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PHYSICAL ACTIVITY IN PATIENTS WITH TYPE 1 DIABETES: CARBOHYDRATE REQUIREMENTS FOR EXERCISE AND HYDRATION STRATEGIES

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ABSTRACT

INTRODUCTION: Maintaining homeostasis can be particularly complicated in diabetic individuals, especially during physical activity. Glucose is the primary metabolic fuel, and T1DM individuals have to adapt their therapy and diet in order to reduce the risk of glycemic perturbations, such as hypoglycemia or hyperglycemia. Fluid balance is relevant to preserve physical and cognitive performance, and elevated blood glucose levels are likely to increase fluid losses in urine.

METHODS: Four studies will be presented combining T1DM, exercise, and hydration science. ECRES algorithm has been proposed to estimate the proper amount of carbohydrate (CHO) for exercise in diabetic individuals. In the first part of this thesis, ECRES algorithm was compared with individual strategies during a 24x1-h relay run; then it was compared with a reference method (REF) during moderate intensity walk on a treadmill. Hydration during training was investigated using a survey in both healthy and T1DM athletes, querying about their individual characteristics, sport characteristics, therapy (in T1DM individuals), and fluid intake behaviour.

RESULTS: ECRES was found to suggest an amount of CHO similar to the amount actually consumed by T1DM when they were free to decide their own strategy (median, 38 and 30 g respectively, p = NS). Preliminary results from the validation study suggest that glycemic responses during exercise may be similar when estimated using ECRES or REF to estimate CHO needs. The hydration survey in healthy Italian athletes indicated that fluid intake during training was smaller than the volume suggested by international guidelines, and it is primarily influence by the number of pauses to drink, training duration, and coaches' encouragement to drink. From preliminary results in T1DM individuals, fluid intake seems to be similar to healthy individuals, despite almost 1/4 of the sample reported to start the training with blood glucose above 180 mg/dL (10.0 mMol).

CONCLUSIONS: Results from this thesis support the validity of ECRES algorithm in estimating the proper amount of CHO to prevent glycemic imbalances during exercise. Fluid balance is currently underrated in T1DM, despite its effects on performance and health: specific guidelines should be developed for diabetic athletes considering their behaviours and fluid requirements.

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1. INTRODUCTION

"No one, unless he is grossly ignorant of what science has done for mankind, can entertain any doubt of the incalculable benefits which will hereafter be derived from physiology"
(C. Darwin)

This dissertation will introduce the effects that exercise and physical activity induce on people with insulin-dependent diabetes, or type 1 diabetes mellitus (T1DM), and its consequent responses. Included in this summary will be an overview of aspects related to exercise physiology in patients with T1DM, with specific interest in muscle metabolism and carbohydrate oxidation. Since hypoglycemia represents one of the main issues in T1DM, and above all during physical activity, blood glucose management during exercise is one of the key points to this work. Not only glycemic management, but also hydration and fluid balance maintenance represent a particular characteristic of homeostasis during physical activity, in both healthy individuals and patients with T1DM. The aim of this dissertation is to determine interactions between T1DM, exercise and hydration in order to present a complete overview about this topic (**Figure 1)**.

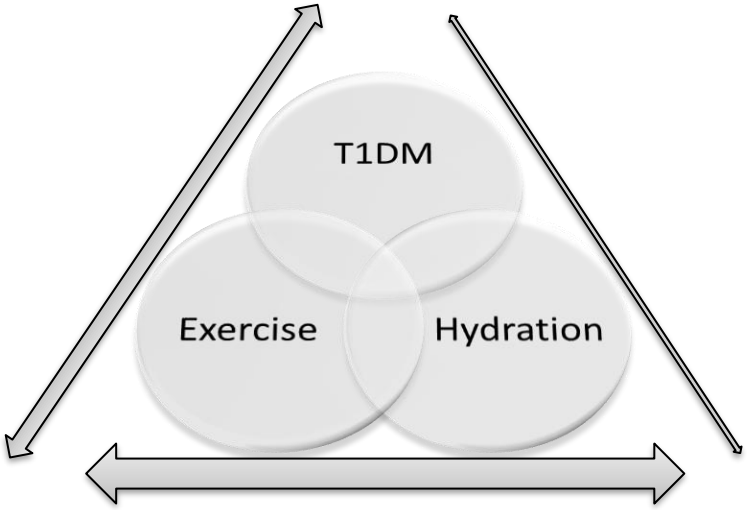

Figure 1: Graphic interaction of dissertation topics

Graphic interaction between the three key aspects of this doctoral dissertation: Diabetes T1 (T1DM) and Exercise, Exercise and Hydration, Diabetes T1 (T1DM) and hydration. Arrow size represent the amount of scientific literature available between the two linked variables.

Following the general literature review, where basic concepts of exercise physiology, diabetes pathophysiology and fluid homeostasis will be presented, this thesis will discuss the results from three specific studies and based on the interactions discussed above.

The first part of this dissertation will describe the Exercise Carbohydrate Requirement Estimating Software (ECRES), a novel algorithm that incorporates specific information about patients' therapy, diet, duration, time and intensity of exercise, and through these parameters it estimates the correct amount of carbohydrate (CHOs) a T1DM patient needs in order to prevent exercise-induced hypoglycaemia (Francescato et al., 2015). The ECRES algorithm has been already validated in a number of conditions, and is currently under a validation protocol with a greater number of subjects (currently N= 23, target population N= 50). This thesis will also communicate results obtained during a study which involves a peculiar exercise protocol: a 24 h relay marathon where a single athlete runs for 1-h. During this fundraising event, 24 T1DM patients ran for 1 h on an urban track. Participants' physical activity intensity (i.e., heart rate), and glycemia levels before,

during and after their run, were measured (Buoite Stella et al., 2016). In a post-hoc fashion, the ECRES algorithm was used to estimate the amount of carbohydrate athletes would have needed to prevent hypoglycemia during their run, and those results have been compared with the amount of carbohydrate that the patient-athletes actually consumed.

The second part of this dissertation will discuss the relationship between hydration and physical activity in healthy individuals. In many sports, fluid ingestion and hydration practices are often overlooked by athletes and coaches, whilst its scientific relevance represents a key challenge for researchers involved in ergonomics and sports science. Indeed, water is essential for many physiological functions.

> "Water is both a reactant and a product, the basis by which the volume of cells, tissues and organs is maintained; it is a shock absorber, the medium for mass-flow transport of different substances, a thermal reservoir, and the substrate for sweating" (Cotter et al., 2014).

Even if the effects of dehydration on physical performance have not been clearly elucidated in the current sport-science context, there remain many brilliant debates still emerging in the scientific literature on this topic (Sawka et al., 2007), where scientists extoll the benefits of maintaining good hydration habits in order to perform exercise better and reduce the risk of heat-related injuries. Leaving aside for the moment the direct impact of dehydration on exercise, it is also important to observe how athletes of different sports hydrate themselves and whether their habits to drinking are affected by various factors, including their competitive sport level, age, sex, and how they manage their fluid balance during their training. Hydration practices during exercise can be also influenced directly by the athletes' coaches. It has been shown that a short educational intervention in young

athletes leaded to reduced urine USG (i.e., urine specific gravity) and better aerobic performance (Kavouras et al., 2012). This means that if you "train" the athletes, they may be better able to manage their own hydration practices. In addition, it has been observed that coaches' own knowledge about nutrition and hydration in sport can often be weak (Torres-McGehee et al., 2012); consequently some coaches prefer to not give any advice because they do not feel prepared enough (Zinn et al., 2006), and many athletes receive no coaching advice on hydration during their training. Moreover, in many instances hydration was one of the most popular improperly given advices (Couture et al., 2015).In Italy, current state of the art information about hydration practices is lacking, thus an *ad hoc* designed questionnaire has been proposed to collect information about athletes drinking habits during sport training, and how coaches stimulate their athletes to follow a careful fluids replacement protocol (Buoite Stella et al., 2016).

The last part of this dissertation will consider the relationship between T1DM and fluid hydration status. It is commonly accepted that patients suffering from T1DM are at a greater risk of dehydration (i.e. lower total body water), mainly when their blood glucose levels are higher than the usually accepted upper limit for euglycemia (i.e., 10.0 mMol), with increased risk of hyperosmolarity (Yardley et al., 2013A). However, there is a lack of scientific literature about TIDM fluid replacement strategies, in particular whether they are subject to additional heat stress, which may be exacerbated during exercise (Yardley et al., 2013B). For T1DM patients, dehydration could not only have negative effects on their physical performance, but it could also represent a threatening situation for their physiological functions because of hypovolemic hyperosmolarity. Thus, the third section of this dissertation will compare the results obtained from a hydration surveillance questionnaire and contrast the data between the healthy athlete population to data from athletes diagnosed with T1DM.

SUMMARY

This dissertation will communicate to the reader an overview of the interactions between i) fluid intake and exercise, ii) fluid intake and a clinical population as T1DM patients during exercise, and iii) the benefits of physical activity in T1DM patients, reducing the risks for health due to improper blood glucose management. By combining different experiences and competences from a variety of research disciplines (from medicine to exercise and sports science, nutrition and thermoregulation), it is envisioned that this thesis will propose the most effective solution for maintaining healthy hydration and exercise practices in the insulin-dependent diabetic population.

1.1 Exercise physiology and carbohydrate oxidation

The human body is a "machine" requiring energy to work. This energy comes from different metabolic pathways, aerobic and anaerobic, involving different substrates: proteins, fatty acids and glucose. However, since the net utilization of proteins is negligible during exercise (di Prampero, 1981), fat and carbohydrate remain the main substrates for energy production in skeletal muscle during aerobic exercise in well-fed humans (Åstrand, 2003). Beyond blood glucose, carbohydrate stores are represented by glycogen, a multi-branched polysaccharide of glucose, primarily found in the cells of the liver and the muscles, to act as long-term energy source (Kreitzman et al., 1992). In aerobic conditions, the type and amount of substrate oxidized depends on the intensity of the exercise, usually expressed as percentage of the maximal oxygen consumption ($\dot{V}O_{2MAX}$) and can be measured through the respiratory quotient (RQ) or, indirectly, by the respiratory exchange ratio (RER) (Pirnay et al., 1982; Romijn et al., 1993; Romijn et al., 2000) (**Figure 2**).

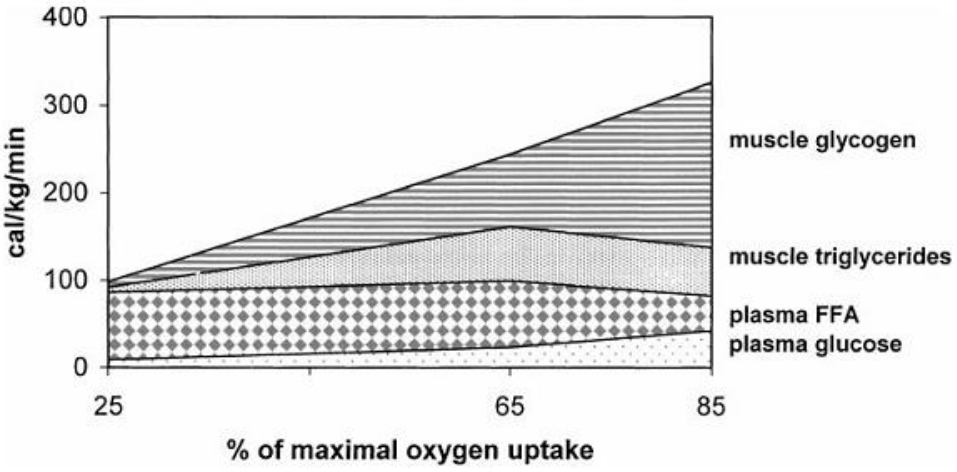

Figure 2: Substrate oxidation according to exercise intensity

Maximal caloric contribution of plasma free fatty acids (FFA) and glucose and minimal contribution of muscle triglyceride and glycogen stores in relation to exercise intensity.

(From Romijn et al., 2000)

Below the anaerobic threshold, carbohydrate oxidation and heart rate increase linearly as a function of exercise intensity (Åstrand, 2003). The correlation between glucose oxidation and heart rate is significant in both healthy subjects and patients with T1DM, and has been recently defined as the "glucose pulse" (Francescato et al., 2005). Interestingly, the glucose pulse, expressed as glucose oxidized per heartbeat, is lower in aerobically trained people (Francescato et al., 2005). Additionally, carbohydrate utilization depends also on carbohydrate stores in the body: the lower the stores, the lower will be carbohydrate contribution to the energy demand (di Prampero, 1981).Differences in metabolism are observable if the exercise is performed in fasting or postprandial conditions. In healthy individuals, fasting elicits a mobilization of triglycerides reserves and decreases carbohydrate utilization in order to preserve blood glucose concentration, essential for central nervous system and red cells metabolism (Maughan et al., 2010).

A scheme of insulin and glucagon regulation to maintain blood glucose levels, and its effects on pancreas and liver is shown in **Figure 3**.

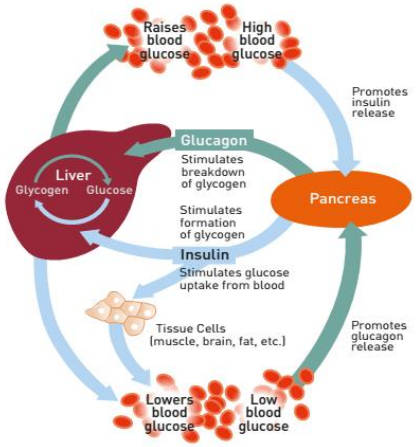

Figure 3: Insulin and glucagon regulation

Low blood glucose promotes pancreatic glucagon release, and glucagon stimulates hepatic breakdown of glycogen that raises blood glucose. Conversely, high blood glucose promotes insulin release from pancreas ß-cells. Insulin stimulates glucose uptake from blood both in liver and tissue cells, thus lowering blood glucose levels.

(From IDF Atlas, 6th edition, 2014)

Exercise induces metabolic adaptations, such as the secretion of catecholamines and cortisol, and an increase in glucagon concentration. These hormones promote hepatic glycogen degradation and a decrease in insulin resistance (Thompson et al., 2001).

The performance of postprandial exercise seems to increase the metabolism of fatty acids during the day after the physical activity (Barwell et al., 2009), while fasting seems to determine a greater mobilization of fat during exercise (Paoli et al., 2011). During submaximal exercise, blood glucose behaviour is mediated by insulin and glucagon response, the increase in glucagon together with the decrease in insulin concentration stimulating gluconeogenesis and glycogenolysis (Goodwin, 2010). In the post absorptive state, exercise initially requires a small contribution from blood glucose, that increases as the exercise continues and intramuscular glycogen stores are depleted (Rose & Richter, 2005).

Skeletal muscle glucose uptake is regulated by three linked steps: a) glucose delivery to skeletal muscle cells, b) glucose transport and c) intracellular metabolism (Rose & Richter, 2005).Glucose delivery (a) is commonly defined as the product between blood glucose concentration and blood flow, and during physical activity is mainly increased through the greater muscular blood perfusion (Richter, 1996). Furthermore, elevated glucose concentrations during exercise increases skeletal muscle glucose uptake, even when a rise in insulin concentration is prevented (Richter, 1996; Zinker et al., 1993). Additionally, it was observed that when blood glucose concentration decreases during exercise, glucose uptake also decreases (Ahlborg et al., 1974). These results suggest that blood flow seems to cope sufficiently with muscular metabolic needs, and it does not represent a limiting factor to glucose uptake; nevertheless, blood glucose concentration seems to greatly influence the amount of glucose uptake in skeletal muscle (Rose & Richter, 2005).

Glucose transport into the cells of different tissues is determined by multiple isoforms of glucose transporters (Ploug & Ralston, 1998). Skeletal and striated muscle tissues, as well as adipose tissue and cardiac muscle tissue, are characterised by a relevant presence of the glucose transporter type 4 protein (GLUT4), and are strongly insulin-dependent (Lenzi et al., 2008). In the skeletal muscle tissue, glucose transport through the GLUT4 is greater in slow twitch muscle fibres compared to other muscle fibre types (Luzi, 2010). Insulin stimulates GLUT4 to move from intracellular vesicles to the plasma membrane, increasing glucose absorption and acting as transporters (Cushman & Wardzala, 1980; Rao et al., 2013). When glucose is transported into the cells it is rapidly phosphorylated to glucose-6-phosphate. Glucose-6-phosphate cannot diffuse back out of the cell, thus it is polymerized into glycogen or enters glycolysis (Watson et al., 2004). A schematic representation of the processes involved for glucose transport into the cell, and the consequent utilization is depicted in **Figure 4**.

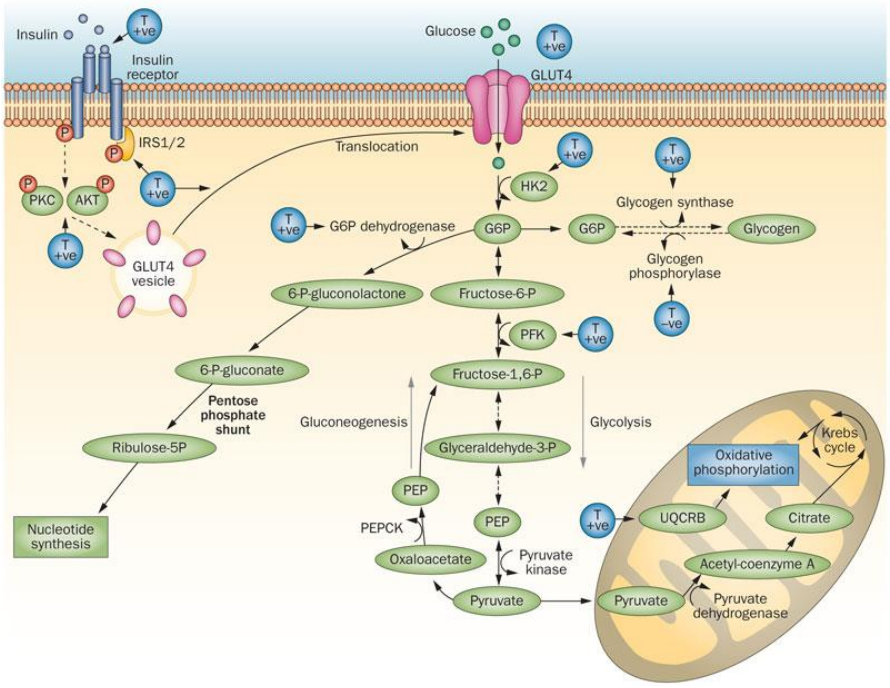

Figure 4: Glucose transport into the cell

Transport mechanisms and metabolic processes involving glucose utilization into the cell, through glycolysis for energy production or glycogen synthesis. GLUT4, Glucose transporter type 4; G6P, Glucose-6-phosphate; HK2, Hexokinase 2; IRS, Insulin receptor substrate; T, Testosterone; P, Phosphate; PEP, Phosphoenolpyruvate; PEPCK, Phosphoenolpyruvatecarboxykinase; PFK, Phosphofructokinase; PKC, Protein kinase C; UQCRB, Ubiquinol cytochrome c-reductase-binding protein.

(From Rao et al., 2013)

In cardiac muscle, and with a lesser extent in the skeletal muscle, muscle contraction itself stimulates GLUT4 to reach the surface and transport glucose independent from insulin concentration (Lund et al., 1995). In addition, exercise seems to increase both the number of GLUT4 activated and individual transporters activity (Rose & Richter, 2005). Since intramuscular glucose does not accumulate except perhaps in the initial minutes of the exercise, glucose transport could represent a limiting factor for glucose uptake in skeletal muscle during moderate exercise (Richter et al., 1998).

Liver tissue is not completely dependent on insulin concentration, as GLUT4 is not the only glucose transporter found in these cells. GLUT2, present in different organs, such as the liver,

pancreatic ß-cells, the kidneys, and the small intestine, participates in the glucose sensing process, and acts as both introducing or liberating glucose in and from the cell, depending on blood glucose concentration (glycemia) (Arienti, 2003). Other active (SGLUT1 and SGLUT2) and passive (GLUT1, GLUT2, GLUT3, and GLUT5) glucose transporters are involved in glucose regulation in different tissues, but they won't be examined in detail in this dissertation.

Inside the cell, phosphorylation of glucose (c) is determined by hexokinase activity (HK) (Halseth et al., 2001). Muscle glucose delivery is a function of the muscle blood flow and diffusion distance, and removal of glucose from the inner sarcolemma surface is determined by diffusion and glucose phosphorylation (O'Doherty et al., 1998). In rat, it was demonstrated that physiological increments in insulin induce an increase in sarcolemmal permeability that is not accompanied by a proportional increase in glucose delivery and/or phosphorylation (O'Doherty et al., 1998). Slow twitch fibers (type I) are characterised not only by a greater capillary density, blood flow, and GLUT4 protein expression, but also a greater HK activity, as compared to fast twitch fibers (Halseth et al., 2001). In the rat model, it has been shown that in fast twitch fibers (type II) muscles, glucose uptake is primarily determined by glucose transport and glucose delivery to the sarcolemma. In type I fibers' characterised muscle, as the soleus, results indicate phosphorylation to not represents a strong limiting factor to muscle glucose metabolism (Halseth et al., 2001).

SUMMARY

Glucose supply, transport, and phosphorylation are relevant regulatory steps of skeletal muscle glucose uptake and consumption during exercise. It is difficult to determine in healthy individuals which factor is the most limiting for glucose uptake, since all steps are likely to be closely coupled to the metabolic state of the muscle fibre and fibres recruitment. Additionally, it is important to note that muscular contractions seem to independently regulate glucose uptake (Rose & Richter, 2005). This chapter highlighted the importance of glucose metabolism during exercise, and carbohydrate supplementation should be an important factor to consider when facing physical activity in healthy and diabetic athletes.

1.2 Hydration and dehydration during exercise

Water is an essential nutrient for human body (Marcos et al., 2014), and maintaining the fluid balance can be a challenge during physical activity, under environmental heat stress, or in pathological conditions.

A schematic representation of typical body fluid compartments distribution is illustrated in **Figure 5**.

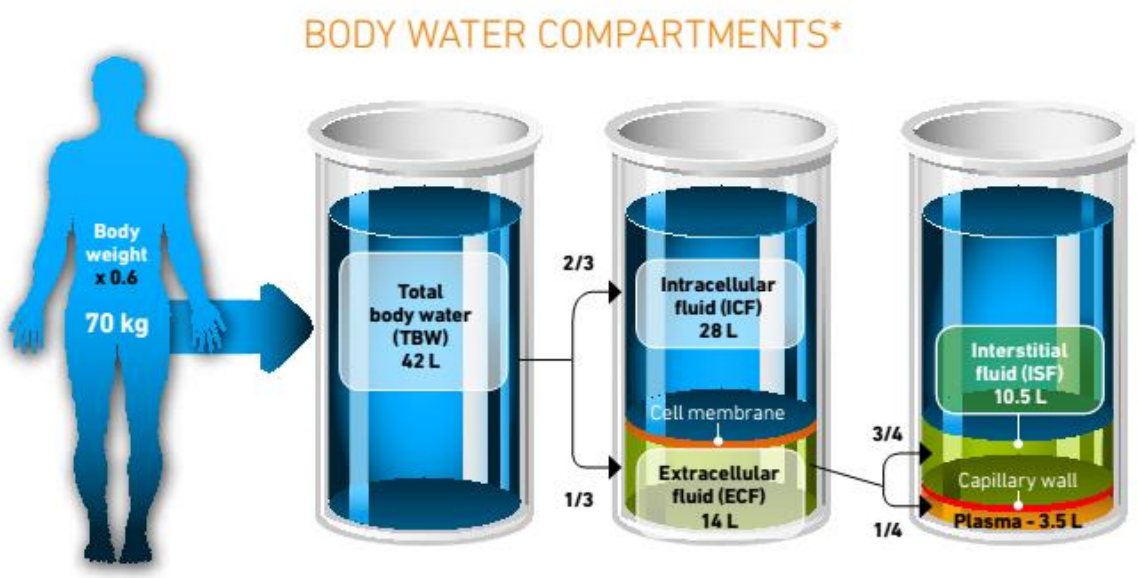

Figure 5: Body fluid compartments

Schematic figure of typical body fluid compartments for a 70-kg adult fit male. Total body water (TBW) 42 L, Intracellular Fluid (ICF) 28 L, Extracellular Fluid (ECF) 14 L, Interstitial Fluid (ISF) 10.5 L, Plasma Volume ~3.5 L (From Key Hydration Tips, European Hydration Institute)

Total body water (TBW) is usually divided between intracellular fluid (ICF) and extracellular fluid (ECF). Extracellular fluid compartments are further categorized between interstitial fluid volume (ISF) and plasma volume (PV). Even if total body water depends on several factors, as individual characteristics, fitness, body fatness and glycogen storage, it is possible to assume that TBW represents about 60% of total body mass for an adult man, and ICF is about 40% of total body

mass (Cheung, 2010). Thus, a typical male of 70 kg would have 42 L of water, with ICF of 28 L and ECF of 14 L. Blood volume divides into plasma volume (PV) and cell volume, where PV can range from 70 $mL \cdot kg^{-1}$ to 100 $mL \cdot kg^{-1}$ in a healthy young male, with higher values in trained and lean individuals (Sawka et al., 1992).

In fluid balance studies, dehydration is commonly defined as a dynamic process in which body water is lost without adequate replacement, leading to a state defined as "hypohydration", and is usually defined in relation to a reference body mass (Cheung, 2010). In most cases fluid loss is mainly caused by sweating, a physiological thermoregulatory process that dissipates heat from the skin to prevent hyperthermia (Cheung, 2010). Sweat rate can differ based on several factors, mainly individual (e.g., age, sex, body fat, etc.), environmental (e.g., temperature, humidity, wind speed, etc.), ergonomic (e.g., clothing, equipment, etc.), or sports-related (e.g., intensity, duration, characteristics, etc.). During an ultra-endurance cycling event (7.0 ± 2.1 h) performed in a hot environment (35.5 ± 6.5 °C), total sweat loss ranged from 4.9 to 12.7 L, which was not correlated with thirst ratings or performance (Armstrong et al., 2015). In a different sport such as tennis, characterised by self-determined and self-controlled pacing to avoid dangerous levels of physiological strain, it was possible to observe a mean sweat rate in the heat up to 2.6 L/h (Bergeron, 2014). In a team sport such as soccer, adult players lost up to 3.1 ± 0.6 L of sweat during a 90-min game performed in warm humid conditions (34.3 ± 0.6 °C, RH 64 ± 2 %) (Kurdak et al., 2010). In similar but less humid environmental conditions (32.0 ± 3.0 °C, RH 20 ± 5 %), professional soccer players lost 2.2 ± 0.4 L of sweat during a 90-min training, showing relevant individual differences between athletes, with losses ranging from 1.7 to 3.1 L (Shirreffs et al., 2005). In non-competitive conditions, such as adults exercising freely in a gymnasium, total sweat loss was reported at lower levels, 0.79 ± 0.39 L for 1-h of training (Peacock et al., 2011).

Heat stress alone has been proposed to be a limiting factor for aerobic performance, and its effects seem to be exacerbated by hypohydration (Cheuvront et al., 2010). Indeed, dehydration may induce a reduction in muscle blood flow (Gonzalez-Alonso et al., 1999; Gonzalez-Alonso et al., 1997), and may generate skeletal muscle metabolism impairments (Febbraio, 2000), such as an increased glycogen depletion (Logan-Sprenger et al., 2015). However, this finding is still debated, and recent results suggest a minor role of water deficit in glycogen use during exercise (Fernandez-Elias et al., 2015).

It is commonly accepted that hypohydration has detrimental effects on aerobic performance, although the specific mechanisms of action are still subject to debate, and usually divides between physiological aspects and thirst perception (Kenefick & Cheuvront, 2012). Some authors suggest a major role of thirst sensation as the main limiting factor for aerobic performance, and argue that the greatest part of the studies on dehydration have been conducted by forcing the participants to unnatural behaviours (Sawka & Noakes, 2007). Indeed, these authors suggest it would be unnatural to consume diuretics, or lay in a heat chamber to increase sweat rate before a cycling trial (Sawka & Noakes, 2007). These behaviours, therefore, may affect performance mainly because of psychological factors, physiological factors having only a secondary role. In particular, diuretics should not be used in these studies on dehydration because of the possible detrimental effects the drug itself could induce on exercise performance, independently from hypohydration (Cotter et al., 2014; Sawka & Noakes, 2007). Not only the methods to induce dehydration may force the participant to unnatural behaviours, but also unrealistically low airflows, as observed in many laboratory settings, may affect results (Cotter et al., 2014). Other possible bias may derive by the absence of blinding to the hypohydration protocol, and no familiarisation to the stress of its imposition (Cotter et al., 2014). It is suggested that being submitted to a pre-exercise dehydration protocol, or knowing that no fluids will be available during the exercise, may provoke an

anticipatory mechanism in the brain that automatically reduce the intensity of the exercise and impairs performance to prevent an excessive rise of blood osmolality (St Clair Gibson & Noakes, 2004; Tucker et al., 2006). Additionally, it is argued that the currently accepted theory relies on a too simplistic model of exercise physiology (Noakes et al., 2005). Different papers (Below et al., 1995; McConell et al., 1997) suggest there is not a dehydration threshold after which performance is impaired, whereas it mainly depends on thirst sensation. Thus, *ad libitum* fluid consumption seems to be sufficient to prevent a fall in performance, even when dehydration levels are greatly over the common threshold of 2 % of body mass (BM) loss (Cotter et al., 2014; Noakes, 2007). Therefore, supporters of this theory suggest that results reported by many reference papers on exercise dehydration could be actually influenced by psychological more than physiological factors, and those factors should be carefully taken in account. A different and opposite opinion comes from supporters of the principal theory (i.e., dehydration impairs aerobic performance through direct physiological effects, when a specific hypohydration threshold is surpassed), suggesting that the multifactorial model usually referred to is not simplistic at all (Sawka & Noakes, 2007). Dehydration impairs physical performance, augments hyperthermia (Cheuvront et al., 2004), increases cardiovascular strain (Montain et al., 1998), reduces skeletal muscle blood flow (Gonzalez-Alonso et al., 1999; Gonzalez-Alonso et al., 1997), alters skeletal muscle metabolism (Hargreaves et al., 1996) and EMG patterns (Bigard et al., 2001). Thirst perception mechanisms, as hyperosmolality and hypovolemia, are the same mechanisms involved in the augmented hyperthermia and cardiovascular strain, and it seems difficult to be considered separately. In a recent attempt to control for confounding factors, as described above (Cheung et al., 2015), dehydration slightly > 3 % BM was obtained by cycling prior to a time trial (TT) performance. Performance was compared in different blinded conditions, as i) euhydration with mouth rinse, ii) euhydration without mouth rinse, iii) dehydration with mouth rinse, and iv) dehydration without

mouth rinse. This novel design considered both synergically and separately hydration/dehydration (real or sham IV infusion) and thirst perception (mouth rinse). Results indicate that dehydration elicits minimal thermal or cardiovascular effects during steady-state cycling, whilst thermo-physiological strain was elevated during the consecutive 20-km self-paced cycling TT; dehydration, however, did not significantly influence aerobic performance (Cheung et al., 2015).

