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TITLE OF THE DOCTORAL THESIS

Exercise, inflammation and vascular function in aging and obesity.

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Exercise, inflammation and vascular function in aging and obesity — Anna Pedrinolla
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Abstract

Background. While aging is a non-modifiable process, obesity is a reversible condition. However, both are characterized by a low-grade inflammatory profile and vascular dysfunction. Exercise is a non-pharmacological strategy able to counteract the negative effect of aging as well as of obesity. Nonetheless, its short-term and long-term effect on inflammation and vascular function in obese and non-obese elderly individuals are still matter of debate. Still not clear are the differences in the acute inflammatory and vascular response to different types and intensities of exercise in sedentary subjects.

Aims. The primary aim was to examine how the inflammatory profile and vascular function in non-obese and obese elderly individuals is affected. A secondary aim was to understand the acute inflammatory and vascular response to different exercise types (i.e. aerobic, A; resistance, R) and intensity (i.e. high, H; low, L) in sedentary obese individuals (OB) compared with normal weight (NW) subjects.

Methods. Seventy individuals who attended a structured exercise program (30/40-f/m; 75±5 years; 5±2 years of regular training) were enrolled in study 1 and tested for vascular function (flow-mediated dilation; FMD) and inflammatory profile (plasma CRP, IL-1β, IL-1ra, IL-6, IL-8, IL-10, TNF-α, MCP-1). Subjects were stratified for age and BMI. Correlations between age, BMI and the measured variables were investigated. In study 2, still ongoing, 5 NW (54±7 years; 24.2±0.7 BMI) and 5 OB (53±6 years; 33.7±1.2 BMI) subjects were included and tested for FMD and inflammatory profile before and after 4 different exercise sessions.

Results. In study 1 inverse correlations were found between age and IL-β (r -0.232; p<.05); IL-1ra (r -0.181; p<.05); IL-6 (r -0.255; p<.05); and IL-8 (r -0.248; p<.05). Direct correlations were found between BMI and CRP (r 0.155; p<.05), MCP-1 (r 0.217; p<.05); and TNF-α (r 0.184; p<.05). An inverse correlation was also found between BMI and FMD (r -0.433; p<.01). In a preliminary analysis of data of study 2, different types and intensities of exercise seem to elicit different acute inflammatory responses in NW and OB. However, most differences did not reach statistical significance. FMD showed a significant increase in the post exercise period for all the
exercise sessions in both groups (p<.05) with smaller increases in OB as compared with NW. No significant differences between exercise sessions were found.

**Conclusion.** Sustained, regular exercise can counteract the deleterious effects of aging but not of obesity on vascular function and inflammatory profile. Preliminary results of study 2 lead us to speculate that exercise, both aerobic and resistance exercise as well as both high and low intensity, do not seem to affect adversely the inflammatory profile and the acute vascular response in either obese or non-obese individuals. However, further research is needed to confirm these findings.
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Experimental study - Inflammation and vascular function in aging and obesity: chronic and acute exercise-induced adaptations.

Exercise-induced chronic adaptations of inflammatory profile and vascular function during aging and in obesity.

1. Hypothesis

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5. Conclusion

Acute inflammatory and vascular response to different type and intensity of exercise in obese subjects.

1. Hypothesis

2. Methods

- Subjects
- Experimental design
- Control session
• Familiarization session
• Maximal exercise testing
• Blood samples analysis
• Flow-mediated dilation (FMD)
• Exercise sessions
• Statistical analysis

3. Results

• Inflammatory response
• Vascular response

4. Discussion

5. Conclusion

References
Aging, Obesity, and Physical Activity

1. Aging

1.1 Definition

Aging is inevitable (1). By definition, aging includes a progressive physiological degeneration resulting in decline in function of organ systems and reduction of physiological reserve. It can be thought of as “primary aging” or “secondary aging”. The former refers to the inevitable deterioration of cellular structure and function, independent of the disease and environment. The latter refers to aging caused by disease and environmental factors (2).

However, aging affects all physiological processes by means of progressive deterioration involving delicate and irreversible changes in the function of most organs (3). The rapidity of the decline in function varies with the organ system under consideration but is relatively constant within a given system. Therefore, the rate of aging is the same for a 45-year-old man as it is for an 85-year-old man. The difference is that by 85 years of age more age-related changes have accumulated (3).

1.2 Epidemiology

Demographics. Over the past century, remarkable changes have been observed in the health of older persons throughout the World, and these changes have strongly affected society. The growth of the older population has resulted mostly from a general increase in overall population size but is also strongly influenced by major declines in leading causes of mortality (4). This demographic transformation resounds in society, increasing medical care and social needs, which are expected to increase sharply in the next years (5). In 1900 only 4.1% of the 76 million persons in the United States were aged 65 years or older, and among those in this age group only 3.2% were aged 85 years and older. By 1950 more than 8% of the population was aged 65 years and older, and by 2000 this percentage had increased to 12.6% (4). Change in the proportion of
a population that is elderly depends on changes in the survival of older persons and in the birthrate. Improved survival at older ages and a low birthrate have resulted in European countries, such as Germany and Italy, having oldest populations in the World, and almost one of the four Europeans projected to be aged 65 years or older by 2030 (6).

Mortality. Life expectancy at birth in Europe was only 47.3 years in 1900 and rose to 68.2 years by 1950, affected largely by improvements in infant and child mortality. Life expectancy continued to rise through the second half of the twentieth century, driven mainly by increases in survival in middle and old age (4). The increasingly greater life expectancy of the population has been driven in part by reduced mortality at older ages. The five leading causes of death (heart disease, cancer, stroke, chronic lower respiratory tract disease, and Alzheimer’s disease) account for 69.5% of all death (7). However, an exponential increase is present for all causes of death, and parallel increases are seen for heart disease, cerebrovascular disease, pneumonia, and influenza. Diabetes mortality rates also do not show an exponential increase with advancing age (4).

Disease status. Among people aged 65 years and older, the most common reported condition is hypertension, followed by coronary heart disease and stroke. Arthritis and chronic joint symptoms are reported by a large proportion of older persons, and these conditions have a large impact on overall health and quality of life but do not appear on the list of the most common conditions causing death (8). An important consideration of disease status that distinguishes the older population from the younger population is the high rate of co-occurrence of multiple chronic conditions, comorbidities. The concept of comorbidity is useful in considering the burden of disease in older people. However, the standardization of a definition for comorbidity depends on the number of conditions being ascertained and the intensity of the diagnostic effort to identify prevalent disease. The longer the list of conditions of prevalent diseases, the greater the prevalence of comorbidity (4).

Disability. The National Long-Term Care Survey performed assessments disability from 1982 to 2005. The findings show that the decline in disability observed for the first 12 years of the study continued and actually accelerated from 1994 to 2005 (9). Physical limitations include basic tasks such as standing, reaching, and grasping. These
tasks represent the building blocks of functional but are not specific measures of disability. Activities of daily living (ADLs) are basic self-care tasks. Instrumental ADL (IADLs) are tasks that are physically and cognitively somewhat more complicated and difficult than self-care tasks and are necessary for independent living in the community. ADLs and IADLs are measures of disability and reflect how an individual’s limitations interacts with the demands of the environment (10). The prevalence of ADLs disability, defining as needing help with three or more ADLs, increases with increasing age. Because disability status is a good way of representing overall health status in older persons with complex patterns of disease, and because disability also has direct implications for the long-term care needs of an older person, there has been much interest in evaluating disability trends over the time.

1.3 Physiological aspects.

In the last decades, the study of aging has expanded rapidly both in depth and width. Biological, epidemiological, and demographic data have generated a number of theories that attempt to identify a cause or process to explain aging. In recent years, the search for a single cause of aging, such as a single gene or the decline of a key body system, has been replaced by the view of aging as an extremely complex, multifactorial process (11). However, since aging is characterized by the declining ability to response to stress and by increasing homeostatic imbalance and incidence of pathology, death remains the ultimate consequence of aging (11). The biological basis of aging is incompletely understood with many theories being divided into two main categories: “programmed” theories and “damage” theories (2). According to the “programmed” theories, aging depends on biological clocks regulating the timetable of the life span through the stages of growth, development, maturity, and old age. This regulation would depend on genes sequentially switching on and off signals to the nervous, endocrine, and immune systems responsible for maintenance of homeostasis and for activation of defense responses (2). The “damage” theories identifies environmental insults to living organisms that induce progressive damage at various levels, such as mitochondrial DNA damage, oxygen radicals accumulations, cross-linking [2,11]. However, during aging several processes may interact simultaneously and may operate at many levels of functional organization. Consequently, different theories are not
mutually exclusive and may adequately describe some or all features of the aging process alone or in combination with other theories (11).

Below some of the physiological changes associated with the aging process on specific organ systems will be outlined

Respiratory system. The structure of the upper airway changes little with aging, however there are age-related structural lung changes including decreased elastic rebound of the lung, increased chest wall rigidity and decreases force-generation capacity of the respiratory muscles. These changes lead to a reduction in forced vital capacity, forced expiratory volume in 1 second (FEV$_1$) and vital capacity, and an increase in functional residual capacity (FRC) [2,3]. Furthermore, arterial oxygen pressure shows a progressive decrease with aging, thus increasing the alveolar-arterial oxygen difference (A-a)O$_2$. Most of this decrease in arterial oxygen pressure results from a mismatch of ventilation and perfusion that together with diminished elastic recoil of the lungs leads to a greater tendency for airways to collapse (3).

Cardiovascular system. Cardiac output decreases linearly with the aging process, about 1% per year in normal subjects or subjects free of cardiac disease. Consequently, the cardiac output of an 80-year-old subject is approximately half that a 20-year-old. This may be due to one of several factors. First, senescent cardiac muscle has decreased inotropic response to catecholamines, both endogenous and exogenous. Second, with aging there is an associated increase in diastolic and systolic myocardial stiffness, which might be due to increased interstitial fibrosis in the myocardium. Third, there is a progressive stiffening of the arteries with age leading to an increased afterload of the heart (Table 1) (3).

Another aspect to consider in the aging process is hypertension. A progressive increase in blood pressure after the first decade of life has long been regarded as normal consequences of aging and was the basis for ignoring the presence of hypertension in the elderly. The elevation with age is more pronounced for systolic than diastolic pressure (3). Aging is associated with stiffening of the large elastic arteries (i.e. aorta and carotid arteries). Enhanced pulse wave velocity, about 40-50%, and prolonged ejection augment antegrade and retrograde arterial waves, thus elevating systolic and
pulse pressure, cardiac work and oxygen demand (2). Left ventricular hypertension ensues as does tissue damage as a result of the increase in pulsatile flow, especially in high-flow organs, resulting in cerebrovascular events and renal impairment (2). Delayed or impaired baroreceptor response causes blood pressure lability, postural and postprandial hypotension and loss of sinus arrhythmia. In addition, with aging there is a reduction in \( \beta \)-adrenoceptor sensitivity with a reduced response to exogenous \( \beta \)-agonists. This baroreflex deterioration is multifactorial: reduced arteriole compliance, blunted transduction of stretch signals, altered central neural processing, altered baseline efferent autonomic outflows, and dampened end-organ responsiveness (Table 1) (2).

Furthermore, as consequences of all this processes arteriosclerosis and coronary artery disease often develop during the aging process. Indeed, thickening of the arteries walls with hyperplasia of the intima, collagenization of the media and accumulation of calcium and phosphate in elastic fibers progressively occurs with aging. Additionally, the lipid content of nonatherosclerotic portion of vessels increases, particularly of cholesterol (2).

<table>
<thead>
<tr>
<th>Aging effects on major structural and functions characteristics of the cardiovascular system.</th>
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<td>Cardiac changes</td>
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<td>Early diastolic filling</td>
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<td>End-diastolic filling</td>
</tr>
</tbody>
</table>

Table 1. ↓, diminished; ↑, augmented; =, unchanged (2).

**Endocrine system.** Increasing age results in a progressive deterioration in the number and the function of insulin-producing beta cells. As a consequence, the capacity of these cells to recognize and respond to changes in glucose concentration is impaired (3). In the elderly subjects, a greater proportion of the insulin released into the circulation in
response to a glucose challenge is in the form of the inactive precursor proinsulin than in younger individuals. In addition, with age there is a progressive peripheral insulin resistance. Compared with younger persons the elderly have a relative decrease in lean body mass with a relative increase in adiposity. Since little change in the total number of fat cells occurs with age, the increased adiposity appears due to an increase in fat cell size. In general, as adipocytes enlarge they turn off some insulin receptors. Consequently, even in non-obese elderly persons there is peripheral insulin resistance due to increased size of adipocytes with a relative decrease in insulin receptors. The combination of abnormal beta cell function with peripheral insulin resistance leads to increased glucose intolerance in normal aged persons (3).

Musculoskeletal system. The age-related decline in lean body mass is well known and is primarily due to loss and atrophy of muscle cells. Age-dependent changes also occurs in the innervation of muscles but the exact pathologic process is not well understood. Furthermore, degenerative joint disease occurs in 85% of persons older than 70 years of age and is a major cause of disability. It affects both, peripheral and axial skeleton and is characterized by degeneration of cartilage, subchondral bone thickening and remodeling of bone with formation of marginal spurs and subarticular bone cysts (3).

Summary. Age-dependent changes occurs at structural, functional and molecular level throughout the body systems declining linearly with the aging process. These changes result in reduced physiological functional capacity, diminished cardiovascular responsiveness and decreased autonomic homeostasis.
2. Obesity

2.1 Definition

The word obesity (from the Latin ob-esum, meaning on account of having eaten) is a lay term which means the same as fatness but with moderately abusive overtones. Obesity is a condition with International Classification of Disease code E66 (WHO) (12). Obesity generally is defined as excess body fat (13). However, the definition of excess is not clear-cut. Adiposity is a continuous trend not marked by a clear division into normal and abnormal. Moreover, it is difficult to measure body fat directly. Consequently, obesity often is defined as excess body weight rather than as excess of fat (13). Conventionally, obesity is measured and classified by means of the body weight index (BMI) calculated as weight in kilograms divided by eight in meters squared. In adults, the BMI of 25 was approximately equivalent to the upper end of the weight range for large frame size in the 1959 Metropolitan Life tables, defined obesity as a BMI of 30 or more for men and of 28.6 or more for women (Table 2)(13).

<table>
<thead>
<tr>
<th>BMI</th>
<th>Classification</th>
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<tbody>
<tr>
<td>&lt;18.5</td>
<td>Underweight</td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>Normal weight</td>
</tr>
<tr>
<td>25-29.9</td>
<td>Overweight</td>
</tr>
<tr>
<td>30-34.9</td>
<td>Obesity grade I</td>
</tr>
<tr>
<td>35-39.9</td>
<td>Obesity grade II</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Obesity grade III</td>
</tr>
</tbody>
</table>

*Table 2. Classification of obesity based on the BMI*

2.2 Epidemiology

In United States in 2003-2004, 32.9% of adults 20-74 years old were obese. In the early 1960s, the prevalence of obesity was 11% among men and 16% among women. The prevalence changes relatively little over the period from 1960 to 1980. However, the prevalence of obesity between 1980 and 1994 increased considerably, to about 21% in
men and to about 26% in women. By 2003-2004 the prevalence had increased to almost 32% in man and 34% in women (13). The prevalence of overweight and obese appears to increase with age. In 1999-2004, older adults were more likely to be obese than their younger counterparts. Among adults 20-39 years of age, 26.8% were obese. Among 40-to 59 year-old-adults 34.8% were obese, and among 60-70 year-old adults 35.2% were obese (13).

At global level, United States is not the only country experiencing increases in the prevalence of obesity. The current epidemic of obesity has been reported in several but not all regions globally. The highest rate of obesity has been reported in the Pacific Islands and the lowest rates have been reported in Asia (14). In England, the prevalence of obesity among women 25-34 years of age increased from 12% to 24% in only 9 years between 1993 and 2002. In Portugal, increases in overweight among school-age children also have been reported and less-developed countries have seen increases in obesity as well [13,14]. Differences in the prevalence of obesity between countries in Europe or between race-ethnic groups in the United States tend to be more pronounced for women than for men. However, the WHO estimates that in 2005 approximately 1.6 billion people worldwide were overweight and that at least 400 million adults were obese (14).

**Health implications.** Obesity is associated with increased risk of death. Studies estimated the risk of death in a prospective cohort of more than 500,000 U.S. men and women after 10 years of follow-up, and reported that, among patients who had never smoked, the risk of death is increased by 20% to 40% in overweight patients and by 2- to 3-fold in obese compared with normal-weight patients (14). Obesity is also associated with increased risk for numerous chronic diseases, including diabetes, hypertension, heart disease, and stroke and is further linked to several digestive disease, including gastroesophageal reflux disease and its complications, colorectal polyps and cancer, and liver disease [13-15].

**2.3 Pathophysiological aspects**

The development of obesity occurs when the caloric intake is disproportionate to the energy expended. Three metabolic factors have been reported to be predictive of
weight gain: a low adjusted sedentary energy expenditure, a high respiratory quotient (carbohydrate-to-fat oxidation ratio), and a low level of spontaneous physical activity (15). Several other factors are associated with being overweight, but it is not clear why or how they have an impact. Sex, age, race, and socioeconomic status have an impact on weight gain, with overweight and obesity being more likely among women, older individuals, ethnicity and those of low socioeconomic status (15).

Obesity appears to have a pivotal role in the dysregulation of cellular metabolism increasing the risk of many disorders that are associated with high mortality and morbidity including diabetes mellitus type 2, hypertension, coronary heart disease, dyslipidemia, gallbladder disease, and certain malignancies [16,17]. Moreover, excess adipocytes secrete numerous cytokines that contribute to vascular dysfunction in hypertension and dyslipidemia, as manifested by hypercholesterolemia and triglyceridemia. These conditions may contribute to significant atherosclerosis, and when associated with obesity and/or diabetes and insulin resistance, they contribute to metabolic syndrome (16).

Below some of the pathophysiological aspects associated with the obesity will be outlined.

Lipotoxicity and Insulin resistance. Excessive fat storage that creates obesity eventually leads to the release of excessive fatty acids from enhanced lipolysis, which is stimulated by the enhanced sympathetic state existing in obesity. The release of these excessive free fatty acids then incites Lipotoxicity, as lipids and their metabolites create oxidant stress to the endoplasmic reticulum and mitochondria. This affects adipose as well as nonadipose tissue, accounting for its pathophysiology in many organs, such as liver and pancreas, and in the metabolic syndrome. The free fatty acids released from excessively stored triacylglycerol deposits also inhibit lipogenesis, preventing adequate clearance of serum triacylglycerol levels that contributes to hypertriglyceridemia. Release of free fatty acids by endothelial lipoprotein lipase from increased serum triglycerides within elevated β lipoproteins causes Lipotoxicity that results in insulin-receptor dysfunction. The consequent insulin-resistant state creates hyperglycemia with compensated hepatic gluconeogenesis. The latter increases hepatic glucose
production, further accentuating the hyperglycemia caused by insulin resistance. Free fatty acids also decrease utilization of insulin-stimulated muscle glucose, contributing further to hyperglycemia. Lipotoxicity from excessive free fatty acids also decreases secretion of pancreas \( \beta \)-cell insulin, which results in \( \beta \)-cell exhaustion (16).

**Inflammation and immune dysfunction.** Adipocytes not only store triacylglycerol in fats depots in various body sites to provide energy reserve, but in aggregate constitute the largest endocrine tissue that constantly communicates with other tissues by adipocytes-released secretagogues, such as the proteohormones lectin, adiponectin, and visfatin. Visceral fat depots release inflammatory adipokines that along with free fatty acids provide the pathophysiologic basis for comorbid conditions associated with obesity. Visceral adipokines are transported by the portal vascular system to the liver, enhancing nonalcoholic steatohepatitis (NASH), and also by systemic circulation to another diverse site. Along with fatty acid Lipotoxicity, visceral adipokines also contribute to the adipokines inflammatory injury that leads to pancreatic \( \beta \)-cell dysfunction [17,18].

Dyslipidemia, hypertension, and atherogenesis are comorbid conditions, in addition to insulin resistance, that are associated with obesity and adversely influenced by the secretion of diverse inflammatory adipokines, particularly from the white adipose tissue in visceral fat depots. Specific adipokines enhance endothelial vasomotor tone by secreting renin, angiotensinogen, and angiotensin II, but when secreted from adipocytes, enhance hypertension in obese subjects. Tumor necrosis factor (TNF)-\( \alpha \) secretion increases in proportion to increased total body-fat mass and enhances inflammation in fatty livers and fat depots elsewhere, particularly pancreas, mesentery, and gut visceral fat. Furthermore, inflammatory markers that are increased in obesity commonly contribute to inflammatory conditions such as NASH and in the bronchial tree of patients with obstructive sleep apnea [16-18].

