FINAL DEGREE PROJECT

"What are the limiting factors during an Ultra-Marathon?: A systematic review of the scientific literature"

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INDEX

INTRODUCTION

There are lots of sports worldwide and one of the most popular one is running, in all of its modalities (from short distances – 60m sprints – to the quite unknown Ultra Marathons), with 17,114,800 individuals participating in the USA´s events (Running USA 2016) and 10.5 million runners participating in UK events (Sports Marketing Survey´s Inc. 2015). It is believed that the main attraction of the running events is their variety: distances (as mentioned above), road events, trail events, one-day races, multi-day events… It is not usual to achieve success in different modalities due to the differences in biomechanical, physiological and technical requirements of each distance and modality (Thompson 2017).

Although the differences on the mechanisms leading to success on each of the distances are well documented by Thompson (*Physiological and biomechanical mechanisms of distance specific human running performance*, M.A. Thompson 2017), basically the performance is dependent on the time required to cover a certain distance, which can be expressed as the average velocity over the event duration as well. Velocity is settled by the ratio of metabolic power (P_{met}) and energy cost of running (C) (di Prampero et al. 1986):

$$v = \frac{P_{met}}{C}$$

P_{met} is the energy production of both aerobic and anaerobic paths together over the whole duration of the event. The other part of the equation (C) depends on factors such as acceleration, terrain, wind speed and so on (apparently several biomechanical factors affect this part of the equation).

The purpose of this review is to understand what the limiting factors of the performance during Ultra-Marathons are (Ultra-Marathons and Mountain Ultra-Marathons as well). In order to clarify this issue, a systematic review of the scientific literature has been performed in the well-known PubMed database.

It is considered an Ultra-marathon when a foot race is performed involving longer distances than the classic marathon (42.195m) (Millet and Millet, 2012). In addition to the Ultra-marathons, there are some ultra-endurance races that are carried out in a mountainous environment, those are known as Mountain Ultra-Marathons (MUMs) (Vernillo et al. 2014, 2015). More precisely, the shortest ultra-marathon races are around 50 km (in which elite athletes have a time about 3h) while the longest or hardest races

can last up to several days (Thompson 2017). And not only the distance to cover is greater, it´s been told that the energetic fluctuation and demand (whether we are talking about Ultra-Marathons or MUMs) is extreme, really close to the human limits (Millet and Millet, 2012).

Table 1. Different distances in ultra-marathons and MUMs based on Thompson, 2017. The races presented here are only examples of different distances (adapted from www.runultra.co.uk). *MD = multi-day event

NAME OF THE RACE	DISTANCE COVERED
Gran Ridge Trail Runs	50km
Çekmeköy International ultra trail marathon	60km
Marathon du Mont-Blanc	80km
Wilson´s Promontory	100km
Patagonia Run	120km
Ultra Fiord (MD)	163km
Trail Menorca Camí de Cavalls	185km
Lake Balaton Supermarathon (MD)	194km
Great Naseby water race	200km
The Lost Island ultra (MD)	230km
La ultra – The High (MD)	333km

METHODS

Experimental Approach to the Problem

A literature search was conducted on March 31, 2018. The following database was searched: PubMed. The previously named database was searched from inception to March 2018, with language limitations: only peer reviewed articles in English were selected. Citations from scientific conferences were excluded.

Literature Search

In the database the title and abstract search fields were searched. The following MeSH terms and key words, combined with the Boolean operators (AND, OR), were used: (((((("Running"[Mesh]) AND ultra-marathon) OR ultra marathon)) AND "Athletic

Performance"[Mesh]) AND "Humans"[Mesh]. No additional filters or search limitations were used.

Inclusion Criteria

Studies were eligible for further analysis if the following inclusion criteria were met; a) studies were written in English; b) studies analysed ultra-marathon distances from 42.2km to 101km. If any study analysed more than one distance, it was included in the review as long as at least one of the distances analysed fell into the range mentioned above.

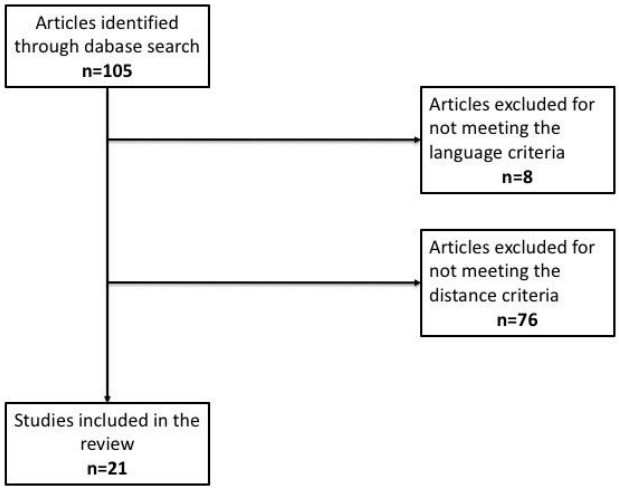

Figure 1 Flow chart

Quality assessment

Oxford's level of evidence[12] and the Physiotherapy Evidence Database (PEDro) scale[13] were used by two independent observers in order to assess the methodological quality of the articles included in the review. Oxford's level of evidence ranges from 1a to 5, with 1a being systematic reviews of high-quality randomised controlled trials (RCT) and 5 being expert opinions. The PEDro scale consists of 11 different items related to scientific rigor. The items include random allocation; concealment of allocation; comparability of groups at baseline; blinding of subjects, researchers and assessors; analysis by intention to treat; and adequacy of follow-up. Items 2-11 can be rated with 0 or 1, so the highest rate in the PEDro scale is 10, and the lowest, 0. Zero points are awarded to a study that fails to satisfy any of the included items, and 10 points to a study that satisfies all the included items.

Table 2 Physiotherapy Evidence Database (PEDro) ratings and Oxford evidence levels of the included studies.

	PEDro ratings*											
Study	1	2	3	4	5	6	7	8	9	10	11	**Total**	**Evidence level**
Sansoni et al.	yes	0	0	0	0	0	0	0	0	1	1	2	2b
Bonsigniore et al.	yes	0	0	1	0	0	0	1	1	1	1	5	2b
Chan-Dewar et al.	yes	0	0	1	0	0	0	1	1	1	1	5	2b
Da Ponte et al.	yes	0	0	0	0	0	0	1	1	0	1	3	2b
Martinez et al.	yes	0	0	0	0	0	0	1	1	0	1	3	2b
Lazzer et al.	yes	0	0	0	0	0	0	1	1	0	1	3	2b
McKune et al.	yes	0	0	0	0	0	0	0	1	0	1	2	2b
Knechtle et al. (2010)	yes	0	0	0	0	0	0	1	1	0	1	3	2b
Knechtle et al. (2011)	yes	0	0	0	0	0	0	0	1	1	1	3	2b
Leonard	yes	0	0	1	0	0	0	1	1	1	1	5	2b
Noakes et al.	yes	0	0	0	0	0	0	1	1	1	1	4	2b
Balducci et al.	yes	0	0	0	0	0	0	1	1	1	1	4	2b
Rehrer et al.	yes	0	0	0	0	0	0	1	1	1	1	4	2b
Brow et al.	yes	0	0	1	0	0	0	1	1	1	1	5	2b
Millet et al.	no	0	0	0	0	0	0	0	0	0	0	0	5
Cona et al.	yes	0	0	0	0	0	0	1	1	1	1	4	2b
Guillaume Y. Millet	no	0	0	0	0	0	0	0	0	0	0	0	5
Murray et al	no	0	0	0	0	0	0	0	0	0	0	0	5
Guillaume Y. Millet	no	0	0	0	0	0	0	0	0	0	0	0	5
Schwellnus et al.	yes	0	0	1	0	0	0	1	1	1	1	5	2b
Ker et al.	yes	0	0	0	0	0	0	1	1	1	1	4	2b

