Nature and Nurture: The Effects of Genetics, Dietary Composition, and Training on Endurance Performance

Andreas Kreutzer

Bachelor of Arts in English (Writing)

Master of Science in Kinesiology (Exercise Physiology)

Harris College of Nursing & Health Sciences

Texas Christian University

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Dedication

This work is dedicated to my family for their love, support, and inspiration.

Chapter I: Introduction

1.1 Endurance Performance – Definition and Measurement

Endurance can be defined as "the ability to sustain a prolonged stressful effort or activity".[1] When addressing human endurance exercise, the term is often used to describe activities powered mainly by aerobic energy production or oxidative phosphorylation (OXPHOS). However, these activities exist on a wide spectrum: OXPHOS becomes the predominant metabolic pathway for energy production in activities lasting more than 90-120 seconds[2] and can provide energy for activities lasting hours to days.[3,4] This dissertation investigates events lasting ~5 minutes (one-mile run in recreationally active participants) to ~60 minutes (30-km cycling time trial (TT) in recreationally active cyclists). Additionally, this document examines the maximal metabolic steady-state (critical power (CP)) in cyclists and triathletes as a marker of endurance performance potential.[5] Positive performance outcomes include 1) maximizing mechanical power output or speed over a given time, distance, or at the maximal metabolic steady-state, 2) maximizing time to task failure during constant-load exercise, and 3) minimizing time to complete a given distance or a prespecified amount of work (typically measured in kilojoules).[6,7]

1.2 Determinants of Endurance Performance

The factors influencing human endurance performance can be grouped into system-wide concepts and their underlying cellular and molecular processes; the three most important system-wide determinants of endurance performance are maximal oxygen consumption ($\dot{V}O_2max$), the ability to maintain a high fractional utilization of $\dot{V}O_2max$ during prolonged exercise – often represented by a threshold concept (e.g., lactate thresholds, ventilatory thresholds, CP, etc.), and gross mechanical efficiency or movement economy.[8] An additional emerging factor in the determination of endurance performance, which is traditionally not included in the determinants

of endurance performance, is fatigue resistance or durability.[9] Underlying organ-level, cellular, and molecular factors mainly reflect the body's ability to deliver oxygen to the working muscles (cardiovascular), extract oxygen from the blood (cardiovascular and cellular), and use oxygen, nutrients, and reducing equivalents efficiently for energy production (metabolic).[2]

1.2.1 Maximal Oxygen Consumption ($\dot{V}O_2max$)

An individual's $\dot{V}O_2max$ represents the greatest rate of oxygen the individual's body can consume, which is typically measured during incremental exercise to exhaustion.[10] $\dot{V}O_2max$ is governed by the components of the Fick equation

$$\dot{V}O_2 = \dot{Q} \times a - v\,O_2 difference$$

where $\dot{Q}$ is the cardiac output and the a-v O_2 difference (arterio-venous oxygen difference) is the difference in oxygen content between the arteries and veins. $\dot{Q}$ is the product of heart rate (HR) - the number of heart beats per minute – and stroke volume (SV) – the amount of blood ejected with each heart beat measured in milliliters (mL).[10] Thus, $\dot{Q}$ represents the body's ability to deliver blood to the working muscles, while the a-v O_2 difference represents the body's ability to extract oxygen.

While not the sole determinant of athletic success, $\dot{V}O2max$ is one of the major factors determining endurance performance, especially in heterogenous populations.[11,12] In those situations, individuals with a higher $\dot{V}O_2max$ perform better than those with a lower $\dot{V}O_2max$.[13,14] However, among groups of elite athletes with similar $\dot{V}O_2max$ values, aerobic capacity becomes less of a factor.[15,16] Thus, it stands to reason that $\dot{V}O_2max$ is a better predictor of endurance performance among recreational athletes, so-called age-group athletes, whose aerobic capacity might exhibit large inter-individual differences. Among elite athletes, who might have to achieve a certain $\dot{V}O_2max$ as a prerequisite for reaching their elite status, the homogeneity of aerobic

capacity decreases the predictive value of $\dot{V}O_2max$ for performance. In the latter, other factors, including fractional utilization of $\dot{V}O_2max$ and gross mechanical efficiency, become more important.

1.2.2 Fractional Utilization of $\dot{V}O_2max$

The ability to maintain a high workload over extended periods of time lies at the heart of endurance exercise performance.[13] This ability is governed by an individual's capacity to fuel exercise using OXPHOS.[13] Several different threshold concepts have been applied to demarcate the exercise intensity at which the body begins relying on anaerobic energy production to the extent that fatigue processes are accelerated and time to task failure becomes shorter.[17] Originally conceptualized by Wasserman[18] as the anaerobic threshold (AT), thresholds concepts now include ventilation-based thresholds (e.g., ventilatory threshold (VT) and respiratory compensation point (RCP)), power/speed-based thresholds (e.g., the critical power/speed (CP/CS) and functional threshold power (FTP)), and blood lactate-based thresholds (e.g., lactate threshold (LT), lactate turnpoint (LTP), and maximal lactate steady-state (MLSS)).[17]

The highest sustainable fractional utilization of $\dot{V}O_2max$ – is a strong predictor of endurance performance: the higher the intensity at which an individual can achieve a metabolic steady-state[5,19], the better they can perform in an endurance event, as they can maintain higher work outputs for longer periods of time. The fractional utilization of $\dot{V}O_2max$ at the LT correlates strongly with time to task failure;[20] similarly, fractional utilization capability, measured in the laboratory as the power output at VT, was a strong positive predictor for time to completion in three Tour de France time trials performed by professional cyclists.[21] Elite marathon runners exhibit extremely high fractional utilization – up to 92% of $\dot{V}O2max$ at the LTP.[22] To break the coveted 2-hour mark in a marathon, runners have to achieve a metabolic steady-state at a running

speed of 21.1 km, a feat which only the highly elite can attain.[22] This evidence shows that fractional utilization is an important factor in determining endurance exercise performance. Physiologically, a high fractional utilization capability is positively correlated with greater skeletal muscle capillary density[20], mitochondrial density, and mitochondrial fat oxidation rates.[23]

1.2.3 Mechanical Efficiency and Movement Economy

The final major factor in determining endurance performance is gross mechanical efficiency.[24] Gross mechanical efficiency is typically measured using a cycle ergometer;[25] it is the ratio of the work generated to the total metabolic cost of the activity. Thus, it is a measure of work achieved for the amount of energy expended; this translates directly to performance, as a higher efficiency allows more of the overall energy cost to produce work rather than heat. Cycling gross efficiency ranges approximately from 18-23% of total energy expenditure in trained cyclists, and is strongly correlated with fiber type composition of the working muscle: the greater the percentage of Type I (oxidative) fibers, the higher the gross efficiency.[26] Variation in gross efficiency in cycling explains approximately 30% of the variation in power output during cycling time trials.[24]

In running, gross efficiency cannot be directly measured, since work output can only be estimated; thus, running economy (RE) is used as a similar measure: RE measures energy expenditure ($\dot{V}O_2$ in mL of oxygen per kilometer or per minute) at a given running speed.[27] Better RE (lower energy expenditure) has been shown to directly affect running performance: Hoogkammer et al.[28] showed that a 1.1% increase in energy expenditure equates to a reduction in performance of 0.78%. Similarly, di Prampero et al.[29] reported that an improvement of 5% in RE lead to an improvement of 3.8% in running performance. Observational studies have shown that elite athletes have better RE than highly trained and recreationally trained runners[27,30], and that East African runners have better RE than European athletes.[31] RE is affected by morphological

factors such as calf circumference[31], limb length body mass distribution, and Achilles tendon moment arm.[27] Physiological factors influencing RE include muscle fiber type composition, neural signaling and motor programming, and elastic energy storage.[27] Stride length, foot strike patterns, vertical oscillation, lower body kinematics, and footwear can also affect RE.[27,28]

1.2.4 Durability

The traditional determinants of endurance performance are typically measured in a well-rested state, with participants in studies and athletes in the applied setting refraining from heavy exercise prior to testing. However, due to the nature of many prolonged endurance events and tactical considerations, endurance performance in races often requires athletes to perform multiple spurts of high-intensity efforts interspersed throughout an event ("high-intensity repeatability") and/or a finishing surge at the end of an event ("fatigue resistance").[9,32–35] Therefore, recent research efforts have begun to investigate the effects of prior acute and/or chronic exercise on the traditional determinants of endurance performance and on the ability to repeatedly perform and recover from high-intensity efforts within a longer bout of submaximal exercise.[9,36–41]

In the first study examining the effect of acute exercise on the maximal metabolic steady-state, Clark et al. found that CP and work capacity above CP (W') were reduced following two hours of heavy-intensity exercise by approximately 8% and 20%, respectively.[37] In follow-up studies they confirmed the reductions in CP and W' and showed that carbohydrate supplementation during the heavy-intensity bout attenuated this effect;[36] however, the change in CP and W' was not correlated with decreases in muscle glycogen.[42] Similarly, in an analysis of race data, Leo et al. found that in under-23 year old (U23) cyclists, maximal mean power output (MMP) over five, 10, and 30 seconds, as well as over one, two, five and 12 minutes decreased significantly after 1,000 kJ accumulated work.[41] Their 20-minute MMP dropped significantly after 1,500 kJ

accumulated work and 30-minute MMP after 2,500 kJ. Among professional riders in the same race, only 5- and 12-minute MMP decreased after 1,000 kJ, with MMP over all other durations not dropping until 2,000 or 3,000 kJ accumulated work.

To investigate the effects of chronic workload accumulation over a season on MMP over 10 seconds, and one, five, and 20 minutes, van Erp et al. analyzed training and race data from 26 professional cyclists over eight seasons.[38] They showed that 10-second MMP declined following 30 kJ/kg body mass accumulated work in successful and less successful climbers and sprinters. However, 1-minute, 5-minute and 20-minute MMP declined after less accumulated work in unsuccessful climbers (10-20 kJ/kg) and sprinters (10-30 kJ/kg) when compared with successful climbers (40-50 kJ/kg) and sprinters (10-50 kJ/kg). This difference was greater among climbers, suggesting a heightened importance of durability among this rider type. Similarly, in a field-based study of professional cyclists, Rodríguez-Marroyo et al. found decreases in $\dot{V}O_2max$, power output and HR at $\dot{V}O_2max$, and power output at VT and RCP following participation in a three-week cycling grand tour (Vuelta a España) when compared to pre-competition values.[43]

These studies demonstrate the importance of durability for endurance performance. Additional research is needed to better understand the effect of prior work on the traditional determinants and other markers of endurance performance. Additionally, studies should investigate how to improve durability in a range of sports and populations.

1.3 Factors Affecting the Determinants of Endurance Performance

1.3.1 Genetics

While there is no current evidence of a common genetic profile specific to world class endurance athletes[44], there is no doubt that there is a sizeable influence of genetics on human athletic performance and its determinants.[45] Based on data from family and twin studies,

approximately 40-93% of the variation in baseline $\dot{V}O_2$max can be explained by genetics with a recent meta-analysis estimating heritability at approximately 72%.[46–51] Additionally, at least 97 genes have been identified that appear to predict the trainability of $\dot{V}O_2$max, i.e., the increase in $\dot{V}O_2$max in response to endurance training.[52] Bouchard et al. were among the first to employ a family study to investigate the effect of genetics on the response of $\dot{V}O_2$max to a standardized 20-week exercise training program; they discovered a panel of 21 single-nucleotide polymorphisms (SNPs) that accounted for approximately 47% of the variance in $\dot{V}O_2$max trainability.[53] Participants who carried at least 19 of the favorable alleles associated with these SNPs improved their $\dot{V}O_2$max significantly more (0.60 L/min) than those carrying less than 10 favorable alleles (0.22 L/min). While additional genetic markers for $\dot{V}O_2$max trainability have been found in more recent investigations, only 13 genetic variants have been reproduced by more than two studies.[52] Thus, additional research is needed to further elucidate the effect of genetics on $\dot{V}O_2$max trainability.

Similar to $\dot{V}O_2$max and its trainability, there appears to be a genetic component determining RE. As described above, cycling gross efficiency[26] and RE[27] are positively correlated with the proportion of Type I fibers present in skeletal muscle. A study by Simoneau and Bouchard showed that approximately 45% of the variance in fiber type composition can be attributed to genetics.[54] Gene variants associated with increased proportions of Type I muscle fibers include the following alleles: *ACE I*, *ACTN3 577X*, *HIF1A Pro582*, *PPARA rs4253778 G*, *VEGFR2* 472*Gln*.[55] In twin studies, the heritability estimates of oxygen consumption at submaximal intensities, i.e., RE, ranges from 3.5% to 67%;[47,56] the wide range of heritability estimates can in part be attributed to methodological variation with some experimental conditions including exercise at intensities that might have elicited a significant anaerobic component of energy production. Nevertheless,

there appears to be a moderate to strong influence of genetics on RE, which needs to be further investigated.

Mitochondrial content, an important correlate of fractional utilization capacity, is influenced by genetics as well.[23] Curren et al. estimated the heritability of mitochondrial content to be approximately 33%.[57] Another factor in the ability to maintain high percentages of $\dot{V}O_2max$ during submaximal exercise, is capillary density in skeletal muscle. Capillary length density appears to be influenced by the *ACE* rs1799752 and *TNC* rs2104772 SNPs. A direct investigation of the heritability of fractional utilization capacity has not been performed.

In general, there are several SNPs and polygenic profiles that appear to be beneficial for endurance exercise performance. In rodent studies, the heritability of endurance performance has been estimated around 39-50%.[58,59] Studies investigating the effect of genetics on endurance performance in humans have generally looked at the over- or underrepresentation of certain genotypes in groups of highly endurance trained or elite endurance athletes compared with strength/power athletes and/or control groups from the general population. Ben-Zaken et al. found that the *IGF-1R* (rs1464430) AA genotype was significantly overrepresented among endurance athletes (49%) when compared with power athletes (33%), but not when compared with general population controls (46%).[60] The A allele in this SNP is related to left ventricular (LV) hypertrophy, which is beneficial for endurance performance.[61] Further, Guilherme et al. showed a decreased frequency of the *FTO* (rs9939609) AA genotype in long-distance athletes (9.5%) compared with middle distance athletes (14.6%) and controls (14.2%).[62] The A allele in this SNP is related to increased fat mass and obesity, which would be detrimental to endurance performance in weight-dependent sports such as running, cycling, and triathlon.[63–65] Another allele overrepresented among elite endurance athletes is *ACE* (rs4341) I. [66,67] The presence of this allele

has been linked to lower angiotensin-I converting enzyme (ACE) activity.[68] ACE breaks down vasodilator kinins and upregulates the formation of the vasoconstrictor angiotensin II, thereby increasing vasoconstriction.[69] If this occurs in the working muscle, it is detrimental to exercise performance; thus, the downregulation of ACE by the *ACE* I allele is proposed to be beneficial for endurance performance.

While several studies, including the aforementioned, have compared allele and genotype frequencies between elite athlete cohorts and non-athlete controls, these studies only give a glimpse at possible genetic determinants of endurance performance. Few studies have directly compared endurance performance or markers of endurance performance/capacity between individuals with different genotypes. Falahati and Arazi found no association of the *ACE* I allele with $\dot{V}O_2$max among trained and untrained men.[70] Jin et al. reported greater 20m shuttle-run distance, a marker of endurance performance, in individuals with the *PPARGC1A* (rs8192678) GG genotype compared with the other two genotypes.[71] Presence of the G allele appears to be beneficial for lipid oxidation, providing a mechanism for improved endurance performance.[72] Additional studies comparing direct measures of endurance performance between individuals with different genotypes implicated in elite endurance athlete status are required to further elucidate the effects of these genes on actual performance.

1.3.2 Nutrition

The goal of nutrition interventions to improve endurance performance is to prolong the fatigue process at submaximal intensities, i.e., to increase an individual's ability to maintain a high fractional utilization of $\dot{V}O_2$max for long periods of time, repeatedly throughout and event, or following chronic and/or acute prior heavy exercise. [9,73,74] Undoubtedly, the most studied and most important nutritional factor for endurance performance is carbohydrate availability to the

working muscle.[75] While fat oxidation typically predominates at intensities below the LTP, carbohydrate oxidation from muscle glycogen and blood glucose prevails at intensities above the LTP;[76,77] given the nature of most endurance sports events, the ability to resist fatigue at these higher intensities is the principal determinant of endurance performance. Muscle glycogen functions as the body's most important fuel storage site to provide energy substrates at higher exercise intensities, and additionally fulfills regulatory roles, including the acute regulation of metabolic substrate use and catecholamine release, as well as the chronic adaptation to training.[78] In low muscle glycogen states, circulating catecholamines (epinephrine and norepinephrine) are elevated[79], muscle protein breakdown is increased to facilitate amino acid release, and plasma free fatty acid concentration and whole-body fat metabolism are upregulated.[80–82] Training in glycogen depleted states appears to increase the body's ability to metabolize fat, specifically of muscle-derived triacylglycerol.[83] However, it remains unclear whether this improved fat oxidation capability translates to improved endurance performance.[83,84] Crucially, acute glycogen depletion is one of the best-established causes for fatigue and diminished performance in endurance activities.[85–88]

Glycogen depletion and hypoglycemia causes fatigue and affect performance in two general ways: 1) it causes fatigue associated with the central nervous system (CNS), so-called central fatigue and 2) it causes fatigue associated with alterations in metabolism and skeletal muscle contraction, so-called peripheral fatigue.[75,89] The major central fatigue mechanism associated with low glycogen and blood glucose states is a decrease in central motor drive (CMD), i.e., a reduction in the neural activation signal sent to the exercising muscle which leads to a decline in force production and thus diminished performance.[90] Three mechanisms have been suggested to play a role in the decreased CMD associated with reduced glycogen states during exercise: 1)

Group III and IV afferent nerve fibers in the locomotor muscles inhibit CMD in response to metabolic disturbances[91–93], 2) a decrease in brain glycogen and blood glucose during prolonged endurance exercise reduces the CMD[89,90,94], and 3) reduced blood glucose and muscle glycogen increase the perceived effort leading to a conscious down-regulation of power output or movement speed and thus decreased performance.[95] The major peripheral fatigue mechanisms include 1) the inability to maintain the necessary rate of ATP synthesis at higher exercise intensities as muscle carbohydrate availability is reduced and ATP production from free fatty acids is too slow to match the demand of the working muscle[96], and 2) the negative impact of glycogen depletion on the excitability of the muscle membrane and the release of calcium from the sarcoplasmic reticulum.[75,97]

Researchers and athletes have employed a variety of acute and chronic nutritional strategies to maximize carbohydrate availability to the working muscle and brain during endurance exercise.[73] Acute interventions include carbohydrate loading – increased carbohydrate consumption in the days leading up to an endurance event with or without prior glycogen depletion – and carbohydrate intake during the event.[74,98] Following carbohydrate loading, muscle glycogen stores, which typically range from 80-120 mmol/kg of muscle wet weight (ww), can increase to above 200 mmol/kg ww.[99,100] This in turn has been shown to improve performance in events lasting longer than 90 minutes by 2-3% compared with lowered or normal glycogen availability.[101–103] Ergogenic effects of carbohydrate consumption during an endurance event have been described for exercise bouts lasting more than 60 minutes.[73,74] While some studies show reduced muscle glycogen use with carbohydrate intake during endurance exercise[104], i.e., muscle glycogen sparing, this does not appear to be universally the case.[105] However, there is strong evidence that acute carbohydrate intake does increase plasma glucose concentration and carbohydrate oxidation

during exercise[106] with greater amounts of carbohydrate consumed leading increased plasma glucose concentrations and oxidation rates.[105] Similarly, performance appears to improve in a dose-dependent manner.[107] Recommendations for carbohydrate intake during exercise suggest intakes of up to 30 g/hr are appropriate for events lasting one to two hours, up to 60 g/hr for events lasting 2-3 hours, and up to 90 g/hr for events of longer duration.[73] While 60 g/hr appears to be the upper limit for glucose absorption in the intestinal tract[108], the inclusion of alternative carbohydrate sources, e.g., fructose, is necessary to successfully achieve these higher supplementation rates;[109] in fact, a recent study showed that intakes of 120 g/hr of combined glucose and fructose are tolerable.[110] During shorter events lasting up to one hour, specifically when performed in low glycogen states, carbohydrate mouth rinse interventions, have been effective to improve exercise performance.[111]

In contrast to the more traditional approach of maximizing carbohydrate intake and oxidation, endurance athletes and researchers have attempted to employed an opposing strategy: they minimize carbohydrate intake by following extremely low carbohydrate – ketogenic – diets or training in low-glycogen states to induce so-called fat adaptation.[112–115] The proposed benefit of fat adaptation is an improved ability to oxidize fat as the main energy substrate at exercise intensities where carbohydrate oxidation typically predominates.[115] This would in essence give an individual access to near limitless energy resources – the body can store more than 74,000 kcal in subcutaneous, visceral, and intramuscular fat – and would practically eliminate the negative effects of glycogen depletion on endurance performance.[116] While studies have consistently shown increased fat oxidation in response to low-carbohydrate diets, the effect of these interventions on performance are less clear.[113,117–124]. It appears that in endurance events at intensities above 70%

of $\dot{V}O_2$max, potential benefits of increased fat oxidation are negated by impaired economy, thus leading to a failure to improve performance.[119,120]

In addition to chronic low-carbohydrate diets, athletes and researchers have experimented with performing individual sessions in low-carbohydrate states to improve metabolic flexibility, i.e., the body's capability to change energy substrate utilization based on needs and availability.[125,126] Mitochondrial adaptations suggesting improved metabolic flexibility are greatest when pre-exercise muscle glycogen concentration is less than 300 mmol/kg dry weight (dw); however, pre-exercise muscle glycogen levels less than 200 mmol/kg dw might impair training intensity and thereby negate some of these beneficial adaptations.[127] Thus, there appears to be a glycogen window in which to perform these low-carbohydrate training sessions, which should be employed sparingly to avoid low energy availability and impaired adaptations in the long-term.[128] An additional strategy employed by athletes and researchers to increase fat oxidation and improve endurance performance is the ingestion of exogenous ketone ester supplements.[129–132] While exogenous ketone consumption increases intramuscular fat oxidation during exercise, even when adequate muscle glycogen is present, it is unclear whether this positively influences endurance performance.[129,131]

Other supplements used commonly by endurance athletes include caffeine and nitrates.[133] Caffeine is a well-established ergogenic aid, which improves endurance performance, muscle strength, and power.[134] Some studies suggest that a SNP in the *CYP1A2* gene might moderate the ergogenic effect of caffeine on endurance performance.[135,136] However, the effects presented in these studies are small and inconsistent.[137] The mechanisms of caffeine erogenicity are poorly understood.[138] Original investigations suggested an increase in the mobilization and oxidation of free fatty acids allowing the sparing of muscle glycogen; however, this hypothesis lacks sufficient

support.[139] An alternative mechanism is the effect of caffeine on the central nervous system and its role as an adenosine-receptor antagonist in delaying central fatigue.[140] Finally, caffeine has consistently been shown to increase catecholamine release, which could potentially have a beneficial effect on endurance performance.[141–144] Dietary nitrates, often administered as beetroot juice, have been shown to increase the bioavailability of nitric oxide in the body, thus improving vasodilation and potentially the blood flow and oxygen delivery to working muscle.[145] Early studies described improvements in exercise economy following three days of nitrate supplementation.[146,147] While some studies demonstrate improved endurance performance following acute and chronic nitrate supplementation, others do not.[145] Although these and other supplements appear to have beneficial effects on performance in certain situations, it seems clear that the biggest improvements in endurance performance can be made by manipulating dietary carbohydrate intake to optimize exercise metabolism; additional studies are needed to further elucidate the role of low-carbohydrate diets, acute low-carbohydrate training, and exogenous ketone supplementation.

