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THE IMPACT OF PHYSICAL FITNESS AND PHYSICAL ACTIVITY LEVEL ON BIOMECHANICAL AND METABOLIC PARAMETERS OF TREADMILL WALKING IN NORMAL-WEIGHT AND OBESE YOUNG ADULTS

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To my family

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ABSTRACT

Obesity and physical inactivity are classified as *"noncommunicable disease"* and causes every year millions of deaths in the world. Walking has been proposed as a valid way to fight against these diseases because it can produce substantial health benefits and also because people can easily perform it every day.

AIMS: At first, we assessed if physical activity (PA) or physical fitness (PF) could affect walking economy (Net EC) and efficiency in a group of healthy normal-weight adults, separately for men and women. Secondly, we assessed the same variables in a group of obese young adults.

MATERIALS AND METHODS: 30 young normal-weight (14F,16M; 18.5<BMI<25 kg·m^{-2}) and 14 obese (7F,7M; BMI>30 kg·m^{-2}) subjects were recruited in the study. Economy (Net EC) was analyzed with indirect calorimetry and, simultaneously, total mechanical work (W_{tot}) was assessed with an optoelectronic system at 3.5/ 4.5/ 5.5 km·h^{-1}. Efficiency was calculated as W_{tot}/Net EC. To assess PA, subjects wore an activity monitor for a whole week, inferring time spent in sedentary (SED, <1.5 METs), or moderate to vigorous (MVPA, >3 METs) physical activity. MVPA were calculated both as overall daily minutes ($MVPA_{all}$) and also in bouts of at least 10 min ($MVPA_{bouts}$). To assess PF, a maximal $V'O_{2max}$ test was performed on a treadmill with indirect calorimetry, isometric maximal voluntary contraction (iMVC) of lower limbs was measured by two force plates on horizontal leg press, flexibility (Flex) was assessed by the V-sit and reach test, and % fat mass (%FM) was evaluated with skinfold thickness (in normal-weight) or with girth measurements (in obese subjects).

RESULTS: In normal-weight adults, no significant associations, adjusted for age and body mass index, were identified between PA and gait economy or efficiency. Regarding PF, in females iMVC and Flex affected efficiency (R^2 = 0.73, F= 12.95, p<0.001), whereas $V'O_{2max}$ and iMVC were associated with economy (R^2 = 0.69, F= 15.13, p<0.001).

In the obese group, at a speed near the preferred one, SED (with %FM, iMVC and Flex) was positively associated with Net EC (R^2 = 0.54, F= 4.04, p<0.05), whereas $MVPA_{all}$ was positively associated with gait efficiency (R^2 = 0.32, F= 4.09, p<0.05).

CONCLUSIONS: The main findings of this study were that in normal-weight adults PA did not affect neither economy nor efficiency of treadmill walking, whereas in obese individuals SED reduced gait economy and $MVPA_{all}$ enhanced gait efficiency. These findings might to be considered for exercise prescription.

Key words: economy, efficiency, physical activity, physical fitness, gait, walking

LIST OF ABBREVIATIONS

%FM	% Fat mass
ACC	Accelerometry
ACSM	American college of sports medicine
AH	Actiheart
BD	Body density
BM	Body mass
BMI	Body mass index
CLM	Combined Limbs Method
CoM	Centre of mass
CVD	Cardiovascular disease
EC	Energy cost
ECG	Electrocardiogram
Gross EC	Gross energy cost
Net EC	Net energy cost
Eff	Mechanical efficiency
E_{tot}	Total mechanical energy
$FeCO_2$	Fraction of carbon dioxide in expired air
FeO_2	Fraction of oxygen in expired air
Flex	Flexibility
GC	Gait cycle
HDL	High density lipoprotein
HR	Heart rate
HR_{max}	Maximal heart rate
ILM	Individual Limbs Method
iMVC	Isometric maximal voluntary contraction
IPAQ	International physical activity questionnaire

KE	Kinetic energy
LDL	Low density lipoprotein
MET	Metabolic equivalent of task
$MVPA_{all}$	Total moderate and vigorous physical activity
$MVPA_{bouts}$	Bouts of moderate and vigorous physical activity
PA	Physical activity
PAR-Q	Physical activity readiness questionnaire
PE	Potential energy
PF	Physical fitness
PWS	Preferred walking speed
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
SED	Minutes of sedentary time
SHR	Sleeping heart rate
SMR	Standing metabolic rate
TEE	Total energy expenditure
$V'CO_2$	Carbon dioxide production
$V'O_2$	Oxygen consumption
$V'O_{2max}$	Maximal oxygen uptake
$V'O_{2peak}$	Peak oxygen uptake
VE	Ventilation
W_{ext}	External mechanical work
W_{int}	Internal mechanical work
W_{tot}	Total mechanical work

1. INTRODUCTION

In the current occidental society the improvement of technology leads to higher levels of sedentary lifestyle, that generates a higher incidence of health-related pathologies (Manson et al., 1999; Kesaniemi et al., 2001; Thune et al., 2001; Yaffe et al., 2001; Manson et al., 2002; Petrović-Oggiano et al., 2010). Also obesity prevalence and the associated risks for comorbidities are a growing health problem.

According to a 2009 WHO report about the leading global risks for mortality, physical inactivity and obesity are ranked in the 4th and 5th place, after hypertension, smoking and high blood glucose, and every year cause 3.2 (6%) and 2.8 (5%) millions of deaths in the world, respectively (Fig.1.1).

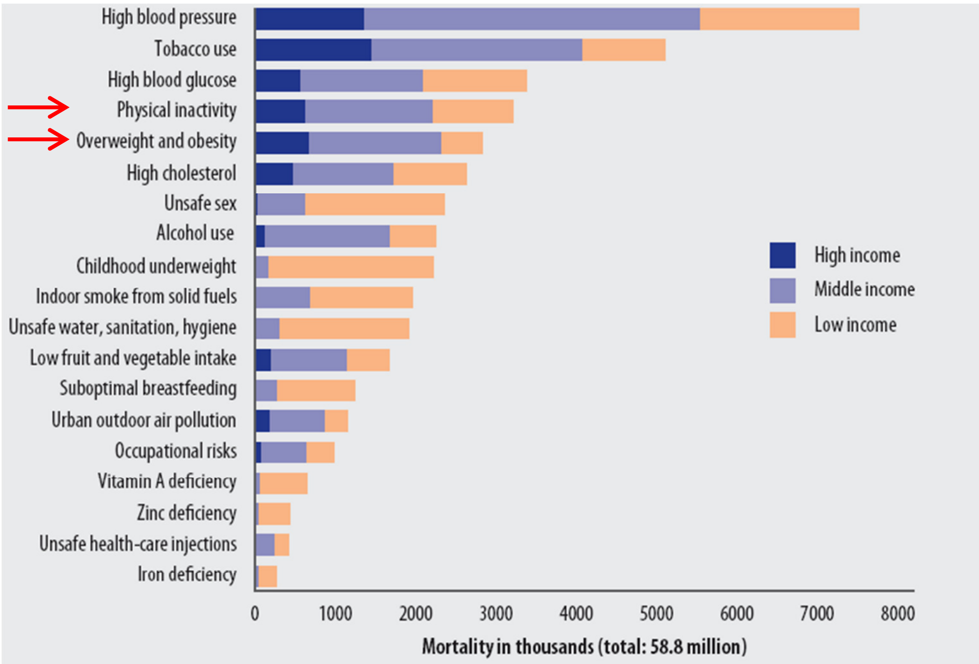

Fig.1.1 Deaths attribute to main risk factors. (In WHO, 2009).

Physical activity leads to numerous health benefits (Warburton et al., 2006) and in older adults can influence the performance of habitual daily tasks, mobility and locomotion (Mian et al., 2007; den Ouden et al., 2013).

The American College of Sports Medicine suggests that to obtain health benefits healthy adults should perform at least 30 min/day for 5 or more days/week of at least moderate intensity exercise in bouts lasting no less than 10 min (Garber et al., 2011).

Walking is the most popular activity that people can easily perform every day and that can produce substantial health benefits (Lee et al., 2008). It allows to enhance physical activity level and, if performed constantly and at right intensities, to improve cardiovascular fitness and to meet exercise recommendations (Murtagh et al., 2002).

In sedentary and obese people, even walking at preferred walking speed (PWS) can be very demanding, and this can quickly bring to the exertion.

It is well established that PWS is very close to the so called 'optimal walking speed', where the energy cost (energy spent per unit distance covered) is minimized (Cavagna and Kaneko, 1977; Rose and Gamble, 2006). If the physical demand of PWS of this population falls formerly in the moderate intensity, it means that the minimum rate of perceived exertion is already demanding.

Humans are inclined to save their energies, so sedentary individuals will probably become more and more inactive, establishing thus an insane vicious circle. In the long term, this could bring functional limitations and a premature loss of independence.

Although gait has been largely investigated, human walking is still an actual topic.

The improvement in technologies and the even more simply accessibility to the instrumentations now permit to analyze what human walking is from a global point of view.

For this reason, in the last years many investigators developed a multifactorial approach, analyzing simultaneously both biomechanical and physiological domains. In particular, metabolic cost and mechanical work produced by muscles, that are assumed to be directly proportional to the work done by the body (Browder and Wilkerson, 1993), are two common but different measures that relate biomechanics with bioenergetics.

Also, a very interesting topic, that reflects the interrelationships between metabolic and mechanical work, is the evaluation of the efficiency of human movement, that is the ability to convert physiological energy into mechanical work. In particular, gait efficiency is defined by the ratio of total mechanical work and net energy cost of walking (Cavagna and Kaneko, 1977).

1.1 OBESITY

The universal accepted obesity classification is based on Body Mass Index (BMI) assessment, that is the ratio between weight in kg and height in m^2. People affected with obesity are those with BMI values that exceed 30 kg/m^2 (WHO, 2000). Obesity may impair health enhancing the risk for many heart and chronic diseases as hypertension, type 2 diabetes, stroke, respiratory disease, some kind of cancers (especially endometrial, breast, and colon) and osteoarthritis (Jakicic et al., 2001). Quality of life is often compromised in this medical condition, with greater impairments on physical domain (Fontaine and Barofsky, 2001).

Obesity, characterized by an abnormal accumulation of body fat, is grouped in a so-called *"noncommunicable diseases"* that are the main causes of global death in the world (Alwan et al., 2011). In 1997 the World Health Organization recognized obesity as a global epidemic: basing on BMI data of 2008, more than half a billion adults worldwide (nearly 11%) were obese (Alwan et al., 2011). Unbelievably, the "*65% of the world's population lives in countries where overweight and obesity kills more people than underweight"* (Alwan et al., 2011).

The leading cause of obesity is an energy imbalance between calories introduced with food and calories expended. The rapid increase in obesity prevalence suggests that this phenomenon is principally due to environmental and social influences: Fig 1.1.1 shows different levels of determinants that can affect population energy balance. It suggests that food intake and physical activity can be largely manipulated by contextual scenarios often independently from people, that lead beyond an individual's rational control. Therefore, a possible solution to this social epidemic lies in policies and actions that modify those contextual features (Huang et al., 2009).

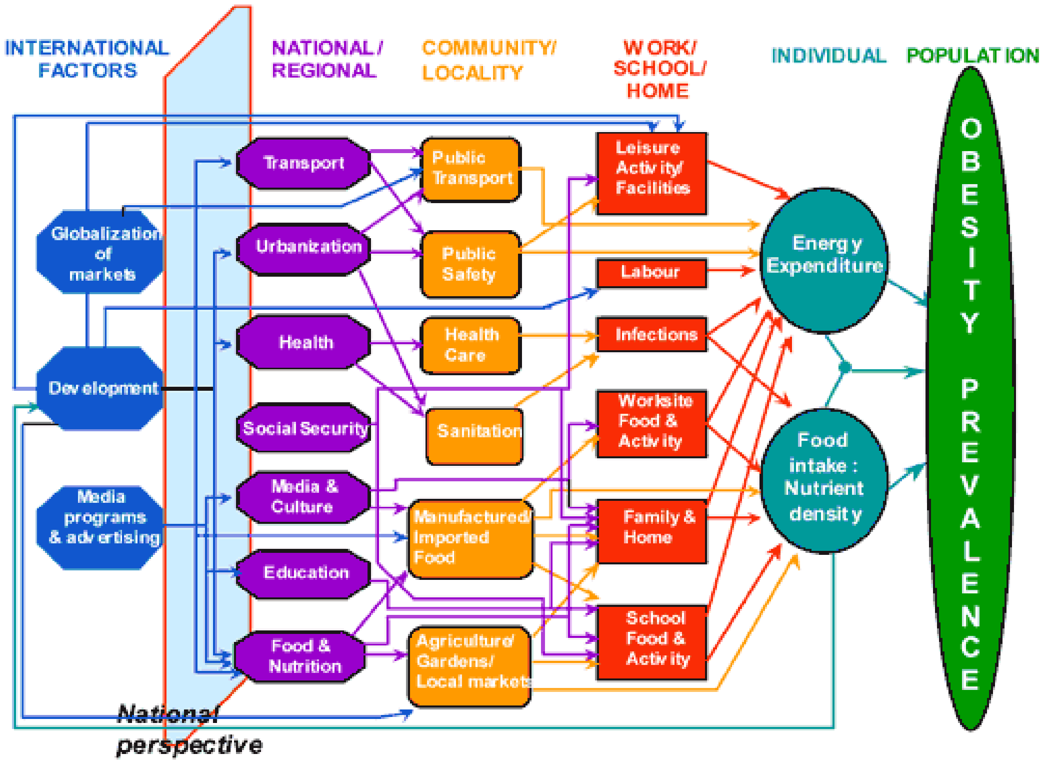

Fig. 1.1.1 Levels of determinants and sectors of society implicated in the complex systems of obesity (From Kumanyika et al., 2002)

At individual level, it is easy to understand that diet and PA behaviors play a fundamental role in weight loss and in the prevention of weight gain, especially if these strategies are combined together (Donnelly et al., 2009).

As an example, a normal, healthy young adult woman with a sedentary lifestyle and an office work usually needs about 1900 calories/day. This means eating approximately 2 dishes of pasta, 1 of meet with potatoes, 1 portion of bread, 1 apple and 1 yogurt. All the other food intakes (snacks, cakes, alcohol, etc.) include extra calories that should be consumed at soon as possible. Otherwise, they will be converted into fat tissue. Unfortunately, one kilogram of body fat corresponds to about 7000 Kcal, so to burn them one should run for 112 km, or ride a bicycle for 290 km, all activities that are very difficult to organize and perform in our towns.

Indeed, the principal statement about the efficacy of physical activity on weight loss affirms that *"exercise alone produces modest weight losses"* (NIH, 1998; Donnelly et al., 2009), and that generates a smaller magnitude of weight loss than nutritional modifications (Jakicic, 2001). Despite this, it is has been demonstrated that regular moderate physical activity is a fundamental and high-priority component for any weight-management program (Hill and Wyatt, 2005).

In fact, in addition to promote weight loss, regular exercise has been associated to numerous health benefits (summarized in Table 1.1.1), well described in many consensus statements for Primary Care (EASO, 2004).

Table 1.1.1 Benefits of physical exercise. Modified from EASO, 2004.

Physical activity benefits in obese people	
↓ body and abdominal fat	↑ lean (muscle and bone) mass
↓ loss of lean body mass due to dietary restriction	↑ diet-induced weight loss
↓ blood pressure	↔ ↑ resting energy expenditure
↓ elevated blood insulin	↑ glucose tolerance improvement
↓ anxiety and depression	↑ lipid profile improvement
	↑ emotional state and mental health.
	↑ general feeling of well-being and self-esteem
	↑ fitness
	↑ compliance to the dietary regimen
	↑ long-term weight maintenance.

To increase overall levels of physical activity and energy expenditure, the majority of expert panels recommend to adopt an active lifestyle changing habitual daily sedentary behaviors: climbing stairs rather than take the elevator, ride a bicycle for small travels instead of using a car, gardening or walking with a dog, are all examples of positive changes.

One of the most debated question is *"how much physical activity do obese people need to perform?"*

Basically it depends on the main goal that they want to achieve: the position stands of the ACSM (Jakicic et al., 2001 and Donnelly et al., 2009) deal with this topic, defining how much PA is necessary to prevent weight gain, to promote weight loss, and to maintain gain after weight loss. Table (1.1.2).

Table 1.1.2 PA guidelines for obese people based on ACSM's position stand (Donnelly et al., 2009)

Main objective	PA amount	Energy consumption
Weight gain prevention (within 3%)	From 150 to 250 min/wk	1200-2000 kcal/wk
Weight loss	Minimal: <150 min/wk Modest: >150 min/wk (2-3 kg/month) Optimal: >225-420 min/wk (5-7 kg/month)	
Weight maintenance after weight loss	A minimum of 200-300 min/wk	2000 kcal/wk
General recommendations	A minimum of 30 min/day of moderate PA (3-5.9 METs)	

Another approach that is effective to promote physical activity is to diminish sedentary behaviors. Both of these actions turn into the same goal: a more active individual. Clearly the most difficult challenge is to be successful in the change of wrong lifestyle routine: for this reason obese people should be guided and helped in starting or increasing exercise. Physicians should construct ad hoc programs, following patient's ability and health, and focusing on a safe and enjoyable gradual increase of physical activity levels. In some cases (depression, excessive stress, anxiety and more) the aid of a psychological support and/or treatment was fundamental to a successful obesity management (EASO, 2004).

1.2 SEDENTARISM

The term *'sedentarism'* refers to those activities that require very low energy consumption, like sitting, lying down, sleeping, watching television and many more (Fox, 2012). An individual who accumulates a high quantity of these activities can be characterized as a sedentary person.

Then, it is clear that sedentarism is not only a lack of physical exercise and it doesn't represent people who don't perform at least 150 minutes of moderate and vigorous PA (MVPA) per week, that is the minimum threshold of PA recommendations (Garber et al., 2011).

Indeed, Hamilton et al. (2008) reported that in a group of adults that meets exercise guidelines, excessive sedentary time not only negated the expected benefits of exercise, but also worsen the global health of the tested group. Katzmarzyk and collegues (2009) demonstrate that a dose-response association between sitting time and mortality from all causes, but especially for cardiovascular disease (CVD) exists, and that is independent of leisure time physical activity.

So researchers indicates that sedentarism is associated with physiologic deterioration, disease, and death and it may be an important predictor of future health (Fox, 2012). The actual society and the natural development of technology lead people to have less need for physical activity. Machines are replacing humans in performing heavy works, so sedentary jobs (<1.5 METs) and light activities (1.5-2.9 METs) include the majority of actual occupations. These tendencies have diminished daily energy consumption of more than 100 kcal that, if multiplied for 365 day give 36500 kcal, that are the equivalent of more than 5 kg in one year (1kg=7000 Kcal, 36500 kcal/7000 = 5.2 kg of weight gain).

To fight against sedentarism, some big companies are helping their workers with some ergonomic facilities (like standing desks) and with corporate wellness solutions. In addition, people should increase energy consumption engaging in leisure time activities avoiding sedentary behaviors like television viewing (Fox, 2012).

1.3 PHYSICAL ACTIVITY

Physical activity is a complex behavior defined as *"any bodily movement produced by skeletal muscles that results in energy expenditure"* (Caspersen et al., 1985). Everyone in daily life performs PA in a variety of situations to sustain life: occupational, household tasks, leisure time activities and sports are some examples of domains that contribute to enhance PA energy consumption. Generally, the prevalent portion of total energy expenditure (TEE) is due to the resting metabolic rate (60-70% of TEE), 10% is derived from the diet, and the residual 20-30% from physical activity (Bouchard et al., 2007). PA is the only component that may differ considerably from person to person, from 10% of TEE until 80% in very active subjects (McArdle, 1996). PA, quantified as the total amount of energy expenditure or as minutes passed in different intensities categories, is determined by the frequency, duration and intensity of muscular contractions and activity type (Howley et al., 2001). Type, frequency and duration are easily assessed, but intensity, that is the energy expended per unit of time (e.g. $kcal \cdot min^{-1}$, $mLO_2 \cdot kg^{-1} \cdot min^{-1}$ etc.), is more difficult to quantify. Metabolic Equivalent of Task (MET) is one of the most common measure that well represents the intensities of specific activities. One MET is defined as "*the ratio of the associated metabolic rate for a specific activity divided by the resting metabolic rate"* (Ainsworth et al., 1993). By convention, 1 MET is also the equal to 1 $kcal \cdot kg^{-1} \cdot h^{-1}$ or 3.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ (Ainsworth et al., 1993; 2000). A specific MET value can be assigned to every kind of task, expressing activity intensity as a multiple of the resting energy expenditure: for example, a walking pace with a MET value of 2 means that a person expends twice the energy required for resting values. MET intensity classifications (listed in Table 1.3.1) were provided and validated by Pate et al. (1995).

Table 1.3.1 MET classification

MET Cutoff	Activity Intensities
<1.5	Sedentary
1.5 – 3	Light activities
3 – 6	Moderate activities
>6	Vigorous activities

PA can be measured with objective (physiological markers like heart rate, indirect calorimetry, and motion sensors) or subjective (questionnaires, diaries, etc.) methods. Objective measures are more accurate and reliable but some of these are too expensive and/or not so practical, especially in daily living settings (e.g. doubly labelled water method, direct calorimetry, etc.). Indirect measures have the opposite problem: even if validated against gold standard methods, they are less precise, but very cheap and less invasive, so they are largely used for epidemiological studies, especially to categorize people with high vs low physical activity levels (Jørgensen et al., 2009).

During the last couple of decades, the use of wearable activity monitors has exponentially grown up. These devices represent probably the best compromise to objectively assess free-living PA, because they produce very accurate data combining two or more physiological signals, with a minimum invasivity at sustainable costs. Accurate measurements in field settings are essential to gather additional information about relevant features of physical activity behaviors in order to better develop PA programs and interventions to improve health.

It is well documented that physical activity produces substantial health benefits in healthy people, in those who are at risk for chronic disease and also in individuals with chronic disease: for this reason, the majority health promotion associations include exercise in their general recommendations (CDC, 1996; Haskell et al., 2007) as a fundamental lifestyle component.

It has been observed that (HHS, 2008):

- Regular physical activity reduces the risk to incur in many adverse health outcomes.
- Some physical activity is better than none, more is better.
- To increase health benefits, people have to perform PA at higher intensity, with greater frequency, and/or longer duration.
- A minimum of 150 min/wk of moderate intensity physical activity seems to be the lowest dose to obtain health benefits. Additional benefits occur adding more physical activity.
- Both aerobic and muscle-strengthening components of PA produce health improvements.
- Health benefits occur for adults, older adults, children, adolescents, and also in people with disabilities.

PA assessments based on surveys population generally find that (HHS, 2008):

- women are less active than men;
- age is inversely related with activity levels and adiposity;
- activity levels are consistently higher in people with higher education and income;
- activity levels are inversely associated with adiposity;

- in the long term, physical inactivity is not associated with the development of obesity, but obesity may lead to physical inactivity (Petersen et al., 2004).

Many researchers highlighted the importance of walking as a physical activity to improve health, change sedentary behaviors and enhance PA levels (Morris and Hardman, 1997). In particular, Eyler et al., (2003) have demonstrated the efficacy of walking to reduce cardiovascular risks, some cancers, type 2 diabetes, osteoarthritis and osteoporosis.

Jeon and colleagues (2007) found that the risk of type 2 diabetes was substantially reduced following recommendations to participate in moderate intensity physical activities, such as brisk walking. In obese people, walking is an effective, safe and cheap way to increase energy expenditure (Browning et al., 2009), and walk briskly or uphill allows to reach main PA recommendations, lose weight and improve cardiovascular fitness (Ehlen, 2011).

1.4 PHYSICAL FITNESS

Physical fitness (PF) is *"the ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample energy to enjoy leisure-time pursuits and to meet unforeseen emergencies"* (Caspesen et al., 1985). Practically, it defines a set of individual characteristics that people have or achieve, in relation to perform physical activity.

These individual characteristics can be quantified by different components that can be listed in two principal groups, one related to health, and the other related to technical skills.

In this thesis, we focused our attention on health-related components, listed in Tab.1.4.1.

Table 1.4.1 Main physical fitness subdivision components

Physical Fitness	
→ **Health-related components**	**Skill-related components**
Cardiorespiratory fitness	Agility
Muscular fitness	Balance
Body composition	Coordination
Flexibility	Speed
	Power
	Reaction time

The most important element of PF is unquestionably cardiorespiratory endurance ($V'O_{2max}$), that refers to "*the ability to circulatory and respiratory systems to supply fuel during sustained PA and eliminate fatigue products after supplying fuel"* (Caspersen et al., 1985).

In clinical practice $V'O_{2max}$ is an essential health indicator for healthy and unhealthy (symptomatic and asymptomatic) people (Gibbons et al., 2002; Myers et al., 2002).

Higher levels of $V'O_{2max}$ are associated to:

- lower risks of premature death for all-cause mortality, and especially for cardiovascular disease (Blair et al., 1989; 1995; Church et al., 2005; ACSM, 2009). Moreover, changes over time in cardiorespiratory fitness produce changes in mortality risks, with higher risks occurring in more unfit people (Lee et al., 2010)

- higher levels of physical activity, that is related to numerous health benefits (ACSM, 2009)

In the modern society, lean persons are often considered healthy persons, whereas obesity is a synonymous of illness: it is not always true.

Indeed, in the recent years it has been developed the “fit but fat” concept, that demonstrates that high levels of physical activity and/or cardiovascular fitness attenuate health risks associated with overweight and obesity (Lee et al., 1999; 2005; Farrell et al., 2002). In a study of Wei and colleagues (1999), deaths and CVD were twice more frequent in normal-weight but unfit persons than in obese people with moderate V’O2max.

