Unsteady physiology and perception during long “steady state” runs. What role do training characteristics play?

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Kristian Tjørnhom

ABBREVIATIONS

In order of appearance

LIT	Low intensity training
LT_1	First lactate turn point
LT_2	Second lactate turn point
HIT	High intensity training
LT	Lactate threshold
HR	Heart rate
CV drift	Cardiovascular drift
VO_{2max}	Maximal oxygen uptake ($ml \cdot kg^{-1} \cdot min^{-1}$)
SV	Stroke volume
C_r	Cost of running
RPE	Rating of perceived exertion (Borg scale, 6-20)
EMG	Electromyography
HV group	High-volume group
LV group	Low-volume group
RER	Respiratory exchange ratio
VO_{2peak}	Peak oxygen uptake ($ml \cdot kg^{-1} \cdot min^{-1}$)
LR90	Long run performed at 90% of LT_1 speed
LR100	Long run performed at 100% of LT_1 speed
VM	Vastus Medialis
BF	Biceps Femoris
RMS	Root mean square
MDF	Median frequency
HD group	High-drift group
LD group	Low-drift group

ABSTRACT

PURPOSE: To contribute to a better understanding of low-intensity (<LT_1) endurance training by quantifying and explaining the physiological mechanisms associated with cardiac drift and fatigue during low-intensity running and relate them to training characteristics.

METHODS: Twenty-two well trained runners (16 male, 6 female) (37 ± 9 yrs, 69 ± 10 kg, 176 ± 9 cm, peak oxygen uptake 63.6 ± 6.8 $ml \cdot kg^{-1} \cdot min^{-1}$) participated in this study. Based on their weekly training volume and weekly long-run duration, runners were categorized into a high-volume group (HV, n=11) and a low-volume group (LV, n=11). Runners conducted two 120 minute long runs performed at 90% and 100% of LT_1 speed (LR90 and LR100) with repeated measurements every 30 minutes.

RESULTS: There was a significant drift for heart rate, oxygen consumption, ventilation, RER and RPE during both runs ($P<0.05$). There was no difference in drift between groups for any measurements, except for running economy during LR100. The drift was similar during LR90 and LR100 for all measurements except RER, which was significantly greater during LR90 ($P<0.05$).

CONCLUSION: The present study demonstrates that an upward drift occurs for HR, RPE, ventilation, RER, and oxygen consumption for well-trained runners when running at an intensity corresponding to below or approximating LT_1 for 120 minutes. The study also demonstrates that while wide differences are seen at the individual level, there is no consistent difference in the magnitude of physiological or perceptual changes observed between two groups differing by XX% in weekly training volume.

KEYWORDS: Endurance, running, low-intensity training, cardiac-drift, fatigue, electromyography

SAMMENDRAG

HENSIKT: Bidra til en bedre forståelse av lavintensitets (<LT_1) utholdenhetstrening ved å kvantifisere og forklare de fysiologiske mekanismene knyttet til cardiac drift og fatigue ved lavintensitets løping, og relatere dem til treningskarakteristikk

METODE: Tjue-to godt trente løpere (16 menn, 6 kvinner) (37 ± 9 år, 69 ± 10 kg, 176 ± 9 cm, peak oksygen opptak 63.6 ± 6.8 $ml \cdot kg^{-1} \cdot min^{-1}$) deltok i denne studien. Basert på deres ukentlige treningsvolum og varighet på lengste ukentlige langtur ble deltakerne fordelt en høy-volum gruppe (HV, n=11), og en lav-volum gruppe (LV, n=11). Løperne gjennomførte to 120 minutter lange løpeøkter, gjennomført på 90% og 100% av LT_1 fart (LR90 og LR100), med repeterte målinger hvert 30. minutt.

RESULTATER: Det var en signifikant drift for hjertefrekvens, oksygenforbruk, ventilasjon, RER og RPE under begge øktene ($P<0.05$). Det var ingen forskjell i drift mellom gruppene for noen av målingene, bortsett fra for løpsøkonomi under LR100. Driften var lik mellom LR90 og LR100 for alle målinger, bortsett fra RER, som var signifikant større for LR90 ($P<0.05$)

KONKLUSJON: Denne studien viser at en stigende drift skjer for hjertefrekvens, ventilasjon, RER, RPE, og oksygenforbruk for godt trente løpere som løper på en intensitet tilsvarende under eller på LT_1 fart, i 120 minutter. Studien viser også at mens det er store individuelle forskjeller, er det ingen konsekvent forskjell i graden av fysiologiske og perseptuelle endringer observert mellom to grupper som er XX% forskjellige i ukentlig treningsvolum.

NØKKELORD: Utholdenhet, løping, lavintensitets trening, kardiovaskulær drift, fatigue, elektromyografi

STRUCTURE OF THESIS

This thesis consists of three parts:

Part 1 presents the theoretical background for the experiment, a methodological chapter of how the study was performed, and a chapter discussing the methodology.

Part 2 presents a research paper, written in accordance with the guidelines from the open access journal Frontiers in Physiology. This part consists of methods, results, discussion regarding results and conclusion.

Part 3 consists of appendices.

Results, discussion, and conclusions from the study are only presented in the research paper due to word-limitations.

PART 1

THEORETICAL BACKGROUND AND METHODS

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1.0 Introduction

The importance of large amounts of training to perform at a high level is well documented among elite athletes in endurance sports (Fiskerstrand & Seiler, 2004; Seiler, 2010; Stöggl & Sperlich, 2015; Tønnessen et al., 2014). The performance of repeated bouts of exercise over a long period of time causes numerous physiological changes that result in improved performance in endurance activities (Jones & Carter, 2000). The training response depends on the duration, the frequency and the intensity of these repeated bouts of exercise (Jones & Carter, 2000). While scientists are in general agreement about the physiological factors limiting endurance performance (Coyle, 1995; Esteve-Lanao, Foster, Seiler, & Lucia, 2007; Hawley & Stepto, 2001; Pate & Kriska, 1984), there is still debate about how the daily training components should be distributed to best improve performance (Seiler & Kjerland, 2006). When planning endurance training it is important to include different intensities (Fiskerstrand & Seiler, 2004; Laursen, 2010; Stöggl & Sperlich, 2015). For example, low intensity training (LIT), lactate threshold training, and high-intensity interval training, are well-known training methods used to exercise within different regions of the intensity scale (Seiler, 2010). Several recent studies examining training intensity distribution have used the first and second lactate and ventilatory turn points (LT^1/VT^1 and LT^2/VT^2) to define three intensity zones (Zone 1, Zone 2 and, Zone 3) (Esteve-Lanao, San Juan, Earnest, Foster, & Lucia, 2005; Hofmann & Tschakert, 2010; Meyer, Lucia, & Earnest, 2005; Seiler & Kjerland, 2006; Zapico et al., 2007). This intensity scale defines zone 1 as intensity below LT_1, zone 2 as intensity between LT_1 and LT_2, and zone 3 as intensity above LT_2 (Seiler, 2010) (figure 1).

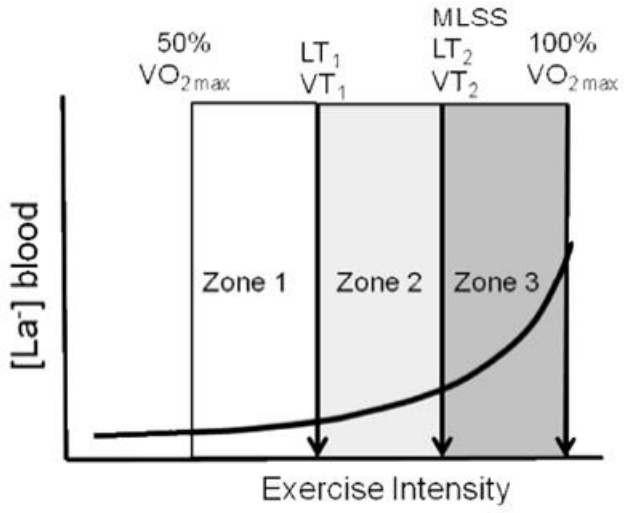

Figure 1. A three-intensity-zone model based on ventilatory and lactate thresholds (Seiler, 2010).

There are several different training models being used by athletes. One model can be described as a *threshold*-training model, where a large part of the total training volume is training performed very near or at the lactate threshold (LT). This method has been shown in several studies to significantly improve physiological markers and performance in untrained subjects (Denis, Dormois, & Lacour, 1984; Gaskill et al., 2001; Kindermann, Simon, & Keul, 1979; Londeree, 1997). Another model has been described as *polarized*-training, where training is predominantly performed either clearly below or clearly above their LT intensity (Seiler & Kjerland, 2006). This model has emerged from studies of world class rowers (Steinacker, Lormes, Lehmann, & Altenburg, 1998), time-trial cyclists (Schumacher & Mueller, 2002), and elite marathoners (BILLAT, DEMARLE, SLAWINSKI, PAIVA, & KORALSZTEIN, 2001). It has also been shown that many athletes employ what has been termed a *pyramid*al-training model, with extensive volume spent in zone 1 (>70%), less time in zone 2, and very little in zone 3 (BILLAT et al., 2001; Seiler & Kjerland, 2006; Tønnessen et al., 2014). The latter 2 intensity distribution models are most commonly used among high performing endurance athletes. Common to both is the observation that athletes execute ~70-80% of their training sessions below the LT_1, that is, LIT (BILLAT et al., 2001; Esteve-Lanao et al., 2007; Neal et al., 2013; Seiler & Kjerland, 2006; Seiler & Tønnessen, 2009; Steinacker et al., 1998). Interestingly, High intensity training (HIT) has been shown to have a positive effect on aerobic performance for both recreational athletes and elite athletes (Laursen, 2010;

Stöggl & Sperlich, 2015), and is frequently investigated in laboratory studies. However, considering that most of the training for a highly trained endurance athletes is performed at a "low" intensity relative to maximal capacity, there is need for more research on this part of the training. The range of intensities that fall into the zone 1 region is broad and creates a very interesting discussion about optimizing intensity and duration in this intensity zone. This is underscored by Esteve-Lanao et al. (2007) that describes heart rate ranges for intensity zone 1 as 50-80% of maximum heart rate (HR). When performing a long-duration, LIT session at a constant-pace or power, fatigue processes do still occur. For example, HR increases slowly during exercise at a fixed constant work rate (Mattsson et al., 2010). Changes in the central circulation during prolonged exercise are well established (Mattsson et al., 2010). This phenomenon is known as cardiovascular drift (CV drift), and is characterized by a rise in HR over time during a constant-rate submaximal exercise at an intensity of 50-75% of maximal oxygen uptake (VO_{2max}) (Coyle & Gonzalez-Alonso, 2001; Wingo, Ganio, & Cureton, 2012). It is crucial to control not only intensity, but also the duration for any specific intensity to induce an appropriate training effect while avoiding excessive fatigue (Viru, Smirnova, ICKarelson, & Viru, 1996).

1.1 Overall goal and hypothesis

The internal workload, or physiological cost, associated with maintaining a given external workload (a running pace or cycling power, for example) tends to shift upward over time during low intensity sessions. This study aims to quantify and explain this shift and associated metabolic, cardiac, and kinematic "drift" happening during a long-duration LIT running session, exploring potential physiological mechanisms and possible associations with subjects' training characteristics. By quantifying the fatigue patterns associated with long, low-intensity training sessions, and relating them to individual training characteristics, we hope to provide useful information for optimizing the prescription of LIT, as a tool in developing the physiological determinants associated with endurance performance.

<u>Main purpose</u>

1. To comprehensively quantify physiological and perceptual changes during long LIT running bouts and contribute to a better understanding of the physiological mechanisms associated with cardiac drift and fatigue during LIT.

2. To detect possible differences in cardiac drift and fatigue between two groups differing in training characteristics.
3. To quantify differences in cardiac drift and fatigue rate between a long run performed at clearly below LT_1 and one performed right at LT_1.

Hypothesis:

Prior training characteristics affect the rate of cardiac drift and fatigue during a submaximal low intensity run.

2.0 Theoretical framework

2.1 Physiological factors influencing endurance performance.

Endurance can be defined as "the capacity to sustain a given velocity or power output for the longest possible time" (Jones & Carter, 2000). Endurance exercise, in the form of running, is continuous training or competition of approximately 5 to 240 minutes duration (Jones & Carter, 2000). Running performance in long events depends on a complex interplay of factors (Joyner, 1991). Endurance training results in adaptations in the pulmonary, cardiovascular and neuromuscular systems that improves both maximal oxygen delivery capacity to the working muscles and their capacity to consume that delivered oxygen, a combination consistently associated with improved endurance performance (Hawley, 1995; Jones & Carter, 2000). There are three key physiological factors that are considered the most important for endurance performance (Jones & Carter, 2000). These physiological factors include VO_2max, fractional utilization rate of VO_{2max}, and the efficiency of mechanical work performed relative to metabolic cost, termed "economy" in distance running (Conley & Krahenbuhl, 1980; COSTILL, THOMASON, & ROBERTS, 1973; Farrell, Wilmore, Coyle, Billing, & Costill, 1979; Jones & Carter, 2000; Jones & Doust, 1998; Pollock, 1977; Svedenhag & Sjodin, 1985).

2.1.1 Maximal oxygen uptake (VO_{2max})

VO_{2max} is one of the most common measurements made in exercise physiology laboratories (Howley, Bassett, & Welch, 1995). The term maximal oxygen uptake was used first by Hill et al. in 1923, and is traditionally defined as "the maximal rate at which oxygen can be taken up and used by the body during exercise" (Hill & Lupton, 1923). VO_{2max} is generally accepted as a gold-standard measure of the functional limit of the cardiovascular system (Rowell, 1974), and is commonly used as an index of cardiorespiratory fitness (Howley et al., 1995). Already in the 1930s, very high values for VO_{2max} in athletes were found and identified as a marker for elite performance (Robinson, Edwards, & Dill, 1937). VO_{2max} values for male elite athletes typically range from 70 to 85 $ml \cdot kg^{-1} \cdot min^{-1}$, with extreme values exceeding 90 $ml \cdot kg^{-1} \cdot min^{-1}$ (Costill, 1986; Daniels, 1974; Haugen, Paulsen, Seiler, & Sandbakk, 2018; Pollock, 1977; Saltin & Astrand, 1967). The most striking adaptations to training that contribute to high VO_{2max} values include increased stroke volume (SV), increased blood volume, increased capillary density and increased mitochondrial density in the trained muscles (Costill, Fink, &

Pollock, 1976). Several studies have shown that the VO_{2max} is highly related to running performance (BILLAT et al., 2001; COSTILL et al., 1973; Esfarjani & Laursen, 2007; Foster, Costill, Daniels, & Fink, 1978; Saltin & Astrand, 1967). Although important, the VO_{2max} is only one of the factors that determine success inn endurance competitions. A study by Sjodin & Svedenhag (1985) reported large variations in running capacity between runners of equal VO_{2max} and vice versa.

2.1.2 Running economy

Until recently, the importance of running economy on endurance performance has been given less attention than the other factors for endurance performance (Barnes & Kilding, 2015), despite awareness of its importance since at least the late 1970s (Lucia et al., 2006). Running economy is defined as the steady-state oxygen consumption at a given running velocity (C_r – Cost of running). C_r reflects the energy demand of running at a constant submaximal speed (Conley & Krahenbuhl, 1980; Daniels, 1985; Saunders, Pyne, Telford, & Hawley, 2004) and is typically quantified as ml $O_2 \cdot kg^{-1} \cdot km^{-1}$ (Bassett & Howley, 2000; Brueckner et al., 1991; Jones et al., 2021; Lucia et al., 2006). Runners with a good running economy use less oxygen compared to runners with a poorer economy at the same submaximal constant speed (THOMAS, Fernhall, & GRANAT, 1999). Improvement in running economy indicates a lower oxygen consumption for a given absolute running speed (Lucia et al., 2006). Differences in running economy across individuals are substantial and have been associated with anthropometric, physiological, metabolic, biomechanical, and technical factors (Bailey & Pate, 1991). Running economy has been shown to be better in trained athletes than untrained individuals (Morgan et al., 1995). Further, the best endurance athletes are usually the most efficient (Conley & Krahenbuhl, 1980; Daniels, 1974; Noakes, 1988). In addition to this, a lower energy cost of running (C_r) has been shown in Kenyan runners, and other runners from Africa compared to runners of European descent (Saltin et al., 1995; WESTON, MBAMBO, & MYBURGH, 2000). Further, a study investigating the running economy of Eritrean runners, found running economy for the Eritrean runners to be among the best ever reported (Lucia et al., 2006).

A study by Weston et al., (2000) comparing running economy in African and Caucasian runners found that the two groups had similar 10km performance. In this comparison, the African runners had a significantly lower VO_{2max}, but the similarity in race performance was explained by their significantly better running economy. A similar finding was reported by

Scrimgeour et al., (1986), demonstrating that in a group of marathon runners with similar VO_{2max} and % VO_{2max} the best performers showed a significantly better running economy compared to the other groups.
In this regard, numerous factors can determine better running economy, such as comprehensive training background, muscle fiber type, and particularly, biomechanics (Saunders, Telford, et al., 2004).

2.1.3 Fractional utilization of VO_{2max}

Fractional utilization of VO_{2max} refers to "the percentage of an athlete's VO_{2max} that can be utilized at a specific work rate" (Hawley, 1995). The "threshold velocity" integrates VO_{2max}, fractional utilization and running economy, which are known to separately contribute to better performance (Svedenhag & Sjodin, 1985). The "threshold velocity" or work intensity corresponding to LT is the highest exercise intensity an athlete can sustain for an extended period without lactate accumulating (Wells & Pate, 1988). This intensity can be calculated through both fixed lactate concentrations and individualized lactate thresholds (Christopher, 2000). With sufficient training containing both LIT and HIT a rightward shift of the LT curve to a higher velocity may occur, meaning that one can work at a higher velocity without further accumulation of lactate (Jones & Carter, 2000). Well-trained athletes usually have a higher fractional utilization rate than athletes less well-trained (Jones & Carter, 2000). The fractional utilization of VO_2max has been shown to be significantly correlated with running performance in distances from 5 km to marathon (COSTILL et al., 1973; Sjodin & Schele, 1982; Sjodin & Svedenhag, 1985). The fractional utilization of VO_{2max} has been shown to increase more through training than the actual VO_{2max} (Bassett & Howley, 2000). Åstrand and Rodahl provided an early characterization of the impact that training has on one's ability to maintain a high percentage of VO_{2max} during prolonged exercise (Åstrand & Rodahl, 1970). They reported that trained individuals could maintain 87% and 83% percent of their VO_{2max} for 1h and 2h respectively, while untrained subjects maintained only 50% and 35% of their VO_{2max} for 1h and 2h (Bassett & Howley, 2000; Åstrand & Rodahl, 1970). Studies have demonstrated that high-level marathon runners may sustain an average of 80-85% of VO_{2max} during a marathon (Costill, 1972; Sjodin & Svedenhag, 1985) whereas marathon runners on a lower level may only sustain an average of 60-75% of VO_{2max} during a marathon (Farrell et al., 1979; Sjodin & Svedenhag, 1985; Wells, Hecht, & Krahenbuhl, 1981). A recent study published on world-class runners training to break the 2h barrier in the marathon estimated

that the best performers could sustain ~90% of their maximal oxygen consumption for 2 hours (Jones et al., 2021).

2.2 Low intensity training

Although the underlying physiological adaptations associated with improved endurance performance are well known, there is still debate about how an athlete should train to induce these adaptations and improve performance (Esteve-Lanao et al., 2007). A key issue of the debate is how the training should be distributed regarding intensity (Esteve-Lanao et al., 2007). The large amount of training performed by successful endurance athletes in different sports is well documented (Fiskerstrand & Seiler, 2004; Seiler, 2010; Stöggl & Sperlich, 2015; Tønnessen et al., 2014), and it is the combination of the training volume and the intensity that it is performed at, that determines the impact of training on an athlete (Seiler & Tønnessen, 2009). Over a considerable range, increases in total training volume correlate well with improvements in physiological variables and performance (Seiler, 2010). Several authors, including Foster at al. (1977) and Slovic (1977) have suggested the volume of training (miles/week) during the months before a competition to be of high importance for competitive running performance. Recent studies have shown a correlation between average weekly mileage during the last two months preceding a marathon and performance (Foster et al., 1977). It seems that sufficient volumes of relatively low-intensity training are a crucial part of competitive endurance training programs (Esteve-Lanao et al., 2007; Esteve-Lanao et al., 2005; Fiskerstrand & Seiler, 2004; Ingham, Carter, Whyte, & Doust, 2008; Zapico et al., 2007). The high volume and frequency of LIT stimulates adaption throughout the body, but may also provide a platform for additional adaptions that occur in response to HIT (Esteve-Lanao et al., 2007; Laursen, 2010). Elite endurance athletes achieve good results when accumulating a high training volume, where the major part of the volume is prolonged training bouts of about 60 to 180 min performed at a low intensity, in combination with a modest proportion of training performed at higher intensities (A. Hawley, Myburgh, Noakes, & Dennis, 1997; Seiler, 2010). Esteve-Lanao et al. describes heart-rate ranges for intensity zone 1 as 50-80% of HR_{max} (2007), making it a somewhat broad-ranged zone considering heart-rate differences. Considering the importance of high training volume in endurance sports, and that approximately 80-90% of this total training volume (~80% of training sessions and up to 90% of "time in zone") seems to be performed as LIT (Seiler, 2010), this underlines how much training an athlete actually performs as LIT throughout a career, and

further underlines the importance of enhancing our knowledge of the physiological and perceptual responses that occur during LIT.

LIT is training performed at an intensity below LT_1. LT_1 is the lowest intensity at which there is a sustained increase in blood lactate concentration above resting values (Christopher, 2000). Training below LT_1 is so-called aerobic training. The aerobic energy system is characterized by the combustion of carbohydrates and fat in the presence of oxygen (Gastin, 2001). The determination of the exact intensity corresponding to LT_1 has been described using different methods, for example a fixed blood lactate concentration of 2 $mmol.L^{-1}$ (Kindermann et al., 1979), the workload associated with a 0.4 $mmol.L^{-1}$ increase above baseline values (Christopher, 2000), or a workload associated with a 1.0 $mmol.L^{-1}$ increase above baseline values (Farrell et al., 1979). This intensity can also be estimated using rating of perceived exertion (RPE), HR as a percentage of HRmax or oxygen consumption (Bourdon et al., 2017). Some of the benefits of LIT include an increased number of capillaries within the trained muscles, increases in myoglobin concentration, proliferation of the number and size of mitochondria, and a large increase in oxidative enzyme activity (Wilmore & Knuttgen, 2003). LIT involves mainly slow twitch motor unit recruitment (Enoka & Duchateau, 2008; Laursen, 2010).

The magnitude and importance of LIT (zone 1) in endurance sports have been shown above, but less is known about how to prescribe and distribute this large training volume within the Low intensity zone (Z1 in a 3-zone model). Training duration is rarely prescribed by individual measures, and rather just a product of experience and "what everyone else does" (Hofmann & Tschakert, 2017). The duration of a session within the different training zones is important, as the exercise duration interacts with intensity to determine the different kinds of cellular stressors, degree, types of fatigue, and training effect (Hofmann & Tschakert, 2017). Still, explicit prescription guidelines for individually optimal duration and intensity combinations are missing (Hofmann & Tschakert, 2017). Small differences in intensity slightly below or above LT_1/VT_1 are usually not detected and recognized by athletes although they may be quite important with respect to the maximal duration, grade of fatigue, and the subsequent recovery time (Burnley & Jones, 2018). An intensity just slightly above LT_1 has shown to increase lactate values which could indicate that the lactate clearance rate for the working muscle has been exceeded, and therefore, markedly different hormonal and cardio-respiratory responses may be induced (Moser et al., 2015). Therefore, an individual and accurate intensity prescription seems to be crucial even at a work intensity below LT_1, since

intensities just 10% above LT_1, has shorten the time to fatigue by ~40% (Hofmann & Tschakert, 2017). A consequence of too high intensity during LIT training is an increased rate and magnitude of fatigue, which could lead to athletes not being able to repeat a high volume of training on a daily basis (Hofmann & Tschakert, 2017). Based on this it is suggested that both intensity and duration for any specific intensity is crucial to induce training effect or to avoid overload (Tremblay, Copeland, & Van Helder, 2005; Viru et al., 1996).