1.3.3 Training

Adaptations to endurance or aerobic training include central and peripheral processes,[148] the main central adaptations are morphological changes to the heart, while peripheral adaptations comprise changes in the vasculature and skeletal muscles, including improvements in their cellular and molecular makeup.[149–152]

1.3.3.1 Central Adaptations

A major central adaptation to endurance training is cardiac remodeling, specifically an increase in the size of the heart muscle and its compliance.[149] Endurance trained individuals consistently exhibit healthy LV hypertrophy, which includes greater LV chamber size, LV wall

thickness, and LV mass compared with untrained individuals.[153–156] Additionally, Arbab-Zadeh et al. showed that previously sedentary individuals significantly increased their LV chamber size, wall thickness and mass in response to one year of progressively increasing volume and intensity of endurance training.[149] Further, these individuals experienced an improvement in myocardial compliance, a measure of the heart's ability to stretch and quickly accept incoming blood. This increase in compliance, along with an increase in plasma volume, allowed participants to raise their maximal SV from 98 mL to 115 mL. It has been shown that endurance training leads to faster LV filling,[157–159] which can at least partially be attributed to this increase in compliance and a change in the pressure gradient between the left atrium (LA) and LV.[160] In the study by Arbab-Zadeh, the increase in SV led to an improvement in maximal $\dot{Q}$ from 20 L/min to 22 L/min.[149] As described above, the increase in $\dot{Q}$ is the major reasons for improved $\dot{V}O_2$max following endurance training; indeed, participants in the study increased their $\dot{V}O_2$max from 40.3 mL/kg/min to 47.4 mL/kg/min.

1.3.3.2 Peripheral Adaptations

Endurance training leads to several adaptations of the vascular system. It has been shown that endurance athletes' arteries have a greater diameter and decreased wall thickness compared with sedentary individuals.[161] This allows for increased blood flow to the working muscle and thus improved oxygen delivery. In addition to the diameter and wall-thickness of arteries, endurance trained individuals show an increase in the number and density of skeletal muscle capillaries, which again improves blood and oxygen delivery to the working muscle.[162,163] This increase in capillary density is strongly correlated with improved exercise performance.[20] Andersen and Henriksson showed a 20% increase in capillary density along with a 16% increase in $\dot{V}O_2$max following eight weeks of endurance training.[162]

The most important hematological adaptation to endurance training is increased total blood volume, which is almost entirely driven by increased plasma volume.[164] Elite athletes can have blood volume of more than 50% larger than those of sedentary individuals; typical blood volumes for untrained individuals is approximately 5 L, whereas trained individuals can reach 6 L and elite athletes up to 8 L.[165] Plasma volume expansion can occur within 24 hours of a single training session[166] and typically plateaus after 10-14 days.[164] While hematocrit initially falls due to the rapid plasma volume expansion, erythrocyte volume follows this increase within 30 days[164], but hematocrit typically remains below pre-training levels and lower than that of untrained individuals.[165] As discussed above, hemoglobin mass does not further increase based on sea-level training in normothermia, but altitude training and heat training can increase hemoglobin mass, and thus hematocrit, in athletes.[167–169] Thus, the major hematological factor improving endurance performance due to training is increased blood volume, which leads to increased SV, and thus increased oxygen delivery to the blood based on an increased $\dot{Q}$. This allows for an increase in $V\dot{O}_2max$, which is beneficial to endurance performance. Coyle et al.[170] showed that 2-4 weeks of detraining in endurance trained men led to a 9% decrease in blood volume, which lead to a 12% decrease in SV resulting in a 6% decrease in $\dot{V}O_2max$; restoration of blood volume by infusing saline resulted in almost complete recovery of $\dot{V}O_2max$.

Cellular and metabolic adaptations to endurance training include, among others, mitochondrial biogenesis, improved skeletal muscle buffering capacity, and mitochondrial enzyme activity.[152,171,172] While the exact mechanisms of mitochondrial biogenesis are still debated, it appears clear that mitochondria cannot be synthesized de novo.[173] Nevertheless, endurance training has consistently been shown to increase mitochondrial volume density in skeletal muscle, specifically intermyofibrillar mitochondria.[172] This increase in volume density is achieved by

increased mitochondrial cross sectional area and length.[172] The increase in mitochondrial density has been shown to be an important factor in improved exercise performance;[174] it improves muscle respiratory capacity and fat oxidation capability, which in turn improve fractional utilization and performance in prolonged efforts.[171,172] This improvement in fractional utilization is represented by a right-shift of the lactate curve in incremental exercise tests showing the body's ability to rely on OXPHOS at higher intensities. [175]

Endurance-trained muscle exhibits increased respiratory capacity, specifically the capacity to oxidize fatty acids, ketones, and pyruvate.[171] One mechanism explaining this increase is the heightened activity of mitochondrial enzymes. Spina et al.[176] showed that 7-10 days of endurance training increased the activities of beta-hydroxyacyl-CoA dehydrogenase, mitochondrial thiolase, and carnitine acetyltransferase by approximately 30% which coincided with an increase in $\dot{V}O_2$max of approximately 9% and a reduction in lactate at four different submaximal intensities. In another study, citrate synthase increased significantly following 12-weeks of endurance training in young and older men[177]. Wibom et al.[178] reported a 40% increase in citrate synthase, a 78% increase in cytochrome-c oxidase, an 18% increase in succinate cytochrome c reductase, and a 45% increase in glutamate dehydrogenase following six weeks of endurance training; these changes were concomitant with a 70% increase in the mitochondrial ATP production rate. Thus, increased mitochondrial enzyme activity leads to increased $\dot{V}O_2$max, increased fractional utilization, and improved ATP production during submaximal exercise.

Human skeletal muscle contains three muscle fiber types: Type I (oxidative) fibers, Type IIa (oxidative-glycolytic) fibers, and Type IIx (glycolytic fibers), which are typified by their myosin heavy chain (MHC).[179] Type I fibers, who possess the greatest oxidative potential, are beneficial for endurance performance; individuals with greater Type I fiber content display greater

gross efficiency[26], running economy[180], and improved oxygen uptake kinetics.[181] All of these are mechanisms that can explain the positive link between greater Type I muscle fiber content and endurance performance.[182] Endurance training has been shown to lead to a transformation of Type IIX fibers toward Type IIa fiber characteristics, which includes increased oxidative capacity, making them more like Type I fibers.[183,184] While a transformation from Type II to Type I MHC in response to endurance exercise has not been experimentally demonstrated in humans, Schantz and Dhoot[185] showed the co-existence of MHCI and MHCII proteins in single muscle fibers following a prolonged endurance task (800 km mountain skiing in 36 days); the authors called these fibers "intermediate fibers". Additionally, the participants in the same study, reported no change in Type I fiber distribution, but a significant increase in intermediate fiber content following the exercise task; at the same time, Type IIa and Type IIx distribution decreased.[186] This suggests that, while a complete transition of Type II to Type I fibers might not be induced by endurance training, Type II fibers will acquire some of the same properties as their Type I counterparts.

1.3.3.3 The Influence of Training Parameters on Endurance Training Adaptations

Endurance training parameters include the volume (distance or time), intensity (absolute or relative workload), and frequency (number of sessions over a given time) of training. The following section details the influence of these parameters on endurance performance. It is important to note that these factors are interconnected and in practice are difficult to manipulate in complete isolation; thus, the distribution (volume and frequency) of exercise at different intensities is discussed.

When considered in isolation, increasing exercise volume progressively leads to greater adaptations in $\dot{V}O_2max$ with proportionally increasing improvements in $\dot{V}O_2max$, maximal O_2

pulse (a correlate of SV), and time to task failure with increasing exercise duration.[187,188]. Hickson et al. showed that reducing training duration while keeping frequency and intensity constant had negative effects on cycling time to task failure, but not on $\dot{V}O_2$max.[189] More recently, some laboratories have begun investigating the minimal effective duration of exercise for improvements in $\dot{V}O_2$max with as little as ten minutes of exercise including three 20-second sprints – three times per week showing marked improvements in central and peripheral factors.[190] Following the exercise intervention, participants increased their $\dot{V}O_2$max by 12% concomitant with an increase in citrate synthase and ß-hydroxy acyl CoA dehydrogenase activity and cytochrome oxidase 4 as well as glucose transporter type 4 (GLUT-4) protein content. When frequency and intensity are held constant, it appears that increasing duration will produce proportionally increasing adaptations in $\dot{V}O_2$max and submaximal exercise performance.

When considering training frequency in isolation, it appears that increasing frequency leads to progressively greater adaptations up to six sessions per week.[30,187,191] Pollock et al. showed that training 30-45 minutes at the same intensity two, three or four times a week elicited progressively larger improvements in $\dot{V}O_2$max.[192] Similarly, training one, three, or five times per week for 30 minutes at 85-95% of HRmax over the course of 20 weeks produced augmented adaptations in the higher frequency groups compared to the lower frequency groups.[191] Wenger and Bell argued that the optimal frequency for all intensities of training is four times per week.[187]

With the ever-increasing use and popularity of high-intensity interval training (HIIT) and sprint interval training (SIT), the effects of exercise intensity on endurance performance and physiological adaptations are an important topic in the prescription of endurance training.[193] Similar to volume and frequency, when intensity is studied in isolation it has been shown that increasing exercise intensity in the range from 50% to 100% $\dot{V}O_2$max leads to progressively

increasing adaptations in aerobic capacity.[187] When holding frequency and duration steady, training at 50-70% of maximal oxygen uptake improves $\dot{V}O_2$max by approximately 4.5 mL/kg/min, whereas training at 90-100% improves $\dot{V}O_2$max by approximately 7 ml/kg/min over the same duration of training. It appears that using supramaximal intensities, i.e., intensities above those eliciting $\dot{V}O_2$max, improve $\dot{V}O_2$max to a lesser degree (~5.5 mL/kg/min) compared with training close to or at $\dot{V}O_2$max.[187,194]

When work is matched between moderate-intensity continuous training (MICT) and HIIT or SIT, the training with higher intensities (HIIT and SIT) produced greater training adaptations compared with lower intensities.[151] When work is not matched between HIIT and MICT and SIT, HIIT and SIT have been shown to require less exercise volume to elicit similar changes to MICT.[151] Interestingly, it does appear that MICT and HIIT lead to greater central adaptations when compared with SIT, whereas the latter produces more peripheral changes.[195,196] In summary, exercise intensity is an important regulator of the adaptive response to endurance exercise. It appears that an intensity of at least 50% of $\dot{V}O_2$max is necessary to elicit adaptations in maximal aerobic capacity[197,198] and that the greatest adaptations are in $\dot{V}O_2$max are achieved with intensities of 90-100% of maximal oxygen uptake.[187] Additionally, it appears that HIIT and SIT elicit superior adaptations compared with lower intensity training when work is matched and can elicit similar adaptations to MICT even with reduced exercise volume.[151]

Based on the above discussion about volume, intensity, and frequency it would be prudent to say that increasing and maximizing all three of these training parameters would lead to the greatest adaptations. Thus, more frequent, longer training sessions at higher intensities would appear to be the best training prescription based on purely looking at the variables in isolation. However, as discussed before, in practice these variables are interconnected and cannot be seen in

isolation.[187] Additionally, maximizing all three parameters is impossible and inadvisable in practice: 1) time constraints limit athletes to a certain frequency and volume of training[199], 2) overtraining and injuries can result from too much volume, too much intensity, and too little recovery, leading to injury and illness[200,201], and 3) even if illness and injury can be avoided, too much or too frequent high-intensity training can lead to inadequate autonomic recovery, which could lead to blunted adaptations.[202] Thus, it is critical to find the right balance in the manipulation of training variables to maximize performance and minimize the risk of overtraining and maladaptation.

When prescribing training intensities, coaches and athletes often use training intensity zones based on physiological parameters from exercise testing (e.g., LT, LTP, MLSS, CP, FTP).[203] A variety of models have been proposed including 3-zone, 5-zone, 6-zone, and 7-zone models.[203–206] In a simplified 3-zone model, Zone 1 typically refers to exercise below LT or VT, Zone 2 comprises intensities between LT/VT and LTP or MLSS, and Zone 3 includes intensities above MLSS; in a 5-zone model, Zones 1 & 3 are further divided into transitionary zones, which often don't correspond to a directly measured physiological event.[206] For the rest of this document, a 3-zone model will be referenced for simplicity.

Historically, training at the upper end of Zone 2, i.e., threshold training, was considered one of the most potent stimuli to improve endurance performance, specifically by improving fractional utilization.[207] However, recent research has shown that this type of training makes up only a minimal amount of the overall training regimen of highly successful elite athletes.[203] These studies showed that typical training distributions among elite athletes comprise approximately 80% low-intensity (Zone 1) training and 20% high-intensity (Zone 3) training in a variety of endurance sports including cross-country skiing[208], rowing[209,210], running[211,212], orienteering[213], and

triathlon[214]. In fact, Seiler coined the term "polarized training" to describe this training pattern.[215] Several training studies have confirmed improved adaptations and exercise performance following polarized training when compared with interventions relying more heavily on threshold training.[216–220] Thus, it appears that polarized training, i.e., spending approximately 80% of training time in Zone 1 and 20% of training time in Zone 3 leads to improved adaptations and better direct indicators of endurance performance than threshold-focused training. Important considerations in the interpretation of the amount of training performed in each zone include the variable used to determine zones (HR, power, speed) and the strategy to quantify the training volume in each zone. The latter can be approached from a session-goal perspective, i.e., how many of the total sessions are targeted at high-intensity, medium-intensity, or low-intensity exercise, or from a time-in-zone perspective, i.e., how many minutes does an individual spend in each zone.[221] The aforementioned 80-20% distribution is more appropriate for the session-goal approach, whereas the percentage of high-intensity training in a time-in-zone approach can be much lower (e.g., 2-4% vs. ≥ 90% low-intensity training).[203,212] However, there is active debate on whether TID in the original studies investigating this paradigm was mischaracterized and whether a polarized TID is indeed optimal for endurance athletes.[222,223]

1.3.4 Environment

Environmental factors, including altitude and temperature, and their influence on exercise performance. $\dot{V}O_2max$ exhibits a linear decrease with increasing altitude due to the decrease in partial pressure of oxygen and the resulting decline in arterial hemoglobin oxygen saturation; specifically, $\dot{V}O_2max$ decreases by approximately 6-11% per 1,000 m of altitude.[116,224] The hypoxia experienced at altitude leads to a reduction in absolute power output at LT and LTP, but due to the concomitant decrease in $\dot{V}O_2max$, the relative exercise at which LT and LTP occur

remain the same [225]. Thus, fractional utilization capacity does not change with acute altitude exposure. However, the absolute power output or running speed eliciting this fractional utilization decreases. In the laboratory setting, cycling gross efficiency and running economy at altitude appear to be similar to sea-level values.[226,227] However, it could be argued that due to the decrease in air density at altitude, the resulting loss of aerodynamic drag, and the subsequent decrease in energy requirements to maintain the same exercise intensity, field-based gross efficiency and running economy are improved at altitude.[228] This decrease in air density also leads to improved performances in sprint and power sports as well as shorter running distance at altitude.[229,230] In endurance sports, however, the decreased arterial oxygen saturation leads to performance decrements that become larger the longer the distance of the event.[230] Physiological adaptations to living and/or training in hypoxic conditions, mainly an increase in hemoglobin mass, have the potential to improve performance at altitude and at sea-level;[231,232] however, this is outside of the scope of this document.

Analogous to altitude, $\dot{V}O_2max$ is decreased in hot conditions; this is most likely due to increased skin blood flow, which 1) reduces the portion of $\dot{Q}$ perfusing the working muscle, and 2) reduces venous return and subsequently cardiac output [233]. This reduction in $\dot{V}O_2max$ remains even after heat acclimation.[233] The increase in skin blood flow has also been linked to decreased cycling gross efficiency high ambient temperatures.[234] As with altitude, the relative intensity at LT and LTP remains the same in hot environments, but the absolute intensity is decreased.[235] Concomitantly, endurance performance is decreased in hot environments: hyperthermia, dehydration, and physiological and mental heat stress can reduce performance by approximately 6-7%.[236,237] Researchers and athletes have used a variety of strategies, including heat acclimation protocols, hydration protocols, and cooling protocols, to reduce the ergolytic effect of hot

environments on endurance performance.[238,239] Additionally, heat training has been proposed as a means to improve exercise performance in normothermic environments.[238] However, these approaches are outside of the scope of this document.

1.4 Summary of Aims, Purpose, and Hypotheses

The purpose of this dissertation was to examine the influence of genetics, nutrition, and training on endurance exercise performance. Studies include investigations of the effects of *ACTN3* genotype, diet composition, and training parameters on running and cycling performance.

Study 1 (Chapter 2) examined the association of *ACTN3* genotype with self-reported one-mile and 5-km running personal records (PRs) in 94 recreationally active men and women using a cross-sectional design. We hypothesized that those with the *ACTN3* XX genotype would report faster running PRs compared with those exhibiting the RX and RR genotypes.

Study 2 (Chapter 3) investigated the effects of diet composition (habitual vs. high-carbohydrate vs. ketogenic diet) on cycling performance in a simulated 30-km TT in recreationally competitive cyclists and triathletes. This study employed a randomized cross-over design with two-week diet intervention periods. We hypothesized that the high-carbohydrate diet would significantly improve cycling performance when compared with the ketogenic diet.

Study 3 (Chapter 4) analyzed raw training and racing data provided by 232 male and female Strava© and Golden Cheetah users to investigate the training intensity distribution among recreational cyclists and triathletes. Additionally, this study examined the effects of age, and training characteristics (volume, intensity, and intensity distribution) on cycling performance. We hypothesized that, when adjusting for age, performance may be predicted from greater total volume, greater average intensity, and greater training polarization.

