic involution, the age-related reduction in thymus size and activity, and also reduction of the hyperstimulation of both innate and adaptive immune systems. The net result of these processes is increased susceptibility to diseases and increased morbidity and mortality due to infections and other age-associated diseases [(12)].

Thymic involution results in significantly reduced levels of naïve T cells at older ages. While the adaptive immune response to previously seen antigens dependent on memory T cells remains functional, although in a reduced capacity, the ability to respond to new infectious agents, requiring naïve T cells, is severely impaired. Poor immune function, combined with continued exposure to antigens, results in chronic activation of macrophages and other pro-inflammatory cells and contributes to chronic low level inflammation common in older age [(20)]. In addition, senescent cells demonstrate significant increases in production and secretion of many pro-inflammatory cytokines. Chronic inflammation, therefore, not only results from, but also drives immunosenescence [(21)].

Endocrinosenescence*:*

In addition to immunosenescence, the endocrine system also experiences age-related declines in function (endocrinosenescence) most notably affecting sex steroid production[(13)].Levels of *growth hormone* and *dehydroepiandrosterone* (DHEA) and its primary circulating form dehydroepiandrosterone sulfate (DHEAS) decrease with age as well. However, *cortisol* production is increased due to over-stimulation of the hypothalamic-pituitary-adrenal (HPA) axis [(22)].

Chronic over-stimulation of the hypothalamic-pituitary-adrenal axis leads to immune dysregulation and contributes to immunosenescence [(23)]. Decreased levels of DHEA and growth hormone also likely play a role in immunosenescence. Both DHEA and growth hormone enhance the proliferation and activity of cellular mediators of immunity and DHEA reduces inflammatory cytokine production . Reduction in levels of these hormones and increasing levels of cortisol with age would therefore lead to increased inflammation[(24)].

Sex hormones also modulates the production of inflammatory cytokines. Increasing in interleukin-6 and other pro-inflammatory cytokines in women subsequent to menopause. As levels of these steroid hormones decrease with age, levels of inflammatory cytokines are increased contributing to chronic inflammation, cellular senescence, and other age-related diseases [(13)].

Aging process in:

A. The musculoskeletal system:

Can be summarized ***(Figure 2)*** as follows:

*Skeletal muscle fibers become smaller in diameter,*this reduction in size reflects primarily a decrease in the number of myofibrils. In addition, the muscle fibers contain

smaller ATP, creatine phosphate(CP), and glycogen reserves and less myoglobin. The overall effect is a reduction in skeletal muscle size, strength, and endurance, combined with a tendency to fatigue rapidly. Because cardiovascular performance also decreases with age, blood flow to active muscles does not increase with exercise as rapidly as it does in younger people. *Skeletal muscles become less elastic* as aging skeletal muscles develop increasing amounts of fibrous connective tissue, a process called **fibrosis** ,which makes the muscle less flexible, and the collagen fibers can restrict movement and circulation. *Reduction in the number of muscle capillaries*; which is a factor that can alter muscle strength, as it reduces blood flow to active muscles and consequently decreases oxygen and nutrients supply and removal capacity for metabolites and heat[25]. *Age-related apoptotic motor neuron loss* is proposed to directly attenuate strength, rate of force development, and muscular power, and eventually leads to declines in muscle fiber number and physiological cross-sectional area [26].

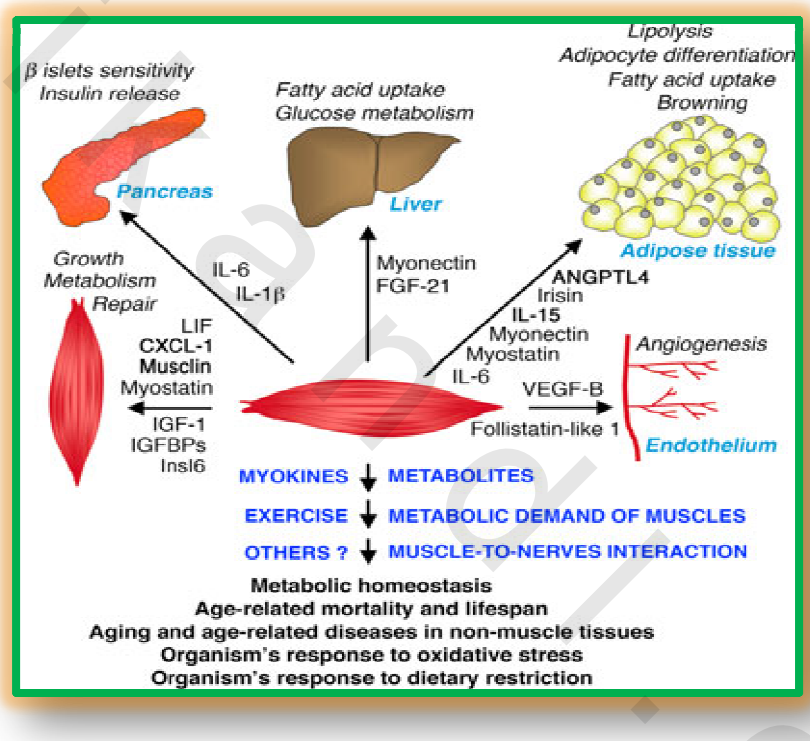

Figure 2*:* Systemic regulation of metabolism and aging by skeletal muscle [27,28].

Muscle protein metabolism in the elderly:

Skeletal muscle proteins are constantly and simultaneously synthesized and degraded. ***Net protein balance*** is defined as the difference between skeletal muscle protein synthesis (MPS) and breakdown (MPB). Thus, a significant rise in MPS (anabolism) and/or a reduction in MPB (catabolism), such that net protein balance remains *positive* can result in the accretion of skeletal muscle proteins. Conversely, in elderly a *negative* net protein balance, arising from a reduction in MPS and/or increase in MPB, will result in a loss of skeletal muscle protein. ***Net protein balance*** is maintained by ingestion of *PROTEIN-CONTAINING MEALS* which results in systemic hyperaminoacidemia that is stimulatory for the synthesis of new proteins. In addition to protein consumption,

EXERCISE increases rates of MPS, thereby improving net protein balance[25].Therefore, the synergistic effect of protein ingestion with exercise potentiates the muscle synthetic response, swinging net balance in favour of muscle protein accretion when practiced frequently over time[26,29]. Only overloading of muscle with weight-lifting exercise (resistance training) may prevent losses of muscle mass (and also strength) in older individuals depending on the subject characteristics and intensity of the program[27,28,30].

Sarcopenia as a geriatric syndrome:

A grave change associated with human ageing is progressive decline in skeletal muscle mass, a downward spiral that may lead to decreased strength and functionality. In 1989, Irwin Rosenberg proposed the term '**Sarcopenia**' (Greek 'sarx' or flesh + 'penia' or loss) to describe this age-related decrease of muscle mass [31-33]. It may be likewise helpful to recognise sarcopenia as a geriatric syndrome because this view promotes its identification and treatment even when the exact causes remain unknown [34-37].

- **What is sarcopenia?**

Sarcopenia is a syndrome characterised by progressive and generalised loss of skeletal muscle mass and strength with an impaired state of health with a high mobility disorders, increased risk of falls and fractures, impaired ability to perform activities of daily living, disabilities, loss of independence and increased risk of death [38-42].