Items in the PEDro scale: 1 = eligibility criteria were specified; 2 = subjects were randomly allocated to groups; 3 = allocation was concealed; 4 = the groups were similar at baseline regarding the most important prognostic indicators; 5 = blinding of all subjects; 6 = blinding of all therapists who administered the therapy; 7 = blinding of all assessors who measured at least 1 key outcome; 8 = measures of 1 key outcome were obtained from 85% of subjects initially allocated to groups; 9 = all subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least 1 key outcome were analyzed by "intention to treat"; 10 = the results of between-group statistical comparisons are reported for at least 1 key outcome; 11 = the study provides both point measures and measures of variability for at least 1 key outcome

RESULTS

Studies Selected

The search strategy yielded 105 total citations as presented in Figure 1. Reviewing those 105 articles, 22 met the inclusion criteria. Excluded studies had at least one of the following characteristics: the article was not written in English, the distance covered in the race (no matter whether it was single-day or multi-day event) was higher than 101 km. The overall sample included 17 cohort studies and 4 experts opinion (Table 2).

Level of Evidence and Quality of the Studies

Seventeen of the 21 included studies had a level of evidence 2b (individual cohort study). The 4 remaining studies has a level of evidence of 5 (expert opinion). Also, mean score in the PEDro scale was 3.05 ± 1.78, with values ranging from 0 to 5 (Table 2).

Characteristics of the Participants

Participants were characterised as experienced or well-trained athletes in all of the studies due to the training volume they had until the day of the race. A summary of participants´ characteristics is presented in Table 3. The total number of participants was 959 (860 men, 76 women and 23 unknown).

Table 3 Included studies

Study	Participants			
	Number (M/F)	Age	Level	Main Outcome
Sansoni et al.	20 (20/0)	38.8 ± 7.2	Experienced	Bone turnover
Bonsigniore et al.	21 (15/6)	39.8 ± 8.3	Well-trained	Arterial compliance
Murray et al.	-	-	-	-
Guillaume Y. Millet	-	-	-	Neuromuscular fatigue
Chan-Dewar et al.	19 (16/3)	41 ± 9	Well-trained	Electro-mechanical delay
Da Ponte et al.	22 (22/0)	46.1 ± 10.8	Well-trained	Cardiac and muscle biomarkers
Martinez et al. (trail)	109 (98/11)	35 ± 8.4	Well-trained	Macronutrient and water intake
Martinez et al. (marathon)	53 (41/12)	36.6 ± 8.0	Well-trained	Macronutrient and water intake
Lazzer et al.	11 (11/0)	40.5 ± 8.4	Experienced	Metabolic cost of transport
McKune et al.	25	43 ± 10	Experienced	Immunoglobulin types
Cona et al.	30 (30/0)	43 ± 8.6	Well-trained	Cognitive function
Ker et al.	10 (8/2)	38.2 ± 7.9	Well-trained	Respiratory muscle fatigue
Knechtle et al.	95 (95/0)	44.5 ± 10.0	Well-trained	Hyponatremia
Leonard W.	11 (8/3)	43.7 ± 8.6	Well-trained	Plasma B-6 vitamin
Noakes et al.	23 (Unkown)	28 ± 1 / 25 ± 4	Experienced / Novice	Serum Biochemical parameters
Balducci et al.	26 (26/0)	41.7 ± 9.5	Well-trained	Performance factors
Rehrer et al.	170 (158/12)	40 (M) / 35 (F)	Well-trained	Gastro-intestinal disturbance
Schwellnus et al. (EAMC group)	20 (19/1)	40.8 ± 11.7	Well-trained	Muscle cramps
Schwellnus et al. (CON group)	29 (23/6)	40.2 ± 9.2	Well-trained	Muscle cramps
Shave et al. (Study 1)	11 (11/0)	42 ± 11	Well-trained	Cardiac disfunction and cTnT
Shave et al. (Study 2)	26 (26/0)	41 ± 10	Well-trained	Cardiac disfunction and cTnT
Brown et al. (TT)	21 (16/5)	38.5 ± 9.6	Well-trained	COL5A1
Brown et al. (TC)	32 (25/7)	42.5 ± 9.3	Well-trained	COL5A1
Brown et al. (CC)	18 (10/8)	40.6 ± 9.2	Well-trained	COL5A1
Millet et al.	-	-	-	-
Knechtle et al.	157 (157/0)	45.7 ± 9.6	Experienced	Hyponatremia

Evidence talking about physiological limitations

Physiological factors that are commonly associated with endurance running ability include (but are not limited to) muscle capillary density, maximal heart rate, anthropometry, substrate utilization and aerobic enzzyme activity (Joyner et al., 2008;). In this review we are going to analyse some of the just mentioned and some more.

Sansoni et al. mentioned how one of the keys of endurance sports is the capacity of the aerobic exercise to mimic the effects of the insulin increasing glucose uptake into skeletal muscles and liver via insulin-independent membrane translocation of glucose transporter (GLUT)-4. Elevate glucose levels always return to their normal values no matter what, and this control prevents us from severe dysfunctions (i.e. loss of consciousness due to hypoglycaemia). The main cellular mechanism for the dispatch of an exogenous glucose load is insulin-stimulated glucose transport into skeletal muscle. It is well known that skeletal muscle stores glucose as glycogen and oxidizes it to produce energy as well (Huang et al., 2007). There are 13 sugar transporter proteins (Joost and Thorens, 2001; Wood and Trayhurn, 2003) and the most significant one is GLUT4 (Gene name, SLC2A4; protein name: GLUT4), which contains 12-transmembrane domains (Figure 2).

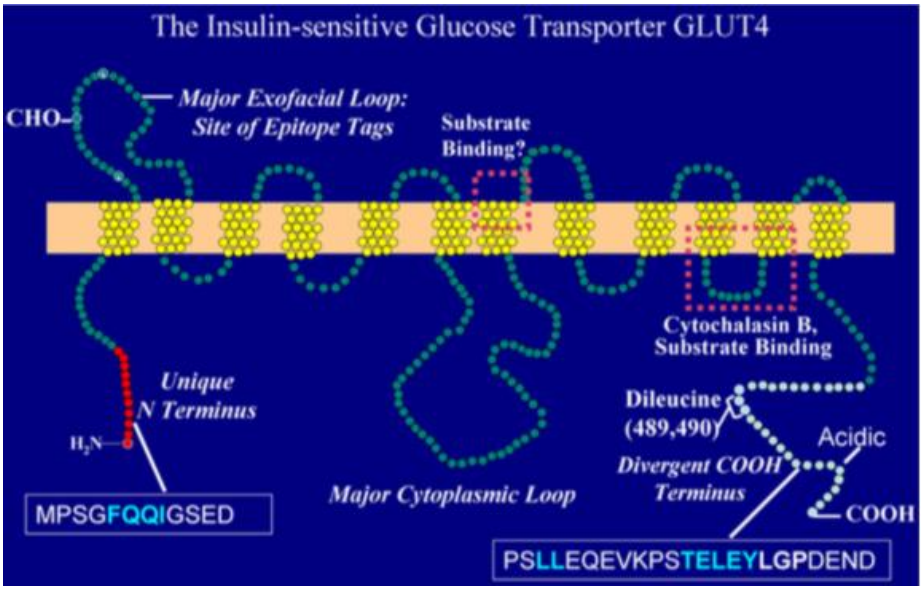

Figure 2 Structural Features of the Insulin-Regulated GLUT4 Glucose Transporter Protein (Huang et al., 2007)

Although there are more effects of the endurance exercise and, in general, of the physical activity in the human body, Sansoni et al. focused on the bone mass and metabolism, because physical activity (strength training and activity that supposes repeated impacts specially, due to the mechanical strain) is one of the determinants of those parameters. Consequently, runners have a higher bone mass than cyclists, for example, because those repeated impacts favour bone anabolism (Lombardi et al., 2012(a), 2012(b)).