The progressive inflammatory state resulting from increased obesity that promotes insulin resistance also perpetuates atherogenesis throughout its development, from endothelial fatty streaks to late-plaque formation, rupture and thrombosis. Moreover, endothelial and adipose cell lipoprotein lipase activity are also decreased by inflammatory cytokines such as interleukin-6 (IL-6), so that by inhibiting lipolysis they increase serum triacylglycerol levels accentuating hyper-triglyceridemia. Furthermore,
as atherosclerosis progresses with macrophage and smooth-muscle cell infiltration, there is additional secretion of other cytokines, such as monocytes chemoattractant protein 1 (MCP-1), macrophage migration inhibiting factor (MMIF), and endothelin-1, that enhance the evolving inflammatory lesions of atherosclerotic plaques within the vascular wall. Progression of atherosclerotic plaque formation and remodeling of collagen results from the action of matrix metalloproteinases also secreted by adipocytes. This activity causes atheroma cap thinning and plaque rupture that precipitates release of the tissue factor, also promoting intravascular thrombosis (16).

Clinical manifestation. Comorbidities result from the burden of weight and space-occupying effects of obesity. This include: diabetes mellitus type 2, endothelial dysfunction and hypertension, dyslipidemia. As explained before, these comorbidities and the effects of fatty acid Lipotoxicity culminate to promote atherogenesis, including coronary artery disease. All these disorders are adversely affected by enhanced upregulation of NF-kB from visceral white adipose tissue inflammatory adipokines. Other conditions that are linked to obesity include chronic renal disease, obstructive sleep apnea, and non-alcoholic fatty-liver disease [12,16,17].

Furthermore, obesity is a major risk factor for many forms of cancer, including breast, colon, endometrial, esophageal, hepatocellular, renal, and prostate cancer. Mechanisms of carcinogenesis or tumor growth include perturbed cellular proliferation, dedifferentiation and/or apoptosis, angiogenesis, and chronic adipokines-associated inflammation, along with effects of cancer genes and/or environmental toxins that enhance inflammation [12,17]. Other comorbidities related to obesity include joint disease, obstructive sleep apnea, asthma, pulmonary embolism, cholesterol gallstone disease, polycystic ovarian syndrome. Furthermore, obesity is a risk factor for preeclampsia and eclampsia of pregnancy. However, many of these disorders improve or even disappear with the elimination of obesity (16).
3. Physical Activity

3.1 Definition

Physical activity, exercise, and physical fitness are terms that describe different concepts. However, they are often confused with one another, and the terms are sometimes used interchangeably. Physical activity is defined as any bodily movement produced by skeletal muscles that results in energy expenditure. Physical activity in daily life, can be categorized into occupational, sports, conditioning, household, or other activities. Exercise, is a subset of physical activity that is planned, structured, and repetitive and has as a final or an intermediate objective the improvement or maintenance of physical fitness. Physical fitness is a set of attributes that are either health- or skill-related (17).

Physical activity is complex behavior, and may be meaningfully partitioned into other categories mutually exclusive of each other. For example, one might divide them according to intensity: light, moderate, or heavy intensity; those that are intentional or compulsory; or regularly such as: daily or weekend activities [19,20].

3.2 Epidemiology of Physical (in)Activity

Since the industrial revolution, the development of new technologies has enabled people to reduce the amount of physical labor needed to accomplish many tasks in their daily lives. As the availability of new devices has continued to increase, the effects of physical labor and human energy expenditure have grown to include many aspects of the lives of more and more people. The use of many of these technologies has been driven by the goal of increased individual worker productivity and reduces physical hardships and disabilities caused by jobs entailing continuous heavy labor. However, the human body has evolved in such a way that most of its systems (skeletal, muscle, metabolic, and cardiovascular) do not develop and function in an optimum way unless stimulated by frequent physical activity (18). Although the technological revolution has been of great benefit to many populations throughout the World, it has come at a major cost in terms of the contribution of physical inactivity to the worldwide epidemic of non-communicable diseases. In 2009, physical inactivity was identified as the fourth
leading risk factor for non-communicable diseases and accounted for more than 3 million preventable deaths (18).

From the WHO global health observatory repository. In 2012, the WHO obtained comparable estimates for physical inactivity in adults from 122 countries worldwide. The combined population of these 122 countries represents 88.9% of the World’s population. Physical inactivity was defined as not meeting any of three criteria: 30 min of moderate-intensity physical activity on at least 5 days week, 20 min of vigorous-intensity physical activity on at least 3 days week, or an equivalent combination achieving 600 metabolic equivalent (MET)-min per week. Results shown that worldwide, 31.1% of adults are physically inactive. The frequency of inactivity varied greatly between WHO regions: 27.5% of people are inactive in Africa, 43.4% in the Americas, 43.3% in the eastern Mediterranean, 34.8% in Europe, 17.0% in Southeast Asia, and 33.7% in the western Pacific. Women are generally more inactive than are men, 33.9% versus 27.9%. Inactivity increases with age in all WHO regions, which is a pattern known to have a strong biological basis. Moreover, physical inactivity is more common in countries of high income than in those of low income (18).

Walking is a common, accessible, inexpensive form of physical activity and is an important component of total physical activity in adult populations. It is aerobic and necessitates use of large skeletal muscles, and confers the multifarious health benefits of physical activity with few adverse effects. Interventions have been implemented to increase population levels of walking and have proven this activity’s effectiveness. Indeed, 64.1% of adults report walking for at least 10 min consecutively on 5 or more days per week. Variation between WHO regions is modest: 57.0% report such walking in Africa, 65.6% in the Americas, 66.9% in the eastern Mediterranean, 66.9% in Europe, 67.2% in southeast Asia, and 65.0% in the western Pacific. Additionally, patterns of walking hardly differ between men and women and between age groups.

Another aspect of the human movement range that has received attention is sedentary behavior, which is usually defined as the time spent sitting. Overall, the proportion of adults spending 4 or more hours per day sitting is 41.5%. The value varied greatly in WHO regions: 37.8% of individuals sit for 4 or more hours per day in Africa, 5.2% in the Americas, 41.4% in the eastern Mediterranean, 64.1% in Europe, 23.8% in southeast Asia, and 39.8% in the western Pacific. For adults aged 15-59 years, the
proportions spending 4 hours or more sitting does not vary substantially, and both sexes are similar; for individuals aged 60 years or older, the frequency increased (18).

*Direct cost of inactivity to a health plan.* In 2004, Garret and colleagues published an interesting study aimed to estimate the total medical expenditures attributable to physical inactivity patterns among members of a large health plan, Blue Cross Shield of Minnesota (19). This study used a cost-of-illness approach to attribute medical and pharmacy costs for specific diseases to physical inactivity in 2000. Relative risk calculated from patient data confirmed that heart disease, stroke, hypertension, type 2 diabetes, colon cancer, breast cancer, osteoporosis, depression, and anxiety are directly related to individual physical activity patterns in adults. Results of the study shown that nearly 12% of depression and anxiety and 31% of colon cancer, heart disease, osteoporosis, and stroke cases were attributable to physical inactivity. Heart disease was the most expensive outcome of physical inactivity within the health plan population, costing 35.3$ million in 2000. Total health plan expenditure attributable to physical inactivity were 83.6$ million, or 56$ per member. This study confirmed the growing body of evidence quantifying physical inactivity as a serious and expensive public health problem (19).

### 3.3 Benefits of Physical Activity

The benefits of regular physical activity are extensive. Indeed, physical activity reduces risk of cardiovascular disease, thromboembolic stroke, hypertension, type 2 diabetes mellitus, osteoporosis, obesity, colon cancer, breast cancer, anxiety, and depression (20). Moreover, of particular interest to older adults, there is substantial evidence that physical activity reduces risk of falls and injuries from falls, prevents of mitigates functional limitations, and is effective therapy for many chronic diseases. Additionally, clinical practice guidelines identify a significant therapeutic role of physical activity in coronary artery disease, hypertension, peripheral vascular disease, type 2 diabetes, obesity, elevated cholesterol, osteoporosis, osteoarthritis, claudication, and chronic obstructive pulmonary disease. Also, physical activity has a role in the management of depression and anxiety disorders, dementia, pain, congestive heart failure, syncope, stroke, prophylaxis of venous thromboembolism, back pain, and constipation. Other
evidence shown that physical activity may help in prevent or delaying cognitive impairment and disability, and improves sleep [20,24].

3.4 Recommendations

*Physical activity for healthy adults and older adults.* Several studies have supported a dose-response relationship between chronic physical activity levels and health outcomes; such greater benefit is associated with higher amounts of physical activity. Epidemiologic studies have estimated the volume of physical activity needed to achieve specific health benefits typically expresses as kilocalories per week, Metabolic Equivalent for Task (MET)-minute per week, or MET-hour per week. Studies of diverse populations clearly show that an energy expenditure of approximately 1000 kcal•week\(^{-1}\) of moderate-intensity physical activity is associated with lower rates of cardiovascular disease and premature mortality [20,25]. In the general population, this 1000 kcal•week\(^{-1}\) volume of physical activity is accumulated through a combination of physical activities and exercise of varying intensities.

However, to promote and maintain health, all healthy adults aged 18-65 years need moderate-intensity aerobic physical activity for a minimum of 30 min on five days a week or vigorous-intensity aerobic activity for a minimum of 20 min on three days a week. Also, a combination of moderate-and vigorous-intensity activity can be performed to meet this recommendation. Furthermore, to promote and maintain good health and physical independence, adults will benefit from performing activities that maintain or increase muscular strength and endurance for a minimum of two days a week. It is recommended that 8-10 exercises be performed on two or more nonconsecutive days each week using the major muscle groups. To maximize strength development, a weight should be used that allows 8-12 repetitions of each exercise resulting in volitional fatigue [20,26].

*Physical activity for weight loss and preventing weight regain in overweight and obese people.* The clinical significance of weight maintenance and weight loss is often questioned in studies that provide marginal results. To provide context to a discussion of physical activity for weight maintenance, weight loss, or prevention or weight regain after weight loss weight maintenance was defined as a change of <2.3kg, or <3% change in
body weight (22). However, a primary prevention of obesity starts with maintenance of current weight, not weight reduction. The risk for weight gain may vary across time, and the need for physical activity to prevent weight gain may also vary. In general, guidelines suggest that moderately vigorous physical activity of 150 to 250 min\(\cdot\)week\(^{-1}\) with an energy equivalent of \(~1200\) to \(2000\) kcal\(\cdot\)week\(^{-1}\) is sufficient to prevent a weight gain greater than 3% in most adults (22). In order to promote weight loss, a negative energy balance generated by physical activity has to be applied, and the larger the negative energy balance, the greater the weight loss. Any increase in physical activity has the potential for weight loss, however, it seems that physical activity <150 min\(\cdot\)week\(^{-1}\) results in minimal weight loss compared with greater amount. Physical activity > 150 min\(\cdot\)week\(^{-1}\) results in modest weight loss of \(~2\) to \(3\)kg, while physical activity between 225 and 420 min\(\cdot\)week\(^{-1}\) results in 5-to 7.5kg weight loss (22). For weight maintenance after weight loss, some studies support the value of \(~200\) to \(300\) min\(\cdot\)week\(^{-1}\) of physical activity in order to reduce weight regain. However, there are no correctly designed, adequately powered, energy balanced studies to provide evidence for the amount of physical activity to prevent weight regain (22). The American College of Sport and Medicine (ACSM) Position emphasized diet restriction and endurance exercise. Resistance training was not assigned a major role by the authors because it was believed that evidence was insufficient. Although the energy expenditure associated with resistance training is not large, resistance training may increase muscle mass which may in turn increase 24-hour energy expenditure. However, resistance training may increase loss of fat mass when combined with aerobic exercise compared to resistance training alone. Unfortunately, no evidence currently exists for prevention of weight regain after weight loss of for a dose effect for resistance training and weight loss (22).
Inflammatory response

1. Definition

The word inflammation comes from the Latin *inflammare* (to set on fire) and reflects a physiological protective response which is generally tightly controlled by the body at the site of injury [1,2]. Most pathologists would agree that inflammation “represents a response of living tissue to local injury”; or “is a basic way in which the body reacts to infection, irritation or other injury, the key feature being redness, warmth, swelling and pain”; or “the basic process whereby tissues of the body respond to injury” (24). Indeed, at present inflammation is defined by the presence of five macroscopic pathological phenomena, four of them proposed by Celsus as early as 2000 years ago. There are: swelling of the tissue, elevated tissue temperature, blood-color like redness of vascularized tissue at the inflammation site, intensive sensation of a noxious stimulus, and impaired function of the organ affected (24). All signs have been regarded as secondary to one primary pathophysiological event: enhancement of vasculature permeability as a direct consequence of tissue injury (24). This definition of inflammation recognizes what we would today known as “classical” acute inflammatory response, defining inflammation according to clinical signs and symptoms. Yet, in most cases the cellular processes and signals that underlie the cardinal signals occur at a subclinical level and not give rise to any heat, redness, swelling or pain (25).

Two centuries after Celsus, Galen was important in promoting the humoral view of inflammation. In this model, inflammation was part of the beneficial response to injury, rather than a superimposed pathology (25). Later, in 1871 with the advances in microscopy and cell biology, Virchow viewed inflammation as inherently pathological giving rise to a cell based definitions of inflammation, and by the end of the 19th century it was acknowledged that changing cell populations arising from both the blood and local proliferation were a key feature of many model of inflammation (25). Finally, a prominent German biologist, Neumann, defined inflammation more loosely as a “series of local phenomena developing as the result of primary lesions to the tissues and that tend to restore their health”, defining inflammation as a necessary phase in
the repair response after injury (25). However, the significance of this discovery has been largely neglected, leaving considerable confusion the cause-effect relationship in pathogenesis and in the approaches to treatment of inflammation disease. In particular, it is very often noted that, although inflammation is a defensive, therefore useful process, its exaggeration or prolonged action may harm the body (24).

2. Physiology of inflammation

The innate ability of the body to defend itself is based on three elements: external barriers against invasion and tissue injuries, non-specific systems against foreign pathogens and debris, and antigen-specific responses to foreign pathogens (23). Inflammation is the body’s initial non-specific response to tissues injury produced by mechanical, chemical or microbial stimuli and it is a highly amplified controlled humoral and cellular response: the complement, kinin, coagulation and fibrinolytic cascades are triggered in tandem with activation of phagocytes and endothelial cells. There are four major events in the inflammatory process: a) vasodilation, b) increased microvascular permeability, c) cellular activation/adhesion and e) coagulation. Vasodilation and increased microvascular permeability at the site of injury increase locally available oxygen and nutrients, and produce heat, swelling and tissue edema, giving the rise to the five classical symptoms previously mentioned (23). Furthermore, the normal physiological response to stress and injury results in a series of cardiovascular changes and neuroendocrine changes, such as increases in heart rate, contractile and cardiac output, increased release of catecholamines, cortisol, antidiuretic hormone, growth hormone, glucagon, and insulin. The major metabolic change that occurs in response to inflammation is an initial increase in oxygen consumption [1,4].

3. Causes/inducers of inflammation

Inducers of inflammation can be exogenous or endogenous (26). Exogenous inducers can be classified into two groups: microbial and non-microbial. However, we will focus on the endogenous inducers which are signals produced by stressed, damaged or otherwise malfunctioning tissues. The identity and characteristics of these signals are
poorly defined, but they probably belong to various functional classes according to the nature and the degree of tissue anomalies in which they report. One common theme detecting acute tissue injury is the sensing of the desequestration of cells of molecules that are normally kept separate in intact cells and tissues. The desequestration of these components is afforded by the various types of compartmentalization that occur in normal tissue.

**Cell-derived inducers.** During necrotic cell death, for example, the integrity of the plasma membrane is disrupted, resulting in the release of certain cellular constituents such as ATP, K\(^+\) ions, uric acid, and several others. ATP binds to purinoceptors at the surface of macrophages, resulting in K\(^+\) efflux, and can cooperate with other signals to activate other inflammasomes. Also, ATP activates nociceptors reporting tissue injury to the nervous system (26).

**Tissue-derived inducers.** In intact tissues, epithelial cells and mesenchymal cells are normally separated from each other by the basement membrane, and the disruption of this barrier results in “unscheduled” epithelial-mesenchymal interactions. These interactions indicate the presence of tissue damage and consequently initiate tissue-repair responses (26).

**Plasma-derived inducers.** Damage to the vascular endothelium allows plasma proteins and platelets to gain access to extravascular spaces. A key plasma-derived regulator of inflammation, the Hageman factor, becomes activated by contact with collagen and other components of the extracellular matrix acting like a sensor of vascular damage and initiates the four proteolytic cascades that generate inflammatory mediators: the kallikrein-kinin cascade, the coagulation cascade, the fibrinolytic cascade and the complement cascade. Platelets are also activated by contact with collagen and produce various inflammatory mediators, including thromboxane and serotonin (26).

**Reactive oxygen species (ROS).** ROS produced by phagocytes in tissue injury also have a role inflammatory process (26). When tissues are injured by ischemia or anoxia, their ability to control the metabolism of oxygen is compromised and the species that are generated activate a superoxide-dependent chemoattractant process. This leads to an influx of leukocytes which generates still more ROS. Yet, ROS can initiate and amplify
the inflammatory process by upregulation of several proinflammatory cytokines (IL-2, IL-6 and TNF-α) and adhesion molecules (23).

4. Most important mediators of inflammation

Cytokines are the physiological mediators of the inflammatory response. Cytokines are the physiological messenger of the inflammatory response and the principal molecules involved are tumor necrosis factor (TNF)-α, interleukins, interferons and colony stimulating factors (CSFs), while monocytes/macrophages and endothelial cells are the cellular effectors of the inflammatory response (23). Leukocyte activation leads to increased leucocytes aggregation and tissues infiltration within the microcirculation where these leukocytes undergo respiratory burst, with an increase in their oxygen consumption and production of cytokines and other inflammatory mediators. Endothelial cells, exposed to this milieu of humoral and leucocytes-derived factors also become activated, and commence the expression of several adhesion molecules and receptors in their surface along with the synthesis and secretion of additional cytokines and secondary inflammatory mediators, including prostaglandins, leukotrienes, thromboxane, platelet activating factor, oxygen free radicals, nitric oxide and proteases. The presence of activated endothelial cells and the enhanced cytokines milieu results in activation of the coagulation cascades which leads to local thrombosis minimizing blood loss and the walling off of injured tissues, attempting physiologically to isolate the inflamed areas[1,4].

Of the multitude of mediators operating in the inflammatory response there are some that appears to be more influential than others, as follows:

TNF-α Several cells produce TNF-α. Its expression is tightly controlled both at transcriptional and translational levels. Specific receptors for this cytokine are found on a wide variety of cells and a maximal biological response is elicited by occupancy of as few as 5% of these receptors. The systemic and tissue-specific cellular mechanisms of TNF-α are dependent on its direct effect as well as the release of other soluble mediators from host cells. TNF-α elicits the release of neutrophil margination by inducing expression of adhesion molecules,
promoting their trans-endothelial passage and activation. It promotes differentiation of monocytes and macrophages, and induces the activation of macrophages. It stimulates the synthesis of acute-phase proteins and activates the common pathway of the coagulation and complement systems. TNF-α produces a dose-dependent increase in endothelial procoagulant activity and may inhibit thrombomodulin expression at the endothelial cell surface. It induces IL-1 release from endothelial cells and macrophages, while IL-1 subsequently stimulates the biosynthesis of other cytokines (23).

**IL-1.** This cytokine appears to be released either in parallel or in response to TNF-α. IL-1 consists of two different molecules, IL-1α and IL-1β which are structurally related polypeptides. Most IL-1α remains in the cytosol in a precursor form or is associated with the cell membrane in a biologically active form. The presence of a cell-associated form of IL-1 can explain the capability of activated macrophages to induce natural killer cell cytotoxicity, T cell proliferation and other functions by cellular contact in the absence of any releasable IL-1. It is a strong inducer of granulocytes, macrophages, and hepatic acute-phase protein synthesis. Excessive IL-1 release produces excessive margination of activated neutrophils into the vascular wall, stimulates endothelial cell procoagulant activity and increases leucocytes binding (23).

**IL-6.** IL-6 is a family of at least six differentially modified phosphoglycoproteins that are released rapidly within an hour in response to injury. IL-6 interacts synergistically with IL-1 to affect thymocyte proliferation. The temporal relationship of IL-6 appearance within the cytokine cascade suggests a strong relationship to antecedent TNF-α or IL-1 activity stimulation. Transcription and production are enhanced in response to TNF-α and IL-1. When TNF-α or IL-1 activity is attenuated, the subsequent IL-6 response is decreased (23).