Substantially, all these studies suggest that highest $V'O_{2max}$ produce health benefits independently to body mass, and that being “fit and fat” was better than to be lean and unfit (Lee et al., 2005).

Muscular fitness, composed by muscle strength and endurance, refers to the ability of muscles to generate force (muscular strength) or to resist to subsequent contractions for an extended period of time (muscular endurance).

Higher levels of muscular fitness are related with lower risks for poorer health, and in particular with better cardiometabolic risk factors profiles, lower risk of CVD events and all-cause mortality, lower risk to incur in functional limitations and other disease (Garber et al., 2011).

Moreover, improvements in bone health, body composition, blood pressure, insulin sensitivity and blood glucose level were checked. Importantly, it has been proved that resistance training can significantly help to treat and prevent the metabolic syndrome and osteoporosis (Malik et al., 2004;

Maïmoun and Sultan, 2011).

Flexibility can be improved at all ages. Benefits are related to balance and postural stability enhancement, particularly if performed in combination with resistance exercise (Bird et al., 2011). In contrast with popular beliefs, regular flexibility workouts are not associated with DOMS reductions of musculotendinous injuries, delay onset muscular soreness and low back pain prevention (Garber et al., 2011).

Regarding body composition, greater fat free mass has a protective effect on the risk of all-cause mortality (Bigaard et al., 2005) and it can enhance resting metabolic rate. Moreover, abdominal obesity is associated with high CVD (Garber et al., 2011).

1.5 BASICS OF WALKING: THE GAIT CYCLE

Human locomotion is a combination of complex coordinated movements where the passive dynamics of musculoskeletal system and the active control produced by the central nervous system work together to maintain stability and balance while the body continuously moves from one leg to another (Donelan et al., 2004). The main characteristics of this gait is that at least one foot is always in contact with the floor. When we are walking, all the body is involved in this task, but the main players in this travel from a place to another are lower limbs. Muscles represent the engine of the locomotor system, and they must work in a precise sequence to assure a normal forward motion without losing our balance.

Walking is characterized by the same repetitive movements: for this reason researchers have defined a functional unit of gait that they called *'gait cycle'* (or *'stride'*). This cycle describes the motions from two successive contact of the same foot on the ground (Perry and Burnfield, 2010). Each stride is divided into 2 different phases (fig. 1.4.1):

- the **stance phase**, where the foot is in contact with the ground;
- the **swing phase**, where the limb is advancing in the air.

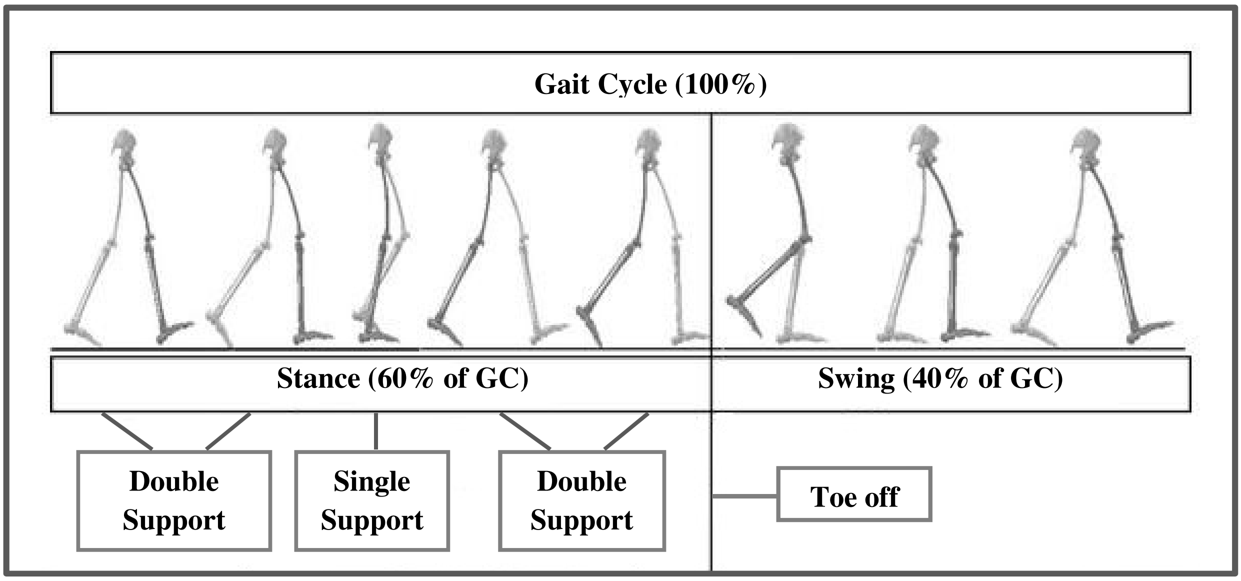

Fig.1.4.1 Gait cycle division (in: Hartmann et al., 2010)

These phases are normally expressed as a percentage of the total stride duration (0%-100%): this normalization is essential to allow comparisons with different populations or conditions because every person has a different gait cycle time (Perry and Burnfield, 2010). The stance phase, that normally represents 60% of the gait cycle (GC), begins with the heel contact until the toe off of the same foot. For the swing phase, that lasted for the additional 40% of the GC, it is exactly the opposite: the beginning is at toe off and the end is represented by heel contact of the ipsilateral limb. Gait speed modifies these ratios: higher velocities lengthen swing disadvantaging the stance phases and vice-versa (Stoquart et al., 2008).

Stance can be subdivided into 3 sections, that comprise initial double stance, single limb stance and terminal double limb stance. Each double stance period (also called *'double support'*) is then repeated 2 times in a gait cycle and each period accounts for 10% of the GC (20% total), whereas single stance normally represents 40%.

In a gait cycle, it is possible to recognize spatial and temporal features. Temporal parameters include:

- **stance phase**;
- **swing phase**;
- **double support phase**;
- **cadence** (or **step frequency**), the number of steps per minute. It could be expressed also as the number of cycles per second (Kirtley, 2006);
- **step time**, time elapsed between two subsequent contacts of different feet (e.g, from right to left heel strike);
- **stride time**, time elapsed between two subsequent contacts of the same feet.

Spatial parameters include (fig.1.4.2):

- **stride length,** the distance between the initial contact of one foot to the subsequent initial contact of the ipsilateral foot;
- **step length**, the distance between the initial contact of one foot to the subsequent initial contact of the contralateral foot (Perry and Burnfield, 2010);
- **step width**, the medial-lateral distance between consecutive left and right contacts (Owings and Grabiner, 2004).

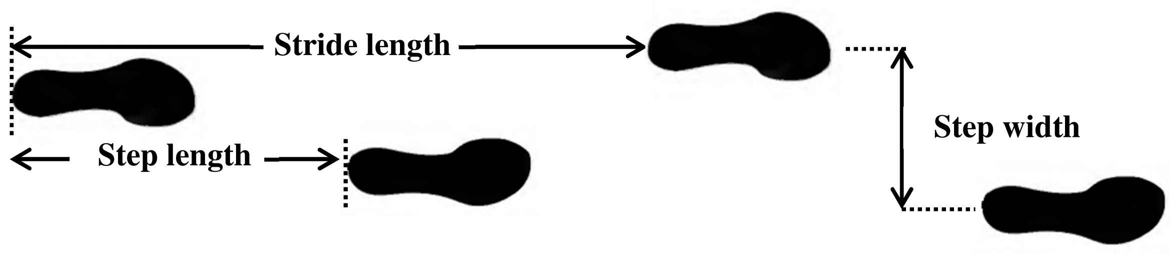

Fig.1.4.2 Spatial characteristics of a gait cycle (modified from Perry and Burnfield, 2010)

Functionally speaking, it is possible to recognize 8 key events in a stride, where 5 take place in the stance phase, and the last 3 occur during swing (fig.1.4.3):

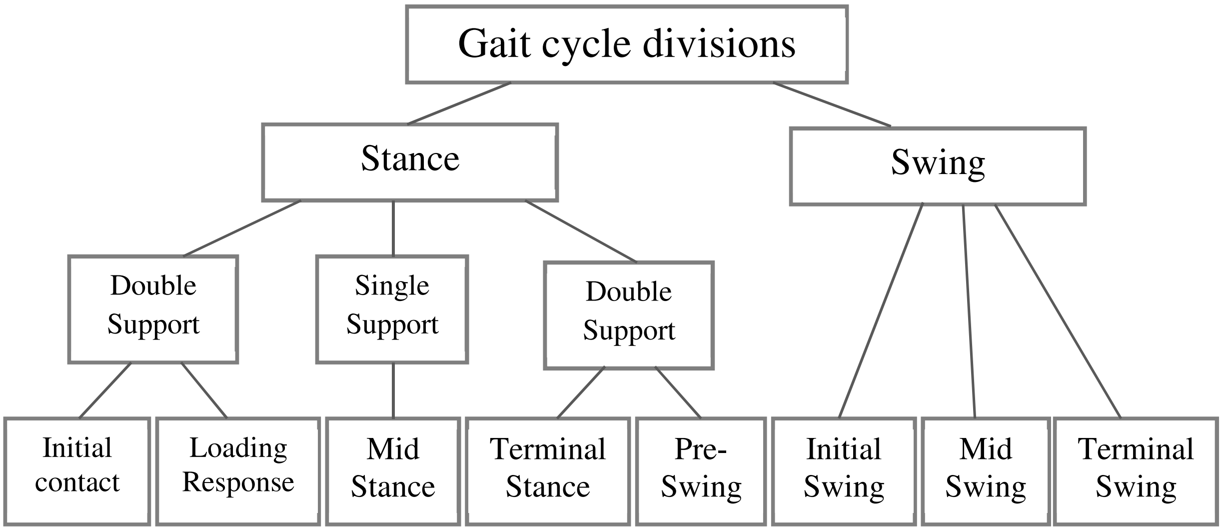

Fig.1.4.3 Functional division of the gait cycle (modified from Perry and Burnfield, 2010)

1- The *'initial contact'* (0-2% GC), where the whole body decelerates and that generally represents the 0% of the gait cycle
2- The *'loading response'* (2-12% GC)

These two phases constitute the first double support period.

3- The *'mid stance'* (12-31% GC)
4- The *'terminal stance'* (31-50% GC)
5- The *'pre-swing'* (50-62% GC)
6- The *'initial swing'* (62-75% GC)
7- The *'mid swing'* (75-87% GC)
8- The *'terminal swing'* (87-100% GC)

In the mid and terminal stance phases there's a progression of the body centre of mass (CoM) over the support foot. The pre-swing starts with the end of double support and ends with toe-off of the ipsilateral limb.

Phases 6-7-8 are swing subdivisions: in the initial swing the limb reaches the maximum knee flexion, and in the mid swing the leg of the flowing limb arrives at the vertical position. The terminal swing is comprised between the mid swing and the initial contact phases (Cuccurullo, 2004).

Human walking is affected by numerous factors, like:

- Speed of walking (Cavagna, 1976; 1988; Chung and Wang, 2010);
- Gradient of walking (Saibene and Minetti, 2003);
- Age (Mian et al., 2006; Van de Walle et al., 2010);
- Gender (Kerrigan et al., 1998; Cho et al., 2004);
- Anthropometric parameters (Schuch et al., 2011; Wearing et al., 2006);
- Different surfaces of the ground (Lejeune et al., 1998; Marigold et al., 2005; Menant et al., 2009; Parvataneni, 2009);
- Added weight (Browning et al., 2007; Huang and Kuo, 2013)
- Gravity (Cavagna, 2000; Saibene and Minetti, 2003).

1.5 MECHANICAL WORK OF LOCOMOTION

Mechanical work is a complex biomechanical variable. Total mechanical work is represented by the sum of external work (W_{ext}, about 60% of total work), that is "*the work done to raise and accelerate the body CoM within the environment*" and internal work (W_{int}), that is "*the work associated with the acceleration of body segments (mainly limbs) with respect to the CoM*" (Saibene and Minetti, 2003). From these definitions, it results clear that all depends from CoM calculation, so it is extremely important to define what is CoM.

CoM is an imaginary point where all the mass is concentrated (Winter, 1979), so it reflects movements of all the body or the segment at which it refers to.

As illustrated in figure 1.5.1, walking has been usually modelled as an inverted pendulum paradigm (Margaria, 1976), where continuous accelerations and decelerations occur and where velocity falls to zero at each step because of the heel impact to the ground: this is the reason why legged locomotion is not particularly efficient compared with other gait patterns (Saibene and Minetti, 2003).

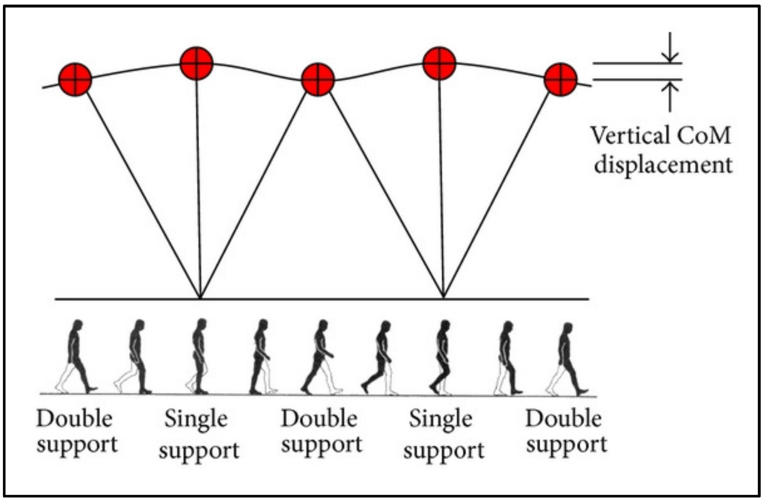

Fig.1.5.1 Inverted pendulum model of gait, showing how the CoM rises during the single support and falls during the double support (from Lobet et al., 2013)

In this paradigm, total mechanical energy is represented by the sum of potential (PE) and kinetic energy (KE) that continuously interchange at each step. In particular, at every heel strike the CoM is located behind the point of contact: the heel impact produces a forward deceleration, detectable in a decrease of the KE and in an increase of the PE (Cavagna, 1988): to reduce the negative effect of this deceleration, the CoM begins to raise up to reach its maximum height in the single support phase (Lobet et al., 2013). After that, a phase of forward acceleration begins, and the CoM reaches its lowest position; simultaneously KE increases at the expense of PE, that decreases.

Thus, at each step KE and PE are out of phase (Cavagna, 1988). This mechanism allows to reduce the variations of total mechanical energy (= mechanical external work) of the body CoM, because the resulting curve oscillates usually less than each one of the two components (fig. 1.5.2).

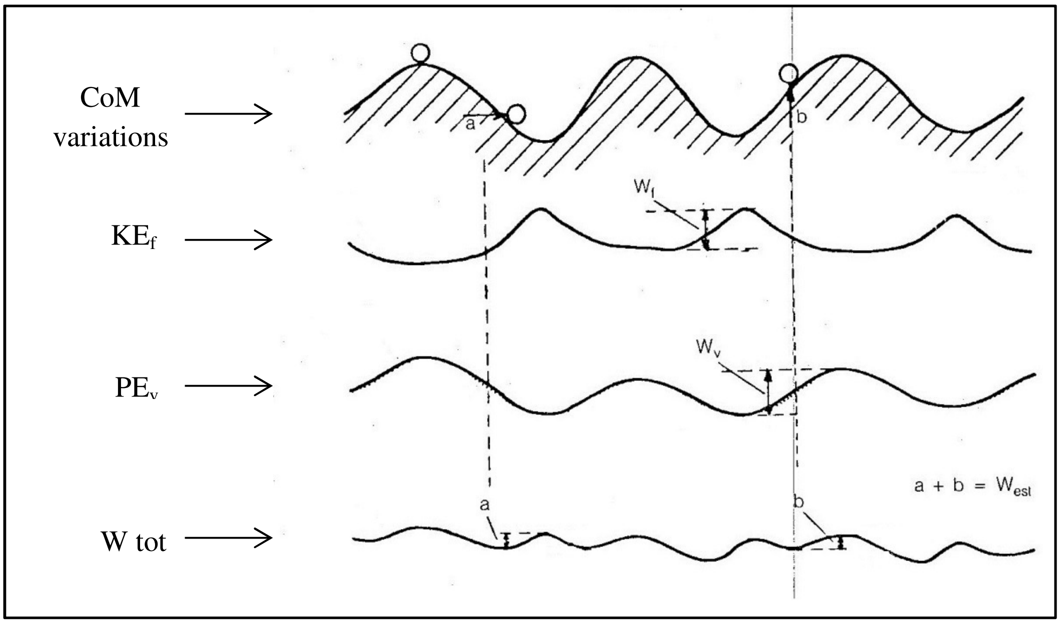

Fig.1.5.2 (modified from Cavagna, 1988). Variations of CoM mechanical energy during walking. The lower curve is the sum of the previous two (KE+PE). It runs clear that the sum of positive work a+b of the W tot curve is less than the sum of the forward work (W_f) in KE curve with the vertical work (W_v) in the PE curve.

Regarding external mechanical work, it has been shown that:

- W_{ext} (expressed for meter traveled) shows a curvilinear relationship with walking speed: as a consequence, there is one speed, that is the most economical one and that is near the preferred walking speed of the subject, where W_{ext} reaches its lowest value (Cavagna et al., 1963);
- as for walking speed, W_{ext} is minimized near to the freely chosen step frequency (Cavagna et al., 1997; Umberger et al., 2007);
- age does not affect W_{ext} (Mian et al., 2006; Ortega et al., 2007; Hernandez et al., 2009);
- W_{ext}, expressed in absolute values ($J \cdot step^{-1}$ or $J \cdot m^{-1}$) is affected by body mass, with higher values with higher body masses. If normalized per body mass ($J \cdot kg^{-1} \cdot m^{-1}$ or $J \cdot kg^{-1} \cdot m^{-1}$) no differences were detected (Browning et al., 2009; Malatesta et al., 2009);
- W_{ext} is one of the primary determinants of the energy cost of walking (Donelan et al., 2002);
- it seems that at same speeds of walking, moderately obese adults do not alter their gait in order to decrease the external mechanical work of the CoM (Browning et al., 2009);
- An increase of 50% in step width can contribute to increase W_{ext} (Donelan et al., 2001).

As previously explained, W_{int} reflects the acceleration of body segments whose movements are not directly connected with a variation in CoM location (Minetti, 1998).

To obtain W_{int} it is necessary at first to sum the kinetic energy curves of each single segments allowing energy transfer only between segments of the same limbs, but not among different limbs or between the limbs and CoM (Willems et al., 1995). Then the final value was achieved by summing all the energy increases in the resulting curves (Minetti, 1998).

Generally, mechanical work assessment has some disadvantages, because it does not take into account several factors, like co-contractions of antagonistic muscles, different muscle fiber types, elastic storage of energy in muscles and ligaments, isometric contractions (Burdett et al., 1983). For this reason, the measure of mechanical work is always lower than the measure of metabolic work.

Regarding internal mechanical work, it has been established that:

- W_{int} increases with higher step frequencies (Cavagna et al., 1986; Schepens et al., 2001);
- W_{int} increases nearly as the square of walking speed (Cavagna et al., 1977);
- W_{int} increases with age (Mian et al., 2006).

1.6 METABOLIC WORK OF LOCOMOTION

Metabolic cost of locomotion has been extensively studied from researchers (Margaria, 1976; di Prampero 1986). Gross EC reflects the energy consumed *per* unit of covered distance ($J \cdot kg^{-1} \cdot m^{-1}$), and shows a U-shaped relationship when plotted versus walking speed (di Prampero, 1986; Cavagna, 1988; Browning and Kram, 2005). Hence, similarly to W_{ext}, there's a speed close to PWS where energy cost shows its minimum value (Margaria, 1976). This means that when walking, people automatically move their body saving energy, minimizing the amount of metabolic work to go from a place to another. For normal weight adults, the minimum value of Gross EC occurs at about 1.4 m/s (Margaria, 1976; Cavagna, 1988), and corresponds to measured preferred walking speed (Inman et al., 1981). Regarding obese adults, Browning and Kram (2005) demonstrated that the minimum Gross EC (that occurs at 1.33 m/sec) is not affected by moderate obesity, but they speculated that in individuals with greater levels of adiposity this could be not true, because of an observed shift of a U-shaped curve slightly toward slower velocities.

At the same walking speeds, obese adults displayed higher absolute Gross EC than normal weight adults, but, if normalized per body mass, differences were reduced or eliminated, which suggests that the primary determinant of the cost of walking is represented by total body weight (Browning and Kram, 2005).

The net energy cost of walking (Net EC, calculated as gross steady-state $V'O_2$-standing $V'O_2$) reflects gait economy, and is affected by numerous factors, like speed, stride length and rate (Martin and Morgan, 1992), step width and leg swing circumduction (Spyropulos et al., 1991), muscle fiber type (Hunter et al. 2001) and the ability to store and reuse elastic energy from eccentric muscle contractions (Cavanagh and Kram, 1985).

Also obesity influences Net EC of walking: at the same speed, obese persons displayed greater Net EC compared to non-obese adults (Foster et al., 1995; Browning and Kram, 2005; Browning et al., 2006), due in part to their smaller mass-specific standing metabolic rate (SMR). This suggests that economy is reduced by obesity in level walking. In addition, obese women have higher values of Net EC than obese men (+10%), even if this difference is not explained by different body fat distribution (e.g. heavier legs) that generally occurs between genders (Browning et al., 2006).

For years many researchers has drawn their attention on a very interesting question: *'can physical activity (PA) or physical fitness (PF) influence walking economy?'*

For what concern PF, all health-related qualities can affect the economy of walking.

Browning et al. (2006) showed that Net EC was positively associated with percentage body fat (= less economical people were those with higher %FM).

Gleim et al. (1990) found that less flexible healthy adults were more economical while walking or running on a treadmill, probably for the greater elastic energy contributions that diminished unproductive movements. Hunter and colleagues (2008) confirm that walking economy (less aerobic demand) was independently and positively related with lower flexibility in conjunction with increased strength in a group of post-menopausal women following weight loss.

Hunter et al. (2005) reported an inverse relationship between $V'O_{2max}$ and walking economy in sedentary women. This was strengthened also by Sawyer et al. (2010), that reported that in a range of walking speeds from 2.0 to 4.0 mph (3.2-6.4 $km \cdot h^{-1}$), higher $V'O_{2peak}$ led to greater increases in $V'O_2$ between velocities in a group of healthy active adults.

Basing on these findings, some people can conclude that having higher values of cardiorespiratory fitness is disadvantageous for walking economy: this is not completely true because they compare EC at the same absolute walking speed, where physiological intensities were not the same. In fact, for exercise physiologists it is crucial to record EC and to make comparisons at the same relative intensities, where levels of metabolic demands are the same.

The approach used by Pintar and colleagues (2006) was in line with this way of thinking: they demonstrated that different levels of cardiorespiratory fitness can influence the relative intensity of PWS: it means that at same workloads (represented by speed of walking), people with lower maximal oxygen uptake ($V'O_{2max}$) exhibited higher relative intensities (worked at a higher percentage of $V'O_{2max}$) with respect to fitter counterparts.

PA effects on walking economy are less studied: to our knowledge, only Martin et al. (1992) investigated the impact of PA, subjectively assessed, on aerobic demand in young and older adults at 7 speeds, but results showed no effects of PA.

1.7 GAIT EFFICIENCY

If economy of walking has been largely investigated, the same does not apply to gait efficiency.
First of all, it's useful to distinguish the concept of efficiency from those of economy because there is often confusion over their meaning and usage.
As previously explained, gait economy refers to the metabolic energy expended to walk.
Instead, gait efficiency is the ratio of total mechanical work done to metabolic energy consumed during walking. Efficiency is often expressed as a percentage, following the subsequent formula:

$$Eff = (\text{Total mechanical work}/ \text{Net metabolic energy expended}) \cdot 100 \quad \text{(Eq.1)}$$

Therefore, economy is the denominator of efficiency.
Diminishing energy cost or increasing in mechanical work will augment efficiency. Cavagna and Kaneko (1977) showed that maximum values of gait efficiency occurred from 5 to 7 $km \cdot h^{-1}$, ranging between 35% and 40%.
Efficiency is affected by a great number of factors. The main are represented by structural factors (body mass distribution, point of muscle insertion from joint centers, muscle fiber orientation and length), that Cavanagh and Kram described them in a detailed paper of 1985.
Regarding this topic, a question is still opened: *'are active or fitter people more efficient than sedentary individuals in performing common physical activities, such as walking?'*
Literature is not exhaustive: efficiency was studied in clinical populations (Waters and Mulroy, 1999), in older adults (Mian et al., 2006), in common task performed in daily living (Kaneko, 1990) and in athletes during running (Cavagna and Kaneko, 1977).
Studying efficiency in terrestrial locomotion may gain insights into PA and PF effects on the biomechanical elements associated with the net metabolic cost of walking.

1.8 AIMS OF THE STUDY

As previously explained, many researchers have drawn their attention to understand if physical fitness can influence walking economy (see Par. 1.6). However, nobody has explored if also different levels of objectively measured physical activity might influence the Net EC of walking.
Very few studies calculated mechanical efficiency of locomotion, as very few authors assessed the total mechanical work done by muscles, which is required for the determination of efficiency.
Many researchers attempted to explain increases in metabolic costs of walking in children, older adults or pathological populations, using measures of mechanical work to understand if some changes occur in the pattern and the neuromuscular control of locomotion (Frost et al., 2002; Detrembleur et al., 2003).
If so, a possible explanation could be due to a different mechanics of the task, reflected in abnormal trajectories of the CoM and the limbs, which may return in an increase of mechanical work.
As previously clarified, efficiency of work production relates W_{tot} and Net EC, and needs simultaneous measurement of metabolic cost and total mechanical work (Par. 1.8).
It is well known that different levels of PF can result in a different walking economy, but it is not known if mechanics of walking can be affected by PA and/or PF, and if it can result in a better efficiency.

