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SCHOOL OF MEDICINE AND SCHOOL OF SCIENCE

Prevention-treatment of metabolic syndrome based on CPET (Cardio-Pulmonary Exercise Test)

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CHAPTER 1

1. GENERAL INTRODUCTION

A number of highly related metabolic disorders, including abdominal adiposity, insulin resistance, hyperglycemia, dyslipidemia, and hypertension characterize and predict type 2 diabetes and cardiovascular disease (CVD). This cluster of factors has been referred as syndrome X, insulin resistance syndrome, and metabolic syndrome (MetS), the last term recently is adopted by the World Health Organization (WHO) and the National Cholesterol Education Program Adult Treatment Panel III (NCEP). This high-risk pre pathological state is characterized by several definitions and subjected to multivariate statistical approaches, although none has achieved wide acceptance in the literature. WHO and NCEP recently have presented criteria to define the MetS , and they reported that there is a high prevalence of the syndrome in the general population (Roberts et al. 2013 Rev).

Generally the individuals with MetS have insulin resistance (IR), this notion is based on the results of many studies that have documented IR in subjects with dyslipidemia and abdominal adiposity (other components of the syndrome). In addition, factor analysis of several studies, has been used to document (latent) traits or clusters of these disorders in large groups of subjects.

The metabolic syndrome represents a clinical situation with a high cardiovascular risk and a high social cost (25% of public health spending). This syndrome is often related with lifestyle (overweight, sedentary lifestyle) and with pre-existing pathological situations such as hypercholesterolemia, hypertension, insulin resistance and diabetes type 2. It affects a large percentage of the population worldwide, mainly in old age. This syndrome needs an interdisciplinary therapeutic approach based on diet, drugs therapy and physical exercise, all these act to reduce overweight and prevent diseases related to it. The medical control of metabolic syndrome in individuals with hypertension, type 2 diabetes and hypercholesterolemia, slows the progression of the disease and possibly limit the use of drugs. In 2005 the International Diabetes Federation (IDF) has revised the diagnostic criteria and proposed as a method to identify the disease in the patient the presence of 2 of the following disorders: fasting glucose over 100 mg/dl, hypertension: more than 130/85 mm Hg or drug therapy, hypertriglyceridemia: above 150 mg/dl, reduced HDL cholesterol: < 40 mg/dl in males, < 50 mg/dl in females or lipid-lowering therapy, associated with a waist circumference over 94 cm in males, 80 cm in females for patients of Caucasian ethnicity (the parameters vary by ethnic group).

In Italy, the metabolic syndrome affects about 25% of men and 27% of women, about 14 million of adults. In a first prospective

study, conducted in Finland, a sample of males suffering of MetS have a 3 times higher risk of total mortality and cardiovascular disease in 10 years than those not suffering from this disease. Other studies were done in a general population samples, in which both sexes were represented. The medical community agree that the syndrome is an independent risk factor of cardiovascular morbidity and mortality. It also seems that, the syndrome gives a much greater risk to women than to men. In recent years, the metabolic syndrome plays an important role also in childhood and adolescence this is linked to the increased consumption of soft drinks and energy drinks. In the United States of America from 1965 to 2006 the prevalence of MetS in children increased from 11 to 21%, the prevalence can vary greatly depending on the diagnostic criteria used and go from 30 to 50% in obese children and 4 to 16% in children of normal weight (Pacifico et al. 2011). However, there is still no agreement on cut-off criteria to be used (in adults usually BMI of 30): recently, as biomarkers of simple relief, it has been proposed the circumference of the wrist and the weight/height rate. A ten years old child can develop an increase in insulin levels and insulin resistance that predispose to diabetes in adulthood. The insulin resistance occurring more frequently when is present obesity: of 10 obese children, 8 will be obese or overweight as adults. The waist circumference is a reliable index as in the adult, however, we did not agree on what is the optimal cut-off, and when to attend. Another indicator is the relationship between waist circumference and

stature: > 5 carries a greater risk of developing MetS waist circumference and BMI varies with age, so we need to refer to percentiles. Also the arterial pressure (P.A.) varies with age: a child of 10 years with values of systolic pressure of 130 mm Hg or higher is considered with hypertension, but in very high children this can be considered a normal value. For the IDF in children metabolic syndrome may be considered a disease after 10 years old, the only criticism is the different ethnicity of the subjects: South-American children for example have a higher risk than Caucasian. When there is a diagnosis of MetS, the first therapeutic approach is a correct amount of physical activity and avoid sedentary behaviors (television and sedentary lifestyle). The protein intake in the early of life seems to be associated with MetS (protein diet increases the number of adipocytes). We have to encourage breastfeeding and avoid the excess of soft drinks in children.

Pathogenesis

Insulin resistance

One early consequence of MetS is an increased insulin resistance. The insulin resistance is a condition in which the physiological amounts of insulin produces a reduced biological response, there is a reduction of the early action of insulin on glucose control after a meal, it is associated with an inadequate

insulin suppression during overnight fast, in presence of an insulin preserved synthesis. It follows an alternation between insulin resistance and hyperinsulinemia, we found elevated insulin concentrations in fasting and after meals. The states of pre diabetes and type 2 diabetes mellitus (MetS) are themselves a cardiovascular risk factors. Patients with impaired glucose tolerance or fasting hyperglycemia have a relative risk of developing macrovascular complications (ischemic heart disease) 2-3 times higher than that of healthy subjects of the same ages.

Lipid Metabolism

The alterations of the lipid profile which characterize the metabolic syndrome frequently is secondary to insulin resistance, in fact there is a change in the physiological suppression of the release of fatty acids from the adipose tissue in the postprandial. The greater availability of precursors determines an increase in liver LDL-C synthesis and an increased availability of triglycerides in the systemic circulation. It is possible to imagine that the healthy adipose tissue can become hypertrophic with progressively altered functions due to genetic imprinting, perinatal conditions and chronic overfeeding. This would generate a lipotoxicity (inflammation, mitochondrial dysfunction, insulin resistance related to unfavorable effects due to adipokines). The lipotoxicity is responsible of endothelial dysfunction and then macrovascular damage, this can trigger and maintain the atherosclerotic cascade.

Vascular remodeling in MetS

All clinical conditions that are part of the metabolic syndrome are associated with the development of early atherosclerosis. It can be documented by carotid doppler ultrasound study of large vessels (IMT or intimate-media thickening and plaques) and arterial tonometry (central arterial pressure, normal carotid-femoral pulse wave velocity > 12m/sec). There is evidence that the presence of MetS is associated with a greater degree of damage on the carotid body, the waist circumference and systolic blood pressure have a direct effect on IMT. A dietary and behavioral therapy may decrease the IMT. An accurate measure of arterial stiffness is given by the arterial tonometry that does not have clinical implications, but is only used in research: if we exert more pressure on a vessel it becomes more rigid. With traditional drug therapy we can reduce the brachial but not central pressure (CAFE Trial of 2199 hypertensive patients Annual American Society of Cardiology Congress in 2005). However, many studies consider sessions of aerobic training very important to reduce the central blood pressure.

Fatty liver and MetS

The nonalcoholic fatty liver disease (NAFLD: Non Alcoholic Fatty Liver Disease) is a extremely heterogeneous clinical pathology characterized by the accumulation of triglycerides in the liver (Targher et al. 2013, Marchesini et al. 2003). The clinical and pathological presentation of NAFDL is generally

variable: the less advanced forms is potentially reversible, but it can also move towards the hepatic steatosis associated with inflammation and fibrosis (steatohepatitis), and in more severe cases into cirrhosis. Cytokeratin 18 is an excellent blood chemistry marker of fibrosis. The presence of steatosis facilitates the increase of carotid IMT (intimate media thickness), increased levels of inflammatory markers (CRP, fibrinogen, Von Willebrand factor) and increases the probability of finding electrocardiographic changes. The majority of patients with hepatic steatosis died for cardiac or pulmonary and not hepatic event.

MetS and ischemic heart disease

Individuals with MetS have cardiac ischemia with a frequency at least 3-5 times greater. Furthermore, survival after cardiac ischemia is significantly reduced if there is hyperglycemia and hypertension. The content of coronary calcium is a good predictor of atherosclerosis, this is higher in presence of MetS. The patients with the metabolic syndrome have a higher rate of restenosis, both at the level of native coronary arteries treated with angioplasty and/or stents and at the level of arterial/venous graft (bypass); in this case the use of medicated stents is preferred to traditional metal stents. The INTERHEART study Lancet 2004 indicates that 20% of IMA is related to visceral fat, two latest Italian studies in 2010 linking MetS and acute coronary syndrome, show that having a BMI between 26.5 and 27 increases the relative risk of

cardiovascular mortality. The Japanese study JATOS of 2011 shows that MetS represents a risk factor for mortality under the age of 75, above this threshold the main influences on mortality is the age of the subject. The patient with MetS who used antiplatelet drugs seem to have an increased risk of bleeding.

MetS and nephropathy

In patients with MetS there is an increased risk of nephrolithiasis. The literature contains at least 20 items reporting how patients with biopsy-proven proteinuric nephropathy appears to have a nodular glomerulosclerosis "diabetic like": these patients had metabolic syndrome without diabetes mellitus. Supplementation with vitamin D appears to have a role in preventing kidney stones, kidney disease and proteinuria. Vitamin D also improve glucose tolerance and reduce the secretion of renin (receptor VDR vitamin D in the beta cell).

MetS and COPD

Many patients with COPD, have a metabolic syndrome (about 47%). The pro-inflammatory condition (increase of IL 6, TNF, CRP) may originate from the lungs of COPD or from other peripheral organs, including adipose tissue.

These two beliefs have created a debate in the scientific literature: according to the first assumption, is the inflammation of the lung to spill inflammatory mediators into the bloodstream, while according to another hypothesis, it would be the presence

of an increased production of inflammatory mediators in non-pulmonary compartments. Rehabilitation can be however a solution: physical activity reduces the systemic inflammatory activity.

2. PURPOSE OF THE STUDY and CONCEPTS OF MUSCLE BIOENERGETICS

2.1 PURPOSE OF THE STUDY

We plan to evaluate, in medium term (duration of the study 3 years), the positive effects (decreased body weight, increase aerobic capacity, changes in blood chemistry parameters and in drug therapy), of a program of physical activity builds on an accurate assessment of parameters derived from a cardio-respiratory exercise test (CPET). The main index, more easily understood and well known, we intend to use for a proper prescription of exercise is the anaerobic threshold (AT), which represents the maximum aerobic potential of the subject. This metabolic level represents the main index on which to plan training, in fact above it there is a reduction of cardiac efficiency due to an increase in blood pressure and heart rate.

Hard intensity exercise (elevated heart rate and systolic and diastolic pressure), increases the oxygen consumption (VO2) of the coronary circulation (we have a decrease in diastolic time that is related to oxygen extraction by myocardial cells, especially when there is significant stenosis of the coronary tree and microcirculation). The result is an increased risk of ischemic events. We have an interest in having a complete picture of aerobic metabolism in our subjects in fact we want to

see the benefits (in terms of improvement of some parameters which we will consider) of physical activity correctly prescribed.

The study sample will include patients with risk factors and blood chemistry parameters indicative of metabolic syndrome and subject with metabolic syndrome associated with diseases such as diabetes mellitus type 2, hypertension and hypercholesterolemia, free from major cardiovascular diseases. Our interest in oxidative metabolism concerns in particular in the evaluation of ventilatory threshold (VT/AT), and on the study of the oxygen consumption and heart rate kinetics. Particularly the study of VO2 and HR kinetics during exercise of intensity (watt) lower of 10% of intensity (watt) calculated at the ventilatory threshold.

At this workloads, in fact, the subjects would be able to perform a long slow intensity physical activity, during which optimizes fat consumption that is the target for subjects with metabolic syndrome. The possible occurrence of so-called "slow component", an excess of O2 consumption compared to that predicted in relation to the predetermined workload, represent an increased energy cost, with an anaerobic component and production of lactic acid, in that case the subjects will result to perform physical activity using glucose instead of lipids.

Both the determination of the ventilatory threshold and the analysis of VO2 and HR kinetics at exercise intensities below

the ventilatory threshold in patients with metabolic syndrome is a new proposal, and allows us to identify cardiovascular and metabolic adaptations ever tested in this way in this population. There are no studies in literature dealing with VO2, HR kinetics and AT in subject with metabolic syndrome. We want to value each person as if he/she was an athlete of high level, with the purpose to define precisely the type of "biological motor", with the use of CPET evaluation.

2.2 MUSCLE ENERGY METABOLISM

The cells are able to convert some substrates (fats, proteins, carbohydrates) in energy, through a complex system of chemical reactions:

- the catabolic reactions convert complex molecules into simpler with release of energy and require oxygen to do this
- anabolic reactions synthesize larger molecules from smaller precursors, and require a supply of energy.

The link between the pathways that produce energy and those that use it is adenosine-5'-triphosphate (ATP). During the reaction of hydrolysis of ATP, catalyzed by the enzyme adenosine triphosphatase (ATPase), the external phosphoric

group comes off (inorganic phosphate, Pi), adenosine diphosphate (ADP) is formed and energy is released:

ATPasi

$ATP + H_2O \rightarrow ADP + Pi - 7.3$ kcal/mol (Mc Ardle p.133)

The value of - 7.3 kcal/mol represents the energy available (according to the laws of thermodynamics and is denoted by ΔG) to do useful work. The cell can provide energy by splitting ATP.

The muscle cell has limited amount of ATP (24 mmol/kg of muscle mass) (Casey and Greenhaff, 2000): the ATP concentration decreases a lot when the cellular metabolism increases, so the ATP must be reconstituted from ADP and Pi; the amount of ATP present in the muscle at rest is sufficient to support a little number of contractions. The cells synthesize ATP through the metabolic pathway of phosphocreatine (PCr), through anaerobic glycolysis and oxidative metabolism.

2.2.1 Phosphocreatine (PCr)

This is the most simple and rapid way to produce ATP. Implies the donation of a phosphate group from PCr to ADP, through a reaction of hydrolysis of PCr catalized by the enzyme creatine phosphokinase (CPK).

CPK

PCr + ADP → ATP + Cr

The cells have small quantities of PCr (80 mmol/kg of muscle mass) (Williams and Branch, 1998), and the amount of ATP which can be formed by this way is poor, however, it is sufficient until alternative slower metabolic processes reach adequate speed of production of ATP. The PCr system is characterized by a remarkable power, an high capacity of resynthesis of ATP in unit of time, and provides energy for muscular contraction at the beginning and during short and strong exercises. The PCr mechanism does not require the presence of O2 and therefore is called "anaerobic"; also it does not involve the production of lactic acid, so is called "anaerobic alactacid". The resynthesis of PCr requires ATP and occurs only during rest after exercise.

2.2.2 Anaerobic glycolysis

This metabolic pathway don’t need O2 to run. The glycolysis involves the degradation of glucose with the formation of two molecules of pyruvic acid or lactic acid for each molecule of glucose. The final result of the process is the production of energy. The first five reactions require the use of 2 ATP molecules for the phosphorylation of glucose to glucose-6-phosphate and fructose-6-phosphate to fructose-1,6-bisphosphate.

The next five reactions lead to the formation of 4 ATP, two for each of the two molecules of glyceraldehyde-3-phosphate

derived from fructose-1,6-bisphosphate. Thus there is a gain of 2 moles of ATP for each mole of glucose.

We must also remember that this is true when glucose comes from blood. If glucose results from cellular stores of glycogen we need a single molecule of ATP: through that action the glycogen phosphorylase enzyme, responsible of glycogenolysis, free glucose that enter into the process of glycolysis already as glucose-6-phosphate. The glycolysis, free hydrogen ions (H+) reaction that transforms the glyceraldehyde-3-phosphate in 1,3-diphosphoglycerate, with the formation of NADH from NAD (nicotinamide adenine dinucleotide).

So to continue glycolysis we need NAD as acceptor of H+. The cells can use two ways to do this: if O2 is present, H+ can be transported by specific transport systems inside the mitochondria to produce ATP aerobically (entering the electron transport chain); if the O2 is not available as an acceptor of H+, this role is played by pyruvic acid, with the formation of lactic acid.

The enzyme that catalyzes this reaction is lactic dehydrogenase (LDH). However, lactic acid acidifies the cytoplasm, with inactivation of the enzymes of anaerobic glycolysis. This metabolic pathway is called "anaerobic lactacid", it does not require the presence of O2, and implies the production of lactic acid. During glycolysis only 5% of the total amount of ATP is formed. This ATP is liberated from the complete catabolism of 1 molecule of glucose. The glycolytic pathway releases a significant amount of energy during strenuous exercise,

because the concentration of cytoplasmic enzymes and rate of reaction are high.

2.2.3 ATP aerobic production (oxidative metabolism)

The aerobic production of ATP occurs in the mitochondria and consists of two cooperating pathways: the Krebs cycle and the electron transport chain (respiratory chain). The Krebs cycle has the primary function of complete the oxidation (removal of hydrogen) of carbohydrates, fats, proteins (who access the Krebs cycle as acetyl-coenzyme A), reducing NAD+ and flavin-adenosine-dinucleotide (FAD+). The respiratory chain uses NADH and FADH as electron donors. The transport along the electron transport chain free energy that is used for ATP synthesis (oxidative phosphorylation coupled to the respiratory chain).

Krebs cycle. Acetyl-coenzyme A (acetyl-CoA) is the starting substrate of the Krebs cycle. Acetyl-CoA derived from the degradation of carbohydrates, fats, proteins. In mitochondria the pyruvic acid resulting from the processes of glycolysis is linked to coenzyme A to form the acetyl-CoA; this reaction, catalyzed by the pyruvate dehydrogenase enzyme, releases CO2 and two hydrogen atoms with formation of NADH. NADH is directed to the respiratory chain.

The triglycerides degraded to glycerol and fatty acids can enter in the Krebs cycle and the fatty acids are converted into acetyl-

CoA during the ß-oxidation. The proteins can enter the metabolic energetic pathways after a degradation to amino acids, some of them can be converted into glucose or pyruvic acid or acetyl-CoA or still other intermediates of the Krebs cycle. Protein contribution to the energy production is still quantitatively insignificant. Acetyl-CoA is completely oxidized to CO2 and H2O. For every molecule of acetyl-CoA degraded corresponds the formation of three molecules of NADH, one of FADH and one of guanosine triphosphate (GTP, a molecule that can transfer its terminal phosphate to ADP to give ATP).

Chain electron transport. This process coupled to oxidative phosphorylation allows the aerobic synthesis of ATP. The respiratory chain uses the potential energy available in NADH and FADH, to phosphorylate ADP to ATP. NADH and FADH don't interact directly with the O2: the electrons removed from the hydrogen atoms pass through a series of carriers named cytochromes. Redox reactions take place along the chain of cytochromes so we have sufficient energy to synthesize ATP. The final electron acceptor is O2.

The coupling of the respiratory chain with the oxidative phosphorylation is explained by the chemiosmotic hypothesis: the energy released by the electrons along the chain is used to pump from the mitochondrial matrix through the inner mitochondrial membrane the H+. H+ accumulates in the space between the inner and external mitochondrial membrane; so we have a gradient concentration which drives the H+ to diffuse

through the inner membrane into the mitochondrial matrix from which they originate. The inner mitochondrial membrane is impermeable to H+, so the transition is possible only through specialized channels: the passage of 2 H+ through these channels provides enough energy for the phosphorylation of ATP.

The complete catabolism of one molecule of glucose leads to the formation of 38 ATP (including anaerobic glycolysis and the transformation of pyruvic acid in acetyl-CoA). Whereas the potential energy released by complete oxidation of one mole of glucose is 686 kcal, and that from the complete catabolism of one mole of glucose we obtained 38 moles of ATP, and, that each mole of ATP provides 7.3 Kcal, one can easily calculate that the efficiency of the system is 40%.

While the PCr and anaerobic glycolysis metabolic pathways provide energy for intense and/or short efforts, the possibility of performing prolonged muscular exercises depends strictly on the oxidative metabolism (Fig. 2.1 and 2.2): the oxidative metabolism, provides a less maximum power than the anaerobic mechanisms, but is able to maintain this power for prolonged periods of time. So then the amount of work developable is much greater than that of PCr and glycolysis.

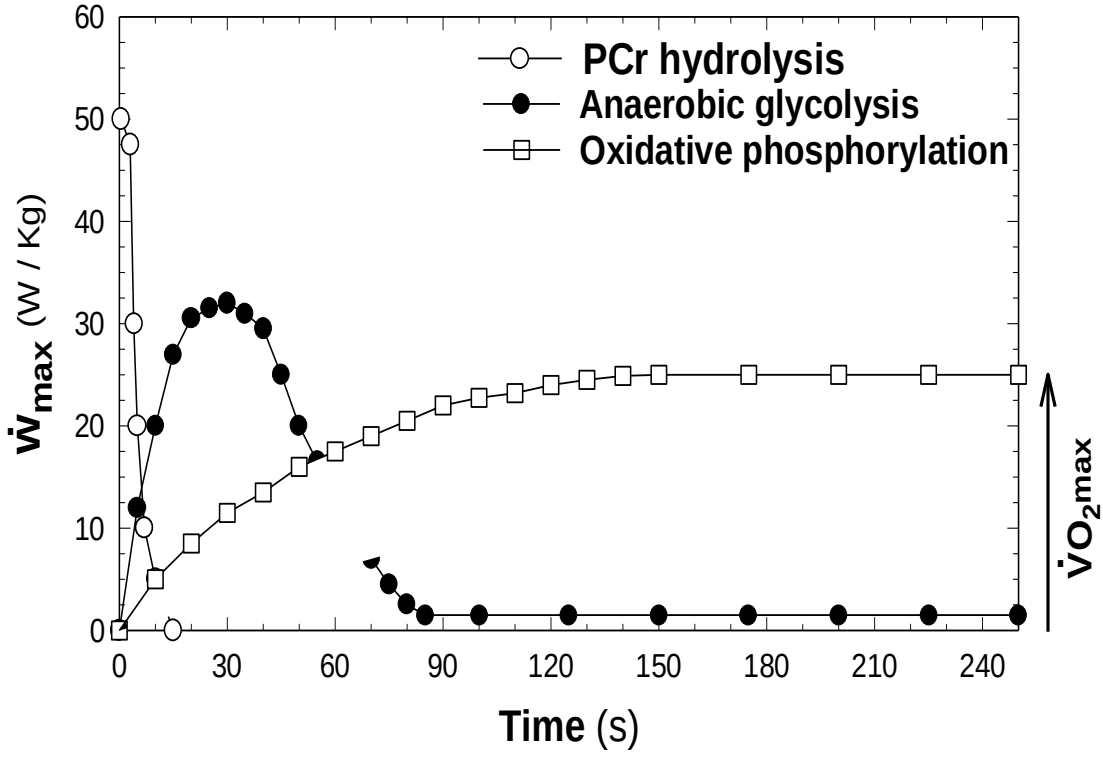

Figure 2.1 - The graph shows the evolution over time of the three metabolic pathways deputies to energy production during exercise. The anaerobic mechanisms are characterized by rapid activation and increased power. The mechanism of aerobic ATP production is rather slow and less "powerful", but ensures energy for prolonged periods. After about 60 seconds of exercise the metabolic mechanism more important for the ATP production is oxidative (Grassi et al. 2003a).

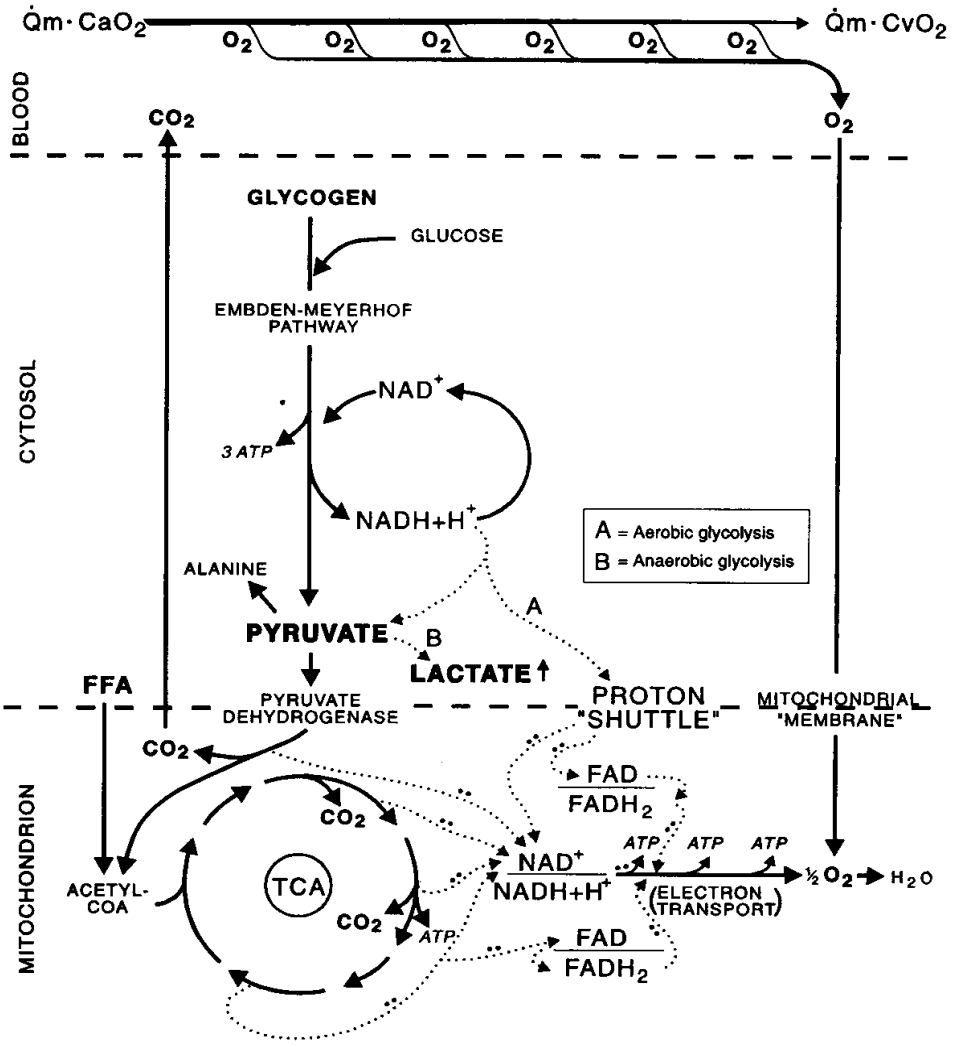

Figure 2.2: Oxidative metabolism, (From Wasserman et al.: Principles of exercise testing and interpretation).