**IL-4 and IL-8.** IL-4 synergistically increases TNF-α or IL-1-induced antigen expression in endothelial cells, but inhibits the increased expression of adhesion molecules by TNF-α, IL-1, or IFN-γ. IL-4 enhances lymphocytes adhesion to the endothelial cells and regulates growth and differentiation of T cells. Furthermore, it induces antigen expression on macrophages and supress IL-8 expression from stimulated monocytes but not from stimulated fibroblasts or endothelial cells. IL-8 is produced by endothelial cells and is chemotactic for both neutrophils and lymphocytes (23).
IFN-γ. It promotes the release of TNF-α, IL-1 and IL-6 by augmenting the effects of endotoxin on macrophages, thereby increasing the expression of adhesion molecules and cellular receptors for TNF-α. It may act synergistically with TNF-α to produce cytotoxic and cytostatic activity, and promotes B cell activation to increase antibody production. Also, IFN-γ enhances adhesion of lymphocytes to endothelial cells, and promotes maturation of macrophages and enhances their activity (23).

5. Inflammation and Aging: “inflamm-aging”

The term “Inflamm-aging” was invented by Franceschi and colleagues to describe the up-regulation of the inflammatory response at older ages resulting in the low-grade chronic systematic pro-inflammatory state that underline the most age-associated diseases (27). This process seems to be mediated by increased circulating levels of pro-inflammatory cytokines (primarily IL-1, IL-6, TNF-α, and IL-1) and it results from the counterbalance between pro- and anti-inflammatory cytokines (Il-4, IL-6, IL-13, and IL-10) that ultimately sees an upregulation of the pro-inflammatory response [6-8].

The concept of “Inflamm-aging” determines that aging, either physiologically or pathologically, can be drive by the pro-inflammatory cytokines and other inflammatory mediators and this potentially harmful pro-inflammatory signals at a later stage of life may act antagonistically to the beneficial role they had in earlier stage of life (28). Moreover, this concept is based on an antagonistic pleiotropy theory programmed during evolution. In fact, aging is accompanied by chronic low-grade inflammation state, showed by a 2 to 4-fold increase in serum levels of inflammatory mediators which act as predictors of mortality independent of pre-existing morbidity. Low-grade inflammation has emerged as critical in the pathogenesis of several age-related chronic disease as Alzheimer’s disease, cardiovascular disease, type 2 diabetes, sarcopenia, frailty and functional disability. These diseases appear to be correlated, leading to the concept of age-related disease, which may be linked by the process of inflammation (28).

Chronic low-grade inflammation may have a rapid or slow onset but it is characterized primarily by its persistence and lack of clear resolution, occurring when the tissue are unable to overcome the effects of the harmful agent. During these chronic
inflammation events, immune responses, tissue injury and healing proceed simultaneously. The inflammatory side effects accumulate slowly and can lead to severe tissue deterioration without any relevant symptoms for years (29). Interestingly, the chronic inflammation state is characterized by the infiltration of various migratory inflammatory cells of macrophage, lymphocytes, and plasma cells due to sustained ROS production, implicating a major role of ROS in the inflammatory reactions (30). ROS causes both oxidative damage and elicit the release of additional inflammatory cytokines, perpetuating a vicious cycle resulting in a chronic pro-inflammatory state where pathophysiological changes, tissue injury and healing mechanisms proceed simultaneously and damage slowly accumulates asymptotically over decades (27).

6. Inflammation and Obesity

Over the past decades, the search for a potential unifying mechanism behind the pathogenesis of obesity-related diseases has revealed a close relationship between nutrient excess and derangements in the cellular and molecular mediators of immunity and inflammation. This has given birth to the concept of “metainflammation” to describe the chronic low-grade inflammatory state of obesity [12,13]. The inflammatory response triggered by obesity involves many components of the classical inflammatory response to pathogens and include systemic increases in circulating inflammatory cytokines and acute phase proteins (i.e., C-reactive protein), recruitment of leukocytes to inflamed tissues, activation of tissues leukocytes, and generation of reparative tissue response (31).

In the recent years, it has become clear that obesity is a chronic and mild systemic inflammatory condition, and there is much evidence that chronic inflammation of White Adipose Tissue (WAT) contributes to the development of insulin resistance, as well as is at the molecular basis of diabetes (32). In the past, white adipose tissue (WAT), was considered to be simply a site for energy storage, however in the recent years it has become better understood at the molecular level (32). Indeed, WAT secretes physiologically active substances, collectively known as adipokines. Thus, WAT is now considered to be one of the tissue that play a critical role in the onset of life-style related disease (32). When adipocytes hypertrophy occurs due to excessive
energy intake or lack of exercise, infiltration of macrophages is observed in WAT increasing the production of pro-inflammatory adipokines, such as TNF-α and monocyte chemoattractant protein-1 (MCP-1), IL-6, and leptina, and decreasing the production of anti-inflammatory adiponectin, causing WAT’s chronic inflammation (32). Specifically, changes in adipocyte and fat pad size lead to physical changes in the surrounding area and modifications of the paracrine function of the adipocyte. Therefore, adipocytes begin to secrete low levels of TNF-α, which can stimulate preadipocytes to produce MCP-1 (33). At the same time, endothelial cells also secrete MCP-1 in response to cytokines and the increased secretion of leptin by adipocytes contribute to macrophage accumulation by stimulating macrophages to adipose tissue and promoting adhesion of macrophages to endothelial cells. It is conceivable also that physical damage to endothelium, could also play a role in macrophage recruitment, similar to that seen in atherosclerosis. Whatever the stimulus to recruit macrophages into adipose tissue is, once these cells are present and active, along with adipocytes and other cell types, could perpetuate a vicious cycle of macrophage recruitment, production of inflammatory cytokines, and impairment of adipocytes function [15,16]. Furthermore, increasing adiposity activates both c-Jun N-terminal Kinase (JNK) and IKappaB kinase (IKKβ) as well as many of the typical inflammatory stimuli simultaneously activate JNK and IKKβ pathway that in addition to pro-inflammatory cytokines and ROS production play a pivotal role in the development of obesity-induced insulin resistance (34). One potential mechanisms in though the activation of NADPH oxidase by lipid accumulation in the adipocytes, which increase ROS production. This mechanism was shown to increase the production of TNF-α, IL-6, and MCP-1 and decrease the production of adiponectin. Lipid accumulation also activates the unfolded protein response to increase endoplasmic reticulum stress which was shown to activate JNK to lead to serine phosphorylation of insulin receptor substrate-1 (34).

7. Inflammation and Exercise

In the past 20 years, research has demonstrated that exercise induces considerable changes in the immune system. The interaction between exercise and the immune
system provided a unique opportunity to evaluate the role of underlying endocrine and cytokine mechanisms. Recent studies showed that exercise provokes an increase in certain cytokines and it was demonstrated that active, but not resting, leg of human released significant amounts of IL-6 into the circulation during prolonged exercise (35). In light of that, it was theorized that IL-6 response may acts as an indicator that muscle glycogen stores are reaching critically low levels and that active muscles reliance on blood glucose as a source of energy is on the increase (36). Furthermore, recent research demonstrated that skeletal muscles might produce and express cytokines belonging to distinctly different families. Since skeletal muscle has the capacity to express cytokines, and muscle contractions play a regulatory role in the muscular expression of these cytokines, the term “myokines” was suggested (35). A bout of exercise provokes the appearance of several cytokines, including IL-6, IL-1ra, IL-8, I-10, whereas TNF-α is only stimulated by very intense exercise (35).

Myokines. All cytokines and other peptides expressed, produced, and released by muscle fibers and which exert either paracrine or endocrine effects should be classified as myokines (35).

Increased circulating levels of IL-6 have been seen after prolonged exercise that seem to be independent on concomitant muscle damage. The level of IL-6 increases in an exponential fashion in response to exercise, and it declines in the post exercise period. The magnitude by which plasma IL-6 increases, is related to exercise duration, intensity, the muscle mass involved in the mechanical work, and the endurance capacity. IL-6 is most often classified as a proinflammatory cytokine, although data also suggest that IL-6 regulates acute-phase proteins are anti-inflammatory and immunosuppressive and that they may negatively regulate the acute phase response [20,21]. In response to exercise, IL-6 may be released in significant amounts from the working muscles in the circulation, where it can exert its effect in other organs in a hormone-like fashion. The exercise-induced increase in plasma IL-6 is followed by increasing levels of anti-inflammatory cytokines such as IL-1ra and IL-10 meanwhile TNF-α appears to be suppressed [18,21]. Also, IL-6 gene is silent in resting muscles, but it is rapidly activated by contractions. The transcription rate is faster than reported for any other gene in muscles, and the fold increase of the transcript is massive. IL-6 production is modulated by the carbohydrate availability in skeletal muscles, suggesting
that IL-6 acts as an “energy sensor”. IL-6 released from contracting muscles into the circulation may enhance lipolysis and gene transcription in abdominal subcutaneous fat via its effect on adipose tissue. Lastly, muscle-derived IL6 is likely to inhibit low-grade TNF-α production and thereby TNF-α-induced insulin resistance and it may be a player in mediating the beneficial health effects of exercise [18,21].

IL-8 is a known chemokine that attracts primarily neutrophils as well as acts as an angiogenic factor. IL-8, like IL-6, responds to exercise and its plasma concentration increases in response to exhaustive exercise such as running, which involved eccentric muscle contractions. The physiological function of IL-8 within the muscle is still unknown. The main part of the systemic increase in IL-8 as seen during exercise with eccentric component is most likely due to an inflammatory response. However, just a small and transient release of IL-8 was noted after a concentrically exercise limb, which did not result in an increase in the systemic IL-8 plasma concentration. A high local IL-8 expression take place in working muscle with only a small transient release could indicate that muscle-derived IL-8 acts locally and exerts its effects in an endocrine or paracrine fashion. A plausible function of IL-8 would be chemoattraction of neutrophils and macrophages when in concentric exercise is little or no accumulation of neutrophils or macrophages in skeletal muscle [18,22]. A more likely function of muscle-derived IL-8 is to stimulate angiogenesis. Indeed, IL-8 associated with the CXC receptor 1 and 2 induces its chemotactic effects via CXCR1, whereas CXCR2 is the receptor responsible for IL-8-induced angiogenesis (35).

IL-15 has been identified as an anabolic factor, which is highly expressed in skeletal muscle. Moreover, IL-15 seems to play a role in muscle-adipose tissue interaction. In human skeletal muscle myogenic culture, IL-15 indicates an increase in accumulation of the protein myosin heavy chain in differentiated muscle cells, suggesting that IL-15 is an anabolic factor in muscle growth and it stimulates myogenic differentiation independently of insulin-like growth factors (IGFs). Furthermore, in opposition to IGF-1, IL-15 has effects on fully differentiated myoblast (35).

Cytokines responses to exercise. In severe infections, the cytokine cascade consist of: TNF-α, IL-1β, IL-6, IL-1ra, sTNF-R, and IL-10 (Figure 1). The first two cytokines in the
cytokines cascade are TNF-α and IL-1β, which are produced locally. These cytokines are usually referred to as proinflammatory cytokines. TNF-α and IL-1 stimulate the production of IL-6. The cytokine response to exercise vary from that elicited by severe infections. Indeed, the classic pro-inflammatory cytokines, TNF-α and IL-1β in general do not increase with exercise (Figure 1) (37). Typically, IL-6 is the first cytokine present in the circulation during exercise and its level increases in an exponential fashion. Furthermore, in response to exercise circulating levels of anti-inflammatory cytokines and cytokines inhibitors such as IL-1ra and TNF-R are increased. Taken together, exercise provokes an increase primarily in IL-6, followed by an increase in IL-1ra and IL-10 (37).

![Figure 1. Different pattern of inflammation cascade during severe infection (A) (i.e. sepsis) and exercise (B) (37).](image)

**Anti-inflammatory effects of myokines.** Data suggest that IL-6 exerts inhibitory effects on TNF-α and IL-1 production. IL-6 inhibits lipopolysaccharide induced TNF-α production both in cultured human monocytes and in the human monocytic line, indicating that circulating IL-6 is involved in the regulation of TNF-α levels. The anti-inflammatory effects of IL-6 are also demonstrated by the fact that IL-6 stimulates the production of IL-1ra and IL-10. As well as it stimulates the release of TNF-α receptors, but not IL-1β or TNF-α, and appears to be primary inducer of the hepatocyte-derived acute phase proteins, many of which have anti-inflammatory properties [20,23].

IL-10 and IL-1ra in response to exercise also contribute to mediate the anti-inflammatory effects of exercise. IL-10 inhibits the production of IL-1α, IL-1β, and TNF-α as well as the production of chemokines, including IL-8 and macrophage
inflammatory protein-\(\alpha\). Taken together, these observations suggest that IL-10 plays a pivotal role in orchestrating the inflammatory reaction. IL-10 also prevents cytokine synthesis by posttranscriptional mechanisms, where IL-1\(\alpha\), IL-1\(\beta\), and TNF-\(\alpha\) release induced by LPS is a direct consequence of mRNA degradation of their corresponding genes (37).

The biological role of IL-1ra is to inhibit signaling transduction through the IL-1 receptor complex. The IL-1ra is a member of the IL-1 family that binds to IL-1 receptor but does not induce any intracellular response (37). C-reactive protein (CRP), the most frequently studies biomarker of chronic inflammation, has a role both in the induction of anti-inflammatory cytokines in circulating monocytes and in the suppression of the synthesis of pro-inflammatory cytokines in tissue macrophages. A small increase of CRP levels is seen the day after exercise of longer duration (37).

7.1 Exercise-induced chronic adaptations

*General considerations.* Exercise has anti-inflammatory effects, and therefore, in the long term, regular physical activity can protect against the development of chronic disease [24-28]. This anti-inflammatory effects may be mediated not only via a reduction in visceral fat mass, with subsequent decreased production and release of pro-inflammatory adipokines, but also by induction of an anti-inflammatory environment with each bout of exercise, as well as by a reduced expression of Toll-like receptors on monocytes and macrophages (38). Furthermore, there could be other mechanisms involved in the exercise-induced anti-inflammatory effects such as the inhibition of monocyte and macrophages infiltration into adipose tissue and the phenotypic switching of macrophages within adipose tissue [24,29].

Observational data from large population cohort studies consistently show an association between physical activity and inflammation. Specifically, lower inflammatory biomarker concentrations are observed in individuals who report performing more frequent and more intense physical activity [30,31]. At present, there is strong evidence for an inverse, independent, dose-response relationship between systemic CRP concentration and level of physical activity [32,33]. Furthermore, it has been shown that being engaged in physical activity more than 22 times per month is
associated with a 37% reduction in risk for an elevated CRP compared to engaging in activity less than three times per month [30,34]. Importantly, this inverse relationship between CRP and physical activity is seen across a wide age range, including the elderly. Even though CRP is the most frequently studied biomarker of chronic inflammation, there are data regarding the association of other inflammatory markers with physical activity such as IL-6, TNF-α, and others. It seems that in elderly men both IL-6 and TNF-α were negatively related to the number of reported hours per year of moderate and strenuous exercise. Furthermore, the lowest concentrations of both CRP and IL-6 were found in elderly persons with the highest levels of recreational activity (39). Evidence has shown a significant dose-response relationship between absolute amount of physical activity reported an unadjusted inflammatory biomarker expression, such that individuals who exercise more than 4 hours per week have 4% lower soluble TNF factor receptor 1 and 2, 6% lower IL-6, and 49% lower CRP than those exercising less than half an hour per week (39). Moreover, physically active individuals with metabolic syndrome compared with sedentary persons shown 30% lower IL-6, 15% lower TNF-α, 19% lower serum amyloid A, and 15% lower white blood cell counts [30,35].

Concluding, information from observational studies shows that the greater volume of reported physical activity, the lower is the risk of elevated levels of inflammatory biomarkers.

In Aging. In 2005, Elosua and colleagues (41) published an interesting study aimed to determine the association between physical activity and physical performance, and inflammatory biomarkers in elderly people. The study included thousand-four subjects aged 65 years or older, and information on self-reported physical activity during the previous year was collected. Eight-hundred and forty-one participants performed a 400-meter walking test to assess physical performance and plasma concentrations of inflammatory biomarkers were determined. As compared to sedentary men, men practicing light and moderate physical activity had a significantly lower erythrocyte sedimentation rate, fibrinogen level, CRP level, while only those practicing moderate-high physical activity had significantly lower uric acid level, IL-6, and TNF-α level (41). In women, those participating in light and moderate-high physical activity had significantly lower uric acid, and IL-6 levels, but only those performing moderate-high
physical activity had significantly lower CRP level (41). This study supports the hypothesis that regular and constant physical activity is associated with lower inflammatory biomarkers in the elderly.

On the contrary, Beavers and colleagues in 2011 (42) published a study aimed to determine the effect of 12-month physical activity intervention on inflammatory biomarkers in elderly men and women. In the study 424 elderly, age 70 to 89 years were included and randomized to either 12-month moderate-intensity physical activity intervention or a successful aging health education intervention. Biomarkers of inflammation were measured at baseline, 6 and 12 months (42). Results show that IL-8 was the only inflammatory biomarker affected by an intervention of physical activity. Overall, data of these study did not provide a further evidence for an effect of regular exercise on inflammatory biomarkers in older adults (42).

Another interesting study by Della Gatta and colleagues (43) aimed to examine the expression of cytokines both at rest and following a bout of isokinetic exercise performed before and after 12 weeks of resistance exercise training in young and elderly men. Protein expression of various cytokines was determined in muscle homogenates. Results show that the inflammatory response were not significantly different between young and elderly men, either before or after 12 weeks of exercise training. However, compared with the young men, the expression of pro-inflammatory cytokines 2 hours post-exercise tended to be greater in the elderly men prior to training, but training attenuated this difference (43). Authors concluded that the inflammatory response to unaccustomed exercise increase with age, but regular exercise training may help to normalize the inflammation response which could have important implications for muscle regeneration and adaptation in the elderly (43).

Given the contrasting results from different studies, future research should aim to determine whether type, intensity, and duration of exercise are important to intervention success. Furthermore, researchers should also be careful to note study details, such as the specific parameters being studied, body weight status, and baseline inflammatory status, as variations in such characteristics may yield conflicting results and inherent confusion in the literature.
In Obesity. Christiansen and colleagues in 2010 (44) published an interesting study aimed to investigate the effect of exercise training and diet-induced weight loss alone or in combination on inflammatory biomarkers. The study included 79 obese subjects, randomized into three groups for a 12-week intervention: exercise only, EXO; diet-induced weight loss using a very low energy diet, DIO; and exercise and diet-induced weight loss combined, DEX. In the EXO group, the weight loss was 3.5 kg and in the DIO and DEX group, it was 12 kg in both. Furthermore, in the DIO e DEX groups, circulating levels of MCP-1, MIP-1α, IL-15, and IL-18 were decreased, and adiponectin was increased. In the EXO group, only MCP-1 was decreased (44). By combining the weight loss in all three groups, authors found a correlation between the degree of weight loss and improvement in several of the inflammatory markers. Authors concluded that rather large weight losses (<5-7%) were found to have beneficial effects on circulating inflammatory markers in obese subjects. Furthermore, aerobic exercise for 12 weeks, was found to have no effects on circulating inflammatory markers in these subjects, suggesting that a more intensive exercise may be necessary to affect systemic inflammation in obese (44).

Another study Brunn and colleagues published in 2006 (45) aimed to investigate the effect of a 15-week lifestyle intervention (hypocaloric diet and daily exercise) on inflammatory biomarkers in 27 severely obese subjects. The intervention reduced body weight and increased insulin sensitivity, as well as increased plasma adiponectin while CRP, IL-6, IL-8, and MCP-1 levels decreased. In agreement with the previous study, authors concluded that the combination of hypocaloric diet and moderate physical activity resulted in a significant general decrease in the level of inflammation (45).

In 2003, Pischon and colleagues (46) investigated the relationship between physical activity and the obesity-related inflammatory markers CRP, IL-6, and soluble TNF-receptors (sTNF-Rs) 1 and 2. Authors examined even the relationship between physical activity and insulin sensitivity and whether inflammatory markers mediate this association. In the study, 405 healthy men and 454 healthy women were included and information about physical activity and other variables were assessed by questionnaires. Results shown that physical activity was inversely associated with plasma level of sTNF-R1 and 2, IL-6, and CRP but after having adjusted for BMI and leptin (as a surrogate of fat mass), most of these association were no longer significant.
Physical activity was also inversely related to insulin and C-peptide level, but level of inflammatory markers explained only very little of this inverse relationship. Authors suggest that the frequent physical activity is associated with lower systemic inflammation and improves insulin sensitivity and this association can be partially explained by a lower degree of obesity in physically active subjects. However, although inflammatory markers may mediate obesity-dependent effects of physical activity in inflammatory related disease, this study suggests that inflammatory-related disease does not directly account for the beneficial effects of physical activity on insulin resistance (46).