Results from the study above, showing power output during TT in different experimental conditions are presented in **Figure 6**.

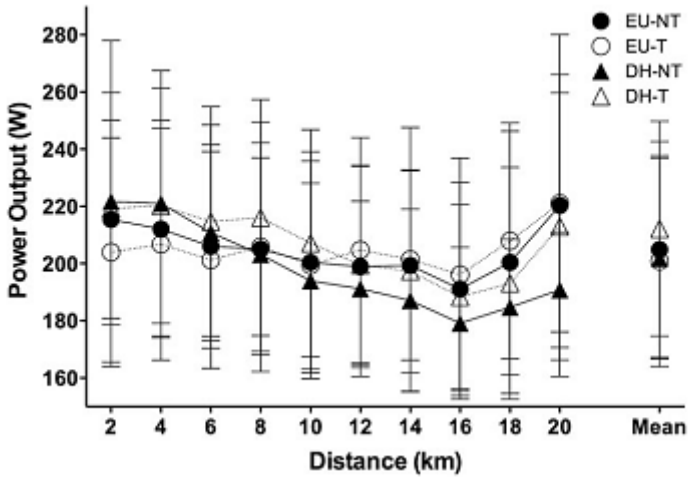

Figure 6: Dehydration and TT power output

Power output responses during a 20-km time trial in hot (35 °C, 10% relative humidity) conditions. DH, dehydrated; EU, euhydrated; NT, not thirsty; T, thirsty. No significant difference was observed.
(From Cheung et al., 2015)

Dehydration not only may decrease physical and cognitive performance, but it may represent a risk factor for several diseases (El-Sharkawy et al., 2015; Ferry, 2005; Marcos et al., 2014; Riebl & Davy, 2013).

Vasopressin (AVP), or antidiuretic hormone, is secreted in response to an increase in plasma osmolality or a reduction in plasma volume, being an index of fluid balance (Melander, 2016). AVP acts on different receptors, by enhancing renal water reabsorption and promoting vasoconstriction. As a result, urine output is reduced and urine is characterised by a greater osmolality (Melander, 2016). Since measuring AVP is complicated, a common marker has been identified in a C-terminal part of the AVP precursor pre-provasopressin (pre-proAVP), named "copeptin" (Dobsa & Edozien, 2013). Copeptin reflects AVP concentration in plasma, and high concentrations have been associated with various diseases. In specific, it is associated with an increased risk of cardiovascular and metabolic diseases (Dobsa & Edozien, 2013). Copeptin may promote development of obesity, dyslipidemia, elevated concentration of triglycerides and low-HDL cholesterol (Dobsa & Edozien, 2013). In addition, elevated copeptin concentration at baseline predicts the incidence of diabetes mellitus and abdominal obesity during a long-term follow-up, independently from obesity itself or other risk factors (Enhorning et al., 2010). Inhibition of AVP secretion emerges as a novel attractive strategy to prevent diabetes and its associated cardiovascular complications (Melander, 2016).

SUMMARY

As described in this chapter, hypohydration seems to impair physical performance, however its role remains unclear. Current guidelines recommend to avoid a dehydration level > 2 % of BM loss. These suggestions are based on a great part of studies on this topic, where aerobic performance impairment and thermal strain were associated with dehydration greater than that level. Nevertheless, results are difficult to interpret since they may be potentially influenced by several confounding factors, namely i) unrealistic airflow (low airflow could negatively influence thermal balance and increase heat strain), ii) unnatural drinking or sweating behaviours, iii) absence of blinding and/or familiarisation trials, and iv) a negative psychological attitude knowing that no fluid or restricted fluids will be provided during exercise. Although still debated, *ad libitum* (i.e., drinking how much and whenever you need) fluid replacement seems to be sufficient to prevent performance impairment and heat-related injuries. It is important, however, to consider specific conditions in particular when fluids are not easily available or thirst perception could be altered. We agree with the complexity of mechanisms underlying the effects of dehydration on exercise, and it seems difficult to identify the best strategies to optimize performance in both healthy and even more in type 1 diabetes mellitus (T1DM) athletes. Furthermore, a proper hydration-induced reduction of AVP secretion may be a safe and cost-effective intervention to prevent diabetes mellitus and cardiovascular events.

1.3 Diabetes type 1

Diabetes occur when the body cannot produce enough of the hormone insulin or cannot use insulin effectively (International Diabetes Federation, 2015). Type 1 and type 2 diabetes mellitus (T1DM and T2DM) are characterized by high blood glucose levels (hyperglycemia) that can cause serious health complications as ketoacidosis, kidney failure, heart disease, stroke, and blindness (Van Belle et al., 2011). Common diagnostic symptoms are excessive thirst, urination and hunger, resulting from the underlying hyperglycemia and the insufficient insulin functionality (Van Belle et al., 2011). Globally, the estimated diabetes prevalence for adults (20-79 years old) in 2015 was 415 million, and statistics estimate an increase up to 642 million people by 2040 (International Diabetes Federation, 2015). In addition, 318 million adults worldwide suffer from impaired glucose tolerance, putting them at high risk of developing diabetes in the future.

T1DM usually accounts for 10% of total diabetes patients (International Diabetes Federation, 2015), with estimates of 41.5 million people suffering from this form of diabetes.

A schematic view of the distribution of diabetes (type 1, type 2, and gestational diabetes) in the world, based on epidemiological results in 2015, and estimates for 2040, is shown in **Figure 7**.

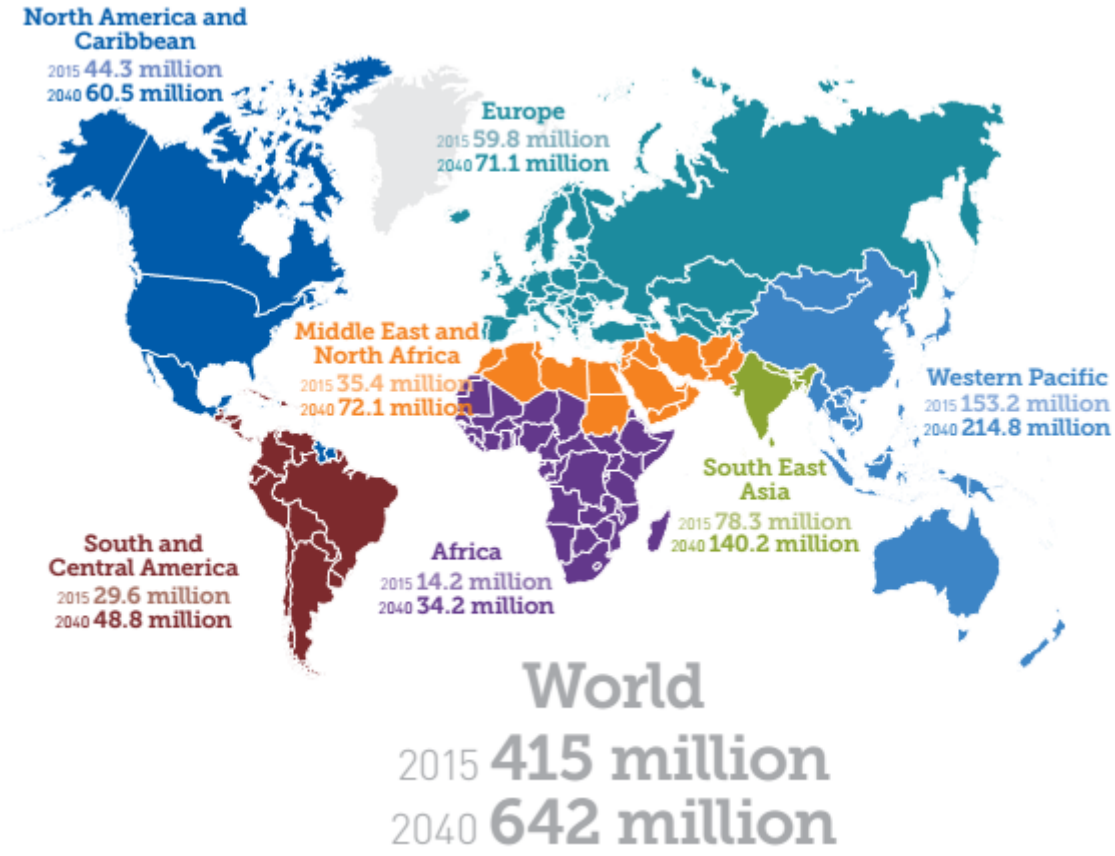

Figure 7: Diabetes world epidemiology

Epidemiology of diabetes worldwide and in different regions in 201,5 and estimates for 2040. Values expressed as number of cases (millions).

(From IDF Atlas, 7th edition, 2015)

Type 1 diabetes mellitus (T1DM) is a chronic disease characterized by pancreatic inability to secrete sufficient insulin for blood glucose regulation (Bhattacharyya et al., 2009). While T2DM is mainly associated with obesity and older age, T1DM usually affects people younger than 30 years old (it is known also as "juvenile diabetes"), although it can occur at any age (Van Belle et al., 2011). T1DM is defined as a chronic autoimmune disorder that precipitates in genetically susceptible individuals by environmental factors (Atkinson & Eisenbarth, 2001). It is considered as an autoimmune pathology since own immune system attacks the beta-cells in the islets of

Langerhans of the pancreas, destroying or damaging them sufficiently to reduce and eventually completely suppress insulin production (Van Belle et al., 2011).

T1DM is rarely caused by mutational defects in a single gene (Van Belle et al., 2011), while it is suggested that the HLA region on chromosome 6p21 (commonly known as IDDM1, meaning insulin-dependent diabetes mellitus locus), is a critical susceptibility locus for many immune diseases as T1DM (Bertrams, 1984). Moreover, there are over 50 non-HLA regions that significantly affect the risk for type 1 diabetes (Pociot et al., 2010). Other T1DM susceptibility genes are for example the IDDM2 locus on chromosome 11 (insulin gene region), PTPN22 (which encodes the lymphoid tyrosine phosphatase), allelic variation in the interleukin-2 receptor-α (IL2RA), and the gene encoding cytotoxic T lymphocyte-associated protein 4 (CTLA-4) in the IDDM12 region (Van Belle et al., 2011). As stated above, genetic factors predispose for T1DM development, that is usually associated with environmental triggers. Viral infections seem to be linked with T1DM, even if there is still no direct evidence for a particular viral strain being causative (Van Belle et al., 2011). Enteroviruses are identified as prime viral candidates that can trigger T1DM development (Filippi & von Herrath, 2008). Rodents studies found bacteria as linked with T1DM (Bach, 2002). Collectively, it appears that antibiotics and probiotics may influence T1DM development by altering the balance of gut microbiota toward either a tolerogenic or non tolerogenic state, depending on constitution of the intestinal microflora at the time of administration (Vaarala et al., 2008). Those alterations seem to be mainly located at intestinal level, and other substances could alter mucosal immune system, leading to T1DM (Van Belle et al., 2011). Cow's milk could promote T1DM in genetically susceptible people, however convincing arguments are rough (Harrison & Honeyman, 1999). Even wheat proteins have been suspected to be related to T1DM, but no strong evidence was found (Hummel et al., 2002). Conversely vitamin D seems to

have a protective function, and people at risk of T1DM should avoid vitamin D deficiency (Mathieu et al., 2002).

The new therapeutic approach is moving toward an early diagnosis of diabetes, in order to prevent a complete loss of pancreatic ß-cells, and replacement of destroyed islets (Van Belle et al., 2011). However, insulin substitution is the main pharmacological therapy proposed to T1DM patients, as it substantially improves quality of life. For sure, insulin is not a cure, but it remains the major treatment in the short term and potentially in the long term, as for the "artificial pancreas" (Hirsch, 2009). There are different types of insulin analogs, short acting and long acting, which can be administered with multiple injection protocols or continuous infusion.

T1DM unfortunately may lead to several long-term complications that affect the eyes, kidneys, feet, and peripheral and autonomic nervous system (Nathan, 1993). Cardiovascular diseases is more prevalent among patients with diabetes, even 10-fold greater than in not diabetic individuals (Laing et al., 2003). These results have been confirmed by the scientific literature, identifying the glycemic imbalances as the main phenomenon that increases the risk of complications (Nathan, 1993).

Glycated hemoglobin (HbA1c) is a useful biomarker to assess average plasma glucose concentration during periods of time. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases. In patients with T1DM, high HbA1c values indicates a poor control of blood glucose levels, and it seems to be associated with cardiovascular diseases, nephropathy and retinopathy (Larsen et al., 1990). The American Diabetes Association recommends for most of patients to maintain HbA1c levels below 53 mmol/mol (7.0%) (American Diabetes Association, 2009; Rodriguez et al., 2009).

In healthy individuals, glycemia is usually < 5.6 $mmol \cdot L^{-1}$ in the fasting period, and about < 7.5 $mmol \cdot L^{-1}$ in the postprandial period (Rossetti et al., 2008). Hypoglycemia is commonly defined by

the development of autonomic or neuroglycopenic symptoms, blood glucose concentration < 3.9 $mmol \cdot L^{-1}$, and the reversal of symptoms when glycemia returns to normal values (Rossetti et al., 2008). Since brain is completely dependent on blood glucose delivery for its metabolism, hypoglycemia would result in possible neurological impairment (Rossetti et al., 2008). Depending on severity of hypoglycemia, physical, intellectual and psychological symptoms are observable (Rossetti et al., 2008). Additionally, hypoglycemia can contribute to morbidity and mortality associated with diabetes (Rossetti et al., 2008).

Hyperglycemia mainly acts inducing chronic cellular and molecular impairment of neural and vascular structure and function (Lotfy et al., 2015). When diabetes is not diagnosed, or patients' blood glucose management is ineffective, hyperglycemia results in diabetic ketoacidosis and hyperosmolar hyperglycemic state (Kitabchi et al., 2009). Diabetic ketoacidosis and hyperosmolar hyperglycemia not only represent a risk for chronic damages, but may provoke severe reactions leading to coma and death (Kitabchi et al., 2009).

Exercise represents one major challenge for T1DM patients, since it may increase the risk of severe hypoglycaemia during and even several hours after the effort. However, physical activity is strongly recommended not only for specific benefits related to diabetes, but also for its important protective effects against cardiovascular and metabolic diseases (Roberts & Taplin, 2015).

SUMMARY

In conclusion, T1DM is spread worldwide, and experts estimate an increase of people affected by diabetes in next years. Pancreatic inability to secrete insulin induces higher levels of blood glucose. Symptoms may be severe, both acutely and chronically, with a greater risk of complications and cardiovascular diseases, potentially leading to death. Since a cure is not yet available, substitute insulin represents the major therapy, with appropriate diet. However, since insulin cannot be regulated by the organism, hypoglycemia frequently occurs, and in particular during physical activity. Regular exercise is however suggested to help preventing tissue damages and reducing the risk of cardiovascular diseases. Nevertheless, exercise should be performed avoiding glycemic imbalances, and this seems to be particularly hard for many patients.

1.3.1 Exercise in T1DM: ECRES algorithm

Physical activity is highly recommended in healthy individuals and for many different diseases (Kesaniemi et al., 2010), and it is strongly suggested also for patients with T1DM (Chimen et al., 2012). Its main effects are identified as a reduced risk of cardiovascular disease (Laaksonen et al., 2000) and an improved insulin sensitivity (Chiang et al., 2014). However, the independent effect of physical activity in improving glycemic control has not fully been demonstrated (Kennedy et al., 2013). Physical activity not only produces positive effects on the physiopathological aspects of diabetes, but also it enhances psychological well-being by increasing self-esteem and quality of life (Steppel & Horton, 2003).

Nevertheless, patients are often reluctant to participate in constant physical activity programs, because of different barriers that depend on specific group characteristics (Bauman et al., 2002; Brazeau et al., 2008). Since physical activity greatly influences blood glucose levels (Briscoe et al., 2007), it is important for T1DM to adapt their diet and therapy in order to avoid exercise-induced hypoglycemia and hyperglycemia (Dube et al., 2005). Hypoglycemia, defined as a blood glucose concentration < 3.9 $mmol \cdot L^{-1}$, seems to be the major barrier to physical activity (Brazeau et al., 2008) and it limits volitional exercise in T1DM patients.

Aerobic exercise generally increases the risk of hypoglycaemia in T1DM, while recent studies suggest that anaerobic forms of exercise may reduce this risk (Yardley, Sigal, et al., 2013).

The principal physical adaptations during aerobic exercise in T1DM patients and healthy individuals are summarized in **Table 1**.

Table 1*: Effects of aerobic exercise on physiological functions and physical parameters. (From Yardley et al, 2013)*

Exercise response	No T1DM	T1DM
Change in glucose uptake	↑↑↑	↑↑↑
Initial blood glucose response	↓	↓
Insulin response	↓↓	↔
Glucagon response	↑↑	↑ or ↔
Catecholamine response	↑	↑
Hepatic glucose production	↑↑↑	↑
Resulting blood glucose levels	↔ (euglycemia)	↓↓ (hypoglycemia)

High intensity exercise increases the level of catecholamines (epinephrine and norepinephrine) inducing the liver to release glucose into blood (Kjaer et al., 1986) often exceeding the rate of use and resulting in an increase in blood glucose concentration (Calles et al., 1983; Kjaer et al., 1986). After the cessation of exercise, glucose production does not diminish as quickly as glucose uptake, and since in T1DM there is not endogenous insulin secretion, hyperglycemia could persist for several hours after exercise (Sigal et al., 1994).

Resistance training relies mainly on anaerobic processes; **Table 2** summarize physiological responses to this type of exercise.

Table 2*: Effects of anaerobic exercise on physiological functions and physical parameters. (From Yardley et al, 2013)*

Exercise response	No T1DM	T1DM
Change in glucose uptake	↑↑↑	↑↑↑
Initial blood glucose response	↑↑↑↑	↑↑↑↑
Insulin response (exercise)	↓ or ↔	↔
Glucagon response (exercise)	↑↑	↑ or ↔
Catecholamine response	↑↑↑	↑↑↑
Hepatic glucose production	↑↑↑↑	↑↑↑↑
Insulin response (post-exercise)	↑↑	↔
Resulting blood glucose levels	↔ (euglycemia)	↑↑ (hyperglycemia)

Combining aerobic and anaerobic training is recommended to prevent an excessive fall in blood glucose levels. Indeed, resistance training before aerobic training may attenuate the decrease in blood glucose concentration usually observed during moderate aerobic activity (Yardley, Sigal, et al., 2013). It improves glycemic stability throughout exercise, and reduces the duration and severity of post-exercise hypoglycemia for T1DM patients (Yardley, Sigal, et al., 2013).

Exercise-induced hypoglycemia could be prevented adapting patients' therapy, namely reducing the insulin dose (West et al., 2011), albeit it often results in high blood glucose levels before the exercise (Campbell et al., 2014). The most commonly used method to manage exercise in T1DM consists of an increased ingestion of carbohydrate (CHO) before and during the effort (Grimm et al., 2011). Even an excessive CHO ingestion, however, could provoke a detrimental and excessive increase of blood glucose concentration (Perry & Gallen, 2009).

Current guidelines are usually designed for activities lasting up to 1 h (Francescato et al., 2015), and are commonly defined as a simple approximation that patients need to correct and improve with

their own experience (Perry & Gallen, 2009). Prescriptions exist also to adapt the dose of insulin for exercise, which have also to be adapted by individual patients.

The simplest method to estimate the amount of extra CHO completely disregards any potential contribution of the liver in blood glucose homeostasis, and the CHO ingested are quantified to match the amount of muscular glucose disposal (Perkins & Riddell, 2006). A general recommendation for carbohydrate consumption is to eat 15 to 30 g of CHO every 30 to 60 min of exercise (Perkins & Riddell, 2006). This method does not require any insulin dose correction, but may be rough since the amount of glucose oxidized is widely dependent on the intensity of exercise and patient's body mass.

Standardized tables have been proposed to suggest the correct amount of CHO for exercise, based on patient's body mass and type of exercise (Perkins & Riddell, 2006). This kind of tables are useful and easy to read, although the suggested intake does not take in account patient's fitness level, initial glycemia, time when exercise is performed, or blood insulin concentration (not even considered when suggesting CHO consumption up to 15-30 $g \cdot h^{-1}$), factors strongly contributing to CHO needs.

CHO supplements for different activities in T1DM, based on the best known standardized table, are summarized in **Table 3**.

Table 3*: CHO supplements (g) per hour of training in different physical activities and different body masses (kg). (From Perkins and Riddell, 2006)*

Activity		CHO (g/h)		
		Body Mass (kg)		
		45	68	90
Baseball		25	38	50
Basketball	moderate	35	48	61
	vigorous	59	88	117
Bicycling	10 km/h	20	27	34
	16 km/h	35	48	61
	22 km/h	60	83	105
	29 km/h	95	130	165
	32 km/h	122	168	214
Dancing	moderate	17	25	33
	vigorous	28	43	57
Digging		45	65	83
Eating		6	8	10
Golf with pull cart		23	35	46
Handball		59	88	117
Jump Rope (80/min)		73	109	145
Mopping		16	23	30
Mountain Climbing		60	90	120
Outside Painting		21	31	42
Raking Leaves		19	28	38
Running	8 km/h	45	68	90
	13 km/h	96	145	190
	16 km/h	126	189	252
Shoveling		31	45	57
Skating	moderate	25	34	43
	vigorous	67	92	117
Skiing (cross country)	8 km/h	76	105	133
Downhill Skiing		52	72	92
Water Skiing		42	58	74
Soccer		45	67	89
Swimming	slow crawl	41	56	71
	fast crawl	69	95	121
Tennis	moderate	23	34	45
	vigorous	59	88	117
Volleyball	moderate	23	34	45
	vigorous	59	88	117
Walking	5 km/h	15	22	29
	7 km/h	50	45	59

A novel method has been proposed to estimate glucose supplements in T1DM patients through an individualized algorithm. ECRES algorithm (Exercise Carbohydrate Requirement Estimating Software) has been developed by our group from University of Udine, laying its foundation on the almost linear relationship between heart rate and glucose oxidation, as introduced in chapter 1.1. This concept that was defined as "Glucose Pulse", is characterised by a significant difference between sedentary and trained individuals, where trained individuals use less glucose while exercising at the same intensity of sedentary individuals (Francescato et al., 2005; Francescato et al., 2008). No differences were observed between T1DM patients and healthy subjects.

The relationship between glucose oxidation and heart rate in trained and sedentary individuals, is illustrated in **Figure 8**.

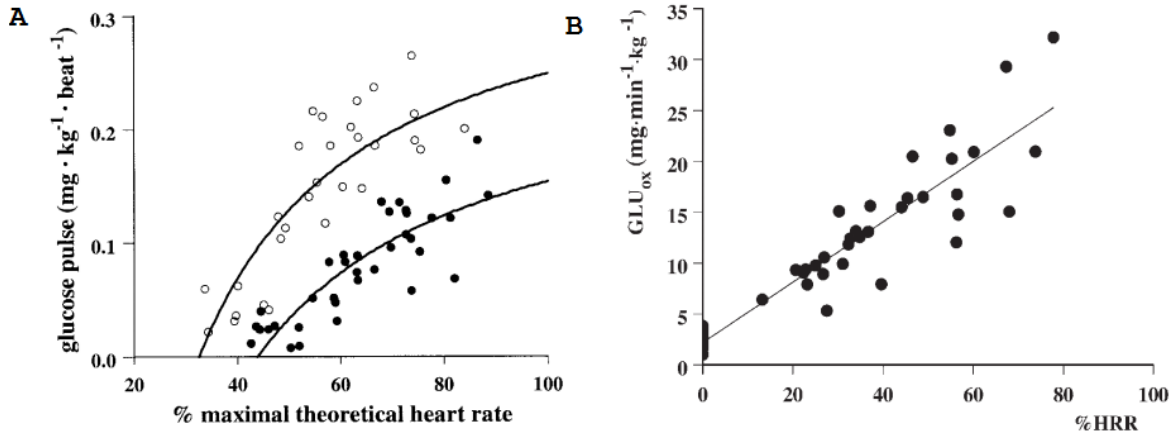

Figure 8: Glucose Pulse

(A) glucose "pulse" oxidation expressed as mg per kg body mass per heartbeat, in relation with percentage of maximal theoretical heart rate (220-age), in trained (full dots) and sedentary(empty dots) individuals (From Francescato et al., 2005). (B) glucose oxidation expressed as mg per kg body mass per minute, in relation with percentage of the heart rate reserve. (From Francescato et al., 2008)

Despite higher insulin levels throughout almost the whole day, patients with T1DM behave metabolically like healthy individuals during aerobic exercise, and in particular when the activity is performed under euglycemic conditions (Jenni et al., 2008). In healthy individuals, blood insulin levels fall promptly after exercise onset. Conversely, in T1DM patients insulinemia depends on time after the injection, and it stays constant during exercise (Francescato et al., 2004; Wallberg-Henriksson, 1989).

Blood insulin levels during exercise performed at different times of day, in healthy individuals and T1DM patients, are illustrated in **Figure 9**.

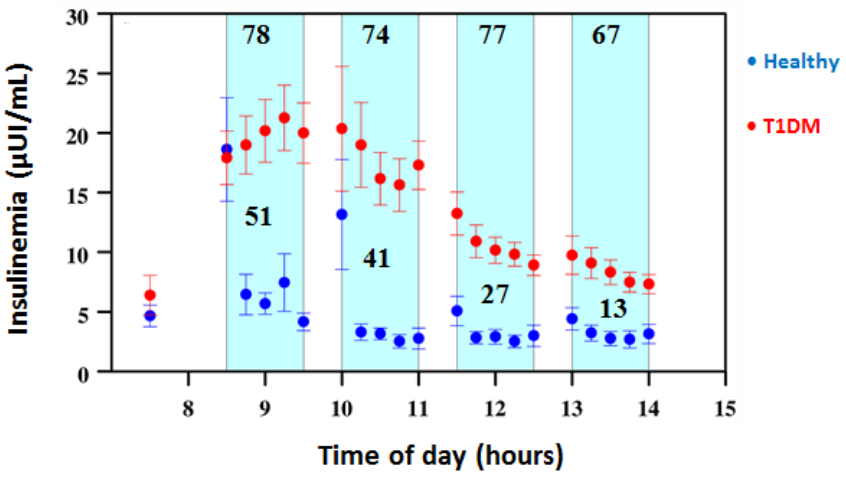

Figure 9: Blood insulin ad different times of day

Blood insulin concentration (µUI/mL) measured in healthy individuals (blue dots) and T1DM patients (red dots), during exercise performed at different time of day (i.e, different time after insulin injection in T1DM patients). (Modified from Francescato et al., 2004)

Since T1DM patients have lost the capability to regulate and produce endogenous insulin, two main conditions may be identified. If exercise is performed soon after insulin injection, blood insulin concentration is high throughout the effort, and this leads to an insufficient glucose transfer from the liver to blood (i.e., inhibited gluconeogenesis), not sufficient to match muscular glucose consumption, resulting in hypoglycemia. Conversely, if exercise is performed late after insulin injection, insulinemia will be low inducing an upregulated hepatic gluconeogenesis, resulting in hyperglycemia (Wallberg-Henriksson, 1989).

The phenomena that happen during aerobic exercise in healthy individuals and in T1DM patients, soon after insulin administration or after long time intervals from insulin injection, are shown in **Figure 10**.