The purposes of this thesis are fourfold:

1- To study the influence of PA and PF on Net EC of treadmill walking in a group of healthy adults, separately for men and women;
2- The same aim on the same population was proposed for gait efficiency.

Results of 1 and 2 are presented in the 1st study (Par. 3.1).

3- To verify if PA and PF can affect Net EC of treadmill walking in a group of obese adults;
4- To assess if efficiency is affected by different levels of PA and PF.

Results of 3 and 4 are presented in the 2nd study (Par. 3.2).

2. MATERIALS AND METHODS

2.1 RECRUITMENT

The main objective of the recruitment was to include 40 normal-weight and 40 obese subjects, in particular 20 males and 20 females for each group. We submitted every person to a meticulous pre-participation screening, because all of them had to perform a maximal V'O_2max test.
For this reason, we had met many obstacles in the recruitment phase, especially for obese people. Furthermore, study design was too demanding for many of them, both in terms of time (they had to return for 3 different days in 2 different laboratories quite far one from another), and also because they were too scared to perform a V'O_2max test until exhaustion.

2.1.1 PRE-PARTICIPATION SCREENING

In the recruitment phase, we examined every risk factor that could affect the safety of the V'O_2max test. The **first step** was to administer a flow chart to exclude people at high risk. In particular, the objective was to had some detailed information about the individual's health history and current health (see Attachment 1), to investigate if there were some typical signs or symptoms suggestive of cardiovascular, metabolic or pulmonary disease. We also considered 8 possible risk factors for cardiovascular disease, that allowed us to classify the participant at low or at moderate risk (ACSM, 2009). Every positive response to items listed below were counted as a risk:

Age

Men ≥45 year; Women ≥55 year.

Family History

Myocardial infarction (heart attack), coronary revascularization (heart bypass or angioplasty) OR sudden death (attributed to a stroke or cardiovascular disease) before 55 years of age in men considered immediate family (father, brother, son) OR before 65 in women considered immediate family (mother, sister, daughter).

Smoking

Current smoking OR those who quit within the previous six months.

Hypertension

Systolic blood pressure >140mm OR diastolic blood pressure >90mm, confirmed by measurements on at least two separate occasions OR on antihypertensive medications.

Dyslipidemia

LDL >130 mg/dL, HDL <40 mg/dL OR on lipid-lowering medication
If serum cholesterol is all that is available, use serum cholesterol >200mg/dL.

Impaired Fasting Glucose

Fasting blood sugar >100 mg/dl, confirmed on at least two separate occasions.

Obesity

BMI >30 kg/m^2 OR

Waist girth >102 cm for men and >88 cm for women OR

Waist/hip ratio >0.95 for men and >0.86 for women.

Physical Inactivity

Persons not participating in a regular exercise program or not meeting the minimal recommendations of the 1996 Surgeon General's Report (at least 30 min/day of moderate physical activity).
People with ≤1 risk factor was at low risk for an acute cardiovascular event, even in a maximal performance. On the other hand, people with ≥2 risk factors were at moderate risk: this means that they could be included, but a medical examination before the V'O_2max test (see par.2.4.5) was necessary.

The **second step** was to be sure that they met the following inclusion criteria:

- BMI between 18.5 and 24.99 kg/m^2 for normal-weight condition, and BMI>30 kg/m^2 for obesity;
- age from 18 to 40 years: the upper limit was set at 40 years because we would be sure to exclude any kind of metabolic changes due to age (pre-menopausal state);
- low or moderate cardiovascular risk;
- no neurological or musculoskeletal pathologies affecting gait (Danion et al., 2003; Whittington et al., 2009);
- stable weight (<2.5 kg change) over the previous 6 months (Browning and Kram, 2005);

- no medications known to influence metabolism or heart rate (see Attachment 1);
- no diabetes;
- not to be an athlete playing in a sport federation;
- negative PAR-Q (physical activity readiness questionnaire, see Attachment 2).

If every condition was satisfied, the subject could be recruited.
We had also included a **third step,** where every subject had to pass the medical examination described in par. 2.4.5 before the execution of the $V'O_{2max}$ test.
If the third step wasn't satisfied, the subject didn't perform the test, or, if it was possible, he/she performed a submaximal $V'O_{2max}$ test.

2.2 STUDY DESIGN

All the measures were made in 3 different days on two different laboratories.

DAY 1, Applied Exercise Physiology Laboratory, Università Cattolica del S.Cuore, Milan:

- Health-related quality of life questionnaire;
- Physical activity questionnaire;
- Anthropometric measurements;
- Body composition assessment;
- Resting metabolic rate;
- Maximal isometric strength test;
- Flexibility test;
- Preferred walking speed assessment;
- Physical activity level evaluation over 7 consecutive days of normal life (the subject received the instrument and was dismissed from the laboratory).

DAY 2, L.A.F.A.L. Laboratory of Functional Anatomy of the Locomotor System, Università degli Studi di Milano:

- Kinematic and metabolic analysis of treadmill walking at three different speeds.

DAY 3, Applied Exercise Physiology Laboratory, Università Cattolica del S.Cuore, Milan:

- Medical examination;
- $V'O_{2max}$ test.

To correctly assess the physical activity level, a minimum of 7-day period passed between day 1 and day 2. To avoid any source of error for metabolic measurements, every subject had to respect the following indications for all the 3 days of testing:
- food, drink, medications and smoke, restriction for at least 3 hours before the test;
- avoid any kind of vigorous activity for at least 48 hours before the test;
- if possible, a minimum of 8 hours sleeping.

2.3 SUBJECTS

Study 1:

42 normal-weight (23 males and 19 females) participated in the study. Of the 42 recruited and tested individuals, we discarded 12 subjects for incomplete or invalid data. A total of 30 subjects (14 females and 16 males) were included in the study.

The mean characteristics are presented in table 2.3.1.

Table 2.3.1 Descriptive characteristics of the normal-weight population (Mean±SE)

	Overall (n=30)	**Males (n=16)**	**Females (n=14)**
Age (y)	26.5±0.8	27.1±1.2	25.9±1.1
Weight (kg)	65.7±1.9	73.1±1.8**	57.2±1.8**
Height (m)	1.72±0.02	1.80±0.02**	1.63±0.02**
BMI ($kg\cdot m^{-2}$)	22.1±0.4	22.7±0.5	21.5±0.5
PWS ($km\cdot h^{-1}$)	5.6±0.1	5.6±0.2	5.5±0.2
IPAQ ($METs\cdot min\cdot wk^{-1}$)	1662±239	1966±330	1282±335
SMR ($mL\cdot kg^{-1}\cdot min^{-1}$)	4.14±0.13	4.23±0.21	4.04±0.12
RMR ($MJ\cdot day^{-1}$)	7.44±0.21	8.30±0.19***	6.46±0.17***
Risk Factors (n)	0.7±0.1	0.7±0.2	0.8±0.2

BMI: body mass index; PWS: preferred walking speed; IPAQ: international physical activity questionnaire; SMR: standing metabolic rate; RMR: resting metabolic rate.

**significant differences with p<0.001, ***p<0.0001 (one-way ANOVA) between males and females.

Generally, men were taller and heavier than women (p<0.001, Tab. 3.1.1), whereas females had a lower resting metabolic rate than their counterparts (p<0.0001). No statistically significant effect were found for other variables.

Study 2:

14 obese subjects (7 males and 7 females) were included and tested in the study.
The overall main characteristics were presented in Table 2.3.2.

Table 2.3.2: Descriptive statistics of the obese group (mean± SE).

	Overall (n=14)
Age (y)	27.9±1.6
Weight (kg)	110.3±3.9
Height (m)	1.68±0.02
BMI (kg·m^{-2})	39.3±1.5
Risk factors (n)	2.6±0.3
PWS (km·h^{-1})	5.4±0.2
IPAQ (METs·min·wk^{-1})	2611±874
SMR (mL·kg^{-1}·min^{-1})	3.81±0.19
RMR (MJ·day^{-1})	8.7±0.3

BMI: body mass index; PWS: preferred walking speed; IPAQ: international physical activity questionnaire; SMR: standing metabolic rate; RMR: resting metabolic rate.

Before the participation, every subject was informed about every procedure and gave his/her full consent (see Attachment 3).

2.4 MEASUREMENTS

2.4.1 QUESTIONNAIRES

In addition to the PAR-Q, another questionnaire was administered.

2.4.1.1 IPAQ SHORT FORM

We used the Italian translation of the short last 7-day recall of the International Physical Activity Questionnaire (IPAQ) to obtain an estimate of subject's physical activity: this was essential for our study, because we needed people with various levels of physical activity, from sedentary to very active condition. This self-administered survey, composed by 7 questions, was validated against obese people (Tehard et al., 1995) and assessed the frequency and the duration of the participation in vigorous, moderate, walking and sedentary activities, related to the past 7 days. To score the questionnaire, the minutes per week of every activity were multiplied by a specific MET factor (8 for vigorous, 4 for moderate, 3.3 for walking) that suggested the intensity of the exercise. One MET is the equivalent of the resting metabolic rate and is conventionally set at 3.5 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$ (Ainsworth et al., 1993): it means that a 2 MET activity would require twice the energy that an average person consumes at rest.

The sum of all the three activity scores gave an indicator of total physical activity.

In particular, we considered:

- subjects with low activity who didn't reach 600 $MET \cdot min \cdot wk^{-1}$;
- subjects with moderate activity who performed a minimum of 3 day of vigorous activity of at least 20 min/day OR a minimum of 5 d accumulating a total ≥ 600 $MET \cdot min \cdot wk^{-1}$;
- subjects with high activity who performed at least 1500 $MET \cdot min \cdot wk^{-1}$.

A sample of the questionnaire is available in the Attachment 4.

2.4.2 ANTHROPOMETRIC MEASUREMENTS

2.4.2.1 WEIGTH, HEIGHT AND BODY MASS INDEX

Body mass (kg) and height (m) of each subject were recorded with a mechanical column scale with stadiometer (SECA 201, Deutschland). All the measures were made without shoes and with underwear.

Dividing the weight (in kg) by the squared height (in m^2) we obtained the body mass index (BMI), that allowed us to classify individuals as underweight, normal weight, overweight or obese.

The recommended classifications for BMI (NHLBI, 1998) are shown in Table 2.4.2.1.1.

Table 2.4.2.1.1 Cut off points for BMI classification and associated disease risk. Adapted from NHLBI, 1998.

CLASSIFICATIONS FOR BMI	BMI	DISEASE RISK (for type 2 diabetes, hypertension and CVD)	
		Men ≤102 cm Women ≤88 cm	Men ≥102 cm Women ≥88 cm
Underweight	<18.5 $kg \cdot m^{-2}$	-	-
Normal weight	18.5-24.9 $kg \cdot m^{-2}$	-	-
Overweight	25-29.9 $kg \cdot m^{-2}$	Increased	High
Obesity (Class I)	30-34.9 $kg \cdot m^{-2}$	High	Very High
Obesity (Class II)	35-39.9 $kg \cdot m^{-2}$	Very High	Very High
Obesity (Class III)	>40 $kg \cdot m^{-2}$	Extremely High	Extremely High

2.4.2.2.WAIST CIRCUMFERENCE

Waist circumference is strongly related to the excess abdominal fat, and it is an important, independent risk factor for disease (ACSM, 2009). For this reason, it was measured in both groups. In fact, waist circumferences ≥88 cm for women and ≥102 cm for men, could be a marker for increased risk, even in normal weight people. It was measured at the midpoint between the lowest lateral portion of the rib cage and iliac crest, and anteriorly, midway between the xyphoid process of the sternum and the umbilicus with an ergonomic circumference measuring tape (SECA 201, Deutschland) at the end of a normal expiration paying attention to not compress the skin.

The relationship between BMI and waist circumference for defining risk is shown in table 2.4.2.2.1.

Table 2.4.2.2 .1 From Brian GA, 2004

WAIST CIRCUMFERENCE (cm)		
RISK CATEGORY	WOMEN	MEN
Very Low	<70	<80
Low	70-89	80-99
High	90-109	100-120
Very High	>110	>120

2.4.3 BODY COMPOSITION

2.4.3.1 SKINFOLD THICKNESS

This indirect technique is based on the assumption that 50% of total body fat is located in the subcutaneous region, and it is proportional of the total amount of body fat (ACSM, 2009). A Harpenden skinfold caliper (Baty, UK), a precision instrument with a standard measuring pressure of 10 g/mm^2 and an accuracy of 99%, was used. The method requires the measurement of a set of subcutaneous skinfolds, whose values are used in a pre-calibrated regression equation calculated using some gold standard for body composition. Skinfold location depends on the regression equation; we decided to use a three-site formula, where skinfold sites were different according to sex (Tables 2.4.3.1.1 and 2.4.3.1.2).

Table 2.4.3.1.1 Skinfold three-sites for men. Adapted from ACSM, 2009

SKINFOLD SITE	DESCRIPTION
Chest	Diagonal fold; one half the distance between the anterior axillary line and the nipple.
Abdominal	Vertical fold; 2 cm to the right side of the umbilicus.
Thigh	Vertical fold; on the anterior midline of the thigh, midway between the proximal border of the patella and the inguinal crease (hip).

Table 2.4.3.1.2 Skinfold three-sites for women. Adapted from ACSM, 2009

SKINFOLD SITE	DESCRIPTION
Triceps	Vertical fold; on the posterior midline of the upper arm, halfway between the acromion and olecranon processes, with the arm held freely to the side of the body.
Suprailiac	Diagonal fold; in line with the natural angle of the iliac crest taken in the anterior axillary line immediately superior to the iliac crest.
Thigh	Vertical fold; on the anterior midline of the thigh, mid-way between the proximal border of the patella and the inguinal crease (hip).

To take skinfolds, we used the followed procedures (adapted from Heyward, 2010):

- All measurements were made on the right side of the body with the subject standing upright.
- The caliper was placed 1 cm away from the thumb and finger, perpendicular to the skinfold, and half way between the crest and the base of the fold.
- A pinch was maintained while reading the caliper.
- 1 to 2 seconds have been passed before reading caliper.
- Duplicate measures at each site were taken, if values were not within 1 to 2 mm we took another measurement. After that, the mean of the two nearest values was calculated.
- To regain normal texture and thickness, measurement were taken not in consecutive order.

Body density (BD) was estimated via three site skinfold methodology according to the following equations:

For men (Jackson & Pollock, 1978):

$$BD = 1.10938 - 0.0008267 \cdot (\text{sum of three skinfolds}) + 0.0000016 \cdot (\text{sum of three skinfolds})^2 - 0.0002574 \cdot (\text{age}).$$

(Eq.2)

For women (Jackson et al., 1980):

$$BD = 1.099421 - 0.0009929 \cdot (\text{sum of three skinfolds}) + 0.0000023\ (\text{sum of three skinfolds})^2 - 0.0001392 \cdot (\text{age}).$$

(Eq.3)

To predict the % body fat (%FM), we used the following equation (Brozek et al., 1963):

$$\%\ FM = (457/BD) - 414.2$$

(Eq.4)

We used this method only for the normal weight group, because obese subjects results may be less accurate and specific than when using other techniques (Kuczmarski et al., 1987). Source of error is ±3.5% (Heyward and Stolarczyk, 1996).

2.4.3.2 GIRTH MEASUREMENTS

We used girth measurements method to predict body composition in the obese group, because we found population-specific equations with prediction errors < 4% body fat (Weltman et al., 1988). A cloth tape measure (SECA 201, Deutschland), placed on the skin surface without compressing the subcutaneous adipose tissue, was used for the following girth circumferences:

- waist (see par. 2.4.2.2);
- abdomen: laterally, at the level of the iliac crests, and anteriorly, at the umbilicus.

Every circumference was assessed twice, in a rotational order. If the difference between the two measurements exceeded 5 mm, another measure was done, and the mean of the two nearest values was calculated.

To assess % body fat, the following equations were used:

For women (Weltman et al., 1988):

% FM = 0.11077 · (mean value between waist and abdomen in cm) - 0.17666 · (height in cm) + 0.l4354 · (weight in kg) + 51.03301.

(Eq.5)

For men (Weltman et al., 1987)

% FM = 0.31457 · (mean value between waist and abdomen in cm) - 0.10969 · (weight in kg) + 10.834.

(Eq.6)

2.4.4 RESTING METABOLIC RATE

Resting metabolic rate (RMR) is defined as the amount of daily energy expenditure at rest, in the post-absorptive state in a neutrally temperate environment, and represents 60-70% of total energy expenditure of general population (Bouchard et al., 2007).

To accurately assess RMR, participants had to follow some restrictions that might cause some estimation errors (Compher et al., 2006):

- a minimum of 6-h of restriction of food, alcohol and caffeine;
- a minimum of 2-h of restriction of nicotine;
- a minimum of 48-h of restriction of physical exercise;
- the individual should not move arms or legs during the test;
- medications taken should be noted, such as stimulants or depressants.

Furthermore, it was important to maintain a quiet surroundings when the test was in progress.

Before the measure, subjects rested awake for 15 min in a supine position on a bed in a 22-24°C conditioning room. After the rest period, RMR was measured using a previously calibrated metabolic system (Fitmate, Cosmed, Italy) that assessed oxygen consumption ($V'O_2$) in real-time sampling data every 15 sec. During the test the individual was interfaced with a metabolic measurement system by means of a facemask. The test lasted 15 min: we discarded the first 5 minutes and took the mean value of the last 10 minutes, paying attention that coefficient of variation of $V'O_2$ was ≤10%, as suggested in published recommendations (Compher et al., 2006).

The Fitmate, validated with the gold-standard Douglas bag method, calculated RMR on the basis of a Weir equation using a fixed respiratory exchange ratio (RER) of 0.85 (Nieman et al., 2006):

$$\mathbf{RMR\ (Kcal/day) = 5.675 \cdot V'O_2\ (in\ ml/min) + 1.593 \cdot V'CO_2 (in\ ml/min) - 21.7}$$

(Eq.7)

$V'CO_2$ represents the volume of expired carbon dioxide, and was estimated from RER as follows:

$$\mathbf{V'CO_2 = V'O_2 \cdot RER}$$

(Eq.8)

2.4.5 $V'O_{2max}$

$V'O_{2max}$ was evaluated with the gold standard method: a maximal treadmill test (RunRace, Technogym, Italy) with breath-by-breath indirect calorimetry (Quark CPET, Cosmed, Italy).
The Quark CPET measures the main respiratory parameters like ventilation, oxygen consumption ($V'O_2$), carbon dioxide production ($V'CO_2$), averaged expiratory concentration of O_2 (FeO_2) e CO_2 (FeO_2).
This system consisted of a main unit (Fig.2.4.5.1) that contained all the analyzers and the electronics, at which the flowmeter was attached.

Fig. 2.4.5.1 The Quark CPET system

A flowmeter, consisting of a bidirectional turbine of 28 mm Ø, an optoelectronic reader and a sampling tube, were connected to the front panel of the main unit. The main unit was directly connected to a PC that, with a dedicated software, showed in real time all the desired parameters (in our case, VE, $V'O_2$, $V'CO_2$, HR, RER).
Before every measurement, the Quark CPET had to warm-up for at least 5 minutes. Afterward, the system needed the calibration of:

- the turbine, set with a 3-litre syringe to ensure accurate volume measurements;
- the O_2 and CO_2 analyzers, with a reference gas of known composition (15.93% O_2 and 4.92% CO_2);
- the room air calibration, performed automatically before each test, to be sure that CO_2 and O_2 registered coincided with theoretical atmospheric values.

A silicon face mask (Hans Rudolph Inc., USA) was used to collect the expired air. Through the flowmeter, directly attached on the mask, the expired air was sent to gas analyzers.

Before the test, every participant had to pass a medical examination where a medical history, an ECG and a blood pressure measure at rest were assessed. To allow a safe and effective test execution, obese subjects were ECG-monitored also during the V'O_2max test.

After a familiarization with the ergometer, subjects performed a warm up of 12-min as shown below:

- 4 min at 3.5 km·h^{-1};
- 4 min at 4.5 km·h^{-1};
- 4 min at 5.5 km·h^{-1}.

Every stage lasted 4 minutes because we wanted to reach the steady-state condition: this allowed us to build the HR/V'O_2 individual relationship, useful for the activity monitor calibration (see par. 2.4.8). Furthermore, if some people failed to reach almost three of the five criteria for a maximal test determination, we mathematically extrapolated V'O_2max to the subject's age-predicted maximal HR (HR_{max}). HR_{max} was estimated with Tanaka's formula (Tanaka et al., 2001):

$$\mathbf{HR_{max} = 208\text{-}(0.7 \cdot age)} \quad \text{(Eq.9)}$$

After the warm-up, there was a minute of rest: then, the maximal test began.

Three different protocols were used:

- Balke treadmill protocol for normal-weight sedentary or low active people.
- Modified Balke treadmill protocol for obese people.
- Bruce treadmill protocol for normal-weight active people.

The Bruce treadmill test employed large increments between stages. This protocol was better suited for physically active individuals, because it offered an adequate stimulus to reach the volitional fatigue in 10-15 min. We administered this protocol to individuals that reported more than 1500 METs · min · $sett^{-1}$ from IPAQ questionnaire.

On the other hand, deconditioned individuals and patients with chronic diseases (like obesity) needed protocols with smaller increments, because they were less able to adjust their physiological responses to big workloads.

Balke protocol (Fig. 2.5.4.2) maintained a fixed speed (5.3 km·h^{-1} for normal-weight and 4.5 km·h^{-1} for obese), and the treadmill slope increased every minute as shown in tab. 2.4.5.1.

Table 2.4.5.1: Balke protocol for normal weight individuals

Step	Speed (km·h^{-1})	Slope (°)
1 (0'-1')	5.3	0
2 (1'-2')	5.3	2
3 (2'-3')	5.3	3
4 (3'-4')	5.3	4
↓	↓	↓

In the Bruce protocol, speed and slope increased every 3 min, as shown in tab. 2.4.5.2

Table 2.4.5.2: Bruce protocol

Step	Speed (km·h^{-1})	Slope (°)
1 (0'-3')	2.7	10
2 (3'-6')	4	12
3 (6'-9')	5.5	14
4 (9'-12')	6.8	16
5 (12'-15')	8.1	18
6 (15'-18')	8.9	20
↓	↓	↓

The test finished when subjects reached the maximal exhaustion. After 3-5-7-9 min, a blood sample was taken from the ear lobe to assess the lactate peak with the Biosen C-Line analyzer (EKF, Germany).

Almost three of subsequent criteria were used to confirm that a maximal effort has been elicited during graded exercise testing (ACSM, 2009):

- achievement of ±5 of age-predicted HR_{max};
- a plateau in oxygen uptake (or failure to increase oxygen uptake by 150 mL · min^{-1}) between the final two workloads;
- a respiratory exchange ratio ≥ 1.1;
- post exercise blood lactate concentrations ≥ 8 mmol · L^{-1};
- a rating of perceived exertion ≥ 9 on the 0 – 10 Borg scale.

To calculate $V'O_{2max}$, we averaged all data every 30 sec, and then we took the mean value of the last minute of the test.

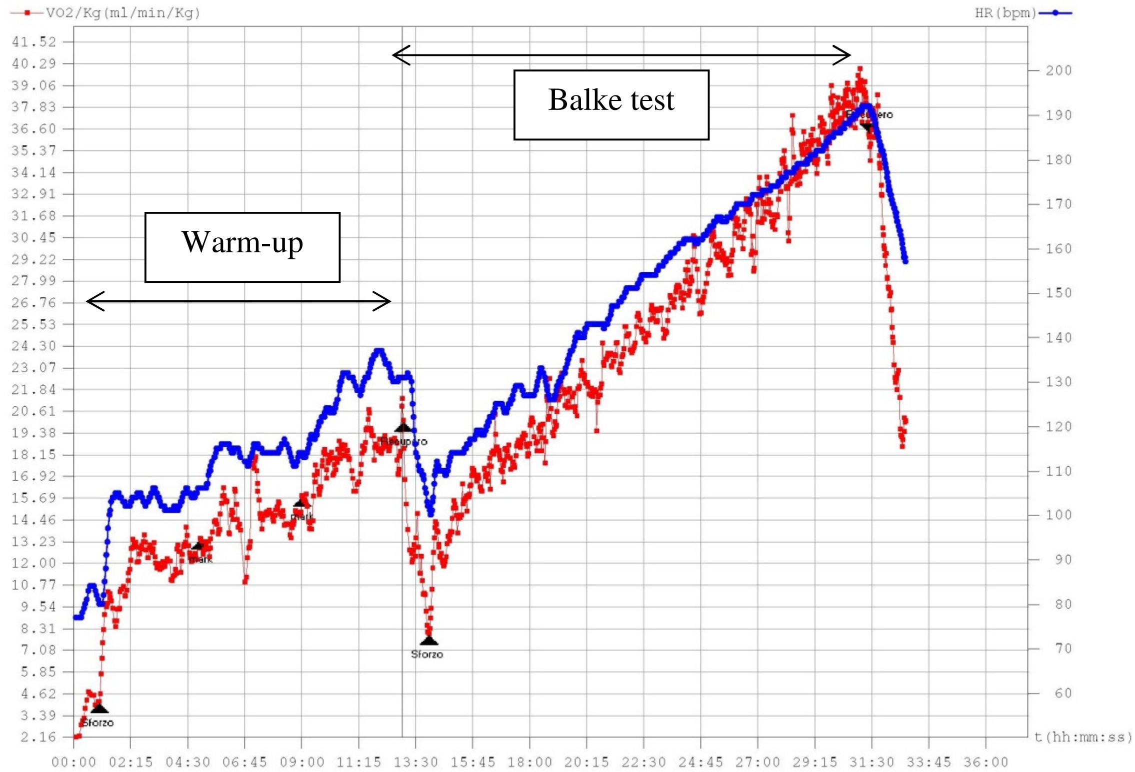

Fig. 2.4.5.2. Graphical representation of HR and $V'O_2$ curve of a Balke test.