As already stated previously, given the contrasting results from different studies, future research should aim to determine whether type, intensity, and duration of exercise are important to intervention success.

7.2 Exercise-induced acute response

General considerations. Inflammatory response happens while exercising. Acute exercise has an effect on cytokine response and inflammation in healthy individuals. The intensity, duration and type of exercise, as well as acute vs. chronic exercise can all influence various immune parameters, which are also associated with chronic inflammatory disease. Acute exercise has an effect both during and after exercise in the immune system. During acute exercise muscles releases IL-6 and levels can increase significantly. Furthermore, leukocyte subset as neutrophils, lymphocytes and monocytes, as well as plasma concentrations CRP and both pro- and anti-inflammatory cytokines TNF-α, IL-1, IL1ra, IL-10 and sTNF-r can increase to various magnitudes during a bout of exercise [42-45]. Following the cessation of intense exercise, neutrophils and monocytes can continue to increase into the recovery period. During this same time, other leukocyte subsets decrease in number, while plasma concentrations of the above-mentioned cytokines stay elevated for some hours. Strenuous and eccentric exercise seem to exert the most prominent changes in immune parameters. While extreme exercise such as marathon, and frequently executed training programs have been associated with a depression in immune function, which may increase the elite athlete’s susceptibility to infection (47). The pro-inflammatory
markers TNF-α and IL-1β do not seem to increase in short period of moderate intense exercise, although conflicting results have been documented (48). It is therefore clear that acute bouts of exercise exert various effects on the immune system and are typically transient in nature (47). However, as mentioned previously there are several possible explanations for the variable results on pro-inflammatory and anti-inflammatory responsive cytokines in relation to exercise. These include: a) the type of physical activity as well as the intensity and duration of the exercise, b) the specificity and sensitivity of the assays (48).

In Aging. Very few studies focused their attention of the inflammatory response to a single bout of exercise in older adults. Some study aimed to investigate the gene expression of growth and remodeling factors, as well as gene expression of some inflammatory biomarkers in response to resistance training in older adults.

For example, Dennis and colleagues in 2008 (49) published a study aimed to compare expression of genes that function in inflammation and stress, cell structures and signaling, or remodeling and growth in skeletal muscle of young and elderly healthy subjects before and after a bout of resistance leg exercise. The protocol included 10 min of light cycling for warm-up followed by three sets of eight repetitions followed by a fourth set to voluntary failure for each of the three exercises with resistance of 80% 1-RM. Results shown that level of mRNAs (IGF-1, IGFEBP5, ciliary neurotrophic factor, and MMP2, were significantly affected by aging and were greater in the young than elderly muscle. However, although this mRNAs were affected by exercise in young group, elderly muscles did not display any significant changes in gene expression post-exercise. Thus, aging muscle shows decreased levels at rest and in impaired response to exercise for various mRNAs for factor potentially involved in muscle growth and remodeling (49).

Another interesting study by Przybyla and colleagues (50) tested the hypothesis that aging alters the abundance and properties of skeletal muscle macrophages that will influence their functional response to acute resistance exercise. Total macrophages, as well as pro- and anti-inflammatory subpopulations and associated cytokines mRNAs were quantified in vastus lateralis biopsies from young and elderly males pre- and 72
hours post-exercise. Results show that in pre-exercise, young muscles to have a greater number of macrophages, whereas elderly muscle possess higher level of IL-1β, IL-1ra, and IL-10. Post-exercise, total macrophages did not change in either group, however the number of pro-inflammatory and anti-inflammatory subpopulations cells increased 55 and 29% respectively, but only in the young group. IL-1β, IL-10, and AMAC-1 also increased only in the young group. In summary, both pro- and anti-inflammatory subpopulation increased only in the young group suggesting that aging may be associated with a defective regulation of muscle macrophage function, both at baseline and in response to resistance exercise, that may limit muscle hypertrophy in older adults (50).

In 2001, Jozsi and colleagues (51) compared the gene expression profile as well as inflammatory profile of skeletal muscle form elderly compared to younger men at baseline and in response to a single bout of resistance exercise. A biopsy sample from the vastus lateralis was obtained from all the subjects one week prior and 24 hours after the single bout of exercise. The resistance exercise protocol consisted of five lower body exercises including the left and right knee extension, bilateral knee flexion, leg press, and squat exercise. Each subject completed 3x8 repetitions on each exercise at an intensity equivalent to 80% of 1-RM (51). Results shown a 2 to 4-fold higher of genes encoding the stress-responses proteins as well as proteins involved in cytoskeletal reorganization and DNA repair in older man. On the other hand, the expression of these genes was largely unchanged in old muscle following exercise. However, the expression of the majority of these genes increased 3 to 5-fold in the muscle of younger men in response to acute exercise such that expression in young and old muscle became comparable (51). Furthermore, the expression of several molecules characteristic of inflammation/injury that were not differentially expressed between old and young at baseline increased more robustly in muscle of younger as compared to older men following acute resistance exercise. Authors, shown 1.5 to 4 -fold increases in the expression of the inflammatory cytokine genes encoding IL-1β, macrophage colony-stimulating factor and RANTES in the muscle of younger as compared to older men after acute resistance exercise. Taken together, these results highlight the inability of muscles from elderly subjects to respond to resistance exercise (51).
In conclusion, even though not so many studies have investigated the inflammatory response to a single bout of different type and intensities of exercise in older people, the few evidence including gene expression in response to acute resistance exercise are all in one direction. Indeed, all the studies reported here have shown that older people are characterized by a higher gene expression of stress, injury and inflammatory biomarkers as well as total macrophages compared with younger people. However, the response of all this variables to single bouts of resistance training appeared blunted in older people compared with younger people, highlighting the inability of old muscle to respond adaptively to resistance exercise.

In Obesity. Even in the field of obesity, very few studies focused their attention on the acute inflammatory response to single bouts of different exercise types and intensities. Christiansen and colleagues in 2013 (52) publishes a study aimed to investigate if overweight and obese people, compared to lean individuals, displayed differences in level of inflammatory markers in circulation after acute exercise. The study enrolled 15 lean subjects and 16 overweight or obese individuals who completed 120 min of ergometer bicycling at 55-60% of maximal heart rate. Blood samples were obtained at baseline, after 60 and 120 min of exercise (52). Results shown that circulating IL-6, TNF-α, IL-8 and IL-15 all increased after 120 min where IL-6 and IL-15 increased in all subjects, but only IL-6 was significantly higher in overweight and obese individuals. IL-6 was positively correlated with body fat percentage. Also, IL-8 and TNF-α increased in overweight and obese but not in lean subjects. Authors concluded that systemic inflammatory response to acute exercise is different in lean compared to overweight and obese subjects, with more pronounced increase in inflammatory markers in overweight and obese individuals (52).

Van Pelt and colleagues (53) have recently published an interesting study aimed to examine the effects of acute exercise on mRNA expression of markers of lipid metabolism, inflammation, fibrosis, and hypoxia/angiogenesis in subcutaneous adipose tissue, as well as adipocyte cell size. This study included 8 active overweight and 12 sedentary overweight individuals. Abdominal subcutaneous adipose tissue biopsy samples were obtained before and one hour after one single session of aerobic
exercise (60 min at 65% VO$_2$peak). Results shown that the exercise session increased subcutaneous adipose tissue mRNA expression of VEGF in both groups. In addition the active group had greater mRNA expression of IL-6 compared with the sedentary group. Authors concluded that aerobic exercise may alter processes related to whole-body metabolic outcomes in obesity, such as angiogenesis and immune response, in the subcutaneous adipose tissue of overweight and obese adults (53).

Accattato and colleague (54), recently published a study aimed to assess antioxidant and inflammatory parameters, both at rest and after acute exercise, in sedentary young men with or without obesity. Thirty sedentary males were included and divided into 3 groups based on the BMI. Blood samples were collected before and after a 20-min run at 70% of their VO2max and measured for glutathione reductase, glutathione peroxidase, superoxidase dismutase, total antioxidant status and cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, IL-1α, IL-1β, TNF-α, MCP-1, VEGF, IFNγ, EGF) (54). Inter-group comparison demonstrated significantly higher glutathione reductase activity in severely obese subjects in the post-exercise period, as well as higher EGF levels in normal weight individual either before and after exercise. Intra-group comparison showed that the acute stress induced a significant increase in glutathione reductase activity in severely obese subjects only, a significant increase in MCP-1 in the normal weight and a decrease in EGF levels in all groups. Authors concluded that in sedentary individuals with different ranges of BMI, glutathione reductase and distinct cytokines are differently involved into the adaptive metabolic changes and redox responses induced by physical activity (54).

In summary, although there are very few study investigating the inflammatory response to single bouts of exercise in obese individuals, it seems that the results go in the same direction. Obese individuals are characterized by higher circulating cytokines and inflammatory gene expression compared with lean subjects, on the other hand the acute response to exercise seems to be higher in overweight and obese than in lean subjects. However, future research should aim to distinguish between the different inflammatory response to different type, intensity, and duration of exercise.
Endothelial Function

1. Endothelium

The endothelium is a single layer of cells that lines the entire cardiovascular system and produces vasoactive molecules including NO, which is responsible for smooth muscle cell relaxation and artery vasodilation (55). Endothelium regulates vascular homeostasis by elaborating a variety of paracrine factors that act locally in the blood vessel wall and lumen (56). Under normal conditions, the sum total effect of these endothelial factors is to maintain normal vascular tone, blood fluidity, and limit vascular inflammatory and smooth cell proliferation [2,3]. Endothelial cells also function as a selective sieve to facilitate bidirectional passage of macromolecules and blood gases to and from tissue and blood (57). Consequently, endothelium is a key regulator of the elastic properties of conduit vessels and its dysfunction has been identified as an early event playing a role in the development of atherosclerosis, and early cardiovascular disease [4,5].

2. Endothelial function

The importance of the endothelium was first recognized by its effect on vascular tone. This is achieved by production and release of several vasoactive molecules that relax or constrict the vessels, as well as by response to and modification of circulating vasoactive mediators such as bradykinin and thrombin (59). This vasomotion plays a direct role in the balance of tissue oxygen supply and metabolic demand by regulation of vessel tone and diameter, and is also involved in the remodeling of vascular structure and long-term organ perfusion. It as been long time since when was discovered and endothelium-derived relaxing factor: the nitric oxide (NO). NO is generated from L-arginine by the action of endothelial NO synthase (eNOS), this gas diffuses to the vascular smooth muscle cells and activates guanylate cyclase, which leads to cGMP-mediated vasodilation. Shear stress is a key activator of eNOS in normal physiology, and this adapts organ perfusion to changes in cardiac output. In addition, the enzyme may be activated by signaling molecules such as bradykinin, adenosine, vascular endothelial growth factor and serotonin (59). The endothelium also mediates
hyperpolarization of vascular smooth muscle cells via a NO-independent pathway, which increase potassium conductance and subsequent propagation of depolarization of vascular smooth muscle cells, to maintain vasodilator tone. Furthermore, endothelium modulates vasomotion, not only by release of vasodilator substances, but also by an increase on constrictor tone via generation of endothelin and vasoconstrictor prostanoids, as well as via conversion of angiotensin I to angiotensin II at the endothelial surface. These vasoconstrictor agents predominantly act locally, but may also exert some systemic effects and have a role in the regulation of arterial structure and remodeling [3,6]. Moreover, in normal vascular physiology, NO plays a key role to maintain the vascular wall in a quiescent state by inhibition of inflammation, cellular proliferation, and thrombosis as well as limiting oxidative phosphorylation in mitochondria (59). Laminar shear stress is probably the major factor that maintains this quiescent, NO-dominated, endothelial phenotype (59).

3. Endothelial dysfunction

The definition of endothelial dysfunction should probably be considered an endothelial activation, which may contribute to arterial disease when certain conditions are fulfilled. Endothelial activation represents a switch from a quiescent phenotype toward one that involves the host defense response. Interestingly, most cardiovascular risk factors activate molecular machinery in the endothelium that results in expression of chemokines, cytokines, and adhesion molecules designed to interact with leukocytes and platelets and target inflammation to specific tissue to clear microorganisms (59). The fundamental change involved in this process is a switch in signaling from an NO-mediated silencing of cellular processes toward activation by redox signaling. Reactive oxygen species (ROS) commonly lead to generation of hydrogen peroxide, which like NO, can diffuse rapidly throughout the cell and react with cysteine groups in proteins to alter their function. Because of the different chemistry involved, this results in very different consequences, such as phosphorylation of transcription factors, induction of nuclear chromatin remodeling and transcription genes, and protease activation. Also, eNOS which normally helps maintain the quiescent state of the endothelium, can switch to generate ROS in appropriate circumstances as part of endothelial activation (59).
In certain circumstances, chronic production of ROS may exceed the capacity of cellular enzymatic and non-enzymatic anti-oxidants, and thus contribute to vascular disease by induction of sustained endothelial activation. An important source of ROS is probably the mitochondrion, in which production of ROS and the dismuting capacity of mitochondrial superoxide dismutase are typically carefully balanced during oxidative phosphorylation. This may be disturbed during hypoxia or conditions of increased substrate delivery, such as occurs in obesity-related metabolic disorders or type I diabetes, which are characterized by hyperglycemia and increased circulating free fatty acids. Again, endothelial ROS signaling may be initiated by exposure to inflammatory cytokines and growth factor, and the interaction of the endothelium with leukocytes. Regardless of their source, the interaction between OS and NO sets up a vicious circle, which result in further endothelial activation and inflammation (59).

Prolonged or repeated exposure to cardiovascular risk factors can ultimately exhaust the protective effect of endogenous anti-inflammatory systems within endothelial cells. Therefore, the endothelium not only becomes dysfunctional, but endothelial cells can also lose integrity, progress to senescence, and detach into the circulation [6,7].

4. Assessment of endothelial function

Brachial artery ultrasound is a widely used, non-invasive measure of endothelial cell function, in particular of endothelium-dependent flow-mediated dilation (FMD)[3,7]. Practically, the forearm blood flow is occluded for 5 minutes using a blood pressure cuff maintained at a standard pressure. When the pressure is release, reactive hyperemia occurs. This results in shear stress-induced NO release and subsequent vasodilation (57). This method uses a stimulus that is particularly relevant physiologically for endothelium-dependent vasodilation (i.e. increases in laminar shear stress), the tangential force exerted by blood flow over the surface of endothelium (60).
5. Endothelial function in Aging

Several lines of experimental evidence indicate that vascular endothelial dysfunction develops with aging in humans in absence of clinical cardiovascular risk factors or cardiovascular disease. Impaired endothelial function, together with reduced fibrinolytic function, increased leucocyte adhesion and other markers of endothelial dysfunction have been observed in older adults (61). Moreover, evidence shows that FMD is impaired in older compared with younger healthy adults [10-12].

NO bioavailability. Data in both, human and animal studies, indicate that the impaired endothelial function with aging is mediated by a decrease in NO bioavailability. This is supported by the facts that the reduction in endothelial-function produced by pharmacological inhibition of NO production by eNOS is smaller with advancing age, and there no longer are significant age group difference in endothelial dilation in the absence of NO synthesis (62). The mechanisms underlying reduced NO-mediated endothelial dilation with age could involve deceased stimulus-evoked NO production, increased NO removal, or both. NO production is reduced in older compared with young adults under baseline resting conditions, as indicated by reduced vasoconstriction in response to infusion of L-NMMA. However, despite consistent observations of reduced NO bioavailability, analysis of arterial tissue in experimental animals indicated decreased, increased or unchanged eNOS expression and activation with aging [10,11]. In healthy humans, eNOS protein expression tends to be greater in vascular endothelial cells obtained from the brachial artery of older compared with young adults, whereas eNOS phosphorylated at Ser is significantly increased, suggesting a greater state of activation of the enzyme with aging. If so, such activation with age in healthy adults may represent an attempt to compensate for low NO bioavailability (62).

Endothelin-1 (ET-1). ET-1 is the most potent vasoconstrictor molecule produced by the vascular endothelium. Plasma ET-1 concentrations increase with age in some adults, ET-1 mediated vasoconstriction is augmented in older adults and synthesis of ET-1 is greater in cultured aortic endothelial cells obtained from older compared with young donors (62).
Inflammation. In vascular endothelial cells obtained from the brachial artery and/or antecubital veins of humans, expression of the proinflammatory nuclear transcription factor NF-κB, and pro-inflammatory cytokines IL-6, TNF-α, and MCP-1 are increased in older adults. Expression of MCP-1 and matrix metalloproteinases are greater in the thickened arterial intima of older compared with young adult donors obtained during autopsy (62). Moreover, among middle-aged and older adults, brachial FMD is inversely related to plasma markers of inflammation, including CRP, IL-6, and ICAM-1. Furthermore, in healthy overweight and obese middle-ages and older adults, inhibition of NF-κB signaling improves brachial artery FMD to near-normal young control levels by reducing oxidative stress, whereas inhibition of TNF-α restored endothelial-dilation in older rodents. Taken together, these observations suggest that inflammation contributes to the tonic suppression of endothelial-dilation with aging, perhaps by inducing oxidative stress (62).

Prostaglandins. Endothelial-dilation in response to prostacyclin is impaired in older compared with young adult, and this seems to be NO-dependent as since the difference is abolished by L-NMMA. Basal prostanoid vasodilation also is reduced in older adults. These observations suggest that the mechanisms involved may include increased expression of prostanoid vasoconstrictor proteins, and altered COX and prostaglandin H synthase activities (62).

In summary, aging is associated with vascular endothelial-dysfunction which appears to be mediated by reduced NO bioavailability and also possibly by decreased responsiveness to endothelial-released vasodilatory prostaglandins. Oxidative stress and inflammation are major “macro mechanisms” by which aging leads to reduced NO-bioavailability and consequent endothelial dysfunction. Vascular oxidative stress develops with aging as a result of increased production of ROS in the face of unchanged or reduced antioxidant defenses. Increases in the endothelial vasoconstrictor molecule ET-1 also seems to contribute to impaired endothelial dysfunction with aging.
6. Endothelial function in Obesity

Obesity is closely related with the development of type 2 diabetes, hypertension, dyslipidemia, and cardiovascular disease, among other medical problems. An important mechanism by which obesity leads to the development of all of the above metabolic and vascular diseases is the development of insulin resistance, which is typically defined as reduced insulin action in peripheral tissues (63). Indeed, adipose tissue is associated with increased production of free fatty acids, TNF-α, IL-6, resistin, and leptin that are known to reduce insulin action, as well as decreased production of adiponectin, a mediator known to improve insulin sensitivity (63). Furthermore, there is growing evidence that some of these mediators also have some direct and indirect effects on the vascular wall. In combination, all of the above abnormalities create a state of constant and progressive damage to the vascular wall, manifested by a low-grade progressive inflammatory process and endothelial dysfunction (63). The mechanisms by which insulin resistance leads to endothelial dysfunction are certainly multiple and complex. All major abnormalities that are part of the insulin resistance syndrome, such as hyperglycemia, hypertension, dyslipidemia, and altered coagulation/fibrinolysis, are directly and independently linked to endothelial dysfunction [13-16].

Free fatty acids (FFAs). An increased efflux of FFAs from the more lipolytically active intra-abdominal adipocytes leads to decreased insulin action in liver and skeletal muscle through mechanisms that may affect the intracellular insulin signaling cascade. Studies demonstrated that this process is closely associated with endothelial dysfunction in obese (63).

Insulin resistance. Insulin resistance also contributes to this complex cascade of events. In individuals with insulin resistance, and increase in insulin production by pancreatic β-cells usually occurs as an attempt to maintain normal plasma glucose levels. Hyperinsulinemia may promote lipogenesis and weight gain as fat cells are usually more sensitive to insulin action than skeletal muscle (63). Insulin is known to have a direct vasodilatory effect mediated through stimulation on NO production in endothelial cells. In the insulin resistant state, the ability of insulin to stimulate NO production in endothelial cells is diminished. Interestingly, the stimulation of NO production in endothelial cells and the stimulation of glucose uptake in muscle and fat tissue by
insulin occur through the phosphatidylinositol 3-kinase and Akt pathway. In contrast, other effects of insulin action on the vasculature, including the stimulation of migration and growth of smooth muscle cells and the production of PI-1, are mediated through the mitogen-activated protein kinase pathway. Theoretically, one could speculate that individuals who exhibit insulin resistance syndrome may have an abnormality in NO production by endothelial cells and, at the same time, a constant stimulation of proatherogenic changes in the vasculature in response to hyperinsulinemia that frequently accompanies the syndrome (63).