3. EVALUATION OF OXIDATIVE METABOLISM

In the functional evaluation of muscle oxidative metabolism are of particular interest three variables:

1. The VO2max (maximum oxygen consumption represents the maximum aerobic power, the maximum amount of energy that can be available, using only oxidative processes. VO2max is the maximum ability to use O2 by mitochondrial enzymes of respiratory chain.

2. The VT (ventilatory threshold), defined as the intensity of exercise at which lactic acid begins to accumulate, and its production exceeds the capacity of removal.

3. The speed of adjustment of oxidative metabolism and heart rate to new workloads (test at constant load and on-kinetic of O2 consumption and HR).

3.1 MAXIMUM AEROBIC POWER (VO2max)

VO2max is a key factor, it influence the ability to sustain high intensity exercise for periods longer than 4 to 5 minutes.

When the oxidative substrates are sufficiently present, the factors that may limit VO2max are: pulmonary factors (alveolar ventilation, gases respiratory diffusing capacity across the alveolar-capillary membrane), blood factors (Hb concentration), cardiovascular factors (cardiac output, capacity of the peripheral circulation), tissue factor (diffusion capacity of O2 from the capillaries to mitochondria of muscle cell, the ability of mithocondria to use the O2).

Under physiological conditions the main factor that determines the VOmax is the cardiac output (maximum capacity of the heart to pump blood rich in O2 to the working muscles). The determination of VO2max is considered one of the main variables for the assessment of the functional capacity of muscle, cardiovascular and respiratory system.

The values of VO2max of sedentary adults vary between 40 and 50 ml O2/kg/min (Powers et al., 1989, Lacour et al., 1977), and are influenced by sex and age.

These values are significantly higher in men (> 30%) than women (Yoon et al., 2007; Åstrand 1952), due to the greater percentage of body fat in women (Von Döblen 1956), and decrease with age (Ferri and coll., 2007, Drinkwater et al., 1975, Hermansen and Saltin, 1969; Åstrand 1960).

VO2max is usually measured during an incremental test on a cycle ergometer or treadmill: the workload is increased every minute or with a ramp protocol until exhaustion of the subject. The VO2 increases with increasing load. We have VO2max when at an increase of the load no longer corresponds an increase of VO2 (VO2 plateau).

The energy to sustain the load over VO2max derived from glycolysis, with lactic acid accumulation and rapid fatigue. In pathological subjects, elderly, or even in normal untrained subjects, the identification of the plateau of VO2 during incremental exercise is difficult or impossible. In these cases we have VO2peak, defined as the value of VO2 detected at physical exhaustion of the subject (Howley et al., 1995; Cooper et al., 1984; Davis et al., 1982).

3.2 THE VENTILATORY THRESHOLD (VT, AT)

The incremental test is used to calculate the lactic acid (LT) and ventilatory threshold (VT).

Physical activity affects the oxygen consumption (VO2), carbon dioxide production (VCO2), pulmonary ventilation (VE) and the lactic acid accumulation, and there is a close relationship between VO2, VE, and lactic acid production.

During an incremental exercise (considering the production of lactic acid and VO2, Fig. 3.1) we can see:

1) During lighter workloads we don't observe an increase in lactic acid in the blood: it remains constantly on values comparable to those of rest. These workloads can be sustained for long time, virtually indefinitely. During lighter workload VE and VO2 increases consensually.

2) During moderate exercise we observe at a 'threshold intensity of exercise', an increase of the concentration of blood lactic acid around values of 4 mmol /L (Van Schuylenbergh et al., 2005; Seip et al., 1991; Yoshida et al., 1987), this is the result of an equilibrium between production and reuse of lactic acid.
Lactic acid has to be buffered to prevent that muscle fiber microenvironment becomes excessively acid. Bicarbonate is the primary buffer, according to the reaction:

$$H^+La^- + NAHCO_3 \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O + Na^+ + La^-$$

The CO2 produced and that derived from the oxidation of energy substrates is then eliminated by pulmonary ventilation, increasing workload causes a greater increase in VE than VO2. Pulmonary ventilation, have to eliminate the CO2 produced by oxidation of energy substrates, and a surplus of CO2 produced by bicarbonate buffering of the lactic acid, therefore the CO2 increases more than the VO2, the increase of VO2 depends only by the workload.

3) At the maximum workload we have a continuous increase in blood lactate concentration to values greater than 4 mmol/L. These workloads can be sustained for limited periods of time, because of early onset of muscle fatigue.

The more lactic acid accumulates in the blood, the lower is the concentration of bicarbonate. When the lactic acid reaches the value of 5 mmol /L the bicarbonates system is no longer able to properly perform the buffering action (Wasserman et al., 1999): the lactic acid accumulates more and more so pH in the muscle fibers and in the blood decreases. The accumulation of H+, causes a state of metabolic acidosis.

Metabolic acidosis stimulates the carotid glomus, so there is a compensatory hyperventilation: VE increases even more than it does VO2; pulmonary ventilation must respond not only to the need to expel the carbon dioxide resulting from the oxidation of energy substrates and from the buffering of lactic acid, but also to hyperactivation of ventilatory stimulus coming from the carotid bodies that react to metabolic acidosis.

The muscle fiber during incremental test (ramp test) increase lactic acid production. This has essentially two reasons:

- If glycolysis proceed at a speed greater than that of use of the pyruvate in the Krebs cycle the lactic acid can accumulate in the muscle cell and in blood during exercise.

- When the NADH + H+, reduced during glycolysis, it is not rapidly reoxidized by shuttle protons of the inner mitochondrial membrane, that reoxidation is made in the cytosol by pyruvate according to the reaction:

  Pir + NADH + H+ → Lac + NAD+

  This mechanism happens when the oxygen supply is not sufficient for the reoxidation of NADH + H+, and it is the primarily responsible for lactic acid accumulation.

The increase of lactic acid takes place at a given subjective workload; with increasing the workload beyond that threshold value we have an increase in blood lactic acid concentration, a decrease of bicarbonate, and an increase in carbon dioxide production of a surplus in addition to that resulting from the oxidation of energy substrates due to lactic acid buffering.

The oxygen consumption at this workload is defined LT. LT can be detected by analysis of gas exchange during incremental test (see data analysis section), and then takes the name of ventilatory threshold (VT), LT and VT have the same physiological meaning, indicating the point at which, during an incremental test, the production of lactic acid exceeds its removal.

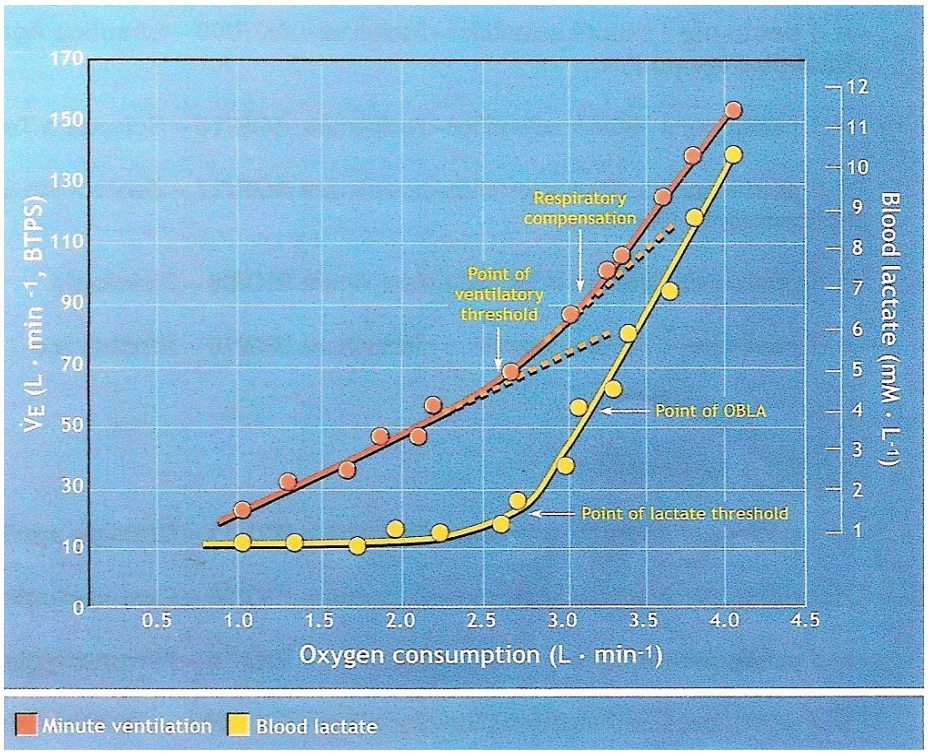

Figure 3.1 - VE, blood lactate concentrations, and VO2 during an incremental test. The lactic acid threshold represents the intensity of exercise associated with blood concentrations of lactic acid less than 4 mmol/L. The lactic acid threshold and the VT occurs at the same intensity of exercise (the point at which the relationship between VE and VO2 presents the first inflection). Obla (onset of blood lactate accumulation) represents the point at which the concentration of lactic acid in the blood reaches and exceeds the value of 4 mmol/L. The point of respiratory compensation shown by the flection of the relationship between VE and VO2, is in concomitance of lowering of plasma pH during exercises near maximal workloads. Mc Ardle et al. (2001).

VT is usually expressed as a percentage (%) of the VO2max. The average value of VT in healthy young untrained corresponds to 53-55% VO2max (Davis et al., 1997, Jones et al., 1985; Davis et al., 1979), with a dispersion which is 49-63% (Davis et al., 1997, Jones et al., 1985; Nery et al., 1983; Orr et al., 1982).

Jones et al. (1985), Davis (1997), Sanada et al. (2007) have also studied the effect of age on VT value: they found that absolute VT value decreases with age, but less than the decrease of the absolute value of VO2max, so the value of VT expressed as a percentage of VO2max will tend to increase in elderly subjects (58-60% on average for 70 years of healthy sedentary men).

In trained resistance athlete VT assumes values greater than in the sedentary (Wilber et al., 1997; Whipp, 1994), with peaks of up to 80-90% of VO2max (Tschakovsky and Hughson, 1999).

The metabolic cause of accumulation of lactic acid in the muscle fiber and into the blood stream is still matter of debate, there seems to be greater agreement regarding the factors that determine the percentage of VO2max corresponding to the VT. It can be said that the peripheral factors, such as the composition of the muscle fibers, the capillary density, the quantity and size of mitochondria of muscle fiber and the concentration of the enzymes of oxidative metabolic pathway (Weltman, 1995; Coggan et al., 1992; Holloszy and Coyle, 1984) are major determinants of VT value.

The value of VT is closely related to aerobic capacity of the subject, many authors have already shown how VT has significant predictive value on endurance performance (Nicholson and Sleivert, 2001, Bishop et al., 1998; Speechly et al., 1996; Coyle and coll., 1988).

In a study by Hagberg and Coyle (1983) conducted on athletes who practiced the march they had shown a strong correlation between VT and performance time 20 km distance, the speed at VT had predictive value on performance, with a gap of 0,6%. Similar results were highlighted by Coyle et al. (1991) on cyclists, in which the mechanical power expressed in correspondence of VT was highly correlated (r = 0.93) with the power maintained during a maximal test of one hour on a cycle ergometer.

3.3.1 KINETICS OF VO2 ADAPTATION (TAU VO2)

The kinetics of adjustment of oxidative metabolism to new constant workloads depend on the load in fact is useful to consider the kinetics of VO2 under moderate load (moderate exercise), heavy (heavy exercise) and severe (very heavy exercise).

Moderate exercise. This is a intensity of muscular exercise performed below VT/LT. Under these loading conditions, VO2 increases, up to reach a steady state (SS) in about 3 min (Fig.

3.2), according to a monoexponential function (Hill and Lupton 1923) of the type:

$$y(t) = y_{\mathrm{Bas}} + \mathrm{A_p}\cdot[1 - e^{-(t-\mathrm{TD_p})/\tau_\mathrm{p}}]$$

as:

- bas is baseline VO2 value, before the beginning of exercise;
- Ap is the difference between the value of VO2 to the attainment of steady-state and the value of VO2 baseline;
- τp is the time constant (time required to attain 63% of the difference between the asymptotic level and level of rest of VO2). Within this category of exercise the time constant τp does not vary for load changes, however, is lower in trained subjects (Cerretelli et al., 1979) and may be decrease in the same subject with training (Hagberg et al., 1980). The time constant τp has different values depending on age, and is higher in elderly than in younger (Heberstreit et al., 1998, Babcock et al., 1994, Paterson et al., 1990; Åstrand, 1960; Berg, 1947), indicating a slower rate of adjustment to the workload, this indicates an increase in slow oxidative metabolic pathways to adapt to exercise. Even in elderly subjects, however, the exercise improves the adaptation of VO2 kinetics, in fact there is a decrease of τp with training (Fukuoka et al., 2001; Babcock et al., 1994).
- TDp (time delay) the latency time to onset of exponential trend of increase of VO2 starting from the situation of rest.

The kinetics of VO2 consist of three phases, which have a different physiological significance. The phase 1 is the increase of VO2 within the latency time to onset of exponential trend (TDp); it reflects the increase in cardiac output and therefore of the pulmonary blood flow (Wasserman et al., 1974; Krogh and Lindhard, 1913). This phase, said cardiodynamic phase, does not express a metabolic muscle phenomenon, but is simply due to the increase blood flow in the lungs. The delay TDp, corresponding to the duration of phase 1, is the time taken by venous blood, coming from the exercising muscles, to reach the lungs. Phase 2 is the increase in VO2 according to the exponential function. It's the most interesting part because it reflects the kinetics of activation of muscle oxidative processes. The increase in VO2 during phase 2 represents the increased muscle extraction of O2, and the continuous increase of the flow itself (Grassi et al. 1996; Barstow, 1994). After the monoexponential phase follows the steady state (phase 3), in which there is a balance between the consumption of ATP and its aerobic resynthesis; if there is lactic acid production this is oxidized, or converted back into glucose in liver and kidneys. Within this category of physical exercise the value of VO2 at steady state is a linear function of the exercise intensity (Henson et al., 1989; Roston et al., 1987; Whipp and Wassermann, 1972).

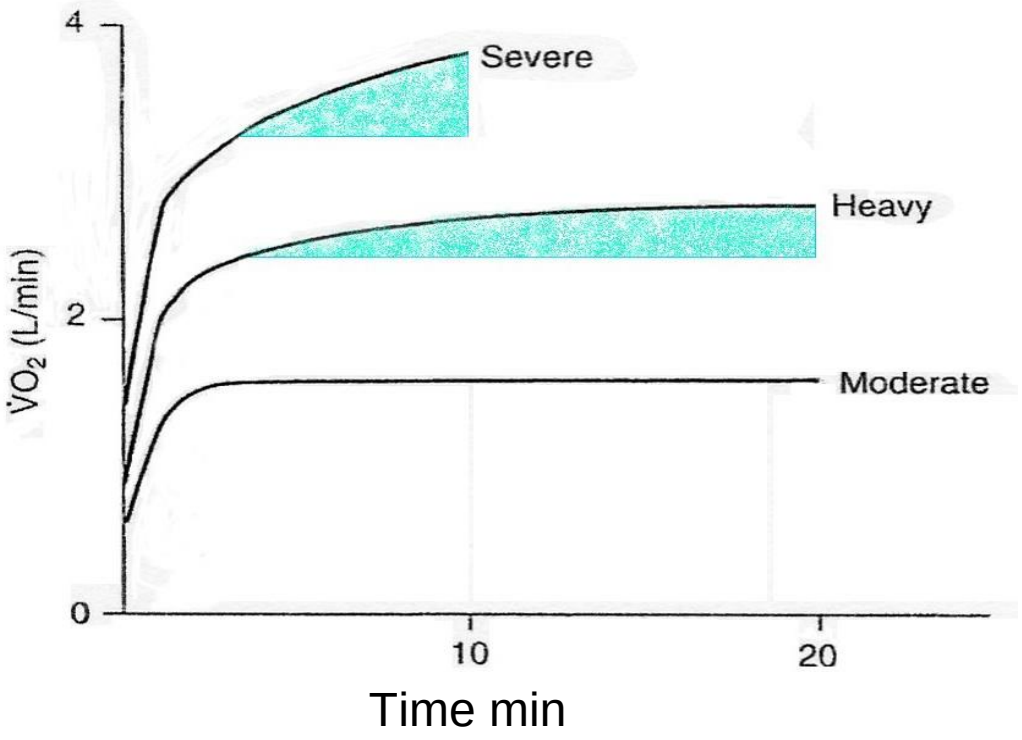

Figure 3.2 - Schematic representation of VO2 during constant load exercise of moderate, heavy and severe intensity. The cardiopulmonary phase was omitted.

During moderate exercise VO2 increases according to a monoexponential function to reach steady state. The attainment of steady state during heavy exercise is reached later due to the slow component (gray bleu area), which is present only after exceeding the lactic acid threshold. During severe exercise the stationary state is never achieved. From Poole and Richardson (1997).

When you switch from a resting state to exercise, the steady state is reached, after 3 min, according to the exponential model.

This delay indicates that in step, resting → exercise the muscle use the non-oxidative energy mechanisms (PCr and glycolysis) to produce ATP, the amount of O2 needed to produce the same amount of energy supplied by anaerobic system is the so-called O2 debt (Fig. 3.3), which is repaid at the end of exercise, when the O2 consumption is not instantaneously lowered, but decrease in according to an exponential kinetics similar to that of the rest → exercise passage.

We can conclude that the more rapid the adaptation of oxidative metabolism, the less the muscle will have to use some other mechanism, with positive effects in some phenomena (less depletion of muscle glycogen, less accumulation of lactic acid) associated with muscle fatigue (as in trained subjects, for example fig. 3.4): the kinetics of adjustment of VO2 has, in fact, functional consequences.

The characteristics of VO2 kinetics may depend on:

1. the kinetics of adjustment of the intake of O2 to the muscle (Hughson et al. 2001).
2. an intrinsic inertia of muscle oxidative metabolism (Whipp and Mahler 1980).

The debate on which of the two factors mentioned above is the predominant is still in progress. Many studies have shown a kinetic adjustment of HR and cardiac output faster than that of the pulmonary VO2 for sudden workloads imposed, that is, an excess of O2 transported than that used (Bangsbo et al. 2000; Grassi et al. 1996). Other studies have shown as a facilitation of convective and diffusive O2 intake in in situ isolated skeletal muscle does not significantly alter the kinetics of VO2 in the muscle (Grassi et al. 1998a; Grassi et al. 1998b; Grassi et al. 2000). Observations of this kind favor the hypothesis that intrinsic inertia of muscle oxidative capacity is the most important factor in determining the characteristics of VO2 kinetics. In agreement with this hypothesis, the VO2 kinetics should be determined primarily by the concentration levels of cellular mediators and/or activation of certain enzymes of the oxidative metabolic process. We considered as possible factors involved in that control the state of activation of pyruvate dehydrogenase complex and the cell concentration of NO (nitric oxide) (Kindig et al. 2001) which would act as an inhibitor of cellular respiration. Other recent studies have reduced the importance of these two factors (Grassi et al. 2003c; Grassi et al. 2002). One of the main factors involved in the control of the VO2 on kinetics could be the concentration of PCr or some intermediate of this pathway: Creatine (Cr) which is liberated from the hydrolysis of PCr at the beginning of the exercise would fit in mitochondrial outer space where it is reacted with ATP, to replenish PCr. The ADP resulting from this reaction can

reach the mitochondrial matrix and stimulate cell respiration and oxidative resynthesis of ATP (Whipp and Mahler 1980). Many studies have shown how subjects suffering from diseases of muscle O2 supply, such as metabolic myopathies (which really affect the oxidative metabolism) (Grassi et al., 2002), congestive heart failure (Sietsema et al., 1994), chronic respiratory diseases (Nery et al., 1982), diseases of the peripheral vascular system (Bauer et al. 1999), type 2 diabetes (Regensteiner et al., 1998), or cardiac transplant (Cerretelli et al., 1988, Grassi et al., 1997), show lower VO2 kinetics compared to healthy subjects, in association with early fatigue, so these diseases have a negative effect on the distribution of O2 to the peripheral tissues (and therefore also to the working muscles during exercise), and/or directly on the oxidative metabolic processes.

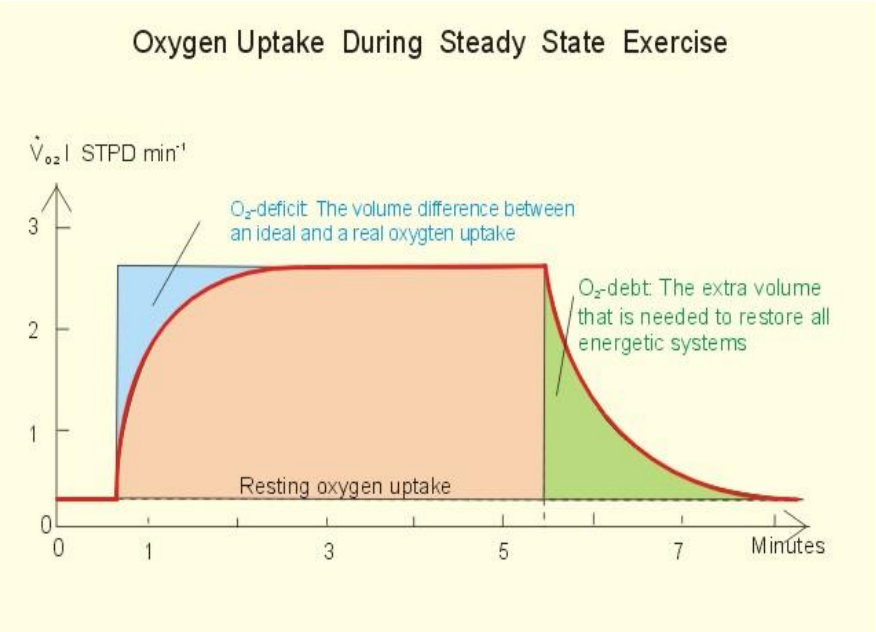

Figure 3.3 - Evolution of VO2 during constant load exercise. When there is a switch from rest to exercise the steady state is not achieved instantaneously. In step resting → exercise the muscle produce ATP in non-oxidative way (PCr and glycolysis), the amount of O2 needed to produce the same amount of energy provided by anaerobic is the so-called O2 debt (O2 deficit in the figure), which is restored at end of the exercise, when the consumption of O2 is not instantaneously lowered, but is reduced according to exponential kinetics similar to kinetic of rest → exercise (O2 debt in the figure).

