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# Ultra-endurance triathlon: heart rate-based intensity profile, energy balance, muscle damage and race performance

Anna Barrero Franquet

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# ULTRA-ENDURANCE TRIATHLON

heart rate-based intensity profile,  
energy balance, muscle damage  
and race performance



Anna Barrero Franquet

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# **Ultra-endurance triathlon: heart rate- based intensity profile, energy balance, muscle damage and race performance**

TESI DOCTORAL PRESENTADA PER:  
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DIRIGIDA PER:  
**Dr. JORDI PORTA MANZAÑIDO**

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*It is not the mountain we conquer but ourselves.*

Sir Edmund Hillary





Als meus pares, Pilar i Antonio.  
Tot el que tinc us ho dec a vosaltres.



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## LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BIA	Bioelectrical impedance analysis
BM	Body mass
CHO	Carbohydrates
CO <sub>2</sub>	Carbon dioxide
ECW	Extracellular water
EE	Energy expenditure
EI	Energy intake
HR	Heart rate
HR <sub>peak</sub>	Peak heart rate
HR <sub>diff</sub>	Difference between heart rates
HSD	Honestly significant differences
ICW	Intracellular water
O <sub>2</sub>	Oxygen
RER	Respiratory exchange ratio
rpm	Revolutions per minute
SD	Standard deviation
T1	First transition: swim-to-cycle
T2	Second transition: cycle-to-run
TBW	Total body water
TRIMP	Training impulse
UET	Ultra-endurance triathlon
VCO <sub>2</sub>	Carbone dioxide production
VE	Respiratory ventilation
VO <sub>2</sub>	Oxygen uptake
VO <sub>2peak</sub>	Peak oxygen uptake
VT	Ventilatory tresholds
VT <sub>1</sub>	First ventilatory treshold
VT <sub>2</sub>	Second ventilatory treshold
W	Watts



# **1. INTRODUCTION**

Physiology has made a significant contribution to the understanding of what the human body goes through during physical training and athletic competition, with an end goal of learning how to maximize the potential of athletes. In the sport of triathlon, however, knowledge of the physiological demands during competition is far from being extensive, likely due to the complexity of studying a sport that consists of multiple modes of exercise and covers a multitude of distances. Answers to the most fundamental question of what factors determine the performance of a sport can only be obtained by studying real competition. Thus, the main purpose of this research is to identify and describe the physiological response, energy balance and muscle damage during an ultra-endurance triathlon (UET) competition.

## **1.1. Triathlon**

### **1.1.1. Brief history of triathlon**

Triathlon is considered by some to have rooted in France in the 1920-1930's. Competitors participated in a tri-sport race called variously "Les trois sports", "La Course des Débrouillards", and "La course des Touche à Tout"— which consisted of crossing the channel Marne, a 12 km bike ride and a 3km run. Those three parts were done without any break. Nowadays, this race is held every year at Joinville le Pont, in Meulan and Poissy (France) (Tinley, 1999).

But the modern triathlon is considered to have been conceived in San Diego (California, USA) in 1974 under the direction of Jack Johnstone and Don Shanahan. The first 'Mission Bay Triathlon' was held on September 25, 1974, a date considered by many to be the 'birthday' of triathlon. The course was a 6 miles of running, 5 miles of biking and 500 yards swimming (in this order), and welcomed 46 athletes. John Collins, the Ironman founder, was among the athletes competing that day (Tinley, 1999).

Fifteen years later, the International Triathlon Union (ITU) was founded in 1989 in Avignon, France, the site of the first official World Championship. Since then, the sport has grown rapidly and now has over 140 affiliated National Federations around the world. As the youngest International Federation in the Olympic Games, triathlon made its debut at the Sydney Games in 2000, and was competed over what is typically referred to as the Olympic Distance course (1.5 km swim – 40 km bike – 10 km run). The growth in popularity and interest of the sport of triathlon differs between countries, and Spain is no exception.

The first historical reference of triathlon in Spain is an event called "Ciclo-Nata-Cross" which consisted of a 1.2 km bike, 0.2 km swim and 1.3 km run organized as a contest in 1963 in Castro Urdiales. But it is not until 1984 when in Guadalajara took place the first modern triathlon (Ballesteros, 1987). The Spanish Triathlon Federation (Federación Española de Triatlón, FETRI) was not established until 1999.

### *The origin of ultra-endurance triathlon events*

The origin of the ultra-endurance triathlon dates back to 1978 when John Collins, a US Marines commander and a group of athletes discussed what type of athlete was the best, the toughest, and the fittest. From those discussions, Collins set a challenge: to combine the three hardest Hawaiian events into one ultimate athletic challenge. The Waikiki Rough Water swim (3.8 km), the Around Oahu Bike Race (180 km), and the Honolulu Marathon (42.2 km) were the competitions that were combined into a single race. Prior to the race Collins had declared that, “whoever finishes first we will call the Iron man” (Ballesteros, 1987). This is how the trademark Ironman was born (Tinley, 1999). Without marketing efforts, the race gathered 50 athletes in 1979 (Figure 1).



**Figure 1.** Pioneering first triathletes at an aid station, Hawaii, 1979 (source: [www.ironman.com](http://www.ironman.com)).

Currently there are more than 20 sanctioned Ironman events throughout the world under the Ironman World Triathlon Corporation trademark that are considered qualifiers for the Hawaii Ironman World Championship in Kona on Hawaii's "Big Island". In Spain, to the best of our knowledge, the oldest ultra-endurance triathlon event is the 'Ironman Lanzarote'. The first edition was held in 1992 in the southern town of Playa Blanca. The competition was hosted by Club La Santa, and had 148 participants, 116 of whom were finishers. In the present, due to triathlon's growth and popularity around the world, new events with the same distance, but different name, are being created.

### **1.1.2. General characteristics of triathlon**

Triathlon is a unique endurance sport that comprises a sequential swim, swim-to-cycle transition (T1), cycle, cycle-to-run transition (T2) and run (see Figure 2). Competitions are usually performed over the short (Sprint and Olympic) distance, or over the long (Ironman) distance (Table 1).





**Figure 2.** The five sequential sections that comprise a triathlon: swim, swim-to-cycle transition, cycle, cycle-to-run transition and run.

The specific rules and regulations for triathlon can vary depending on the competition. There are some universal rules such as the prohibition of the use of performance enhancing drugs or procedures, and the prohibition of external assistance during the race. Other rules can vary from race to race like the allowance, obligation or prohibition to use wetsuits during the swimming leg (depending on the water temperature).

**Table 1.** Discipline distance breakdown for common triathlon events.

Event	Swim	Bike	Run
Sprint	0.75 km	20 km	5 km
Olympic	1.5 km	40 km	10 km
‘Ironman’	3.8 km	180 km	42.2 km

The following describes the legs and transitions of an ultra-endurance ‘Ironman’ triathlon, without lack of generality. Also, the most relevant rules are discussed (rules and regulations are available at Ironman (2014)).

### ***Swim course***

The triathlon starts with an open water swim (lakes, oceans, etc.). It is common that triathletes use their legs less vigorously than other swimmers, preserving their leg muscles for the next courses. To closely follow a competitor during swimming (drafting) is allowed and may represent a gain in performance (Delextrat et al., 2003). Further, athletes must wear the head cap provided by the race organizers, and may wear swim goggles or facemasks. Finally, the use of wetsuits is permitted if the water temperature is below or equal 24.5 degrees Celsius, and prohibited if greater.

### ***Cycle course***

The cycle course takes place on asphalt roads. Triathletes can use road or triathlon bicycles, usually optimized for aerodynamics. It is mandatory to wear a bike helmet with the number on the front, and a clearly visible number fixed to the bike frame.

Maybe the most influential rule in this leg is about drafting, which determines whether or not a cyclist can ride directly behind (less than 7 m) another cyclist. In the case of ultra-endurance triathlons, drafting is not allowed. The no drafting rule results in more individual and less tactical race. Conversely, the International Triathlon Union has adopted the style of draft-legal racing for the elite competitors, a rule that promotes pack style racing.

### ***Run course***

The run course takes place on asphalt, dirt roads and gravel tracks. Athletes must wear a shirt or racing top and the number in front of them clearly visible. The run course will officially close at midnight (17 hours after the event start).

### ***Swim-to-cycle transition (T1) and cycle-to-run transition (T2)***

There is a transition area designated to change over from discipline to discipline and to keep the material (bicycle, helmet, bike shoes, running shoes, hydration and other gear) ready to use. There are specific rules affecting the transitions (e.g., a helmet is required both exiting and entering the transition area) that athletes should be aware of as violating these rules may result in a penalty or disqualification.

### **1.1.3. Performance factors in ultra-endurance triathlon**

Ultra-endurance triathlon (UET) is an extremely tough competition consisting of a 3.8 km swim, 180 km of cycling and 42.2 km of running. Average completion time is around 16 h for slower triathletes and approximately 8 h for faster, more successful triathletes (Lepers et al., 2012).

With the growth and popularity of UET, the interest in optimal training and performance strategies has also increased (Laursen, 2011). Successful ultra-endurance performance relies on long-term physical preparation, sufficient nutrition, accommodation of environmental stressors, and psychological toughness (Zaryski & Smith, 2005). The study of the body's physiological response to UET's exercise stimulus may offer insight into the important factors related to its performance, ultimately as a means to optimize performance and recovery.

### ***Food energy and water requirements***

One of the main goals for being successful in a UET is to manage the consumption of food and drinks throughout the race (Laursen & Rhodes, 2001) so as to enhance performance while maintaining body homeostasis. An adequate energy intake is essential for optimal performance in ultra-endurance athletes as an insufficient caloric intake can lead to chronic fatigue, weight loss and impaired physical performance (Applegate, 1991). A proper balance between energy expenditure and energy intake and the capacity to generate energy at a high rate are important factors related to UET performance (O'Toole & Douglas, 1995).

In addition to managing energy intake, ensuring proper hydration status is also important. Water constitutes ~73% of muscle weight, and during exercise sweat evaporation is the major physiologic defense against overheating. If not properly rehydrated, sweating can represent a serious loss of body water. Thus, proper fluid replacement maintains plasma volume to optimize the circulatory and sweating response (McArdle et al., 2011).

### ***Thermoregulatory demands***

Macronutrients from food and drink are converted to mechanical energy that powers the triathlete during the UET. This energy exchange is accompanied by the release of energy in the form of heat (~75%). Heat energy, although necessary to maintain temperatures required for life, may negatively affect the performance of triathletes in an uncompensable thermal environment. When the rate of heat production exceeds that of the triathlete's ability to release it from the body, core body temperature increases (Laursen, 2011). A range between 34.2 and 40.5°C of core body temperatures have been recorded during and after UET (Laursen, 2011). The high body temperatures and increased water loss due to sweating likely reduce the performance (i.e. speed) during the race (Laursen, 2011). The best UET performances in a hot environment are typically from triathletes that have acclimatized to exercise in those conditions.

Heat acclimation has been found to successfully improve performance (Lorenzo et al., 2010). Lorenzo et al. (2010) found that heat acclimation induces positive physiological adaptations such as reduced oxygen uptake at a given power output, muscle glycogen sparing, reduced blood lactate at a given power output, plasma volume expansion, improved myocardial efficiency and increased ventricular compliance. So, as most of the UET are usually scheduled during the hotter months of the year, a heat acclimation program prior to competition can be helpful in order to perform better during races in hot environments.

### ***Muscle damage***

Another key point that affects UET performance is the muscle damage suffered by athletes. In spite of the symptoms associated with the exercise-induced muscle damage, detrimental consequences of this damage include a decrease in force production, a decrease in muscle power, and a decrease in exercise performance. During competition, muscle damage can impair the athlete's optimal performance, especially in endurance events (Thiebaud, 2012).

Ultra-endurance triathletes are exposed to efforts which inevitably produce this muscle damage, but it could be reduced with an oriented strength training program in order to build muscle adaptations to the muscular fibers that are most affected. The problem is that the origin of such muscle damage still remains elusive.

## **1.2. Cardiovascular profile of ultra-endurance triathletes**

### **1.2.1. Introduction to cardiovascular profile in endurance sports**

The cardiovascular system, or circulatory system, is composed by the heart, arteries, veins and capillaries. During physical activity it delivers oxygen and nutrients to active tissues, aerates blood returned to the lungs, and transports hormones and heat throughout the body (McArdle et al., 2011). Exercise training induces adaptations on the cardiovascular system to meet body's oxygen demands (Baggish et al., 2011).

Cardiac output, the product of heart rate and the quantity of blood ejected with each stroke, provides the most important indicator of body's functional capacity to meet the demands for physical activity. In exercise conditions, endurance athletes present higher cardiac output than sedentary individuals, but at lower heart rate. It can be deduced from the above equation, that endurance athletes have a greater stroke volume to transport more oxygen to muscles. Thus, exercise training induces an increase in stroke volume, an increase in maximum cardiac output that directly improves the capacity to transport oxygen, and an increase in the maximal rate of oxygen consumption ( $VO_{2max}$ ) (McArdle et al., 2011).

Initial reports from Darling (1899) and Henschen (1899) describing this adaptive physiology in athletes dates back to the late 1890's. Thanks to advances in cardiovascular diagnostic techniques, it is now well established that repetitive vigorous training results in significant changes in the heart structure (Baggish et al. 2011). For instance, Parker et al. (1978) performed a non-invasive cardiac evaluation of twelve long-distance runners that were compared to normal control subjects. The athletes showed a higher frequency of gallop rhythm with lower heart sound. Echocardiographic examination revealed increased wall thickness, left ventricular muscular mass, diastolic volume, and ventricular function. Some of these variations were also reported in long-distance walkers (Kaimal et al., 1993). For recent reviews on the science of cardiac remodelling in athletes see Naylor et al. (2008) and Baggish et al. (2011).

### **1.2.2. Cardiovascular profile of ultra-endurance triathletes**

In the case of triathlon, the literature contains several studies of simulated and real competitions (Cuddy et al., 2010; Díaz et al., 2009; González-Haro et al., 2005; Hausswirth & Lehenaff, 2001; Kohrt et al., 1987; Kreider et al., 1988; Laursen & Rhodes, 2001; Laursen et al., 2002; Laursen et al., 2005; Millet & Bentley, 2004; Millet et al., 2003; Perrey, 2003); however, relative to individual metabolic capacities, there is no clear knowledge of the physiological demands for an entire triathlon of any distance. Although an incomplete knowledge of the physiological response during triathlon exists, evidence from the study of singular modes of exercise, according to the principle of testing specificity, may be helpful to understand triathlon performance.

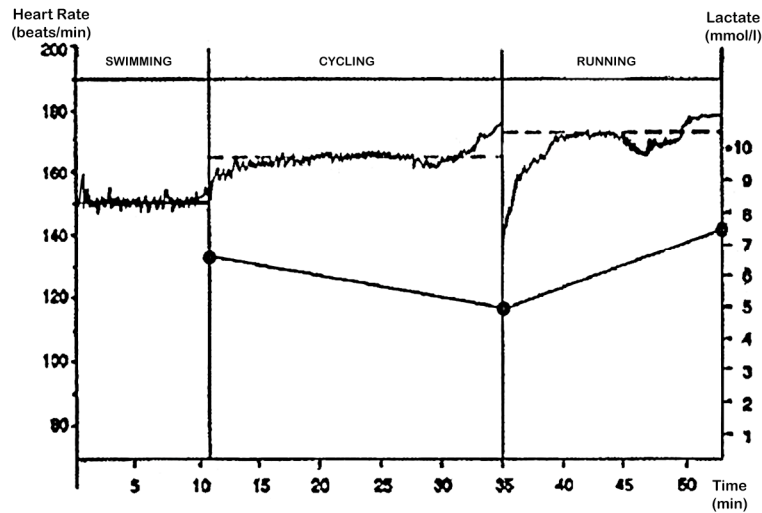
Previous studies report that the peak heart rate ( $HR_{peak}$ ) and peak oxygen uptake ( $VO_{2peak}$ ) are lower during swimming than during running or cycling as presumably less muscle mass is activated (i.e. swimming is predominantly an upper body exercise, with minimal activation of the relatively large muscle mass of the legs. Further, the haemodynamics associated with a horizontal body position and the effect of gravity makes it easier for blood to move to and from the heart. Finally, reflex bradycardia is smaller (i.e. the autonomic nerve reflex lowers heart rate and blood pressure) (Hauber et

al., 1997; Holmér & Astrand, 1972; Magel et al., 1975; McArdle et al., 1978). However, more recent results showed that  $VO_{2peak}$  in a maximal 400-m swim is not different from that achieved during maximal cycling and running in competitive swimmers despite attaining a lower  $HR_{peak}$  (Rodriguez, 2000).

While important to consider the different metabolic demands between the three modes of exercise in a UET, more important is the relative intensity with which triathletes perform in each mode. Field-based research has shown that well-trained triathletes perform the cycling stage of a UET at 80-83% of maximum HR ( $HR_{max}$ ) (Laursen et al., 2005; O'Toole & Douglas, 1995) and 55% of peak power output (Abbiss et al., 2006). Other studies show a metabolic intensity of around 55% of  $VO_{2peak}$  (Laursen et al., 2002; O'Toole et al., 1987).

A more resolute analysis shows that the intensity of amateur triathletes during a UET tends to decrease as the race progresses (Abbiss et al., 2006). That is, the intensity at the beginning of the competition is higher than at the end. Evidence supporting this notion (Laursen et al., 2002; O'Toole et al., 1998; O'Toole et al., 1987), describe 6-7% reductions in the heart rate (HR) measured in the cycling and running stages of UET (Laursen et al., 2002; O'Toole et al., 1998; O'Toole et al., 1987). However, it does not seem reasonable to assume that exercise intensity in high-level triathletes should decrease during the running stage of a UET.

In contrast, according to Berbalk et al. (1997) the HR in a sprint distance triathlon (0.75-20-5 km) tends to increase as the race progresses. They designed a laboratory test that simulated the conditions of a triathlon and reported lower HR values in the swimming stage than in the cycling and running stages (Figure 3).



**Figure 3.** Heart rate and lactate concentration in a simulated sprint triathlon (0.75-20-5 km) (Berbalk et al., 1997). Figure adapted from Cejuela et al., 2007.

In a sprint triathlon, where drafting is allowed in the cycling leg, emerging from the swim in the leading group may be crucial as it allows the athletes to cycle in a pack. Riding in a pack avails the athletes an opportunity to ‘shield’ themselves from air resistance behind other competitors during the cycling stage, which can reduce the energy cost by up to 30 % (Faria, 1992). In fact, the advantage of being in the first pack out of water significantly increases the triathlete’s chances of winning an event (Landers et al., 2008).

Conversely, in non-drafting events like UET, it may be considered that the swim leg plays a less important role due to its shorter duration compared to the other legs. Further, the swim is less of a factor because the triathletes cannot get the advantage of emerging earlier in order to be in the first cycling pack. Thus, if during a sprint triathlon the HR is found to be lower than the rest of the portions (Berbalk et al., 1997), then, apparently the HR during the swimming portion of a UET, which has less ‘importance’, would be as well lower compared to the other portions.

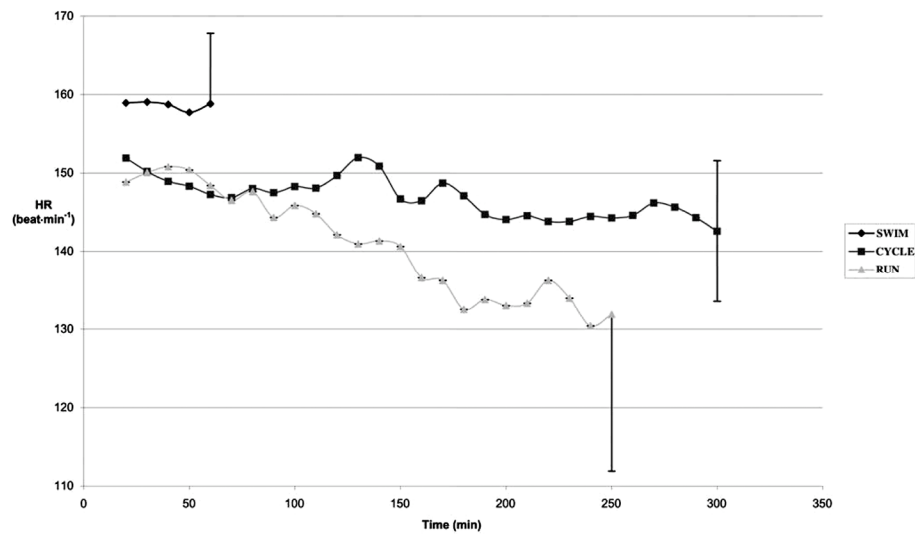
Whyte (2006) states that for the same relative intensity of work, heart rate was lower during swimming than cycling or running, and stroke volume, blood pressure and



blood lactate were all higher. The author postulates that these results were a result of the body being horizontal submerged in water and the use of the arm musculature (which is smaller than lower body musculature groups) as the primary means of locomotion in swimming exercise.

The study by Laursen et al. (2005) is the only one, to the best of our knowledge, assessing physiological demands during an UET by measuring HR in a real competition (Figure 4). This study of well-trained triathletes shows HR values during the cycling stage (148 (9) beats·min<sup>-1</sup>) and during the running stage (143 (13) beats·min<sup>-1</sup>) that were significantly lower than the second ventilatory threshold (VT<sub>2</sub>) calculated in the laboratory (160 (13) beats·min<sup>-1</sup> and 165 (14) beats·min<sup>-1</sup>, respectively). Nevertheless, the HR values during the cycling and running stages were significantly related to, and not significantly different from, the first ventilatory threshold (VT<sub>1</sub>). Further, the difference between the HR during the cycling stage and the HR at VT<sub>1</sub> was correlated with the marathon time and the total triathlon time, such that the higher HR difference, the slower the marathon and total time. Finally, in contrast to the theory that swimming is less metabolically demanding than cycling or running, the authors also reported higher HR values in the swimming stage than in the cycling or running.

Despite being a pioneering work, the study had some methodological limitations that should be taken into account. Firstly, the laboratory measurements were taken on various ergometers and metabolic measurement systems that were not calibrated with one another. Secondly, competition data were gathered from three different UET (Ironman) events, each with different environmental and track conditions. Thirdly, the time pattern did not take into account the progressive decrease of the sample size along the race as athletes ran into transitions or finished. Finally, HR during the swimming stage was not properly described and related to the testing profile of the athletes during swimming.



**Figure 4.** Heart rate response during the swim, cycle and run phases of a UET (Laursen et al., 2005).

Despite these methodological limitations, and although Laursen et al. (2005) reported higher HR values during the swimming than the cycling and running stages (Figure 4), we hypothesized that, according to the previous studies above mentioned, the HR values in the swimming stage would be significantly lower than in the cycling and running stages, and that the exercise intensity in the three disciplines would be around  $VT_1$ .

## 1.3. Nutritional behavior in ultra-endurance triathlon

### 1.3.1. Introduction to nutritional behavior in endurance sports

The human body needs support in the form of proper nutrition and hydration. The carbohydrate, lipid, and protein macronutrients consumed daily supply the energy to maintain bodily functions. During endurance exercise, the majority of energy comes from glycogen stored in muscles, and once glycogen stores are depleting the much slower energy supply from fat becomes crucial. At this point bloodborne glucose becomes the major source of the limited carbohydrate energy (McArdle et al., 2011).

Meeting macronutrient demands can help in preventing hypoglycaemia and is an important priority for successful participation in ultra-endurance exercise (Peters, 2003).

The nutritional strategy during ultra-endurance exercise is one of the main concerns of athletes competing in this kind of event. A negative energy balance leads to a reduced blood glucose concentration and glycogen depletion during endurance exercise, and is associated with fatigue (Robins 2007). Therefore, due to the importance of carbohydrates (CHO) to avoid fatigue, it is important to examine the limitations and characteristics of CHO absorption.

Gastric emptying, which determines the rate at which nutrients are emptied into the duodenum (where CHO can be absorbed into the blood-stream), is regulated by a number of stimuli such as particle size, dietary fiber, meal volume, meal temperature and osmolality (Laursen & Rhodes, 2001). It has been demonstrated that electrolytes increase the rate of gastric emptying and are necessary for hydrolysis and absorption, and sodium has a role in glucose and water absorption. In addition, particles larger than 1 mm in diameter are refused by the pylorus, and therefore solid food should be reduced to this particle size. Additionally, meals rich in fiber content delay gastric emptying and transit of the small intestine. Furthermore, a liquid meal empties more quickly than solid meal, which apparently makes a liquid diet more convenient than solid foods. Moreover a liquid meal has reduced bulk and can supply water, CHO, protein, vitamins and minerals. Ingesting cold liquids has been shown to increase the rate of gastric emptying. (Laursen & Rhodes, 2001). All this provides reasons for the endurance athlete to consume cold CHO-electrolyte beverages during ultra-endurance events.

Fluid ingestion is not only important for performance, but is also necessary to maintain a proper cardiovascular homeostasis and to guarantee the athlete's health during endurance events (O'Toole & Douglas, 1995). In the field of ergogenic aids there is a *before & after* of what happened in the female Marathon run of Los Angeles 1984 Olympic Games. The dramatic last four hundred meters of Gabriela Andersen Schiess, five minutes forty four seconds of zigzag walking with her torso twisted, her left arm limp and her right leg mostly seized, felling many times and waving away any medical

assistance, horrified the crowd. Before Gabriela's marathon, the dilemma among sport physiologists was only 'to drink or not to drink'. After, despite a short period when many athletes and sports physiologists fell into the error of recommending an exaggerated water intake -favoring hyponatremia, there is consensus about the complexity of fluid replacement during ultra-endurance sports. It is not only a problem of the amount or volume of water and composition of the energetic products to ingest, but also the timing of everything.

In 1996, the American College of Sports Medicine issued the position statement "runners should be encouraged to replace their sweat losses or consume 150-300ml every 15 minutes". Other research work has recommended that athletes should drink early and as much fluid as tolerable (Sawka & Coyle, 1999) or an amount that matches sweat rates (Convertino et al., 1996). However, Laursen et al. (2011) state that the best advice for athletes competing in long distance events is to drink according to thirst.

### **1.3.2. Nutritional behaviour in ultra-endurance triathlon**

A UET is a very demanding event that, besides the specific training and the recommendations according to the literature described above, requires nutritional practice. Very little information is available on the nutritional pattern of triathletes during training for or competing in real UET events. This information would be useful in order to face the two essential energy balance problems: to maintain energy balance on a daily basis during prolonged training and to generate energy at a high rate during triathlon racing (O'Toole & Douglas, 1995).

For the preparation of the nutrition, it is important to consider the energy balance and to align dietary strategy with energy substrate usage. For this, the physiological demands and the energy expenditure (EE) of such events are important to be considered in order to calculate the required energy intake (EI) (Robins, 2007). It has been estimated that the energy expenditure for a UET may range from 8,500 to 11,500 Kcal (Kimber et al., 2002; Kreider, 1991; Laursen & Rhodes, 2001). However, to the best of

our knowledge, only two previous studies (Kimber et al., 2002; Cuddy et al., 2010) have investigated this issue under field conditions.

Kimber et al., (2002) assessed the energy balance of a UET using heart rate-VO<sub>2</sub> regression equations during cycling and running as well as a multiple regression equation during the swimming section. They estimated an EE of 10,036 kcal and 8,550 kcal in ten males and eight females, respectively. However, the energy intake was only 3,940 kcal and 3,115 kcal in both groups showing an energy deficit above 60% through the race.

The other study was done by Cuddy et al. (2010). They combined in a case study two different approaches to assess the EE; indirect calorimetry and doubly labeled water. The data from both assessments were similar indicating that the EE of the athlete was ≈9,000 kcal. Nevertheless, this study did not assess the dietary consumption and fluid ingestion of the athlete during the event and, consequently, the EI and the energy deficit were not shown. Therefore, given these limitations and the dearth of research relating to the nutritional practice in ultra-endurance triathletes, it seems important to investigate the energy demands and the nutritional pattern of triathletes during real events.

It is also important to consider that the reduction of the body stores of energy can also explain changes in the body mass (BM) of athletes after ultra-endurance events. For instance, there is evidence indicating a significant decrease in muscle density (glycogen loss) and fat mass of athletes after a UET (Knechtle et al., 2010; Knechtle et al., 2011; Knechtle et al., 2008; Mueller et al., 2013). In addition, Laursen et al. (2006) reported that a BM loss of up to 3% was not linked with thermoregulatory failure in 10 triathletes performing a UET, suggesting that part of the BM reduction occurred due to losses of glycogen and fat.

Another explanation for the overall reduction in BM is that due to sweating. Sweat was estimated to account for 6% to 8% of the loss in body mass in marathon runners (McArdle et al., 2011). The evaporation of sweat while exercising releases about 600 kcal of heat energy per litre of sweat evaporated from the skin (McArdle et

al., 2011), which makes humans able to perform ultra-endurance exercise (Laursen, 2011). However high sweat rates means that water is removed from our body at high rates, and must therefore be replaced. This makes fluid ingestion one of the key factors for the UET performance. Nevertheless, as shown by Sharwood et al., (2004) dehydration does not necessarily lead to detriments in exercise performance, and subtle dehydration does not cause increases in rectal temperature during a UET in moderate ambient conditions.

On the other hand, previous studies by Speedy et al. (Speedy, Noakes, et al., 2000; Speedy et al., 1999; Speedy et al., 2001; Speedy, Rogers, et al., 2000) have shown that triathletes performing a UET may suffer from exercise-associated hyponatremia even despite modest fluid intakes. This fact can be linked to renal function disturbances (Rüst et al., 2012; Shephard, 2011), which may induce an overload of extracellular water (ECW). However, changes in BM during longer events are not only related to fluid balance as explained above.

We hypothesized that triathletes performing a UET would incur a substantial energy deficit of > 70% due to the high energy demands of these types of events and the limited food intake of athletes through the race. We also hypothesized that sweat losses during UET under hot environmental conditions (over 25°C and 80% humidity) would not be compensated by fluid ingestion.

## **1.4. Muscle damage induced by an ultra-endurance triathlon**

### **1.4.1. Introduction to muscle damage in endurance exercise and its markers**

Long duration and unaccustomed exercise induces muscle damage that is characterized by myocellular morphological changes and enzyme leakage (Armstrong, 1986; Van Rensburg et al., 1986). Due the fact that the total volume of a race is never included in one training bout, endurance sports such as triathlon are unaccustomed exercise even for the more trained athletes (Margaritis et al., 1999).

Serum levels of skeletal muscle enzymes are markers of the functional status of muscle tissue and vary widely in both pathological and physiological conditions (Brancaccio et al., 2007). An increase in these enzymes may represent an index of cellular necrosis and tissue damage following acute and chronic muscle injuries. To assess the amount of skeletal muscle damage, plasma creatine kinase activity and plasma myoglobin levels have been widely used as markers for muscle injury (Sorichter et al., 1999). Though creatine kinase and myoglobin are two of the most useful serum markers of muscle injury, they are not totally specific for skeletal muscle and they do not discriminate the type of fiber damaged.

On the basis of muscle fiber enzymatic and contractile properties, there are two classes of fibers distinguishable in human skeletal muscle, which are designated as slow-twitch and fast twitch. There is great variability in the percentage of fiber types among athletes. However, it is well known that endurance athletes are characterized for having a greater proportion of slow-twitch fibers (Burke et al., 1977; Costill, et al., 1976; Ricoy, et al., 1998). These fibers are recruited for aerobic activities and therefore have many characteristics needed for endurance, such as perfusion with a large network of capillaries to supply oxygen, lots of myoglobin to transport oxygen, and lots of mitochondria—the aerobic factories that contain enzymes responsible for aerobic metabolism. True to their name, slow fibers contract slowly but are very resistant to fatigue.

Myosins, which are microscopic molecules that use chemical energy to power the movement (both voluntary and involuntary) of the muscles, present an ideal profile as a parameter to study and is directly assignable to the grade of the lesion since, because of its high molecular weight, its appearance in blood can only be explained by a fiber lesion (Guerrero et al., 2008).

Myosins from white fibers (fast myosins) and from red fibers (slow myosins) have different biochemical properties (Samaha et al., 1970). Fast myosin is characteristic of fast skeletal muscle only, while slow myosin is common to skeletal and cardiac muscle. As there are mixed muscles (slow fibers and fast fibers), lesions allow

the entrance of both slow and fast myosins into the blood. However the two types of fibers have different resistance to lesions. The level of slow or fast myosin in the blood depends on the type of fiber damaged (Guerrero et al., 2008). Therefore an evaluation of fast and slow myosin levels in blood after exercise is a useful aid able to differentiate injuries by measuring myosins from fast and slow muscle fibers.

#### **1.4.2. Muscle damage induced by an ultra-endurance triathlon**

Muscle damage can result in muscle function impairment (Gibala et al., 1995) and has been reported to occur in UET races (Margaritis et al., 1999; Neubauer et al., 2008). This damage occurs irrespective of training level, and may actually be higher in well trained athletes due to the higher run speeds, greater muscle mass engaged, and reduced amount of walking relative to less trained triathletes (Laursen, 2011). Indeed, the majority of the muscle damage that occurs during a long distance triathlon originates from the eccentric-based contractions of the run phase (Farber et al., 1991). The damage produced is characterized by muscle soreness, loss of muscle function and reductions in aerobic capacity (Nosaka et al., 2010; Suzuki et al., 2006).

Suzuki et al. (2006) showed reductions in muscle strength and jump height, with accompanying increases in muscle soreness, blood markers of muscle damage, and inflammation in the 24 h period following a UET triathlon. Further, muscle breakdown was highlighted as one of the most relevant sources of muscle fatigue during a triathlon by Coso et al. (2012). The authors analyzed the myoglobin and creatine kinase concentrations after a half UET (1.9km swimming, 90 km cycling and 21.1 km running). In spite of all these studies, none of these discriminated the type of fiber damaged.

Myosins, as stated above, are a good marker to assess the injuries from fast and slow muscle fibers separately. We hypothesized that triathletes performing a UET would have a higher damage in the slow fibers than in the fast fibers, and that this damage would be related to the hydration status.





## 2. AIMS

The aims for the Study I were:

- To provide the first comprehensive characterization of the heart rate-based intensity profile during an entire UET race estimated from the HR-VO<sub>2</sub> relationship assessed during specific graded tests in each of the three exercise modes for each individual subject.
- To relate laboratory and field physiological parameters to race performance.
- To determine the ability of different variables to predict the race performance.

The aims for the Study II were:

- To provide a proper characterization of the energy and fluid intake of a group of male triathletes during a whole UET race.
- To estimate the EE and the fluid balance (intra and extracellular stores) of triathletes throughout the race using three different locomotion-specific individualized equations.
- To assess the muscle damage after a UET race through the evaluation of fast and slow myosin serum levels and creatine kinase concentration.
- To relate the muscle damage to hydration status.
- To determine the ability of different variables to predict the race performance.



## 3. METHODS

### 3.1. Design and schedule

This thesis has been designed to study the physiological response, energy balance and muscle damage induced by a UET. To perform this, two weeks before the race subjects carried out three incremental tests to volitional exhaustion in each discipline. On the day of the race, pre-race measurements of BM and bioelectrical impedance were assessed, and a venous blood sample was collected. During the race, HR was continuously monitored and nutritional data collected. After the race, BM and bioelectrical impedance were assessed again. Lastly, 48 h after the race, BM and bioelectrical impedance were measured a third time, and a second venous blood sample was collected. Table 2 summarizes this schedule.

**Table 2.** Schedule of the study.

Dates	Procedures and tests
May 1-16, 2011	Recruitment of subjects
May 16 - 29, 2011	Swimming tests
May 16 - 29, 2011	Cycling tests
May 16 - 29, 2011	Running tests
June 5, 2011	Pre-race measurements
June 5, 2011	Ultra-endurance triathlon race measurements
June 5, 2011	Post-race measurements
June 7, 2011	48 h post-race measurements

### 3.2. Subjects

We placed an advertisement on the triathlon race webpage to recruit non-professional male triathletes. The inclusion criteria were to train at least 10 h per week and the participation in at least one UET during the past 3 years.

We chose fifteen triathletes of regional and national level from among those who volunteered to participate in the UET race and the test measurements designed for the study. The average experience of participants in UET and ultra-endurance events was of  $10 \pm 3$  years and they had been training regularly for approximately 15-18 hours a week for at least the three previous years.

The characteristics of the subjects that comprised each study are summarized in Table 3. Because we experienced technical problems with some data measurements (malfunctioning of prototype Polar RCX5 portable monitors) and some subjects failed to attend to all the programmed assessments, each study was composed of a subset of the fifteen subjects. Before participating in the study, all subjects undertook a medical examination which included a physical examination (body composition), a medical questionnaire, and an electrocardiogram within the same year to ensure that each participant was in good health and gave their informed written consent, which was in accordance with legal requirements and the Declaration of Helsinki, and approved by the Catalan Sports Council's Ethics Committee.

**Table 3.** Subject characteristics in the two studies which compose this thesis.

Study	n	Age (yr)	Body mass (kg)	Height (cm)	Body mass index (kg·m <sup>-2</sup> )	VO <sub>2max</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )
Study I	9	36.7 (5.5)	75.2 (6.7)	174 (6)	24.8 (1.9)	67.0 (4.3)
Study II	11	36.8 (5.1)	75.5 (6.4)	174 (6)	24.8 (1.7)	66.9 (4.1)
Study II*	11	37.2 (4.6)	74.3 (6.7)	174 (6)	24.5 (1.9)	67.5 (4.2)

\*This sample was used for the analysis of the muscle damage and its relationship with the hydration status.