The role of protein turnover in age-related sarcopenia reported that muscle wasting in the elderly was due to a decline in basal rates of MPS, elevated basal rates of MPB, or a combination of the two processes resulting in a negative net protein balance[43].

Sarcopenia has multiple contributing factors; initiating with the ageing process over the life course, early life developmental influences, less-than-optimal diet, bed rest or sedentary lifestyle, chronic diseases as osteoporosis, insulin resistance and arthritis and finally certain drug treatments [44-46] .

The European Working Group on Sarcopenia in Older People (EWGSOP) included representatives from four participant organisations, i.e. the European Geriatric Medicine Society, the European Society for Clinical Nutrition and Metabolism, the International Association of Gerontology and Geriatrics European Region and the International Association of Nutrition and Aging have developed a practical clinical definition and consensus diagnostic criteria for age-related sarcopenia;

- **Criteria for the diagnosis of sarcopenia:** [47]

Diagnosis is based on documentation of criterion 1 plus (criterion 2 or criterion 3):

1. Low muscle mass
2. Low muscle strength
3. Low physical performance

The EWGSOP recommends using the presence of both low muscle mass and low muscle function (strength or performance) for the diagnosis of sarcopenia [48,49]. The rationale for use of two criteria is: muscle strength does not depend solely on muscle mass, and the relationship between strength and mass is not linear. Thus, defining sarcopenia

only in terms of muscle mass is too narrow and may be of limited clinical value. Some have argued that the term ***dynapenia*** is better suited to describe age-associated loss of muscle strength and function. However, sarcopenia is already a widely recognised term, so replacing it might lead to further confusion [49,50].

- **Mechanisms of sarcopenia:**

Loss of muscle fibre number is the principal cause of sarcopenia, although fibre atrophy(particularly among type II fibres) is also involved. Denervation results in the loss of motor units and thus, muscle fibres. A decrease in the production of anabolic hormones such as: testosterone, growth hormone and insulin-like growth factor-1 impairs the capacity of skeletal muscle to incorporate amino acids and synthesize proteins [47].

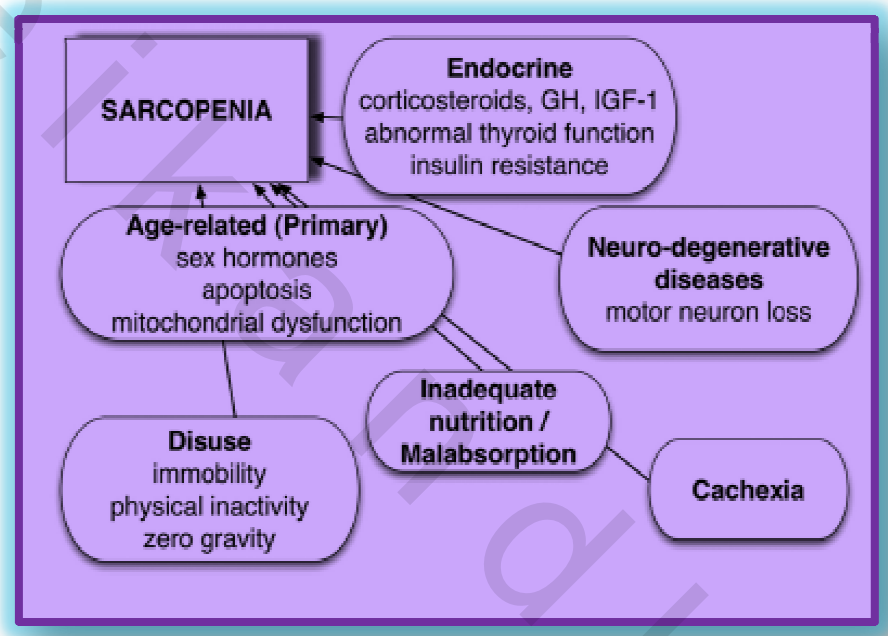

Figure 3: Causes of sacropenia [51] .

- **Sarcopenia categories and stages:**

In some individuals, a clear and single cause of sarcopenia can be identified. In other cases, no evident cause can be isolated. Thus, the **categories** of *Primary Sarcopenia* and *Secondary Sarcopenia* may be useful in clinical practice. Sarcopenia can be considered 'PRIMARY' (or age-related) when no other cause is evident but ageing itself, while sarcopenia can be considered 'SECONDARY' when one or more other causes are evident.

The '**presarcopenia' stage** is characterised by low muscle mass without impact on muscle strength or physical performance. This stage can only be identified by techniques that measure muscle mass accurately.

The '**sarcopenia' stage** is characterised by low muscle mass, plus low muscle strength or low physical performance.

'**Severe sarcopenia'** is the stage identified when all three criteria of the definition are met (low muscle mass, low muscle strength and low physical performance). Recognizing stages of sarcopenia may help in selecting treatments and setting appropriate recovery goals [51].

B. The immune system:

Aging of the immune system, particularly the dysregulation of *T-cell* function, appears to be partly responsible for the comorbidities presented by the elderly population. These individuals are more susceptible to different infectious diseases, autoimmune diseases and cancer and also respond less well to vaccination when compared with a young adult population [52].

Oxidative stress and the molecular inflammatory theory of aging

The ***molecular inflammation hypothesis*** [19,20] provides molecular insights into the interactions between age-related physiological changes and the pathogenesis of many age-related diseases during aging. Increases in oxidative stress associated with aging may also contribute to the development of chronic inflammation and diseases[23].As a result, pharmacological therapies and lifestyle modifications designed to prevent oxidative stress could potentially have a significant impact on this chronic inflammation [24].

Two to four-fold elevations in circulating levels of pro-inflammatory cytokines: such as ***interleukin (IL)-6*** and ***tumor necrosis factor (TNF)-α***, and acute phase proteins such as ***C-reactive protein (CRP)*** and ***serum amyloid A (SAA),*** are typical in the elderly even in the absence of chronic disease . While acute inflammation resolves when the trigger ceases, chronic inflammation remains in response to the homeostatic imbalance; therefore the levels of these inflammatory markers increase as a result of local inflammation in response to an acute infection or trauma and then decrease when the infection or trauma is resolved [53]. A sustained low-level increase in the systemic concentrations of these inflammatory markers is defined as **chronic low-grade inflammation**, which is related to atherosclerosis and also to insulin resistance [54-57].

Furthermore, increased levels of circulating inflammatory mediators may result from a constant, low-grade activation of cytokine producing cells or a dysregulated cytokine response following stimulation. As with all other physiological systems, there are significant declines in immune function with aging that promote inflammation.As people age, the prevalence of conditions associated with inflammation increase, such as; obesity, physical inactivity [54], cardiovascular disease (CVD) [58], diabetes [59] ,chronic kidney disease [60],osteoarthritis [61]and Alzheimer's disease .While inflammatory biomarkers are increased in the elderly individuals in the absence of overt disease ,others have failed to show any evidence of increases in inflammatory biomarkers in the healthy elderly [12,62]. The strong association between low-grade elevations in levels of circulating inflammatory mediators and high mortality risk independently of pre-existing morbidity, suggests that cytokines trigger/exaggerate pathological processes or act as very sensitive markers of subclinical disorders in elderly populations [63].