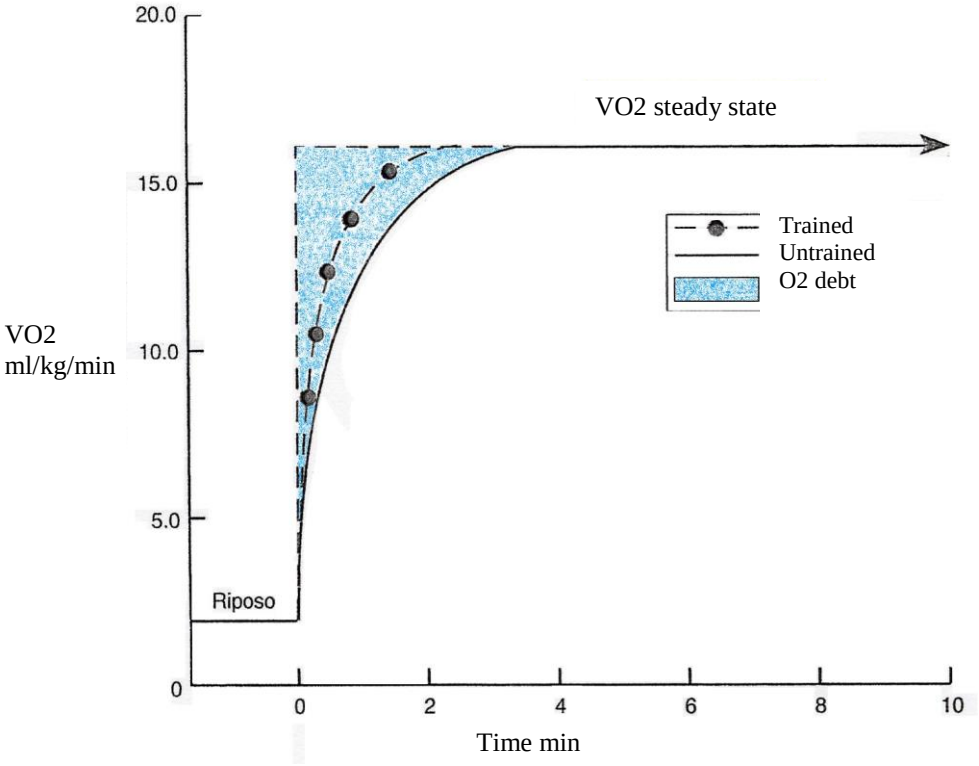

Figure 3.4 - Time trend of VO2 in move from rest to running in a person trained and an untrained. Both reach the steady state, with the same value of VO2. The bleu area indicates the O2 debt. This is the quantity that should be available from the beginning of the exercise if the oxidative mechanism is immediately fit to the workload. In trained subjects the oxidative mechanisms of adaptation to the workload is much faster, and lower the O2 debt.

From "Energy for physical activity", ch. 7 "Release of energy in physical exercise".

Heavy exercise. This category includes exercises in which the increase of lactic acid exceeds its removal (above LT). In this case, after monoexponential phase there is another component ("slow component" of the VO2 kinetics) which describes the increase of VO2 (Poole et al., 1994b; Paterson and Whipp 1991; Whipp 1987; Whipp and Wasserman 1972), which follows the steady state phase (Fig. 3.2), if the subject is able to sustain exercise intensity over time.

Severe exercise. In this case VO2 and lactic acid never stabilize and VO2 increase until the VO2max; this condition is typical of workloads equal to 50% of the difference between LT and VO2max (fig. 3.2). Also in this case there is a slow component, but we don't reach steady state because at this workload VO2 increase virtually since VO2max.

The slow component of VO2 kinetics is still little known, there is still no consensus on the best kinetic (linear vs. exponential) describing the phenomenon (Barstow and Mole 1991, Paterson and Whipp 1991).

There are many factors evaluated as possible causes of the slow component. The increase of lactic acid during exercise was highly correlated with the amplitude of the slow component (Whipp 1987). It's well known that the infusion of lactic acid increases the VO2, both during rest and exercise (Svedmyr, 1966 and Ryan et al., 1979). Lactic acid can stimulate gluconeogenesis in active muscle during exercise, this process

has an energy cost, and could be a cause of the slow component (Whipp, 1994, Talmadge et al., 1989). Wasserman et al. (1991) have considered that the lowering of pH caused by lactic acid accumulation and not lactic acid itself might be in correlation with the slow component: the acidosis lead, for Bohr effect, a rightward shift of the O2 dissociation curve for hemoglobin (Hb) increasing the partial pressure of O2 (PO2) in the capillaries, and thus allowing the increase of VO2.

Another factor that could explain the behavior of VO2 during exercise above LT is the increase in respiratory muscle and heart work: the slow component may depend partly also by O2 consumed to increase pulmonary ventilation and support the increased workload of the heart (Womack el al., 1995).

There is also great interest towards epinephrine, potassium released from the muscle during exercise, and the temperature rise of the muscle. It is well documented that the infusion of epinephrine increases the resting metabolism (Fellows et al., 1985). Plasma concentration of epinephrine increases considerably during heavy and severe exercise (Poole et al., 1991, Poole et al., 1988). Other studies, have shown that, despite the high plasma concentration, epinephrine has no appreciable effect on VO2 (Womack et al., 1995; Gaesser et al., 1994). The temperature of the muscle increases during exercise (Xu and Rhodes, 1999; Poole et al., 1991; Poole et al., 1988; Casaburi et al., 1987; Hagberg et al., 1978) and this could, through the 'Q10' effect in active muscles, contribute to the progressive increase in VO2 during heavy exercise. Poole

et al. (1991, 1988) have estimated that the temperature rise was related to the slow component, this was responsible for a significant proportion (as much as 39%), moreover, in the same studies it was shown how the temperature of muscle can rise despite VO2 of the lower limb remains constant. It is well known from studies of Rowell, 1971, that the increase in body temperature, and therefore also of muscles temperature, does not increase the pulmonary VO2 during exercise.

It was shown that potassium causes an increase in metabolism in isolated muscles of a frog (Barnes 1988). During heavy exercise the muscles release potassium, with an increase in plasma concentration. In one study, Yasuda et al. (1992) found a strong correlation between serum potassium and VO2, but in this case is not possible to draw definitive conclusions. Poole et al. (1991) observed that potassium increases at the beginning of the exercise, but after three minutes it remains stable until the end, whereas the VO2 continues to increase.

Some studies indicate that the slow component could be attributed to the pattern of recruitment of motor units (MUs). During exercises above the threshold we recruited also fast fibers, type II (less oxidative efficient) (Kushmerick et al., 1992), and this would increase O2 consumption. Shinoara and Moritani (1992) have shown that in fact, between the 4° and 7° minute of vigorous exercise on a cycle ergometer, the amplitude of surface electromyography (EMG) is correlated to the increase of VO2; the EMG amplitude reflects motor unit recruitment and/or frequency of discharge, these authors argue

that the slow component could be due to the recruitment of more fast twitch muscle fibers during exercise.

The recruitment increase could be due to:
1. active muscle increasing temperature, which compromises the efficiency of oxidative phosphorylation (Willis and Jackman 1994)
2. muscle fibers lowering pH or lactic acidosis (Hogan et al., 1995), which can adversely affect contractile function.

Other recent works (Scheuermann et al., 2000), examining VO2 and EMG during exercise on a cycle ergometer above threshold, however, have come to different conclusions, showing no correlation between muscle activity and the slow component of VO2, these study concluded that the slow component must be coupled with an increasing demand for ATP by the MUs already recruited rather than the recruitment of new MUs.
It is clear, that there is still not clarity on the physiological processes responsible for the slow component. This physiological process, whatever, should be located in the same muscles used during exercise (Poole 1994). Measuring simultaneously the pulmonary VO2 and the VO2 of the lower limbs during exercise on a cycle ergometer, Poole et al. (1991) have shown that approximately 86% of the increase in pulmonary VO2 occurs above the third minute of exercise and

can be justified by lower limbs VO2 increase. Other factors beyond muscle contribute minimally to the slow component.
Regardless the mechanism that is responsible for the slow component, this component bringing the subject to his VO2max, and contribute to muscle fatigue, or at least is associated with phenomena (recruitment of less efficient fibers, lactic acid accumulation, lowering pH, muscle glycogen stores depletion) that are closely associated with muscle fatigue.

3.3.2 KINETICS OF HR ADAPTATION (TAU HR)

In addition to the evaluation of the kinetics of O2 consumption, other parameters can be evaluated with the same method. In particular, it can be evaluated the kinetics of heart rate, as an index of cardiodynamics response (Phase 1). In this case are taken into account all the heart rate values from the beginning of steady state exercise (time 0). The phase 1 represent the increase in cardiac output and therefore of the pulmonary blood flow (Wasserman et al., 1974; Krogh and Lindhard, 1913). This phase, said cardiodynamics phase, does not express a metabolic muscle phenomenon, but is simply due to the increase blood flow in the lungs and therefore is expression of heart pump function and of the diffusion capacity of the lung, as well as index of the balance between sympathetic-parasympathetic system.

3.4 TRAINING EFFECTS

The body adaptations to training load is subjected to principle of specificity: anaerobic stimulus (short and intense exercise) induces specific adaptations of anaerobic metabolic pathways, while an aerobic overload (exercise for prolonged period of relatively lower intensity) determines specific adjustments to the aerobic metabolic systems (Tabata et al., 1996; Hurley et al., 1984).

3.4.1 Anaerobic Metabolism

The main changes that come with speed and power exercise training are:

- Increase of the substrates: the muscle biopsies done before and after training showed a significant increase of resting levels of ATP, PCr, creatine, glycogen, together with an increase of the force in trained muscles (MacDougall et al., 1998; Cadefau and coll., 1990; Saltin et al., 1974).
- Increase the amount and activity of anaerobic glycolysis key enzymes (phosphofructokinase, lactate dehydrogenase) (MacDougall et al., 1998). These changes occur on fast fibers, but are not comparable to those involving enzymes of aerobic oxidative processes in slow fibers as a result of resistence training (Jacobs, 1987).

- Increased ability to tolerate higher blood concentrations of lactic acid, due to an increase of energy substrates (glycogen) and the amount and activity of glycolytic enzymes, and for the increased motivation to tolerate the pain of fatigued muscles (Jacobs 1987, Gollnik and Hermansen, 1973).
- Selective hypertrophy of fast muscle fiber.

3.4.2 Aerobic Metabolism

Aerobic training induces functional adaptations of all body systems related to oxygen transport and use.

- The muscle of resistance trained subjects show a greater number and largest size mitochondria in each muscle fiber. It is known that larger mitochondrial have an increased capacity of regenerating ATP (Bizeau et al., 1998), in fact there is an increased amount of mitochondrial enzymes (succinate-dehydrogenase and phpsphofructokinase) (Hickson 1981; Gollnick et al., 1973b).
- Aerobic training results in a selective slow fibers hypertrophy (Gollnick et al., 1973b.)
- In resistance trained subjects, we detect a decrease in heart rate at rest and during submaximal exercise. The heart rate reduction indicates the training effect, it reflects an increase in stroke volume and cardiac output

(Saltin, 1969). The heart rate during sub maximal exercise decreases by about 12-15 beats/min, and decrease of 2-6 beats/min at rest (Wilmore et al., 1996; Seals and Chase, 1989). Already in a study of 1969 Saltin had shown that during an incremental exercise heart rate of untrained subjects increases much faster than that of endurance athletes. The untrained persons shown, for the same VO2 and equal exercise intensity an increase in heart rate respect the athletes: at VO2 of 2 L/min athletes have an heart rate of 70 beats/minute, lower than sedentary subjects. This difference was reduced to 40 beats/minute after a program of aerobic training.

- The increase in heart stroke volume due to training effect is related to 4 factors: an increased volume of the left ventricle; an increased heart compliance; an increase in the diastolic time of filling of the ventricular cavities; probably an increase in intrinsic contractile capacity of the heart muscle (Woodiwiss et al., 1998; Krip et al., 1997; Mier et al., 1997).
- The maximum cardiac output increase, is one of the most important adaptations to training and aerobic exercise. The maximum heart rate achieved during maximal exercise tends to decrease in the trained subjects, the increase in cardiac output is a direct consequence of the increase in stroke volume. The importance of increased cardiac output to have a best

performance was already evident in the study by Saltin, 1969: in this study Saltin showed a linear relationship between maximum cardiac output and VO2max; trained subjects showed increased maximum cardiac output and VO2max.

- Aerobic training results in a significant increase in active muscles oxygen extraction from the bloodstream (Rowell, 1996). The increase in arterio-venous difference results from a more effective distribution of cardiac output to the active muscles, as well as a greater capacity of oxygen extraction and use. After a relatively short period of training (55 days) the arterio-venous difference increases by approximately 13% (from 15 to 17 ml O2/dl) (Saltin, 1969).
- Aerobic training also leads to increased blood flow to active muscles during intense exercise, for three reasons: a higher maximum cardiac output, a better redistribution of cardiac output to the muscles involved in the exercise, an increase of cross section of small arteries and veins, together with the increase (about 10%) of muscle capillarization (Lash et al., 1995).
- Aerobic training probably also determines the increase of Mb in muscles rich in type I fibers (Jacobs, 1987), although recent studies also show opposite results (Masuda et al., 2001).

Aerobic training improves peripheral and central factors that help the transport and use of oxygen in the muscles. These positive effects have a very important functional aspect, resulting in a considerable improvement in the aerobic fitness, which is detected experimentally during an incremental or constant load test.

In fact we have an improvement of the parameters measured:

- An increase of VO2 max during the incremental test to exhaustion;
- An increase of the fraction (%) of VO2 max sustainable aerobically (an increase in VT).
- A decrease of the time constant τp and therefore a more rapid kinetics of O2 consumption and heart rate measured at the mouth during exercise at constant load.

CHAPTER 2

Title: Prevention-treatment of metabolic syndrome based on CPET (Cardio-Pulmonary Exercise Test)

Authors

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In progress

4. MATERIAL AND METHODS

4.1 SUBJECTS

The subjects for the study are recruited in collaboration with the San Carlo Borromeo Hospital (Milan) Operative Unit Dietetics and Clinical Nutrition. They are adults with risk factors or blood chemistry parameters indicative of metabolic syndrome (IDF 2005 criteria), not in drug therapy (subclinical phase of disease) and patients with metabolic syndrome, in therapy for associated diseases such as diabetes mellitus type 2, hypertension and hypercholesterolemia. In 2005 the International Diabetes Federation (IDF) has revised the diagnostic criteria and proposed as a method to identify the disease in the patient the presence of 2 of the following disorders: fasting glucose over 100 mg/dl; Hypertension: more than 130/85 mm Hg or drug therapy; Hypertriglyceridemia: above 150 mg/dl; reduced HDL cholesterol: < 40 mg/dl in males, < 50 mg/dl in females or lipid-lowering therapy, associated with a waist circumference over 94 cm in males, 80 cm in females for patients of Caucasian ethnicity (the parameters vary by ethnic group).

The study was proposed to 19 patients of both sexes (11 males and 8 females), all with the metabolic syndrome aged between 22 and 66 years.

Of the 19 subjects tested, 7 are in drug treatment for hypertension, 1 have diabetes mellitus type 2 in drug therapy with oral hypoglycemic, 1 with hypertriglyceridemia in medication with fibrates and 2 are in pharmacological treatment for hypercolesterolemia.

The subjects enrolled in this group must be free of cardiovascular diseases that preclude the possibility to perform an exercise test.

The anthropometric characteristics of subjects are summarized in Table 4.1.

The metabolic measurements were performed in the Laboratory of Applied Sport Physiology, Old San Gerardo Hospital in Monza and at San Carlo Borromeo Hospital in Milan.

Before attending the trial all subjects were adequately informed about the purposes of the study, the procedures used, and risks associated. All participants gave written informed consent to participate in the study, as required by research protocol.

Tab. 4.1 anthropometric data at the enrolment				
Subjects	age	BMI		Sex
1	55	25,4		F
2	30	30		M
3	62	35,13		F
4	43	29,7		M
5	62	33,12		M
6	57	27		F
7	59	30,8		F
8	54	20,8		F
9	54	26,7		F
10	42	27,3		M
11	48	30,9		M
12	56	39,9		M
13	48	41,3		M
14	50	24,4		F
15	61	31,5		F
16	56	25,4		M
17	22	26		M
18	54	37,8		M
19	66	37,9		M

Intervention

At the time of recruitment for the study subjects must have blood tests (triglycerides, HDL cholesterol, fasting glucose) related to parameters indicative of metabolic syndrome, we conduct an assessment of blood pressure, BMI calculation and measurement of waist circumference.

We evaluated each patient at time 0 with pulmonary function tests (complete spirometry to asses static and dynamic volumes) and two CPET (cardio pulmonary exercise test) led to the minimum value of anaerobic threshold.

Each subject visited the laboratory in different occasions. The first session provided a familiarization test to know the instrument. This test is very important to define the correct ramp for the next test, this test was conducted to exhaustion of the subject (inability to maintain pedaling frequency 60 rpm/min), and possibly to reach Respiratory Quotient (RQ) > 1,12.

Then in a second time each experimental session included two tests. Between the two tests we granted two hours of rest, this period is sufficient to return to baseline heart rate (HR) and blood pressure (BP). The first test was a ramp incremental test (the ramp is defined with familiarization test), the second was a constant workload test of at least 6 minutes, with workload at 90% of watts corresponding to those at VT.

The magnitude of the ramp for each subjects ranged between 8 and 25 watt ramp/min. See Table 4.2.

Tab. 4.2	Ramp (watt/min)	Steady state (watt)
Mean	14,4	68,4
DS	4,51	20,96

We defined for each subjects a personalized exercise program considering functional indexes obtained during CPET. The prescription of exercise consist of 3 training session of exercise every week, lasting 40 minutes each session, at a workload intensity corresponding to 90% of energy expenditure (expressed in terms of VO2 and HR) obtained at anaerobic threshold. The prescription of a low intensity personalized program should potentially ensure a greater compliance by subjects.

We proposed to use for the prescription of exercise, in addition to anaerobic threshold, also the perception of fatigue according to Borg scale rate of perceived exertion (RPE 6-20 value 11 is an exercise of light enough intensity). Each subject was free to perform each training session as he/she prefers, taking into account that the energy expenditure is different depending on the type of exercise performed.

The subjects involved in the study will be reassessed after 6 months of training program. The evaluation will include analysis of all functional parameters, blood chemistry and two CPET. Based on the results obtained from cardiopulmonary exercise testing is likely that the training program set previously should be modified in relation to expected increase in physical capacity of the subjects.

Expected results

- Change of drug therapy taken by the subject at the beginning of the exercise program of study (correlation with the decrease in body weight or improvement of the blood chemistry parameters evaluated).
- Increase in working capacity and aerobic fitness in general.
- Decrease in BMI.
- Improvement of some specific ventilatory parameter evaluated (AT, VO2peak, TAU value, VE. Etc.)

4.2 OPERATIG PROTOCOLS

Each subject performs a full spirometry with measurement of static and dynamic lung volumes. (Sensor Medics Vmax).

All exercise tests were carried out under close medical supervision and the subject were continuously monitored by 12-lead electrocardiography (ECG telemetry Cosmed).

Blood pressure was measured at rest, every 2 min during exercise and during recovery. All tests were performed on a electromagnetically braked cycle ergometer.

VT was determined during the incremental test using the integration of data from the three graphs defined by Wasserman and the method proposed by Beaver et al. (1987) (see fig 4.1).

The analysis of O2 and HR kinetics during exercise at constant load is computed whit a mathematical method (see section data analysis, Graph pad prism 5.00.288).

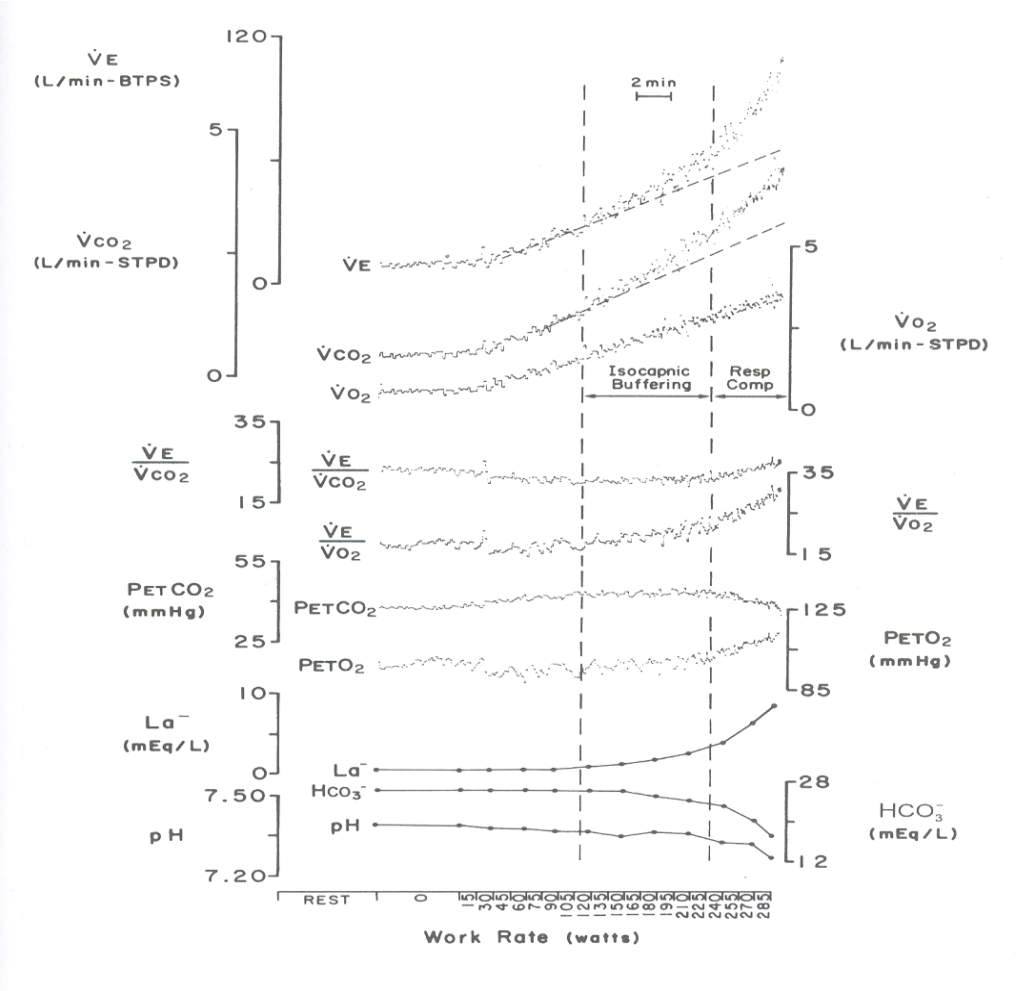


Figure 4.1: Breath-by-breath measurements of minute ventilation (VE), CO2 output (VCO2), O2 uptake (VO2), VE/VO2, VE/VCO2, PetO2, PetCO2, arterial lactate and bicarbonate, and pH during a one-minute incremental exercise test on a cycle ergometer. The AT or VT occurs when lactate increased (left vertical dashed line). This is accompanied by a fall in HCO3- (LAT) and generally an increase in VE/VO2. "Isocapnic buffering" refers to the period when VE and VCO2 increase in a curvilinear at the same rate without an increase in VE/VCO2, thus retaining a constant PetCO2. After the period of isocapnic buffering, PetCO2 decreases and VE/VCO2 increases, reflecting ventilatory compensation of the exercise metabolic acidosis. (From Wasserman et al.: Principle of exercise testing and interpretation).

The incremental test (Fig. 4.2) provides:

- 3 min of rest (subject sitting on the bike);
- 3 min of cycling in absence of load at 60-70 rpm (warm-up);
- Starting loading ramp protocol (ramp defined during the trial test);
- Interruption at muscular exhaustion, ECG criteria, excessive increase of arterial pressure, QR > 1.12, reached maximum predicted HR;
- 3 min of recovery without load.

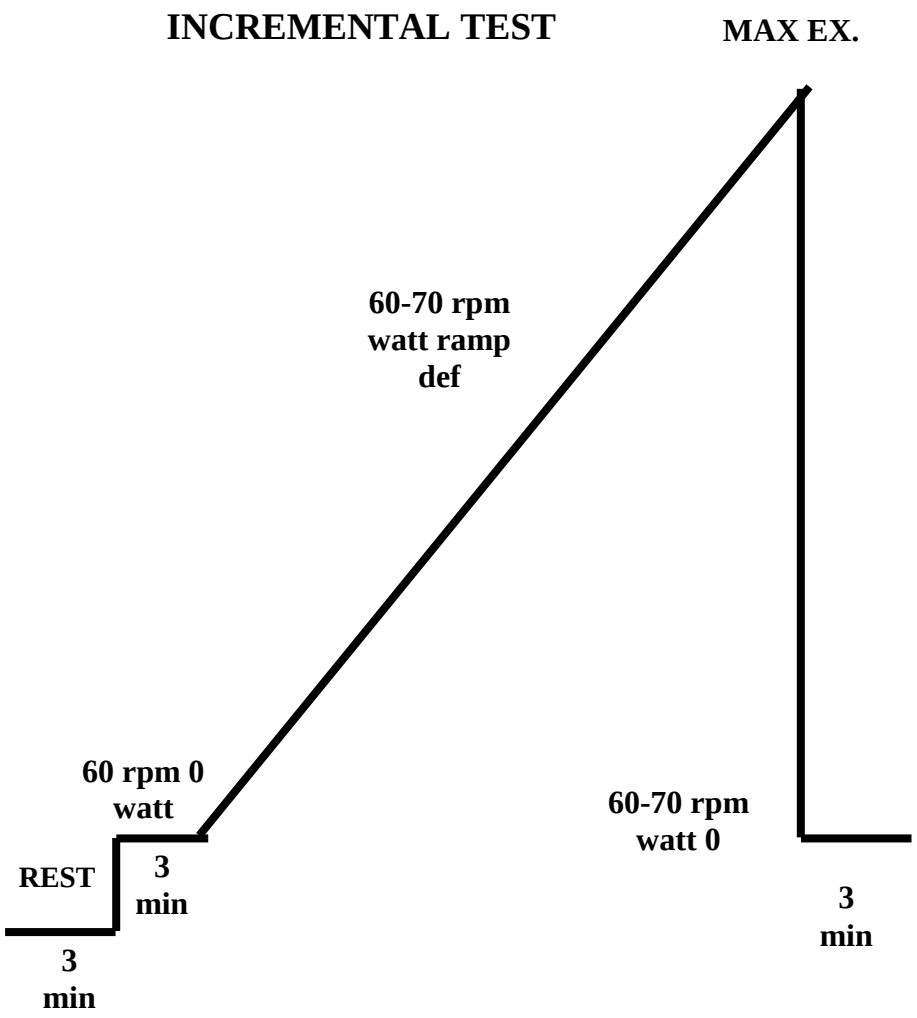


Figure 4.2: Protocol of incremental test. Ramp in Watts/min.