### 3.3. The ultra-endurance triathlon

The chosen event was the *Extreme Man Salou–Costa Daurada* triathlon, held on 5 June 2011. It was chosen for the proximity of the venue to Barcelona (107 km) and the convenience of dates. The willingness of organizers to collaborate with us also greatly facilitated the development of the study.

*Extreme Man Salou–Costa Daurada* triathlon, with more than 370 registered athletes from eleven countries, is among the hardest UET around the world due to 2,600 meters of gain in elevation over the course of the cycling stage. The distance of each stage consisted of a 3.8 km swim, a 180 km cycle, and a 42.2 km marathon run. No drafting was allowed during the cycling stage (for more details about the circuit we refer the reader to *Annex 4*).

Environmental conditions registered during the race indicated that the average ambient air temperature was 26°C (range 13–30°C), the water temperature was 21 °C (range: 20.8–21.2 °C), and the relative humidity was 77% (range: 64–94%). The average wind speed was 1.3 m·s<sup>-1</sup> (range: 0.3–5 m·s<sup>-1</sup>).

### **3.4. Preliminary testing**

Two weeks before the race, all subjects reported three times to the physiology laboratory or to a swimming pool to perform three incremental tests to volitional exhaustion in each discipline under randomised conditions.

The tests consisted of a graded swimming test in a 50-m pool, a graded cycling ergometry test in the laboratory and a graded treadmill running test in the laboratory.

Running and cycling tests were executed under controlled conditions (22 ± 1°C, 40–60% relative humidity, Pb 1013-1027 hPa) separated by at least 48 h.

All the tests were performed at the same time of day to minimize the effects of circadian rhythms. And triathletes were asked to refrain from caffeine, alcohol and heavy exercise on the day before the tests, and to report to the laboratory well hydrated and having eaten more than three hours before the tests.

#### **3.4.1. Swimming test**

The swimming test was conducted in an outdoor 50-m swimming pool. The water temperature was 24 °C.

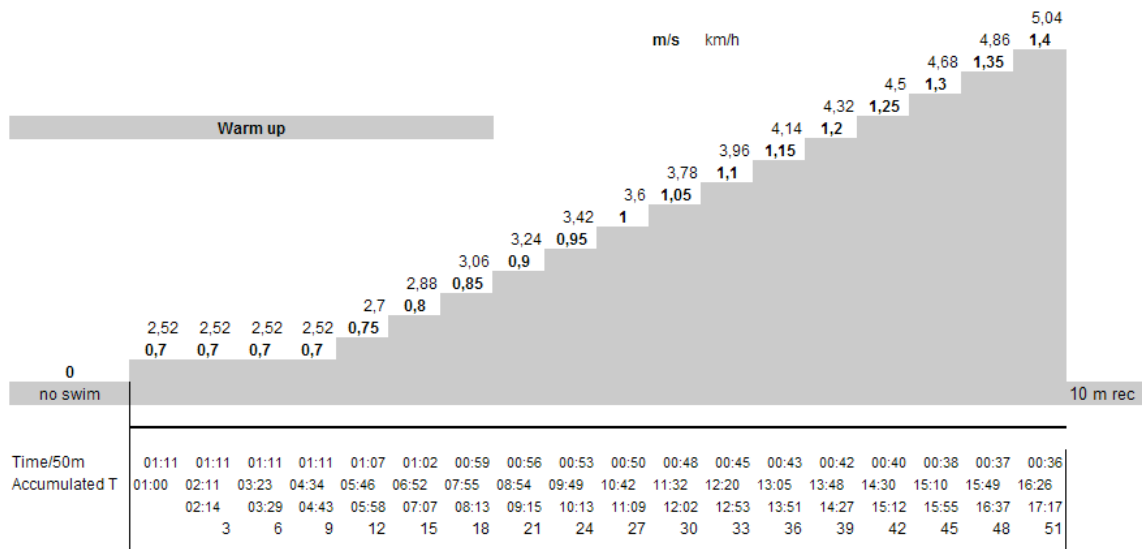
The protocol used was as follows (see Figure 5). After a standardized warm-up of 200 m at  $0.7 \text{ m}\cdot\text{s}^{-1}$ , subjects started at a speed of  $0.75 \text{ m}\cdot\text{s}^{-1}$ , which was increased by  $0.05 \text{ m}\cdot\text{s}^{-1}$  every 50 m until the athlete could not keep up with the imposed pace. Subjects followed a red ribbon hanging from a thread at the top of a hook carried by an assistant. The assistant closely followed the sound signal emitted by a computer using an ancillary sound software program (EZ Sound, Barcelona, Spain) and used cones placed at 5 m intervals as a visual sign.

To better simulate competitive conditions the subjects wore the same wetsuit as in the race. They were also asked to wear a heart rate monitor (see section 3.5), and a portable gas analyzer combined with a respiratory snorkel for breath-by-breath gas analysis was used (see section 3.6). These materials are illustrated in Figure 6.

### **3.4.2. Cycle ergometry**

The cycling test was conducted on an electronically braked cycle ergometer (Excalibur Sport, Lode, The Netherlands) modified with clip-on pedals. The saddle and handlebar positions of the ergometer were adjusted to resemble each triathlete's own bike. The exercise protocol (see Figure 7) started at 25 W and was increased 25 W every minute until exhaustion. The pedaling cadence was individually chosen in the range of 70–100 rpm.

During the test HR was continuously monitored (see section 3.5), and a computerized gas analyzer recorded oxygen uptake, respiratory ventilation, carbon dioxide production and respiratory exchange ratio (see section 3.6). Figure 8 illustrates the equipment used.

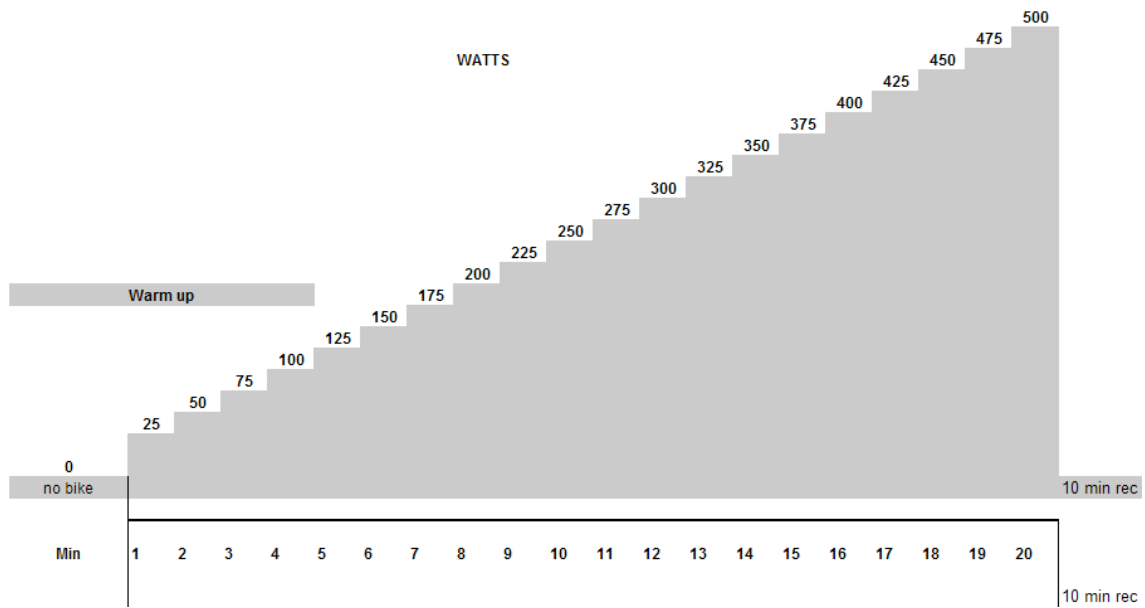


**Figure 5.** Protocol of the swimming test.

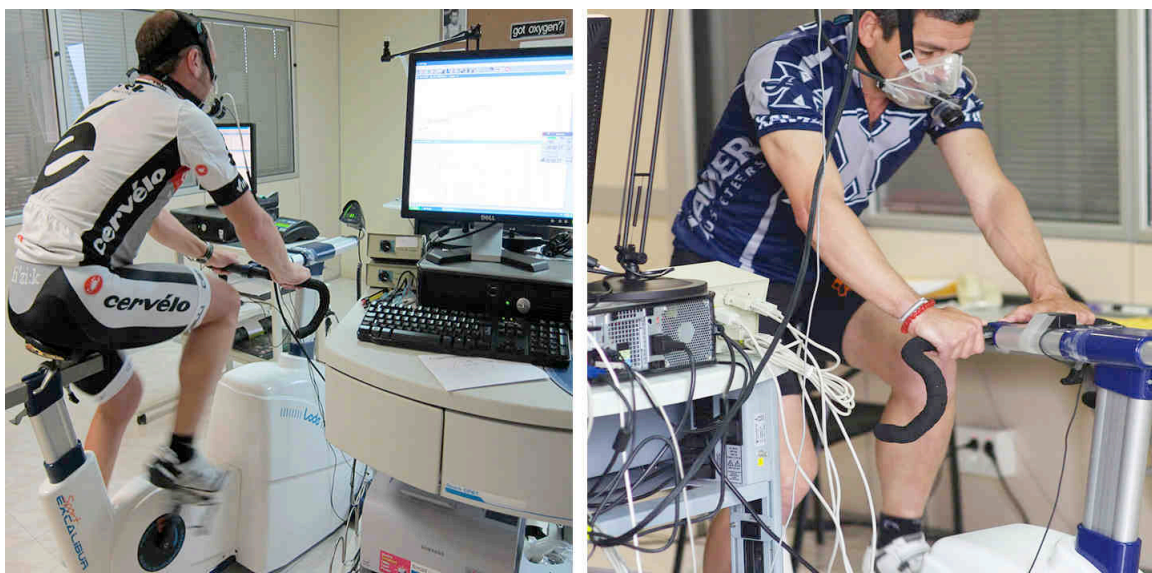


**Figure 6.** Subjects performing the swimming test with the respiratory snorkel and valve system for breath-by-breath gas analysis.





**Figure 7.** Protocol of the cycling test.



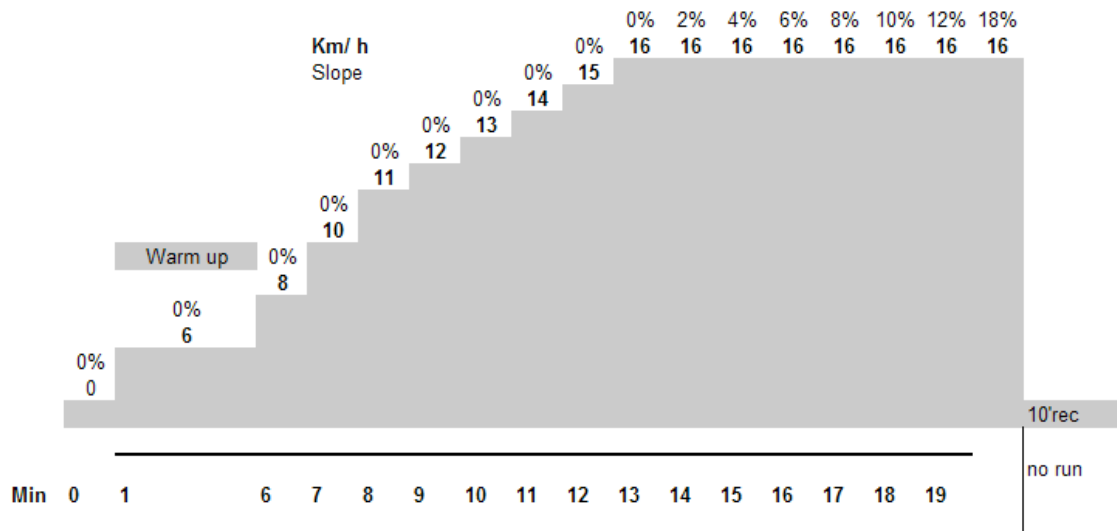
**Figure 8.** Subjects performing the cycling test on the cycle ergometer.

### 3.4.3. Treadmill running

The running test was performed on a motorized treadmill (h/p/Cosmos Pulsar, Germany).

Exercise started at 6 km·h<sup>-1</sup> (0% slope) for 5 min. Subsequently, speed was increased to 8 km·h<sup>-1</sup> (0% slope) and then by 1 km·h<sup>-1</sup> every minute. When 16 km·h<sup>-1</sup> was reached, the grade was increased by 2% every min until exhaustion. See the protocol in Figure 9. For the numerical and statistical analysis of running speed in the upper range of this variable, each increase in grade was converted to running speed using the equations of Margaria et al. (1963), where a 1.5% rise in grade corresponds to an increase in speed of 1 km·h<sup>-1</sup>.

During the test, triathletes wore a portable heart rate monitor (see section 3.5), and a mask connected to a computerized gas analyzer that recorded gas exchange (see section 3.6). These materials are illustrated in Figure 10.



**Figure 9.** Protocol of the running test.



**Figure 10.** Subjects performing the running test on the treadmill.

### **3.5. Heart rate measurement**

During the tests HR was continuously monitored using portable Polar RS800CX (Polar Electro, Finland) monitors for the cycle ergometry and treadmill running tests, and waterproof Polar RCX5 (Polar Electro, Finland) monitors for the swimming tests. During the race HR was continuously monitored using Polar RCX5 portable monitors and averaged at 5 s intervals. Triathletes were allowed to use their own accessories compatible with Polar RCX5, like cadence sensor and GPS, as they do not interfere with the heart rate recordings. Subjects were instructed to change the recording mode (swimming, cycling and running) during the transitions following the manufacturer instructions. Figure 11 illustrates one of the subjects of this study wearing a Polar RCX5 during the first transition of the race. In this study, heart rate measures were used to estimate the oxygen uptake ( $\text{VO}_2$ ) during the competition (see 3.6 for further details on  $\text{VO}_2$  estimation).



**Figure 11.** Polar RCX5 portable monitor during the first transition of the race.

### **3.6. Gas analysis and ventilatory demarcation points**

During the tests, oxygen uptake ( $\text{VO}_2$ ), respiratory ventilation ( $V_E$ ), carbon dioxide production ( $\text{VCO}_2$ ), and respiratory exchange ratio (RER) were measured breath-by-breath using a computerized gas analyzer (Quark CPET, Cosmed, Italy) for cycle ergometer and treadmill running tests. A portable gas analyzer of the same brand and similar operating system (K4 b<sup>2</sup>, Cosmed, Italy) combined with a respiratory snorkel and valve system for breath-by-breath gas analysis (Keskinen et al., 2003; Rodriguez et al., 2008) was used for the measurement of metabolic and ventilatory parameters during the swimming tests.

Before each test, the ambient conditions were measured, and the gas analyzers were calibrated using high precision calibration gases ( $16.00 \pm 0.01\%$   $\text{O}_2$  and  $5.00 \pm 0.01\%$   $\text{CO}_2$ , Scott Medical Products, USA). Ventilatory flow was calibrated using a 3-l syringe (Hans Rudolph, Chicago, USA). After the test, all respiratory data were averaged at 15 s intervals to determine  $\text{VO}_{2\text{peak}}$ , which was taken as the highest 15-s average value.

Criteria for  $\text{VO}_{2\text{max}}$  were:

- a plateau in  $\text{V}'\text{O}_2$  despite increases in workload (main criterion)
- an RR value  $>1.10$
- a maximal heart rate  $>90\%$  of age predicted values.

The first ventilatory threshold ( $\text{VT}_1$ ), and the second ventilatory threshold ( $\text{VT}_2$ ), were estimated by three independent researchers according to methods described by Wasserman et al., (1987) and in accordance with previous reports (Laursen et al., 2005). The reliability of  $\text{VO}_{2\text{peak}}$  (TEM, typical error of measurement: 3.2% [2.5-4.9%]) and ventilatory thresholds determinations (TEM: 3.2% [2.5-4.8%]) during swimming are comparable to that of other exercise modes (Rodriguez et al., 2007).

To estimate  $\text{VO}_2$  during competition, regression equations for each stage developed from the individual HR- $\text{VO}_2$  relationship in each preliminary specific test were used.

To determine the intensity profile we used the HR values registered during the race in relation with the HR corresponding to the  $\text{VO}_{2\text{peak}}$  and each to the ventilatory demarcation points in each of the exercise-specific laboratory and pool tests.

### **3.7. Load of exercise and energy expenditure**

To estimate the total work load of exercise performed by each triathlete we used the training impulse (TRIMP) method (Foster et al., 2001). To establish a reference for heart rate, three zones of physical exertion were identified. These were based on the ventilatory thresholds (VT). The first zone was below  $\text{VT}_1$ , the second zone was between  $\text{VT}_1$  and  $\text{VT}_2$ , and the third zone was above  $\text{VT}_2$ .

To calculate TRIMP, the score for each heart rate zone was calculated by multiplying the accumulated duration in this zone by a multiplier for this particular phase, e.g. 1 min in zone I was given a score of 1 TRIMP ( $1 \times 1$ ), 1 min in zone II was given a score of 2 TRIMP ( $1 \times 2$ ), and 1 min in zone III was given a score of 3 TRIMP ( $1 \times 3$ ). The total TRIMP score was obtained by summing the results of the three

zones [(min of zone I HR [ $< VT1$ ]  $\times 1$ ) + (min of zone II HR [ $> VT1 - < VT2$ ]  $\times 2$ ) + (min of zone III HR [ $> VT2$ ]  $\times 3$ )].

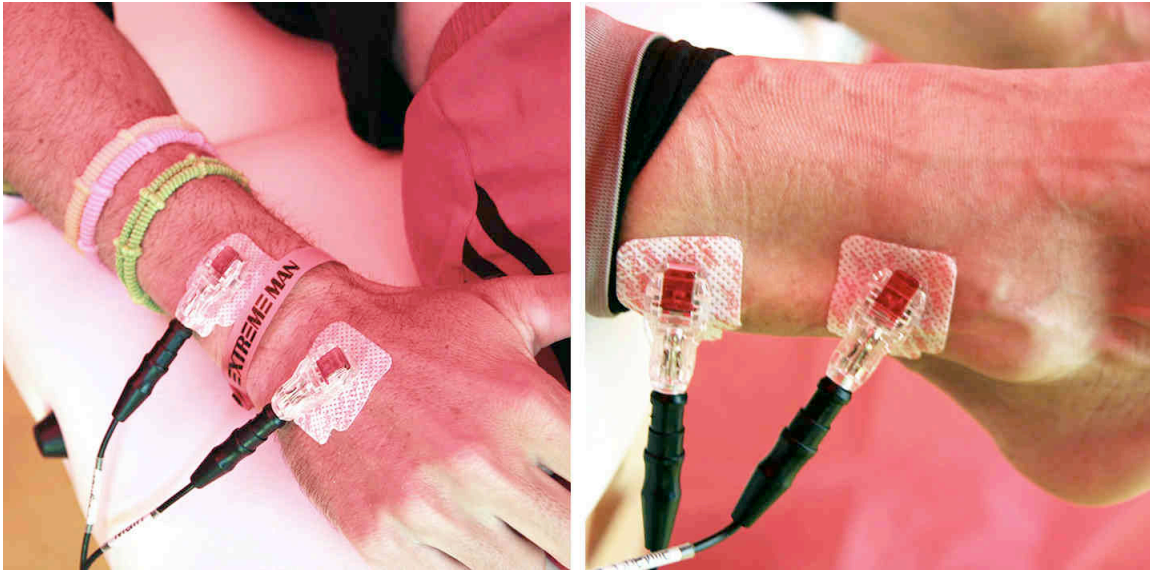
The individually derived linear relationship between heart rate and  $VO_2$  was used to estimate the oxygen cost during the work efforts for each segment. Three different individualized equations were established. These were three linear regression equations derived from data during each incremental exercise test. To estimate energy expenditure during the race, we used an energy equivalent of oxygen based on the mean intensity during racing time, as described in a previous study (Zuntz, 1901).

### **3.8. Body mass and bioimpedance bioelectricity variables**

Body mass (BM) was measured using a Seca 710<sup>®</sup> weighing scale calibrated beforehand (capacity: 200 kg; precision 50 g).

Total body water (TBW), intracellular water (ICW) and extracellular water (ECW) were measured using a multifrequency bioimpedance analyzer (Z-Metrix<sup>®</sup>, BioparHom<sup>®</sup>, Bourget du Lac Cedex, France) before and 30 min after the race (to avoid skin temperature effect on BIA). Another assessment was done further 48 h after. The device was previously calibrated with a circuit of known impedance value provided by the manufacturer. The bioelectrical measurements were repeated until they were stable within 1  $\Omega$  (usually up to three times within an interval of 20 s), and the average value was used in calculations.

The measurements were conducted through the standard tetrapolar electrodes distribution (Red Dot<sup>™</sup> 2660-5, 3M Corporate Headquarters, St. Paul, MN, USA). The inner arm electrode (sensor) was placed on the dorsal surface of the right wrist and between the ulna and the radius. The leg electrode was placed on the anterior surface of the right ankle between the prominent portions of the bones. The external electrodes (source or injector) were placed on the dorsal surface of the third proximal phalanx of the right hand and right foot. Proximal electrodes were separated by 5 cm to avoid interaction between electric fields, which could otherwise lead to an overestimation of the impedance values. The placement of electrodes is illustrated in Figure 12.



**Figure 12.** Electrode placement for impedance measurement to estimate total body water, intracellular water and extracellular water after the race.

### **3.9. Nutritional data**

The triathletes were encouraged to follow their own diet that is typical of the days before and during a competition of this sort. Furthermore, they received no direct nutrition-related instructions prior to or during the event.

During the competition (*Extreme Man Salou–Costa Daurada* triathlon), twenty-five trained researchers and graduate students were divided among the refreshment points (for more details on the placement of refreshment points we refer the reader to *Annex 4*) collecting all the wraps and bottles of each triathlete and keeping them in airtight bags. Immediately after the race, all samples were weighed and recorded by the researchers. Additionally, upon completion the UET triathletes were asked to confirm the data collected during the event by researchers (Figure 13 illustrates this procedure). Then, a nutritional software was used to assess macronutrient intake (CESNID 1.0, Barcelona University, Spain). The information about the nutritional content that was not available in the database was taken from the manufacturer. In addition, information derived from homemade foods such cake or sandwich, was provided directly by the triathletes.



**Figure 13.** Triathlete confirming the nutritional data collected during the race.

### **3.10. Blood tests**

A medical and nursing team took venous blood samples before the start of the *Extreme Man* competition (pre-) and 48 h post-race. Figure 14 illustrates the blood extraction before the race. In both cases, samples were stored in an insulated transport cooler and were immediately taken to the Faculty of Medicine (University of Barcelona) for their analysis. ELISA (Enzyme-Linked Immunosorbent Assay) technique (Gan & Patel, 2013) was used to determine fast and slow myosin levels and creatine kinase activity in serum.





**Figure 14.** Venous blood extraction before the race.

### **3.11. Statistical analysis**

Descriptive data are presented as mean and standard deviation (SD) unless otherwise indicated. A one-way analysis of variance (ANOVA) was used to show differences between tests (swimming test, cycle ergometry and treadmill running) in the variables corresponding to the demarcation points (VT<sub>1</sub>, VT<sub>2</sub> and peak), and also between disciplines (swimming, cycling and running) during competition in the mean HR, mean estimated VO<sub>2</sub> and percentage of time spent in each intensity zone as well as between macronutrient, fluid and sodium intake in each stage (cycling and running).

Furthermore, another ANOVA test was performed to analyze differences between BIA data (TBW, ECW and ICW) and blood data (creatine kinase, slow myosin and fast myosin levels) before and after the race. Also HR corresponding to the demarcations points during the exercise tests was compared to the mean HR in each discipline during competition using ANOVA. Post-hoc analyses were performed using Tukey honestly significant differences (HSD).

Pearson's rank correlation analysis was used to assess the relationship between the individual physiological variables measured and performance in each stage of the

triathlon, as well as the overall race time. This was also used to assess the relationship between the mean race HR in each discipline and performance (i.e. final racing time). A stepwise multiple linear regression analysis was used to determine the best predictors of final racing time after checking the correlation matrix for collinearity. Significance was set at  $P < 0.05$ .

All analyses were performed using PASW Statistics v 18 for Windows.



## 4. RESULTS

In this section the results of each study are presented.

### 4.1. Results of Study I

#### 4.1.1. Preliminary laboratory testing

The results of the three incremental tests are shown in Table 4. Additionally, the individual regression plots and derived linear regression equations are presented in Figure 15, Figure 16 and Figure 17. Significant differences were found between tests in some parameters:  $\text{VO}_2$  and HR at  $\text{VT}_1$  were significantly lower in the swimming test than in the cycling ( $P < 0.001$  and  $P = 0.001$  respectively) and treadmill tests ( $P < 0.001$  and  $P = 0.001$  respectively).  $\text{VO}_2$  and HR at  $\text{VT}_2$  were significantly lower in the swimming test than in the cycling ( $P < 0.001$  and  $P = 0.003$  respectively) and treadmill tests ( $P < 0.001$  and  $P = 0.002$  respectively). Peak  $\text{VO}_2$  and HR were significantly lower in the swimming test than in the cycling ( $P < 0.001$  and  $P = 0.022$  respectively) and treadmill tests ( $P < 0.001$  and  $P = 0.014$  respectively). Relative percent value of  $\text{HR}_{\text{peak}}$  at  $\text{VT}_1$  was lower in the swimming test than in the cycling test ( $P < 0.036$ ).

#### 4.1.2. Ultra-endurance triathlon race performance

Table 5 summarizes the main results for each stage of the race, namely performance, HR, and estimated  $\text{VO}_2$  derived from HR recordings based on the  $\text{VO}_2/\text{HR}$  individual relationship during each of the three graded tests.

Race duration including the transition intervals for each stage and their correlation with the overall racing time are presented in Table 6. The duration of both cycle and run stages was closely related to the final time ( $r = 0.92$ , and  $0.90$ , respectively,  $P = 0.001$ ), while swimming duration was not ( $P = 0.183$ ).

**Table 4.** Graded exercise test variables for the swimming, cycle ergometry and treadmill running tests.

	Test	VO <sub>2</sub> (l·min <sup>-1</sup> )	VO <sub>2</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	% VO <sub>2peak</sub>	HR (beats·min <sup>-1</sup> )	% HR <sub>peak</sub>	Speed (m·s <sup>-1</sup> ) / Power output (W) / Speed (km·h <sup>-1</sup> )
VT <sub>1</sub>	Swimming	2.22 (0.37)*	29.6 (3.7)*	71.1 (4.2)	128.7 (12.4)*	78.7 (3.4)**	0.9 (0.1)
	Cycle ergometry	3.65 (0.45)	48.6 (5.6)	74.0 (5.3)	148.7 (7.8)	84.5 (3.4)	267 (25)
	Treadmill running	3.55 (0.32)	47.4 (4.4)	73.1 (2.7)	147.0 (10.1)	83.5 (5.7)	13.0 (1.5)
VT <sub>2</sub>	Swimming	2.74 (0.49)*	36.4 (5.3)*	87.4 (4.6)	149.3 (10.3)*	91.5 (4.5)	1.1 (0.2)
	Cycle ergometry	4.36 (0.47)	58.0 (4.6)	88.4 (3.2)	164.4 (8.6)	93.4 (2.8)	328 (29)
	Treadmill running	4.31 (0.39)	57.6 (5.6)	88.7 (1.3)	164.6 (6.0)	93.4 (2.6)	16.3 (1.3)
Peak values	Swimming	3.15 (0.59)*	41.8 (6.0)*	100 (0)	163.4 (13.5)*	100 (0)	1.3 (0.1)
	Cycle ergometry	4.94 (0.57)	65.6 (4.8)	100 (0)	176.1 (8.6)	100 (0)	381 (33)
	Treadmill running	4.86 (0.46)	64.9 (6.3)	100 (0)	176.2 (7.9)	100 (0)	18.8 (1.2)

Data are presented as mean (SD). VT<sub>1</sub>: first ventilatory threshold; VT<sub>2</sub>: second ventilatory threshold; Max: maximum; VO<sub>2</sub>: oxygen uptake; % VO<sub>2peak</sub>: percentage of peak oxygen uptake at the test; HR: heart rate; % HR<sub>peak</sub>: percentage of HR peak at the test; W: watts. \* a value for the swimming test that is significantly lower than that for the cycle ergometry and treadmill running tests  $P < 0.05$ . \*\* a value for the swimming test that is significantly lower than that for the cycle ergometry  $P < 0.05$ .

**Table 5.** Swimming, cycling and running duration and intensity variables during the ultra-endurance triathlon race.

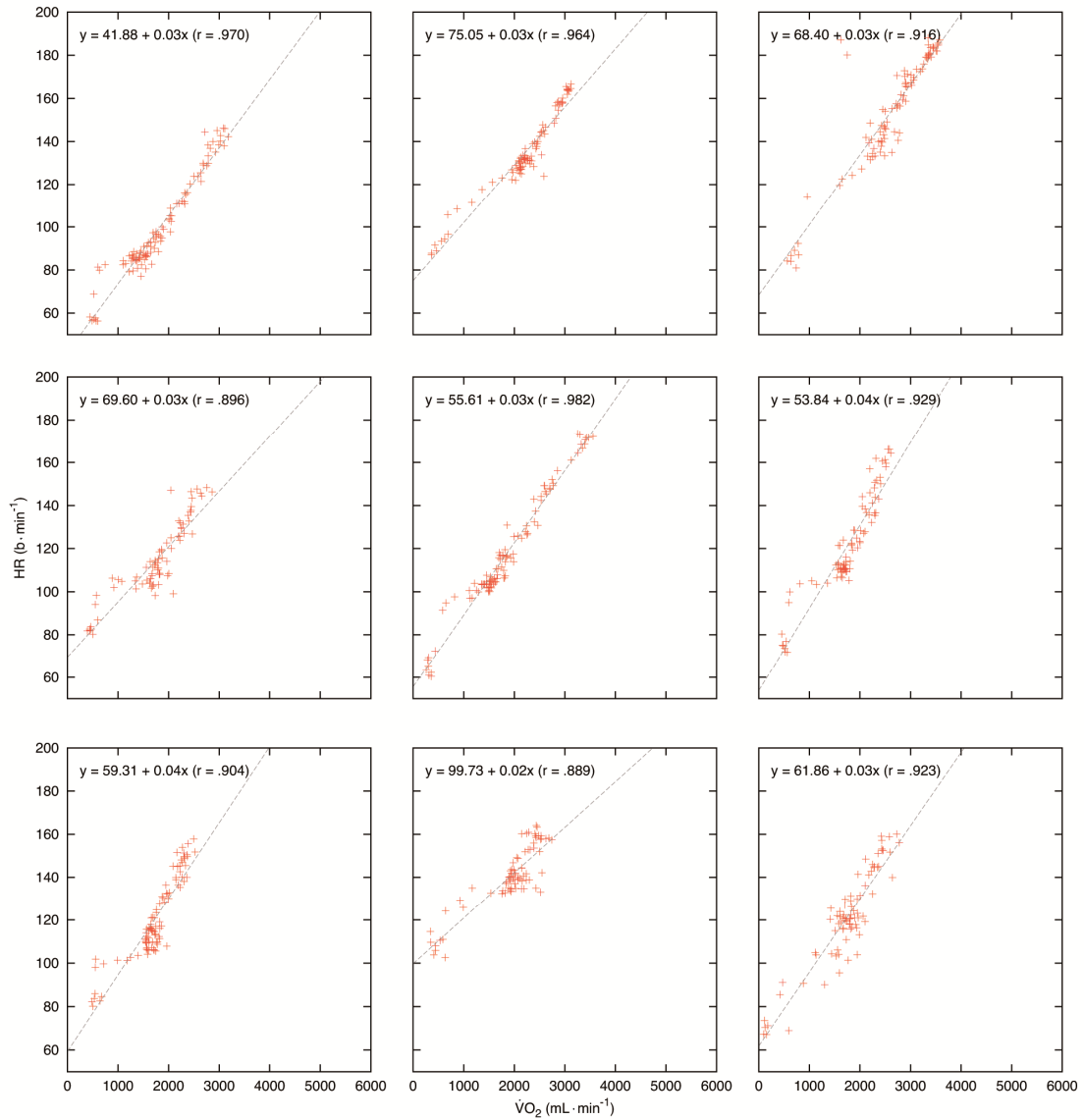
Time (h:min:s)	Percent of total time (%)	Mean speed (m·s <sup>-1</sup> / km·h <sup>-1</sup> )	Mean HR (beats·min <sup>-1</sup> )	Percent of HR <sub>peak</sub> (%)	Mean estimated VO <sub>2max</sub> (ml·min <sup>-1</sup> )	Percent of estimated VO <sub>2max</sub> (%)
Swimming (3.8 km) 01:03:13 (00:08:03)	8.6 (1.1)	0.91 (0.09)	149.2 (10.1)	91.5 (3.9)	2911 (111)	92.4
Cycling (180 km) 07:02:24 (00:40:54)	57.3 (5.6)	25.8 (2.4)	137.1 (5.7)*	77.9 (2.0)**	3079 (413)	62.4**
Running (42.2 km) 04:12:02 (00:35:43)	34.2 (4.8)	10.2 (1.3)	136.2 (10.5)*	77.3 (4.2)**	3080 (239)	63.3**

Data are presented as mean (SD). VO<sub>2</sub>: oxygen uptake; % VO<sub>2max</sub>: percentage of maximum oxygen uptake at the specific test; HR: heart rate. VO<sub>2peak</sub>; % HR<sub>peak</sub>: percentage of peak HR. \*,\*\*: a value for the swimming stage that is significantly higher than that for cycling and running stages at \**P* < 0.05 or \*\**P* < 0.01.

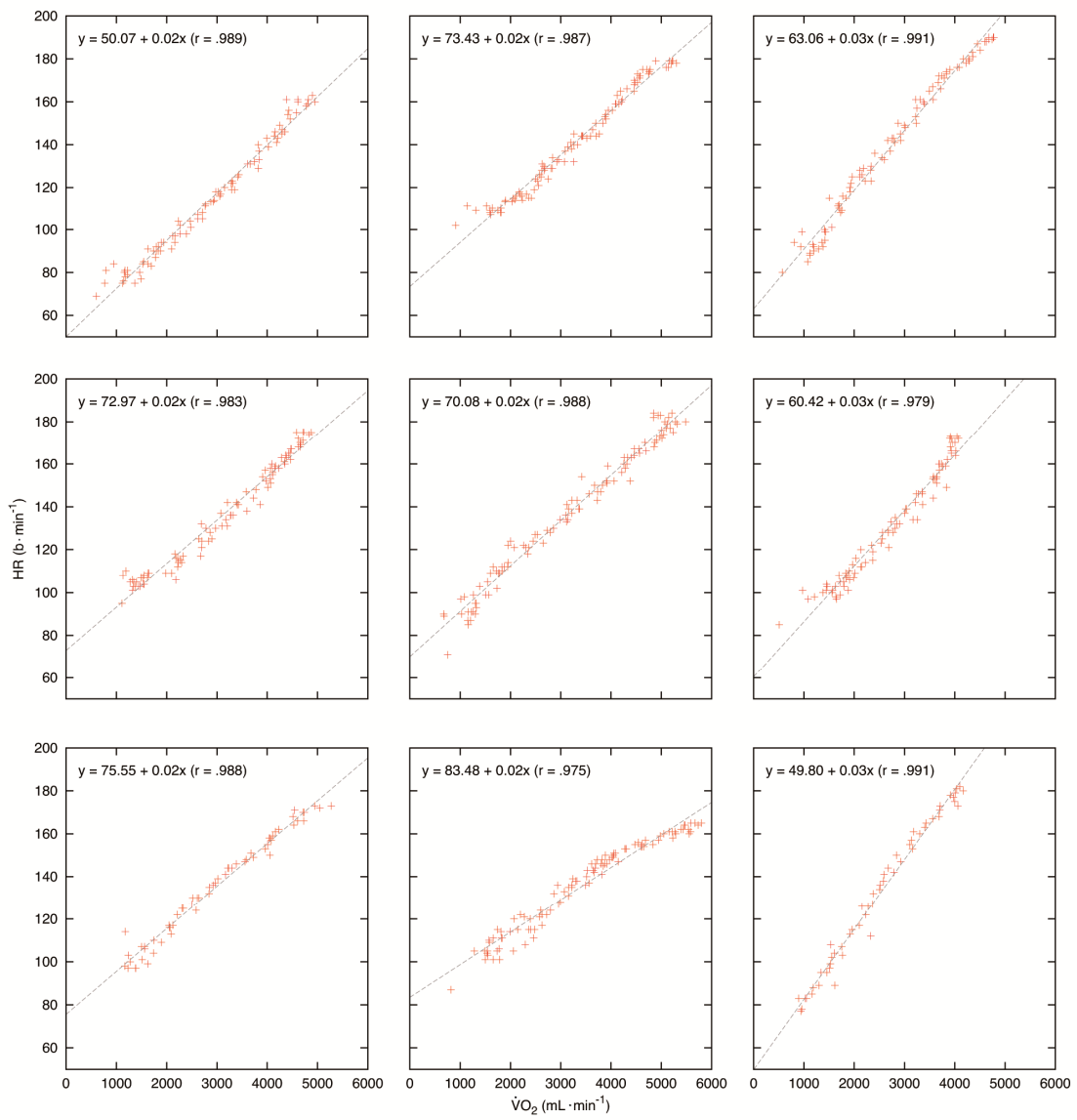
**Table 6.** Swimming, cycling and running stage duration and correlation with final racing time.