To achieve the desired adaptive stimuli at a manageable level of stress during a training session or a training period it is important to prescribe athletes with a correct workload. Training can be quantified through either internal or external workloads (Halson, 2014; Mujika, 2017). Internal workload is defined as "the relative biological (both physiological and psychological) stressors imposed on an athlete during training" (Bourdon et al., 2017). Common measures to assess the internal workload are HR, blood lactate, oxygen consumption and RPE. In contrast external workload is an objective measure of the mechanical work performed by an athlete and is measured independent from the internal workload. Common measures for the external workload are power output (watts) and speed or pace (Bourdon et al., 2017; Mujika, 2017). LIT is performed at an intensity with a relatively low internal and external workload but a high volume or duration. Internal and external workload and their relationship become very interesting when distributing LIT, considering that the combination of frequency, volume, duration, and intensity of LIT plays an important role for the total workload (both external and internal) (Bourdon et al., 2017). Ensuring an appropriate workload is important for decreasing fatigue and optimizing adaptations to training (Halson, 2014; Pyne & Martin, 2011). Assessing the correct combination of internal and external workload is of great importance, considering that a given external workload may not provide the same internal workload at all times, but rather, is highly dependent on an athlete's state of fatigue, recent training history and/or psychological state (Halson, 2014). This is known as "uncoupling" of internal and external load (Halson, 2014). This uncoupling of the internal and external workload can occur as a consequence of the chronic training load and acutely during training sessions over the duration of a training session.(Maunder, Seiler, Mildenhall, Kilding, & Plews, 2021) A study by Maunder et al., (2021) showed that a group of 11 well trained marathon runners showed a relative increase in internal work from after ~ 60% of the total marathon duration, despite running at a sub-maximal pace. Further, during a 4hr constant-power bike ride at 60-65% of self-identified best-effort 60min power, 28 age-group cyclists and triathletes showed moderate to high

decoupling, while 15 subjects showed low decoupling. These data illustrate how physiological responses to prolonged exercise below LT are not constant.

2.3 Physiological responses during long duration running.

Fatigue-related mechanisms has been shown for moderate- and high-intensity training (Hofmann & Tschakert, 2017). For endurance exercise performed below a certain threshold, acute responses seems to only occur after a long duration exercise (Viru et al., 1996). However, fatigue processes do indeed seem to occur during LIT as well.

2.3.1 Cardiovascular drift

Changes in the central circulation during prolonged exercise are well stablished (Mattsson et al., 2010). CV drift is a phenomenon whereby some cardiovascular responses begin a gradual time-dependent drift (Coyle & Gonzalez-Alonso, 2001). CV drift during constant-load, prolonged low intensity exercise is a well-known phenomenon characterized by a rise in HR and a fall in SV (Coyle & Gonzalez-Alonso, 2001; Wingo et al., 2012). This phenomenon may lead to a gradual decrease in physical work capacity during prolonged exercise (Saltin & Stenberg, 1964). There have been two prevailing hypotheses regarding the causes of CV drift. The first and traditional one asserts that CV drift is the consequence of a progressive increase in cutaneous blood flow and it is thought that the rise in cutaneous blood flow may be accompanied by a reduction in central venous pressure, and thereby a reduced SV, and that the increase in HR is most likely a reflex intended to maintain the cardiac output, despite the lowered SV (Rowell, 1986). The second hypothesis asserts that the increased HR lowers ventricular filling time and, thereby, SV (Coyle & Gonzalez-Alonso, 2001). Mean core- and skin temperature has been shown to increase during exercise in both cold and hot ambient temperatures (Gliner, Raven, Horvath, Drinkwater, & Sutton, 1975; Lafrenz, Wingo, Ganio, & Cureton, 2008). It is likely that factors such as increased core temperature and dehydration that occur during long-duration exercise become more pronounced as exercise duration increases (Wingo et al., 2012), and CV drift has been proposed to be affected by increases in these factors. (Jose, Stitt, & Collison, 1970; Montain & Coyle, 1992; Rowell, 1974). Further, CV drift has been shown to be greater in hot versus cool temperatures (Gliner et al., 1975; Lafrenz et al., 2008). However, it is important to note that HR “drift” upward during long LIT can occur independent of either significant dehydration or increased core temperature. HR increases can also reflect muscular fatigue, changes in muscle fiber recruitment, and acute

decreases in work efficiency/economy (Maunder et al., 2021; Scheer, Vieluf, Cramer, Jakobsmeyer, & Heitkamp, 2018; Viru, Karelson, & Smirnova, 1992; Zouhal, Jacob, Delamarche, & Gratas-Delamarche, 2008). CV drift should not be interpreted as a benign response with no relation to actual intensity or stress responses (Wingo et al., 2012), and its consequences are important when prescribing training. A study by Wingo et al., (2012) found that VO_{2max} decreases concurrently with CV drift. This means that a given relative workload may reflect a higher relative metabolic intensity than intended, as the upward CV drift and decrease in VO_{2max} drift leads to the exercise being performed at a higher percent of VO_{2max} (Wingo et al., 2012). According to Lambert et al., (1998) it may be speculated that less CV drift occurs after endurance training, but there are no scientific data to confirm this.

2.3.2 Running economy during long duration running

Some studies investigating the relationship between running economy and fatigue propose that running economy declines during prolonged exercise at 65-80% of VO_{2max}, and that there is an association between the magnitude of decline in running economy and the exercise intensity and duration (Sproule, 1998; Xu & Montgomery, 1995). However, there are also studies suggesting that fatigue does not affect running economy (BROOKS, HITTELMAN, FAULKNER, & BEYER, 1971; Dressendorfer, 1991). The proposed deterioration in running economy associated with prolonged exercise may be related to acute muscle damage, and therefore an increase in muscle fiber recruitment (Davies & Thompson, 1986), alterations in neuromuscular function (Burgess & Lambert, 2010) or/and alterations in kinematic measurements (Kyröläinen et al., 2000; Nicol, Komi, & Marconnet, 1991). It can also be due to a shift in muscle recruitment towards less efficient fast-twitch motor units, which have a larger VO2 and heat production per work unit during low contraction speed, compared to slow-twitch fibers (Barclay, Constable, & Gibbs, 1993).

2.3.3 Muscular fatigue/Electromyography

It is known that muscles and the nervous system are subject to acute fatigue alterations during exercise (Millet, Martin, Martin, & Vergès, 2011). Fatigue can either be classified as central, i.e., nervous or peripheral i.e., muscle (Enoka & Stuart, 1992). Neuromuscular fatigue is an exercise-related reduction in maximal voluntary activation, and this leads to an inability to recruit motor units and/or discharge them at a sufficient rate to maintain muscular force (Giandolini et al., 2016). This seems to be of great importance for prolonged exercise (Martin

et al., 2010). Muscular fatigue is not the point of task failure, but a decrease in the power a muscle is able to produce, and it seems to develop gradually during sustained physical activity (Enoka & Duchateau, 2008). A study by Millet and Lepers (2004) showed that prolonged exercise induces significant central fatigue, and the intensity and duration of a activity is likely to be among the most important factors for fatigue (Martin et al., 2010). According to recent studies, running duration seems to determine the amount of central fatigue, whereas average central deficits were found to be -8 and -28% after running bouts of 3 and 8.5 hours, respectively (Millet et al., 2002; Millet, Martin, Lattier, & Ballay, 2003). The effect of running duration on the maximal muscle force has been shown above, however, how duration affects the rate of muscle activation during a LIT session typical of daily training in distance runners is not established. Muscle activation and fatigue are commonly measured through electromyography (EMG) (Cifrek, Medved, Tonković, & Ostojić, 2009). EMG is measuring the myoelectrical signal which is discharged when a muscle is activated (Bhattacharya & McGlothlin, 1996), and yields information about the amplitude and frequency of the recruitment signals (Bhattacharya & McGlothlin, 1996).

This possible drift in different mechanism during prolonged running raises questions concerning the prescription of LIT, considering intensity and duration. As well as how to handle these responses if and when they occur.

3.0 Methods

3.1 Study design

This master's thesis was part of a wider research project conducted with a fellow master's degree candidate in sports science at the University of Agder, Faculty of Health and Sport Science, Department of Sport Science and Physical Education. The project consisted of two master's theses. The study was designed as a quantitative descriptive study with repeated measures of runners performing long duration endurance sessions at a controlled, "sub-threshold", or LIT intensity. After preliminary testing to establish training intensity zones, we prescribed two 120-minute long runs, one clearly below LT_1 and one approximating LT_1, with repeated measures of physiological mechanisms, kinematics, EMG, and RPE performed every 30 minutes to answer the research questions. Prior to the study, the authors conducted extensive pilot testing to optimize the testing process, both in terms of data acquisition and practicality for the runners.

The participants were invited to an information meeting, which was later cancelled due to corona-restrictions, and all information were instead distributed digitally via an information sheet (Appendix 1). All data collection and testing was performed between December 2020 and February 2021.

3.2 Subjects

Subjects were recruited in October 2020 through local running clubs, personal interactions, and announcements on social media. The target group for the study was experienced, well-trained runners. A total of 61 runners (48 male, 13 female) announced their interest in participating and were asked to fill out a questionnaire (Appendix 5) quantifying their training and performance status. The data collected from the questionnaire provided useful information about the subjects and gave the authors the opportunity to achieve a varied group of subjects including a range of ages, sex, and performance level. The high number of interested runners gave us an opportunity to pick a convenience sample well suited to answer the research questions. When choosing the subjects for the study the following inclusion criteria were used to assess whether the runners should be included: (1) aged 18-55 years, 2) absence of disease and injuries 3) running at least 30 km/week for the last eight weeks (4) sufficiently trained to perform a two-hour low intensity running session on a treadmill. The

exclusion criteria used to eliminate potential participants were: (1) Current illness/injuries that could influence their running performance, (2) not having access to objective training data to confirm their training and performance level. Within the limited timeframe and resources, we decided to include 22 subjects (16 male, 6 female) in the study.

The subjects were recruited to the study and organized into two groups based on 1) weekly training volume and 2) typical duration of their weekly long runs for the last 8 weeks; one group was a high-volume long-distance group (HV) and the other was a low-volume short-distance group (LV). To be eligible for the HV group, runners had to have a weekly training volume of at least 70 km/week and a weekly long run duration of 90 minutes or longer. To be eligible for the LV group runners had to run less than 70 km/week and have a weekly long run duration of 75 minutes or shorter. These cut-offs were set based on the answers from the questionnaire. The characteristics of the groups are presented in Table 1.

Table 1. Descriptive data for the subjects

	HV (N=11)	**LV (N=11)**
Age (y)	39 ± 9	35 ± 9
Weight (kg)	68 ± 10	70 ± 11
Height (cm)	175 ± 9	177 ± 9
Training characteristics		
Running experience (y)	11 ± 7	10 ± 9
Training volume ($km \cdot wk^{-1}$)	88 ± 22*	47 ± 11
Duration long run (min)	125 ± 46*	71 ± 6
Personal bests		
10k (min:sec)	36:40 ± 03:39 *	40:02 ± 04:46
Half-marathon (hr:min:sec)	01:22:42 ± 00:08:36*	01:33:02 ± 00:12:31
Marathon (hr:min:sec)	02:58:20 ± 00:15:35 (n=10)	03:55:46 ± 01:29:29 (N=3)

Values are presented as mean ± standard deviation (SD). HV = High volume; LV = Low volume;; n= number of subjects * = HV group significant different from LV group (P= <0.05)

One runner from the LV group was not able to perform the baseline testing because of injury and was therefore excluded from the study. Further, two of the runners from the HV group suffered injuries and illness, respectively, between the third and fourth test day and were therefore forced to withdraw from the study before the last test. These two runners are included in the analysis for the preliminary testing as well as for the threshold run and the

90% long run, but not for the 100% long run. In addition to this, one runner from the HV group was unable to complete the last test because of muscular cramps, resulting in missing data for this subject at the last time point (Figure 2).

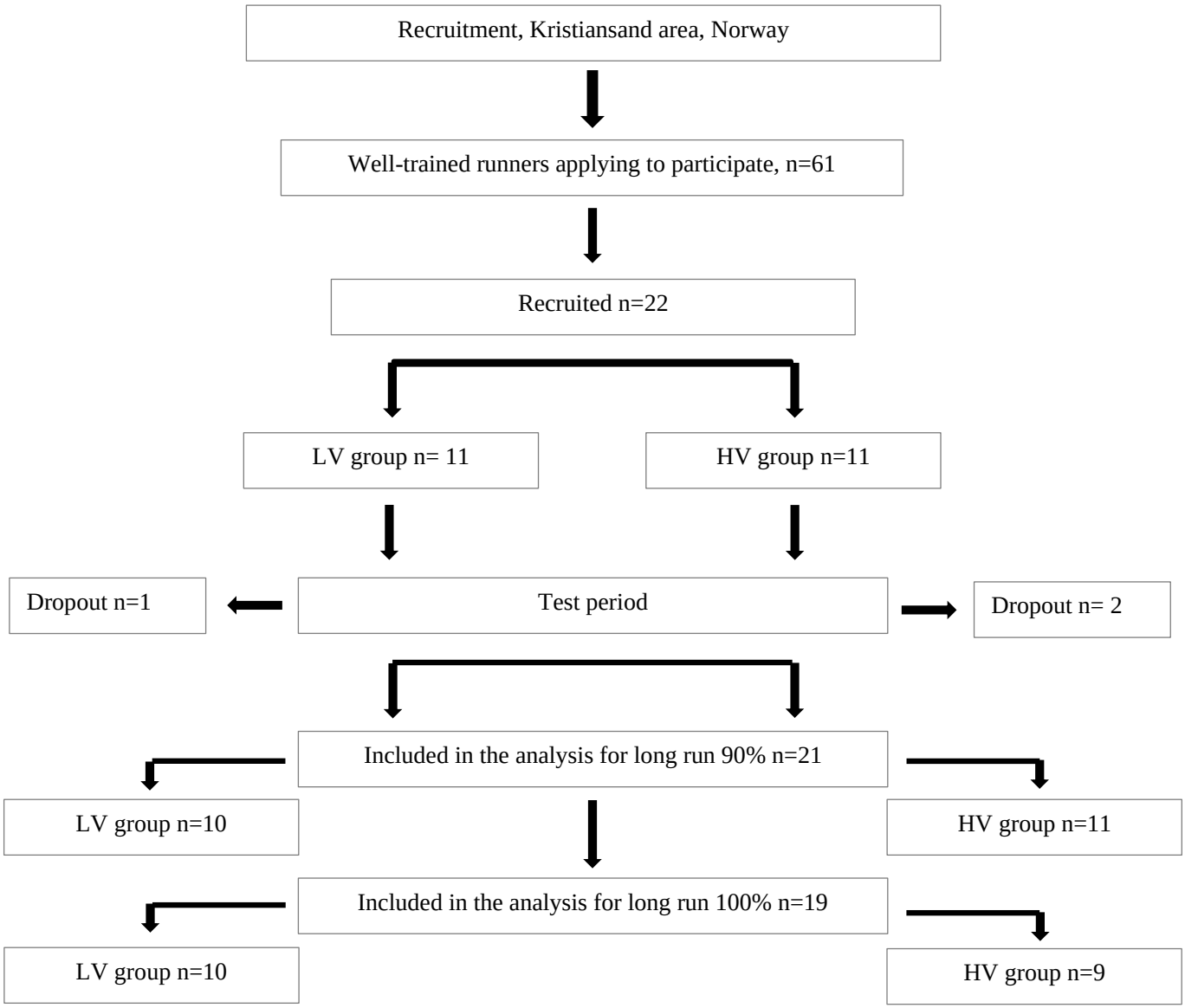

Figure 2. Recruitment of runners, dropouts, and number of subjects for final analysis. LV group = low-volume group, HV group = high-volume group, n = number of runners.

3.3 Testing procedure

The study consisted of four tests performed on four different days, each testing day separated by at least 2 days. The first test day consisted of a VO_{2max} and lactate profile test (Figure 2) and was considered as a preliminary test. The VO_{2max} test was used to determine the fitness

level of the runners, while the lactate profile test was used to determine the runners' LT_1 speed, which would be used for calibrating the intensity of later long runs. The second test day consisted of a 30-minute run in threshold range (Figure 3) with running speed derived from the lactate profile test. The third and fourth test days both consisted of a two-hour long run at low intensity (Figure 4). All runners had to complete preliminary testing before performing tests 2, 3, and 4. All treadmill testing was performed at a treadmill incline of 1% to compensate for the absence of wind resistance (Jones & Doust, 1996).

The two long-duration low-intensity runs were planned to be performed in randomized order, but because of fear of a Covid-19 shutdown we decided to perform them in the same order for all the runners to ensure completion of at least one long run condition. All tests were performed in the same laboratory, with windows and doors open, to ensure airflow. We strived to achieve similar environmental conditions for all the tests, approximately 18-20°C. Each subject performed their respective tests at approximately the same time of the day (± 2h). All runners had to have at least 48 hours of recovery time between test days to ensure sufficient recovery and optimal physiological performance during test days (Parra, Cadefau, Rodas, Amigo, & Cusso, 2000). Further, the runners were instructed to refrain from any intense training during the last 24 hours before testing. Runners were also instructed to consume the same type of meal before the test days and to avoid consumption of products containing caffeine during the last 3 hours preceding testing. All tests and measurements were supervised and performed by the same two test leaders. During tests requiring a maximal effort, strong verbal encouragement was given to stimulate max effort. Each runner wore the same running shoes for all tests.
Additionally, as the menstrual cycle has shown to affect endurance performance we strived to perform testing for the female runners in the follicular phase of their menstrual cycle (de Jonge, 2003). If someone had amenorrhea, were using oral contraceptives, or were in menopause, the menstrual cycle was not accounted for (Appendix 4).

Otherwise, runners were instructed to continue with their regular training routine through the data collection period. Extra focus given explaining to the runners to not change their weekly volume or long-run duration during the data collection period.

3.3.1 Test day 1

The first test day consisted of a submaximal incremental lactate profile test and an incremental test to exhaustion. There was a 10-minute recovery period between tests. Test day 1 is illustrated in Figure 3.

Submaximal incremental test

The first test was a submaximal incremental test, aiming to determine speed, HR, and lactate values at LT_1 and LT_2. The test started with a 10-minute warm-up. During the warm-up, the runners were familiarized with the treadmill and provided information about the test protocol. During the main part of the lactate profile, 5-minute submaximal bouts with increasing speed were performed until LT_2 was reached based on blood lactate sampling quantified in real time during the profile. Runners stood still for 30s with their feet on the side of the moving treadmill between each 5min running bout to execute finger sticks and capillary tube blood draws from the left hand. The starting speed for the first 5min running bout was individualized and based on lactate measurements after the warm-up, as well as discussion between test leaders and runners. Treadmill speed was increased 1 $km \cdot h^{-1}$ after every 5-minute bout, and a total of 6-8 bouts were performed. VO_2, HR, ventilation, and respiratory exchange ratio (RER) were measured during the last 2.5 minutes of each bout. The RPE was recorded at the end of each 5-minute bout, using Borg's 6-20 RPE scale (Borg, 1970). When the [la^{-1}] reached a value clearly exceeding LT_2 the lactate profile test was immediately stopped. LT_1 values were calculated as the speed associated with a blood lactate concentration 0.5 $mmol \cdot L^{-1}$ above the mean of the first 2 blood [la^{-1}] measurements (Christopher, 2000; Hughson & Green, 1982; Pallarés, Morán-Navarro, Ortega, Fernández-Elías, & Mora-Rodriguez, 2016), while LT_2 was calculated as the speed associated with a blood lactate concentration 2.1 mMol·L-1 above the mean of the first 2 blood [la^{-1}] measurements +, based on national testing recommendations from The Norwegian Olympic and Paralympic Confederation of Sport.

Incremental test to exhaustion

After a 10-minute recovery period following the lactate profile test, runners performed an incremental test to exhaustion. This test was used to determine: (1) VO_{2peak}, (2) HR_{peak} (3) treadmill $velocity_{peak}$, and (4) peak blood lactate concentration [$la^{-1}{}_{peak}$]. Testing started with one minute of running at a speed approximately 1 $km \cdot h^{-1}$ below LT_2 speed derived from the

lactate profile testand was subsequently increased 1 km·h^{-1} every minute until voluntary exhaustion. Through the closing stages of the test, runners were given the opportunity to increase the speed with 0.5 km·h^{-1} instead of 1 km·h^{-1} if they felt this allowed them to best mobilize their maximal effort. Additionally, the test leaders gave verbal encouragement throughout the test. A total duration of 8-10 minutes was expected. VO_2 was continuously measured and averaged every 30 seconds. The average of the two highest VO_2 measurements was defined as VO_{2peak}. Immediately after the termination of the test [la^{-1}_{peak}], $HR_{peak,}$ and peak treadmill velocity were determined and recorded.

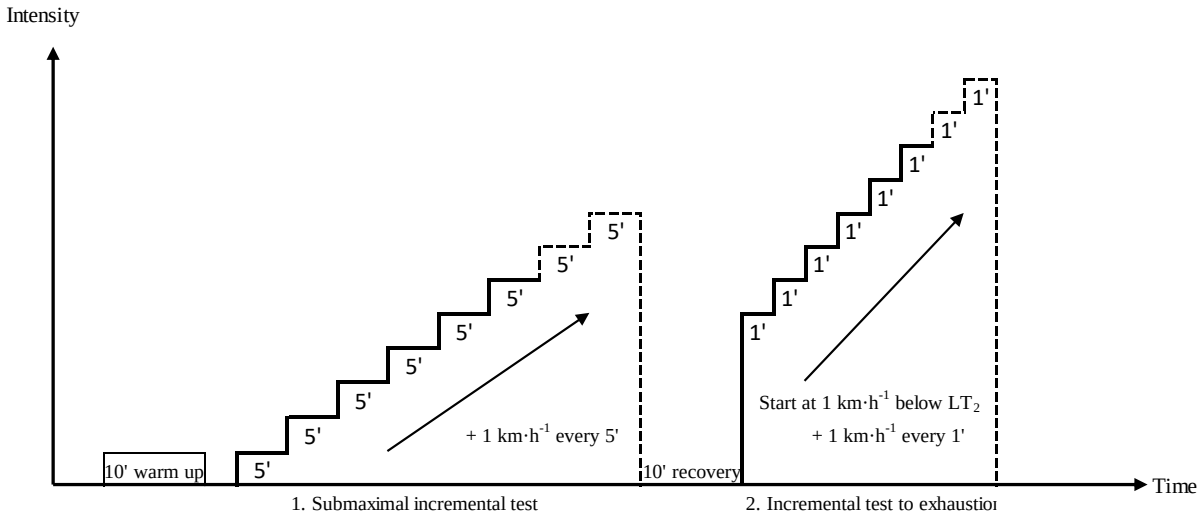

Figure 3. Test day 1. The first test day started with a warm-up, further there was a submaximal 5-min steps incremental test to identify the speed, HR and lactate corresponding to LT_1 and LT_2. After a 10-min recovery period an incremental test to exhaustion was performed to quantify (1) VO_{2peak}, (2) HR_{peak}, (3) $Speed_{peak}$, and (4) peak blood lactate concentration [la^{-1}_{peak}].

3.3.2 Test day 2

30min run between LT_1 and LT_2

The second test day consisted of a 30 min run between LT_1 and LT_2. The test started with a 10-minute warm-up at a speed clearly below LT_1- speed. The main part of the test consisted of 30 minutes of running at a speed corresponding to the mean of LT_1-speed and LT_2-speed, split into two 15-minute bouts. Each of the two 15-minute bouts consisted of the same test protocol. When the runners had been running for 10 minutes, they were told to run with their mouth closed for 2 minutes, and directly after opening their mouth they were asked: “Did

running with your mouth closed feel comfortable?". The runners were instructed to respond with one of three possible answers: (1) "yes", (2) "equivocal", and (3) "no". Immediately after, runners conducted a talk test, where they were instructed to recite a standard paragraph that required 10-15 seconds of speaking. The standard paragraph used for this test was the first verse of the Norwegian national anthem. After reciting the paragraph, the runners were asked: "Did reciting the paragraph feel comfortable?". The runners responded: (1) "yes", (2) "equivocal", or (3) "no". During the last two and a half minutes of the 15-minute bouts, oxygen consumption was measured, and HR were recorded. Right before the end of each bout runners were asked to report their RPE while looking at a large poster of the RPE scale with descriptive anchor (i.e "hard"). There was a short break, of 30 seconds, between the two 15-minute bouts to perform lactate measurements. This test was used mainly for the other master's thesis included in the study. Test day 2 is illustrated in Figure 4.