The constant load test (Fig. 4.3) provides:

- 3 min of rest (subject sitting on the bike);
- 6 min at workload of 90% of workload in watts at AT/VT
- 3 min of recovery without load.

After 6 months the subjects were recalled for a new test session with the same protocol and controls.

STEADY STATE TEST

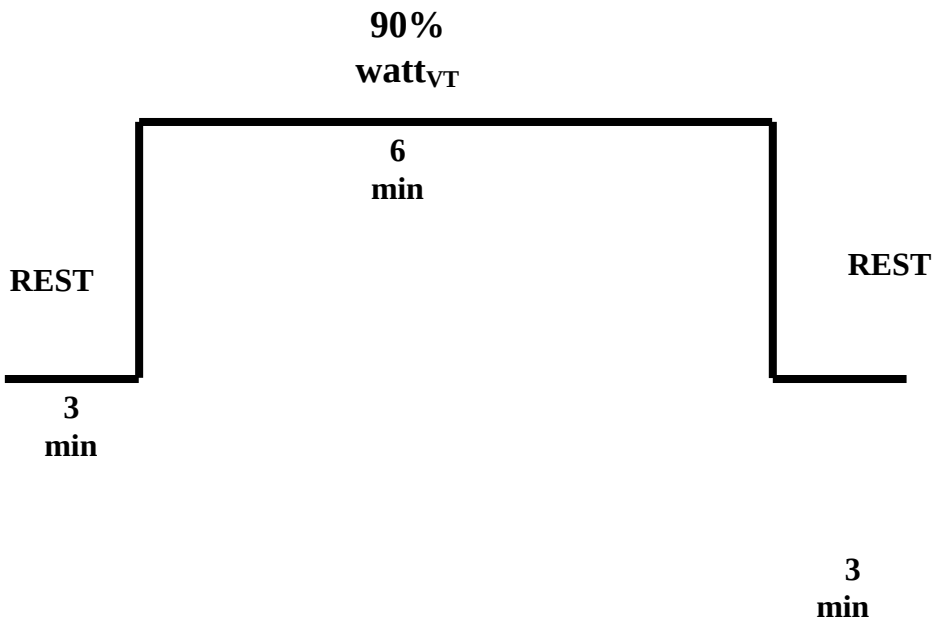


Figure 4.3: Protocol of test at constant load. watts VT is the value of watts at 90% VT.

4.3 MEASUREMENTS CARRIED

The CPET compared to a normal exercise stress test (usually aimed to analyze only the electrocardiogram trace during exercise), allows us to assess the specific efficiency of the oxygen transport-utilization system. The cardiac and pulmonary function have to give an adequate supply of oxygen to support working muscle energy demands. The sequence of events that realize such a supply of oxygen is the following (Fig 4.4):

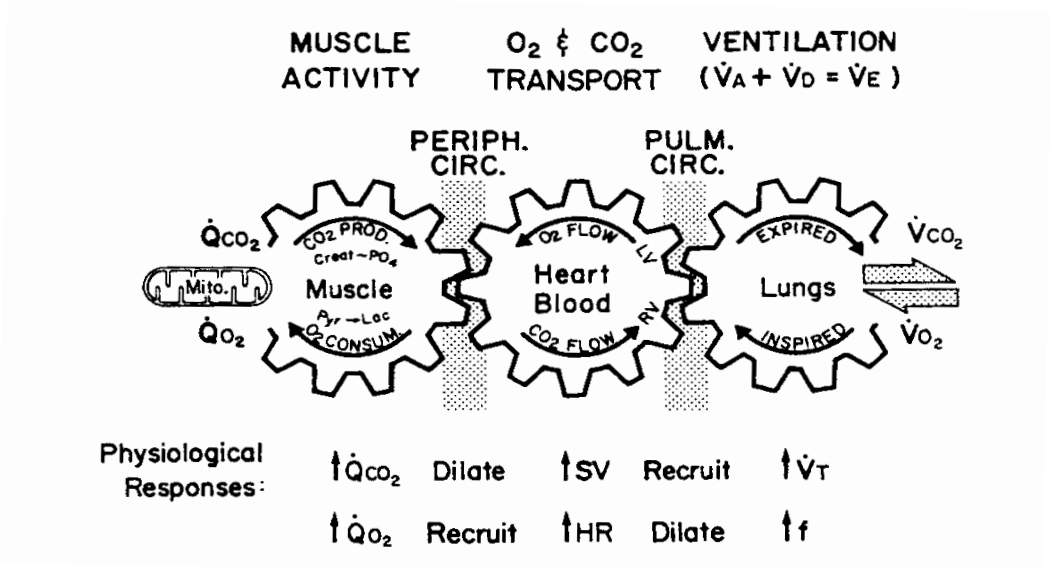


Figure 4.4: Gear of Wasserman (From Wasserman et al.: Principle of exercise testing and interpretation).

These process is guaranteed by:

Environmental Factors

The oxygen partial pressure in ambient air.

Pulmonary factors

The integrity of pulmonary ventilation function and of the alveolar-capillary O2 diffusion.

It is also important that each pulmonary area is adequately ventilated and perfused.

Blood factors

The O2 binding with hemoglobin (the limiting factor is the amount of Hb).

Cardio-circulatory factors

The heart has to pump the O2 in circulation (great importance have the cardiac output = heart rate * stoke volume, CO = HR * SV).

The peripheral vascular system must be patent and able to vasodilatation.

Tissue factor

The O2 must diffuse from capillaries to the cell. The enzymes should adequately support the mitochondrial oxidative metabolism.

The efficiency of the oxygen transport-utilization system is evaluated during CPET from trend analysis of specific cardio-respiratory parameters and compared these values with reference predicted values in relation to gender and age.

During ramp and steady-state exercise test the following variables were determined (Fig. 4.5):

a) VO2max or VO2peak that represent the maximum oxygen consumption, which corresponds to the maximum capacity of the system to use O2 during muscular work (you can compare it to the displacement of an engine), carbon dioxide production (VCO2), pulmonary ventilation (VE). These measurements were carried out breath by breath using a computerized metabolic system (Sensor Medics Vmax) in STPD conditions. The tidal volume (Vt) and VE (in BTPS) were measured by integration of the flow signal recorded at the mouth with a mass flow sensor (hot wire anemometer), previously calibrated at different flows rates by a 3 liters syringe. VO2 and VCO2 were determined by measuring continuously with fast analyzers, fractions of O2 and CO2 at the mouth, and using mass balance equations. The analyzers have been calibrated before each exercise with gas mixtures of known composition. The respiratory quotient (QR) was calculated as VCO2/VO2. The oxygen pulse = VO2/HR in ml/beat is an indirect index of cardiac output (O2 pulse). We have measured the peak values of VO2, VO2/kg, VCO2, VE, RQ, HR, RR (respiratory rate),

PETO2 and PETCO2 (end-tidal O2 and CO2 partial pressure analogue of alveolar pressures at the end of inspiration), and the ventilatory equivalent of O2 (VE/VO2) and CO2 (VE/VCO2). All these parameters have been defined by an average of the measured values over the last 10 seconds of operation prior to the exhaustion. For tests at constant load were also determined resting (average of the last 60 seconds before the start of the exercise) and ‘steady state’ values (average for the last 30 seconds of exercise).

- **Incremental test.** The incremental test was considered maximal, and consequently also the parameters corresponding to the peak value, when at least 2 of the following conditions were met: QR> 1.12, HR> 85% of the HR max estimated based on HR max = 220 - age (years) (Lear et al., 1999), or when the VO2 had reached a plateau, that did not increase after increase in work (watts).

- **VT.** VT or AT in each subject was calculated from the incremental test. Average values of VO2 and VCO2 were calculated every 10 seconds. It represents the value of VO2 that corresponds to a metabolic level above which, the use of anaerobic metabolism, is necessary to produce ATP to perform mechanical work, with a progressive accumulation of lactic acid. The evaluation of AT is determined by the methods based on the

determination of the breaking point of the linear relation between ventilation and oxygen consumption (integrating the values of the three reference charts of Wasserman) for this is called ventilatory threshold (VT). The physiological explanation of this event is the need to compensate metabolic acidosis by hyperventilation and then hypocapnia (Fig. 4.1).

- **Steady state test: Oxygen and HR Tau.** Time required to attain 63% of the difference between the asymptotic level and level of rest of VO2 and HR.

b) HR , continuously, from the ECG signal (telemetric Cosmed ECG).

c) O2 saturation (SaO2) of arterial blood continuously through the finger oximeter (Pulse Oximeter).

d) PA, by manual sphygmomanometer. The PA was determined, during incremental and constant load test, at baseline, during the test every two minutes and at 1, 3, 5 minutes of recovery after the end of the exercise.

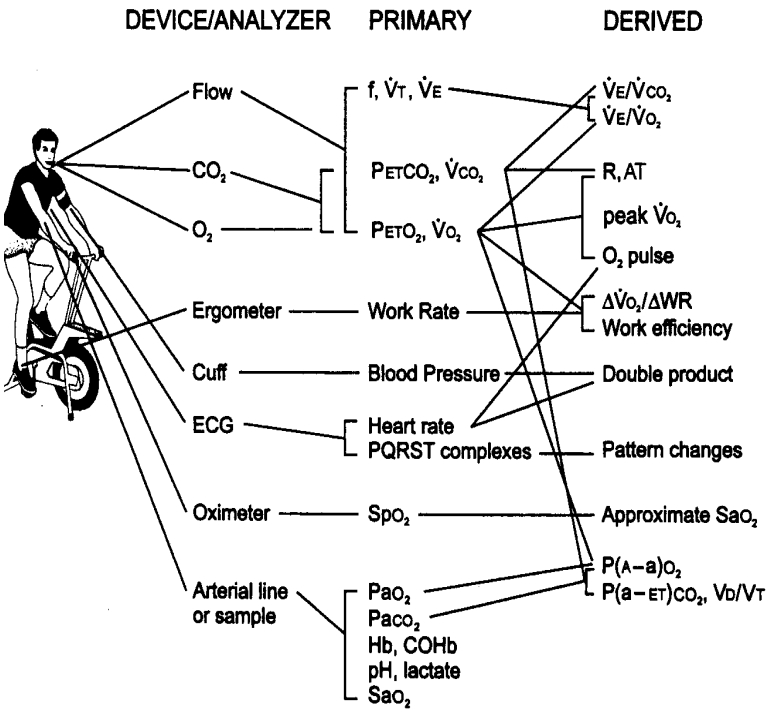


Figure 4.5 Example of experimental set up.

4.4 DATA ANALYSIS

Kinetics analysis. As for pulmonary gas exchange data, breath-by-breath VO_2 values obtained during the constant-load protocol were time aligned, interpolated on a second-by-second basis, and then superimposed for each subject, placing the beginning of the exercise at time zero. Average VO2 values every 10 sec were calculated and utilized for kinetics analysis. Data obtained during the first 20 sec of the transition (corresponding to the "cardiodynamic phase") were excluded from the analysis. For VO2 kinetics analysis mainly dealt with the "phase 2" (or "primary" component) of the response, which should closely reflect gas exchange kinetics at the skeletal muscle level.

To evaluate mathematically the on-kinetics of VO_2, data were fitted by a function of the following type

$$y(t) = y_{\mathrm{Bas}} + \mathrm{A_p}\cdot[1 - e^{-(t-\mathrm{TD_p})/\tau_\mathrm{p}}] \quad (1)$$

and parameter values [primary time delay (TD_p) and time constant (τ_p)] that yielded the lowest sum of squared residuals were determined. In *Eq. 1,* y_{Bas} indicates the baseline value, A_p is the amplitude between y_{Bas} and the steady-state value during the primary component, TD_p is the time delay, and τ_p is the time constant of the function for the primary component. To check

the presence of a "slow component" of the kinetics, data were also fitted by a function of the following type

$$y(t) = y_{\mathrm{Bas}} + \mathrm{A_p}\cdot[1 - e^{-(t-\mathrm{TD_p})/\tau_\mathrm{p}}] + \mathrm{A_s}\cdot[1 - e^{-(t-\mathrm{TD_s})/\tau_\mathrm{s}}] \quad (2)$$

In *Eq. 2*, A_s, TD_s, and τ_s indicate the amplitude, time delay, and time constant, respectively, of the slow component of the kinetics. *Equation 1* or *2* was utilized on the basis of which equation yielded the lowest sum of squared residuals. The slow component, however, does not always follow an exponential function, being sometimes linearly related to the time of exercise; moreover, its τ_s values appear devoid of physiological significance. Thus, among the parameters related to the slow component obtained by *Eq. 2*, only TD_s was considered in the present study. A_s was estimated as the difference between the asymptote of the primary component and an average value obtained during the last 30 s of the constant-load exercise. The percent contribution of the slow component to the total amplitude of the response was also calculated.

The determination of which of the two functions would provide the best fit of the data was carried out by F-test (Graph Pad Prism 5.00.288). If the best fitting derived from the function (2) there was a slow component of the kinetics of VO2, while in cases where the best fitting derived from the monoexponential function (1), the kinetics of VO2 did not present a slow

component (all subject tested fit with monoexponential function 1).
When present the slow component was expressed in ml / min • kg, and a percentage of the total amplitude of the response (amps / AMPP + amps).

We also evaluated with the same methods kinetic analysis of HR in this case we considered all the heart rate values from the beginning of steady state test (time 0). The phase 1 represent the increase in cardiac output and therefore of the pulmonary blood flow (Wasserman et al., 1974; Krogh and Lindhard, 1913). This phase, said cardiodynamics phase, does not express a metabolic muscle phenomenon, but is simply due to the increase blood flow in the lungs and therefore is expression of heart pump function and of the diffusion capacity of the lung, as well as index of the balance between sympathetic-parasympathetic system.

4.5 STATISTICAL ANALYSIS

All data are given as mean ± DS. Comparison of parameters between the subjects were performed using *t* test (homogeneous population tested). Linear and exponential regression was used to correlate some data (see data analysis).

5. RESULTS

5.1 BLOOD CHEMISTRY AND SPIROMETRIC PARAMETERS

At the time of recruitment, the subjects, have done specific blood tests chosen in agreement with Operative Unit Dietetics and Clinical Nutrition of San Carlo Borromeo Hospital, in particular blood tests indicative for the diagnosis of metabolic syndrome according to the IDF 2005 criteria (International Diabetes Federation). The results of these tests associated with anthropometric measures were significant for diagnosis of MetS in all sujects.

All subjects then performed in Sport's Physiology Laboratory located at Old S. Gerardo Monza Hospital or at San Carlo Hospital in Milan, pulmonary static and dynamic function tests, that except in four subjects who showed a mild obstruction of the small airways were normal allowing us to perform a CPET in total safety and quality. The mean anthropometric and spirometric values are shown in Table 5.1 A and 5.1 B.

As you can see there is:

- no relevant variation in waist circumference (n.s.)
- no increase in BMI 100,2% after 6 months (n.s.)

- no significant changes of blood chemistry parameters assessed (fasting blood glucose, total cholesterol and HDL, triglycerides) (n.s.)

Tab 5.1 A Anthropometric values			
	AGE	BMI	HEIGHT
MEDIA pre	51,53	30,58	168,53
DS pre	11,36	5,7	10,69
MEDIA post	51,53	30,64	168,53
DS post	11,36	5,47	10,69
post/pre %	100	100,2	100

Tab 5.1 B Spirometric values (absolute values and % of predicted)		
	MEDIA	DS
FVC (L)	3,89	0,86
% FVC	105,21	16,30
FEV1 (L/SEC)	3,11	0,73
FEV %	102,63	16,09
PEF (L/SEC)	7,30	2,06
PEF %	96,00	18,71
FEV1/FVC	79,83	5,48
FEV1/FVC %	100,78	6,82

5.2 CPET RESULTS

After six months of correctly prescribed exercise we have seen, (normal distribution of the measured variable) on average:

During incremental test, same ramp protocol (Tab. 5.2, 5.3): an increase in:

- + 9,39% VO2 peak ml/kg/min (p<0.05)
- + 6,31%, VO2 peak L/min (p<0.05)
- + 12,06% Watts peak (p<0.01)
- + 13,25% VE peak L/min (p<0.01)
- + 5,81% HR peak (p<0.01)

we can see also an increase of the value at AT:

- + 10,02% VO2 ml/kg/min (p<0.05)
- + 4,48% VO2 L/min (p<0.05)
- + 13,82% Watts (p<0.01)
- + 4,99% HR (p<0.01)
- + 8,66% VE L/min (p<0.01)
- There was no evidence of a significant change in % compared AT values to the peak values for VO2, HR, Watts and QR, there is only a small % decrease in VE value (probably hypoventilation after training program).
- An increase in the time duration and in watts peak of the test with the same ramp protocol.

In Table 5.2 we show the mean and DS values of VO2 peak, VO2 AT, Watts peak, Watts AT, QR peak, QR AT, HR peak and AT, VE peak and AT, O2 pulse peak and AT of the subjects during an incremental test with the same ramp, at time 0 (black) and after six months of the training program (blue). In red is shown the percentage change between post-pre exercise program and the corresponding *t* test values.

The values shown in the tables correspond to those measured during the last 10 seconds of an exhausting incremental load CPET (cardiopulmonary exercise testing).

As you can see the subjects after six months of physical activity correctly prescribed increase peak and AT value and seem to be able to better tolerate fatigue probably due to improved efficiency of the trained muscle.

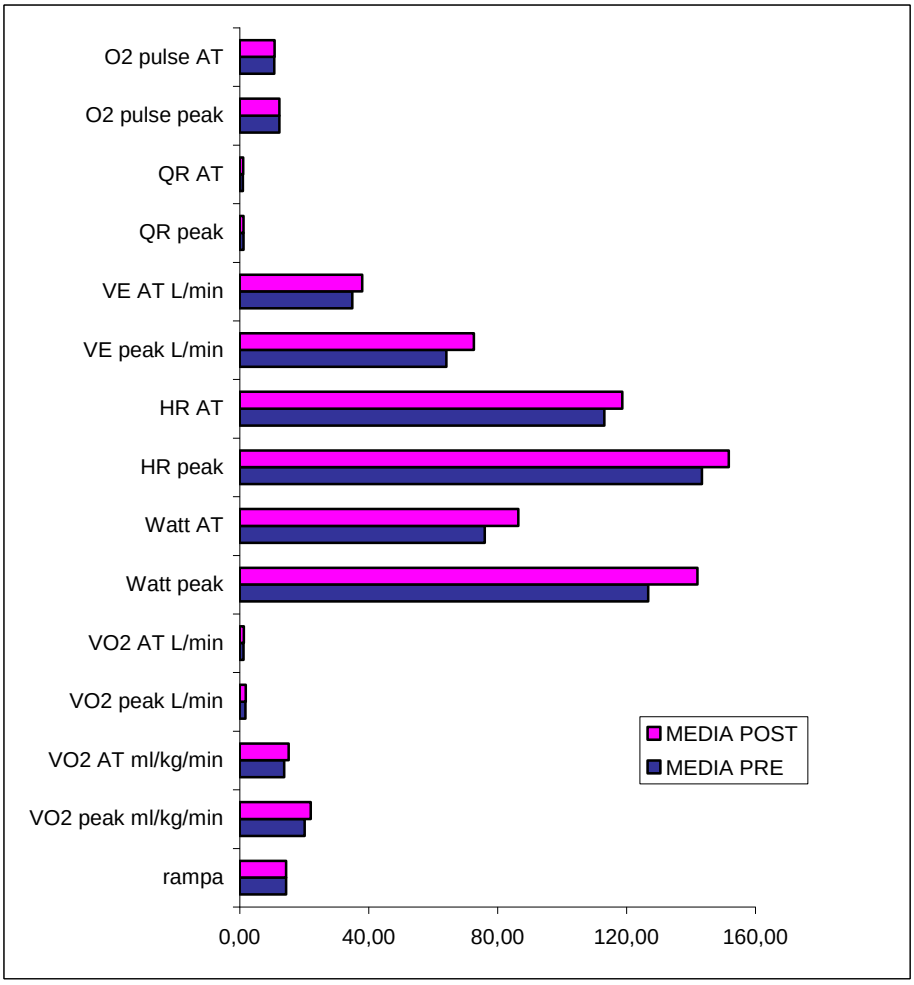
All the tests result maximal for QR attained (QR > 1,12) both at time 0 and after six months of training. The maximality of exercise testing was defined when the subject gets a respiratory quotient considered maximal. We don't considered the maximum heart rate as reliable parameter suffering from an error rate equal to ± 10% than the predicted.

Maximum heart rate derived from the formula HR max = 220 – age. All examined subjects showed poor VO2 peak values.

The increase in heart rate threshold after six months of training allow us to increase the heart rate for training in each subject for the next period (subjects can work at a higher intensity, higher heart rate, continuing to consume essentially lipids as

fuel, and then were able to perform a more intense and more tiring physical exercise without producing early large amounts of lactic acid).

Tab. 5.2	MEDIA PRE	DS PRE	MEDIA POST	DS POST	post/ pre %	*t* test
Ramp watt/min	14,37	4,51	14,37	4,51	100,00	
VO2 peak ml/kg/min	20,14	3,73	22,03	3,79	109,39	p<0.05
VO2 AT ml/kg/min	13,76	2,29	15,14	2,07	110,02	p<0.01
VO2 peak L/min	1,76	0,54	1,87	0,53	106,31	p<0.05
VO2 AT L/min	1,21	0,31	1,26	0,29	104,48	p<0.05
Watt peak	126,79	38,38	142,08	37,67	112,06	p<0.01
Watt AT	76,00	23,31	86,50	19,57	113,82	p<0.01
HR peak bpm	143,42	14,16	151,75	11,59	105,81	p<0.01
HR AT bpm	113,11	8,91	118,75	10,30	104,99	p<0.05
VE peak L/min	64,11	20,60	72,61	22,03	113,25	p<0.01
VE AT L/min	34,95	9,19	37,98	8,29	108,66	p<0.01
QR peak	1,18	0,07	1,24	0,06	104,92	p<0.05
QR AT	1,01	0,07	1,05	0,07	103,99	p<0.05
O2 pulse peak	12,27	3,6	12,34	3,48	100,57	n.s.
O2 pulse AT	10,73	2,9	10,79	2,99	100,56	n.s



Tab. 5.2 figure.

In Tab 5.3 (values at time 0, black and value after six months of training, blue) we show the mean values at peak and at AT of VO2 L/min, VO2 ml/kg/min, Watts, HR, VE L/min, HR and QR of the subjects during an incremental test with the same ramp.
After a program of a properly prescribed exercise period of six months, the rate of VO2 and HR value at AT from the peak is comparable even larger in absolute value. There in only a small decrease in the rate of VE and QR at AT compared to the peak. There is a small increase of watts value at AT compared to the peak.