2.4.6 MAXIMAL STRENGTH

To investigate the muscular strength, we used an isometric maximum voluntary contraction (iMVC) test.

The protocol began with a 5-minute warm-up on a treadmill walking at 4.5 km·h^{-1}.

After the warm-up, subjects sat on a chained horizontal leg press (Technogym, Italy), where two force plates of 240 x 400 mm (Twin plates, Globus, Italy) with a sampling frequency of 1000 Hz were fixed on the foot platform (Fig.2.4.6.1)

Fig.2.4.6.1 The leg press with force plates

The leg press seat was adjusted to obtain a knee angle of 90° and a hip angle of 45°. After the calibration of force plates, subjects placed their feet on the force plates, and were asked to exert maximal force as fast and as hard as possible after a verbal cue. Three trials, each lasting 5 s, were performed with a 3-min of rest between trials. A specific software (Tesys I-metrics, Globus, Italy) automatically built the force/time curve for both lower limbs (Fig. 2.4.6.2).

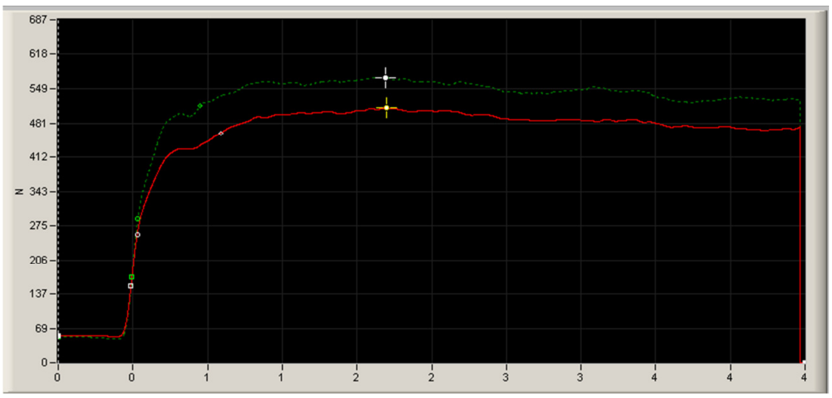

Fig. 2.4.6.2 A force/time curve representation

Maximal strength was defined as the greatest force (in Newton) attained during the 5-s isometric trial of the right lower limb added to the left one. To allow meaningful comparisons between individuals, we normalized the results for kg of body mass (N/kg).

2.4.7 FLEXIBILITY

Hamstrings and lower back flexibility was assessed with a YMCA adult trunk flexion sit and reach test, also called "V-sit&reach" (Cantell et al., 2008).

Subject seated without shoes on a mat, with a tape placed between legs. Heels were 30.5 cm apart and touched a taped line positioned on 38.1 cm, as shown in Fig 2.4.7.1.

Fig.2.4.7.1 Starting position for the V-Sit&reach test

Subjects bended forward slowly with both hands as far as possible while taking an expiration, holding the position for 2 sec. Legs had to be straight, the angle at the ankle was 90°, and fingertips were overlapped and in contact with the measuring tape.

Three trials were performed, and the best one (in cm) was recorded (Morrow et al., 2005).

2.4.8 PHYSICAL ACTIVITY LEVEL

2.4.8.1 ACTIHEART OVERVIEW

To provide an accurate estimation of physical activity level during free-living activities, we used the Actiheart (AH) activity monitor (CamNtech, UK).

This small (diameter 33 mm, wire length 100 mm) and light device (weight 8 g) comprised two sensors connected by a short cable which simply clip onto two standard ECG electrodes (Fig. 2.4.8.1.1).

Fig. 2.4.8.1.1 The Actiheart activity monitor

The instrument is minimally invasive and it is able to combine HR and movement monitor signals. This has some advantages because one can supply to the limitations of one method to another. Indeed, both HR and movement sensors have limitations in the estimation of PA. In particular, accelerometers (ACC), typically worn on the hip, are unable to assess all physical activities, such as upper body movements, walking at different slopes or other activities (such as cycling) where the body centre of mass (CoM) is static. The main result, supported by numerous studies (Valanou et al., 2006), is an underestimation of PA in free-living context. By contrast, even if an individual relationship was made, HR can overestimate PA, because HR is influenced by many factors under resting conditions (stress and anxiety, medications use, smoke, caffeine, environmental factors).

The piezoelectric uniaxial accelerometer, placed in the larger round clip, works with a frequency range of 1–7 Hz and a sampling rate of 32 Hz, and measures acceleration along the body longitudinal axis. The ECG signal is sampled at 128 Hz and has a sensitivity of 0.250 mV.

Free-living HR and ACC could be measured in 15s, 30s or 1-min epochs. AH can assess activity energy expenditure and duration and intensity of physical activity (Brage et al., 2005; Crouter et al., 2008; Barreira et al., 2009).

2.4.8.2 PRELIMINARY OPERATIONS

To improve skin conductivity for ECG signal, we shaved (if necessary) the area where electrodes were placed and cleaned and scrubbed the skin with a mild abrasive gel (NuPrep, Weaver and Company, Aurora, CO, USA). This procedure removed dry surface skin and moistened the corneum skin layer.

To avoid activity underestimation or overestimation, it was vital to place AH with the cable exit as near the horizontal as possible, as shown in Fig 2.4.8.2.1 (Brage et al., 2005): for this reason, the electrodes were positioned to stretch the cable to its full length.

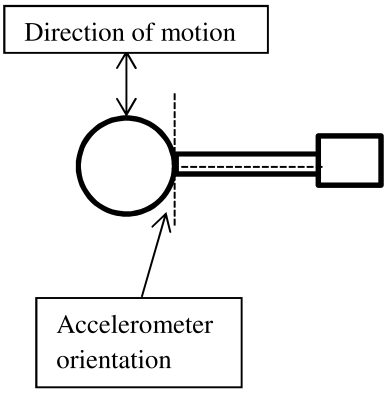

Fig. 2.4.8.2.1 Correct placement of the AH unit (Adapted from "The actiheart guide to getting started", V.4.0)

A couple of standard ECG electrodes to which the AH unit was clipped, were applied to the participant's left side of the chest. Two positions (suggested by the manufacturer) were used (Fig. 2.4.8.2.2):

- upper position: at the level of the third intercostal space
- lower position: just below the apex of the sternum

The medial electrode was placed in a vertical line with the umbilicus, and the lateral one was positioned 12–13 cm horizontally from there.

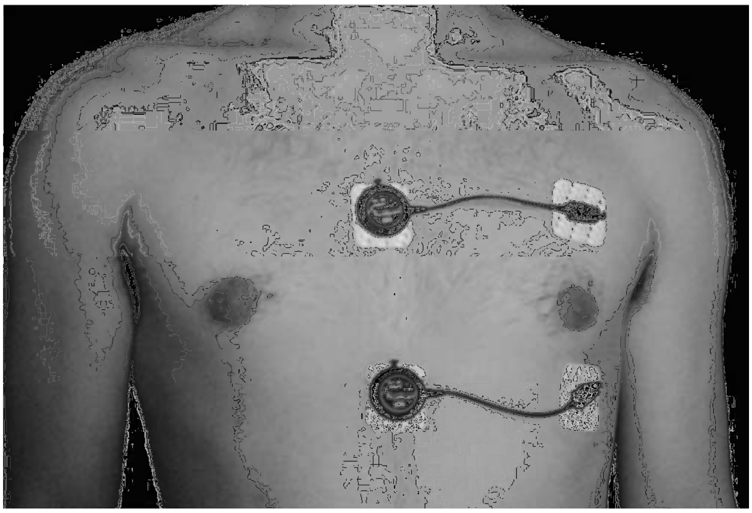

Fig. 2.4.8.2.2 Upper and lower position of the AH unit

The first choice was the lower position, because might yield cleaner HR data (Brage et al., 2006) but especially in obese subjects the clip often detached from the electrodes, so the upper position was used.

Before the recording, a short signal test was done to check HR signal integrity and to avoid artefacts due to noise.

Once a signal test has been successfully completed, the AH could be set to long-term recording with epoch length of 1-min.

2.4.8.2 DATA ACQUISITION

The AH unit was worn on day 1 of the study design. Every subject had to wear the device for almost 7 complete and consecutive days, with the exception of bathing. The participants were requested to carry on with their habitual lifestyle while wearing the monitor.

On day 2 (after a week or more), we removed AH, and we immediately downloaded the stored data: in this way we detected lost HR signals.

To be representative of a "typical week", it was necessary to have good ACC and HR signals of at least 3 weekdays and 1 weekend day, with 10 or more consecutive hours of the awake time (Ekelund et al., 2004).

Subjects who did not meet these criteria, had to wear the AH device for a second time, and repeat the same past iter from day 2 to day 3. At the end of the week, data were downloaded into a database using the commercial software. Individuals that did not satisfy the criteria previously described were excluded from the analysis.

2.4.8.3 INDIVIDUAL CALIBRATION

Individual calibration was made to increase the precision of the estimate of PA level. This operation could be done after data collection.
The subsequent values were entered in the software:

Sleeping heart rate
Sleeping heart rate (SHR) was set as the highest value of the 30 lowest minute-by-minute HR readings during the 24-h period, disregarding observations where HR=0 bpm.
The software determined the average sleeping heart rate for all nights of the analysis and then provided an overall average for all acceptable days.

Measured RMR

Once RMR was assessed (see par. 2.4.4), we obtained a value expressed on kcal/day that was transformed in $MJ \cdot day^{-1}$. It is known that 1cal = 4,186 J (Brockway, 1987), so we convert the RMR as follows:

$$\mathbf{1\ MJ = 1kcal \cdot 4.186 / 1000.} \tag{Eq.9}$$

Measured $V'O_{2max}$
The measured $V'O_{2max}$ value, expressed in $ml \cdot kg^{-1} \cdot min^{-1}$, was entered in the AH software

Measured HR_{max}
The highest value reached on the $V'O_{2max}$ test was entered in the AH software. If a maximal test was not performed, the age-predicted HR_{max} was used (Tanaka et al., 2001).

$HR/V'O_2$ individual relationship
The assumption to build the individual $HR/V'O_2$ relationship is that a linear relationship exists between HR and $V'O_2$: to build it, we used the warm-up of the $V'O_{2max}$ test, as described in par.2.4.5. In the last minute of steady-state of each of the three stages, we obtained the averaged values of HR, $V'O_2$ and RER.
For the AH calibration, values had to be entered in $J \cdot kg^{-1} \cdot min^{-1}$, so we transformed $V'O_2$ values from $ml \cdot kg^{-1} \cdot min^{-1}$ to $J \cdot kg^{-1} \cdot min^{-1}$ with Garby and Astrup formula (1987):

$$\mathbf{V'O_2\ (J \cdot kg^{-1} \cdot min^{-1}) = [(4.94 \cdot RER) + 16.04] \cdot V'O_2\ (ml \cdot kg^{-1} \cdot min^{-1})} \tag{Eq.10}$$

For each subject, we plotted HR against V'O_2 values. The equation of the line generated from the plotted point represents the individual HR/V'O_2 relationship (Fig 2.4.8.3.1), and allows to extrapolate V'O_2 values from a known HR to put in the AH software (Fig. 2.4.8.3.2).

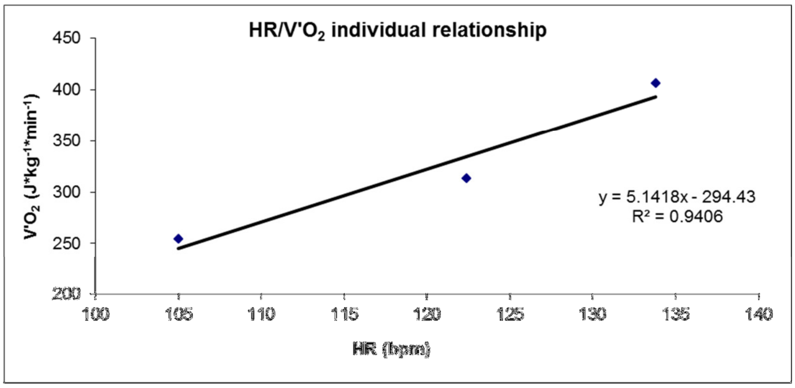

Fig. 2.4.8.3.1 Example of an individual HR/V'O_2 relationship.

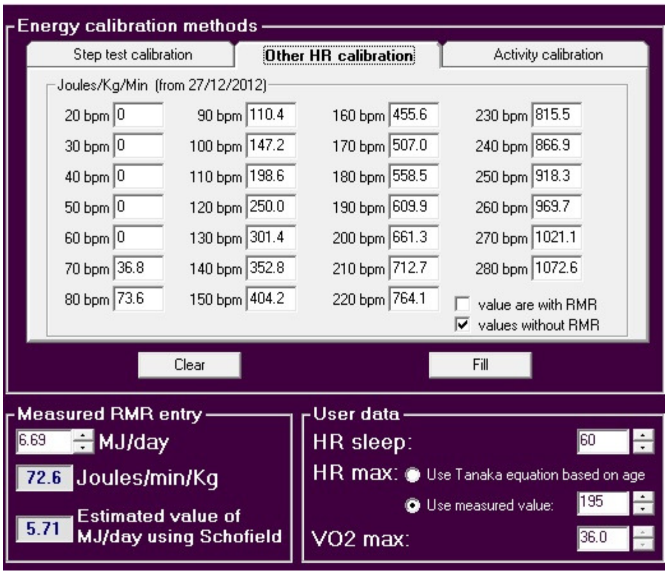

Fig. 2.4.8.3.2 Example of an individual calibration of the AH software.

2.4.8.4 DATA ANALYSIS

To estimate PA, we decided to use only values where accelerometer and HR were combined.

The Actiheart software was used to clean and recover or interpolate noisy and missing HR data <5 min (for detailed information, see "The actiheart guide to getting started, V.4.0").

To assess the averaged minutes spent in different intensities, Brage et al. (2004) studied a branched model equation that improved measurement precision of PA estimation, weighting the relative contribution of activity and HR according to different counts and HR thresholds (Brage et al., 2007). Essentially, when both ACC and HR values are low, ACC has more weight, whereas when ACC and HR values are high, the HR predominates.

The energy expenditure modality with branched model algorithm, that took into account the individual calibrations previously added, returned the following parameters for every day of the acquisition:

- Activity Energy Expenditure (kcal/day)
- Total Energy Expenditure (kcal/day)
- Physical Activity Level
- Minutes spent in various METs activities (min/day)

In particular, the classification proposed by Pate et al. (1995) was used to categorize MET intensities of PA (table 2.4.8.4.1) .

Table 2.4.8.4.1 MET intensities classification

Intensity	MET Classification
Sedentary	<1.49
Light	1.5-2.99
Moderate	3-5.99
Vigorous	>6

To define minutes passed in sedentary intensities, we subtracted the minutes of sleeping from the numerical value <1.5 METs given by the software for every selected day.

Sleeping hours were calculated with the advanced energy expenditure modality, where it was possible to detect sleeping phases from awake periods. Substantially, when absolute heart rate was lower and the accelerometer signal equals to zero, the subject was considered asleep (Fig. 2.4.8.4.1).

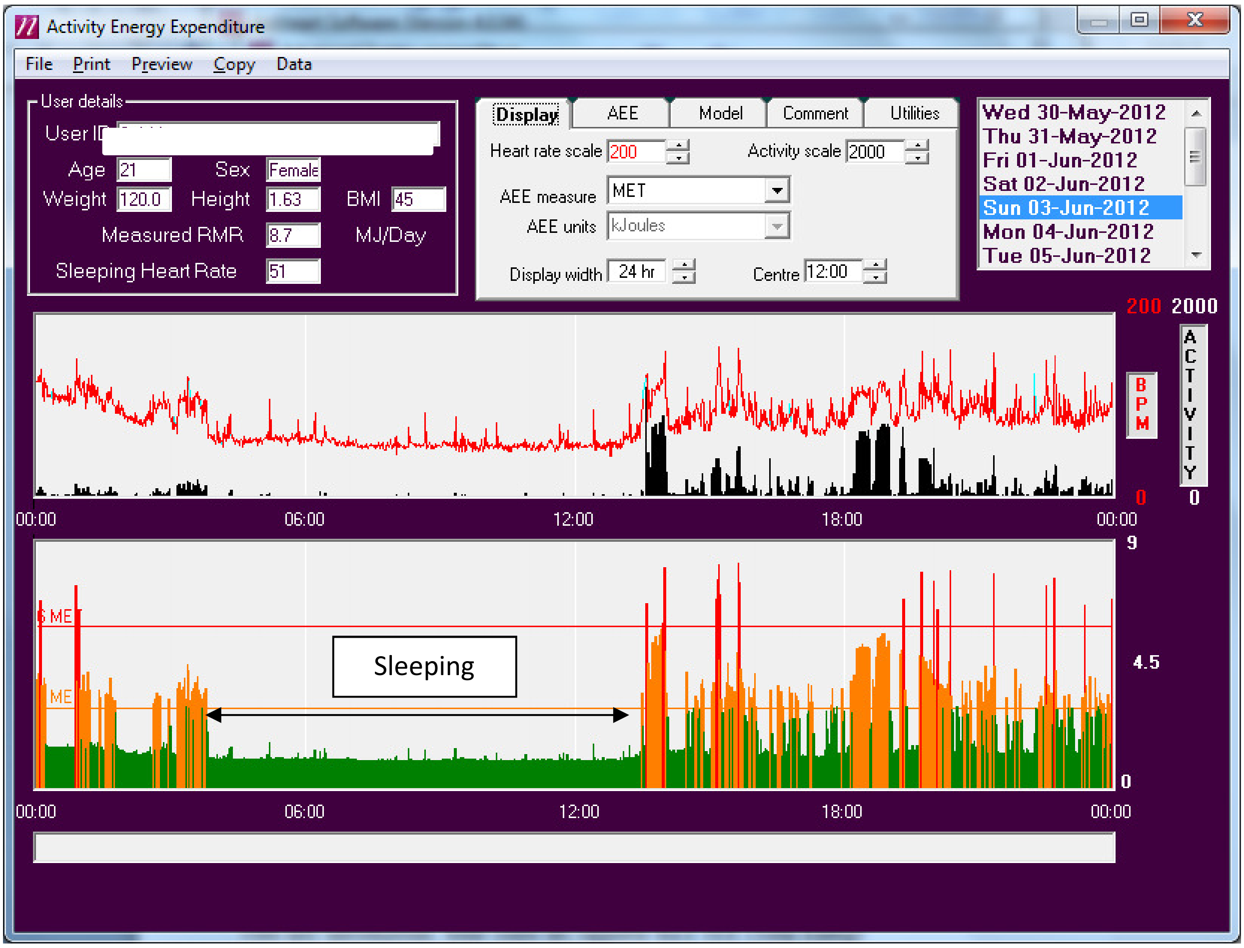

Fig. 2.4.8.4.1 Example of a sleeping time detection of a single day from the AH software

To obtain minutes passed in moderate and vigorous PA intensities (MVPA), we added minutes with values > 3METs.

Then, for every variable, the averaged value of all the days with available data was computed.

Since PA guidelines recommends to accumulate MVPA in bouts of at least 10 minutes, MVPA were analyzed in bouts of at least 10 consecutive min ($MVPA_{bouts}$) and in overall minutes ($MVPA_{all}$).

2.4.9 PREFERRED WALKING SPEED

The preferred walking speed (PWS) was assessed with the Racetime 2 Light Radio kit (Microgate, Italy) equipped by two photocells (Polifemo Radio Light, Microgate, Italy) and a chronometer (Racetime 2, Microgate, Italy) that measured the time required to cover 50 m on an athletic track (Fig. 2.4.9.1). Subjects were instructed to walk six times along a line 70-m section at a comfortable walking pace. We discarded the initial and final 10 m to avoid periods of accelerations or decelerations. To obtain PWS, the last five trials were averaged. All preferred speed measurements were made during clement weather (Browning, 2005).

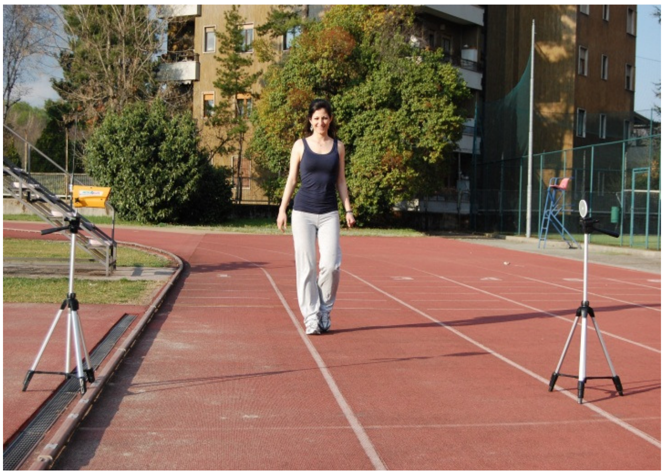

Fig. 2.4.9.1 PWS assessment on the athletic track

2.4.10 GAIT ASSESSMENT

To evaluate gait, we simultaneously assessed both biomechanical and metabolic data at three different speeds (3.5 – 4.5 – 5.5 km·h^{-1}) on a motor driven treadmill (525ex, Pro Form, USA). The choice of these velocities was made because, according to literature, the PWS of obese persons is between 4.25 km·h^{-1} (Malatesta et al., 2009) and 5.04 km·h^{-1} (Browning and Kram, 2005), with a gait transition around 6 km·h^{-1} (Ilić et al., 2012). Therefore, a “preferred” velocity of 4.5 km·h^{-1} was coupled with a faster and a lower speed, remaining below the transition phase. We tested only three speeds to limit the duration of the acquisition protocol.
For biomechanical analysis we calculated:

- Main spatial-temporal gait parameters
- Angular kinematics
- Gait variability
- Mechanical external, internal and total work

From metabolic analysis, we calculated:

- Gross and Net energy cost of walking

From the ratio of total mechanical work and the Net EC, we also calculated the efficiency of walking.

2.4.10.1 INSTRUMENTATION FOR BIOMECHANICAL ASSESSMENT

Biomechanical data were captured with a high precision optoelectronic system (Smart-E, BTS, Italy), consisting of nine infrared TV cameras positioned around a working volume of about 1650 x 2200 x 1320 mm. In short, the system uses the principles of stereophotogrammetry, and allows the three-dimensional reconstruction of the position of selected body landmarks during time, starting from the two-dimensional images obtained by pairs of cameras recording the same landmark with different viewpoints. The landmarks are identified by light weight markers.
The accuracy of BTS Smart system was <0.2mm, and the resolution was at 800x600 pixels.
Every camera (Fig. 2.4.10.1.1) was surrounded by an array of LED (Light-Emitted Diodes) mounted around the lens, emitting a stroboscopic light with a wavelength of 880 nm.

Fig. 2.4.10.1.1 BTS SMART camera

The lens focused the light on a CCD (Charge Coupled Device) sensor, that transmits and processes all data to reconstruct the three-dimensional position of retro-reflective markers (1.5 cm diameter) with respect to a reference system with the so-called "spatial triangulation" method, combining the 2D recordings data of at least 2 cameras.

To allow the identification of the relative position and orientation of every marker, the system was previously calibrated with a static and a dynamic phase.

In the first one, called "axes sequence", the static position and orientation of a reference system (Fig. 2.4.10.1.2) , made by three axes (x, y, z) with known distances between markers, was recorded to define the work volume, where the movement was to be performed.

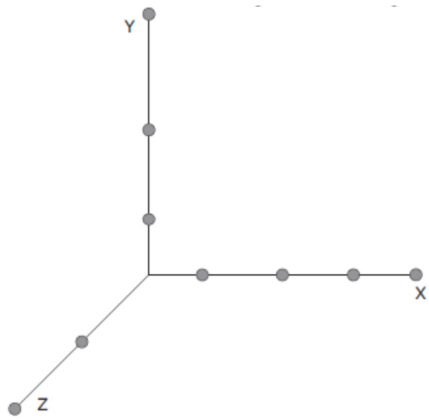

Fig 2.4.10.1.2 Global reference system for axes sequence

In the dynamic calibration phase, called "wand sequence", the operator had to move for a minimum of 90 sec the Y axis (40 cm length) in every way throughout the work volume, while all cameras recorded its motion.

After that, the system returned the calculation of 2 values, that provided the quality of the calibration:

- the mean error, that determined the accuracy of the system
- the standard deviation of the mean error, that was an estimate of the system precision

In this study, the accuracy was below 0.4 mm.

The sampling frequency of the SMART system was set at 60 Hz; the optional sampling rate was 120 Hz. After some trials we decided for the first option because gait was characterized by cycling and relatively slow patterns, and this sampling rate was already used in gait studies (Lai et al., 2008; Bovi et al., 2011). Furthermore, data were more clear, with few artifact, so the processing phase was relatively less time-consuming.

2.4.10.2 INSTRUMENTATION FOR METABOLIC ASSESSMENT

Energy cost of walking (EC) was assessed with $K4b^2$ (Cosmed, Italy), a portable, light (<1 kg) breath-by-breath gas analysis system (McLaughlin et al., 2001). This device, like Quark CPET, can measure the main respiratory parameters.

The $K4b^2$ consists of a portable unit (Fig. 2.4.10.2.1) that contains O_2, CO_2 analyzers, and other sensors and electronics. It is powered by a rechargeable battery fixed on a special harness wore by the subject (Fig. 2.4.10.2.2).