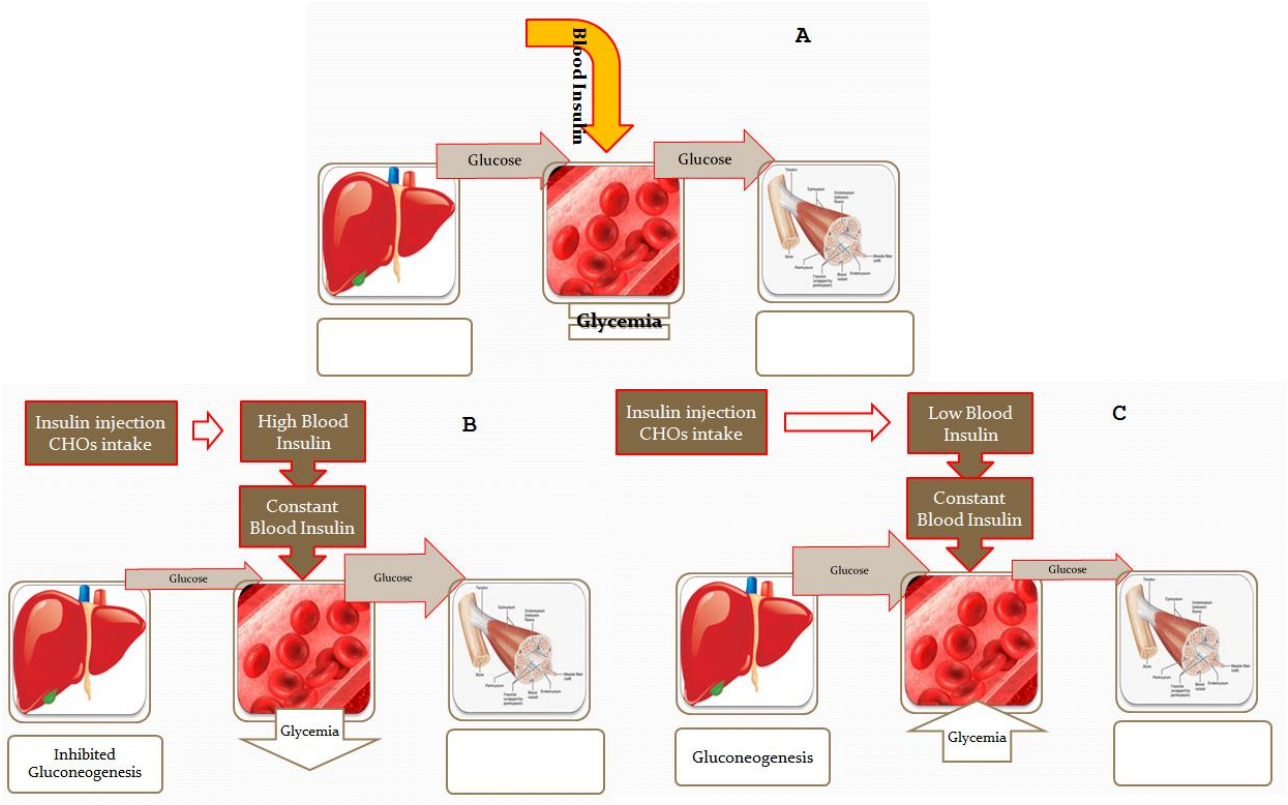

Figure 10: Glucose regulation during exercise at different times following insulin injection

(A) In an healthy individual, aerobic exercise induces a fall in blood insulin that regulates glucose from the liver to blood and from blood to muscles in order to maintain glycemia constant. (B) In T1DM patients, soon after insulin injection, blood insulin concentration is high and remains high during the exercise. High insulin inhibits liver gluconeogenesis that cannot match glucose uptake from the muscles, decreasing blood glucose levels. (C) In the opposite condition, far from insulin injection, blood insulin level is low and remains low throughout exercise, stimulating liver gluconeogenesis in excess, increasing glycemia.
(Modified from Wallberg-Henriksson, 1989)

ECRES algorithm shows many peculiarities, such as i) it is based on patient's habitual therapy, and no changes in insulin dose are mandatory; ii) patient's insulin sensitivity is taken in account through the individual dietary carbohydrate-to-insulin ratio; iii) patient's physical fitness level is considered to estimate the correct glucose pulse; iv) exercise intensity and duration are taken into account to estimate the amount of glucose oxidized, and v) exercise can be performed at any time of the day since the algorithm takes in account blood insulin level (Francescato et al., 2011). As

previously described in detail (Francescato et al., 2011), the algorithm estimates the patient's daily total insulin concentration profile, based on the usual therapy and standard pharmacokinetic curves of insulin analogs, as reported in the literature. Standard insulin curves are then adapted and re-scheduled based on patient's individual therapy. Later, patient's carbohydrate-to-insulin ratios are computed for three daily intervals (morning, afternoon and evening), and an efficacious insulin profile is calculated. Finally, each time point of the efficacious insulin curve is converted to the percentage of glucose oxidation rate required to maintain euglycemia. The relationship between blood insulin level and glucose supplement needed to prevent hypoglycaemia during moderate exercise has been found to be linear (Francescato et al., 2004). The last part of the algorithm consists in merging the results from the "insulinemic profile" with the estimated glucose oxidation rate during exercise, based on its intensity and duration (Francescato et al., 2005). Finally, the algorithm is ready to predict the amount of CHO to ingest prior to exercise, corrected for the eventual excess/lack glucose solved in the body, measured as the product between the difference between actual glycemia and the theoretical glycemia the patient should have at the time of exercise, and assuming an extracellular fluid volume of 0.27 $L \cdot kg^{-1}$ in males and 0.225 $L \cdot kg^{-1}$ in females. The excess/lack glucose solved in the body is finally subtracted/added to the amount of CHO to be actually consumed.

ECRES validity has been tested both in laboratory and field conditions.

During laboratory testing, 27 T1DM patients walked at constant intensity (i.e., constant heart rate) in three trials after different times following insulin injection (81 walks overall). Extra CHO were ingested based on ECRES algorithm estimation for each patient and time of the day, while glycemia was measured every 15 min. This study showed that, independently from the time of day, in 70.4 % of trials ECRES estimation adequately allowed T1DM patients to complete the exercise with a blood glucose level between 3.9 and 10 $mmol \cdot L^{-1}$ (Francescato & Carrato, 2011).

Distribution of participants depending on the final glycemia after exercise is illustrated in **Figure 11**.

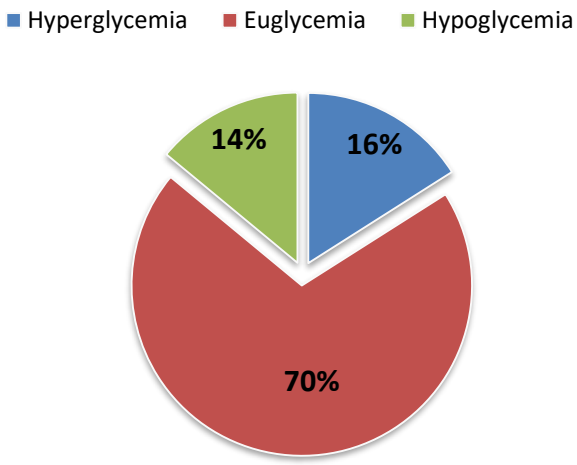

Figure 11: Final glycemia during ECRES validation

Independently from the time of day, ECRES estimation allowed T1DM patients to complete the exercise in euglycemia (3.9 and 10 mmol·L-1), hypoglycaemia (< 3.9 mmol·L-1) or hyperglycemia (> 10 mmol·L-1).
(From Francescato et al., 2011)

Physically activity is not always pre-planned, and it could be useful for patients with T1DM to have a portable instrument to estimate extra CHO. Thus, ECRES algorithm has been implemented in a smartphone application to test its validity on field conditions (Vuattolo et al., 2012). Smartphones are now widely diffused, and in a quite recent review it has been reported there are 137 mobile applications for diabetes management (Chomutare et al., 2011). However, at the time no application existed to offer decision support for management of glycemia during physical activity (Vuattolo et al., 2012). The mobile application has been developed for Android operative system, in order to allow a real time connection with other devices such as heart rate belts or glucose monitors. First, patients filled the system with their therapy and daily diet on a web server (with their specialist physician), where ECRES algorithm was uploaded. Forecasting version (not real time) has been tested simulating the same activity, therapy and diet observed in the laboratory based

validation (Francescato & Carrato, 2011) on the 27 patients' data sets, and the results were similar in both studies (Vuattolo et al., 2012). A version using SMS technology was developed, automatically sending a message with the suggested amount of CHO to the user; the results have been published on the "Associazione Medici Diabetologi" journal.

Further studies are necessary to validate ECRES algorithm on portable devices during different activities, conditions and time of day.

SUMMARY

Physical activity in T1DM patients is warmly recommended. However glycemic management represents a challenge for physicians, specialists and patients themselves. The risk of hypoglycemia is seen as a barrier for exercise, and many patients prefer to limit their volitional engagement in physical activity. On the other side, a common strategy to prevent hypoglycaemia consists in ingesting an excessive amount of CHO, resulting in initial hyperglycemia. In scientific literature it is possible to find specific tables and suggestions aimed at maintaining a good control on blood glucose levels, both adjusting insulin therapy (usually reducing insulin dose before exercise) or suggesting extra amounts of CHO. However, these findings are mainly rough and are not designed for specific cases, with patients needing to implement those suggestions through their own experience. ECRES algorithm estimates extra CHO for exercise, based on patients' specific characteristics and physical activity. In the first part of the algorithm, patient's usual therapy, diet and individual fitness level are uploaded in the system. Secondarily, for each use patients can adapt their current therapy or diet variations, and specify exercise duration and intensity (heart rate). The algorithm computes the amount of extra CHO suggested to prevent hypoglycemia without incurring in hyperglicemia. In a first laboratory validation, ~71 % of trials were successful. This system seems to be primarily useful for patients that are not able to manage their blood glucose during exercise, as sedentary patients, and the possibility to use it through a portable device could encourage those patients to be more active.

1.3.2 Hydration and dehydration in T1DM

It is supposed that hyperglycemia may influence hydration status in diabetic patients, because it could alter fluids reabsorption in the kidneys. When blood glucose concentration is within the normal physiological range, almost all of glucose in the filtrate is reabsorbed in the proximal tubule, and the amount of glucose in urine is negligible (Yardley & Riddell, 2016). However, when blood glucose concentration is above certain values, glucose is excreted with urine. This is well explained since average tubular maximum transport for glucose reabsorption is around 375 mg/min (although inter-individual variability was found) and average glomerular filtration rate (GFR) is 1.25 dL/min; thus, the renal threshold for glucose is approximately 300 mg/dL (16.7 mMol) (Johansen et al., 1984). However, since there are differences between nephrons maximum transport, some glucose molecules in the filtrate escape the carrier molecules even if they are not completely saturated, and glucose can be found in the urine before the renal threshold for glucose is reached. This usually occurs once blood glucose levels reach 9-10 mMol (162-180 mg/dL) (Johansen et al., 1984).

Schematic approximate values of glucose reabsorption and excretion in the kidneys, depending on blood glucose concentration are illustrated in **Figure 12**.

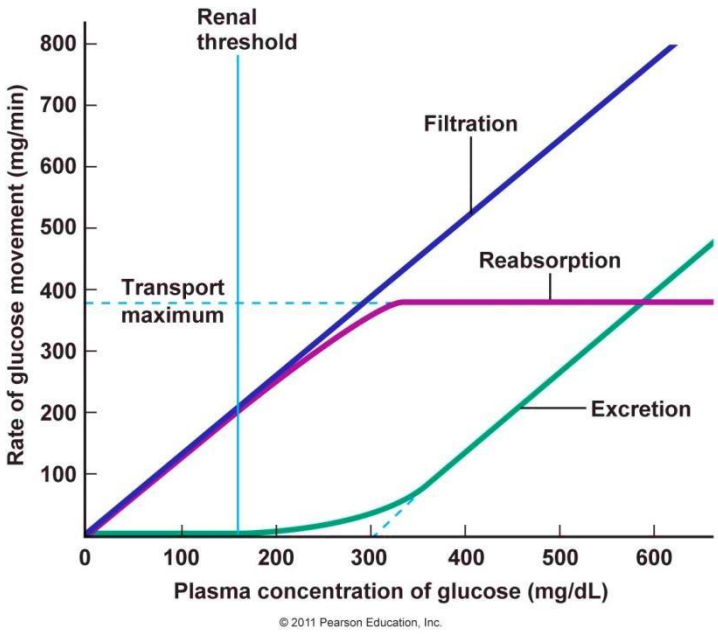

Figure 12: Renal glucose filtration, excretion and reabsorption

Renal glucose reabsorption and excretion with different plasma glucose concentration (mg/dL). When plasma glucose concentration is greater than ~180 mg/dL (10.0 mMol), glucose is not completely reabsorbed and part of it is excreted in urine. When glycemia is above 300 mg/dL (16.7 mMol), transport maximum for glucose of 375 mg/min is surpassed: glucose reabsorption stays constant and glucose excretion increases linearly with the amount of glucose filtered. (From Pearson Education Inc., 2011)

Fluid balance may be compromised in T1DM, since glycosuria (i.e., the abnormal presence of glucose in urine) can substantially increase water loss through osmotic diuresis (Dhatariya, 2008), increasing the risk of dehydration if fluid losses are not adequately compensated. Furthermore, in T1DM patients poor blood glucose control may induce a renal resistance to vasopressin, decreasing the kidneys' ability to retain water during exercise (McKenna et al., 2000).

T1DM and altered blood glucose concentration may also influence electrolyte balance. Hyperinsulinemia causes sodium retention in healthy individuals (Quinones Galvan et al., 1995; Sechi & Bartoli, 1996), and persistent hyperglycemia may lead to sodium retention through the activation of sodium/glucose cotransporter in the proximal tubule (Korner et al., 1994). The condition of hyperglycemia lasting no longer than 2 h seems to not largely influencing plasma

concentration of sodium, chloride, and calcium, but it might increase potassium concentration, potentially leading to muscle weakness and cardiac arrhythmia (Caduff et al., 2011). In addition, high blood glucose concentration may be associated with greater urinary magnesium excretion, potentially leading to muscle cramps (Djurhuus et al., 2000).

Dehydration effects on physiological responses and performance have been discussed in the previous chapters; however, it is not completely clear if dehydration affects CHO oxidation. It has been observed that the RER (an index of substrate utilization) was greater during progressive dehydration up to 2.9 % of BM loss, both during cycling and running, when compared to a well hydrated condition, suggesting a greater whole body CHO oxidation (Fallowfield et al., 1996; Hargreaves et al., 1996). These results have been disputed in a more recent study suggesting that dehydration > 2% BM does not influence glucose oxidation in healthy individuals (Logan-Sprenger et al., 2013). Nevertheless, it must be noted that the same authors found a trend to a greater CHO oxidation when participants were hypohydrated > 1.5% BM, which became significant after the first 60 min of exercise (Logan-Sprenger et al., 2015).

Respiratory exchange ratio (RER) values during constant exercise in a progressive dehydration trial or during a well-hydrated control condition are shown in **Figure 13**.

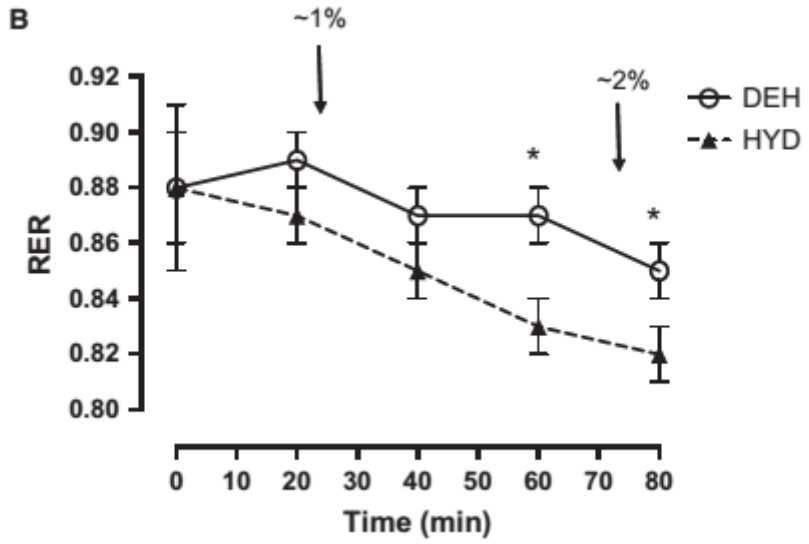

Figure 13: Respiratory Exchange Ratio during dehydration

*Respiratory Exchange Ratio (RER) during cycling at 65% for 90 min, in Hydrated (HYD) and Dehydrated conditions (DEH). Arrows (↓) indicate 1% and 2% BM loss in DEH. Greater RER indicates a greater utilization of carbohydrate as fuel for exercise. *Significantly greater than HYD trial (P<0.05).*

(From Logan-Sprenger et al., 2015)

It was suggested that glycogen metabolism may also be affected by dehydration. Indeed, it has been observed that muscle glycogen utilization during a 2 h cycling was 24% greater during a progressive dehydration protocol with a fluid loss > 2% of body mass, when compared with the control condition (i.e., maintaining euhydration) (Logan-Sprenger et al., 2013).

Glycogen depletion during a constant power cycling and the subsequent time trial while participants where in a well-hydrated or dehydrated condition is illustrated in **Figure 14**.

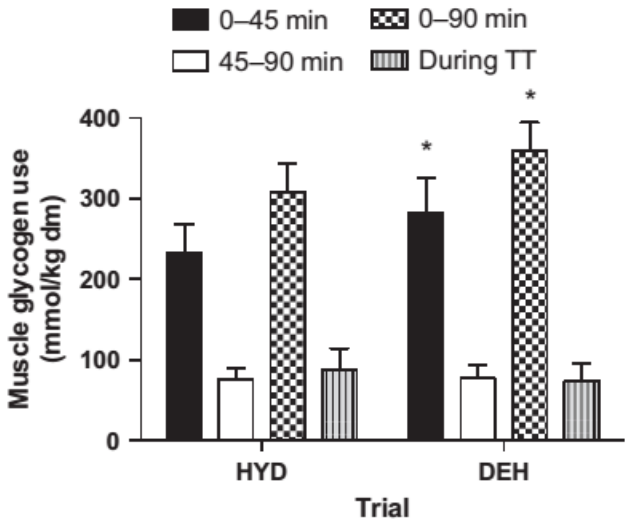

Figure 14: Glycogen depletion during dehydration

*Muscle glycogen use during cycling at 65% for 90 min, and after the consequent Time Trial, in Hydrated (HYD) and Dehydrated conditions ~ 3% BM (DEH). *Significantly greater than HYD trial (P<0.05).*

(From Logan-Sprenger et al., 2013)

Actually, there are few studies investigating hydration status and fluid intake in T1DM patients, and their behaviours during physical activity. Based on the results from the above presented studies, it is possible to suppose that T1DM patients are at a greater risk of becoming dehydrated during physical activity. Fluid and carbohydrate provision during prolonged exercise is critical for safety and performance in T1DM. Excessive carbohydrate concentration seems to decrease the rate of gastric emptying and absorption because of the increased osmolality, lessening fluid transition into the bloodstream (Osterberg et al., 2010) and possibly causing gastric distress (Gisolfi et al., 1998). It may be indicated to consume an 8-10% carbohydrate drink with electrolytes, ingested before and during exercise, to maintain fluid, electrolytes and carbohydrate in T1DM athletes. Post exercise ingestion of beverages with high carbohydrate concentration may increase glycogen storage, enhancing intracellular fluid retention, and should be considered when evaluating both fluid balance and glycemia (Olsson & Saltin, 1970; Tarnopolsky et al., 1997).

SUMMARY

In this chapter, some concepts behind fluid homeostasis during exercise have been discussed in reference for individuals with T1DM. It was observed as alterations in blood glucose concentration may affect renal function, potentially decreasing water reabsorption and increasing urine production. Diabetes may also affect electrolytes balance, possibly leading to alterations of normal physiological functions, including fluid balance. The interaction between diabetes and hydration should be carefully taken into account not only for diabetes influence on fluid homeostasis, but also for the effects dehydration may elicit on glucose uptake and metabolism. Some authors report a greater utilization of CHO during exercise while hypohydrated, but results are still debated and need further studies to better understand dehydration effects on metabolism. Actually, no studies on hydration status, fluid intake, or hydration habits in T1DM physically active patients have been found. If results presented above would be confirmed, adequate hydration in T1DM athletes may be of great importance not only for fluid balance, but also for maintaining blood glucose concentration.

2. AIMS

During the first half of my PhD activity I was involved in research about physical activity in insulin-dependent diabetic patients, and about their carbohydrate needs for preventing glycemic imbalances. This research topic, that combines nutrition and exercise physiology in a clinical environment, was based on the potential benefits of the ECRES (Exercise Carbohydrate Requirements Estimating Software) algorithm, already developed and validated in this department. One of the key aspects of this algorithm is its ability to predict CHOs needs for exercise based on both patients' characteristics, and exercise intensity, duration, and time when it is performed. Thus, aim of the first part of my study was to further test and validate ECRES estimation in a peculiar condition, as the 24x1h relay race event involving a team of T1DM patients running for 1 h in a day. At a later time I focused on the physiological differences in mechanical efficiency and CHOs oxidation during exercise between healthy individuals and patients with T1DM.

In the second half of my PhD I deepened my knowledge and my interests about hydration science, and its relevance for health and performance. Although this research topic has been well investigated all around the world, it was not possible to find references about hydration during exercise in the Italian population. My research then consisted in investigating hydration habits in a large sample of Italian athletes, to define fluid intake behaviours in this population and differences among sexes, age, or sports characteristics. With a similar protocol, the last part of my PhD consisted in combining hydration science and the experience with T1DM patients, investigating hydration habits in diabetic athletes, to better understand their needs and to compare their behaviours with healthy individuals from the third part study.

3.RESULTS

3.1 Strategies for preventing hypoglycemia during a 24x1-h marathon, and comparison with ECRES algorithm

Patients walked/ran an average distance of 10.4±2.8 km (range 6.5 km to 15.6 km). An average heart rate of 167±11 bpm (corresponding to 90±6% of HR_{max}) was reached in a few minutes and thereafter remained quite stable over time (Friedman test, F = 6.04, P = 0.812).

At the start of the exercise, median blood glucose concentration was 9.3 mmol·L^{-1} (IQR 7.3 mmol·L^{-1} to 13.3 mmol·L^{-1}), as illustrated in **Figure 15**.

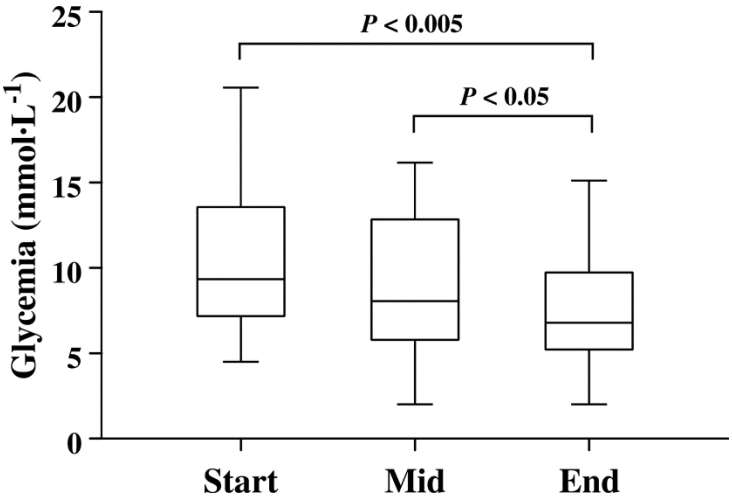

Figure 15: Blood glucose during 1 h runs

Box-and-whisker plot comparing the blood glucose levels measured before, at the middle and at the end of the 1-h runs

After about 30 min, glycaemia was not changed significantly (median 8.1 mmol·L^{-1}, IQR 6.6 mmol·L^{-1} to 11.9 mmol·L^{-1}, Wilcoxon test, P = 0.523), whereas at the end of the run glycaemia was significantly lower than both the preceding values (median 6.8 mmol·L^{-1}, IQR 5.6 mmol·L^{-1} to 9.2 mmol·L^{-1}, Wilcoxon test, P = 0.006 and P = 0.011 for the start and intermediate glycaemia,

respectively). The fall in glycaemia from the start to the end of the exercise amounted to a median value of -1.8 mmol·L^{-1} (IQR -0.4 mmol·L^{-1} to -5.3 mmol·L^{-1}). An increase in glycaemia was observed in 3 patients, all of them exercising at least 4.5 hours after the last meal.

Figure 16 illustrates, for each of the three measuring time points, the number of patients where glycaemia was on target, too low (< 3.9 mmol·L^{-1}), or too high (> 10 mmol·L^{-1}).

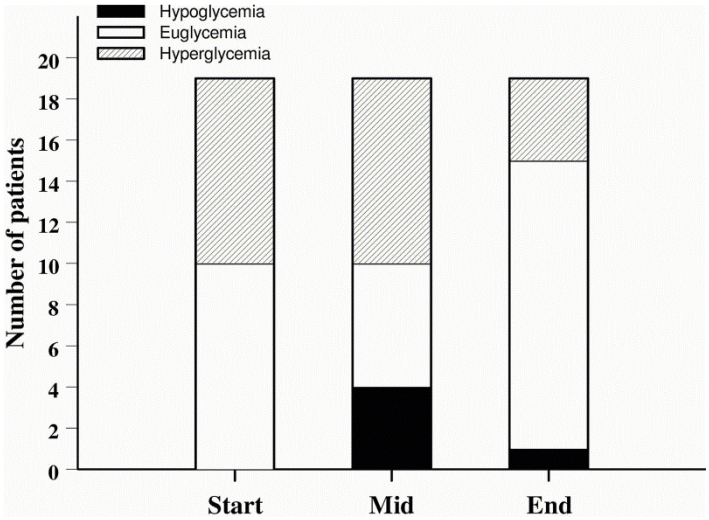

Figure 16: Blood glucose classification during 1 h runs

Histogram illustrating the number of patients showing excessively low glycaemia (<3.9 mmol·L^{-1}; black bars), on target glycaemia (3.9-10.0 mmol·L^{-1}; white bars) or excessively high glycaemia (>10.0 mmol·L^{-1}; hatched bars) before, at the middle and at the end of the 1-h runs

About half of the patients (9 out of 19) started the exercise with hyperglycaemia. At the middle of the run hyperglycaemia was observed in 9 patients (6 of them being so also at the start), while at the end of exercise only 4 patients (21%) showed hyperglycaemia. None of the patients started exercising with too low glycaemia. Hypoglycaemia was observed in 4 patients at the middle of the run, with only one being unable to recover before the end. Glycaemia was observed to be on target in 14 patients (74%) at the end of the exercise. No significant differences were observed in the blood glucose levels measured at the three time points by comparing patients under MDI or insulin

pump, as well as by comparing patients having received (or not) extra carbohydrate (Mann-Whitney test, $P > 0.254$ for all the comparisons).

Patients consumed a median of 30 g of extra carbohydrate (IQR 0 g to 71 g) during the last meal, just before and/or during the exercise. No significant difference was detected between patients on MDI and insulin pump users (Mann-Whitney test, $P = 0.709$). Five patients on MDI did not consume extra carbohydrate; two patients (out of the remaining 5) reduced their insulin bolus to 60% with the last meal while consuming the usual amount of carbohydrate. Only one patient among the insulin pump users did not consume extra carbohydrate, having completely suspended the basal infusion rate during exercise. Four patients among the other insulin pump users reduced their basal infusion rate to 60% during their races.

The amount of extra carbohydrate estimated by the ECRES algorithm (median 38 g; IQR 24 g to 68 g) was not significantly different from the corresponding quantity actually consumed by patients (Wilcoxon test, $P = 0.653$), the two quantities being significantly related to each other ($R = 0.646$, $n = 19$, $P = 0.003$).

The CHO consumption as estimated by the ECRES algorithm, is compared to the amount actually consumed in **Figure 17**.

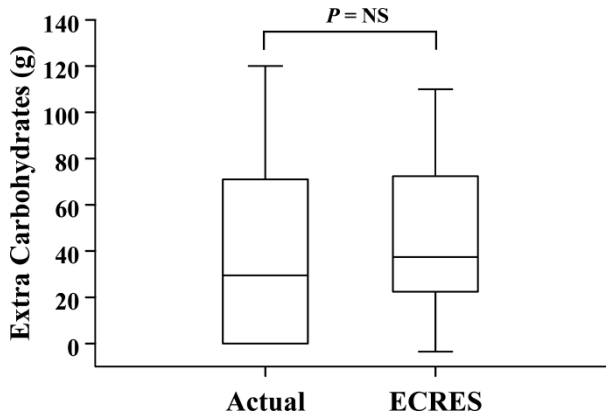

Figure 17: CHO consumption during 1 h runs

Box-and-whisker plot comparing the amounts of extra carbohydrate actually consumed by the group of 19 patients with T1D and the quantities estimated by the ECRES algorithm

Of note, the ECRES algorithm estimated a greater amount of extra carbohydrate (+17 g) for the only patient who experienced hypoglycaemia by the end of his/her exercise. Conversely, the amount of extra carbohydrate estimated by the ECRES algorithm for the patients who were hyperglycaemic by the end of their races was lower than the amount actually consumed (about -13 g; with the exception of one patient, who showed a null value for both the amount consumed and the estimated amount of carbohydrate).

3.2 ECRES algorithm validation

Participants' recruitment for this study is still underway. Until now, 23 individuals (M: 13 F: 10) with insulin-dependent diabetes (T1DM) voluntary participated to the validation of ECRES algorithm. Among them, 15 were classified as "Athletes" (ATH) and 8 were classified as "Sedentary" (SED) participants.

Participants characteristics are listed in **Table 4**.

Table 4: *Descriptive statistics of participants (n= 23), comparison between athletes (ATH) and sedentary (SED) T1DM patients. Data are presented as mean ± sd.*

	ATH n= 15	SED n= 8	TOTAL n= 23	p
Age (y)	45 ± 11	38 ± 14	43 ± 12	NS
BM (kg)	73 ± 12	75 ± 19	74 ± 14	NS
ECF (L)	18.9 ± 4.2	18.7 ± 5.2	18.9 ± 4.4	NS

Notes: Age (y), Body Mass (BM, kg), Extracellular Fluid (ECF, L) estimated by validated methods. p: Significance Level, NS: No significant differences were observed between groups.