Bones act in energy metabolism via osteocalcin (OC), a hormone that works on pancreatic β-cells and adipocytes (Otani et al., 2015). OC is a type of vitamin K-dependent protein synthetized by osteoblasts, and its function is to bind hydroxyapatite[1] and to regulate bone mineralization. OC is presented in two forms: its carboxylated form (Gla-OC) or the undercarboxylated form (Glu-OC), and whether it is carboxylated or not it regulates glucose metabolism (Lombardi et al. 2015).

Sansoni et al., in their study, measured Glu-OC, Gla-OC and PINP (procollagen type I N-terminal propeptide[2]) through blood samples that were collected in the morning, the day before the race (both in experimental and control groups) and only in the experimental group after the MUM. Osteoblast anabolic activity, revealed as PINP serum concentration, was 50% greater in the experimental group at rest compared to control group (p=0.001).

Otherwise, no differences were found in Gla-OC but, the undercarboxylated form of the osteocalcin (p=0.001), and consequently the Glu-OC-to-Gla-OC ratio (p=0.001), were significantly lower in the experimental group than in control group. Changes in Glu-OC were directly related to modifications in adiponectin and inversely related to leptin and energy expenditure (Lombardi et al., 2012). When they compared pre- and post-race values, they saw a 15% decrease in PINP (p=0.020) even though it remained higher than in control group (p=0.017). On the other hand, Gla-OC was unchanged while Glu-OC was 50% lower after the MUM (p=0.020) and compared to the control group, its serum

[1] **Hydroxyapatite:** A group of compounds with the general formula $M_{10}(PO_4)_6(OH)_2$, where M is barium, strontium, or calcium. The compounds are the principal mineral in phosphorite deposits, biological tissue, human bones, and teeth.[Hydroxyapatites. (1975). Retrieved from https://www.ncbi.nlm.nih.gov/mesh/68006882]

[2] **Procollagen type I N-terminal propeptide:** As we know bones are dynamically and metabolically active organs, they are constantly subjected to two processes: resorption and formation (both togehter called bone turnover) (Kučukalić-Selimović et al., 2013). Each of those two processes has their own biomarkers and PINP is the standard bone formation marker (Lee et al., 2012).

concentrations were reduced by 2/3 (p=0.017). Glu-OC/Gla-OC ratio followed the same trend as Glu-OC. Speaking about other metabolic changes after the MUM, Sansoni et al. saw that C-peptide and insulin were lower after the race although only C-peptide reached significance (p=0.005). Glucagon and GLP-1 increased significantly (p = 0.002 and p = 0.005, respectively) in the experimental group after the race.

Bonsigniore et al. analysed the influence of race length on arterial compliance. They had shown before how the vascular function had changed after an ultra-marathon due to the chronic exposure to prolonged endurance exercise with an increase in resting arterial stiffness when it was compared to age-matched recreational athletes (Burr, Dury et al., 2014). Not only that, they also demonstrated changes in vascular function (arterial stiffness) from pre- to post-race (Burr et al., 2012). In the study that is mentioned for this review, they compared the changes in arterial compliance as well as body composition, and no changes on any parameter between subjects of the same category (80km race –this data was analysed for those review– and 195km race –this data was excluded due to the unfulfilment of the inclusion criteria–). When analysing data from pre- to post-race there are some meaningful differences though: Diastolic blood pressure (mmHg) and large artery (LA) compliance (mmHg) changed from 75.8 ± 9.6 to 74.2 ± 11.1 (p= 0.008) and from 16.1 ± 3.0 to 17.4 ± 4.0 (p=0.02) respectively.

Other factor to take into account is the electro-mechanical delay (EMD) produced by the acute exhausting exercise (Horita et al., 1987; Zhou 1996). Using the Tissue-Doppler imaging (TDI) technique there is the possibility to asses global and segmental myocardial tissue as well as the EMD from electrical signal (onset of the QRS) to peak of systole (S´) or early diastole (E´) wall motion. Reduced peak tissue velocity has been appreciated after prolonged exercise sessions (George et al.2005; Neilan et al., 2006a; La Gerche et al., 2008). Even though there were some measurements performed by other authors (Yu et al., 2003; Ng et al., 2008) to assess both intra-ventricular and inter-ventricular synchrony in healthy subjects and subjects with heart failure, there were not any studies assessing synchrony changes within or between ventricles in such long distances until Chan-Dewar et al. (2009) carried out this study. They made 16 male ultra-marathon runners undergo echocardiographic scans up to 24 h prior to the race (The Comrades Marathon, 89 km) and within 60 min time after the race. After the race they

observed a small decrease in body mass but more important, the observed a drop in systolic blood pressure (117 ± 11 to 105 ± 6 mmHg; $p < 0.05$)[3].

Extended exercise (i.e. marathon running) done at moderate intensity leads to an increase in muscular and cardiac biomarkers (Frassl et al., 2008; Mohlenkamp et al., 2014). Among the most interesting biomarkers to focus on, Creatine kinase (CK) and myoglobin are two of the main markers used to measure cell damage. Talking about cardiac damage the most used biochemical markers are CK (MB cardiac isoform) and the cardiac troponin (cTn) (Shave et al., 2010). cTn levels are elevated after a marathon the transitory inflammation is one of the factors that helps to it (Saravia et al., 2010). Shave et al. (2002) carried out a study to examine cardiac function during an extended exercise bout (2 day Lowe Alpine Mountain Marathon) and they found out that left ventricular systolic function (stroke volume, ejection fraction and fractional shortening) was significantly ($p<0.05$) reduced after the completion of the event. Left ventricular diastolic function was also significantly decreased as shown by the reduced early filling velocity ($p<0.05$) and so, the decrease in the E:A ratio ($p<0.05$). All subjects were below the upper reference level for total CK and cTnT prior to the event and all but one (4%) were below the upper reference limit for CK-MB_{mass} . 96% of the subjects were above the upper reference limit for total CK and 85% were above the upper reference limit for CK-MB_{mass} . Da Ponte et al. conducted a study to evaluate changes in cardiac troponin l levels (cTnl) and the main biomarkers of skeletal muscle damage after the "Supermaratona dell´Etna" (43km, 0 – 2850m). They found that Aspartate aminotransferase (AST)[4], Lactate dehydrogenase (LDH) and CK values increased were significantly higher after the race (42.0%, 37.4% and 191.5%, respectively; $p < 0.001$). LDH and CK increased above normal values (+8% and +71% respectively, $p < 0.001$), AST remained between normal values though. Not only that, cTnl also increased significantly by +900.0% ($p < 0.001$) with a high inter-individual variability after the race. Mb, creatinine and cortisol followed the same tendency and increased significantly (999.1&, +30.5% and +291.4%; $p < 0.001$, $p < 0.001$, $p < 0.001$ respectively) after the race.