Cytokine-mediated bone damage is primarily driven by the effects of these mediators on the differentiation and activity of the bone-resorbing cell; the osteoclast [64].Osteoclasts are hematopoietic cell stemming from the monocyte lineage, which undergo a series of differentiation steps to become mature bone-resorbing cells [65].Several pro-inflammatory cytokines (such as TNFα, IL-1, IL-6 and IL-17) are major triggers for osteoclast activation explaining the enhanced bone loss during inflammation. On the other hand other cytokines such as interluken-12 (IL-12), IL-18, IL-33 and interferons (IFN) are strong suppressors of osteoclast differentiation and inhibit bone loss[64].

Given the detrimental effects of acute and chronic inflammation on skeletal muscle mass and protein metabolism, age-associated inflammation may affect the anabolic sensitivity of older muscles.***TNF-α***, which is one of the main inducers of the acute-phase response, plays an important role in alterations of muscle protein metabolism [66].

i. Tumor necrosis factor-alpha (TNF-α)

Tumor necrosis factor-alpha (TNF- α) is a pleiotropic inflammatory cytokine. In 1891, the success story of William Coley in using supernatant extract of heat-killed mixtures of streptococcus pyogenes and serratiam arcescens bacteria to treat tumors may be in fact the first discovery of TNF. Human tumor necrosis factor was cloned in 1975, and recombinant TNF was shown to induce the haemorrhagic necrosis of transplanted methylcholanthrene induced sarcomas [67]. The cytokine possesses both *growth stimulating* properties and *growth inhibitory* processes, and it appears to have self regulatory properties as well. TNF has since been implicated in a diverse range of *inflammatory, infectious and malignant* conditions, and the importance of TNF in inflammation has been highlighted by the efficacy of anti-TNF antibodies or administration of soluble TNF receptors (TNFRs) in controlling disease activity in rheumatoid arthritis and other inflammatory conditions[68].

Originally sepsis was believed to result directly from the invading bacteria itself, but it was later recognized that host system proteins, such as TNF- α induced sepsis in response. Exogenous and endogenous factors from bacteria, viruses, and parasites stimulate production of TNF- α and other cytokines. Lipopolysaccharide(LPS) from bacterial cell walls is an especially potent stimulus for TNF-α synthesis. When cytokine production increases to such an extent that infection enters the bloodstream, sepsis ensues[69].

If TNF- α remains in the body for a long time, it loses its anti tumor activity. This can occur due to polymerization of the cytokine, shedding of TNF receptors by tumor cells, excessive production of anti-TNF antibodies, found in patients with carcinomas or chronic infection. Prolonged overproduction of TNF- α also results in a condition known as cachexia, and which occurs in illnesses such as cancer and AIDS. Cachectin and TNF- α were once considered different proteins, but in 1985 researchers discovered that the two proteins were homologous [70].

Structure and production of TNF- alpha:

TNF-α is a protein encoded within the major histocompatibility complex. TNF is a non-glycosylated protein of noncleaved 27kd precursor form also existed in transmembrane form, with 157 amino acids and belongs to a family of peptide ligands that activate a corresponding set of structurally related receptors [71].It belongs to a super family of

ligand/receptor proteins called the ***TNF/TNF receptor (TNF/TNFR)*** superfamily proteins. TNFRs are either constitutively expressed (TNFR1) or inducible (TNFR2)[72]. TNF possesses a trimeric symmetry with a structural motif called the *TNF homology domain (THD),* which is shared with all other members of the TNF proteins. This THD binds to the *cysteine rich domains (CRDs*) of the TNFRs, and variations of these CRDs lead to heterogeneity of the TNFRs .There are roughly 20 ligand receptor pairings now recognized for the TNF superfamily [73].

TNF is a key signalling protein in the immune system. As a regulatory cytokine, TNF orchestrates communication between immune cells and controls many of their functions .TNF is best known for its role in leading immune defenses to protect a localized area from invasion or injury . Although cells of the ***monocyte/macrophage lineage*** are the main source of TNF in inflammatory disease, a wide range of cells can produce TNF, including ; ***mast cells***, ***T and B lymphocytes***, ***natural killer (NK) cells, neutrophils, endothelial cells, smooth*** and ***cardiac muscle cells, fibroblasts*** and ***osteoclasts*** [74].

TNF converting enzyme (TACE, also known as *ADAM-17*) mediates release of TNF from the cell surface, but is involved in processing several cell membrane-associated proteins, including TNF receptors, which are released by its action to produce soluble forms that can neutralize the actions of TNF. TACE may therefore be either pro-or anti-inflammatory, depending on whether it acts on an effector (e.g. macrophage) or target (e.g. endothelial cell), releasing ligand or receptors, respectively [75].

TNF signal transduction:

TNF signal transduction pathways are complex and still not fully understood. The ***transcription factor(Nuclear Factor) NF-κB*** is a central regulator of the immune response whose actions are rapidly induced by proinflammatory stimuli such as TNF-α . NF-κB also acts to promote cellular growth, staving off programmed cell death by pro-apoptotic stimuli such as TNF-α. Regulation of the ***transcription factor NF-κB*** is a key component of TNF signal transduction [76].

TNF-R1 adaptor proteins:

All known responses to TNF are triggered by binding to one of two distinct receptors ***(Figure 4),*** designated ***TNFR1*** and ***TNFR2*** which are differentially regulated on various cell types in normal and diseased tissue[77]. Surface-expressed ***TNFR1*** exist as trimers associated through ***pre-ligand assembly domains (PLADs***) which reside within the membrane distal ***cysteine-rich domain(CRD)***. In unstimulated receptors the cytoplasmic domain is pre-associated with a cytoplasmic protein designated ***silencer of death domain (SODD)*** [78].

Subsequent signalling events involve recruitment and activation of different mitogen activated protein kinase kinase kinases (MAP3Ks)[79,80]. Cystosolic I*κ*B proteins from a complex with the NF-*κ*B family transcription factors, masking nuclear localization signals within NF-*κ*B, and I*κ*B degradation allows NF-*κ*B to enter the nucleus and initiate gene transcription [81,82]. In addition to mediating cell survival and proinflammatory signals through NF-*κ*B. TNFR1 can also initiate cell death signalling pathways,this involves the binding of ***Fas-associated DD protein (FADD)*** to ***Tumor necrosis factor receptor type 1-associated death domain protein (TRADD)*** to the subsequent *TRADD–FADDcomplex*[83].