	3.8 km swim	180 km cycle	42.2 km run	Final time
Time (h:min:s)	01:03:13 (00:08:03)	07:02:24 (00:40:54)	04:12:02 (00:35:43)	12:30:12 (01:12:13)
Correlation with final time	0.49	0.92**	0.90**	
<i>P</i> -value	0.183	0.001	0.001	

Data are presented as mean (SD). \*\* *P* < 0.01.

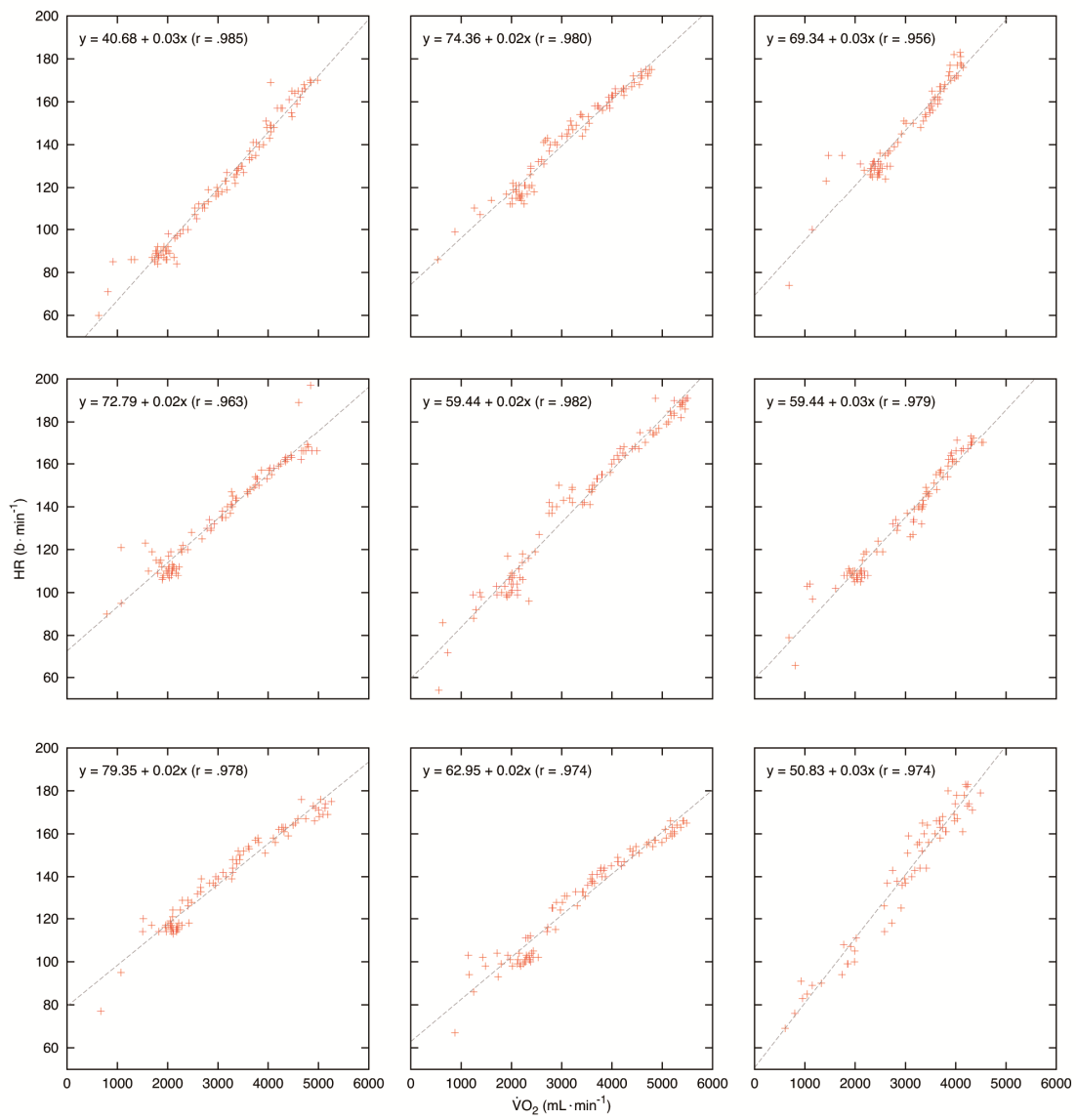


**Figure 15.** Individual regression plots and linear regression equations of the swimming tests.



**Figure 16.** Individual regression plots and linear regression equations of the cycle ergometry tests.

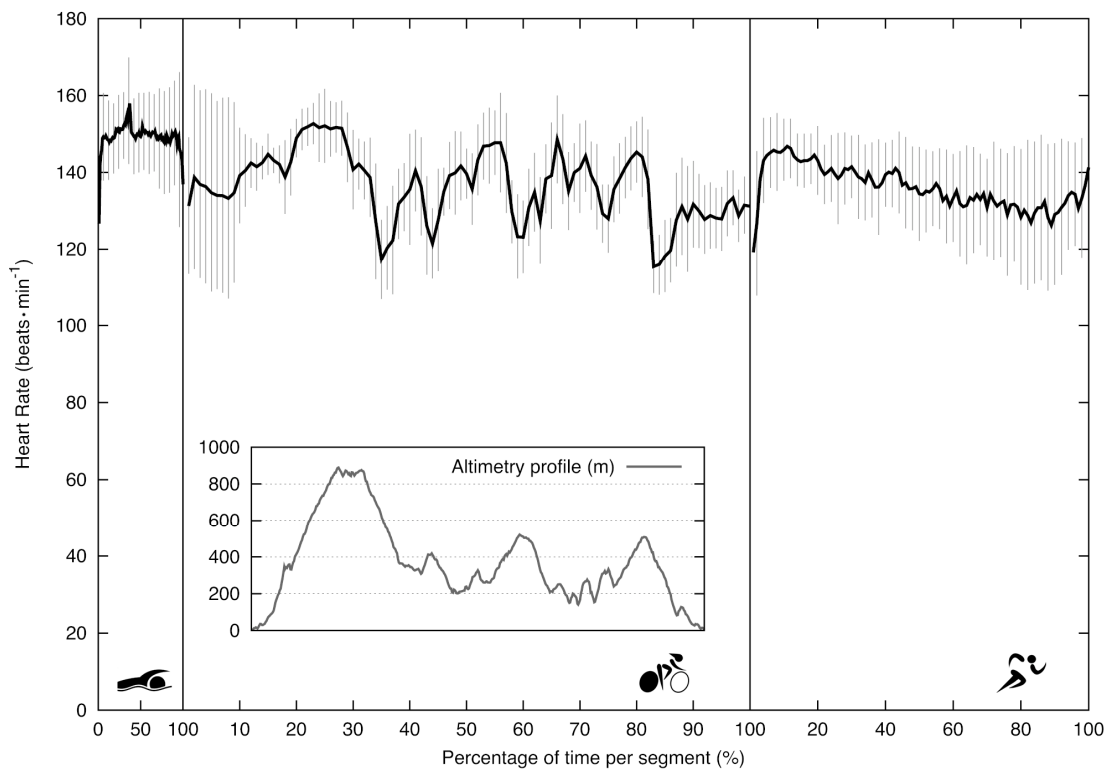




**Figure 17.** Individual regression plots and linear regression equations of the treadmill running tests.

### 4.1.3. Heart rate response during the race

Figure 18 shows the HR group profile during the race. For the sake of proportionality, data in the x-axis are expressed as percent of total time per stage, and the length of each stage was scaled relative to the overall time. This takes into account the relative duration of each stage for each individual, and avoids calculating the last intervals with a progressively decreasing number of cases. In the swim stage a peak HR can be identified corresponding to the out-of-the-water running interval between two consecutive swimming laps. In the cycling stage the effect of slope changes can also be seen and related to the altimetry profile (inbox).



**Figure 18.** Heart rate group profile during the ultra-endurance triathlon race.

Data are mean (thick line) and upper and lower SD error bars at 5% (swim), 1% (cycle) and 2% (run) intervals for clarity. The altimetry profile (inner panel) of the cycling stage is shown. For the sake of proportionality, the x-axis data are expressed as percent of total race time per stage, and the length of each stage was scaled relative to the overall time.

Average swimming HR was significantly higher than the cycle ( $P = 0.024$ ), run ( $P = 0.013$ ) and overall HR ( $P = 0.025$ ) during the race, but there was no difference between the cycling and running stages ( $P = 0.995$ ) (Table 5, Figure 18). Subjects attained a higher percentage of each test's  $HR_{peak}$  during swimming than during the cycling ( $P < 0.001$ ) and running stages ( $P < 0.001$ ), which were not different among them ( $P = 0.915$ ) (Table 5, Figure 19).

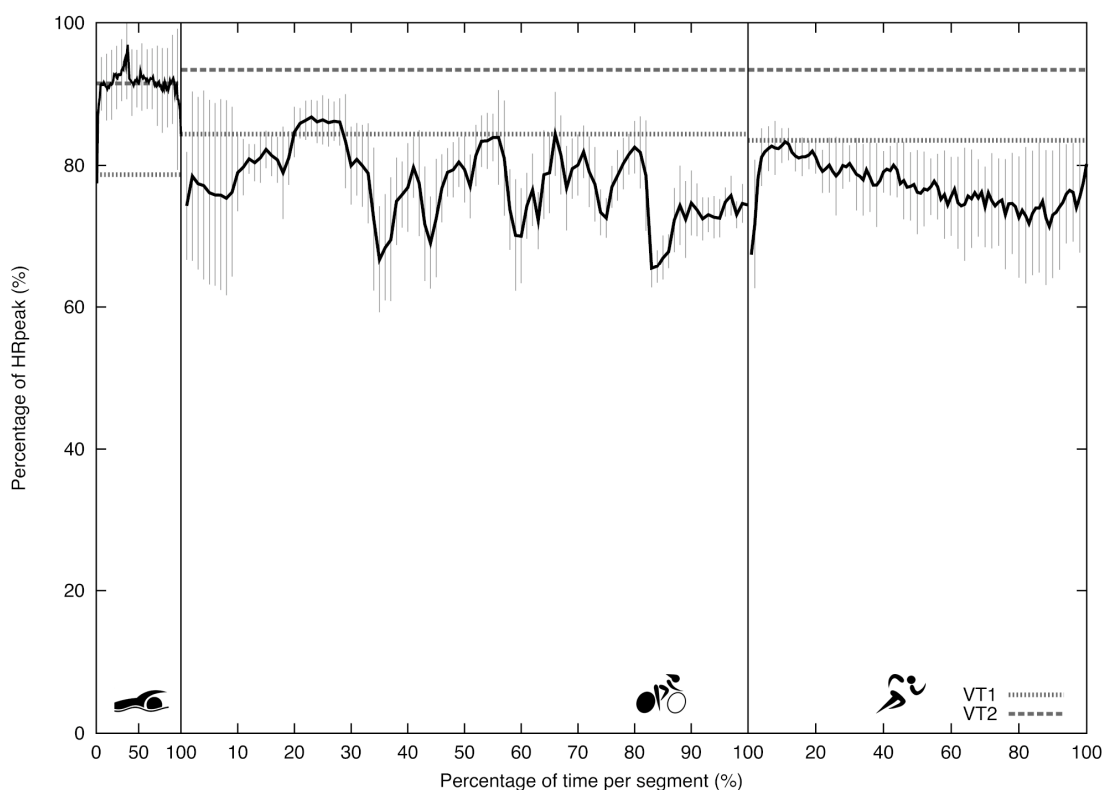
Figure 19 shows the relative HR response group profile during the race (y-axis), expressed as the percentage of each specific test's  $HR_{peak}$ . The group-average value for  $VT_1$  and  $VT_2$  corresponding to each specific test is depicted within each stage for visual reference. Swimming relative HR ( $\% HR_{peak}$ ) was above HR at  $VT_1$  (Table 4) during the swimming test, while cycling and running relative HR were below HR at  $VT_1$  ( $P < 0.05$ ).

The percentage of time spent in each intensity zone (Zone I: below to the  $VT_1$ ; zone II; between the  $VT_1$  and the  $VT_2$ ; zone III: above to the  $VT_2$ ) is presented in Table 7. During the swimming stage, the triathletes spent significantly more time over the  $VT_2$  (92%;  $P < 0.01$ ). During the cycling and running stages, they spent significantly more time exercising under the  $VT_1$  (75% and 78% respectively;  $P < 0.01$ ). The largest percentage of total racing time was spent under the  $VT_1$  (70% of total;  $P < 0.01$ ).

**Table 7.** Percentage of time spent in each intensity zone during the ultra-endurance triathlon.

	Percent time spent in		
	Zone I	Zone II	Zone III
Swimming	7.55 (18.44)	0.01 (0.04)	92.43 (0.04)**
Cycling	75.23 (16.69)**	22.92 (15.96)	1.85 (5.3)
Running	78.28 (36.14)**	21.59 (34.09)	0.13 (0.36)
Total	70.40 (20.02)**	20.48 (20.45)	9.12 (3.19)

Data are presented as mean (SD). Zone I: below to the first ventilatory threshold ( $VT_1$ ); zone II; between the first ventilatory threshold ( $VT_1$ ) and the second ventilatory threshold ( $VT_2$ ); zone III: above to the second ventilatory threshold ( $VT_2$ ). \*\* The time value is significantly higher in this zone than that in the others zones at  $*P < 0.01$ .

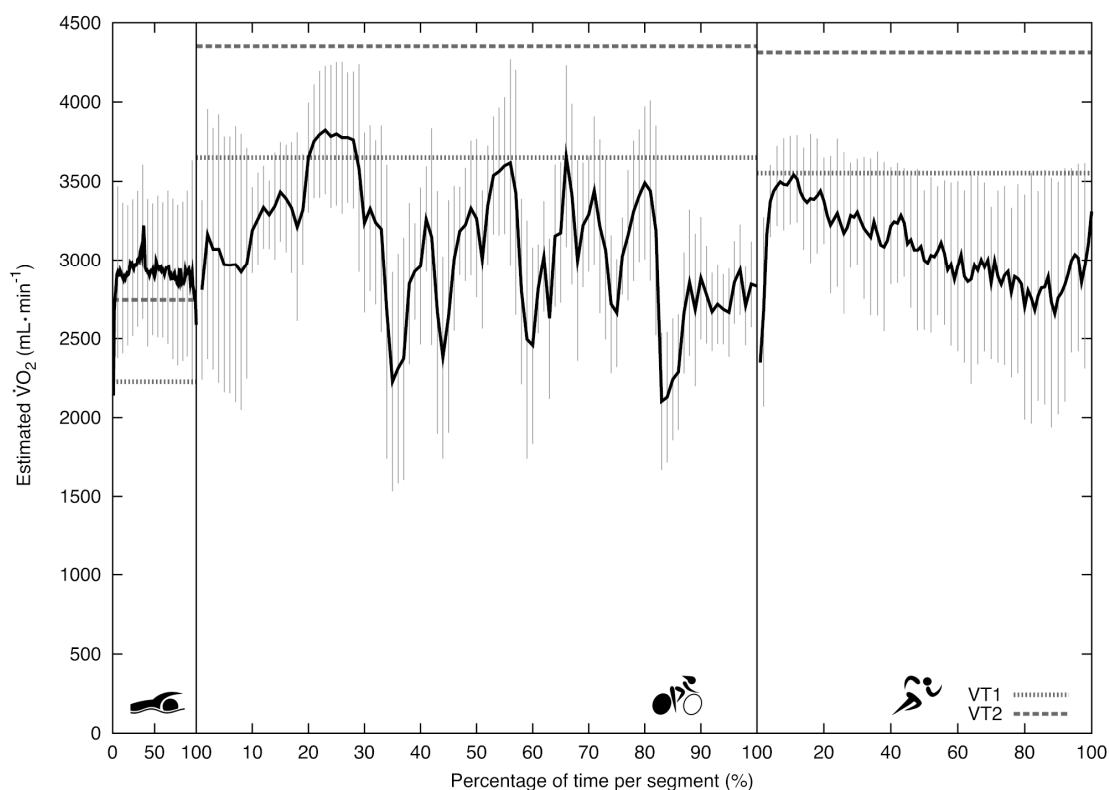


**Figure 19.** Heart rate group profile during the ultra-endurance triathlon race, expressed as percentage of peak heart rate at the specific exercise test.

VT<sub>1</sub>: first ventilatory threshold; VT<sub>2</sub>: second ventilatory threshold; % HR<sub>peak</sub>: percentage of peak heart rate. Data are mean (thick line) and upper and lower SD error bars at 5% (swim), 1% (cycle) and 2% (run) intervals for clarity. Mean group heart rate at VT<sub>1</sub> and VT<sub>2</sub> during each of the preliminary exercise tests are depicted for reference.

#### 4.1.4. Estimated oxygen uptake during the race

Figure 20 shows the estimated VO<sub>2</sub> group profile during the race in relation to group-average VO<sub>2</sub> at VT<sub>1</sub> and VT<sub>2</sub> as measured in each of the specific tests (Table 4). There was no difference in estimated VO<sub>2</sub> across the three stages (Table 5). The percentage of estimated VO<sub>2max</sub> attained during the race was higher during swimming than in the cycling and running stages ( $P < 0.001$ ), but these two did not differ ( $P = 0.872$ ).



**Figure 20.** Estimated oxygen uptake group profile during the ultra-endurance triathlon race.

VT<sub>1</sub>: first ventilatory threshold; VT<sub>2</sub>: second ventilatory threshold;  $\dot{V}O_2$ : oxygen uptake. Data are mean (thick line) and upper and lower SD error bars at 5% (swim), 1% (cycle) and 2% (run) intervals for clarity. Mean group estimated oxygen uptake at VT<sub>1</sub> and VT<sub>2</sub> during each of the preliminary exercise tests are depicted for reference.

#### 4.1.5. Relationship between racing performance and physiological parameters

Table 8 shows the relationship between HR during the three stages of the race and HR corresponding to both VT. Mean HR in the swimming stage was closely related to HR at VT<sub>1</sub> ( $r = 0.82$ ,  $P = 0.007$ ) and VT<sub>2</sub> ( $r = 0.77$ ,  $P = 0.015$ ), but was significantly higher than HR at VT<sub>1</sub> ( $P = 0.002$ ) and coincident with HR at VT<sub>2</sub>. During the cycling and running stages, HR also correlated with HR at VT<sub>2</sub> ( $r = 0.74$  and  $0.75$ ,  $P = 0.023$  and  $0.019$ , respectively), but not with HR at VT<sub>1</sub>. Average HR was below both VT<sub>1</sub> (~11%) and VT<sub>2</sub> (~27-28%).

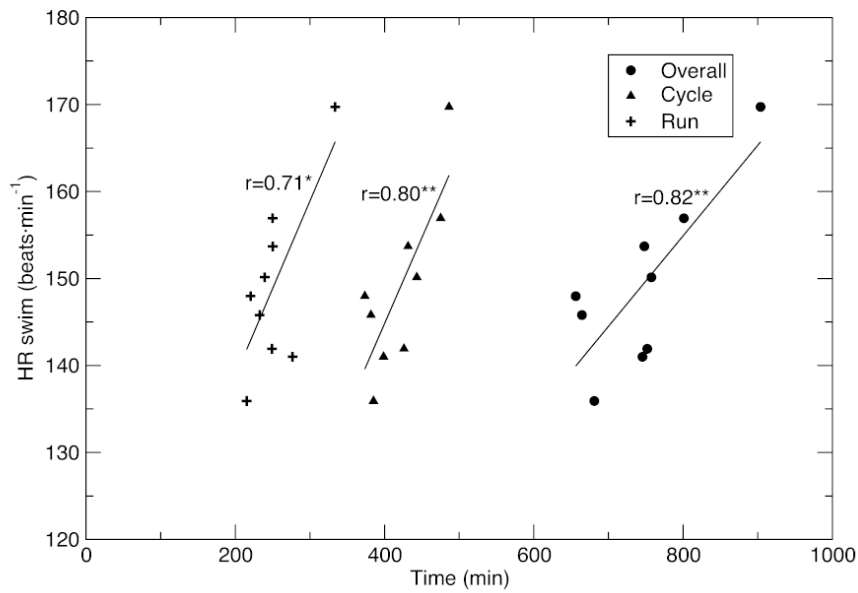
**Table 8.** Relationship ( $r$ ) and difference (race HR – test HR) between HR during the ultra-endurance triathlon and the HR corresponding to the ventilatory thresholds (VT<sub>1</sub> and VT<sub>2</sub>).

	HR Swim	HR Cycle	HR Run
Correlation with HR@VT <sub>1</sub>	0.82 <sup>††</sup>	0.59	0.32
Difference with HR@VT <sub>1</sub> (beats·min <sup>-1</sup> )	20.6 (7.1)**	-11.5 (6.4)**	-10.8 (12.0)*
Correlation with HR@VT <sub>2</sub>	0.77 <sup>†</sup>	0.74 <sup>†</sup>	0.75 <sup>†</sup>
Difference with HR@VT <sub>2</sub> (beats·min <sup>-1</sup> )	-0.1 (6.9)	-27.3 (5.9)**	-28.4 (7.2)**

Data are presented as mean (SD). HR: HR; VT<sub>1</sub>: first ventilatory threshold; VT<sub>2</sub>: second ventilatory threshold; Difference with HR@VT<sub>1</sub>; difference between HR at the first ventilatory threshold and the mean HR during a stage during the race (swimming, cycling or running) of triathlon; Difference with HR@VT<sub>2</sub>; difference between HR at the second ventilatory threshold and the mean HR during a stage during the race (swimming, cycling or running) of triathlon. Statistical differences: \* $P < 0.05$ ; \*\* $P < 0.01$ ; Correlation <sup>†</sup> $P < 0.05$ ; <sup>††</sup> $P < 0.01$ .

Figure 21 shows the significant correlations found between swimming HR and performance times for the three stages and the final racing time. Swimming HR was closely related to the completion time for cycling and running ( $r = 0.80$ ,  $P = 0.009$  and  $r = 0.71$ ,  $P = 0.031$ , respectively), and to the final time ( $r = 0.82$ ,  $P = 0.007$ ). Thus, swimming HR was inversely related to cycling, running, and overall racing times.

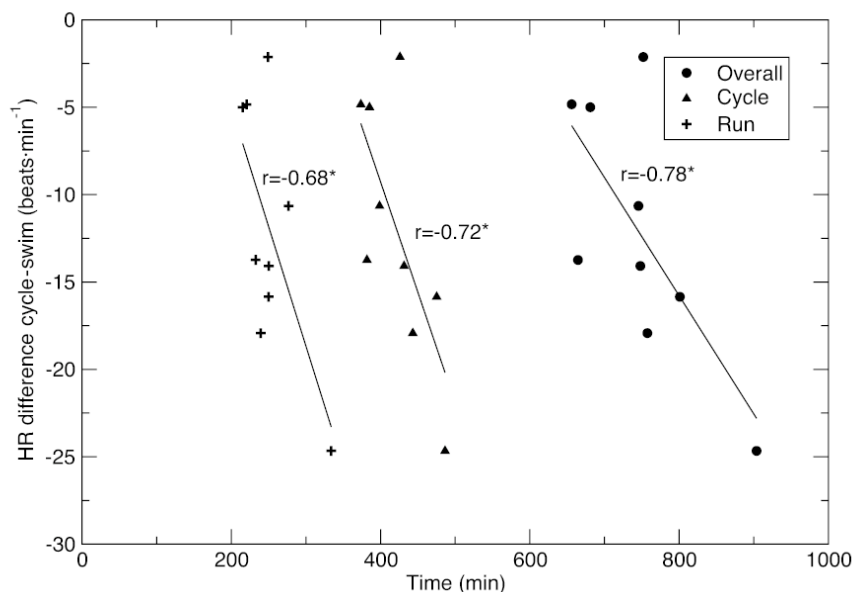
We also wanted to examine the relationships between the HR at the two ventilatory demarcation points in the maximal tests and the average HR during the race, as well as how they related to performance in each of the stages and to overall racing performance. Therefore, we calculated the pairwise differences between mean HR in each stage and HR at the ventilatory thresholds (HR<sub>diff</sub>). In the case of swimming, the difference was calculated using VT<sub>2</sub>, which is the threshold that competitors are closest to during competition (i.e. HR<sub>diff</sub> [swim-VT<sub>2</sub>]). In the case of cycling and running, the difference was calculated using VT<sub>1</sub>, as the intensity tends to be below the corresponding VT<sub>1</sub> in competition (i.e. HR<sub>diff</sub> [cycle-VT<sub>1</sub>] and [run- VT<sub>1</sub>]).



**Figure 21.** The relationship between HR swim and performance times for cycling and running stages and for the overall race.

HR swim: heart rate at the swimming stage. Significant correlations: \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

In addition, calculations of the differences between mean HR in the cycling and swimming stages ( $HR_{diff}$  [cycle-swim]), the running and swimming stages ( $HR_{diff}$  [run-swim]), and the running and cycling stages ( $HR_{diff}$  [run-cycle]) were made, as well as how these differences related to performance (expressed as racing time) in each stage and in the entire event. Among all these parameters, relationships were found between  $HR_{diff}$  [cycle-swim] and the time in the cycling stage ( $r = -0.72$ ,  $P = 0.030$ ), the running stage ( $r = -0.68$ ,  $P = 0.046$ ), and the final time ( $r = -0.78$ ,  $P = 0.013$ ; Figure 22). These relationships indicate that the larger the  $HR_{diff}$  between the swimming and the cycling, the lower the performance in the following stage and in the entire race.



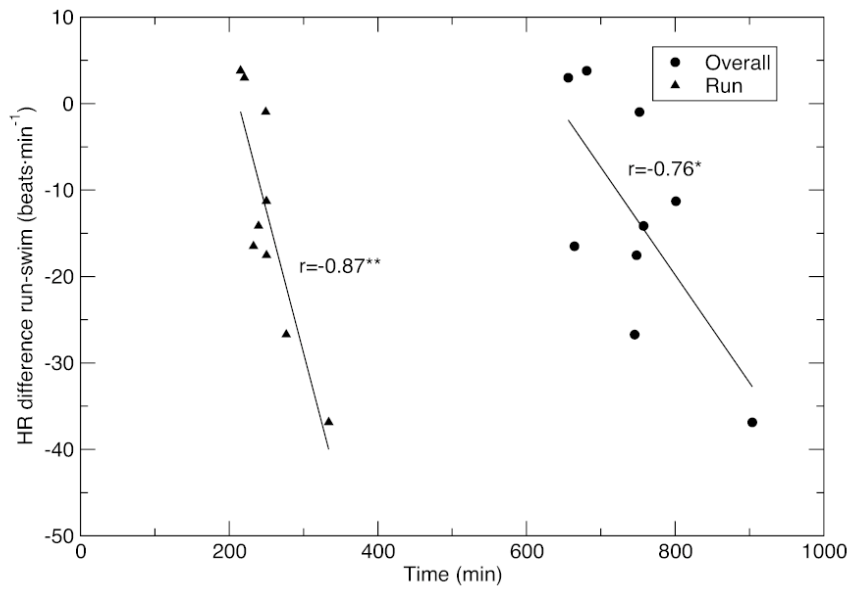
**Figure 22.** The relationship between the cycle-to-swim heart rate difference and performance times.

Cycle-swim  $HR_{diff}$ : difference between HR in the cycling and swimming stages. Significant correlations: \* $P < 0.05$ .

Furthermore, there were negative relationships between  $HR_{diff}$  [run-swim], and the time in the running stage ( $r = -0.87$ ,  $P = 0.003$ ) and the overall time ( $r = -0.76$ ,  $P = 0.016$ ; Figure 23), as well as between  $HR_{diff}$  [run-cycle] and the time in the running stage ( $r = -0.80$ ,  $P = 0.010$ ; Figure 24). This indicates that a large  $HR_{diff}$  during swimming or cycling and running is related to a lower running performance, and a lower overall performance also in the case of swimming.

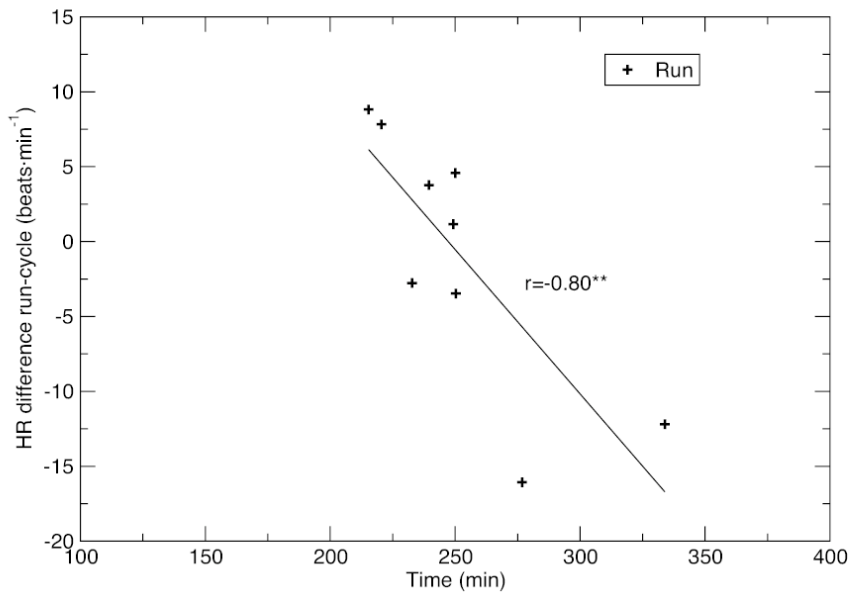
Stepwise multiple regression analysis was also performed finding that the best predictors of total racing time were the weight-adjusted  $VO_{2max}$  ( $\beta = -0.51$ ,  $SE = 206.8$ ,  $P = 0.047$ ) and the  $HR_{diff}$  [cycle-swim] ( $\beta = -0.53$ ,  $SE = 123.1$ ,  $P = 0.041$ ), which accounted for 81% ( $r^2 = 0.81$ ) of the final time variance. The other parameters that correlated linearly with the finishing time were excluded due to collinearity with  $HR_{diff}$  [run-swim].





**Figure 23.** The relationship between the run-to-swim heart rate difference and performance times.

Run-swim HR<sub>diff</sub>: difference between heart rate in the running and swimming stages. Significant correlations: \*  $P < 0.05$ ; \*\*  $P < 0.01$ .



**Figure 24.** The relationship between run-to-cycle heart rate difference and running performance time.

Run-cycle HR<sub>diff</sub>: difference between HR in the running and cycling stages. Significant correlation: \*\*  $P < 0.01$ .

## 4.2. Results of Study II

### 4.2.1. Macronutrient intake

Food and fluids consumed during the UET were mainly those provided at the aid stations by the triathlon organizers. Table 9 summarizes the percent contribution from food and fluids consumed by athletes during the event.

**Table 9.** Percentage of energy contribution from food and fluids during the ultra-endurance triathlon race.

Food and fluids	Energy contribution (%)		
	Cycling	Running	Total
Sport gels	20.2	62.6	35.3
Sport bars	34.4	6.2	24.4
Sport drinks	20.3	13.5	17.9
Sandwich (parma jam and cheese)	13.9	2.0	9.7
Dried fruits (almonds and nuts)	4.2	3.6	4.0
Caffeinated drinks	1.9	6.0	3.3
Fruits (banana, apple and orange)	1.1	6.1	2.9
Cereals	3.1	--	2.0
Others (protein supplements...)	0.8	--	0.5

Table 10 summarizes the consumption of macronutrients during the race. Subjects consumed 927 (178) g (6.2 (1.3) g·kg<sup>-1</sup>; 84 (18) g·hour<sup>-1</sup>; 89.9 (3.5) %) of CHO, which provided the main source of energy consumed during the race ( $P < 0.001$ ). The consumption of solid CHO (697 (147) g) was higher than the consumption of fluid CHO (229 (67) g;  $P < 0.001$ ). Macronutrient intake was significantly greater in the cycling stage compared with the running stage ( $P < 0.001$ ). However, regarding CHO, there were no statistical differences between both stages (cycling: 1.4 (0.5); running: 1.3 (0.3) g·min<sup>-1</sup>).

**Table 10.** Macronutrient intake during the ultra-endurance triathlon race.

		Cycling	Running	Total
Carbohydrates	Solids (g)	405.7 (147.8)	291.5 (60.8)	697.2 (147.2) <sup>†</sup>
	Fluids (g)	177.8 (67.0)	51.6 (24.3)	229.4 (67.0)
	Total (g)	583.5 (176.3) <sup>§</sup>	349.0 (73.3)	926.6 (177.5) <sup>*</sup>
	g/kg <sup>a</sup>	7.8 (2.3)	4.7 (1.2)	6.2 (1.3)
	g/hour <sup>b</sup>	84 (30)	78 (18)	84 (18)
	% <sup>c</sup>	83.2 (5.6)	96.6 (3.8)	89.9 (3.5)
Proteins	Total (g)	40.7 (11.5) <sup>§</sup>	4.6 (3.4)	45.4 (12.0)
	g/kg	0.6 (0.2)	0.1 (0.0)	0.3 (0.1)
	%	6.4 (2.9)	1.3 (1.0)	3.8 (1.6)
Lipids	Total (g)	69.1 (18.0) <sup>§</sup>	7.7 (10.5)	76.8 (20.4)
	g/kg	0.9 (0.3)	0.1 (0.1)	0.5 (0.1)
	%	10.5 (3.6)	2.2 (3.0)	6.3 (2.4)

Data are presented as mean (SD). <sup>a</sup> Ratio between total macronutrient intake (g) and body mass (kg); <sup>b</sup> Ratio between total carbohydrate intake (g) and total racing time (min); <sup>c</sup> Macronutrient percentage of the total energy intake in each segment and in total

<sup>\*</sup> The amount of carbohydrates was significantly higher than the amount of protein and lipids ( $P < 0.001$ )

<sup>†</sup> The consumption of solid CHO was higher than that of fluid CHO ( $P < 0.001$ )

<sup>§</sup> The intake amount was significantly higher in the cycling stage than in the running stage ( $P < 0.001$ ).

#### 4.2.2. Fluid and sodium intake

Table 11 summarizes the fluid balance and the sodium intake during the UET. In absolute values, fluid and sodium intakes were significantly higher during the cycling stage than in the running stage ( $P < 0.001$ ). However, when comparing relative values (fluid intake/time of exercise), fluid intake was significantly greater during the running period compared to the cycling stage (395 (183) and 362 (172) mL/h respectively;  $P < 0.001$ ).

**Table 11.** Fluid and sodium intake during the ultra-endurance triathlon.

	Cycling	Running	Total <sup>a</sup>
Fluid intake (mL)	2530.9 (1255.9) <sup>*</sup>	1657.3 (717.4)	4188.2 (1836.9)
Fluid intake rate (mL/h)	361.6 (171.8) <sup>†</sup>	394.5 (183.2)	366.6 (146.9)
Sodium (mg)			
From fluids	485.2 (307.5) <sup>*</sup>	232.0 (208.5)	2152.2 (1124.2)
From food	1135.9 (953.9) <sup>*</sup>	299.1 (369.3)	

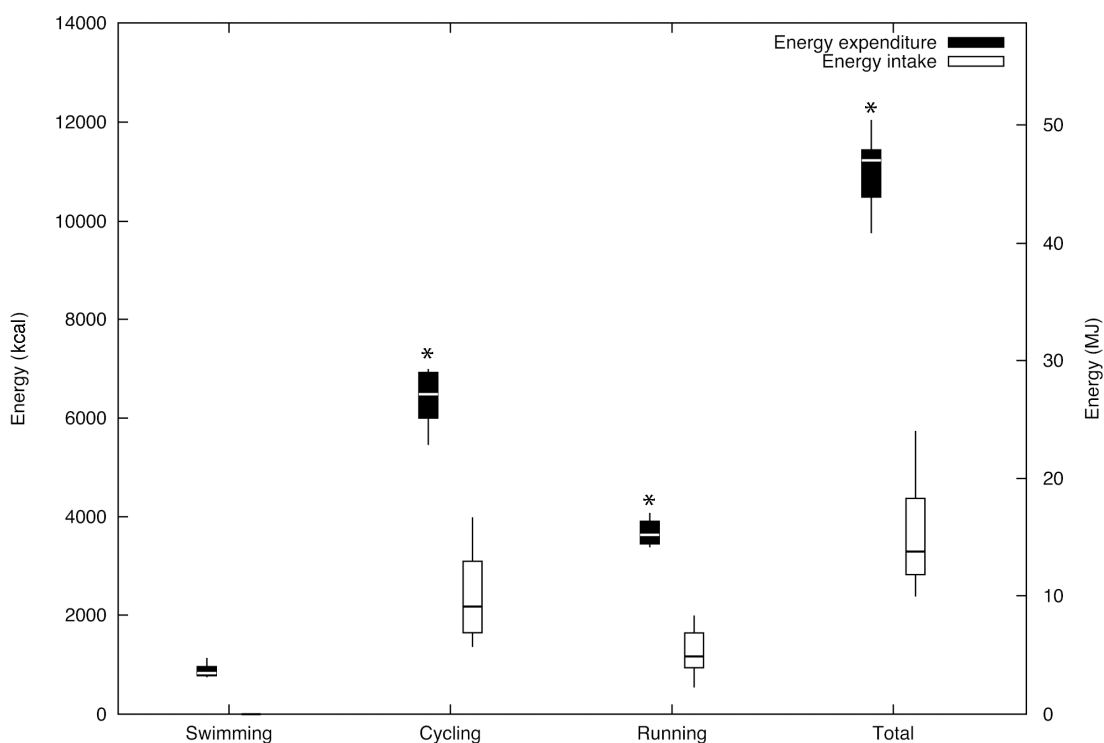
Data are presented as mean (SD). <sup>a</sup> Includes time of swim and race transitions.