[3] **The times presented here are measurements taken based on R-R interval timing.**
[4] **Aspartate aminotransferase:** a transaminase enzyme that catalyzes the conversion of aspartate and alpha-kletogutarate to oxalacetate and glutamate. (Aspartate aminotransferase https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/aspartate-transaminase)

Martinez et al. (2017), analysed the effects of distance on energy, macronutrient and water intake during the race. For that porpoise, they performed an observational study during the "2015 Ultra Mallorca Serra de Tramuntana" in Mallorca. They evaluated the total kcal (as well as the $kcal \cdot h^{-1}$, $kcal \cdot kg^{-1 \cdot w}h^{-1}$), the carbohydrate intake (% total energy), protein intake (% total energy) and lipids intake (% total energy) and the found out that no differences were found in energy intake expressed per hour of exercise but there was a significant difference for the percentage of energy coming from lipids (with higher values for those participating in longer distances; $p = 0.034$). Also, a significant difference was appreciated in water intake (not in sodium intake though) expressed per hour of competition as expected.

When taking any drink or food competitors must take into account gastro-intestinal (GI) disturbances though. There are some several factors affecting this, for example dehydration at levels of 4-5% loss of body mass (Neufer et al. 1989). Rehrer et al. (1991) made a study to investigate the prevalence of GI dysfunction and to find out the correlation with dehydration and physiological stress using the data of the participants of The Swiss Alpine Marathon (67 km). Men´s body mass loss was of 2.4 ± 0.1 kg and women´s 2.3 ± 0.2 kg (3.3% and 4.0% of body mass respectively) and fluid intake was of 3335 ± 107 ml and 2746 ± 338 ml respectively (most commonly consumed fluid was water). GI distress was reported by 42% of men and 57% of women.

As mentioned above, performance depends on time required to cover a certain distance, and there are three physiological factors that are going to determine that time widely: (1) the value of the maximal oxygen uptake (VO_{2max} ml $O_2 \cdot kg^{-1} \cdot min^{-1}$), (2) the fraction we use of that VO_{2max} (F, %) and (3) the value of metabolic cost of transport (CoT, ml $O_2 \cdot kg^{-1} \cdot m^{-1}$) (di Prampero et al., 1986):

$$v_{end} = F \cdot VO_{2max} \cdot CoT^{-1}$$

The most common mistake that some trainers commit is focusing on the improvement of the VO_{2max} exclusively, this is based on the correlations found between VO_{2max} and running performance in heterogeneous-level runners (Billat et al., 2003; Maughan et al., 1983). But not only that determines the performance of an athlete, if that was so, two athletes with the same VO_{2max} always would have the same performance. Several studies showed that, in elite distance runners (so it is supposed that they have very similar VO_{2max} values), F, which is associated to adaptations coming from prolonged training (Holloszy et al.,1984) is a crucial factor to determine performance. Going back to the supposition that two athletes have the same VO_{2max}values, the one with higher F

value will be victorious over the other one, due to his improved ability to use the oxygen he inhales. Lastly, we have CoT, usually expressed as the amount of energy spent to transport 1 kg of body mass (M_b) over a distance of 1m. Strength (Støren et al., 2008) and plyometric (Spurrs et al., 2003) training allows muscles and tendons to utilize more elastic energy and to reduce the amount of energy wasted in braking forces. This is related to one of the big questions of the moment: muscle and tendons stiffness and stretching before the race. Schwellnus and Collins (2011) investigated the presence of one of a specific alleles of the gene COL5A1 in endurance runners, a gene related to inflexibility, and they discovered that those runners with the aforementioned gene allele were considerably more economic than those without that gene (Baxter et al., 2016). Talking about stretching (acute stretching) and performance, the main problem is that the majority of studies are performed measuring explosive efforts as bench press, leg press or sprints, not endurance exercise. The most economical runners showed a higher triceps-surae tendon stiffness (k_{tendon}) compared to the less economical runners. Brown et al. (2011) performed a study to know the rol of the COL5A1 gene genotypes during a 56 km ultra-marathon. Range of motion (ROM) is related to running economy (Gleim et al., 1990; Craib et al., 1996), that is a measurable marker of ultra-endurance performance (Laursen et al., 2001). In elite athletes the sit-and-reach ROM test (SR ROM) has a negative relation with running economy (Jones AM, 2002). The CC genotype of the COL5A1 was significantly associated with increased SR ROM (Brown et al., 2011) and, on the other hand, Posthumus et al. (2011) reported that the TT genotype of the COL5A1 was associated with improved endurance running ability in the 42.2 km run of the 226 South African Ironman triathlon due to the changes in musculotendinous stiffness. Time to complete the race had a tendency to be different (p=0.053) among genotypes. Individuals with TT genotype (341 ± 41, n=21) were significantly (p=0.014) faster that individuals with TC or CC genotype (365 ± 39, n=50) (Figure 3).

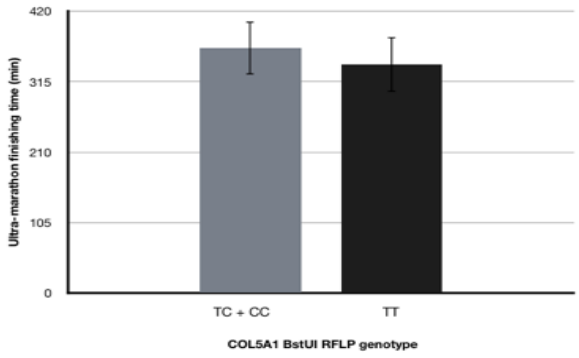

Figure 3 COL5A1 genotype and finishing time. (Adapted from Brown et a. (2011)).

The magnitude of the change in performance between those with TT genotype and those with either a TC or CC genotype was "moderate" (effect size = 0.61). Brown et al. (2011) tried to elucidate what factors contributed to variance in race finishing and the best model they could do consisted on: age, weight and COL5A1 genotype (p<0.001, SEE = 32.76), which accounted for 35%. Of those, only weight (p=0.018) and gneotype (p=0.038) contributed significantly to the race time model. Also, they defined some quadrants using values of SR ROM and time to complete the race. Participants with more ROM than the median were termed *flexible* and those with less *inflexible*. Similarly, those faster than the median were named *fast* and the slower than the median *slow*. So, they were divided into four quadrants:

- *Inflexible-fast:* T alelle 69% ; C alelle 31%
- *Inflexible-slow:* T alelle 50% ; C alelle 50%
- *Flexible-fast:* T alelle 53% ; C alelle 47%
- *Flexible-slow:* T alelle 36% ; C alelle 64%

After the quadrants were made and after each participant of the study was assigned to one quadrant depending on his/her genotype, Brown et al. (2011) found that T alelle was significantly over-represented in participants within *inflexible-fast* group.

Lazzer et al. (2013) carried out a study to investigate the role of VO_{2max}, F and CoT in determining the performance of runners who participated in the "Magraid" race, a 93 km multi-day race. At the same time, they evaluated the relationship between CoT, k_{tenon} and the morphological properties of the gastrocnemius medialis (GM). The results they got are the following: they did a multiple regression between VO_{2max}, CoT, F, and $v_{end,mean}$ (r = 0.77, 0.36 and -0.30 respectively). Analysing the CoT more closely, some relationships with the CoT were revealed, such as (1) P_{max} (r=-0.74, p<0.001) and (2) the vertical stiffness (k_{vert}, r=-0.65, p<0.05). Additionally, direct relationships between CoT and footprint index (FPI, r=0.70, p<0.05), step frequency (f, r=0.62, p<0.05) and external work per unit of distance (W_{ext}, r=0.60, p<0.05). FPI increased at the end of each stage significantly (11.9 ± 9.1, 31.6 ± 24.6 and 22.2 ± 21.2%, respectively, p<0.001) and GRF decreased at the end of the first and second stage (-4.0 ± 4.6 and -3.8 ± 4.9%, respectively, p<0.05).