Fig. 2.4.10.2.1 $K4b^2$ system

Fig. 2.4.10.2.2 The $K4b^2$ harness

The flowmeter is connected to the front panel of the portable unit. An additional external slight case containing sensors for temperature, humidity and heart rate detection is linked on a front panel. The portable unit is also provided with a small display, that shows the main respiratory parameters as well as temperature, barometric pressure and battery charge level in real time.

Before every measurement, $K4b^2$ has to warm-up for at least 45 minutes. Afterward, the system needs the same calibrations described for Quark CPET, plus the delay calibration, performed at the suggested intervals to compensate for the time elapsed between the expiratory flow measurement and gas analyzers.

During the experiment, the heart rate (HR) was measured continuously with a Polar heart rate monitor (Polar, Finland).

After the calibration process and before test commencement, ambient humidity and physical characteristics of the participant were entered into the K4b^2.
Data were stored in an internal storage, and subsequently transferred to a PC for the analysis.

2.4.10.3 PROTOCOL

The acquisition, including the time for the subject's preparation, lasted about 90 min.
Before markers placement, subjects had to perform a familiarization period of about 20 minutes (Lavcanska et al., 2005). Then, they had to wear the HR transmitter to the thorax, wetting the grooved electrode area to allow good HR signal. Afterwards, the K4b^2 device was fixed on its harness, and a silicon mask (Hans Rudolph, Inc., USA) was mounted on the subject's face. Care was taken to eliminate possible leaks. For the correct markers placement for the biomechanical assessment, every subject had to wear close-fitting clothes (or a swimsuit) and habitual low sports shoes, not covering the ankle joints. The markers were firmly attached to the skin near bone prominences with a double-sided adhesive tape to avoid soft tissue artifacts; all markers were positioned by the same expert operator to reduce variability.
In particular, we followed a biomechanical model composed by 27 markers: 23 placed on specific anatomical landmarks listed in Table 2.4.10.3.1 plus 4 marker positioned on the treadmill base (Fig. 2.4.10.3.1).

Tab. 2.4.10.3.1 Paired landmarks were positioned on the right and left side of the body.

Marker number	Paired/ unpaired	Anatomical Landmark
1-3	Paired	Tragi
2	Unpaired	Glabella
5	Unpaired	Spinous process of the 7th cervical vertebra
4-6	Paired	Acromia
7-9	Paired	Lateral epicondyles of the humerus
8-10	Paired	Ulna styloid processes
12	Unpaired	Sacrum
11-13	Paired	Anterosuperior Iliac spines
14-15	Paired	Greater trochanters
16-17	Paired	Femoral lateral epicondyles
18-19	Paired	Lateral malleoli
20-22	Paired	Heel
21-23	Paired	Toe, midpoint of the shoe
B1-B2-B3-B4		Treadmill base

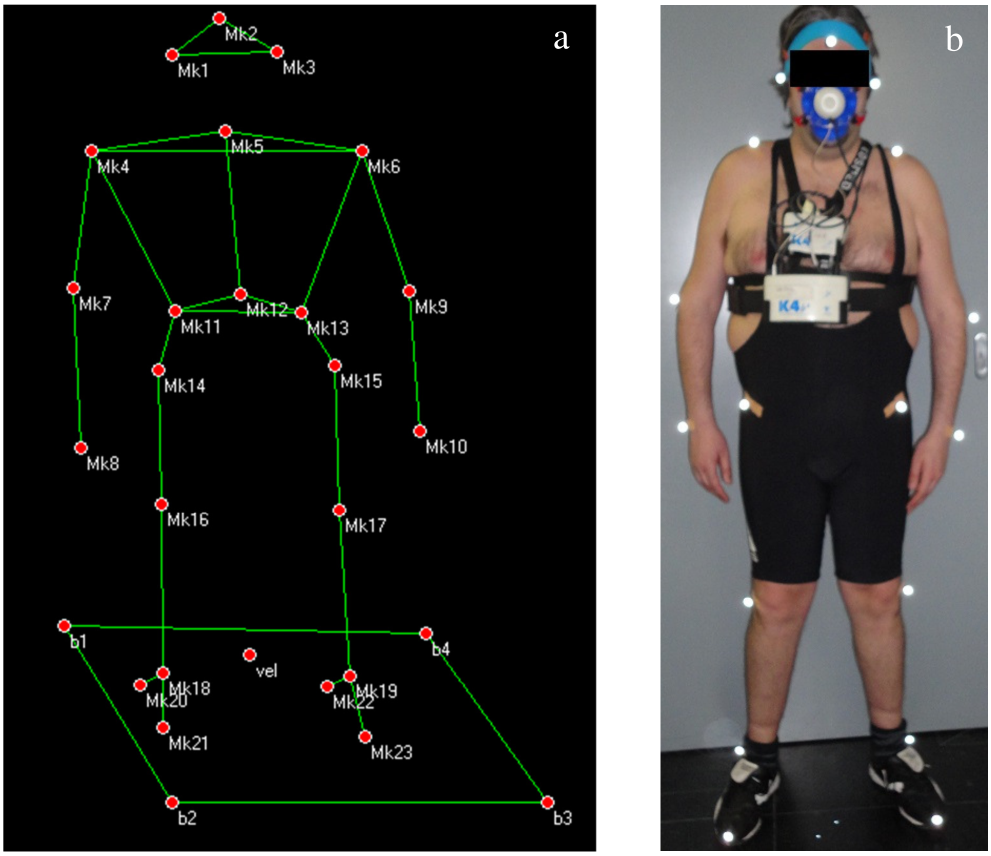

Fig 2.4.10.3.1. a: The biomechanical model; **b:** An obese subject ready for the acquisition

The choice of anatomical landmarks was based on a recent investigation of Mapelli et al. (2014), that used 14 landmarks for CoM calculation, (see also the par. on mechanical external work The advantage of this protocol is its high accuracy obtained with a small number of markers: the lower the markers, the lesser the risk of impeding or blocking movements when wearing them.

Markers on the feet (from 20 to 23) were added to define the gait cycles and spatial-temporal parameters, whereas B1-B2-B3-B4 were added to define the reference floor, useful for CoM's height and to detect feet contact phases on the treadmill belt.

Markers 2, 5 and 12 were added to determine angular kinematics.

Before the beginning of the test, age, height, weight, gender and relative humidity were entered into the Cosmed K4b^2, a room air calibration was performed and then the subject was ready to start the acquisition.

The protocol comprised:

- 10 minutes standing on the treadmill: in this phase we adjusted camera's thresholds to obtain good data, we captured the subject in a standing position for 5 sec to provide the reference for CoM evaluation and angular kinematics, and we assessed standing metabolic rate, useful for the calculation of net energy cost.

- 10 minutes walking at 3.5 km·h^{-1}: the subject had to walk continuously without any support.
- 5 min of sitting: this rest period was added to allow metabolic values to return to basal conditions, avoiding the so-called "EPOC" condition (excess post exercise oxygen consumption), due to the previous phase.
- 10 minutes walking at 4.5 km·h^{-1}
- 5 min of sitting.
- 10 minutes walking at 5.5 km·h^{-1}

Metabolic data were collected continuously, whereas for biomechanical acquisitions we captured 10 gait cycles/minute from the 4th to the 9th minute of each speed test, with a total of 50 cycles for speed.

2.4.10.4 DATA ANALYSIS

Preliminary operations for biomechanical analysis

After the movement recording, we obtain a cloud of unmarked points that a skilled operator had to label following a previously created biomechanical model (see par 2.4.10.3). Thanks to this procedure (called "tracking"), the system could recognize every single marker for the subsequent frames, except for the following not ordinary cases, where the operator had to manually correct the error:

- one marker was exchanged with a near one or confused with an artifact image;
- a marker disappeared for several frames, and was not labeled when it reappeared.

For each subject, we obtained 15 tracked files (one file for each minute from the 4th to the 9th minute = 5 files x 3 speeds). The files were then analyzed by the SMART Analyzer program; after filtering all files with a second-order Butterworth low-pass filter with a cut off frequency of 20 Hz, gait cycle events were automatically identified. Subsequently, an expert operator controlled the correct position of heel strike and toe off events automatically detected by the algorithms. For this study, a total of about 16.500 events were controlled or corrected. The final step was the automatic calculation of the variables of interest using the previously defined biomechanical model.

Mechanical Work

External Work and Recovery

To calculate the whole CoM, we used the segmental kinematic centroid method described elsewhere (Mapelli et al., 2014).

Briefly, this method divided the body in 10 segments whose inertial parameters were studied by Zatiorski et al. (1990). Subsequently, it was possible to calculate the CoM of each segment. The whole-body CoM was represented by the weighted average of all the 10 segments.

The displacement of CoM was analyzed in vertical (y), horizontal (x), and mediolateral (z) components. During walking, the potential (PE) and the kinetic energy (KE) fluctuations of CoM within each step can be obtained as described by Cavagna (1988) as follows:

$$\text{PE (Joules)} = m \cdot g \cdot h \tag{Eq.12}$$

where m is the body mass in kilograms; g is the acceleration due to gravity ($9.81 \text{ m} \cdot \text{s}^{-2}$); h is the vertical position of the CoM in meters; y is the vertical position of the CoM in meters;

$$\text{KE} = 0.5 \cdot [m(x^{\cdot})^2 + (y^{\cdot})^2 + (z^{\cdot})^2] \tag{Eq.13}$$

$x^{\cdot}$ is the horizontal CoM velocity of the horizontal displacements of CoM in m/s, $y^{\cdot}$ is the vertical velocity of CoM computed of the vertical displacements of CoM in m/s, and $z^{\cdot}$ is the lateral velocity of CoM of the vertical displacements of CM in m/s.

Then, the total mechanical energy (Etot) of the CoM increments was obtained summing kinetic and potential energies:

$$\text{Etot} = \text{KE} + \text{PE} \tag{Eq.14}$$

The work performed per stride (W_{ext} stride) was calculated as the sum of the positive increments in the total mechanical energy in a cycle, neglecting negative work. To obtain external work in J/step, we divided the W_{ext} stride for 2, whereas Wext expressed in $\text{J} \cdot \text{kg}^{-1} \cdot \text{m}^{-1}$ was calculated as follows:

$$W_{ext}\ (\text{J} \cdot \text{kg}^{-1} \cdot \text{m}^{-1}) = \frac{\text{Wext}\ (\frac{\text{J}}{\text{step}})/\text{m(kg)}}{\text{v}(\frac{\text{m}}{\text{s}})/(2*\text{Cad})} \tag{Eq.15}$$

where "v" represented the treadmill speed expressed in $m \cdot s^{-1}$ and "Cad" was the cadence expressed in cycles/s.

Internal Work

The calculation of internal work (W_{int}) was more complicated than the external one. This was because it required the analysis of the energy changes of the CoM of a finite number of body segments from their movements relative to the whole CoM as described in Willems et al. (1995).
The kinetic energy of W_{int} was obtained by the following equation:

$$KE\ (J \cdot kg^{-1} \cdot m^{-1}) = \sum_{i=1}^{n}(\frac{1}{2} m_i\ v_{r,i}^2 + \frac{1}{2} m_i K_i^2 \omega_i^2)$$

(Eq.16)

where m_i was the mass of i^{th} rigid segment, $V_{r,i}$ is the linear velocity of the centre of mass of the i^{th} segment relative to the whole CoM, ω_i and K_i are the angular velocity and the radius of gyration of the i^{th} segment around it's CoM.
The PE of each segment was not calculated because, as indicated by Cavagna et al. (1976), was considered negligible (W_{int} underestimation of about 17.5%), especially at low walking speeds.
As for Wext, Wint performed per stride was assessed as the sum of the positive increments in the total mechanical energy in a cycle. Then, to obtain W_{int} in J/step, we divided the previous value for 2. W_{int} expressed in $J \cdot kg^{-1} \cdot m^{-1}$ was calculated with the same equation shown for W_{ext} (Eq.15), changing the W_{ext} (J/step) at the numerator with W_{int} (J/step).

Total Work

Total mechanical work was represented by the sum of external and internal work:

$$W_{tot} = W_{ext} + W_{int}$$

(Eq.17)

To allow direct comparison with metabolic energy, we expressed W_{tot} in $J \cdot kg^{-1} \cdot m^{-1}$.

<u>Metabolic Work</u>

Data stored on $K4b^2$ were downloaded and analyzed with the dedicated software. After filtering data (6 point smoothing), we calculated standing metabolic rate (SMR) analyzing generally from 4^{th} to 9^{th} min of the first 10 minutes of the acquisition. Since SMR showed a lot of variability, we decided to consider only the interval of 5 consecutive minutes where $V'O_2$ changed by <10% (Cadena et al., 2005).

EC was calculated for every speed considering the averaged 5 minutes of $V'O_2$ steady state data (expressed in $mL \cdot min^{-1}$) from the 4^{th} to the 9^{th} minute. (Fig. 2.4.10.5.1)

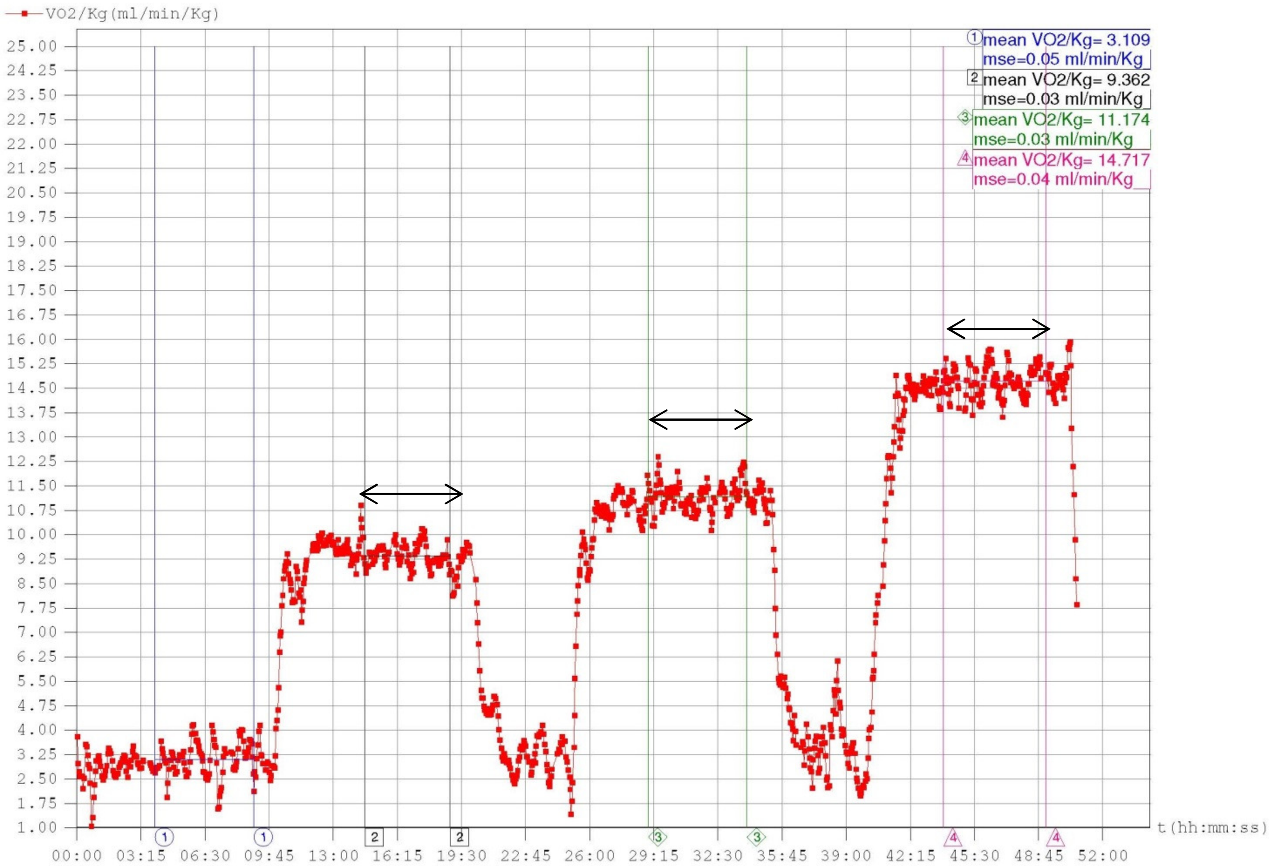

Fig. 2.4.10.5.1 Example of a steady state graphical representation

The same operation was made for RER.

Then, we converted $V'O_2$ from $mL \cdot min^{-1}$ to $J \cdot min^{-1}$ with the following equation (Garby and Astrup, 1987)

$$V'O_2\,(J \cdot min^{-1}) = V'O_2\,(ml \cdot min^{-1}) \cdot (4.94 \cdot RER + 16.04)$$

(Eq.18)

Subsequently, to obtain the relative values we divided for body mass (BM):

$$V'O_2\,(J \cdot kg^{-1} \cdot min^{-1}) = V'O_2\,(J \cdot min^{-1}) \,/\, BM\,(kg)$$

(Eq.19)

To obtain gross EC we divided $V'O_2$ for speed, expressed in m/min:

$$\text{Gross EC } (J \cdot kg^{-1} m^{-1}) = V'O_2 \, (J \cdot kg^{-1} \cdot min^{-1}) \, / \, \text{speed } (m \cdot min^{-1})$$

(Eq.20)

Finally, to obtain net EC, we subtracted SMR from Gross EC:

$$\text{Net EC } (J \cdot kg^{-1} \cdot m^{-1}) = (V'O_2 \, (J \cdot kg^{-1} \cdot min^{-1}) - \text{SMR } (J \cdot kg^{-1} \cdot min^{-1})) \, / \, \text{speed } (m \cdot min^{-1})$$

(Eq.21)

Efficiency

Efficiency, expressed as a percentage, was calculated with the following equation:

$$\text{Eff} = W_{tot} \, (J \cdot kg^{-1} \cdot m^{-1}) \, / \, \text{Net EC } (J \cdot kg^{-1} \cdot m^{-1}) \cdot 100$$

(Eq.22)

2.4.11 STATISTICAL ANALYSIS

Statistical analyses were performed using StatView statistical program (version 5.0.1 for Windows; SAS Institute Inc.).

Descriptive statistics were presented as mean ± standard error (SE).

For all studies, a Kolmogorov-Smirnov test was used to determine if variables were normally distributed.

In this dissertation, we present 2 different studies: for this reason, statistical analyses will be described in the subsequent sections.

2.4.11.1 *FIRST STUDY*

A two-way ANOVA (speed x gender) with repeated measures on speed with Tukey/Kramer post hoc analysis were conducted to assess the variation of mechanical and metabolic gait parameters across the three speeds for overall population. On variables where main effects of gender or gender x speed interactions were observed, simple effects of sex were tested at the different levels of speed using one-way ANOVA test. The single effect of speed on each group of metabolic and mechanical parameters was analyzed with a one-way ANOVA with repeated measures on speed with Tukey/Kramer post hoc analysis.

Pearson's correlation coefficients indicated relationships with physical activity and fitness parameters with main gait outcomes (mechanical, metabolic work, economy and efficiency).

Multiple backward step-wise regression analyses adjusted for age and BMI were conducted separately for men and women and for the three different walking speeds to ascertain how physical activity and physical fitness influenced gait efficiency or economy. Efficiency or economy (= Net EC) were set as the dependent variable, whereas SED or $MVPA_{all}$ or $MVPA_{bouts}$ and %FM, iMVC, $V'O_{2max}$, and Flex were entered as independent variables. Significance was set at $p<0.05$.

2.4.11.2 *SECOND STUDY*

A repeated measures ANOVA with Tukey/Kramer post hoc analysis was used to assess the variation of mechanical and metabolic gait parameters at the three speeds tested.

Pearson's correlation coefficients indicated relationships with physical activity and fitness parameters with main gait outcomes (mechanical, metabolic work, economy and efficiency).

Multiple backward step-wise regression analyses adjusted for age and BMI were made for the three different walking speeds to ascertain how physical activity and physical fitness influenced gait efficiency or gait economy. Efficiency or economy were set as the dependent variable, whereas

SED or $MVPA_{all}$ or $MVPA_{bouts}$ and %FM, iMVC, $V'O_{2max}$, and Flex were entered as independent variables. Significance was set at $p<0.05$.

3. RESULTS

3.1 FIRST STUDY:

Are Gait Economy, Total Mechanical Work and Efficiency affected by Physical Fitness and Physical Activity Level?

All the presented variables were normally distributed (Kolmogorov-Smirnov test, p>0.05).

3.1.1 Gender differences for physical activity and physical fitness

Table 3.1.1.1 shows that men were more physically active and less sedentary than women ($p<0.01$ and $p<0.05$ respectively), and that they had greater relative values of $V'O_{2max}$ and iMVC than women ($p<0.01$). In contrast, women were more flexible and showed more %FM than men ($p<0.01$).

Table 3.1.1.1 Differences between genders for physical activity and physical fitness components

	Overall	Males	Females
Physical Activity			
SED (min·day^{-1})	607±16	578±22*	641±21
MVPA$_{all}$ (min·day^{-1})	113±16	156±2**	63±7
MVPA$_{bouts}$ (min·day^{-1})	40±9	61±14**	16±2
Physical Fitness			
$V'O_{2max}$ (mL·kg^{-1}·min^{-1})	43.6±1.3	47.0±1.8**	39.7±1.4
iMVC (N·kg^{-1})	22.1±0.7	23.7±0.8**	20.3±0.9
Flex (cm)	35.9±2.3	30.0±3.1**	42.6±2.3
FM (%)	18.4±1.2	15.2±1.5**	22.1±1.3

SED: sedentary time; $MVPA_{all}$: overall moderate and vigorous physical activity; $MVPA_{bouts}$: bouts of moderate and vigorous physical activity; $V'O_{2max}$: maximal oxygen uptake; iMVC: isometric maximal voluntary contraction; Flex: flexibility; FM: fat mass.
* $p<0.05$ and ** $p<0.01$ (one-way ANOVA) between males and females

3.1.2 Differences between gender and speeds for metabolic and mechanical variables

In general, energy values were higher in females than in males (on average across all three speeds, 8.71% for Gross EC and 14.24% for Net EC), even if one-way ANOVA showed significant gender differences only for Gross EC at 5.5 km·h^{-1} ($p<0.05$) and for Net EC (J·kg^{-1}·m^{-1}) at 4.5 km·h^{-1}and at 5.5 km·h^{-1} ($p<0.01$ for both; Fig.3.1.2.1).

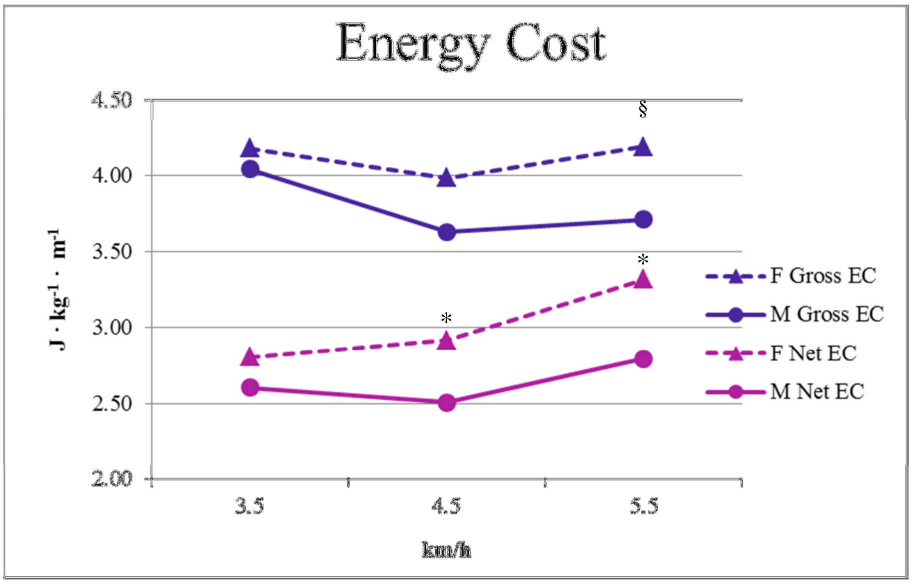

Fig. 3.1.2.1 Gross (blue lines) and net (purple lines) energy costs as a function of speed. Continuous curves = males, dashed curves = females. $^{§}p<0.05$, $^{*}p<0.01$, (one-way ANOVA).

The intensity of exercise expressed in % $V'O_{2max}$, was lower for males at all speeds (3.5 km·h^{-1}: $p<0.01$; 4.5 and 5.5 km·h^{-1}: $p<0.0001$; Tab. 3.1.2.2).

As expected, speed had an influence on all metabolic parameters: ($p<0.0001$), except for Net EC from 3.5 to 4.5 km·h^{-1}.

For Energy values, a significant speed x gender interaction was observed only for Net EC ($p<0.05$) and for %$V'O_{2max}$ ($p<0.0001$).

There was no significant difference between genders for all mechanical components of work and efficiency, even if females exhibited somewhat higher values (+6.7% on average for mechanical work, and -6.2% for efficiency; Tab. 3.1.2.2).

As for metabolic parameters, speed determined the main effect between all velocities ($p<0.0001$), except for W_{ext} from 3.5 km·h^{-1} to 4.5 km·h^{-1}. No significant speed x gender interaction was observed for all mechanical components of work and efficiency (Fig.3.1.2.2 and Fig.3.1.2.3).