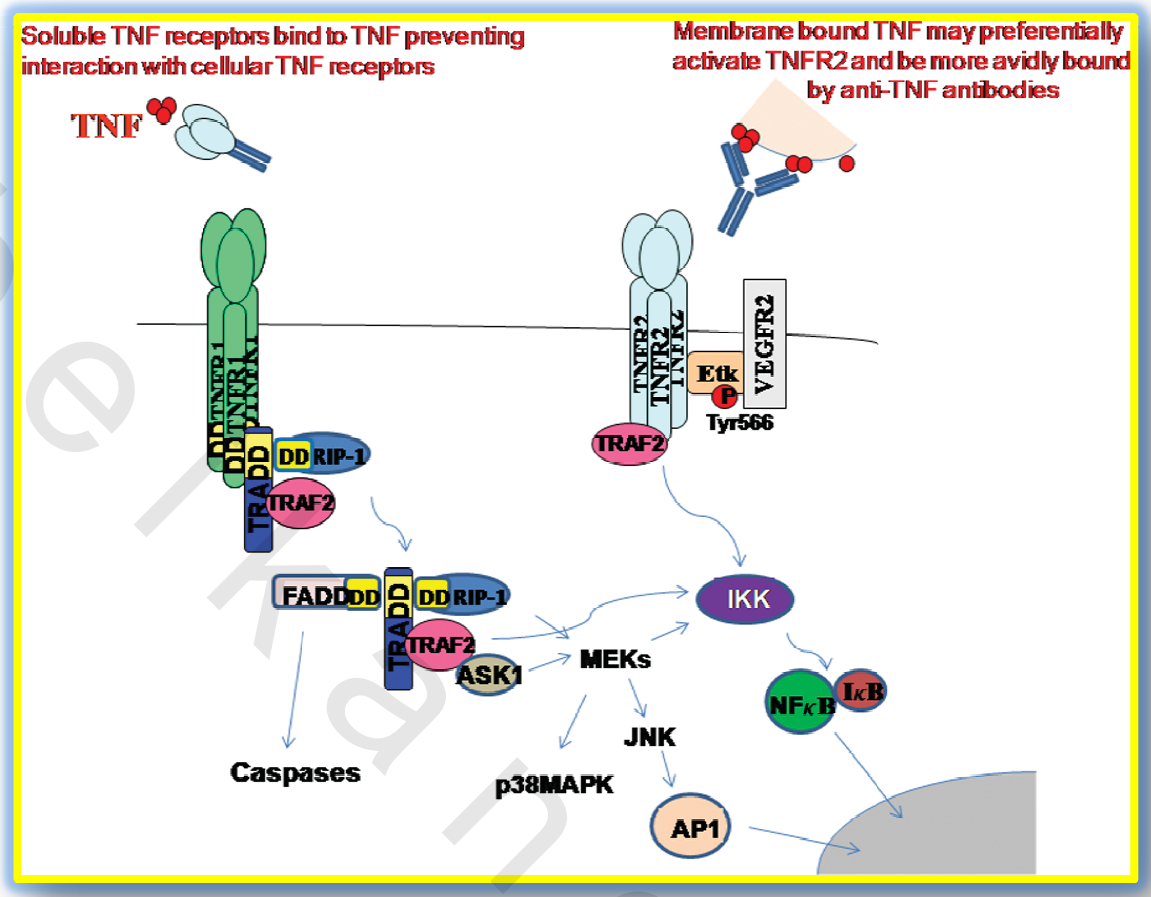

Figure 4: Signalling pathways leading to the main cellular responses of TNF. Soluble TNF receptors or monoclonal anti-TNFantibodies, which prevent TNF interacting with its receptors and activating these pathways,can be used to treat inflammatory disease [76].

Biological effects of TNF-ALPHA in inflammatory response:

Although TNF receptors are differentially expressed on a wide range of cells and tissues, many of the proinflammatory effects of TNF can be explained on the basis of TNF's effects on vascular endothelium and endothelial leukocyte interactions. In response to TNF, endothelial cells promotes inflammation by displaying, in a distinct temporal, spatial and anatomical pattern,different combinations of adhesion molecules for leukocytes, including E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [84].

In addition, many of the classical features of inflammation can be produced by local effects of TNF on endothelial cells. TNF-induced expression of cyclo-oxygenase 2 can increase extracellular production of vasodilatory prostaglandins 2 (PGI2) resulting in vasodilatation . Also, TNF-induced expression of pro-coagulant proteins, such as tissue factor, and down-regulation of anticoagulant protein, such as thrombomodulin so TNF, can cause intravascular thrombosis [84].

TNF-alpha and bone metabolism:

The key cytokines: receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), TNF, interleukin 6 (IL-6), interferons (IFNs), and CRP affects the osteoclastogenesis and bone resorption. As, ***TNF*** exerts its effect on osteoclastogenesis[85,86] by acting *directly* on osteoclast precursors, as well as *indirectly* on nonosteoclast lineage cells, by upregulating the production of macrophage colony-stimulating factor (M-CSF) and RANKL on mesenchymal cells [87]. RANKL; a member of TNF superfamily, is the key osteoclastogenic cytokine .Although there remains debate regarding whether TNF can induce osteoclastogenesis independently of RANKL, indicating that RANKL is required for TNF-induced osteoclast formation [88-90].

Interestingly, pro-inflammatory cytokines (TNF-α, IL-1) suppresses OPG expression,while simultaneously enhancing RANKL expression [90].TNF assails bone by inhibiting the expected homeostatic response of new bone formation. Furthermore, it also impairs the function of bone-forming osteoblasts in three ways: by

(1) Suppressing mature osteoblast function such as the production of a matrix that is competent for mineralization,
(2) Blocking the differentiation of new osteoblasts from their progenitors, and
(3) Inducing osteoblast resistance to vitamin D, 1,25 dihidroxyvitamin D (1,25(OH)2D3)[91].

TNF- α and clinical applications:

TNF- α seems to serve as a mediator in various pathologies. A few such examples include: *septic shock, cancer, AIDS, transplantation rejection, multiple sclerosis, diabetes, rheumatoid arthritis, trauma, malaria, meningitis, ischemia-reperfusion injury, and adult respiratory distress syndrome.*

One hypothetical advantage of treatment with anti-TNF-α antibodies results from its role in multiple types of *inflammation* .Strategies for preventing TNF- α activity include neutralization of the cytokine via either anti-TNF antibodies, soluble receptors, or receptor fusion proteins , supression of TNF- α synthesis via drugs such as ; ***cyclosporine A, glucocorticoides or cytokine IL-10***; reduction of responsiveness to TNF- α via repeated low dose stimulation and lastly, by inhibition of secondary mediators such as IL-1, IL-6, or nitric oxide [92].

Therapeutic agents for TNF blockade:

Three drugs:

i. **Humira (ADALIBUMAB)**; is a human anti-human TNF antibody produced by phage display.

ii. **Remicade (INFLIXIMAB)**; is a human–murine chimeric IgG1 monoclonal anti-TNF antibody which has been used to treat *Crohn's disease* as; soluble TNF-R will also neutralize TNFα before it can bind to its target cell receptor [92,93].

iii. **Enbrel (ETANERCEPT)** ; is TNF blocking agents used to treat *rheumatoid arthritis ,ankylosing spondylitis and Crohn's disease*.It is is a recombinant human soluble fusion protein in which TNFR2 is coupled to the Fc portion of IgG [93].

ii. C-reactive protein(CRP)

CRP is one of several proteins that are often referred as **acute phase reactants** and is used to monitor changes in inflammation associated with many infectious and autoimmune diseases [94].C- reactive protein (CRP) was so named because it was first discovered as a substance in the serum of patients with acute inflammation that reacted with the C-(capsular) polysaccharide of pneumococcus. Discovered by *Tillett* and *Francis* in 1930[95], but it was not actually isolated until 1941.Its plasma concentration increases during inflammatory states, a character that has long been employed for clinical purposes. CRP is a pattern recognition molecule, binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. It is rapidly increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response[94] .