Tab. 5.3						
	Mean peak value pre	Mean AT value pre	Mean peak value post	Mean AT value post	% value at AT pre	% value at AT post
VO2 L/min	1,76	1,21	1,87	1,26	68,75	67,38
VO2 ml/kg/min	20,14	13,76	22,03	15,14	68,33	68,72
Watt	126,79	76,00	142,08	86,50	59,94	60,88
VE L/min	64,11	34,95	72,61	37,98	54,51	52,31
HR	143,42	113,11	151,75	118,75	78,86	78,25
QR	1,18	1,01	1,24	1,05	85,54	84,68

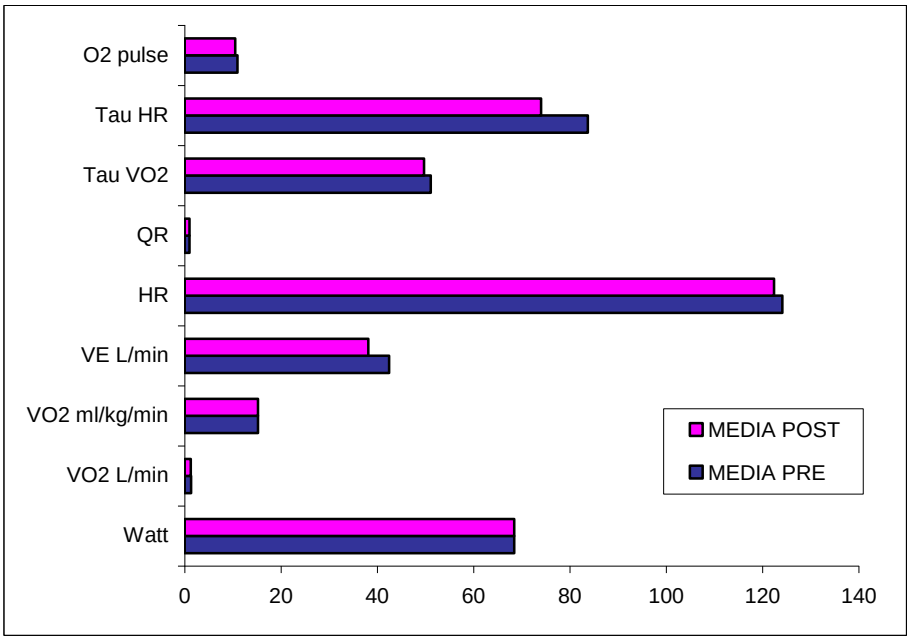
During steady state test, same SS watts value (Tab 5.4):
a reduction in:

- rate of perceived exertion (Borg scale) – 25,84%. ($p<0.01$)
- – 6,62% VO2 L/min (n.s.)
- – 10,23% VE L/min ($p<0.05$)
- – 2,71% QR (n.s.)
- – 2,7% Tau VO2 (n.s.)
- - 11,66% Tau HR ($p<0.05$)
- not significant % variation in HR (n.s.)

In Table 5.4 are shown the mean values of watts, VO2 L/min, VO2 ml/kg/min, VE L/min, QR, HR bpm, O2 pulse, Tau and Borg scale value (6-20) of the subjects during tests at constant load of at least 6 minutes (constant load equal to 90% of the load in watts given at the value of AT during the first test) the values were mediated during last 30 seconds of constant load at the recruitment of subjects (black) and after six months of training program (blue). In red is shown the percentage change between post-pre exercise program and the corresponding *t* test values.
These results show how the same intensity of workload after six months of training, is completed with an increased use of lipids (QR media <1). The subjects after six months are more trained and at equal workload have a best performance in terms of efficiency with a reduction in perceived exertion. Table 5.5

reports Tau VO2 and HR values of a general healthy population (DeLorey DS et al. 2005).

Tab. 5.4						
	MEDIA PRE	DS PRE	MEDIA POST	DS POST	post/ pre %	t-test
Watt	68,42	20,96	68,42	20,96	100,00	n.s.
VO2 L/min	1,36	0,34	1,27	0,27	93,38	n.s.
VO2 ml/kg/min	15,24	3,13	15,23	2,76	99,92	n.s.
VE L/min	42,45	10,83	38,11	9,42	89,77	p<0.05
HR	124,11	10,67	122,42	13,64	98,64	n.s.
QR	1,02	0,06	0,99	0,07	97,29	n.s.
O2 pulse	10,97	2,7	10,53	2,7	97,29	n.s.
τ VO2	51,07	15,35	49,69	15,12	95,98	n.s.
τ HR	83,72	46,52	74,04	32,64	88,44	p<0.05
borg	17,42	0,96	12,92	1,68	74,16	p<0.01



Tab. 5.4 figure.

Tab. 5.5	τ_p (sec) VO2 exercise < AT	τ_p (sec) HR exercise < AT
Young	29 ± 4	36 ± 8
Older	49 ± 8	71 ± 31

On a scale of value between 0 to 10 individuals report being able to perform the exercise program prescribed (adherence) by a numerical index on average equal to 7,8, DS 1,29.

The figure 5.1 shows the VO2 ml/kg/min values indicative of different levels of fitness, all our subjects fall in the deconditioned category, subjects that in absence of cardiac or respiratory disease have a low VO2 peak, these subject surely would have an improvement if subjected in a well structured training program.

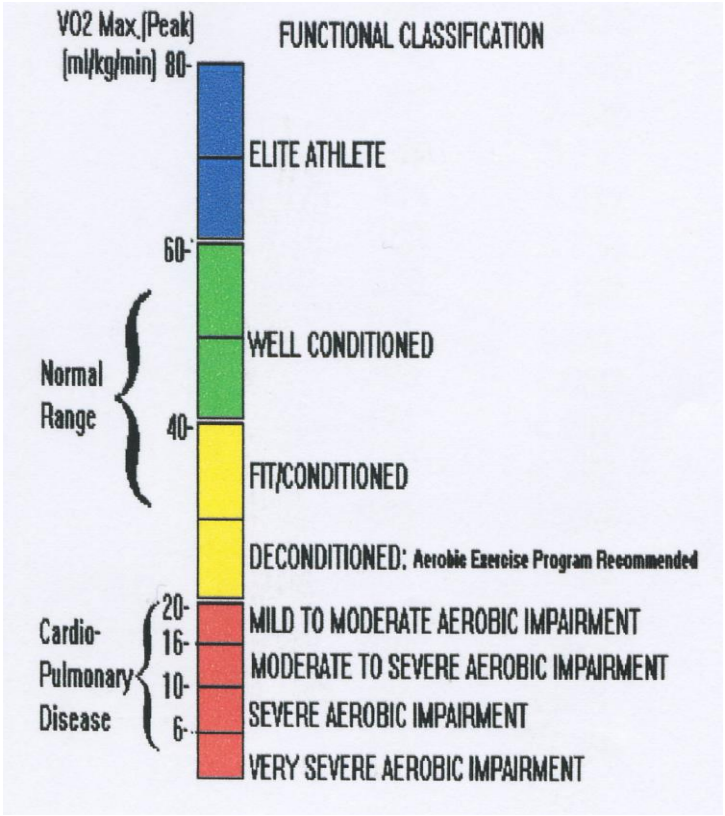


Figure 5.1: Values of VO2 and functional level.

In Table 5.6 we show the average range values of oxygen consumption in sedentary middle-aged male and female (homogeneous population tested).

Tab. 5.6	VO_2 max (ml/kg/min)
Women middle aged sedentary	25 – 35
Men middle aged sedentary	35 – 44

At the end of the incremental and constant load test each subject has to express a numerical value from 6 to 20 indicative of the level of the rate of perceived exertion (RPE) exercise (Borg scale Fig. 5.2). At the end of the first experimental session (time 0), all subjects described the incremental test very hard and the 6 min constant load test as moderately strenuous.

All subjects tested after six months of personal exercise training perceived the incremental test very tiring, but finish the 6 min of constant load test done with a identical load of the first experimental session, with a lower level of physical exertion, rather tiring, light enough.

Borg scale
(rate of perceived exertion, RPE)

6
7 Extremely light
8
9 Very light
10
11 Light enough
12
13 Rather tiring
14
15 Tiring
16
17 Very tiring
18
19 Extremely tiring

Figure 5.2: Borg scale.

CHAPTER 3

6. SUMMARY CONCLUSION AND FUTURE PERSPECTIVES

Population studies relative to MetS and exercise suggest that only few people take adequate exercise. Long-term participation in exercise program is usually poor, with a drop out rate in the order of 20-50% from supervised program (Eriksson et al. 1997 Rev, Katzmarzyk PT et al. 2003).
In particular, the type of exercise prescribed may influence adherence to the training program in fact, despite the results presented in some scientific papers (see Tjonna AE et al. 2008) an interval training protocol may not be readily accepted to the general population of patients with MetS, in fact this type of training implies an exercise of higher intensity (intensity equal to 90% of VO2 peak-max) and shorter duration, with an high risk of musculoskeletal injuries.
Surely you will get better results in terms of adherence to the program with an aerobic physical activity for longer periods.

This means that much emphasis has to be put on the development of exercise program (we proposed a personalized program based on an accurate CPET evaluation). The exercise

should be enjoyable, affordable and accessible, individuals who exercise regularly report improved mood and sense of well-being. An important point is that it does not matter what type of physical activity is performed.
Regular exercise as a lifestyle behavior must be emphasized, and therefore exercise probably is most likely to be effective when taken in young individuals, before the onset of irreversible damage, and when lifestyle changes may be more acceptable. To maximize the benefits of physical training, proper nutrition and healthy habits must be combined.

Low cardiorespiratory fitness is a strong and independent predictor of MetS in women and men. Clinicians should consider the potential benefits of greater cardiorespiratory fitness also in primary prevention of MetS, particularly among patients who have already begun the cluster MetS components (LaMonte et al. 2005). The use of a CPET is the key to quantify cardiorespiratory fitness, in fact the functional capacity of the subjects depend not only on the age and sex of the subjects but also derived from muscular and genetic variables that are often unrecognized.
Regular physical activity is a low cost, safe therapy with minimal adverse side effect if correctly prescribed.

Many studies deal with oxygen uptake kinetics, maximal oxygen consumption and ventilatory threshold in healthy subject (young and old subject) (Bangsbo J el al. 2000, Bunc V et al. 1993,

Chilibeck PD et al. 1996, Ferri A et al. 2007, Henson LC et al. 1989, Fukuoka Y et al. 2001, Grassi B et al. 1999-2000-2003) and in subject with cardiovascular and pulmonary disease (Bauer TA et al 1999, Cerretelli P et al. 1988, Chiappa GR et al. 2008, Grassi B et al. 1997, Koike A et al. 1994, Nery LE et al. 1982-1983, Sietsema KE et al. 1986-1994, Palange P et al. 1995, Petersen ES et al. 1983, Regensteiner JG et al. 1998).
And other works deal with training effect in healthy (Bateman LA et al. STRRIDE-AT/RT, Cerretelli P et al. 1979, Edgett BA et al. 2013, Huonker M et al. 1996, Inbar O et al. 2013) and pathological subjects (Eriksson J et al. 1997 Rev, Johnsonn JL et al. 2007, Katzmarzyk PT et al. 2003, Raditisa P et al. 2013).

All studies generally demonstrate benefits of different entities in the considered parameters.

Now it has not been done any study that includes measure of Tau HR and VO2 and a proper exercise prescription through CPET evaluation (as in elite athletes) pre and post training in subjects with metabolic syndrome.

CONSIDERATIONS ON THE BASIS OF COLLECTED DATA

If we evaluate in our study, the data of individual subjects we noted a greater variability also relatable to slight variations in terms of adherence to the exercise prescription proposal (Fig. 6.1).

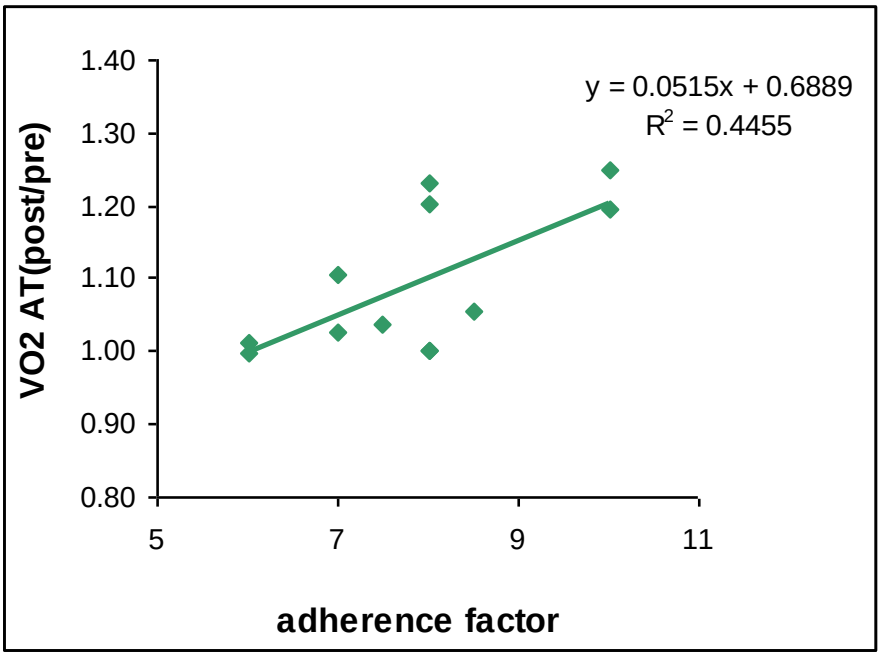


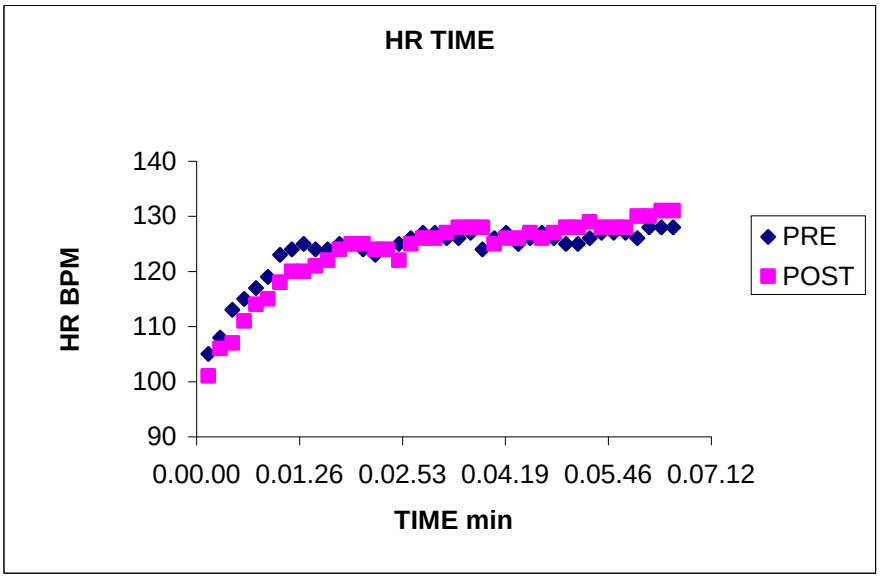
Figure 6.1 : Relation between adherence factor and VO2 AT and (post/pre) in all single subjects.

In particular, we show below the graphs relating to heart rate and VO2 (L/min) during a constant load test of identical intensity expressed in watts in two subjects at time 0 and after 6 months of training according to the requirements defined.

You can see how in the first subject (Fig. 6.2), there has not been a change in terms of improving performance, the second subject (Fig. 6.3), instead shows an evident considerable reduction both of the heart rate and of VO2 at equal intensity of the workload. Both subjects did not vary significantly their BMI.

The only difference in these subjects was in adherence to the proposed protocol of physical activity. The first subject in a scale from 1 to 10 report being adherent to the prescription with a numerical value of 6, the second, 10.

Certainly, the adherence to the personal training protocol is a key element to achieve improvements, as well as for professional athletes, improved performance stems from a workout performed correctly and consistently with the right amount of intensity and duration of training. Adherence to therapy is a fundamental key of all medical practice whether it is taking a drug, a dietary prescription or an exercise program.



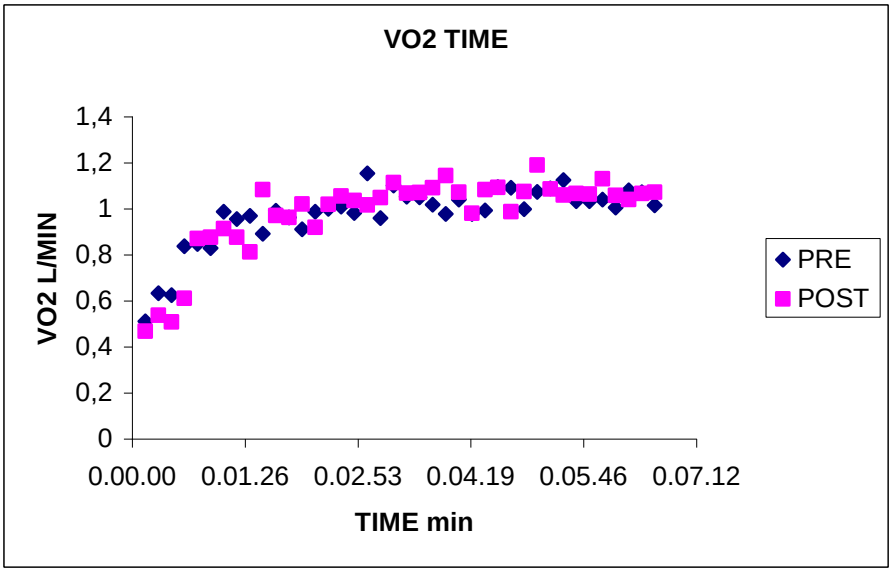
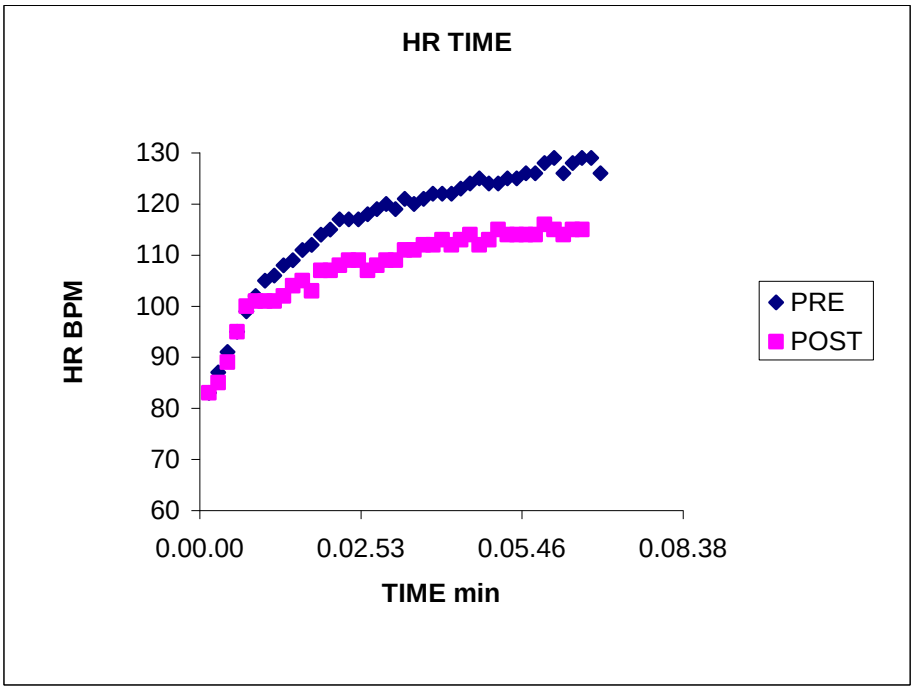


Figure 6.2: subject adherence to the program at numerical level of 6.


HR TIME
HR BPM
130
120
110
100
90
80
70
60
0.00.00
0.02.53
0.05.46
0.08.38
TIME min
PRE
POST

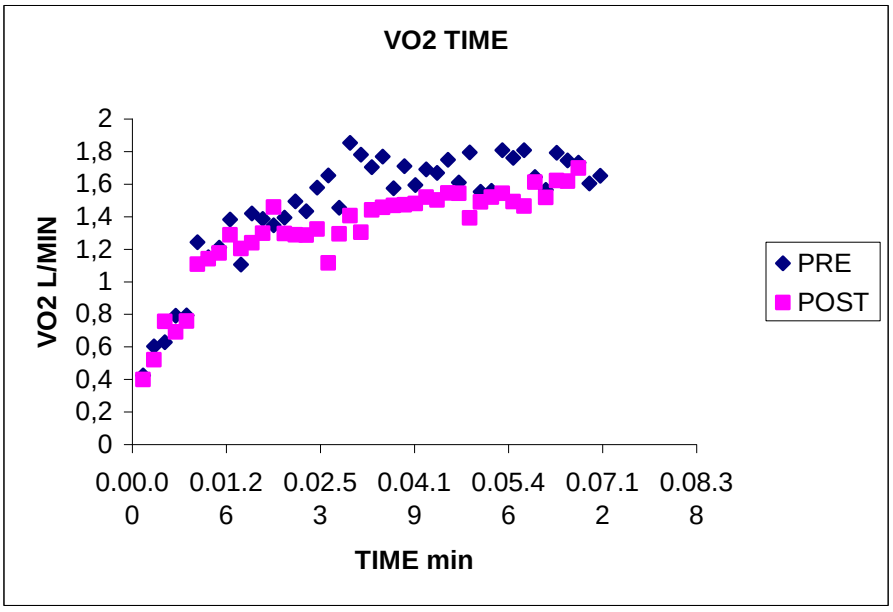

VO2 TIME
VO2 L/MIN
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0.00.0 0
0.01.2 6
0.02.5 3
0.04.1 9
0.05.4 6
0.07.1 2
0.08.3 8
TIME min
PRE
POST

Figure 6.3: subject adherence to the program at numerical level of 10.

Regarding the incremental test (Subject of Fig. 6.3), you can see how there is an hypoventilation (Fig. 6.4).

The duration of the test, and then the maximum load reached after 6 months of training is increased.

In particular the increase in performance was accompanied by an hypoventilation at the same workload (Fig. 6.5).

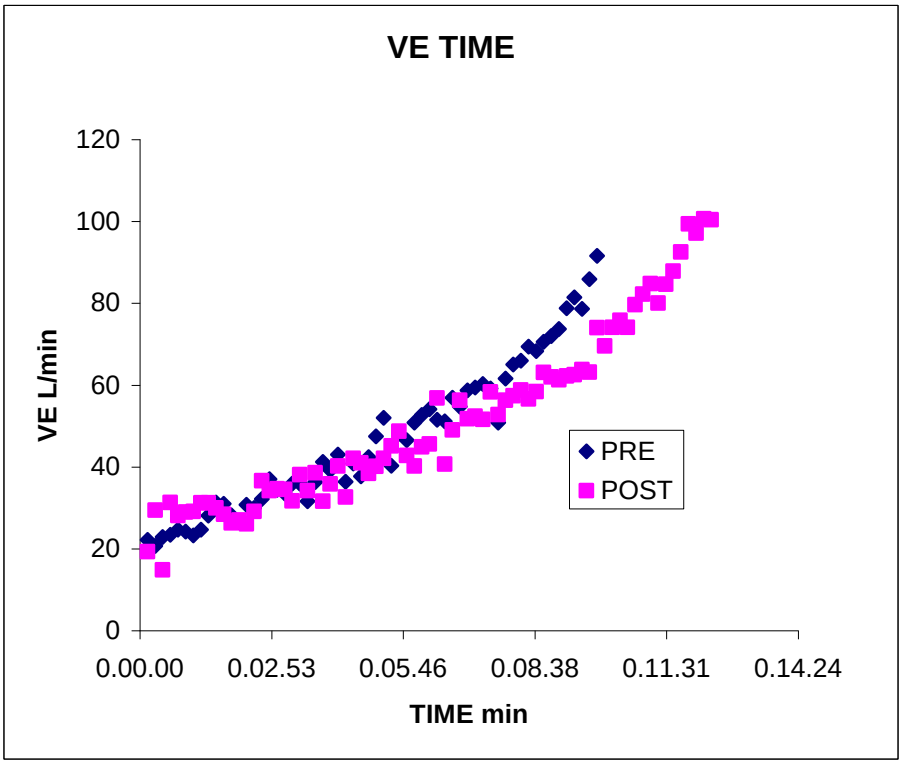


Figure 6.4: performance of ventilation during incremental test before and after the training program.

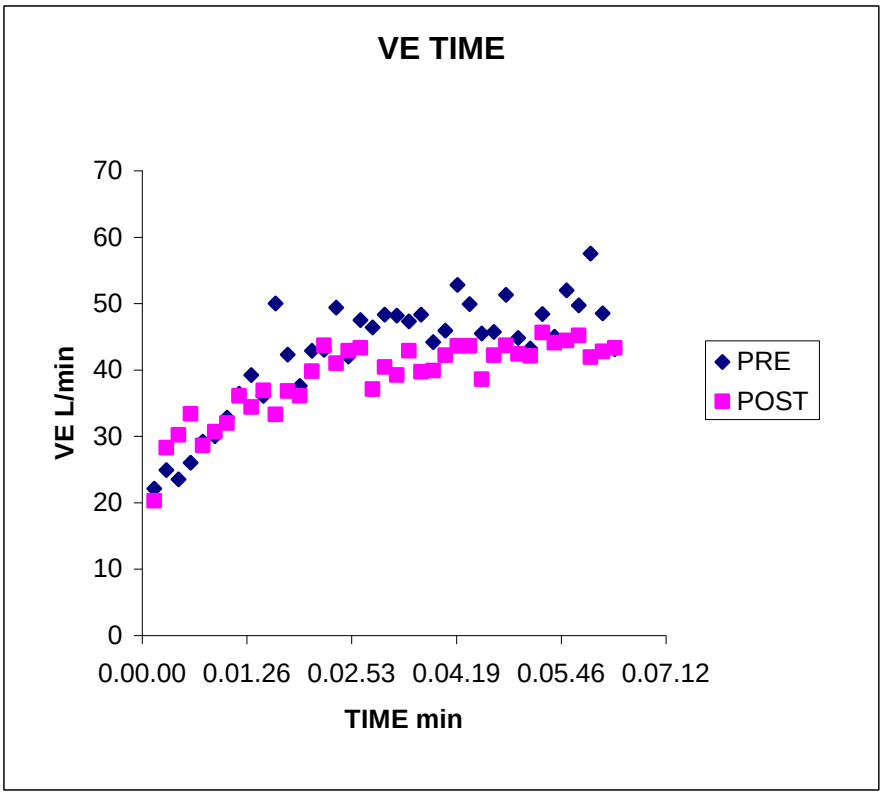


Figure 6.5: performance of ventilation during steady state test before and after the training program.