While using the reference method (REF) for carbohydrate (CHO) need estimation, ATH began their exercise with a blood glucose concentration of 131 ± 36 mg/dL (7.3 ± 1.9 mMol), and for SED it was 128 ± 16 mg/dL (7.1 ± 0.9 mMol). At the end of the exercise, glycemia was 155 ± 83 mg/dL (8.6 ± 4.6 mMol) for ATH and 147 ± 62 mg/dL (8.2 ± 3.4 mMol) for SED, with a mean difference between pre and post exercise of -21 ± 70 mg/dL (-1.2 ± 3.9 mMol) and -20 ± 66 mg/dL (-1.1 ± 3.7 mMol) for ATH and SED respectively. Reference method indicated to participants to consume an amount of extra CHO before the exercise of 23 ± 4 g for ATH, and 24 ± 5 g for SED. None of the presented variables was significantly different between ATH and SED during REF.

When ECRES was used for carbohydrate (CHO) need estimation, ATH began their exercise with a blood glucose concentration of 151 ± 50 mg/dL (8.4 ± 2.8 mMol), and for SED it was 136 ± 39 mg/dL (7.6 ± 2.2 mMol). At the end of the exercise, glycemia was 128 ± 53 mg/dL (7.1 ± 2.9 mMol) for ATH and 140 ± 79 mg/dL (7.8 ± 4.4 mMol) for SED, with a mean difference between pre and post exercise of 24 ± 50 mg/dL (1.2 ± 3.9 mMol) and -4 ± 73 mg/dL (0.2 ± 4.1 mMol) for ATH and SED respectively. Reference method indicated to participants to consume an extra amount of CHO before the exercise of 10 ± 10 g for ATH, and 23 ± 16 g for SED. While using ECRES, extra CHO consumption was significantly greater for SED than for ATH ($p < 0.05$).

CHO oxidation (CHOox) during exercise, determined by the sum of extra CHO and difference in blood glucose concentration, indicated that during REF, ATH oxidised 19 ± 14 g, while SED oxidised 20 ± 13 g. During ECRES, ATH oxidised 14 ± 9 g and SED oxidised 24 ± 8. No significant difference was observed between REF and ECRES in both groups.

Table 5 summarizes the results for glycemia at the start and at the end of exercise, CHO consumption, and CHO oxidation while using REF or ECRES, in ATH and SED T1DM patients.

Table 5*: Participants power output, carbohydrate consumption, and carbohydrate oxidation during different steps of the incremental exercise protocol (n= 50), with comparison between healthy individuals (CTRL) and insulin-dependent diabetic patients (T1DM). Data are presented as mean ± sd.*

		ATH n= 15	SED n= 8	p
Glycemia Pre (mg/dL)	REF	131 ± 36	128 ± 16	NS
	ECRES	151 ± 50	136 ± 39	NS
Glycemia Post (mg/dL)	REF	155 ± 83	147 ± 62	NS
	ECRES	128 ± 53	140 ± 79	NS
Δ Glycemia (mg/dL)	REF	-21 ± 70	-20 ± 66	NS
	ECRES	24 ± 50	-4 ± 73	NS
extraCHO (g)	REF	23 ± 4**	24 ± 5	NS
	ECRES	10 ± 10**	23 ± 16	< 0.05
CHOox (g)	REF	19 ± 14	20 ± 13	NS
	ECRES	14 ± 9	24 ± 8	< 0.05

Notes: Blood glucose concentration at the start of exercise (Glycemia Pre, mg/dL), blood glucose concentration at the end of exercise (Glycemia Post, mg/dL), difference in blood glucose concentration at the begin and at the end of exercise (Δ Glycemia, mg/dL), extra carbohydrate consumption before exercise (extraCHO, g), and amount of carbohydrate oxidised during exercise (i.e., the sum of extraCHO and Δ Glycemia) (CHOox, g). Reference method (REF) and ECRES algorithm, T1DM individuals classified as athletes (ATH) and sedentary (SED). p: Significance Level between groups, NS: No significant differences were observed between groups. ** p< 0.01 significance between trials.

A tendency for a significantly different Δ Glycemia was found only in ATH individuals, with a tendency to increased blood glucose level during REF than during ECRES (difference between start and end of exercise glycemia: -21 ± 70 mg/dL and 24 ± 50 mg/dL, respectively; p = 0.055). Negative values indicate an increase in blood glucose concentration. Significant difference was not observed for Δ Glycemia in SED individuals.

Only in ATH the amount of suggested extra CHO was found to be significantly greater during REF than during ECRES (23 ± 4 g and 10 ± 10 g, respectively; $p < 0.01$).

Figure 18 shows extra CHO consumption between REF and ECRES in ATH and SED individuals.

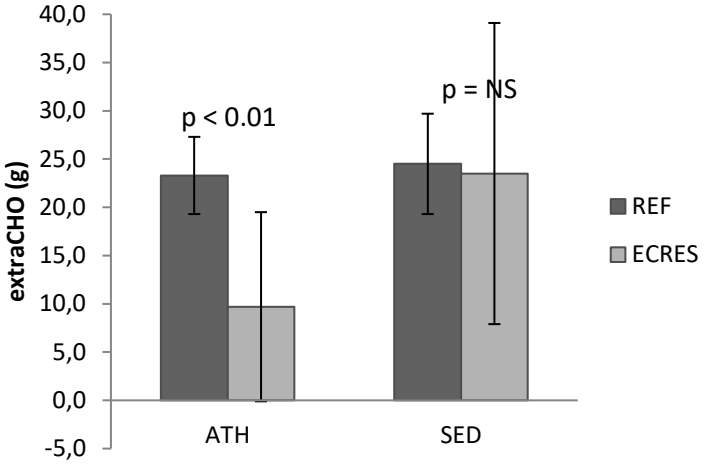

Figure 18: CHO consumption using the reference method or ECRES

Difference in extra carbohydrate consumption (extraCHO, g) between the reference method (REF, dark grey) and ECRES (light grey) in athletes (ATH) and sedentary (SED) T1DM individuals. p values indicate the statistical significance level for difference between ATH and SED. Significance for $p < 0.05$.

When REF was used to estimate extra CHO, 4 patients classified as ATH concluded exercise with blood glucose above 180 mg/dL and 1 patient concluded his exercise with blood glucose below 70 mg/dL. As a consequence, 10 ATH individuals (~66 %) were characterised by a blood glucose level considered euglycemic (70-180 mg/dL). Reference method applied to SED induced hyperglycemia (blood glucose > 180 mg/dL) in only one patient, while 7 SED individuals (~88%) terminated exercise with a good blood glucose level (70-180 mg/dL).

When ECRES was used to estimate extra CHO, 3 patients classified as ATH terminated exercise with blood glucose above 180 mg/dL and 1 patients terminated exercise with blood glucose below

70 mg/dL. As a consequence, 11 ATH individuals (~73 %) were characterised by a blood glucose level considered euglycemic (70-180 mg/dL). ECRES method applied to SED induced hyperglycemia (blood glucose > 180 mg/dL) in 2 patients, and hypoglycemia (blood glucose < 70 mg/dL) in 2 patients, while 4 SED individuals (50%) terminated exercise with a good blood glucose level (70-180 mg/dL).

For ATH and SED participants pooled together, when REF was used to estimate extra CHO, 17 individuals (74 %) concluded exercise with euglycemic glucose level, while 6 individuals (26 %) were classified as hypoglycemic or hyperglycemic. Conversely, when ECRES was used to estimate extra CHO, 15 individuals (65 %) ended exercise with euglycemic glucose level, while 8 individuals (35 %) were classified as hypoglycemic or hyperglycemic. No significant difference was observed in the proportion of participants presenting euglycemia while REF or ECRES methods were used to estimate extra CHO.

The frequency distribution of T1DM patients who were classified as euglycemic, hypoglycemic, or hyperglycemic at the end of exercise, depending on the method used to estimate extra CHO is shown in **Figure 19**.

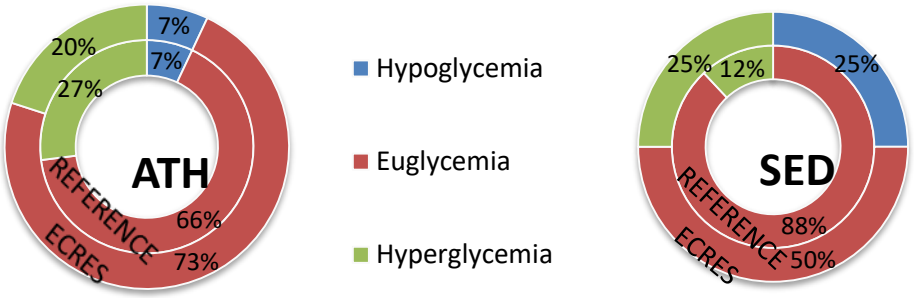

Figure 19: Blood glucose classification when using the reference method or ECRES

Frequency distribution of T1DM patients who were classified as euglycemic (70-180 mg/dL), hypoglycemic (< 70 mg/dL), or hyperglycemic (> 180 mg/dL) at the end of exercise, in physical active (ATH) and sedentary (SED) individuals, depending on the method used to estimate extra carbohydrate for exercise: internal circle reference method (Perkins & Riddell, 2006), external circle ECRES algorithm (Buoite Stella et al., unpublished results)

3.3 Hydration habits in Italian athletes

From the survey we obtained results from a sample of 352 healthy sportsmen, almost equally distributed between males and females (M: 191 [54 %], F: 161 [46 %]). Participants age ranged from a minimum of 8 y to a maximum of 63 y (median: 21 ± 13 y, 95 % CI: 20 to 22), with 230 of them younger than 18 y. Mean body mass was 61 ± 16 kg, while mean body height was 1.68 ± 0.13 m, resulting in a BMI of 21.1±3.4 $kg \cdot m^{-2}$. All the participants were within healthy guidelines, and passed the medical examination for competitive sports. All dependent variables apart from body height, body mass and BMI violated normality assumptions. Males were 4±1 y older than females ($P < 0.05$, Cohen's $d = 0.309$), with greater body mass (9 ± 2 kg, $P < 0.01$, Cohen's $d = 0.612$) and height (0.08 ± 0.01 m, $P < 0.01$, Cohen's $d = 0.603$).

Participants characteristics according to gender and competitive level are summarized in **Table 6**.

Table 6*: Participants' anthropometrics (n= 289), comparison between males and females, and between different sport competitive level. Data are presented as mean ± sd.*

Personal Characteristics	Male			Female		
	INT n=5	NAT n=72	REG n=80	INT n=6	NAT n=48	REG n=78
Age (y) *	19±6	21±12	26±16	18±6	18±10	20±12
Mass (kg) *	78 ± 10	66 ± 17	65 ± 16	58 ± 8	57 ± 15	54 ± 13
Height (m) * †	1.80±0.09	1.73±0.14[b]	1.70±0.13[b]	1.68±0.60	1.65±0.10[b]	1.62±0.11[b]
BMI ($kg \cdot m^{-2}$) *	24.9±1.9	21.3±3.4	21.9±3.8	20.8±2.0	20.8±3.7	20.3±3.1

Notes: INT, international competition level athletes; NAT, national competition level athletes; REG, regional athletes. (*) Significant difference between males and females within a given competition level, (†) significant difference between competition level within-sex ($P < 0.05$). With males and females pooled, ([b]) indicates greater values in national level athletes compared to regional athletes.

Questionnaire responses indicated that 3.1 % of the athletes were involved in international-level competitions, 34.1 % in national competitions, and 44.9 % in regional competitions, whilst 17.9 %

did not report their competition level. The most commonly played sports were basketball (n = 58, M: 44 F: 14), volleyball (n = 54, M: 3 F: 51), tennis (n = 31, M: 24 F: 7) and athletics distance events (n = 29, M: 10 F: 19).

Figure 20 illustrates competitive level distribution (A) and participants' sport type played (B) according to gender.

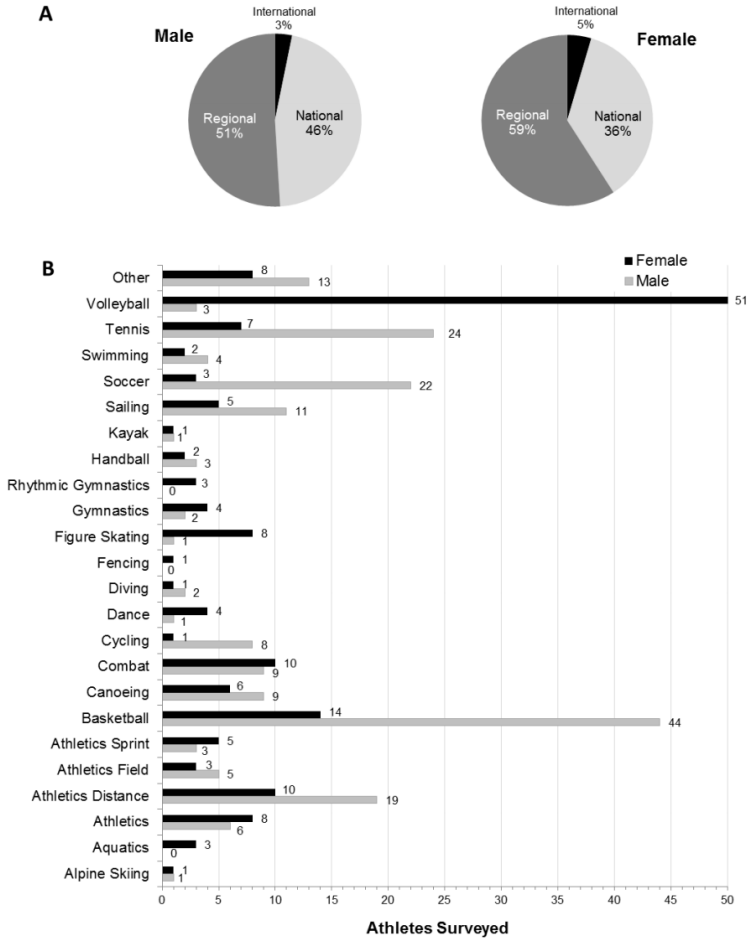

Figure 20: Participants sport level and played sport

(A) Athlete distribution in different competitive levels for males and females (%). (B) Sport frequencies (counts) for males and females. "Aquatics" includes: diving, fin swimming, synchronised swimming; "Combat" includes: karate, judo, kick boxing; "Athletics field" includes: jumping and throw events; "Athletics sprint" includes: 100 m, 110 m hurdles, 200 m track events), Athletics distance (any running distances over 400 m). Athletics alone indicates participants who did not report a specific speciality.

About sport training characteristics, total training volume per week was 6.5 ± 3.2 h, and single training bout duration was 1.7 ± 0.5 h. On average, participants reported to have 1.7 ± 1.4 pauses or breaks where they could drink. Fluid intake during the training bout was reported to be 0.46 ± 0.36 L, or 0.28 ± 0.21 $L \cdot h^{-1}$ when normalized per training duration; fluid intake normalized per unit of body mass was 4.8 ± 3.6 $mL \cdot (kg \cdot h)^{-1}$.

There were no significant differences between males and females in terms of total training volume per week, single training bout duration, and number of pauses per training to drink, or total fluid intake, even when normalised by total training hours or body mass. The Kruskal-Wallis ANOVA indicated, however, the International athletes exercised 6.5±0.9 h longer than National level athletes ($P < 0.01$, Cohen's d = 1.308) each week; in turn these last trained 1.9 ± 0.4 h longer than the Regional athletes ($P < 0.01$, Cohen's d = 0.690).

A single training bout was longer for International athletes by 0.6 ± 0.2 h compared to National level athletes ($P < 0.01$, Cohen's d = 0. 949), and National level athletes reported longer training durations by 0.2 ± 0.1 h compared to Regional athletes ($P < 0.01$, Cohen's d = 0. 283).

Only a minor part (21.9 %) of the sample reported they did not feel thirsty enough to drink something at the end of their training. Most athletes (41.2 %) reported 'no encouragement to drink' by their coaches, whereas a smaller percentage (28.3 %) reported the most consistent encouragement rank.

Training characteristics, fluid intake, and hydration habits in the sample population are summarized in **Table 7**.

Table 7: *Participants' (n= 289) answers to training and hydration-specific questions from the survey, results compared between different sport competitive level.*

Training and Hydration-Specific Questions		INT (n=11)	NAT (n=120)	REG (n=158)
How many hours do you practice each week? *	(hours)	13.8±6.4 [a]	7.3±2.9[b]	5.4±2.6
How long does a typical training bout last? *	(hours)	2.3±0.8[a]	1.7±0.4[b]	1.6±0.6
How many pauses or breaks to drink do you have during a typical training?	(count)	2.2±1.3	1.9±1.7	1.6±1.2
What type of beverage do you normally drink before, during or after training?	Sport Drink Water Fruit Juice Tea Nothing (%)	18.2 72.7 0 0 9.1	8.3 73.3 0 0 18.4	7.6 77.2 0.6 1.3 13.3
How much fluid (volume) did you drink in your last training session?	(L)	0.76±0.65	0.49±0.43	0.43±0.27
At the end of your training, did you feel thirsty enough to drink something? (yes/no)	Yes (Y) No (N) (%)	72.7 27.3	77.5 22.5	80.4 19.6
Does your coach actively encourage you to drink at some point during the training session?	Never Sometimes Always (%)	27.2 36.4 36.4	30.4 41.0 28.6	27.0 39.0 34.0

Notes: INT, international competition level athletes; NAT, national competition level athletes; REG, regional athletes. Mean ± standard deviation. (*) Significant difference between competition levels ($P < 0.05$). ([a]) indicates greater values in international level compared to national and regional level athletes, ([b]) indicates greater values in national level athletes compared to regional athletes.

Mean fluid intake when coach's encouragement was 'never' amounted to 0.19 ± 0.17 $L \cdot h^{-1}$; it was 0.28 ± 0.18 $L \cdot h^{-1}$ if the coach's encouragement was ranked 'sometimes', and 0.38 ± 0.24 $L \cdot h^{-1}$

if ranked as 'always'. Athletes who were 'always' encouraged to drink reported significantly greater fluid intakes than athletes who were 'never' encouraged to drink (by 0.19 ± 0.03 $L \cdot h^{-1}$; $P < 0.01$). Fluid intake for the most popular sports in males were: basketball 0.29 ± 0.13 $L \cdot h^{-1}$, tennis 0.52 ± 0.28 $L \cdot h^{-1}$, soccer 0.20 ± 0.14 $L \cdot h^{-1}$, and athletics "distance" events 0.18 ± 0.18 $L \cdot h^{-1}$. Fluid intake for the most popular sports in females were: volleyball 0.34 ± 0.16 $L \cdot h^{-1}$, basketball 0.28 ± 0.08 $L \cdot h^{-1}$, combat sports 0.08 ± 0.08 $L \cdot h^{-1}$, and athletics "distance" events 0.24 ± 0.29 $L \cdot h^{-1}$. The Kruskal-Wallis ANOVA indicated fluid intake was not significantly different between the most popular sports within-sex. The Mann-Whitney U test indicated fluid intake was not significantly different between males and females in their most popular sports.

Mean fluid intake during training in the most popular played sports in both males and females is illustrated in **Figure 21** according to gender.

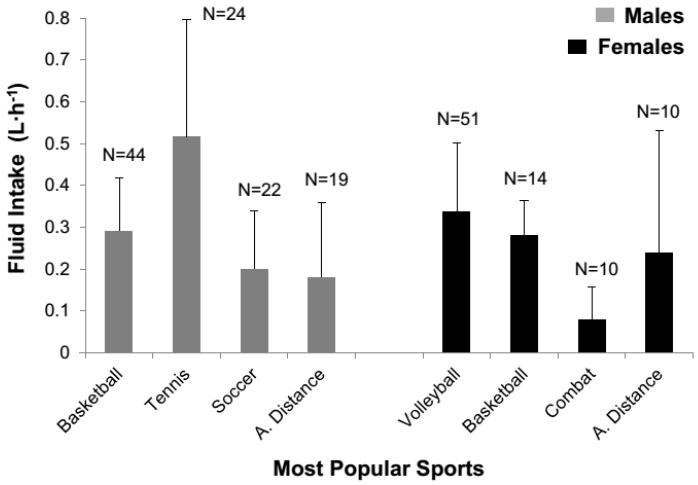

Figure 21: Most popular played sports

Mean fluid intake (L·h-1) and standard deviations during training between the most popular sports, differentiated by sex. Most popular: M: basketball n=44, F: volleyball n=51; second most popular: M: tennis n=24, F: basketball n=14; third most popular: M: soccer n= 22, F: combat sports n=10; fourth most popular for both sexes: athletics "distance" events, M: n=19, F: n=10. No significant difference in fluid intake was observed either between-sport or between-sex within a given preferred sport.

Water was most commonly ingested during training (0.28 ± 0.21 $L \cdot h^{-1}$), irrespective of the athletes' sex or competitive level. Only 23 athletes (6.5 % of total sample) reported drinking more than 0.6 $L \cdot h^{-1}$, in accordance to ACSM guidelines.

Participants' fluid intake distribution in reference with well-established international position stands for fluid replacement during exercise is shown in **Figure 22**.

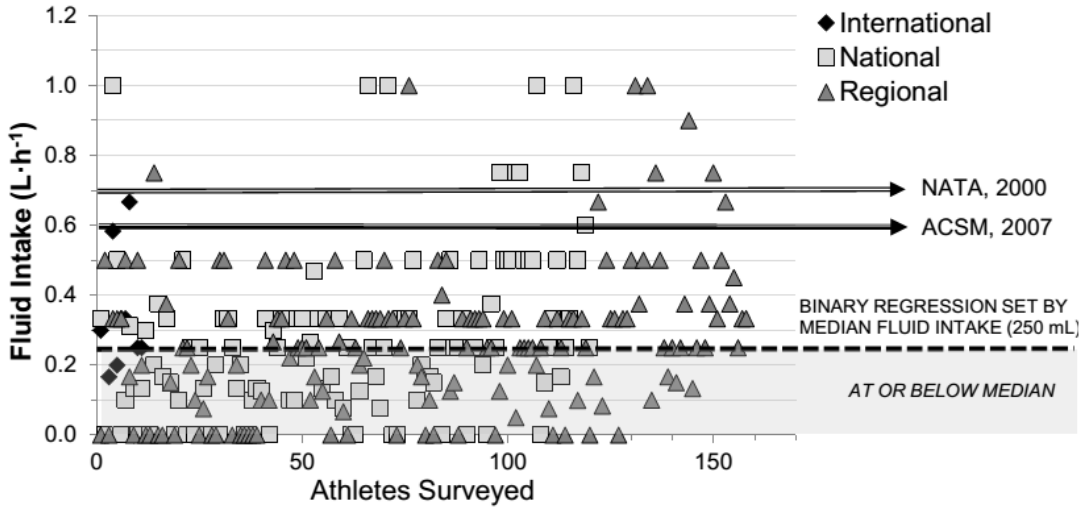

Figure 22: Fluid intake distribution for different sport levels

Individual fluid intake values (L) during exercise training, differentiated at the International (black diamonds), National (light grey squares) and Regional (grey triangles) competitive level. Horizontal arrows indicate the suggested fluid volume intake values during exercise from the National Athletic Trainers Association (NATA, Casa et al. 2000) and American College of Sports Medicine (ACSM) position stands (Sawka et al. 2007). Dotted horizontal line indicates the median fluid intake during training (0.25 L) used in the binary logistic regression model.

A binary logistic regression was performed to assess the association between independent variables and the binary high or low fluid intake values during training, as shown in **Table 8**. The full model contained predictors for: age, sex, competitive level, pauses to drink, training per week, training duration, and coaching stimuli; the model was statistically significant, $\chi 2$ (8, n=352,

=70.55, P < 0.01), indicating it could distinguish between respondents who reported fluid intake above or below the median. The Hosmer and Lemeshow test was not significant, indicating a goodness of fit for this model. The model explained between 24.4 % (Cox and Snell R square) and 32.7 % (Nagelkerke R squared) of the common variance in fluid intake, and had the strength to discriminate and correctly classify 68.7 % of all cases. According to the model, the log-of-the-odds of an athlete being classified in the 'high' or 'low' fluid intake group was positively related to the number of pauses to drink (Wald test: 22.150, P < 0.01), negatively related to training duration (Wald test: 17.269, P < 0.01), and positively related to coaching stimuli (Wald test: 8.592, P < 0.05).

Table 8: *Binary logistic regression summary.*

	B	S.E.	Wald	df	P.	Exp(B)	95% CI	
							Lower	Upper
Age	-0.13	0.13	1.067	1.0	0.302	0.987	0.962	1.012
Sex	-0.528	0.301	3.069	1.0	0.080	0.590	0.327	1.065
Competition level	-0.060	0.291	0.431	1.0	0.836	0.942	0.532	1.665
Pauses to drink	0.771	0.164	22.150	1.0	**0.00**	2.162	1.568	2.981
Training per week (h)	0.013	0.065	0.042	1.0	0.838	1.013	0.893	1.150
Training duration (min)	-2.237	0.538	17.269	1.0	**0.000**	0.107	0.037	0.307
Coaches stimuli	0.601	0.205	8.592	1.0	**0.03**	1.824	1.220	2.726
Rehydration	-.021	0.356	0.320	1.0	0.572	0.818	0.407	1.643
Constant	2.784	1.209	5.301	1.00	0.21	16.184		

Notes: Age (years), Sex (1, males; 2, females), Competition level (1, International; 2, National; 3, Regional), Pauses to drink (counts), Training per week (hours), Training duration (minutes), Coaches stimuli (1, "never"; 2, "sometimes"; 3, "always"), Rehydration (i.e., does the athlete need to consume fluids after the training; 0, "no"; 1, "yes"). P: significance level.

3.4 Hydration habits in T1DM athletes

Data collection for this study is currently underway, in cooperation with the University of Alberta (Canada) and the University of Primorska (Slovenia). Therefore, statistical analysis will present only descriptive results. To date, 40 individuals (32 ± 9 y, M: 18 F: 22) with insulin-dependent diabetes mellitus (T1DM), from Italy (n= 10) and Canada (n= 30), voluntarily participated to the study and completed the online survey.

Anthropometric characteristics and diabetes characteristics of the sample are presented in **Table 9**.

Table 9: *Participants' anthropometrics (n= 40), comparison between males and females. Data are presented as mean ± sd.*

Personal Characteristics	Male n= 18	Female n= 22	Total n= 40
Age (y)	33 ± 8	31 ± 9	32 ± 9
Mass (kg)	76 ± 9	65 ± 10	70 ± 11
Height (m)	1.88 ± 0.06	1.66 ± 0.04	1.72 ± 0.08
BMI ($kg \cdot m^{-2}$)	24.0 ± 2.4	23.8 ± 4.0	23.9 ± 3.3
Hb1Ac (%)	7.6 ± 1.4	7.2 ± 0.7	7.4 ± 1.1
Diagnosis (y)	15 ± 11	20 ± 10	18 ± 11
Insulin Dose (unit)	40 ± 5	40 ± 16	40 ± 15

Notes: Participants' reported age (y) Body Mass (Mass, kg), body height (Height, m), body mass index (BMI, $kg \cdot m^{-2}$), glycated haemoglobin (Hb1Ac, %), time from first diagnosis of T1DM (Diagnosis, y), mean daily insulin dose (Insulin Dose, unit). Results summarized for males and females, and the total sample.

Among this sample, 20 individuals (50 %) received their insulin therapy with a multiple daily injection (MDI), while 19 individuals (47.5 %) received their insulin therapy with the insulin pump. One participants did not answer to the question. Twenty-eight participants (70.0 %) reported to begin their exercise with a blood glucose concentration between 70 and 180 mg/dL (3.9 – 10.0 mMol), while 11 individuals (27.5 %) start their training with glucose concentration between 180

and 300 mg/dL (10.0 – 16.7 mMol). One participant did not answer to this question. Blood glucose concentration at the end of the training was reported to be below 70 mg/dL (3.9 mMol) in 2 individuals (5.0 %), between 70 and 180 mg/dL (3.9 – 10.0 mMol) in 28 individuals (70.0 %), between 180 and 300 mg/dL (10.0 – 16.7 mMol) in 9 individuals (22.5 %). One participant did not answer to this question.

The reported blood glucose concentration at the start and at the end of the training is illustrated in **Figure 23**.

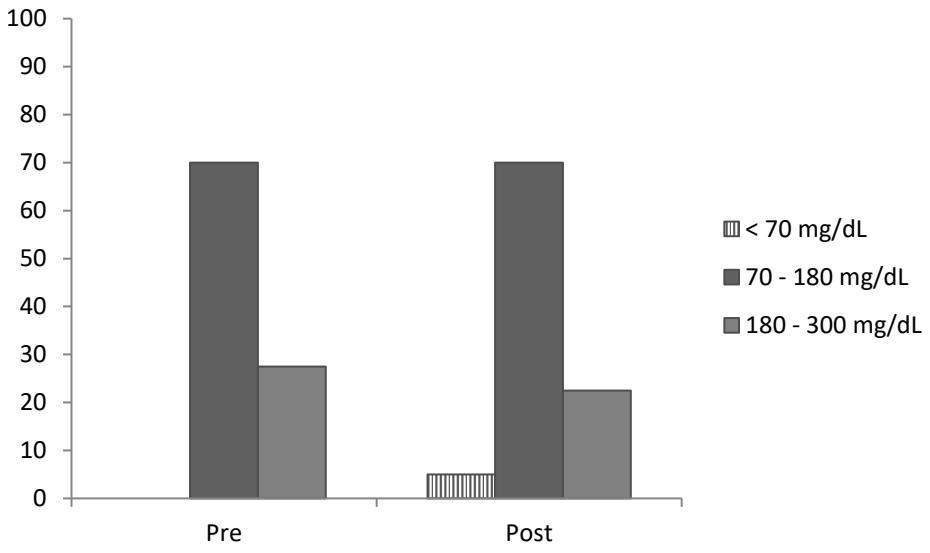

Figure 23: Reported blood glucose during training

Frequency distribution of the reported blood glucose concentration at the begin (Pre) and at the end (Post) of the training in T1DM individuals. <70 mg/dL (< 3.9 mMol, vertical grey stripes), 70 – 180 mg/dL (3.9 – 10.0 mMol, dark grey), 180 – 300 mg/dL (10.0 – 16.7 mMol, light grey).