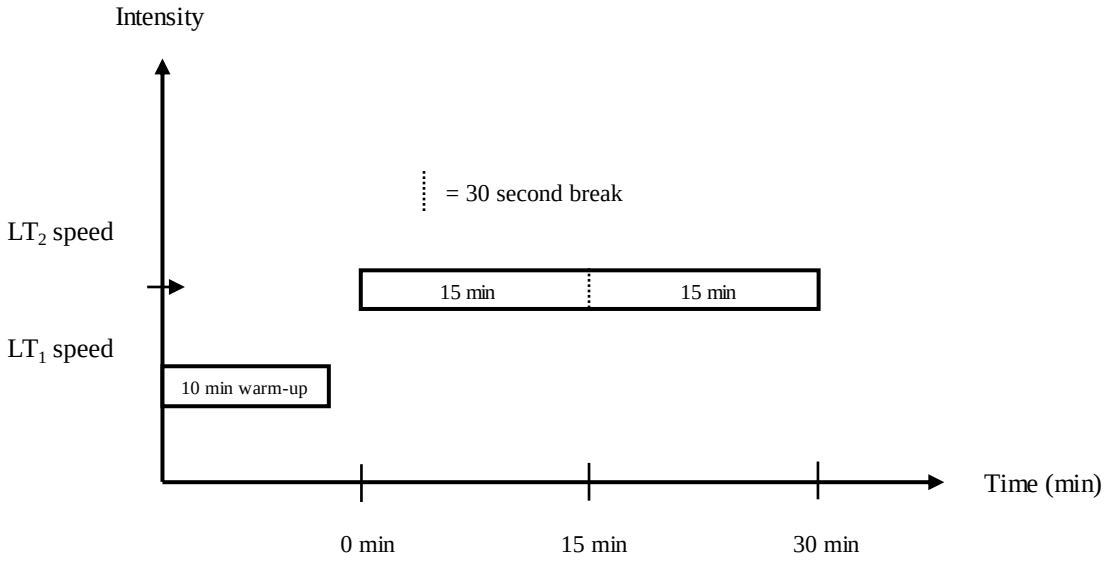

Figure 4. Test day 2. The second test day started with a 10 min warm-up clearly below LT_1. The main part of the test was a 30min run at a speed corresponding to the median of LT_1 and LT_2. The 30-minute run was split into two 15-minute parts with a 30-sec recovery period between for measurements. Metabolic measurements were made in the final 2.5min of each 15min running period.

3.3.3 Test day 3 and 4

2-hour low-intensity long runs

The third and fourth test days consisted of 120-minute low-intensity long runs, performed at 90% of LT_1 speed (LR90) and 100% of LT_1 speed (LR100), respectively. The 120-minute run

was split into four 30-minute segments, with a 1-minute recovery period between each segment for measurement purposes (Figure 5). Each of the four bouts consisted of the same measurement protocol (Figure 6) and represent a time point in the results-chapter. The runners ran undisturbed for the first 22 minutes of each 30min block, before data acquisition during the last 8 minutes of each bout. After 22 minutes of running and 24 minutes of running, respectively, runners performed the same mouth-closed test and talk-test as described for the second test day. EMG data was collected from 24-26min of each time block, while VO_2 was recorded from 26 to 28min. HR and kinematic factors were recorded and averaged for the last 4.5 minutes of each bout. The average of the values recorded in the sample periods was used to quantify physiological changes across the 120min run (defined as 30-, 60-, 90-, and 120-min time points). Just before the end of each 30min running bout, RPE was reported verbally with the help of a Borg Scale poster made clearly visible in front of the treadmill. During the 1-minute measurement periods between 30min running blocks, blood lactate, skin temperature, and core temperature measurements were made. Skin temperature was measured two times on the back and two times on the legs and then averaged, providing one value for the back and one for the legs. Core temperature was self-measured by the runners through the tympanic membrane.

The runners were weighed before and after each of the long runs to quantify weight loss due to dehydration. To make the test as similar as possible to the runners' behavior during regular long runs, they were encouraged to drink *ad libitum*. The test leaders encouraged the runners to drink during runs to limit dehydration. Further, they were given the possibility to listen to music or watch television during the run. However, due to the possible enhanced performance and endurance due to music (Karageorghis & Terry, 1997), the runners had to perform the long runs either with no music at all, or with music throughout the entire run, to not affect the state negatively or positively during the run.

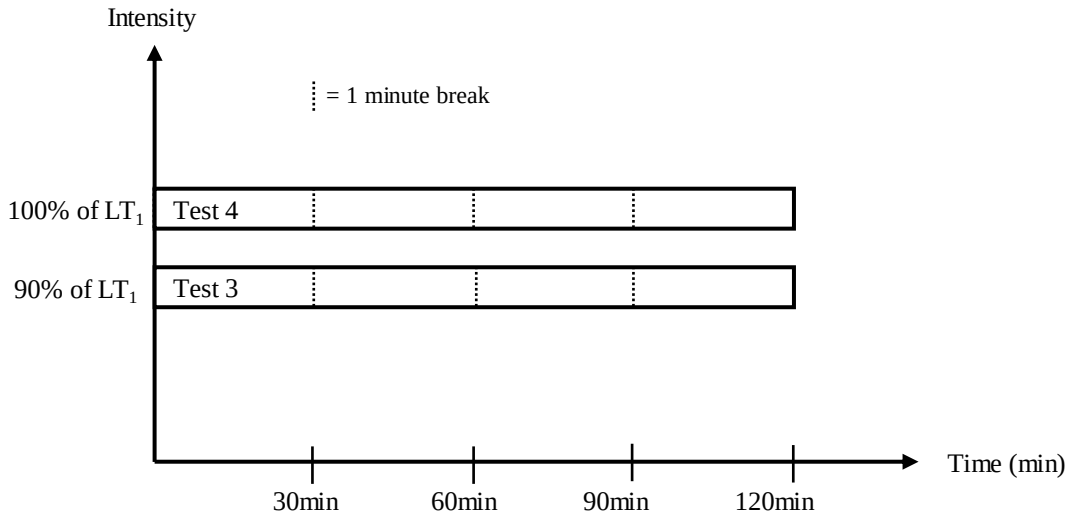

Figure 5. Test day 3 and 4. The third and fourth test days consisted of a two-hour run at 90% of LT_1 and 100% of LT_1, respectively. There was a 1-min break every 30-minute bout, to do measurements.

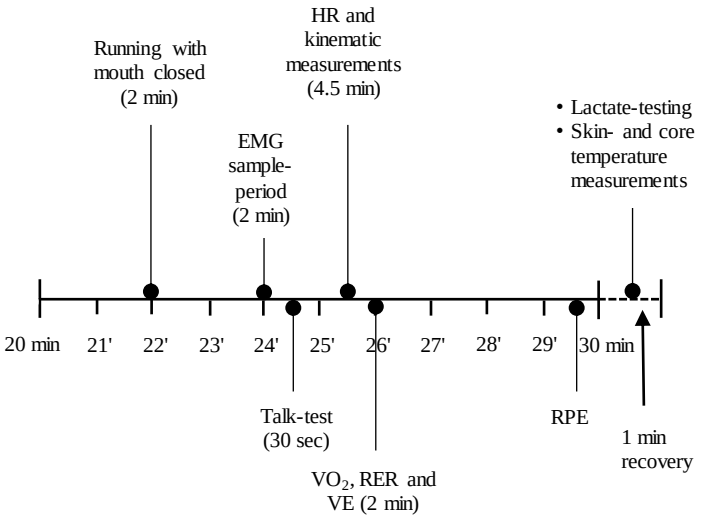

Figure 6. Timeline for testing during the last 10-minutes of each bout on the 120-minute low-intensity long run.

3.4 Testing instruments

All tests were performed on the same treadmill, Lode Katana Sport (Lode B. V., Groningen, Netherlands). The treadmill was calibrated on a regular basis. VO_2 measurements during all tests were measured using Oxycon Pro™ with mixing chamber and 30 second sampling time (Oxycon, Jaeger GmbH, Hoechberg, Germany). Gas sensors were calibrated via an automated process using certified calibration gases of known concentrations before every test, as well as half-way through the long runs. Blood [la-] during all tests were analyzed using a stationary

lactate analyzer (EKF BIOSEN, EKF diagnostic, Cardiff, UK) which was calibrated every 60 minutes. HR was measured using Polar V800 (Polar Elektro Oy, Kempele, Finland). Core temperature was measured using Braun IRT6520 ThermoScan® 7 Age precision® (Braun, Kronberg im Taunus, Germany). Skin temperature was measured using a Flir TG267 Thermal Camera® (Flir Systems, Inc. Wilsonville, Oregon, US). Kinematic variables were measured using a Stryd™ foot pod (Stryd, Boulder, Colorado, US). EMG was measured using Delsys Trigno Wireless EMG System (Delsys, Natick, Massachusetts, US). Before and after each test subjects were weighed using a Seca model 713 (Seca, Hamburg, Germany).

3.5 Electromyography

A large part of the methodological development work leading into the main data collection was focused on how to best optimize our EMG testing. EMG was measured using surface electrodes (Trigno Wireless EMG systems; Delsys, Boston, MA, USA). Electrodes were placed at the following lower limb muscles during both 120min runs; m. Vastus Medialis (VM) and m. Biceps Femoris (BF). Placement and location of the electrodes were performed according to the recommendation of SENIAM (Surface EMG for Non-Invasive Assessment of Muscles (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). Briefly, electrodes were placed parallel to the muscle fibers on the belly of the muscle. Prior to placing the electrodes, the skin was shaved and cleaned with alcohol. To prevent detachment of electrodes due to sweat during the long runs, they were fixed with additional tape. The same test leader was assigned to place the electrodes each time to ensure consistency in the placement.

Based on preliminary testing and data analysis, EMG measurements were performed for 120s at the end of each 30-minute bout on both long runs, with a sampling rate of 2000Hz. EMGworks Version 4 analysis software (Delsys) and MATLAB was used to analyze EMG data. The Raw EMG signal was filtered using a fourth-order Butterworth band-pass filter at 30-500Hz (Hsu et al., 2017). This allowed noise or movement interference below 30Hz and other non-physiological signals above 500Hz to be removed. Root Mean Square (RMS) and Median Frequency (MDF) were used for analyses and comparison across time. RMS was calculated to obtain the EMG amplitude, using a moving window of 100ms. Each repetition was identified through the RMS signal, and the median of the peak RMS of each contraction was calculated. Further, all valid contractions for each recording were Fast Fourier transformed and the MDF was obtained. Ultimately, each 30min time period during the long

runs was described by a single value for MDF and a single value for RMS for each muscle at each time point. Only valid measurements were included in the final analysis, and outliers caused by sweat or sensors partially detaching during running were removed from the analysis (Appendix 8).

EMG data was analyzed for all subjects as a combined group in SPSS using a repeated measures ANOVA. Because of the relatively high amount of data lost because of sensors partially detaching during running or being corrupted by sweat accumulation at the skin-electrode interface, EMG data was analyzed separately from the rest of the data. Only subject long runs with uncorrupted recordings and values for all 4 timepoints were included in the final analysis using SPSS.

3.6 Statistical analysis

All statistical analysis was performed using SPSS (version 25, IBM, Chicago, IL, USA) and data are presented as mean ± standard deviation. Tables and figures were made using Microsoft Word version 16.0 (MS, Redmond, WA, USA) and Microsoft Excel version 16.0 (MS, Redmond, WA, USA). Subjects' characteristics and training characteristics were compared using a One-way between-groups analysis of variance (ANOVA), with an LSD post-hoc test. A General Linear Model repeated measures model (ANOVA) was used to compare the changes in physiological, kinematic, and perceptual factors across the long run duration between groups. When Mauchly's test of Sphericity[a] was statistically significant, indicating lack of sphericity in the data, a Greenhouse Geiser correction was applied. A paired-samples T-test was used to compare the two long runs to each other, both for the first time point and for the absolute change from the first to the last time point. An independent samples T-test was used to compare changes between the high-drift group and the low-drift group. A value of $p<0.05$ was considered statistically significant in all analyses.

3.7 Ethical considerations

This study was performed on healthy adults, all of whom were physically active with no known health problems. All subjects received written information prior to the study, explaining the magnitude of the testing, the purpose of the research, the potential risks involved, the potential benefits of participating, a statement explaining that their data was collected anonymously and that they at any point during the research period could choose to

withdraw their participation without any reason. On the first day of testing, all subjects signed a consent form (Appendix 2). The test leaders spent a lot of time before the study itself, on learning and practicing how to carry out safe tests, both in terms of measures to avoid accidents, and for how to react if accidents did occur. In addition, the test leaders observed extra Covid-19 precautions, using facemasks and rubber gloves during all testing, as well as washing all surfaces with antibacterial agents between tests. When the subjects arrived for the tests, they completed a Covid-19 self-declaration (Appendix 3), confirming that they did not have any symptoms.

The study was approved by the ethics committee of the Faculty for Health and Sport Science, University of Agder (Appendix 6), and by the Norwegian Center for Research Data (NSD) (Appendix 7).

4.0 Methodological discussion

When doing scientific research, methodological challenges arise. Several factors are essential in testing during scientific research: 1) that the test is valid and reliable, 2) control of the work conditions, 3) accurate measures from the equipment, and 4) a standardized protocol before, during, and after the test (Thomas, Nelson, & Silverman, 2015).

4.1 Study design

This study was conducted as a descriptive study with repeated measures. A descriptive research method examines the situation as it exists in the current state, and involves the identification of a particular phenomenon based on an observational basis, or exploring the relationship between two or more phenomena (Williams, 2007). The main purpose of a descriptive study is to provide an "overall" picture of a phenomenon or a population that is relatively new or just simply needs to be described (Rubin & Babbie, 1997; Thomlison, 2001). We used a repeated-measures design to assess changes over time within subjects. A repeated measures design is one where multiple measurements are made on each experimental unit, these measurements may be performed under different experimental conditions or at different points in time (Sullivan, 2008), as we did in this study. A strength of applying a repeated measures design is that a relatively small number of subjects is needed (Sullivan, 2008) to achieve adequate statistical power. With the main target of this study being to explore possible shifts in physiological mechanisms and possible differences between groups during prolonged exercise, a descriptive study with repeated measures was deemed and appropriate approach.

4.2 Subjects

The study sample in this study included 22 well-trained runners (16 male and 6 female) aged 37 ± 9 years. We strived to include 15-20 subjects in the final analysis, and anticipating the possibility of dropouts, we included 22 subjects. Three subjects had to drop out during the test phase due to injuries or illness, however, the number of subjects included in the final analysis was still inside what we wanted (n=19). A large N is considered essential to increase the statistical power (Polit & Beck, 2014). Considering the research design, the extensive testing protocol, and the duration of the tests, as well as the limited timeframe and resources, we concluded that a total of approximately 20 subjects was sufficient.

The age limit was set to between 18-55 years. The lower limit was set to be able to conduct the study without having to obtain permission from the subjects' parents, while the upper limit was set arbitrarily to minimize health and injury risk. Even though there is limited evidence that age affects the injury risk, there are some studies that have shown a higher age to may lead to higher injury risk (Hirschmüller et al., 2012; Wen, Puffer, & Schmalzried, 1997, 1998). Also, we thought this population group (18—55 years) would represent the majority of people running several times a week. We strived to include both male and female runners as previous studies have demonstrated that the overall physiological impact of endurance training is not gender-dependent (Skinner et al., 2001).

Another inclusion criterion for participating was that the runners had been running at least 30 km/week the last 8 weeks, as well as being able to run for two hours without stopping. We set these criteria to increase the likelihood that that the runners would be at a sufficient training level to be able to successfully complete the study

4.3 Testing procedure

This study consisted of four test days for each subject. All tests were performed on the same treadmill. All tests had the same treadmill incline to make the measurements transferable between tests. Further, to make the data from the tests as transferable as possible to a regular long run, an incline of 1% was set, as a 1% incline is the incline that most accurately reflects the energy cost of outdoor running (Jones & Doust, 1996). Each test day had to be separated by at least 48 hours, as 48 hours of recovery has been reported to be sufficient in order to achieve recovery from normal training sessions (Parra et al., 2000). The runners were instructed to not perform any kind of intense exercise the last 24 hours before testing. In addition, they were allowed to choose which day they wanted to be tested. Therefore, it is reasonable to assume that the runners were recovered between test days. Each subject had to perform their respective tests at the same time in their circadian rhythm, meaning each test had to be performed at approximately the same time of day (± 2h). Several recent studies have shown that most physiological parameters demonstrate cyclic variations throughout the day under resting conditions (Deschenes et al., 1998). Further, the effects of time of day have been reported to affect VO_2 kinetics (Hill, 1996) and the blood lactate-intensity relationship (Deschenes et al., 1998; Madsen & Lohberg, 1987).

Both hot and cold conditions has been shown to affect optimum athletic performance during prolonged exercise, for example, the marathon (Martin & Buoncristiani, 1999). High ambient

temperature have also been shown to may induce increased blood lactate concentrations (MAcDOUGALL, Reddan, Layton, & Dempsey, 1974), therefore windows and doors were held open to achieve a standardized ambient temperature of between 18 and 20°c for all tests. In addition, runners ran with a motorized fan turned on and directed at their front torso to provide better evaporative cooling.

The nutritional status of a runner may affect their lactate responses during exercise, as it seems to decrease at any given intensity when muscle glycogen stores are depleted prior to exercise. Lactate measurements made when athletes have depleted glycogen stores may overestimate endurance capacity (Maassen & Busse, 1989). Therefore, the runners were instructed to consume the same type of meal and have similar preparation before all test days to achieve similar nutritional status for all tests.

Further, due to the rapid evolution in shoe technology, with the increasingly popular carbon-plated shoes, we instructed the runners to wear the same running shoes for all tests. The relatively new Nike Vaporfly shoe collection, which is the most known carbon-plated shoe, has shown a roughly 4% reduction in energy expenditure during running compared to other popular racing shoes without carbon-plates (Barnes & Kilding, 2019; Hoogkamer et al., 2018). Therefore, we were careful to ensure consistent shoe choice, to ensure that the shoe choice would not affect the data between tests.

Based on the measures done it is reasonable to assume that the runners were performing all their tests under as similar circumstances as possible. However, we could not control if all the guidelines provided to the athletes were followed.

4.4 Test instruments

In a repeated-measures study where data are being compared through different time points, it is important that the instruments used during testing provide stable measurements.

4.4.1 Oxygen consumption

All oxygen consumption measurements in this study were acquired using the Oxycon Pro™ mixing chamber. The Oxycon Pro™ system has been shown to be a reliable and accurate system for measuring oxygen, with a margin error of +3% (Carter & Jeukendrup, 2002; Foss & Hallen, 2005; Rietjens, Kuipers, Kester, & Keizer, 2001). The Oxycon Pro™ system was calibrated before the start of tests, as well as halfway through the long runs to ensure reliable

measurements.

4.4.2 Lactate

Lactate measurements were performed using a stationary EKF Biosen lactate analyzer. This analyzer has been widely used in other studies measuring lactate (Alis et al., 2015; Gabrys, Garnys, Szmatlan-Gabrys, & Mróz, 2015; Salam, Marcora, & Hopker, 2018; Sylta et al., 2016). The same analyzer was used for all tests throughout the project, seeing that some studies have documented different lactate values using different analyzer from the same blood samples (Medbø, Mamen, Holt Olsen, & Evertsen, 2000). The lactate analyzer was automatically calibrated every 60th minute.

4.4.3 Kinematic measurements

Kinetics were measured using a Stryd™ foot pod. The most used device for assessing human locomotion is the OptoGait (García-Pinillos, Roche-Seruendo, Marcén-Cinca, Marco-Contreras, & Latorre-Román, 2021). The assessment results of this gait analysis system have been validated for both walking (Lee et al., 2014) and running (Lussiana, Hébert-Losier, Millet, & Mourot, 2016). However, considering the limited resources we chose to assess the relatively new and field friendly device, the Stryd™ foot pod, to assess running gait and kinematic measurements. A recent study by F. Garcia-Pinillos et al., (2021) evaluated the reliability and validity of the stryd™ foot pod and compared data from the stryd™ foot pod with the OptoGait. The study demonstrated adequate reliability for running assessment for the stryd, and thereby data from this device can be compared over time, however, there are limitations of directly comparing data reported by the stryd™ foot pod against data from OptoGait (García-Pinillos et al., 2021).

4.4.4 Core temperature

Traditionally, to measure an accurate core-temperature, invasive methods such as introducing the sensors into deep body tissues such as the esophagus or rectum have been employed (Binkley, Beckett, Casa, Kleiner, & Plummer, 2002; Casa, Armstrong, Ganio, & Yeargin, 2005; Moran & Mendal, 2002). A new and accurate technology used to measure core-temperature is the ingestible telemetry pill sensor (Ganio et al., 2009; Teunissen, De Haan, De Koning, & Daanen, 2012), and these measurements have been shown to reflect both rectal

and esophageal temperature measurements (Easton, Fudge, & Pitsiladis, 2007). However, these methods are expensive, uncomfortable, and highly invasive. Therefore, we decided to use a Braun Thermoscan™, measuring the tympanic temperature, which is a non-invasive, affordable, and practical alternative for temperature assessment during running. A study by Douglas J. Casa et al., (2007) found tympanic measurements to significantly underestimate core temperature compared to rectal measurements. While more recent studies by Moran-Navarro et al., (2019) and Fenemor et al., (2020) showed that measurements made using Braun™ thermometers registered similar temperatures as ingestible pills, both in rest and during exercise. This demonstrates that the technological advances in the design and specificity of tympanic membrane thermometer have the potential to limit the differences between measurements methods (Morán-Navarro et al., 2019).

4.4.5 Skin temperature

Skin temperature measurements during research settings have historically been achieved through contact devices (de Andrade Fernandes et al., 2014; Kelechi, Michel, & Wiseman, 2006), however measurements using contact method presents several methodological challenges, for example, wire entanglement (Bach, Stewart, Disher, & Costello, 2015) or loss of sensor contact (Buono, Jechort, Marques, Smith, & Welch, 2007). Considering the impracticality and high cost of this method, we chose to assess skin temperature through a more affordable and practical method. Therefore, the present study used a portable infrared camera to assess skin temperature. The authors were aware of the questionable validity of this method with several studies showing low reliability between these two methods for skin measurements (Bach et al., 2015; de Andrade Fernandes et al., 2014; James, Richardson, Watt, & Maxwell, 2014; Li et al., 2015; van den Heuvel, Ferguson, Dawson, & Gilbert, 2003). However, due to the skin-temperature measurements not causing any extra strain on either the runners or the test leaders, we chose to assess skin temperature through infrared camera, hoping to get consistent measurements. It is also interesting to note that, at the time of designing the study, this same non-contact technology was being actively used internationally during the onset of the Covid-19 pandemic to screen individuals for elevated core temperature potentially indicative of viral infection.

4.4.6 Electromyography

EMG measurements can be made using either surface EMG or indwelling (needle)EMG. A study by Watanabe & Akima (2011) demonstrated that there were no significant differences observed between measurements done using needle EMG and surface EMG. Surface EMG is a far less invasive method for EMG measurements than needle EMG, and therefore, the present study utilized surface EMG to assess muscular fatigue.