Following the sutdies of Di Prampero and Lazzer, Balducci analysed the Interlacs Trail (75 km long, positive elevation = + 3930m; negative elevation = -3700m) in order to clarify some of the performance factors of ultra-marathons. In this study he observed 26 athletes, of those 24 finished the race, and for that he performed some tests such as an

incremental test 1 week before the MUM and one day prior and immediately after the MUM CoT at 0 and 10% slope, kinematics, stiffness, knee extensors (KE) force and jump height were assessed. The results in this study showed that MUM performance in a heterogeneous group is correlated mainly with: Maximal aerobic speed (MAS), fraction of MAS (F_{MAS}) sustained, knee extensors force and force loss.

Intense bouts of long exercise increase the risk of upper respiratory tract infection (URTI) (Nieman et al., 1990; Peters et al., 1983). This increased risk, so, suggests that a suppression or immunity alteration happens due to that bout of exercise. Humoral arm of adaptive immunity is associated with circulating antibodies/immunoglobulins (Roitt et al., 2001). T cells and antigens activate naive B cells, that later on differentiate into short lived plasma cells, long lived plasma cells or memory B cells (Crotty et al., 2004). The first response to a threat are the short-lived B cells, that provide a fast and specific response. This involves an early rise in antibodies of the antigen specific IgM isotope, followed by affinity maturation, isotope switching and a rise in antigen specific IgG, IgA and/or IgE antibodies (Boes et al., 2000). The aim of the study conducted by McKune et al. (2005) was to determine alterations in serum concentrations of immunoglobulin isotopes and subclasses after a 90 km ultra-marathon. They found that all serum immunoglobulin concentrations before and after the race were among clinical normal ranges, individually speaking IgD concentration decreased immediately after the race (-51%, p=0.04) and 24 hours later (-41%, p=0.04) but differences three hours after did not present significant changes (p=0.15). 72 hours after concentrations had returned to baseline. IgM, 24 hours after the race a significant decrease (-23%, p=0.04) was appreciated. Talking about IgG, immediately after the ace was increased (+12%, p=0.05) ad 24 and 72 hours later concentrations returned to baseline. IgG measurements after exercise is not clear yet, Poortmans (1971) found an increase of 12% in serum IgG after a progressive cycle ergometer test, Nieman et al. (1991) found a decrease (-7.6% in the lowest point) and Poortmans et al. (1979) reported a significant 7% increase in IgG after a 100 km ultra-marathon. Now focusing on training induced changes, decreases in IgG have been seen. Mashiko et al. (2004) found a significant decrease of an 8% after 20 days of rugby training six times a week. Finally, in IgA there were some changes, but they were not significant.

Exercise-associated hyponatraemia (EAH) is another factor to take care of, it is defined as serum [Na^+] due to excessive water intake. Some risk factors are known, such as, weight gain, exercise duration of more than 4h, a slow exercise pace, gender (females

more), low body mass, >1.5 L/h drinking while racing, pre-exercise hyperhydration, abundant availability of drinking fluids at the event, nonsteroidal anti-inflammatory drugs and extreme environments (excessive hot or cold) (Hew-Butler et al., 2005; 2008; Irving et al., 1991; Noakes et al., 2005; Rosner, 2009; Rosner et al., 2007). The aim of the investigation carried out by Knechtle et al. (2010) was to investigate the prevalence of EAH in 145 male ultra-runners (157 originally, 17 drop outs) during the "100 km run" in Biel, Berne, Switzerland. Based on the classification made by Noakes et al. (2005) for the serum [Na^+] concentration, two of the finishers in the study of Knechtle et al. (2010) were classified as hypernatremic (1.4%), seven as hyponatremic (4.8%) and the remaining 136 as normonatremic (93.8%). Surprisingly, hyponatremic subjects did not drink more than non-hyponatremic subjects.

Following with the plasma biochemical responses, Noakes et al. (1982) analysed changes in biochemical parameters after a 56 km race in novice (n=5) and experienced (n=18) runners. The major finding was the difference between groups in plasma CK and AST, the novice, or non-experienced, runners´ rise of CK and AST were significantly greater. The only parameter significantly higher in experienced runners than in novice ones, was serum calcium.

During exercise (and especially long bouts of exercise) both muscle and liver glycogen are catabolized to glucose, and certain aminoamides are deaminated to be able to go through the gluconeogenesis. Both glycogenolysis and gluconeogenesis from amino acid deamination require vitamin B-6, as pyridoxal 5´-phosphate (Leklem, 1985). Leonard (1999) analysed plasma B-6 vitaminic changes following a 50 km ultra-marathon and for that, he provided the same breakfast to all the athletes involved in the study (around 500 kcal and 0.47 mg of B-6 vitamin). Some food was available during the race, and that food was chosen because of their B-6 vitamin content. The main result of the study was the decrease in PLP concentration: 41.1 ± 14.2^{AB} (Pre-race), 28.2 ± 10.8^{A} (Post race) and 23.2 ± 9.9^{B} (Post 1 h) (A is significant to A and B to B, $p<0.001$).

During such a long race, exercise-associated muscle cramps (EAMC) are one of the most common medical problems (Schwellnus MP, 2007). EAMC can be defined as syndrome of involuntary painful skeletal muscle spasms that occur during or immediately after physical exercise (Schwellnus et al, 1997; Schwellnus MP, 1999) and clinically presents as painful localised muscle cramping that occurs spasmodically in different exercising muscle groups (Schwellnus MP, 1999; Schwellnus MP, 2009). Scwellnus et al. (2011) analysed 49 runners participating in a 56 km race to identify the risk factors

associated to EAMC in ultra-runners. They found that those runners within the EAMC group ran the first half of the race significantly faster (144.3 ± 20.2 min) than those in CON group (157 ± 14.3 min; p=0.029).

Evidence talking about cognitive limitations

Cona et al. (2015) explored cognitive functioning using two modified versions of two computerized tasks: The Inhibitory Control Task (ICT; Black letter were shown for 500 ms without inter-stimulus interval, in the center of a white background computer screen. Interspersed within the other letters, the target letters X and Y were presented and during the first sessions the participants were instructed to respond by pressing the spacebar for every X and Y. During the second session, participants were instructed to press the spacebar only when X and Y were alternating -go trials- and to inhibit their response when X and Y were repeated -nogo trials-) and a dual-task paradigm with emotional stimuli. To better explore the impact of executive functions on running, they performed a dual-task paradigm, in which two distinct tasks, heavily dependent on frontal executive processes, needed to be executed at the same time. It consisted of an ongoing activity, a working memory 2-back task (participants were told to decide whether the picture on the screen was same or different from the picture presented two trials before), and a Prospective Memory (PM) task (they had to press a key when a pre-memorized picture, namely PM cue, occurred on the screen amid the ongoing trials) (Cona et al., 2014). In the ICT test, post hoc comparisons revealed that faster runners outperformed slower runners selectively in the nogo trials (p<0.001), whereas did not differ from slower runners in the detect and go trials (p>0.05). On the other hand, in the dual-task paradigm post hoc comparisons showed that, as compared with faster runners, slower ones tended to have increased reaction times in ongoing trials when they had to monitor for pleasant and unpleasant PM cues (both p=0.05).