1.5 Significance

Endurance sports, including running, cycling, and triathlon, are popular among recreational athletes in the USA. Prior to the COVID-19 pandemic the number of race registrations in running and triathlon alone was estimated between 22 and 30 million annually.[240] USA Cycling (USAC) members amassed over 300,000 racer days in 2019.[241] According to the Outdoor Industry Association, Americans spend close to $14 billion per year on cycling gear and almost $83 billion on cycling-related travel.[242] Competitive recreational cyclists spend on average 12.04 hours per week across 5.3 days for pleasure and to improve their performance.[243] Runners have been shown to spend approximately $1,000 on the preparation for and participation in a single marathon. These recreational endurance athletes strive to perform their best in so-called age-group races and spend a significant amount of their time and disposable income to improve their performance. Thus, it is important to investigate the influence of genetics, nutrition, and training on performance in this population and to provide these athletes with the most accessible and actionable information to optimize their performance.

1.6 References

1. Merriam-Webster.com dictionary. Endurance. In: *Merriam-Webster.Com Dictionary*. Accessed May 19, 2021. https://www.merriam-webster.com/dictionary/endurance

2. Brooks GA, Fahey TD, Baldwin KM. *Exercise Physiology: Human Bioenergetics and Its Applications*. 4th ed. McGraw Hill; 2004.

3. Savoldelli A, Fornasiero A, Trabucchi P, et al. The Energetics during the World's Most Challenging Mountain Ultra-Marathon - A Case Study at the Tor des Geants®. *Front Physiol*. 2017;8. doi:10/ggkf94

4. Vernillo G, Savoldelli A, Skafidas S, et al. An Extreme Mountain Ultra-Marathon Decreases the Cost of Uphill Walking and Running. *Front Physiol*. 2016;7. doi:10/gj428t

5. Jones AM, Burnley M, Black MI, Poole DC, Vanhatalo A. The Maximal Metabolic Steady State: Redefining the 'Gold Standard.' *Physiol Rep*. 2019;7(10):e14098. doi:10/ggx7x3

6. Amann M, Hopkins WG, Marcora SM. Similar Sensitivity of Time to Exhaustion and Time-Trial Time to Changes in Endurance. *Med Sci Sports Exerc*. 2008;40(3):574-578. doi:10/cqv58j

7. Hopkins WG, Schabort EJ, Hawley JA. Reliability of Power in Physical Performance Tests. *Sports Med*. 2001;31(3):211-234. doi:10/c2mfh9

8. Coyle EF. Physiological determinants of endurance exercise performance. *J Sci Med Sport*. 1999;2(3):181-189. doi:10/cq69wx

9. Maunder E, Seiler S, Mildenhall MJ, Kilding AE, Plews DJ. The Importance of "Durability" in the Physiological Profiling of Endurance Athletes. *Sports Med*. 2021;51(8):1619-1628. doi:10/gnz7qq

10. Levine BD. VO2max: What Do We Know, and What Do We Still Need to Know? *J Physiol*. 2008;586(1):25-34. doi:10/d25scn

11. Bergh U, Ekblom B, Astrand PO. Maximal Oxygen Uptake "Classical" Versus "Contemporary" Viewpoints. *Med Sci Sports Exerc*. 2000;32(1):85-88. doi:10/dztrx8

12. di Prampero PE. Factors Limiting Maximal Performance in Humans. *Eur J Appl Physiol*. 2003;90(3-4):420-429. doi:10/dm3gwx

13. Costill DL, Thomason H, Roberts E. Fractional Utilization of the Aerobic Capacity During Distance Running. *Med Sci Sports Exerc*. 1973;5(4):248-252. doi:10/fchvpv

14. Joyner MJ. Modeling: Optimal Marathon Performance on the Basis of Physiological Factors. *J Appl Physiol (1985)*. 1991;70(2):683-687. doi:10/gfxfxj

15. Bassett DRJ, Howley ET. Maximal Oxygen Uptake: “Classical” Versus” Contemporary” Viewpoints. *Med Sci Sports Exerc*. 1997;29(5):591-603. doi:10/fr6vp3

16. Noakes TD. Maximal Oxygen Uptake: “Classical” Versus “Contemporary” Viewpoints: A Rebuttal. *Med Sci Sports Exerc*. 1998;30(9):1381-1398. doi:10/d3w67b

17. Poole DC, Rossiter HB, Brooks GA, Gladden LB. The Anaerobic Threshold: 50+ Years of Controversy. *J Physiol*. 2021;599(3):737-767. doi:10/ghhfrq

18. Wasserman K. The Anaerobic Threshold Measurement to Evaluate Exercise Performance. *Am Rev Respir Dis*. 1984;129(2 Pt 2):S35-40. doi:10/gj428m

19. Jamnick NA, Pettitt RW, Granata C, Pyne DB, Bishop DJ. An Examination and Critique of Current Methods to Determine Exercise Intensity. *Sports Med*. 2020;50(10):1729-1756. doi:10/gg64rw

20. Coyle EF, Coggan AR, Hopper MK, Walters TJ. Determinants of Endurance in Well-Trained Cyclists. *J Appl Physiol (1985)*. 1988;64(6):2622-2630. doi:10/gjxz44

21. Lucia A, Hoyos J, Pérez M, Santalla A, Earnest CP, Chicharro JL. Which laboratory variable is related with time trial performance time in the Tour de France? *Br J Sports Med*. 2004;38(5):636-640. doi:10/d8pgrq

22. Jones AM, Kirby BS, Clark IE, et al. Physiological Demands of Running at 2-Hour Marathon Race Pace. *J Appl Physiol (1985)*. 2020;130(2):369-379. doi:10/ghkg96

23. Joyner MJ, Coyle EF. Endurance Exercise Performance: The Physiology of Champions. *The Journal of Physiology*. 2008;586(1):35-44. doi:10/c6fj7p

24. Jobson SA, Hopker JG, Korff T, Passfield L. Gross Efficiency and Cycling Performance: A Brief Review. *J Sci Cycl*. 2012;1(1):3-8.

25. Ettema G, Lorås HW. Efficiency in Cycling: A Review. *Eur J Appl Physiol*. 2009;106(1):1-14. doi:10/cd83zj

26. Coyle EF, Sidossis LS, Horowitz JF, Beltz JD. Cycling Efficiency Is Related to the Percentage of Type I Muscle Fibers. *Med Sci Sports Exerc*. 1992;24(7):782-788.

27. Barnes KR, Kilding AE. Running Economy: Measurement, Norms, and Determining Factors. *Sports Med Open*. 2015;1(1):8. doi:10/gftjwx

28. Hoogkamer W, Kipp S, Spiering BA, Kram R. Altered Running Economy Directly Translates to Altered Distance-Running Performance. *Med Sci Sports Exerc*. 2016;48(11):2175-2180. doi:10/f9bgrd

29. di Prampero PE, Capelli C, Pagliaro P, et al. Energetics of Best Performances in Middle-Distance Running. *J Appl Physiol (1985)*. 1993;74(5):2318-2324. doi:10/gj477p

30. Pollock ML. Submaximal and Maximal Working Capacity of Elite Distance Runners. Part I: Cardiorespiratory Aspects. *Ann N Y Acad Sci*. 1977;301(1):310-322. doi:10.1111/j.1749-6632.1977.tb38209.x

31. Foster C, Lucia A. Running Economy: The Forgotten Factor in Elite Performance. *Sports Med*. 2007;37(4-5):316-319. doi:10/bp932c

32. Tucker R, Lambert MI, Noakes TD. An Analysis of Pacing Strategies During Men's World-Record Performances in Track Athletics. *Int J Sports Physiol Perform*. 2006;1(3):233-245. doi:10/gfxgw2

33. Sanders D, van Erp T. The Physical Demands and Power Profile of Professional Men's Cycling Races: An Updated Review. *Int J Sports Physiol Perform*. 2020;16(1):3-12. doi:10/ghnvdz

34. Impellizzeri FM, Marcora SM. The Physiology of Mountain Biking. *Sports Med*. 2007;37(1):59-71. doi:10/bp7j8x

35. Stapelfeldt B, Schwirtz A, Schumacher YO, Hillebrecht M. Workload Demands in Mountain Bike Racing. *Int J Sports Med*. 2004;25(4):294-300. doi:10/cz9mf2

36. Clark IE, Vanhatalo A, Thompson C, et al. Dynamics of the Power-Duration Relationship During Prolonged Endurance Exercise and Influence of Carbohydrate Ingestion. *J Appl Physiol (1985)*. 2019;127(3):726-736. doi:10.1152/japplphysiol.00207.2019

37. Clark IE, Vanhatalo A, Bailey SJ, et al. Effects of Two Hours of Heavy-Intensity Exercise on the Power-Duration Relationship. *Med Sci Sports Exerc*. 2018;50(8):1658-1668. doi:10/gn2xv8

38. Van Erp T, Sanders D, Lamberts RP. Maintaining Power Output with Accumulating Levels of Work Done Is a Key Determinant for Success in Professional Cycling. *Med Sci Sports Exerc*. 2021;53(9):1903-1910. doi:10/gn2xwc

39. Muriel X, Valenzuela PL, Mateo-March M, Pallarés JG, Lucia A, Barranco-Gil D. Physical Demands and Performance Indicators in Male Professional Cyclists During a Grand Tour: WorldTour Versus ProTeam Category. *Int J Sports Physiol Perform*. 2022;17(1):22-30. doi:10.1123/ijspp.2021-0082

40. Leo P, Spragg J, Mujika I, Menz V, Lawley JS. Power Profiling in U23 Professional Cyclists During a Competitive Season. *Int J Sports Physiol Perform*. 2021;16(6):881-889. doi:10.1123/ijspp.2020-0200

41. Leo P, Spragg J, Mujika I, et al. Power Profiling, Workload Characteristics, and Race Performance of U23 and Professional Cyclists During the Multistage Race Tour of the Alps. *Int J Sports Physiol Perform*. Published online March 31, 2021:1-7. doi:10/gm58nb

42. Clark IE, Vanhatalo A, Thompson C, et al. Changes in the Power-Duration Relationship Following Prolonged Exercise: Estimation Using Conventional and All-Out Protocols and

Relationship With Muscle Glycogen. *Am J Physiol Regul Integr Comp Physiol*. 2019;317(1):R59-R67. doi:10/gn2xv7

43. Rodríguez-Marroyo JA, Villa JG, Pernía R, Foster C. Decrement in Professional Cyclists' Performance After a Grand Tour. *Int J Sports Physiol Perform*. 2017;12(10):1348-1355. doi:10.1123/ijspp.2016-0294

44. Rankinen T, Fuku N, Wolfarth B, et al. No Evidence of a Common DNA Variant Profile Specific to World Class Endurance Athletes. *PLoS One*. 2016;11(1):e0147330. doi:10/f8qjk3

45. Guth LM, Roth SM. Genetic Influence on Athletic Performance. *Curr Opin Pediatr*. 2013;25(6):653-658. doi:10/f5hrcv

46. Klissouras V. Heritability of Adaptive Variation. *J Appl Physiol (1985)*. 1971;31(3):338-344. doi:10/gntvrt

47. Fagard R, Bielen E, Amery A. Heritability of Aerobic Power and Anaerobic Energy Generation During Exercise. *J Appl Physiol (1985)*. 1991;70(1):357-362. doi:10/gntvrx

48. Maes HH, Beunen GP, Vlietinck RF, et al. Inheritance of Physical Fitness in 10-Yr-Old Twins and Their Parents. *Med Sci Sports Exerc*. 1996;28(12):1479-1491. doi:10/c4zxtd

49. Mustelin L, Latvala A, Pietiläinen KH, et al. Associations Between Sports Participation, Cardiorespiratory Fitness, and Adiposity in Young Adult Twins. *J Appl Physiol (1985)*. 2011;110(3):681-686. doi:10/bsw4b7

50. Bouchard C, Lesage R, Lortie G, et al. Aerobic Performance in Brothers, Dizygotic and Monozygotic Twins. *Med Sci Sports Exerc*. 1986;18(6):639-646.

51. Bouchard C, Daw EW, Rice T, et al. Familial Resemblance for VO2max in the Sedentary State: The HERITAGE Family Study. *Med Sci Sports Exerc*. 1998;30(2):252-258. doi:10/btg8ts

52. Williams CJ, Williams MG, Eynon N, et al. Genes to Predict VO2max Trainability: A Systematic Review. *BMC Genomics*. 2017;18(8):831. doi:10.1186/s12864-017-4192-6

53. Bouchard C, Sarzynski MA, Rice TK, et al. Genomic Predictors of the Maximal O2 Uptake Response to Standardized Exercise Training Programs. *J Appl Physiol (1985)*. 2011;110(5):1160-1170. doi:10/d4nkw6

54. Simoneau JA, Bouchard C. Genetic Determinism of Fiber Type Proportion in Human Skeletal Muscle. *FASEB J*. 1995;9(11):1091-1095. doi:10/gntvrz

55. Ahmetov II, Vinogradova OL, Williams AG. Gene Polymorphisms and Fiber-Type Composition of Human Skeletal Muscle. *Int J Sport Nutr Exerc Metab*. 2012;22(4):292-303. doi:10/f344r2

56. Rodas G, Calvo M, Estruch A, et al. Heritability of Running Economy: A Study Made on Twin Brothers. *Eur J Appl Physiol Occup Physiol*. 1998;77(6):511-516. doi:10/dtn2kv

57. Curran JE, Johnson MP, Dyer TD, et al. Genetic Determinants of Mitochondrial Content. *Hum Mol Genet*. 2007;16(12):1504-1514. doi:10/db56f9

58. Barbato JC, Koch LG, Darvish A, Cicila GT, Metting PJ, Britton SL. Spectrum of Aerobic Endurance Running Performance in Eleven Inbred Strains of Rats. *J Appl Physiol (1985)*. 1998;85(2):530-536. doi:10.1152/jappl.1998.85.2.530

59. Koch LG, Meredith TA, Fraker TD, Metting PJ, Britton SL. Heritability of Treadmill Running Endurance in Rats. *Am J Physiol*. 1998;275(5):R1455-1460. doi:10/gntvrv

60. Ben-Zaken S, Meckel Y, Nemet D, Eliakim A. IGF-I Receptor 275124A>C (rs1464430) Polymorphism and Athletic Performance. *J Sci Med Sport*. 2015;18(3):323-327. doi:10/f7dpv5

61. Horio T, Kamide K, Takiuchi S, et al. Association of Insulin-Like Growth Factor-1 Receptor Gene Polymorphisms With Left Ventricular Mass and Geometry in Essential Hypertension. *J Hum Hypertens*. 2010;24(5):320-326. doi:10/ccdkts

62. Guilherme JPLF, Egorova ES, Semenova EA, et al. The A-allele of the FTO Gene rs9939609 Polymorphism Is Associated With Decreased Proportion of Slow Oxidative Muscle Fibers and Over-represented in Heavier Athletes. *J Strength Cond Res*. 2019;33(3):691-700. doi:10/gntvrp

63. Hinney A, Nguyen TT, Scherag A, et al. Genome Wide Association (GWA) Study for Early Onset Extreme Obesity Supports the Role of Fat Mass and Obesity Associated Gene (FTO) Variants. *PLoS One*. 2007;2(12):e1361. doi:10/bxzg23

64. Scuteri A, Sanna S, Chen WM, et al. Genome-Wide Association Scan Shows Genetic Variants in the FTO Gene Are Associated With Obesity-Related Traits. *PLoS Genet*. 2007;3(7):e115. doi:10/c7qz33

65. Frayling TM, Timpson NJ, Weedon MN, et al. A Common Variant in the FTO Gene Is Associated With Body Mass Index and Predisposes to Childhood and Adult Obesity. *Science*. 2007;316(5826):889-894. doi:10/fntk4p

66. Myerson S, Hemingway H, Budget R, Martin J, Humphries S, Montgomery H. Human Angiotensin I-converting Enzyme Gene and Endurance Performance. *J Appl Physiol (1985)*. 1999;87(4):1313-1316. doi:10.1152/jappl.1999.87.4.1313

67. Gayagay G, Yu B, Hambly B, et al. Elite Endurance Athletes and the ACE I Allele--The Role of Genes in Athletic Performance. *Hum Genet*. 1998;103(1):48-50. doi:10.1007/s004390050781

68. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An Insertion/Deletion Polymorphism in the Angiotensin I-converting Enzyme Gene Accounting for Half the Variance of Serum Enzyme Levels. *J Clin Invest*. 1990;86(4):1343-1346. doi:10/b77vq7

69. Kem DC, Brown RD. Renin–From Beginning to End. *N Engl J Med*. 1990;323(16):1136-1137. doi:10/cnt5c5

70. Falahati A, Arazi H. Association of ACE Gene Polymorphism With Cardiovascular Determinants of Trained and Untrained Iranian Men. *Genes Environ*. 2019;41(1):8. doi:10/gntvrr

71. Jin HJ, Hwang IW, Kim KC, et al. Is There a Relationship Between PPARD T294C/PPARGC1A Gly482Ser Variations and Physical Endurance Performance in the Korean Population? *Genes Genom*. 2016;38(4):389-395. doi:10/gntvrn

72. Vega RB, Huss JM, Kelly DP. The Coactivator PGC-1 Cooperates With Peroxisome Proliferator-Activated Receptor Alpha in Transcriptional Control of Nuclear Genes Encoding Mitochondrial Fatty Acid Oxidation Enzymes. *Mol Cell Biol*. 2000;20(5):1868-1876. doi:10/dk645r

73. Jeukendrup AE. Nutrition for Endurance Sports: Marathon, Triathlon, and Road Cycling. *J Sports Sci*. 2011;29 Suppl 1:S91-99. doi:10/b52hvj

74. Burke LM, Hawley JA, Wong SHS, Jeukendrup AE. Carbohydrates for Training and Competition. *J Sports Sci*. 2011;29 Suppl 1:S17-27. doi:10/czm3qj

75. Ørtenblad N, Westerblad H, Nielsen J. Muscle Glycogen Stores and Fatigue. *J Physiol*. 2013;591(Pt 18):4405-4413. doi:10/f2z259

76. Romijn JA, Coyle EF, Sidossis LS, et al. Regulation of Endogenous Fat and Carbohydrate Metabolism in Relation to Exercise Intensity and Duration. *Am J Physiol*. 1993;265(3 Pt 1):E380-391. doi:10/ggxrfb

77. Brooks GA, Mercier J. Balance of Carbohydrate and Lipid Utilization During Exercise: The "Crossover" Concept. *J Appl Physiol (1985)*. 1994;76(6):2253-2261. doi:10/ggkgcw

78. Philp A, Hargreaves M, Baar K. More Than a Store: Regulatory Roles for Glycogen in Skeletal Muscle Adaptation to Exercise. *Am J Physiol Endocrinol Metab*. 2012;302(11):E1343-E1351. doi:10/f34z9t

79. Watt MJ, Hargreaves M. Effect of Epinephrine on Glucose Disposal During Exercise in Humans: Role of Muscle Glycogen. *Am J Physiol Endocrinol Metab*. 2002;283(3):E578-E583. doi:10.1152/ajpendo.00098.2002

80. Steensberg A, van Hall G, Keller C, et al. Muscle Glycogen Content and Glucose Uptake During Exercise in Humans: Influence of Prior Exercise and Dietary Manipulation. *J Physiol*. 2002;541(Pt 1):273-281. doi:10/cqhmvf

81. Weltan SM, Bosch AN, Dennis SC, Noakes TD. Preexercise Muscle Glycogen Content Affects Metabolism During Exercise Despite Maintenance of Hyperglycemia. *Am J Physiol*. 1998;274(1):E83-88. doi:10.1152/ajpendo.1998.274.1.E83

82. Blomstrand E, Saltin B. Effect of Muscle Glycogen on Glucose, Lactate and Amino Acid Metabolism During Exercise and Recovery in Human Subjects. *J Physiol*. 1999;514(Pt 1):293-302. doi:10/frmh7p

83. Hulston CJ, Venables MC, Mann CH, et al. Training With Low Muscle Glycogen Enhances Fat Metabolism in Well-Trained Cyclists. *Med Sci Sports Exerc*. 2010;42(11):2046-2055. doi:10/d7djxc

84. Maunder E, Plews DJ, Kilding AE. Contextualising Maximal Fat Oxidation During Exercise: Determinants and Normative Values. *Front Physiol*. 2018;9:599. doi:10/gdjcdm

85. Balsom PD, Gaitanos GC, Söderlund K, Ekblom B. High-Intensity Exercise and Muscle Glycogen Availability in Humans. *Acta Physiol Scand*. 1999;165(4):337-345. doi:10/fkpw74

86. Iaia FM, Perez-Gomez J, Nordsborg N, Bangsbo J. Effect of Previous Exhaustive Exercise on Metabolism and Fatigue Development During Intense Exercise in Humans. *Scand J Med Sci Sports*. 2010;20(4):619-629. doi:10/dqmd6n

87. Shulman RG, Rothman DL. The "Glycogen Shunt" in Exercising Muscle: A Role for Glycogen in Muscle Energetics and Fatigue. *Proc Natl Acad Sci U S A*. 2001;98(2):457-461.