Molecular structure and phylogeny of CRP:

CRP belongs to the pentraxin family of calcium-dependent ligand-binding plasma proteins, the other member of which in humans is serum amyloid P component (SAP). The human CRP molecule is composed of five identical nonglycosylated polypeptide subunits , each containing 206 amino acid residues. The protomers are non covalently associated in an annular configuration with cyclic pentameric symmetry (***Figure 5)*** [96].

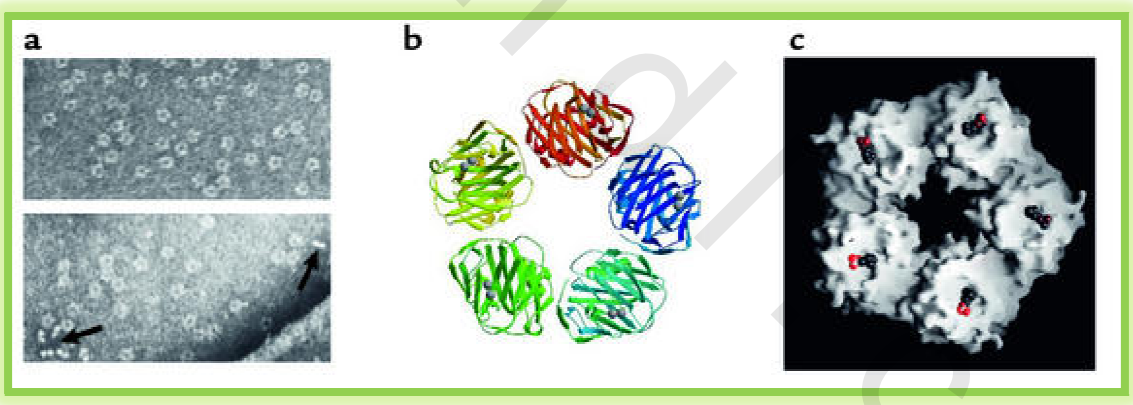

Figure 5: Molecular structure and morphology of human CRP. (**a**) Negatively stained electron micrograph showing the typical pentameric disc-like structure face-on and side-on (arrows). (**b**) Ribbon diagram of the crystal structure, showing the lectin fold and the two calcium atoms(spheres) in the ligand-binding sites of each protomers .(**c**) Space filling model of the CRP molecule ,showing a single phosphocholine molecule located in the ligand –binding sites of each protomers[96].

Function:

Displays several functions associated with ***host defense*** . A major function of CRP,is a ***component of innate immune system*** ,as it promotes ; agglutination, bacterial capsular swelling, phagocytosis (CRP initiates the activation of the complement cascade), induction

of inflammatory cytokines and tissue factor in monocytes, also CRP recruits monocytes by receptor mediated chemotaxis into the arterial wall , and complement fixation through its calcium-dependent binding to phosphorylcholine.

Large amounts of CRP are produced by hepatocytes in response to circulating cytokines, such as tumor necrosis factor (TNF)-α and IL-1, produced at the site of tissue destruction. This CRP production (as shown in ***Figure 6***) by hepatocytes occurs at the expense of albumin and other constitutive proteins, a process labeled "***Reprioritization***" of hepatic protein synthesis. However, competing demands for protein synthesis in cases of acute, overwhelming inflammation can lead to anomalous short-term changes in acute-phase reactant [97].

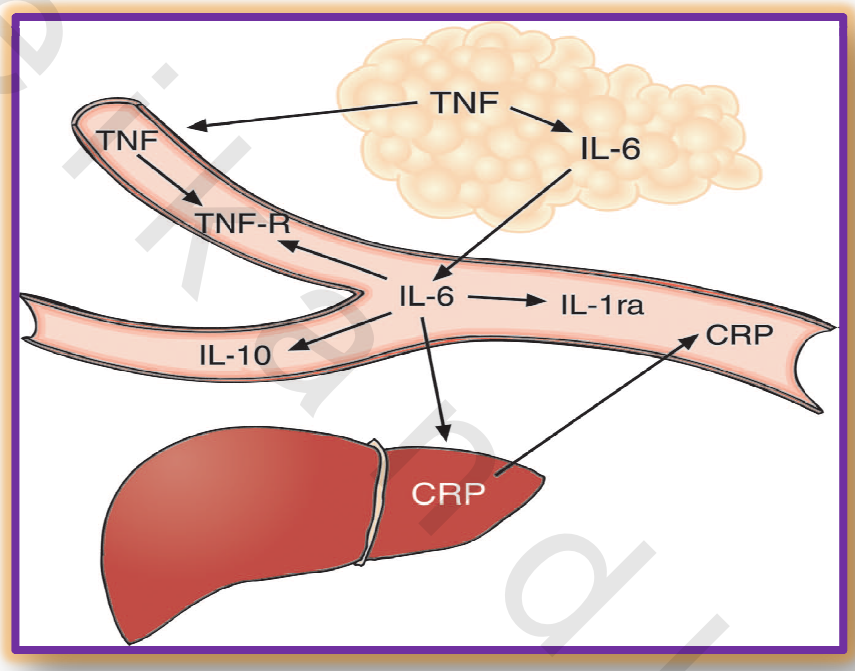

Figure 6: In chronic low-grade systemic inflammation; TNF- ***α*** is produced in adipose tissue and stimulates the production of IL-6 in adipose tissue and blood mononuclear cells. IL-6 enhances the systemic levels of IL-1 receptor antagonist (IL-1ra), soluble TNF receptor (TNF-R), IL-10, and C-reactive protein (CRP) [97].

Table 1: Changes in concentrations of plasma protein in the acute phase reactant[97].

	Increased	Decreased
Proteinase inhibitors	α_1-Antitrypsin α_1-Antichymotrypsin	Inter-α-antitrypsin
Coagulation proteins	Fibrinogen Prothrombin Factor VIII Plasminogen	
Complement proteins	C1s C2 B C3 C4 C5 C1 inhibitor	Properdin
Transport proteins	Haptoglobin Hemopexin Ceruloplasmin	
Miscellaneous	CRP SAA Fibronectin α_1-acid glycoprotein Gc globulin	Albumin Transthyretin HDL LDL

Tests of C-reactive protein:

There are *two different tests* for CRP:

A. ***The standard test*** measures a much wider range of CRP levels but is less sensitive in the lower ranges.

B. The high-sensitivity CRP (hs-CRP) test:

This test can detect more accurately lower concentrations of the protein (it is *more sensitive*), which makes it more useful than the standard one [98].

Circulating CRP concentrations:

The reference range for C-reactive protein is 0-10 mg/L for the standard CRP,while (hs-CRP) remains < 3 mg/L.CRP plasma levels increases within 4-6 h after initial tissue injury and continue to increase several hundred fold within 24-48 h. CRP remains elevated during the acute phase response and returns to normal with restoration of tissue structure and function. The rise in CRP is exponential, doubling every 8-9 h. The half-life is less than 24 h [99].

The amount of CRP produced by the body varies from person to person, and this is affected by individual's genetic and lifestyle factors. Higher levels are found in *late pregnant women, active inflammation, bacterial infection, tissue injury*

(postoperation),trauma and burns. *Smoking* and *obesity* are positively correlated with CRP levels, whereas weight loss and cessation of smoking decrease CRP values[94].