Evidence talking about neuromuscular and muscular limitations

Not only peripheral muscles´ fatigue matters, prolonged increased demands on the respiratory system, could lead to fatigue too (Macklem P.T., 1980). Ker et al. (1996) hypothesized that increased demand on the respiratory muscles during an ultra-marathon could lead to prolonged fatigue. For that reason, they recruited 10 athletes and performed some tests before and after an 87 km ultra-marathon. They measured the maximum

inspiratory pressure (MIP) and values were compared to age matched normal individuals, and no significant difference was found. The main result of the study was that no significant result was found in MIP measured 3 days after the race between those athletes that ran the race and age matched individuals. Hill et al. (Hill et al., 1991) demonstrated a 26% reduction in MIP immediately after a triathlon. They also measured the endurance time of each subject, in which athletes had to inspire to a give target pressure (Pi, the 75% of the MIP) and hold on there for 5 seconds (Ti). In this case, they found a significant (p<0.002) difference between pre-race and post-race endurance times (T-limit), demonstrating that inspiratory muscles do actually fatigue with intensive exercise.

DISCUSSION

As it can easily be seen, the great majority of studies carried out are about physiological limitations. The most important findings in this review are those made by Lazzer et al. where he found that (1) high level performance in long-distance running depends on high VO_{2max}, high F and low CoT_{mean}; (2) low CoT values before the race are related to high P_{max} and k_{vert}, and low FPI, f and W_{ext}; and finally, (3) around 50% of the increase in CoT during the stages of the competition is related to changes in FPI, making it the most important factor in order to improve the CoT. A low FPI means that the pressure of the centre of the foot (foot centre of pressure, CoP) remains as close as possible from the foot axis, suggesting a better ankle stability (Huang et al., 2011; Willems et al., 2005), what means better energy absortion through the foot axis (Ker et al., 1987). They also found that low W_{ext} and low f were directly related to low CoT. On average, Lazzer et al. (2013) concluded that two biomechanical parameters could significantly change CoT at the end of each stage they analysed: FPI and maximal GRF. These results are in line with Morin et al. (2011). Balducci continued with this matter in his study, in which he concluded that in this case CoT was not correlated with performance, and this finding does not support other findings such as those made by Vernillo et al. (2015). On the other side, MAS was strongly correlated to performance in the study carried out by Balducci et al. (2017). This is not surprising considering that running performance during long distances rely on several factors, such as VO_2max and F_{MAS}. This last one, was highly related to performance, the higher the fraction, the better the performance (r = -0.89, p<0.001). Stiffness and jump height on CMJ were not related

to MUM performance. KE force capability and fatigability appear to be determining factors assessing MUM performance because during uphill running there are lots of concentric and eccentric contractions (Yokozawa et al., 2007).

Not less important are those findings made by Bonsigniore et al. (2016). The novel finding of this investigation was that the race length actually influences acute changes in resting arterial compliance (Bonsigniore et al., 2016). It is well known that beneficial changes in vascular function happen due to chronic aerobic exercise, for example, Kingwell et al. (1997) demonstrated that an acute session of moderate intensity aerobic exercise leads to improvements in arterial compliance, even though one hour after the stimuli the values came back to baseline. Other authors (Burr et al., 2014; Nickel et al., 2011; Wilson et al., 2015) have backed up these findings. The specific mediators responsible for those changes in vascular function have not been identified yet, but it is thought that these changes are vessel specific and are mediated by more than one mechanism (neuro-hormonal, metabolic and endothelial changes) (Gielen et al., 2010). This study highlights the finding about large, but not small, resting arterial function. Small vessels tend to be more sensitive to changes in oxygen perfusion and metabolic sensors, while large vessels are more susceptible to heightened oxidative stress and inflammation (Dierich et al., 2000; Kroll et al., 1997). Long endurance events lead to increases in pro-inflammatory markers and changes in endothelial function because of the increased blood flow and shear stress in order to satisfy the increased oxygen demand of the heart and skeletal muscles. Besides, that shear stress leads to induction of the PI3K[5] and ERK pathways, which induces endothelial nitric oxide synthase (eNOS) expression and nitric oxide (NO) production, what ends up with arterial dilation (Dimmeler et al., 1999). Lastly, they hypothesized that changes in pressure of the race because of the changes in elevation (2300m in the 80km race) and fluctuations in temperature, helped too with the changes in arterial compliance. Continuing the investigations of cardiovascular function and adaptations due to this kind of races, Horita et al. after their study concluded that one of the main findings was that they perceived a longer time from onset of QRS to peak S´ and E´ after the race, with an increase on EMD (Chan-Dewar et al., 2009). As Horita and

[5] **PI3K:** This pathway is crucial in some cellular aspects such as cell growth. This pathway is activated because of the stimulation of the tyrosine kinase membrane receptors that phospohorylize the insulin receptor substrate (IRS). This, phophorylizes the p85 of the PI3K.

Ishiko (1987) and Zhou (1996) stated, after exercise, skeletal muscle EMD lengthens. This could be for reduced skeletal muscle excitability, reduction in cytosolic Ca^{2+} concentration, reduced myofibrillar Ca^{2+} sensitivity and/or metabolic derangement (Zhou, 1996). After the findings on their study, Chan-Dewar et al. (2009) proposed same mechanisms could be working on myocardial delay (they excluded the "membrane excitability" option because they did not appreciate any alterations on QRS). They also observed a decline in E´ and S´. About the decline in E´, it is widely documentated how E´ declines after a 42 km race (George et al., 2005; Oxborough et al., 2006; Neilan et al. 2006a, b; Hart et al., 2007). The drop in S´ appreciated in the study included in this review (Chan-Dewar et al., 2009) could be due to the extra effort that supposes the 89 km distance.

There are two studies included talking about biomarkers, Shave et al. (2010) and Da Ponte et al. (2017). It can be said that the main result of the study conducted by De Ponte et al. clarifies that after an uphill ultra-marathon is an increase in cardiac and skeletal muscle blood biomarkers of injury. Talking about the release of skeletal muscle damage biomarkers, they deduced that effort length and intensity prevail over the contraction type. They base this deduction on the race they studied, because even though there were no too much downhill zones (it is mainly an uphill race, so the amount of eccentric contractions were not too many) they found out a huge increase in damage biomarkers (Da Ponte et al., 2017). Talking about cardiac muscle´s behaviour during this uphill ultra-marathon, due to the predominance of repeated and simultaneous concentric contractions of the muscle, a higher cardiac afterload and vascular resistance, resembling a higher-pressure situation somehow (Kim et al., 2013). cTnl is a well-known marker of cardiac damage, which means that an uphill ultra-marathon supposes a great effort to our body, no matter age, training or either performance status, but the increase in cTnl does not seem to have any correlation with athletes´ race times.