88. Bangsbo J, Nørregaard L, Thorsøe F. The Effect of Carbohydrate Diet on Intermittent Exercise Performance. *Int J Sports Med*. 1992;13(2):152-157. doi:10/bc6skh

89. Matsui T, Soya M, Soya H. Endurance and Brain Glycogen: A Clue Toward Understanding Central Fatigue. *Adv Neurobiol*. 2019;23:331-346. doi:10/gnx4g9

90. Nybo L. CNS Fatigue and Prolonged Exercise: Effect of Glucose Supplementation. *Med Sci Sports Exerc*. 2003;35(4):589-594. doi:10/ccxh29

91. Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA. Implications of Group III and IV Muscle Afferents for High-Intensity Endurance Exercise Performance in Humans. *J Physiol*. 2011;589(21):5299-5309. doi:10/ccmm59

92. Amann M, Secher NH. Point: Afferent Feedback From Fatigued Locomotor Muscles Is an Important Determinant of Endurance Exercise Performance. *J Appl Physiol (1985)*. 2010;108(2):452-454; discussion 457; author reply 470. doi:10/d3w7ms

93. Gandevia SC. Spinal and Supraspinal Factors in Human Muscle Fatigue. *Physiol Rev*. 2001;81(4):1725-1789. doi:10/ggdd5r

94. Matsui T, Omuro H, Liu YF, et al. Astrocytic Glycogen-Derived Lactate Fuels the Brain During Exhaustive Exercise to Maintain Endurance Capacity. *Proc Natl Acad Sci U S A*. 2017;114(24):6358-6363. doi:10/gbjh9r

95. Marcora SM, Staiano W, Manning V. Mental Fatigue Impairs Physical Performance in Humans. *J Appl Physiol (1985)*. 2009;106(3):857-864. doi:10/cg5wtb

96. Sahlin K, Tonkonogi M, Söderlund K. Energy Supply and Muscle Fatigue in Humans. *Acta Physiol Scand*. 1998;162(3):261-266. doi:10/d5s9dg

97. Allen DG, Lamb GD, Westerblad H. Skeletal Muscle Fatigue: Cellular Mechanisms. *Physiol Rev*. 2008;88(1):287-332. doi:10.1152/physrev.00015.2007

98. Hawley JA, Schabort EJ, Noakes TD, Dennis SC. Carbohydrate-loading and exercise performance. An update. *Sports Med*. 1997;24(2):73-81. doi:10/fhwwhg

99. Sherman WM, Costill DL, Fink WJ, Miller JM. Effect of Exercise-Diet Manipulation on Muscle Glycogen and Its Subsequent Utilization During Performance. *Int J Sports Med*. 1981;2(2):114-118. doi:10/cjxrw4

100. Bergström J, Hultman E, Roch-Norlund AE. Muscle Glycogen Synthetase in Normal Subjects. Basal Values, Effect of Glycogen Depletion by Exercise and of a Carbohydrate-Rich Diet Following Exercise. *Scand J Clin Lab Invest*. 1972;29(2):231-236. doi:10/fdr845

101. Bergström J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen and physical performance. *Acta Physiol Scand*. 1967;71(2):140-150. doi:10/cwvpf7

102. Ahlborg B, Bergström J, Brohult J. Human Muscle Glycogen Content and Capacity for Prolonged Exercise After Different Diets. *Foersvarsmedicin*. Published online 1967:85-99.

103. Christensen EH, Hansen O. Arbeitsfahigkeit und Errichtung. *Skandinavische Archiv für Physiologie*. 1939;8:160-171.

104. Stellingwerff T, Boon H, Gijsen AP, Stegen JHCH, Kuipers H, van Loon LJC. Carbohydrate Supplementation During Prolonged Cycling Exercise Spares Muscle Glycogen but Does Not Affect Intramyocellular Lipid Use. *Pflugers Arch*. 2007;454(4):635-647. doi:10/c94wqz

105. Mitchell JB, Costill DL, Houmard JA, Fink WJ, Pascoe DD, Pearson DR. Influence of Carbohydrate Dosage on Exercise Performance and Glycogen Metabolism. *J Appl Physiol (1985)*. 1989;67(5):1843-1849. doi:10.1152/jappl.1989.67.5.1843

106. Coyle EF, Coggan AR, Hemmert MK, Ivy JL. Muscle Glycogen Utilization During Prolonged Strenuous Exercise When Fed Carbohydrate. *J Appl Physiol (1985)*. 1986;61(1):165-172. doi:10.1152/jappl.1986.61.1.165

107. Smith JW, Zachwieja JJ, Horswill CA, et al. Evidence of a Carbohydrate Dose and Prolonged Exercise Performance Relationship: 855: June 4 8:00 AM - 8:15 AM. *Med Sci Sports Exerc*. 2010;42(5):84. doi:10/cb4d89

108. Radziuk J, Bondy DC. Abnormal Oral Glucose Tolerance and Glucose Malabsorption After Vagotomy and Pyloroplasty. A Tracer Method for Measuring Glucose Absorption Rates. *Gastroenterology*. 1982;83(5):1017-1025.

109. Jentjens RLPG, Moseley L, Waring RH, Harding LK, Jeukendrup AE. Oxidation of Combined Ingestion of Glucose and Fructose During Exercise. *J Appl Physiol (1985)*. 2004;96(4):1277-1284. doi:10/dcj5tr

110. Viribay A, Arribalzaga S, Mielgo-Ayuso J, Castañeda-Babarro A, Seco-Calvo J, Urdampilleta A. Effects of 120 g/h of Carbohydrates Intake during a Mountain Marathon on Exercise-Induced Muscle Damage in Elite Runners. *Nutrients*. 2020;12(5):E1367. doi:10/gnzbv3

111. Silva T de A e, de Souza MEDCA, de Amorim JF, Stathis CG, Leandro CG, Lima-Silva AE. Can Carbohydrate Mouth Rinse Improve Performance during Exercise? A Systematic Review. *Nutrients*. 2013;6(1):1-10. doi:10/gchnjw

112. Burke L. Low Carb High Fat (LCHF) Diets for Athletes – Third Time Lucky? *J Sci Med Sport*. 2017;20 Suppl 1:S1. doi:10/ghp7b8

113. Carey AL, Staudacher HM, Cummings NK, et al. Effects of Fat Adaptation and Carbohydrate Restoration on Prolonged Endurance Exercise. *J Appl Physiol (1985)*. 2001;91(1):115-122. doi:10.1152/jappl.2001.91.1.115

114. Lambert EV, Speechly DP, Dennis SC, Noakes TD. Enhanced Endurance in Trained Cyclists During Moderate Intensity Exercise Following 2 Weeks Adaptation to a High Fat Diet. *Eur J Appl Physiol Occup Physiol*. 1994;69(4):287-293. doi:10.1007/bf00392032

115. Lambert EV, Hawley JA, Goedecke J, Noakes TD, Dennis SC. Nutritional Strategies for Promoting Fat Utilization and Delaying the Onset of Fatigue During Prolonged Exercise. *J Sports Sci*. 1997;15(3):315-324. doi:10.1080/026404197367326

116. Kenney WL, Wilmore JH, Costill DL. *Physiology of Sport and Exercise*. 7th ed. Human Kinetics; 2020.

117. Volek JS, Freidenreich DJ, Saenz C, et al. Metabolic Characteristics of Keto-Adapted Ultra-Endurance Runners. *Metabolism*. 2016;65(3):100-110. doi:10.1016/j.metabol.2015.10.028

118. Burke LM, Angus DJ, Cox GR, et al. Effect of Fat Adaptation and Carbohydrate Restoration on Metabolism and Performance During Prolonged Cycling. *J Appl Physiol (1985)*. 2000;89(6):2413-2421. doi:10.1152/jappl.2000.89.6.2413

119. Burke LM, Ross ML, Garvican-Lewis LA, et al. Low Carbohydrate, High Fat Diet Impairs Exercise Economy and Negates the Performance Benefit From Intensified Training in Elite Race Walkers. *J Physiol*. 2017;595(9):2785-2807. doi:10.1113/JP273230

120. Burke LM, Sharma AP, Heikura IA, et al. Crisis of Confidence Averted: Impairment of Exercise Economy and Performance in Elite Race Walkers by Ketogenic Low Carbohydrate, High Fat (LCHF) Diet Is Reproducible. *PLoS One*. 2020;15(6):e0234027. doi:10/gg23h5

121. Durkalec-Michalski K, Nowaczyk PM, Siedzik K. Effect of a Four-Week Ketogenic Diet on Exercise Metabolism in Crossfit-Trained Athletes. *J Int Soc Sports Nutr*. 2019;16(1):16. doi:10.1186/s12970-019-0284-9

122. McSwiney FT, Wardrop B, Hyde PN, Lafountain RA, Volek JS, Doyle L. Keto-Adaptation Enhances Exercise Performance and Body Composition Responses to Training in Endurance Athletes. *Metabolism*. 2018;81:25-34. doi:10.1016/j.metabol.2017.10.010

123. Prins PJ, Noakes TD, Welton GL, et al. High Rates of Fat Oxidation Induced by a Low-Carbohydrate, High-Fat Diet, Do Not Impair 5-km Running Performance in Competitive Recreational Athletes. *J Sports Sci Med*. 2019;18(4):738-750.

124. Stepto NK, Carey AL, Staudacher HM, Cummings NK, Burke LM, Hawley JA. Effect of Short-Term Fat Adaptation on High-Intensity Training. *Med Sci Sports Exerc*. 2002;34(3):449-455. doi:10.1097/00005768-200203000-00011

125. Burke LM. Fueling Strategies to Optimize Performance: Training High or Training Low? *Scand J Med Sci Sports*. 2010;20(s2):48-58. doi:10/dfcdrj

126. Morton JP, Croft L, Bartlett JD, et al. Reduced Carbohydrate Availability Does Not Modulate Training-Induced Heat Shock Protein Adaptations but Does Upregulate Oxidative Enzyme Activity in Human Skeletal Muscle. *J Appl Physiol (1985)*. 2009;106(5):1513-1521. doi:10/dm2dg3

127. Impey SG, Hearris MA, Hammond KM, et al. Fuel for the Work Required: A Theoretical Framework for Carbohydrate Periodization and the Glycogen Threshold Hypothesis. *Sports Med*. 2018;48(5):1031-1048. doi:10/gdwk98

128. Impey SG, Hammond KM, Shepherd SO, et al. Fuel for the Work Required: A Practical Approach to Amalgamating Train-Low Paradigms for Endurance Athletes. *Physiol Rep*. 2016;4(10):e12803. doi:10.14814/phy2.12803

129. Cox PJ, Kirk T, Ashmore T, et al. Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. *Cell Metab*. 2016;24(2):256-268. doi:10/bm8z

130. Egan B, D'Agostino DP. Fueling Performance: Ketones Enter the Mix. *Cell Metab*. 2016;24(3):373-375. doi:10/gmj347

131. Pinckaers PJM, Churchward-Venne TA, Bailey D, van Loon LJC. Ketone Bodies and Exercise Performance: The Next Magic Bullet or Merely Hype? *Sports Med*. 2017;47(3):383-391. doi:10/f92sp9

132. Dearlove DJ, Faull OK, Clarke K. Context Is Key: Exogenous Ketosis and Athletic Performance. *Curr Opin Physiol*. 2019;10:81-89. doi:10/gnz7qz

133. Vitale K, Getzin A. Nutrition and Supplement Update for the Endurance Athlete: Review and Recommendations. *Nutrients*. 2019;11(6):1289. doi:10/gg2bd4

134. Grgic J, Grgic I, Pickering C, Schoenfeld BJ, Bishop DJ, Pedisic Z. Wake Up and Smell the Coffee: Caffeine Supplementation and Exercise Performance—An Umbrella Review of 21 Published Meta-Analyses. *Br J Sports Med*. Published online 2019:bjsports-2018-100278. doi:10.1136/bjsports-2018-100278

135. Guest N, Corey P, Vescovi J, El-Sohemy A. Caffeine, CYP1A2 Genotype, and Endurance Performance in Athletes. *Med Sci Sports Exerc*. 2018;50(8):1570-1578. doi:10.1249/MSS.0000000000001596

136. Womack CJ, Saunders MJ, Bechtel MK, et al. The Influence of a CYP1A2 Polymorphism on the Ergogenic Effects of Caffeine. *J Int Soc Sports Nutr*. 2012;9(1):7. doi:10.1186/1550-2783-9-7

137. Grgic J, Pickering C, Del Coso J, Schoenfeld BJ, Mikulic P. CYP1A2 Genotype and Acute Ergogenic Effects of Caffeine Intake on Exercise Performance: A Systematic Review. *Eur J Nutr*. 2021;60(3):1181-1195. doi:10/gnz7qx

138. Graham TE. Caffeine and Exercise: Metabolism, Endurance and Performance. *Sports Med*. 2001;31(11):785-807. doi:10/fhqpch

139. Acheson KJ, Zahorska-Markiewicz B, Pittet P, Anantharaman K, Jequier E. Caffeine and Coffee: Their Influence on Metabolic Rate and Substrate Utilization in Normal Weight and Obese Individuals. *Am J Clin Nutr*. 1980;33(5):989-997. doi:10.1093/ajcn/33.5.989

140. Davis JM, Zhao Z, Stock HS, Mehl KA, Buggy J, Hand GA. Central Nervous System Effects of Caffeine and Adenosine on Fatigue. *Am J Physiol Regul Integr Comp Physiol*. 2003;284(2):R399-404. doi:10/gnz7qw

141. Greer F, Friars D, Graham TE. Comparison of Caffeine and Theophylline Ingestion: Exercise Metabolism and Endurance. *J Appl Physiol (1985)*. 2000;89(5):1837-1844. doi:10/gnz7qv

142. Greer F, McLean C, Graham TE. Caffeine, Performance, and Metabolism During Repeated Wingate Exercise Tests. *J Appl Physiol (1985)*. 1998;85(4):1502-1508. doi:10/gnz7qt

143. Graham TE, Spriet LL. Metabolic, Catecholamine, and Exercise Performance Responses to Various Doses of Caffeine. *J Appl Physiol (1985)*. 1995;78(3):867-874. doi:10/gnfrtg

144. Van Soeren MH, Graham TE. Effect of Caffeine on Metabolism, Exercise Endurance, and Catecholamine Responses After Withdrawal. *J Appl Physiol (1985)*. 1998;85(4):1493-1501. doi:10/gnz7qr

145. Jones AM, Thompson C, Wylie LJ, Vanhatalo A. Dietary Nitrate and Physical Performance. *Annu Rev Nutr*. 2018;38:303-328. doi:10/gns7js

146. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Effects of Dietary Nitrate on Oxygen Cost During Exercise. *Acta Physiol (Oxf)*. 2007;191(1):59-66. doi:10/bj3fs8

147. Bailey SJ, Winyard P, Vanhatalo A, et al. Dietary Nitrate Supplementation Reduces the O2 Cost of Low-Intensity Exercise and Enhances Tolerance to High-Intensity Exercise in Humans. *J Appl Physiol (1985)*. 2009;107(4):1144-1155. doi:10/fmfbwr

148. Nadel ER. Physiological Adaptations to Aerobic Training: The Increase in Blood Volume Induced by Aerobic Conditioning Is a Critical Factor in Improving Performance and Promoting Resistance to Fatigue. *Am Sci*. 1985;73(4):334-343.

149. Arbab-Zadeh A, Perhonen M, Howden E, et al. Cardiac remodeling in response to 1 year of intensive endurance training. *Circulation*. 2014;130(24):2152-2161. doi:10/f6sdnd

150. Bangsbo J, Gunnarsson TP, Wendell J, Nybo L, Thomassen M. Reduced Volume and Increased Training Intensity Elevate Muscle Na+-K+ Pump alpha2-Subunit Expression As Well as Short- and Long-Term Work Capacity in Humans. *J Appl Physiol (1985)*. 2009;107(6):1771-1780. doi:10/bwh6gz

151. MacInnis MJ, Gibala MJ. Physiological Adaptations to Interval Training and the Role of Exercise Intensity. *J Physiol*. 2017;595(9):2915-2930. doi:10/ggj7rq

152. Weston AR, Myburgh KH, Lindsay FH, Dennis SC, Noakes TD, Hawley JA. Skeletal Muscle Buffering Capacity and Endurance Performance After High-Intensity Interval Training by Well-Trained Cyclists. *Eur J Appl Physiol Occup Physiol*. 1997;75(1):7-13. doi:10/dtk997

153. Pelliccia A, Maron BJ, Spataro A, Proschan MA, Spirito P. The Upper Limit of Physiologic Cardiac Hypertrophy in Highly Trained Elite Athletes. *N Engl J Med*. 1991;324(5):295-301. doi:10/bns7sj

154. Pelliccia A, Culasso F, Di Paolo FM, Maron BJ. Physiologic Left Ventricular Cavity Dilatation in Elite Athletes. *Ann Intern Med*. 1999;130(1):23-31. doi:10/gj5sn7

155. Pressler A, Haller B, Scherr J, et al. Association of Body Composition and Left Ventricular Dimensions in Elite Athletes. *Eur J Prev Cardiol*. 2012;19(5):1194-1204. doi:10/fk8xjd

156. Spirito P, Pelliccia A, Proschan MA, et al. Morphology of the "Athlete's Heart" Assessed by Echocardiography in 947 Elite Athletes Representing 27 Sports. *Am J Cardiol*. 1994;74(8):802-806. doi:10/dn88pp

157. Baggish AL, Yared K, Weiner RB, et al. Differences in Cardiac Parameters Among Elite Rowers and Subelite Rowers. *Med Sci Sports Exerc*. 2010;42(6):1215-1220. doi:10/d8cjtf

158. Naylor LH, Arnolda LF, Deague JA, et al. Reduced Ventricular Flow Propagation Velocity in Elite Athletes Is Augmented With the Resumption of Exercise Training. *J Physiol*. 2005;563(Pt 3):957-963. doi:10/d8cr57

159. Prasad A, Popovic ZB, Arbab-Zadeh A, et al. The Effects of Aging and Physical Activity on Doppler Measures of Diastolic Function. *Am J Cardiol*. 2007;99(12):1629-1636. doi:10/fhbtxv

160. Yellin EL, Nikolic S, Frater RW. Left Ventricular Filling Dynamics and Diastolic Function. *Prog Cardiovasc Dis*. 1990;32(4):247-271. doi:10/b67ww5

161. Green DJ, Spence A, Rowley N, Thijssen DHJ, Naylor LH. Vascular Adaptation in Athletes: Is There an 'Athlete's Artery'? *Exp Physiol*. 2012;97(3):295-304. doi:10/fwgfz9

162. Andersen P, Henriksson J. Capillary Supply of the Quadriceps Femoris Muscle of Man: Adaptive Response to Exercise. *J Physiol*. 1977;270(3):677-690. doi:10/gj5tj6

163. Cocks M, Shaw CS, Shepherd SO, et al. Sprint Interval and Endurance Training Are Equally Effective in Increasing Muscle Microvascular Density and eNOS Content in Sedentary Males. *J Physiol*. 2013;591(3):641-656. doi:10/bnhf

164. Convertino VA. Blood Volume Response to Physical Activity and Inactivity. *Am J Med Sci*. 2007;334(1):72-79. doi:10/bdvb5r

165. Heinicke K, Wolfarth B, Winchenbach P, et al. Blood Volume and Hemoglobin Mass in Elite Athletes of Different Disciplines. *Int J Sports Med*. 2001;22(7):504-512. doi:10/dqxvst

166. Gillen CM, Lee R, Mack GW, Tomaselli CM, Nishiyasu T, Nadel ER. Plasma Volume Expansion in Humans After a Single Intense Exercise Protocol. *J Appl Physiol (1985)*. 1991;71(5):1914-1920. doi:10/gj5t33

167. Nummela A, Eronen T, Koponen A, Tikkanen H, Peltonen JE. Variability in Hemoglobin Mass Response to Altitude Training Camps. *Scand J Med Sci Sports*. 2021;31(1):44-51. doi:10/gj428r

168. Rønnestad BR, Hamarsland H, Hansen J, et al. Five Weeks of Heat Training Increases Haemoglobin Mass in Elite Cyclists. *Exp Physiol*. 2021;106(1):316-327. doi:10/gj428p

169. Steiner T, Maier T, Wehrlin JP. Effect of Endurance Training on Hemoglobin Mass and VO2max in Male Adolescent Athletes. *Med Sci Sports Exerc*. 2019;51(5):912-919. doi:10/gj428q

170. Coyle EF, Hemmert MK, Coggan AR. Effects of Detraining on Cardiovascular Responses to Exercise: Role of Blood Volume. *J Appl Physiol (1985)*. 1986;60(1):95-99. doi:10/gj6tzs

171. Holloszy JO, Coyle EF. Adaptations of Skeletal Muscle to Endurance Exercise and Their Metabolic Consequences. *J Appl Physiol Respir Environ Exerc Physiol*. 1984;56(4):831-838. doi:10/ghk6sm