Twenty-one participants (52.5 %) reported to currently compete in a sport. One participant did not answer to the question. The most commonly played sports are: running (15.0 %), ice hockey (10.0 %), cycling and soccer (7.5 %), and were played at an international (2.5 %), national (10.0 %) or regional (37.5 %) level.

Training intensity was reported to be somewhat hard in 15.0 % of the sample, moderately hard in 62.5 %, and hard in 17.5 %. Two individuals did not answer to the question.

Participants reported to train for 7.3 ± 3.0 h per week, and the usual training bout was reported to last 1.5 ± 0.5 h.

Participants stated to have 2 ± 2 breaks during training with 37 participants (92.5 %) reporting to have the opportunity to drink something. Two participants did not answer to the question. The most preferred beverage was water (67.5%) or sports drinks (25.0%). Other participants reported to drink other beverages, as fruit juice or tea. Fluid volume consumed during training was reported to be 0.71 ± 0.46 L, or 0.6 ± 0.5 $L \cdot h^{-1}$. Participants reported to drink from bottles which volume was 0.76 ± 0.40 L, up to a maximum of 2 bottles per training. Those bottles were reported to be consumed for the 25 % (7.5 %), 50 % (10.0 %), 75 % (12.5 %) and 100 % (55 %) of its volume. A small percentage (2.5 %) of the sample reported to not drink anything from the bottle, 12.5 % did not answer to the question.

In 50% of the cases, participants reported to not check their hydration status or fluid balance. Colour of urine was used by the 37.5 % of the cases, as well variation in body mass was used by 5.0 % of the participants. 7.5 % of the participants did not answer to the question.

About two thirds of the sample (60 %) reported to train without the supervision of a coach for the major part of training time, while 2 participants did not answer to the question. Those who reported to train under a coach supervision rated the perceived encouragement to drink as “never” (31.2 %), “sometimes” (43.8 %) and “always” (25.0%). Fluid replacement protocol was reported to be decided “a priori” (7.5 %), “ad libitum” (57.5%), or “ad libitum only when coach set a break” (22.5 %). A small amount of the sample (12.5 %) of the participants reported to use other methods to decide their own fluid balance protocol, or they did not answer to the question. For the 3

participants who reported to decide their fluid balance strategy a priori, the protocol was designed by themselves. Five patients (12.5 %) declared to not feel thirsty at the end of the training, 57.5 % was somewhat thirsty, while 25.0 % was very thirsty. Two participants did not answer to the question.

When participants were asked to identify the influence of diabetes in their fluid requirements and fluid balance as compared to healthy individuals, 50.0 % of the sample did not consider T1DM as influencing their fluid requirements, 42.5 % reported T1DM may influence hydration by increasing patients' fluid requirements, while 2.5 % considered T1DM decreasing their fluid requirements.

Some participants suggested that their fluid requirements, fluid intake, and thirst sensation may be influenced by their blood glucose concentration, outlining how elevated blood glucose level may increase the need for fluid replacement.

Sport characteristics, hydration habits and behavioural characteristics are summarized in **Table 10.**

Table 10: *Participants' (n= 40) answers to training and hydration-specific questions from the survey*

Training and Hydration-Specific Questions		
How many hours do you practice each week?	(hours)	7.3 ± 3.3
How long does a typical training bout last?	(hours)	1.5 ± 0.5
How many pauses or breaks to drink do you have during a typical training?	(count)	2.0 ± 2.0
What type of beverage do you normally drink before, during or after training? (%)	Sport Drink Water	25.0 67.5
How much fluid (volume) did you drink in your last training session?	(L)	0.71 ± 0.46
Do you regularly check your hydration status? (%)	No Colour of Urine Body Mass	50.0 37.5 5.0
Does your coach actively encourage you to drink at some point during the training session? (%)	Never Sometimes Always	31.2 43.8 25.0
How do you decide your fluid replacement protocol? (%)	Set a priori "Ad libitum" "Ad libitum – Coach"	7.5 57.5 22.5
At the end of your training, did you feel thirsty enough to drink something?	Not Thirsty Somewhat Thirsty Very Thirsty	12.5 57.5 25.0
Do you think T1DM influence somehow your fluid requirements, compared with healthy individuals? (%)	No Yes, increasing needs Yes, decreasing needs	50.0 42.5 2.5

Notes: Mean ± standard deviation or percentage (%).

4. DISCUSSION

4.1 Strategies for preventing hypoglycemia during a 24x1-h marathon, and comparison with ECRES algorithm

Results of the present study showed that about half of the patients started their exercise with high blood glucose concentration. It should be emphasized, however, that preloading with carbohydrate does not always exempt patients from the need of further extra amounts (Grimm et al., 2004; West et al., 2011). Of note, the reduced basal infusion rate in the insulin pump users helped patients in maintaining glycaemia on target, but all of them (except one) still required some extra carbohydrate. In addition, detrimental effects might be induced by the high glycaemia, thus reducing some of the health benefits of exercise (Gallen et al. , 2011; Tamborlane, 2007).

Current guidelines for the prevention of exercise-induced glycaemic imbalances in patients with T1DM are based mainly on small studies and observational evidence (Garcia-Garcia et al., 2015). Patients have to discover their own strategy, and fear of hypoglycaemia still remains the strongest barrier to physical activity (Brazeau et al., 2008; Lascar et al., 2014; Yardley & Sigal, 2015). Consequently, a large percentage of patients with T1D do not reach the recommended levels of physical activity (Lascar et al., 2014; Waden et al., 2008). Moreover, there is evidence that several factors, such as plasma insulin concentration during the effort, the time of day that the exercise is performed, and the patient's degree of fitness (Garcia-Garcia et al., 2015; Tamborlane, 2007), are important in determining the carbohydrate requirement. Patients often have difficulty in handling all these factors together to establish the most appropriate countermeasure and/or amount of extra carbohydrate. The ECRES algorithm (Francescato et al., 2011) was designed to fill this gap, providing patients with a tool that can estimate the requirement on the basis of a few simple data (i.e. exercise intensity and duration, and starting glycaemia), while automatically taking into

account as many factors as possible. Indeed, results of the present work show that the ECRES algorithm, even under challenging physical activity conditions (i.e. the 24 x 1-hour Telethon relay marathon, where each athlete exercised for one hour starting at one hour intervals all over the day), would have suggested the same amounts of extra carbohydrate as those actually consumed by patients following the advice of expert physicians. Interestingly, the percentage of patients who ended their race on target was similar to the 70% that finished the 1-hour controlled laboratory exercises with glycaemia on target (Francescato et al., 2011). It should be remembered, however, that the risk of hypoglycaemia continues in the 12-24 hours following the exercise. Future studies are thus warranted to investigate the possible effects of a more stable glycaemia during exercise on the post-exercise glucose levels.

Finally, exercise has been identified as a major obstacle even in current control algorithms of the artificial pancreas (van Bon et al., 2011) and maintenance of optimal glucose control during and after exercise may be challenging despite dual-hormone systems (Basu et al., 2014). Indeed, the lack of comprehensive models of insulin action and glucose uptake during exercise represents a significant knowledge gap to appropriately quantify the effects of exercise on overall glucose homeostasis (Kudva et al., 2014). Moreover, as discussed by Basu et al. (Basu et al., 2014), taking in mind that even healthy individuals without diabetes need to resort to periodic carbohydrate ingestion during and after exercise, to date it would be impractical to imagine relying solely on closed-loop control systems (with dual-hormone insulin and glucagon) to prevent exercise-induced hypoglycaemia without carbohydrate ingestion. Accordingly, it can be expected that the ECRES algorithm might continue being helpful while using closed-loop systems.

Several different implementations of the algorithm can be foreseen, ranging from single patient's use versions (e.g. apps for smart phones) to web-based solutions. The algorithm can also be easily integrated into many different devices, such as training equipment or glucose monitoring systems.

Finally, a real-time version can be easily implemented on portable devices, designed to estimate in real-time the extra carbohydrate still available on the basis of actual exercise intensity. As such, they would be able to warn patients of the actual risk of hypoglycaemia during their physical activities. Such a device might be very intriguing, since it might help patients with T1DM to keep more constant blood glucose levels on each exercise occasion and thus to safely enjoy all the benefits of physical activity, in particular if it is spontaneous, thereby liberating them from the fear of exercise-induced hypoglycaemia. So far, an international version of the algorithm in the form of an app is under implementation in order to make it easily accessible and user-friendly for both clinicians and patients.

In summary, results of the present work showed that the consumption of extra carbohydrate was required by the majority (13/19) of patients to avoid the possible exercise-induced hypoglycaemia. Modification of the insulin dosage did not completely exempted patients from the consumption of extra carbohydrate. The ECRES algorithm provided good estimates of the carbohydrate required to avoid exercise-induced glycaemic imbalances that were almost equal to the quantities consumed by patients following medical advice, independent of the time of day that the exercise was performed. These results further open the prospect of a simple device to help patients avoid exercise-induced hypoglycaemia.

4.2 ECRES algorithm validation

Physically activity has been classically indicated helping to prevent cardiovascular diseases and to improve blood glucose control in type 1 diabetes mellitus (T1DM) (Gallen et al., 2011; Lascar et al., 2014; Roberts & Taplin, 2015). Nevertheless, fear of hypoglycemic events often discourage patients to exercise regularly, representing an important barrier for physical activity (Brazeau et al., 2008). To date, many T1DM individuals adapt their therapy and diet for exercise, based on personal experience. However, this experience requires long time to be achieved, during which patients may face several hypoglycemic or hyperglycemic events, following a trial-and-error approach. Additionally, unusual exercise characteristics (e.g., type of exercise, time of day, intensity, etc.) or recent changes in insulin therapy or diet, may require to restart the learning process.

Several guidelines have been developed suggesting different strategies to help patients maintain a good blood glucose concentration during exercise, adjusting insulin dose and/or suggesting extra amount of carbohydrate (CHO) (Franc et al., 2015; Hopkins, 2004; Kemmer, 1992; Perrone et al., 2005; Rabasa-Lhoret et al., 2001). Some authors suggested to introduce high-intensity exercises to prevent blood glucose fall below euglycemic levels (Bussau et al., 2006; Guelfi et al., 2007; Yardley & Sigal, 2015; Yardley et al., 2013), because of its effects on the counteregulatory hormones and on the hepatic gluconeogenesis (Miller et al., 2002).

All these strategies may be subject to limitation since they are often designed for only one-time interval following insulin administration, and exercise intensity may be rough.

The Exercise Carbohydrate Requirements Estimating Software (ECRES) is an algorithm that was designed to suggest the amount of extra CHO based on patient's individual characteristics,

therapy, diet, exercise intensity, exercise duration, and time interval following insulin administration (Francescato & Carrato, 2011). Carbohydrate oxidation rate (CHOox) may be different depending on aerobic fitness level, and it is often difficult to discriminate between trained and sedentary individuals (and different levels of training) (Francescato et al., 2011). ECRES algorithm has been tested both in laboratory studies with researchers directly applying the algorithm, and on field by using an automatic short massage system (SMS) suggesting extra CHO for exercise. On average, 70 % of the participants prevented immediate exercise-induced glycemic imbalances, independent of the time distance from insulin injection and without requiring any trial-and-error approach (Francescato et al., 2015; Francescato et al., 2011; Vuattolo et al., 2012).

Results from these studies however are unlikely comparable with other methods already validated in the literature, because of the different characteristics of sample, therapy, diet, and exercise characteristics. This study, currently underway, aims to compare exercise-induced glycemic fluctuation when extra CHO were estimated using ECRES algorithm or a reference validated method (REF).

A quantitative method for extra CHO was proposed, based on standardized tables (Perkins & Riddell, 2006). This strategy has been chosen among many others because, although not tested in a clinical trial setting, it represents a popular resource among physically active T1DM individuals (Perkins & Riddell, 2006).

Study protocol has been developed in order to reproduce the same conditions during both trials (REF or ECRES), in a double-blinded randomized cross-over design; nevertheless, it should be noted that unlikely it will be possible to perfectly mimic insulinemic and glycemic conditions in the two trials, mainly because participants were left in a free-living condition. Participants were

categorised between trained (ATH) and sedentary (SED), based on a brief interview. Preliminary results from 23 T1DM patients suggested similar personal characteristics, such as age and body mass, between paritcipants who were classified as athletes or sedentary individuals. Experimental conditions (i.e., while using the reference method or ECRES) were comparable as blood glucose concentration at the start of exercise was similar for both groups. As also indicated in the methods section and in the inclusion criteria, patients had to begin the exercise protocol with blood glucose between 70 and 180 mg/dL, and the days before experimental session they were followed and instructed by their diabetes specialist physician and dietician, in order to better stabilize their therapy and diet. Similarly, blood glucose level at exercise cessation was similar while using reference method or ECRES in both groups. On the contrary, a trend for a different blood glucose behaviour was observed only in athletes ($p = 0.055$); indeed, the difference between blood glucose at the start and at the end of exercise indicated a tendency to 21 ± 71 mg/dL increased glycemia at the end of exercise if participants were using the reference method, or a 24 ± 50 mg/dL decreased glycemia if participants were using ECRES method.

The amount of extra CHO suggested for physical activity in sedentary individuals was not significantly different between the reference method and ECRES estimation. Conversely, in physically active T1DM individuals, ECRES estimated less CHO to be ingested prior to exercise as compared to the amount proposed by the reference method (10 ± 10 g vs 24 ± 50 g, respectively; $p < 0.01$). Generally, patients classified as physically active, or athletes, are characterised by different responses during exercise, and ECRES algorithm suggests specific amounts of extra CHO. In contrast, the reference method does not differentiate between trained and sedentary individuals.

Preliminary results indicated a similar proportion of cases where patients concluded the exercise protocol with good blood glucose (70-180 mg/dL), independently from the method used to estimate

extra CHO. In specific, about only 1/3 of the sample presented blood glucose levels below 70 mg/dL (hypoglycemia) or above 180 mg/dL (hyperglycemia). In ATH, however, significantly lower amounts of CHO were administered.

Results from ECRES are consistent with previous observations, supporting validity of this method when compared with an international popular method based on a standardized table. To date, a greater sample size is required to definitely confirm trends and observations, and validate ECRES algorithm as a better method for extra CHO estimation.

4.3 Hydration habits in Italian athletes

This is the first study to characterise current hydration habits in Italian athletes from a wide range of sporting backgrounds, ages and abilities. The data indicate that i) total fluid intake values are significantly lower than currently published guidelines, and ii) athletes' perception of their coach's encouragement to drink directly influences the total volume of fluid consumed during that training bout, regardless of the age, sex, sport type, or competition level of the athlete.

To our knowledge, this is the first study to examine hydration practices in such a large, and diverse sample of athletes (n= 352), ranging from children to older adults (8 to 63 y) in free-living conditions. Males and females were evenly-represented, including a balanced distribution between international, national and regional athletes. Females were principally involved in indoor sport (e.g. volleyball, basketball, combat sport), whereas males were more involved in outdoor sport (e.g. tennis, soccer, athletics), after the most popular sport, basketball. Sailing athletes reported the longest training bout duration (3-h) and total training hours per week (11-h). Other sports with (relatively) longer training durations were: alpine skiing, athletics (field), canoeing, diving and kayak, each averaging ~2-h per training bout, and ~11-h total training per week. In contrast, basketball and volleyball reported 1.5-2.0-h per training bouts for 6-7-h of training per week. The practice duration and total training hours per week did not affect overall fluid intake of athletes when expressed as mean fluid intake per hour exercise. Previous literature has typically focused on matching fluid replacement to sweat rate in an attempt to maintain a euhydrated state. Mean sweat rates are typically suggested to be between 0.5 to 2.0 $L \cdot h^{-1}$ in most athletes (Sawka et al., 2007). However, many athletic groups report drinking far less liquid than can be replaced from sweat loss alone. For example, male rowers may sweat on average 1.98 $L \cdot h^{-1}$ (from 0.99 to 2.92 $L \cdot h^{-1}$), yet

consume mean fluid intakes of 0.96 $L \cdot h^{-1}$ (from 0.41 to 1.49 $L \cdot h^{-1}$) (Burke, 2007). In an indoor sports such as basketball, the literature has reported summer training sweat rates to be in the order of 1.37 $L \cdot h^{-1}$ and fluid intakes of only 0.80 $L \cdot h^{-1}$ (Broad et al., 1996). Indeed, the average fluid consumption rate in the present study was 0.28 ± 0.21 $L \cdot h^{-1}$. There were no significant differences in fluid intake values between males and females even when normalised per hour of training, or normalised per kg body mass.

The current ACSM position stand on hydration espouses that athletes should not lose more than 2 % BM (Sawka et al., 2007) during a given training/competition. Authors recommend individuals should periodically drink (as opportunities allow) during exercise, and the amount should be determined based on individuals' estimated sweat loss during a particular exercise task with respect to the weather conditions (Sawka et al., 2007). For marathon runners, the ACSM position stand suggests a fluid intake *ad libitum* from 0.4 to 0.8 $L \cdot h^{-1}$. For a more generic exercise, the position stand states that athletes typically sweat at a rate of ~1.2 $L \cdot h^{-1}$ for a 70 kg athlete. Given that the current sample found mean fluid intakes of 0.28 ± 0.21 $L \cdot h^{-1}$, this fluid intake rate would result in a probable loss of ~1.3 % BM (equivalent to 0.9-L of uncompensated sweat loss) for every hour of training, with athletes reaching their 2 % BM threshold for 'dehydration' after a common training bout of as short as 1.5 h. For example, the basketball players in this study reported drinking 0.29 ± 0.12 $L \cdot h^{-1}$, whereas basketball players from other literature have reported drinking up to 0.80 $L \cdot h^{-1}$ (Broad et al., 1996). Supposing a similar sweat rate for both samples, a 70-kg basketball player could lose 1.7 % BM for every hour of training in this study, and since mean basketball training bouts lasted ~1.5 h, it would have been possible to observe fluid losses greater than 2 % BM in this sample for this popular sport.

When individualised fluid replacement protocols based on individual sweat rates are not available, NATA and ACSM guidelines suggest consuming ~0.20 L fluid every 15-20 min, or ~0.4-0.8 $L \cdot h^{-1}$ (Casa et al., 2000; Sawka et al., 2007). The current sample (across all sports) reported rates of 0.28 ± 0.21 $L \cdot h^{-1}$ (range: 0 to 1 $L \cdot h^{-1}$), irrespective of the athletes' sex or competitive level. Thus, athletes' mean fluid intake is 65 % lower than volumes suggested by NATA, and 53 % less than volumes proposed by ACSM. Only 23 athletes (6.5 % of total sample) reported drinking at or above 0.6 $L \cdot h^{-1}$. Guidelines for hydration practices during exercise have developed from general recommendations to the adoption of individualised strategies to partially or fully replace sweat losses (Garth & Burke, 2013). However, other studies have found that *ad libitum* drinking to thirst should dictate the amount of fluid consumed during exercise (Cheung et al., 2015; Cotter et al., 2014; Noakes, 2010; Sawka & Noakes, 2007), leading to disagreement between scientists, and confusion amongst athletes. Since this study did not determine hydration status via direct measures or include an assessment of performance outcomes *per se*, it cannot be speculated on whether a lower fluid intake during training may result in significant athletic impairment for a given sport. This study did find that training bout duration negatively predicted fluid intake behaviour, since longer activities were associated with lower overall fluid intake when normalised per hour of training. Thus, fluid intake during training may be (at least partially) influenced by psychological factors related to fluid availability and overall training duration.

Notably, it has been demonstrated that when a coach's knowledge about hydration and nutrition practices may be classified as 'weak' (measured via self-reported questionnaire), coaches preferred to not give any advice to their athletes regarding this topic whatsoever (Torres-McGehee et al., 2012). Fluid intake is one of the most common improperly given advices, even when coaches are reluctant to offer misinformed or outdated recommendations (Couture et al., 2015). Therefore,

educational interventions may increase a coaches' knowledge about the most current hydration practices which they can later impart to their athletes (Gianotti et al., 2010). Indeed, an athlete's knowledge on nutrition and hydration practices is also reported as 'weak' in the sport-science literature, with only 9 % of athletes reporting that they feel sufficiently prepared on this topic (Torres-McGehee et al., 2012). Thus, a short, educational intervention may be useful to both coaches and athletes, since it has been shown that athletes themselves do alter fluid consumption behaviour after educational interventions or short training sessions on the topic (Kavouras et al., 2012; McDermott et al., 2009). Some authors have reported that poor fluid intake may be related to a weak knowledge of the international guidelines, and thus, short-term educational interventions may help improve fluid replacement practices (Cleary et al., 2012; Nichols et al., 2005). In the current study, 92 athletes (26.1 %) ranked their coaches as 'always' encouraging them to drink during training, and 99 (28.1 %) ranked their coaches as 'never' encouraging them to drink. Therefore, although this study cannot comment on whether coaches were espousing international hydration guidelines directly, it is apparent that athletes are not receiving uniform advice on the topic, regardless of the sport they compete in. Finally to consider, it is established that weight-retention from voluntary fluid intake after dehydration is greater when one consumes a carbohydrate/electrolyte-containing beverage compared to pure water (Park et al., 2012). Thus, fluid composition should also be factored into any hydration advice prescribed to athletes. Hydration guidelines are constantly evolving to include the most recent research findings. Considering that current (and previous) literature espouses vastly different practices regarding hydration for sport performance, it is imperative that both coaches and athletes are aware of current best-practices for their sport and competition level.

This study utilised a retrospective self-report to query hydration habits in a large and variable sample of Italian athletes. There were no direct measurements of fluid intake or sweat rate conducted during trainings. However, the aim of the present study was not to determine dehydration effects during exercise *per se*, but to characterise the typical hydration strategies athletes employed within a field-based scenario, and to determine which factors may influence fluid intake within this unique cohort. Although retrospective self-report is not considered as accurate as direct measurement, it is an appropriate methodology when larger sample sizes are investigated (Passe et al., 2007; Wilson et al., 2015). Considering the sample size, the conservative statistical approach employed, and the fact that hydration practices have not previously been investigated in this population, the current study provides valuable information regarding hydration practices in an ecologically-valid (i.e. not laboratory) test environment.

4.4 Hydration habits in T1DM athletes

Results from this study are preliminary and can only show a trend of T1DM patients' hydration habits during training. To date, participants were equally distributed between males and females, but the majority of the sample came from Canada, with a smaller fraction of participants from Italy (CAN n= 30, ITA n= 10). Preliminary analysis indicated a longer duration of training bouts (CAN: 1.1 ± 0.5 h, ITA: 2.0 ± 0.8 h, $p < 0.01$) but similar total fluid volume intake in the Italian population when compared with Canadians (CAN: 0.76 ± 0.51 L, ITA: 0.58 ± 0.26 L, p = NS). This resulted in a smaller fluid intake, when normalized per training duration, in Italian T1DM individuals (CAN: 0.73 ± 0.54 $L \cdot h^{-1}$, ITA: 0.30 ± 0.14 $L \cdot h^{-1}$, $p < 0.01$). Being the sample size from the two countries not equal, it may be hasty to define regional difference, and comparing actual results from T1DM with the results from Italian healthy individuals presented in previous research (Buoite Stella et al., 2016) may be rash. Approximately, fluid intake in Italian healthy individuals was similar to fluid intake indicated by Italian T1DM individuals (healthy: 0.28 ± 0.21 $L \cdot h^{-1}$, T1DM: 0.30 ± 0.14 $L \cdot h^{-1}$).

One of the primary outcomes from this study shows that a relevant proportion of T1DM individuals (27.5 %) reported to start their training with a blood glucose concentration above 180 mg/dL (10.0 mMol), threshold above which glucose can be usually found in urine (Johansen et al., 1984). The remaining fraction of participants reported to start their exercise in euglycemic conditions, between 70 and 180 mg/dL (3.9 – 10.0 mMol). At the end of the exercise, the fraction of patients who declared to conclude their exercise with euglycemic concentration was the same, while 22.5% of participants reported blood glucose levels greater than 180 mg/dL (10.0 mMol). Only a small fraction of participants indicated to usually end exercise with hypoglycemia (blood glucose < 70 mg/dL, < 3.9 mMol). Without continuous blood glucose monitoring it is not possible to determine precise blood glucose behaviour during training; nevertheless, it may be speculated that

~1/4 of the participants are characterised by high blood glucose levels throughout the whole training, enhancing urine production.

T1DM individuals are characterised by reduced sweat rate, especially beyond certain levels of heat stress or in poorly controlled diabetes (Carter et al., 2014; Yardley et al., 2013). However, it must be noted that during light to moderate intensity exercise, well-controlled T1DM patients showed local heat loss responses of sweating similar to matched healthy individuals (Stapleton et al., 2013). In 62.5 % of the sample, exercise intensity was self-reported to be "moderately hard", and mean Hb1Ac was 7.4 ± 1.1 %, characteristics similar to the sample and protocol of the study by Stapleton at al. (2013) (7.4 ± 1.1 % for the present study and 7.7 ± 0.3 % for the reference study respectively).

To our knowledge, no studies tried to measure sweat rate, fluid intake, and urine output in hyperglycemic T1DM individuals during exercise, and from this study it was not possible to determine if participants properly consumed fluids to prevent dehydration. On a theoretical basis it may be possible to speculate that participants in this study would be characterised by greater fluid losses (because of an increased urine output) not properly compensated by the Italian T1DM sample. It is mandatory to stress that these are preliminary results of an international study aiming at collecting data from a large sample of T1DM patients, in order to determine regional differences and to better speculate fluid balance variation between healthy and T1DM individuals, based on reference literature. A logistic regression will be used to determine the factors primarily influencing fluid intake, such as blood glucose levels, therapy, or training characteristics.

4.5 General Discussion

The aim of my PhD was to better understand how physical activity influences glucose and fluid balance homeostasis, in particular in a chronic metabolic disease such as insulin-dependent diabetes mellitus (T1DM).

Fear of hypoglycemia has been clearly reported to be one of the major barriers for physical activity in diabetic individuals (Brazeau et al., 2008), while regular exercise is strongly recommended to reduce cardiovascular risk factors and to improve insulin sensitivity and glucose management (Francescato et al., 2011). From a physiological point of view, patients with T1DM have similar characteristics to healthy individuals (data from our laboratory). Indeed, we tried to characterise physiological responses during physical activity by using two parameters representing the metabolic response to exercise. Efficiency (η) is the ratio between the mechanical power and the metabolic power, and is related to the metabolic cost of locomotion (Ferrer-Roca et al., 2016). Carbohydrate oxidation (CHOox) is an estimation of substrate consumption and may predict glycemic fluctuations in T1DM individuals (Francescato et al., 2005). Preliminary results indicate similar trends for both η and CHOox in healthy individuals and T1DM patients, suggesting similar physiological responses. As a consequence, it may be possible to suppose that well-controlled T1DM individuals who can properly manage their blood glucose levels, may perform at the same level of well-matched healthy athletes. Consequently, healthy individuals may be recruited to predict carbohydrate oxidation rate also in T1DM individuals, when large samples are required (Francescato et al., 2008).

When patients are free to choose their strategy to avoid hypoglycemia during exercise, our study showed that a relevant proportion of T1DM individuals (47%, 9 on 19 participants) began their physical activity with a blood glucose concentration above 180 mg/dL (10.0 mMol) (Buoite Stella

et al., 2016). This study represents a valid picture of patients' behaviour in free-living conditions, since they did not receive any indication on how to regulate their therapy and diet. When patients were asked how they adjusted their therapy and diet for exercise, the most common method consisted in increasing carbohydrate (CHO) intake, and/or reducing insulin dose. One participant removed her insulin pump during the exercise. In general, participants to this study were trained or physically active, and the glucose management strategy used mainly came from personal experience rather than reference equations or tables.