Other mechanisms. All inflammatory markers as well as production of ROS have direct effect in the vasculature. TNF-α may have a direct effect in the vasculature. IL-6 is a potent stimulus for the production of CRP in the liver, which may have some direct deleterious effects in the vascular wall. CRP is considered an excellent marker of low-grade inflammation in the vascular wall and a well-recognized mechanism in the development of atherosclerosis. Yet, activation of renin-angiotensin system and elevation of ET-1 contribute to endothelial dysfunction in obese individuals (63).

7. Endothelial function and Exercise

Early studies on hemodynamic forces highlight the fact that increased blood flow causes more branching in blood vessels, thus fewer branches develop in blood vessel where blood flow is lower. This early observation suggested that hemodynamic forces, broadly defined as mechanical forces associated with flowing blood (i.e., shear stress and pressure), were important in adaptation of the vasculature (64). More recently, the endothelium has provided a focus for research given its strategic placement between the flowing blood and artery wall and crucial role in the progression and development of atherosclerosis. It is now understood that vascular adaption is dependent on an intact, functional endothelium and that hemodynamic stimuli induce functional and structural changes in the arterial wall via endothelial cell signal transduction (64).

It has been observed that the increase in cardiac output as a result of exercise raining is accompanied by a corresponding rise in vascular conductance, the latter mediated by functional and structural adaptations in conduit, resistance, and microvessels. Changes in the vasculature are associated with decreased cardiac afterload at rest and
during submaximal exercise, which enhances ventricular function and myocardial oxygen demand. This integrative physiological perspective emphasizes the key role played by changes in the vasculature in response to exercise training.

**Pressure effects.** Exercise increases systolic pressure, and as arterial waves propagate, pulse pressure changes due to interactions between the segmental arterial compliance and pressure wave harmonics. Blood pressure can influence vascular cells in at least two ways. First, affecting the growth rate of endothelial cell. Second, by distending the arteries, thereby stretching vascular cells in the wall. Because arteries are compliant, changes in pressure consequently produce circumferential stress (64). In response to cyclic circumferential strain resulted from the pulsatile nature of arterial blood pressure, endothelial cells respond morphologically with alignment of cells perpendicular to the force vector, subsequently followed by phenotypic changes. The mechanisms by which the endothelium recognizes and transduces mechanical stimuli involves various signaling systems. This complex system of mechanosensors converts mechanical stimuli into chemical signals that lead to the activation of intracellular signaling cascade, activating genes that regulate the fate of the endothelial cells and smooth muscle cells (i.e., proliferation, migration, and apoptosis) (64).

**Endothelial shear stress.** Any increase in flow that increases the drag subsequently triggers acute dilation, a functional change that tends to homeostatically modify the initial increase in shear. When exposed to prolonged periods of change in flow and shear, vessel remodeling can occur, whereby local drag is returned toward the norm by virtue of structural arterial modification (65). The endothelium is essential in mediating structural arterial adaptations. Indeed, through NO-dependent pathways, plays a role in remodeling of vessel diameter in response to increases shear stress. Mechanotransduction at the luminal surface of the endothelium is initiated by shear stress detection by ion channels (K⁺, Ca²⁺, Na⁺, Cl⁻), cell membrane receptors, G protein, caveolae, and the plasma membrane lipid bilayer. Furthermore, the lumen is lined with glycocalyx that is responsible for the shear stress-induced NO production. A possible explanation for the involvement of multiple, distinct types of mechanotransduction is that shear stress, in contrast with pressure, is a relatively weak force. Therefore, highly sensitive mechanisms seem necessary to sense the shear stress, including the detection of complex patterns of shear (64).
Thus, exercise has complex effects on hemodynamics that result in increased blood flow and shear stress, increased frequency of pulsatile changes in pressure and flows, and increased arterial systolic and pulse pressure. These complex hemodynamic effects of exercise can contribute to the expression of pro-atherogenic vascular phenotypes.

7.1 Exercise-induced chronic adaptations

*General considerations.* Studies in subjects who exhibit impaired endothelial function, such as those possessing cardiovascular risk, or with established cardiovascular disease, have typically revealed improvement in conduit artery function, measured as the FMD, following exercise training [17,19-21]. The strong inverse relation between pretraining FMD and improvement in FMD suggested that conduit artery endothelial function is highly amenable to improvement. Furthermore, exercise is able to improve coronary artery diameter, coronary blood flow responses to intracoronary administration of acetylcholine, and coronary blood flow reserve to adenosine infusion. The mechanisms underlying the positive adaptation of vascular function to exercise may be based on the upregulation of eNOS expression. Indeed, studies aimed to understand the hemodynamic stimuli on vascular adaptation to training, revealed that after 4 weeks of exercise, arteries exhibited 2-fold increase in eNOS expression and 4-fold higher eNOS Ser phosphorylation. The upregulation of eNOS Ser is of particular relevance, since phosphorylation of eNOS at position Ser is linked to shear stress transduction (66). These data suggest that exercise causes activation of eNOS, through a shear stress-induced Akt-dependent increase in eNOS phosphorylation on Ser, ultimately leading to improvement in endothelial function.

Other mechanisms by which exercise could induce positive adaptation in vascular function include the upregulation of the vasodilator prostacyclin (PGI2), and the inhibition of ET-1. Studies have demonstrated the 8 weeks of exercise training in hypertensive participants increased the formation of interstitial adenosine and PGI2, which may contribute to improved vascular responses after exercise. Furthermore, although ET-1 and angiotensin II do not importantly contribute to the regulation of baseline vascular tone, it has been demonstrated that exercise training is also associated with decreased plasma and muscle levels of ET-1 and angiotensin II.
In summary, exercise is a potent stimulus able to induce chronic adaptation at vascular level. The majority of the mechanisms involved in the long-term adaptation to exercise seem to involve endothelium and NO-mediated endothelial function.

*In Aging.* Landers-Ramos and colleagues in 2005 (67) published an interesting study demonstrating that already 10 consecutive days of exercise may improve endothelial function in older adults. The study included 11 healthy subjects (61±2 years) who took part in 60 minutes of aerobic exercise training at 70% of maximal oxygen consumption for 10 consecutive days. Before and after the training endothelial function was measured as FMD of the brachial artery. After training FMD was significantly improved (10%±1.3 before vs. 16%1.4% after training). Authors concluded that even short-term aerobic exercise training can have significant impact on endothelial function and consequent cardiovascular risk disease in aged population (67).

Another interesting study published in 2011 by Pierce and colleagues (68) indicated that habitual aerobic exercise affects endothelial-dependent dilation in middle-aged and older men and post-menopausal women. Participants (55-79 years) took part in 8 weeks of brisk walking (6 days/week for 50 min/day), before and after brachial FMD was measured. Results show that in sedentary middle-aged and older adults without cardiovascular diseases, 8 weeks of brisk walking increased brachial artery FMD by 50%, but did not change FMD in the post-menopausal women. Authors concluded that regular aerobic exercise is consistently associated with enhanced brachial artery FMD in middle-aged/older men, but not in post-menopausal women (68).

Slightly differently, in 2005 Franzoni and colleagues (69) published a research evaluating the relationship between long term physical activity, plasma antioxidant status, and conduit artery endothelial function in younger and older healthy men. The study included 16 young and 16 older athletes and a group of age-matched sedentary counterparts. Brachial artery FMD was measured as well as endothelium-independent response to glycerol trinitrate (GTN), plasma malondialdehyde (MDA) and antioxidant capacity as total oxyradical scavenging capacity (TOSC). Results indicated that FMD was lower in sedentary older subjects as compared with older athletes and both sedentary and athletically trained young subjects. Furthermore, sedentary older
subjects showed higher MDA levels and lower plasma antioxidant capacity as compared with the other groups, whereas in older athletes MDA levels and antioxidant capacity were similar to those observed in the young groups. In the whole groups, FMD but not GTN was negatively related to age and directly related to maximal oxygen consumption and TOSC. Authors concluded that regular physical activity is associated with preserved antioxidant defenses and endothelial function in older individuals (69).

A similar study by Pierce and colleagues (70) demonstrated that habitually exercising older men do not show age-associated vascular endothelial oxidative stress which is related to the preserved endothelium-dependent dilation. The study included 13 older habitually exercising men (62±2 years) and 28 sedentary men (63±1 years). Results show that exercising men had higher physical activity and maximal oxygen consumption as compared with the sedentary counterparts. Brachial artery FMD was greater, as well as marker of oxidative stress (nitrotyrosine) was 51% lower in the exercising group vs. sedentary older men. Furthermore, this was associated with lower endothelial expression of the oxidant enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the redox-sensitive transcription factor nuclear factor kappa B (NFkB). Author concluded that older men who exercise regularly do not demonstrate vascular endothelial oxidative stress, and this may be a key molecular mechanisms underlying their reduced risk of cardiovascular disease (70).

In an animal study, Durrant and colleagues (71) demonstrated that voluntary exercise restores endothelial function in conduit arteries of old mice. The study included young and old cage control and voluntary exercising mice. Age-related reduction in maximal carotid artery endothelial-dependent dilation to acetylcholine and NO-dependent endothelial-dilation were restored after 14 weeks of voluntary exercise in older mice. Nitrotyrosine, a marker of oxidative stress, was increased in aorta with age, but was markedly reduced in old voluntary exercising mice. Furthermore, aortic superoxide dismutase (SOD) activity was greater, whereas NADPH oxidase protein expression and activity were lower in old voluntary exercising mice vs. old cage control mice. Authors concluded that voluntary aerobic exercise restores the age-associated loss of endothelial-dependent dilation by suppression of oxidative stress via stimulation of
SOD antioxidant activity and inhibition of NADPH oxidase superoxide production (71).

In Obesity. Kelly and colleagues (72) demonstrated that 8 weeks of stationary cycling improves fitness, HDL cholesterol and endothelial function in a group of overweight children. Twenty-five overweight children were included in the study and assessed for brachial artery FMD, nitroglycerin-induced dilation, CRP, lipids, glucose, insulin, oral glucose tolerance, body composition, aerobic fitness, and blood pressure. Participants were randomly assigned to either 8 weeks of stationary cycling (30 min at 50-60% of peak oxygen consumption, 4 times per week) or to a non-exercising control group. After the training protocol, significant improvements were observed in the exercise group compared with the control group for HDL and FMD but not for the other variables (72).

In 2011, Vinet and colleagues (73) investigated the effect of short-term low intensity exercise training in middle-ages obese men. Ten individuals were recruited and tested for brachial FMD before and after 8 weeks of individualized low-intensity program including three 45-min sessions of walking or cycling at home, without any dietary intervention. Compared with normal-weight men exhibited poorer FMD (5.7±0.4% vs. 3.3±0.5%). However, exercise training normalized FMD values in the obese group (3.3±0.4% before vs 5.3±0.5% after training). Authors concluded that in obese middle-aged men conduit vessel reactivity is depressed, but a short-term low intensity exercise training improves endothelium-dependent vasodilation (73).

Another recent study by Dow and colleagues (74) show that regular aerobic exercise reduces ET-1-mediated vasoconstrictor tone in overweight and obese adults. The aims of the study included to determine if regular aerobic exercise training reduced ET-1-mediated vasoconstrictor tone as well as to determine if the reduction of ET-1-mediated vasoconstriction contributed to exercise-induced improvement in endothelium-dependent vasodilation in overweight and obese adults. Forearm blood flow (FBF) in response to intra-arterial infusion of selective ET<sub>A</sub> receptor blockade (BQ-123), acetylcholine in the absence and presence of ET<sub>A</sub> receptor blockade were determined before and after a 3-month aerobic exercise training intervention in 25
overweight/obese adults. The vasodilator response to BQ-123 was significantly lower (25%) and the FBF responses to acetylcholine were 35% higher after exercise training. Before the exercise intervention, the co-infusion of acetylcholine plus BQ-123 resulted in a greater vasodilator response that acetylcholine alone. However, after the exercise intervention the FBF response to acetylcholine was not significantly increased by ET_A receptor blockade. The authors concluded that regular aerobic exercise reduces ET-1-mediated vasoconstrictor tone in previously sedentary overweight and obese adult and this mechanism may be very important in the exercise-induced improvement in endothelium-dependent vasodilator function in this population (74).

7.2 Exercise-induced acute response

General considerations. At the onset of exercise, blood flow and shear stress markedly increase in active regions in an exercise-intensity-dependent manner to meet increased metabolic demand [31,32]. Moreover, local vasodilator mechanisms along with increases in arterial pressure and cardiac output contribute to exercise hyperemia, leading to significant increases in shear stress in the active areas during exercise (76). Evidence have demonstrated that acute exercise can lead to an immediate increase in endothelium-mediated dilation (76). Tinken and colleagues in 2010 (65) have examined brachial artery vasodilator function, using the FMD test, before and after 30-min handgrip exercise, cycle exercise, and forearm heating. After successfully increasing shear stress levels, FMD significantly improved. Given the marked differences between the three interventions in pulse pressure and pulse frequency, these results highlight the importance of shear stress in mediating acute change in endothelium-mediated dilation. Moreover, given the intensity-dependent relationship between exercise and hyperemia, higher intensity exercise may lead to incremental increases in post-exercise vascular function. However, most studies that have examined this hypothesis have reported decrease in vascular function immediately after high-intensity cycle exercise, which may be followed by a rebound recovery of function one or more hours after the cessation of the bout [32,33]. In addition, to increases in shear stress, strenuous exercise also mediated other effects such as the production of ROS and activation of the sympathetic nervous system that may mitigate beneficial shear stress effect right after exercise.
Thus, although long-term exercise-induced vascular adaption adaptation is generally accepted; the acute response to exercise is still matter of debate. Certainly, the type, intensity, and duration of exercise is crucial in determining acute endothelium-dependent vascular response to exercise.

In Aging, Iwamoto and colleagues (77) recently published a study aimed to investigate the potential intensity-dependent effects of an acute bout of exercise on conduit and resistance artery function in healthy older adults. Eleven healthy older adults (66±1 years) were included in the study and completed 30 minutes of recumbent cycling at 50-55% (low intensity) and 75-80% (high intensity) of their age-predicted maximal heart rate on two separate days. Brachial artery FMD and reactive hyperemia (RH) were taken at baseline, ten minutes post-exercise, and one-hour post exercise. Results show that FMD was enhanced 10 minutes after high intensity, but not low-intensity exercise. Peak and total blood flow during RH were enhanced 10 mins pot-exercise for both intensities. However, the magnitude of change in peak and total blood flow were not different between exercise intensities. Independent of exercise intensity, FMD returned to baseline one-hour after exercise. The authors concluded that high-intensity exercise acutely enhances conduit artery function in healthy older adults as well as an acute bout of exercise enhances artery function independent of intensity (77).

Another recent study by Bailey and colleagues (78) examined the hypothesis that exercise intensity alters the brachial FMD response in elderly men and is modulated by maximal oxygen consumption (VO2max). The study included 47 elderly men who were stratified into lower (VO2max=24.3±2.9 ml•kg⁻¹•min⁻¹) and higher fit group (VO2max=35.4±5.5 ml•kg⁻¹•min⁻¹) after a cycling peak power output (PPO) test. In randomized order, participants undertook moderate-intensity continuous exercise (40% PPO) or high-intensity interval cycling exercise (70% PPO), or no exercise control. Brachial FMD was assessed at rest, 10 and 60 min after exercise. FMD increased after moderate-intensity exercise in both groups and normalized after 60 min. in the higher fit group, FMD was unchanged immediately after high-intensity and increased after 60 min, which was correlated with VO2max. Authors concluded that VO2max modulates the FMD response following high-intensity exercise but not following moderate-intensity exercise. Moreover, the sustained decrease in FMD in
the lower fit group following high-intensity exercise may represent a signal for vascular adaptation or endothelial fatigue (78).

McGowan and colleagues in 2006 (79) investigated the acute vascular response to unilateral isometric handgrip exercise in older adults with hypertension. Seventeen subjects were recruited and performed 2-min unilateral handgrip contraction at 30% of maximal voluntary effort. Pre- and post-exercise FMD was measured. Results shown that FMD decreased following an acute bout of isometric handgrip exercise. Authors concluded that acute isometric handgrip exercise attenuated brachial FMD which could be attributed to anti-hypertensive medications (79).

In Obesity. In 2008, Harris and colleagues (80) conducted a study investigating the interaction of IL-6 and TNF-α on endothelial function in response to acute exercise in overweight men exhibiting different physical activity profiles. Sixteen overweight men (8 active and 8 inactive) performed three different intensity acute exercise treatments. Brachial FMD and subsequent blood samples were taken pre-exercise and one-hur following the cessation of exercise. Independent of exercise intensity, the active group displayed a 24% increase in FMD following acute exercise compares to a 32% decrease in the inactive group. Elevated concentrations of IL-6 following moderate, and high intensity acute exercise were observed in both groups. TNF-α levels were unchanged in response to acute exercise. Authors concluded that FMD response to acute exercise is enhanced in active men who are overweight, whereas inactive men who are overweight exhibit an attenuated response. The interaction of IL-6 and TNF-α did not provide insight into the physiological mechanisms associated with the disparity of FMD observed between groups (80).

Franklin and colleagues (81) recently published a study aimed to determine if a single bout of strenuous weight lifting (SWL) reduced endothelium-dependent vasodilation among sedentary obese women. This study included 9 obese and 8 lean sedentary young women. Brachial FMD was measured immediately before and after SWL. Sublingual nitroglycerin (NTG) was used to determine brachial artery endothelium-independent vasodilation following SWL. Results show that Brachial FMD was significantly reduced in obese and lean women after SWL. There was no difference in
the magnitude of change pre-and post-exercise between groups. Dilation to NTG was lower in obese compared to lean subjects and associated with body weight. Author concluded that endothelium-dependent vasodilation is reduced in women after acute resistance exercise. Dilation to NTG were lower in obese compared to lean woman and associated with body weight suggesting that changes in sensitivity of blood vessel to NO occurs during obesity (81).

Hallmark and colleagues, in 2014 (82) examine the effects of exercise intensity on acute changes in endothelial function in lean and obese adults. Sixteen lean and ten obese physically inactive adults were recruited and studied during 3 randomized sessions, control session, moderate-intensity exercise, and high-intensity exercise. FMD was assessed at baseline, and 1, 2, and 4 hours post-exercise. Results shown that lean subject exhibited greater FMD than obese subjects. Moreover, in the obese group a trend was observed for increases in FMD at 2-, and 4-hours after moderate-intensity exercise. For lean subjects, FMD was significantly elevated at all time point after high-intensity exercise. Authors concluded that in lean adults, high-intensity exercise acutely enhances endothelial function while moderate-intensity exercise has no significant effect above that seen in the absence of exercise. The FMD response of obese adults is blunted compared to lean adults (82)
Experimental Study.

Inflammation and vascular function in aging and obesity: chronic and acute exercise-induced adaptations.

Although exercise is recognized to induce long-term positive adaptation in aging and other chronic conditions, results about its effects on inflammatory profile and vascular functions are still mate of debate. Also, the acute inflammatory and vascular response to exercise in obese people is not clear. In light of that, this study, throughout two sub-studies provides evidence about the long-term effect of exercise practiced for several years on inflammation and vascular function in elderly non- and obese subjects, as well as it provides preliminary data about the acute inflammatory and vascular response to different kind of exercise in an obese population.

Therefore, in the first sub-study a cohort study was applied to investigate the effect of regular structured exercise on inflammatory profile and vascular function in non-obese and obese elderly individuals who regularly exercise.

Additionally, since several studies report that inflammatory and endothelial response to exercise depend on intensity, type and duration of the exercise conflicting results from different studies might be due to different exercise (type, intensity, and duration) performed by subjects. Thus, in the second sub-study a crossover study was used to investigate the inflammatory and vascular response to different exercise sessions that differ for types and intensity, in obese individuals, compared with normal weight individuals.
Exercise-induced chronic adaptations of inflammatory profile and vascular function during aging and in obesity.

1. Hypothesis

Our working hypothesis was that regular structured exercise would have helped in maintaining a low-inflammatory profile as well as maintaining vascular function in non-obese and obese elderly individuals. Moreover, we hypothesized that regular structured exercise would have helped in contrasting the malefic effects of obesity on endothelial and inflammatory profile.

2. Methods

Subjects. The study population included 70 physically active older adults (30 females, 40 males, mean age 70±5 years) (Table 1) who habitually took part in a structured, supervised exercise program. Subjects were included in the study in the absence of smoking history, atherosclerotic vascular disease, heart failure and liver, renal or inflammatory disease. Subjects were informed about testing procedures, possible risks and discomfort that might ensue and gave their written informed consent to participate in accordance with the Declaration of Helsinki, as part of a protocol approved by the Institutional Review Board of the Azienda Ospedaliera Universitaria Integrata of Verona, Italy.