172. Lundby C, Jacobs RA. Adaptations of Skeletal Muscle Mitochondria to Exercise Training. *Exp Physiol*. 2016;101(1):17-22. doi:10/f77bzd

173. Ryan MT, Hoogenraad NJ. Mitochondrial-Nuclear Communications. *Annu Rev Biochem*. 2007;76:701-722. doi:10/fh7c6m

174. King A. Could Mitochondria Help Athletes to Make Gains? *Nature*. 2021;592(7852):S7-S9. doi:10/gj5v5v

175. Bosquet L, Léger L, Legros P. Blood Lactate Response to Overtraining in Male Endurance Athletes. *Eur J Appl Physiol*. 2001;84(1):107-114. doi:10/bdckk2

176. Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, Holloszy JO. Mitochondrial Enzymes Increase in Muscle in Response to 7–10 Days of Cycle Exercise. *J Appl Physiol (1985)*. 1996;80(6):2250-2254. doi:10/gj6vqp

177. Murias JM, Kowalchuk JM, Ritchie D, Hepple RT, Doherty TJ, Paterson DH. Adaptations in Capillarization and Citrate Synthase Activity in Response to Endurance Training in Older and Young Men. *J Gerontol A Biol Sci Med Sci*. 2011;66A(9):957-964. doi:10/fvgc4r

178. Wibom R, Hultman E, Johansson M, Matherei K, Constantin-Teodosiu D, Schantz PG. Adaptation of Mitochondrial ATP Production in Human Skeletal Muscle to Endurance Training and Detraining. *J Appl Physiol (1985)*. 1992;73(5):2004-2010. doi:10.1152/jappl.1992.73.5.2004

179. Wilson JM, Loenneke JP, Jo E, Wilson GJ, Zourdos MC, Kim JS. The Effects of Endurance, Strength, and Power Training on Muscle Fiber Type Shifting. *J Strength Cond Res*. 2012;26(6):1724-1729. doi:10/b5v7b5

180. Kyröläinen H, Kivelä R, Koskinen S, et al. Interrelationships between Muscle Structure, Muscle Strength, and Running Economy. *Med Sci Sports Exerc*. 2003;35(1):45-49. doi:10/d9km89

181. Barstow TJ, Jones AM, Nguyen PH, Casaburi R. Influence of Muscle Fiber Type and Pedal Frequency on Oxygen Uptake Kinetics of Heavy Exercise. *J Appl Physiol (1985)*. 1996;81(4):1642-1650. doi:10/gj6wff

182. Inbar O, Kaiser P, Tesch P. Relationships Between Leg Muscle Fiber Type Distribution and Leg Exercise Performance. *Int J Sports Med*. 1981;2(3):154-159. doi:10/c6c32s

183. Andersen P, Henriksson J. Training Induced Changes in the Subgroups of Human Type II Skeletal Muscle Fibres. *Acta Physiol Scand*. 1977;99(1):123-125. doi:10/fnscxn

184. Green HJ, Thomson JA, Daub WD, Houston ME, Ranney DA. Fiber Composition, Fiber Size and Enzyme Activities in Vastus Lateralis of Elite Athletes Involved in High Intensity Exercise. *Eur J Appl Physiol Occup Physiol*. 1979;41(2):109-117. doi:10/bw2p8p

185. Schantz PG, Dhoot GK. Coexistence of Slow and Fast Isoforms of Contractile and Regulatory Proteins in Human Skeletal Muscle Fibres Induced by Endurance Training. *Acta Physiol Scand*. 1987;131(1):147-154. doi:10/bbjv5w

186. Schantz P, Henriksson J. Increases in Myofibrillar ATPase Intermediate Human Skeletal Muscle Fibers in Response to Endurance Training. *Muscle Nerve*. 1983;6(8):553-556. doi:10/b5jx3z

187. Wenger HA, Bell GJ. The Interactions of Intensity, Frequency and Duration of Exercise Training in Altering Cardiorespiratory Fitness. *Sports Med*. 1986;3(5):346-356. doi:10/fbqzcf

188. Milesis CA, Pollock ML, Bah MD, Ayres JJ, Ward A, Linnerud AC. Effects of Different Durations of Physical Training on Cardiorespiratory Function, Body Composition, and Serum Lipids. *Res Q*. 1976;47(4):716-725. doi:10/gj7hm8

189. Hickson RC, Kanakis C, Davis JR, Moore AM, Rich S. Reduced Training Duration Effects on Aerobic Power, Endurance, and Cardiac Growth. *J Appl Physiol Respir Environ Exerc Physiol*. 1982;53(1):225-229. doi:10/gj7hnb

190. Gillen JB, Percival ME, Skelly LE, et al. Three Minutes of All-Out Intermittent Exercise per Week Increases Skeletal Muscle Oxidative Capacity and Improves Cardiometabolic Health. *PLoS One*. 2014;9(11):e111489. doi:10.1371/journal.pone.0111489

191. Gettman LR, Pollock ML, Durstine JL, Ward A, Ayres J, Linnerud AC. Physiological Responses of Men to 1, 3, and 5 Day per Week Training Programs. *Res Q*. 1976;47(4):638-646. doi:10/gj7ht8

192. Pollock ML, Miller HS, Linnerud AC, Cooper KH. Frequency of Training as a Determinant for Improvement in Cardiovascular Function and Body Composition of Middle-Aged Men. *Arch Phys Med Rehabil*. 1975;56(4):141-145.

193. Thompson WR. Worldwide Survey of Fitness Trends for 2021. *ACSMs Health Fit J*. 2021;25(1):10-19. doi:10/gj7hwg

194. Sloth M, Sloth D, Overgaard K, Dalgas U. Effects of Sprint Interval Training on VO2max and Aerobic Exercise Performance: A Systematic Review and Meta-Analysis. *Scand J Med Sci Sports*. 2013;23(6):e341-352. doi:10/f5nmm4

195. Macpherson REK, Hazell TJ, Olver TD, Paterson DH, Lemon PWR. Run Sprint Interval Training Improves Aerobic Performance but Not Maximal Cardiac Output. *Med Sci Sports Exerc*. 2011;43(1):115-122. doi:10.1249/MSS.0b013e3181e5eacd

196. Rosenblat MA, Granata C, Thomas SG. Effect of Interval Training on the Factors Influencing Maximal Oxygen Consumption: A Systematic Review and Meta-Analysis. *Sports Med*. Published online January 18, 2022. doi:10.1007/s40279-021-01624-5

197. Davies CTM, Knibbs AV. The Training Stimulus. *Int Z Angew Physiol Einschl Arbeitsphysiol*. 1971;29(4):299-305. doi:10/cjfj3m

198. Gaesser GA, Rich RG. Effects of High- and Low-Intensity Exercise Training on Aerobic Capacity and Blood Lipids. *Med Sci Sports Exerc*. 1984;16(3):269-274.

199. Burlot F, Richard R, Joncheray H. The Life of High-Level Athletes: The Challenge of High Performance Against the Time Constraint. *Int Rev Sport Sociol*. 2018;53(2):234-249. doi:10/gc2ndz

200. Lehmann M, Foster C, Keul J. Overtraining in Endurance Athletes: A Brief Review. *Med Sci Sports Exerc*. 1993;25(7):854-862. doi:10/cfw52f

201. Mountjoy M, Sundgot-Borgen J, Burke L, et al. The IOC consensus statement: beyond the Female Athlete Triad--Relative Energy Deficiency in Sport (RED-S). *Br J Sports Med*. 2014;48(7):491-497. doi:10/f5zq8r

202. Seiler S, Haugen O, Kuffel E. Autonomic Recovery After Exercise in Trained Athletes: Intensity and Duration Effects. *Med Sci Sports Exerc*. 2007;39(8):1366-1373. doi:10.1249/mss.0b013e318060f17d

203. Seiler KS, Kjerland GØ. Quantifying Training Intensity Distribution in Elite Endurance Athletes: Is There Evidence for an "Optimal" Distribution? *Scand J Med Sci Sports*. 2006;16(1):49-56. doi:10/btm8mw

204. Coggan AR. Power Training Zones for Cycling. TrainingPeaks. Published April 29, 2016. Accessed May 26, 2021. https://www.trainingpeaks.com/blog/power-training-levels/

205. San Millán I. Zone 2 Training For Endurance Athletes. TrainingPeaks. Published April 2, 2014. Accessed May 26, 2021. https://www.trainingpeaks.com/blog/zone-2-training-for-endurance-athletes/

206. Sylta Ø, Tønnessen E, Seiler S. From Heart-Rate Data to Training Quantification: A Comparison of 3 Methods of Training-Intensity Analysis. *Int J Sports Physiol Perform*. 2014;9(1):100-107. doi:10.1123/ijspp.2013-0298

207. Janssen PGJM. *Lactate Threshold Training*. 1st ed. Human Kinetics; 2001.

208. Solli GS, Tønnessen E, Sandbakk Ø. The Training Characteristics of the World's Most Successful Female Cross-Country Skier. *Front Physiol*. 2017;8. doi:10/ggkf9x

209. Guellich A, Seiler KS, Emrich E. Training Methods and Intensity Distribution of Young World-Class Rowers. *Int J Sports Physiol Perform*. 2009;4(4):448-460. doi:10/ghk2ct

210. Fiskerstrand A, Seiler KS. Training and Performance Characteristics Among Norwegian International Rowers 1970–2001. *Scand J Med Sci Sports*. 2004;14(5):303-310. doi:10/dnqrth

211. Billat VL, Demarle A, Slawinski J, Paiva M, Koralsztein JP. Physical and Training Characteristics of Top-Class Marathon Runners. *Med Sci Sports Exerc*. 2001;33(12):2089-2097. doi:10.1097/00005768-200112000-00018

212. Esteve-Lanao J, Juan AFS, Earnest CP, Foster C, Lucia A. How Do Endurance Runners Actually Train? Relationship With Competition Performance. *Med Sci Sports Exerc*. 2005;37(3):496-504. doi:10.1249/01.mss.0000155393.78744.86

213. Tønnessen E, Svendsen IS, Rønnestad BR, Hisdal J, Haugen TA, Seiler KS. The Annual Training Periodization of 8 World Champions in Orienteering. *Int J Sports Physiol Perform*. 2015;10(1):29-38. doi:10/f8mmgt

214. Muñoz I, Cejuela R, Seiler KS, Larumbe E, Esteve-Lanao J. Training-Intensity Distribution During an Ironman Season: Relationship With Competition Performance. *Int J Sports Physiol Perform*. 2014;9(2):332-339. doi:10/f5v8x8

215. Seiler KS. What Is Best Practice for Training Intensity and Duration Distribution in Endurance Athletes? *Int J Sports Physiol Perform*. 2010;5(3):276-291. doi:10/ggkgpc

216. Seiler KS, Jøranson K, Olesen BV, Hetlelid KJ. Adaptations to Aerobic Interval Training: Interactive Effects of Exercise Intensity and Total Work Duration. *Scand J Med Sci Sports*. 2013;23(1):74-83. doi:10/dm2db5

217. Esteve-Lanao J, Foster C, Seiler S, Lucia A. Impact of Training Intensity Distribution on Performance in Endurance Athletes. *J Strength Cond Res*. 2007;21(3):943-949. doi:10/bkd6qs

218. Muñoz I, Seiler KS, Bautista J, España J, Larumbe E, Esteve-Lanao J. Does Polarized Training Improve Performance in Recreational Runners? *Int J Sports Physiol Perform*. 2014;9(2):265-272. doi:10.1123/ijspp.2012-0350

219. Neal CM, Hunter AM, Brennan L, et al. Six Weeks of a Polarized Training-Intensity Distribution Leads to Greater Physiological and Performance Adaptations Than a Threshold Model in Trained Cyclists. *J Appl Physiol (1985)*. 2012;114(4):461-471. doi:10/gfvw42

220. Stöggl T, Sperlich B. Polarized Training Has Greater Impact on Key Endurance Variables Than Threshold, High Intensity, or High Volume Training. *Front Physiol*. 2014;5:33. doi:10.3389/fphys.2014.00033

221. Stöggl TL, Sperlich B. The Training Intensity Distribution Among Well-Trained and Elite Endurance Athletes. *Front Physiol*. 2015;6:295. doi:10.3389/fphys.2015.00295

222. Burnley M, Bearden SE, Jones AM. Polarized Training is Not Optimal for Endurance Athletes. *Med Sci Sports Exerc*. Published online February 9, 2022. doi:10.1249/MSS.0000000000002869

223. Foster C, Casado A, Esteve-Lanao J, Haugen T, Seiler S. Polarized Training is Optimal for Endurance Athletes. *Med Sci Sports Exerc*. Published online February 9, 2022. doi:10.1249/MSS.0000000000002871

224. Wehrlin JP, Hallén J. Linear Decrease in VO2max and Performance With Increasing Altitude in Endurance Athletes. *Eur J Appl Physiol*. 2006;96(4):404-412. doi:10.1007/s00421-005-0081-9

225. Faulhaber M, Gröbner K, Rausch L, Gatterer H, Menz V. Effects of Acute Hypoxia on Lactate Thresholds and High-Intensity Endurance Performance—A Pilot Study. *Int J Environ Res Public Health*. 2021;18(14):7573. doi:10.3390/ijerph18147573

226. van Erck D, Wenker EJ, Levels K, Foster C, de Koning JJ, Noordhof DA. Cycling at Altitude: Lower Absolute Power Output as the Main Cause of Lower Gross Efficiency. *Int J Sports Physiol Perform*. 2019;14(8):1117-1123. doi:10.1123/ijspp.2018-0221

227. Bailey DM, Davies B, Romer L, Castell L, Newsholme E, Gandy G. Implications of Moderate Altitude Training for Sea-Level Endurance in Elite Distance Runners. *Eur J Appl Physiol*. 1998;78(4):360-368. doi:10.1007/s004210050432

228. Levine BD, Stray-Gundersen J, Mehta RD. Effect of Altitude on Football Performance. *Scand J Med Sci Sports*. 2008;18(s1):76-84. doi:10.1111/j.1600-0838.2008.00835.x

229. Hamlin MJ, Hopkins WG, Hollings SC. Effects of Altitude on Performance of Elite Track-and-Field Athletes. *Int J Sports Physiol Perform*. 2015;10(7):881-887. doi:10.1123/ijspp.2014-0261

230. Buskirk ER, Kollias J, Akers RF, Prokop EK, Reategui EP. Maximal Performance at Altitude and on Return From Altitude in Conditioned Runners. *J Appl Physiol*. 1967;23(2):259-266. doi:10.1152/jappl.1967.23.2.259

231. Lundby C, Millet GP, Calbet JA, Bärtsch P, Subudhi AW. Does 'Altitude Training' Increase Exercise Performance in Elite Athletes? *Br J Sports Med*. 2012;46(11):792-795. doi:10.1136/bjsports-2012-091231

232. Lundby C, Robach P. Does 'Altitude Training' Increase Exercise Performance in Elite Athletes? *Exp Physiol*. 2016;101(7):783-788. doi:10.1113/EP085579

233. Sawka MN, Young AJ, Cadarette BS, Levine L, Pandolf KB. Influence of Heat Stress and Acclimation on Maximal Aerobic Power. *Eur J Appl Physiol Occup Physiol*. 1985;53(4):294-298. doi:10.1007/BF00422841

234. Hettinga FJ, De Koning JJ, de Vrijer A, Wüst RCI, Daanen HAM, Foster C. The Effect of Ambient Temperature on Gross-Efficiency in Cycling. *Eur J Appl Physiol*. 2007;101(4):465-471. doi:10.1007/s00421-007-0519-3

235. Lorenzo S, Minson CT, Babb TG, Halliwill JR. Lactate Threshold Predicting Time-Trial Performance: Impact of Heat and Acclimation. *J Appl Physiol (1985)*. 2011;111(1):221-227. doi:10.1152/japplphysiol.00334.2011

236. Pryor RR, Casa DJ, Adams WM, et al. Maximizing Athletic Performance in the Heat. *Strength Cond J*. 2013;35(6):24-33. doi:10.1519/SSC.0000000000000016

237. Mitchell JB, Rogers MM, Basset JT, Hubing KA. Fatigue During High-Intensity Endurance Exercise: The Interaction Between Metabolic Factors and Thermal Stress. *J Strength Cond Res*. 2014;28(7):1906-1914. doi:10.1519/JSC.0000000000000319

238. Lorenzo S, Halliwill JR, Sawka MN, Minson CT. Heat Acclimation Improves Exercise Performance. *J Appl Physiol (1985)*. 2010;109(4):1140-1147. doi:10.1152/japplphysiol.00495.2010

239. Mitchell JB, Schiller ER, Miller JR, Dugas JP. The Influence of Different External Cooling Methods on Thermoregulatory Responses Before and After Intense Intermittent Exercise in the Heat. *J Strength Cond Res*. 2001;15(2):247-254.

240. Run Signup. *Annual Industry Report 2020*.; 2021.

241. Vandivort M. State of the sport: USAC racing in 2019 by the numbers. To Be Determined. Published 2019. Accessed November 22, 2020. https://www.tobedetermined.cc/journal/state-of-the-sport-usac-racing-in-2019

242. Outdoor Industry Association. *The Outdoor Recreation Economy*.; 2017. Accessed November 21, 2020. https://outdoorindustry.org/wp-content/uploads/2017/04/OIA_RecEconomy_FINAL_Single.pdf

243. Frederick-Recascino CM, Schuster-Smith H. Competition and Intrinsic Motivation in Physical Activity: A Comparison of Two Groups. *J Sport Behav*. 2003;26(3):240-254.

Chapter II: The Effect of ACTN3 Polymorphism on Self-Reported Running Times

Authors:

Andreas Kreutzer[1], Christopher A. Martinez[1], McKensie Kreutzer[2], Jason D. Stone[1], Joel B. Mitchell[1], and Jonathan M. Oliver[1]

Author Affiliation:

[1]Exercise & Sport Performance Laboratory, Texas Christian University, Fort Worth, TX

[2]Beutler Lab, University of Texas Southwestern Medical Center, Dallas, TX

Corresponding Author:

Andreas Kreutzer, M.S., Department of Kinesiology, Texas Christian University, Fort Worth, TX 76129, USA. Email: a.kreutzer@tcu.edu. Tel: 817-257-6859

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2.1 Abstract

This investigation examined the effect of ACTN3 genotype on self-reported distance running personal records (PR). Out of ninety-four (n = 94) recreationally active men and women, eighty-two (f = 42, m = 40; age: 22.6 ± 4.5 years; BMI: 23.5 ± 3.4 kg/m2) reported one-mile running personal records (PRs), while fifty-seven (f = 33, m = 24; age: 23.4 ± 5.3 years; BMI: 22.9 ± 9.3 kg/m2) reported 5K running PRs. Subjects were grouped by presence (ACTN3+) or absence (ACTN3-) of α-actinin-3, as well as by individual genotype (RR, RX, XX). Among female participants, ACTN3- reported 64.5 seconds faster (p = .048) one-mile PRs compared with their ACTN3+ counterparts. No differences were found when comparing 5K PRs between genotypes. Our study confirms a reportedly greater prevalence of XX benefits for endurance performance in females when compared with males, but fails to strongly link ACTN3 genotype to endurance performance. Practitioners should continue to be cautious when using genetic information for talent identification and sport selection.

Key words: endurance performance; exercise genetics; alpha-actinin-3; R577X

2.2 Introduction

Alpha-actinins serve as Z-disk proteins that form a crosslink between actin filaments of adjacent sarcomeres.[1] In addition to their structural function, α-actinins are involved in signaling and metabolic pathways, where their roles are determined based on the isoform presen.t[2] One isoform of particular interest in humans, α-actinin-3, is encoded by the *ACTN3* gene and only present in fast-twitch type II fibers.[3] A polymorphism in the *ACTN3* gene results in a cytosine to thymine transition that converts arginine (R) to a stop-codon (X) and is referred to as R577X.[4] Homozygosity for the 577X allele (XX genotype) results in complete deficiency of α-actinin-3 and compensatory upregulation of α-actinin-2, whereas heterozygosity (RX genotype) and homozygosity for the 577R allele (RR genotype) provide for the production of α-actinin-3.[5–7] RR homozygotes have been reported to possess a greater number and greater relative Type IIx fiber surface area than XX homozygotes.[8]

Recent research efforts have demonstrated relationships between different *ACTN3* genotype frequencies in human populations and geographic location as well as athletic performance.[9–15] An association between *ACTN3* and athletic performance was first established in 2003 when Yang et al. demonstrated that allele frequencies differed significantly between elite sprint and endurance athletes.[16] The authors reported fewer XX genotypes among elite sprint athletes with zero prevalence in those sprinters competing at the Olympic level. The XX genotype was overrepresented in female endurance athletes when compared to controls and sprint/power athletes. Similarly, Ben-Zaken et al. reported a significant difference in R577X polymorphism frequencies between long-distance runners and sprinters.[11] Among short distance runners, RR and RX genotypes were expressed by 83.3% of subjects, whereas only 64.6% of the long-distance

runners expressed at least one R allele. Conversely, 35.4% of the long-distance runners expressed the XX genotype, which was significantly greater than the 16.7% frequency in sprinters.