Subject with MetS have generally an increased RR (respiratory rate) at peak of exercise with as you can see reduced values of VO2 peak. Arterial carbon dioxide tension (PaCO2) homeostasis during exercise was normal but the ventilatory cost of carbon dioxide excretion was increased in MetS as in heart failure (Lewis at al. 2007, Casaburi et al. 1987). PetCO2

measurements correlate only poorly with PaCO2 in individual with MetS, providing a possible explanation based on PetCO2 of inappropriate hyperventilation. MetS patients show hyperventilation that was appropriate to maintain normal PaCO2. These was reduced after training.

After only four week of training we can see an increase in VO2 peak, a decrease in HR during same steady state exercise, a reduction of RR and Tau VO2 time (Edgett BA et al., 2013) in healthy subjects.

It was also noted an increase in the value of AT calculated with ventilatory methods (Wasserman K. plots see Fig. 6.6, 6.7, 6.8A, 6.8B single subject). (See Davis et al. 1979, Dustin et al. 2005, Ferri et al. 2007, Gollnick et al. 1973a,b, 1972, Holloszy 1984).

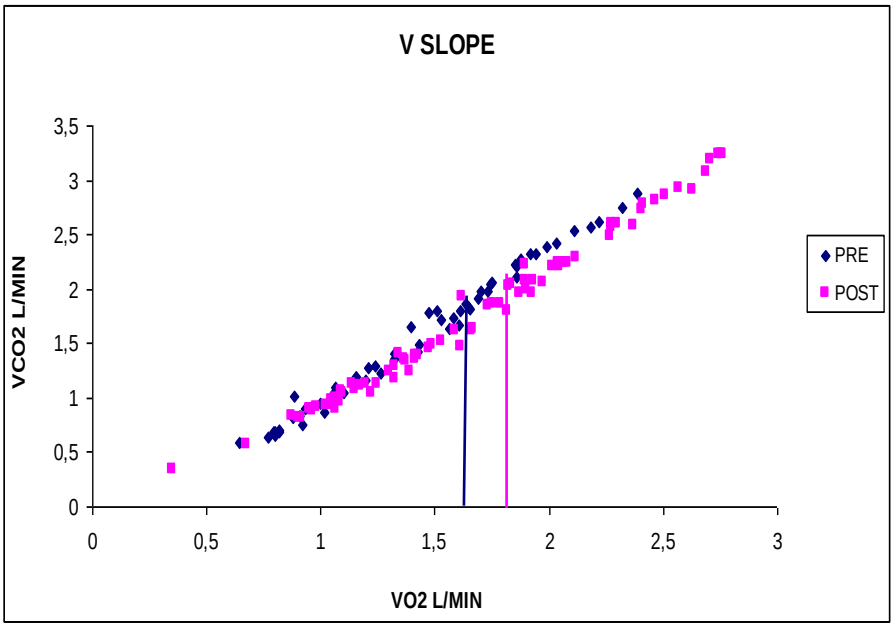


Figure 6.6: V slope before and after the training program.

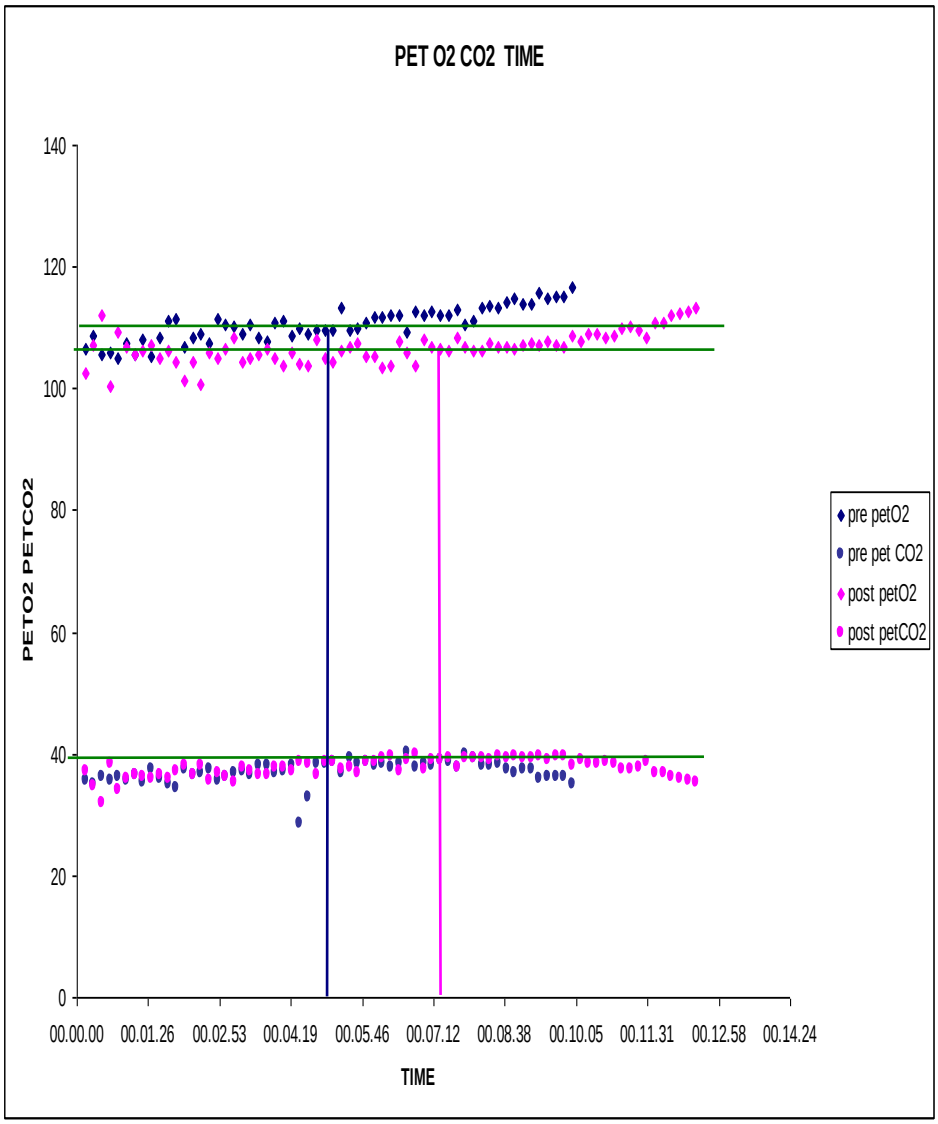
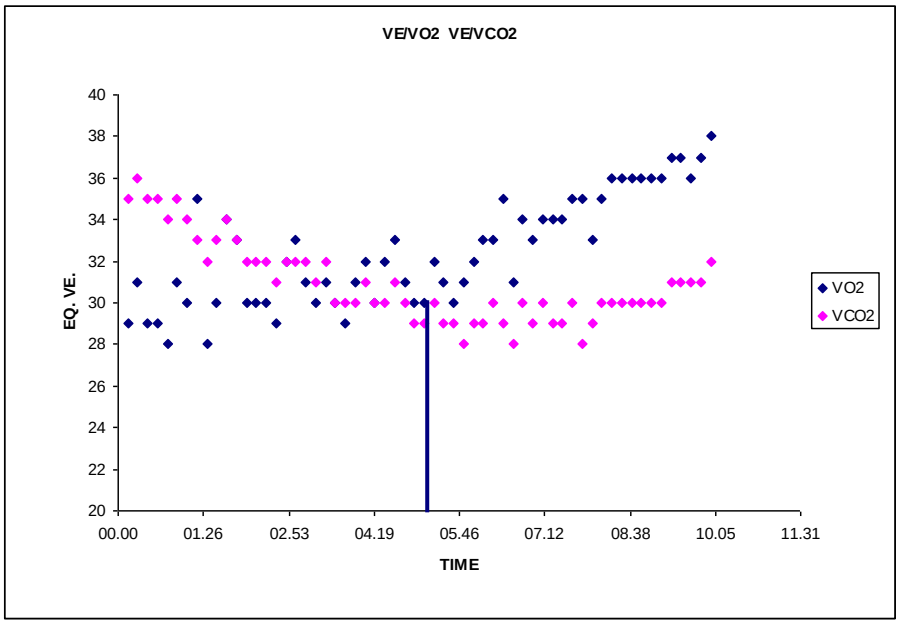
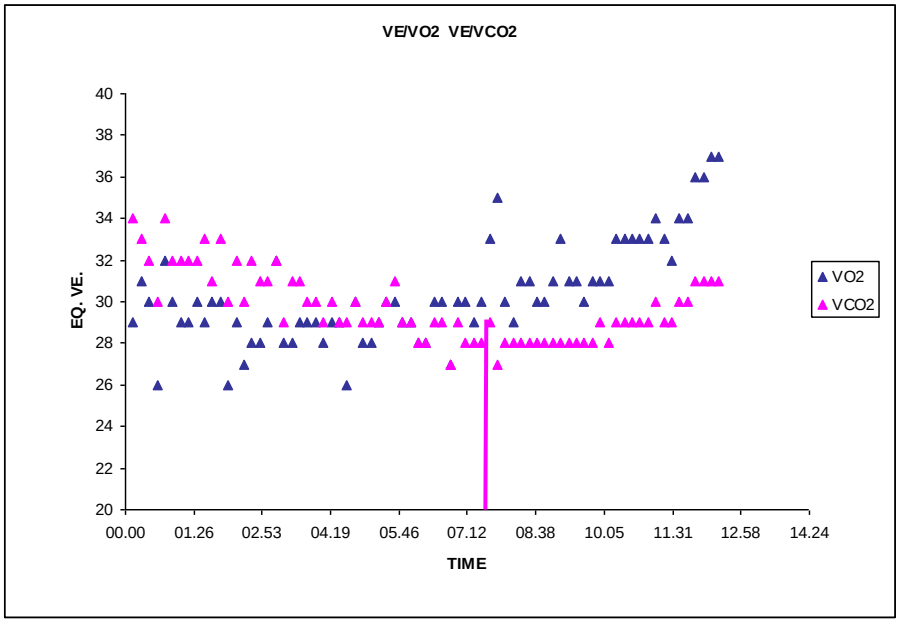


Figure 6.7: trend of PetO2 and PetCO2 before and after the training program.



A pre



B post

Figure 6.8 A,B: trend of VE/VO2 and VE/VCO2 before (A) and after (B) the training program.

On average, the value of tau VO2 (early fitness index linked to an improvement in chain of transport-use of oxygen) pre and post the 6 months of training was similar.

Actually going to evaluate the individual subjects, it is seen that a significant improvement has occurred in individuals who were more adherent to the proposed program (Fig. 6.9 A-B) (See Fukuoka et al. 2001, Inbar et al. 2013, Grassi et al. 1996, Henson et al. 1989).

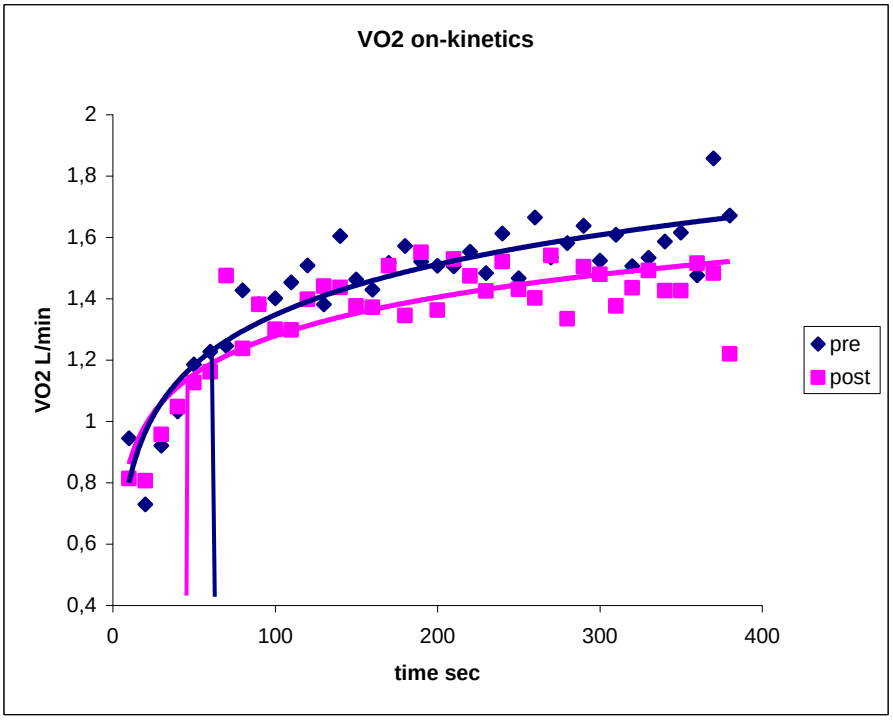


Figure 6.9 A: trend of tau VO2 in a subject with high adherence to training program.

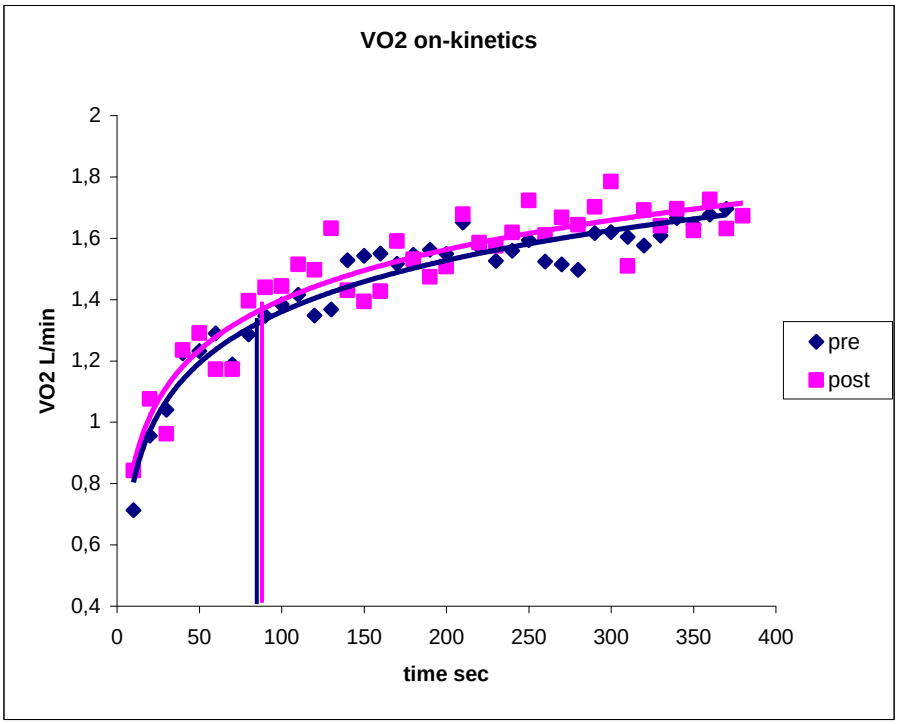


Figure 6.9 B: trend of tau VO2 in a subject with low adherence to training program.

As you can see tau values of VO2 kinetics in subject with metabolic syndrome are high compared to the general population. There is in fact an impairment (especially in subjects with diabetes mellitus type 2) of oxidative phosphorylation and mitochondrial biogenesis (Ren J. et all. 2010, Padilla et al., 2007, Raditisa et al., Regensteiner et al. 1998). There is also a mithocondrial dysfunction that predisposes to lipids accumulation in heart and skeletal muscle that leads to peripheral myopathy and metabolic

cardiomyopathy caracterized by diastolic dysfunction. Tau values are high also in subjects with chronic heart failure (Grassi et al. 1997, Sietsema et al. 1994, Cerretelli et al. 1988).

On average, also the value of HR tau is decrease after six months of training (in this case does not appear to be correlation with the adhesion to the training program proposed). As shown in figure 6.10 A-B the trend of two individual subjects.

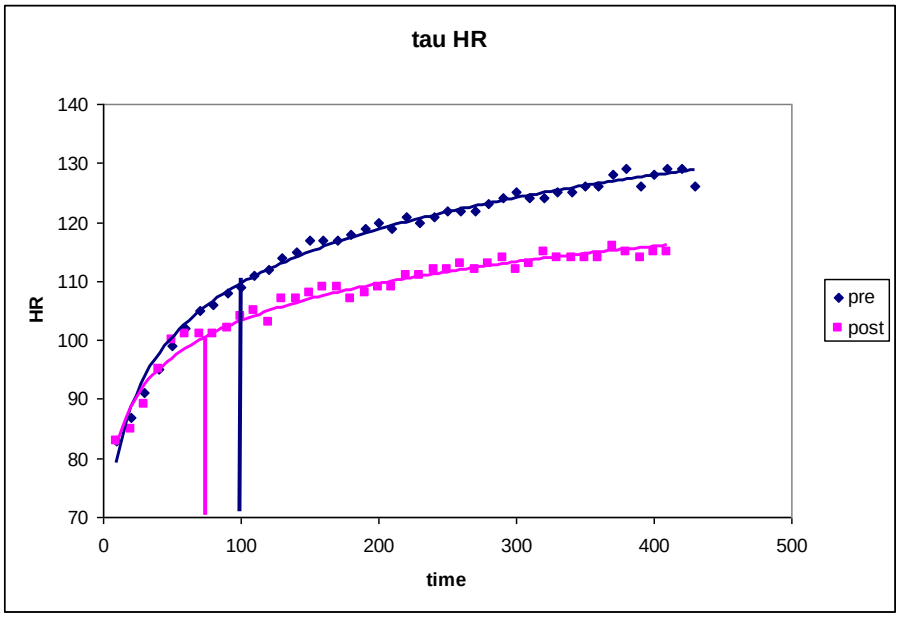


Figure 6.10 A: trend of tau HR in a subject with decreased tau value post training program.

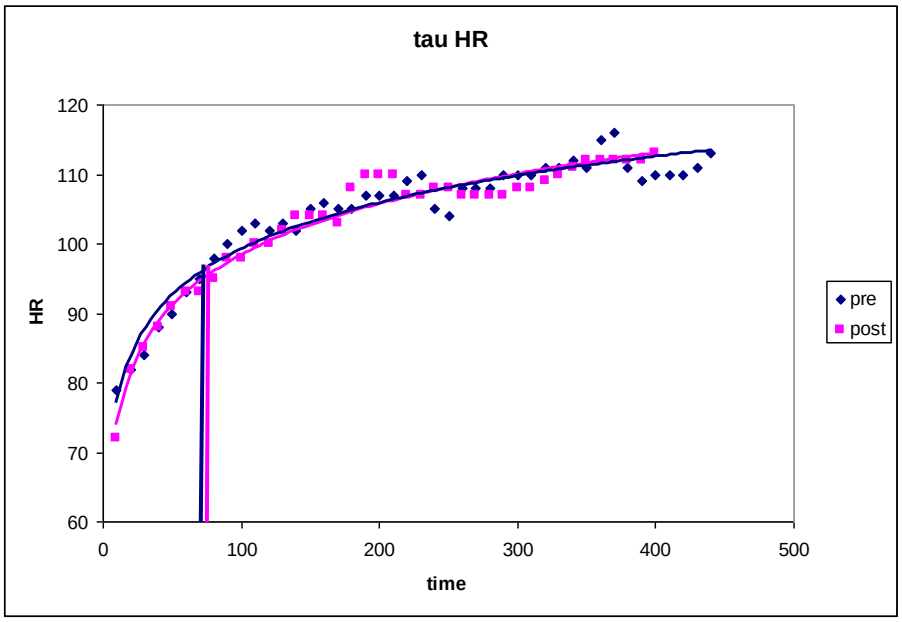
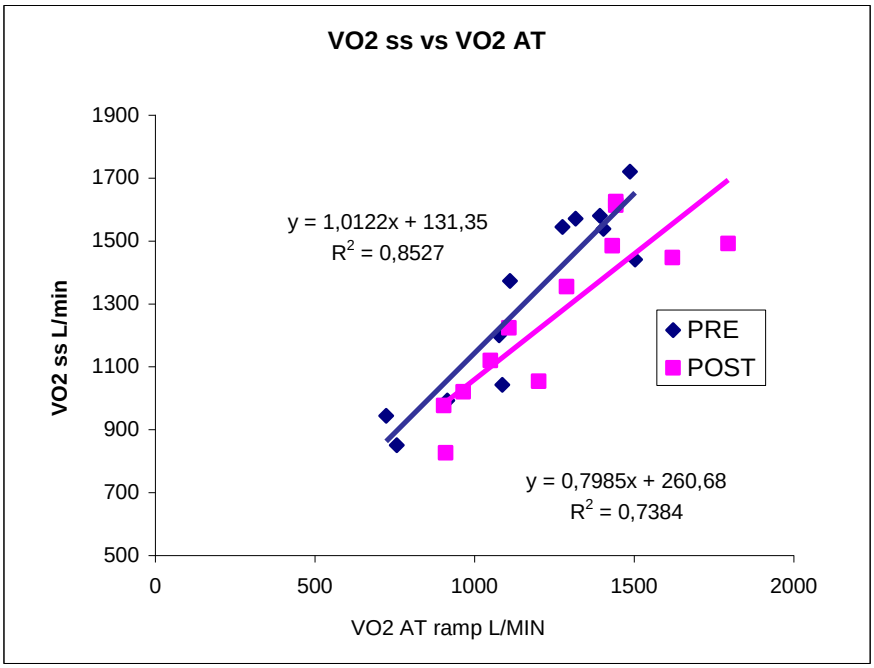


Figure 6.10 B: trend of tau HR in a subject with equal tau value after training program.

Since no cardio respiratory systems pathology occurred in tested subjects (normal spirometry values, normal O2 saturation, normal trend of VO2 pulse, normal arterial pressure trend, normal E.C.G. trace at baseline and during incremental CPET), the variability that has been identified in the HR tau trend can be correlated with a different adaptation in some subjects of the relationship between the sympathetic and parasympathetic system after six months of training. In particular a slower adaptation of HR kinetics could depend on age related changes in arteriolar vasodilatation or autonomic imbalance (DeLorey DS et al. 2005).

In figure 6.11 A, B, C, D, we show the trend, of VO2 AT, HR AT, VE AT and Watt AT in respect of the values of the same parameters at steady state (Watts equal at steady state in the two test) in all of the subjects before and after 6 months of training. As you can see the trend is improved in all subjects after six months, (see linear regression and R2 in fig 6.11).