Talking about immunoglobulin isotypes, McKune et al. (2005) found an explanation to the increase in antibodies, it could be that after an exercise bout non-systemic immunoglobulins are flushed out of secondary lymph "storages" and/or enter the circulation because of increased lymphatic flow (Nieman et al., 1991). McKune et al. (2005) propose that those increases in IgG concentration may have something to do with antibody switching. This is a process recently described and involves a switch in immunoglobulin isotope from IgM to IgG, thus, a decrease in measured IgM was expected (Coffman et al., 1993). That isotype switching is coordinated by T helper 2

cytokines and the hypothalamic pituitary adrenal axis and sympathetic nervous system (Elenkov et al., 2000). For the concentrations of IgM found in the Study of McKune et al. (2005) they explain that it could be due to two reasons: (1) isotype switching may have occurred as part of a fast secondary response or (2) the decrease reflect the interaction of IgM with the innate immune system in response to the injured tissue after the race (Nieman et al., 1991). IgD measurements are not in agreement with Petibois et al (2003). McKune et al. (2005) explain that duringthe early stages of the immunoglobulin response Little IgD concentrations are found in plasma, as naive B cells undergo maturation and isotype switching from IgM to IgG (Janeway et al., 2001). So, IgD represents a marker of naive B cells and if its concentration decreases it means the initiation of an immunoglobulin isotype switching.

Leonard´s investigation is in contrast with other studies (Hatcher et al., 1982; Hofman et al., 1991; Leklem et al., 1983; Manore et al., 1987) that reported increase in plasma PLP post exercise. The explanation Leonard gives to that contradiction is that the experiment he carried out was much more stressful for the body than the previous ones, Leklem et al. (1983) suggested that the release of LPL into the plasma is just a homeostatic control process. Their hypothesis is that vitamin B-6 is stored in rat muscle coupled to glycogen phosphorylase, this is based on the work made by Black et al. (1977; 1978). During exercise the muscle is the main B-6 vitamin source and as the duration of exercise increases and the liver tends to gluconeogenesis, the liver PLP requirement increases due to its use as a coenzyme for amino acid transaminations (Leklem et al., 1983; Leklem, 1985). On the other hand, Hofman et al. (1991) suggested that increased plasma PLP is destined for use in skeletal muscle. In conclusion, Lonard hypothesized that during endurance exercise the body´s homeostatic control mechanism may be to release PLP during the first moments of exercise for subsequent usage by the liver and/or muscle. If the PLP released into the plasma is not used during the exercise, it is taken back to storage pools.

Strategies during this sort of races include eating and drinking strategies, where and how much an athlete should eat or drink. Glace et al. (2002), Clemente-Suarez (2015), Kruseman et al. (2005) and Stuempfle et al. (2011) clarify how only around 50% of energy expenditure is covered during races. This phenomena is said to be due to more than one factor, like increased levels of satiety, appetite suppression and gastrointestinal discomfort (Costa et al., 2014). It has been suggested that from 30g to 60g·h^{-1} of CHO provides benefits by contributing to muscle fuel needs and maintaining blood glucose

(Rodriguez et al., 2009). However, Martinez et al. discovered that in the observational study they conducted, the values obtained were slightly above the minimal CHO intake recommendation. Closely related to drinking strategies, a couple of studies taling about EAH are mentioned. In those, seven runners developed an asymptomatic EAH but it seems that generally ultra-marathon runners are less likely to develop EAH compared to marathon runners (Stuempfle et al., 2002). In the "100 km run" in Biel the participants had an average of one aid station every 6 km more or less, 17 during the whole race, and in some of those aid stations they were able to drink soup. It has been shown by Twerenbold et al. (2003) that EAH risk can be reduced using fluids of high sodium concentrations while running for 4 h. They also mention the study of Sanders et al. (1999) that underlines the potential advantage of sodium supplementation in a hot environment. In the study conducted by Rehrer et al., as expected, PVC[6] and Hb increases were observed produced by dehydration. Post-race plasma cortisol concentrations were significantly correlated with lower finishing times (faster runners). There was a trend for post-race noradrenaline to be less in sufferers (2047 ± 139) than in non-sufferers (p=0.08). Plasma potassium but not sodium was significantly increased, and the author have some possible explanations for that: (1)When glucose is limited at a membrane or tissue the ionic pump may be impaired, thus sodium collects intracellularly and potassium extracellularly from active muscle tissue. Intracellular reaccumulation of potassium may not keep pace with potassium released through repolarisation (Rehrer et al. 1991); (2) an increased utilization of liver and muscle glycogen in those individuals may have contributed to the plasma potassium increase since potassium is stored with glycogen (Fenn, 1940), but these theories do not explain the significant increase in potassium in those with not GI distress. And to finish studies related to plasma biochemical changes, Noakes et al. (1982) found three possible explanations for that differences for their results:

1. The $5^{1/2}$ hour time thresholds proposed by Berg and Haralambie (1978). In the study of Noakes et al. (1982) the values that novice runners reach should only occur after $12^{1/2}$ h exercise following Ber and Haralambie´s theory (1978). This difference could be because the values of the original theory are taken from walking and in the study

[6] **PVC: Packed cell volume, volume of the blood cells in a sample of blood after it has been centrifuged in the hematocrit. Don´t misunderstand with hematocrit: the proportion by volume of blood occupied by erythrocytes (red blood cells). (** https://medical-dictionary.thefreedictionary.com/packed+cell+volume**)**

carried out by Noakes et al. (1982) the values are taken from runners, but this explanation was not enough for Noakes et al. so they dismissed this explanation.

2. Novice runners ran at higher intensity. The exercise intensity is a well-known factor determining the degree to which plasma enzyme activities rise (Gardner et al. 1964; Fowler et al. 1968; Schmidt et al., 1969; Shapiro et al., 1973), but this explanation is not possible because only experienced runners are able to maintain a high intensity of exercise.
3. It is due to the lower fitness level of the novice runners. This is the most likely explanation.

Schwellnus et al. (2011) found that those athletes performing the first half faster ran at a higher relative intensity, and this finding is similar to another study analysing Ironman athletes (Schwellnus et al., 2011). There was also a significant difference (p=0.026) between those runners stretching before exercise who experienced EAMC (92.9 % stretched before the race) compared with the CON group (54.6%). After the race, CK concentrations were significantly (p=0.066) in the EAMC group. In this study is of particular interest the finding of serum pre-race CK concentrations, that was higher in EAMC runners (p=0.066), what means a greater degree of subclinical pre-race muscle damage. This higher pre-race damage combined with the higher relative intensity during the first half of the race could have been one of the triggers of EAMC.

Although physiological factors concerning ultra-marathon performance are not totally clear yet, those factors are much more clear than cognitive and mental aspects and their effect on performance (Starkes et al., 2003). Outstanding atheletes were more able at making decisions and at extrapolating relevant information in order to anticipate future events and outcomes (Holyoak et al., 1991; Williams et al., 1999). Focus of attention has been deeply explored across sporting domains, and typically focus is divided into external (to direct attention towards the effect of a movement) and internal (to direct attention towards the performance of movements) focus (Wulf G., 2007). Overall, external focus of attention appears to be more beneficial for a successful performance (Castaneda et al., 2007; Jackson et al., 2006). Talking about strategy, there are not only pacing strategies or nutritional strategies during a race, there are also cognitive strategies: associative strategies (implies directing attention towards task-relevant stimuli and physiological sensations experienced during exercise) and dissociative strategies (implies directing

attention towards distracting thoughts) (Masters et al., 1998). Generally, some studies showed that runners adopting associative strategies ran faster than those adopting dissociative strategies (Morgan et al., 1997; Masters et al,. 1998). For all these reasins, Cona et al. performed such an elaborated test, to asses as deep as possible the cognitive functioning of runners. The results they got mean that faster runners have a greater capacity to inhibit a dominant, but inappropriate, response. Thus, they have an enhanced motor inhibition, and that might be a crucial point for performance. Motor inhibition might have a key role in some physical skills, as agility for example (Verburgh et al., 2014).