During one-day preceding tests, the subjects were refrained from training and maintained their normal diet. Laboratory tests were carried out on the same day in similar conditions. Room temperature was 22-24°C and relative humidity was 50%. All the ultrasound studies were performed by a single experienced vascular sonographer who was unaware of the clinical and laboratory characteristics of the subjects. Height and weight were recorded by means of scale and stadiometer (Seca, Hamburg, Germany) and Body Mass Index (BMI) was calculated as weight(kg)/height(m)^2.

Blood samples and analysis. Venous blood samples were drawn from an antecubital vein and collected into EDTA tubes for analysis of inflammatory profiles. The samples were centrifuged for 10 minutes at +4°C with 2500xg. Plasma was kept at -80°C until
analyzed for IL1-α, IL-1β, IL-6, IL-8, IL-10, TNF-α, MCP-1, which were determined by means of commercially available MILLIPLEX multi-analyte panel (Merk Millipore, Darmstadt, Germany) following the manufacture’s recommendation. CRP was measured using an ELISA commercial kit (DBC-Diagnostics Biochem Canada Inc., London, Canada).

*Flow-mediated dilation (FMD) test.* FMD test was performed in a quiet room in abstinence from alcohol, and caffeine for at least 12 h (83). High-resolution ultrasound was used to image the brachial artery at rest and after 5 min of ischemia. All the FMD were performed with the participant in the supine position, with the right arm extended at an angle of ~90° from the torso. The brachial artery was imaged using a high-resolution ultrasound system Logiq-7 ultrasound Doppler system (General Electric Medical Systems, Milwaukee, WI, USA). The ultrasound Doppler system was equipped with a 12-14 MHz linear array transducer. The brachial artery was imaged 5-10 cm above the antecubital fossa in the longitudinal plan, and the diameter was determined at 90° angle along the central axis of the scanned area. When an optimal image was acquired, the position was maintained for the whole test and all scans were stored for later analysis. After baseline brachial artery imaging, a blood pressure cuff was placed around the forearm and inflated to 100 mmHg above systolic pressure for 5 min. Brachial artery images were obtained continuously 30s before and 2 min after cuff release (84). The brachial artery images were analyzed by a blinded investigator by means of FloWave.US (85). Arterial diameter was measured as the distance (mm) between the intima-lumen interfaces for the anterior and posteriors walls. FMD was expressed in relative changes in post-reactive hyperemia diameter compared to the baseline diameter.

*International Physical Activity Questionnaire (IPAQ).* The IPAQ questionnaire is a self-report questionnaire that estimates physical activity in the last seven days. Using the IPAQ scoring system, the total number of days and minutes of physical activity were calculated for each participants. The IPAQ records the activity in four intensity levels: sitting, walking, moderate intensity (i.e., leisure cycling), and vigorous intensity (i.e., running or aerobics) (86).

*Supervised exercise training.* Individuals included in the study habitually exercised at the “Silver Fitness” program, going on at the Department of Neurosciences, Biomedicine
and Movement Sciences, Section of Movement Sciences, University of Verona, Italy. All individuals exercised at least twice a week for 90 minutes combining moderate intensity endurance and resistance training. Sessions usually started with 15 minutes of warm up, which included active joint mobilization and walking on treadmill or cycling at preferred speed. Then, individuals performed two 15-minute endurance exercises (either on cycle ergometer, or treadmill, or arm cranking ergometer) at 70% of maximal heart rate (calculated using the Karvonen formula: 220-age in years). Subsequently, individuals performed 3 sets of 8 to 15 reps of resistance exercises at 60-75% of 1 repetition maximum (1RM). 1RM was determined by means of the Brzycki method for all the isotonic ergometers included in the training (i.e.: chest press, lat machine, leg press and others, Technogym, Gambettola, Italy) during the first training session of the year. Exercise sessions ended with stretching exercises for all the muscle involved in the training. All training sessions were supervised by kinesiologists, with a ratio of 2:12.

Statistical analysis. Data are expressed as mean ± SD and minimum-maximum range. Analysis of variance was used to assess mean differences between groups. Differences were considered significant at values of p<0.05. Interaction between variables were calculated by correlation and multiple regression analysis. All statistical analysis were performed with Sigma STAT 4.0 (Systat Software, Chicago, IL).

3. Results

Characteristics of the participants. Table 1 displays the main characteristics of the subjects participating in the study, including amount of physical activity, physical performance, vascular function and inflammatory profile. Females and males did not differ for any of the parameters measured except for weight, height, and performance at the 6MWT (Table 1), with male being little bit faster than females. On average, individuals have been taking part in the exercise program since 5±1 years, involved 3±1 times per week, for a total amount of 270±60 minutes per week.

Subgroups analysis. The whole group was first stratified for age in tertiles: I tertil, 65±3 years (n=23; f=12; m=11), II tertil, 72±2years (n=24; f=13; m=11), and III tertil, 76±2 (n=23; f=5; m=18) (Table 1). The three subgroups did not differ for BMI, blood
pressure, amount of physical activity in terms of years of activity, times a week, or minutes a week, and performance. Vascular function did not differ significantly between groups. About the inflammatory profile, circulating CRP was not significantly different between groups. The III tertile subgroup showed significant differences in IL-10 levels, as compared with the I and II tertile subgroups, showing significantly lower level of IL-10. Plasma II-1ra and II-1β showed the same trend as IL-10, indeed significant differences were found between III tertile and I and II tertiles. IL-6 exhibited a similar trend, although statistical difference was found even between I and II tertiles. Plasma IL-8 was not significantly different between groups. MCP-1 was found to be significantly higher in III tertile compared with I tertile. Finally, TNF-α did not exhibit significant differences between groups (Table 1).

The group was then stratified for BMI in three subgroups: normal weight, BMI=18-24.9 (n=25; f=18; m=7); overweight, BMI= 25-29.9 (n=35; f=9; m=26); and obese, BMI>30 (n=10; f=3, m=7) (Table 1). The three groups did not differ for age, nor for the amount of physical activity. Significant differences between the three groups were found as regards the performance, with lower speed for higher BMI. The same trend was found for vascular function, with significantly lower FMD for higher BMI. As regards the inflammatory profile, plasma CRP, MCP-1, and TNF-α were significantly different between groups, with higher values for higher BMI. II-1ra, II-1β, and II-8 levels did not exhibit differences between groups. IL-6 was significantly lower in the obese subgroup as compared with both normal weight and overweight subjects (Table 1).

Correlations. Table 2 and Figure 1 show the correlations between age, BMI, amount of physical activity and the other variables examined, including blood pressure, vascular function, physical performance, and inflammatory profile. Age, showed significant inverse linear correlations with 6MWT (Figure 1, panel A), as well as with II-1ra, II-1β, II-6, and II-8 levels indicating a decrease in all this variables with aging (Table 2). As regards BMI, significant direct linear correlations were found with systolic and diastolic blood pressure, FMD (Figure 1, panel D), 6MWT (Figure 1, panel B), CRP (Figure 1, panel F), as well as with MCP-1 (Figure 1, panel H), and TNF-α (Figure 1, panel I) levels, indicating an increase in all these variables with body weight increase (Table 2). The amount of physical activity, summarized as minutes per week, showed...
significant linear correlations with FMD (Figure 1, panel N), plasma IL-ra, and IL-8 (Table 2).
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Note: BMI: body mass index, kg/(m$^2$). PA: physical activity (min/week); Sys: systolic blood pressure, mmHg; Dia: diastolic blood pressure, mmHg; FMD: flow-mediated dilation, %; 6MWT: 6-minute walking test, km/h; CRP: C-reactive protein, pg/mL; IL: interleukin, ng/mL; MCP-1: monocyte chemoattractant protein-1, ng/mL; TNF-a: tumor-necrosis factor-a, ng/mL.

$r$ = Pearson's correlation coefficient;
$p$ = p-value
Figure 1. Pearson's correlations between variables. Age (panels A, C, E, G, I), BMI (panels B, D, F, H, I), and level of activity (panels M, N, O, P, Q) were plotted against 6MWT, FMD, CRP, MCP-1, and TNF-α.
4. Discussion.

Although the beneficial effect of exercise on healthy aging as well as on chronic conditions has already been investigated, the effect of aging and obesity on exercise-induced adaptation still need to be clarified. In particular, the exercise-induced adaptations of endothelial and inflammatory profile in aging and obesity need to be understood. In the present study, we measured endothelial function and inflammatory profile in habitually exercising obese and non-obese older adults who have been taking part in a structured, supervised exercise program for several years. Partially in accordance with our hypothesis, the effects of aging on endothelial function and inflammatory profile were remarkably blunted in exercising individuals but on the other hand, the effect of obesity on the investigated variables did not seem to be counteracted by the exercise. To the best of our knowledge, this is the first study to demonstrate that regular, structured supervised physical activity not only can reduce the age-associated loss in endothelium dependent vasodilation but also can blunt the development of a pro-inflammatory status in non-obese elderly individuals.

Evidence about the efficacy of exercise on vascular functions during aging. Emerging literature has underlined the positive effects of exercise in the aging process including the reduction of cardiovascular risk factors, as well as endothelial-dependent vasodilation (70,74,87,88). For instance, DeSouza et al. (74) examined forearm blood flow responses to intra-arterial infusion of acetylcholine and sodium nitroprusside in young and older adults who were either sedentary or endurance exercise-trained. Authors reported a reduction of about 25% in the older sedentary subjects compared with the young group. In contrast, there was no age-related difference in the vasodilatory response to acetylcholine among the endurance-trained men. The forearm blood flow at the highest acetylcholine dose was almost identical in the older adults and young endurance-trained groups. Furthermore, Welsh et al. (89) examined brachial artery FMD in a group of elderly individual who were classified by their performance on the physical functional performance (PFP-10) test (Class I, Class I, and Class III). Authors reported that brachial artery FMD was associated with total PFP-10 score and age, and it was significantly different between the three Classes. Our results are in accordance with those studies (74,89). Indeed, in our group of individuals who have been taking part in a structured, supervised- exercise program the endothelial-dependent brachial
arterial FMD does not decline linearly with the advancing age. Moreover, results suggest a linear relationship between brachial artery FMD and the quantity of physical activity, independently from aging.

Those results may be due to different reasons. First of all, mechanistically peripheral artery function changes when endothelium repeatedly experiences elevated levels of shear stress (90). When this stimulus is constantly repeated over the weeks, months or even years, positive adaptation may occur and can be maintained. Exercise has a complex effect on hemodynamics that results in increased blood flow and shear stress, increased frequency of pulsatile changes in pressure and flows and increased arterial systolic and pulse pressure. These complex hemodynamic effects of exercise can contribute to the expression of pro-atherogenic vascular phenotypes over the time (64). Indeed, mechanotransductions at the luminal surface of the endothelium is initiated by shear stress detection by ion channels, cell membrane receptors, G proteins, and the plasma membrane lipid bilayer, as well as specific structures responsible for shear stress-induced NO production. Moreover, about this Hambrecht et al. (66) studied the impact of cycle exercise training on the internal mammary artery of CAD patients. Data from the study showed a 2-fold increase in eNOS expression and 4-fold higher eNOS Ser phosphorylation after the period of training. The upregulation of eNOS Ser is of particular interest, since phosphorylation of eNOS at position Ser is linked to shear stress transduction. Also, a correlation was present between improvement in endothelial function in vivo and shear-dependent eNOS phosphorylation. These results suggest that exercise causes activation of eNOS, through a shear stress-dependent increase in eNOS phosphorylation Ser, ultimately leading to improvement in endothelial function (66).

Another study by Taddei et al. (87) investigated the effect of long-term physical activity on age-related endothelial dysfunction comparing sedentary and active elderly subjects with younger subjects. Authors investigated the forearm blood flow modification induced by intrabrachial acetylcholine, at baseline and during infusion of L-NMMA, which is a nitric oxide-synthase inhibitor. The results of this study suggested that regular physical activity can at least in part prevent the age-induced endothelial dysfunction, and the beneficial effect of exercise might be related to preservation of
NO availability by a mechanisms probably linked to oxidative stress and the consequent NO breakdown (87).

Our data are in agreement with the results achieved in other studies including not only older active subjects but even older endurance athletes showing that long-term regular physical activity can lead to positive adaptation of vascular system helping in the maintenance of vascular health during the aging process and reducing cardiovascular risk factors.

Evidence about the efficacy of exercise on inflammatory profile during aging. Recent literature started to focus on long-term physical activity and inflammatory biomarkers in older individuals, but results are still conflicting (39,41,42). Ellosua et al. (41) investigated the association between physical activity and physical performance, and inflammatory biomarkers in older individuals. The study included thousand-four subjects aged 65 years or older, and information on self-reported physical activity during the previous year were collected. Eight-hundred forty-one participants performed a 400-meter walking test to assess physical performance and plasma concentrations of inflammatory biomarkers were determined. Results showed that compared to sedentary men, men practicing light and moderate physical activity had a significantly lower CRP level, while only those practicing moderate-high physical activity had significantly lower IL-6, and TNF-α level (41). In women, those participating in light and moderate-high physical activity had significantly lower IL-6 levels, but only those performing moderate-high physical activity had significantly lower CRP level (41). At the contrary, Beavers et al. (42) studied the effect of 12-month physical activity intervention on inflammatory biomarkers in elderly men and women. In the study 424 elderly, age 70 to 89 years were included and randomized to either 12-month moderate-intensity physical activity intervention or a successful aging health education intervention. Biomarkers of inflammation were measured at baseline, 6 and 12 months (42). Results shown that IL-8 was the only inflammatory biomarker affected by an intervention of physical activity. More over, Della Gatta et al. (43) examined the expression of cytokines both at rest and following bout of isokinetic exercise performed before and after 12 weeks of resistance exercise training in young and elderly men. Protein expression of various cytokines was determined in muscle homogenates. Results showed that the inflammatory response were not significantly
different between young and elderly men, either before or after 12 weeks of exercise training. However, compared with the young men, the expression of pro-inflammatory cytokines 2 hours post-exercise tended to be greater in the elderly men prior to training, but training attenuated this difference (43). Authors concluded that the inflammatory response to unaccustomed exercise increase with age, but regular exercise training may help to normalize the inflammation response (43).

Our results are partially in agreement with previous findings. Indeed, in the group taken into account in this study several pro-inflammatory cytokines such as CRP, IL-6, TNF-α, did not increase with the advancing aging. On the other hand, cytokines known to have anti-inflammatory role such as IL-1ra, IL-1β, and IL-10 seemed to decrease with the advancing aging not reflecting an exercise-induced effects.

A cross-sectional study demonstrated an association between physical activity and low-grade systemic inflammation in elderly people but these correlation data do not provide any information with regard to a possible causal relationship (91). However, other longitudinal studies found that regular training induces a reduction of CRP level (38,92). Moreover, Petersen at al. (37) developed a model of “low-grade inflammation” in which they injected a low dose of Escherichia coli endotoxin to healthy volunteers, who had been randomized to either rest or exercise before endotoxin administration. Results showed that in resting subjects, endotoxin induced a 2 to 3-fold increase in circulating TNF-α. In contrast, when the subjects performed 3 hours of aerobic exercise and received the endotoxin bolus at 2.5 hours, the TNF-α response was totally blunted (37). Therefore, there is the possibility that with regular exercise, the anti-inflammatory effects of an acute bout of exercise will protect against chronic systemic low-grade inflammation, but such a link has not been proven yet. Certainly, exercise protects against several diseases associated with chronic low-grade inflammation, and an alternative explanation would be that regular exercise, which offer protection against several conditions, indirectly offers protections against a pro-inflammatory status. This long-term effect of exercise can be attributed to the anti-inflammatory response elicited by an acute bout of exercise which is partially mediated by muscle-derived IL-6 (35,93). Physiological concentrations of IL-6 stimulate the appearance in the circulation of the anti-inflammatory cytokines IL-1ra and IL-10 and inhibit the production of the pro-inflammatory cytokines CRP and TNF-α (37,93). This process
would explain why, the long-term exercise may promote a reduced low-grade inflammation profile characterized primarily by reduced level of CRP, TNF-α and MCP-1, and not by increased level of anti-inflammatory cytokines like we found in our subjects.

Evidence about the efficacy of exercise on vascular function in obesity. Although several studies have already investigated the effects of exercise intervention on vascular functions in obese individuals supporting a positive exercise-induced adaptation, the majority of the studies investigated short-term exercise program, administered to previous sedentary obese subjects (73,74). Kelly et al. (72) demonstrated that 8 weeks of stationary cycling improves HDL and endothelial function in a group of overweight children. Vinet et al. (73) investigated the effect of short-term low intensity exercise training in middle-ages obese men. Ten individuals were recruited and tested for brachial FMD before and after 8 weeks of individualized low-intensity program including three 45-min sessions of walking or cycling at home, without any dietary intervention. Compared with normal-weight men exhibited poorer FMD (5.7%±0.4% vs. 3.3%±0.5%). However, exercise training normalized FMD values in the obese group (3.3%±0.4% before vs 5.3%±0.5% after training). Authors concluded that a short-term low intensity exercise training improves endothelium-dependent vasodilation in sedentary middle-age obese men (73). Another study by Dow at el. (74) shown that regular aerobic exercise reduces ET-1-mediated vasoconstrictor tone in overweight and obese adults. Forearm blood flow (FBF) in response to intra-arterial infusion of selective ETₐ receptor blockade (BQ-123), acetylcholine in the absence and presence of ETₐ receptor blockade were determined before and after a 3-month aerobic exercise training intervention in 25 overweight/obese adults. The vasodilator response to BQ-123 was significantly lower (25%) and the FBF responses to acetylcholine were 35% higher after exercise training. Before the exercise intervention, the co-infusion of acetylcholine plus BQ-123 resulted in a greater vasodilator response that acetylcholine alone. However, after the exercise intervention the FBF response to acetylcholine was not significantly increased by ETₐ receptor blockade. Authors concluded that regular aerobic exercise reduces ET-1-mediated vasoconstrictor tone in previously sedentary overweight and obese adult and these mechanisms may be very
important in the exercise-induced improvement in endothelium-dependent vasodilator function in this population.

Unfortunately, our results are not in agreement with the mentioned studies. Indeed, individuals included in our study show significantly decreased vascular function linearly related with advancing BMI, even though the subjects have been exercising regularly for several years and there was no statistical difference in the amount of physical activity practiced during the week in the three subgroups. Only one study, by Lind et al. (94) demonstrated that increased level of self-reported physical activity does not fully eliminate the deleterious cardiovascular consequences associated with overweight and obesity, supporting our findings. One reason supporting our findings may be that our subjects were not sedentary in the last years and they may have gained weight over the time, limiting the positive exercise-induced effect on vascular function. Another reason may reside directly in the physiology of adipose tissue. Indeed, adipose tissue functions as an endocrine organ that releases bioactive molecules known as adipokines that in obesity are overexpressed leading to a pro-inflammatory status. Among adipose tissue depots, the perivascular adipose tissue (PVAT) displays a unique physiological role that is a paracrine regulation of vascular function. At physiological level, PVAT exerts regulatory effects on metabolism and inflammatory response via local release of hormones, cytokines and reactive oxygen and nitrogen species. Also, PVAT carries an anti-contractile property that influences arteriolar responses to agonist contributing to the regulation of blood flow, nutrient uptake and tissue homeostasis. However, PVAT is susceptible to inflammation and in obesity infiltration of immune cells into the PVAT is aggravated, as well as the amount of PVAT alongside large and small vessels is augmented exerting an anti-contractile effects on conduit and resistance arteries (95). Consequently, PVAT seems to be a candidate for explaining how impaired adipose tissue homeostasis affects vascular functions, even in active obese individuals.

Evidence about the efficacy of exercise on inflammatory profile in obesity. Recent literature started to focus on long-term physical activity and inflammatory biomarkers in obese subjects, but results are still contrasting. Christiansen et al. (44) aimed to investigate the effect of exercise training and diet-induced weight loss alone or in combination on inflammatory biomarkers. The study included 79 obese subjects, randomized into a 12-week intervention: exercise only, EXO; diet-induced weight loss using a very low
energy diet, DIO; and exercise and diet-induced weight loss combined, DEX. By combining the weight loss in all three groups, authors found a correlation between the degree of weight loss and improvement in several of the inflammatory markers. Authors concluded that rather large weight losses (<5-7%) were found to have beneficial effects on circulating inflammatory markers in obese subjects. Furthermore, aerobic exercise for 12 weeks, was found to have no effects on circulating inflammatory markers in these subjects, suggesting that a more intensive exercise may be necessary to affect systemic inflammation in obese (44).