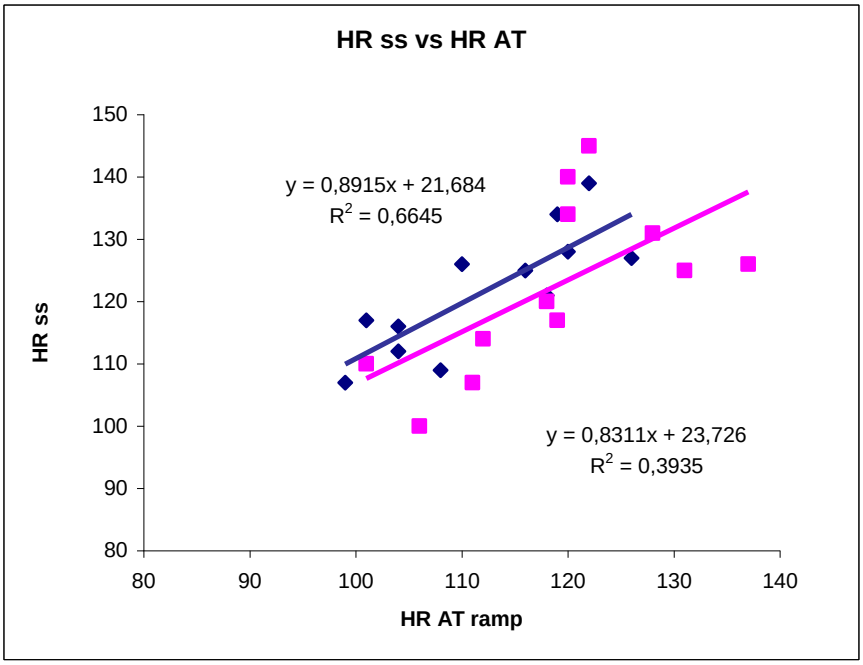
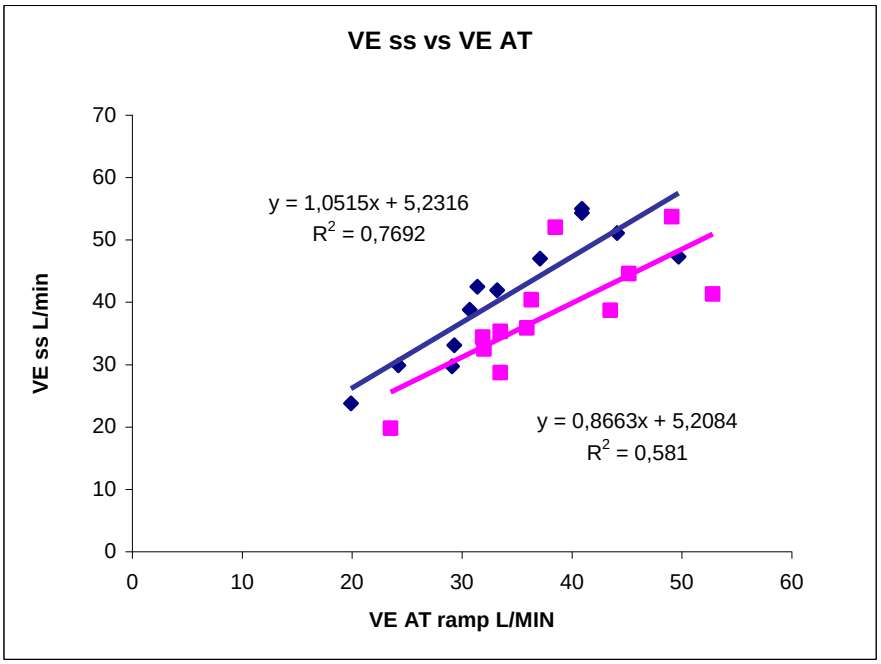


Figure 6.11 A,B: trend of VO2 AT vs VO2 SS and of HR AT vs HR SS in all the subjects before and after the training program.



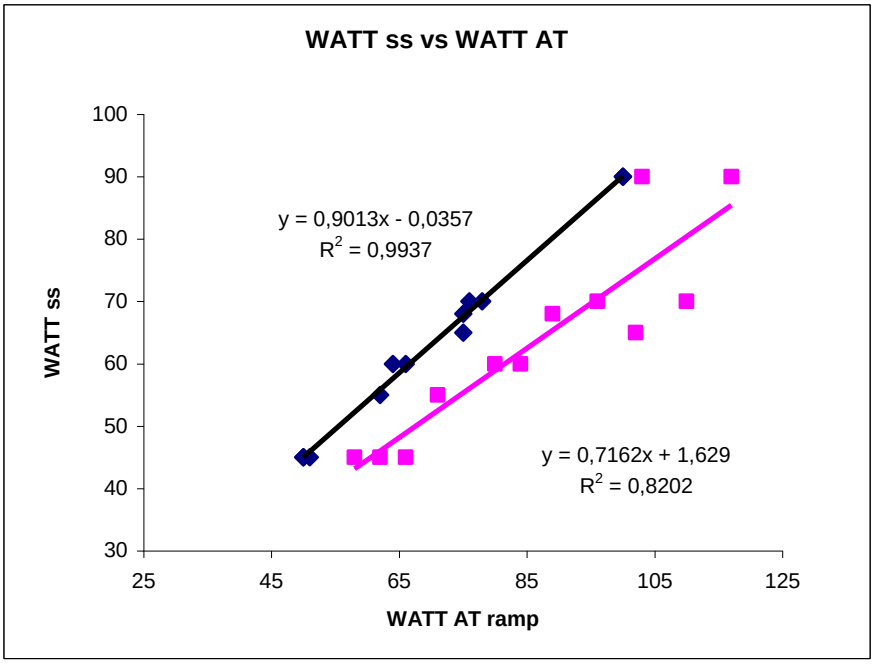


Figure 6.11 C,D: trend of VE AT vs VE SS and of watts AT vs watts SS in all the subjects before and after the training program.

STRENGHTS AND WEAKESS OF THE PROJECT

As often happens in clinical trials, one of the main problems lies in adherence to the project of the recruited subjects at time 0. Of our small group of subjects, 35% did not recur after six months to repeat the proposed tests. The subject that have recurred for the functional evaluation on a scale from 1 to 10 have been reported to be adherent to the exercise program custom prescribed with an average numerical value of 7,8.

The subjects report that they had no difficulty to perform the exercise program prescribed by monitoring heart rate with the aid of a polar heart rate monitor and with a level of exercise intensity equal to 10-11 on the Borg scale

As for the adhesion, we can be satisfied, the subjects reported that they were be able to perform the type of exercise prescribed in a pleasant way and without difficulty. All of the subjects have not experienced disconfort or injuries, performing the physical activity at the intensity defined previously from the results of the CPET.

Certainly, the economic cost of this functional assessment is not trivial, the subjects who compartecipated in the study on average had an improvement in their fitness level, an improvement of mood, and, in combination to diet program (that alone is usually unsuccessful) not gained their weight.

Perhaps a best results could be obtained, if the subjects were followed constantly in their exercise program with a personal trainer (which would increase the cost of the project yet), the optimal solution may be come from the organization of exercise group, even if, in our way the single subject can make his training session at any time of the day and with not obligation to attend a gym, this could in the long term increase the compliance of the subjects.

In order to know what was the real efficiency and impact of a proper prescription of exercise on the basis of data provided by a CPET, we should expand the study on a large numbers of subjects, at least applying in a systematic manner on subject with MetS the first part of assessment, the part based on the correct prescription of training in relation to the value of the ventilatory anaerobic threshold (reducing the time and cost of the test). Surely this field of study is very interesting for the future, in relation to the constant increase in the average age of the population and increase in the prevalence of diseases such as MetS in western sedentary populations, with a high social cost.

To better understand any peripheral or central limitation to oxygen consumption may be interesting to combine the assessment proposed with an indirect assessment of the peripheral extraction of oxygen through the NIRS, as well as an assessment of baseline and under stress cardiac output.

LIST OF ABBREVIATIONS

ADP = adenosine diphophate.
ATP = adenosine-5'-triphophate
ATPasi = adenosine triphosphatase enzyme
BMI = body mass index
CO_2 = carbon dioxide
CPK = creatine phosphokinase enzyme
Δ_{av} = arterio venous difference
ECG = electrocardiogram
FAD = flavin-adenosine-dinucleotide.
FE = ejection fraction
RR = respiratory rate
FVO_{2max} = LT.
GTP = guanosine triphophate
Hb = hemoglobine
HbO_2 = oxygenated hemoglobine
HR = heart rate
LA = lactic acid
LDH = lactic dehydrogenase enzyme
LT = lactate threshold
NAD = nicotinamide-adenine-dinucleotide.
O_2 = oxygen
OBLA = onset of blood lactate accumulation
PA = arterial pressure
PCr = phosphocreatinine
$PetO_2$ = end-tidal oxygen pressure
$PetCO_2$ = end-tidal carbon dioxide pressure
PO_2 = oxygen partial pressure
Q = cardiac output
QR = respiratory quotient
SaO_2 = arterial oxygen saturation
SV = stroke volume
TD_p = increase in VO2 within the latency time of occurrence of exponential kinetics of VO2
τ_{off} = time constant of off-kinetic
τ_p = time constant of VO2 kinetic
UM = motor unit
VCO_2 = carbon dioxide production
VE = pulmonary ventilation

VO_{2max} = maximum oxygen consumption
VO_{2peak} = peak oxygen consumption
VT = ventilatory threshold
Vt = tidal volume
MetS = metabolic syndrome

REFERENCES

- Aloulou I, Varlet-Marie E, Mercier J (2006). Hemorheologic effects of low intensity endurance training in sedentary patients suffering from the metabolic Syndrome. Clin Hemorheol Microcirc. (1-2): 333-9.

- Astrand PO, Rodahl K (1986) *Textbook of work physiology, physiological bases of of exercise*. 3rd ed. New york: Mc Graw Hill.

- Astrand PO, Rodahl K (1977) *Textbook of work Physiology*. New York: Mc Graw Hill Co. pp 583-586.

- Åstrand I (1960) Aerobic work capacity in men and women with special reference to age. *Acta Physiol. Scand.,* 49 (suppl. 169): 1-89.

- Åstrand PO (1952) Experimental studies of physical working capacity in relation to age and sex. Munskgaard, Copenhagen.

- Babcock MA, Paterson DH, Cunningham DA, Dickinson JR (1994) Exercise on-transient gas exchange kinetics are slowed as a function of age. *Med. Sci. Sport Exerc.,*vol.26, no 4, pp. 440-446.

- Bangsbo J., Krustrup P., Gonzalez-Alonso J., Boushel R., saltin B. (2000) Muscle oxygen kinetics at onset of intense dynamic exercise in humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* 279: 899-906.

- Bar-Or O, Dotan R, Inbar O (1980) Anaerobic capacity and muscle fiber composition in man. *Int. J. Sports Med.*, 1: 82-85.

- Bassett DR, Howley TH (1997) Maximal oxygen uptake: "classical" versus "contemporary" viewpoints. *Med. Sci. Sports Exerc.*, 29: 591-603.

- Bateman LA, Slentz CA, Willis LH, Tamylin Shields A, Piner LW, Bales CW, Houmard JA, Kraus WE. Comparison of aerobic versus resistence training effects on metabolic syndrome (from the studies of a targeted risk reduction intervention through defined exercise – STRRIDE-AT/RT).

- Barnes WS (1988) Depressing effect of calcium antagonist in isolated skeletal muscle on oxygen consuption during potassium depolarisation. *Ca. J.Appl. Physiol.,* 66: 836-840.

- Barstow TJ (1994) Characterization of VO_2 kinetics during heavy exercise. *Med. Sci. Sports Exerc*., 26: 1327-1334.

- Barstow BJ, Mole PA (1991) Linear and non linear characteristic of oxygen uptake kinetics during heavy exercise. *J. Appl. Physiol.,* 71: 2099-2106.

- Bauer TA., Regensteiner JG, Brass EP, Hiatt WR (1999) Oxygen uptake kinetics during exercise are slowed in patients with peripheral arterial disease. *J. Appl. Physiol.,* 87: 809-816.

- Berg W (1947) Individual differences in respiratory gas exchange during recovery from moderate exercise. *Am. J. Physiol.,* 149: 597-610.

- Billat V, Beillot J, Jan J, Rochcongar P, Carre F (1996b) Gender effect on the relationship of time at 100% VO_{2max} with other bioenergetic characteristics. *Med. Sci Sports Exerc*., 28: 1049-1055.

- Bishop D, Jenkins DG, Mackinnon LT (1998) The relationship between plasma lactate parameters, Wpeak and 1-h cycling performance in women. *Med. Sci. Sports Exerc.*, 30 (8): 1270-1275.

- Bizeau ME, Willis WT, Hazel JR (1998) Differential responses to endurance training in sub-sarcolemmal and intermyofibrillar mitochondria. *J. Appl. Physiol.*, 85 (4): 1279-1284.

- Brambilla P. et al. International Journal of Obesity (2011) 35, 16-28.

- Brooks GA (1986) The lactate shuttle during exercise and recovery. *Med. Sci. Sports Exerc*., 18: 360-368.

- Bunc V, Heller J.J (1993) Ventilatory threshold in young and adult female athletes. *Sports Med. Phys. Fitness*., 33 (3): 233-238.

- Cadefau J, Casademont J, Grau JM, Fernandez J, Balaguer A, Vernet M, Cusso R, Urbano-Marquez A (1990) Biochemical and histochemical adaptation to sprint training in young athletes. *Acta Physiol. Scand.*, 140 (3): 341-351.

- Calle e alt. N Engl J Med 1999; 341: 1097-1105.

- Carter H, Jones AM, Barstow TJ, Burnley M, Williams CA, Doust JH (2000) Effects of endurance training on oxygen uptake kinetics during treadmill running. *J. Appl. Physiol.*, 89: 1744-1752.

- Casaburi R, Storer TW, Wasserman K (1987) Mediation of reduced ventilatory response to exercise after endurance training. *J. Appl. Physiol.,* 63: 1533-1538.

- Claudiu Avram, Mihaela Oravitan, Lucian D. Hoble, Bogdan Almajan-Guta. Cardipulmonary exercise testing – effective method for evaluation and recommendation of individualized exercise training in patients with metabolic syndrome. *Timiscara Physical Education and Rehabilitation Journal. Vol. 4 Issue 7 2001*.

- Casey A, Greenhaff PL (2000) Does dietary creatine supplementation play a role in skeletal muscle metabolism and performance? *Am. J. Clin. Nutr*., 72 (2 Suppl): 607-617.

- Cerretelli P, Grassi B, Colombini A, Carù B, Marconi C (1988) Gas exchange and metabolic transients in heart transplant recipients. *Respir. Physiol.,* 74: 355-371.

- Cerretelli P, Di Prampero PE (1987) Gas exchange in exercise. In: Fishman AP, Fahri LE, Tenney SM (eds) *Handbook of physiology*. Bethesda, MD: American Physiology society, 297-339.

- Cerretelli P, Pendergast DR, Paganelli WC, Renne DW (1979) Effects of specific muscle training on the VO_2 on-response and early blood lactate. *J. Appl. Physiol.,* 47: 761-769.

- Chance B, Dait MT, Zhang C, Hamaoka T, Hagerman F (1992) Recovery from exercise-induced desaturation in the quadriceps muscles of élite competitive rowers. *Am. J. Physiol.*, 262 (3 Pt 1): C766-C775.

- Chiappa GR, Borghi-Silva A, Ferreira LF, Carrascosa C, Carneiro Oliveira C, Maia J, Gimenes AC, Queiroga F Jr, Berton D, Ferreira EMV, Nery LE, Neder A. Kinetics of muscle deoxygenetion are accelerated at the onset of heavy-intensity exercise in patients with COPD: relationship to central cardiovascular dynamics. *Journal Appl. Physiol 104: 1341-1350, 2008.*

- Chilibeck PD, Paterson DH, Petrella RJ, Cunningham DA (1996) The influence of age and cardiorespiratory fitness on kinetics oxygen uptake. *Can. J: Appl. Physiol.*, 21 (3): 185-196.

- Coggan AR, Kohrt WM, Spina RJ, Kirwan JP, Bier DM, Holloszy JO (1992) Plasma glucose kinetics during exercise in subjects with high and low lactate thresholds. *J. Appl. Physiol.*, 74 (5): 1873-1874..

- Cooper DM, Weiler-Ravell D, Whipp BJ, Wasserman K (1984) Aerobic parameters of exercise as a function of body size during growth in children. *J. Appl. Physiol.,* 56: 628-635.

- Costill Dl, Fink WJ, Getchell LH, Ivy JL, Witzmann FA (1979) Lipid metabolism in skeletal muscle of endurance-trained males and females. *J. Appl. Physiol.*, 47: 787-791.

- Costill DL, Daniels J, Evans W, Fink WJ, Krahenbuhl G, Saltin B (1976b) Skeletal muscle enzymes and fiber composition in male and female track athletes. *J. Appl. Physiol*., 40: 149-154.

- Costill DL (1976c) The relation between selected physiological variables and distance running performance. *J. Sports Med. Phys. Fitness*, 7: 61-66.

- Coyle EF, Feltner ME, Kautz SA, Hamilton MT, Montain SJ, Baylor AM, Abraham LD, Petrek GW (1991) Physiological and biomechanical factors associated with élite endurance cycling performance. *Med. Sci. Sports Exerc.*, 23 (1): 93-107.

- Coyle EF, Coggan AR, Hopper MK, Walters TJ (1988) Determinants of endurance in well trained cyclists. *J. Appl. Physiol.*, 64 (6): 2622-2630.

- Davis JA, Storer TW, Caiozzo VJ (1997) Prediction of normal values for lactate threshold estimated by gas exchange in men and women. *J. Appl. Cardiol.*, 76: 157-164.

- Davis JA, Whipp BJ, Lamarra N, Huntsman DJ, Frank MH, Wasserman K (1982) Effect of ramp slope on measurements of aerobic parameters from the ramp exercise test. *Med. Sci. Sports Exerc.,* 14: 339-343.

- Davis JA, Frank MH, Whipp BJ, Wasserman K (1979) Anaerobic threshold alterations caused by endurance training in middle-aged men. *J. Appl. Physiol.*, 46 (6): 1039-1046.

- DeLorey DS, Kowalchuk JM, Paterson DH. Adaptation of pulmonary O2 uptake kinetics and muscledeoxygenation at the onset of heavy-intensity exercise in young and older adults. *J Appl. Physiol 98:1697-1704, 2005.*

- Donovan CM, Pigliassotti MJ (2000) Quantitative assessment of pathways for lactate disposal in skeletal muscle fiber types. *Med. Sci. Sports Exerc.*, 32: 772-777.

- Drinkwater BL, Horvath SM, Wells CL (1975) Aerobic power of females, ages 10 to 68*. J. Gerontol.* 30: 385-394.

- Dudley GA, Tullson PC, Terjung RL (1987) Influence of mitochondrial content on the sensivity of respiratory control. *J. Biol. Chem*., 262: 9109-9114.

- Dustin S. Hittel, William E. Kraus, Chuck J. Tanner, Joseph A. Houmard and Eric P. Hoffman. Exercise training increases electron and substrate shuttling proteins in muscle of overweight men and women with the metabolic syndrome. *J Appl Physiol* 98:168-179, 2005.

- Edgett BA, Ross JED, Green AE, MacMillan NJ, Gurd BJ. The effects of recreational sport on VO2 peak, VO2 kinetics and sunmaximal exercise performance in male and females. *European Journal of Applied Physiology, January 2013.*

- Eriksson J, Taimela S, Koivisto VA. Exercise and metabolic syndrome. *Review Diabetologia 1997;40:125-135.*

- Fagard R, Aubert A, Staessen J, Eynde EV, Vanhees L, Amery A (1984) Cardiac structure and function in cyclists and runners. Comparative echocardiographic study. *Br. Heart J.*, 52 (2): 124-9.

- Fellows IW, Bennett T, Macdonald IA (1985) The effects of adrenaline upon cardiovascular and metabolic functions in man. *Clin. Sci.,* 69: 215-222.

- Ferri A, Adamo S, Longaretti M, Marzorati M, Lanfranconi F, Marchi A, Grassi B (2007) Insights into central and peripheral factors affecting the "oxidative performance" of skeletal muscle in aging. *Eur. J. Appl. Physiol.*, 100 (5): 571-579.

- Fitts RH (1994) Cellular mechanisms of muscle fatigue. *Physiol. Rev.*, 74: 49-94.

- Foster C, Costill DL, Daniels T, Fink WJ (1978) Skeletal muscle enzymes activity, fiber composition and VO_{2max} in relation to distance running performance. *Eur. J. Appl. Physiol.*, 39: 73-80.

- Freund H, Zouloumian P (1981) Lactate after exercise in man. *Eur. J. Appl. Physiol.*, 46: 121-176.

- Fukuoka Y, Grassi B, Conti M, Guiducci D, Sutti M, Marconi C, Cerretelli P (2001) Early effects of exercise training on VO_2 on- and off-kinetics in 50-year-old subjects. *Eur J. Physiol.,* 443: 690-697.

- Gaesser GA, Ward S., Baum VC, Whipp BJ (1994) Effects of infused epinephrine on slow phase of O_2 uptake kinetics during heavy exercise in humans. *J. Appl. Physiol*., 77: 2413-2419.

- Gaesser GA, Poole D (1996) The slow component of oxygen uptake kinetics in humans. *Exerc. Spors Sci. Rev*., 24: 35-7.

- Gollnick PD (1982) Peripheral factors as limitations to exercise capacity. *Can J. Appl. Sports Sci.*, 7: 14-21.

- Gollnick P, Hermansen L (1973a) Biochemical adaptation to exercise: anaerobic metabolism. *Exerc. Sport Sci. Rev.*, 1: 1-43.

- Gollnick P, Armstrong RB, Saltin B, Saubert CW 4th, Sembrowich WL, Shepherd RE (1973b) Effects of training on enzyme activity and fiber composition of human skeletal muscle. *J. Appl. Physiol.*, 34 (1): 107-111.

- Gollnick P, Armstrong R, Saubert C IV, Piehl K, Saltin B (1972) Enzyme activity and fibre composition in skeletal muscle of untrained and trained men. *J. Appl. Physiol*., 33: 312-319.

- Grassi B (2003a) Oxygen uptake kinetics: old and recent lessons from experiments on isolated muscle in situ". *Eur J Appl Physiol.*, 90 (3-4): 242-249.

- Grassi B, Pogliaghi S, Rampichini S, Quaresima V, Ferrari M, Marconi C, Cerretelli P (2003b) Muscle oxygenation and gas exchange kinetics during cycling exercise on-transitions in humans. *J. Appl. Physiol.*, 95: 149-158.

- Grassi B, Hogan MC, Kelley RM, Hawlett RA, Pattillo RE, Gladden LB (2003c) Effects of L-nome during metabolic transitions in isolated canine muscle. *Med. Sci. Sports Exerc.*, 35: 5227.

- Grassi B, Hogan MC, Kelley KM, Aschenbach WG, Hamann JJ, Evans RK, Pattillo RE, Gladden LB (2000) Role of convective O_2 delivery in determining VO_2 on-kinetics in canine muscle contracting at peak VO_2. *J. Appl. Physiol.,* 89: 1293-1301.

- Grassi B, Morandi L, Pogliaghi S, Rampichini S, Marconi C, Cerretelli P (2002) Functional evaluation of metabolic myopathies during exercise*. Med. Sci. Sports Exerc.* 34: 878.

- Grassi B, Quaresima V, Marconi C, Ferrari M, Cerretelli P (1999) Blood lactate accumulation and muscle deoxygenation during incremental exercise. *J. Appl. Physiol.*, 87: 348-355.

- Grassi B, Gladden LB, Richardson RS, Samaja M, Stary CM, Hogan MC (1998a) Faster adjustment of O_2 delivery does not affect VO_2 on-kinetics in isolated in situ canine muscle. *J. Appl. Physiol.,* 85: 1394-1403.

- Grassi B, Gladden LB, Stary CM, Hogan MC, Wagner PD (1998b) Peripheral O_2 diffusion does not affect VO_2 on-kinetics in isolated in situ canine muscle. *J. Appl. Physiol.,* 85: 1404-1412.

- Grassi B, Marconi C, Meyer M, Rieu M, Cerretelli P (1997) Gas exchange and cardiovascular kinetics with different exercise protocols in heart transplant recipients. *J. Appl. Physiol.,* 85: 1394-1403.

- Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, Wagner PD (1996) Muscle O_2 kinetics in human: implication for metabolic control. *J. Appl. Physiol.,* 80: 988-998.

- Hagberg JM, Coyle EF (1983) Physiological determinants of endurance performance as studied in competitive racewalkers. *Med. Sci. Sports Exerc*., 15 (4): 287-289.

- Hagberg JM, Hickson RC, Ehsani AA, Hollosky JO (1980) Faster adjustment to and recovery from submaximal exercise in the trained status. *J. Appl. Physiol.,* 48: 218-221.

- Hagberg JM, Mullin JP, Nagle FJ (1978) Oxygen consumption during constant-load exercise. *J. Appl. Physiol,.* 45: 381-384.

- Heberstreit H, Kriemler S, Hughson RL, Bar-Or O (1998) Kinetics of oxygen uptake at the onset of exercise in boy and men. *J. Appl. Physiol.*, 85: 1833-1841.

- Henson LC, Poole DC, Whipp BJ (1989) Fitness as a determinant of oxygen uptake response to costant-load exercise. *Eur. J. Appl. Physiol*., 59: 21-28.

- Hepple RT (2000) Skeletal muscle: microcirculatory adaptation to metabolic demand. *Med. Sci. Sports Exerc*., 32 (1): 117-123.

- Hermansen L, Saltin B (1969) Oxygen uptake during maximal treadmill and bicycle exercise. *J. Appl. Physiol.,* 26: 31-37.

- Hickson RC (1981) Skeletal muscle cytochrome c and myoglobin, endurance, and frequency of training. *J. Appl. Physiol.*, 51 (3): 746-749.

- Hill AV (1927) *Muscular movement in Man*. New York: Mc Graw-Hill.

- Hill AV, Lupton H (1923) Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Q. J. Med.,* 16: 135-171.

- Holloszy JO, Coyle EF (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.*, 56 (4): 831-838.

- Hogan MC, Gladden LB, Kurdak S, Poole DC (1995) Increase [lactate] in working dog muscle reduces tension development independent of pH. *Med. Sci. Sports Exerc.,* 27: 371-377.

- Hough TJ, Johnson GO, McDowell SL (1992) The relationship between anaerobic running capacity and peak plasma lactate. *J. Sports Med. Phys. Fitness*, 32: 117-122.