<sup>\*</sup> Values are significantly higher during cycling stage than in the running stage ( $P < 0.001$ )

<sup>†</sup> Values are significantly lower during running stage than in the cycling stage ( $P < 0.001$ ).

#### 4.2.3. Energy balance

Figure 25 shows the box-and-whisker plot of the estimated EI and EE during each stage of the competition and in overall terms. EE (11,009 (664) kcal; 46.1 (2.8) MJ) was significantly higher than EI (3,643 (1,219) kcal; 15.3 (5.1) MJ;  $P < 0.001$ ) meaning that an energy deficit of 7,365 (1,286) kcal (30.8 (5.4) MJ; 66.9 (11.7) %) occurred. Solid food significantly provided more energy than fluids (2,812 (1,150) kcal; 11.8 (4.8) MJ) (831 (668) kcal; 3.5 (2.8) MJ;  $P < 0.001$ ). Absolute EI was higher during the cycling stage (2,391 (82.9) kcal; 10.0 (0.4) MJ; 65.63 %) compared to the running stage (1,252 (43.1) kcal; 5.24 (0.18) MJ; 34.4 %;  $P < 0.001$ ). Mean ratios between EI and EE during the cycling, running and swimming stages (i.e. including the swimming stage and transitions times) were 0.37 (0.14), 0.34 (0.13) and 0.33 (0.11), respectively.



**Figure 25.** Swimming, cycling, running and overall energy intake and energy expenditure.

Data are smallest value, first quartile (Q1), median, third quartile (Q3) and largest value of energy intake and energy expenditure during each stage and overall competition.

\* Energy expenditure was significantly greater than energy intake ( $P < 0.001$ ).

#### 4.2.4. Body mass and body water content variables

BM decreased significantly after the race (-4.3 (1.4) kg; -5.7 (1.9) %,  $P < 0.001$ ). Table 12 summarizes the differences in the TBW, ECW and ICW before competition and after competition. TBW, ECW and ICW also decreased significantly after the race (-8.4 (2.9) %, -10.8 (3.7) % and -6.8 (2.9) %, respectively,  $P < 0.001$ ). The decrease in ECW was significantly greater than the decrease in ICW ( $P < 0.001$ ). The decrease in BM was closely related to the decrease in TBW ( $r = 0.98$ ), ECW ( $r = 0.93$ ) and ICW ( $r = 0.85$ ) ( $P \leq 0.001$ , respectively).

**Table 12.** Body water content variables.

	Total body water		Extracellular body water		Intracellular body water	
	Liters	%	Liters	%	Liters	%
Pre-race	45.9 (3.4)	61.3 (3.8)	18.1 (1.3)	39.5 (0.7)	27.8 (2.2)	60.5 (0.7)
Post-race	42.0 (3.3)*	59.6 (4.0)*	16.1 (1.1)*	38.4 (1.2)*	25.9 (2.3)*	61.6 (1.2)*
Difference	3.8 (1.4)	8.4 (2.9)	2.0 (0.7)	10.8 (3.7)	1.9 (0.8)	6.8 (2.9)

Data are presented as mean (SD)

\* Lower values post-race compared to pre-race ( $P < 0.001$ ).

#### 4.2.5. Relationship between racing performance and parameters assessed during the race

The decrease in TBW ( $r = 0.61$ ,  $P = 0.046$ ) and ECW ( $r = 0.72$ ,  $P = 0.01$ ) was related to the cycling time. CHO intake (g/min) was inversely related to the time ( $r = -0.71$ ), and directly related to the speed ( $r = 0.70$ ), during the cycling stage ( $P = 0.02$ ). The stepwise multiple linear regression analysis identified the amount of CHO (g/min) ingested during the race and the % of ECW loss during the race ( $\beta = -0.52$ ,  $SE = 60.8$  and  $\beta = 0.47$ ,  $SE = 4.4$  respectively,  $P < 0.05$ ) as the best predictors of total racing time, accounting for 60% ( $r^2 = 0.60$ ) of the final time variance.

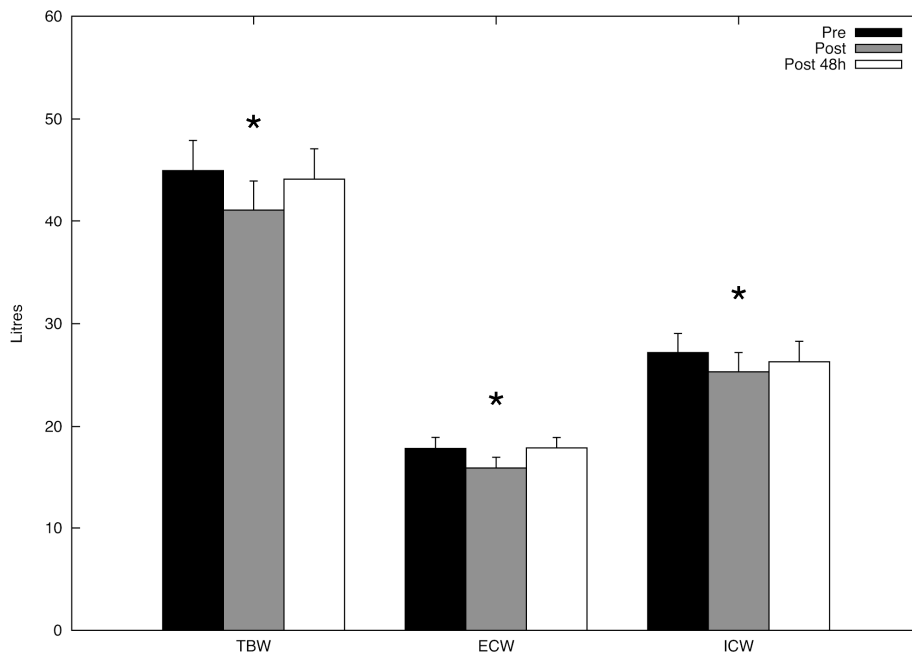
#### 4.2.6. Body water and muscle damage markers

As shown in Figure 26, TBW, ECW and ICW decreased after the race (-8.5 (2.9) %, -10.8 (3.7) % and -7.0 (2.8) respectively,  $P < 0.001$ ), but not further 48 h after.

Slow myosin (1016 (228)  $\mu\text{g/l}$ ), fast myosin (1091 (374)  $\mu\text{g/l}$ ), and creatine kinase activity (202 (87) U/l) largely increased after the race (170 (93) %, 16 (37) % and 600 (476) %, respectively,  $P < 0.001$ ) (Figure 27).

The increase in the slow myosin levels was closely related to the increase in creatine kinase levels ( $r=0.72$ ,  $P = 0.01$ ).

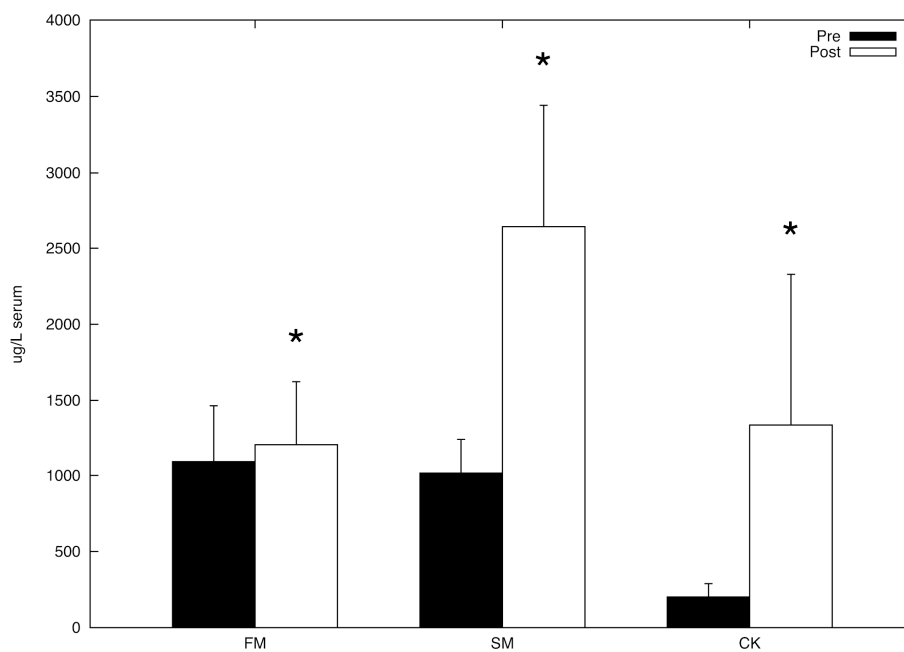
The increase in SM levels was related with the post-race decrease in TBW ( $r=0.71$ ) and ICW ( $r=0.74$ ) ( $P = 0.01$ ), but not in ECW ( $r=0.56$ ,  $P = 0.08$ ).



**Figure 26.** Estimated total, extracellular and intracellular body water content before, and 30 minutes and 48 h post-race.

TBW: Total body water; ECW: extracellular water; ICW: intracellular water.

\* Lower values 30 minutes post-race compared to pre-race ( $P < 0.001$ ).



**Figure 27.** Fast and slow myosin levels and CK activity pre- and post-race.

FM: fast myosin levels; SM: slow myosin levels, CK: creatine kinase levels.

\* Higher values post-race compared to pre-race ( $P < 0.001$ ).

## **5. GENERAL DISCUSSION**

### **5.1. Study I**

Study I of this thesis examined the HR response during a UET race in relation with HR-based intensity markers derived from specific swimming, cycling and running incremental tests.

We found that, contrary to what was believed until now, the absolute or relative HR (expressed as percentage of  $HR_{peak}$ ) is greater in the swimming stage than in the cycling and running stages. It was also observed that swimming at a higher intensity inversely correlated with performance during the following stages and the overall racing performance. Moreover it was found that 81% of the variance in total racing time was explained by the weight-adjusted  $VO_{2max}$  and the  $HR_{diff}$  between the cycling and swimming stages. Furthermore, our data support the concept of an “oxygen consumption ultra-endurance threshold”.

To our knowledge, only one study has previously described the HR response during a UET event (Laursen et al., 2005). However, unlike in the present research, and probably due to technical limitations (i.e. testing during graded swimming with direct  $VO_2$  measurements to assess the individual profile was not performed), HR during the swimming stage was not related to the individual testing profile of the athletes during graded swimming.

In this study, moreover, more adequate scaling (i.e. expressing HR in relation to the percentage of time spent in each segment during the race in the group of subjects) allowed to better characterize the time pattern of HR during the whole event.

#### **5.1.1. Preliminary exercise testing**

Current  $VO_{2peak}$  values from cycle ergometry and treadmill running tests in ultra-endurance athletes are slightly above initial studies from the 1980's (around  $59 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) (Kohrt et al., 1987; O'Toole et al., 1987) and comparable to subsequent



studies involving other triathlon distances (Basset & Boulay, 2000; Caputo & Denadai, 2006; Laursen et al., 2002). Regardless of dissimilarities in testing protocol and techniques, these values are very similar to those reported by Basset & Boulay (2000) (64.6 and 66.9 ml·kg<sup>-1</sup>·min<sup>-1</sup> in the cycle ergometer and the running treadmill, respectively), and Caputo & Denadai (2006) (61.4 and 63.7 ml·kg<sup>-1</sup>·min<sup>-1</sup>, respectively), and slightly lower than Laursen et al., (2002) in the cycle ergometer (67.5 ml·kg<sup>-1</sup>·min<sup>-1</sup>) and Carey et al. (2009) (68.4 ml·kg<sup>-1</sup>·min<sup>-1</sup>). In short, paying attention to a review (Suriano & Bishop, 2010) it can be seen that values reported in this thesis were similar to those reported in other studies with similar subject samples (highly trained) and lower than those studies done with elite triathletes or specialists (elite runners and cyclists).

Regarding VT, in a study done with triathletes of UET distance, Laursen et al. (2005) found values of VT<sub>1</sub> and VT<sub>2</sub> in the cycle ergometer (69% and 81% of VO<sub>2peak</sub>, respectively) which are lower than in our study. VT values in the treadmill running (68% and 81% of VO<sub>2peak</sub>, respectively) were also lower. They also reported lower VO<sub>2peak</sub> values (4.72 and 4.75 l·min<sup>-1</sup> in cycle ergometry and treadmill running, respectively) which means that apparently our subjects were of higher level.

In this study, peak physiological values during the laboratory and pool tests – including VO<sub>2peak</sub>, HR<sub>peak</sub>, absolute VT – were lower during swimming as compared to cycling and running. These results are in agreement with previous studies reporting lower HR<sub>peak</sub> and VO<sub>2peak</sub> during swimming than during land-based exercises as less muscle mass is activated and the haemodynamics associated with a horizontal body position are different (Hauber et al., 1997; Holmér & Astrand, 1972; Magel et al., 1975; McArdle et al., 1978).

The previous finding that VO<sub>2peak</sub> in a maximal 400-m swim was not different from that achieved during maximal cycling and running in competitive swimmers despite attaining a lower HR<sub>peak</sub> (Rodriguez, 2000), suggests that our triathletes may have not reached their full cardiorespiratory capacity, and that peripheral fatigue could have been the main limiting factor in their swimming test performance. Moreover, unlike cycling and running, the performance in swimming is a subtle combination of conditioning and technical abilities (Olbrecht, 2011).

A previous study (Kohrt et al., 1987) also shows lower values in a swimming tethered test than in a cycling and running test, and these values did not relate with the swimming time in a half-triathlon as they did with the other two disciplines. Therefore, we suggest that other factors, such as efficiency, may strongly influence swimming performance. Subjective observations of our subjects suggest that those triathletes with a better swimming technique were able to attain higher speed at the incremental test.

### **5.1.2. Ultra-endurance triathlon race performance**

As in previous studies (Laursen & Rhodes, 2001; Laursen et al., 2005), we found that the swimming time did not correlate with the final racing time, while cycling and running times did correlate closely with finishing time (Table 6). It may be argued that this is due to the shorter duration of the swimming stage (8.5% of total time) compared to cycling (57%) and running (34%). Therefore, not being a good swimmer does not appear to be a limiting factor in UET performance, whereas it could be the case for a sprint or Olympic triathlon, where swimming accounts for 15% and 19% of the overall duration, respectively (Cejuela et al., 2007), and where success may depend on an earlier exit from the water, closer to the leading group during the cycling stage (González-Parra & Díaz-Rodríguez, 2009).

### **5.1.3. Heart rate response and estimated oxygen uptake during the race**

The average HR in the swimming stage (Figure 18, Table 5) was higher than in the cycling and running stages. These results are in agreement with a previous report in which this response was explained in part due to anxiety at the start of the race and to the stress of swimming in open water surrounded by a large number of competitors (Laursen et al., 2005). An alternative explanation is that fatigue affecting the intensity in the cycling and running legs does not affect the swimming leg to the same extent due to its shorter duration (Laursen et al., 2000; O'Toole & Douglas, 1995).

It can be argued that triathletes swim the initial stage of the race at a quicker pace in order to emerge in the leading group, gaining an advantage for the following

stages (Cala & Cejuela, 2011). Considering the competitive level of our participants, a factor that could better explain this difference is that proposed by Olbrecht (2011). He stated that triathletes very often suffer from a lack of technique, resulting in expending considerable energy in the swimming stage –with little propulsive energy in return– which is then no longer on hand for the rest of the race.

In this study, since the incremental swimming test was performed using the same wetsuit as in competition in a pool at 24 °C, we cannot rule out a certain tachycardic effect of thermoregulatory mechanisms on exercise HR (Rhoades & Bell, 2012), though it is not likely to substantially affect our results due to the relatively short duration of the test (ca. 15 min on average) and the relatively similar water temperature during the race (range: 20.8–21.2 °C).

The intensity of the race with respect to  $HR_{peak}$  was on average 78% in the cycling stage, which is a little lower than the intensity found by Laursen et al. (2005) (83%). We ascribe this difference to the 2,600 meters of elevation gain in the cycling stage of this race. The intensity in the running stage was 77%, very similar to that found by Laursen et al. (2005). O'Toole et al., (1998) described values close to 80% with a drop of 6-7% in the final part of the cycling and running stages. Laursen et al., (2005), found that this decrease was only significant in the running stage. However, in these studies the duration of the test was not normalized, meaning that in the final stage there were only the slowest triathletes.

In our study, we normalized the duration expressed in time percentage (Figure 18) and a novel intensity profile can be observed. When properly scaled, HR showed a clearly declining trend at the final part of each of the three stages, which is likely, the result of skeletal and cardiac muscle fatigue. Only at the final stage of the running stage did HR rise up to previous levels, which probably reflects the last effort before the end of the race. In addition, the intensity expressed as a percentage of the peak HR was significantly higher in the swimming stage (92%) than in the cycling and running stages (78 and 77%, respectively), despite attaining a similar  $VO_2$  (Figure 19, Table 5). This novel value for the swimming stage is in agreement with the HR racing response reported by Laursen et al. (2005) and challenges the hypothesis that the HR would be

lower during the swimming leg than during the land-based segments for an equivalent level of  $\text{VO}_2$  due to a shift to the right in the HR vs.  $\text{VO}_2$  regression line during swimming. In our triathletes, the regression was, in fact, shifted to the left (Figure 15) in all subjects as compared to cycling and running exercises.

The characterization of the HR response during the entire race in relation with the physiological parameters derived from specific exercise testing and of the intensity pattern during the swimming stage, as well as a more adequate description of the time pattern response during the whole race, add new information concerning the intensity profile and cardiovascular demands of an ultra-endurance triathlon race.

From the physiological point of view an ultra-endurance sport event can be described as a predominantly aerobic effort throughout its duration (O'Toole, 1989; Sleivert & Rowlands, 1996). A previous study (Neumayr et al., 2003) had shown that an ultra-endurance athlete who did a 460 km cycling stage spent 99.6% of the total time (i.e., 20 h 46 min) working under aerobic conditions with just 0.4% of the remaining time at a high-intensity work rate. Although that study stated that it was applicable to the UET, a recent review (Laursen, 2011) has defined “ultra-endurance” as moderate to high-intensity (intensities between  $\text{VT}_1$  and  $\text{VT}_2$ ) exercise performed for durations longer than 4 hours.

As the results of the present study show, the overall race intensity was mainly under that corresponding to  $\text{VT}_1$  (70%). In this zone the body mainly uses fat as a fuel. However the intensity during the swimming stage did not follow the same pattern since the intensity was above the  $\text{VT}_2$  (92% of swimming time). This also differed from what was initially hypothesized, where intensity was expected to be around the  $\text{VT}_1$  in all three stages.

Some studies hypothesize the existence of an ultra-resistance metabolic threshold based on HR (Laursen & Rhodes, 2001; Laursen et al., 2002; O'Toole et al., 1998; O'Toole et al., 1987). As this study shows, however, this threshold cannot be based on HR, since it varies in the three stages (swimming, cycling and running).

In the swimming stage, it may be reasoned that peripheral muscular fatigue occurred due to lack of strength and endurance in the upper body compared to the lower

body, and to less specific training of these body segments. It is also important to consider the energy substrate, given that glycogen depletion will lead to a consequent drop in performance over time.

Hydration, temperature, cardiovascular drift and cardiac fatigue are also factors that may affect HR during exercise (Achten & Jeukendrup, 2003) and therefore challenge this notion of a threshold based on HR. It is also important to take into account that HR is a poor indicator of psychological strain in competition (Esteve-Lanao et al., 2008).

When looking at Figure 20, it can be seen that  $\text{VO}_2$  is similar in all three stages, which suggests an ultra-endurance threshold based on estimated  $\text{VO}_2$  that is around  $3,000 \text{ ml}\cdot\text{min}^{-1}$ . This value is above the  $\text{VT}_2$  for the swimming and below the  $\text{VT}_1$  for the cycling and running stages. Therefore, estimated  $\text{VO}_2$  can be used as a practical guide during an UET.

#### **5.1.4. Relationship between race performance and physiological parameters**

A close relationship between HR at the  $\text{VT}_1$  measured in graded exercise tests and HR during the cycling and running stages has been previously reported (Laursen et al., 2005). Although a similar pattern was found in this study, particularly in the running stage, participating subjects tended to work below  $\text{VT}_1$ . In contrast, HR during the swimming stage was remarkably higher (close to  $\text{VT}_2$ ) when compared to the cycling and running stages, thus clearly indicating the higher intensity of the first stage. These new values for the swimming stage represent a new reference of intensity.

Unlike Laursen et al. (2005), no correlation was found between exercising under  $\text{VT}_1$  (or  $\text{VT}_2$  in the case of swimming in the present study) and the completion time for each triathlon stage or the overall event. A negative correlation was found between the difference in HR in the cycling and running stages and the marathon time ( $r = -0.8$ ). This seems to indicate that the lower the HR during cycling, the better the performance during running, but with the trade-off that an improvement in running time does not compensate for a lower performance in the cycling stage. This may be the reason why

no correlation was found between the difference in HR in the running and cycling stages and the overall racing time. Laursen et al. (2005) are also in line with these findings.

Other studies (Chatard et al., 1998; Delextrat et al., 2003; Delextrat et al., 2005; Kreider et al., 1988) show that dropping the relative intensity in the swimming stage using neoprene or drafting may provide a reserve of metabolic energy, which could further influence overall race success. In a later study (Peeling et al., 2005) it is said that swimming at an intensity below that of a time trial effort significantly improves subsequent cycling and overall triathlon race performance.

In Olympic distance, Vleck et al. (2006) showed that a lower swimming performance can result in a tactic that involves greater work in the initial stages of the cycling portion, and may influence subsequent running performance. Another study (Peeling & Landers, 2009) highlights the importance of regulating the swimming intensity during sprint and Olympic distance triathlons and how it impacts on the ensuing cycle and run.

But, what happens with the intensity in an UET? On the one side, Millet et al. (2003) demonstrated a significantly faster swim time in short-distance triathletes than in long-distance triathletes while physiological characteristics measured in cycling and running in laboratory trials were all similar. On the other side, a previous report concluded that 3,000 m of swimming had no significant performance effect (in terms of power output) on subsequent 3-h cycling performance in UET athletes (Laursen et al., 2000).

In this study the differences in HR between the swimming and cycling stages correlated with the cycling time, marathon time and the overall time. Triathletes with the greatest difference in HR in the swimming and cycling stages had the worst times in the next portions. As stated by Laursen et al. (2005), working beyond capacity in one stage makes recovery difficult, leading to lower performance in the next stage. This correlation can also be seen in HR differences for the swimming and running stages, as a large difference in values is associated with a reduction in the marathon time and consequently in overall racing time. Therefore, the present results support the view of Peeling & Landers (2009) that, though performed at a relatively lower intensity as

compared to shorter distance triathlon races, swimming intensity may also affect the physiology of subsequent cycling and overall triathlon performance. In any case, it seems advisable to improve the ultra-endurance triathlete's swimming efficiency and to consider using strategies for manipulating the relative swimming intensity (e.g. drafting and using wetsuits or speedsuits) without compromising absolute performance (Peeling & Landers, 2009), as well as adequately pace the swimming leg according to the triathlete's swimming ability.

Some studies have analyzed which factors can best predict performance in a triathlon. In Olympic distance triathlon, Baldari et al. (2007) showed that velocity at the power output of optimal lactate removal was the variable most related to overall performance. A further study on the Olympic distance (De Vito, 1995) provided evidence that the  $VO_{2peak}$  and the  $VT_2$  were good predictors of performance in the cycling and running stages.

In a study (Hue, 2003) it has been shown that lactate concentration noted at the end of the cycle stage and the distance covered during the running part in a submaximal test (i.e. cycle-run transition) correlate with overall time. In the same way, it has been shown that the greater  $VO_{2max}$  during maximal exercise tests and the smaller increment of  $VO_2$  during a simulated laboratory test triathlon –indicating good economy– were good predictors of performance in an Olympic distance triathlon (Miura, 1997). Schabort et al. (2000) showed that peak treadmill running velocity and blood lactate concentration measured during steady-state cycling were the variables that best predicted the performance in Olympic distance triathlon.

Regarding predictors in UET, Knechtle et al. (2010) recently found that the three variables that best explained final time (64% of the variance) were the speed in running during training, the personal best marathon time, and the personal best time in an Olympic distance triathlon, although strong collinearity can be expected to lay behind this association.

In this study it was found that the best predictors of overall triathlon time were the weight-adjusted  $VO_{2max}$  and the  $HR_{diff}$  [cycle-swim], which accounted for 81% of the variance in UET race time. To the best of our knowledge, such a relationship has not

yet been reported and would be important to take into account as it suggests that the  $\text{VO}_{2\text{max}}$  may be as important as it may be with shorter distance triathlons and that the race strategy may also play a substantial role in UET race performance.

## **5.2. Study II**

This study provided proper characterization of the energy and fluid intake, as well as the estimated energy expenditure, of a group of male triathletes during an entire UET race.

The estimated EE was ~11000 kcal (46 MJ), whereas EI was only ~3600 kcal (15 MJ), which resulted in an energy deficit of almost 70%. This result partially confirms our hypothesis and demonstrates the challenging metabolic demands of a UET. Triathletes and coaches should be aware of the unique stresses involved by participating in such events in order to prepare an adequate training, nutritional and hydration plan, and adequate race strategies taking into account the individual physiological profile.

The ability to fuel these high levels of output during long periods of time is a major factor of success. This study emphasizes the importance of being economic and efficient (i.e. to improve the ability to generate high levels of energy from fat across a broad intensity range), of maximizing CHO absorption and oxidation, and of increasing the absorptive capacity of the gut during training to perform better during competition.

In this study it was as well assessed the muscle damage induced by a UET and its relationship with the hydration status.

### **5.2.1. Macronutrient intake and energy balance**

As shown by two previous studies (Cuddy et al., 2010; Kimber et al., 2002), a high negative energy balance seems to be common in UET. The high energy deficit in UET events can be explained by the lower EI of athletes in comparison with the higher energy demands of such events. The average of EE ( $11,009 \pm 664$  kcal) in this study was higher than in these previous studies. This fact could be explained by the positive



elevation that triathletes had to overcome during the cycling stage in the current UET which induced a higher EE.

In this study, athletes consumed an average of  $927 \pm 178$  g of carbohydrates (90 % of the overall EI). In relative terms, this amount corresponds to  $\approx 84 \text{ g}\cdot\text{h}^{-1}$ . However, while these values can be considered within the current recommendations for longer events (Smith et al., 2013), it must be noted that CHO ingestion was heterogeneous between participants. For instance, five participants consumed less than  $70 \text{ g}\cdot\text{h}^{-1}$  of CHO while two of them ingested more than  $90 \text{ g}\cdot\text{h}^{-1}$ . The remaining 4 triathletes consumed an amount within the range of  $70\text{--}90 \text{ g}\cdot\text{h}^{-1}$ . Such differences between CHO ingestion were also linked with exercise performance on the bike. We found that triathletes ingesting higher amounts of CHO on the bike were able to perform better than those with low rates of CHO ingestion. This confirms the well-known fact that CHO supply plays a key role in order to improve exercise performance of athletes during endurance events (Rodriguez et al., 2009).

Furthermore, a part of the amount of CHO, another key point related to the CHO intake is the type of CHO. It has been shown that the combination of different types of CHO (glucose-fructose-maltodextrin) with a consistent ratio (1:1:1) optimizes CHO oxidation and exercise performance (Smith et al., 2013). Although it is difficult to estimate such ratio in this study due to the variety of food that athletes ingested during the event (Table 4), the combination of sport gels, sport bars, sport drinks and sandwiches may indicate that athletes ingested multiple CHO types.

Although the amount of CHO could be associated to an improved performance, knowing that the body is physiologically limited, it seems logical to not exceed the gut's capacity to absorb more CHO. Only those athletes with below average intakes might therefore expect to enhance their performance by increasing their intake. For this reason it is important to focus on finding strategies to enhance the macronutrient oxidation capacity and to try to improve the physiological threshold. But the problem arises when we have to program the nutrition for the trainings bouts since there is no clear evidence on how to do it. What seemed an easy task as drinking from the bottle or eating a bar,

should be planned and studied carefully, as proven, because in a UET any small detail could mark the difference between reaching one's performance target or not.

The average protein intake per triathlete was  $45 \pm 12$  g in total (4 % of total EI). This amount was higher than previous data reported in triathletes (Kimber et al., 2002). However, to date, there is not a consensus about the protein needs during ultra-endurance events. Under laboratory conditions, it has been shown that the ingestion of  $0.25 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  in combination with carbohydrates can improve protein balance by increasing protein synthesis and decreasing protein breakdown during long efforts (6h) (Koopman et al., 2004). Considering the data provided by Koopman et al., (2004), along with the amount of CHO intake by the athletes in this study, the 'ideal' amount of protein ingestion would have been 236 g. This amount of protein would increase the energy intake (> 900 kcal) and it would help to decrease the energy deficit of athletes.

The consumption of fat was  $77 \pm 20$  g during the whole event. These values are lower compared with those presented in a previous study (Kimber et al., 2002). Like protein intake, currently there is no evidence supporting that the ingestion of lipids in form of either long-chain triacylglycerol (LCT) or tolerated amounts of medium-chain triacylglycerols increases performance during prolonged exercise (Coyle, 2004).

Although the human body stores of fat are so large and they will not become depleted after prolonged events, some stores, such as intramyocellular triglycerides (IMTG), can play a key role during exercise (Knechtle et al., 2004). These lipids are located directly to the site of contraction to ensure immediate substrate availability for physical activity (Van Loon, 2004). Under conditions in which muscle glycogen availability is severely challenged, IMTG can compensate (at least in the endurance trained individual) by providing an alternative fuel source of similar potential energy to glycogen, as a means to maintain moderate intensity physical activity (Johnson et al., 2004). However, while early research of the role of muscle glycogen in endurance exercise provided clear prescriptive information for the endurance-trained athlete, no such direction for optimising exercise performance is yet apparent from research concerning IMTG. On the other hand, the inclusion of fat in the diet of ultra-endurance athletes could also be interesting to satisfy the taste of foods.

It has been shown that training with low glycogen may be beneficial for improving muscle oxidative capacity of fats (Psilander et al., 2012). However it has also been shown that exogenous CHO oxidation rate is increased after a high CHO diet during 28 days compared to the control group (Cox et al., 2010). It should be useful to guide the training programs to increase the capacity to oxidize fat when muscle glycogen stores are depleted. However it can be in detriment of the capacity to oxidize CHO. Therefore, a lack of CHO during exercise may mean an improvement in the fat oxidation capacity but in detriment of an improvement of CHO oxidation.

First question is: How important is it to increase fat metabolism at the expense of the decrease in CHO metabolism? And the second question is: is “train on low CHO, compete on high CHO” a good strategy for ultra-endurance triathletes? We may suggest that it would be more important to be efficient at burning fat, as we found that the triathletes in this study were working mainly under the first ventilatory threshold where the body uses mainly fat as fuel and the total EI did not provide the amount of energy necessary to deal with the UET.

In a recent review (Gonzalez & Stevenson, 2012) the authors suggest that pre-exercise low glycaemic index meals that lead to a greater rate of fat oxidation during sub-maximal, continuous exercise, are limited to endurance events where optimal nutrient intake during competition may be limited like the swim leg of a UET. Then the key seems to be looking for further adaptations to increase the ability to generate energy from fat.

Further research is needed in order to investigate such different macronutrient training strategies and methods as well as the use of other supplements like caffeine or nitrates that may enhance fat oxidation. Moreover, while an increase of lipid and protein consumption during UET would reduce the energy deficit of athletes, it is unknown how this would affect other key physiological responses such as gastric emptying and intestinal absorption during the race. Consequently, more research is also needed in order to investigate the protein and lipid needs in ultra-endurance events.

### **5.2.2. Body mass, fluid and sodium intake**

In our athletes, the BM decreased significantly after the UET (-6 (2) %). Decreases in BM up to as much as 11% of the total body weight have been shown previously (Cuddy et al., 2010; Hew-Butler et al., 2008; Laursen et al., 2006; Sharwood et al., 2004; Speedy et al., 1997). These BM losses can be explained by high energy deficits which induce a decrease in muscle density (glycogen loss) and fat mass of the body of athletes participating in ultra-endurance events (Knechtle et al., 2010; Knechtle et al., 2011; Knechtle et al., 2008; Mueller et al., 2013).

In the current study we found a fluid deficit of 1.3 L which was far from the mean loss of body weight. Therefore, we assume that most of the loss in BM was due to losses of glycogen and fat stores. However we can not report the values of the changes in the fat mass and muscle mass due the fact that these devices are good to analyse body water but they have several limitations to assess body composition. BIA analyzers use an electrical current via electrodes to measure resistance. Fat mass has high impedance, while free fat mass has low impedance. Importantly, resistance which is a component of impedance is converted to total water and this value is used to estimate fat free mass. Thus, since BIA body composition relies on total body water, hydration changes can affect BIA estimates. For this reason, we think that the use of these devices to estimate body composition after endurance events is not valid, especially, in studies like this one showing significant losses of body water.

Additionally, a significant and negative correlation between losses of TBW and ECW and performance during the cycling stage was found in this study. Although this fact corroborates the well-known actuality that hydration is a key point for exercise performance, it is also important to note that losses of TBW were mainly linked to a reduction of ECW.

An overload of ECW is a risk factor to develop hyponatremia (Speedy, Noakes, et al., 2000; Speedy et al., 2001; Speedy, Rogers, et al., 2000). Importantly, in the current study none of the athletes showed an increase of ECW. Furthermore, despite the fact that hyponatremia is an electrolyte disorder affecting serum sodium concentration

(<135 mmol·L<sup>-1</sup>), the consumption of high amounts of sodium during exercise does not reduce the risk of developing hyponatremia (Hew-Butler et al., 2006).

Fluid overload is considered the main risk factor in the pathogenesis of hyponatremia, and this is controlled by fluid intake during exercise as well as by the activity of renal hormones such as vasopressin (Rüst et al., 2012; Shephard, 2011).

The mean sodium intake reported by the current triathletes (2,152 (1,124) mg) was within the Recommended Dietary Intake and above the mean sodium ingestion indicated by Kimber et al., (2002) in a previous study. This can be explained by the higher content of sodium of some sport food and sport drinks consumed by participants of this study during the event. None of the participants consumed sodium in form of supplementation. This finding shows that it is possible to meet sodium recommendations through normal food and drinks in ultra-endurance events and, consequently, sodium supplementation is not needed. These data are in agreement with another recent study investigating sodium intake of ultra-endurance runners during a multi-stage race in a hot environment (Costa et al., 2013).

### **5.2.3. Muscle damage and its relationship with the hydration status**

While plasma creatine kinase content is believed to be a reliable marker of muscle damage (Brancaccio et al., 2010), the elevated slow myosin levels that the subjects in this study presented suggest specific damage in slow muscle fibers, which are largely predominant in ultra-endurance athletes (García-Manso et al., 2011) and have a high mitochondrial density and oxidative enzyme capacity which allows the majority of energy production to come from aerobic metabolism.

Our results, beyond confirming fiber muscle damage induced by an UET race, strongly suggest that, as hypothesized, cellular structural damage predominantly affects slow fibers, and that muscle damage is related to the hydration status.

Given this result and knowing that the majority of the muscle damage occurs during the run phase of a UET (Farber et al., 1991), an appropriate training program that can result in muscular adaptations in running following subsequent bouts of swimming and cycling exercise would help. Such adaptations would create more resilient and

elastic muscle fibers and tendons, lessen muscle damage and pain, and improve run performance ability (Laursen, 2011). In time, fibers are regenerated, and adaptations in the connective tissue would provide greater resilience to insult during the critical long distance triathlon event (Ebbeling & Clarkson, 1989). It would be appropriate as well, during the UET, to keep the body in a euhydrated state to minimize the potential for muscle damage.



## 6. CONCLUSIONS

The sums of the findings from the two studies that comprise this thesis support the following conclusions:

- Information resulting from the measurement of the physiological profile during UET can be used to change training and race strategies as a means to improve performance.
- Exercising at a higher intensity (i.e. higher HR values) during the swimming stage -in relation to the intensity during the cycling and running stages- of a UET has a negative relation on performance during the following stages and the overall racing performance, so it may be advantageous to lower swimming intensity.
- Weight-adjusted  $VO_{2max}$  measured in laboratory tests and the  $HR_{diff}$  between the cycling and swimming stages during the UET explain 81% of the variability in UET performance.
- The similar  $VO_2$  data found in all three stages of a UET, support the concept of an “oxygen consumption ultra-endurance threshold”, which is situated at ~3000 mL.
- The high energy demands (~11000 kcal) induced by UET exceed by far the EI (~3600) of triathletes despite consuming an adequate amount of CHO. It corresponds to an energy deficit close to 70%.
- Athletes competing in a UET show significant BM and fluid losses (TBW, ECW and ICW). These fluid losses are more related to a reduction of extracellular



fluids than intracellular fluids. The TBW and ECW losses produced during a UET are related to a worse performance in the cycling stage.