The *clearance rate* of CRP is constant, therefore the level of CRP in the blood is regulated solely by synthesis. Importantly, acute-phase CRP values show no diurnal variation .Liver failure impairs CRP production, but no other intercurrent pathologies and very few drugs can reduce CRP values [100].

Clinical applications of CRP:

The CRP concentration is thus a ***very useful non specific biochemical marker of inflammation***, measurement of which contributes importantly to screening for organic disease, monitoring the response to treatment of inflammation, and detecting of intercurrent infection in immunocompromised individuals, and in the few specific diseases characterized by modest or absent acute-phase responses as shown in ***Table 2*** [101].

CRP is a more sensitive and accurate reflection of the acute phase response than the **ESR**(erythrocyte sedimentation rate). CRP appears and disappears more quickly than changes in ESR. Therefore, CRP level may drop to normal following successful treatment, whereas ESR may remain elevated for a longer period . In the first 24 h, ESR may be normal while CRP elevated [101].

Table 2: Major CRP response and modest or absent CRP response[101].

Major CRP acute-phase response	
Infections	Bacterial
	Systemic/Severe fungal, mycobacterial, viral
Allergic complications of infection	Rheumatic fever
	Erythema nodosum
Inflammatory disease	Rheumatoid arthritis
	Juvenile chronic arthritis
	Ankylosing spondylitis
	Psoriatic arthritis
	Systemic vasculitis
	Polymyalgia rheumatica
	Reiter disease
	Crohn disease
	Familial Mediterranean fever
Necrosis	Myocardial infarction
	Tumor embolization
	Acute pancreatitis
Trauma	Surgery
	Burns
	Fractures
Malignancy	Lymphoma
	Carcinoma
	Sarcoma
Modest or absent CRP acute-phase response	
	Systemic lupus erythematosus
	Scleroderma
	Dermatomyositis
	Ulcerative colitis
	Leukemia
	Graft-versus-host disease

Causes of decreased CRP level:[102]

1. Exercise.
2. Weight loss.
3. Moderate alcohol consumption.
4. Medications like: statins, niacin and fibrates.

Factors affecting high CRP levels:

A. *Non steroidal anti-inflammatory drugs: (NSAIDs* like aspirin, ibuprofen, and naproxen)
B. Statins: Both anti-inflammatory drugs and statins may help to *reduce the inflammation, thus reducing CRP*. However, there are ***natural treatments*** that can help reduce inflammation in the blood,as:
C. *Fish Oil Omega 3 Fatty Acids*: Fish oil contains two of the most therapeutic Omega 3 Fatty Acids the DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). These two fatty acids are the most readily absorbed by the body and can help reduce inflammation in the blood .
D. *Ginger:* Ginger can also help reduce inflammation, as it relaxes the muscles surrounding blood vessels and facilitates blood flow throughout the body.
E. *MSM*: (Methyl Sulfonyl Methane), is a naturally occurring sulfur compound found in some vegetables. MSM has strong anti-inflammatory properties [103,104].

Strategies to reduce inflammation in elderly

Due to the strong correlation between inflammation and the development and progression of many age-related chronic diseases, a variety of strategies have been utilized to minimize inflammation associated with aging;

Pharmacological Interventions may provide alternative one and it has been suggested that medications like: fibrates, statins, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers, and non-steroidal anti-inflammatory drugs (NSAIDS) may have a clinical role in reducing inflammation [105].

Lifestyle Interventions such as *exercise training and dietary modifications* may provide a low cost and long-term alternative to limit inflammation and slow declines in the elderly. In short, reductions in caloric intake resulting in weight loss may provide one mechanism to dampen age-related inflammation [106] ***(Figure 7)***.

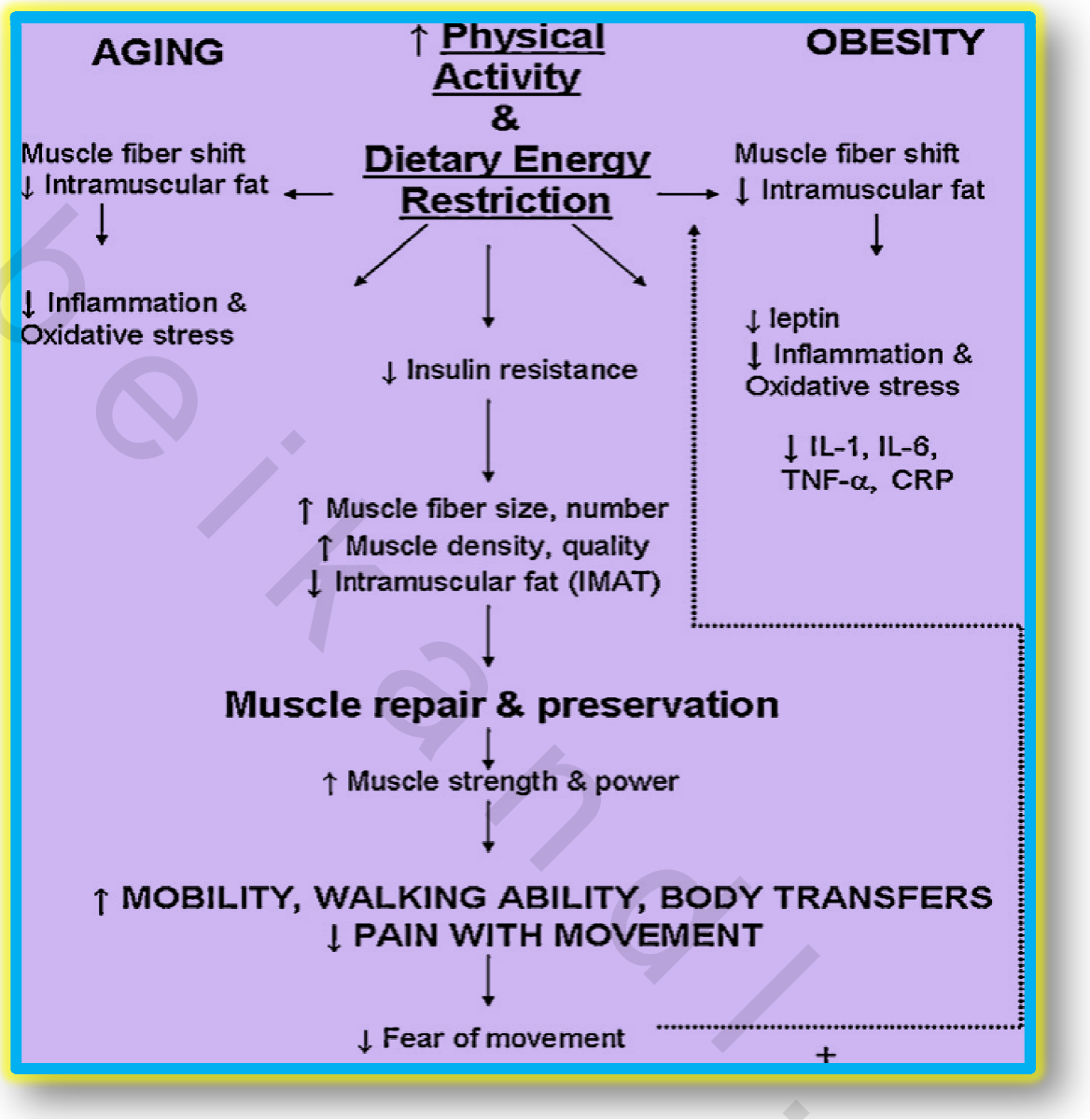

Figure 7: Additional benefits of exercise in elderly.Several physiological pathways that contribute to sarcopenia in the obese older adult may be favorably altered by exercise, such as inflammation and oxidative stress[107] .