- Howley ET, Bassett DR Jr, Welch HG (1995) Criteria for maximal oxygen uptake: review and commentary. *Med. Sci. Sports Exerc.*, 27 (9): 1292-1301.

- Hughson RL, Tschakoswsky ME, Houston ME (2001) Regulation of oxygen consumption at the onset of exercise. *Exerc. Sport Sci. Rev.* 29: 129-133.

- Huonker M, Halle M, Keul J (1996) Structural and functional adaptations of the cardiovascular system by training. *Int. J. Sports med.*, 17 (S3): 164-172. J. of Cardiopulmonary Rehabilitation 2005 25: 226-232.

- Inbar O, Faina M, Demarie S, Whipp BJ. VO2 kinetics during moderate effort in muscle of different masses and training level. Physiology vol.. 2013.

- Irving BA, Rutkowski J, Brock DW, Davis CK, Barret EJ, Gaesser GA, Weltman. Comparison of Borg- and OMNI-RPE as Markers of the Blood Lactate Response to Exercise. Medicine & Science in Sport & Exercise 2006.

- Jacobs I (1987) Sprint training effects on muscle myoglobin, enzymes, fiber types, and blood lactate. *Med. Sci. Sports Exerc.*, 19 (4): 368-374.

- Jacobs I, Lithell H, Sjodin B, Wallesten R (1980) Some features of the ultra-marathoner: a case study. *Int. J. Sports Med.*, 1: 523-527.

- J. of Cardiopulmonary Rehabilitation 2005 25: 226-232.

- Johnson JL, Slenz CA, Houmard JA, Samsa GP, duscha BD, Aiken LB, McCartney JS, Tanner CJ, Kraus WE. Exercise training amount and intensity effects on metabolic syndrome (from studies of a targeted risk reduction intervention through defined exercise). *American Journal of Cardiology. Vol 100, Issue 2, December 2007*.

- Jones NL, Makrides L, Hitchcock C, McCartney N (1985) Normal standards for an incremental progressive cycle ergometer test. *Am. Rev. Respir. Dis.*, 131: 700-708.

- Juel C (1997) Lactate-proton co-transport in skeletal muscle. *Physiol. Rev*., 77: 31-358.

- Juel C (1996) Lactate/proton co-transport in skeletal muscle: regulation and importance of pH omeostasis. *Acta Physiol. Scand.*, 156: 369-374.

- K.Cusi Curr Diab Rep 2010; 10:206-315.

- Katch VL Weltman A (1979) Interrelationship between anaerobic power output and aerobic power. *Ergonomics*, 22: 325-332.

- Katzmarzyk PT, Leon AS, Wilmore JH, Skinner JS, Rao DC, Rankinen T, Bouchard C. Targeting the metabolic syndrome with exercise: evidence from the HERITAGE family study. *Medicine & Science in Sport & Exercise 2003.*

- Kindig CA, McDonough P, Erickson HH, Poole DC (2001) Effect of L-nome on oxygen uptake kinetics during heavy-intensity exercise in the horse. *J. Appl. Physiol.,* 91: 891-896.

- Koike A, Hiroe M, Adachi H, Yajima T, Yamauchi Y, Nogami A, Ito H, Miyahara Y, Korenaga M, Marumo F. Oxygen uptake kinetics are determined by cardiac function at onset of exercise rather than peak exercise in patients with prior myocardial infarction. *Circulation. 1994;90:2324-2332.*

- Krip B, Gledhill N, Jamnik V, Warburton D (1997) Effect of alterations in blood volume on cardiac function during maximal exercise. *Med. Sci. Sports Exerc.*, 29 (11): 1469-1476.

- Krogh A, Lindhard J (1913) The regulation of respiration and circulation during the initial stages of muscular work. *J. Appl. Physiol. (Lond.)* 47: 112-136.

- Kushmerick MJ, Meyer RA, Brown TR (1992) Regulation of oxygen consumption in fast- and slow-twitch muscle. *J. Appl. Physiol. (Cell. Physiol.),* 263: C598-C606.

- Lacour JR, Bouvat E, Barthelemey JC (1990) Post-competition blood lactate concentrations as indicators of anaerobic energy expenditure during 400-m and 800-m races. *Eur. J. Appl. Physiol.*, 6: 172-176.

- Lacour JR, Flandrois R (1977) Le role du metabolisme aerobic dans le exercise intense de longue duree. *J. Physiol. (Paris)*, 73: 89-130.

- Lamarra N, Whipp BJ, Ward SA, Wasserman K (1987) Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. *J. Appl. Physiol.,* 62 (5): 2001-2012.

- LaMonte MJ, Barlow CE, Jurca R, Kampert JB, Church TS, Blair SN. Cardiorespiratory fitness is inversely associated with the incidence of metabolic syndrome: a prospective study of men and women. *Circulation 2005; 112:505-512. 2005*.

- Lash JM, Nixon JC, Unthank JL (1995) Exercise training effects on collateral and microvascular resistance in rat model of arterial insufficiency. *Am. J. Physiol.*, 268 (1 pt 2): H125-H137.

- Laughlin MH, McAllister RM (1992) Exercise training-induced vascular adaptations. *J. Appl. Physiol.*, 73 (6): 2209-2225.

- Lavoie NF, Mercier TH (1987) Incremental and constant load determinations of VO_{2max} and maximal constant load performance time. *Can. J. Appl. Sports Sci.*, 12: 229-232.

- Lear SA, Brozic A, Myers JN, Ignaszewski A (1999) Exercise stress testing. An overview of current guidelines. *Sports Med.,* 27: 285-312.

- Lewis NP, Hutchinson SJ, Willis H, Henderson AH. Syndrome X and hyperventilation. *Br Heart J. 1991 Feb; 65 (2): 94-6.*

- MacDougall JD, Hicks AL, MacDonald JR, McKelvie RS, Green HJ, Smith KM (1998) Muscle performance and enzymatic adaptations to sprint interval training. *J. Appl. Physiol.*, 84 (6): 2138-2142.

- Mancini DM, Wilson JR, Bolinger L, Li H, Kendrick K, Chance B, Leigh S (1994) In vitro magnetic resonance spectroscopy measurement of deoxymyoglobin during exercise in patients with heart failiure. Demonstration of abnormal muscle metabolism despite adequate oxygenation. *Circulation*, 90: 500-508.

- Marchesini G, Bugianesi E, Forlani G, et al. Non alcoholic fatty liver, steathepatitis and metabolic syndrome. Hepatology 2003; 37:917-23.

- Margaria R (1968) Capacity and power of the energy process in muscle activity : their practical relevance in athletics. *Int. Z. Angew. Physiol.*, 25: 352-360.

- Masuda K, Okazaki K, Kuno S, Asano K, Shimojo H, Katsuta S Endurance training under 2500-m hypoxia does not increase myoglobin content in human skeletal muscle. *Eur. J. Appl. Physiol.*, 85 (5): 486-490.

- Mc Ardle DW, Katch FI, Katch VL (2001) *Exercise Physiology. Energy, nutrition and human performance*. 5th Ed. Lippincott Williams & Wilkins, pp. 290

- McBride PE, Einerson JA, Grant H, Sargent C, Underbakke G, Vitcenda M, Zeller L. Stein JH. Putting the diabetes prevention program into practice: a program for weight loss and cardiovascular risk reduction for patients with metabolic syndrome or type II diabetes mellitus. *The Journal of Nutrition, Health & Aging, December 2008, Vol 12, Issue 10.*

- McCully KK, Vanderbone K, DeMeirleir K, Posner JD, Leigh JSJ (1992) Muscle metabolism in track athletes, using 31 P magnetic resonance spectroscopy. *Can. J. Physiol. Pharmacol*., 70: 1353-1359.

- Messonnier L, Freund H, Denis C, Dormois D, Dufour AB, Lacour JR (2002) Time to exhaustion at VO_{2max} is related to the lactate exchange and removal abilities. *Int J. Sports Med.*, 23: 433-438.

- Messonnier L, Freund H, Bourdin M, Belli A, Lacour JR (1997) Lactate exchange and removal abilities in rowing performance. *Med. Sci. Sports Exerc.*, 29: 396-401.

- Mier CM, Turner MJ, Ehsani AA, Spina RJ (1997) Cardiovascular adaptations to 10 days of cycle exercise. *J. Appl. Physiol.*, 83 (6): 1900-1906.

- Miyatake N, Saito T, Wada J, Miyachi M, Tabata I, Matsumoto S, Nishikawa H, Makino H, Numata T. Comparison of ventilatory threshold and exercise habits between Japanese men with and without metabolis syndrome. *Diabetes Res Clin Pract 2007 Aug; 77 (2): 314-9 Epub 2007 Jan 4.*

- Moritani T, Nagata A, De Vries HA, Muro M (1981) Critical power as a measure of physical working capacity and anaerobic threshold. *Ergonomics*, 24: 339-350.

- Nery LE, Wasserman K, French W, Oren A, Davis JA (1983) Contrasting cardiovascular and respiratory responses to exercise in mitral valve and chronic obstructive pulmonary deseases. *Chest*, 83: 446-453.

- Nery LE, Wasserman K, Andrews JD, Huntsman DJ, Hansen JE, Whipp BJ (1982) Ventilatory and gas exchange kinetics during exercise in chronic obstructive pulmonary disease. *J. Appl. Physiol.* 215: 523-531.

- Nevill AM, Brown D, Godfrey R, Johnson PJ, Romer R, Stewart AD, Winter EM (2003) Modelling maximal oxygen uptake of élite endurance athletes. *Med Sci. Sports Exerc*., 35: 488-494.

- Newsholme EA (1986) Application of principles of metabolic control to the problem of metabolic limitations in sprinting, middle distance, and marathon running. *Int. J. Sports Med.*, 7: 66-70.

- Nicholson RM, Sleivert GG (2001) Indices of lactate threshold and their relationship with 10-km running velocity. *Med. Sci. Sports Exerc*., 33 (2) 339-342.

- Norris SR, Petersen SR (1998) Effects of endurance training on transient oxygen uptake response in cyclists. *J. Sports Sci.*, 35: 488-494.

- Okura T, Nakata Y, Ohkawarw K, Numao S, Katayama Y, Matsuo T, Tanaka K. Effects of aerobic exercise on metabolic syndrome improvement in response to weight reduction. *Obesity Vol. 15 N. 10 October 2007.*

- Orr GW, Green HJ, Hughson RL, Bennett GW (1982) A computer linear regression model to determine ventilatory anaerobic threshold. *J. Appl. Physiol.*, 52: 1349-1352.

- Pacifico L et al. Menagement of metabolic syndrome in children and adoloscents Nutrition ,Metabolism e Cadiovascular Diseases 2011; 21: 455-66.

- Padilla DJ, McDonough P, Behnke BJ, Kano Y, Hageman KS, Musch TI, Poole DC. Effects of type II diabetes on muscle microvascular oxygen pressures. *Respiratory Physiology & Neurobiology. May 2007.*

- Paganini AT, Foley GM; Meyer RA (1997) Linear dependence of muscle phosphocreatine kinetics on oxidative capacity. *Am. J. Physiol.*, 272: 501-510.

- Palange P, Galassetti P, Mannix ET, Farber MO, Manfredi F, Serra P, Carlone S. Oxygen effect on O2 deficit and VO2 kinetics during exercise in obstructive pulmonary disease. *Journal of App. Physiol. 1995 June.*

- Paterson DH, Cunningham DA, Babcock MA (1990) Oxygen kinetics in the elderly. Faculty of Physical Education and Centre for Activity and Ageing. The University of Western Ontario. London, Canada.

- Paterson DH, Whipp BJ (1991) Asymmetries of oxygen uptake transients at the on-set and off-set of heavy exercise in human. *J. Physiol. (Lond.)* 443: 575-586.

- Petersen ES, Whipp BJ, Davis JA, Huntsman DJ, Brown HV, Wasserman K. Effects of beta-adrenergic blocade on ventilation and gas exchange during exercise in human. *J Appl. Physiol. 1983; 54:1306-1313.*

- Poole DC, Richardson RS (1997) Determinants of Oxygen uptake-Implication for exercise tesing. *Sports Med.* 24 (5): 308-320.

- Poole DC (1994) role of exercising muscle in slow component of VO_2. *Med. Sci. Sports Exerc.* 26: 1335-1340.

- Poole DC, Barstow TJ, Gaesser GA, Willis WT, Whipp BJ (1994b) VO_2 slow component: physiological and functional significance. *Med. Sci. Sports Exerc.*, 26: 1354–1358.

- Poole DC, Schaffartzik W, Knight DR, Derion T, Kennedy B, Guy HJ, Prediletto R, Wagner PD (1991) Contribution of exercising legs to the slow component of oxygen uptake kinetics in humans. *J. Appl. Physiol.* 71: 1245-1253.

- Poole DC, Ward SA, Gardner GW, Whipp BJ (1988) Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* 31: 1265-1279

- Powers SK, Lawler J, Dempsey JA, Dodd S, Landry G (1989) Effects of incomplete pulmonary gas exchange on VO_{2max}. *J. Appl. Physiol.*, 66 (6): 2491-1495.

- Regensteiner JG, Bauer TA, Reusch JEB, Brandenburg SL, Sippel JM, Vogelsong AM, Smith S, Wolfel EE, Eckel RH, Hiatt WR (1998) Abnormal oxygen uptake kinetics in women with type II diabetes mellitus. *J. Appl. Physiol.,* 85: 310-317.

- Ren J, Pulakat L, Whaley-Connel A, Sowers JR. Mitochondrial biogenesis in the metabolic syndrome and cardiovascular disease. *Journal of Molecular Medicine, October 2010*.

- Renoux JC, Petit B, Billat V, Koralsztein JP (1999) Oxygen deficit is related to the exercise time to exhaustion at maximal aerobic speed in middle distance runners. *Arch. Physiol. Bioch.*, 107: 280-285.

- Respiratory Medicine 2007 101, 2447-2453

- Reusch JEB, Bridenstine M, Regensteiner JG. Type 2 diabetes mellitus and exercise impairment. *Reviews in Endocrine and Metabolic Disorders. March 2013, Vol. 14, Issue 1, pp77-86.*

- Roberts CK, Hevener AL, Barnard RJ. Metabolic syndrome and insuline resistance: underlying causes and modification by exercise training. Comprehensive physiology 2013 Rev.

- Robert H, Fagard RH (1997) Impact of different sports and training on cardiac structure and function. *Cardiol. Clin.*, 3: 397-412.

- Roditisa P, Dimoupoulosb S, Sakellarioua D, Sarafogloub S, Kaldarab E, Venetsanakosb J, Anastasiou-Nanab M, Roussosa C, Nanasa S. The effects of exercise training on the kinetics of oxygen uptake in patients with chronic heart failire. European Journal of Preventional Cardiology 2013.

- Roston WL, Whipp BJ, Davis JA, Cunningham DA, Effros RM, Wasserman K (1987) Oxygen uptake and lactate concentration during exercise in human. *Am. Rev. Respir. Dis.,* 135: 1080-1084.

- Rowell LB (1994) *Human cardiovascular control*. Cary, NC: Oxford University Press.

- Rowell LB (1971) Cardiovascular limitation to work capacity. Simonsen E. (Ed). *Physiology of work capacity and fatigue*. Springfield, Charles C. Thomas, 132-169.

- Ryan WJ, Sutton RJ, Toews CJ, Jones NL (1979) Metabolism of infused L(+) lactate during exercise*. Clin. Sci.,* 55: 139-146.

- Saltin B, Strange S (1992) Maximal oxygen uptake: "old" and "new" arguments for a cardiovascular limitation. *Med. Sci. Sports Exerc.*, 24 (1): 30-37.

- Saltin B, Wahren J, Pernow B (1974) Phosphagen and carbohydrate metabolism during exercise in trained middle-aged men. *Scand. J. Clin. Invest.*, 33 (1): 71-77.

- Saltin B (1969) Physiological effects of physical conditioning. *Med. Sci. Sports Exerc.*, 1: 50.

- Saltin B, Stenberg J (1964) Circulatory response to prolonged severe exercise. *J. Appl. Physiol.*, 19: 833-838.

- Sanada K, Kuchiki T, Miyachi M, McGrath K, Higuchi M, Ebashi H (2007) Effects of age on ventilatory threshold and peak oxygen uptake normalised for regional skeletal muscle mass in Japanese men and women aged 20-80 years. *Eur. J. Appl. Physiol.*, 99 (5): 475-483.

- Seals DR, Chase PB (1989) Influence of physical training on heart rate variability and baroreflex circulatory control. *J. Appl. Physiol.*, 66 (4): 1886-1895.

- Scheuermann BW, Barstow TJ, Hoelting BD, Noble ML (2000) The slow component of O_2 uptake is not accompanied by changes in muscle EMG during repeated bouts of heavy exercise in humans. *Journal of Physiology,* 53: 245-256.

- Seip RL, Snead D, Pierce EF, Stein P, Weltman A (1991) Perceptual responses and blood lactate concentration: effect of training state. *Med. Sci. Sports Exerc.*, 23 (1): 80-87.

- Seiyama A, Hazeki O, Tamura M (1988) Non invasive quantitative analysis of blood oxygenation in rat skeletal muscle. *J. Biochem.*, 103: 419-424.

- Shapiro LM (1997) The morphologic consequences of systemic training. *Cardiol. Clin.*, 15 (3): 373-379.

- Shinoara M, Moritani T (1992) Increase in neuromuscolar activity and oxygen uptake during heavy exercise. *Ann. Physiol. Anthropol.,* 11: 257-262.

- Sietsema KE, Ben-Dov I, Zhang YY, Sullivan C, Wasserman K (1994) Dynamics of oxygen uptake for submaximal exercise and recovery in patients with chronic heart failure. *Chest,* 105: 1693-1700.

- Sietsema KE, Cooper DM, Perloff JK, Rosove MH, Child JS, Canobbio MM, Whipp BJ, Wasserman K. Dynamic of oxygen uptake in adults with cyanotic congenital heart disease. *Circulation. 1986;73:1137-1144.*

- Sjodin B, Thorstensson A, Frith K, Karlsson J (1976) Effect of physical training on LDH activity and LDH isoenzyme pattern in human skeletal muscle. *Acta Physiol. Scand*., 97: 150-157.

- Souraty P. Nat Clin Prct Nephol. 2009.

- Speechly DP, Taylor SR, Rogers GG (1996) Difference in ultra-endurance exercise in performance-matched male and female runners. *Med. Sci. Sports Exerc.*, 28 (3): 359-365.

- Sung J, Choi YH, Park JB. Metabolic syndrome is associated with delayed heart rate recovery after exercise*. J Korean Med Sci 2006; 21:621-6.*

- Svedmyr N (1966) Metabolic effects of infused L-(+)-lactate in man before and after triiodothyronine treatment. *Acta Physiol. Scand*., 67: 229-235.

- Tabata I, Nishimura K, Kouzaki M, Hirai Y, Ogita F, Miyachi M, Yamamoto K (1996) Effects of moderate–intensity endurance and high-intensity intermittent training on aerobic capacity and VO_{2max}. *Med. Sci. Sports Exerc.*, 28 (10): 1327-1330.

- Talmadge RJ, ScheideJI, Silverman H (1989) Glycogen synthesis from lactate in a chronical active muscle. *J. Appl. Physiol.,* 66: 2231-2238.

- Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with non alcoholic fatty liver desease. N Engl Med 2010;363: 1341-50.

- Terjung RL (1976) Muscle fiber involvement during training of different intensities and durations. *Am. J. Physiol.*, 230: 946-950.

- Tjonna AE, Lee SJ, Rognmo O, Stolen TO, Bye A, Haram PM, Loennechen JP, Al-Share QY, Skogvoll E, Slordahl SA, Kemi OJ, Najjar SM, Wisloff U. Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation 2008; 118:346-354*.

- Trial CAFE Circulation 2006 ; 113:1213-1225.

- Tschakovsky ME, Hughson RL (1999) Interaction of factors determining oxygen uptake at the onset of exercise. *J. Appl. Physiol.*, 86 (4): 1101-1113.

- Turpeneim AK, Kuikka JT, Vanninem E, Vainio P, Litmanen H,Vanninem M (1996) Athletic heart: a metabolic, anatomical and functional study. *Med. Sci. Sports Exerc.*, 28 (1): 33-40.

- Van Schuylenbergh R, Vanden Eynde B, Hespel P (2005) Effect of exercise-induced dehydration on lactate parameters during incremental exercise. *Int. J. Sports Med.*, 26 (10): 854-858.

- Vollestad NK, Blom PC (1995) Effect of varying exercise intensity on glycogen depletion in human muscle fibres. *Acta Physiol. Scand.*, 125 (3): 395-405.

- Von Döblen W (1956) Human standard and maximal metabolic rate in relation to fat-free body mass. *Acta Physiol. Scand. 37* (suppl. 126).

- Wasserman K, Hansen JE, Sue DY, Casaburi R, Whipp BJ (1999) *Principles of exercise testing and interpretation*. Third ed., Lippincot Williams & Wilkins,.

- Wasserman K, Hansen JF, Sue DY (1991) Facilitation of oxygen consumption by lactic acidosis during exercise. *New Physiol. Sci.,* 6: 29-34.

- Wasserman K, Whipp BJ, Castagna J (1974) Cardiodynamic hyperpnea: hyperpnea secondary to cardiac output increase. *J. Appl. Physiol.,* 36: 457-464.

- Weltman A (1995) The blood lactate response to exercise. *Champaign, IL: Human Kinetics*.

- Whipp BJ, Rossiter HB, Skasick A, Ward SA (2001) The kinetics of VO_2 as a determinant of maximum aerobic performance during exercise in humans. In: *Proceedings of the International Union Physiological Sciences*, Christchurch, New Zealand.

- Whipp BJ (1994) The slow component of O_2 uptake kinetics during heavy exercise. *Med. Sci. Sports Exerc.*, 26 (11): 1319-1326.

- Whipp BJ (1987) Dynamics of pulmonary gas exchange. *Circulation,* 76 (Suppl. VI): 18-28.

- Whipp BJ, Ward SA, Lamarra N, Davis JA, Wasserman K (1982) Parameters of ventilatory and gas exchange dynamics during exercise. *J. Appl. Physiol.,* 52 (6): 1506-1513.

- Whipp BJ, Malher M (1980) Dynamics of pulmonary gas exchange during exercise. In West J.B. (ed) *Pulmonary gas exchange,* vol. 2 Academic Press, New York, pp 33-96.

- Whipp BJ, Wasserman K (1972) Oxygen uptake kinetics for various intensities of constant-load work*. J. Appl. Physiol.,* 33: 351-356.

- Wilber RL, Zawadzki KM, Kearney JT, Shannon MP, Disalvo D (1997) Physiological profiles of élite off-road and road cyclists. *Med. Sci. Sports Exerc.*, 29 (8): 1090-1094.

- Williams CA, Carter H, Jones AM, Doust JH (2001) Oxygen uptake kinetics during treadmill running in boys and men. *J. Appl. Physiol.*, 90: 1700-1706.

- Williams MH, Branch JD (1998) Creatine supplementation and exercise performance: an update. *J. Am. Coll. Nutr*., 17 (3): 216-234.

- Willis WT, Jackman MR (1994) Mitochondrial function during heavy exercise. *Med Sci. Sports Exerc.,* 26: 1347-1354.

- Wilmore JH, Stanforth PR, Gagnon J, Leon AS, Rao DC, Skinner JS, Bouchard C (1996) Endurance exercise training has a minimal effect on resting heart rate: the Heritage study. *Med. Sci. Sports Exerc.*, 28 (7): 829-835.

- Womack CJ, Davis SE, Blumer JL, Barrett E, Weltman AL, Gaesser GA (1995) Slow component of O_2 uptake during heavy exercise: adaptation to endurance training. *J. Appl. Physiol.,* 79: 838-845.

- Woodiwiss AJ, Oosthuyse T, Norton GR (1998) Reduced cardiac stiffness following exercise is associated with preserved myocardial collagen characteristics in the rat. *Eur. J. Appl. Physiol*., 78 (2): 148-154.

- Xu F, Rhodes EC (1999) Oxygen uptake kinetics during exercise. *Sports Med.*, 27 (5): 313-327.

- Yasuda Y, Ishida K, Miyamura M (1992) Effects of blood gas, Ph, lactate, potassium on the oxygen uptake time courses during costant-load bicycle exercise. *Japanese J. Physiol.,* 42: 223-237.

- Yoon BK, Kravitz L, Robergs R (2007) VO_{2max}, protocol duration, and the VO_2 plateau. *Med. Sci. Sports Exerc.*, 39 (7): 1186-1192.

- Yoshida T, Udo M, Iwai K, Yamaguchi T (1993) Physiological characteristics related to endurance running performance in female distance runners. *J. Sports. Sci.*, 11: 57-62.

- Zoladz LA, Grassi B, Majerczak J, Szkutnik Z, Korostynsky M, Karasinski J, Kilarski W, Korzeniewski B. Training-induced acceleration of O2 uptake on.kinetics precedes muscle mitochondrial biogenesis in humans. *Experimental Physiology, April 2013*.