Individual experience may be the most accurate method to decide personal strategy to prevent blood glucose fluctuations during exercise, but it needs time and errors before a good knowledge is achieved and patients may face several hypoglycemic or hyperglycemic events. Furthermore, changes in both therapy, diet, or exercise characteristics, may require new trials to adapt and find the optimal strategy. The Exercise Carbohydrate Requirements Estimating Software (ECRES) is an algorithm developed by the University of Udine and our group to reduce the time needed to obtain a proper experience, by suggesting the amount of extra CHO to ingest without altering the insulin therapy (Francescato et al., 2011). Previous laboratory and field studies have tested ECRES accuracy to prevent glycemic fluctuations during exercise, reporting a proper estimation in 70 % of cases (Francescato et al., 2015; Francescato et al., 2011; Vuattolo et al., 2012). However, to better determine ECRES validity, a comparison study with a reference method (REF) is currently underway. One of the most reported methods to predict CHO needs for exercise consists in a table in which different activities are listed (such as walking, running, swimming, etc.), and the corresponding amount of suggested extra CHO is indicated depending on individual's body mass and exercise duration (Perkins & Riddell, 2006). From the results obtained until now it was possible to observe a similar trend in blood glucose during exercise between the two methods (REF and ECRES); however, a trend for a smaller amount of extra CHO estimated by ECRES was observed:

when compared to REF, in particular in T1DM athletes but not in sedentary patients. Preliminary results suggest that ECRES is an equally valid method to prevent hypoglycemia as the reference method, even by reducing the total amount of extra CHO ingested. Notably, greater amounts of extra CHO may lead to an increase in blood glucose level, in particular during specific conditions such as resistance training (Garcia-Garcia et al., 2015; Yardley & Sigal, 2015; Yardley, Sigal, et al., 2013), or when blood insulin level is low (Francescato et al., 2004). The REF method does not seem to take into account the time after insulin injection, but it may be designed for different types of exercises (i.e., anaerobic or aerobic); conversely, ECRES algorithm is currently designed only for moderate intensity (between 55 and 70 % of HRmax) aerobic training, although it more accurately considers insulin therapy, as insulin type and dose, and time from injection.

If glucose homeostasis represents a relevant characteristic for exercise performance in both healthy individuals and T1DM patients, fluid homeostasis evenly influences both exercise performance and health. Great fluid losses through sweating because of exercise or high ambient temperatures may lead to a deficiency in total body water (TBW), altering physiological and cognitive functions (Cheuvront & Kenefick, 2014; Riebl & Davy, 2013; Sawka et al., 2015). To prevent these alterations, a proper fluid intake should be maintained before, during, and after exercise, following specific guidelines or the stimulus of thirst (Casa et al., 2000; Cotter et al., 2014; Noakes, 2010; Sawka et al., 2007). Even if some authors still debate about the optimal fluid replacement strategy (in order to avoid a fluid deficit) (Sawka & Noakes, 2007), it is possible to conclude that athletes need to be properly educated about hydration, and fluids should be available during exercise. Ecologically-valid epidemiological descriptive studies show a picture of a population, and its behaviour in specific conditions. Hydration science is characterised by both experimental and observational studies, in order to quantify or estimate hydration status and fluid intake (Baillot & Hue, 2015; Nichols et al., 2005; Wilson et al., 2015; Zetou et al., 2008). Regional

differences have been observed for example in daily fluid intake (Malisova et al., 2016; Mora-Rodriguez et al., 2016), and age or sex may predict fluid consumption for exercise. To the best of our knowledge, there is no study analysing hydration strategies during training in free-living conditions, in a large sample of athletes from different age, sex, and sport. Furthermore, we found a lack of studies about hydration habits, both during exercise and daily living, in the Italian population. We developed a survey and gave it to a large sample (n = 352) of athletes, asking them to consider their recent usual training characteristics and behaviours (Buoite Stella et al., 2016). Fluid intake was shown to be accurately determined also without direct measures, but with an *"a posteriori"* self-report (Wilson et al., 2015). From these results it was possible to observe that in general, participants reported a mean fluid intake below the most common suggested volume for exercise (Rodriguez et al., 2009). It must be noted that we don't have direct measures of sweat rate and fluid intake, thus biases are possible and proper hydration may not reflect the international guidelines. Nevertheless, from this study it was possible to determine the main factors influencing voluntary fluid intake during training in free-living athletes, which were identified as the number of pauses during which athletes could drink, coach's encouragement to drink, and training duration.

Dehydration may not only be determined by sweat loss and fluid intake; indeed, other osmotic factors may influence fluid balance. As shown before, from our results it was possible to observe that a great proportion of T1DM individuals may choose to start their physical activity with high blood glucose concentration, and even if reference methods would be used to maintain a better glycemic control, specific conditions may lead to glycemic unbalance. High blood glucose levels have been suggested to increase fluid losses because of an increased production of urine, through an osmotically induced mechanism (Yardley & Riddell, 2016; Yardley et al., 2013). Thus, it seems mandatory for T1DM that proper attention should be given not only to glucose homeostasis, but also to body fluid balance, in order to prevent both hypoglycemia and dehydration. To our

knowledge, there is no study trying to identify hydration habits during exercise in T1DM individuals, nor specific guidelines for a proper fluid intake for this population (Yardley, Stapleton, et al., 2013). With a protocol similar to the survey used for the healthy athletes, we started a self-report data collection about hydration habits in physically active T1DM individuals. Results obtained until now in a small sample (n = 40) suggest a similar self-reported fluid intake between T1DM and healthy individuals. Little less than 1/3 of the sample indicated to start their training session with a blood glucose level between 180 and 300 mg/dL (10.0 – 16.7 mMol), almost confirming our field-study results. In general, participants perceived that T1DM increased their fluid requirements when compared with healthy athletes, in particular when their blood glucose was high, pushing them to consume a greater amount of fluids.

In conclusion, results obtained from the different studies conducted during my PhD tried to give a picture of glucose and fluid homeostasis in both healthy individuals and T1DM patients. Hydration is a multidisciplinary topic that is strongly related to metabolic disorders, and may be important for people with diabetes. Chronic dehydration, characterised by an increase in blood osmolarity and the consequent increased secretion of vasopressin (AVP), is considered to be a risk factor for developing diabetes (Enhorning et al., 2010; Melander, 2016). Diabetic individuals are at higher risk of incurring in excessive fluid losses, and to our knowledge there is a lack of studies showing whether patients are able to balance those losses with proper fluid intake.

Hypoglycemia is a well-known adverse event for people with T1DM during physical activity, and many strategies may be adopted to prevent glycemic fluctuation. However, the risk of dehydration seems to be an underrated complication, as shown by the absence of specific guidelines for fluid replacement during exercise in diabetic individuals. The take-home message of this

dissertation is an invitation for athletes, coaches, and researchers, to further investigate the complex interaction between fluid balance and blood glucose homeostasis in both healthy individuals and a spreading disease such as insulin-dependent diabetes mellitus.

5.MATERIALS AND METHODS

5.1 Strategies for preventing hypoglycemia during a 24x1-h marathon, and comparison with ECRES algorithm

The Telethon 24x1h marathon

The Telethon 24 x 1-hour relay marathon is a non-competitive fund-raising event lasting a whole day that involves teams composed of 24 members. Each participant starts at 1-hour intervals and is free to run (or walk) at his/her own pace along the urban track for 1 hour. The number of laps performed (thus the distance covered) by each participant and by each team is recorded.

Experimental procedure

Patients were advised to check their glycaemia carefully during the preceding 24 hours in order to avoid also mild hypoglycaemic events. All of them were then free to choose how to counteract the risk of exercise-induced hypoglycaemia. Participants received further specific advice by the medical staff, given on the basis of current guidelines (Perkins & Riddell, 2006) and on the patients' own experiences for similar exercises. Medical staff gave their advice without using the ECRES algorithm.

Patients reached the medical staff (which provided assistance during the whole event) at least one hour before the individual start time. At this point, they signed the informed consent and answered questions related to their usual therapy and diet, as well as any change to these in anticipation of the effort. Patients were then equipped with the belt of a heart rate monitor (Polar, Finland) to record heart rate (HR) throughout the race. Known amounts of simple carbohydrate were administered before and/or during the exercise, when appropriate, and quantities were recorded in detail.

Just before the start, at the middle and at the end of the race, capillary blood glucose concentrations were measured using a hand-held glucometer (AccuCheck Aviva, Roche). Glycaemia in the range from 3.9 $mmol \cdot L^{-1}$ to 10.0 $mmol \cdot L^{-1}$ was defined as "on target".

After the whole marathon was completed, the information provided by the patients and average measured heart rate were used to calculate, by means of the ECRES algorithm (Francescato & Carrato, 2011; Francescato et al., 2011), the estimated amount of required carbohydrate per patient.

The ECRES algorithm

The following information was uploaded in the algorithm for each patient (Francescato et al., 2011):

1) his/her usual therapy (i.e. insulin types, doses and time scheduling, together with the dietary carbohydrate);

2) the time of day the patient exercised;

3) the capillary blood glucose measured just before the start;

4) the average HR measured throughout the race.

In brief, the algorithm calculates the amount of carbohydrate required to avoid glycaemic imbalances (*reqCHO*) as a fraction (*Ft*) of the overall amount of carbohydrate burned during the exercise (*CHOox*), and further correcting it by subtracting (or adding) the excess (or lack) of glucose contained in the extra-cellular compartment (*Gb*) as follows:

$$reqCHO = (CHOox \cdot Ft) \pm Gb.$$

In turn, *Gb* is inferred from the actual capillary glucose level measured before the start of the exercise (*aGL*), depending on the volume of the extra-cellular fluid compartment (*ECF*):

$$Gb = (aGL - theoGL(t)) \cdot ECF$$

where *theoGL(t)* is the theoretical glycaemia the patient should have at the time of day the exercise is performed.

The *CHOox* is computed as the product of exercise duration (*exD*) and the whole-body carbohydrate oxidation rate, in turn estimated on the basis of the expected exercise intensity (represented by the average heart rate; *HR*) and the patient's fitness level ("sedentary" or "active").

$$CHOox = (m \cdot HR + q) \cdot exD$$

where the *m* and *q* values are those reported in a previous work (Francescato et al., 2005) for trained and untrained patients.

Finally, as described in detail previously (Francescato et al., 2011), Ft is almost proportional to the prevailing insulin concentration throughout the effort (*IC(t,j)*). This is estimated on the basis of the patient's usual therapy data and on "standard" pharmacokinetic profiles of the insulin analogues loaded in the system. It is also corrected for the patient's insulin sensitivity (*Sens(j)*), assumed to be represented by the patient's usual dietary carbohydrate to insulin ratio:

$$Ft = (IC(t,j) * Sens(j)) \cdot e + f$$

where *t* is the time of day and *j* is the day period (i.e. morning, afternoon or evening) the exercise is performed while the *e* and *f* values were previously reported (Francescato et al., 2011).

Changes in usual therapy (either in the scheduling and/or the dosage of the last insulin injection) in anticipation of the exercise can be appropriately accounted for.

Data analysis

Heart rate data acquired for each patient throughout the race were first averaged over 10 min periods. Percentage maximal heart rate (HR_{max}) was then calculated for each patient as the ratio between the average of measured HR values and the theoretical maximal HR (HR_{max} = 220 – age, expressed in years).

Results were analysed using Systat vs.11 software. Normally distributed data were presented as mean and standard deviation (SD), whereas skewed variables were reported as median and interquartile range (IQR; i.e. the difference between the 75th and the 25th percentile). The non-parametric Friedman analysis of variance for repeated measures and the Wilcoxon test (or the Mann-Whitney test) were used, as appropriate, to detect significant differences. The Pearson correlation coefficient was used to look for associations between the study parameters. Statistical significance was set to $P < 0.05$.

5.2 ECRES algorithm validation

This study is currently underway.

Participants

We aim at recruiting 50 patients with type 1 diabetes mellitus (T1DM), of both sexes, aged between 18 and 55 y. Patients were recruited on a voluntary basis from the Health Centres for Diabetes "Triestina" and "Bassa Friulana-Isontina" (Italy). Inclusion criteria to participate to the study were: first diagnosis of T1DM from at least 2 y, body mass index (BMI) < 28.0, Basal/Bolus insulin therapy, 6.0 % < Hb1Ac < 8.0 %, Hirsch Index: 2SD < mean blood glucose concentration, absence of other medical complications, no alterations in the last year ECG, not regular use of medications other than insulin, absence of contraindications for physical activity. The study protocol has been approved by the Ethical Board of Friuli-Venezia Giulia, and was designed in respect of the declaration of Helsinki.

Study Protocol

A double-blinded, cross-over randomized trial was used to assess the effect of two different methods to estimate carbohydrate (CHO) needs for exercise. Participants were asked to attend to the laboratory 2 times, with one week of rest between trials. Experimental procedure took place at the same time of the day, after 4 h from lunch and insulin injection. Participants were asked to arrive to the laboratory (Sports Medicine centre in Trieste, Italy) at least 60 min before experimental procedure, and to avoid hypoglycemia or hyperglycemia the 2 days before experiments. They were also asked to avoid physical activity the day before measurements. Insulin type, dose and diet were stabilised the days before experimental sessions. A continuous glucose monitoring system (DexcomG4, Dexcom Inc.) was used by patients starting from 3 days before the first trial; this was

removed 24 h after the second trial, in order to record glycemic fluctuations the days before and after the exercise.

Within 10 min of patients' arrival to the laboratory, research staff recorded their usual therapy and diet, and if they modified it the day of the experiment. A trained nurse measured the capillary blood glucose with a portable glucometer (AccuCheck Aviva, Roche, Switzerland). The experimental session was postponed if patients report hypoglycemic events < 70 mg/dL (3.9 mMol) in the previous 24 h, or if blood glucose at the start of the exercise was lower than 120 mg/dL (6.7 mMol) or greater than 180 mg/dL (10.0 mMol). Later, the nurse inserted a venous catheter to obtain a blood sample at different times: 30 min before exercise (-30), at the start of exercise (0), after the first 30 min of exercise (+30), at the end of exercise (+60), and 1 h after the end of exercise (+120). Samples were kept in an ice-filled box, and sent to the laboratory for glucose concentration analysis, following the proper methodologies.

The exercise protocol consisted in 60 min walking on a treadmill at a constant heart rate. Speed and slope were adapted during the first minutes of exercise in order to obtain the 65 % of the theoretical maximum heart rate for the individual (HRmax, 220 – age) (Tanaka et al., 2001). Results from a pilot study indicated 65 % HRmax as corresponding to 5 $km \cdot h^{-1}$ speed (slopes ranging from 4.0 to 6.5 %) for all the individuals. 15 min before exercise patients received an amount of CHO to drink (Glucosprint, Harmonium Pharma, Italy), in a randomized cross-over fashion, based on two different methods for CHO needs estimation: a) a standard validated method, corresponding to 0.31 $g \cdot kg^{-1} \cdot h^{-1}$ if assuming a walking speed of 5 $km \cdot h^{-1}$ (REF) (Perkins & Riddell, 2006); b) ECRES algorithm estimation based on patients' characteristics, therapy, diet, exercise intensity and duration (ECRES) (Francescato et al., 2011). Patients and all the research staff (exception made for 1 researcher, unique responsible for patients' randomization, CHO estimation and administration) were completely unaware of both the CHO amount and the method used. During both conditions, if

blood glucose concentration decreased below a "safety level" of 70 mg/dL (3.9 mMol), exercise was terminated and patients received extra glucose upon return to normal glycemic levels. Independently if exercise was terminated because of hypoglycemia or after 60 min, patients remained in the laboratory 1 h, followed by a trained physician that instructed them on how to prevent and manage exercise-related hypoglycemia.

Statistics and Data analysis

All the measured variables were recorded on a spreadsheet (MS Excel, USA) and lately analysed with a software for statistics (SPSS 19.0). Blood glucose results presented in this analysis have been obtained by the venous blood sample, stored and properly analysed from trained biochemists. Descriptive statistics present results as mean ± standard deviation (SD), or median and range for non-normally distributed variables. Absolute and relative frequency analysis were used for nominal variables. A logistic regression will be used to assess the factors associated with an increased risk of hypoglycemic events. A paired sample t-test was used to define significant differences between the two methods for CHO estimation. A chi-squared test was used for difference between proportions. A significance level of $p < 0.05$ was used.

5.3 Hydration habits in Italian athletes

Participants and Protocol

This study was approved by the Clinical Review Board for Sports Medicine at the Salus Hospital Trieste, Italy, following the declaration of Helsinki. All participants gave written, informed consent prior to volunteering in the study. Youth athletes (≤18 y) had informed consent signed by their parents or legal guardian. Athletes underwent a preliminary physical examination by a sports medicine physician to assess overall health status before participation in the study. Athletes were recruited during their annual sports medicine check-up, a mandatory procedure in Italy for anyone who wants to participate in any kind of sport competition (See: Italian Ministerial Decree on Competitive Sports, 18th February 1982). Height and body mass were measured during the medical check-up by the physician.

After the preliminary examination, athletes were requested to complete on paper a hydration surveillance questionnaire. Data were collected from February to July in Trieste, Italy (ambient temperature: 16±9 °C, range: 12 to 20 °C, relative humidity: 62±6%). In addition, a standard medical history questionnaire was used to exclude participant(s) with any pre-existing conditions, including: evidence of any clinically relevant cardiovascular, hematologic, hepatic, gastrointestinal, renal, pulmonary, endocrine, or psychiatric history of disease. To be included in the analysis, participants needed to exercise at least 2 times per week for not less than 30 min per training bout. Furthermore, they had to officially compete at the regional level (or above) to be considered sufficiently active to continue in the study.

Survey Design

Previously-validated Italian health and physical activity questionnaires regarding hydration strategies during sport training were not found in the review of literature. Self-report for hydration strategies has been suggested as a valid method when large sample sizes are investigated (Wilson et al., 2015). This hydration questionnaire was established by a research team composed of a sports medicine physician, a psychologist, a dietitian, and an exercise physiologist. The questionnaire queries the athlete's age, sex, sport mode, competitive level, and hours of training per week, following suggested methodologies. If more than one sport was practiced, athletes were asked to consider their most important one, defined as the sport involving the greatest number of training hours per week, and then, if equal, the highest competitive level. Competitive level was assessed as 'International' if the participant was currently selected to the Italian National Team, competing in international competitions; they were considered "National" athletes if they were involved in the highest level of competitions within the Italian Championship sport system, or finally, athletes were considered "Regional" if they competed for their regional club, but were not involved in other high-level or national competitions. The athletes were requested to consider both their hours of training per week and the total number of hours for each training bout since a variety of sports had different training schedules. For example, sailing typically involved a greater number of total training hours than other sport modes, but the athletes were sailing less frequently, usually around ~2 days per week.

Hydration-specific questions consisted of determining one's typical self-reported hydration strategies during a regular training session in the previous training session (i.e. not leading up to a particular competition or event). Specifically, athletes were asked to report what type of fluid they

usually drank (water only, sport drink, tea, fruit juice or other), and the quantity (L) ingested during a typical training bout. Athletes were also asked to indicate how many pauses to drink they usually have during their training, and whether at the end of training they felt thirsty enough to drink something (Yes/No). The final section of the questionnaire consisted of a 3-point Likert scale to rank athletes' perception on whether their coach encouraged them to drink (coaching stimuli) during training. They were asked to rate whether they felt the coach adequately instructed them on the fluid consumption needs specific to their sport (Yes/ No), and whether the coach encouraged the athletes to drink during a typical training bout (0=never, 1=sometimes, 2=always).

Statistical Analyses

All analyses were conducted using IBM SPSS Statistics (v.22.0, Chicago, IL, USA). For frequency analysis, some of the more obscure sports were grouped into more general categories, consisting of: aquatics (diving, fin swimming, synchronised swimming), combat sport (karate, judo, kick boxing) and athletics, which was further separated into three specialities: athletics field (jumping and throw events), athletics sprint (100m, 110m hurdles, 200 m track events), and athletics distance (any running distances over 400 m). Data are presented as means ± standard deviations (sd). The Kolmogorov-Smirnov test was used to test normal distribution of the data sets. Independent t-tests were conducted between groups for all dependent variables. Cohen's d as effect size coefficient was included where applicable. Non-parametric tests were conducted for any non-normally distributed variables, namely Mann-Whitney U test for two groups (sex) comparisons, and the Kruskal-Wallis ANOVA was conducted for multiple groups (competitive level) comparison. Univariate associations between nominal and ordinal dependent variables were tested with Chi Square.

A binary logistic regression was applied to determine the independent variables related to fluid consumption during training ($P < 0.05$). The independent variables (predictors) were: age and sex of participants, competitive level, number of pauses to drink, volume of training per week, training bout duration, coaches' stimuli and need to rehydrate after training. For the binary logistic regression, answers concerning 'fluid intake' (dependent variable) were separated into two (binary) groups: (0, Lower fluid intake) athletes who reported a fluid consumption below or equal the median (0.25 L·h-1), and (1, Higher fluid intake) athletes who reported a fluid consumption above the median. There were n= 186 in the lower and n= 150 in the higher fluid intake groups, respectively.

5.4 Hydration habits in T1DM athletes

This study is currently underway.

Participants and Protocol

This study is part of an international project of the University of Udine (Italy), University of Alberta (Canada), and University of Primorska (Slovenia), named "Hydration during Exercise in Diabetic Athletes" (HEDA), and it was approved by the University of Alberta Research Ethical Board (Canada), in respect of the declaration of Helsinki. This study aims to recruit at least 100 T1DM individual, 50 from Canada and 50 from Italy. Slovenian participation is currently under development. All participants read the informed consent prior to volunteering in the study. Participants were recruited from Canada and Italy sharing the recruitment poster among diabetes specialists, patients' associations, and sport clubs. All the anthropometrical characteristics were independently stated by the participants. Data were collected from August to October using an online survey service, hosted on a web server approved by the ethical committee (SurveyMonkey). Anonymity of participants was maintained and personal information were not asked in order to avoid any possible identification of participants. Individuals received the recruitment poster where a summary of study design and methods was presented, together with the inclusion/exclusion criteria and a weblink. Following the weblink patients were redirected to the project website (https://heda2016.wordpress.com/), where they could find the complete informed consent. Patients were instructed to read the informed consent and only after it, to click on a weblink they could find at the bottom of the page, redirecting them to the survey. Inclusion factors were: age between 18 and 50 y, first diagnosis of T1DM for at least 2 y, last glycated haemoglobin (Hb1Ac) < 9.9 %, and participants had to be physically active and training for at least 6 months, a minimum of 3 times per week, and for not less than 30 min per training.

Survey Design

This survey is a modified version of a questionnaire established by a research team composed of a sports medicine physician, a psychologist, a dietitian, and an exercise physiologist, and presented in a paper currently under review. This modified version of the survey has been recently validated for test-retest in a sample of 20 T1DM patients from Italy and Canada. Only glycated haemoglobin (Hb1Ac) failed the validity assumptions.

First question assessed if participants read the informed consent and asked for their nationality. The questionnaire queried the patient's age, sex, sport mode, competitive level, and hours of training per week, following suggested methodologies. If more than one sport was practiced, athletes were asked to consider their most important one, defined as the sport involving the greatest number of training hours per week, and then, if equal, the highest competitive level. Competitive level was assessed as 'International' if the participant was currently selected to the corresponding National Team, competing in international competitions; they were considered "National" athletes if they were involved in the highest level of competitions within the national championship sport system, or finally, athletes were considered "Regional" if they competed for their regional club, but were not involved in other high-level or national competitions. The athletes were requested to consider both their hours of training per week and the total number of hours for each training bout since a variety of sports had different training schedules.

Diabetes specific questions consisted in asking the participants to report their last glycated haemoglobin (Hb1Ac, %), the time from the first diagnosis of T1DM (y), any complication due to diabetes or drugs other than insulin that could influence blood glucose, their insulin injection therapy (multiple daily injection, MDI, or insulin pump), and the mean total dose of insulin per day (units). Patients were also asked to report their usual blood glucose concentration at the start and at

the end of the training, choosing between 4 different ranges: < 70 mg/dL (3.9 mMol), between 70 and 180 mg/dL (3.9 – 10.0 mMol), between 180 and 300 mg/dL (10.0 – 16.7 mMol), > 300 mg/dL (16.7 mMol).

Hydration-specific questions consisted of determining one's typical self-reported hydration strategies during a regular training session in the previous training session (i.e. not leading up to a particular competition or event). Specifically, athletes were asked to report what type of fluid they usually drank (water only, sport drink, or other), and the quantity (L) ingested during a typical training bout. According to previous literature, where self-report for hydration strategies has been suggested as a valid method when large sample sizes are investigated (Wilson et al., 2015), participants were also asked to indicate if they drank from a bottle, the volume of that bottle, the number of bottles, and the percentage of the total volume consumed from the last bottle. Athletes were also asked to indicate how many pauses to drink they usually have during their training, and if they had the possibility to drink during those pauses. Then, participants were asked if they regularly checked their hydration status and what method they used (e.g., colour of urine or body mass variation), as well as what type of protocol for fluid replacement they followed. They could choose between: "a priori" prescribed plan (if they decided before the training how much and when to drink), "ad libitum" (if they could drink whenever and how much they wanted), "ad libitum when coach set a break" (if they could drink how much they wanted but only when the coach set a break), or other. If they declared to use an "a priori" protocol, they were also asked to indicate who designed that protocol (e.g., themselves, coach, physician, etc.). Last questions asked whether at the end of training they felt thirsty in a 3-point fashion (not thirst, somewhat thirsty, very thirsty). Similarly, patients were prompted with a 3-point Likert scale to rank their perception on whether their coach encouraged them to drink (coaching stimuli) during training. They were asked to rate whether the coach encouraged them to drink during a typical training bout (0= never, 1= sometimes,

2= always). At the end, patients were asked to report if they think having diabetes may influence their fluid requirements and hydration habits. If the answer was "yes", they were asked to specify if they perceived diabetes increased or decreased their needs when compared to healthy subjects. A notes section allowed for comments or suggestions.

Clicking on the "submit" button, participants agreed to complete the survey and data were saved in the survey datasheet, accessible only by the research team.

Statistical Analyses

All analyses were conducted using IBM SPSS Statistics (v.22.0, Chicago, IL, USA). Data are presented as means ± standard deviations (sd). Since data collection is still running, results will be presented only as descriptive statistics.

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7. PUBLISHED ARTICLES

LIST OF PUBLICATIONS

Manuscripts in SCI journals

Buoite Stella, A., Assaloni, R., Tonutti, L., Manca, E., Tortul, C., Candido, R., Francescato, M.P. (2016). Strategies used by patients with type 1 diabetes to avoid hypoglycemia in a 24x1-hour marathon: comparison with the amounts of carbohydrates estimated by a customizable algorithm. *Can J Diabetes, in press*.

Buoite Stella, A., Francescato, M.P., Sims, S.T., Morrison, S.A. (2016). Fluid intake behaviour in athletes during typical training bouts. *J Sports Med Phys Fitness, in press*.

Manuscripts in non-SCI journals or abstracts in SCI journals

Venuto, I., **Buoite Stella, A.**, Stel, G., Mazzolini, S., Francescato, M.P., Cauci, S. (2015). Differences in Hydration Status of Adolescent Soccer Players After Training Sessions. *Biochimica Clinica, 39(5):* 415.

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Buoite Stella, A., Vesnaver, M., Gaio, M., Morrison, S.A., Francescato, M.P. Effect of a cooling vest on exercise capacity in patients with multiple sclerosis: A pilot study. *6th International Conference of Physiology and Pharmacology of Temperature Regulation.* 5-8th December 2016, Ljubljana (SLO).

Buoite Stella, A., Francescato, M.P. Thermoregulatory and hydration strategies during exercise in the youth: a review. *1st symposium on Environmental Ergonomics and Physiology*. October 2014, Ljubljana (SLO).

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Original Research

Strategies Used by Patients with Type 1 Diabetes to Avoid Hypoglycemia in a 24×1-Hour Marathon: Comparison with the Amounts of Carbohydrates Estimated by a Customizable Algorithm

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ABSTRACT

Objectives: The preferred countermeasure to avoid exercise-related hypoglycemia was investigated in a group of patients with type 1 diabetes participating in a stressful event, a 24×1-hour relay marathon. The carbohydrates actually consumed were compared to those estimated for each patient by applying a customizable algorithm, Exercise Carbohydrates Requirement Estimating Software (ECRES), based on patient's usual therapy and diet and on the exercise characteristics.
Methods: Glycemia was tested at the start, middle and end of the races. Usual therapies and diets and the adopted countermeasures were recorded in detail.
Results: We studied 19 patients who walked/ran 10.4±2.8 km with a heart rate of 167±11 beats per minute. Of the 19 patients, 7 patients reduced the administered insulin (premeal bolus or basal infusion rate). Glycemia fell by the end of the races (p=0.006; median −1.8 mmol·L^{-1}; interquartile range −0.4 mmol·L^{-1} to −5.3 mmol·L^{-1}), despite 9 patients being hyperglycemic at the start. Of the patients, 14 concluded the race with glycemia on target, and 4 patients were hyperglycemic. Amounts of carbohydrates actually consumed (median 30 g; interquartile range 0 g to 71 g) were not significantly different from those estimated by ECRES (median 38 g; interquartile range 24 g to 68 g), the 2 quantities being significantly related (R=0.64; p=0.003). ECRES estimated lower carbohydrate levels (−13 g) than the amounts actually consumed by the 4 patients who concluded their exercises with hyperglycemia.
Conclusions: Patients preferred to consume extra carbohydrates to avoid the possible exercise-induced hypoglycemia. ECRES would provide satisfactory estimates of the carbohydrate requirements, even for a stressful condition, and almost equal to the quantities consumed following medical advice.