Physical activity

As the number of elderly persons in our country increases, more attention is being given to geriatric healthcare needs and successful ageing is becoming an important topic in medical literature. Sedentary living[108] has assumed epidemic proportions in the industrialized world and therfore ***physical activity (PA)*** is one of the most non-pharmaceutical important modifiable factors that determine the risk of chronic morbidity and mortality in the population in general [109,110].

Concept of ***successful ageing*** is in first line on a preventive approach of care for older people. Moderate but regular physical activity is associated with a reduction in total morbidity among older people, a positive effect on *primary prevention of coronary heart disease* and a significant benefit on the lipid profile. *Improving body composition* with a reduction in fat mass, *reducing blood pressure* and *prevention of stroke*, as well as *type 2 diabetes* [111,112], are also well established. *Prevention of some cancers* (especially that of breast and colon), *increasing bone density* and *prevention of falls* are also reported. Moreover, physical activity is linked to a reduced risk of developing *dementia* and *Alzheimer's disease* in particular[113,114].

However, with aging, a progressive decline in skeletal muscle mass becomes apparent. This age-related loss of muscle protein must be attributed to an imbalance between muscle protein synthesis and breakdown rates, resulting in a ***negative muscle protein balance*** and, over time, a subsequently decline in skeletal muscle mass (***Figure 8***)[115]. Concerning very older subjects (80 years and older), benefits of physical activity are considered in terms of strength, muscular function and more difficult, in terms of disability prevention. As aerobic exercises in endurance are difficult to perform for elderly people, strength exercise programs and resistance one are often chosen. Physical activity must be adapted to the physiological ageing process. The first aim is to improve functional activity without injuries [116].

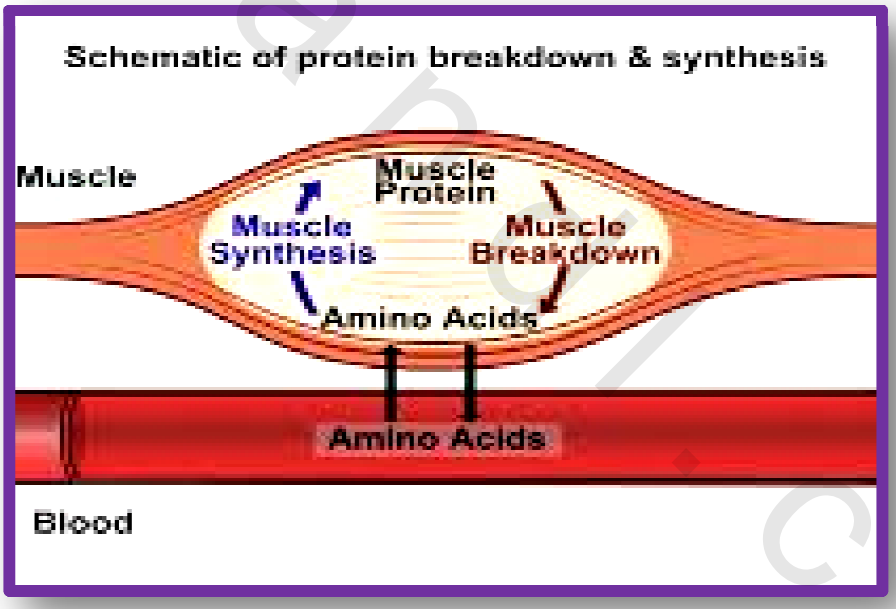

Figure 8: Schematic of protein breakdown &synthesis[115].

Categories of physical activity:

Physical therapy for elderly people may be classified in three categories: firstly, prevention or restoration of the sequelae of disuse in healthy elderly people; secondly, prevention or restoration of the sequelae of disuse in the acute and chronically ill; thirdly, rehabilitation of functional losses caused by trauma and disease [116].

There were a difference in definitions between physical activity and exercise as; ***Physical activity*** refers to body movement that is produced by the contraction of skeletal muscles and that increases energy expenditure. Measured by self-report or interview surveys, direct observations, or electronic monitoring devices. While ***Exercise*** refers to planned, structured, and repetitive movement to improve or maintain one or more components of physical fitness. However ***Physical fitness*** is operationally defined as a state of well-being with a low risk of premature health problems and energy to participate in a variety of physical activities ,it is measured as a physiological variable such as maximal oxygen uptake or heart rate response [117].

Types of exercise: (Table 3)

Table 3: Types of exercise[117].

TYPE OF EXERCISE	*ACTIVITIES RECOMMENDED AS TYPE OF EXERCISE FOR ELDERLY*	*POTENTIAL BENEFITS*
1. Aerobic/endurance	Walking Cycling Aquafit or swimming Low-impact aerobics	Cardio-respiratory Weight loss/control Glycemic control Pain control Mood and cognition Better Sleep
2. Resistance/strength	Free weights Weight machines Elastic bands/tubing Body weight against gravity	Muscle strength Bone mass Physical functioning Mobility
3. Balance and Flexibility	Stretching Yoga Tai-Chi Stair-climbing Balance boards, balls, and elastic bands	Balance Fall prevention Mobility

A. ***Aerobic (endurance) Exercise Training*** **(AET)** refers to exercises in which the body's large muscles move in a rhythmic manner for sustained periods;

B. ***Resistance Exercise Training*** **(RET)** are exercises that causes muscles to work or hold against an applied force or weight;

C. ***Flexibility Exercise*** refers to activities designed to preserve or extend range of motion (ROM) around a joint; and

D. ***Balance Training*** refers to a combination of activities designed to increase lower body strength and reduce falling.

Table 4: Summary of American College of Sports Medicine (ACSM) American Heart Association (AHA) physical activity recommendations for older adults[118]

	Endurance exercise for older adults	Resistance exercise for older adults	Flexibility exercise for older adults
Frequency:	For moderate-intensity activities, accumulate at least 30 or up to 60 (for greater benefit) min·d^{-1} in bouts of at least 10 min each to total 150-300 min·wk^{-1}, at least 20-30 min·d^{-1} or more of vigorous-intensity activities to total 75-150 min·wk^{-1}, an equivalent combination of moderate and vigorous activity.	At least 2 d·wk^{-1}.	At least 2 d·wk^{-1}.
Intensity:	On a scale of 0 to 10 for level of physical exertion, 5 to 6 for moderate-intensity and 7 to 8 for vigorous intensity.	Between moderate(5-6) and vigorous (7-8) intensity on a scale of 0 to 10.	Moderate (5-6) intensity on a scale of 0 to 10.
Duration:	For moderate-intensity activities, accumulate at least 30 min·d^{-1} in bouts of at least 10 min each or at least 20 min·d^{-1} of continuous activity for vigorous-intensity activities.		
Type:	Any modality that does not impose excessive orthopedic stress; walking is the most common type of activity. Aquatic exercise and stationary cycle exercise may be advantageous for those with limited tolerance for weight bearing activity.	Progressive weight training program or weight bearing calisthenics (8-10 exercises involving the major muscle groups of 8-12 repetitions each), stair climbing, and other strengthening activities that use the major muscle groups.	Any activities that maintain or increase flexibility using sustained stretches for each major muscle group and static rather than ballistic movements.