- The amount of CHO (g/min) ingested during the race and the % of ECW loss during the race explain 60% of the variability in UET performance.
- A UET induces structural damage in the muscle, predominantly affecting slow-twitch fibers. This muscle damage is related to the degree of hydration during the race such that as water loss increases muscle damage also increases.

## 7. STRENGTHS AND LIMITATIONS

It is important to acknowledge that this research not only confirms UET heart-rate based intensity, but also represents a pioneering research study on the relative intensity of the swimming stage.

The protocol for the endurance test for this stage should be revised, since it may be the case that triathletes reached the end of the test with local muscle fatigue in the upper body rather than reaching the  $VO_{2max}$  as indicator of general fatigue.

It can be argued that using a different protocol may have allowed triathletes to reach a higher  $VO_{2peak}$  during swimming. However, this would not have an effect on the estimation of race intensity relative to the VT demarcation points.

We are also aware that, as in many other sport-performance related studies (Hopkins et al., 1999), the rather small size of the final sample in these studies –mostly due to instrumental malfunction of the HR monitors during the race– may have enhanced the risk of type-2 error.

Finally, even though the sample in these studies was composed of well-trained, ultra-endurance triathletes, differences in the athletes' rank may yield somewhat different results.

The careful nutritional analysis carried out in a community and setting where little information has been provided could be considered a major strength of this thesis. However, we should also acknowledge some limitations and caveats.

Perhaps, a limitation was the method used to estimate energy expenditure (relationship between HR- $VO_2$ ). It is known that this method can be affected by several physiological and environmental factors such as dehydration and temperature (Hiilloskorpi et al., 1999). However, there is also evidence indicating good correlation between the doubly labelled water method (which is considered as the gold standard) and the use of equations based on the linear relationship between HR and  $VO_2$  under field conditions (Cuddy et al., 2010). Furthermore, a study performed in laboratory conditions showed that the ratio  $VO_2/HR$  increased 7% after 12 hours of continuous

exercise (Mattsson et al., 2010). These data suggest that the current study underestimated the EE in a range of 700-800 kcal, and consequently, there was an even higher energy deficit.

It would be interesting to perform new studies in athletes of higher (e.g. professional triathletes) and lower (e.g. leisure competitors, youth, etc.) ability levels.

It would be interesting as well to carry out the same study in other UET races with different terrain and environmental conditions as the present results may be related to the particular race conditions.

Moreover, it would also be of interest to assess if other factors such as the catecholamine levels or emotional stress during competition can have any influence in the HR response during competition, as it could alter the HR-VO<sub>2</sub> relationship assessed in the preliminary tests.

Finally, it should also be taken into account that UET undoubtedly imposes demands that are different in many aspects to those of other triathlon modalities such as Olympic and sprint, or super-sprint. Further research regarding the specific physiological requirements is needed to guide triathletes in these modalities.

## 8. SUMMARY IN CATALAN

En aquesta tesi es presenten els resultats de 2 estudis d'investigació relacionats amb les demandes fisiològiques, el balanç energètic i el dany muscular en els triatlons d'ultra-resistència.

L'èxit en el triatló d'ultra-resistència està regit per la capacitat de mantenir una velocitat absoluta superior per una donada distància, en comparació amb els altres competidors (Zaryski & Smith, 2005). Per optimitzar el rendiment i determinar els factors de rendiment és necessari conèixer la resposta fisiològica i els requeriments energètics durant la competició. El perfil d'intensitat durant un triatló pot ajudar a entendre les demandes fisiològiques i proporcionar informació essencial per a l'entrenament òptim dels triatletes que competeixen en aquests tipus d'esdeveniments. A més a més, donada la llarga durada d'aquests esdeveniments esportius, un dels principals objectius per als atletes és la gestió del consum d'aliments i begudes durant la cursa (Laursen & Rhodes, 2001) per tal de millorar el rendiment i el manteniment de l'homeòstasi del cos. La informació en aquest camp és escassa o gairebé nul·la.

Un altre punt clau que afecta aquests triatlons és el dany muscular que pateixen els atletes, que pot disminuir el rendiment muscular i, per tant, afectar el rendiment final del triatló. Un programa d'entrenament de força orientat a produir adaptacions musculars podria reduir aquest dany, però primer cal conèixer quines són les fibres musculars afectades.

D'acord als punts exposats, els **objectius** plantejats en aquesta tesi han sigut:

- Proveir, per primera vegada, una caracterització comprensiva del perfil d'intensitat basat en la freqüència cardíaca durant un triatló d'ultra-resistència. Aquest perfil d'intensitat s'estimarà en funció de la relació freqüència cardíaca-consum d'oxigen obtinguda en tests específics en cadascun dels tres modes d'exercici.

- Relacionar els paràmetres fisiològics de laboratori i de camp mesurats amb el rendiment en la competició.
- Proveir una caracterització del consum d'energia i líquids durant la totalitat d'un triatló d'ultra-resistència.
- Estimar la despesa energètica i el balanç de fluids (dipòsits intra i extracel·lulars) al llarg de la competició utilitzant les tres equacions individualitzades obtingudes de cada triatleta.
- Avaluar el dany muscular produït per un triatló d'ultra-resistència mitjançant l'avaluació dels nivells de sèrum de les miosines ràpides i lentes, i dels nivells d'activitat de la creatine kinase.
- Relacionar el dany muscular amb el nivell d'hidratació dels atletes.

Els **resultats i conclusions** derivats d'aquests dos estudis que conformen la tesis han sigut:

La freqüència cardíaca mitjana durant la competició va ser superior durant el segment de natació (149.2 (10.1) batecs·min<sup>-1</sup>) que durant el segments de ciclisme (137.1 (5.7) batecs·min<sup>-1</sup>) i cursa a peu (136.2 (10.5) batecs·min<sup>-1</sup>). Durant aquests dos últims segments la freqüència cardíaca va estar per sota dels dos llindars ventilatoris (~11% i ~27-28%) mentre que en el segment de natació la freqüència cardíaca es va situar al voltant del segon llindar ventilatori.

Les diferències en la freqüència cardíaca entre el segment de natació i el segment de ciclisme van obtenir una forta correlació amb els temps dels segments de ciclisme i cursa a peu, així com també amb el temps final; com més gran va ser la diferència entre la natació i el ciclisme, pitjors resultats van obtenir els subjectes. Per tant, es pot afirmar que el segment de natació en un triatló d'ultra-resistència es desenvolupa en una intensitat relativa superior als altres dos segments i que aquesta intensitat està relacionada amb un pitjor rendiment als següents segments.

El consum d'energia va ser de 3.643 (1.219) kcal i la despesa energètica estimada va ser de 11.009 (664) kcal. En conseqüència, els atletes van mostrar un dèficit d'energia de 7.365 (1.286 kcal (66,9 (11,7%))) posant de manifest les altes demandes

energètiques d'aquests tipus de competició, que no són compensades per la ingesta de nutrients i líquids, resultant en un dèficit energètic de grans dimensions.

La massa corporal va disminuir significativament després de finalitzar el triatló i també es van trobar pèrdues significatives en l'aigua total corporal. Aquestes pèrdues van estar més relacionades amb la reducció dels fluids extracel·lulars que amb la reducció dels fluids intracel·lulars.

Els paràmetres que millor prediuen el rendiment (expressat com a temps final de cursa) en els triatlons d'ultra-resistència són el consum màxim d'oxigen relatiu i la diferència entre la freqüència cardíaca entre el segment de ciclisme i natació.

Una caracterització del patró d'intensitat durant tota la cursa, especialment del segment de natació, afegeix nova informació del perfil d'intensitat i les demandes cardiovasculars d'un triatló d'ultra-resistència, la qual cosa remarca la importància de l'especificitat en els tests per avaluar el perfil fisiològic de cara a preparar els entrenaments i l'estratègia de competició.

Un triatló d'ultra-resistència provoca dany muscular afectant principalment les fibres musculars 'lentes'.

Un programa d'entrenament orientat a la producció d'adaptacions a les fibres musculars lentes, pot ajudar a reduir el dany muscular produït durant un triatló d'ultra-resistència.

El dany muscular produït en triatlons d'ultra-resistència està relacionat amb el grau d'hidratació durant la competició i, per tant, els triatletes haurien d'evitar la deshidratació per, entre altres coses, prevenir el deteriorament del múscul.



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## **ANNEX 1. MANUSCRIPT I**

**Intensity profile during an ultra-endurance triathlon in relation to testing and performance**



# Intensity Profile during an Ultra-endurance Triathlon in Relation to Testing and Performance

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## Key words

- heart rate
- oxygen uptake
- ventilatory threshold
- swimming
- cycling
- running

## Abstract



We examined the heart rate (HR)-based intensity profile during an ultra-endurance triathlon (UET) estimated from the individual HR-oxygen uptake ( $\dot{V}O_2$ ) relationship during specific graded tests, relating it to race performance. 9 male ultra-endurance triathletes completed the study. Before racing, subjects performed graded exercise tests involving cycle (C) ergometry, treadmill running (R) and free swimming (S) for peak  $\dot{V}O_2$  and HR at ventilatory thresholds (VT). Exercise-specific HR- $\dot{V}O_2$  regression equations were developed. Mean race HR was higher during S (149.2 (10.1) bpm) than during C (137.1 (5.7)

bpm) and R (136.2 (10.5) bpm). During C and R, HR was below both VT (11% and 27–28%). HR differences between S and C correlated with C, R and final times. The greatest differences between S and C were related to the worst times in the next stages. These ultra-endurance triathletes performed S at a higher relative intensity, which was inversely correlated with performance in the following stages. The best predictors of final racing time (81%) were relative  $\dot{V}O_{2max}$  and HR difference between C and S. A more adequate characterization of the time pattern during the whole race, especially during S, adds new information concerning the intensity profile and cardiovascular demands of an UET race.

## Introduction



While triathlon is rising in popularity, witnessing an increase in the number of triathletes and competitions especially since its debut in the 2000 Olympics in Sydney, knowledge of the physiological demands during competition is far from being extensive. One reason for this is the complexity of studying sports such as triathlon involving more than one discipline. In the case of ultra-endurance triathlon (UET), consisting of 3.8 km swimming, followed by 180 km cycling and 42.2 km running, average completion times stand at around 8 h for faster triathletes and approximately 16 h for slower triathletes [27]. To optimize training and determine performance factors, it is necessary to know the physiological response and requirements during competition. An exercise intensity profile can promote greater understanding of the physiological demands of UET and provide essential information for optimal training of triathletes. Although the literature contains several studies of simulated and real competitions [5,9,12,16,21–25,32,33,40], there is no clear knowledge of the physiological demands for an entire triathlon at the different

distances and in relation to the individual metabolic capacities of triathletes assessed according to the principle of testing specificity.

Previous studies report that the peak heart rate ( $HR_{peak}$ ) and peak oxygen uptake ( $\dot{V}O_{2peak}$ ) are lower during swimming than during running or cycling as less muscle mass is activated, and the hemodynamics associated with a horizontal body position are different [15,17,28,30]. However, more recent results showed that  $\dot{V}O_{2peak}$  in a maximal 400-m swim is not different from that achieved during maximal cycling and running in competitive swimmers despite attaining a lower  $HR_{peak}$  [42]. Field-based research has shown that well-trained triathletes perform the cycling leg of an Ironman triathlon at 80–83% of maximum HR ( $HR_{max}$ ) [24,34] and 55% of peak power output ( $\dot{P}_{peak}$ ) [1]. Other studies show a metabolic intensity of around 55% of  $\dot{V}O_{2peak}$  [23,36]. According to Abbiss et al., [1], the intensity during an UET tends to decrease as the race progresses, i.e. the intensity (and the speed) is high at the beginning of the competition, while dropping at the end. Some authors [23,35,36] describe 6–7% reductions in the HR measured in the cycling and running legs of UET. However, it does not seem

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reasonable to assume that exercise intensity in high-level triathletes should decrease during the running leg of an UET.

The study by Laursen et al. [24] is the only one, to the best of our knowledge, assessing physiological demands during an UET by measuring HR in a real competition. This study of well-trained triathletes shows HR values during the cycling leg (mean 148 (9) beats·min<sup>-1</sup>) and during the running leg (143 (13) beats·min<sup>-1</sup>) that were significantly lower than the second ventilatory threshold (VT<sub>2</sub>) calculated in the laboratory (160 (13) beats·min<sup>-1</sup> and 165 (14) beats·min<sup>-1</sup>, respectively). Nevertheless, the HR during the cycling and running legs were significantly related to, and not significantly different from, the first ventilatory threshold (VT<sub>1</sub>). The difference between the HR during the cycling leg and the HR at VT<sub>1</sub> was correlated with the marathon time and the total triathlon time. Higher HR values are reported in the swimming leg than in the cycling or running. Despite being a pioneering work, the study had some methodological limitations that should be taken into account. Firstly, the laboratory measurements were taken on various ergometers and metabolic measurement systems that were not calibrated with one another. Secondly, competition data were gathered from three different UET (Ironman) events, each with different environmental and track conditions. Thirdly, the time pattern did not take into account the progressive decrease of the sample size along the race as athletes ran into transitions or finished. Finally, HR during the swimming leg was not properly described and related to the testing profile of the athletes during swimming. In fact, according to the results reported in this stage, if it were to be assumed that HR is lower as less muscle mass is activated, the hemodynamics associated with a horizontal body position are different, and the effect of gravity and reflex bradycardia is smaller, then it would seem reasonable to hypothesize that the HR values in the swimming leg would be significantly lower than in the cycling and running legs.

Therefore, the aim of this study is to provide the first comprehensive characterization of the heart rate-based intensity profile during an entire UET race estimated from the HR- $\dot{V}O_2$  individual relationship assessed during specific graded tests in each of the three exercise modes. A further aim is to relate laboratory and field physiological parameters to race performance.

## Materials and Methods



### Subjects

9 well-trained, nonprofessional ultra-endurance male triathletes of regional to national level (mean (SD): age 36.7 (5.5) years, body mass 75.2 (6.7) kg, height 174.0 (0.6) cm, BMI 24.8 (1.9) kg·m<sup>-2</sup>,  $\dot{V}O_{2max}$  5.03 (0.4) l·min<sup>-1</sup>, 67.0 (4.3) ml·kg<sup>-1</sup>·min<sup>-1</sup>) volunteered to participate in an UET race and the test measurements designed for the study. The average experience of participants in UET and ultra-endurance events was of 10 (3) years and they had been training regularly for 15–18 h a week for at least the three previous years. All participants had passed a medical examination and gave their informed written consent before participating in the study, following the legal requirements and the Declaration of Helsinki [14]. The study had received previous approval from the local Ethics Committee [blinded].

### The ultra-endurance triathlon race

The chosen event was the Extreme Man Salou-Costa Daurada triathlon, held on 5 June 2011. With more than 370 registered

athletes from eleven countries, this event is among the hardest UET due to the 2600 m of elevation gain in the cycling leg. The three stages consisted of a 3.8 km swim, a 180 km cycle (no drafting allowed), and a 42.2 km marathon run. Environmental conditions during the race were: average ambient air temperature 26 °C (13–30 °C), water temperature 21 °C (20.8–21.2 °C), relative humidity 77% (64–94%), and average wind speed 1.3 m·s<sup>-1</sup> (0.3–5 m·s<sup>-1</sup>). HR was continuously monitored during the entire race using waterproof Polar RCX5 (Polar Electro, Finland) portable monitors and averaged at 5-s intervals.

### Preliminary testing

2 weeks before the race, all subjects reported 3 times to the physiology laboratory (or swimming pool) to perform three incremental tests to volitional exhaustion. The tests consisted of a graded swimming test, a graded cycling ergometry test, and a graded treadmill running test. All tests were done randomly under controlled conditions (22 ± 1 °C, 40–60% relative humidity, Pb 1013–1027 hPa) separated by at least 48 h. The tests were performed at the same time of day to minimize the effects of circadian rhythms. Triathletes were asked to refrain from caffeine, alcohol and heavy exercise on the day before the tests, and to report to the laboratory well hydrated and having eaten more than three hours before the tests [11].

### Swimming test

The swimming test was conducted in an outdoor 50-m swimming pool. The water temperature was 24 °C. After a standardized warm-up of 200 m at 0.7 m·s<sup>-1</sup>, subjects started at a speed of 0.75 m·s<sup>-1</sup>, which was increased by 0.05 m·s<sup>-1</sup> every 50 m until the athlete could not keep up with the imposed pace. Subjects followed a red ribbon hanging from a thread at the top of a hook carried by an assistant. The assistant closely followed the sound signal emitted by a computer using an ancillary sound software program (EZ Sound, Barcelona, Spain) and used cones placed at 5 m intervals as a visual sign. To better simulate competitive conditions, the subjects wore the same wetsuit as in the race.

### Cycle ergometry

The cycling test was conducted on an electronically braked cycle ergometer (Excalibur Sport, Lode, The Netherlands) modified with clip-on pedals. The saddle and handlebar positions of the ergometer were individually adjusted. The exercise protocol started at 25 W and was increased 25 W every minute until exhaustion. Pedalling cadence was individually chosen in the range of 70–100 rpm.

### Treadmill running

The running test was performed on a motorized treadmill (h/p/ Cosmos Pulsar, Germany). Exercise started at 6 km·h<sup>-1</sup> (0% slope) for 5 min. Subsequently, speed was increased to 8 km·h<sup>-1</sup> (0% slope) and then by 1 km·h<sup>-1</sup> every minute. When 16 km·h<sup>-1</sup> was reached, the grade was increased by 2% every minute until exhaustion. For the numerical and statistical analysis of running speed in the upper range of this variable, each increase in grade was converted to running speed using the equations of Margaria et al. [29], where a 1.5% rise in grade corresponds to an increase in speed of 1 km·h<sup>-1</sup>.

### Gas analysis and ventilatory demarcation points

During the tests, oxygen uptake ( $\dot{V}O_2$ ), minute ventilation ( $\dot{V}_E$ ), carbon dioxide production ( $\dot{V}CO_2$ ), and respiratory exchange ratio (RER) were measured breath-by-breath using a computerized gas analyser (Quark CPET, Cosmed, Italy) for cycle ergometer and treadmill running tests. A portable gas analyser of the same brand and similar operating system (K4 b<sup>2</sup>, Cosmed, Italy) combined with a respiratory snorkel and valve system for breath-by-breath gas analysis [19,41] was used for the swimming tests. Before each test the ambient conditions were measured, and the gas analysers were calibrated using high precision calibration gases ( $16.00 \pm 0.01\%$  O<sub>2</sub> and  $5.00 \pm 0.01\%$  CO<sub>2</sub>, Scott Medical Products, USA). Ventilatory flow was calibrated using a 3-l syringe (Hans Rudolph, Chicago, USA). After the test, all respiratory data were averaged at 15 s intervals to determine  $\dot{V}O_{2peak}$ , which was taken as the highest 15-s average value. Criteria for  $\dot{V}O_2$  max were: 1) a plateau in  $\dot{V}O_2$  despite increases in workload (main criterion), 2) an RR value > 1.10, and 3) a maximal heart rate > 90% of age-predicted values. The VT<sub>1</sub> and VT<sub>2</sub>, were estimated by three independent researchers according to methods described by Wasserman et al. [46] and in accordance with previous reports [24]. The reliability of  $\dot{V}O_{2peak}$  (TEM, typical error of measurement: 3.2% [2.5–4.9%]) and ventilatory thresholds determinations (TEM: 3.2% (2.5–4.8%)) during swimming are comparable to that of other exercise modes [43]. In addition, HR was continuously recorded beat-by-beat using portable Polar RS800CX (Finland) monitors for the cycle ergometry and treadmill running tests, and Polar RCX5 (Finland) monitors for the swimming tests. To estimate  $\dot{V}O_2$  during competition, regression equations for each stage developed from the individual HR- $\dot{V}O_2$  relationship in each preliminary specific test were used. To determine the intensity profile we used the HR values registered during the race in relation with the HR corresponding to the  $\dot{V}O_{2peak}$  and each to the ventilatory demarcation points in each of the exercise-specific laboratory and pool tests.

### Statistical analysis

Descriptive data are presented as mean and standard deviation (SD) unless otherwise indicated. A one-way analysis of variance (ANOVA) was used to show differences between tests (swimming test, cycle ergometry and treadmill running) in the variables corresponding to the demarcation points (VT<sub>1</sub>, VT<sub>2</sub> and peak) and also between disciplines (swimming, cycling and running) during competition in the mean HR, mean estimated  $\dot{V}O_2$  and percentage of time spent in each intensity zone. Also HR corresponding to the demarcations points during the exercise tests was compared to the mean HR in each discipline during competition using ANOVA. Tukey's post-hoc test was used to identify where the differences lie. Pearson's product-moment coefficient was used to assess the relationship between the individual physiological variables corresponding to the demarcation points measured in the laboratory and pool tests and performance in each stage of the triathlon race as well as the overall (final) race time. This was also used to assess the relationship between the mean race HR in each discipline and performance (i.e. final racing time). A stepwise multiple linear regression analysis was used to determine the best predictors of final racing time after checking the correlation matrix for collinearity. Significance was set at  $P < 0.05$ , and all analyses were performed using PASW Statistics v 18 for Windows.

### Results



#### Preliminary laboratory testing

The results of the 3 incremental tests are shown in **Table 1**.

#### Ultra-endurance triathlon race performance

**Table 2** summarizes the main results for each stage of the race, namely performance, HR, and estimated  $\dot{V}O_2$  derived from HR

**Table 1** Graded exercise test variables for the swimming, cycle ergometry and treadmill running tests.

Test		$\dot{V}O_2$ (l·min <sup>-1</sup> )	$\dot{V}O_2$ (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	% $\dot{V}O_{2peak}$	HR (beats·min <sup>-1</sup> )	% HR <sub>peak</sub>	Speed (m·s <sup>-1</sup> )/ Power output (W)/ Speed (km·h <sup>-1</sup> )
VT <sub>1</sub>	swimming	2.22 (0.37)*	29.6 (3.7)*	71.1 (4.2)	128.7 (12.4)*	78.7 (3.4)**	0.9 (0.1)
	cycle ergometry	3.65 (0.45)	48.6 (5.6)	74.0 (5.3)	148.7 (7.8)	84.5 (3.4)	267 (25)
	treadmill running	3.55 ± (0.32)	47.4 (4.4)	73.1 (2.7)	147.0 (10.1)	83.5 (5.7)	13.0 (1.5)
VT <sub>2</sub>	swimming	2.74 (0.49)*	36.4 (5.3)*	87.4 (4.6)	149.3 (10.3)*	91.5 (4.5)	1.1 (0.2)
	cycle ergometry	4.36 (0.47)	58.0 (4.6)	88.4 (3.2)	164.4 (8.6)	93.4 (2.8)	328 (29)
	treadmill running	4.31 (0.39)	57.6 (5.6)	88.7 (1.3)	164.6 (6.0)	93.4 (2.6)	16.3 (1.3)
peak values	swimming	3.15 (0.59)*	41.8 (6.0)*	100 (0)	163.4 (13.5)*	100 (0)	1.3 (0.1)
	cycle ergometry	4.94 (0.57)	65.6 (4.8)	100 (0)	176.1 (8.6)	100 (0)	381 (33)
	treadmill running	4.86 (0.46)	64.9 (6.3)	100 (0)	176.2 (7.9)	100 (0)	18.8 (1.2)

Data are presented as mean (SD). VT<sub>1</sub>: first ventilatory threshold; VT<sub>2</sub>: second ventilatory threshold; Max: maximum;  $\dot{V}O_2$ : oxygen uptake; %  $\dot{V}O_{2peak}$ : percentage of peak oxygen uptake at the test; HR: heart rate; % HR<sub>peak</sub>: percentage of HR peak at the test. \* a value for the swimming test that is significantly lower than that for the cycle ergometry and treadmill running tests  $P < 0.05$ . \*\* a value for the swimming test that is significantly lower than that for the cycle ergometry  $P < 0.05$

**Table 2** Swimming, cycling, running and overall duration and intensity variables during the ultra-endurance triathlon race.

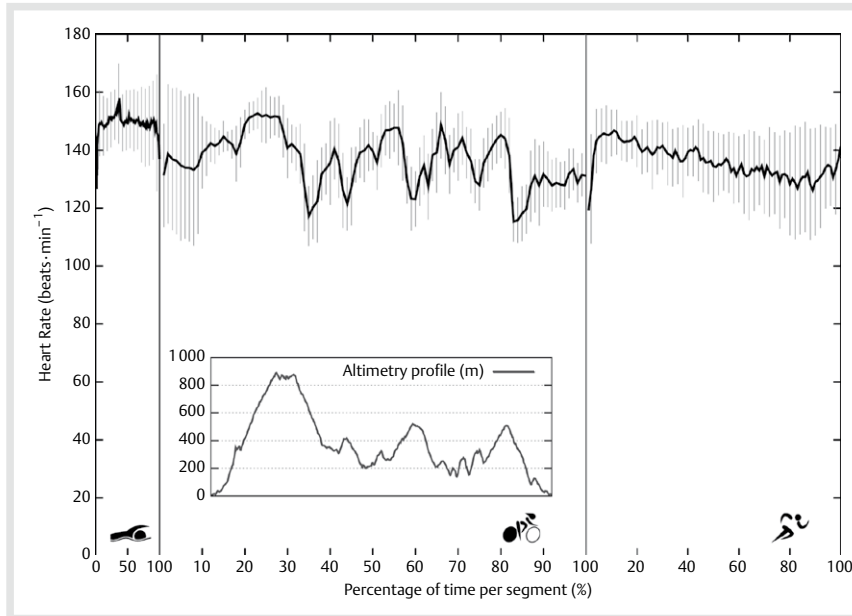
	Time (h:min:s)	Percent of total time (%)	Mean speed (m·s <sup>-1</sup> /km·h <sup>-1</sup> )	Mean HR (beats·min <sup>-1</sup> )	Percent of HR <sub>peak</sub> (%)	Mean estimated $\dot{V}O_{2max}$ (ml·min <sup>-1</sup> )	Percent of estimated $\dot{V}O_{2max}$ (%)
<b>swimming (3.8 km)</b>	01:03:13 (00:08:03)	8.6 (1.1)	0.91 (0.09)	149.2 (10.1)	91.5 (3.9)	2911 (111)	92.4
<b>cycling (180 km)</b>	07:02:24 (00:40:54)	57.3 (5.6)	25.8 (2.4)	137.1 (5.7)*	77.9 (2.0)**	3079 (413)	62.4**
<b>running (42.2 km)</b>	04:12:02 (00:35:43)	34.2 (4.8)	10.2 (1.3)	136.2 (10.5)*	77.3 (4.2)**	3080 (239)	63.3**

Data are presented as mean (SD).  $\dot{V}O_2$ : oxygen uptake; %  $\dot{V}O_{2max}$ : percentage of maximum oxygen uptake at the specific test; HR: heart rate.  $\dot{V}O_{2peak}$ ; % HR<sub>peak</sub>: percentage of peak HR. \*, \*\*: a value for the swimming stage that is significantly higher than that for cycling and running stages at \*  $P < 0.05$  or \*\*  $P < 0.001$

**Table 3** Swimming, cycling and running stage duration and correlation with final racing time.

	3.8 km swim	180 km cycle	42.2 km run	Final time
mean (SD) (h:min:s)	01:03:13 (00:08:03)	07:02:24 (00:40:54)	04:12:02 (00:35:43)	12:30:12 (01:12:13)
correlation with final time	0.49	0.92 **	0.90 **	
p-value	0.183	0.001	0.001	

Data are presented as mean (SD). \*\*  $P < 0.001$



**Fig. 1** Heart rate group profile during the ultra-endurance triathlon race. Data are mean (thick line) and upper and lower SD error bars at 5% (swim), 1% (cycle) and 2% (run) intervals for clarity. The altimetry profile (inner panel) of the cycling leg is shown. For the sake of proportionality, the x-axis data are expressed as percent of total race time per stage, and the length of each stage was scaled relative to the overall time.

recordings based on the  $\dot{V}O_2$ /HR individual relationship during each of the 3 graded tests.

• **Table 3** shows race duration including the transition intervals for each stage, and their correlation with the overall racing time. The duration of both cycle and run stages was closely related to the final time ( $r=0.92$ , and  $0.90$ , respectively,  $P=0.001$ ), while swimming duration was not ( $P=0.183$ ).

### Heart rate response during the race

• **Fig. 1** shows the HR group profile during the race. For the sake of proportionality, data in the x-axis are expressed as percent of total time per stage, and the length of each stage was scaled relative to the overall time. This takes into account the relative duration of each stage for each individual, and avoids calculating the last intervals with a progressively decreasing number of cases. In the swim stage a peak HR can be identified corresponding to the out-of-the-water running interval between 2 consecutive swimming laps. In the cycling leg the effect of slope changes can also be seen and related to the altimetry profile (inbox).

Average swimming HR was significantly higher than the cycle ( $P=0.024$ ), run ( $P=0.013$ ) and overall HR ( $P=0.025$ ) during the race, but there was no difference between the cycling and running legs ( $P=0.995$ ) (• **Table 2**, • **Fig. 1**). Subjects attained a higher percentage of each test's  $HR_{peak}$  during swimming than during the cycling ( $P < 0.001$ ) and running legs ( $P < 0.001$ ), which were not different among them ( $P=0.915$ ) (• **Table 2**, • **Fig. 2**).

• **Fig. 2** shows the relative HR response group profile during the race (y-axis), expressed as the percentage of each specific test's  $HR_{peak}$ . The group-average value for  $VT_1$  and  $VT_2$  corresponding to each specific test is depicted within each stage for visual reference. Swimming relative HR (%  $HR_{peak}$ ) was above HR at  $VT_1$

(• **Table 1**) during the swimming test, while cycling and running relative HR were below HR at  $VT_1$  ( $P < 0.05$ ).

### Estimated oxygen uptake during the race

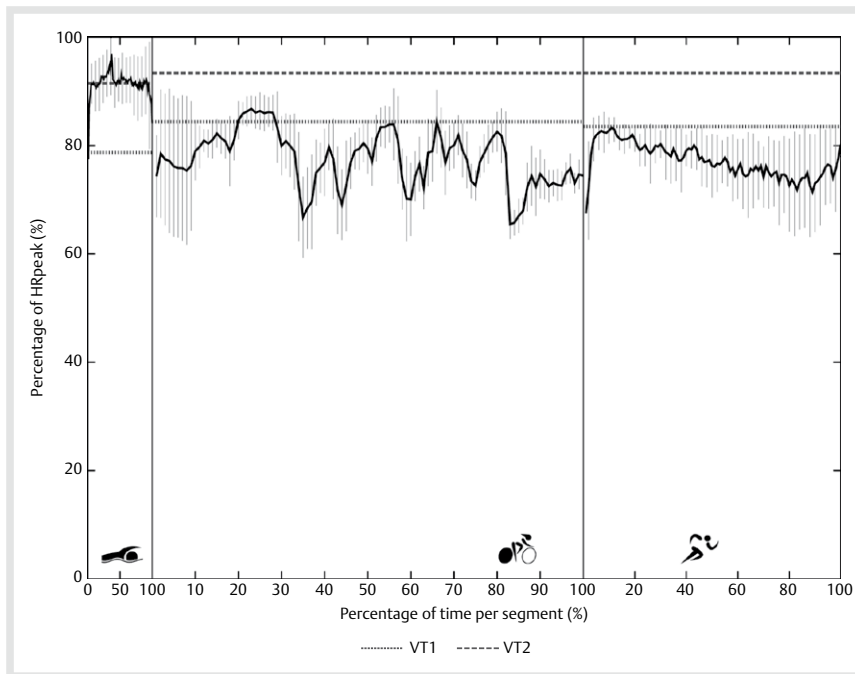
• **Fig. 3** shows the estimated  $\dot{V}O_2$  group profile during the race in relation to group-average  $\dot{V}O_2$  at  $VT_1$  and  $VT_2$  as measured in each of the specific tests (• **Table 1**). There was no difference in estimated  $\dot{V}O_2$  across the 3 stages (• **Table 2**).

### Relationship between racing performance and physiological parameters

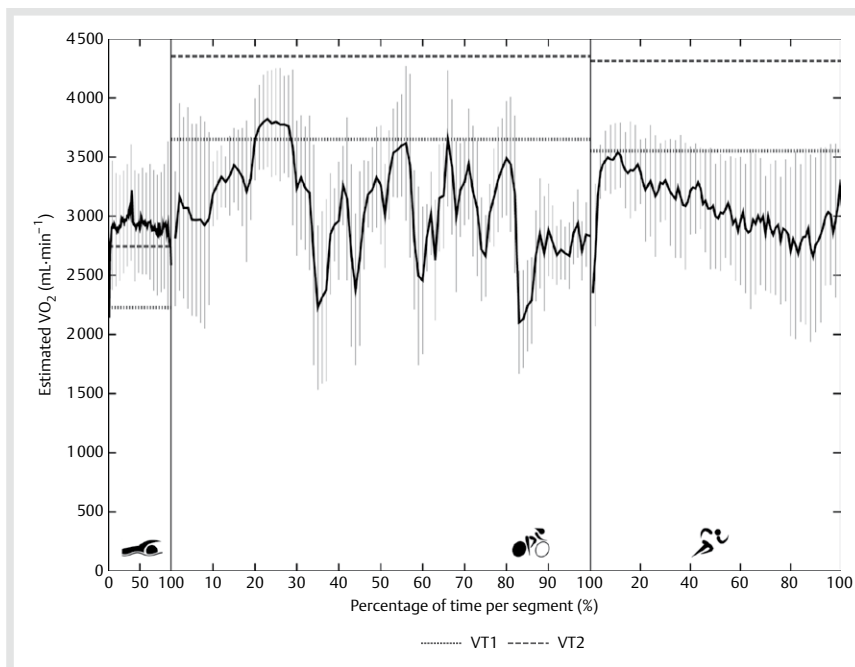
• **Table 4** shows the relationship between HR during the three stages of the race and HR corresponding to both VT.

• **Fig. 4** shows the significant correlations found between swimming HR and performance times for the 3 stages and the final racing time. Swimming HR was closely related to the completion time for cycling and running ( $r=0.80$ ,  $P=0.009$  and  $r=0.71$ ,  $P=0.031$ , respectively), and to the final time ( $r=0.82$ ,  $P=0.007$ ). Thus, swimming HR was inversely related to cycling, running, and overall racing times.

We also wanted to examine the relationships between the HR at the 2 ventilatory demarcation points in the maximal tests and the average HR during the race, as well as how they related to performance in each of the stages and to overall racing performance. Therefore, we calculated the pairwise differences between mean HR in each stage and HR at the ventilatory thresholds ( $HR_{diff}$ ). In the case of swimming, the difference was calculated using  $VT_2$ , which is the threshold that competitors are closest to during competition (i.e.  $HR_{diff}$  [swim- $VT_2$ ]). In the case of cycling and running, the difference was calculated using  $VT_1$ , as the intensity tends to be below the corresponding  $VT_1$  in competition (i.e.  $HR_{diff}$  [cycle- $VT_1$ ] and [run- $VT_1$ ]).



**Fig. 2** Heart rate group profile during the ultra-endurance triathlon race, expressed as percentage of peak heart rate at the specific exercise test. VT<sub>1</sub>: first ventilatory threshold; VT<sub>2</sub>: second ventilatory threshold; % HR<sub>peak</sub>: percentage of peak heart rate. Data are mean (thick line) and upper and lower SD error bars at 5% (swim), 1% (cycle) and 2% (run) intervals for clarity. Mean group heart rate at VT<sub>1</sub> and VT<sub>2</sub> during each of the preliminary exercise tests are depicted for reference.



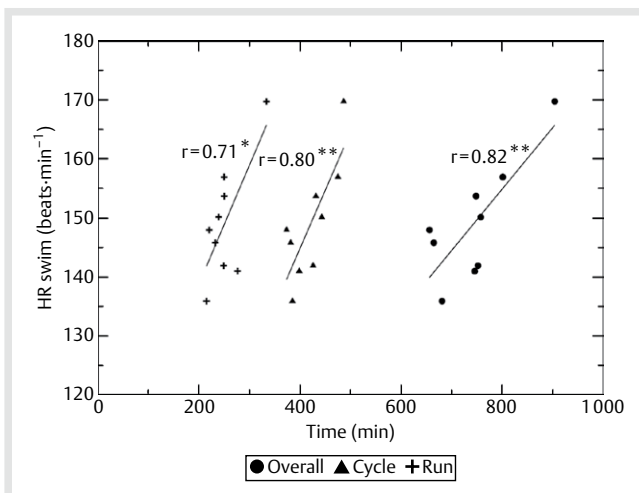
**Fig. 3** Estimated oxygen uptake group profile during the ultra-endurance triathlon race. VT<sub>1</sub>: first ventilatory threshold; VT<sub>2</sub>: second ventilatory threshold;  $\dot{V}O_2$ : oxygen uptake. Data are mean (thick line) and upper and lower SD error bars at 5% (swim), 1% (cycle) and 2% (run) intervals for clarity. Mean group estimated oxygen uptake at VT<sub>1</sub> and VT<sub>2</sub> during each of the preliminary exercise tests are depicted for reference.