When choosing the EMG system to use throughout this study we initially examined both the Trigno Wireless EMG system and the MuscleLab EMG module. Through extensive pilot testing using both systems there were similar consistency for the measurements. We decided to use the Trigno system due to it not having any wires attached to the electrodes, and thereby, making it easier to attach, less troublesome for the runners while running, and, easier to tape on. Several other studies have also used the Trigno system to measure EMG signals during running (Chumanov, Wille, Michalski, & Heiderscheit, 2012; Hunter, Seeley, Hopkins, Carr, & Franson, 2014; Semciw, Freeman, Kunstler, Mendis, & Pizzari, 2015).

4.5 Test protocol

4.5.1 Preliminary testing

Submaximal incremental test

Individual thresholds was used to determine LT_1 and LT_2, for the reason that blood lactate concentrations can vary greatly among individuals (Stegmann, Kindermann, & Schnabel, 1981). Some studies have suggested that a duration period of at least 5 to 7 minute work bouts are necessary to attain steady-state lactate concentrations, and thereby determine an accurate lactate turn point (Foxdal, Sjödin, & Sjödin, 1996; Foxdal, Sjödin, Sjödin, & Östman, 1994; Rieu, Miladi, Ferry, & Duvallet, 1989; Stegmann & Kindermann, 1982), therefore, the present study used 5 minute work bouts during the submaximal incremental test. Further, not only the duration of the work bout, but also the duration of the recovery between bouts can affect the determination of the lactate turn points. Longer breaks tend to lead to overestimation of the threshold intensity, (Heck, Mader, HESS, Mucke, & Muller, 1985) with 1 min recovery being a possible maximum (Bourdon, 2000). The present study therefore used a standardized 30 second break between bouts. Studies have shown that the blood sampling site may affect the measurement of the relationship between intensity and blood lactate concentration (Dassonville et al., 1998; Feliu et al., 1999; Foxdal, Sjödin, Östman, & Sjödin,

1991; Foxdal et al., 1990), therefore, the blood sampling site was standardized, and all blood samples were consistently taken from the fingertip.

Incremental test to exhaustion

The incremental test to exhaustion was performed on the same day as the submaximal incremental test, with a 10-minute recovery between, according to the standard protocol used by the Norwegian Olympic Center. Performing the incremental test to exhaustion right after the submaximal incremental test can be a disadvantage as the runners may be exhausted before the start, and therefore, not be able to perform with maximum effort. However, with the submaximal incremental test lasting no more than approximately 5-8 bouts, as well as having a 10-minute recovery period between tests, the runners were expected to be able to perform the incremental test to exhaustion with maximum effort.
During the incremental test to exhaustion in the present study, the speed was increased by 1 $km \cdot h^{-1}$ every minute, and the total duration was ~7-10 min for all runners, which approximates the 8-12 minute criterion previously established as the optimal duration of VO_{2max} testing (Buchfuhrer et al., 1983). The most accepted criterion for confirming attainment of VO_2max is a plateau in oxygen consumption despite increasing workload (Astorino, 2009), however, in the present study not all runners reached a clear VO_2 plateau, and therefore, the term VO_{2peak} was used instead.

4.5.2 30min run between LT_1 and LT_2

The duration of this test was set at 30 minutes. This was done so that the threshold test would correspond to the first 30-minute period of the low-intensity long runs, performed later in the project. By doing this, the data collected from the threshold test, performed above LT_1, could be compared with data from the long runs performed below and right at LT_1. The intensity, halfway between the velocities associated with LT_1 and LT_2, or in other words, clearly above LT_1, was chosen to hopefully ensure a running intensity that would differ markedly from the data retrieved during the low intensity long runs, and thus testing the validity of talk-test and the mouth-closed test as a tool for controlling intensity during running.

4.5.3 Low intensity long runs

The duration of the long runs was chosen based on practical experiences from the running environment, both locally and nationally. A 20-30 km or 90-120 min duration long run seems

to be a duration being performed regularly by experienced runners, and the duration was therefore set to 120 minutes. We chose a duration in the upper part of the range of what is common to try to mimic a normal long run, and also push the athlete into a fatigued condition. Jack Daniels (1989) suggests 120-150 min to be a long enough long run for experienced runners.

As for the intensity of the runs, 90% of LT_1 and 100% of LT_1 were chosen to make the two runs significantly different from each other, while both still were categorized as low-intensity, and were inside zone 1 from the start (i.e not above LT^1) (Esteve-Lanao et al., 2005). Throughout the testing, we experienced that the duration of the runs was manageable and that a longer duration at the prescribed intensities would be too demanding for some of the runners. However, some runners expressed that the intensity was hard early in the run, and the test leaders also perceived that some of the runners were struggling earlier and more than expected during the low intensity runs, while the same intensity was relatively easy for other runners. This was the case for both the LR90 and LR100. In hindsight a larger difference in intensity between the two runs may have been more ideal.

Measurement challenges during long runs

EMG measurements were done on m. Vastus Medialis (VM) and m. Biceps Femoris (BF). Several other studies have assessed these muscles when measuring EMG while running (Albertus-Kajee, Tucker, Derman, Lamberts, & Lambert, 2011; Guidetti, Rivellini, & Figura, 1996; Kyröläinen, Avela, & Komi, 2005; Montgomery III, Pink, & Perry, 1994). VM and BF are important muscles during the running gait cycle (Novacheck, 1998) along with several other muscles. During pilot testing in the lab, the test leaders experienced that VM and BF were the two muscles providing us with the most consistent measurements. In addition to this, using these muscles provided us with measurements from both the stance phase (VM and BF) and the swing phase (BF) (Novacheck, 1998). BF is a biarticular muscle and hence it plays an important role in both the eccentric and concentric functions while running (Novacheck, 1998). Further, both muscles were easily accessible when placing the surface electrodes, which was done by the same test leader each time to ensure consistency in the placements. A challenge when measuring EMG through surface electrodes during exercise is sweat accumulation. The influence of sweat accumulation has been shown to influence the amplitude of EMG signals (Abdoli-Eramaki, Damecour, Christenson, & Stevenson, 2012; Ray & Guha, 1983). These effects can result in interpretation errors of EMG findings in

human performance studies (Abdoli-Eramaki et al., 2012). Little is known about the amount of sweat needed before signal fidelity deteriorates. However, sweating has been reported after as short as 10 min of low-intensity exercise (Takano et al., 1996), while a study by Ray and Guha (1983) performing 100 min of exercise reported significant differences in EMG signal with only a small layer of sweat compared to dry conditions. In a 120 min long exercise trial, as in the present study, sweat accumulation is inevitable. We used tape around the electrodes to prevent sweat dripping down on the electrodes. However, this did not address the challenge of sweat being released from the sweat glands beneath the electrodes. Therefore, before analyzing the EMG data from the long runs, we performed control-testing where we measured EMG data with respectively, dry conditions, saltwater applied above the electrodes, and saltwater applied beneath the electrodes (Appendix 8). Measurements with saltwater applied beneath the electrodes were clearly disturbed by the salt water and were easy to identify. Therefore, the measurements during the long runs that were showing similar tendencies as the ones disturbed by sweat, and therefore likely disturbed by sweat, were excluded from the final analysis.

Skin temperature measurements derived from the long runs resulted in inconsistent measurements ranging over 5 degrees within-subjects during long runs, both higher and lower compared to baseline measurements. There are many limitations to measuring skin temperature using a thermal camera, and measurement errors can occur if measurement point, angle, and distance are not held constant (James et al., 2014). Measurements were done according to recent studies, with the camera hand-held at ~ 1m, with the camera at a 90° to the body (Fenemor et al., 2020). Further, the onset of sweat-pools has been shown to affect skin temperature measurements during exercise (Johnstone, Ford, Hughes, Watson, & Garrett, 2012). The equivocal skin-temperature measurements in this study can possibly be attributed to the questionable validity of the thermal camera (de Andrade Fernandes et al., 2014; James et al., 2014), together with the test leaders' lack of understanding of the small margins associated with the aforementioned measurements errors. Due to the equivocal and inconsistent data from the skin temperature measurements, these were not included in the final analysis.

4.6 Strength and limitations

The main strengths of the present study were: (a) a well-controlled design with a carefully standardized and extensive test protocol, (b) a high number of runners willing to participate in

the study, which gave the authors the opportunity to choose the most suitable sample to answer the research questions, (c) a relatively high number of participants, despite the time consuming and extensive test-protocol, (d) a low drop-out rate, and (e) well-established objective measurement methods and equipment for the main measurements during testing.

However, there were also limitations and challenges in this study. Despite investigators being able to allocate subjects to 2 distinct training volume groups based on the questionnaire, the groups are still relatively homogenous relative to the entire range of long-distance runners. Further, some of the equipment used during the long runs, such as the Flir™ thermal camera, Stryd™ foot pod, and Braun™ tympanic temperature sensor, were not gold-standard methods, but rather relatively untested and experimental methods for this purpose. Therefore, the reliability and validity of this equipment can be questioned. In addition, collecting high quality surface EMG signals from the thigh and hamstrings of runners during 120 minutes of continuous running has not, to our knowledge, been previously reported. Further testing and research on how to optimize running EMG measurements over such a long period of time is needed.

We believe that the present subject sample and findings are representative for well-trained, "recreational" runners aged 18-55 years, but not necessarily representative for other subject-groups training substantially less, or more. Based on the results from the long runs, we speculate that a larger difference in intensities between the two long runs, with one being performed at a significantly lower intensity may have induced different results.

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PART 2

RESEARCH PAPER

Unsteady physiology and perception during long “steady state” runs. What role do training characteristics play?

The following paper is written according to the standards of the following journal:

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Kristian Tjørnhom

University of Agder

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Unsteady physiology and perception during long “steady state” runs. What role do training characteristics play?

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ABSTRACT

PURPOSE: To contribute to a better understanding of low-intensity (<LT_1) endurance training by quantifying and explaining the physiological mechanisms associated with cardiac drift and fatigue during low-intensity running and relate them to training characteristics.

METHODS: Twenty-two well trained runners (16 male, 6 female) (37 ± 9 yrs, 69 ± 10 kg, 176 ± 9 cm, peak oxygen uptake 63.6 ± 6.8 $ml \cdot kg^{-1} \cdot min^{-1}$) participated in this study. Based on their weekly training volume and weekly long-run duration, runners were categorized into a high-volume group (HV, n=11) and a low-volume group (LV, n=11). Runners conducted two 120 minute long runs performed at 90% and 100% of LT_1 speed (LR90 and LR100) with repeated measurements every 30 minutes.

RESULTS: There was a significant drift for heart rate, oxygen consumption, ventilation, RER and RPE during both runs ($P<0.05$). There was no difference in drift between groups for any measurements, except for running economy during LR100. The drift was similar during LR90 and LR100 for all measurements except RER, which was significantly greater during LR90 ($P<0.05$).

CONCLUSION: The present study demonstrates that an upward drift occurs for HR, RPE, ventilation, RER, and oxygen consumption for well-trained runners when running at an intensity corresponding to below or approximating LT_1 for 120 minutes. The study also demonstrates that while wide differences are seen at the individual level, there is no consistent difference in the magnitude of physiological or perceptual changes observed between two groups differing by XX% in weekly training volume.

KEYWORDS: Endurance, running, low-intensity training, cardiac-drift, decoupling, fatigue, electromyography

INTRODUCTION

The importance of large volumes of training to perform at a high level in endurance sports is well documented (1-4). The performance of repeated bouts of exercise over months to years induces numerous physiological changes that result in improved performance in endurance activities (5). The training response depends on the duration, frequency, and intensity of these repeated bouts of exercise and how they interact over time (5). While the physiological factors limiting endurance performance are generally agreed upon (6-9), there is still debate about how the daily training components should be distributed to best improve performance (10). When planning endurance training it is important to include different intensities (1, 3, 11); this can be achieved, for example, by prescribing low intensity training (LIT), lactate threshold training, or high-intensity interval training. These are all well-known training methods used to exercise within different, reasonably distinguishable regions of the intensity scale (2). Several recent studies examining training intensity distribution have used the first and second lactate or ventilatory turn points (LT_1/VT_1 and LT_2/VT_2) to define three intensity zones (iZone 1, iZone 2 and, iZone 3) (10, 12-15). This intensity scale defines zone 1 as below LT_1, zone 2 as between LT_1 and LT_2, and zone 3 as above LT_2 (2) (Figure 1).

There are several different training intensity distribution models described in the research literature. The *threshold*-training model (16-19), the *polarized*-training model (10), and the *pyramid*-training model (4, 10, 20) are all established models describing endurance training intensity distribution and potential optimization strategies. It seems that independent of training model, it is quite common for endurance athletes to perform a large percentage of their training at a relatively low intensity, that is, below their LT_1 (9, 10, 20-23). High intensity training (HIT) and threshold training are shown to have a positive effect on aerobic performance for both recreational athletes and elite athletes (3, 11) and the physiological and perceptual responses associated with executing these higher intensity sessions are well described in the research literature. However, considering that most of the training of successful endurance athletes is actually performed at a “low” intensity relative to their maximal capacity, there is need for a deeper understanding of this part of the training distribution. It seems that total training volume plays an important role in improving endurance performance (2, 24), and with LIT constituting up to 80% of this total training volume, this makes LIT crucial to performance development (9, 12, 25). Compared to Threshold and HIT training, the range of intensities that fall into the LIT region (zone 1) is

broad and this catalyzes many questions about optimizing intensity and duration in this intensity zone. This is underlined by Esteve-Lanao et al. (9) when they describe heart-rate (HR) ranges for intensity zone 1 as "50-80% of maximum heart rate". Both duration and intensity play an important role when planning LIT; their interaction likely impact the stress responses imposed, the degree and type of fatigue, and the training effect (26). Excessive intensity or duration during LIT could lead to elevated fatigue, in turn resulting in athletes not being able to maintain a sufficient training volume or properly execute higher intensity training bouts (26).

Fatigue processes seem to occur during long duration LIT (27). For example, HR has been shown to increase slowly during exercise, even when performed at a constant, initially "comfortable" sub-maximal work rate (28). This phenomenon is known as cardiovascular drift (CV drift), and is characterized by a rise in HR with or without a fall in stroke volume (SV) over time during a constant-rate submaximal exercise at an intensity of 50-75% of VO_{2max} (29, 30). However, HR can also increase due to muscular fatigue, changes in muscle recruitment and/or decrease in work efficiency, independent of dehydration and associated SV decline (31). Further, a study by Millet and Lepers (32) showed that prolonged exercise induces significant central fatigue, and the intensity and duration of an activity is likely to be among the most important factors for fatigue (33). It is crucial to control not only intensity, but also the duration for any specific intensity to induce training effects while avoiding overload (27).

Overall goal and purpose

The current study aimed to quantify and explain the physiological and perceptual response shift occurring during constant load, long duration LIT, and examine the magnitude of these changes in relation to subject training characteristics. By quantifying the fatigue patterns associated with long, low intensity training sessions, and relating them to individual training characteristics, we hope to provide useful information for optimizing the prescription of LIT, as a tool in developing the physiological mechanisms associated with endurance performance.

Main purpose

1. To comprehensively quantify physiological and perceptual changes during long LIT running bouts and contribute to a better understanding of the physiological mechanisms associated with cardiac drift and fatigue during LIT.

2. To detect possible differences in cardiac drift and fatigue between two groups differing in training characteristics.
3. To compare cardiac drift and fatigue rate between a long run performed at clearly below LT_1 and one performed right at LT_1.

MATERIALS AND METHODS

Study design

This study was part of a wider research project conducted at the University of Agder, Department of Sport Science and Physical Education. The study was designed as a quantitative descriptive study with repeated measures of runners performing long duration endurance session at a controlled, "sub-threshold" or low intensity. After preliminary testing, we prescribed two 120-minute long runs, one clearly below LT_1 and one approximating LT_1, with repeated measures of physiological, kinematic, electromyographical (EMG), and perception of exertion (RPE) performed every 30 minutes to answer the research questions. Prior to the study, the authors conducted extensive pilot testing to optimize the testing process, both in terms of data acquisition and practicality for the runners.

The participants were invited to an information meeting, which was later cancelled due to corona-restrictions, and all information were instead distributed digitally via an information sheet. All data collection and testing were performed between December 2020 and February 2021.

Subjects

Subjects were recruited in October 2020 through local running clubs, personal interactions, and announcements on social media. The target group for the study was experienced, well-trained runners. A total of 61 runners announced their interest in participating and were asked to complete a digital questionnaire about their training and performance status. The data collected from the questionnaire provided the investigators a basis for assigning athletes to one of two groups differing in training characteristics, but matched for age and sex. When choosing the subjects for the study the following inclusion criteria were used: 1) aged 18-55 years, 2) absence of reported disease and injuries 3) running at least 30 km/week for the last eight weeks 4) able to perform a two-hour low intensity running session on a treadmill. The exclusion criteria used to eliminate potential participants were: 1) illness/injuries that could influence their running performance, 2) not having access to daily training data to confirm

their training and performance level. With these criteria and considering laboratory and time constraints, 22 runners (16 male, 6 female) were included in the study.

The subjects were recruited to the study and organized into two groups based on 1) weekly training volume and 2) typical long run duration for the last 8 weeks. One group was a high-volume long-distance group (HV) and the other was a low-volume short-distance group (LV). To be eligible for the HV group, runners had to have a weekly training volume of at least 70 km/week and a weekly long run duration of 90 minutes or longer. To be eligible for the LV group runners had to run less than 70 km/week and have a weekly long run duration of 75 minutes or shorter. These cut-offs were set based on the answers from the questionnaire. The characteristics of the groups are presented in Table 1.

One runner from the LV group was not able to perform the baseline testing because of injury and was therefore excluded from the study. Further, two of the runners from the HV group suffered injuries and illness, respectively, between the third and fourth test day and were therefore forced to withdraw from the study before the last test. These two runners are included in the analysis for the preliminary testing as well as for the threshold run and the 90% long run, but not for the 100% long run. In addition, one runner from the HV group was unable to complete the last 2-hour running test because of muscular cramps, resulting in missing data for this subject at the last time point (Figure 2).

Testing procedure

The study consisted of four tests performed on four different days, with each test separated by at least 2 days. The first test day consisted of a preliminary VO_{2max} and lactate profile test. The VO_{2max} test was used to determine the fitness level of the runners, while the lactate profile test was used to determine the runners' LT_1speed, which would be used for calibrating the intensity of later long runs. The second test day consisted of a 30-minute run in threshold range. The third and fourth test days consisted of a two-hour long run just below or at LT_1. All runners had to complete preliminary testing before performing tests 2, 3, and 4. All treadmill testing was performed at a treadmill incline of 1% to compensate for the absence of wind resistance (34).

The two long-duration low-intensity runs were planned to be performed in randomized order, but because of fear of a Covid-19 shutdown we decided to perform them in the same order for all the runners to ensure completion of at least one long run condition. All tests were

performed in the same laboratory, with windows and doors open, to ensure maximal exchange of room air. In addition, athletes ran with a large, motorized fan blowing air towards their torso throughout the test tuns. We strived to achieve similar environmental conditions for all the tests, approximately 18-20°C. Each subject performed their respective tests at approximately the same time of the day (± 2h). All runners had to have at least 48 hours of recovery time between test days to ensure sufficient recovery and optimal physiological performance during test days (35). Further, the runners were instructed to refrain from any intense training during the last 24 hours before testing. Runners were also instructed to consume the same type of meal before each test day and to avoid consumption of products containing caffeine during the last 3 hours preceding testing. All tests and measurements were supervised and performed by the same two test leaders. During tests requiring a maximal effort, strong verbal encouragement was given throughout. Due to the rapid evolution in shoe technology, each runner wore the same running shoes for all tests, to make the tests valid and reliable.

Additionally, we strived to perform testing of female runners in the follicular phase of their menstrual cycle. If a subject reported amenorrhea, was using oral contraceptives, or were in menopause, the menstrual cycle was not accounted for.

Otherwise, runners were instructed to continue with their regular training routine through the data collection period. Extra focus was given explaining to the runners to not change their weekly volume or long-run duration during the data collection period.

Test-day 1

The first test day consisted of a submaximal incremental lactate profile test and an incremental test to exhaustion. There was a 10-minute recovery period between tests. Test day 1 is illustrated in Figure 3.

Submaximal incremental test

The first test was a submaximal incremental test, aiming to determine speed, HR, and lactate values at LT_1 and LT_2. The test started with a 10-minute warm-up. During the warm-up, the runners were familiarized with the treadmill and provided information about the test protocol. During the main part of the lactate profile, 5-minute submaximal bouts with increasing speed were performed until LT_2 was reached based on blood lactate sampling quantified in real time during the profile. Runners stood still for 30s with their feet on the side of the moving

treadmill between each bout to execute finger sticks and capillary tube blood draws from the left hand. The starting speed for the first 5min running bout was individualized and based on lactate measurements after the warm-up, as well as discussion between test leaders and runners. Treadmill speed was increased 1 $km \cdot h^{-1}$ after every 5-minute bout, and a total of 6-8 bouts were performed. VO_2, HR, ventilation, and respiratory exchange ratio (RER), were measured during the last 2.5 minutes of each bout. RPE was recorded at the end of each 5-minute bout, using Borg's 6-20 RPE scale (36). When [la^{-1}] reached a value clearly exceeding LT_2 the lactate profile test was immediately stopped. LT_1 values were calculated as the treadmill speed corresponding to the mean of the first 2 blood [la^{-1}] measurements + 0.5 $mmol \cdot L^{-1}$ (37-39), while LT_2 was calculated as the treadmill speed corresponding to the mean of the first 2 blood [la^{-1}] measurements + 2.1 $mmol \cdot L^{-1}$, based on the recommendations from The Norwegian Olympic and Paralympic Confederation of Sport.

Incremental test to exhaustion

After a 10-minute recovery period, runners performed an incremental test to exhaustion. This test was used to determine: (1) VO_{2peak}, (2) HR_{peak} (3) Treadmill $velocity_{peak}$, and (4) peak blood lactate concentration [$la^{-1}{}_{peak}$]. Testing started with one minute of running at a speed approximately 1 $km \cdot h^{-1}$ below LT_2 speed determined from the lactate profile test. Treadmill speed was subsequently increased 1 $km \cdot h^{-1}$ every minute until voluntary exhaustion. Through the closing stages of the test, runners were allowed to increase treadmill speed by 0.5 $km \cdot h^{-1}$ instead of 1 $km \cdot h^{-1}$ if they deemed this best for mobilizing their highest oxygen consumption before exhaustion. Additionally, test leaders gave verbal encouragement to ensure maximal effort. A total test duration of 8-10 minutes was expected. VO_2 was continuously measured and averaged every 30 seconds. The average of the two highest 30s VO_2 measurements was defined as VO_{2peak}. Immediately after the termination of the test [$la^{-1}{}_{peak}$], HR_{peak}, and peak treadmill velocity were determined and recorded.

Test day 2

30 min run between LT_1 and LT_2

The second test day consisted of a 30min run between LT_1 and LT_2. The test started with a 10-minute warm-up at a speed clearly below LT_1- speed. The main part of the test consisted of 30 minutes of running at a speed corresponding to the mean of LT_1-speed and LT_2-speed, split into two 15-minute bouts. Each of the two 15-minute bouts consisted of the same test protocol. When the runners had been running for 10 minutes, they were told to run with their

mouth closed for 2 minutes, and directly after opening their mouth they were asked: “Did running with your mouth closed feel comfortable?”. The runners were instructed to respond with: (1) “yes”, (2) “equivocal”, and (3) “no”. Immediately after, runners conducted a talk test, where they were instructed to recite a standard paragraph that required 10-15 seconds of speaking outloud. The standard paragraph used for this test was the first verse of the Norwegian national anthem. After reciting the paragraph, the runners were asked: “Did reciting the paragraph feel comfortable?”. The runners responded: (1) “yes”, (2) “equivocal”, or (3) “no”. During the last two and a half minutes of the 15-minute bouts, oxygen consumption and HR were recorded. Approaching the end of each bout, runners reported their RPE with the aid of a large RPE poster visible from the treadmill. A short break of 30s was interjected between the two 15-minute bouts for blood lactate measurements. This test was used mainly for a second research study. Test day 2 is illustrated in Figure 4.