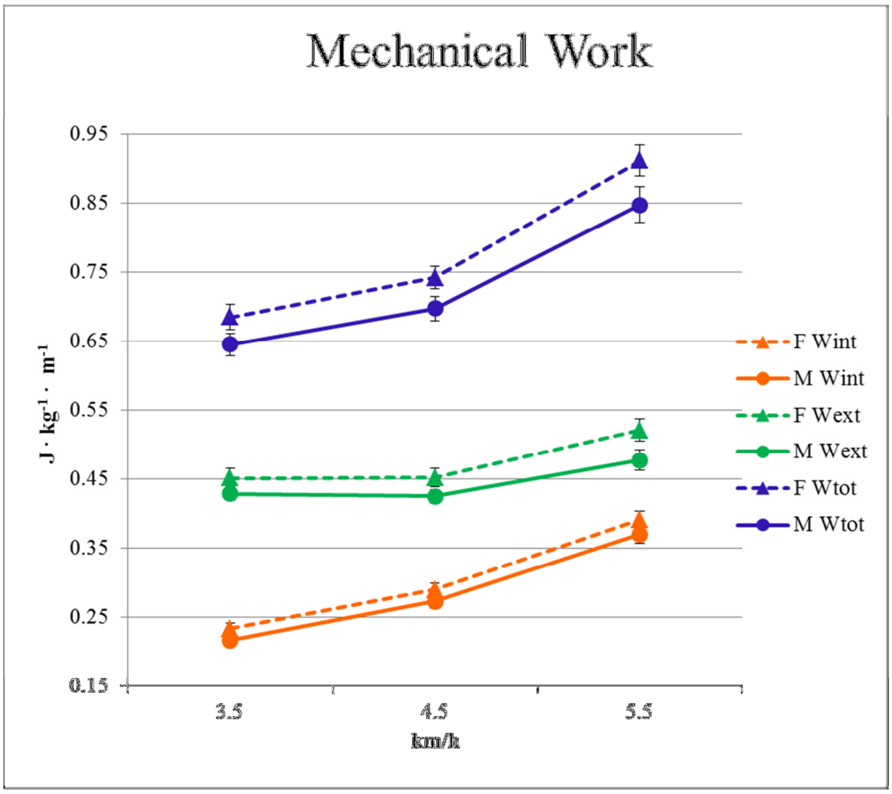

Fig. 3.1.2.2 Mechanical work normalized to body mass as a function of speed

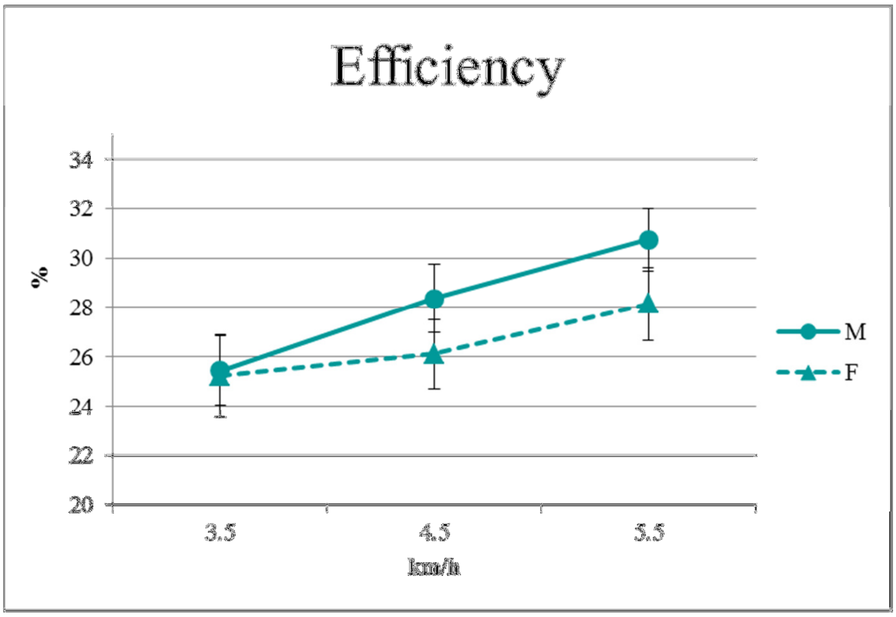

Fig. 3.1.2.3 Walking efficiency at the three speed tested.

Table 3.1.2.2 Metabolic and mechanical values at three different speeds for males and females

	Overall			**Males**			**Females**		
	3.5 km·h^{-1}	**4.5 km·h^{-1}**	**5.5 km·h^{-1}**	**3.5 km·h^{-1}**	**4.5 km·h^{-1}**	**5.5 km·h^{-1}**	**3.5 km·h^{-1}**	**4.5 km·h^{-1}**	**5.5 km·h^{-1}**
Gross EC (J·kg^{-1}·m^{-1})	4.11±0.10	3.80±0.10	3.94±0.10	4.05±0.14	3.63±0.11	3.71±0.11§	4.18±0.15	3.99±0.15	4.19±0.16§
Net EC (J·kg^{-1}·m^{-1})	2.70±0.08	2.70±0.08	3.04±0.09	2.60±0.09	2.51±0.09*	2.80±0.08*	2.81±0.12	2.92±0.13*	3.32±0.14*
Exercise intensity (%V'O$_{2max}$)	28.24±0.94	33.36±1.16	42.12±1.50	25.64±1.13*	29.36±1.22***	36.65±1.61***	31.22±1.13*	37.93±1.22***	48.36±1.34***
W$_{int}$ (J·kg^{-1}·m^{-1})	0.22±0.01	0.28±0.01	0.38±0.01	0.22±0.01	0.27±0.01	0.37±0.01	0.23±0.01	0.29±0.01	0.39±0.01
W$_{ext}$ (J·kg^{-1}·m^{-1})	0.44±0.01	0.44±0.01	0.50±0.01	0.43±0.01	0.42±0.01	0.48±0.01	0.45±0.01	0.45±0.01	0.52±0.02
W$_{tot}$ (J·kg^{-1}·m^{-1})	0.66±0.01	0.72±0.01	0.88±0.02	0.65±0.02	0.70±0.02	0.85±0.03	0.68±0.02	0.74±0.02	0.91±0.02
Eff (%)	25.33±10.7	27.31±0.99	29.52±0.98	25.43±1.40	28.36±1.38	30.73±1.27	25.21±1.70	26.10±1.39	28.14±1.47

Gross EC: gross energy cost; NetEC: net energy cost; V'O$_{2max}$: maximal oxygen uptake; W$_{int}$: internal work; W$_{ext}$: external work; W$_{tot}$: total work; Eff:Efficiency.
§p<0.05; *p<0.01, **p<0.001, ***p<0.0001 (one-way ANOVA).

3.1.4 Regressions for economy

Regressions showed that in overall population (Tab. 3.1.4.1):

- PA had no effect on Net EC at all speeds;
- iMVC significantly influenced Net EC across all speeds;
- Flex significantly influenced Net EC across all speeds;
- BMI was as a possible confounders, except for 3.5 km·h^{-1}.

In females:

- PA had no effect on Net EC at all speeds;
- iMVC significantly influenced Net EC across all speeds;
- V'O_{2max}, significantly influenced Net EC at 4.5 and 5.5 km·h^{-1};
- Flex significantly influenced Net EC at 3.5 and 4.5 km·h^{-1};
- BMI was as a possible confounders, but also for 3.5 and 4.5 km·h^{-1}.

In males, no significant associations were detected.

Table 3.1.4.1. Multiple backward step-wise regression analyses for walking economy in overall population and in females

Females				**Overall**			
	R^2	**F**	**p-value**		**R^2**	**F**	**p-value**
3.5 km·h^{-1}				**3.5 km·h^{-1}**			
iMVC and Flex BMI	0.68	10.16	<0.01	iMVC and Flex	0.21	4.88	<0.05
4.5 km·h^{-1}				**4.5 km·h^{-1}**			
iMVC, Flex and V'O_{2max} BMI	0.77	11.69	<0.01	iMVC and Flex BMI	0.43	8.39	<0.001
5.5 km·h^{-1}				**5.5 km·h^{-1}**			
iMVC and V'O_{2max} BMI	0.69	15.13	<0.001	iMVC and Flex BMI	0.39	7.26	<0.01

3.1.5 Regressions for efficiency

Regressions for efficiency showed that in overall population (Table 3.1.5.1):

- %FM can affect efficiency at 3.5 $km \cdot h^{-1}$;
- iMVC can affect efficiency only at 3.5 $km \cdot h^{-1}$;
- Flex had an influence on efficiency at 4.5 and 5.5 $km \cdot h^{-1}$;
- BMI was a possible confounders only at 4.5 $km \cdot h^{-1}$.

In females:

- iMVC and flexibility can affect efficiency at all speeds;
- BMI was a possible confounders across all velocities.

In males:

- $MVPA_{all}$ or $MVPA_{bouts}$ and Flex affect efficiency at 3.5 $km \cdot h^{-1}$;
- Flex had an influence on efficiency at 5.5 $km \cdot h^{-1}$.

Table 3.1.5.1 Regressions for efficiency

Females				**Males**				**Overall**			
	R^2	**F**	**p-value**		**R^2**	**F**	**p-value**		**R^2**	**F**	**p-value**
3.5 km·h^{-1}				**3.5 km·h^{-1}**				**3.5 km·h^{-1}**			
iMVC and Flex, BMI	0.78	16.61	<0.001	$MVPA_{all}$, Flex	0.32	4.58	<0.05	%FM, iMVC	0.18	4.17	<0.05
				$MVPA_{bouts}$, Flex	0.31	4.31	<0.05				
4.5 km·h^{-1}				**4.5 km·h^{-1}**				**4.5 km·h^{-}**			
iMVC and Flex, BMI	0.76	14.86	<0.001	-	-	-	-	Flex BMI	0.21	4.84	<0.05
5.5 km·h^{-1}				**5.5 km·h^{-1}**				**5.5 km·h^{-1}**			
iMVC and Flex, BMI	0.73	12.95	<0.001	Flex	0.23	5.59	<0.05	Flex	0.16	5.12	<0.05

3.2 SECOND STUDY:

Influence of Physical Fitness and Physical Activity Level on Total Mechanical Work and Economy of Treadmill Walking in Obese Adults

All the analyzed variables were normally distributed (Kolmogorov-Smirnov test, $p>0.05$).

3.2.1 Descriptive statistics for physical activity and physical fitness

Descriptive statistics of physical activity and physical fitness parameters are presented in Tab.3.2.1.1 as mean ± SE.

Generally, the analyzed subjects spent about ¼ of their day (excluding sleeping) in sedentary activities, almost 3 hours in moderate and vigorous physical activities of which 45 min in bouts of at least 10 minutes.

All physical fitness parameters, expressed for kg of body mass, are below the mean normative values.

Table 3.2.1.1. Physical activity and physical fitness components (Mean±SE)

	Overall (7M, 7F)
Physical Activity	
SED (min·day^{-1})	572±37
MVPA$_{all}$ (min·day^{-1})	175±21
MVPA$_{bouts}$ (min·day^{-1})	45±10
Physical Fitness	
V'O$_{2max}$ (mL·kg^{-1}·min^{-1})	31.3±1.6
iMVC (N·kg^{-1})	15.3±0.8
Flex (cm)	26.6±3.2
FM (%)	43.5±2.1

3.2.2 Differences between speeds for metabolic and mechanical variables

Repeated measures ANOVA showed that energy values were strongly dependent on speed: Gross EC significantly decreased from 3.5 km·h^{-1} to 4.5 km·h^{-1} ($p<0.001$, Fig. 3.2.2.1), whereas from 4.5 km·h^{-1} to 5.5 km·h^{-1} the opposite was observed (with $p<0.01$).

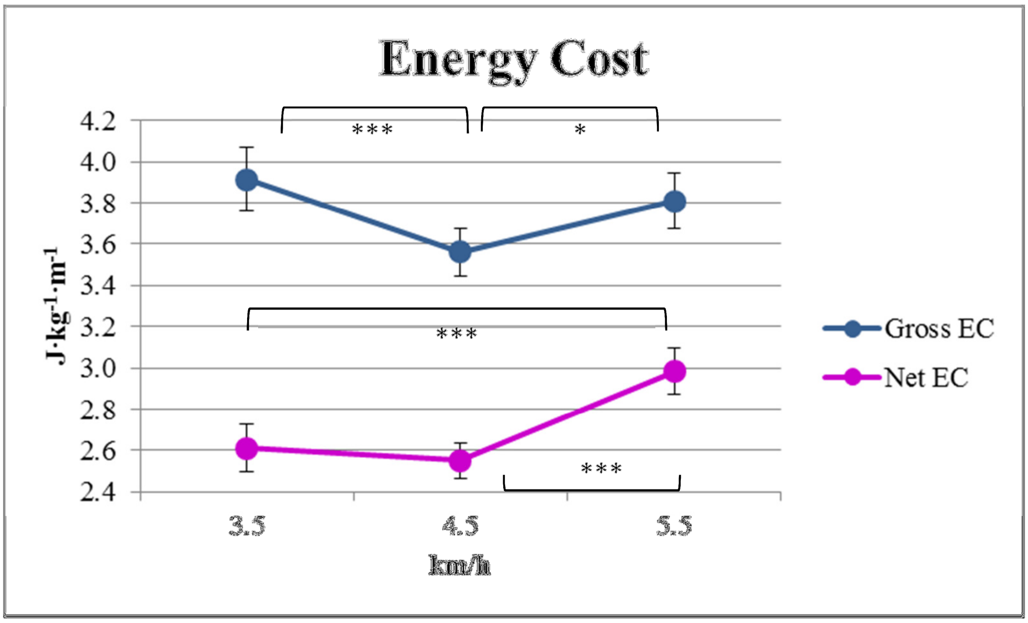

Fig.3.2.2.1 Energy values as a function of speed. $^{*}p<0.01$, $^{***}p<0.0001$

Also Net EC decreased from 3.5 to 4.5 km·h^{-1}, but not significantly. On the contrary, significant differences were detected between 3.5 and 5.5 km·h^{-1}, and between 4.5 and 5.5 km·h^{-1} (Fig.3.2.2.1). Exercise intensity, expressed as a percentage of $V'O_{2max,}$ increased significantly across all velocities ($p<0.001$ for all speeds).

Regarding mechanical values, W_{int} and W_{tot} increased significantly with speed, except for W_{tot} from 3.5 to 4.5 km·h^{-1} (Fig. 3.2.2.2). No differences were found for W_{ext} between any speed.
Efficiency increases with speed, but not significantly (Fig. 3.2.2.3; Tab. 3.2.2.2).

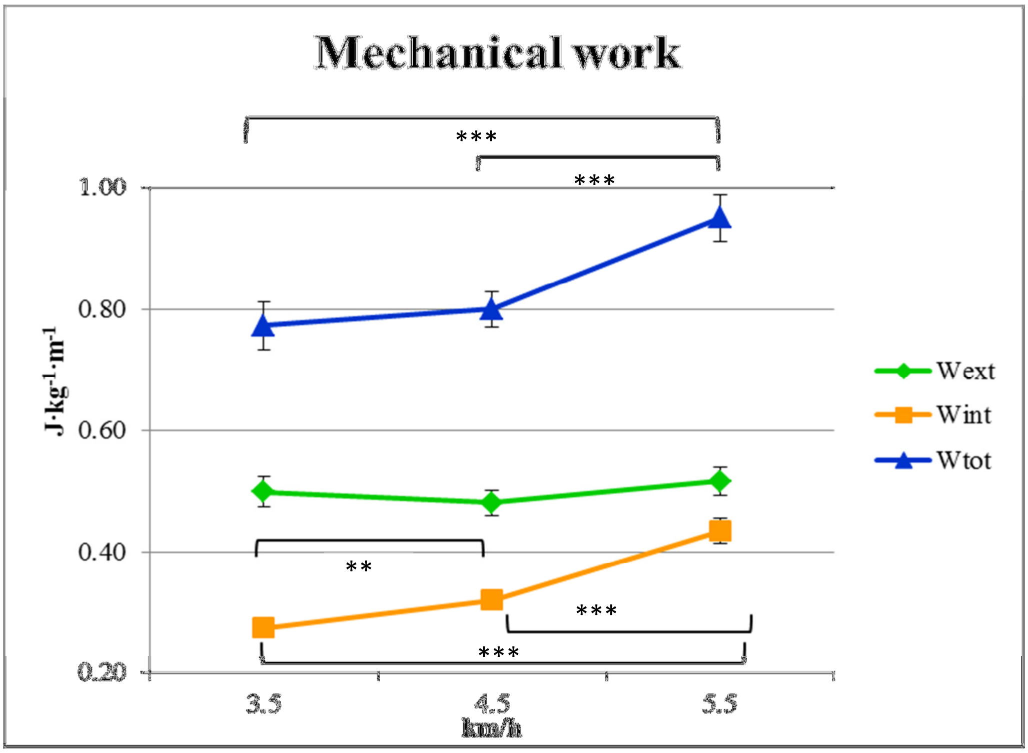

Fig.3.2.2.2 Mechanical work, and its components as a function of speed. **p<0.001, ***p<0.001

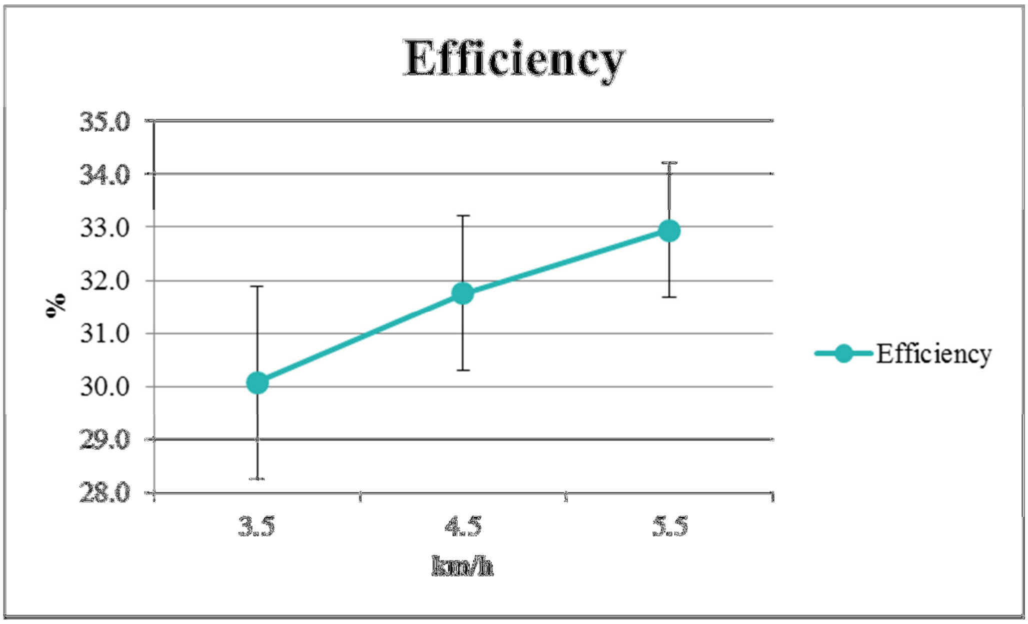

Fig.3.2.2.3 Efficiency as a function of speed

Table 3.2.2.2 Energy values as a function of walking speed (Mean±SE)

	3.5 km·h^{-1}	**4.5 km·h^{-1}**	**5.5 km·h^{-1}**
Gross EC (J·kg^{-1}·m^{-1})	3.91±0.16	3.56±0.12	3.81±0.14
Net EC (J·kg^{-1}·m^{-1})	2.61±0.11	2.55±0.08	2.98±0.11
Exercise intensity (%V'O$_{2max}$)	37.87±2.36	43.93±2.40	56.61±3.05
Wint (J·kg^{-1}·m^{-1})	0.27±0.02	0.32±0.01	0.43±0.02
Wext (J·kg^{-1}·m^{-1})	0.50±0.02	0.48±0.02	0.52±0.02
Wtot (J·kg^{-1}·m^{-1})	0.77±0.04	0.80±0.03	0.95±0.04
Eff (%)	30.1±1.81	31.8±1.46	32.9±1.27

3.2.3 Regressions for economy

Regressions showed that (Table 3.2.3.1):

- at 3.5 km·h^{-1} neither PA nor PF variables affected the economy of treadmill walking;
- at 4.5 km·h^{-1} only %FM had an influence on Net EC;
- at 5.5 km·h^{-1} %FM, iMVC, and Flex and SED affected economy. In the model with SED, age was a possible confounder, whereas in regression models with $MVPA_{bouts}$ or $MVPA_{all}$ (which did not affect Net EC), age and BMI were possible confounders (Table 3.2.3.1).

Table 3.2.3.1 Regressions for Net ECat all speeds

	R^2	F	p
3.5 km·h^{-1}			
-	-	-	ns
4.5 km·h^{-1}			
%FM	0.32	7.00	<0.05
5.5 km·h^{-1}			
%FM, iMVC, Flex, SED age	0.54	4.04	<0.05
%FM, iMVC, Flex BMI, age	0.54	4.01	<0.05

3.2.4 Regressions for efficiency

Regression analysis showed that only at 5.5 km·h^{-1} $MVPA_{all}$ could have a small influence on efficiency. BMI was a confounder (Table 3.2.4.1).

Table 3.2.4.1 Regressions for efficiency at 5.5 km·h^{-1}

	5.5 km·h^{-1}		
	R^2	**F**	**p-value**
5.5 km·h^{-1}			
$MVPA_{all}$	0.32	4.09	<0.05
BMI			

4. DISCUSSION AND CONCLUSIONS

4.1 MAIN FINDINGS

4.1.1 FIRST STUDY

4.1.1.1 Differences between speeds

Metabolic parameters

In good accord with literature (Ralston, 1958; Margaria, 1976), the Gross EC - speed relationship displayed a U-shaped curve both for men and women. It is well accepted that the minimum value of this curve, where energy cost is minimized, should correspond to a speed next to the subject's preferred walking speed (Rose et al., 1994). Metabolic values indicated that for females this minimum was reached next to 4.5 $km \cdot h^{-1}$ because the decrease (from 3.5 to 4.5 $km \cdot h^{-1}$) and the increase (from 4.5 to 5.5 $km \cdot h^{-1}$) of Gross EC were statistically significant.

For males the trend was slightly different: the absence of a significant variation of Gross EC from 4.5 to 5.5 $km \cdot h^{-1}$ seems to suggest that the more economical speed could be placed somewhere between 4.5 and 5.5 $km \cdot h^{-1}$, even if, from available data, it was unclear if the shift was downward or upward. To verify this hypothesis, it will be useful to study additional intermediate speeds.

Our measured preferred speeds (5.5 $km \cdot h^{-1}$ for females and 5.6 $km \cdot h^{-1}$ for males) were faster if compared to previous reports of about 5 $km \cdot h^{-1}$ (Ralston, 1958; Bohannon, 1997).

Matching data of Gross EC with those of the measured PWS of the two groups, we observed that in females the speed of EC minimization (4.5 $km \cdot h^{-1}$) did not correspond to their habitual walking speed (5.5 $km \cdot h^{-1}$). Also in males, which had a PWS of 5.6 $km \cdot h^{-1}$, the lowest value was not so clear, but it was quite surely between 4.5 and 5.5 $km \cdot h^{-1}$. Several hypotheses can be proposed to explain these findings.

In our opinion, this upward shift could be principally due to the dissimilar surfaces of the ground and secondly to the different protocol used to test the PWS: indeed, our subjects walked on an athletic track made by tartan, where the elastic contribution might be the main responsible of the increased speed. Moreover, measurements were made outdoors and with shoes, while other studies assessed PWS indoor (Bohannon, 1997), on a treadmill (Malatesta et al., 2009) or barefoot (Chung et al., 2010). Finally, all the overground protocols for the PWS determination were based on very small lengths, typically less than 10 m (Bohannon, 2008): in this way, subjects could not have the time to appropriately adjust the self-selected speed to the more similar speed maintained in daily life.

Net EC represented the true metabolic cost of walking because it did not include standing metabolic rate. If Net EC was plotted vs speed, it displayed the same "U-shaped" relationship described for Gross EC. Generally the point where EC was minimized was shifted downward with respect to that of Gross EC, so they did not coincide as previously studied by some authors (Snaterse et al., 2011). Typically, the net cost of transport reached a minimum at about 1.05 $m \cdot s^{-1}$. (Saibene et al., 2003), that was equal to 3.8 $km \cdot h^{-1}$. Our results are in line with previous reports: in fact, for females the minimum point was found at about 3.5 $km \cdot h^{-1}$, whereas for males it was 4.5 $km \cdot h^{-1}$.

Mechanical work

Generally, our values agree with literature data for both W_{ext} and W_{int} (Cavagna et al., 1988; Willems et al., 1995; Saibene et al., 2003; Mian et al., 2006; Ortega et al., 2007; Hernandez et al., 2009). Cavagna et al. (1976) demonstrated that the W_{ext} showed its minimum value at intermediate speeds, as for the Net EC. Our results are in agreement with them: in fact, the minimization of W_{ext} occurred for both groups between 3.5 and 4.5 $km \cdot h^{-1}$, that reflected the same behavior of the Net EC. On average, our W_{ext} values were comprised between 0.43 and 0.45 $J \cdot kg^{-1} \cdot m^{-1}$, that were slightly different when compared to literature data. A possible explanation may that our subjects walked on a treadmill and not overground as done in previous studies (Cavagna et al., 1976; Ortega et al., 2007; Hernandez et al., 2009). Additionally, we used an optoelectronic system instead of force plates to estimate W_{ext} (Cavagna et al., 1976).

We found significant increments of W_{int} at all speeds for both groups: these results were expected, because normally W_{int} increases with increasing walking speed (Cavagna et al., 1988): a higher speed requires more movement (e.g. swinging) of upper and lower limbs relative to the CoM. Our values were very similar to those reported by Mian et al. (2006) in level walking, ranging from 0.22 to 0.39 $J \cdot kg^{-1} \cdot m^{-1}$.