Human association studies have consistently shown an underrepresentation of the XX genotype among sprint/power athletes,[17–20] whereas an overrepresentation of the X allele among endurance athletes has been found in some,[11,16,17,21] but not all cohorts.[22–24] A number of researchers have studied *ACTN3* genotype and allele frequencies in different types of sports[11,25,26] and competition levels.[17,27] However, fewer studies have investigated the association between *ACTN3* genotype and quantitative measures of sprint/power[14,28] and endurance performance in humans.[24,29] Papadimitriou et al. found no associations between *ACTN3* polymorphisms and distance running times in Caucasian endurance athletes.[29] Conversely, in the animal model, MacArthur et al. showed greater intrinsic exercise capacity in *ACTN3* knockout (KO) mice genetically modified to be devoid of α-actinin-3.[7] KO mice on average ran 33% further than Wild Type (WT) mice, whose Type IIx muscle fibers contained α-actinin-3, in a treadmill test to exhaustion. Further, Hogarth et al. reported a dose-dependent effect of *ACTN3* genotype on endurance capacity in mice.[5] Heterogeneous (HET) mice, generated by crossing KO with WT, showed intermediate endurance running capacity compared to KO and WT mice in accordance with intermediate expression of α-actinin-2 and α-actinin-3 on the muscle level. Based on the paucity of similar endurance performance-related studies in humans and the equivocal findings in human association studies, further investigation of the effect of *ACTN3* genotype on human endurance performance is warranted.

Thus, the primary purpose of this study was to investigate the effect of *ACTN3* on self-reported distance running records (PR) in a diverse sample of young, recreationally active men

and women. We hypothesized that participants with the XX genotype would report faster distance running PRs than those with the RX and RR genotypes.

2.3 Methods

2.3.1 Experimental Approach to the Problem

To test our hypothesis that the XX genotype would report faster distance running times than those with the RX and RR genotypes, we asked subjects to self-report PRs for a variety of distances. Further, we determined their *ACTN3* genotype from buccal swabs, and compared mean running types based on genotypes. Subjects were recruited from Kinesiology courses, activity classes, local running races, and running groups. Data were collected either in the laboratory or in the field. Prior to inclusion in the study, subjects completed an informed consent form and a medical history questionnaire including details on current physical activity level to determine activity status. Those qualifying as recreationally active provided a buccal swab for later genotyping as well as a running PR questionnaire detailing personal records for distances ranging from the 100m dash to the marathon. PRs were accepted from races and personal training. Due to the scope of our study and the availability of data, independent validation of self-reported running PRs was not performed. Sufficient data for statistical analysis were reported only for the one-mile and 5K distances. DNA extraction and *ACTN3* genotyping were performed in batches and are detailed in the following sections. Subjects were informed of their *ACTN3* genotype following analysis.

2.3.2 Subjects

This study was approved by the Institutional Review Board for use in human subjects. All subjects were informed of the benefits and risks of the study before signing an approved informed consent form. Ninety-four recreationally active men and women between the ages of 18 and 35

years participated in the study. To be classified as recreationally active, subjects must have engaged in a minimum of three 40-60-minute exercise sessions per week as verified by a questionnaire. Subjects reported participation in a wide range of moderate to high intensity physical activity including recreational resistance training, running, and a variety of individual and team sports. Eighty-two subjects (African American: n = 2; Asian: n= 1; Caucasian: n = 67; Hispanic: n = 11; Native American: n = 1) reported one-mile running personal records (PR), while fifty-seven (Asian: n= 1; Caucasian: n = 47; Hispanic: n = 9) reported 5K running PRs. Subjects characteristics are presented in **Table 2.1**. For analysis, subjects were grouped by presence ($ACTN3^{+}$) or absence ($ACTN3^{-}$) of the *ACTN3* R allele to test the effect of α-actinin-3 on running times, as well as by individual genotype (RR, RX, XX).

2.3.3 Procedures

2.3.3.1 Buccal Swabs

Subjects refrained from eating, drinking, and nicotine use for 30 minutes prior to buccal swab collection. Researchers inserted a sterile flocked collection device (Puritan® PurFlock® Ultra, Puritan Diagnostics, Guilford, ME) into subjects' mouths and performed rigorous ten-second swabs of the inside of each cheek. Swabs were stored at 3-5°C for subsequent DNA isolation in batches.

Table 2.1 Subject Characteristics

Subjects reporting One-Mile Personal Records					
	n	**Age (yr)**	**Height (cm)**	**Weight (kg)**	**BMI (kg/m^2)**
All	82 (f=42, m=40)	22.6±4.5	172.8±11.2	70.6±14.3	23.5±3.4
ACTN3^{+a}	63 (f=29, m=34)	22.9±4.8	172.8±12.1	71.6±15.1	23.8±3.5
ACTN3^{-b}	19 (f=13, m=6)	21.5±3.4	172.1±8.0	67.3±10.5	22.4±2.4
RR	12 (f=6, m=6)	22.3±3.3	171.5±11.5	68.1±11.0	23.0±2.11
RX	51 (f=23, m=28)	23.0±5.1	173.1±12.3	72.4±15.9	23.9±3.8
XX	19 (f=13, m =6)	21.5±3.4	172.1±8.0	67.3±10.5	22.4±2.4
Subjects reporting 5K Personal Records					
	n	**Age (yr)**	**Height (cm)**	**Weight (kg)**	**BMI (kg/m^2)**
All	57 (f=33, m=24)	23.4±5.3	171.5±10.7	67.7±13.0	22.9±3.3
ACTN3^{+a}	42 (f=22, m=20)	24.2±5.6	171.9±11.5	68.8±13.9	23.1±3.6
ACTN3^{-b}	15 (f=11, m=4)	21.3±3.6	170.2±8.0	64.4±9.8	22.1±2.2
RR	7 (f=5, m=2)	22.3±4.2	168.4±10.2	61.9±10.5	21.7±2.5
RX	35 (f=17, m=18)	24.5±5.9	172.6±11.8	70.2±14.2	23.4±2.5
XX	15 (f=11, m=4)	21.3±3.6	170.2±8.0	64.4±9.8	22.1±2.2

[a]R allele (α-actinin-3) present (RR+RX)
[b]R allele (α-actinin-3) not present (XX)

2.3.3.2 DNA Isolation

DNA isolation was performed using a kit based (QIAamp® DNA Mini Kit, QIAGEN, Germany) extraction procedure. Samples were processed in batches with each batch including a negative control sample. The tip of each swab was transferred to a 2.0 mL microtube, and 400 µL Phosphate Buffered Saline (AMRESCO, Solon, OH) was added. Subsequently, 20 µL Proteinase K Solution (QIAGEN) and 400 µL lysis buffer (Buffer AL, QIAGEN) were added to each tube,

which were then incubated at 56°C for 10 minutes. The microtubes were then centrifuged briefly to remove drops from inside the lid. Thereafter, 400 µL absolute ethanol (MilliporeSigma, Billerica, MA) was added to all tubes.

Approximately 700 µL of the resulting mixture was applied to a QIAamp Mini spin column, which was subsequently centrifuged at 6000 x g for one minute. The resulting filtrate was discarded and the spin column was placed in a new collection tube. This step was repeated with the remaining mixture resulting in centrifugation of a total of approximately 1,100 µL per sample. Following the addition of 500 µL wash buffer 1 (Buffer AW1, QIAGEN) and centrifugation at 6000 x g for one minute, 500 µL wash buffer 2 (Buffer AW2, QIAGEN) was added to the spin column, which was subsequently centrifuged at 20,000 x g for three minutes. Centrifugation at 20,000 x g was repeated for an additional minute with a new collection tube to eliminate the change of Buffer AW2 carryover. For the final step, the QIAamp Mini spin column was placed in a clean 1.5 mL microcentrifuge tube. After addition of 150 µL of elution buffer (Buffer AE, QIAGEN), samples were incubated at room temperature for one minute and subsequently centrifuged at 6000 x g for one minute. Eluate containing the DNA was stored at -20°C in the 1.5 mL microcentrifuge tube until *ACTN3* genotyping.

2.3.3.3 ACTN3 Genotyping

ACTN3 genotype was determined using a four-primer polymerase chain reaction (PCR) protocol and gel electrophoresis as previously described by Schadock et al.[30] Briefly, PCR was performed using external primers hACTN3f (5'-CGCCCTTCAACAACTGGCTGGA-3') and hACTN3r (5'-GATGAGCCCGAGACAGGCAAGG-3') at 0.5 µM, and internal primers hACTN3Tif (5'-CAACACTGCCCGAGGCTGACTG-3') and hACTN3Cir (5'-CATGATGGCACCTCGCTCTCGG-3') at 0.125 and 0.25 µM respectively. All primers were

manufactured by Integrated DNA Technologies, Inc. (Coralville, IA). Ten microliters of primer mix, 20 µL of 2x GoTaq® Green Matermix (Promega, Madison, WI), and 10 µL of DNA sample were combined in PCR tubes and subjected to the following PCR conditions on a Bio-Rad T100™ Thermal Cycler (Bio-Rad, Hercules, CA): 95°C for 2 minutes, 35 cycles at 95°C for 10 seconds, 68°C for 10 seconds, and 72°C for 45 seconds, with a final step of 72°C for 2 minutes. PCR product was analyzed in a 2% agarose gel stained with 1:10,000 SYBR® Safe DNA gel stain (Invitrogen, Carlsbad, CA) at 120V for 45 minutes, and compared to a 100-bp ladder (Invitrogen).

2.3.3.4 Statistical analysis

For statistical analysis, subjects were grouped by sex, presence ($ACTN3^{+}$) or absence ($ACTN3^{-}$) of the *ACTN3* R allele, as well as by individual genotype (RR, RX, XX). Group means among individual genotype were compared using a one-way analysis of variance (ANOVA) with *Tukey's post-hoc test* to elucidate significant differences between genotype groups. Independent-samples t-tests were employed to compare $ACTN3^{+}$ and $ACTN3^{-}$. The alpha level was set at 0.05. Statistical tests were performed using SPSS® Statistics Version 24 (IBM, Armonk, North Castle, NY) and R Statistics Version 3.5.1 with RStudio Version 1.1.456.[31,32] Effect sizes were calculated using spreadsheets provided by Lakens, while 95% Confidence Intervals for the effect sizes were calculated using the MBESS package in R.[33,34] To address the suggested sex-difference in the effect of *ACTN3* polymorphisms on endurance performance, we performed additional analyses of genotype effects within male and female cohorts.[16] Further, to address possible effects of training status, sub-group analyses were performed on a more homogenous, faster group of subjects reporting one-mile times <420 seconds. Similar to Papadimitriou et al. sub-groups with very small sample sizes ($n < 6$) were not analyzed.[29] Thus, we performed further analyses on one-mile PRs

among fast subjects, and within male and female cohorts, but were unable to do the same for 5K PRs.

Along with all t-tests, two one-sided tests (TOST) were performed to test for statistical equivalence of the means as described by Lakens using the TOSTER package in R.[35] Briefly, the TOST procedure specifies lower and upper bounds, "such that results falling within this range are deemed equivalent to the absence of an effect that is worthwhile to examine".[35] The alpha-level for TOST was set at 0.05, and a significant p-value in this test was considered indicative of statistical equivalence[35]. The lower and upper bounds for smallest effect size of interest (SESOI) in race PRs was calculated based on publically available results of age-group road race USA national championships in the respective distances. Based on the public availability of results, finishing times in the one-mile national championships from 2014-2017 and finishing times in the 5K national championships from 2007-2017 were included in the analysis. To calculate threshold values, time differences between the top three finishers in every year were averaged. When comparing all subjects by genotype, time differences among male and female top three finishers were included, whereas time differences between males only and females only were averaged to obtain threshold values for within-sex analysis respectively.

2.4 Results

Self-reported one-mile PRs are shown in **Table 2.2**. No statistically significant differences [$F(2,79) = 0.075$, $p = 0.928$, $\eta^2_p = 0.002$ (95% CI of η^2_p: 0.000 – 0.026)] were found between individual genotypes when comparing PRs among all subjects. Similarly, no difference [$t(80) = 0.386$, $p = 0.701$, Cohen's $d = 0.102$ (95%CI for Cohen's d: -0.413 – 0.614] was found between the $ACTN3^-$ and $ACTN3^+$ groups. In a subset of faster subjects, who reported one-mile PRs <420 seconds, no differences [$F(2,48) = 0.790$, $p = 0.460$, $\eta^2_p = 0.032$ (0.000 – 0.043)] were found

between individual genotypes. Further, the independent t-test revealed no difference [t(49) = 1.257, p = 0.215, Cohen's d = 0.452 (-0.256 – 1.138)] between $ACTN3^{-}$ and $ACTN3^{+}$.

In the within-sex sub analysis (**Table 2.3**), ANOVA revealed no significant difference between individual genotypes [F(2,37) = 0.357, p = 0.702, η^2_p = 0.019 (0.000 – 0.129)] among males. Similarly, the independent t-test revealed no differences [t(38) = -0.852, p = 0.400, Cohen's d = 0.387 (-1.247 – 0.497)] between $ACTN3^{-}$ and $ACTN3^{+}$

Among female subjects, those in the $ACTN3^{-}$ group reported significantly faster [t(40) = 2.041, p = 0.048, Cohen's d = 0.698 (0.006 – 1.348)] one-mile PRs (-64.5, ±53.0 seconds) than those in the $ACTN3^{+}$ group. ANOVA showed no significant differences [F(2,39) = 2.120, p = 0.134, η^2_p = 0.098 (0.000 – 0.264)] based on individual genotypes among females.

TOST equivalence testing of reported one-mile PRs revealed that none of the detected effects were equivalent to zero. **Figure 2.1** shows all TOST results regarding one-mile PRs.

Table 2.2. Self-Reported One-Mile Personal Records - All

All subjects							
	n	**male**	**female**	**time[a] (sec)**	**95% CI M (sec)**	**95% CI M_{diff} (sec)**	**p**
ACTN3[+c]	63	34	29	418.7±99.1	393.7 – 443.6	-40.9 – 60.5	.701
ACTN3[-d]	19	6	13	408.8±91.0	365.0 – 452.7		
RR	12	6	6	417.4±83.4	364.4 – 470.4	-77.7 – 94.9	.969
XX	19	6	13	408.8±91.0	365.0 – 452.7		
RR	12	6	6	417.4±83.4	364.4 – 470.4	-76.6 – 73.5	.999
RX	51	28	23	419.0±103.2	389.9 – 448.0		
RX	51	28	23	419.0±103.2	389.9 – 448.0	-52.8 – 73.0	.922
XX	19	6	13	408.8±91.0	365.0 – 452.7		
Fast subjects (<420 sec)							
	n	**male**	**female**	**time[a] (sec)**	**95% CI M (sec)**	**95% CI M_{diff} (sec)**	**p**
ACTN3[+b]	41	31	10	362.6±42.3	349.2 – 375.9	-11.7 – 50.8	.215
ACTN3[-c]	10	4	6	343.0±51.4	306.2 – 379.8		
RR	7	6	1	359.9±52.4	311.4 – 408.3	-36.3 – 70.0	.725
XX	10	4	6	343.0±51.4	306.2 – 379.8		
RR	7	6	1	359.9±52.4	311.4 – 408.3	-48.0 – 41.5	.983
RX	34	25	9	363.1±40.9	348.9 – 377.4		
RX	34	25	9	363.1±40.9	348.9 – 377.4	-18.7 – 58.9	.427
XX	10	4	6	343.0±51.4	306.2 – 379.8		

[a]Mean time ± standard deviation
[b]R allele (α-actinin-3) present (RR+RX)
[c]R allele (α-actinin-3) not present (XX)

Table 2.3. Self-Reported One-Mile Personal Records By Sex

Male subjects					
	n	**time[a] (sec)**	**95% CI M (sec)**	**95% CI M_{diff} (sec)**	**p**
ACTN3[+c]	34	365.3±57.3	345.3 – 385.3		
				-89.2 – 36.4	.400
ACTN3[-d]	6	391.7±124.8	260.7 – 522.6		
RR	6	367.5±52.9	312.0 – 423.0		
				-77.7 – 94.9	.826
XX	6	391.7±124.8	260.7 – 522.6		
RR	6	367.5±52.9	312.0 – 423.0		
				-76.7 – 73.5	.996
RX	28	364.8±59.1	341.9 – 387.7		
RX	28	364.8±59.1	341.9 – 387.7		
				-52.8 – 73.0	.826
XX	6	391.7±124.8	260.7 – 522.6		
Female subjects					
	n	**time[a] (sec)**	**95% CI M (sec)**	**95% CI M_{diff} (sec)**	**p**
ACTN3[+b]	29	481.3±101.8	442.6 – 520.0		
				0.6 – 128.4	.048
ACTN3[-c]	13	416.8±75.6	371.1 – 462.5		
RR	6	467.4±80.8	382.5 – 552.1		
				-64.5 – 165.6	.538
XX	13	416.8±75.6	371.1 – 462.5		
RR	6	467.4±80.8	382.5 – 552.1		
				-124.4 – 89.3	.915
RX	23	484.9±107.8	438.3 – 531.5		
RX	23	484.9±107.8	438.3 – 531.5		
				-12.7 – 149.0	.113
XX	13	416.8±75.6	371.1 – 462.5		

[a]Mean time ± standard deviation (sec)
[b]R allele (α-actinin-3) present (RR+RX)
[c]R allele (α-actinin-3) not present (XX)

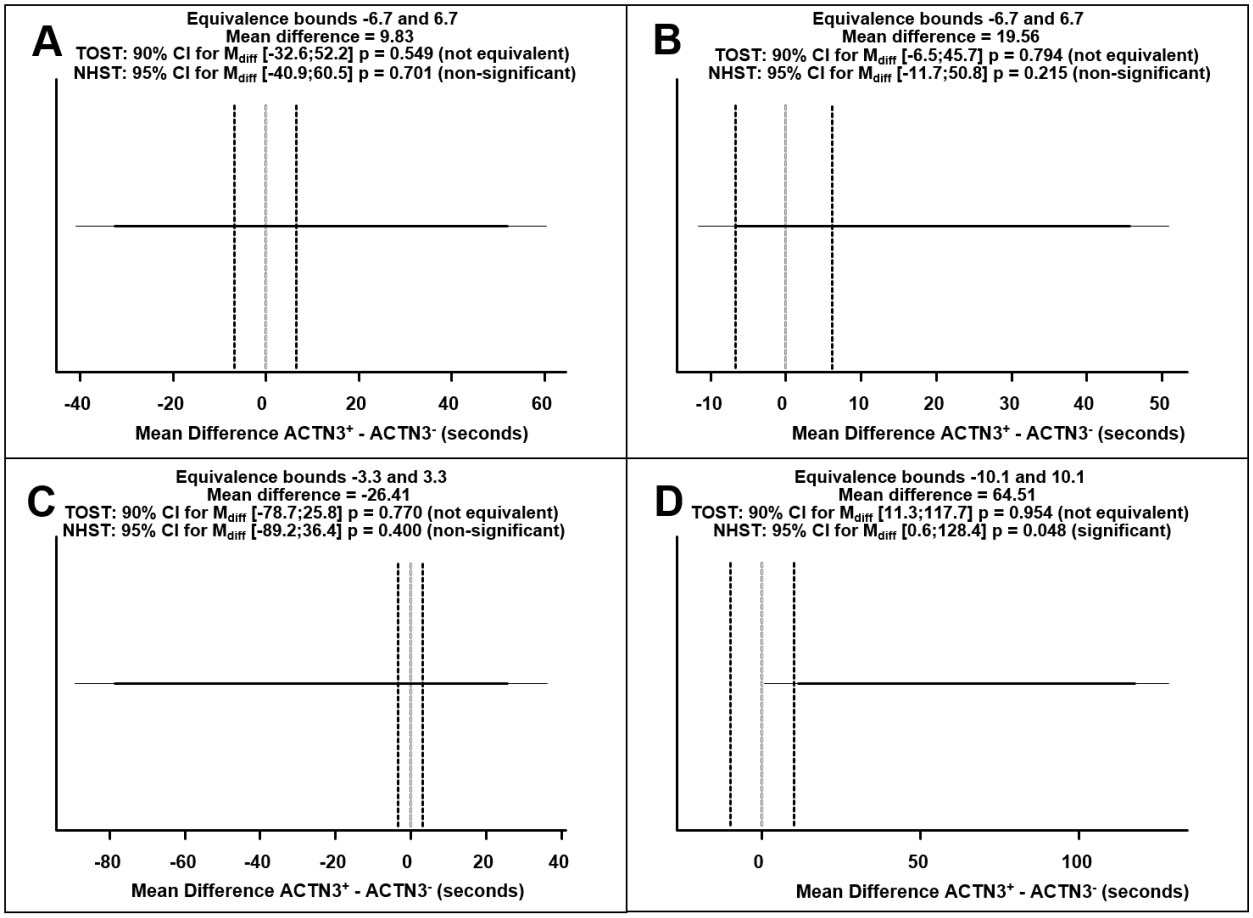

Figure 2.1 One-Mile PR Equivalence Tests. Mean difference in self-reported one-mile Personal Records (black squares), 90% confidence intervals (CIs; thick horizontal lines), and 95% CIs (thin horizontal lines) with equivalence bounds (dark dashed vertical lines) comparing $ACTN3^{+}$ and $ACTN3^{-}$ in (A) all subjects reporting one-mile times, (B) fast subjects reporting one-mile times <420 seconds, (C) male subjects reporting one-mile times, and (D) female subjects reporting one-mile times. If a NHST interval does not cross zero, the test is statistically significant. If the TOST interval lies within its equivalence bounds the effect is considered statistically equivalent to zero.