**Table 4** Relationship (r) and difference (race HR – test HR) between HR during the ultra-endurance triathlon and the HR corresponding to the ventilatory thresholds (VT<sub>1</sub> and VT<sub>2</sub>).

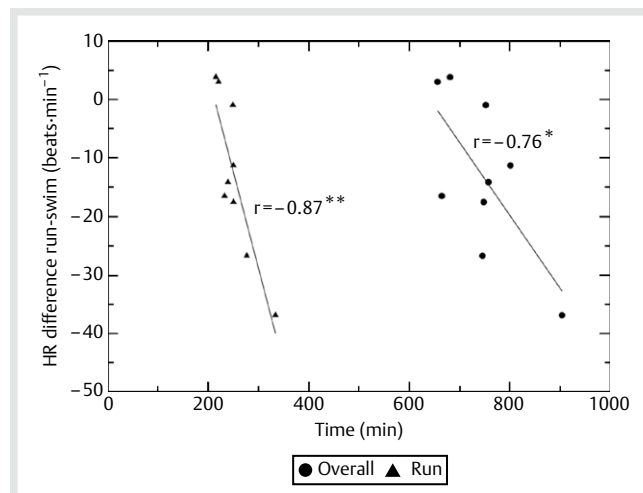
	HR during		
	Swim	Cycle	Run
<b>Correlation with HR@VT<sub>1</sub></b>	0.82 <sup>††</sup>	0.59	0.32
<b>Difference with HR@VT<sub>1</sub> (beats·min<sup>-1</sup>)</b>	20.6 (7.1)**	-11.5 (6.4)**	-10.8 (12.0)*
<b>Correlation with HR@VT<sub>2</sub></b>	0.77 <sup>†</sup>	0.74 <sup>†</sup>	0.75 <sup>†</sup>
<b>Difference with HR@VT<sub>2</sub> (beats·min<sup>-1</sup>)</b>	-0.1 (6.9)	-27.3 (5.9)**	-28.4 (7.2)**

Data are presented as mean (SD). HR: HR; VT<sub>1</sub>: first ventilatory threshold; VT<sub>2</sub>: second ventilatory threshold; Difference with HR@VT<sub>1</sub>; difference between HR at the first ventilatory threshold and the mean HR during a stage during the race (swimming, cycling or running) of triathlon; Difference with HR@VT<sub>2</sub>; difference between HR at the second ventilatory threshold and the mean HR during a stage during the race (swimming, cycling or running) of triathlon. Statistical differences: \*P<0.05; \*\*P<0.01; Correlation <sup>†</sup>P<0.05; <sup>††</sup>P<0.01

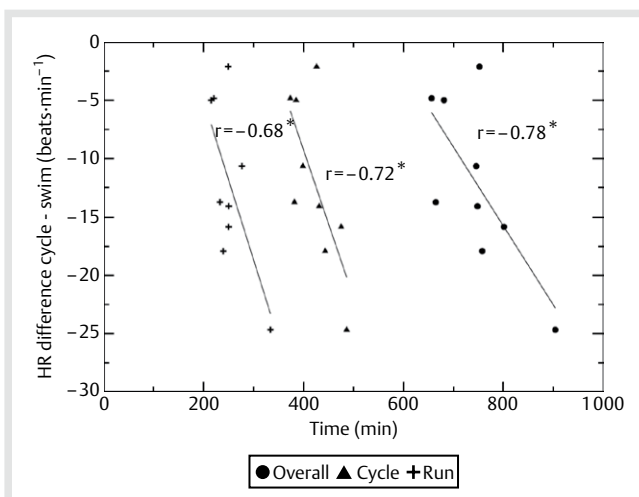
In addition, calculations of the differences between mean HR in the cycling and swimming legs ( $HR_{diff}$  [cycle-swim]), the running and swimming legs ( $HR_{diff}$  [run-swim]), and the running and cycling legs ( $HR_{diff}$  [run-cycle]) were made, as well as how these differences related to performance (expressed as racing time) in each stage and in the entire event. Among all these parameters, relationships were found between  $HR_{diff}$  [cycle-swim] and the time in the cycling leg ( $r=-0.72$ ,  $P=0.030$ ), the running leg ( $r=-0.68$ ,  $P=0.046$ ), and the final time ( $r=-0.78$ ,  $P=0.013$ ; ● Fig. 5). Furthermore, there were negative relationships between  $HR_{diff}$  [run-swim], and the time in the running leg ( $r=-0.87$ ,  $P=0.003$ ) and the overall time ( $r=-0.76$ ,  $P=0.016$ ; ● Fig. 6), as well as between  $HR_{diff}$  [run-cycle] and the time in the running leg ( $r=-0.80$ ,  $P=0.010$ ; ● Fig. 7). This indicates that a large  $HR_{diff}$  during swimming or cycling and running is related to a lower running performance, and a lower overall performance also in the case of swimming.



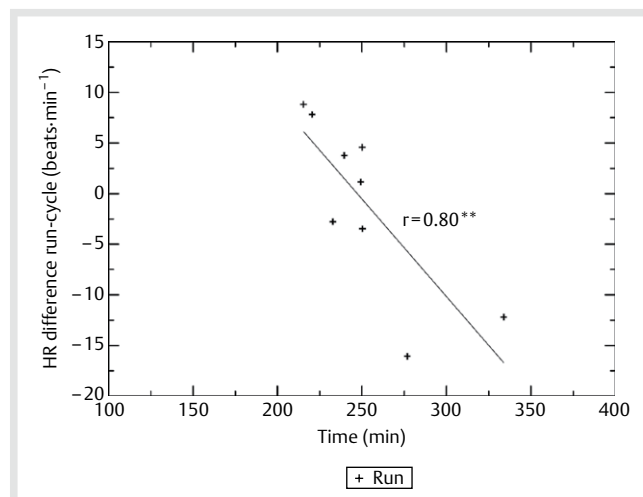
**Fig. 4** The relationship between HR swim and performance times for cycling and running legs and for the overall race. HR swim: heart rate at the swimming leg. Significant correlations: \* $P<0.05$ ; \*\* $P<0.01$ .



**Fig. 6** The relationship between the run-to-swim heart rate difference and performance times. Run-swimming  $HR_{diff}$ : difference between heart rate in the running and swimming legs. Significant correlations: \* $P<0.05$ ; \*\* $P<0.01$ .



**Fig. 5** The relationship between the cycle-to-swim heart rate difference and performance times. Cycle-swimming  $HR_{diff}$ : difference between HR in the cycling and swimming legs. Significant correlations: \* $P<0.05$ .



**Fig. 7** The relationship between run-to-cycle heart rate difference and running performance time. Run-cycle  $HR_{diff}$ : difference between HR in the running and cycling legs. Significant correlation: \*\* $P<0.01$ .

Stepwise multiple regression analysis was also performed finding that the best predictors of total racing time were the weight-adjusted  $\dot{V}O_{2max}$  ( $\beta=-0.51$ ,  $SE=206.8$ ,  $P=0.047$ ) and the  $HR_{diff}$  [cycle-swim] ( $\beta=-0.53$ ,  $SE=123.1$ ,  $P=0.041$ ), which accounted for 81% ( $r^2=0.81$ ) of the final time variance. The other parameters that correlated linearly with the finishing time were excluded due to collinearity with  $HR_{diff}$  [run-swim].

## Discussion

This study examined the HR response during an UET race in relation with HR-based intensity markers derived from specific swimming, cycling and running incremental tests. We found that, contrary to what was believed until now, the absolute and relative HR (expressed as percentage of  $HR_{peak}$ ) are greater in the swimming leg than in the cycling and running legs. It was also

observed that swimming at a higher intensity inversely correlated with performance during the following stages and the overall racing performance. Moreover it was found that 81% of the variance in total racing time was explained by the weight-adjusted  $\dot{V}O_{2max}$  and the  $HR_{diff}$  between the running and swimming legs.

### Preliminary exercise testing

Paying attention to a review [44] it can be seen that  $\dot{V}O_{2peak}$  values from cycle ergometry and treadmill running tests in ultra-endurance athletes in this study are similar to those reported in other studies with similar subject samples (highly trained) and lower than those studies done with elite triathletes or specialists (elite runners and cyclists). Laursen et al. [24] found values of  $VT_1$  and  $VT_2$  in the cycle ergometer (69% and 81% of  $\dot{V}O_{2peak}$ , respectively) which are lower than in our study.  $VT$  values in the treadmill running (68% and 81% of  $\dot{V}O_{2peak}$ , respectively) were also lower. They also reported lower  $\dot{V}O_{2peak}$  values (4.72 and 4.75  $l \cdot min^{-1}$  in cycle ergometry and treadmill running, respectively). In this study, peak physiological values during the laboratory and pool tests including  $\dot{V}O_{2peak}$ ,  $HR_{peak}$ , absolute  $VT$ , and  $\dot{V}_E$ , were lower during swimming as compared to cycling and running. These results are in agreement with previous studies reporting lower  $HR_{peak}$  and  $\dot{V}O_{2peak}$  during swimming than during land-based exercises as less muscle mass is activated and the haemodynamics associated with a horizontal body position are different [15, 17, 28, 30].

### Ultra-endurance triathlon race performance

As in previous studies [24, 25], we found that the swimming time did not correlate with the final racing time, while cycling and running times did correlate closely with finishing time (Table 3). It may be argued that this is due to the shorter duration of the swimming leg (8.5% of total time). Therefore, not being a good swimmer does not appear to be a limiting factor in UET performance, whereas it could be the case for a sprint or Olympic triathlon, where swimming accounts for 15% and 19% of the overall duration, respectively [4], and where success may depend on an earlier exit from the water, closer to the leading group during the cycling leg [13].

### Heart rate response and estimated oxygen uptake during the race

The average HR in the swimming leg (Fig. 1, Table 2) was higher than in the cycling and running legs. These results are in agreement with a previous report in which this response was explained in part due to anxiety at the start of the race and to the stress of swimming in open water surrounded by a large number of competitors [24]. An alternative explanation [26, 34] is that fatigue affecting the intensity in the cycling and running legs does not affect the swimming leg to the same extent due to its shorter duration. It can be argued that triathletes swim the initial stage of the race at a quicker pace in order to emerge in the leading group, gaining an advantage for the following stages [3]. Considering the competitive level of our participants, a factor that could better explain this difference is that proposed by Olbrecht [37]. He stated that triathletes very often suffer from a lack of technique, resulting in expending considerable energy in the swimming leg – with little propulsive energy in return – which is then no longer on hand for the rest of the race. The intensity of the race with respect to  $HR_{peak}$  was on average 78% in the cycling leg, which is a little lower than the intensity

found by Laursen et al. [24] (83%). We ascribe this difference to the 2600 meters of elevation gain in the cycling leg of this race. The intensity in the running leg was 77%, very similar to that found by Laursen et al. [24]. O'Toole et al. [35] described values close to 80% with a drop of 6–7% in the cycling and running legs. Laursen et al. [24] found that this decrease was only significant in the running leg. However, in these studies the duration of the test was not normalized, meaning that in the final stage there were only the slowest triathletes.

In our study, we normalized the duration expressed in time percentage (Fig. 1) and a novel intensity profile can be observed. When properly scaled, HR showed a clearly declining trend at the final part of each of the 3 stages, which is likely the result of fatigue. Only at the final stage of the running leg did HR rise up to previous levels, which probably reflects the last effort before the end of the race. In addition, the intensity expressed as a percentage of the peak HR was significantly higher in the swimming leg (92%) than in the cycling and running legs (78 and 77%, respectively), despite attaining a similar  $\dot{V}O_2$  (Fig. 2, Table 2). This novel value for the swimming leg is in agreement with the HR racing response reported by Laursen et al. [23] and challenges the hypothesis that the HR would be lower during the swimming leg than during the land-based segments for an equivalent level of  $\dot{V}O_2$  due to a shift to the right in the  $HR/\dot{V}O_2$  regression line during swimming.

Some studies hypothesize the existence of an ultra-resistance metabolic threshold based on HR [23, 25, 35, 36]. As this study shows, however, this threshold cannot be based on HR, since it varies in the 3 legs (swimming, cycling and running). In the swimming leg a muscular limiting factor was found. It may be reasoned that this peripheral fatigue is due to lack of strength and endurance in the upper body compared to the lower body due to less specific training of these body segments. It is also important to consider the energy substrate, given that glycogen depletion will lead to a consequent drop in performance over time. Hydration, temperature, cardiovascular drift and cardiac fatigue are also factors that may affect HR during exercise [2] and therefore question this threshold based on HR. It is also important to take into account that HR is a poor indicator of psychological strain in competition [10]. When looking at Fig. 3, it can be seen that  $\dot{V}O_2$  is similar in all three stages and there is thus an ultra-endurance threshold based on estimated  $\dot{V}O_2$  that is around 3000  $ml \cdot min^{-1}$ , which is above the  $VT_2$  for the swimming and below the  $VT_1$  for the cycling and running legs. Therefore, HR-estimated  $\dot{V}O_2$  can be used as a practical guide during an UET.

### Relationship between race performance and physiological parameters

A close relationship between HR at the  $VT_1$  measured in graded exercise tests and HR during the cycling and running legs has been previously reported [24]. Although a similar pattern was found in this study, particularly in the running leg, participating subjects tended to work below  $VT_1$ . In contrast, HR during the swimming leg was remarkably higher when compared to the cycling and running legs and close to  $VT_2$ , thus clearly indicating the higher intensity of the first stage. Unlike Laursen et al. [24], no correlation was found between exercising under  $VT_1$  (or  $VT_2$  in the case of swimming) and the completion time for each triathlon stage or the overall event. These new values for the swimming leg represent a new reference of the intensity throughout a UET race.

A strong negative correlation was found between the difference in HR between the swimming leg and the cycling ( $r = -0.7$ ) and running legs and the marathon time ( $r = -0.9$ ). Laursen et al. [24] are also in line with these findings. Other studies [6–8, 22] show that dropping the relative intensity in the swimming leg using neoprene or drafting may provide a reserve of metabolic energy, which could further influence overall race success. In a later study [38] it is said that swimming at an intensity below that of a time trial effort significantly improves subsequent cycling and overall triathlon race performance. In Olympic distance, Vleck et al. [45] showed that a lower swimming performance can result in a tactic that involves greater work in the initial stages of the cycling portion, and may influence subsequent running performance. Another study [39] highlights the importance of regulating the swimming intensity during sprint and Olympic distance triathlons and how it impacts on the ensuing cycle and run.

But what happens with the intensity in a UET? On the one side, Millet et al. [32] demonstrated a significantly faster swim time in short-distance triathletes than in long-distance triathletes, while physiological characteristics measured in cycling and running in laboratory trials were all similar. On the other side, a previous report concluded that 3000 m of swimming had no significant performance effect (in terms of power output) on subsequent 3-h cycling performance in UET athletes [26]. In this study the differences in HR between the swimming and cycling legs correlated with the cycling time, marathon time and the overall time. Triathletes with the greatest difference in HR in the swimming and cycling legs had the worst times in the next portions. As stated by Laursen et al. [24], working beyond capacity in one stage makes recovery difficult, leading to lower performance in the next stage. This correlation can also be seen in HR differences for the swimming and running legs, as a large difference in values is associated with a reduction in the marathon time and consequently in overall racing time. Therefore, the present results support the view that, though performed at a relatively lower intensity as compared to shorter distance triathlon races, swimming intensity may also affect the physiology and biomechanics of subsequent cycling and overall triathlon performance [39]. In any case, it seems advisable to improve the ultra-endurance triathlete's swimming efficiency and to consider using strategies for manipulating the relative swimming intensity (e.g. drafting and using wetsuits or speedsuits) without compromising absolute performance [39], as well as adequately pace the swimming leg according to the triathlete's swimming ability.

Regarding factors that can best predict performance in a UET, Knechtel et al. [20] recently found that the three variables that best explained (64%) final time were the speed in running during training, the personal best marathon time, and the personal best time in an Olympic distance triathlon, although strong collinearity can be expected to lay behind this association. In this study it was found that the best predictors of overall triathlon time were the weight-adjusted  $\dot{V}O_{2max}$  and the  $HR_{diff}$  [cycle-swim], which accounted for 81% of the variance in UET race time. To the best of our knowledge, such a relationship has not yet been reported and would be important to take into account as it suggests that the  $\dot{V}O_{2max}$  may not be as important as it may be with shorter distance triathlons, and that the race strategy may also play a substantial role in UET race performance.

### Limitations and practical applications

It is important to acknowledge that this is a confirmatory study on UET heart-rate based intensity but also a pioneering research study on the swimming leg and that the protocol for the endurance test for this stage should be revised, since it may be the case that triathletes reached the end of the test with muscular fatigue in the upper body rather than reaching the  $\dot{V}O_{2max}$ . It can be argued using a different protocol may have allowed triathletes to reach a higher  $\dot{V}O_{2peak}$  during swimming. However, this would not have an effect on the estimation of race intensity relative to the VT demarcation points. We also acknowledge that, as in many other sport-performance related studies [18], the rather small size of the final sample in this study – mostly due to instrumental malfunction of the HR monitors during the race – may have enhanced the risk of type-2 error. It is also important to recognize that, even if the sample in this study was composed of well-trained UET specialists, differences in the athletes' calibre may yield somewhat different results. New studies are warranted in athletes whether of higher (e.g. professional triathletes) or lower (e.g. leisure competitors, youth, etc.) level. It would be interesting as well to carry out the same study in other UET races with different terrain and environmental conditions as the present results may be partly related to the particular race conditions. Moreover, it should also be taken into account that UET undoubtedly imposes demands that are different in many aspects to those of other triathlon modalities such as Olympic and sprint, or super-sprint. Further research in the specific physiological requirements is needed to guide triathletes with regard to these modalities.

### Conclusions

▼ The characterization of the HR response during the entire race in relation to the physiological parameters derived from specific exercise testing and of the intensity pattern during the swimming leg, as well as a more adequate description of the time pattern response during the whole race, add new information concerning the intensity profile and cardiovascular demands of an ultra-endurance triathlon race. The relevance of specific testing in the assessment of the physiological requirements during competition is emphasized. The present results suggest that UET performance is closely related to weight-adjusted  $\dot{V}O_{2max}$  and the HR difference between the cycling and swimming legs. Further research is needed to better understand the physiological demands of this multi-sport, ultra-endurance sport.

### Author Contributions

▼ ■[blinded]■

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## **ANNEX 2. MANUSCRIPT II**

**Nutritional behaviour of triathletes during an ultra-endurance event**



# Nutritional behavior of triathletes during an ultra-endurance event

## Abstract

### Background

The nutritional strategy during an ultra-endurance triathlon (UET) is one of the main concerns of athletes competing in such events. The purpose of this study is to provide a proper characterization of the energy and fluid intake during real competition in male triathletes during a complete UET and to estimate the energy expenditure (EE) and the fluid balance through the race.

### Methods

Eleven well-trained non-professional ultra-endurance triathletes (mean  $\pm$  SD: age  $36.8 \pm 5.1$  years, mass  $75.5 \pm 6.4$  kg, height  $174 \pm 6$  cm, BMI  $24.8 \pm 1.7$  kg/m<sup>2</sup>,  $\dot{V}O_{2\max}$   $66.9 \pm 4.13$  mL  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) performed a UET. All food and drinks ingested during the race were weighed and recorded in order to assess the energy intake (EI) during the race. The EE was estimated from heart rate (HR) recordings during the race, using the individual HR-  $\dot{V}O_2$  regressions developed from three incremental tests on the 50-m swimming pool, cycle ergometer, and running treadmill. Additionally, body mass (BM), total body water (TBW) and intracellular (ICW) and extracellular water (ECW) were assessed before and after the race using a multifrequency bioimpedance device (BIA).

## Results

Mean competition time and intensity was  $755 \pm 69$  min and  $137 \pm 6$  beats  $\cdot$  min<sup>-1</sup>, respectively. Mean EI was  $3,643 \pm 1,219$  kcal and the estimated EE was  $11,009 \pm 664$  kcal. Consequently, athletes showed an energy deficit of  $7,365 \pm 1,286$  kcal ( $66.9 \pm 11.7\%$ ). BM decreased significantly after the race ( $-4.3 \pm 1.4$  kg;  $-5.7 \pm 1.9\%$ ) and significant losses of TBW were found ( $-8.4 \pm 2.9\%$ ). Such losses were more related to a reduction of extracellular fluids ( $-10.8 \pm 3.7\%$ ) than intracellular fluids ( $-6.8 \pm 2.9\%$ ).

## Conclusions

Our results confirm the high energy demands of UET races, which are not compensated by nutrient and fluid intake, resulting in a large energy deficit.

## Keywords

Triathlon, Energy expenditure, Energy intake, Macronutrient consumption

## Background

The popularity of ultra-endurance triathlon (UET) races (3.8-km swim, 180-km cycle, 42.2-km run) has greatly increased since the first Ironman was held in 1978 [1]. Given the long duration of these sport events, one of the main goals for athletes is to manage the consumption of food and drinks throughout the race [2] so as to enhance performance while maintaining body homeostasis. It has been estimated that the energy expenditure (EE) for a UET may range from 8,500 to 11,500 Kcal [2-4]. However, to the best of our knowledge, only two previous studies have investigated this issue under field conditions. Kimber et al., [3] assessed the energy balance of a UET using heart rate-  $\dot{V}O_2$  regression equations during cycling and running as well as a multiple regression equation during the swimming section. They estimated an EE of 10,036 kcal and 8,550 kcal in ten males and eight females, respectively. However, the energy intake

(EI) was only 3,940 kcal and 3,115 kcal in both groups showing an energy deficit above 60% through the race. In a case study, Cuddy et al., [5] combined two different approaches to assess the EE; indirect calorimetry and doubly labeled water. The data from both assessments were similar indicating that the EE of the athlete was  $\approx 9,000$  kcal. Nevertheless, this study did not assess the dietary consumption and fluid ingestion of the athlete during the event and, consequently, the EI and the energy deficit were not shown. Therefore, given this limitation of data, it seems important to address new research investigating the energy demands and the nutritional pattern of triathletes during real events.

Another key point for exercise performance in UET is fluid ingestion, which is not only important for performance, but also necessary to maintain a proper cardiovascular homeostasis and to guarantee athlete's health during ultra-endurance events [6]. Previous studies by Speedy et al. [7-10] have shown that triathletes performing a UET may suffer from exercise-associated hyponatremia even despite modest fluid intakes. This fact can be linked to renal function disturbances [11,12] which may induce an overload of extracellular water (ECW). However, changes in body mass (BM) during longer events are not only related with fluid balance. The reduction of the body stores of energy can also explain changes in the BM of athletes after ultra-endurance events. For instance, there is evidence indicating a significant decrease in muscle density (glycogen loss) and fat mass of athletes after a UET [13-16]. In addition, Laursen et al. [17] reported that a body mass loss of up to 3% was not linked with thermoregulatory failure in 10 triathletes performing a UET suggesting that part of the BM reduction occurred due to losses of glycogen and fat.

Accordingly, the main aim of this study was to provide a proper characterization of the energy and fluid intake of a group of male triathletes during a whole UET. A second aim was to estimate the EE and the fluid balance (intra and extracellular stores) of triathletes throughout the race using three different locomotion-specific individualized equations. We hypothesized that triathletes performing a UET would incur a substantial energy deficit of  $> 70\%$  due to the high energy demands of these types of events and the

limited food intake of athletes through the race. We also hypothesized that sweat losses during UET under hot environmental conditions would not be compensated by fluid ingestion.

## **Materials and methods**

### **Subjects**

We placed an advertisement on the triathlon race webpage to recruit non-professional male triathletes. The inclusion criteria were to train at least 10 h per week and the participation in a minimum of one UET during the past 3 years.

Eleven triathletes volunteered to participate in the study (mean  $\pm$  SD: age  $36.8 \pm 5.1$  years, BM  $75.5 \pm 6.4$  kg, height  $1.74 \pm 0.06$  m, BMI  $24.8 \pm 1.7$  kg/m<sup>2</sup>,  $\dot{V}O_{2\max}$   $5.03 \pm 0.4$  L  $\cdot$  min<sup>-1</sup>,  $66.9 \pm 4.1$  mL  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>). Triathletes had an average of  $10 \pm 3$  years of experience in UET and ultra-endurance events, and they had been training regularly for approximately 15–18 h per week for at least the three previous years. Before participating in the study, all subjects undertook a medical examination which included a physical examination (body composition), a medical questionnaire, and an electrocardiogram within the same year to ensure that each participant was in good health and gave their informed written consent, which was in accordance with legal requirements and the Declaration of Helsinki, and approved by the Catalan Sports Council's Ethics Committee.

### **The ultra-endurance triathlon**

The Extreme Man Salou–Costa Daurada triathlon was held on the 5th of June 2011 with more than 370 participants from eleven different countries. Importantly, this event was considered among the hardest UET due to positive elevation over 2,600 m during the cycling stage. The distance of each stage consisted of a 3.8 km swim, a 180 km cycle (no drafting allowed), and a 42.2 km marathon run. The average (range) ambient

temperature was 26°C (13–30°C), the water temperature was 21°C (20.8–21.2°C) and the relative humidity was 77% (64–94%). The mean wind speed was 1.3 m · s<sup>-1</sup> (range 0.3–5 m · s<sup>-1</sup>). HR was monitored during the entire race using waterproof Polar RCX5 (Polar Electro, Finland) portable monitors and averaged at 5 s intervals.

### **Preliminary testing**

Two weeks before the race, all subjects reported three times to the physiology laboratory (or to a swimming pool) to perform three incremental tests to volitional exhaustion in each discipline under randomised conditions separated by at least 48 hours. The tests consisted of a graded swimming test in a 50-m pool, a graded cycling ergometry test and a graded treadmill running test. Running and cycling tests were executed randomly under controlled conditions (22 ± 1°C, 40–60% relative humidity, Pb 1013–1027 hPa). All the tests were performed at the same time of day to minimize the effects of circadian rhythms. Athletes were asked to refrain from caffeine, alcohol and heavy exercise on the day before the tests, and to report to the laboratory well hydrated after having eaten more than three hours before.

### **Swimming test**

The swimming test was conducted in an outdoor 50-m swimming pool. The water temperature was 24°C. After a standardized warm-up of 200 m at 0.7 m · s<sup>-1</sup>, subjects started at a speed of 0.75 m · s<sup>-1</sup>, which was increased by 0.05 m · s<sup>-1</sup> every 50 m until the athlete could not keep up with the imposed pace. Subjects followed a red ribbon hanging from a thread at the top of a hook carried by an assistant. The assistant closely followed the sound signal emitted by a computer using an ancillary sound software program (EZ Sound, Barcelona, Spain) and used cones placed at 5 m intervals as a visual signal. To better simulate competitive conditions the subjects wore the same wetsuit as in the race.



## **Cycle ergometry**

The cycling test was conducted on an electronically-braked cycle ergometer (Excalibur Sport, Lode, The Netherlands) modified with clip-on pedals. The saddle and handlebar positions of the ergometer were adjusted to resemble each triathlete's own bike. The exercise protocol started at 25 W and was increased 25 W every minute until exhaustion. The pedaling cadence was individually chosen in the range of 70–100 rpm.

## **Treadmill running**

The running test was performed on a motorized treadmill (h/p/Cosmos Pulsar, Germany). Exercise started at 6 km · h<sup>-1</sup> (0% slope) for 5 min. Subsequently, the speed was increased to 8 km · h<sup>-1</sup> (0% slope) and then by 1 km · h<sup>-1</sup> every minute. When 16 km · h<sup>-1</sup> was reached, the grade was increased by 2% every min until exhaustion. For the numerical and statistical analysis of running speed in the upper range of this variable, each increase in grade was converted to running speed using the equations of Margaria et al. [18], where a 1.5% rise in grade corresponds to an increase in speed of 1 km · h<sup>-1</sup>.

## **Gas analysis and ventilatory demarcation points**

During the tests, oxygen uptake ( $\dot{V}_{O_2}$ ), minute ventilation ( $\dot{V}_E$ ), carbon dioxide production ( $\dot{V}_{CO_2}$ ), and respiratory exchange ratio (RER) were measured breath-by-breath using a computerised gas analyzer (Quark CPET, Cosmed, Italy) for cycle ergometer and treadmill running tests. A similar portable gas analyser and operating system (K4 b<sup>2</sup>, Cosmed, Italy), combined with a respiratory snorkel and valve system for breath-by-breath gas analysis [19,20], was used for the measurement of metabolic and ventilatory parameters during the swimming tests. Before each test, the ambient conditions were measured (22 ± 1°C, 40–60% relative humidity, Pb 1013–1027 hPa), and the gas analysers were calibrated using high-precision calibration gases (16.00 ± 0.01% O<sub>2</sub> and 5.00 ± 0.01% CO<sub>2</sub>, Scott Medical Products, USA). Ventilatory flow was

calibrated using a 3-L syringe (Hans Rudolph, Chicago, USA). After the test, all respiratory data were averaged at 15 s intervals to assess  $\dot{V}O_{2_{peak}}$ , which was taken as the highest 15-s average value. Ventilatory thresholds (AT = VT<sub>1</sub> and RCP = VT<sub>2</sub>) were estimated as previously described [21,22]. In addition, HR was recorded continuously beat-by-beat using portable Polar RS800CX (Finland) monitors for cycle ergometry and treadmill running tests, and Polar RCX5 (Finland) monitors for swimming tests. Regression equations as previously described by Bescós et al., [22] were used to estimate  $\dot{V}O_2$  during the competition.

### **Nutritional data**

After the tests triathletes were encouraged to follow their own diet scheduled usually in this sort of competition the days before and during the competition.

During the race, twenty-five trained researchers were divided among the refreshment points collecting all the wraps and bottles of each triathlete. Additionally, upon completion the UET, triathletes were asked to confirm the data collected during the event by researchers. Then, software was used to assess macronutrient intake (CESNID 1.0, Barcelona University, Spain).

### **Body mass and bioimpedance bioelectricity variables**

BM was measured using a Seca 710® weighing scale calibrated beforehand (capacity: 200 kg; precision 50 g).

TBW, ICW and ECW were measured using a multifrequency bioimpedance analyzer (Z-Metrix®, BioparHom®, Bourget du Lac Cedex, France) before and 30 min after the race (to avoid skin temperature effect on BIA). The device was previously calibrated with a circuit of known impedance value provided by the manufacturer. The bioelectrical measurements were repeated until they were stable within 1 Ω (usually up to three times within an interval of 20 s), and the average value was used in calculations.

The measurements were conducted through the standard tetrapolar electrodes distribution (Red Dot™ 2660–5, 3 M Corporate Headquarters, St. Paul, MN, USA). The inner arm electrode (sensor) was placed on the dorsal surface of the right wrist and between the ulna and the radius. The leg electrode was placed on the anterior surface of the right ankle between the prominent portions of the bones. The external electrodes (source or injector) were placed on the dorsal surface of the third proximal phalanx of the right hand and right foot. Proximal electrodes were separated by 5 cm to avoid interaction between electric fields, which could otherwise lead to an overestimation of the impedance values.

### **Load of exercise and energy expenditure**

To estimate the total work load of exercise performed by each triathlete we used the training impulse (TRIMP) method as previously described by Bescós et al., [21]. The individually derived linear relationship between HR and  $\dot{V}O_2$  was used to estimate the oxygen cost during the work efforts for each segment. Three different individualized equations were established. These were three linear regression equations derived from data during each incremental exercise test. To estimate energy expenditure during the race, we used an energy equivalent of oxygen based on the mean intensity during racing time, as described in a previous study [23].

### **Statistical analysis**

Descriptive data is presented as mean  $\pm$  standard deviation unless otherwise indicated. A one-way analysis of variance (ANOVA) was used to show differences between disciplines (swimming, cycling and running) during competition in the mean HR and percentage of time spent in each intensity zone as well as between macronutrient, fluid and sodium intake in each stage (cycling and running). Furthermore, another ANOVA test was performed to analyze differences between BIA data (TBW, ECW and ICW) before and after the race. Post-hoc analyses were performed using Tukey HSD. Pearson's rank correlation analysis was used to assess the relationship between the

individual physiological variables measured and performance in each stage of the triathlon, as well as the overall race time. A stepwise multiple linear regression analysis was used to determine the best predictors of final racing time after checking the correlation matrix for collinearity. Significance was set at  $P < 0.05$  and all analyses were performed using PASW Statistics v 18 for Windows.

## Results

### Performance during the ultra-endurance triathlon

All subjects successfully finished the race. Table 1 summarizes the main outcomes in each stage of the competition. As expected, time performed within zone I was significantly higher (69%) than in zone II (22%) and zone III (9%) ( $P < 0.001$ ).

**Table 1 Swim, cycle, run and overall variables during the ultra-endurance triathlon race**

Stages	Racing time (min)	TRIMP (a.u.)	Average HR (bpm)	Time spent in zone I (min)	Time spent in zone II (min)	Time spent in zone III (min)	Average speed (km/h)
Swimming	63.1 (8.6)	186.0 (33.9)	149.0 (9.1)	4.0 (10.9)	0.01 (0.03)	60.7 (13.7)	3.7 (0.5)
Cycling	417.2 (38.4)	553.7 (109.7)	137.7 (5.4)	302.6 (84.2)	114.1 (76.8)	7.6 (20.5)	26.1 (2.3)
Running	257.3 (36.3)	313.9 (86.0)	133.9 (10.8)	209.2 (90.9)	51.7 (79.9)	0.4 (0.9)	10.0 (1.3)
<b>Total</b>	754.6 (68.8)	1053.6 (173.9)	136.7 (6.3)	515.8 (137.2)*	165.8 (138.0)	68.7 (22.4)	

Data are presented as mean (SD). TRIMP: training impulse (a.u., arbitrary units); HR: heart rate;  $\dot{V}O_2$  : oxygen uptake; Time spent in zone I: below to the first ventilatory threshold; zone II; between the first ventilatory threshold and the second ventilatory threshold; zone III: above to the second ventilatory threshold.

\* The time spent in zone I is significantly longer than the time value in zone II and zone III ( $P < 0.001$ ).

## Macronutrient intake

Food and fluids consumed during the UET were mainly those provided at the aid stations by the triathlon organizers. Table 2 summarizes the percent contribution from food and fluids consumed by athletes during the event.

**Table 2 Percentage of energy contribution from food and fluids during the ultra-endurance triathlon race**

Food and fluids	Energy contribution (%)		
	Cycling	Running	Total
Sport gels	20.2	62.6	35.3
Sport bars	34.4	6.2	24.4
Sport drinks	20.3	13.5	17.9
Sandwich (parma jam and cheese)	13.9	2.0	9.7
Dried fruits (almonds and nuts)	4.2	3.6	4.0
Caffeinated drinks	1.9	6.0	3.3
Fruits (banana, apple and orange)	1.1	6.1	2.9
Cereals	3.1	--	2.0
Others (protein supplements.)	0.8	--	0.5

Table 3 summarizes the consumption of macronutrients during the race. Subjects consumed  $927 \pm 178$  g ( $6.2 \pm 1.3$  g  $\cdot$  kg<sup>-1</sup>;  $84 \pm 18$  g  $\cdot$  hour<sup>-1</sup>;  $89.9 \pm 3.5\%$ ) of carbohydrates (CHO), which provided the main source of energy consumed during the race ( $P < 0.001$ ). The consumption of solid CHO ( $697 \pm 147$  g) was higher than the consumption of fluid CHO ( $229 \pm 67$  g;  $P < 0.001$ ). Macronutrient intake was significantly greater in the cycling stage compared with the running stage ( $P < 0.001$ ). However, regarding CHO, there were no statistical differences between both stages (cycling:  $1.4 \pm 0.5$ ; running:  $1.3 \pm 0.3$  g  $\cdot$  min<sup>-1</sup>).

**Table 3 Macronutrient intake during the ultra-endurance triathlon race**

		<b>Cycling</b>	<b>Running</b>	<b>Total</b>
<b>Carbohydrates</b>	<b>Solids (g)</b>	405.7 (147.8)	291.5 (60.8)	697.2 (147.2) <sup>†</sup>
	<b>Fluids (g)</b>	177.8 (67.0)	51.6 (24.3)	229.4 (67.0)
	<b>Total (g)</b>	583.5 (176.3) <sup>§</sup>	349.0 (73.3)	926.6 (177.5) <sup>*</sup>
	<b>g/kg<sup>a</sup></b>	7.8 (2.3)	4.7 (1.2)	6.2 (1.3)
	<b>g/hour<sup>b</sup></b>	84 (30)	78 (18)	84 (18)
	<b>%<sup>c</sup></b>	83.2 (5.6)	96.6 (3.8)	89.9 (3.5)
<b>Proteins</b>	<b>Total (g)</b>	40.7 (11.5) <sup>§</sup>	4.6 (3.4)	45.4 (12.0)
	<b>g/kg</b>	0.6 (0.2)	0.1 (0.0)	0.3 (0.1)
	<b>%</b>	6.4 (2.9)	1.3 (1.0)	3.8 (1.6)
<b>Lipids</b>	<b>Total (g)</b>	69.1 (18.0) <sup>§</sup>	7.7 (10.5)	76.8 (20.4)
	<b>g/kg</b>	0.9 (0.3)	0.1 (0.1)	0.5 (0.1)
	<b>%</b>	10.5 (3.6)	2.2 (3.0)	6.3 (2.4)

Data are presented as mean (SD).