Brunn et al. (45) aimed to investigate the effect of a 15-week lifestyle intervention (hypocaloric diet and daily exercise) on inflammatory biomarkers in 27 severely obese subjects. The intervention reduced body weight and increased insulin sensitivity, as well as increased plasma adiponectin while CRP, IL-6, IL-8, and MCP-1 levels decreased. In agreement with the previous study, authors concluded that the combination of hypocaloric diet and moderate physical activity resulted in a significant general decrease in the level of inflammation (45). Furthermore, Pischon et al. (46) investigated the relationship between physical activity and the obesity-related inflammatory markers CRP, IL-6, and soluble TNF-receptors (sTNF-Rs) 1 and 2. Authors examined even the relationship between physical activity and insulin sensitivity and whether inflammatory markers mediate this association. In the study, 405 healthy man and 454 healthy women were included and information about physical activity and other variables were assessed by questionnaires. Results shown that physical activity was inversely associated with plasma level of sTNF-R1 and 2, IL-6, and CRP but after have adjusted for BMI and leptin (as a surrogate of fat mass), most of these association were no longer significant. Authors suggested that the frequent physical activity is associated with lower systemic inflammation and improves insulin sensitivity and this association can be partially explained by a lower degree of obesity in physically active subjects. However, although inflammatory markers may mediate obesity-dependent effect of physical activity in inflammatory related disease, this study suggest that inflammatory-related disease do not directly account for the beneficial effects of physical activity on insulin resistance (46).

Our results are partially in accordance with the mentioned studies. First, even though obese individuals included in the study are considered very active, no exercise-induced
adaptation on inflammatory profile is seen, showing a linear significant increase of pro-inflammatory cytokines with the increase of the BMI. As previously mentioned, although our subjects have been active already for several years and there is no differences in the amount of physical activity among groups, they could have gained weight over the time, limiting the effects of the regular physical activity they practice. However, a physiological explanation always exist. As said before, adipose tissue is not only a storage tissue but it has an endocrine function. In obesity, an increased fat mass is accompanied by alterations in the cellular composition and physiology of adipose tissue. Hypertrophy and increased distribution of adipocytes, together with inflammation and dysregulated adipokines secretion characterize a dysfunctional adipose organ which contributed to the development of several obesity-associated morbidities (95). Furthermore, in obesity the interplay between adipocytes and immune system components changes. Immune cells secrete cytokines that enhance adipose tissue inflammation, and at the same time, adipocytes express classical macrophage features. As adipose tissue expands, macrophages, mast cells, B cells, and T-cell populations increase considerably. As a consequence there is an increased secretion of the inflammatory adipokines TNF-α and CRP and reduction of anti-inflammatory adipokines (95).

Although exercise can be considered an anti-inflammatory treatment as seen in many studies, it appears that if exercise is not accompanied by an important weight lost, the effect of exercise on the inflammatory profile is poor. Indeed, only when exercise decreases body fat and adipocytes hypertrophy the number of inflammatory cells contained within the adipose tissue decline (95). This could explain the trend found in the individuals included in our study. While they are very active, accumulating about 250 min of physical activity a week, this does not seem to be enough to counteract the maleficial effect of fat mass on inflammatory profile in overweight and obese people (46,96).

**Limitations.** Although this is the first study aimed to measure vascular function and inflammatory profile in very active subjects who have been exercising in a structured, supervised—exercise class for several years, some limitations have to be underlined. First, a control group of inactive, age- and BMI-matched counterparts was not included in the study limiting the interpretations of the results. Also, even though we
measured several variables including vascular function and a wide inflammatory profile with anti- and pro-inflammatory cytokines we did not perform measures of oxidative stress, ROS, NO bioavailability, or immune system components. Consequently, we can only speculate about the reasons why subjects included in our study exhibited this trend based on aging and BMI, making the interpretation of the results an assumption based on the existing literature.

5. Conclusion.

The aim of this study was to investigate the effects of regular, supervised-exercise performed for years on vascular function and inflammatory profile in obese and non-obese older individuals. Albeit limitations are consistent, a clear trend of the measured variables has been seen in both, aging and obesity. Results of our study demonstrated that prolonged, regular, supervised-exercise can counteract the deleterious effects of aging but not the maleficent effects of obesity on vascular function and inflammatory profile. Further studies are needed in order to understand the mechanisms underlying the exercise-induced physiological processes that ensure protections against the aging-effects as well as studies focused on efficient exercise-induced physiological effects against obesity-related vascular dysfunction and pro-inflammatory profile.
Acute inflammatory and vascular response to different types and intensities of exercise in obese subjects.

1. Hypothesis

Our hypothesis was that higher intensity would have elicited higher inflammatory and vascular response to acute bouts of exercise compared with lower intensity, as well as resistance exercise would have triggered higher inflammatory and vascular responses compared to aerobic exercise.


Subjects. Obese (n=5) and lean (n=5) individuals between ages of 40 and 60 years volunteered to participate in this study. Obese individuals had to have a BMI greater than 30 kg·m⁻², while lean individuals had to have a BMI between 18 and 25 kg·m⁻². All the participants had a stable BMI (less than 0.7 kg·m⁻² of variation in the last six months), and were sedentary or little active (less than 600 MET/week). Exclusion criteria included smoking, specific medications (i.e. aspirin, FANS, corticosteroids, and β-blockers), documented inflammatory disease (i.e. arthritis, arthrosis, psoriasis, dermatitis, or others), documented cardiovascular disease (i.e. coronary artery disease, cerebrovascular and/or peripheral vascular disease), orthopedic problems inhibiting performing exercise, resting systolic pressure greater than 160mmHg, and resting diastolic pressure greater than 110mmHg. All experiments were conducted after informed consent was obtained from participants in accordance with the Declaration of Helsinki, as part of a protocol approved by the Institutional Review Board of the Azienda Ospedaliera Universitaria Integrata of Verona, Italy.

Experimental design. We designed a crossover study to investigate the acute inflammatory and vascular response to different type and intensities of exercise in obese individuals (OB) compared with lean subjects (NW). All participants attended the laboratories eight times including a control session, a familiarization session, two maximal exercise-testing sessions, and four training sessions. Control session and all the exercise sessions were performed the same day of the week and at the same time.
of the day, 2.5-3 hours after ingestion of a light meal (ca. 300 kcal, 15% proteins, 25% lipids and 60% carbohydrates) agreed with an expert nutritionist. Blood samples were collected from the antecubital vein right before, right after and 2 hours after the exercise trainings. Vascular function was assessed by means of brachial flow mediated-dilation (FMD) test right before exercise training and 2 hours after the end of the training, as well as during the control session with the same timing as training sessions (Figure 1).

**Figure 2. Experimental design.** Study participants came to the lab eight times including: a control session, a familiarization session, two testing session, and four exercise sessions. Exercise sessions were performed in random order and included low intensity aerobic training, high intensity aerobic training, low intensity resistance training, high intensity resistance training. Right before (T0), right after (T1), and two hours after (T2) the training and in the control session blood samples were collected. Flow-mediated dilation (FMD) test was performed at T0 and T2 only.
**Control session.** The very first session was a control session, where participants did not exercise. They came to the lab, following the same schedule as the other sessions, 2.5-3 hours after ingestion of a light meal. First, FMD was performed and participants remained at the lab but they did not exercise. They were free to read, work at the laptop, or doing any activity, which did not include movement. The FMD was repeated with the same timing as training session, about 4 hours later.

**Familiarization session.** All participants took part in an 80-minute exercise session of low-moderate intensity (55-65% HRmax, calculated using the Tanaka formula: 208-0.7 x age in years) in order to get confident with exercise equipment and the effort feeling. This session included a 15-minute warm up with joints mobilization and self-paced treadmill walk followed by resistance exercise including 2 sets of 10-12 repetitions at 3 of the 6 isotonic machines included in the training sessions. Consequently, participants performed 15 minutes of aerobic exercise at the cycle ergometer followed by resistance exercise including the remaining three isotonic machines included in the training sessions. The session ended with 15 minutes of aerobic exercise at the elliptical ergometer or at the arm ergometer and 10 minutes cool down at the treadmill.

**Maximal exercise testing.** Maximal exercise testing were performed to determine the VO2, HR, and percentage of 1RM required to elicit the desired exercise intensity during the different training sessions. A continues incremental cycle-ergometer test was used to assess peak oxygen uptake (VO2peak). Test started with two minutes of resting conditions record, followed by a warm up consisting of 3 3-minute bouts at 30, 40 and 50 Watts. Consequently, the intensities was increased of 10 Watts in 1-min intervals until volitional fatigue was reached (97). A calibrated PFT Quark breath-by-breath metabolimeter (Cosmed, Rome, Italy) was used to obtain metabolic measurements. Hear rate (HR) was monitored and recorded using a 9-lead ECG. Rating of perceived exertion and blood pressure were collected at the end of each 1-min step. Volitional fatigue was defined as achieving two of the following criteria: 1) respiratory exchange ratio greater than 1.0, (2) HR at least 15 bpm less than the age-predicted maximal heart rate, (3) maintenance of oxygen consumption within 2 ml/kg/min despite an increase in workload (97).
One-repetition maximum (1RM) was used to obtain strength measurements. 1RM was measured bilaterally for all the equipment included in the strength exercise sessions (leg press, leg curl, leg extension, chest press, lat machine, and shoulder press) (76).

**Blood samples and analysis.** Venous blood samples were drawn from an antecubital vein right before (T0), right after (T1), and 2 hours after (T2) the exercise sessions. Venous blood was collected into EDTA tubes for analysis of inflammatory profiles. The samples were centrifuged for 10 minutes at +4°C with 2500xg. Plasma was kept at -80°C until analyzed for IL1-α, IL-1β, IL-6, IL-8, IL-10, TNF-α, MCP-1 were determined by means of commercially available MILLIPLEX multi-analyte panel (Merk Millipore, Darmstadt, Germany) following the manufacture’s recommendation. CRP was measured on a measured on ELISA (DBC-Diagnostics Biochem Canada Inc., London, Canada).

**Flow-mediated dilation.** FMD test was performed in a quiet room right before (T0) and 2 hour after (T2) each training session as well as during the control session, 2.5-3 hours after a light meal and in abstinence from alcohol, and caffeine for 12 h (83). High-resolution ultrasound was used to image the brachial artery at rest and after 5 min of ischemia. All the FMD were performed with the participant in the supine position, with the right arm extended at an angle of ~90° from the torso. The brachial artery was imaged using a high-resolution ultrasound system Logiq-7 ultrasound Doppler system (General Electric Medical Systems, Milwaukee, WI, USA). The ultrasound Doppler system was equipped with a 12-14 MHz linear array transducer. The brachial artery was imaged 5-10 cm above the antecubital fossa in the longitudinal plan, and the diameter was determined at 90° angle along the central axis of the scanned area. When an optimal image was acquired, the position was maintained for the whole test and all scans were stored for later analysis. After baseline brachial artery imaging, a blood pressure cuff was placed around the forearm and inflated to 100 mmHg above systolic pressure for 5 min. Brachial artery images were obtained continuously 30s before and 2 min after cuff release (84). The brachial artery images were analyzed by a blinded investigator by means of FloWave.US (85). Arterial diameter was measured as the distance (mm) between the intima-lumen interfaces for the anterior and posteriors walls. FMD is expressed in relative changes in post-reactive hyperemia diameter.
compared to the baseline diameter. Shear rate and FMD normalized for Shear rate until the peak (FMD/Shear) were also calculated (98).

Exercise sessions. All the participants performed four exercise sessions of different kind and intensity in random order. All the session lasted 80 minutes including warm up and cool down, common for all the exercise sessions. Warm up included joints mobilization and 10-minute self-paced treadmill exercise; cool down included 5-minute self-paced treadmill exercise and stretching. In order to allow the correct rest and recovery, there were 5-day break in between training sessions.

The different training sessions included the following: (1) Low intensity aerobic training (LA) including 30 minutes at the cycle ergometer followed by 30 minutes either at the arm ergometer or at the elliptical ergometer. The intensity of both exercises was maintained between 40 and 50% of VO$_2$peak. Participants were free to variate the intensity over the exercise time with an average intensity of 45% VO$_2$peak. (2) High intensity aerobic training (HA) including 3 5-minute bouts at 75% VO$_2$peak with 3 minutes active break at 55% VO$_2$peak at the cycle ergometer followed by 3 5-minute bouts at 75% VO$_2$peak with 3 minutes active break at 55% VO$_2$peak either at the arm ergometer or at elliptical ergometer. The average intensity of the session was 65% VO$_2$peak. (3) Low intensity resistance training (LR) included 3 sets of 15 repetitions at 60% of 1RM with 3-minute rest in between sets. (4) High intensity resistance training (HR) included 3 sets of 6 repetitions at 85% of 1RM with 4-minute rest in between sets. LR and HR included leg press, leg curl, leg extension, lat machine, chest press, and shoulder press.

Statistical Analysis. Descriptive characteristics were calculated and independent t-tests were used to define if any group differences existed at baseline. Between and within group pre- and post-training inflammatory parameters, FMD, Shear Rate, and FMD/Shear data were compared with a three-way analysis of variance (ANOVA) with repeated measures [sessions(5) x time (2) x group (2)]. Post hoc analyses were used if significant interactions were found. Friedman Repeated Measures Analysis of Variance on Ranks and All Pairwise Multiple Comparison Procedures (Tukey Test) was adopted to study differences between groups. Data are presented as the mean ± SD. Statistical significance was set as P <0.05. All statistical analysis were performed with Sigma STAT 4.0 (Systat Software, Chicago, IL).
3. Results.

Subject’s baseline characteristics are presented in Table 1. The groups were similar in age, and level of physical activity, measured by mean of International Physical Activity Questionnaire (IPAQ). OB had ~25% greater BMI (p<0.05) and ~25% poorer FMD (p<0.05), as well as ~35% poorer VO₂peak (p<0.05) than NW. Moreover, OB showed significantly lower value of IL-1β (~49%, p<0.05), IL-6 (~90%, p<0.05), and IL-10 (~37%, p<0.05), as well as higher values of CRP (400%, p<0.05), while IL-1ra, MCP-1 and TNF-α did not exhibit any difference between groups (Table 1).

Table 1. Subjects characteristics*.

<table>
<thead>
<tr>
<th></th>
<th>NW (n=5)</th>
<th>OB (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female - n. (%)</td>
<td>3 (43)</td>
<td>4 (66)</td>
</tr>
<tr>
<td>Age - years</td>
<td>54±7</td>
<td>53±6</td>
</tr>
<tr>
<td>Weight - kg</td>
<td>68.3±11.2</td>
<td>87.6±13.3*</td>
</tr>
<tr>
<td>Height - m</td>
<td>1.67±0.09</td>
<td>1.61±0.12</td>
</tr>
<tr>
<td>VO₂peak - ml<em>min⁻¹</em>kg⁻¹</td>
<td>32.2±4.8</td>
<td>20.5±1.8*</td>
</tr>
<tr>
<td>VO₂peak - l*min⁻¹</td>
<td>2.5±0.4</td>
<td>1.8±0.3*</td>
</tr>
<tr>
<td>BMI - kg*m⁻²</td>
<td>24.2±0.7</td>
<td>33.7±1.2*</td>
</tr>
<tr>
<td>CRP – ng/mL</td>
<td>686.2±189.6</td>
<td>3606.9±1787.6*</td>
</tr>
<tr>
<td>IL-1β – pg/mL</td>
<td>6.8±2.2</td>
<td>3.5±1.1*</td>
</tr>
<tr>
<td>IL-1ra – pg/mL</td>
<td>21.8±5.5</td>
<td>25.3±8.4</td>
</tr>
<tr>
<td>IL-6 – pg/mL</td>
<td>17.0±12.3</td>
<td>1.5±0.7*</td>
</tr>
<tr>
<td>IL-8 – pg/mL</td>
<td>7.0±2.7</td>
<td>2.4±2.3*</td>
</tr>
<tr>
<td>IL-10 – pg/mL</td>
<td>9.1±2.9</td>
<td>5.7±2.8*</td>
</tr>
<tr>
<td>TNF-α – pg/mL</td>
<td>18.1±2.9</td>
<td>18.7±2.9</td>
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<tr>
<td>MCP-1 – pg/mL</td>
<td>441.2±53.8</td>
<td>392.0±112.8</td>
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<tr>
<td>FMD - %</td>
<td>10.0±1.7</td>
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</tr>
<tr>
<td>IPAQ - MET</td>
<td>524±203</td>
<td>540±250</td>
</tr>
</tbody>
</table>

* Plus-minus values are means±SD.
* significant difference between groups, p<0.05
**Note.** NW: normal weight; OB: obese; BMI: Body Mass Index; CRP: C-reactive protein; IL: interleukin; TNF-α: tumor-necrosis factor-α; MCP-1: monocyte chemoattractant protein-1
FMD: Flow Mediated Dilation; IPAQ: International Physical Activity Questionnaire.

**Inflammatory response.**

**Control Session.** Both, NW and OB did not show any significant difference within T0, T1, and T2 for any inflammatory marker during the control session. However, NW group exhibited a slightly higher MCP-1 at T2 compared with T1, as well as OB group showed a slightly higher IL-1ra at T2 compared with T1 (Table 2). Considering between groups differences, OB exhibited markedly higher CRP values than NW at any time, as well as lower IL-1β at T2, lower IL-6 and IL-8 at any time, and lower IL-10 at T1 and T2 (Table 2). TNF-α and MCP-1 did not differ between groups at any time.

**High intensity aerobic training.** Both, NW and OB did not show any significant difference within T0, T1, and T2 concerning CRP (Figure 2, panel A). However, NW exhibited an increasing trend at T2 (Table 2). Again, comparison between groups highlighted a higher CRP values in OB than in NW at each time. IL-1β exhibited a different trend between groups: NW shown an increase at T2, while OB exhibited a decrease at T1 and a significant increase of this cytokine at T2. Comparison between groups shown a higher values in NW compared with OB, significantly different only at T2 (Table 2). IL-1ra increased significantly at T1 and decreased significantly at T2 in NW, while in OB there were no significant difference within the group (Figure 2, panel B). Comparison between groups exhibited a markedly higher IL-1ra values in OB than in NW at T0 and T2, but not T1 (Table 2). IL-6 and IL-8 increased at T1 and T2 in NW group, while in the OB group it increased slightly only at T2. Between groups comparison exhibited a markedly lower IL-6 (Figure 2, panel C) and IL-8 values in OB group (Table 2). IL-10 did not differ at any time in NW nor in OB and again, between groups comparison shown higher values of this cytokine in NW than in OB (Table 2). TNF-α did not change between T0 and T1, while it increased significantly at T2 in OB. No between groups differences were detected (Table 2). MCP-1 increased slightly at T2 in NW group. No between groups differences were detected (Table 2).
Low intensity aerobic training. No differences between times were detected in both groups for CRP (Figure 2, panel A). Again, comparison between groups highlighted a higher CRP values in OB than in NW at each time (Table 2). IL-1β slightly decrease at T2 in NW group, while it increased at T1 and significantly decreased at T2 in OB group. Comparison between groups shown a higher values in NW compared with OB, significantly different only at T2 (Table 2). IL-1ra, slightly increased in NW group throughout T0, T1, and T2 while in the OB group it increased at T1 and decreased significantly at T2 (Figure 2, panel B). Between groups differences were detectable only at T1 and T2 (Table 2). IL-6 did not change between T0 and T1, while it decreased significantly at T2 in NW group. At the contrary, in OB group it increased slightly at T1 and significantly at T2 (Figure 2, panel C). Between groups differences were detectable at each time (Table 2). In NW group, IL-8 exhibited a decreasing trend throughout T0, T1 and T2 while in the OB group it did not change. Again, OB group exhibited markedly lower IL-8 value than NW group (Table 2). IL-10 did not change in the NW group throughout the time, while it decreased significantly at T2 in the OB group. Between group differences were detected at each time (Table 2). TNF-α slightly decreased at T2 in the NW group, while it increased at T1 and significantly decreased at T2 in OB group. MCP-1 did not change in the two group throughout the time (Table 2).

High intensity resistance training. No differences between times were detected in both groups for CRP. (Figure 2, panel A). Again, between group differences were noted at each time (Table 2). IL-1β did not change at any time in both group. Between group differences were not detected (Table 2). IL-1ra increase significantly at T1 and T2 in the NW group (Figure 2, panel B). In OB group it increased significantly only at T2. Between groups differences were detected at T0 and T1 (Table 2). IL-6 did not change in NW group. In OB group it increase at both T1 and T2 (Figure 2, panel C). Between group differences were detected at each time (Table 2). IL-8 did not change in any of the two groups but between groups differences were detected at each time (Table 2). IL-10 significantly increase in T2 for NW while it did not change for OB. Between group differences were detected only at T2 (Table 2). TNF-α did not change between T0, T1 and T2 in the both group. No difference was detected between groups. MCP-1 significantly increase in NW group between T1 and T2 while in the OB group it
slightly increased throughout the time (Table 2). In T0 and T2 OB group shows lower level than NW.