No statistically significant differences were found when comparing self-reported 5K running PRs between groups in the ANOVA [$F(2,54) = 0.645$, $p = 0.529$, $\eta^2_p = 0.023$ (0.000 – 0.081)] or the t-test [$t(55) = 0.261$, $p = 0.795$, Cohen's $d = 0.080$ (-0.512 – 0.668)]. All 5K running PRs among all subjects are shown in **Table 2.4**.

Table 2.4. Self-Reported 5K Personal Records - All

All subjects							
	n	**male**	**female**	**time[a] (sec)**	**95% CI M (sec)**	**95% CI M_{diff} (sec)**	**p**
ACTN3[+b]	42	20	22	1421.5±327.1	1319.5 – 1523.4		
						-180.3 – 234.2	.795
ACTN3[-c]	15	4	11	1394.5±388.7	1179.2 – 1609.7		
RR	7	2	5	1290.6±292.6	1019.6 – 1561.5		
						-492.0 – 284.2	.787
XX	15	4	11	1394.5±388.7	1179.2 – 1609.7		
RR	7	2	5	1290.6±292.6	1019.6 – 1561.5		
						-508.1 – 194.0	.515
RX	35	18	17	1447.6±331.1	1333.9 – 1561.4		
RX	35	18	17	1447.6±331.1	1333.9 – 1561.4		
						-208.5 – 314.8	.871
XX	15	4	11	1394.5±388.7	1179.2 – 1609.7		

[a]Mean time ± standard deviation
[b]R allele (α-actinin-3) present (RR+RX)
[c]R allele (α-actinin-3) not present (XX)

While no statistically significant differences were found in reported 5K PRs when comparing $ACTN3^{+}$ and $ACTN3^{-}$, TOST revealed that the observed effect was not statistically equivalent to zero (**Figure 2.2**).

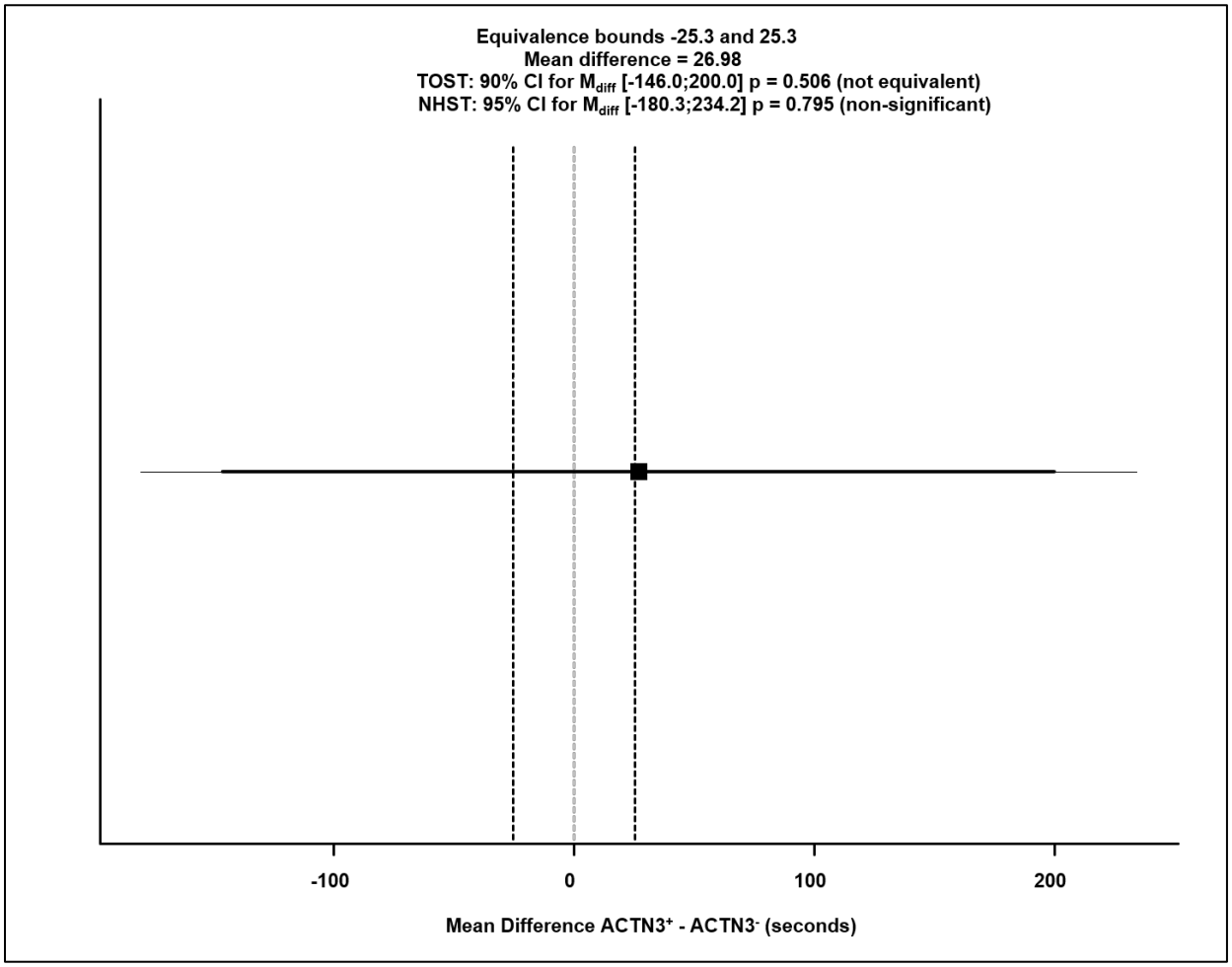

Figure 2.2. 5-km PR Equivalence Test. Mean difference in self-reported 5K Personal Records (black squares), 90% confidence intervals (CIs; thick horizontal lines), and 95% CIs (thin horizontal lines) with equivalence bounds (dark dashed vertical lines) comparing ACTN3+ and ACTN3- in all subjects reporting 5K times. If a NHST interval does not cross zero, the test is statistically significant. If the TOST interval lies within its equivalence bounds the effect is considered statistically equivalent to zero.

2.5 Discussion

Our results show that *ACTN3* genotype may influence self-reported one-mile running times in recreationally active women, with those subjects devoid of α-actinin-3 reporting significantly faster one-mile PRs than those with α-actinin-3 present. Similar genotype effects were not present among male subjects. While Papadimitriou et al. reported no effect of *ACTN3* genotype on personal best times for distances from 1,500m to the marathon among male and female Caucasian endurance athletes, the present study is the first to directly assess the relationship between *ACTN3*

genotype and distance running PRs in a racially diverse, recreationally active sample.[29] Saunders et al. reported no association of *ACTN3* genotype with Ironman triathlon performance in Caucasian male triathletes.[24] However, the authors did not directly analyze genotypic effects on individual race times, but rather divided their subjects into fast, middle of the field, and slow triathletes and examined genotype frequency within those groups. Further, they did not investigate the effects of *ACTN3* genotype in female triathletes.

Our results are partially in agreement with observations in rodents that complete deficiency of α-actinin-3 is associated with increased endurance exercise capacity.[5,7] Further, Hogarth et al. reported a dose-dependent effect of *ACTN3* genotype on endurance exercise capacity, such that mice who were heterozygous for the R577X polymorphism showed intermediate endurance running capacity compared to KO and WT mice in accordance with intermediate expression of α-actinin-2 and α-actinin-3 on the muscle level.[5] Our data did not confirm a dose-dependent effect of *ACTN3* polymorphisms on endurance exercise performance in humans. This is potentially due to inter-species variations and the exercise task at hand. Hogarth et al. employed a time-to-exhaustion protocol with increasing treadmill speed and compared total distance run between mice with differing genotypes.[5] Conversely, the present study analyzed self-reported running PRs, a time-to completion task. Bertuzzi et al. reported that total energy production explained 84.1% of the shared variance in time-to-exhaustion at velocity corresponding to $\dot{V}O_2max$ in recreational long-distance runners.[36] In rodents, α-actinin-3 deficiency results in structural and metabolic changes in fast twitch fibers, such that Type IIx fibers of knockout (KO) mice experience a shift toward the metabolic properties of slow twitch fibers, as evidenced by increased activity of the mitochondrial enzymes citrate synthase, succinate dehydrogenase, and cytochrome c oxidase.[7,37,38] Additionally, KO mice display increased activity of the glycolytic enzymes hexokinase and

glyceraldehyde-6-phosphate, but decreased activity of the anaerobic metabolism enzyme lactate dehydrogenase. These phenotypic differences alter energy production processes and thus might provide an explanation for the dose-dependent effect reported by Hogarth et al., particularly considering the intermediate muscle characteristics found in *ACTN3* heterozygotes. Conversely, in a heterogeneous sample of recreationally active subjects similar to the one in the present study, maximal oxygen uptake ($\dot{V}O_2max$) has been shown to predict approximately 72% of 5K running performance men and 64% in women.[39] Baseline $\dot{V}O_2max$ and $\dot{V}O_2max$ trainability are strongly influenced by genetic variation, with as many as 97 genes implicated in trainability.[40] Thus, differences in $\dot{V}O_2max$ may have masked a dose-dependent relationship in our sample.

Similar to prior research, our results demonstrate an effect of *ACTN3* genotype on endurance phenotype in female subjects, but not in male subjects.[16,41] While prior investigations have reported this relationship between *ACTN3* genotypes and endurance performance in elite female athletes, our study established a similar finding in recreationally active individuals.[16,41,42]

We have shown that the *α-actinin-3* deficiency is associated with endurance running performance as assessed by self-reported one-mile PRs in female runners. One limitation of our investigation is the nature of our performance data. While no scientific data are available on the validity of self-reported running times, an investigation of the validity of self-reported training duration in recreationally active adults showed that 24% of subjects overestimated while 17% of subjects underestimated time spent training in their sport.[43] Further, differences in course profiles, environment, and motivational factors influencing self-reported PRs could not be accounted for. Due to the scope of our study and the availability of data, independent validation of self-reported running PRs was impossible. Future research should attempt to collect prospective performance data or attempt to independently validate reported times. Further, the heterogeneity of our sample

might have introduced additional variables, such as $\dot{V}O_2$max phenotype and training status, which could have influenced our results.

In conclusion, our results suggest that the *ACTN3* XX genotype, i.e. the absence of α-actinin-3, might be beneficial for one-mile running performance in female runners. Further, none of the observed effects comparing $ACTN3^+$ and $ACTN3^-$ were statistically equivalent, warranting additional investigation of the influence of α-actinin-3 deficiency on endurance performance. Future research should examine this relationship in a larger, racially diverse, but athletically more homogeneous sample, such as recreational runners or a diverse sample of athletes, to further elucidate the role of genetic polymorphisms on endurance performance.

2.5.1 Practical Applications

Our study failed to strongly link *ACTN3* genotype with human endurance performance. The current scientific consensus among sport and exercise genetics researchers is that genetic tests are not a satisfactory tool for talent identification or individualized training prescription, specifically when employed as Direct-to-Consumer testing without adequate genetic counselling.[44] While knowledge in the field of exercise genetics and genomics is continuously evolving, our current understanding of the association between genetics and performance-related phenotypes is insufficient to predict individual responses. Thus, practitioners should continue to be cautious when using genetic information for talent identification and sport selection.

2.6 References

1. Foley KS, Young PW. The Non-muscle Functions of Actinins: An Update. *Biochem J*. 2014;459(1):1-13. doi:https://doi.org/10.1042/BJ20131511

2. Ogura Y, Kakigi R, Naito H. Alpha-Actinin Isoform and Skeletal Muscle Activity. *J Sports Med Phys Fitness*. 2013;2(2):229-231. doi:10.7600/jpfsm.2.229

3. Mills M, Yang N, Weinberger R, et al. Differential Expression of the Actin-Binding Proteins, Alpha-Actinin-2 and -3, in Different Species: Implications for the Evolution of Functional Redundancy. *Hum Mol Genet*. 2001;10(13):1335-1346. doi:10.1093/hmg/10.13.1335

4. North KN, Yang N, Wattanasirichaigoon D, Mills M, Easteal S, Beggs AH. A Common Nonsense Mutation Results in Alpha-Actinin-3 Deficiency in the General Population. *Nat Genet*. 1999;21(4):353-354. doi:10.1038/7675

5. Hogarth MW, Garton FC, Houweling PJ, et al. Analysis of the ACTN3 Heterozygous Genotype Suggests That α-Actinin-3 Controls Sarcomeric Composition and Muscle Function in a Dose-Dependent Fashion. *Hum Mol Genet*. 2016;25(5):866-877. doi:10.1093/hmg/ddv613

6. Lek M, Quinlan KG, North KN. The Evolution of Skeletal Muscle Performance: Gene Duplication and Divergence of Human Sarcomeric α-Actinins. *Bioessays*. 2010;32(1):17-25. doi:10.1002/bies.200900110

7. MacArthur DG, Seto JT, Raftery JM, et al. Loss of ACTN3 Gene Function Alters Mouse Muscle Metabolism and Shows Evidence of Positive Selection in Humans. *Nat Genet*. 2007;39(10):1261-1265. doi:10.1038/ng2122

8. Vincent B, De Bock K, Ramaekers M, et al. ACTN3 (R577X) Genotype Is Associated With Fiber Type Distribution. *Physiol Genomics*. 2007;32(1):58-63. doi:10.1152/physiolgenomics.00173.2007

9. Amorim CEG, Acuña-Alonzo V, Salzano FM, Bortolini MC, Hünemeier T. Differing Evolutionary Histories of the ACTN3*R577X Polymorphism among the Major Human Geographic Groups. *PLoS ONE*. 2015;10(2):e0115449. doi:10.1371/journal.pone.0115449

10. Atanasov P, Djarova T, Kalinski M, et al. ACTN3 and AMPD1 Polymorphism and Genotype Combinations in Bulgarian Athletes Performing Wingate Test. *J Sports Sci*. 2015;3(3):1-10. doi:10.17265/2332-7839/2015.01.001

11. Ben-Zaken S, Eliakim A, Nemet D, Rabinovich M, Kassem E, Meckel Y. ACTN3 Polymorphism: Comparison Between Elite Swimmers and Runners. *Sports Med Open*. 2015;1(1):13. doi:10.1186/s40798-015-0023-y

12. Ginszt M, Michalak-Wojnowska M, Gawda P, et al. ACTN3 Genotype in Professional Sport Climbers. *J Strength Cond Res*. 4AD;32(5):1311-1315. doi:10.1519/JSC.0000000000002457

13. Orysiak J, Busko K, Mazur-RoZycka J, et al. Relationship Between ACTN3 R577X Polymorphism and Physical Abilities in Polish Athletes. *J Strength Cond Res*. 2015;29(8):2333-2339. doi:10.1519/jsc.0000000000000880

14. Papadimitriou ID, Lucia A, Pitsiladis YP, et al. ACTN3 R577X and ACE I/D Gene Variants Influence Performance in Elite Sprinters: A Multi-Cohort Study. *BMC Genomics*. 2016;17(1):285. doi:10.1186/s12864-016-2462-3

15. Yang R, Shen X, Wang Y, et al. ACTN3 R577X Gene Variant Is Associated With Muscle-Related Phenotypes in Elite Chinese Sprint/Power Athletes. *J Strength Cond Res*. 2017;31(4):1107-1115. doi:10.1519/jsc.0000000000001558

16. Yang N, MacArthur DG, Gulbin JP, et al. ACTN3 Genotype Is Associated With Human Elite Athletic Performance. *Am J Hum Genet*. 2003;73(3):627-631. doi:10.1086/377590

17. Eynon N, Duarte JA, Oliveira J, et al. ACTN3 R577X Polymorphism and Israeli Top-Level Athletes. *Int J Sports Med*. 2009;30(9):695-698. doi:10.1055/s-0029-1220731

18. Papadimitriou ID, Papadopoulos C, Kouvatsi A, Triantaphyllidis C. The ACTN3 Gene in Elite Greek Track and Field Athletes. *Int J Sports Med*. 2008;29(4):352-355. doi:10.1055/s-2007-965339

19. Roth SM, Walsh S, Liu D, Metter EJ, Ferrucci L, Hurley BF. The ACTN3 R577X Nonsense Allele Is Under-Represented in Elite-Level Strength Athletes. *Eur J Hum Genet*. 2008;16(3):391-394. doi:10.1038/sj.ejhg.5201964

20. Yang N, MacArthur DG, Wolde B, et al. The ACTN3 R577X Polymorphism in East and West African Athletes. *Med Sci Sports Exerc*. 2007;39(11):1985-1988. doi:10.1249/mss.0b013e31814844c9

21. Niemi AK, Majamaa K. Mitochondrial DNA and ACTN3 Genotypes in Finnish Elite Endurance and Sprint Athletes. *Eur J Hum Genet*. 2005;13(8):965-969. doi:10.1038/sj.ejhg.5201438

22. Döring FE, Onur S, Geisen U, et al. ACTN3 R577X and Other Polymorphisms Are Not Associated With Elite Endurance Athlete Status in the Genathlete Study. *J Sports Sci*. 2010;28(12):1355-1359. doi:10.1080/02640414.2010.507675

23. Muniesa CA, González-Freire M, Santiago C, et al. World-Class Performance in Lightweight Rowing: Is It Genetically Influenced? A Comparison With Cyclists, Runners and Non-athletes. *Br J Sports Med*. 2010;44(12):898-901. doi:10.1136/bjsm.2008.051680

24. Saunders CJ, September AV, Xenophontos SL, et al. No Association of the ACTN3 Gene R577X Polymorphism With Endurance Performance in Ironman Triathlons. *Ann Hum Genet*. 2007;71(6):777-781. doi:10.1111/j.1469-1809.2006.00385.x

25. Ben-Zaken S, Eliakim A, Nemet D, Meckel Y. Genetic Variability Among Power Athletes: The Stronger vs. the Faster. *J Strength Cond Res*. Published online January 29, 2016. doi:10.1519/jsc.0000000000001356

26. Eynon N, Ruiz JR, Femia P, et al. The ACTN3 R577X polymorphism across three groups of elite male European athletes. *PLoS One*. 2012;7(8):e43132. doi:10.1371/journal.pone.0043132

27. Chiu LL, Wu YF, Tang MT, Yu HC, Hsieh LL, Hsieh SS. ACTN3 Genotype and Swimming Performance in Taiwan. *Int J Sports Med*. 2011;32(6):476-480. doi:10.1055/s-0030-1263115

28. Moran CN, Yang N, Bailey ME, et al. Association analysis of the ACTN3 R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks. *Eur J Hum Genet*. 2007;15(1):88-93. doi:10.1038/sj.ejhg.5201724

29. Papadimitriou ID, Lockey SJ, Voisin S, et al. No Association Between ACTN3 R577X and ACE I/D Polymorphisms and Endurance Running Times in 698 Caucasian Athletes. *BMC Genomics*. 2018;19(1):13. doi:10.1186/s12864-017-4412-0

30. Schadock I, Schneider A, Silva ED, et al. Simple Method to Genotype the ACTN3 r577x Polymorphism. *Genet Test Mol Biomarkers*. 2015;19(5):253-257. doi:10.1089/gtmb.2014.0299

31. R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2018. https://www.R-project.org/

32. R Studio Team. *RStudio: Integrated Development for R*. RStudio, Inc.; 2016. http://www.rstudio.com/

33. Lakens D. Calculating and Reporting Effect Sizes to Facilitate Cumulative Science: A Practical Primer for T-tests and ANOVAs. *Front Psychol*. 2013;4(863). doi:10.3389/fpsyg.2013.00863

34. Kelley K. *MBESS: The MBESS R Package*.; 2018. Accessed April 13, 2022. https://CRAN.R-project.org/package=MBESS

35. Lakens D. Equivalence Tests: A Practical Primer for t Tests, Correlations, and Meta-Analyses. *Soc Psychol Personal Sci*. 2017;8(4):355-362. doi:10.1177/1948550617697177

36. Bertuzzi R, Bueno S, Pasqua LA, et al. Bioenergetics and Neuromuscular Determinants of the Time to Exhaustion at Velocity Corresponding to VO2max in Recreational Long-Distance Runners. *J Strength Cond Res*. 2012;26(8):2096-2102. doi:10.1519/JSC.0b013e31823b8721

37. MacArthur DG, Seto JT, Chan S, et al. An Actn3 Knockout Mouse Provides Mechanistic Insights Into the Association Between α-Actinin-3 Deficiency and Human Athletic Performance. *Hum Mol Genet*. 2008;17(8):1076-1086. doi:10.1093/hmg/ddm380

38. Quinlan KG, Seto JT, Turner N, et al. Alpha-Actinin-3 Deficiency Results in Reduced Glycogen Phosphorylase Activity and Altered Calcium Handling in Skeletal Muscle. *Hum Mol Genet*. 2010;19(7):1335-1346. doi:10.1093/hmg/ddq010

39. Ramsbottom R, Nute MG, Williams C. Determinants of Five Kilometre Running Performance in Active Men and Women. *Br J Sports Med*. 1987;21(2):9-13. doi:10.1136/bjsm.21.2.9

40. Williams CJ, Williams MG, Eynon N, et al. Genes to Predict VO2max Trainability: A Systematic Review. *BMC Genomics*. 2017;18(8):831. doi:10.1186/s12864-017-4192-6

41. Shang X, Huang C, Chang Q, Zhang L, Huang T. Association Between the ACTN3 R577X Polymorphism and Female Endurance Athletes in China. *Int J Sports Med*. 2010;31(12):913-916. doi:10.1055/s-0030-1265176

42. Ma F, Yang Y, Li X, et al. The Association of Sport Performance with ACE and ACTN3 Genetic Polymorphisms: A Systematic Review and Meta-Analysis. *PLoS One*. 2013;8(1):e54685. doi:10/gf48ht

43. Borresen J, Lambert M. Validity of Self-Reported Training Duration. *Int J Sports Sci Coach*. 2006;1(4):353-359.

44. Webborn N, Williams A, McNamee M, et al. Direct-to-Consumer Genetic Testing for Predicting Sports Performance and Talent Identification: Consensus Statement. *Br J Sports Med*. 2015;49(23):1486-1491. doi:10.1136/bjsports-2015-095343

Chapter III: Ketogenic and High-Carbohydrate Diets in Cyclists and Triathletes: Performance Indicators and Methodological Considerations From a Pilot Study

Authors:

Andreas Kreutzer[1*], Austin J. Graybeal[1], Petra P. Rack[1], Kamiah Moss[1], Garrett R. Augsburger[1], Jada L. Willis[2], Robyn Braun-Trocchio[1], and Meena Shah[1*]

Author Affiliation:

[1]Department of Kinesiology, Harris College of Nursing & Health Sciences, Texas Christian University, Fort Worth, TX 76129, USA.