<sup>a</sup> Ratio between total macronutrient intake (g) and body mass (kg);.

<sup>b</sup> Ratio between total carbohydrate intake (g) and total racing time (min);.

<sup>c</sup> Macronutrient percentage of the total energy intake in each segment and in total.

<sup>\*</sup> The amount of carbohydrates was significantly higher than the amount of protein and lipids ( $P < 0.001$ ).

<sup>†</sup> The consumption of solid CHO was higher than that of fluid CHO ( $P < 0.001$ ).

<sup>§</sup> The intake amount was significantly higher in the cycling stage than in the running stage ( $P < 0.001$ ).

## Fluid and sodium intake

Table 4 summarizes the fluid balance and the sodium intake during the UET. In absolute values, fluid and sodium intakes were significantly higher during the cycling stage than in the running stage ( $P < 0.001$ ). However, when comparing relative values (fluid intake/time of exercise), fluid intake was significantly greater during the running

period compared to the cycling stage ( $395 \pm 183$  and  $362 \pm 172$  mL/h respectively;  $P < 0.001$ ).

**Table 4 Fluid and sodium intake during the ultra-endurance triathlon**

	<b>Cycling</b>	<b>Running</b>	<b>Total<sup>a</sup></b>
<b>Fluid intake (mL)</b>	2530.9 (1255.9) <sup>*</sup>	1657.3 (717.4)	4188.2 (1836.9)
<b>Fluid intake rate (mL/h)</b>	361.6 (171.8) <sup>†</sup>	394.5 (183.2)	366.6 (146.9)
<b>Sodium (mg)</b>			
<b>From fluids</b>	485.2 (307.5) <sup>*</sup>	232.0 (208.5)	2152.2 (1124.2)
<b>From food</b>	1135.9 (953.9) <sup>*</sup>	299.1 (369.3)	

Data are presented as mean (SD).

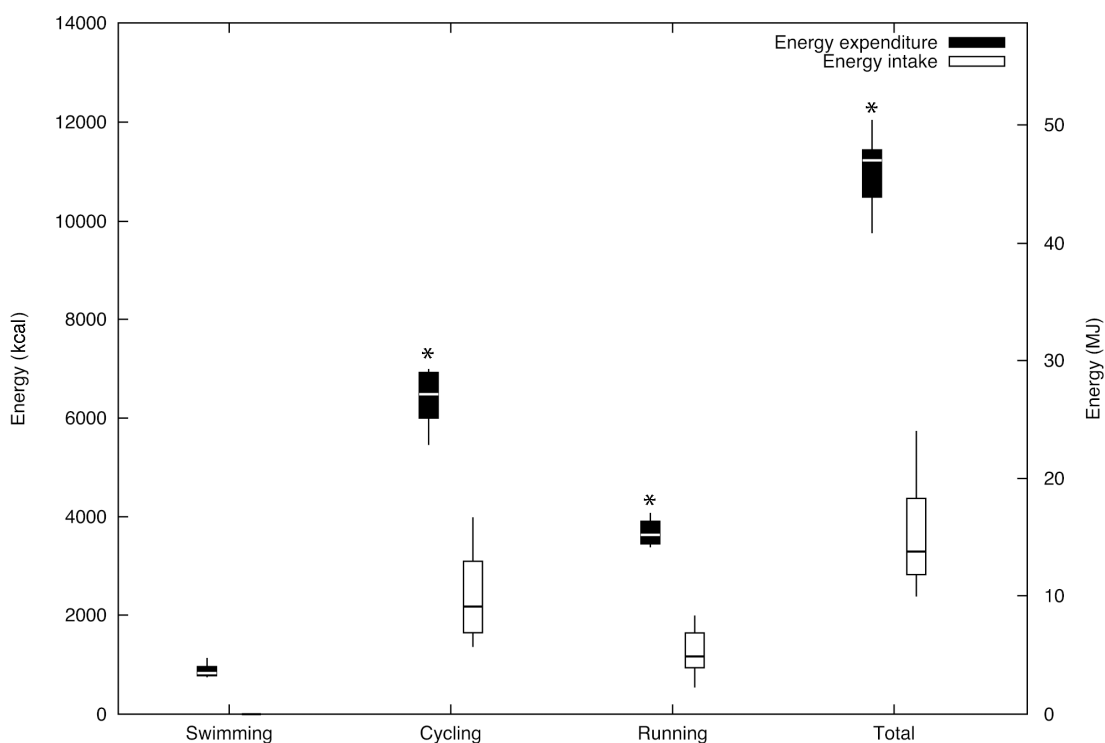
<sup>a</sup> Includes time of swim and race transitions.

<sup>\*</sup> Values are significantly higher during cycling stage than in the running stage ( $P < 0.001$ ).

<sup>†</sup> Values are significantly lower during running stage than in the cycling stage ( $P < 0.001$ )

## Energy balance

Figure 1 shows the box-and-whisker plot of the estimated EI and EE during each stage of the competition and in overall terms. EE ( $11,009 \pm 664$  kcal;  $46.1 \pm 2.8$  MJ) was significantly higher than EI ( $3,643 \pm 1,219$  kcal;  $15.3 \pm 5.1$  MJ;  $P < 0.001$ ) meaning that an energy deficit of  $7,365 \pm 1,286$  kcal ( $30.8 \pm 5.4$  MJ;  $66.9 \pm 11.7\%$ ) occurred. Solid food significantly provided more energy than fluids ( $2,812 \pm 1,150$  kcal;  $11.8 \pm 4.8$  MJ) ( $831 \pm 668$  kcal;  $3.5 \pm 2.8$  MJ;  $P < 0.001$ ). Absolute EI was higher during the cycling stage ( $2,391 \pm 82.9$  kcal;  $10.0 \pm 0.4$  MJ;  $65.63\%$ ) compared to the running stage ( $1,252 \pm 43.1$  kcal;  $5.24 \pm 0.18$  MJ;  $34.4\%$ ;  $P < 0.001$ ). Mean ratios between EI and EE during the cycling, running and swimming stages (i.e. including the swimming stage and transitions times) were  $0.37 \pm 0.14$ ,  $0.34 \pm 0.13$  and  $0.33 \pm 0.11$ , respectively.



**Figure 1** Swimming, cycling, running and overall energy intake and energy expenditure. Data are smallest value, first quartile (Q1), median, third quartile (Q3) and largest value of energy intake and energy expenditure during each stage and overall competition. \* Energy expenditure was significantly greater than energy intake ( $P < 0.001$ ).

### Body mass and bioimpedance bioelectricity variables

BM decreased significantly after the race ( $-4.3 \pm 1.4$  kg;  $-5.7 \pm 1.9\%$ ,  $P < 0.001$ ). Table 5 summarizes the differences in the TBW, ECW and ICW before competition and after competition. TBW, ECW and ICW also decreased significantly after the race ( $-8.4 \pm 2.9\%$ ,  $-10.8 \pm 3.7\%$  and  $-6.8 \pm 2.9\%$ , respectively,  $P < 0.001$ ). The decrease in ECW was significantly greater than the decrease in ICW ( $P < 0.001$ ). The decrease in BM was



closely related to the decrease in TBW ( $r = 0.98$ ), ECW ( $r = 0.93$ ) and ICW ( $r = 0.85$ ) ( $P \leq 0.001$ , respectively).

**Table 5 Bioimpedance bioelectrical variables**

	Total body water		Extracellular body water		Intracellular body water	
	Liters	%	Liters	%	Liters	%
Pre-race	45.9 (3.4)	61.3 (3.8)	18.1 (1.3)	39.5 (0.7)	27.8 (2.2)	60.5 (0.7)
Post-race	42.0 (3.3)*	59.6 (4.0)*	16.1 (1.1)*	38.4 (1.2)*	25.9 (2.3)*	61.6 (1.2)*
Difference	3.8 (1.4)	8.4 (2.9)	2.0 (0.7)	10.8 (3.7)	1.9 (0.8)	6.8 (2.9)

Data are presented as mean (SD).

\* Lower values post-race compared to pre-race ( $P < 0.001$ ).

### **Relationship between racing performance and parameters assessed during the race**

The decrease in TBW ( $r = 0.61$ ,  $P = 0.046$ ) and ECW ( $r = 0.72$ ,  $P = 0.01$ ) was related to the cycling time. CHO intake (g/min) was inversely related to the time ( $r = -0.71$ ), and directly related to the speed ( $r = 0.70$ ), during the cycling stage ( $P = 0.02$ ). The stepwise multiple linear regression analysis identified the amount of CHO (g/min) ingested during the race and the % of ECW loss during the race ( $\beta = -0.52$ ,  $SE = 60.8$  and  $\beta = 0.47$ ,  $SE = 4.4$  respectively,  $P < 0.05$ ) as the best predictors of total racing time, accounting for 60% ( $r^2 = 0.60$ ) of the final time variance.

## **Discussion**

This study provided proper characterization of the energy and fluid intake, as well as the estimated energy expenditure, of a group of male triathletes during an entire ultra-endurance triathlon race. The estimated EE was ~11000 kcal (46 MJ), whereas EI was

only ~3600 kcal (15 MJ), which resulted in an energy deficit of almost 70% which partially confirms our first hypothesis.

### **Macronutrient intake and energy balance**

The high energy deficit can be explained by the lower EI of athletes in comparison with the higher energy demands of the current UET. In this study, athletes consumed an average of  $927 \pm 178$  g of carbohydrates (90% of the overall EI). In relative terms, this amount corresponds to  $\approx 84 \text{ g} \cdot \text{h}^{-1}$ . However, while these values can be considered within the current recommendations for longer events [24], it must be noted that CHO ingestion was heterogeneous between participants. For instance, five participants consumed less than  $70 \text{ g} \cdot \text{h}^{-1}$  of CHO while two of them ingested more than  $90 \text{ g} \cdot \text{h}^{-1}$ . The remaining four triathletes consumed an amount within the range of 70–90  $\text{g} \cdot \text{h}^{-1}$ . Such differences between CHO ingestion were also linked with exercise performance on the bike. We found that triathletes ingesting higher amounts of CHO on the bike were able to perform better than those with low rates of CHO ingestion. This confirms the well-known fact that CHO supply plays a key role in order to improve exercise performance of athletes during endurance events [25].

Furthermore, a part of the amount of CHO, another key point related CHO intake is the type of CHO. It has been shown that the combination of different types of CHO (glucose-fructose-maltodextrin) with a consistent ratio (1:1:1) optimises CHO oxidation and exercise performance [24]. Although it is difficult to estimate such ratio in this study due to the variety of food that athletes ingested during the event (Table 2), the combination of sport gels, sport bars, sport drinks and sandwich may indicate that athletes ingested multiple CHO types.

The average protein intake by triathletes was  $45 \pm 12$  g in total (4% of total EI). This amount was higher than previous data reported in triathletes [3]. However, to date, there is not a consensus about the protein needs during ultra-endurance events. Under laboratory conditions, it has been shown that the ingestion of  $0.25 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  in

combination with carbohydrates can improve protein balance by increasing protein synthesis and decreasing protein breakdown during long efforts (6 h) [26]. Considering the data provided by Koopman et al., [26], along with the amount of CHO intake by the athletes in this study, the 'ideal' amount of protein ingestion would have been 236 g. This amount of protein would increase the energy intake (>900 kcal) and it would help to decrease the energy deficit of athletes.

The consumption of fat was  $77 \pm 20$  g during the whole event. These values are lower compared with a previous study [3]. Like protein intake, currently there is no evidence supporting that the ingestion of lipids in form of either long-chain triacylglycerol (LCT) or tolerated amounts of medium-chain triacylglycerols increases performance during prolonged exercise [27]. Although the human body stores of fat are so large and they will not become depleted after prolonged events, some stores such as intramyocellular triglycerides (IMTG) can play a key role during exercise [28]. These lipids are located directly to the site of contraction to ensure immediate substrate availability for physical activity [29]. Under conditions in which muscle glycogen availability is severely challenged, IMTG can compensate (at least in the endurance trained individual) by providing an alternative fuel source of similar potential energy to glycogen, to enable prolonged moderate intensity physical activity to be maintained [30]. However, while early research of the role of muscle glycogen in endurance exercise provided clear prescriptive information for the endurance-trained athlete, no such direction for optimising exercise performance is yet apparent from research concerning IMTG. On the other hand, the inclusion of fat in the diet of ultra-endurance athletes could also be interesting to satisfy the taste of foods.

As shown by two previous studies [3,5], a high negative energy balance seems to be common in UET. The average of EE ( $11,009 \pm 664$  kcal) in this study was higher than in these previous studies. This fact could be explained by the positive elevation that triathletes had to overcome during the cycling stage in the current UET which induced a higher EE.

## **Body mass, fluid and sodium intake**

In our athletes, the BM decreased significantly after the UET ( $-6 \pm 2\%$ ). Decreases in BM up to as much as 11% of the total body weight have been shown previously [5,17,31-33]. These BM losses can be explained by high energy deficits which induce a decrease in muscle density (glycogen loss) and fat mass of the body of athletes participating in ultra-endurance events [13-16]. In the current study we found a fluid deficit of 1.3 L which was far from the mean loss of body weight. Therefore, we assume that most of the loss in BM was due to losses of glycogen and fat stores.

Additionally, a significant and negative correlation between losses of TBW and ECW and performance during the cycling stage was found in this study. Although this fact corroborates the well-known fact that hydration is a key point for exercise performance, it is also important to note that losses of TBW were mainly linked to a reduction of ECW. An overload of ECW is a risk factor to develop hyponatremia [7-9]. Importantly, in the current study none of the athletes showed an increase of ECW. Furthermore, despite the fact that hyponatremia is an electrolyte disorder affecting serum sodium concentration ( $<135 \text{ mmol} \cdot \text{L}^{-1}$ ), the consumption of high amounts of sodium during exercise does not reduce the risk of developing hyponatremia [34]. Fluid overload is considered the main risk factor in the pathogenesis of hyponatremia and this is controlled by fluid intake during exercise as well as by the activity of renal hormones such as vasopressin [11,12]. The mean sodium intake reported by the current triathletes ( $2,152 \pm 1,124 \text{ mg}$ ) was within the Recommended Dietary Intake and above the mean sodium ingestion indicated by Kimber et al., [3] in a previous study. This can be explained by the higher content of sodium of some sport food and sport drinks consumed by participants of this study during the event. None of the participants consumed sodium in form of supplementation. This finding shows that it is possible to meet sodium recommendations through normal food and drinks in ultra-endurance events and, consequently, sodium supplementation is not needed. These data are in

agreement with another recent study investigating sodium intake of ultra-endurance runners during a multi-stage race in a hot environment [35].

### **Strengths and limitations**

A major strength of this study is the careful nutritional analysis which was carried out in a community and setting where little information has been provided. Another strength is the testing during graded swimming that has not been done before with triathletes. However, we should also acknowledge some limitations and caveats in this study. Perhaps, the main limitation was the method used to estimate energy expenditure (relationship between HR-  $\dot{V}O_2$ ). It is known that this method can be affected by several physiological and environmental factors such as dehydration and temperature [36]. However, there is also evidence indicating good correlation between the doubly labelled water method (which is considered as the gold standard) and the use of equations based on the linear relationship between HR and  $\dot{V}O_2$  under field conditions [5]. Furthermore, a study performed in laboratory conditions showed that the ratio  $\dot{V}O_2$  /HR increased 7% after 12 hours of continuous exercise [37]. These data suggest that the current study underestimated the EE in a range of 700–800 kcal, and consequently, there was and even higher energy deficit.

### **Practical applications**

In this study we showed that triathletes were working mainly under the first ventilatory threshold where the body uses mainly fat as fuel and that the total EI did not provide the amount of energy necessary to deal with the UET, so the key seems to be looking for further adaptations to increase the ability to generate energy from fat. Accordingly it should be useful to guide the training programs to increase the capacity to oxidize fat when muscle glycogen stores are depleted.

It would also be advisable to prepare the nutritional race strategy previously, according to the individual sweat rates and oxidation capacity.

## **Conclusions**

The energy demands induced by UET exceed by far the energy intake of amateur triathletes despite consuming an adequate amount of CHO. The energy deficit induced by these events can be close to 70%. While an increase of lipid and protein consumption during UET would reduce the energy deficit of athletes, it is unknown how this would affect other key physiological responses such as gastric emptying and intestinal absorption during the race. Consequently, more research is needed in order to investigate the protein and lipid needs in ultra-endurance events. Furthermore, this study showed significant fluid losses (TBW, ECW and ICW) of athletes participating in the UET. A significant and negative relationship was found between losses of TBW and losses of ECW. Not only could the loss of body fluids potentially decrease exercise performance, it may also compromise the cardiovascular function and athlete's health through the race.

## **Competing interests**

For the remaining authors no conflicts of interest are declared, including professional relationship with any company or manufacturer who will benefit from the results of the present study.

## **Authors' contributions**

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Authors: Anna Barrero

Title: Nutritional behavior of triathletes during an ultra-endurance event

Journal: Journal of the International Society of Sports Nutrition

MS : 1506426989121133

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Peer review of your manuscript (above) is now complete and we are delighted to accept the manuscript for publication in Journal of the International Society of Sports Nutrition.

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## **ANNEX 3. CONFERENCE PRESENTATIONS**



**7<sup>th</sup> European Congress of the Fédération Internationale de Education Physique.  
Barcelona, Spain, 7-9 June 2012.**

## **Heart Rate response during an ultra-endurance triathlon**

*Barrero A.<sup>1</sup>, Chaverri D.<sup>1</sup>, Erola P.<sup>2</sup>, Iglesias X.<sup>1</sup>, Rodríguez F.A.<sup>1</sup>*

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### **Introduction**

Triathlon is becoming a very popular sport but, even if the number of triathletes and competitions has increased exponentially, our knowledge of the physiological demands during competition is far from being extensive. To optimize training and determine performance factors in a triathlon, we first need to know the physiological demands during competition. However, the requirements for an entire ultra-endurance race related to the individual metabolic capacities of triathletes have not fully been characterized yet. Here we aimed to provide the first comprehensive characterization of the heart rate response during an entire ultra-endurance triathlon in relation with the individual profile assessed during specific graded tests in each of the three exercise modes.

### **Methods**

Nine well-trained ultra-endurance triathletes of regional and national level (mean  $\pm$  SD: age  $36.7 \pm 5.5$  years, mass  $75.2 \pm 6.7$  kg, height  $1.74 \pm 0.06$  m, BMI  $24.8 \pm 1.9$  kg/m<sup>2</sup>, VO<sub>2max</sub>  $5.03 \pm 0.4$  l·min<sup>-1</sup>,  $67.0 \pm 4.3$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) participated in the study, which comprised preliminary testing and heart rate (HR) monitoring during an ultra-distance triathlon (3.8 km swimming, 180 km cycling, 42.2 running). To assess the exercise



intensity during the race HR was continuously recorded during using portable waterproof monitors (RCX5 Polar, Finland). Prior to the race subjects performed three graded exercise tests using a portable gas analyzer (K4 b<sup>2</sup> Cosmed, Italy): ergometer cycling (Excalibur Sport, Lode, Netherlands), treadmill running (h/p Cosmos Quasar Med, Germany), and free swimming on a 50-m outdoor pool. Main variables measured were peak oxygen uptake ( $VO_{2peak}$ ), first and second ventilatory thresholds ( $VT_1$  and  $VT_2$ ), and their corresponding HR values.

## Results

All nine triathletes successfully finished the ultra-endurance event. The mean HR during the swimming stage was significantly higher than during cycling ( $P = 0.024$ ), running ( $P = 0.013$ ) and the overall ( $P = 0.025$ ) mean HR. There was no difference in mean HR between the cycling and running stages ( $P = 0.995$ ; Table1; Figure 1).

Table 1. Swim, cycle, run and overall triathlon performance variables

	Time (h:min:s)	% Total time (%)	Mean speed (m·s <sup>-1</sup> / km·h <sup>-1</sup> )	Mean HR (beats·min <sup>-1</sup> )
Swimming (3.8 km)	01:03:13 (00:08:03)	8.6	1.00	149.2 (10.1)
Cycling (180 km)	07:02:24 (00:40:54)	57.3	25.6	137.1 (5.7)*
Running (42.2 km)	04:12:02 (00:35:43)	34.2	10.1	136.2 (10.5)*

HR: heart rate; Data are mean (SD).

Value for the swimming stage that is significantly higher than that for cycling and running stages at \* $P < 0.05$ .

Figure 1 shows the HR response related to percentage of time spent in each stage.

Mean HR during the swimming stage was closely related to both HR at  $VT_1$  ( $r = 0.82$ ,  $P = 0.007$ ) and  $VT_2$  ( $r = 0.77$ ,  $P = 0.015$ ). HR during the cycling stage was related to HR at  $VT_2$  ( $r = 0.74$ ,  $P = 0.023$ ). HR during the running stage was related to HR at  $VT_2$  ( $r = 0.75$ ,  $P = 0.019$ ).

HR during swimming was significantly higher than the HR at  $VT_1$  ( $P = 0.002$ ), but there were no differences between mean HR during swimming and HR at  $VT_2$ . HR during

cycling and running stages were significantly lower than the HR at VT<sub>1</sub> ( $P = 0.009$  and  $P = 0.048$ , respectively) and VT<sub>2</sub> ( $P = 0.000$  in both cases).

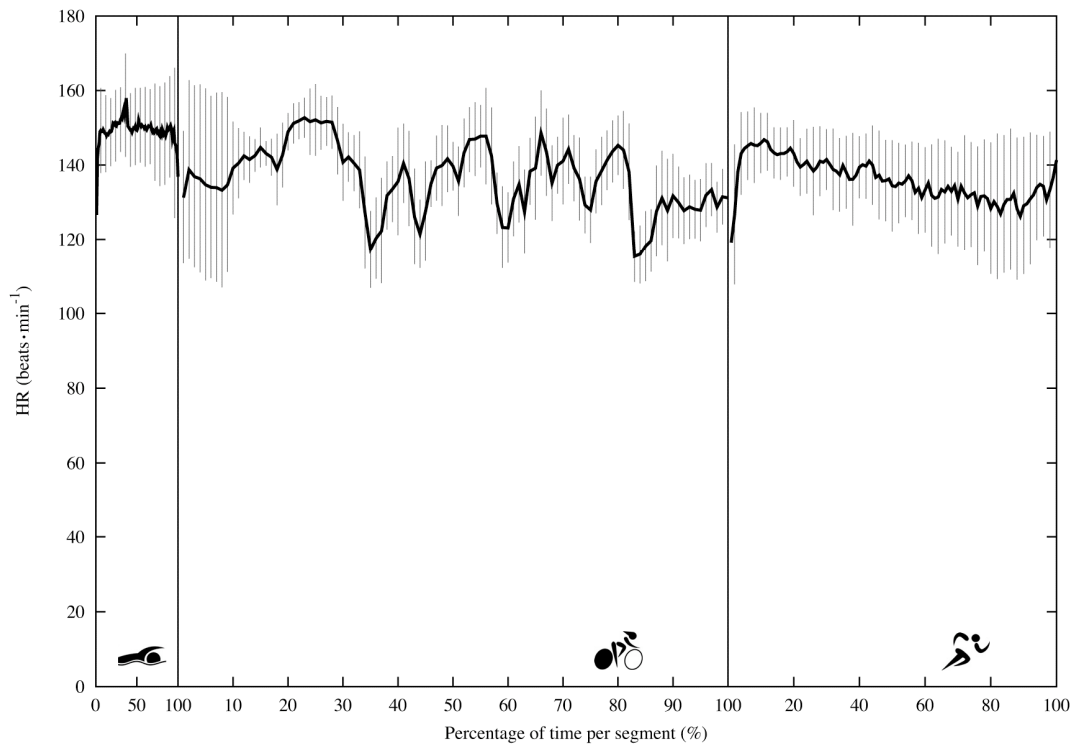


Figure 1. Heart rate response during an ultra-distance triathlon event. Mean HR in the swimming stage was significantly greater than during the cycling and running stages ( $P < 0.05$ ). Mean and SD are depicted

## Discussion

To our knowledge, only a study (P. B. Laursen et al., 2005) has previously described the HR response during an ultra-distance triathlon event. However, unlike in the present research, and probably due to technical limitations, HR during the swimming stage was not properly described and related to the testing profile of the athletes during graded swimming. Additionally, more adequate scaling (i.e. expressing HR in relation to the percentage of time spent in each segment during the race in all subjects), allowed to better characterize the time pattern of HR during the whole event.

A close relationship between HR at the  $VT_1$  measured in graded exercise tests and HR during the cycling and running stages ( $r = 0.76, P < 0.01$ ;  $r = 0.66, P < 0.01$ ) has been previously reported in the above mentioned study (P. B. Laursen et al., 2005). Although we found a similar pattern, particularly in the running stage, our subjects tended to work below  $VT_1$ . In contrast, we found that HR during the swimming stage was remarkably higher as compared to the cycling and running segments, and in fact not different from HR at  $VT_2$ , thus clearly showing the higher intensity of the first stage, likely to be due to its much shorter duration, the stressful conditions derived from swimming in open waters surrounded by other competitors, and the eventual advantage given by a favoured position during the following stage.

When properly scaled, HR showed a clear trend to decline at the final part of each of the three stages, likely to be the result of skeletal and cardiac muscle fatigue. Only at the final segment of the running stage HR rises up to previous levels, likely to reflect the last effort before the end of the race.

In conclusion, the characterization of HR during the swimming stage and a more adequate description of the time pattern response during the race adds new information concerning the cardiovascular demands of an ultra-endurance triathlon. The relevance of specific testing in the assessment of the physiological requirements during competition is to be emphasized. Further research is needed to better understand the physiological demands of this multi-sport, ultra-endurance sport.

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## Physiological demands in an Ironman triathlon

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### Introduction

To optimize training and determine performance factors in a triathlon, we need to know the bioenergetic requirements and physiological response during competition. However, the physiological demands for an entire triathlon in relation to the individual metabolic capacities of triathletes have not been characterized yet. Here we aimed to provide the first comprehensive description of the physiological demands of an entire ultra-endurance triathlon.

### Methods

Ten well-trained ultra-endurance triathletes (mean  $\pm$  SD: age  $37.1 \pm 5.3$  years, mass  $74.9 \pm 6.4$  kg, height  $1.74 \pm 0.06$  m, BMI  $24.7 \pm 1.8$  kg/m<sup>2</sup>, VO<sub>2</sub>peak =  $4.92 \pm 0.5$  l·min<sup>-1</sup>) participated in the study. To investigate exercise intensity, heart rate (HR) was recorded during a competition using portable monitors. Before the ultra-endurance triathlon, subjects performed graded exercise tests involving cycle ergometry, treadmill running and swimming to determine peak oxygen uptake (VO<sub>2</sub>peak) and heart rate corresponding to the first and second ventilatory thresholds (VT1 and VT2).

### Results

The HR in the swimming stage was closely related to the HR at VT1 ( $r = 0.77$ ,  $P = 0.01$ ) and related to the HR at VT2 ( $r = 0.68$ ,  $P = 0.03$ ). The HR in the cycling stage was

not related to the HR at VT1 and VT2. The HR in the running stage was related to the HR at VT2 ( $r = 0.64$ ,  $P = 0.048$ ) but not to the HR at VT1.

The HR during the swimming stage was significantly higher than the HR at VT1 ( $P = 0.00$ ), but there were no differences between the HR during the swimming stage and the HR at VT2. The HR in the cycling and running stages were significantly lower than the HR corresponding to VT1 ( $P = 0.01$  and  $P = 0.01$ , respectively) and VT2 ( $P = 0.00$  and  $P = 0.00$ , respectively).

### **Discussion**

This study is the first to characterise the relative intensity (HR related to ventilatory thresholds) during the race of the three stages of a triathlon.

The differences in HR between the swimming and cycling stages correlate with the marathon time and the overall time. The triathletes with the greatest difference in HR in the swimming stage and HR during the cycling stage had the worst cycling times. As stated in Laursen et al. (2005), working beyond capacity in one stage makes recovery difficult, leading to worse performance in the next stage. This correlation can also be seen in HR differences for the swimming and running stages, as a large difference in values is associated with a reduction in the marathon time.

### **References**

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18th Annual Congress of the ECSS. Barcelona, Spain, 26-29 June 2013.

## Energy balance during an ultra-endurance triathlon

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### Introduction

The popularity of ultra-endurance triathlon (UET) races (3.8-km swim, 180-km cycle, 42.2-km run) has greatly increased since the first Ironman was held in 1978. The primary determinant of success is the ability to sustain a high rate of energy expenditure (EE) for prolonged periods of time (O'Toole & Douglas, 1995). We could find only one study reporting data from real competition (Kimber et al., 2002), though lacking direct assessment of the swimming section. This study aimed to provide the first comprehensive characterization of the energy balance during real competition in male triathletes during a complete UET race.

### Methods

Eleven well-trained non-professional ultra-endurance triathletes (mean  $\pm$  SD: age  $36.8 \pm 5.1$  years, mass  $75.5 \pm 6.4$  kg, height  $174 \pm 6$  cm, BMI  $24.8 \pm 1.7$  kg/m<sup>2</sup>, VO<sub>2</sub>max  $66.9 \pm 4.1$  mL·kg<sup>-1</sup>·min<sup>-1</sup>) competed in the Extreme Man Salou-Costa Daurada UET 2011. EE was estimated from heart rate (HR) recordings during the race (Polar RCX5), using the individual HR-VO<sub>2</sub> regressions developed from three incremental tests on the cycle ergometer (Excalibur Sport, Lode, Netherlands), running treadmill (h/p/Cosmos Pulsar, Germany), and 50-m swimming pool. VO<sub>2</sub> was measured using a portable gas analyser (K4 b<sup>2</sup>, Cosmed, Italy). An observational design, which included weighing and recording all food and fluid ingested and posterior nutritional analysis, was used to assess the energy intake (EI) during the race.

## **Results**

Mean competition time was  $755 \pm 69$  min at an average HR of  $137 \pm 6$  beats/min. Mean EE ( $46.1 \pm 2.8$  MJ,  $11,009 \pm 664$  kcal) was significantly greater than EI ( $16.9 \pm 4.8$  MJ,  $4,043 \pm 1,141$  kcal;  $P < 0.001$ ). Mean EE rate was  $3.8 \pm 4.8$  MJ/h ( $738 \pm 69$  kcal/h). Energy deficit was  $37 \pm 0.1\%$  of total EE. Solid food provided 83% of EI, and fluids the remaining 17%.

## **Discussion**

Both total EE and EI were greater than previously reported by Kimber et al. (2002) during an Ironman race (42.0 and 15.5 MJ, respectively), but energy deficit was smaller (40%). Compared to data on a 24-h cycling race competitor (Bescós et al. 2012), EE and EI were smaller (65.0 and 23.3 MJ, respectively), but EE rate was greater (2.7 MJ/h), resulting in a similar energy deficit (36% of EE). Our results confirm the high energy demands of UET races, which are not compensated by nutritional and fluid intake, resulting in a large energy deficit and dehydration which is likely to lead to early fatigue and impaired physical performance, particularly at the end of the race.

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18th Annual Congress of the ECSS. Barcelona, Spain, 26-29 June 2013.

## **Muscle damage induced by an ultra-endurance triathlon affects slow fibres and is related to dehydration**

*Barrero, A.<sup>1</sup>, Cadefau, J.A.<sup>1</sup>, Irurtia, A.<sup>1</sup>, Chaverri, D.<sup>1</sup>, Guerrero, M.<sup>2</sup>; Carmona, G.<sup>2</sup>, Cussó, R.<sup>2</sup>, Iglesias, X.<sup>1</sup>, Rodríguez, F.A.<sup>1</sup>*

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### **Introduction**

Ultra-endurance triathlon (UET) races (3.8-km swim, 180-km cycle, 42.2-km run) induce muscle damage (Neubauer et al., 2008). Fast (FM) and slow myosin (SM) assessment in blood 48 h after exercise is a useful marker for the detection of skeletal muscle damage (Guerrero et al., 2008). This study aimed to assess muscle damage after an UET race as indicated by FM, SM and creatine kinase (CK) serum levels and to relate them to the hydration status.

### **Methods**

11 well-trained non-professional ultra-endurance triathletes (mean  $\pm$  SD: age  $37.2 \pm 4.6$  years, body mass  $74.3 \pm 6.7$  kg, height  $174 \pm 6$  cm, BMI  $24.5 \pm 1.9$  kg/m<sup>2</sup>, VO<sub>2</sub>max  $67.5 \pm 4.2$  mL/(kg·min)) competed in the Extreme Man Salou-Costa Daurada UET 2011. Venous blood samples were collected up to 30 min before and 48 h after finishing the race. ELISA method was used to determine myosin concentration in serum. Body mass (BM) and bioimpedance (BIA) bioelectrical variables (Z-Metrix<sup>®</sup>, BioparHom Co, France) were obtained before, 30 min post-race, and 48 h after. Total (TBW),



intracellular (ICW), and extracellular body water (ECW) content was estimated using the instrument software.

## **Results**

Race time was  $759 \pm \text{SD } 64$  min. Initial SM levels ( $1016 \pm 228$   $\mu\text{g/l}$ ), FM levels ( $1091 \pm 374$   $\mu\text{g/l}$ ), and CK activity ( $202 \pm 87$  U/l) largely increased post-race ( $170 \pm 93\%$ ,  $16 \pm 37\%$  and  $600 \pm 476\%$ , respectively,  $p < 0.001$ ). SM increase was closely related to the increase in CK ( $r = 0.72$ ,  $p = 0.013$ ). BM ( $74.0 \pm 6.8$  kg) decreased post-race ( $-5.8 \pm 1.9\%$ ) and 48 h after ( $-1.4 \pm 1.2\%$ ) (ANOVA,  $p < 0.001$ ), as well as TBW, ECW and ICW ( $-8.5 \pm 2.9\%$ ,  $-10.8 \pm 3.7\%$  and  $-7.0 \pm 2.8$ , respectively,  $p < 0.001$ ), with no further changes 48 h after. BM decrease was closely related to the reduction in TBW, ECW, and ICW ( $r = 0.98$ ,  $0.95$ , and  $0.85$ , respectively,  $p \leq 0.001$ ). The increase in SM levels was related with the decrease in TBW ( $r = 0.71$ ,  $p = 0.014$ ) and ICW ( $r = 0.74$ ,  $p = 0.01$ ), but not in ECW ( $r = 0.56$ ,  $p = 0.08$ ).

## **Discussion**

While CK increased activity is believed to be a reliable marker of muscle damage, elevated SM levels suggest specific damage in slow muscle fibres, which are largely predominant in ultra-endurance athletes. Our results, beyond confirming fibre muscle damage induced by an UET race, strongly suggest that cellular structural damage predominantly affects slow fibres, and that muscle damage is influenced by hydration status.