Test day 3 and 4

The third and fourth test days each consisted of a 120-minute low-intensity long run performed at 90% of LT_1 speed and 100% of LT_1 speed, respectively. The 120-minute run was split into four 30-minute periods, with a 1-minute recovery period between for measurement purposes (Figure 5). Each of the four bouts consisted of the same measurement protocol (Figure 6) and represent a comparison time point for assessing physiological and perceptual changes across the duration of the long runs. The runners ran undisturbed for the first 22 minutes of each 30min period, before data acquisition during the last 8 minutes of each bout. After 22 minutes of running and 24 minutes of running, respectively, runners performed the same mouth-closed test and talk-test as described for the second test day. EMG data was collected from 24-26min of each time block, while VO_2 was recorded from 26 to 28min. HR and kinematic factors were recorded for the last 4.5 minutes of each bout. The average of the values recorded in the sample periods was used to quantify physiological changes across the 120min run (defined as 30-, 60-, 90-, and 120-min time points). Right before the end of each 30min running period, RPE was reported verbally with the help of a Borg Scale poster made clearly visible in front of the treadmill. During the 1-minute measurement periods between 30min running blocks, blood lactate, skin temperature, and core temperature measurements were made. Skin temperature was measured two times on the back and two times on the legs and then averaged, providing one value for the back and one

for the legs. Core temperature was self-measured by the runners through the tympanic membrane.

The runners were weighed before and after each of the long runs to quantify weight loss due to dehydration. To make the test as similar as possible to the runners' behavior during regular long runs they were encouraged to drink *ad libitum*. The test leaders encouraged the runners to drink regularly to limit dehydration. Further, they were given the possibility to listen to music or watch television during the run. However, due to the possible enhanced performance and endurance due to music (40), runners had to perform the test either with no music at all, or with music throughout the run.

Test day 3 and 4 are illustrated in Figure 5.

Testing instruments

All tests were performed on the same treadmill, Lode Katana Sport (Lode B. V., Groningen, Netherlands). The treadmill was calibrated on a regular basis. VO_2 measurements during all tests were measured using Oxycon Pro™ with mixing chamber and 30 second sampling time (Oxycon, Jaeger GmbH, Hoechberg, Germany). Gas sensors were calibrated via an automated process using certified calibration gases of known concentrations before every test, as well as half-way through the long runs. Blood [la-] during all tests were analyzed using a stationary lactate analyzer (EKF BIOSEN, EKF diagnostic, Cardiff, UK) which was calibrated every 60 minutes. HR was measured using Polar V800 (Polar Elektro Oy, Kempele, Finland). Core temperature was measured using Braun IRT6520 ThermoScan® 7 Age precision® (Braun, Kronberg im Taunus, Germany). Skin temperature was measured using Flir TG267 Thermal Camera® (Flir Systems, Inc. Wilsonville, Oregon, US). Kinematic variables were measured using a Stryd™ foot pod (Stryd, Boulder, Colorado, US). EMG was measured using Delsys Trigno Wireless EMG System (Delsys, Natick, Massachusetts, US). Before and after each test runners, were weighed using a Seca model 713 (Seca, Hamburg, Germany).

Electromyography (EMG)

A large part of the methodological development work leading into the main data collection was focused on how to best optimize EMG measurements under difficult data collection conditions. EMG was measured using surface electrodes (Trigno Wireless EMG systems; Delsys, Boston, MA, USA). Electrodes were placed at the following lower limb muscles during both 120min runs; m. Vastus Medialis (VM) and m. Biceps Femoris (BF). Placement

and location of the electrodes were performed according to the recommendation of SENIAM (Surface EMG for Non-Invasive Assessment of Muscles (41). Briefly, electrodes were placed parallel to the muscle fibers on the belly of the muscle. Prior to placing the electrodes, the skin was shaved and cleaned with alcohol. To prevent the detachment of electrodes due to sweat during the long runs, they were fixed with additional tape. The same test leader was assigned to place the electrodes each time to ensure consistency in the placement.

Based on preliminary testing and data analysis, EMG measurements were performed for 120s at the end of each 30-minute bout on both long runs, with a sampling rate of 2000Hz. EMGworks Version 4 analysis software (Delsys) and MATLAB was used to analyze EMG data. The Raw EMG signal was filtered using a fourth-order Butterworth band-pass filter at 30-500Hz (42), this allowed noise or movement interference below 30Hz and other non-physiological signals above 500Hz to be removed. Root Mean Square (RMS) and Median Frequency (MDF) were used for analyses and comparison across time. RMS was calculated to obtain the average EMG amplitude, using a moving window of 100ms. Each repetition was identified through the RMS signal, and the median of the peak RMS of each contraction was calculated. Further, all valid contractions for each recording were Fast Fourier transformed and the MDF was obtained. Ultimately each 30min time period during the long runs was described by a single value for MDF and a single value for RMS for each muscle at each time point. Outliers caused by sensors partially detaching during running were removed from the analysis.

EMG data was analyzed for all subjects as a combined group in SPSS using a repeated measures ANOVA. Because of the relatively high amount of data lost because of sensors partially detaching during running or being corrupted by sweat accumulation at the skin-electrode interface, EMG data was analyzed separately from the rest of the data. Only subject long runs with completely uncorrupted recordings and values for all 4 timepoints were included in the statistical analysis.

Statistical analysis

All statistical analysis was performed using SPSS (version 25, IBM, Chicago, IL, USA) and data are presented as mean ± standard deviation. Subjects' characteristics and training characteristics were compared using a One-way between-groups analysis of variance (ANOVA), with an LSD post-hoc test. A General Linear Model repeated measures model

(ANOVA) was used to compare the changes in physiological, kinematic, and perceptual factors across the long run duration between groups. When Mauchly's test of Sphericity[a] was statistically significant, indicating lack of sphericity in the data, a Greenhouse Geiser correction was applied. A paired-samples T-test was used to compare the two long runs to each other, both for the first time point and for the absolute change from the first to the last time point. An independent samples T-test was used to compare changes between the high-drift group and the low-drift group. A value of $p<0.05$ was considered statistically significant in all analyses.

Ethical considerations

This study was performed on healthy adults, all of whom were physically active with no known health problems. All subjects received written information prior to the study, explaining the magnitude of the testing, the purpose of the research, the potential risks involved, the potential benefits of participating, a statement explaining that their data was collected anonymously and that they at any point during the research period could choose to withdraw their participation without any reason. On the first day of testing, all subjects signed a consent form detailing these conditions. In addition, the test leaders observed extra Covid-19 precautions, using facemasks and rubber gloves during all testing, as well as washing all surfaces with antibacterial agents between tests. When the runners arrived for the tests, they completed a Covid-19 self-declaration, confirming that they did not have any symptoms.

The study was approved by the ethics committee of the Faculty for Health and Sport Science, University of Agder, and by the Norwegian Center for Research Data (NSD).

RESULTS

Characteristics for the groups

Characteristics for the training volume groups are shown in Table 1. As intended, there were significant differences between the two groups for training volume and long run duration. The groups were also different regarding personal bests for the 10 k and half-marathon (P= <0.05). There were no significant differences between the groups for age, weight, height, or running experience. Preliminary testing showed that the high-volume group achieved significantly higher treadmill velocity at both LT_1 and LT_2 (P= <0.05), as well as a slightly higher VO_{2peak} and peak treadmill velocity compared to the low-volume group, albeit not

significant (Table 2). Results from the first time point at LR90 and LR100 respectively, showed that the two groups had somewhat different kinematic characteristics (Table 3), with the HV group self-selecting a significantly higher cadence and displaying shorter ground contact time compared to the LV group (P=<0.05).

The influence of duration and training characteristics on physiological, perceptual, and kinematic changes during a low intensity long run

Significant changes were observed from the first to the last time point for both groups for the physiological and perceptual factors measured: HRR (HV: +6.3 ± 4.3% and LV: +7.1 ± 2.8%), oxygen consumption (HV: +1.0 ± 2.50 $ml \cdot kg^{-1} \cdot min^{-}1$ and LV: +2.0 ± 1.6 $ml \cdot kg^{-1} \cdot min^{-1}$), ventilation (HV: +2.2 ± 6.0 L/min and LV: +2.9 ± 2.6 L/min), RER (HV: -0.09 ± 0.03 and LV: -0.09 ± 0.03), and RPE (HV: +2.2 ± 1.6 and LV: +2.2 ± 0.9) during the LR90. (all p<0.05) (Figure 7A, 7C, 7G, 7I, 7K). During the LR100, similar changes were observed : (HV: +6.7 ± 3.0% and LV: +7.5 ± 3.4%), oxygen consumption (HV: +1.9 ± 1.5 $ml \cdot kg^{-1} \cdot min^{-1}$ and LV: +2.5 ± 1.1 $ml \cdot kg^{-1} \cdot min^{-1}$), ventilation (HV: +5.2 ± 9.4 L/min and LV: +5.0 ± 4.4 L/min), RER (HV: -0.04 ± 0.05 and LV: -0.08 ± 0.04), and RPE (HV: +1.9 ± 1.5 and LV: +2.1 ± 1.3) + (all p<0.05) (Figure 7B, 7D, 7H, 7J, 7L). There was a significant change from the first to the last time point during the LR100 for blood lactate concentration (HV: +0.28 ± 0.80 $mMol \cdot L^{-1}$ and LV: +0.70 ± 0.70 $mMol \cdot L^{-1}$), and for cost of running (C_r) (HV: 3.9 ± 6.7 $ml\ O_2 \cdot kg^{-1} \cdot km^{-1}$ and LV: 12.8 ± 5.8 $ml\ O_2 \cdot kg^{-1} \cdot km^{-1}$) (P=<0.05) (Figure 7F, Figure 8H), while no time-effect was found for Lactate or for C_r during LR90. No time effect was shown for any of the other kinematic measurements, such as cadence, vertical oscillation, or ground contact time in either long run (Figure 8).

There was no significant difference between groups for the change from the first to the last time point for any factors (Figure 7, Figure 8), except for C_r at LR100, where there was a significantly greater increase in the LV group versus the HV group (LV: 12.8 ± 5.8 $ml\ O_2 \cdot kg^{-1} \cdot km^{-1}$ versus HV: 3.9 ± 6.7 $ml\ O_2 \cdot kg^{-1} \cdot km^{-1}$) (P=<0.05) (Figure 8H).

No significant difference was found between groups or across time during long runs for the control-variable core temperature.

Comparison between rate of fatigue for LR90 and LR100

Comparisons of baseline measurements made at the first time point as well as changes from the first to the last time point for both the LR90 and the LR100 are presented in Table 4. As

expected, the physiological cost and kinematic characteristics of the 2 long runs were initially different; with higher % of heart-rate reserve, oxygen consumption, ventilation, and stride cadence, decreased ground-contact time, and higher RPE (P=<0.05). However, when comparing the magnitude of change during the two runs, we only observed a significant difference in the change in RER (P=<0.05).

Comparison of "high-drift" group versus "low-drift" group

Due to there being a limited difference between the high-volume group and the low-volume group, as well as a limited difference between the two runs, the data for both LR90 and LR100 were merged and thereafter split into two new groups based on the magnitude of increase in %HRR, a high-drift group (HD) (n=20 subject runs), and a low-drift group (LD) (n=20 subject runs). The HD and LD groups were significantly different for elevation in %HRR (HD: 9.7 ± 1.9 % versus LD: 4.3 ± 3.4 %), initial bodyweight (HD: 74.3 ± 9.4 kg versus 63.40 ± 13.7 kg), ventilation (HD: 87.3 ± 13.6 L/min versus LD: 71.4 ± 11.8 L/min), running cadence (HD: 169 ± 6 steps/min versus LD: 176 ± 10 steps/min), and vertical oscillation (HD: 7,53 ± 0,7 cm versus LD: 6,98 ± 0,9 cm) (P=<0.05). There was a significantly greater change from the first to the last time point in the HD group compared to the LD group for RPE (HD: 2.6 ± 1.2 versus LD: 1.7 ± 1.3), ventilation (HD: 5.6 ± 6.6 L/min versus LD: 2.09 ± 5.8 L/min), core temperature (0.16 ± 0.38 degrees versus LD: -0.09 ± 0.34 degrees), and weight loss (HD: -1.7 ± 0.4 kg versus LD: -1.4 ± 0.5 kg) (P=<0.05) (Figure 9). The high and low heart rate decoupling groups were not significantly different for changes in blood lactate, oxygen consumption, RER, cadence, ground contact-time, C_r, or vertical oscillation during the long runs.

Electromyography evolution during a low intensity long run

Evolution in EMG signals throughout the long runs are presented in Table 5. RMS and MDF for VM did not change significantly at either the LR90 (RMS: -2.2 ± 23.4% and MDF: -1.2% ± 12.5%) or the LR100 (RMS: -11.3 ± 16.0% and MDF: 2.5 ± 9.2%). RMS for BF increased significantly at LR100 (5.1 ± 3.0%) (P=<0.05), but not at LR90 (6.1 ± 14.3%). MDF increased significantly at both the LR90 (5.7 ± 4.3%), and the LR100 (3.6 ± 1.6%) (P=<0.05).

DISCUSSION

The purpose of this study was to experimentally investigate and explain cardiac drift and its related physiological and perceptual mechanisms during low intensity running, and to investigate if weekly training volume and long-run duration is associated with the rate of cardiac drift and fatigue.

The original hypothesis in this study was that the runners training less weekly volume would show a systematically greater degree of physiological and perceptual stress and cardiac decopling than high training volume runners during a 120min "long run.". We hypothesized that because the HV group ran more each week and performed longer weekly "long runs", they would demonstrate better resistance to fatigue during these controlled 120min efforts in the laboratory. Several recent studies have aimed to compare cardiac drift and fatigue during low intensity running to race performance (43-45), however, to the author's knowledge this is the first study examining the relationship between cardic drift and fatigue during long sub-LT_1 training sessions and athlete training characteristics.

The main finding of the present study was that during 2-hour low-intensity long runs performed at 90% of LT_1 and 100% of LT_1 speed, there was a significant increase in HR, oxygen consumption, ventilation, RER, and RPE for both the HV group and the LV group during both runs. However, the present study demonstrates that there was no generalizable difference in response during these runs between the groups, whether comparing cardiac decoupling, or several other relevant physiological and perceptual variables during either of the runs. That is, our findings do not support our initial hypothesis.

The substantial increase in relative heart rate reserve utilized during both runs in this study (LR90: 9.9% and, LR100: 9.6%) is consistent with recent literature, showing a significant drift in HR during low- or moderate-intensity prolonged exercise (29, 30). A study by Maunder et al., (31), reported that out of 43 cyclists performing a low-intensity 4 hr bike ride, 28 cyclists showed moderate to high cardiac drift, while 15 cyclists showed little cardiac drift. This is consistent with the findings in the present study; substantial cardiac drift is the norm but there is large individual variation in how athlete physiology and perception evolves during long runs. The change in %HRR observed ranged from a 5% decrease in %HRR to a a 15% increase. This underscores the large individual differences related to physiological

deterioration during long-duration low intensity running and highlights the importance of assessing the physiological and perceptual cost of long, low intensity training sessions an individual characteristic of the athlete to be considered during training prescription and monitored over time.

The significant increase in both physiological and perceptual measurements during low intensity runs performed below LT_1 in this study supports the the practical training concept that no indefinite physiological "steady-state" exists (46). Maunder et al., (31) proposed that exercise physiologists would benefit from better understanding each athlete's "durability", in this case, defined as the time of onset and magnitude of deterioration in physiological measurements during prolonged exercise. The present study confirms this deterioration, and therefore the importance of understanding the durability of the individual athlete. A better understanding of deterioration of physiological and perceptual measurements during low-intensity running can allow coaches and physiologists to prescribe athletes with more specific intensity regulation during low-intensity running.

Hofman and Tschakert (26) recently described how exercise duration is an under-appreciated variable in training prescription. The findings in the present study, demonstrating significant physiological deterioration for both groups underscores the importance of duration during the prescription of LIT. Findings from the present study demonstrated that during a run performed at LT_1 speed, both HR, lactate, and RPE values all drifted from a value equivalent to LT_1 after 30 minutes of running, to a value approximating the mean of LT_1 and LT_2 after 120 minutes of running. This change can be interpreted as meaning that during the last part of the run the "effective" intensity was no longer equivalent to LT_1, but rather at an intensity more typical of "threshold training." According to Hofman and Tschakert (26), an intensity just 10% above LT_1 may shorten time to fatigue by ~40% and therefore increase the rate and magnitude of fatigue. This demonstrates the importance for both coaches and physiologists to be aware of the intensity x duration interaction during "easy, steady state workouts", and prescribe training sessions that anticipate the desired degree of fatigue that will be induced.

In the present study, the HV group, in addition to their higher training volume, also reported faster personal bests in the 10k and half-marathon. They were therefore considered as better runners. We expected that the HV group would demonstrate greater resilience to deterioration

during the long runs. However, this was not the case. This may be explained in part by the fact that, despite the groups being clearly different according to our training criteria, they were still relatively homogenous in the "broader context". It is possible that a similar study examining groups with athletes training significantly more mileage may provide different results. Another aspect regarding the groups in the present study is that while they were different in weekly training volume and weekly long run duration, the majority of runners in both groups identified as marathon runners and therefore likely had been doing long runs on a regular basis on an earlier point of their running "career". They may have accumulated a high amount of slow-twitch, fatigue-resistant muscle fibers, and therefore employed these efficient muscle fibers during the runs (47), independent of which group they were in. It has recently been discussed that faster marathon runners may complete races at a higher percentage of their estimated critical speed than slower runners, due to their greater resilience to deterioration in their physiological profiles (43). A recent study by Merry et al., (44) examining cardiac drift in six trained cyclists (VO_{2peak} = 64, training six times per week) and six untrained cyclists (VO_{2peak} = 45, training one time per week) performing 40min of cycling at 70% of VO_{2max} demonstrated a significantly greater cardiac drift in the untrained cyclists as compared to the trained subjects both while euhydrated and hypohydrated. The group of trained cyclists had similar VO_{2max} (63.6 $ml \cdot kg^{-1} \cdot min^{-1}$) and showed a similar cardiac drift (10% increase) as shown in the present study. Further, a study by Shimazu et al., (45), examining marathon runners at a similar level to the runners in our study found no significant correlation between cardiac "cost" and marathon performance. These findings indicate that individual differences in cardiac drift between subjects are typical, but not clearly correlated with present training characteristics or physiological capacity.

We did not observe an increase in C_r during LR90, but measured a significant increase in C_r during LR100. This increase in C_r indicates a deteriorating running economy during the LR100. This finding is consistent with the majority of studies investigating the relationship between running economy and fatigue. Running economy seems to decline during prolonged exercise and this change has been connected to both mechanical and metabolic factors (48). However, in the present study, there was no significant change in kinematic measurements for either group, during either of the runs. From the author's own perspective this was an interesting finding, considering a number of subjects reported that they felt that their stride was changing towards the end of the run when they started to fatigue. The data collected did

not directly support this altered proprioceptive "feeling" given no actual change in running gait occured. The absence of change in running kinematics in this study, despite deterioration in other physiological factors, may be due to the long-run being performed on a treadmill, and not outdoors. A study by Elliot and Blanksby (49) reported a shorter flight phase, decreased stride length, and increased cadence while running on a treadmill compared to overground running. We suspect that running on a motorized treadmill with a belt moving under the legs at a fixed velocity may "entrain" a certain running gait, despite fatigue. Future research reproducing this study during constant-pace overground running (i.e on a track), instead of on a treadmill, could reveal important differences between the two common running conditions in how kinematic changes relate to fatigue

Due to the limited difference between the original groups, they were merged, and the runs were thereafter compared with each other. The other main finding of this study is that, except for blood lactate concentration and RER, there was no significant difference in the cardiac drift or the drift for any other associated physiological, kinematic and perceptual factors, when running at 90% of LT_1 speed, compared to running at 100% of LT_1 speed. Despite the "starting values" being significantly higher for the LR100 for several variables.

There was a significant decrease in RER values during both runs, however, the change was significantly greater during the LR90 run compared to the LR100 run. For the LR90 run, RER decreased from 0.89 after 30 minutes of running to 0.80 after 120 minutes of running. And since RER only varies between approximately 0.7 and 1.0 this change is huge and equal to about 30% of the entire range. The change demonstrated in the present study corresponds to a shift in the proportions of fat/carbohydrates use from 37% fat and 63% carbohydrates to 67% fat and 33% carbohydrates during the LR90. This finding supports the finding by Costill (50), who demonstrated a similar decrease in RER, from 0.88 to 0.80, during a 2 hr exhaustive run, performed by highly trained runners. This is consistent with the literature, as fat oxidation is known to increase and carbohydrate oxidation to decrease as the exercise duration increases (51). This increased fat oxidation happening during prolonged exercise is likely caused by a reduction in muscle glycogen stores towards the later stages of the long runs. This reduction in muscle glycogen where glycogen concentration reaches low levels and carbohydrate oxidation cannot be maintained at a sufficient rate to maintain ATP resynthesis may be responsible for the fatigue seen in the present study (51).

Further, there was a significant increase in lactate during both runs, however, there was a significantly greater increase during the LR100, compared to the LR90. This may indicate that the runners were utilizing more fast-twitch motor units towards the end of their LR100 run, as the metabolic profile of fast-twitch motor units has shown to favor glycolytic energy production (52). This is supported by the higher RER during the LR100 compared to LR90. The significant difference in lactate increase during the LR100 compared to LR90 makes sense, seeing that LT1 is the lowest intensity where there is a sustained increase in blood lactate concentration (37). This indicates that the LR90 run was performed at a low enough intensity to not induce a significant increase in lactate, while the LR100 run as expected, saw a significant increase.

HR, oxygen uptake, ventilation, and RPE increased significantly during both runs. There was no difference in the change between the runs for these measurements. The present study demonstrates how HR (~10%) and RPE (~20%) increases more during a low-intensity long-run than the oxygen uptake (~3.8%) and ventilation (~4%). This finding is consistent with a study by Ekelund (53), examining cardiac drift during 60 minutes of moderate cycling, showing a higher drift in HR, compared to oxygen uptake.

After analyzing the original groups and finding limited significant differences between the groups, they were merged and then split into two new groups based on the drift found during the runs. Each run was handled as one sample, giving us a total of 40 runs. The 20 runs with the most cardiac drift were placed in the HD group, while the other 20 runs were placed in the LD group. The HD group had a cardiac drift of 9.7 ± 1.9%, while the LD group had a cardiac drift of 4.3 ± 3.4%. The other variables that changed significantly more in the HD group versus the LD group were RPE, ventilation, core temperature, and weight loss. A higher weight loss in the HD group would indicate the subjects in this group getting more dehydrated, and dehydration are known to induce cardiac drift (30). However, the higher weight loss initially observed in the HD group versus the LD group, were actually non-existent when weight loss relative to total bodyweight were accounted for. Therefore, the differences in drift between the groups may not be attributed to dehydration.
Different characteristics between the groups were that the HD group had a significantly higher body weight and ventilation, as well as a significantly higher vertical oscillation and a significantly lower cadence. Some studies report that a relatively high step frequency

(approximately 180 steps/min) and a limited vertical oscillation, are among other factors, known to be associated with a better running economy (54, 55). The LD group in the present study was considerably closer to the 180 steps/min than the HD group, however, a freely chosen stride frequency has also been associated with a better running economy (55). Considering the ambiguous meanings regarding step frequency in literature, and the absence of a significant difference in change in running economy between groups in the present study, there is no evidence that the difference in cardiac drift between the groups can be attributed to a poorer work economy or a larger deterioration in work economy in the HD group compared to the LD group.

The significantly higher body weight in the HD group compared to the LD group in the present study is interesting. The lower body weight may exhibit greater fatigue resistance. African runners have shown better fatigue resistance than caucasian runners (56), and african marathon runners typically have a very low body weight compared to caucasian runners (56). The superiority of african runners in the marathon, a distance where fatigue resistance is very prominent, can possibly be due to their lower body weight, among other factors. Therefore it is worth noticing this difference as a possible explanatory difference between the HD group and the LD group. However, the weight difference between the two groups may be due to the fact that the LD group contained 9 tests performed by females, while the HD group contained only 3. And as female runners typically are lighter than men, this observation makes it possible to speculate if the change in cardiac drift between groups can be attributed to gender differences, rather than to bodyweight. Recently, a number of exceptional performances are seen by female athletes in ultra-endurance sports (57), and this raises the question if women may be more restitant to fatigue during prolonged exercise.