Mots clés :
maladie chronique
compétition
glycémie
métabolisme
exercice

RÉSUMÉ

Objectifs : La contre-mesure privilégiée pour éviter l'hypoglycémie liée à l'exercice a été examinée chez un groupe de patients atteints de diabète de type 1 participant à une épreuve stressante, un marathon à relais 1 heure×24 heures. Les glucides réellement consommés ont été comparés à ceux estimés pour chaque patient en appliquant un algorithme personnalisé, l'ECRES (*Exercise Carbohydrates Requirement Estimating Software*), fondé sur les traitements et les régimes alimentaires habituels des patients et sur les caractéristiques de l'exercice.
Méthodes : La glycémie a été vérifiée au début, au milieu et à la fin des courses. Les traitements et les régimes alimentaires habituels et les contre-mesures adoptées ont été notés minutieusement.
Résultats : Nous avons étudié 19 patients qui ont marché ou couru 10,4±2,8 km à une fréquence cardiaque de 167±11 battements par minute. Parmi les19 patients, 7 patients ont diminué leur administration d'insuline (bolus avant le repas ou débit basal). La glycémie a chuté à la fin des courses (p=0,006; médiane

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–1,8 mmol L^{-1}; intervalle interquartile –0,4 mmol L^{-1} à –5,3 mmol L^{-1}), en dépit du fait que 9 patients ont montré une hyperglycémie au début. Parmi les patients, 14 ont montré une glycémie dans les valeurs cibles à la fin de la course, et 4 ont montré une hyperglycémie. Les quantités de glucides réellement consommés (médiane 30 g; intervalle interquartile 0 g à 71 g) n'ont pas été significativement différentes de celles estimées par l'ECRES (médiane 38 g; intervalle interquartile 24 g à 68 g), les 2 quantités étant significativement liées (R=0,64; p=0,003). L'ECRES a estimé des concentrations plus faibles de glucides (–13 g) que les quantités réellement consommées par les4 patients qui ont montré une hyperglycémie à la fin de leur exercice.

Conclusions : Les patients ont privilégié la consommation de glucides supplémentaires pour éviter la possible hypoglycémie induite par l'exercice. L'ECRES offrirait des estimations satisfaisantes des besoins en glucides, même pour les états de stress, et presque égales aux quantités consommées à la suite des conseils médicaux.

Introduction

Regular moderate-intensity exercise is recommended for patients with type 1 diabetes (1,2) because it can reduce the risk for cardiovascular disease (3,4), enhance insulin sensitivity (5–7) and improve psychological well-being (8). Unfortunately, there is no evidence of long-term benefits of exercise on blood glucose control (9,10). Management of exercise, however, is a challenge for patients with type 1 diabetes; indeed, the desire to exercise is restricted by previous adverse experiences (11) and by fear of hypoglycemia (12–15), i.e. glycemia <3.9 mmol/L (16).

To avoid the exercise-induced hypoglycemia, patients using insulin pumps are usually counselled to temporarily reduce their basal infusion rates (17). A reduced mealtime insulin bolus is often suggested for patients on basal-bolus routines (18–20), although the improved glucose profile is commonly obtained at the cost of hyperglycemia before the effort (19,21). Of note, the latter strategy is useful essentially when exercise occurs early after the bolus (e.g. within 90 minutes) (15) and does not always exempt patients from extra carbohydrates (18,22). According to Gallen (15), however, the mainstay of exercise management remains an increased ingestion of carbohydrates before and during exercise. Indeed, many studies have investigated the category, timing and/or quantity of carbohydrates necessary to prevent hypoglycemia (22–27). It should be noted that excessive carbohydrate ingestion might result in a detrimental increase in blood glucose concentration (28), which might decrease some of the benefits of exercise itself (29).

Recently, a customizable algorithm has been proposed: Exercise Carbohydrates Requirement Estimating Software (ECRES) (26,30). This algorithm deals with several of the factors likely to affect the carbohydrate requirement and is being developed to estimate it on an individual- and situation-specific basis. The algorithm was designed to deal with all patients' therapies. Consequently, it can be applied for patients using any currently available insulin formulations and following either basal-bolus or insulin-pump regimens. Furthermore, it provides appropriate temporary corrections for changes in the usual insulin dosage or time scheduling applied by patients in anticipation of effort. The amount of carbohydrate estimated by the algorithm enabled a group of 27 patients on basal-bolus regimens to complete 1 hour of laboratory-based exercise, performed at 3 different postmeal intervals, with good glycemia in more than 70% of cases (26).

Information concerning patients' preferred strategies to avoid the possible exercise-induced hypoglycemia in real-life settings are, however, scarce (31,32), in particular when patients are engaged in challenging events.

The present investigation aimed to evaluate the freely chosen strategy of a group of patients participating in a particular field-based stressful exercise, the Telethon 24×1-hour relay marathon, in which participants were consecutively involved for a whole day. In addition, the amounts of carbohydrate actually consumed, following the specific advice of patients' physicians, was compared to those estimated for each patient using the ECRES algorithm.

Subjects

Patients with type 1 diabetes were recruited from those attending the regional diabetes units; all gave their voluntary written consent after having been informed about the nature, purpose and possible risks involved. The study was approved by the ethics committee of the Friuli-Venezia Giulia region (Italy) and was conducted according to the Declaration of Helsinki. Patients had been diagnosed with type 1 diabetes for at least 2 years and did not suffer from any other diseases or complications contraindicating physical activities.

The analysis included 19 patients (11 males, 8 females; 36±10 years of age; body mass 68±11 kg); 10 patients were taking multiple daily insulin injections (MDIs); the other 9 were insulin pump users. All participants showed relatively well-controlled diabetes (glycated hemoglobin [AlC] levels of 7.5±0.9%; 58±9 mmol/mol), were fit and were moderately trained.

Methods

The Telethon 24×1-hour relay marathon

The Telethon 24×1-hour relay marathon is a noncompetitive fund-raising event lasting a whole day and involving teams composed of 24 members each. Participants start at 1-hour intervals and are free to run or walk at their own paces along the urban track for 1 hour. The number of laps performed (thus, the distance covered) by each participant and by each team are recorded.

Experimental procedures

Patients were advised to check their glycemia carefully during the preceding 24 hours in order to avoid mild hypoglycemic events. All of them were then free to choose how to counteract the risk for exercise-induced hypoglycemia. Participants received further specific advice by the medical staff, given on the basis of the current guidelines (33) and on the patients' own experiences in similar exercises. Medical staff gave their advice without using the ECRES algorithm.

Patients reached the medical staff (which provided assistance during the whole event) at least 1 hour before the individuals' start times. At that point, they signed the informed consent and answered questions related to their usual therapies and diets as well as any changes in them in anticipation of the effort. Patients were then equipped with the belt of a heart rate (HR) monitor (Polar, Kempele, Finland) to record HR throughout the race. Known amounts of simple carbohydrates were administered before and/or during the exercise, when appropriate, and quantities were recorded in detail.

Just before the start, at the middle and at the end of the race, capillary blood glucose concentrations were measured using a hand-held glucometer (Accu-Chek Aviva, Roche Diagnostics, Indianapolis, Indiana, United States). Glycemia in the range of 3.9 mmol·L^{-1} to 10.0 mmol·L^{-1} was defined as being on target.

After the marathon had been completed, the information provided by the patients and the average measured heart rates were used to calculate, by means of the ECRES algorithm (26,30), the estimated amount of required carbohydrates per patient.

The ECRES algorithm

The following information was uploaded in the algorithm for all patients (26):

1) their usual therapy (i.e. insulin types, doses and time scheduling, together with the dietary carbohydrates)
2) the times of day the patients exercised
3) the capillary blood glucose levels measured just before the start
4) the average HRs measured throughout the race.

In brief, the algorithm calculates the amount of carbohydrates required to avoid glycemic imbalances (reqCHO) as a fraction (Ft) of the overall amount of carbohydrates burned during the exercise (CHOox) and further corrects it by subtracting (or adding) the excess (or lack) of glucose contained in the extracellular compartment (Gb) as follows:

$$reqCHO=(CHOox\cdot Ft)\pm Gb.$$

In turn, Gb is inferred from the actual capillary glucose level measured before the start of the exercise (aGL), depending on the volume of the extracellular fluid compartment (ECF):

$$Gb=(aGL-theoGL(t))\cdot ECF,$$

where theoGL(t) is the theoretical glycemia the patient should have at the time of day the exercise is performed.

The CHOox is computed as the product of exercise duration (exD), and the whole-body carbohydrate oxidation rate, in turn estimated on the basis of the expected exercise intensity (represented by the average HR) and the patient's fitness levels (sedentary or active).

$$CHOox=(m\cdot HR+q)\cdot exD,$$

where the m and q values are those reported in a previous work (34) for trained and untrained patients.

Finally, as described in detail previously (26), Ft is almost proportional to the prevailing insulin concentration throughout the effort (IC(t,j)). This is estimated on the basis of the patient's usual therapy data and on standard pharmacokinetic profiles of the insulin analogues loaded in the system. It is also corrected for the patient's insulin sensitivity (Sens(j)), assumed to be represented by the patient's usual dietary carbohydrate-to-insulin ratio:

$$Ft=(IC(t,j)*Sens(j))\cdot e+f,$$

where t is the time of day and j is the day period (i.e. morning, afternoon or evening) when the exercise is performed; the e and f values have been reported previously (26).

Changes in usual therapy (either in the scheduling and/or the dosage of the last insulin injection) in anticipation of the exercise can be accounted for appropriately.

Data analysis

Heart rate data acquired for each patient throughout the race were first averaged over 10-minute periods. Percentage maximal heart rate (HR_{max}) was then calculated for each patient as the ratio between the average of measured HR values and the theoretical maximal HR (HR_{max}=220 – age, expressed in years).

Results were analyzed using Systat Software, v.11 (Erkrath, Germany). Normally distributed data were presented as mean and standard deviation (SD), whereas skewed variables were reported as median and interquartile range (IQR; i.e. the difference between the 75th and the 25th percentile). The nonparametric Friedman analysis of variance for repeated measures and the Wilcoxon test (or the Mann-Whitney test) were used, as appropriate, to detect significant differences. The Pearson correlation coefficient was used to look for associations between the study parameters. Statistical significance was set as $p<0.05$.

Results

Patients walked/ran an average distance of 10.4±2.8 km (range, 6.5 km to 15.6 km). An average HR of 167±11 beats per minute (corresponding to 90±6% of HR_{max}) was reached in a few minutes and thereafter remained quite stable over time (Friedman test, F=6.04; p=0.812).

At the start of the exercise, median blood glucose concentration was 9.3 mmol·L^{-1} (IQR 7.3 mmol·L^{-1} to 13.3 mmol·L^{-1}) (Figure 1). After about 30 minutes, glycemia was not changed significantly (median 8.1 mmol·L^{-1}, IQR 6.6 mmol·L^{-1} to 11.9 mmol·L^{-1}; Wilcoxon test; p=0.523), whereas at the end of the run, glycemia was significantly lower than both the preceding values (median 6.8 mmol·L^{-1}, IQR 5.6 mmol·L^{-1} to 9.2 mmol·L^{-1}; Wilcoxon test; p=0.006 and p=0.011 for the start and intermediate glycemia, respectively). The fall in glycemia from the start to the end of the exercise amounted to a median value of –1.8 mmol·L^{-1} (IQR –0.4 mmol·L^{-1} to –5.3 mmol·L^{-1}). An increase in glycemia was observed in 3 patients, all of them exercising at least 4.5 hours after the last meal. Figure 2 illustrates, for each of the 3 measuring time points, the number of patients in whom glycemia was on target, too low (<3.9 mmol·L^{-1}) or too high (>10 mmol·L^{-1}). About half of the patients (9 out of 19) started the exercise with hyperglycemia. At the middle of the run, hyperglycemia was observed in 9 patients (6 of them being hyperglycemic also at the start), while at the end of exercise, only 4 patients (21%) showed hyperglycemia. None of the patients started exercising with glycemia that was too low. Hypoglycemia was observed in 4 patients at the middle of the run, with only 1 being unable to recover before the end. Glycemia was observed to be on target in 14 patients (74%) at the end of the exercise. No significant differences were observed in the blood glucose levels measured at the 3 time points by comparing patients using MDIs or insulin pumps, as well as by comparing patients having received (or not) extra carbohydrates (Mann-Whitney test; p>0.254 for all the comparisons).

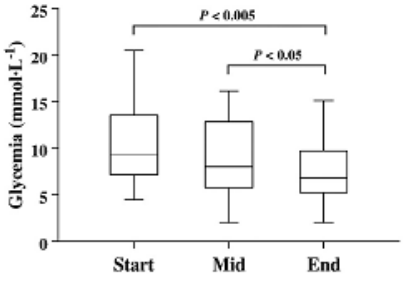

Figure 1. Box-and-whisker plot comparing the blood glucose levels measured before, at the middle and at the end of the 1-hour runs.

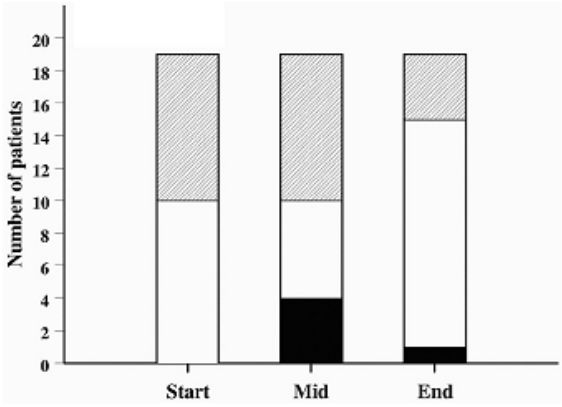

Figure 2. Histogram illustrating the number of patients showing excessively low glycemia (<3.9 mmol·L^{-1}) (black bars); on-target glycemia (3.9 to 10.0 mmol·L^{-1}) (white bars) or excessively high glycemia (>10.0 mmol·L^{-1}) (hatched bars) before, at the middle and at the end of the 1-hour runs.

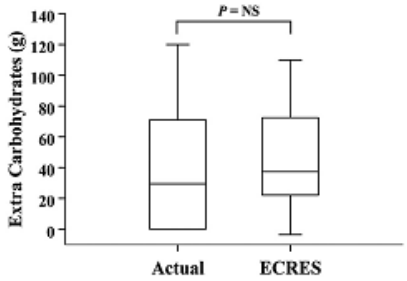

Figure 3. Box-and-whisker plot comparing the amounts of extra carbohydrates actually consumed by the group of 19 patients with type 1 diabetes and the quantities estimated by the ECRES algorithm.

Patients consumed a median of 30 g of extra carbohydrates (IQR 0 g to 71 g) during the last meal, just before and/or during the exercise. No significant difference was detected between patients using MDIs or insulin pumps (Mann-Whitney test; p=0.709). Of the patients on MDIs, 5 did not consume extra carbohydrates; 2 patients (of the remaining 5) reduced their insulin bolus to 60% with the last meal while consuming the usual amount of carbohydrates. Only 1 patient among the insulin pump users did not consume extra carbohydrates, having completely suspended the basal infusion rate during exercise. Among the other insulin pump users, 4 patients reduced their basal infusion rates to 60% during their races.

The amount of extra carbohydrates estimated by the ECRES algorithm (median 38 g; IQR 24 g to 68 g) was not significantly different from the corresponding quantity actually consumed by patients (Wilcoxon test; p=0.653) (Figure 3), the 2 quantities being significantly related to each other (R=0.646, n=19; p=0.003). Of note, the ECRES algorithm estimated a greater amount of extra carbohydrates (+17 g) for the only patient who experienced hypoglycemia at the end of the exercise. Conversely, the amount of extra carbohydrates estimated by the ECRES algorithm for the patients who were hyperglycemic by the end of their races was lower than the amount actually consumed (about –13 g; with the exception of 1 patient, who showed a null value for both the amount consumed and the estimated amount of carbohydrates).

Patients' individual data are summarized in the Supplementary Table A1.

Discussion

The results of the present study showed that about half of the patients started their exercise with high blood glucose concentrations. It should be emphasized, however, that preloading with carbohydrates does not always exempt patients from the need for further extra amounts (18,22). Of note, the reduced basal infusion rate in the insulin pump users helped patients in maintaining glycemia on target, but all of them (except 1) still required some extra carbohydrates. In addition, detrimental effects might be induced by the high glycemia, thus reducing some of the health benefits of exercise (28,29).

Current guidelines for the prevention of exercise-induced glycemic imbalances in patients with type 1 diabetes are based mainly on small studies and observational evidence (35). Patients have to discover their own strategies, and fear of hypoglycemia still remains the strongest barrier to physical activity (11,12,36). Consequently, a large percentage of patients with type 1 diabetes do not reach the recommended levels of physical activity (36,37). Moreover, there is evidence that several factors, such as plasma insulin concentration during the effort, the time of day when the exercise is performed and the patient's degrees of fitness (29,35), are important in determining carbohydrate requirements. Patients often have difficulty in handling all these factors so as to establish the most appropriate countermeasures and/or amount of extra carbohydrate. The ECRES algorithm (26) was designed to fill this gap, providing patients with a tool that can estimate the requirements on the basis of a few simple pieces of data (i.e. exercise intensity and duration and starting glycemia) while automatically taking into account as many factors as possible. Indeed, the results of the present work show that the ECRES algorithm, even under challenging physical activity conditions (i.e. the 24×1-hour Telethon relay marathon, in which each athlete exercised for 1 hour starting at 1-hour intervals during the whole day), would have suggested the same amounts of extra carbohydrates as those actually consumed by patients following the advice of expert physicians. Interestingly, the percentage of patients who ended the race on target was similar to the 70% who finished the 1-hour controlled laboratory exercises with glycemia on target (26). It should be remembered, however, that the risk for hypoglycemia continues during the 12 to 24 hours following the exercise. Future studies are warranted to investigate the possible effects of more stable glycemia during exercise on postexercise glucose levels.

Finally, exercise has been identified as a major obstacle even in current control algorithms for the artificial pancreas (38), and maintenance of optimal glucose control during and after exercise may be challenging despite dual-hormone systems (13). Indeed, the lack of comprehensive models of insulin action and glucose uptake during exercise represents a significant knowledge gap in appropriately quantifying the effects of exercise on overall glucose homeostasis (39). Moreover, as discussed by Basu et al (13), keeping in mind that even healthy individuals without diabetes need to resort to periodic carbohydrate ingestion during and after exercise, it would be impractical, to date, to imagine relying solely on closed-loop control systems (with dual-hormone insulin and glucagon) to prevent exercise-induced hypoglycemia without carbohydrate ingestion. Accordingly, it can be expected that the ECRES algorithm might continue to be helpful while using closed-loop systems as well.

Several different implementations of the algorithm can be foreseen, ranging from versions for single patient use (e.g. apps for smart phones) to web-based solutions. The algorithm can also be easily integrated into many different devices, such as training equipment or glucose monitoring systems. Finally, a real-time version can

easily be implemented on portable devices designed to estimate in real time the extra carbohydrates still available on the basis of actual exercise intensity. As such, they would be able to warn patients of the actual risk for hypoglycemia during their physical activities. Such a device might be intriguing because it might help patients with type 1 diabetes to keep more constant blood glucose levels on each exercise occasion and, thus, to enjoy all the benefits of physical activity safely, in particular if it is spontaneous, thereby liberating them from the fear of exercise-induced hypoglycemia. So far, an international version of the algorithm in the form of an app is under implementation in order to make it easily accessible and user-friendly for both clinicians and patients.

Conclusions

In summary, results of the present work showed that the consumption of extra carbohydrates was required by the majority (13/19) of patients to avoid the possible exercise-induced hypoglycemia. Modification of the insulin dosage did not completely exempt patients from the consumption of extra carbohydrates. The ECRES algorithm provided good estimates of the carbohydrates required to avoid exercise-induced glycemic imbalances that were almost equal to the quantities consumed by patients following medical advice, independent of the time of day when the exercise was performed. These results further open the prospect of a simple device to help patients avoid exercise-induced hypoglycemia.

Acknowledgments

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Author Contributions

RC and MPF drafted the manuscript; ABS, LT, EM and CT assisted in participant recruitment and data collection; ABS, RA, RC and MPF performed the data collection and presentation. All authors approved the final version of the article.

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Appendix: 1

Table A1
Patients' usual regimens, insulin adjustments and extra carbohydrates, either consumed or estimated

	Weight	Therapy	Last meal				Basal insulin		Insulin adjustment	Start time of exercise	Glycemia		Extra cholesterol	
			Time	Insulin		Cholesterol					Start	End	Actual	Estimated
	(kg)				(IU)	(g)		(IU/day)			(mmol/L)	(mmol/L)	(g)	(g)
P01	71	MDI	0:15 PM	Rapid	9.0	100	Glargine	18.0	-	5:00 PM	81	71	71	38
P02	59	Pump	1:00 PM	Rapid	4.2	70	Pump	15.9	-	6:00 PM	233	50	20	37
P03	70	Pump	1:00 PM	Rapid	4.3	100	Pump	12.7	60% basal	8:00 PM	129	127	120	41
P04	64	MDI	8:00 PM	Rapid	10.0	90	Glargine	11.0	60% premeal 1 hour earlier	9:00 PM	168	91	105	113
P05	86	MDI	7:30 PM	Mix 70/30	13.0	100	Glargine	14.0	60% premeal	10:00 PM	317	108	99	87
P06	59	MDI	7:30 PM	Rapid	4.0	80	Glargine	10.0	-	11:00 PM	126	94	0	47
P07	76	MDI	7:30 PM	Rapid	16.0	105	Glargine	26.0	-	12:00 AM	156	189	45	31
P08	80	MDI	9:00 PM	Rapid	6.0	180	Glargine	16.0	-	1:00 AM	208	109	0	80
P09	83	MDI	8:00 PM	Rapid	10.0	100	Glargine	8.0	-	2:00 AM	244	272	0	4
P10	70	Pump	8:00 PM	Rapid	9.	80	Pump	37.5	-	3:00 AM	135	126	30	34
P11	62	MDI	8:00 PM	Mix 60/40	6.5	60	Glargine	19.0	-	6:00 AM	158	117	0	0
P12	75	Pump	7:00 AM	Rapid	7.5	75	Pump	26.1	60% basal	9:00 AM	91	74	52	73
P13	54	Pump	7:00 AM	Rapid	8.5	70	Pump	15.0	-	10:00 AM	185	122	70	63
P14	60	MDI	8:00 AM	Rapid	6.0	48	Glargine	16.0	-	11:00 AM	265	149	0	51
P15	56	Pump	7:00 AM	Rapid	2.0	30	Pump	12.5	60% basal	12:00 PM	229	201	22	10
P16	60	Pump	7:00 AM	Rapid	0.8	15	Pump	18.2	-	1:00 PM	139	192	40	26
P17	78	MDI	1:00 PM	Rapid	3.5	40	Glargine	20.0	Meal 1 hour earlier	2:00 PM	370	175	110	109
P18	86	Pump	12:00 PM	Rapid	11.0	85	Pump	30.1	50% basal	3:00 PM	118	112	10	22
P19	47	Pump	1:00 PM	Rapid	3.0	50	Pump	9.6	Basal suspended	4:00 PM	250	157	0	0

MDI, multiple daily insulin injections.

Fluid intake behaviour in athletes during typical training bouts

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Abstract

BACKGROUND: Hydration habits during training may differ depending on sports mode and individual characteristics. The aim of this study was to assess fluid intake behaviour in a wide sample of Italian athletes during their regular training.

METHODS: Data on hydration habits during training were collected from a random sample of competitive athletes. Hydration strategies and personal characteristics were queried via questionnaire, including athletes' quantity and type of fluid ingested during a typical training bout, sport characteristics (e.g. mode and training duration), and whether their coach encouraged them to drink during trainings.

RESULTS: Three hundred fifty-two competitive athletes participated to the study; two hundred eighty-nine athletes correctly completed all survey items (age: 8-63 y, median: 21±13 y). Athletes were involved in international (3.1%), national (34.1%) and regional (44.9%) competitions. Median fluid intakes during training were 0.25 $L \cdot h^{-1}$; 150 athletes reported fluid intake below the median, whilst 23 athletes (6.5% of total sample) reported fluid intake at or above currently published exercise hydration guidelines (NATA and ACSM). Binary logistic regression indicated that the number of pauses to drink (B: 0.771, significance: 0.000), duration of a typical training bout (B: -2.237, significance: 0.000), and a coach's encouragement to drink (B: 0.601, significance: 0.030) were each associated with fluid consumption above or below the median value.

CONCLUSIONS: Athletes across all disciplines reported drinking less fluid during training than currently espoused in hydration guidelines. A coach's encouragement to drink, the number of pauses during training, and bout duration each influence total fluid volume consumed, regardless of competition level, sex or the age of an athlete.

Abstract word count: 260

Word count: 4040 (without acknowledgements, references, tables and figure captions)

Keywords: behaviour, education, exercise, epidemiology, rehydration solutions

Running title: Ecologically-valid hydration habits

Introduction

Physical activity is a challenge to maintain fluid homeostasis, mainly due to the potential for large amounts of fluid loss via increased sweating, which are not otherwise compensated for by adequate rates of fluid intake.[1] It has classically been demonstrated that moderate hypohydration [on the order of ~2-4% of body mass loss, (BM)] can induce detrimental performance in humans, particularly when core and skin temperatures are concomitantly high. [2, 3] Conversely, several authors have suggested that over-hydration during prolonged exercise may be harmful for health, by increasing the risk of hyponatremia. [4, 5] In addition, drinking beyond thirst may also hinder physical performance. [6]

Currently, guidelines for fluid replacement during exercise have been established for a number of internationally-recognised national associations, including: the National Collegiate Athletic Association (NCAA), the National Federation of State High School Association (NFHS), and the Canadian Society for Exercise Physiology (CSEP), each of which are based on the National Athletic Trainers' Association (NATA) [7] and American College of Sports Medicine (ACSM) [8] published position stands on hydration strategies during exercise. Both the NATA and ACSM guidelines suggest prescribing individualised fluid replacement protocols based on athletes' known sweat rates in order to prevent excessive fluid losses (i.e., >2% BM loss). When no individualised sweat rate protocols are available, these authorities recommend athletes drink between 0.2-0.3 L for every 10-20 min of exercise performed. These recommendations have also been adopted by other expert panels, such as the Academy of Nutrition and Dietetics (formerly known as the American Dietetic Association), Dietitians of Canada [9], and have been highlighted in various scientific reviews. [10] Indeed, the ACSM's latest position stand on hydration report that "drinking to thirst" is not an effective strategy to avoid becoming hypohydrated during exercise (technically defined as BM loss >2%). [8] The underlying assumption here is that the state of hypohydration should be actively avoided, although this logic has been challenged of late. [11, 12] Regardless of whether "drink to thirst" or avoidance of hypohydration is the goal, a

growing body of evidence highlights that educational interventions should be used to promote a better understanding of hydration behaviour during physical activity. [13, 14, 15, 16]

The vast majority of laboratory studies have failed to consider important factors differentiating the effects of hypohydration in lab vs autonomous outdoor exercise, including: intrinsic thirst/drive to drink, training status, airflow speed, blinding participants to hydration status, familiarisation to the stress of experimental trials, exercise pacing, and one's motivation to perform strenuous exercise. Field-based hydration studies have primarily been conducted on adults, and there are relatively fewer studies which also survey children and adolescents in free-living situations. [17, 18] Thus, there is a fundamental lack of field studies investigating hydration status and physical performance in youth. [15, 19, 20] Moreover, several works have reported that even when fluids are freely available during training sessions, both children and adults do not adequately replace their fluid losses. [21, 22] This 'volitional dehydration' has been observed across a variety of sporting contexts and conditions [14, 23, 24], independent of age. [11, 12] Field-based studies usually investigate hydration habits by direct measurement of fluid intake, or using retrospective, questionnaire based self-report, depending on the sample size and study design. [25] When large samples are studied, self-report is generally the most frequently used method to evaluate fluid intake. [26]

Currently, there are few epidemiological data available on fluid habits in ecologically-valid scenarios stratified by sex, age, sport mode and competitive level, and none exist for the Italian population. Therefore, the aim of this investigation was to determine current hydration practices across a range of athletes during their regular training session, and to investigate which factors may affect the fluid volumes ingested. It was hypothesised that the total fluid intake ingested during training would be significantly related to the age, sex, and sport mode of the athlete.

Materials and Methods

This study was approved by the Clinical Review Board for Sports Medicine at the Salus Hospital Trieste, Italy, following the declaration of Helsinki. All participants gave written, informed consent prior to volunteering in the study. Youth athletes (≤18 y) had informed consent signed by their parents or legal guardian. Athletes underwent a preliminary physical examination by a sports medicine physician to assess overall health status before participation in the study. Athletes were recruited during their annual sports medicine check-up, a mandatory procedure in Italy for anyone who wants to participate in any kind of sport competition (See: Italian Ministerial Decree on Competitive Sports, 18th February, 1982). Height and body mass were measured during the medical check-up by the physician.

After the preliminary examination, athletes were requested to complete on paper a hydration surveillance questionnaire. Data were collected from February to July (5 months' total duration) in Trieste, Italy region (ambient temperature: 16±9 °C, range: 12 to 20 °C, relative humidity: 62±6%). In addition, a standard medical history questionnaire was used to exclude participant(s) with any pre-existing conditions, including: evidence of any clinically relevant cardiovascular, hematologic, hepatic, gastrointestinal, renal, pulmonary, endocrine, or psychiatric history of disease. To be included in the analysis, participants needed to exercise at least 2 times per week for not less than 30 min per training bout. Furthermore, they had to officially compete at the regional level (or above) to be considered sufficiently active to continue in the study.

Previously-validated Italian health and physical activity questionnaires regarding hydration strategies during sport training were not found in the review of literature. Self-report for hydration strategies have been suggested as a valid method when large sample sizes are investigated. [25, 27] The hydration questionnaire used in the current investigation was established by a research team composed of a sports medicine physician, a psychologist, a dietitian, and an exercise physiologist. This hydration questionnaire completed a validation process for test-retest purposes; the validation protocol included obtaining data from 20

competitive athletes who completed the survey two times, separated by at least 7 days. All questionnaire items were then evaluated using correlation analysis. Results indicated that all test items reached significance (correlation coefficients >0.85 Pearson's and Spearman's R) with one exception; that question was thus removed from the edited questionnaire version, and eliminated from any further analysis herein.