On average, our total mechanical work was 0.66 $J \cdot kg^{-1} \cdot m^{-1}$ at 3.5 $km \cdot h^{-1}$, 0.72 $J \cdot kg^{-1} \cdot m^{-1}$ at 4.5 $km \cdot h^{-1}$ and 0.88 $J \cdot kg^{-1} \cdot m^{-1}$ at 5.5 $km \cdot h^{-1}$: these results are somewhat higher than those of Mian et al. (2006), that ranged from about 0.59 $J \cdot kg^{-1} \cdot m^{-1}$ to 0.78 $J \cdot kg^{-1} \cdot m^{-1}$ at similar speeds. This was expected, considering our higher values of $W_{ext.}$

Efficiency

Relatively few studies calculated the efficiency of walking in healthy adults (Cavagna and Kaneko, 1977; Waters and Mulroy, 1999; Mian et al., 2006): according to Cavagna and Kaneko (1977) the highest efficiency (35% - 40%) is found at intermediate walking speeds (from 5 to 7 $km \cdot h^{-1}$), and follows an inverted U-shaped relationship.

Our results ranged from about 25% to 31%, and the upper limit seemed to be quite lower than that reported by Cavagna and Kaneko (1977): this could be due to the very small sample size studied by them (only 4 males), to the different method used to calculate mechanical work (with force plates), and to the fitness level of the subjects (2 of those analyzed by the authors were national athletes).

However, we found that our values and trend were very similar to those reported by Mian et al. (2006). We were not able to see (or to recognize) the peak of the curve because of the inadequate number of speeds. To see this peak we should add some tests at faster speeds.

4.1.1.2 Differences between genders

Metabolic parameters

Our data show that women displayed an upward shift both in Gross than in Net EC - speed relationship of treadmill walking if compared to men: this means that at the same walking speed females were less economical than males. In particular, at slow speeds both Gross and Net EC were very similar to male values (4.18 $J \cdot kg^{-1} \cdot m^{-1}$ vs 4.05 $J \cdot kg^{-1} \cdot m^{-1}$), but at 4.5 $km \cdot h^{-1}$ (only for Net EC) and at 5.5 $km \cdot h^{-1}$ (for both), these differences became statistically different.

Previous researchers have established that larger individuals are more economical per unit of body mass than smaller individuals (Martin and Morgan, 1992). In particular, higher stride rates and smaller step length need faster and more frequent movements to cover the same distance: this lead to a greater internal work, a greater recruitment of less economical fast twitch fibers, and so to a higher metabolic demand. Obviously, also maximal oxygen uptake has a great role in influencing exercise economy. For example, Pintar et al (2006) demonstrated that the relative intensity of PWS was influenced by $V'O_{2max}$: at same gait speed, people with higher $V'O_{2max}$ were those who showed lower relative intensities (worked at a lower percentage of $V'O_{2max}$) with respect to less fit counterparts. However, literature (Hunter et al., 2005; Sawyer et al., 2010) reported an inverse relationship between $V'O_{2max}$ and walking economy in sedentary women.

But, does it have sense to understand who is the most economical individual at two different relative intensities? For exercise prescription, definitively not. For this reason, exercise physiologists consider more correct to make comparisons at the same relative speed (the speed where the subject displayed the same relative work intensity, e.g. same % of $V'O_{2max}$), so we made also comparisons between males and females matching similar intensities.

Following this way of thinking, we found that the relative intensity of Net EC of females (in % of $V'O_{2max}$) at 3.5 $km \cdot h^{-1}$ and at 4.5 $km \cdot h^{-1}$ (31% and 38%) were comparable to those of males at 4.5

$km \cdot h^{-1}$ (29%) and at 5.5 $km \cdot h^{-1}$ (37%). Therefore, if we compare Net EC in $J \cdot kg^{-1} \cdot m^{-1}$ at the same % of $V'O_{2max}$ intensity, we confirmed that females were less economical than males but only at 31% of $V'O_{2max}$ ($p<0.05$, one way-ANOVA). In fact, at 31% of $V'O_{2max}$, females had a Net EC of 2.81 $J \cdot kg^{-1} \cdot m^{-1}$, whereas at about the same intensity (29% of $V'O_{2max}$) males consumed 2.51 $J \cdot kg^{-1} \cdot m^{-1}$. At 38% of $V'O_{2max}$, females consumed 2.92 $J \cdot kg^{-1} \cdot m^{-1}$ whereas males 2.80 $J \cdot kg^{-1} \cdot m^{-1}$, but, even if males displayed a smaller value, it was not statistically different from that of females.

Mechanical work

Even if women exhibited a little upward shift for all values at all speeds, no significant differences were found between males and females regarding W_{int}, W_{ext} and W_{tot}.

This finding was unexpected because women were smaller than men ($p< 0.001$)and had higher cadence, factors that usually increase $W_{int.}$

We could only speculate that mechanical work was not the main responsible for the elevation of EC of women, and that others factors, not accounted for in the calculation of W_{tot} like co-contractions, negative work, isometric muscle forces, and elastic contributions to muscle-tendon work also determined the elevation in the metabolic cost of movement.

To the best of our knowledge, no studies examined gender differences in mechanical work components, and further investigations are needed to better understand these data.

Efficiency

Comparing subjects at the same absolute speed, our study showed that mechanical efficiency did not differ between males and females, even if females were inclined to be somewhat less efficient than males.

The logical explanation was in the small increments of female W_{int} and W_{ext}, that led to slightly higher W_{tot} (by an average across speeds of 7% for W_{ext} and 6.5% for W_{int}). However the difference was not statistically significant. The percentage elevation of Net EC was twice larger than W_{tot}: this divergence between elevation in Net EC and W_{tot} caused a lower efficiency in women, even if the reported values were not statistically different. Our data are in agreement with those of Mian et al. (2006), that studied efficiency of walking in young and older men: the efficiency range of their young males vary from 25 to 35% at similar speeds. Unfortunately, we did not find any study for young women, so we could not compare our results with previous publications.

On the other hand, if we compare the two groups at the same relative speed, we found that efficiency at 37-38% of $V'O_{2max}$ was statistically higher in males than in females (26.1% females vs 30.7% males, $p<0.05$, one way ANOVA).

This strengthens the fact that, in order to correctly pursue our aims, it was essential to compare values at the same subject's relative speed.

4.1.1.3 Associations

Economy

It is well established that different levels of PF can influence the economy of a submaximal steady-state exercise, like walking (Gleim et al., 1990; Hunter et al., 2005; Pintar et al., 2006; Hunter et al., 2008; Sawyer et al., 2010). Generally, people who passes high amounts of time in moderate to vigorous PA are also those with higher PF levels (Gutin et al., 2005), so we hypothesized that also PA could affect Net EC of walking.

Contrary to our hypothesis, we found that PA had no effect on Net EC at all speeds for all groups (overall, females, males). Even if this strengths the conclusion of Martin et al. (1992), that found that aerobic demand was not impaired to a greater degree in sedentary individuals than in physically active adults, this result was not expected.

Regarding PF, we found that iMVC and Flex could affect the Net EC of walking in overall population and in females and $V'O_{2max}$ negatively affected Net EC only on females.

Economy was significantly influenced by iMVC across all speeds; we found that stronger people were the most economical. This could be due to a reduced motor units recruitment per muscle to generate force, that might result in the need to recruit less fast-twitch fibers, which are less economical than slow twitch ones (McArdle et al., 1996).

Another factor that influenced Net EC was flexibility, that was negatively associated with economy, except for 5.5 $km \cdot h^{-1}$. Also Gleim et al. (1990) and Hunter et al. (2008) demonstrated that less flexible adults were more economical, probably because of the better reuse of elastic energy that allowed more movements without requiring additional chemical energy.

Also $V'O_{2max}$ influenced EC at 4.5 and 5.5 $km \cdot h^{-1}$: in our case, persons with higher cardiorespiratory fitness were those with higher levels of Net EC.

Normally, training improves both $V'O_{2max}$ and exercise economy.

Even if our findings are in accordance with previous literature (Hunter et al., 2005; Sawyer et al., 2010), that showed an inverse relationship between $V'O_{2max}$ and gait economy, this is a confusing relationship that needs supplementary studies. However, several explanations have been suggested as possible contributors to this conflicting relationship.

People with higher $V'O_{2max}$ generally have more muscles in lower limbs that may need more energy (and thus oxygen) to move the legs during walking with respect to subjects with a greater proportion of their mass in the trunk (Larsen, 2003). Moreover, individuals who have higher

$V'O_{2max}$ are inclined to have higher lipid oxidation rates at submaximal intensities. Fat oxidation needs more oxygen *per* unit of energy expenditure than does carbohydrate metabolism, so people with higher $V'O_{2max}$ probably require more oxygen to perform a task. The result of this process would be higher values of Net EC (Pate et al., 1992). So, it was possible that at a sub-optimal range of intensities, the fitter subjects became "uneconomical" as confirmed by Pate et al. (1992).

We found associations between PF and economy only in women and overall population. We believed that the overall associations were due principally to women's associations, because PF parameters that appeared in relationships were the same for females, and because R^2 decreased from 0.77 to 0.43 at 4.5 $km \cdot h^{-1}$, and from 0.69 to 0.39 at 5.5 $km \cdot h^{-1}$.

Women were less economical than males at 4.5 and 5.5 $km \cdot h^{-1}$: this was an expected behavior because it is well known that smaller individuals consumed more energy per unit of body mass (Martin and Morgan, 1992), because they had to move themselves more than larger ones. Moreover, even if mean values of both groups were good if compared to normative values (60-70 percentile), absolute values were statistically higher for men than for women. It could be interesting to understand if women with similar absolute PF levels of males could compensate their lower economy.

Efficiency

Regarding women, some factors that influenced Net EC were maintained also for efficiency.

In fact, associations revealed that iMVC and flexibility affected Eff at all speeds. In particular, more strength and less flexibility were associated with higher mechanical efficiency. Associations were quite strong, because all these factors, in conjunction with BMI, explained more than 70% of the variance.

However, these associations were also quite strong for economy (R^2 ranges from 0.68 to 0.77), and Net EC was the denominator of Eff, so it seems logical to think that if Net EC was strongly affected from some PF variables, these associations probably will be maintained also for efficiency.

The unexpected results were those found in males: only at 3.5 $km \cdot h^{-1}$ PA and flexibility affected efficiency even if they accounted only for the 30% of variance. In particular, more MVPA (both overall or bouts) and less flexibility were associated with more efficiency.

At 4.5 $km \cdot h^{-1}$ no associations were found, but at 5.5 $km \cdot h^{-1}$ only flexibility was negatively associated with efficiency.

In overall population, Eff was influenced by %FM and iMVC at slower speeds and by Flex at 4.5 and 5.5 $km \cdot h^{-1}$, but these associations accounted only for a very small part of the variance (R^2 = from 16% to 21%).

From available data, it was quite difficult to find effective reasons to explain the behavior of associations in overall population and in males. However it seems that flexibility could have an influence on efficiency of all groups, but to gain better insights on this topic, further investigations with a larger sample size and with wider ranges in PA and PF parameters are mandatory.

4.1.2 SECOND STUDY

<u>*4.1.2.1 Differenced between speeds*</u>

Metabolic parameters

As for normal-weight people, also obese individuals displayed the U-shaped relationship for Gross EC and Net EC vs speed (Browning and Kram, 2005). The minimum value for both was achieved at 4.5 km·h^{-1}. Before and after the intermediate speed, all values were significantly higher, except for Net EC from 3.5 to 4.5 km·h^{-1}. PWS of our obese subjects was at 5.4 km·h^{-1}. The discrepancy with the minimization point seems to be quite larger: however, this could be due to the same methodological differences explained for normal-weight adults (see Par. 4.1.1.1). Moreover, PWS of obese adults is normally shifted slightly upward with respect to the minimization point of Gross EC (Browning and Kram, 2005), thus partly explaining the findings.

Our Gross EC (3.56 J·kg^{-1}·m^{-1}) and Net values (2.55 J·kg^{-1}·m^{-1}, also equal to 3.19 W·kg^{-1}) were quite higher than those reported by Browning and Kram (2005) of about 3.1 J·kg^{-1}·m^{-1} and 2.72 W·kg^{-1} respectively.

Afterward, SMR of our obese individuals was higher than that previously reported by Browning and Kram (2005), maybe for their greater levels of PA and PF, but we have no available data to confirm this hypothesis.

Mechanical work

W_{ext} did not show the typical "U-shaped" curve: across all speeds, values were quite similar, and no significant differences were found, even if the lowest value was that at 4.5 km h^{-1}. Probably, it means that this "plateau" represented the lowest part of the curve, where minimization occurred, and that the region of minimization was more enlarged for our obese subjects. Our W_{ext} at 4.5 km·h^{-1} was larger than literature data: 0.49 J·kg^{-1}·m^{-1} (current values); Malatesta et al. (2009), 0.36 J·kg^{-1}·m^{-1}; Peyrot et al. (2009), 0.41 J·kg^{-1}·m^{-1}. This was unexpected because many papers demonstrated that obese subjects displayed very similar W_{ext} values to normal-weight subjects. This could mean that in addition to higher body mass, the elevation in Net EC could be due to biomechanical changes that could alter the walking pattern.

This is the first study that quantified W_{int} in obese individuals. As for normal-weight adults, we found that across all speeds, W_{int} significantly increased. Browning (2012) speculated that it was possible that the heavier legs associated with obesity would require greater W_{int} during walking if compared with normal-weight counterparts, particularly given that obese individuals swing their legs faster (shorter swing time) and with more lateral circumduction than non-obese individuals. If we compare W_{int} with published data on normal-weight subjects, we can see that our values are quite higher than those previously published, but to confirm Browning's hypothesis, further analyses are necessary.

Efficiency

At increasing speed, efficiency increased from 30% to 33%, but not significantly. As for normal-weight subjects, also in obese the peak of the curve (inverted U-shaped relationship) was not visible for inadequate number of speeds. To our knowledge, two studies calculated walking efficiency in obese adults but took into account only W_{ext} as a numerator (Chen et al., 2004; Peyrot et al., 2009). They found that obese subjects walked less efficiently than normal-weight individuals because for the same W_{ext} they consumed more Net EC.

Since Eff requires the calculation of W_{int}, it was not possible to make direct comparisons with literature.

4.1.2.2 Associations

Economy

The main finding of this analysis was that SED could have an influence on Net EC at a speed near the PWS. In particular, more sedentary lifestyle leads to less economy. Even if SED accounted only for a marginal fraction of the total in the regression analysis, we think that the topic is worth a further study with a larger sample size showing a wider range in physical activity levels. This information could be very useful for exercise prescription in obese adults, because diminishing sedentary time could enhance gait economy, so obese people could perform daily living activities with less effort.

Regarding PF, we found that at 5.5 $km \cdot h^{-1}$ iMVC and Flex could affect the Net EC of walking, as found for normal-weight adults. Moreover, significant positive relationships were found between %FM and Net EC at 4.5 and 5.5 $km \cdot h^{-1}$: practically, obese persons with greater %FM were those with greater energy consumption. This finding is in accordance with previous literature (Browning et al., 2006) that discovered that %BF explained 45% of the variance in the net metabolic rate while walking at 5.5 $km \cdot h^{-1}$: they affirm that adipose tissue have comparable effects on the energy cost of walking. However, our R^2 accounted for 32% at 4.5 $km \cdot h^{-1}$ and only for 19% at 5.5 $km \cdot h^{-1}$, indeed, our subjects had approximately 3% more fat free mass than those investigated by Browning et al. (2006).

Efficiency

To date, this is the first study that assessed efficiency (W_{tot}/Net EC) in obese adults. Contrary to normal-weight adults, PA affected efficiency. Specifically, $MVPA_{all}$ with BMI positively influenced efficiency ($R^2 = 0.32$) at a speed near the preferred one. More efficient individuals are those who perform more $MVPA_{all.}$

This finding is relevant, because it means that obese people could improve efficiency of walking simply increasing time passed in moderate or vigorous PA. Moreover, improvements in efficiency occurred even if the MVPA are accumulated throughout the day in bouts shorter than 10 minutes.

4.2 METHODOLOGICAL DISCUSSION AND MAIN LIMITATIONS

During the experimentation, we noticed lots of limitations:

- Inadequate sample size: theoretically, in the 2nd study we would have to recruit and to test at least 40 obese subjects. This was not possible, because of several problems.
  First of all, the protocol was too demanding (especially in terms of time) for many of them, because it required to be tested in 2 different places (quite far one from another), and for three different days of at least 2 different weeks.
  Second, the inclusion criteria were very restrictive, because it was not so simple to find some obese people completely free from comorbidities or with stable weight since at least 6 months.
  All these difficulties did not allow to reach a greater number of subjects.
  However, other studies too did not recruit a large number of subjects. Browning et al. (2009) investigated only 10 obese subjects and 10 normal weight people. Minetti et al. (1993) studied the mechanical parameters of walking on an inclined surface in 4 people only. Cavagna et al. (1976) reported data on W_{ext} in 10 subjects, and Cavagna and Kaneko (1977) investigated external, internal work and efficiency in 4 subjects.

- Differences between subjects: in the 1st study, the major problem was that in males, PA levels were too high: for example, taking into consideration $MVPA_{all}$ all of them exceeded 30 $min \cdot day^{-1}$, that represents the minimum dose of exercise to obtain health benefits. In the 2nd study, there was the same problem in overall population: indeed, the minimum value of $MVPA_{all}$ was 56 $min \cdot day^{-1}$. Furthermore, the sample was too small with too many obesity typology. Some belonged to first grade of disease, others to second and third grades. Some subjects were affected by android's type obesity, others by gynoid one. It should have been useful to divide the subjects in homogeneous subgroups, and initially we wanted to recruit only 1st class obese, to limit also problems in the calculation of mechanical work (soft tissue artifacts, see below), but difficulties explained in the previous section forced us otherwise.

- Analyzed speeds: Mechanical and metabolic parameters were analyzed only at three speeds (3.5, 4.5 and 5.5 $km \cdot h^{-1}$). We choose these speeds to allow the comparison of normal-weight and obese subjects, and in literature the mean PWS of obese subjects ranged from 4.2 to 5 $km \cdot h^{-1}$ (Browning and Kram, 2005; Malatesta et al., 2009). Averaging them, we obtain 4.6 $km \cdot h^{-1}$, that was our intermediate speed. Speeds slower than 3.5 $km \cdot h^{-1}$ are below PWS for both normal-weight ad obese adults, and above 6 km·h^{-1} the major risk was to incur in the transition speed in which subjects were uncomfortable because they don't know whether to walk or run, thus affecting gait. To verify the complete curve of the energy cost, mechanical work and efficiency, it would have been useful to test further walking speeds, analyzing intermediate speeds between 4.5 and 5.5 km·h^{-1}, faster speeds and the subject's own PWS, both for normal-weight and for obese people.

However, our speeds were in an optimal range, that is typically used for studies of energy expenditure during walking (Malatesta et al., 2003; Hunter et al., 2005). For example, Browning et al. (2009) calculated W_{ext} in obese patients at six different speeds, from 1.8 to 6.3 km·h^{-1}. We did not choose to add other speeds because the acquisition protocol would have been too long: in fact, every speed required 10 min of treadmill walking, with a pause of 5 min within velocities.

- <u>Experimental procedures</u>: as done in the study of obese subjects by Malatesta et al. (2009). an optoelectronic system was used for the calculation of mechanical parameters. Despite the inverse dynamics is a method suitable for analyzing the displacement of the center of mass, an excessive presence of markers on the body may alter or affect the movements of the obese subjects during walking. Moreover, the presence of excessive adipose tissue makes it difficult to identify the landmarks, thereby altering in many cases the calculation of mechanical parameters (soft tissue artifacts). In other researches different methods were used to evaluate the mechanical work of the subjects. For example Peyrot et al. (2009) used the accelerometer to calculate the displacement of the CoM and to calculate W_{ext}., Other investigators used direct dynamics (with force platforms) both in normal-weight (Cavagna et al., 1976) and obese subjects (Browning et al., 2009): this method represents the "gold standard" to assess W_{ext}. Moreover, we assessed W_{ext} with the so called "Combined Limbs Method" (CLM), in which the lower limbs were analyzed as a single force applied to the point mass located in the CoM. With force plates, it is possible to use also the "Individual Limbs Method" (ILM), in which W_{ext} can be calculated as the work performed by the single lower limb (Donelan et al., 2002). With ILM, the values of W_{ext} were 33% higher when compared to those calculated with the CLM (Donelan, 2002). All these differences can lead to different values and different conclusions.

4.3 GENERAL CONCLUSIONS and IMPLICATIONS FOR EXERCISE PRESCRIPTION

This dissertation tried to give answers to some questions:

1- *Can PA and PF affect the Net EC of locomotion in normal weight adults?*

Our research was the first that tried to determine if economy of treadmill walking was influenced by different levels of PA objectively measured taking into account also those of PF. Indeed, considering that many studies revealed that Net EC was affected by some PF qualities, and that generally PA could improve these PF qualities, we aimed to understand if also PA could have an influence on economy, and, if so, to recognize which predominates. Results showed PA has no effect on Net EC and that only some PF parameters (iMVC and Flex) seems to affect walking economy, even if they accounted only for the 39% of the variance. We should note that individuals participating in the current study were healthy and active. Thus, something might change in inactive people.

2- *Can gait efficiency be influenced by different levels of PA and PF?*

From the available data, it is difficult to make conclusive statements. However, it seems that at habitual walking speeds PA does not affect efficiency and that flexibility could have an influence on efficiency of all groups, but further investigations were requested.

Different recommendations for males and females should be given to meet exercise guidelines:

For active females, if the goal is to reach a minimum of 30 $min \cdot day^{-1}$ of MVPA, it is sufficient that they walk at their PWS (5.5 $km \cdot h^{-1}$) because the relative intensity of this activity (48% $V'O_{2max}$) falls in the moderate range. Conversely, for males walking at their self-selected speed is considered a light activity (37% $V'O_{2max}$), so they have to walk faster than 5.5 $km \cdot h^{-1}$. On the other hand, if the main objective is to sustain a prolonged activity, both men and women might walk at a speed near the minimization point (from 3.5 to 4.5 km h^{-1}).

3- *Does something change in the obese population?*

The answer to this question, is a qualified "yes".
The main finding was that SED could have an influence on Net EC and $MVPA_{all}$ affected gait efficiency at a speed near the PWS.

Specifically, we found that less sedentary obese people were more economical than their counterparts, and that a longer time passed in moderate and vigorous PA could enhance gait efficiency.
It is well established that obesity leads to several limitations of daily living activities, including walking. So, if SED was directly associated with Net EC, less time passed in sedentary activities could enhance gait economy making it more enjoyable and less strenuous. This could be the first step of a useful strategy to break the chain of sedentarism and to become more active and healthy.
Additionally, we found that obese people could improve gait efficiency accumulating MVPA throughout the day. Indeed, efficiency was associated only with $MVPA_{all}$ and not with $MVPA_{bouts}$, showing that it was not essential to perform at least 10 min of MVPA (as recommended by PA guidelines), but that, to improve gait efficiency, they have to move themselves as more as they can for at least one consecutive minute (MVPA were calculated on a minimum epoch length of 1 min).
In our sample of obese people, walking at a speed near the PWS required a moderate intensity (more than 50% of $V'O_{2max}$): so, to improve economy and efficiency, and to become more active, the final messages that we could address to obese population were:

- reduce sedentary activities (even change them in light activities) and broke them up with short bouts of physical activity or standing;
- walk at your PWS the more you can;
- be active in as many ways as you can.

4.4 FUTURE DEVELOPMENTS

The effects of PA and PF on gait efficiency were investigated with a reduced statistical power, and no definitive conclusions were obtained. Further investigations should be performed with a larger group of subjects presenting a wider range of PF and PA values (on average, the current subjects were over the normative values). Since only a little part of the variance was explained, other biomechanical variables (like stride length, step frequency, swing and double support time) should be added in the regression model to verify if they could affect Net EC and efficiency.

Direct comparisons with homogeneous normal-weight and obese subject groups are requested to obtained a deeper insight for exercise prescription. Further studies may be focused to detect possible single causes of gait inefficiency, that may be selectively trained and improved, thus increasing the levels of PA and PF.