Low intensity resistance training. CRP, IL-1β and TNF-α did not change in time T0, T1 and T2 in both groups while significant differences were detected between two groups. IL-1ra first increased and then decreased significantly at T2 in NW group. In OB group it did not change throughout the time (Figure 2, panel B). Between group difference were noted only at T1 (Table 2). IL-6 did not change in NW group. In the OB group it increase significantly at both T1 and T2 (Figure 2, panel C). Between group differences were noted at each time (Table 2). IL-8 increased slightly at T1 and T2 in the NW group, while it slightly decrease in the OB group. Between group differences were noted at each time (Table 2). IL-10 decreased throughout the time in the NW group while it first decreased and then increased significantly in the OB group. Between group differences were noted at each time (Table 2). MCP-1 did not change in the NW group, while it decreased significantly between T0 and T1 and increased slightly at T2 (Table 2).
<table>
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<th>IL-1β</th>
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<td>7.5±1.7</td>
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</tr>
<tr>
<td><strong>HR</strong></td>
<td>930.9±177.9</td>
<td>8.5±5.7</td>
<td>9.7±5.3</td>
<td>10.5±7.7</td>
<td>10.7±6.4</td>
<td>11.5±6.6</td>
<td>17.0±12.3</td>
<td>12.0±5.5</td>
<td>9.4±4.6</td>
<td>7.5±1.7</td>
<td>7.5±1.7</td>
<td>7.5±1.7</td>
<td>7.5±1.7</td>
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<tr>
<td><strong>LR</strong></td>
<td>976.6±413.6</td>
<td>8.5±5.7</td>
<td>9.7±5.3</td>
<td>10.5±7.7</td>
<td>10.7±6.4</td>
<td>11.5±6.6</td>
<td>17.0±12.3</td>
<td>12.0±5.5</td>
<td>9.4±4.6</td>
<td>7.5±1.7</td>
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</tr>
</tbody>
</table>

Table 2. CRP, IL-1β, IL-1ra, IL-6, IL-8, IL-10, TNF-a, MCP-1 at T0, T1, and T2.

1. Plus-minus values are mean±SD of T0, T1, and T2 measurements.
2. A three-way, repeated measures ANOVA was performed to assess differences within and between groups for all the conditions.

†. within group difference, p<0.05.
‡. within group difference, T0-T1, p<0.05
§. within group difference, T1-T2, p<0.05

t. within group difference, T2-t, p<0.05

Note. NW: normal weight; OB: obese; Ctrl: control session; HA: high intensity aerobic session; LA: low intensity aerobic session; HR: high intensity resistance session; LR: low intensity resistance training.
Figure 3. CRP (panel A), IL-1ra (panel B), and IL-6 (panel C) during the four different training sessions in normal weight (NW) and obese (OB) subjects.
Vascular response.

Baseline brachial artery diameter pre and post training sessions. **Table 3** presents basal brachial artery diameter variation between T0 and T2, as well as during the control sessions. No statistical differences were noted within group (T0 vs T2) nor between groups (NW vs OB).

<table>
<thead>
<tr>
<th></th>
<th>NW (n=5)</th>
<th>OB (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>0.0±0.00</td>
<td>0.0±0.01</td>
</tr>
<tr>
<td>HA</td>
<td>0.0±0.01</td>
<td>0.0±0.01</td>
</tr>
<tr>
<td>LA</td>
<td>0.0±0.01</td>
<td>0.0±0.00</td>
</tr>
<tr>
<td>HR</td>
<td>0.0±0.01</td>
<td>0.0±0.01</td>
</tr>
<tr>
<td>LR</td>
<td>0.0±0.00</td>
<td>0.0±0.01</td>
</tr>
</tbody>
</table>

# Plus-minus values are means±SD of the variation between T0 and T1 measurements.

† A three way, repeated measures ANOVA was performed to assess differences within and between groups for all the conditions. No statistical differences were noted.

**Note.** NW: normal weight; OB: obese; Ctrl: control session; HA: high intensity aerobic session; LA: low intensity aerobic session; HR: high intensity resistance session; LR: low intensity resistance training.
Control session. No statistical differences within and between groups were noted in FMD%, Shear Rate, nor in the FMD/Shear measured during the control session (Table 4; Figure 3, panel A).

High intensity aerobic training. NW reported a significant increase of FMD% and Shear rate. However, those changes were not accompanied by modification of FMD/Shear. OB reported a significant change in FMD%, and Shear rate. In this group, those variations were accompanied by a significant increase of FMD/Shear (Table 3; Figure 2, panel B). Significant differences between NW and OB were noted for all the parameters: FMD%, Shear rate, and FMD/Shear.

Low intensity aerobic training. NW shown significant increase in FMD%, which was not associated with a change in Shear rate nor in FMS/Shear. OB shown a significant increase in FMD%, which was accompanied by a significant decrease in Shear rate and a significant increase in FMD/Shear (Table 4; Figure 3, panel C). Even in this case, significant differences between NW and OB were noted for all the parameter.

High intensity resistance training. NW exhibited significant increased FMD%, but no significant change in Shear rate, nor FMD/Shear. OB reported significant increase in FMD% accompanied by significant increase in both, Shear rate and FMD/Shear (Table 4; Figure 3, panel D). No significant differences were noted between NW and OB.

Low intensity resistance training. NW reported significant increase in FMD%, but no significant changes in Shear rate, nor in FMD/Shear were noted. OB exhibited significant increased in FMD% accompanied with significant increase in Shear rate. However, no significant change were noted for FMD/Shear (Table 4; Figure 3, panel E). Significant differences between NW and OB were noted only for Shear rate.

Aerobic vs resistance training and high vs low intensity. No significant differences were observed in FMD%, Shear rate, and FMD/Shear between aerobic training and resistance training as well as between high intensity and low intensity.
Table 4. FMD%, Shear Rate, and FMD/Shear at T0 and T1 #$.  

<table>
<thead>
<tr>
<th></th>
<th>NW (n=5)</th>
<th>OB (n=5)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T1</td>
<td>T0</td>
<td>T1</td>
<td></td>
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<tr>
<td>FMD (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ctrl</td>
<td>9.6±0.7</td>
<td>9.8±0.7</td>
<td>6.5±0.02</td>
<td>6.5±0.2 †</td>
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<tr>
<td>HA</td>
<td>10.2±1.2</td>
<td>15.5±1.2 †</td>
<td>6.7±0.01</td>
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<td>LA</td>
<td>10.0±0.7</td>
<td>12.7±0.8 †</td>
<td>6.2±0.4</td>
<td>9.4±0.2 †§</td>
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<tr>
<td>HR</td>
<td>10.4±1.0</td>
<td>13.3±1.1 †</td>
<td>5.0±0.9</td>
<td>8.1±1.1 †</td>
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<tr>
<td>LR</td>
<td>9.6±0.7</td>
<td>12.3±0.8 †</td>
<td>6.5±0.01</td>
<td>9.4±0.2 †</td>
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<tr>
<td>Shear rate</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Ctrl</td>
<td>69675±9343</td>
<td>60966±6101</td>
<td>111221±6277</td>
<td>91698±4157 †§</td>
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<tr>
<td>HA</td>
<td>82164±7709</td>
<td>111666±10523 †</td>
<td>75478±2238</td>
<td>66234±2196 †§</td>
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<tr>
<td>LA</td>
<td>93100±12030</td>
<td>81790±9925</td>
<td>75481±9783</td>
<td>58646±6834 §</td>
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<tr>
<td>HR</td>
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<td>100695±8675</td>
<td>95136±1159</td>
<td>117226±6263 †</td>
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<td>87129±6992</td>
<td>56216±14117</td>
<td>77483±18296 †§</td>
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<tr>
<td>FMD/Shear</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ctrl</td>
<td>0.0186±0.0019</td>
<td>0.0198±0.0014</td>
<td>0.0071±0.0018</td>
<td>0.0088±0.0020 §</td>
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<tr>
<td>HA</td>
<td>0.0117±0.0010</td>
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<td>0.0091±0.0005</td>
<td>0.01631±0.0007 †§</td>
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<td>LA</td>
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<td>0.0077±0.0008</td>
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<td>HR</td>
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<td>0.0044±0.0007</td>
<td>0.0100±0.0027 †</td>
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<tr>
<td>LR</td>
<td>0.0130±0.0030</td>
<td>0.0193±0.0034</td>
<td>0.0318±0.0262</td>
<td>0.0080±0.0005</td>
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</table>

# Plus-minus values are means±SD of the T0 and T1 measurements.

§ A three way, repeated measures ANOVA was performed to assess differences within and between groups for all the conditions.

†, between group difference, p<0.05.

§, within group difference, p<0.05

Note. NW: normal weight; OB: obese; Ctrl: control session; HA: high intensity aerobic session; LA: low intensity aerobic session; HR: high intensity resistance session; LR: low intensity resistance training.
Figure 2. FMD/Shear individual changes during control session (panel A), high intensity aerobic training (panel B), low intensity aerobic training (panel C), high intensity resistance training (panel D), and low intensity resistance training (panel E).
4. Discussion.

Although the beneficial effect of chronic and regular exercise on several different populations has already been investigated, studies about the acute effect of a single bout of exercise still report contrasting results. In particular, the acute inflammatory and vascular response to different types and intensity of exercise in population that are characterized by a high low-grade inflammation and vascular dysfunction needs to be clarified. In the present study, we measured inflammatory profile and vascular response right before, right after and 2 hour after four exercise sessions which differed for type (aerobic and resistance exercise) as well as for intensity (high and low intensity), in sedentary obese compared with sedentary normal weight individuals. Partially in accordance with our hypothesis, different type and intensity of exercise elicited different vascular responses, but we were not able to detect any difference in the inflammatory response. Furthermore, we expected to have higher inflammatory and vascular responses at higher intensity as well as after resistance exercise, which was not confirmed by our results. Indeed, results did not show any trend concerning the inflammatory response and suggested a higher vascular response after aerobic exercise compared with resistance exercise. It is important to note that overall, this four type of exercise training do not appear to significantly affect the circulating cytokines response to 2 hours to acute exercise.

*Evidence about acute inflammatory response to exercise.* Our results are only partially supported by previous findings. First, as expected obese individuals included in our study were characterized by significantly higher CRP levels. However, surprisingly this OB group showed lower level of IL-6, as well as similar values of TNF-α and MCP-1 to the NW group. Moreover, all the measured cytokines did not show different trend after different kind of exercise, and many of them did not change significantly either in the NW or in the OB group. Only IL-1ra exhibited an increasing trend after high intensity resistant exercise in both the groups, while the most popular exercise-related cytokine IL-6 did not change significantly in OB group during any of the exercise sessions. Only it increased in NW after high intensity aerobic exercise and right after low intensity resistance exercise, while oddly in OB it did not change. Thus, we can only confirm that normal obese individuals respond to exercise differently from non-obese individuals.
From the physiological point of view: inflammatory response occurs while exercising. Acute exercise has an effect on cytokine response and inflammation in healthy individuals. The intensity, duration and type of exercise, as well as acute vs. chronic exercise can all influence various immune parameters, which are also associated with chronic inflammatory disease. Acute exercise triggers responses both during and after exercise in the immune system. During acute exercise muscles release IL-6 and levels can increase significantly. Furthermore, leukocyte subset as neutrophils, lymphocytes and monocytes, as well as plasma concentrations CRP and both pro- and anti-inflammatory cytokines TNF-α, IL-1, IL1ra, IL-10 and sTNF-r can increase to various magnitudes during a bout of exercise [14,28,29]. Following the cessation of intense exercise, neutrophils and monocytes can continue to increase into the recovery period. During this post-exercise time, other leukocyte subsets decrease in number, while plasma concentrations of the above-mentioned cytokines stay elevated for some hours. Strenuous and eccentric exercise seem to exert the most prominent changes in immune parameters. While extreme exercise such as marathon, and frequently executed training programs have been associated with a depression in immune function, which may increase the elite athlete’s susceptibility to infection (47). The pro-inflammatory markers TNF-α and IL-1β do not seem to increase in short period of moderate intense exercise, although conflicting results have been documented (48). It is therefore clear that acute bouts of exercise exert various effects on the immune system and are typically transient in nature (47). However, there are several possible explanations for the variable results on pro-inflammatory and anti-inflammatory responsive cytokines in relation to exercise. These include: a) the type of physical activity as well as the intensity and duration of the exercise, b) the timing of sample collections, as well as c) the specificity and sensitivity of the assays (48). Even though we tried to design a study aimed to clarify the behavior of several cytokines in response to different exercise sessions, we were not able to detect any significant difference between type and intensity of exercise in both obese and non-obese individuals. This might be due to several reasons: first, the tissue collected for the analysis, which is blood. In this way, we measured circulating cytokines that not necessarily reflect the real inflammatory response during exercise. Investigating the inflammatory response on muscle fibers or fat tissue might have led to different and clearer results. Furthermore, while exercise sessions were tailored on each individuals, programmed by means of the maximal tests,
the difference in intensity between the sessions may was not enough to detect
difference between high and low intensity on the inflammatory profile. Moreover,
another possible factor which do not allow distinguishing differences between sessions
was that all the high intensity and resistance sessions included a complete recovery
between sets, which may have transformed the total intensity of the exercise session
in a lower intensity that the programmed one.

Evidence about acute vascular response to exercise. Even on the vascular side, several recent
studies have aimed to identify the response to acute exercise. Nevertheless, results are
divergent reporting different vascular response to acute exercise: from no response at all to reduction in vascular response right after the exercise, but even an increased vascular response to acute exercise. However, even in this case different results may be given by different exercise approaches and different timing in the vascular function measure. Franklin et al. (81) determined if a single bout of strenuous weight lifting (SWL) reduced endothelium-dependent vasodilation among sedentary obese women. This study included nine obese and eight lean sedentary young women. Brachial FMD was measured before and right after SWL. Sublingual nitroglycerin (NTG) was used to determine brachial artery endothelium-independent vasodilation following SWL. Results shown that Brachial FMD was significantly reduced in obese and lean women after SWL. There was no difference in the magnitude of change pre-and post-exercise between groups. Dilation to NTG was lower in obese compared to lean subjects and associated with body weight. Author concluded that endothelium-dependent vasodilation is reduced in women after acute resistance exercise. Dilation to NTG were lower in obese compared to lean woman and associated with body weight suggesting that changes in sensitivity of blood vessel to NO occurs during obesity (81).

Our results are partially in accordance with those of Hallmark et al. (82) who found that lean subject exhibited greater FMD than obese subjects and in the obese group increases in FMD at 2-, and 4-hours after moderate-intensity exercise. For lean subjects, FMD was significantly elevated at all time point after high-intensity exercise. Authors concluded that in lean adults, high-intensity exercise acutely enhances endothelial function while moderate-intensity exercise has no significant effect above that seen in the absence of exercise. Furthermore, the FMD response of obese adults is blunted compared to lean adults (82). First, obese individuals showed a reduced
FMD compared to non-obese individuals. Furthermore, they presented blunted FMD compared to non-obese individuals after all exercise sessions. Interestingly, both the groups exhibited augmented FMD two hours after each exercise session, which was not always accompanied by an increased Shear rate, but no significant differences between different exercise sessions were detected.

Physiologically, at the onset of exercise blood flow and shear stress markedly increase in active regions in an exercise-intensity-dependent manner to meet increased metabolic demand [16,30]. Moreover, local vasodilator mechanisms along with increases in arterial pressure and cardiac output contribute to exercise hyperemia, leading to significant increases in shear stress in the active areas during exercise (76). Evidence have demonstrated that acute exercise can lead to an immediate increase in endothelium-mediated dilation (76). Tinken et al. (65) examined brachial artery vasodilator function, using the FMD test, before and after 30-min handgrip exercise, cycle exercise, and forearm heating. After successfully increasing shear stress levels, FMD significantly improved. Given the marked differences between the three interventions in pulse pressure and pulse frequency, these results highlight the importance of shear stress in mediating acute change in endothelium-mediated dilation. Moreover, given the intensity-dependent relationship between exercise and hyperemia, higher intensity exercise may lead to incremental increases in post-exercise vascular function. This was not confirmed by the results of our study that did not show any significant difference in FMD between exercise sessions. However, most studies that have examined this hypothesis have reported decrease in vascular function immediately after high-intensity cycle exercise, which may be followed by a rebound recovery of function one or more hours after the cessation of the bout [16,32]. In addition, to increase in shear stress, strenuous exercise also mediated other effects such as the production of ROS and activation of the sympathetic nervous system that may mitigate beneficial shear stress effect right after exercise. For this reason, we preferred to avoid to measure FMD right after the end of exercise avoiding to have the sympathetic nervous system effect of the vascular response.

Like the inflammatory response, the trend seen in the vascular response may be due to several reason. First, the difference in intensity between the sessions may was not enough to detect difference between high and low intensity. Second, the timing of the
vascular measures. We decided to not to measure FMD right after the exercise, and to take just one measure 2 hours after the end of the exercise sessions. Perhaps, taking more than just one FMD measure at different timing after exercise would have been helpful and would have make results clearer.

Limitations. Although this is the first study aimed to measure acute inflammatory and vascular response to different type and intensity of exercise in obese and non-obese individuals who performed all the sessions, several limitations have to be highlighted. First, the scarce sample size. Due to the severe inclusion criteria and the time required for the participation in the study, we were able to enroll only five subject for each group, which is not enough for such a study. Due to the variability of the measured variables (i.e. cytokines), this might be the first and most important reason for the limited results, leading to a difficult interpretation and discussion. Next, concerning the inflammatory response we decided to measure circulating inflammatory markers, which may be a limit in detecting the real exercise effects on these markers. As we said previously, performing analysis on muscle fibers or fat tissue may have led to different results. Moreover, although we included in the analysis several cytokines both, anti- and pro-inflammatory, we did not measure oxidative stress, ROS, NO bioavailability, or immune system components, which would have supported the interpretation of the results not only of the inflammatory response but also of the vascular response. Again, about the vascular response, we did not included more than just one measure after the end of the exercise session, which may limit our finding.

5. Conclusion.

The first aim of the study was to compare the inflammatory response to different exercise sessions that differ for type and intensity, in obese individuals, compared with normal weight individuals. Second aim of the study was to compare vascular response to different exercise sessions, different for type and intensity in both the groups, obese and normal weight. Although limitations are consistent, results of our study demonstrated that in obesity acute exercise do not provoke nor exacerbate the low-grade inflammation to two hour to any type and intensity of exercise. Moreover, vascular response do not appear to be reduced after any exercise session. In summary,
results of this study let us to speculate that tailored exercise, both aerobic and resistance exercise as well as high and low intensity, including complete recovery between sets or bouts of exercise and lasting 80 to 90 minutes, do not seem to significantly affect negatively the inflammatory profile in both obese and non-obese individuals. Yet, neither the vascular response seems to be negatively affected by any type or intensity of exercise. All together these results suggests that any type and intensity of exercise is safe in obese and non-obese sedentary individuals and it can be included in an exercise program focused on reducing the risk factors for several conditions as well as on the improvement of the quality of life in these populations. However, due to the limited sample size, further studies are needed in order to have more interpretable and generalizable results in order to develop more specific and complete exercise programs for sedentary obese and non-obese individuals.
General conclusion.

The aim of this study was to investigate the chronic and acute exercise-induced effects on inflammatory profile and vascular function in two conditions known to be characterized by high low-inflammatory profile and vascular dysfunction, such as aging and obesity.

Results of the first study, focused on the chronic adaptations induced by exercise, demonstrated that prolonged, regular, exercise can counteract the deleterious effects of aging but not the negative effects of obesity on vascular function and inflammatory profile. Therefore, inflammatory profile and vascular function can be affected by regular exercise, which seems to have a protective effects against the age-related deterioration.

Results of the second study, focused on the acute inflammatory and vascular response to different exercise sessions in obese individuals, demonstrated that acute exercise do not exacerbate the low-grade inflammation to two hour to any type and intensity of exercise. Moreover, vascular response do not appear to be reduced after any exercise session. We have to highlight that this second study was a pilot study and certainly a greater sample size is necessary in order to detect statistically significant results.

Concluding, a single bout of exercise does not seem to exacerbate inflammatory profile and vascular function in obese subjects and any type and intensity of exercise is safe and it can be included in an exercise program focused on reducing the risk factors for several conditions as well as on the improvement of the quality of life in these populations.
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