Athletes in the current study were asked to complete the hydration questionnaireby recalling a typical training session which occurred in the past 7 days. They were given instruction on how to complete the questionnaire by their medical professional, who was also on-hand to answer any questions they might have had when filling out the test items. The hydration questionnaire queries an athlete's age, sex, sport mode, competitive level, and hours of training per week, following suggested methodologies. [25] If more than one sport was practiced, athletes were asked to consider their most important one, defined as the sport involving the greatest number of training hours per week, and then, if equal, the highest competitive level. Competitive level was assessed as 'International' if the participant was currently selected to the Italian National Team, competing in international competitions; they were considered "National" athletes if they were involved in the highest level of competitions within the Italian Championship sport system, or finally, athletes were considered "Regional" if they competed for their regional club, but were not involved in other high-level or national competitions. The athletes were requested to consider both their hours of training per week and the total number of hours for each training bout since a variety of sports had different training schedules. For example, sailing typically involved a greater number of total training hours than other sport modes, but the athletes were sailing less frequently, usually around ~2 days per week.

Hydration-specific questions consisted of determining one's typical self-reported hydration strategies during a regular training session in the previous training session (i.e. not leading up to a particular competition or event). Specifically, athletes were asked to report what type

of fluid they usually drank (water only, sport drink, tea, fruit juice or other), and the quantity (L) ingested during a typical training bout. Athletes were asked to consider/compare the quantity of fluids ingested relative to the volume of the bottles they used during training. Athletes were also asked to indicate how many pauses to drink they usually had during their training, and whether at the end of training they felt thirsty enough to drink something (Yes/No). The final section of the questionnaire consisted of a 3-point Likert scale to rank athletes' perception on whether their coach encouraged them to drink (coaching stimuli) during training. They were asked to rate whether they felt the coach adequately instructed them on the fluid consumption needs specific to their sport (Yes/ No), and whether the coach encouraged the athletes to drink during a typical training bout (0=never, 1=sometimes, 2=always).

Statistical Analyses

All analyses were conducted using IBM SPSS Statistics (v.22.0, Chicago, IL, USA). For frequency analysis, some of the more obscure sports were grouped into more general categories, consisting of: aquatics (diving, fin swimming, synchronised swimming), combat sport (karate, judo, kick boxing) and athletics, which was further separated into three specialities: athletics field (jumping and throw events), athletics sprint (100m, 110m hurdles, 200 m track events), and athletics distance (any running distances over 400 m). Some athletes did not complete all test items, or some answers were illegible. A total of 289 athletes correctly completed all survey items, including the question querying their level of competition status. Thus, for results stratified by competitive level, data have been analysed using the N=289 athletes who correctly completed all test items.

Data are presented as means ± standard deviations (*sd*). The Kolmogorov-Smirnov test was used to test normal distribution of the data sets. Independent t-tests were conducted between groups for all dependent variables. Standard error, 95% confidence intervals of the difference, and Cohen's *d* as effect size coefficients were included where applicable. [28] Non-parametric tests were conducted for any non-normally distributed variables, namely Mann-

Whitney U test for group (sex) comparisons, and the Kruskal-Wallis ANOVA was conducted for group (competitive level) comparison. Univariate associations between nominal and ordinal dependent variables were tested with Chi Square.

Finally, a binary logistic regression was applied to determine the independent variables related to fluid consumption during training ($P < 0.05$). The independent variables (predictors) were: age and sex of participants, competitive level, number of pauses to drink, volume of training per week, training bout duration, coaching stimuli and need to rehydrate after training. For the binary logistic regression, answers concerning 'fluid intake' (dependent variable) were separated into two (binary) groups: (0, Lower fluid intake) athletes who reported a fluid consumption below or equal the median (0.25 $L \cdot h^{-1}$), and (1, Higher fluid intake) athletes who reported a fluid consumption above the median. There were n=186 in the lower and n= 150 in the higher fluid intake groups, respectively.

Results

Sample Characteristics

A total of 352 (M:191 [54%], F:161 [46%]) participants completed the hydration questionnaire, ranging in age from 8 to 63 y (median: 21±13 y, 95% CI: 20 to 22). 230 athletes were ≤ 18 y. Mean body mass (61±16 kg, 95% CI: 59 to 62), height (1.68±0.13 m, 95% CI: 1.66 to 1.69), and BMI (21.1±3.4 $kg \cdot m^{-2}$, 95% CI: 20.8 to 21.5) were within healthy guidelines. Questionnaire responses indicated that 3.1% of the athletes were involved in international-level competitions, 34.1% in national competitions, and 44.9% in regional competitions, whilst 17.9% did not report their competition level (**Figure 1A**). The most commonly played sports were basketball (n=58, M: 44 F: 14), volleyball (n=54, M: 3 F: 51), tennis (n=31, M: 24 F: 7) and athletics distance events (n=29, M: 10 F: 19, **Figure 1B**).

All dependent variables with the exception of body height, body mass and BMI violated normality assumptions. Males were 4±1 y older than females (95%CI of the difference: 1 to

6, P<0.05, Cohen's *d*=0.309), with greater body mass (9±2 kg, 95%CI of the difference: 6 to 12, P<0.01, Cohen's *d*=0.612) and height (0.08±0.01 m, 95%CI of the difference: 0.05 to 0.10, P<0.01, Cohen's *d* = 0.603, **Table 1**).

Training History and Hydration Habits

There were no significant differences between males and females in terms of total training volume per week, single training bout duration, and number of pauses per training to drink, or total fluid intake (**Table 2**), even when normalised by total training hours or body mass. The Kruskal-Wallis ANOVA indicated that the International athletes exercised 6.5±0.9 h (95%CI of the difference: 2.2 to 10.9) longer than National level athletes (P<0.01, Cohen's *d*=1.308) each week, who themselves trained 1.9±0.4 h (95%CI of the difference: 1.2 to 2.6) longer than the Regional athletes (P<0.01, Cohen's *d*=0.690). A single training bout was longer for International athletes by 0.6±0.2 h (95%CI of the difference: 0.2 to 1.0) compared to National level athletes (P<0.01, Cohen's *d*=0. 949), and National level athletes reported longer training durations by 0.2±0.1 h (95% CI of the difference: 0.0 to 0.3) compared to Regional athletes (P<0.01, Cohen's *d*=0. 283).

Water was most commonly ingested during training (0.28±0.21 $L \cdot h^{-1}$, range: 0 to 1 $L \cdot h^{-1}$, 95% CI: 0.26 to 0.30), irrespective of the athletes' sex or competitive level (**Table 2**). Only 23 athletes (6.5% of total sample) reported drinking more than 0.6 $L \cdot h^{-1}$, in accordance to ACSM guidelines. Most athletes (41.2%) reported 'no encouragement to drink' by their coaches, whereas a smaller percentage (28.3%) reported the most consistent encouragement rank. Mean fluid intake when coach's encouragement was 'never' equalled 0.19±0.17 $L \cdot h^{-1}$ (95%CI: 0.16 to 0.23); it was 0.28±0.18 $L \cdot h^{-1}$ (95%CI: 0.25 to 0.31) if the coach's encouragement was ranked 'sometimes', and 0.38±0.24 $L \cdot h^{-1}$ if ranked as 'always' (95% CI: 0.33 to 0.43). Athletes who were 'always' encouraged to drink reported significantly greater fluid intakes than athletes who were 'never' encouraged to drink (by 0.19±0.03 $L \cdot h^{-1}$, 95%CI of the difference: 0.12 to 0.26; P<0.01). Fluid intake for the most popular sports (**Figure 2**)

in males were: basketball 0.29±0.13 $L \cdot h^{-1}$ (95% CI: 0.25 to 0.33), tennis 0.52±0.28 $L \cdot h^{-1}$ (95% CI: 0.40 to 0.64), soccer 0.20±0.14 $L \cdot h^{-1}$ (95% CI: 0.13 to 0.27), and athletics "distance" events 0.18±0.18 $L \cdot h^{-1}$ (95% CI: 0.10 to 0.27). Fluid intake for the most popular sports in females were: volleyball 0.34±0.16 $L \cdot h^{-1}$ (95% CI: 0.29 to 0.39), basketball 0.28±0.08 $L \cdot h^{-1}$ (95% CI: 0.23 to 0.33), combat sports 0.08±0.08 $L \cdot h^{-1}$ (95% CI: 0.02 to 0.14), and athletics "distance" events 0.24±0.29 $L \cdot h^{-1}$ (95% CI: 0.03 to 0.45). The Kruskal-Wallis ANOVA indicated fluid intake was not significantly different between the most popular sports within-sex. The Mann-Whitney U test indicated fluid intake was not significantly different between males and females in their most popular sports.

Logistic Regression Analyses

A binary logistic regression was performed to assess the association between independent variables and the binary high or low fluid intake values during training (**Table 3**). The full model contained predictors for: age, sex, competitive level, pauses to drink, training per week, training duration, coaching stimuli and rehydration; the model was statistically significant, $\chi 2$ (8, n=352, =70.55, P<0.01), indicating it could distinguish between respondents who reported fluid intake above or below the median. The Hosmer and Lemeshow test was not significant, indicating a goodness of fit for this model. The model explained between 24.4% (Cox and Snell R square) and 32.7% (Nagelkerke R squared) of the common variance in fluid intake, and had the strength to discriminate and correctly classify 68.7% of all cases. According to the model, the log-of-the-odds of an athlete being classified in the 'high' or 'low' fluid intake group was positively related to the number of pauses to drink (Wald test: 22.150, P<0.01), negatively related to training duration (Wald test: 17.269, P<0.01), and positively related to coaching stimuli (Wald test: 8.592, P<0.05).

Discussion

This is the first study to characterise current hydration habits in Italian athletes from a wide range of sporting backgrounds, ages and abilities. The data indicate that i) the majority of

athletes are engaged in indoor, team sports, ii) total fluid intake values are significantly lower than currently published guidelines, and iii) athletes' perception of a coach's encouragement to drink directly influences the total volume of fluid consumed during that training bout, regardless of the age, sex, sport type, or competition level of the athlete.

Sport Characteristics of the Surveyed Sample

To our knowledge, this is the first study to examine hydration practices in such a large, and diverse sample of athletes (n=352), ranging from children to older adults (8 to 63 y) in free-living conditions. Males and females were evenly-represented, including a balanced distribution between international, national and regional athletes. Females were principally involved in indoor sport (e.g. volleyball, basketball, combat sport), whereas males were more involved in outdoor sport (e.g. tennis, soccer, athletics), after the most popular sport, basketball. Sailing athletes reported the longest training bout duration (3-h) and total training hours per week (11-h). Other sports with (relatively) longer training durations were: alpine skiing, athletics (field), canoeing, diving and kayak, each averaging ~2-h per training bout, and ~11-h total training per week. In contrast, basketball and volleyball reported 1.5-2.0-h per training bouts for 6-7-h of training per week. The practice duration and total training hours per week did not affect overall fluid intake of athletes when expressed as mean fluid intake per hour exercise. Previous literature has typically focused on matching fluid replacement to sweat rate in an attempt to maintain a euhydrated state. Mean sweat rates are typically suggested to be between 0.5 to 2.0 $L \cdot h^{-1}$ in most athletes. [8] However, many athletic groups report drinking far less liquid than can be replaced from sweat loss alone. For example, male rowers may sweat on average 1.98 $L \cdot h^{-1}$ (from 0.99 to 2.92 $L \cdot h^{-1}$), yet consume mean fluid intakes of 0.96 $L \cdot h^{-1}$ (from 0.41 to 1.49 $L \cdot h^{-1}$ [29]). In indoor sports such as basketball, the literature has reported summer training sweat rates to be in the order of 1.37 $L \cdot h^{-1}$ and yet fluid intakes of only 0.80 $L \cdot h^{-1}$. [30] Indeed, the average rate of fluid consumption in the present study was 0.28±0.21 $L \cdot h^{-1}$. There were no significant differences

in fluid intake values between males and females when normalised per hour of training, or normalised per kg body mass.

Current Hydration Practices Compared to Published Hydration Guidelines

The current ACSM position stand on hydration espouses that athletes should not lose more than 2% BM [8] during a given training/competition. The authors recommend that individuals should periodically drink (as opportunities allow) during exercise, and the amount should be determined based on individuals' estimated sweat lost during a particular exercise task with respect to the weather conditions. [8] For example, for marathon runners, the ACSM position stand suggests a fluid intake *ad libitum* from 0.4 to 0.8 $L \cdot h^{-1}$. For a more generic exercise, the position stand states that athletes will typically sweat at a rate of ~1.2 $L \cdot h^{-1}$ for a 70 kg athlete. Given that our current sample found mean fluid intakes of 0.28±0.21 $L \cdot h^{-1}$, this fluid intake rate would result in a probable loss of ~1.3 % BM (equivalent to 0.9-L of uncompensated sweat loss) for every hour of training, with athletes reaching their 2% BM threshold for 'dehydration' after a common training bout of as little as 1.5 h. To continue, the basketball players in this study reported drinking 0.29±0.12 $L \cdot h^{-1}$, whereas basketball players from other literature have reported drinking up to 0.80 $L \cdot h^{-1}$. [30] Supposing a similar sweat rate for both samples, a 70-kg basketball player could lose 1.7% BM for every hour of training in this study, and since mean basketball training bouts lasted ~1.5 h, it would have been possible to observe fluid losses greater than 2% BM in this sample for this popular sport.

When individualised fluid replacement protocols based on individual sweat rates are not available, NATA and ACSM guidelines suggest consuming ~0.20 L fluid every 15-20 min, or ~0.4-0.8 $L \cdot h^{-1}$. [7, 8] The current sample (across all sports) reported rates of 0.28±0.21 $L \cdot h^{-1}$ (range: 0 to 1 $L \cdot h^{-1}$), irrespective of the athletes' sex or competitive level. Thus, athletes' mean fluid intake is 65% lower than volumes suggested by NATA, and 53% less than volumes proposed by ACSM. Only 23 athletes (6.5% of total sample) reported drinking at or

above 0.6 $L \cdot h^{-1}$. Guidelines for hydration practices during exercise have developed from general recommendations to the adoption of individualised strategies to partially or fully replace sweat losses. [15] However, other studies have found that *ad libitum* drinking to thirst should dictate the amount of fluid consumed during exercise, [11,12, 31, 32] leading to disagreement between scientists, and confusion amongst athletes. As this study did not determine hydration status via direct measures or include an assessment of performance outcomes *per se*, it cannot be speculated on whether a lower fluid intake during training may result in significant athletic impairment for a given sport. This study did find that training bout duration negatively predicted fluid intake behaviour, since longer activities were associated with lower overall fluid intake when normalised per hour of training. Thus, fluid intake during training may be (at least partially) influenced by psychological factors related to fluid availability and overall training duration.

Educational Influence on Hydration Behaviour

Notably, it has been demonstrated that when a coach's knowledge about hydration and nutrition practices may be classified as 'weak' (measured via self-reported questionnaire), coaches preferred to not give any advice to their athletes regarding this topic whatsoever. [33] Fluid intake is one of the most common improperly given advices, even when coaches are reluctant to offer misinformed or outdated recommendations. [34] Therefore, educational interventions may increase a coaches' knowledge about the most current hydration practices which they can later impart to their athletes. [35] Indeed, an athlete's knowledge on nutrition and hydration practices is also reported as 'weak' in the sport-science literature, with only 9% of athletes reporting that they feel sufficiently prepared on this topic. [33] Thus, a short, educational intervention may be useful to both coaches and athletes, since it has been shown that athletes themselves do alter fluid consumption behaviour after educational interventions or short training sessions on the topic. [13, 19] Some authors have reported that poor fluid intake may be related to a weak knowledge of the international guidelines, and thus, short-term educational interventions may help improve fluid replacement practices. [14, 36] In the

current study, athletes ranked 92 (26.1%) coaches as 'always' encouraging them to drink during training, and 99 (28.1%) ranked their coaches as 'never' encouraging them to drink. Therefore, although this study cannot comment on whether coaches were espousing international hydration guidelines directly, it is apparent that athletes are not receiving uniform advice on the topic, regardless of the sport they compete in. Finally to consider, it is established that weight-retention from voluntary fluid intake after dehydration is greater when one consumes a carbohydrate/electrolyte-containing beverage compared to pure water. [37] Thus, fluid composition should also be factored into any hydration advice prescribed to athletes. Hydration guidelines are constantly evolving to include the most recent research findings, and considering that current (and previous) literature espouses vastly different practices regarding hydration for sport performance, it is imperative that both coaches and athletes are aware of current best-practices for their sport and competition level.

Study Considerations

This study utilised a retrospective self-report to query hydration habits in a large and variable sample of Italian athletes. There were no direct measurements of fluid intake or sweat rate conducted during trainings. However, the aim of the present study was not to determine dehydration effects during exercise *per se*, but to characterise the typical hydration strategies athletes employed within a field-based scenario, and to determine which factors may influence fluid intake within this unique cohort. Although retrospective self-report is not considered as accurate as direct measurement, it is an appropriate methodology when larger sample sizes are investigated. [25, 27] Considering the current sample size, the conservative statistical approach employed, and the fact that hydration practices have not previously been investigated in this population, this study provides valuable information regarding hydration practices in an ecologically-valid (i.e. not laboratory) test environment.

Conclusions

Athletes across all disciplines reported drinking less fluid during training than currently espoused in published hydration guidelines. A coach's encouragement to drink, the number of pauses an athlete reports during training, and the training duration can each influence total fluid intake, regardless of competition level, sex or age of the athlete.

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Table 1. Personal characteristics of the study participants (n = 289).

Table 2. Selected hydration awareness questionnaire data of the study participants (n = 289).

Table 3. Binary logistic regression summary.

Figure Captions

Figure 1. (A) Athlete distribution in different competitive levels for males and females (%). (B) Sport frequencies (counts) for males and females. "Aquatics" includes: diving, fin swimming, synchronised swimming; "Combat" includes: karate, judo, kick boxing; "Athletics field" includes: jumping and throw events; "Athletics sprint" includes: 100 m, 110 m hurdles, 200 m track events), Athletics distance (any running distances over 400 m). Athletics alone indicates participants who did not report a specific speciality.

Figure 2. Mean fluid intake ($L \cdot h^{-1}$) and standard deviations during training between the most popular sports, differentiated by sex. Most popular: M: basketball n=44, F: volleyball n=51; second most popular: M: tennis n=24, F: basketball n=14; third most popular: M: soccer n=22, F: combat sports n=10; fourth most popular for both sexes: athletics "distance" events, M: n=19, F: n=10. No significant difference in fluid intake was observed either between-sport or between-sex within a given preferred sport.

Table 1. Personal characteristics of the study participants (n = 289).

Personal Characteristics	Male			Female		
	INT n=5	NAT n=72	REG n=80	INT n=6	NAT n=48	REG n=78
Age (y) *	19±6	21±12	26±16	18±6	18±10	20±12
Mass (kg) *	78 ± 10	66 ± 17	65 ± 16	58 ± 8	57 ± 15	54 ± 13
Height (m) * †	1.80±0.09	1.73±0.14[b]	1.70±0.13	1.68±0.60	1.65±0.10[b]	1.62±0.11
BMI (kg/m^{-2}) *	24.9±1.9	21.3±3.4	21.9±3.8	20.8±2.0	20.8±3.7	20.3±3.1

Notes: INT, international competition level athletes; NAT, national competition level athletes; REG, regional athletes. (*) Significant difference between males and females within a given competition level, (†) significant difference between competition level within-sex ($P < 0.05$). With males and females pooled, ([a]) indicates greater values in international level compared to national and regional level athletes, ([b]) indicates greater values in national level athletes compared to regional athletes, and ([c]) indicated greater values in international level athletes compared to regional athletes.

Table 2. Selected hydration awareness questionnaire data of the study participants (n = 289).

Training and Hydration-Specific Questions		INT (n=11)	NAT (n=120)	REG (n=158)
*How many hours do you practice each week? **	(hours)	13.8±6.4 [a] (CI: 9.5 to 18.1)	7.3±2.9[b] (CI: 6.8 to 7.8)	5.4±2.6 (CI: 5.0 to 5.8)
*How long does a typical training bout last? **	(hours)	2.3±0.8[a] (CI: 1.8 to 2.9)	1.7±0.4[b] (CI: 1.7 to 1.8)	1.6±0.6 (CI: 1.5 to 1.7)
How many pauses or breaks to drink do you have during a typical training?	(count)	2.2±1.3 (CI: 1.4 to 3.2)	1.9±1.7 (CI: 1.6 to 2.2)	1.6±1.2 (CI: 1.4 to 1.8)
What type of beverage do you normally drink before, during or after training?	Sport Drink Water Fruit Juice Tea Nothing (%)	18.2 72.7 0 0 9.1	8.3 73.3 0 0 18.4	7.6 77.2 0.6 1.3 13.3
How much fluid (volume) did you drink in your last training session?	(L)	0.76±0.65 (CI: 0.29 to 1.22)	0.49±0.43 (CI: 0.41 to 0.57)	0.43±0.27 (CI: 0.38 to 0.47)
At the end of your training, did you feel thirsty enough to drink something? (yes/no)	Yes (Y) No (N) (%)	Y=72.7 N=27.3	Y=77.5 N=22.5	Y=80.4 N=19.6
Does your coach actively encourage you to drink at some point during the training session?	Never Sometimes Always (%)	27.2 36.4 36.4	30.4 41.0 28.6	27.0 39.0 34.0

Notes: INT, international competition level athletes; NAT, national competition level athletes; REG, regional athletes. Mean ± standard deviation (CI 95%: Lower Bound to Upper Bound). (*) Significant difference between competition levels ($P < 0.05$). ([a]) indicates greater values in international level compared to national and regional level athletes, ([b]) indicates greater values in national level athletes compared to regional athletes, and ([c]) indicated greater values in international level athletes compared to regional athletes.

Table 3. Binary logistic regression summary.

	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI	
							Lower	Upper
Age	-0.13	0.13	1.067	1.0	0.302	0.987	0.962	1.012
Sex	-0.528	0.301	3.069	1.0	0.080	0.590	0.327	1.065
Competition level	-0.060	0.291	0.431	1.0	0.836	0.942	0.532	1.665
Pauses to drink	0.771	0.164	22.150	1.0	**0.00**	2.162	1.568	2.981
Training per week (h)	0.013	0.065	0.042	1.0	0.838	1.013	0.893	1.150
Training duration (min)	-2.237	0.538	17.269	1.0	**0.000**	0.107	0.037	0.307
Coaches stimuli	0.601	0.205	8.592	1.0	**0.03**	1.824	1.220	2.726
Rehydration	-.021	0.356	0.320	1.0	0.572	0.818	0.407	1.643
Constant	2.784	1.209	5.301	1.00	0.21	16.184		

Notes: Age (years), Sex (1, males; 2, females), Competition level (1, International; 2, National; 3, Regional), Pauses to drink (counts), Training per week (hours), Training duration (minutes), Coaches stimuli (1, "never"; 2, "sometimes"; 3, "always"), Rehydration (i.e., does the athlete need to consume fluids after the training; 0, "no"; 1, "yes"). Sig. $P < 0.05$.

Figure 1

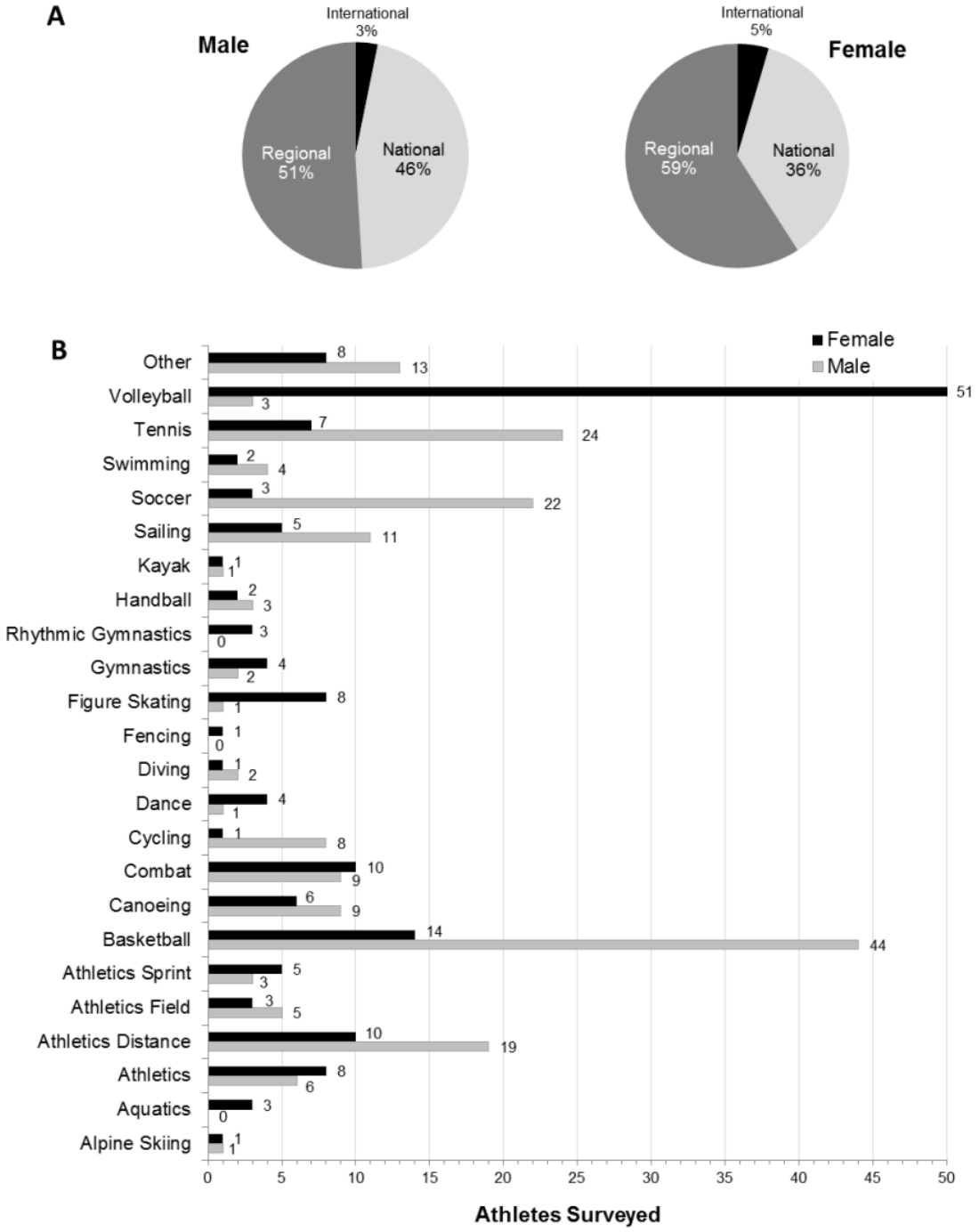
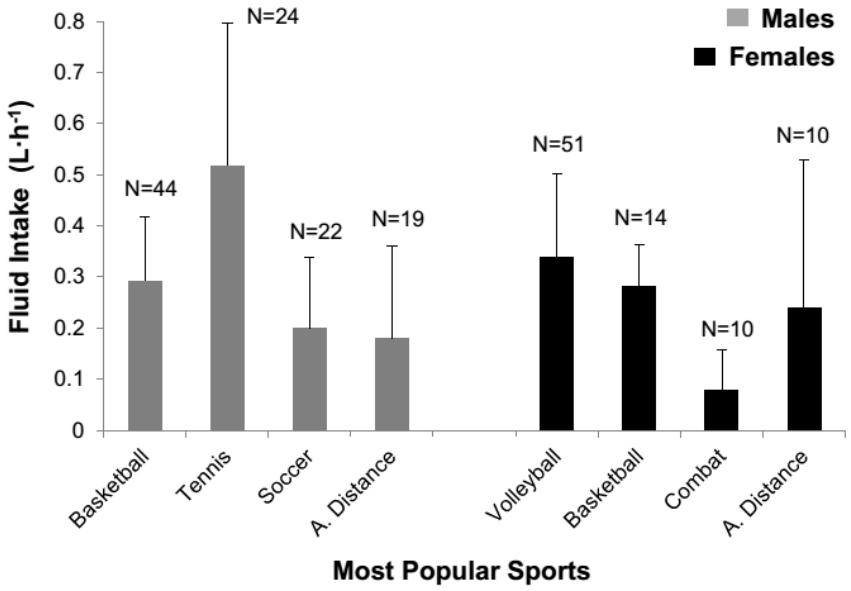

A
Male
International 3%
Regional 51%
National 46%
Female
International 5%
Regional 59%
National 36%
B
Female
Male
Other 8 13
Volleyball 51 3
Tennis 7 24
Swimming 2 4
Soccer 3 22
Sailing 5 11
Kayak 1 1
Handball 2 3
Rhythmic Gymnastics 3 0
Gymnastics 4 2
Figure Skating 8 1
Fencing 1 0
Diving 1 2
Dance 4 1
Cycling 1 8
Combat 10 9
Canoeing 6 9
Basketball 14 44
Athletics Sprint 5 3
Athletics Field 3 5
Athletics Distance 10 19
Athletics 8 6
Aquatics 3 0
Alpine Skiing 1 1
0 5 10 15 20 25 30 35 40 45 50
Athletes Surveyed

Figure 2


Males
Females
Fluid Intake (L·h-1)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
N=44
N=24
N=22
N=19
N=51
N=14
N=10
N=10
Basketball
Tennis
Soccer
A. Distance
Volleyball
Basketball
Combat
A. Distance
Most Popular Sports

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To Simone,
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To my mother, Marina,
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"This thesis is dedicated to all who we recently lost, Samuele, Gianfranco, Antonella, and Mina, and to our hope for the future, Dana Louisa. For a better life through Science"