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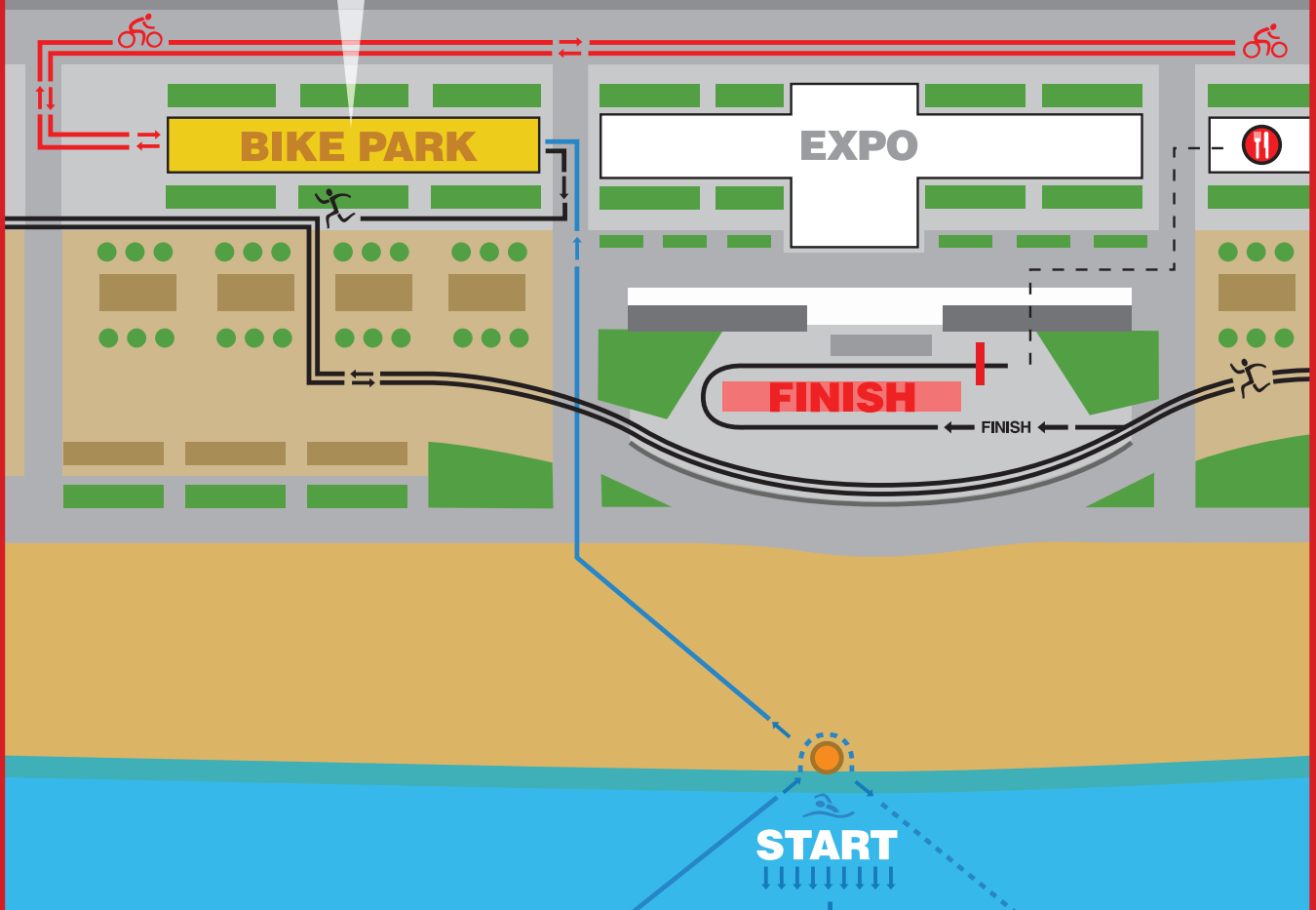
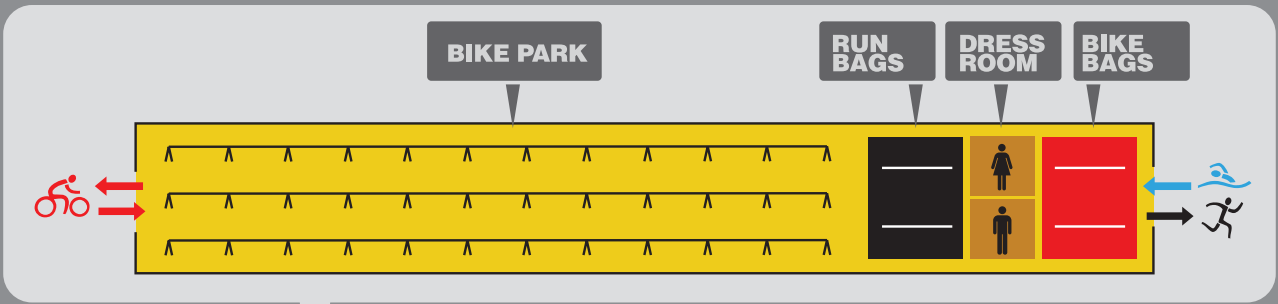
## **ANNEX 4. EXTREME MAN SALOU COSTA- DAURADA 2011**

Characteristics of each race segment

Points of nutritional data collection during the race

(source: Win Sports Factory)





# NATACIÓ. natación / swim



**Recorregut a 2 voltes (1a volta de 1550m i 2a volta de 2250m. Sortida de l'aigua per a iniciar la 2a volta)  
2 embarcacions, 2 motos d'aigua i 10 kaiacs que realitzaran la cobertura aquàtica.**

Recorrido a 2 vueltas (1ª vuelta de 1550m + 2ª vuelta de 2250. salida del agua para iniciar la 2ª vuelta)  
2 embarcaciones, 2 motos de agua y 10 kayaks que realizaran la cobertura acuática.

2 laps circuit (1st loop of 1550m + 2nd loop of 2250m. transition between the 2 laps out of the water)  
2 boats, 2 scooters and 10 kayaks will cover the sector.

06:15 Triatletes a la zona de sortida / Todos los triatletas a la zona de salida / All the triathletes called to the starting area

06:20 Presentació del Triatletes / Presentación de los Triatletas / Triathletes presentation

06:30 Inici de la prova / Inicio de la Prueba / Start of the race

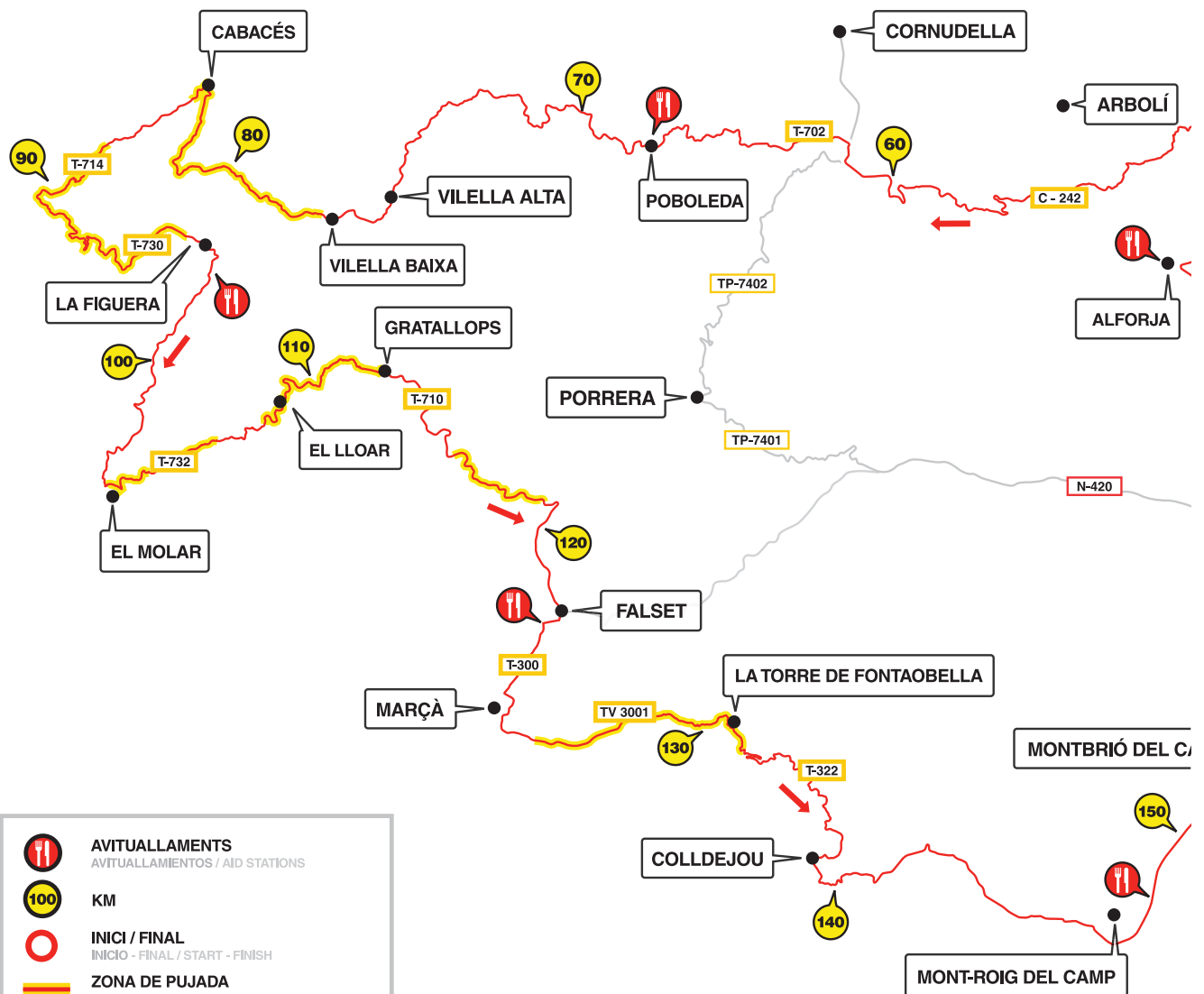
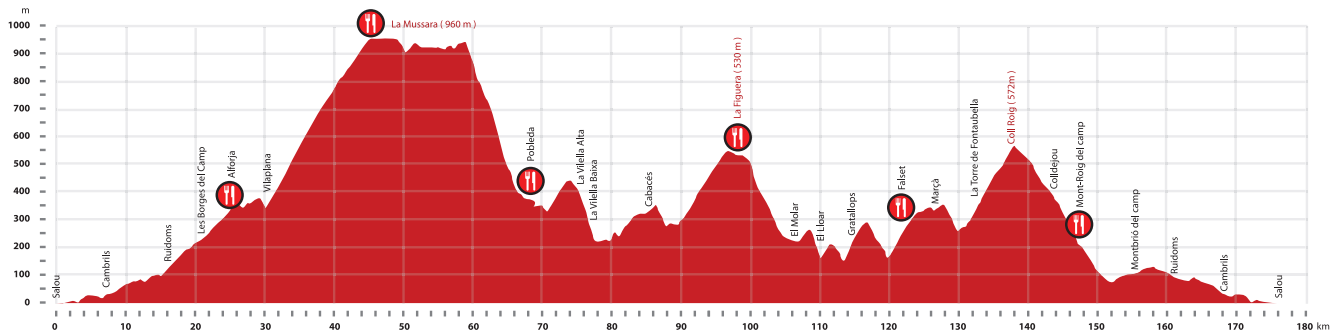
08:30 Temps de tall de natació / Tiempos de corte de natación / Swimming Cut off time



# CICLISME. ciclismo / bike



Una volta de 180km / una sola vuelta / one single loop

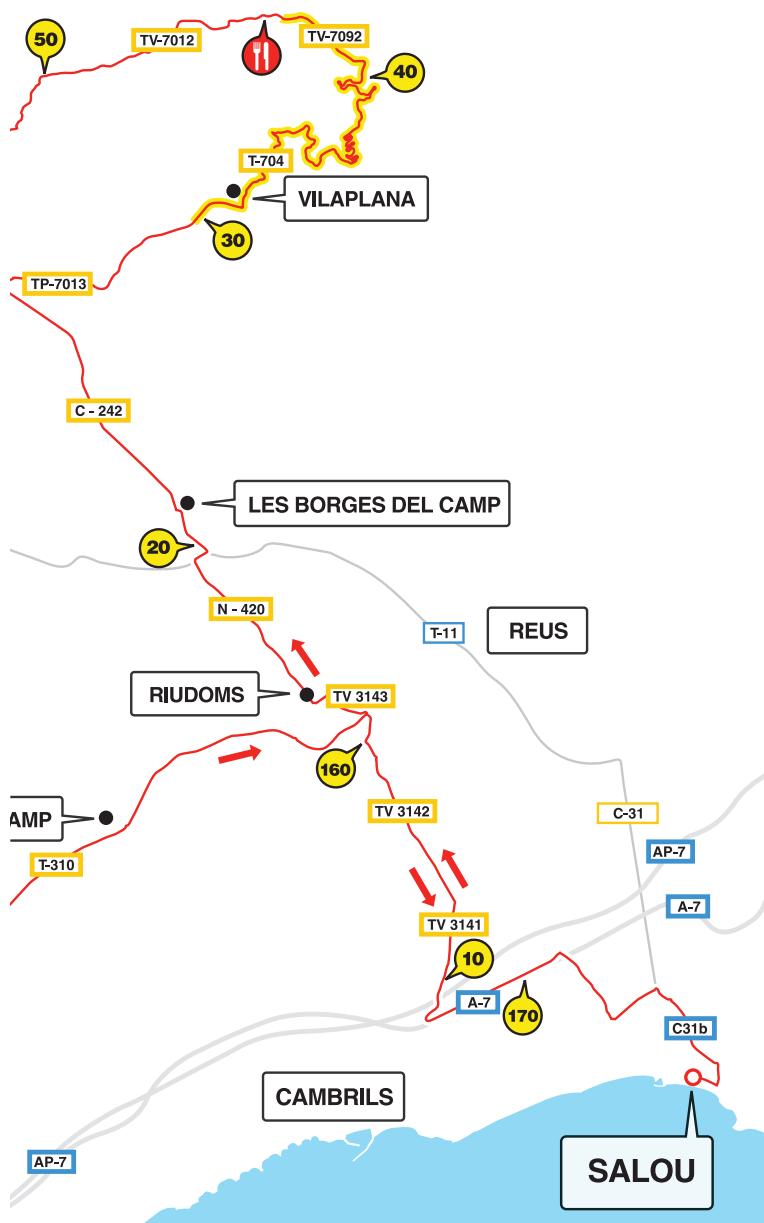


	<b>AVITUALLAMENTS</b> AVITUALLAMIENTOS / AID STATIONS
	<b>KM</b>
	<b>INICI / FINAL</b> INICIO - FINAL / START - FINISH
	<b>ZONA DE PUJADA</b> ZONA DE SUBIDA / CLIMBING ZONE
	<b>CARRETERES</b> CARRETERAS / ROADS NAME

- Obligatori circular SEMPRES pel costat dret de la carretera. (trànsit obert)
- Molta precaució en els trams interns de les poblacions i en les baixades.
- Prohibit llençar residus a la carretera, s'ha de fer en els avituallaments.
- 6 Avituallaments amb aigua (bidó), Gatorade (bidó), barretes energètiques, gels de glucosa Squeezy i WC.
- Cotxe escombra entremig i seguint l'últim corredor.
- **Infraccions penalitzades amb targeta groga i 8 minuts a complir en el Penalty Box:**
  - Tirar residus a la carretera fora dels avituallaments.
  - Envaïr el carril contrari.
  - No mantenir les distàncies reglamentàries de Drafting amb els altres participants.

El Jutge mostrarà la targeta groga al triatleta i li indicarà el dorsal. 2 Targetes grogues són desqualificació directa.

Cada triatleta sancionat és responsable únic de parar-se a la Carpa de Penalty Box per complir la seva sanció. Si el participant no realitza la parada serà desqualificat automàticament.



Es Obligatorio circular **SIEMPRE** por el lado derecho de la carretera. (tránsito abierto)

Mucha precaución en los tramos internos de las poblaciones y en las bajadas.

**Prohibido tirar residuos** a la carretera, hay que realizarlo en los avituallamientos.

**6 Avituallamientos** con agua (bidón), Gatorade (bidón), barras energéticas, geles de glucosa Squeezy y WC. Coche escoba entremedio y siguiendo al último corredor.

**Infracciones penalizadas con tarjeta amarilla y 8 minutos a cumplir en el Penalty Box:**

- Tirar residuos a la carretera fuera de los avituallamientos.
- Invadir el carril contrario.
- No mantener las distancias reglamentarias de Drafting con los otros participantes.

El Juez mostrará la tarjeta amarilla al triatleta y le indicará el dorsal.

2 tarjetas amarillas son la descalificación directa.

Cada triatleta sancionado es el único responsable de pararse en la Carpa de Penalty Box para cumplir su sanción.

Si el participante no realiza la parada será descalificado automáticamente.

**OBLIGATORY** to always circulate on the left side of the road. Be careful when crossing the towns and during the down hills. Forbiden to throw garbages on the road. You have to wait for aid stations. (open transit)

6 aid stations with water (bike bottles), Gatorade (bike bottles), energy bars, gels Squeezy and WC.

2 broom wagons. One intermediate and one final.

**Offenses sanctioned with yellow card and 8 minutes stop & go in the Penalty Box:**

- To throw garbages on the road out of the aid stations areas
- To invade the left side of the road.
- To do not maintain the official distances of drafting with the other participants.

The Marshall will show the yellow card to the Triathlete and will announce his bib number. 2 yellow cards mean direct disqualification.

Each sanctioned triathlete is responsible, on his own, to stop to the Penalty Box and to accomplish his sanction. If the triathlete does not accomplish the sanction, he will be automatically disqualified.

#### COLL DE LA MUSSARA

Distància / Distancia / Distance	10,45 km
km de circuit / km del circuito / km in the loop	Km. 32
Altitud d'arribada / Altura en cima / top height	963 m.
Desnivell / Desnivel / Elevation	602 m.
Pendent mig / Pendiente media / Slope average	5,8 %
Pendent màxim / Pendiente máxima / Max slope	7,3%

#### COLL DE LA FIGUERA

Distància / Distancia / Distance	6 km
km de circuit / km del circuito / km in the loop	Km. 94
Altitud d'arribada / Altura en cima / top height	571 m.
Desnivell / Desnivel / Elevation	287 m.
Pendent mig / Pendiente media / Slope average	2 %
Pendent màxim / Pendiente máxima / Max slope	8%

#### COLL ROIG

Distància / Distancia / Distance	6,2 km
km de circuit / km del circuito / km in the loop	Km. 129
Altitud d'arribada / Altura en cima / top height	415 m.
Desnivell / Desnivel / Elevation	185 m.
Pendent mig / Pendiente media / Slope average	3 %
Pendent màxim / Pendiente máxima / Max slope	7%

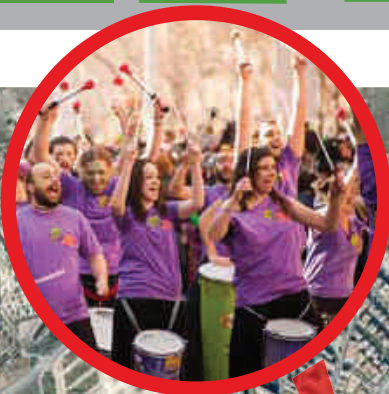
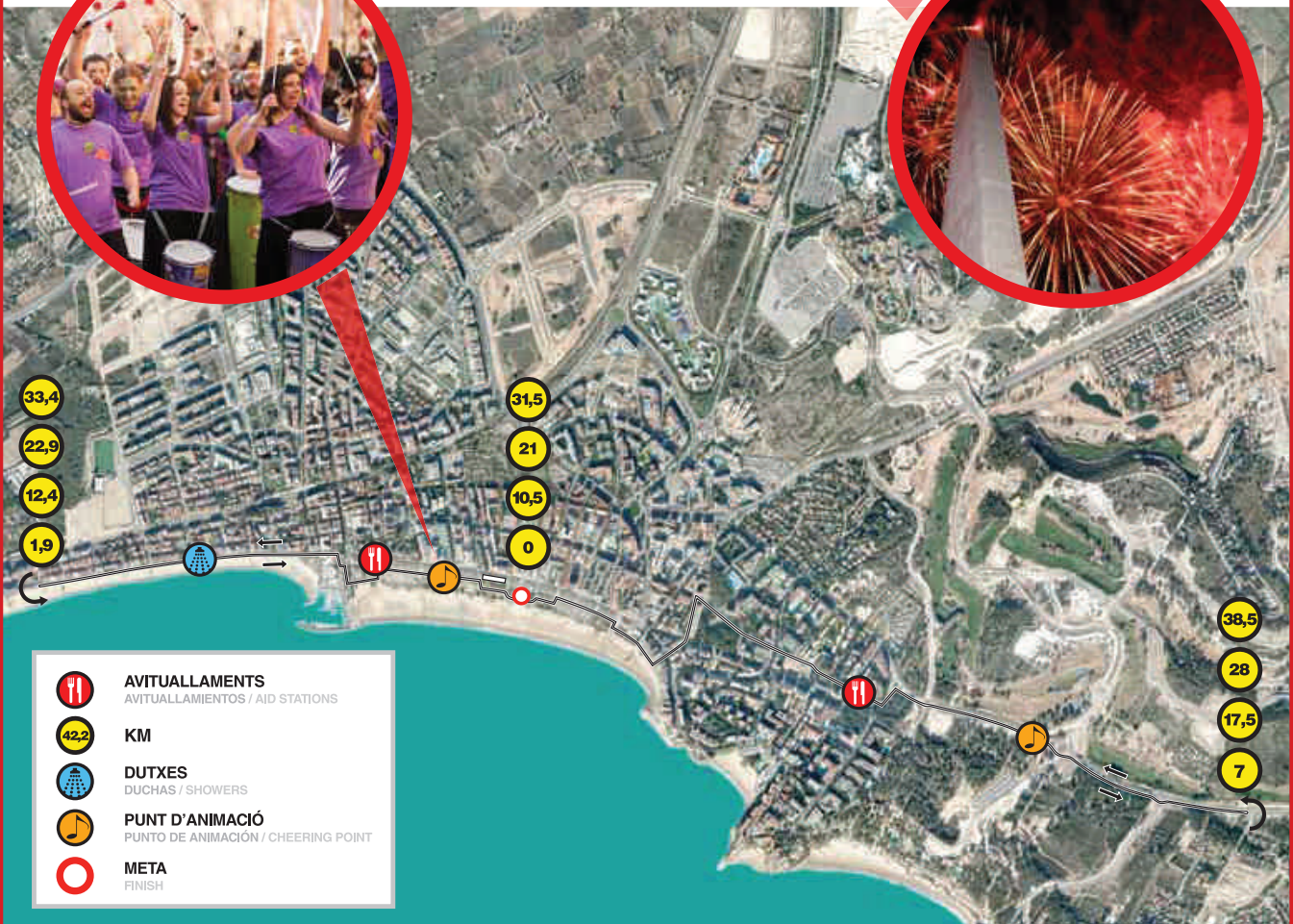
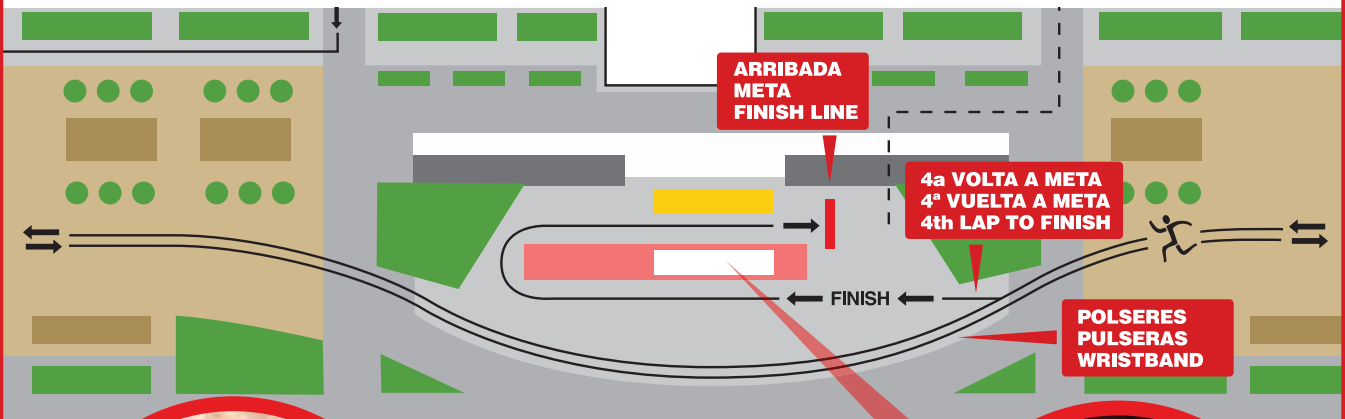
# CURSA A PEU. carrera a pie / run



- Circuit de 4 voltes de 10,5 km per la localitat de Salou.
- Avituallaments cada 2,5 km amb aigua (gots), Gatorade (gots), fruita (taronges i plàtans) i gels de glucosa.
- Al final de cada volta s'entregarà una polsera a cada corredor.
- Prohibit tirar residus fora dels espais dels avituallaments. Penalitzat amb 8 minuts en el temps final.

Circuito de 4 vueltas de 10,5 km por la localidad de Salou.  
 Avituallamientos cada 2,5 km con agua (vasos), Gatorade (vasos), fruta (naranjas y plátanos) y geles de glucosa Squeezy.  
 Al final de cada vuelta se entregará una pulsera a cada corredor.  
 Prohibido tirar residuos fuera de los espacios de los avituallamientos. Penalizado con 8 minutos en el tiempo final.

4 loops circuit of 10,5km each, through Salou streets.  
 Aid stations each 2,5km with water (glasses), Gatorade (glasses), fruits (oranges and bananas) and gels Squeezy.  
 At the end of each lap the participant will receive a wristband.  
 Forbidden to throw garbages out of the aid stations areas.  
 Sanctioned with 8 minutes added to final time.





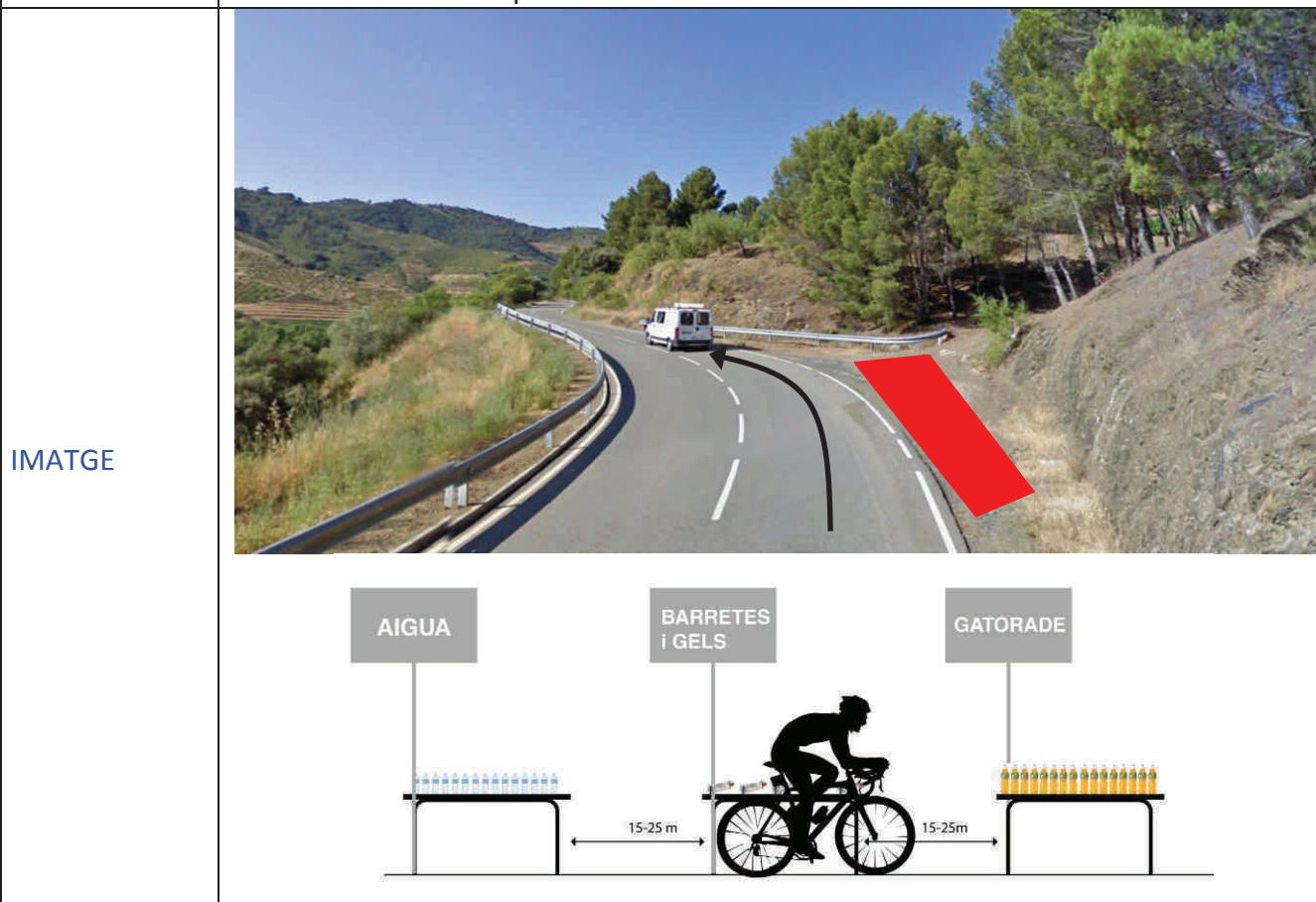






**AVITUALLAMENT 3 CICLISME**

<b>RESPONSABLE</b>	JORDI CATALÀ	<b>TELF. CONTACTE</b>	644.490.153
<b>ENTITAT</b>	VOLUNTARIS DE POBOLEDA	<b>VOLUNTARIS</b>	10
<b>UBICACIÓ</b>	Carretera T-702 (Poboleda) Estació depuradora d'aigües residuals i col·lectors Està a 300 metres del poble direcció Cabacés		



HORA	QUI	QUÈ
07:55	MUNTATGE	El camió arriba en el punt i descarrega tot el material de l'avituellament
07:50	RESPONSABLE	Arribada el punt de l'avituellament per part del responsable i els voluntaris
07:45 – 09:00	VOLUNTARIS	Col·locació de tot el material de l'avituellament: Una taula amb bidons d'aigua i el seu cartell indicatiu (omplir els bidons amb les garrafes) Una taula amb gels, barretes i el seu cartell indicatiu (posar les capses obertes a la taula) Una taula amb bidons de Gatorade i el seu cartell indicatiu (omplir els bidons)
09:10	CAP DE CURSA	Pas del primer ciclista per l'avituellament
09:10 – 12:00	VOLUNTARIS	Entregar els bidons i els aliments als ciclistes Agafar el producte per dalt amb suavitat i que el ciclista l'agafi per la part inferior Netejar el material que hi hagi a la carretera que pugui molestar als ciclistes. Reposar els productes (anar omplint els bidons d'aigua i gatorade)
12:00	ÚLTIM	Pas de l'últim ciclista pel punt d'avituellament
12:00 – 12:30	VOLUNTARIS	Recollir tot el material, deixar-lo tot junt i deixar l'espai net.
12:30	DESMUNTATGE	Pas del camió per emportar-se tot el material de l'avituellament

MATERIAL	BIDONS	AIGUA	GATORADE	BARRETES	GELS	WC	TAULES	PALS FUSTA	CARTELLS
<b>ARRIBAT</b>	400	140 L	140 L	200	250	1	4	3	4
<b>RESTANT</b>									

**AVITUALLAMENT 4 CICLISME**













## **ANNEX 5. REPORT EXAMPLES**

Report example of the laboratory tests

Race report example



Nom:

Data: Juny 2011

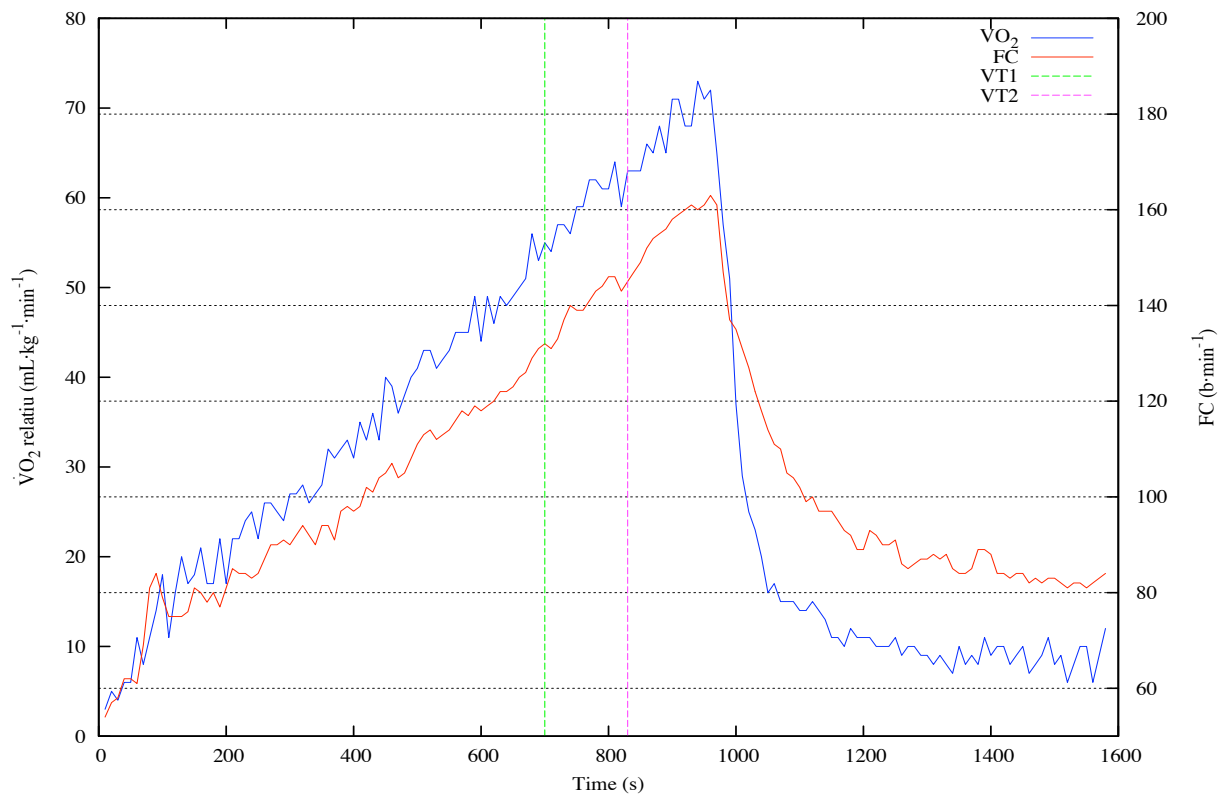
Edat: 34

Talla (cm): 168

Pes (kg): 67.2

Prova: CICLOERGÒMETRE

Valors de la prova d'esforç								
Indicador	REPÒS		Primer Llíndar (VT1) - Llíndar Anaeròbic -		Segon Llíndar (VT2) - Punt Compensació Respiratòria -		MÀXIM	
	bat·min <sup>-1</sup>	%	bat·min <sup>-1</sup>	%	bat·min <sup>-1</sup>	%	bat·min <sup>-1</sup>	%
Freqüència Cardíaca <b>FC</b>	54	33%	132	80%	145	88%	164	100%
Consum Oxigen Absolut <b>VO<sub>2</sub> abs</b>	mL·min <sup>-1</sup>	%	mL·min <sup>-1</sup>	%	mL·min <sup>-1</sup>	%	mL·min <sup>-1</sup>	%
	256	5%	3744	76%	4294	87%	4957	100%
Consum Oxigen Relatiu <b>VO<sub>2</sub> rel</b>	mL·kg <sup>-1</sup> ·min <sup>-1</sup>	%	mL·kg <sup>-1</sup> ·min <sup>-1</sup>	%	mL·kg <sup>-1</sup> ·min <sup>-1</sup>	%	mL·kg <sup>-1</sup> ·min <sup>-1</sup>	%
	3.8	5%	55.7	76%	63.9	87%	73.8	100%
Potència <b>P</b>	watts	%	watts	%	watts	%	watts	%
	0	0%	275	73%	325	87%	375	100%



Col·laboradors



Investigadors





Nom

Data: 5 juny de 2011

Edat

Talla

Pes

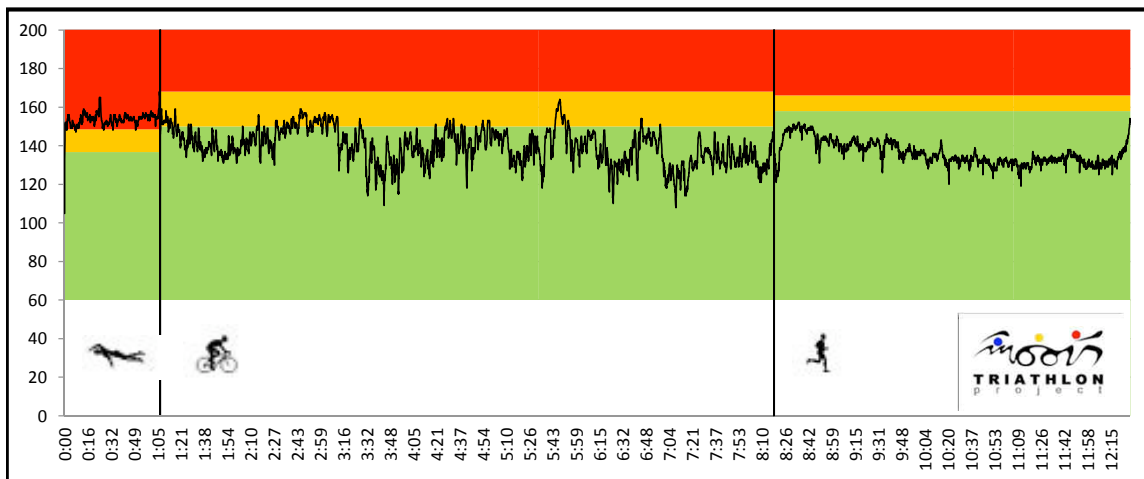
EXTREME MAN SALOU-COSTA DAURADA

### Valors de FC a les proves d'esforç

Disciplina	Primer Llíndar (VT1) - Llíndar Anaeròbic -		Segon Llíndar (VT2) - Punt Compensació Respiratòria -		MÀXIM	
	bat·min <sup>-1</sup>	%	bat·min <sup>-1</sup>	%	bat·min <sup>-1</sup>	%
Natació <b>SWIM</b>	137	80%	148	87%	171	100%
Ciclisme <b>BIKE</b>	150	84%	168	94%	179	100%
Cursa a peu <b>RUN</b>	158	90%	166	95%	175	100%

### Valors de la competició - EXTREME MAN

		Swim	Bike	Run	Totals
FC mitjana	bat·min <sup>-1</sup>	154	140	136	140
FC max	bat·min <sup>-1</sup>	168	164	154	168
FC min	bat·min <sup>-1</sup>	105	108	119	105
Temps	h:mm:ss	1:07:10	7:11:30	4:10:15	12:28:02
Distància	km	3.8	180	42.2	226



Col·laboradors



Investigadors