Resistance exercise

Resistance exercise is generally recommended as the preferred approach to elicit improvements in muscular hypertrophy and strength adaptations. Following even short-term resistance exercise interventions, aging adults may expect improvements in protein synthesis[119,120]. However, low-intensity treadmill walking seems to abolish the age-related insulin resistance of muscle protein synthesis [121] .These data are promising because they reveal that the intensity of contraction required to improve skeletal muscle anabolic sensitivity may be relatively low. Thus, a strategy that may offset, at least in part, the age-related loss of muscle mass is to encourage the elderly to maintain, or rather increase, daily habitual physical activity [122].

Potential mechanisms of exercise training for reducing inflammation in elderly

Regular exercise training protects against atherosclerosis and type 2 diabetes [123] , in addition, evidence exists to support a relationship between regular exercise and improvements in low-grade inflammation[124-127]. Furthermore, inflammation is associated with the age-related loss of muscle mass and muscle strength (e.g., sarcopenia) [128] .High levels of circulating inflammatory markers and/or cytokines are associated with low muscle mass in aged people [129,130].

There are a strong association between elevated systemic inflammatory markers and chronic diseases of the aged,the stimulation of the parasympathetic nervous system, via the efferent vagus nerve, inhibits pro-inflammatory cytokine production and protects against systemic inflammation [131]. They referred to this pathway as the "cholinergic anti-inflammatory pathway," described it as a central homeostatic mechanism by which the sympathetic division of the autonomic nervous system stimulates the inflammatory response through the release of epinephrine and norepinephrine, while the parasympathetic nervous system works reciprocally to suppress this release of proinflammatory cytokine[132]. A primary function of the vagus nerve is to control heart rate, which is typically measured by heart rate recovery (HRR) following exercise and heart rate variability (HRV). A major adaptation to long-term exercise training is a decrease in HRR and HRV. Thus, exercise training may increase efferent vagus nerve activity, and this increased activity may contribute to the anti-inflammatory effect of exercise.Therefore, regular exercise reduces fat mass and adipose tissue inflammation which is known to contribute to systemic inflammation [133,134] .

Resistance exercise prescription and recommendations:

The **ACSM/AHA** Recommendations for Physical Activity are generally consistent with the *2008 Department of Health and Human Services* (**DHHS)** which recommend 150 min/wk^{-1} of physical activity for health benefits. However, the **DHHS** Guidelines note that additional benefits occurs as the amount of physical activity increases through higher intensity, greater frequency, and/or longer duration. The **DHHS** Physical Activity Guidelines stress that if older adults cannot do 150 min of moderate-intensity aerobic activity/wk^{-1}, they should be as physically active as their abilities and conditions allow. Also guidelines for physical activity in elderly recommend 2 or more non consecutive days per week, for 30 to 45 minutes each session, using moderate to high level of effort that allows 10-15 repetitions as "minimum" for "muscle-strengthening activity" [135,136].

The benefits of resistance exercise training:

The effects of age on ***muscle strength*** may be influenced by gender,[137] duration of the training intervention and/or the specific muscle groups trained. However, older adults can substantially increases their strength after RET [138]. Moreover, the age-related loss of ***muscle power*** occurs at a greater rate than the loss of strength most likely owing to a disproportionate reduction in the size of type II fibers[139] . Increasing in muscle power are common after RET in older adults [140-142]. ***Muscle Quality*** (MQ) ; is defined as muscular performance (strength or power) per unit muscle volume or mass [143]. Other factors including; decreased activation of antagonistic muscle groups,[144] alterations in muscle architecture , tendon stiffness,[145] and selective hypertrophy of type II muscle fiber areas[146,147] may also influence MQ [148] . Also physical activity may reduce the development of ***disability*** and ***psychosocial features*** including: social isolation, low self-esteem and efficacy, depressive symptoms, anxiety, smoking and excess alcohol consumption [149-151].

Double faces of exercise:

There were evidences that exercise can ***cause and attenuate inflammation***. Acute, unaccustomed exercise can cause muscle and connective tissue damage, especially if done at high intensities and for prolonged durations. This typically manifests as ***delayed onset muscle soreness*** which is preceded by microstructural skeletal muscle damage, inflammatory cell infiltration and elevation of muscle specific creatine kinase isoforms Inflammatory cytokines can be detected in peripheral blood of people after high intensity, with unaccustomed exercise, especially if lengthening contractions are performed. This damaging response is attenuated if exercise is done repeatedly as the tissue adapts to the new overload stress [152-154].

CAN exercise be an immunotherapy?

Although the impact of exercise on the immune system is an area of extensive research, the immune response to acute exercise is transient and variable, being influenced by a wide range of factors, such as the *intensity, duration and mode of exercise, concentrations of hormones during exercise*, and *changes in body temperature, blood flow, hydration status* and *body position* (upright vs horizontal). Leukocytosis, granulocytosis, slight lymphocytosis and decreases in the proportion of T to B cells usually reflect changes in blood volume, demargination and tissue migration of the peripheral blood cells. Lymphocyte subsets show a decreased helper/suppressor cell ratio and an increase in NK cells [155].

Exercise and obesity:

It is well-known that adipose tissue, especially visceral fat, of obese humans produces pro-inflammatory cytokines that contribute in a large way to systemic inflammation [133]. Adipose tissue from obese subjects contains higher levels of pro-inflammatory macrophages interspersed among adipocytes [156]. These cells can form multinucleate giant cells and can form 'crown-like' structures around dead or dying adipocytes [157,158].

Exercise and osteoporosis:

In elderly, exercise prescription is important for the prevention and treatment of *osteoporosis*. An initial emphasis on weight-bearing aerobic, resistance and balance-enhancing exercises in old age, seems to be optimally address the needs of the musculoskeletal system throughout the life span [159]. An increase in muscle mass is achievable to a significant degree with the progressive resistance training and protein consumption, and also having a potential role in prevention of falls and fractures [160].

Contraindications to exercise:

In elderly, frailty or extreme age is not a contraindication to exercise, although the specific modalities may be altered to accommodate individual disabilities. Acute illnesses, particularly *febrile illnesses, unstable chest pain, uncontrolled diabetes, hypertension, asthma, congestive heart failure, undiagnosed musculoskeletal pain, weight loss* regardless of exercise status must be assessed and controlled before a new exercise regimen is begun[161].

Temporary avoidance of certain kinds of exercise is required *during treatment of hernias, foot ulcers, cataracts, retinopathy and joint injuries*, for example. A small number of untreatable or serious conditions are more permanent exclusions for vigorous exercise, including an ; *inoperable enlarging aortic aneurysm, known cerebral aneurysm, malignant ventricular arrhythmia, critical aortic stenosis, end-stage congestive heart failure or other rapidly terminal illness, or severe behavioral agitation* in response to participation in exercise secondary to *dementia, alcoholism, or neuropsychologic illness* [161].

Finally, moderate and regular physical activity is generally considered to be an important strategy, for the reduction or prevention of functional decline with aging and risk of impaction of chronic diseases [162].