EMG

This study employed EMG to measure muscular activity during long runs. Muscular activity was measured through signal amplitude (RMS) and signal frequency (MDF) to possibly relate the cardiac drift and fatigue experienced by the runners to an increase in muscle fiber recruitment. To the author’s knowledge, no other studies have measured muscular activation during running sessions of similar duration. This makes the EMG testing a unique aspect of this study. The EMG testing provided several methodological challenges. Data from several subjects were lost or deemed invalid due to sensors partially detaching during running, due to

sweat. Due to the high number of lost data, the EMG data were analyzed for all subjects as a combined group, rather than as a HV group and a LV group as initially intended. The challenging nature of EMG testing we experienced, may explain the lack of existing literature on EMG testing during long-duration running.

The findings in this study demonstrated that neither the RMS nor MDF changed significantly for VM during either of the runs. However, there was a significant increase in RMS for BF during the LR100 but not during the LR90. In addition, MDF increased significantly for BF during LR90 and LR100. Muscle fatigue is a reduction in the muscle's ability to produce force (58). During higher intensity running involving fast-twitch motor units (59), fatigue would indicate that the fast-twitch muscles begin to fatigue and replace themselves with slower twitch muscle fibers (58). However, in the present study, the long runs were performed at a low intensity, and therefore, predominantly involved slow-twitch motor unit recruitment (59). When the signal frequency increases during low-intensity exercise, as demonstrated for BF during both runs in this study, this may indicate a trend among the runners to recruit more fast-twitch muscle fibers towards the end of the run (58). However, to test if the reason for the increased frequency actually was due to an increase in fast-twitch fibers, this would require conducting a muscle biopsy, which was not considered a possibility for this study. The increased amplitude of the signal for BF during the LR100 may indicate more slow-twitch fibers being recruited to make up for the loss of power in fatigued fast-twitch fibers (58). The findings in the present study may indicate that BF is more susceptible to fatigue during a long-duration low intensity run than VM. Further, both mean RMS and MDF values retrieved from this study are similar to those done by other studies (60, 61), indicating that the data may be valid, despite the methodological challenges. Apart from the methodological challenges related to EMG testing, another aspect worth taking into consideration is that surface EMG measures activity from the superficial part of the muscle. Research suggests that there is a difference in muscle fiber composition between the superficial, and the deep part of a muscle, with a higher percentage of slow-twitch fibers deep in the muscle (62). Surface EMG do not capture the changes deep in the muscle, and this evolution may differ from the ones seen in the superficial part of the muscle.

The EMG findings in this study were ambiguous, and large individual differences were observed. Based on this, more research is needed regarding EMG activity during low-intensity long-duration running before a conclusion regarding muscle activations' role for

cardiac drift and fatigue can be made.

Strength and limitations

The main strengths of the present study were: (a) a well-controlled design with a carefully standardized and extensive test protocol, (b) a high number of runners willing to participate in the study, which gave the authors the opportunity to choose the most suitable sample to answer the research questions, (c) a relatively high number of participants, despite the time consuming and extensive test-protocol, (d) a low drop-out rate, and (e) well-established objective measurement methods and equipment for the main measurements during testing.

However, there were also limitations and challenges in this study. Despite investigators being able to allocate subjects to 2 distinct training volume groups based on the questionnaire, the groups are still relatively homogenous relative to the entire range of long-distance runners. Further, some of the equipment used during the long runs, such as the Flir™ thermal camera, Stryd™ foot pod, and Braun™ tympanic temperature sensor, were not gold-standard methods, but rather relatively untested and experimental methods for this purpose. Therefore, the reliability and validity of this equipment can be questioned. In addition, collecting high quality surface EMG signals from the thigh and hamstrings of runners during 120 minutes of continuous running has not, to our knowledge, been previously reported. Further testing and research on how to optimize running EMG measurements over such a long period of time is needed.

We believe that the present subject sample and findings are representative for well-trained, "recreational" runners aged 18-55 years, but not necessarily representative for other subject-groups training substantially less, or more. Based on the results from the long runs, we speculate that a larger difference in intensities between the two long runs, with one being performed at a significantly lower intensity may have induced different results.

Practical applications

Substantial volumes of LIT are considered crucial to endurance performance (9, 12) and both duration and intensity play an important role when planning LIT. An individually optimized prescription of LIT will induce the desired amount of stress and training effect (26). However, there is an unsteady physiology and perceptual nature associated with long-duration LIT. Athletes and coaches tend to focus more of their training planning towards higher intensity

”key sessions”, despite the volume and frequency of this training component being relatively limited, compared to LIT. Hopefully, this study can shed some light on the non-steady-state physiology and perception associated with steady tempo LIT. Our observation of large individual variation among athletes with similar training characteristics underscores the importance of being sensitive to these differences and how they evolve during training sessions and over time when prescribing training to athletes.

More research is needed on the area, and future studies should focus on subjecting groups differing more in training level/volume than the groups in this study, in their search to possibly relate training characteristics to cardiac drift and fatigue. Future studies may also investigate if the characteristics separating the HD group from the LD group in this study is incidental, or if these are characteristics affecting the rate of cardiac drift. Lastly, studies should strive to optimize EMG testing during long-duration running, for this to be a useful tool for this purpose.

CONCLUSION

The present study demonstrates that an upward drift occurs for HR, RPE, ventilation, RER, and oxygen consumption for well-trained runners when running at an intensity corresponding to below or approximating LT_1 for 120 minutes. The study also demonstrates that while wide differences are seen at the individual level, there is no consistent difference in the magnitude of physiological or perceptual changes observed between two groups differing by XX% in weekly training volume.

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CONFLICT OF INTERESTS

The authors has no conflicts of interest in this study. The data are represented clearly, honestly, and without fabrication.

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Tables and Figures used in the article are presented below

Article tables and figures

Table 1. Descriptive data for the subjects

	HV (N=11)	**LV (N=11)**
Age (y)	39 ± 9	35 ± 9
Weight (kg)	68 ± 10	70 ± 11
Height (cm)	175 ± 9	177 ± 9
Training characteristics		
Running experience (y)	11 ± 7	10 ± 9
Training volume (km·wk^{-1})	88 ± 22*	47 ± 11
Duration long run (min)	125 ± 46*	71 ± 6
Personal bests		
10k (min:sec)	36:40 ± 03:39 *	40:02 ± 04:46
Half-marathon (hr:min:sec)	01:22:42 ± 00:08:36*	01:33:02 ± 00:12:31
Marathon (hr:min:sec)	02:58:20 ± 00:15:35 (n=10)	03:55:46 ± 01:29:29 (N=3)

Values are presented as mean ± standard deviation (SD). HV = High volume; LV = Low volume;; n= number of subjects * = HV group significant different from LV group (P= <0.05)

Table 2. Physiological characteristics from preliminary testing

	HV (N=11)	**LV (N=10)**
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	65.6 ± 7.7	61.5 ± 5.4
HR_{peak} (bpm)	185 ± 8	191 ± 10
HR_{rest} (bpm)	47 ± 5	52 ± 5
Peak Speed ($km \cdot h^{-1}$)	19.0 ± 2.0	18.0 ± 1.5
Peak VE ($L \cdot min^{-1)}$	152 ± 26	145 ± 29
Peak RER	1.05 ± 0.05	1.06 ± 0.05
Peak RPE (Borg)	19.1 ± 0.8	19.6 ± 0.5
Peak Lactate ($mMol \cdot L^{-1}$)	8.1 ± 1.5*	9.9 ± 1.3
LT_1 HR (bpm)	152 ± 12	158 ± 12
LT_1 HR % HR_{peak}	82.2 ± 5.2	83.0 ± 4.2
LT_1 Speed ($km \cdot h^{-1}$)	13.3 ± 1.3*	11.8 ± 1.1
LT_1 Speed % of peak	69.9 ± 2.8*	65.9 ± 3.7
LT_2 HR (bpm)	165 ± 12	172 ± 12
LT_2 HR % HR_{peak}	89.3 ± 4.7	90.2 ± 3.6
LT_2 Speed ($km \cdot h^{-1}$)	14.8 ± 1.5*	13.5 ± 1.2
LT_2 Pace % of peak	78.0 ± 2.4	75.4 ± 3.4

Values are presented as mean ± standard deviation. HV = High-volume group; LV = Low-volume group; $VO2_{peak}$ = Peak oxygen uptake; HR_{peak} = Peak heart rate; HR_{rest} = Resting heart rate; n = number of runners
* = HV group significantly different from LV group (P= <0.05)

Table 3. Kinematic characteristics from 30 min time point during long runs

	Long run at 90% of LT_1		**Long run at 100% of LT_1**	
	High-volume group (N=11)	Low-volume group (N=10)	High-volume group (N=9)	Low-volume group (N=10)
Cadence (steps/min)	175 ± 9 *	167 ± 7	178 ± 9 *	171 ± 7
Ground contact time (ms)	243 ± 16 *	264 ± 15	227 ± 22 *	249 ±16
Vertical oscillation(cm)	7.1 ± 0.88	7.4 ± 0.89	7.1 ± 0.97	7.4 ±0.93
Stride length (m)	1.14 ± 0.13	1.06 ± 0.12	1.23 ± 0.14	1.16 ±0.12
Cost of running (ml $O_2 \cdot kg^{-1} \cdot km^{-1}$)	220 ±20	227 ± 14	221 ±16	221 ± 14

Values are presented as mean ± standard deviation. LT_1 = First lactate turn point; n= number of runners.
* = HV group significant different from LV group (P= <0.05)

Table 4. Measurements from the first time point and percent change from the first to the last time point for the long run performed at 90% of LT_1 and the long run performed at 100% of LT_1.

			Change	
	TP30	TP120	Change	Change (%)
Physiological factors				
% of heart rate reserve				
Long run 90% of LT1 (n= 21)	67.6 ± 7.6	74.3 ± 7.9	6.6 ± 3.6	9.9 ± 6.1
Long run 100% of LT1 (n= 19)	73.7 ± 7.3 #	80.8 ± 6.7	7.1 ± 3.5	9.6 ± 4.6
Oxygen uptake ($ml \cdot kg^{-1} \cdot min^{-1}$)				
Long run 90% (n= 21)	42.0 ± 4.1	43.5 ± 3.9	1.5 ± 2.1	3.7 ± 5.0
Long run 100% (n=18)	45.4 ± 4.7 #	47.2 ± 4.4	1.7 ± 1.5	3.9 ± 3.3
Lactate ($mmol \cdot L^{-1}$)				
Long run 90% (n= 21)	1.6 ± 0.5	1.8 ± 0.7	0.2 ± 0.6	15.9 ± 43.1
Long run 100 % (n= 19)	1.9 ± 0.5	2.4 ± 0.8	0.5 ± 0.07 *	30.7 ± 46.2
Respiratory Exchange Ratio (RER)				
Long run 90% (n= 21)	0.89 ± 0.04	0.80 ± 0.02	-0.09 ± 0.03 *	-9.0 ± 3.0
Long run 100% (n= 18)	0.89 ± 0.04	0.83 ± 0.03	-0.06 ± 0.05	-7.0 ± 5.0
Ventilation (L/min)				
Long run 90% (n= 21)	75.4 ± 13.4	78.0 ± 14.9	2.6 ± 4.6	3.0 ± 6.0
Long run 100% (n= 18)	82.8 ± 15.0 #	86.50 ± 16.5	3.72 ± 6.7	5.0 ± 8.0
Core temperature				
Long run 90% (n= 21)	36.66 ± 0.64	36.72 ± 0.69	0.06 ± 0.39	0.2 ± 1.1
Long run 100% (n= 19)	36.91 ± 0.57	36.91 ± 0.78	0.00 ± 0.33	0.0 ± 0.1
Cost of running ($ml\ O_2 \cdot kg^{-1} \cdot km^{-1}$)				
Long run 90% (n= 21)	223 ± 17	231 ± 18	8 ± 12	3.7 ± 5.0
Long run 100% (n= 19)	221 ± 15	230 ± 15	8 ± 8	4.1 ± 3.4
Weight loss (kg)				
Long run 90% (n= 21)	68.5 ± 10.1	67.0 ± 9.9	-1.5 ± 0.5	-2.2 ± 0.6
Long run 100% (n= 19)	68.8 ± 10.0	67.2 ± 9.9	-1.6 ± 0.4	-2.2 ± 0.6
Perceptual				
Rated perceived exertion (Borg)				
Long run 90% (n= 21)	10.5 ± 1.0	12.5 ± 1.5	2.0 ± 1.5	22.0 ± 15.0
Long run 100% (n= 19)	12.0 ± 1.0 #	14.0 ± 1.5	2.0 ± 1.5	17.6 ± 11.3
Kinematic				
Cadence				
Long run 90% (n= 21)	171 ± 9	172 ± 8	1 ± 2	0.3 ± 1.2

Long run 100% (n= 19)	174 ± 9 #	176 ± 9	1 ± 2	0.6 ± 1.1
Ground contact time (ms)				
Long run 90% (n= 21)	253 ± 19	253 ± 19	0 ± 4	0.1 ± 1.5
Long run 100% (n= 19)	238 ± 21 #	238 ± 23	0 ± 5	0.1 ± 2.3
Vertical Oscillation (cm)				
Long run 90% (n= 21)	7.20 ± 0.87	7.13 ± 0.83	-0.07 ± 0.27	-0.9 ± 4.0
Long run 100% (n= 19)	7.25 ± 0.93	7.19 ± 0.82	-0.06 ± 0.27	-1.4 ± 3.2

Values are presented as mean ± standard deviation. TP = Time point; n= number of runners; N= 18 for oxygen uptake, RER, VE, and Cost of running for Long-run 100% (Missing data for one subject). # = Significant difference between runs at first time point. * = Significant difference between runs in change from the first to the last time point.

Table 5. EMG measurements from the long runs.

	TP30	TP60	TP90	TP120	% change
m. Vastus Medialis					
LR90 RMS (mV) (n= 12)	127 ± 37	133 ± 42	129 ± 40	124 ± 42	-2.2 ± 23.4
LR100 RMS (mV) (n= 9)	124 ± 39	117 ± 35	116 ± 33	110 ± 35	-11.3 ± 16.0
LR90 MDF (Hz) (n= 12)	82 ± 13	83 ± 15	80 ± 13	81 ± 15	-1.2 ± 12.5
LR100 MDF (Hz) (n= 9)	81 ± 13	83 ± 11	81 ± 13	83 ± 14	2.5 ± 9.2
m. Biceps Femoris					
LR90 RMS (mV) (n= 11)	114 ± 43	120 ± 50	120 ± 49	121 ± 54	6.1 ± 14.3
LR100 RMS (mV) (n = 8)	117 ± 34	123 ± 38	122 ± 39	123 ± 39	5.1 ± 3.0 *
LR90 MDF (Hz) (n= 11)	106 ± 16	111 ± 17	111 ± 17	112 ± 17	5.7 ± 4.3 *
LR100 MDF (Hz) (n= 8)	110 ± 12	113 ± 13	114 ± 13	114 ± 12	3.6 ± 1.6 *

Values are presented as mean ± standard deviation. RMS = Root Means Square; MDF = Median Frequency; TP = Time point; n= Number for runners. * = Significant difference between the first and the last time point.

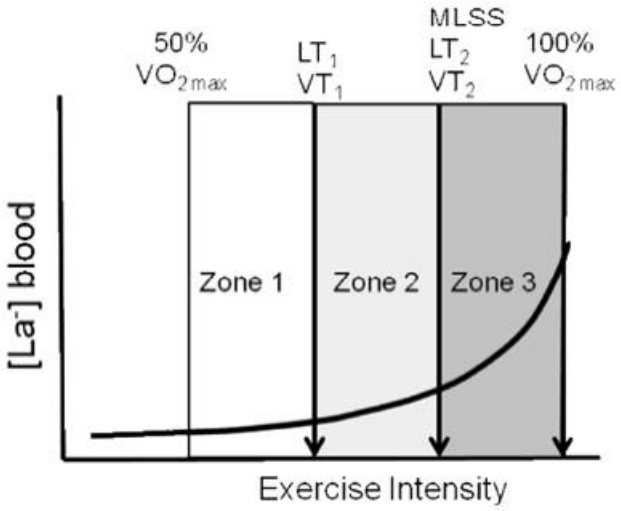


Figure 1. A three-intensity-zone model based on ventilatory and lactate thresholds (Seiler, 2010).

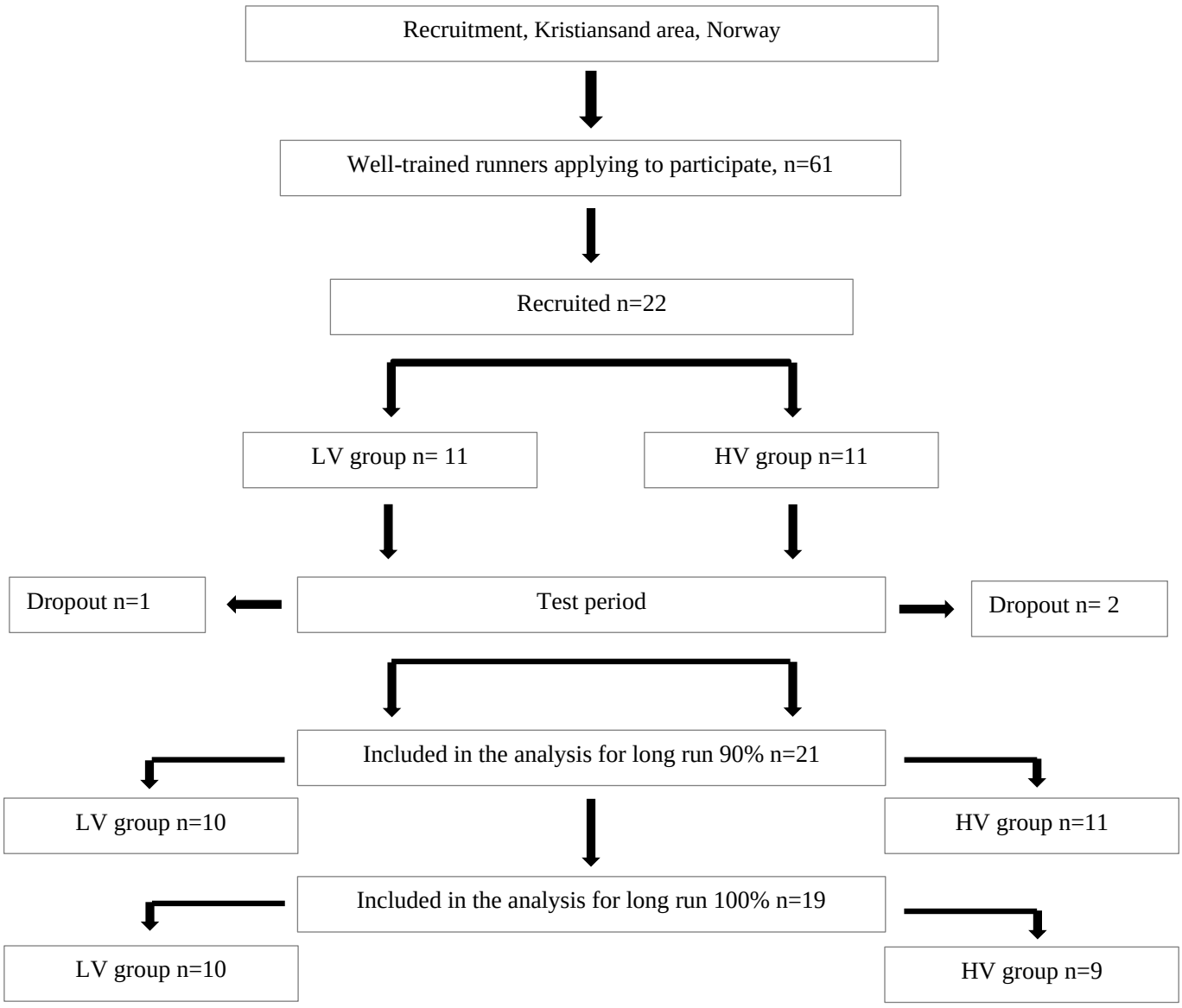


Figure 2. Recruitment of runners, dropouts, and number of subjects for final analysis. LV group = low-volume group, HV group = high-volume group, n = number of runners.

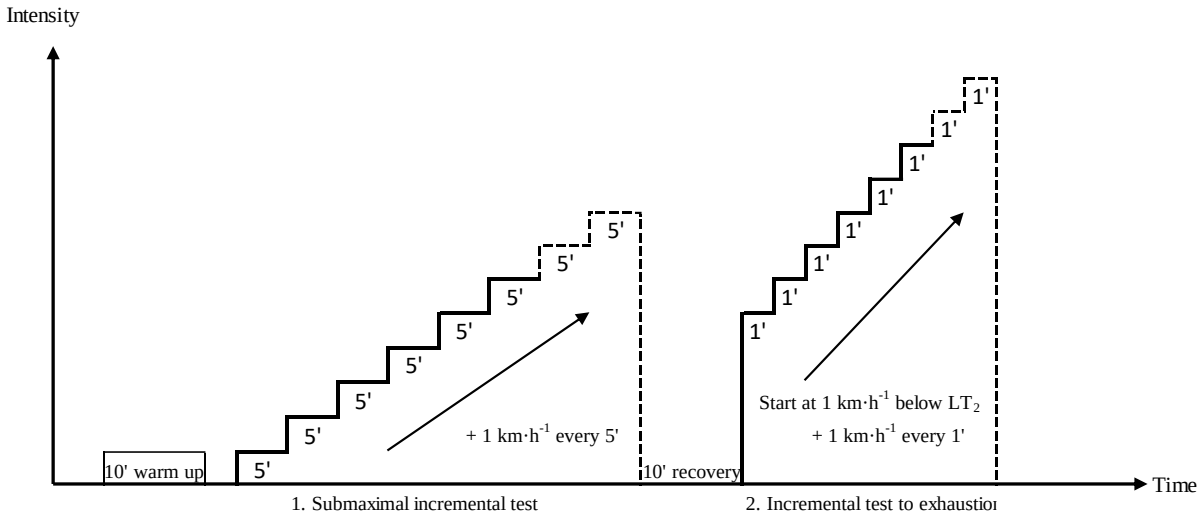


Figure 3. Test day 1. The first test day started with a warm-up, further there was a submaximal 5-min steps incremental test to identify the speed, HR and lactate corresponding to LT_1 and LT_2. After a 10-min recovery period an incremental test to exhaustion was performed to quantify (1) VO_{2peak}, (2) HR_{peak}, (3) $Speed_{peak}$, and (4) peak blood lactate concentration [la^{-1}_{peak}].

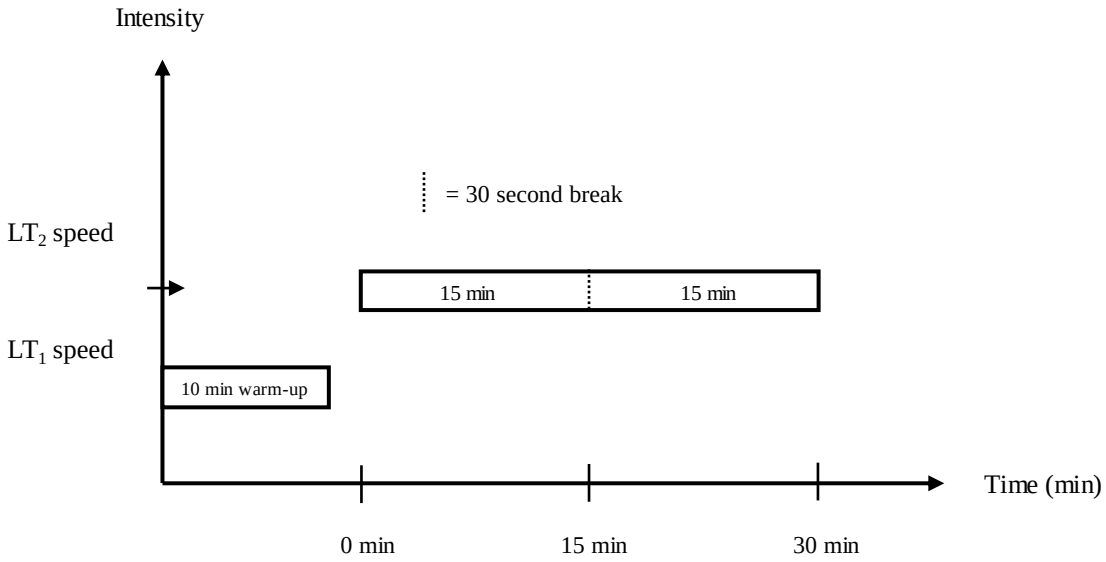


Figure 4. Test day 2. The second test day started with a 10 min warm-up clearly below LT_1. The main part of the test was a 30min run at a speed corresponding to the median of LT_1 and LT_2. The 30-minute run was split into two 15-minute parts with a 30-sec recovery period between for measurements. Metabolic measurements were made in the final 2.5min of each 15min running period.