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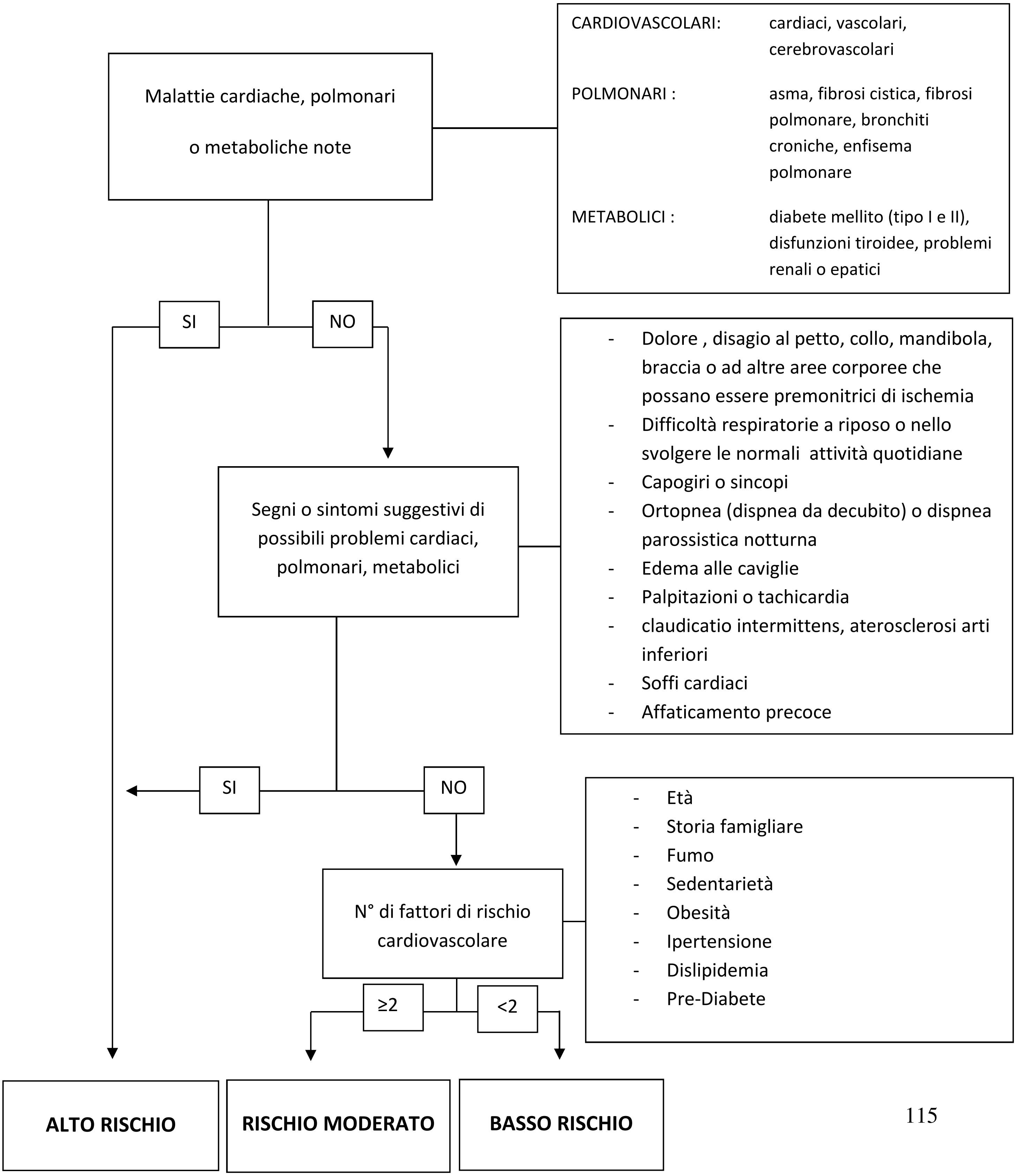
ATTACHMENTS

Attachment 1: Flow chart for the inclusion criteria

STEP 1: FLOW CHART SULLO STATO DI SALUTE

(Per l'idoneità al reclutamento il paziente NON deve essere ad alto rischio)



STEP 2: VERIFICA DEI CRITERI DI INCLUSIONE PER LO STUDIO

1. Età compresa tra 18 e 40 anni **SI** **NO**
2. Alimentazione costante negli ultimi mesi **SI** **NO**
3. BMI è superiore a 30 kg/m^2 **SI** **NO**
4. Assume farmaci prescritti dal Suo medico? **SI** **NO**
   Se si, quali ? ____________________________

   ____

   ____
5. E' incinta ? **SI** **NO**
6. E' tesserato per federazioni sportive **SI** **NO**
7. Ha incapacità fisiche o psichiche ad effettuare un test da sforzo massimale **SI** **NO**

PER L'IDONEITA' AL RECLUTAMENTO :

Il paziente deve aver risposto **SI** alle domande 1-2-3

Il paziente deve aver risposto **NO** alle domande 5-6-7

Per la domanda n°4, se affermativa, **NON** devono essere presenti i seguenti farmaci:

B-bloccanti
Anticoagulanti o antiaggreganti
Agenti steroidei anti-infiammatori
Antidiabetici
Sibutramina
Orlistat

IDONEO AL RECLUTAMENTO **SI** **NO**

Ai sensi del Decreto Legge N°196/03 (Art.7 e 13) relativo alla tutela della persone per il trattamento dei dati personali, il/la Sottoscritto/a ____________________________ autorizza il trattamento dei propri dati personali per scopi di ricerca scientifica.

Data____________________

Firma ______________________________

Attachment 2: PAR-Q

PAR-Q & YOU

(Questionario preliminare alla partecipazione a un programma di attività fisica)

Completate il seguente questionario per valutare se siete in grado di iniziare o intensificare un programma di esercizio fisico.

Un'attività fisica regolare è divertente e salutare e un numero crescente di persone ogni giorno comincia a diventare più attivo. Ciò puo essere fatto in tutta sicurezza nella maggior parte dei casi, ma per alcune persone è consigliabile consultare il proprio medico prima di iniziare o incrementare una propria attività fisica.
Se avete tra 15 e 69 anni, il PAR-Q vi dirà se dovete consultare il vostro medico prima di cominciare.
Se avete più di 69 anni e non siete abituati a un regolare programma di esercizio fisico, consultate comunque il vostro medico.
Il buon senso è la guida migliore nel rispondere a queste domande. Per favore, leggete attentamente e rispondete sinceramente SÌ o NO.

SÌ	NO	
❐	❐	1. Il vostro dottore vi ha mai detto che avete problemi al cuore e che dovete fare un'attività fisica raccomandata dal medico?
❐	❐	2. Sentite dolore al petto quando fate attività fisica?
❐	❐	3. Nell'ultimo mese, avete avuto dolore al petto a riposo?
❐	❐	4. Vi capita di perdere l'equilibrio per vertigine o di essere sul punto di svenire?
❐	❐	5. Avete problemi ossei o articolari (alla schiena, alle ginocchia, alle anche) che potrebbero peggiorare aumentando l'attività fisica?
❐	❐	6. Il vostro medico vi ha prescritto dei farmaci (per esempio diuretici) per problemi di pressione o di cuore?
❐	❐	7. Siete a conoscenza di qualsiasi altra ragione per la quale per voi potrebbe essere controindicato eseguire attività fisica?

Se avete risposto

SÌ a una o più domande

Consultate telefonicamente o di persona il vostro medico PRIMA di iniziare ad aumentare sensibilmente la vostra attività fisica o PRIMA di eseguire un esame medico per valutare il vostro stato di forma. Parlate al vostro medico del PAR-Q e delle domande a cui avete risposto SÌ.

- Siete probabilmente in grado di impegnarvi nell'attività fisica che preferite, a patto di iniziarla lentamente e incrementarla gradualmente. Oppure, potrebbe essere consigliabile che vi limitiate a un programma più adatto alle vostre condizioni. Parlate al vostro medico delle attività cui vorreste partecipare e seguite i suoi consigli.
- Cercate un programma di attività fisica collettivo che sia sicuro e di utilità per voi.

NO a tutte le domande

Se avete risposto sinceramente NO a tutte le domande, potete essere ragionevolmente sicuri di potere:

- cominciare ad aumentare sensibilmente la vostra attività fisica: iniziatela lentamente e incrementatela gradualmente. Questo è il sistema piu facile e sicuro.
- sottoporvi a una valutazione della vostra condizione fisica: è il modo migliore per valutare la vostra forma fisica di base per programmare in modo ottimale l'attività in cui impegnarvi.

ASPETTATE AD AUMENTARE MOLTO LA VOSTRA ATTIVITÀ FISICA:

- se non vi sentite bene a causa di disturbi temporanei come raffreddore o febbre; aspettate fino a che non vi sentirete di nuovo bene;
- se siete o potreste essere in gravidanza, consultate prima il vostro medico.

Ricordate: se la vostra salute cambia e rispondete SÌ ad una delle domande sopra elencate, parlate al vostro medico del vostro problema e chiedetegli se dovete modificare il vostro programma di attività fisica.

Uso informato del PAR-Q. La Canadian Society for Exercise Physiology, la Health Canada e i suoi rappresentanti declinano ogni responsabilita nei confronti di coloro che eseguono un'attività fisica; se siete in dubbio dopo aver completato questo questionario, consultate il vostro medico prima di iniziare il programma di esercizio fisico.

Fonte Physical Activity Readiness Questionnaire (PAR-Q). Riprodotto con il permesso della Canadian Society for Exercise Physiology.

Attachment 3: Informed Consent

Laboratorio di Anatomia Funzionale dell'Apparato Locomotore
Università degli Studi di Milano

Laboratorio di Fisiologia Sperimentale Applicata all'Esercizio Fisico e allo Sport
Università Cattolica del S.Cuore di Milano

Tutor: Prof.ssa Chiarella Sforza
Direttore del Dipartimento di Morfologia Umana e Scienze Biomediche "Città Studi", Università degli Studi di Milano
Professore ordinario di Anatomia Umana, Facoltà di Medicina e Chirurgia dell'Università degli Studi di Milano

Co-Tutor: Prof.ssa Christel Galvani
Docente e Ricercatrice presso l'Università Cattolica del Sacro Cuore di Milano nel CDL in Scienze Motorie delle Attività Preventive e Adattate
Laboratorio di Fisiologia Sperimentale applicata all'esercizio Fisico ed allo Sport

Dottorandi: Dott.ssa Isabella Annoni
Laureata in scienze motorie all' Università Cattolica del Sacro Cuore di Milano
Dottoranda in Scienze Morfologiche presso l'Università degli Studi di Milano
Collaboratrice nel Laboratorio di Fisiologia Sperimentale Applicata all'esercizio fisico e allo sport dell' Università Cattolica del Sacro Cuore di Milano

SCHEDA INFORMATIVA E DICHIARAZIONE DI CONSENSO INFORMATO

Gentile Signor/a,

1. Identificazione della Ricerca:

Presso il Dipartimento di Morfologia Umana e Scienze Biomediche Città Studi dell'Università degli Studi di Milano è in programma una Ricerca scientifica, che vuole andare a valutare i parametri metabolici e biomeccanici della camminata su treadmill tra soggetti adulti normopeso attivi e sedentari.

Il titolo dello Studio è "Differenze biomeccaniche e metaboliche nella camminata su treadmill tra soggetti adulti normopeso in relazione al loro livello di attività fisica e alla loro physical fitness".

Questa Ricerca è svolta dal Laboratorio di Anatomia Funzionale dell'Apparato Locomotore dell'Università Statale di Milano in collaborazione con il Laboratorio di Fisiologia Sperimentale Applicata all'esercizio fisico e allo sport dell'Università Cattolica del Sacro Cuore di Milano.

Per svolgere tale Ricerca abbiamo bisogno della collaborazione e della disponibilità di persone che, come Lei, soddisfino i requisiti scientifici idonei alla valutazione che verrà eseguita, per questo Le proponiamo di partecipare alla presente Ricerca.

Prima però che Lei prenda la decisione di accettare o rifiutare di partecipare, La preghiamo di leggere con attenzione queste pagine e di chiedere chiarimenti, qualora non avesse ben compreso o avesse bisogno di ulteriori precisazioni, alla Dott.ssa Isabella Annoni (tel. 320 0491863, e-mail isabella.annoni@unimi.it), responsabile della parte operativa della Ricerca.

Prima di decidere, qualora lo desiderasse, può chiedere parere sia ai Suoi familiari sia al Suo medico di fiducia.

2. Scopo della ricerca

Questa Ricerca si propone l'obiettivo di comprendere quale sia il maggior predittore di limitazioni funzionali tra le seguenti variabili: indice di massa corporea, livello di attività fisica, livello di fitness, parametri cinematici della camminata su treadmill e velocità di camminata preferita.

3. Procedure dello Studio

Nel caso Lei acconsentisse di partecipare a questa Ricerca, Le sarà chiesto di firmare questo modulo di Consenso Informato.

Il disegno sperimentale di questa Ricerca prevede 3 giornate di studio:

- Giorno 1: valutazione del livello di attività fisica, dello stato di salute e del benessere , valutazioni antropometriche, analisi della composizione corporea, analisi del dispendio energetico a riposo, valutazione della forza massimale isometrica e della flessibilità, rilevazione della velocità di camminata preferita ,monitoraggio dello stile di vita.

Tali valutazioni verranno svolte presso il Laboratorio di Fisiologia Applicata allo sport e all'esercizio fisico dell' Università Cattolica del Sacro Cuore in viale Suzzani 279, Milano.
La rilevazione del dispendio energetico verrà effettuata durante una settimana a partire da Marzo 2011.

- Giorno 2: analisi dei parametri metabolici e cinematici della camminata su treadmill a differenti velocità.

Le valutazioni verranno svolte presso il Laboratorio di Anatomia Funzionale dell'Apparato Locomotore in via L. Mangiagalli 31, Milano, esattamente una settimana dopo la 1° giornata di test.

- Giorno 3: visita medica, ECG a riposo, test per la valutazione del massimo consumo di ossigeno.

Tali valutazioni verranno svolte presso il Laboratorio di Fisiologia Applicata allo sport e all'esercizio fisico dell' Università Cattolica del Sacro Cuore in viale Suzzani 279, Milano.

Non è detto che le 3 giornate di test seguano scrupolosamente questa disposizione: potrà capitare che nella giornata 1 oppure 2 si eseguano i test della giornata 3 e che quindi l'ordine risulti invertito. Ciò verrà comunicato durante la calendarizzazione, compatibilmente coi Suoi impegni di lavoro e personali.

La Ricerca durerà da Marzo 2011 a Luglio 2011.

Per la 1 giornata di test, il soggetto dovrà prevedere circa due ore di permanenza in laboratorio.
Per la 2 giornata, si prevedono al massimo due ore per l'effettuazione dei test.
Per la 3 giornata, l'impegno richiesto è di un'ora e mezza circa.

Saranno coinvolti 40 soggetti normopeso di età compresa tra i 18 e i 40 anni.

Se accetta di partecipare alla presente Ricerca, dovrà firmato il consenso informato, compilare n°3 questionari (PAR-Q, IPAQ, functional limitations), e calendarizzare le giornate successive di test.

Nelle 3 giornate di sperimentazione sarà necessario aver consumato il pasto principale almeno 2 ore prima dell'inizio dei test, mentre bisognerà evitare di fare attività fisica nelle 48 ore precedenti tali date.

Se accetta di partecipare alla Ricerca dovrà rispettare il protocollo complessivo e dettagliato dello Studio, come specificato precedentemente, al fine di ottenere risultati validi e attendibili.

La Sua partecipazione alla presente Ricerca non comporta alcun aggravio di spese, che saranno totalmente a carico del Laboratorio di Anatomia Funzionale Applicata dell'Apparato Locomotore dell'Università degli Studi di Milano e del Laboratorio di Fisiologia Sperimentale Applicata all'esercizio fisico e allo sport dell'Università Cattolica di Milano.

4. Indagini strumentali previste nel Protocollo di Studio:

Giorno 1, nel Laboratorio di Fisiologia Applicata allo sport:

- Analisi del dispendio energetico a riposo,
- Misurazioni antropometriche,
- Valutazione della composizione corporea,
- Test di forza massimale isometrica su leg press orizzontale,
- Test di flessibilità,
- Analisi della self-selected walking speed,
- Monitoraggio dello stile di vita.

L'analisi del dispendio energetico a riposo (REE) verrà svolto con l'ausilio di una strumentazione non invasiva Fitmate (Cosmed, Italia).

Peso e altezza verranno misurati senza scarpe tramite una bilancia meccanica con stati metro (SECA, Deutschland).

La valutazione della composizione corporea avverrà tramite la metodica della plicometria (Harpenden, Baty International, United Kingdom).

La forza massimale isometrica verrà valutata utilizzando 2 pedane di forza (Twin Plates, Globus Italia S.r.l.) fissate ad una leg press orizzontale vincolata (Technogym, Italia).

La flessibilità verrà valutata attraverso il Sit&Reach test.

Per andare a valutare la velocità preferita di camminata, si utilizzeranno due fotocellule poste a 50 m di distanza l'una dall'altra.

Il monitoraggio dello stile di vita verrà svolto con l'ausilio di un activity monitor (Actiheart, CamNtech, United Kingdom), che dovrà essere indossato per 1 settimana. Durante la settimana di monitoraggio ogni soggetto svolgerà la sua vita quotidiana normale, e nel caso sorgesse qualche perplessità , sarà seguito dalla Dott.ssa Annoni con un follow up telefonico.

Nota: Il soggetto sarà seguito da personale specializzato e qualificato durante ogni attività.

ACTIHEART

FITMATE

Giorno 2, nel Laboratorio di Anatomia Funzionale dell'Apparato Locomotore:

- Analisi dei parametri metabolici della camminata su treadmill a differenti velocità tramite calorimetria indiretta,
- Analisi cinematica del cammino su treadmill a differenti velocità,

L'analisi dei parametri metabolici verrà effettuata tramite un metabolimetro portatile ($k4b^2$, Cosmed, Italia) mentre per i dati cinematici, rilevati contemporaneamente ai precedenti, verrà adoperato un sistema optoelettronico a infrarossi (Smart-D, BTS, Italia).

Per andare a valutare la velocità preferita di camminata, si utilizzerà il sistema optoelettronico citato precedentemente.

Giorno 3, nel Laboratorio di Fisiologia Applicata allo sport:

- Visita medica con ECG a riposo e rilevazione della pressione arteriosa,
- Test massimale diretto per la valutazione del massimo consumo di ossigeno.

La visita medica comprenderà un'anamnesi completa effettuata da un medico dello sport, che rileverà la pressione arteriosa a riposo.

Successivamente verrà effettuato anche un ECG a riposo, tramite elettrocardiografo a 12 derivazioni (P8000 power, Esaote, Italia).

Dopo aver accertato le condizioni di salute del soggetto, si effettuerà un test massimale diretto in camminata su treadmill (Run Race, Technogym, Italia) per andare a valutare il massimo consumo di ossigeno. Durante il test il soggetto indosserà un metabolimetro fisso (Quark b^2, Cosmed, Italia) e un cardiofrequenzimetro (Polar, Finland), e dovrà arrivare all'esaurimento.

Il medico effettuerà anche dei prelievi di sangue capillare (dal lobo dell'orecchio) per controllare il picco di lattato raggiunto.

5. Benefici prevedibili della Ricerca:

Per ogni soggetto verrà effettuata una visita medica accurata per escludere la presenza di patologie cardiovascolari. Inoltre verranno forniti gratuitamente i risultati di tutti i test effettuati e verranno confrontati con i valori normativi esistenti in letteratura. Ai soggetti sedentari verranno anche elargiti dei consigli riguardo la giusta dose di attività fisica da intraprendere, in accordo con le linee guida internazionali dell'American College of Sports Medicine.

Infine potrà essere un'occasione altamente educativa, in quanto si frequenteranno laboratori forniti di apparecchiature molto sofisticate, e si verrà a contatto con figure professionali di diverse competenze, ampliando così il proprio bagaglio culturale.

6. Rischi prevedibili della Ricerca

La partecipazione a questa Ricerca potrebbe comportare dei rischi connessi allo svolgimento di un test massimale e all'utilizzo delle attrezzature come il treadmill durante le prove in camminata. Tali rischi saranno ridotti al minimo attraverso la presenza costante di un medico dello sport e/o un operatore qualificato durante tutte le attività svolte.

7. Lei è libero di non partecipare alla presente Ricerca.

8. La Sua adesione a questa Ricerca è completamente volontaria e Lei potrà ritirare il Consenso alla partecipazione in qualsiasi momento.

9. Allo stesso modo la Ricerca potrà essere interrotta in qualsiasi momento se se il medico constaterà effetti collaterali inattesi/indesiderati.

10. Ai sensi del Decreto Legge N°196/03 (Art.7 e 13) relativo alla tutela della persone per il trattamento dei dati personali, La informiamo che i Suoi dati personali verranno raccolti ed archiviati in modo adeguato e saranno utilizzati esclusivamente per scopi di ricerca scientifica.

Firmando il modulo di Consenso Informato Lei autorizza l'accesso a tali dati , che nel caso in cui la Ricerca fosse multicentrica, potranno essere utilizzati e accorpati a dati provenienti da altri centri/Istituti.

Lei ha diritto, se lo vuole, di saper quali informazioni saranno archiviate ed in quale modo.

I risultati della Ricerca a cui Lei parteciperà potranno essere oggetto di pubblicazione, ma la Sua identità rimarrà segreta.

11. Se lei è d'accordo potrebbe risultare utile informare il Suo medico di famiglia della Sua partecipazione a questa Ricerca per evitare interferenze con eventuali farmaci/procedure.

Se lo richiederà alla fine della Ricerca potranno esserLe comunicati i risultati dello studio in generale ed anche in particolare quelli specifici che La riguardano.

12. Per ulteriori informazioni durante lo Studio sarà a Sua disposizione la Dott.ssa Isabella Annoni, tel 320 0491863;

DICHIARAZIONE DI CONSENSO

Questa Dichiarazione deve essere firmata e datata personalmente dal Soggetto a cui sono state lette e spiegate tutte le pagine qui allegate della Scheda Informativa.

Io sottoscritto ……………………………………………………………………………………… (nome e cognome per esteso del Soggetto)

dichiaro di avere ricevuto dal Dottor ……………………………………………… (nome e cognome per esteso del Ricercatore)

esaurienti spiegazioni in merito alla richiesta della Mia partecipazione allo Studio Sperimentale sopra descritto. Copia della presente scheda informativa mi è stata data.

Dichiaro di aver potuto discutere tali spiegazioni, di aver potuto porre domande e di avere ricevuto risposte in merito soddisfacenti.

Dichiaro inoltre di avere avuto la possibilità di informarmi in merito ai particolari dello Studio anche con altre persone di mia fiducia.

Accetto quindi liberamente di partecipare alla Ricerca, avendo perfettamente compreso tutte le informazioni sopra riportate.

Sono consapevole che la Mia partecipazione alla Ricerca sia volontaria e che ho la facoltà di ritirarmi in qualsiasi momento, senza che tale fatto pregiudichi le cure mediche di cui potrei necessitare.

Sono stato informato del Mio diritto di avere libero accesso alla documentazione relativa alla Ricerca.

Sono inoltre consapevole che secondo il rispetto della normativa vigente i Miei dati personali saranno utilizzati esclusivamente per scopi di ricerca scientifica .

Data…………………………

Firma del Soggetto……………………………………………………………………………………

Data………………………

Firma del Ricercatore ………………………………………………………………………………

Attachment 4: IPAQ questionnaire

QUESTIONARIO INTERNAZIONALE SULL'ATTIVITA' FISICA

Siamo interessati a conoscere i tipi di attività fisica che le persone fanno come parte della vita quotidiana. Le domande riguarderanno il tempo che lei ha trascorso in attività fisiche negli **ultimi sette giorni**. Cortesemente, risponda ad ogni domanda anche se non si considera essere una persona attiva. Pensi, per favore, alle attività svolte al lavoro, come parte del lavoro svolto in casa ed in giardino, per spostarsi da un luogo all'altro e nel suo tempo libero come divertimento, esercizio fisico o sport.

Pensi a tutte le attività **vigorose**, energiche che ha svolto negli **ultimi sette giorni.** Le attività fisiche **vigorose** sono quelle che richiedono uno sforzo fisico duro e che la fanno respirare con un ritmo molto più frequente rispetto al normale. Pensi *soltanto* a quelle attività fisiche che lei ha svolto per almeno 10 minuti consecutivamente.

1. Durante gli **ultimi sette giorni**, in quanti giorni lei ha svolto attività fisica vigorosa come sollevare oggetti pesanti, zappare, fare aerobica, o pedalare in bicicletta ad una certa velocità?

_______ **giorni per settimana**

☐ Nessuna attività fisica vigorosa ---------------------→ **Vada alla domanda 3**

2. Quanto tempo in totale di solito trascorre in attività fisiche **vigorose** in uno di quei giorni?

_______ **ore per giorno**

_______ **minuti per giorno**

☐ Non sa / non è sicuro/a

Pensi a tutte quelle attività **moderate** che lei ha svolto negli **ultimi sette giorni**. Le attività moderate sono quelle che richiedono uno sforzo fisico moderato e che la fanno respirare con un ritmo un po' più frequente rispetto al normale. Pensi soltanto a quelle attività fisiche che lei ha svolto per almeno 10 minuti consecutivamente.

3. Durante gli **ultimi sette giorni,** quanti giorni lei ha svolto attività fisica **moderata** come portare pesi leggeri, andare in bicicletta ad un ritmo regolare oppure giocare il doppio a tennis? Non includa il camminare.

______ **giorni per settimana**

☐ Nessuna attività fisica moderata ---------------------→ **Vada alla domanda 5**

4. Quanto tempo lei di solito dedica alle attività fisiche **moderate** in uno di quei giorni?

________ **ore per giorno**

________ **minuti per giorno**

☐ Non sa / non è sicuro/a

Pensi al tempo da lei trascorso **camminando** negli **ultimi sette giorni**. Includa il tempo trascorso sia al lavoro sia a casa, nello spostarsi da un luogo ad un altro e qualsiasi altro cammino che lei ha fatto solo per divertimento, sport, esercizio fisico o per passatempo.

5. Durante gli **ultimi sette giorni,** in quanti giorni lei ha **camminato** per almeno 10 minuti di continuo?

________ **giorni per settimana**

☐ Nessuno ---------------------→ **Vada alla domanda 7**

6. Di solito quanto tempo ha trascorso, in uno di quei giorni, **camminando?**

________ **ore per giorno**

_______ **minuti per giorno**

☐ Non sa / non è sicuro

L'ultima domanda riguarda il tempo trascorso stando **seduto** dal lunedì al venerdì negli **ultimi sette giorni**. Includa il tempo in cui rimane seduto al lavoro, in casa, nello svolgere un corso di formazione, durante il suo tempo libero. Questo può includere il tempo trascorso seduto alla scrivania, nel far visita ad amici, leggendo, o seduto/a o sdraiato/a per guardare la televisione.

7. Durante gli **ultimi sette giorni**, in un giorno della settimana, quanto tempo ha trascorso stando seduto?

_______ **ore per giorno**

_______ **minuti per giorno**

☐ Non sa / non è sicuro

Qui termina il questionario, grazie per la collaborazione.