Magnitude and cause of fatigue depends on the exercise under consideration (Enoka et al., 1992). Critical task variables include the muscle activation pattern, the involved muscle group and type of contraction (Millet, 2011). Alterations in neuromuscular function produced by prolonged running, cycling and skiing were reviewed by Millet and Lepers in 2004 (Millet et al., 2004). They focused on the strength loss in knee extensors (isometric strength loss) after exercise bouts lasting from 30 minutes to several hours and they found that isometric strength loss happens in a non-linear way. Less is known about the decrease in peak power, but it has been reported that the decrease in counter-movement jump performance was around 45-60% of the knee extensors isometric MVC decrease after prolonged running (Petersen et al., 2007; Lepers et al., 1999; Millet et al., 2002; Millet et al., 2000). Central fatigue largely contributes to muscle fatigue during long distance running (Racinais et al., 2007; Saldanha et al., 2008; Millet et al., 2002; Martin et al., 2010; Millet et al., 2003; Place et al., 2004). It is known that the decrease in central activation occurring during exercise can be caused by several factors at the spinal and/or supraspinar levels (Gandevia SC, 2001; Rasmussen et al., 2010; Taylor et al., 2008). Ohta et al. (2005) investigated biochemical modifications during a 24 hour run and from indirect measurements they deduced that this type of exercise induces supraspinal fatigue. It has been suggested for years that the accumulation of serotonin in several brain regions contributes to the development of fatigue during prolonged exercise (Davis et al., 1997). This was thought to be due to an increase in the concentration ratio of free tryptophan to branched-chain amino acids because (i) branched-chain amino acids are oxidized; and (ii) higher plasma free fatty acids during prolonged exercise cause a parallel increase in free tryptophan since the free fatty acids displace tryptophan from their usual binding sites on albumin (Davis et al., 1997). This

in turn increases the concentration of free tryptophan (the serotonin precursor) in the brain. Meeusen et al. (2006) went further suggesting that other neurotransmitters such as dopamine probably also play a significant role in supraspinal fatigue. Another possible explanation is that hyperventilation lowers the arterial CO_2 tension and blunts the increase in cerebral blood flow, which can lead to an inadequate oxygen delivery to the brain and contribute to the development of fatigue (Nybo et al., 2007). But central fatigue cannot explain the entire strength loss, for example, a significant but moderate decrease in high frequency force response has been found for knee extensros after prolonged (Millet et al., 2003; Martin et al., 2010) exercise. Indirect indices of muscle damage suggest somehow the existence of some peripheral alterations (Martin et al., 2010; Ostrowski et al., 1998a, 1998b; Papassotiriou et al., 2008; Koller et al., 1998; Mastaloudis et al., 2006; Skenderi et al., 2006). There is another interesting term, Low-frequency fatigue (LFF), which is a prominent characteristic of exercises involving lengthening contractions of the active muscles such as eccentric- and stretch shortening cycle-type exercises (Martin et al., 2004). With all this information, Millet proposed what he called "The Flush Model", in order to explain how neuromuscular fatigue works. He starts explaining the power output change, pointing that during a self-paced 24 h treadmill run (the participants were told to run as they would during a race) carried out by Martin et al. (Martin et al., 2010) in which they saw a decrease in velocity during the first 16 h before there was a level off. Lambert et al (Lambert et al., 2004) reported that even the best runners reduced their initial speed less and later in the race and that those better runners showed less variation in speed that their slower counterparts. But, even if it has some effect, it does not seem that failure to produce the force required by the exercise limits performance only by itself (Guillaume Y. Millet, 2011). The level of muscle activation is thought to be progressively reduced to maintain the RPE below a maximum tolerated level (Martin et al., 2010). The flush model is based on the central governor model proposed by Noakes et al. (Noakes et al., 2004), but the flush model proposed by Millet also adds some aspects not related to exercise.

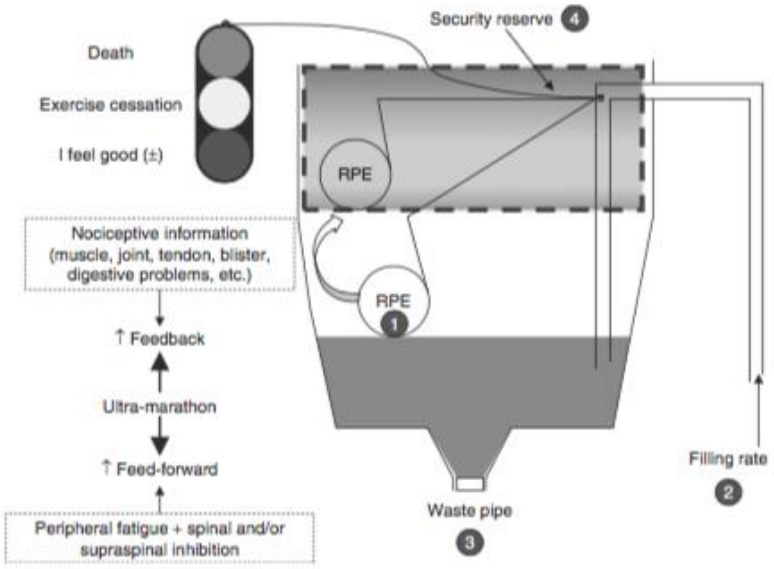

Figure 4 The flush model (retrieved from Guillaume Y. Millet, (2011))

The present model was made to explain fatigue in ultra-marathon running and it consists of four components: (i) the ball: represents the RPE and can increase or decrease based on, (ii) the filling rate and the water evacuated via (iii) waste pipe. Finally, there is a (iv) security reserve too.

The filling rate is influenced by several factors, such as running pace at the beginning of the race. This initial pace is based on a control system that estimated the optimal power output (Ulmer HV, 1996). At the same time, this estimation is based on some different factors: distance, elevation, training status... So, basically this initial pace gives an initial filling rate and the faster the speed, the faster the filling rate (Ulmer HV, 1996). Talking about more investigated fatigue mechanisms, acidosis or inorganic phosphate accumulation is unlikely to occur, but some other mechanisms could happen, like cytokines accumulation due to the structural damage (Ostrowski et al., 1998a; Ostrowski et al., 1998b; Papassotitiou et al., 2008), triggering group III/IV afferent fibres (Ament et al., 2009). Regarding altitude, Amann et al. (2007) showed that peripheral fatigue measured with femoral nerve magnetic stimulation at task failure was substantially less severe in hypoxia compared with normoxia. This was attributed to brain hypoxia effects on effort perception, leading the subjects to stop earlier (Nybo L., 2003). Also, the gain of motoneurones decreases in fatigued conditions such that additional synaptic drive at a premotoneuronal level is required to maintain a constant firing rate (Gandevia SC., 2001). The volume of water in the tank does not only depend on the filling

rate, because it is possible to start running with more water in the tanks than usual (i.e. with a higher RPE) if we had not had enough rest during night for example (Guillaume Y. Millet, 2011).

Talking about the waste pipe (the mechanisms to reduce the water in the tank, to reduce the RPE) Millet says resting is one of the most effective ways, not the only one though, the suitable psychological strategies (Raglin JS., 2007) are effective too, or even nutritional strategies. And the last component of the flush model is the security reserve, why the runner decides to adjust intensity? subjects decide to stop when the task is too difficult or it demands too much effort (Noakes TD., 2004).

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