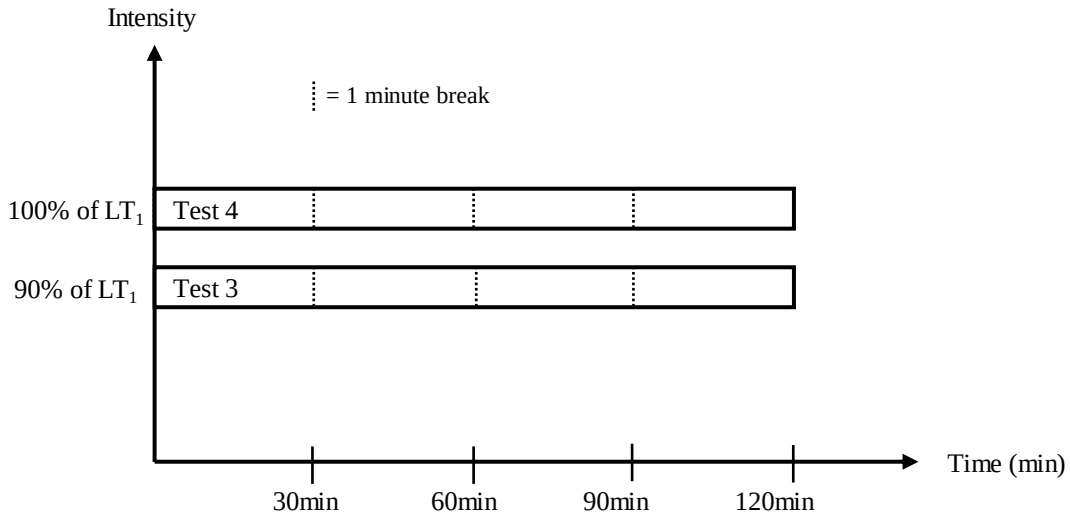


Figure 5. Test day 3 and 4. The third and fourth test days consisted of a two-hour run at 90% of LT_1 and 100% of LT_1, respectively. There was a 1-min break every 30-minute bout, to do measurements.

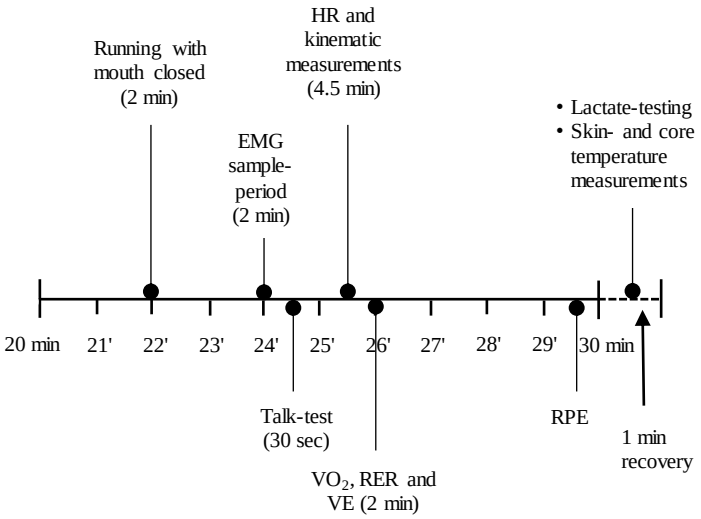
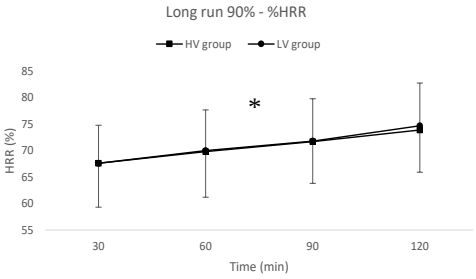
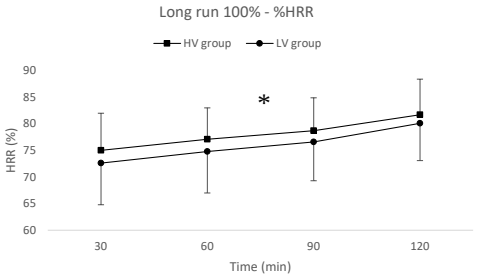


Figure 6. Timeline for testing during the last 10-minutes of each bout on the 120-minute low-intensity long run.

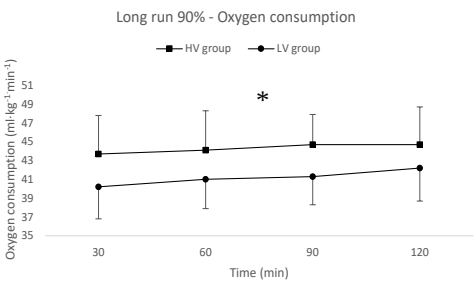
a)


Long run 90% - %HRR
HV group
LV group
HRR (%)
Time (min)
*

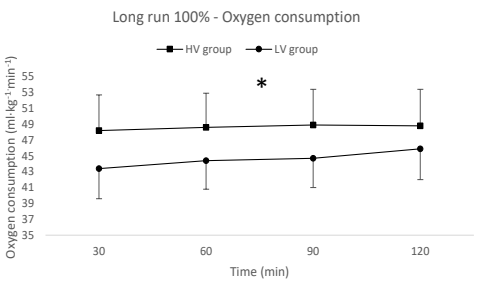
b)


Long run 100% - %HRR
HV group
LV group
HRR (%)
Time (min)
*

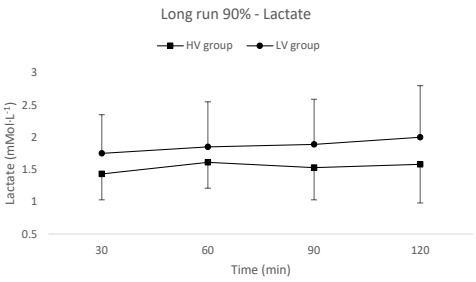
c)


Long run 90% - Oxygen consumption
HV group
LV group
Oxygen consumption (ml·kg⁻¹·min⁻¹)
Time (min)
*

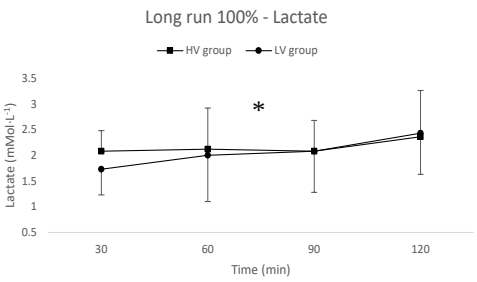
d)


Long run 100% - Oxygen consumption
HV group
LV group
Oxygen consumption (ml·kg⁻¹·min⁻¹)
Time (min)
*

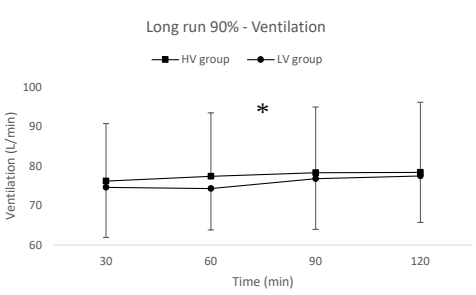
e)


Long run 90% - Lactate
HV group
LV group
Lactate (mMol·L⁻¹)
Time (min)

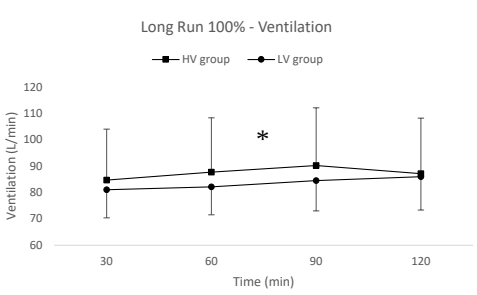
f)


Long run 100% - Lactate
HV group
LV group
Lactate (mMol·L⁻¹)
Time (min)
*

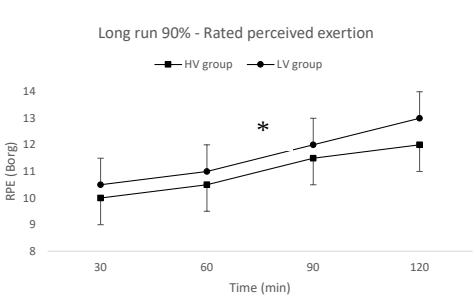
g)


Long run 90% - Ventilation
HV group
LV group
Ventilation (L/min)
Time (min)
*

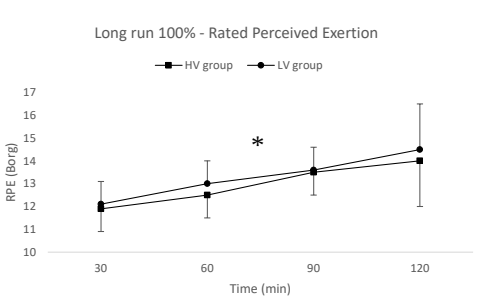
h)


Long Run 100% - Ventilation
HV group
LV group
Ventilation (L/min)
Time (min)
*

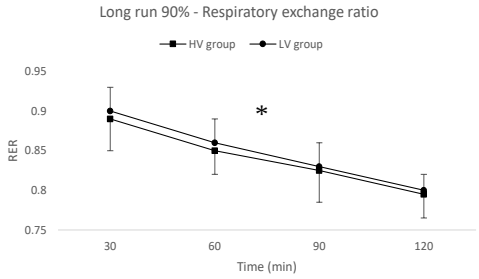
i)


Long run 90% - Rated perceived exertion
HV group
LV group
RPE (Borg)
Time (min)
*

j)


Long run 100% - Rated Perceived Exertion
HV group
LV group
RPE (Borg)
Time (min)
*

k)



l)

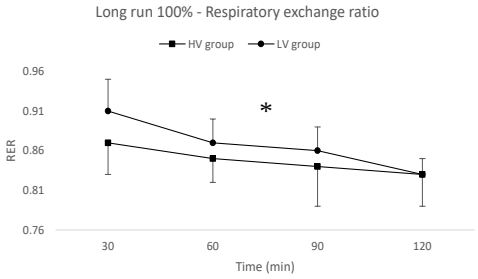


Figure 7. Difference between HV group and LV group and change over time for physiological and perceptual factors (A, B) Heart-rate reserve, (C, D) Oxygen consumption, (E, F) Lactate, (G, H) Ventilation, (I, J) RPE, (K, L) RER. Values are presented as mean ± standard deviation. * = Significant change from first time point to last time point for both groups.

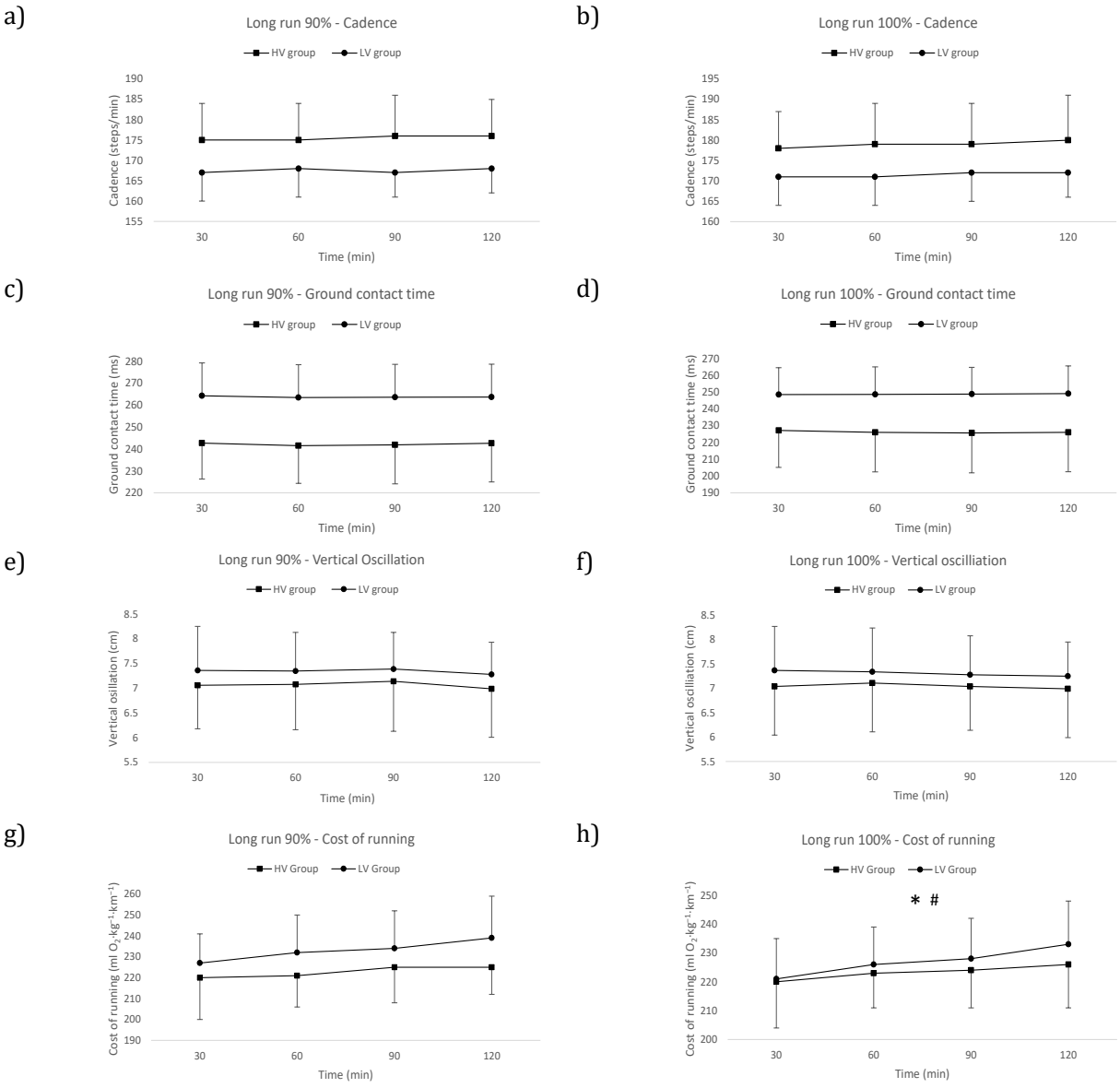


Figure 8. Difference between HV group and LV group and change over time for kinematic factors (A, B) Cadence, (C, D) Ground contact time, (E, F) Vertical oscillation, (G, H) Cost of running. Values are presented as mean ± standard deviation. * = Significant change from first time point to last time point for both groups. # = Significant difference in change between groups.

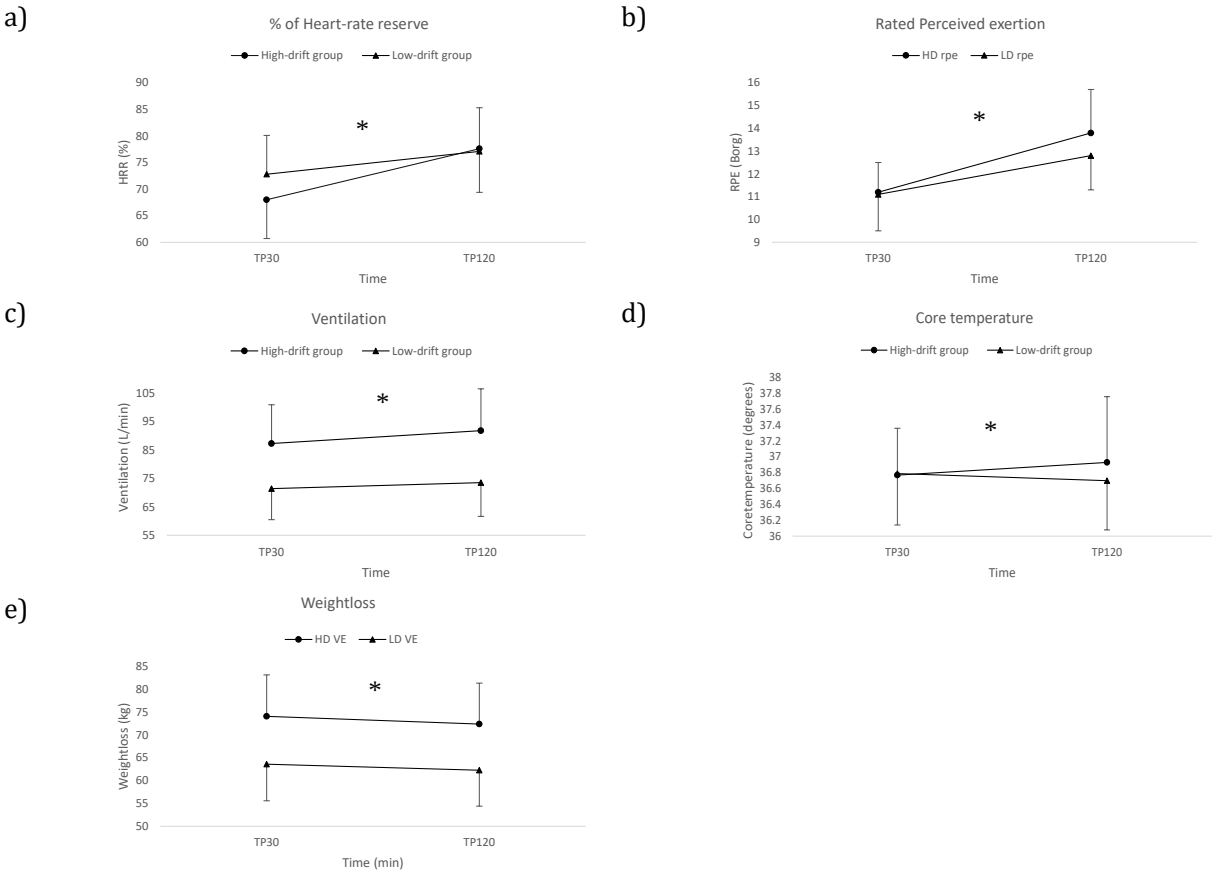


Figure 9. Change from the first time point to the last time point for HD group and LD group for (A) % of Heart-rate reserve, (B) RPE, (C) Ventilation, (D) Core temperature, (E) Weight loss. Values are presented as mean ± standard deviation. * = Significant greater change in HD group versus LD group.

Part 3

Appendices

Contents:

Appendix 1

UiA Fakultet for helse- og idrettsvitenskap

Informasjon og forespørsel om deltakelse i forskningsprosjekt

«Intensitetsstyring og fysiologiske endringer/mekanismer ved langvarige lavintensitets løpeøkter»

- ***En deskriptiv studie i idrettsvitenskap***

Kjære løper!

Vi søker løpere til å bli med på en deskriptiv laboratorie studie i forbindelse med to masteroppgaver i idrettsvitenskap ved Universitetet i Agder (UiA). Her følger informasjon om prosjektet og hva det vil innebære for deg å delta. Det var planlagt et informasjonsmøte der vi skulle gått gjennom denne informasjonen muntlig, men på grunn av Covid-19 velger vi å ikke gjennomføre det. Derfor ber vi deg om å lese dette informasjonsskrivet nøye slik at du får med deg all nødvendig informasjon.

Bakgrunn og hensikt med studien

Forskningsprosjektet har to hensikter:

1. En effektiv metode for å utvikle utholdenhet er å benytte både lav- og høyintensitets treningsøkter over dager, uker, måneder og år. Forskning viser at gode utholdenhetsutøvere sin trening består av cirka 80% rolig trening (intensitetssone 1-2) og 20% høyintensitetstrening (intensitetssone 3-5). Denne fordelingen er godt dokumentert i flere utholdenhetsidretter som løping, langrenn, sykling og roing. De rolige langturene er kanskje den viktigste treningsformen for løpere, men det eksisterer et behov for et mer detaljert fysiologisk bilde over de fysiologiske endringene som skjer når en opplever utmattelse ved langvarige lavintensitets løpeøkter. Hensikten er derfor å kvanitifisere fysiologiske responser ved langvarige

lavintensitets løpeøkter, og gi en bedre forståelse av hvilke fysiologiske mekanismer som er assosiert med kardiorespiratorisk endring og utmattelse ved langvarige lavintensitetsøkter.

2. Det finnes tre metoder for å måle intensitet; eksternt (km/h, watt), internt (hjertefrekvens, laktat) eller subjektivt (selvopplevd anstrengelse). De vanligste metodene løpere bruker for å styre intensitet på rolige langturer er hjertefrekvens, laktat eller selvopplevd anstrengelse. Disse metodene har dog noen begrensninger, da de er avhengig av dagsform. Vi skal undersøke om det finnes andre mer praktiske og kostnadseffektive metoder for måle intensitet ved rolig lankjøring. Derfor vil vi validere og sammenligne ulike feltmetoder opp mot standariserte labmetoder for måling av intensitet.

Hvem er ansvarlig for forskningsprosjektet?

Universitetet i Agder, institutt for idrettsvitenskap og kroppsøving er ansvarlig for prosjektet. Tabellen under viser en oversikt over de som er involvert.

Tabell 1: Oversikt over involverte i forskningsprosjektet.

Institusjon	Navn	Rollebeskrivelse
UiA	Stephen Seiler	Veileder og prosjektansvarlig
UiA	Tor Emil Hansen	Masterstudent
UiA	Kristian Tjørnhom	Masterstudent

Hvorfor får du spørsmål om å delta?

Du får spørsmål om å delta fordi vi ønsker å rekruttere 20 erfarne løpere i alderen 18 til 55 år som løper 30 km i uka eller mer og er i stand til å gjennomføre en rolig langkjøring (120 minutter) på tredemølle.

Hva innebærer det for deg å delta?

Å være med i prosjektet innebærer at vi får hentet ut dine treningsdata for de to siste månedene. I tillegg må du gjennomgå et testbatteri som består av totalt 4 fysiske tester utført på fire forskjellige dager med flere dagers mellomrom. Total tid på testing er cirka seks timer (alle dager inkludert). All testingen i laben vil foregå i tidsrommet desember 2020 -februar 2021. Alt er gratis.

Oversikt over testing

Dag 1:

- Laktatprofiltest (varighet cirka 45 minutter)
- $VO_{2\ max}$ test (varighet 15 minutter)
- Total varighet cirka 1,5 timer

Prosedyre:

- 10min oppvarming
- 5 min drag, 30 sek pause
- Hastigheten økes med 1 km/t for hvert 5 min drag
- Borg-skala etter 4.45 min
- Laktat måles hvert 5. min
- VO_2 måles fra 3-4,5 min på hvert drag
- Testen avsluttes når laktaten er høyere enn 2,1 over hvileverdi

10 min pause før VO_2- maks test

- Kontinuerlig løping (6-10 min)
- Hastighet starter på LT_2-fart
- Hastighet økes med 1 km/h per minutt frem til utmattelse

Dag 2:

- 30 minutter terskeløkt
- Total varighet cirka 1 time
- Her måles: Laktat, VO_2, Hjertefrekvens, Talk Test, Løpe med munnen lukka, Selvopplevd anstrengelse (Borg skala)

Dag 3 og 4:

- Rolig langtur 120 mintutter
- 4x30min (1 min pause) løping på lav intensitet.
- Test 3 gjennomføres på 90% av LT_1 hastighet
- Test 4 gjennomføres på 100% av LT_1 hastighet

På dag 3 og 4 vil vi i løpet av hver 30 minutts periode måle:

- Laktat
- VO_2
- EMG (elektromyografi)
- Hjertefrekvens
- Talk Test
- Løpe med munnen lukka
- Kjernetemperatur (via øre)
- Hudtemperatur (infrarødt kamera)
- Selvopplevd anstrengelse (Borg skala)
- Kortisol (spyttprøve) (Måles kun før og etter)

Forberedelser

- Ikke gjennomføre intensiv fysisk trening 24 timer før testing.
- Kosthold: Innta samme type måltid/innhold før hver test
- Ikke spise siste 2 timer før test.
- Ikke konsumere koffein 3 timer før testing.
- Bruke samme joggesko for hver test.
- Deltakere må utføre testing i shorts (kortbukse) slik at vi får festet EMG-elektroder på Musculus Vastus Lateralis og Musculus Biceps Femoris.

Det er frivillig å delta i prosjektet

Det er frivillig å delta i prosjektet. Hvis du velger å delta, kan du når som helst trekke deg fra studien uten å måtte oppgi grunn om hvorfor. Alle opplysninger om deg, vil da bli anonymisert eller slettet. Det vil ikke ha noen negative konsekvenser for deg hvis du ikke vil delta eller velger å trekke deg på et senere tidspunkt.

Personvern – Oppbevaring og behandling av dine opplysninger

Vi vil bare bruke opplysningene om deg til formålene vi har fortalt om i dette skrivet. Vi behandler opplysningene dine konfidensielt og i samsvar med personvernregelverket. Navn og kontaktopplysninger om deg vil bli anonymisert og kodet slik at dine data ikke er direkte knyttet direkte til ditt navn. All data- og biologisk materiale (spyttprøvene) vil bli lagret på en sikker måte og utilgjengelig for andre enn de som er involvert i prosjektet. Det innebærer at data oppbevares avidentifisert på insituttets passordbelagte PC. Anonymisert data vil kunne bli brukt i forbindelse med publisering av artikkel i tidsskrift eller i undervisning og kongresser.

Hva skjer med opplysningene dine når prosjektet er avsluttet?

Prosjektet skal etter planen avsluttes innen juni 2020. Ved prosjektslutt skal koblingen mellom anonymiserte datafiler og personinformasjon (navn og email adresse) slettes.

Dine rettigheter:

- innsyn i hvilke personopplysninger som er registrert om deg,
- å få rettet personopplysninger om deg,
- få slettet personopplysninger om deg,
- få utlevert en kopi av dine personopplysninger (dataportabilitet),
- å sende klage til personvernombudet eller Datatilsynet om behandlingen av dine personopplysninger.

Hva gir oss rett til å behandle personopplysninger om deg?

Vi behandler personopplysninger om deg basert på ditt samtykke. Studien har blitt godkjent av Fakultetets Etiske Komitè (FEK). I tillegg har Norsk senter for forskingsdata AS (NSD) vurdert at behandlingen av personopplysninger i dette prosjketet er i samsvar med personvernregelverket.

Hvordan bli med?

Dersom du fortsatt ønsker å være en del av dette prosjektet kan du bekrefte din deltakelse ved å sende en mail til toremil96@gmail.com

Hvor kan jeg finne ut mer?

Hvis du har ytterligere spørsmål til studien eller ønsker å benytte deg av dine rettigheter, vennligst ta kontakt med:

- Masterstudent; Tor Emil Hansen, toremil96@gmail.com, telefon 97481860.
- Masterstudent; Kristian Tjørnhom, kristiantjoernhom@hotmail.no, 91157477 telefon
- Vårt personvernombud: Ina Danielsen, Universitetet i Agder, ina.danielsen@uia.no, telefon +47 452 54 401
- NSD – Norsk senter for forskningsdata AS, på epost (personverntjenester@nsd.no) eller telefon: 55 58 21 17.

Med vennlig hilsen

Stephen Seiler, Tor Emil Hansen, Kristian Tjørnhom

Appendix 2

Samtykke til deltakelse i forskningsprosjektet «Intensitetsstyring og fysiologiske endringer/mekanismer ved langvarige lavintensitets løpeøkter»

Ved å signere samtykkeerklæringen bekrefter du at du ikke har noen hjertesykdom eller lidelser/sykdom som medfører at din fastlege har frarådet deg å trene intensivt. Du, som deltaker, er for øvrig også forsikret av UiAs egen forsikringsordning for forskningsprosjekter.

- Jeg samtykker at det innhentes biologisk materiale (spyttprøve)
- Jeg bekrefter å ha fått og forstått informasjon om studien og er villig til å delta

Ja ☐

Nei ☐

(Signert av prosjektdeltaker, dato)

Appendix 3

UiA Institutt for idrettsvitenskap og kroppsøving

NORGE Olympiatoppen Sør

Covid-19: Egenerklæring

- Er du per i dag satt i karantene eller isolasjon?
  - ☐ Ja
  - ☐ Nei

- Har du vært i kontakt med noen som har vært smittet av korona i løpet av de siste 14 dagene?
  - ☐ Ja
  - ☐ Nei

- Har du opplevd noen av følgende symptomer de siste 10 dagene (kryss av for eventuelle symptomer)?
  - ☐ Feber
  - ☐ Hoste
  - ☐ Vond/sår hals
  - ☐ Nei

- Har du opplevd tap av smak- eller luktesans i løpet av de siste 10 dagene?
  - ☐ Ja
  - ☐ Nei

- Har du vært i utlandet/områder med høy smitte de siste 10 dagene?
  - ☐ Ja
  - ☐ Nei

Dato:

Utøver/foresatt:

_____

Appendix 4 - Menstruasjon

Infoskriv for kvinner som skal delta i forskningsprosjektet:

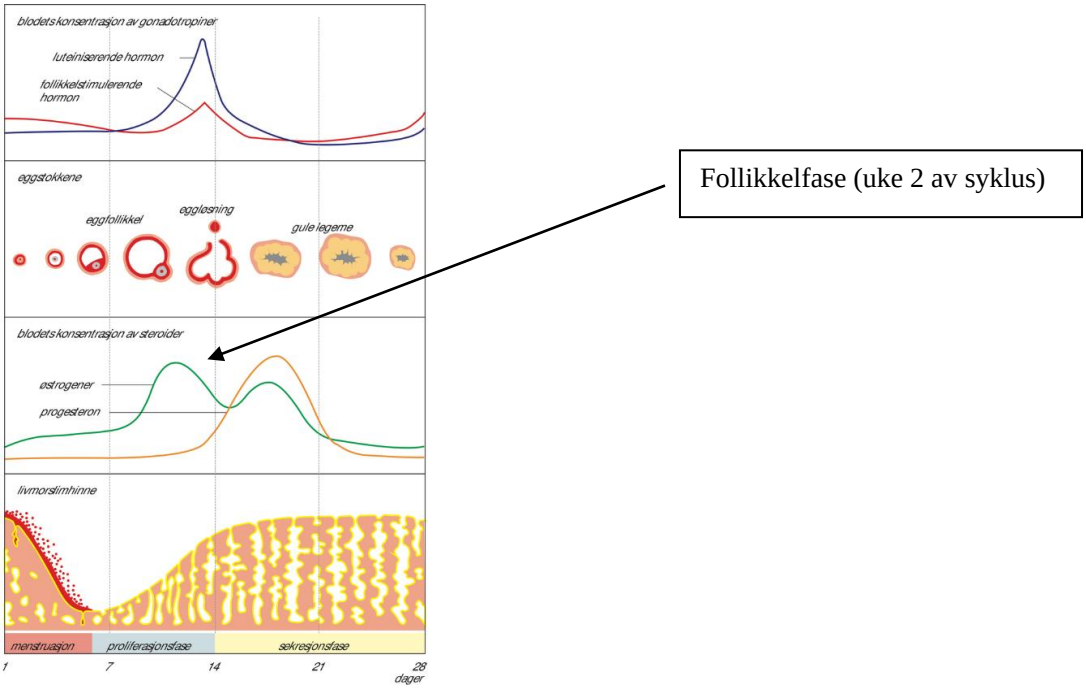
«Intensitetsstyring og fysiologiske endringer/mekanismer ved langvarige lavintensitets løpeøkter»

Kjære løper!

Vi gleder oss til å komme i gang med prosjektet og er glad dere ønsker å være med. Selv om informasjonen som følger i dette skrivet ikke er hovedproblemstillingen i vårt prosjekt, ønsker vi allikevel å ta hensyn til det av metodiske årsaker. Forskning viser nemlig at de hormonelle endringene i menstruasjonssyklus kan påvirke prestasjon og vi ønsker å ta stilling til dette. Fagmiljøet anbefaler at forskere bør kontrollere for menstruasjonsstatus ved fysiologisk testing, og ved repeterte målinger bør det utføres målinger i samme fase av menstruasjonssyklusen. Vi håper at vi kan ha en dialog, hvor dere uttrykker et ønske om når dere vil teste basert på den informasjonen som gis i dette skrivet.

Dersom du går på prevensjonsmidler, eller er i overgangsalderen og ikke har regelmessige blødninger kan du se bort i fra dette infoskrivet, ettersom prevensjonsmidler reduserer de hormonelle endringene.

Vi ønsker så godt det lar seg gjøre, at testene blir utført i follikkelfasen (uke 2 av syklus). Se figur.



Ønsket periode for testing kan sendes på mail til toremil96@gmail.com 😊

Hvis du ønsker mer informasjon om temaet, se kilder under:

Kilder:

- JANSE DE JONGE, XANNE1; THOMPSON, BELINDA1; HAN, AHREUM2. Methodological Recommendations for Menstrual Cycle. Research in Sports and Exercise, Medicine & Science in Sports & Exercise: December 2019 - Volume 51 - Issue 12 - p 2610-2617. Doi: 10.1249/MSS.0000000000002073
- Romance R, Vargas S, Espinar S, Petro JL, Bonilla DA, Schöenfeld BJ, Kreider RB, Benítez-Porres J. Oral Contraceptive Use does not Negatively Affect Body Composition and Strength Adaptations in Trained Women. Int J Sports Med. 2019 Dec;40(13):842-849. doi: 10.1055/a-0985-4373. Epub 2019 Sep 6. PMID: 31491790.
- Thompson B, Almarjawi A, Sculley D, Janse de Jonge X. The Effect of the Menstrual Cycle and Oral Contraceptives on Acute Responses and Chronic Adaptations to Resistance Training: A Systematic Review of the Literature. Sports Med. 2020 Jan;50(1):171-185. doi: 10.1007/s40279-019-01219-1. PMID: 31677121.
- https://sml.snl.no/menstruasjon

Appendix 5 – Questionnaire

Forskningsprosjekt Løping

Intensitetsstyring og fysiologiske endringer ved langvarig løping på lav intensitet.

*Må fylles ut

1. E-postadresse *

_____

2. Navn *

_____

3. Kjønn *

Markér bare én oval.

- ◯ Mann
- ◯ Kvinne

4. Alder *

_____

5. Hvor mange år erfaring har du med løping? *

_____

6. Har du tilgang på treningsdata for de siste 8 ukene gjennom treningsapp? For eksempel i Strava, Polar, Training Peaks eller Garmin. *

Markér bare én oval.

- ◯ Ja
- ◯ Nei

7. Cirka hvor mange kilometer per uke har du løpt de siste 8 ukene? *

_____

8. Hva er typisk varighet (minutter) for din lengste ukentlige langtur de siste 8 ukene? *

_____

9. Har du en personlig rekord på 10 km? *

Markér bare én oval.

- ◯ Ja
- ◯ Nei

10. Hva er din personlige rekord på 10 km?

_____

Eksempel: 4.03.32 (4 timer, 3 minutter, 32 sekunder)

11. Har du en personlig rekord på halvmaraton? *

Markér bare én oval.

- ◯ Ja
- ◯ Nei

12. Hva er din personlige rekord på halvmaraton?

_____

Eksempel: 4.03.32 (4 timer, 3 minutter, 32 sekunder)

13. Har du en personlig rekord på maraton? *

Markér bare én oval.

- ◯ Ja
- ◯ Nei

14. Hva er din personlige rekord på maraton?

_____

Eksempel: 4.03.32 (4 timer, 3 minutter, 32 sekunder)

Appendix 6

Tor Emil Hansen

Besøksadresse:
Universitetsveien 25
Kristiansand

Ref: [object Object]

Tidspunkt for godkjenning: : 17/11/2020

Søknad om etisk godkjenning av forskningsprosjekt - Intensitetsstyring og fysiologiske endringer/mekanismer ved langvarige lavintensitets løpeøkter

Vi informerer om at din søknad er ferdig behandlet og godkjent.

Kommentar fra godkjenner:
Søknaden godkjennes under forutsetning av at prosjektet gjennomføres som beskrevet i søknaden og under forutsetning av godkjenning av NSD. FEK forutsetter at prosjektet er framlagtsvurdert for REK, og ber om at dokumentasjon fra REK og NSD godkjenning sendes på mail til Anne Skisland når dette foreligger.

Hilsen
Forskningsetisk komite
Fakultet for helse - og idrettsvitenskap
Universitetet i Agder

Appendix 7

NSD NORSK SENTER FOR FORSKNINGSDATA

NSD sin vurdering

Prosjekttittel

Validering av feltmetoder for intensitetsstyring, og fysiologiske responser ved langvarige lavintensitets løpeøkter

Referansenummer

915200

Registrert

19.10.2020 av Kristian Tjørnhom - kritjo15@student.uia.no

Behandlingsansvarlig institusjon

Universitetet i Agder / Fakultet for helse- og idrettsvitenskap / Institutt for folkehelse, idrett og ernæring

Prosjektansvarlig (vitenskapelig ansatt/veileder eller stipendiat)

Kerry Stephen Seiler, Stephen.seiler@uia.no, tlf: 91614587

Type prosjekt

Studentprosjekt, masterstudium

Kontaktinformasjon, student

Kristian Tjørnhom, Tor Emil Hansen, kristiantjoernhom@hotmail.no, tlf: 91157477

Prosjektperiode

01.12.2020 - 30.06.2021

Status

05.03.2021 - Vurdert

Vurdering (1)

05.03.2021 - Vurdert

Det er vår vurdering at behandlingen av personopplysninger i prosjektet vil være i samsvar med personvernlovgivningen så fremt den gjennomføres i tråd med det som er dokumentert i meldeskjemaet 05.03.2021 med vedlegg, samt i meldingsdialogen mellom innmelder og NSD. Behandlingen kan starte.

MELD VESENTLIGE ENDRINGER
Dersom det skjer vesentlige endringer i behandlingen av personopplysninger, kan det være nødvendig å melde dette til NSD ved å oppdatere meldeskjemaet. Før du melder inn en endring, oppfordrer vi deg til å lese om hvilke type endringer det er nødvendig å melde:
https://nsd.no/personvernombud/meld_prosjekt/meld_endringer.html

Du må vente på svar fra NSD før endringen gjennomføres.

TYPE OPPLYSNINGER OG VARIGHET
Prosjektet vil behandle særlige kategorier av personopplysninger om helseforhold og alminnelige kategorier av personopplysninger frem til 30.06.2021.

LOVLIG GRUNNLAG
Prosjektet vil innhente samtykke fra de registrerte til behandlingen av personopplysninger. Vår vurdering er at prosjektet legger opp til et samtykke i samsvar med kravene i art. 4 nr. 11 og art. 7, ved at det er en frivillig, spesifikk, informert og utvetydig bekreftelse, som kan dokumenteres, og som den registrerte kan trekke tilbake.

Lovlig grunnlag for behandlingen vil dermed være den registrertes uttrykkelige samtykke, jf. personvernforordningen art. 6 nr. 1 bokstav a, jf. art. 9 nr. 2 bokstav a, jf. personopplysningsloven § 10, jf. § 9 (2).

PERSONVERNPRINSIPPER
NSD vurderer at den planlagte behandlingen av personopplysninger vil følge prinsippene i personvernforordningen om:

- lovlighet, rettferdighet og åpenhet (art. 5.1 a), ved at de registrerte får tilfredsstillende informasjon om og samtykker til behandlingen
- formålsbegrensning (art. 5.1 b), ved at personopplysninger samles inn for spesifikke, uttrykkelig angitte og berettigede formål, og ikke viderebehandles til nye uforenlige formål
- dataminimering (art. 5.1 c), ved at det kun behandles opplysninger som er adekvate, relevante og nødvendige for formålet med prosjektet
- lagringsbegrensning (art. 5.1 e), ved at personopplysningene ikke lagres lengre enn nødvendig for å oppfylle formålet

DE REGISTRERTES RETTIGHETER
Så lenge de registrerte kan identifiseres i datamaterialet vil de ha følgende rettigheter: åpenhet (art. 12), informasjon (art. 13), innsyn (art. 15), retting (art. 16), sletting (art. 17), begrensning (art. 18), underretning (art. 19), dataportabilitet (art. 20).

NSD vurderer at informasjonen som de registrerte vil motta oppfyller lovens krav til form og innhold, jf. art. 12.1 og art. 13.

Vi minner om at hvis en registrert tar kontakt om sine rettigheter, har behandlingsansvarlig institusjon plikt til å svare innen en måned.

FØLG DIN INSTITUSJONS RETNINGSLINJER
NSD legger til grunn at behandlingen oppfyller kravene i personvernforordningen om riktighet (art. 5.1 d), integritet og konfidensialitet (art. 5.1. f) og sikkerhet (art. 32).

For å forsikre dere om at kravene oppfylles, må dere følge interne retningslinjer og eventuelt rådføre dere med behandlingsansvarlig institusjon.

OPPFØLGING AV PROSJEKTET
NSD vil følge opp ved planlagt avslutning for å avklare om behandlingen av personopplysningene er avsluttet.

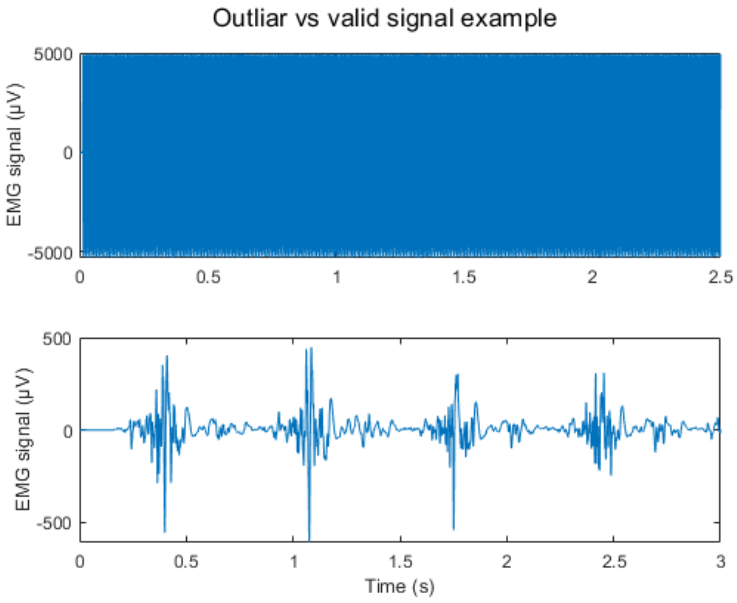
Lykke til med prosjektet!

Kontaktperson hos NSD: Kajsa Amundsen
Tlf. Personverntjenester: 55 58 21 17 (tast 1)

Appendix 8 - EMG

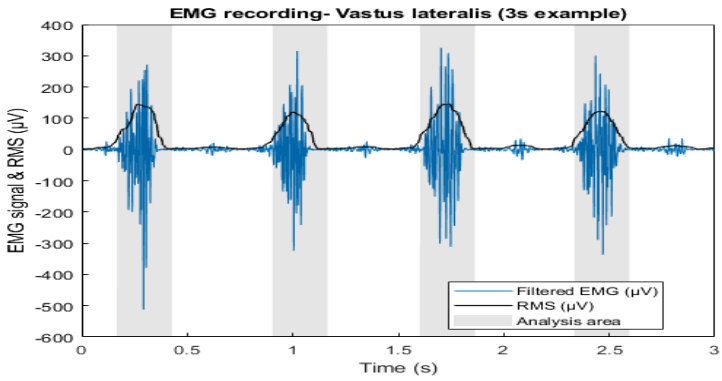
a)

b)



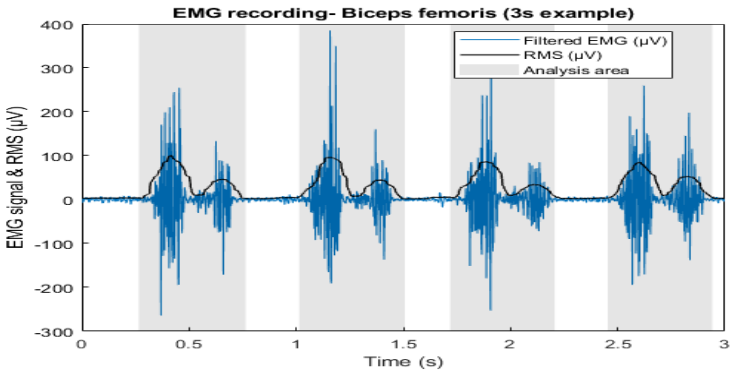
8a. Example of an EMG measurement where sensors detached during running
8b. Example of a valid EMG measurement.

c)



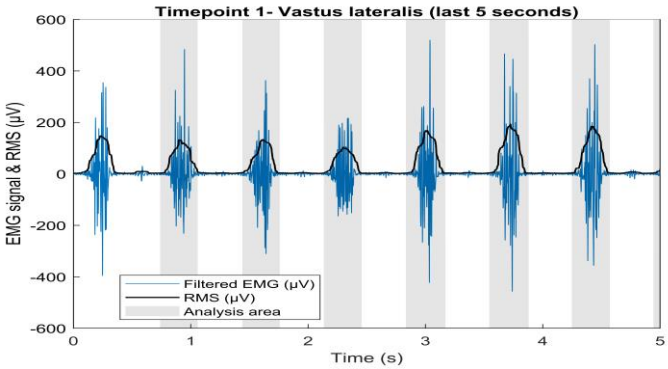
8c. Valid RMS measurement for Vastus Lateralis.

d)



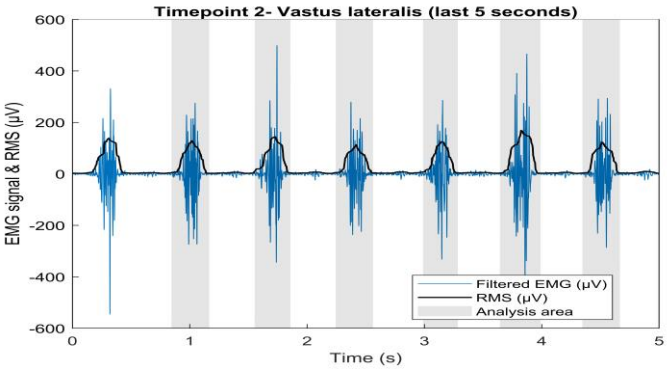
8d. Valid RMS measurement for Biceps Femoris.

e)



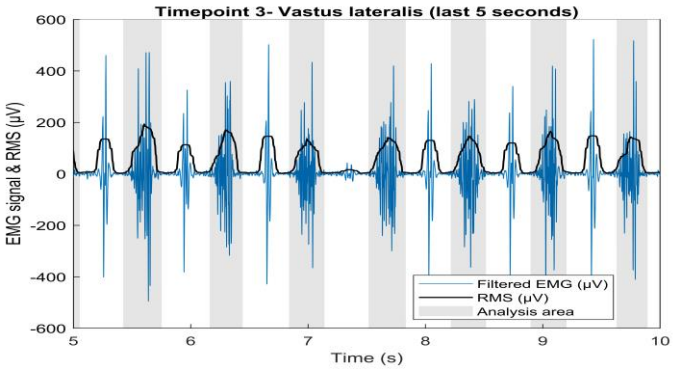
8e. Valid measurement under dry-conditions during control-testing

f)



8f. Valid measurement of sweat applied above the electrodes during control-testing

g)



8g. Example of measurement where signal deteriorated due to sweat beneath the electrodes