[2]Department of Nutritional Sciences, College of Science & Engineering, Texas Christian University, Fort Worth, TX 76129, USA

Corresponding Author:

Andreas Kreutzer, M.S., Department of Kinesiology, Texas Christian University, Fort Worth, TX 76129, USA. Email: a.kreutzer@tcu.edu. Tel: 817-257-6859

Meena Shah, Department of Kinesiology, Texas Christian University, Fort Worth, TX 76129, USA. Email: m.shah@tcu.edu. Tel: 817-257-6871

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Kreutzer A, Graybeal AJ, Rack PP, Moss K, Augsburger GR, Willis JL, Braun-Trocchio R, Shah M. Ketogenic and High-Carbohydrate Diets in Cyclists and Triathletes: Performance Indicators and Methodological Considerations From a Pilot Study. *SportRxiv*. DOI: 10.51224/SRXIV.19

3.1 Abstract

Endurance athletes frequently employ nutritional strategies to enhance performance. While professional organizations recommend high carbohydrate (HC) diets to maximize performance, many athletes, and researchers have recently shown renewed interest in the ketogenic diet (KD) in hopes to promote "fat adaptation", which would allow athletes to make use of the essentially unlimited energy resources from stored body fat. This would circumvent one fatigue mechanism, the depletion of muscle glycogen stores, that has been considered central to performance outcomes in endurance events. The present study investigated the effects of participants' habitual diet (HD), HC, and KD on endurance performance in a 30-km simulated cycling time trial (TT), physiological responses during the TT, and muscle session fuel percentile (SFP) before and after the TT using ultrasonic imaging. Due to the COVID-19 pandemic, data collection ceased after only six recreational cyclists and triathletes (f = 4, m = 6; age: 37.2 ± 12.2; V̇O2max: 46.8 ± 6.8 ml/kg/min; weekly cycling distance: 225.3 ± 64.2 km). Due to the small sample size, we do not report inferential statistics for our primary outcome measure, cycling performance. Participants completed the KD at the lowest power output. Oxygen consumption ($\dot{V}O_2$), heart rate (HR), and perceived exertion (RPE) during the TT were similar in all conditions. FATox rates were highest in the KD condition and lowest in the HC condition. SFP was lower during KD compared with HD and lower following the TT compared with fasted resting values across all conditions. We discuss methodological considerations into the use of exercise equipment, nutritional interventions, and statistical analysis strategies for study designs like the present. Further research is needed to assess the impact of HC and KD on TT performance in this population.

ClinicalTrials.gov Identifier: NCT04097171; OSF preregistration: https://osf.io/ujx6e/

3.2 Introduction

Nutritional interventions remain at the forefront of strategies employed by athletes to enhance their performance.[1] Commonly approaches among endurance athletes include a high daily intake of dietary carbohydrate (CHO; 6-10 g/kg/day) and carbohydrate loading (10-12 g/kg/day) before an event, since low muscle glycogen is a well-established cause of fatigue.[2,3] Contrary to this traditionally favored strategy, endurance athletes and researchers have recently began expressing increased interest in a low carbohydrate, high-fat ketogenic diet (KD) again, for the third time since the 1980s.[4] When following a KD, athletes typically limit their CHO intake to <50 g or 5-10% of their total daily energy intake.[5] The proposed benefit of this diet approach is "fat adaptation", enabling the oxidation of fat as the main energy substrate at exercise intensities (e.g. >70% of maximal oxygen consumption [$\dot{V}O_2max$]) where the oxidation of CHO would typically predominate.[6–8] This would essentially create unlimited energy resources, as the body can store more than 74,000 kcal in subcutaneous, visceral, and intramuscular fat.[9] Despite its recent resurgence in popularity, the KD's restrictive nature counters the current dietary recommendations of several professional organizations, which state that low CHO availability before exercise is a significant component of diminished exercise capacity and performance.[1,10,11]

Two factors influencing the effect of low CHO diets (LCDs) on endurance performance appear to be the length of adaptation and the duration and intensity of the event. Short-term LCDs of one to four days lead to impaired glycogen storage, which can cause substantial decreases in exercise performance.[12,13] However, even with as little as five days of implementing LCDs, increased fat oxidation (FATox) rates have been reported.[14–16] While this increase in FATox is a consistent finding among most studies investigating the effect of LCDs in endurance athletes, the results regarding exercise performance are less clear.[6,17–24]

Recent studies comparing KD to habitual (HD) or mixed control diets have shown decreases[25] or no differences[26] in time to exhaustion (TTE) following prolonged diet adherence. However, early studies employing a direct comparison of KD and high carbohydrate diet (HC) and their effects on prolonged endurance exercise performance have produced ambiguous results.[7,12,27,28] Lambert et al. reported improved TTE at moderate cycling intensity (50% of peak power output [PPO]) following two weeks of KD compared with HC, but not at high intensity (85 % of PPO).[7] Similarly, Burke et al. reported no difference in 7 kJ·kg-1 TT performance immediately following 120 min of steady state cycling at 70% of $\dot{V}O_2$max in eight well-trained male cyclists and triathletes, who adhered to a five-day LCD (2.4 g/kg/day CHO; 4 g/kg/day fat) with one-day CHO restoration compared with an isoenergetic HC (9.6 g/kg/day CHO; 0.7 g/kg/day fat).[18] Prins et al. compared the effects of a 42-day KD and HC on 5 km TT performance at four separate points of each diet in seven male recreational distance runners and found that running time was significantly faster during HC (60–65% CHO; 20% fat) when compared with KD (< 50 g/day CHO; 75-80% fat) on day four of each diet, but not at any other point during the diets.[23] This again indicates that exercise performance might be maintained at higher intensities. However, in a more recent study, Burke et al. compared the effect of a 3-week HC (8.6 g/kg/day CHO; 1.2 g/kg/day fat), a periodized CHO diet (8.3 g/kg/day CHO; 1.2 g/kg/day fat), and a KD (< 50 g/day CHO; 4.7 g/kg/day fat) on 10 km race performance in 21 elite male race walkers; they found that race time improved significantly in the HC and periodized CHO groups, but remained unchanged in the KD group.[19] A recent replication study produced similar results, with HC and periodized CHO leading to performance improvements and KD leading to a performance decrement.[20] Additionally, Burke et al. have elucidated a potential mechanism for performance impairment following a KD at higher intensities; specifically, they showed that exercise economy is reduced following a KD compared to HC and periodized CHO diets.[16,19,20]

While a number of studies have investigated the effect of KD and HC on exercise performance, results remain conflicting, in part due to small sample sizes, limited participation of female athletes across a wide age range, heterogenous interventions, and testing protocols.[7,16,18–20,22,23] Our current study employed a performance assessment (TT) that was representative of the type of races in which our population competes. This approach maximized the external validity of our study while still allowing measurements in a controlled laboratory setting. Finally, to our knowledge, no studies have used a randomized crossover design that directly compares the effects of HD, KD, and HC on prolonged endurance performance.

We intended to address the gaps in the literature with the present study and aimed to collect data from 30 male and female cyclists across a wide age range (18-70 years old). We hypothesized that the HC would lead to improved performance (faster TT completion) compared with the KD and HD. However, due to restrictions on data collection caused by the COVID-19 pandemic, the results presented in the present manuscript should be considered as insights from a pilot study only, i.e., we were unable to address the issues of small sample sizes in this area of research. Since the originally estimated sample size to detect a meaningful difference in performance (see *Power Analysis* section) was not achieved, primary outcomes are presented as means and standard deviations only; reflections on potential inferential statistical analysis techniques and other methodological considerations regarding performance measurement, muscle glycogen estimation in response to the diets using high-frequency ultrasound, and participant adherence to the interventions are presented.[29]

3.3 Method

3.3.1 Study Preregistration

This study was preregistered at Open Science Framework (https://osf.io/ujx6e/) and at ClinicalTrials.gov (NCT04097171).

3.3.2 Experimental Design

The study employed crossover design, where each participant served as their own control. Participants adhered to 14 days each of a KD and an HC in a counter-balanced randomized order. Diet order was randomized employing block randomization in the *blockrand* package in *R*. [30,31] The syntax for the block randomization can be found at https://osf.io/ujx6e/. Participant eligibility, anthropometric measurements, and $\dot{V}O_2$max were determined during two screening visits. During the third visit, all participants completed the experimental procedures following their HD and ingesting a test meal with macronutrient contents similar to a typical American diet.[32] During the KD and HC trials, participants underwent the same procedures, but consumed a test meal corresponding to their diet condition. A diagram showing the experimental design is presented in **Figure 3.1**. The study was approved by the TCU Institutional Review Board (IRB). All procedures were performed according to the Declaration of Helsinki principles for research involving human participants.

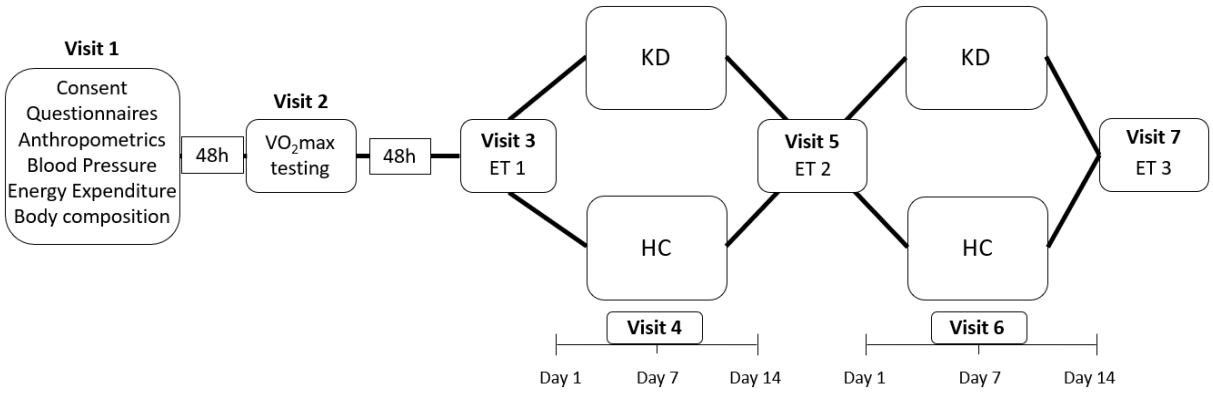

Figure 3.1. Study Design. ET = Experimental Trial; KD = ketogenic diet (<5-10% of total energy intake from carbohydrates); HC= high carbohydrate diet (65-75% of total energy intake from carbohydrates); $\dot{V}O_2$max = maximal oxygen consumption

3.3.3 Participants

Endurance-trained recreational cyclists and triathletes were recruited from the local cycling and triathlon community using flyers, social media, and word of mouth. A total of 46 individuals were assessed for eligibility, 19 of which were unable to begin the study due to COVID-19 restrictions on in-person research. A further six participants started the study, but were unable to finish the entire protocol due to these restrictions. Thus, six participants (m = 2, f =4) completed the study. The study was unable to achieve the originally estimated sample size of 30 participants due to data collection restrictions caused by the COVID-19 pandemic. **Figure 3.2** presents a CONSORT diagram for the present study

Participants were considered endurance trained if they self-reported ≥ 100 km/wk of cycling for the past year and achieved a $\dot{V}O_2$max above the 80th percentile for their sex and age group according to guidelines put forth by the American College of Sports Medicine with a 5% adjustment for comparing cycle ergometry values to the treadmill derived ACSM norms. [33,34]

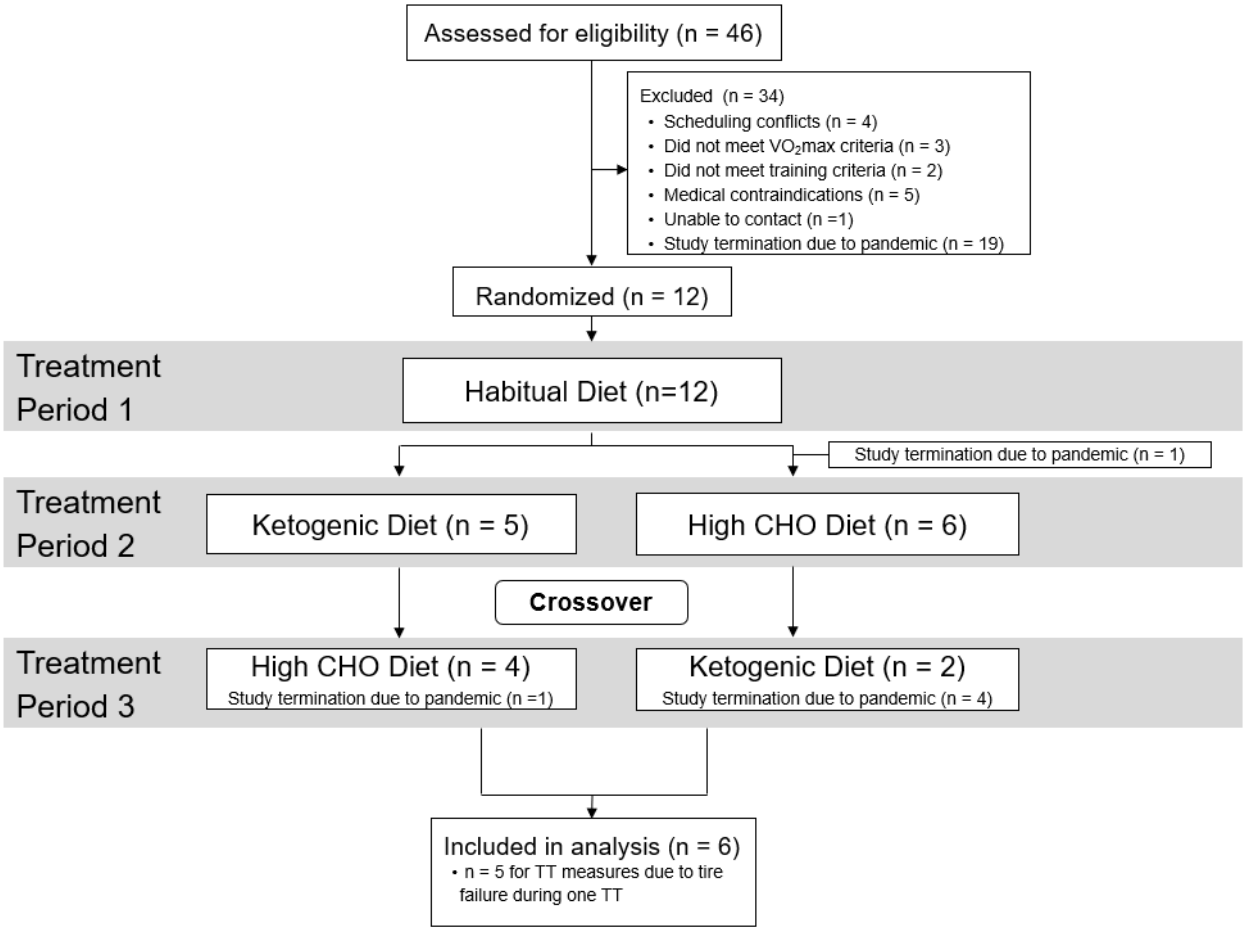

Figure 3.2. CONSORT Diagram. TT = time trial

Participants included one male in Performance Level (PL) 2 and one male in PL 1 as described by De Pauw et al.[35] Further, our study included three female participants in PL 3 and one in PL 1 according to criteria established by Decroix et al.[36] We used relative $\dot{V}O_2$max as the primary criterion for categorization of our participants.[35,36] However, it is important to note that all participants achieved at least PL 3 based on weekly mileage and cycling experience. Further, the male participant classified as PL 2 would have achieved PL 4 or PL 5 based on absolute or relative PPO respectively. Participant characteristics are shown in **Table 3.1** and have in part been previously reported elsewhere.[37]

Table 3.1. Participants Characteristics at Screening.

	Total (n=6)	**Male (n=2)**	**Female (n=4)**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**
Age (y)	37.2 ± 12.2	41.5 ± 20.5	35.0 ± 9.5
Height (cm)	172.3 ± 10.0	183.5 ± 1.0	166.8 ± 5.0
Body mass (kg)	68.5 ± 17.5	89.1 ± 7.1	58.2 ± 8.3
BMI (kg/m^2)	22.7 ± 3.4	26.5 ± 2.3	20.9 ± 2.0
Body fat (%)	21.3 ± 4.6	21.1 ± 7.2	21.4 ± 4.2
Fat-free mass (kg)	53.8 ± 13.2	70.1 ± 0.8	45.6 ± 5.0
Fat mass (kg)	14.7 ± 5.9	19.07 ± 7.9	12.6 ± 4.2
$\dot{V}O_2$max (mL/kg/min)	46.8 ± 6.8	47.2 ± 6.7	46.6 ± 7.9
$\dot{V}O_2$max (L/min)	3.2 ± 0.9	4.2 ± 0.5	2.7 ± 0.2
PPO (W)	295.5 ± 73.1	372.5 ± 74.2	257.0 ± 33.7
PPO (W/kg)	4.4 ± 0.7	4.2 ± 1.2	4.5 ± 0.6
Cycling experience (years)	6.0 ± 4.3	6.5 ± 4.9	5.8 ± 4.8
Cycling frequency (days/wk)	4.5 ± 1.0	4.5 ± 0.7	4.5 ± 1.3
Cycling distance (km/wk)	225.3 ± 64.2	217.0 ± 33.9	229.5 ± 80.0
RMR (kcals/d)	1617.3 ± 314.7	1999.5 ± 68.6	1426.3 ± 132.0

SD = standard deviation; BMI = body mass index; $\dot{V}O_{2max}$ = maximal oxygen consumption; PPO = peak power output; RMR = resting metabolic rate

Exclusion criteria included the self-reported use of medications or supplements to lose weight, following a ketogenic (<10% or less of total energy intake from carbohydrates), a high

carbohydrate diet (>65% of total energy intake from carbohydrate), or weight loss diet. Further, nicotine use or heavy alcohol consumption (>14 drinks/week for males; >7 drinks/week for females) were considered reasons for exclusion. Potential participants were also excluded if they self-reported any food allergies to ingredients used in our test meals. Known cardiovascular disease was cause for exclusion unless participation was approved by the participant's cardiologist. Self-reported presence of diabetes, stroke, anemia, eating disorders, uncontrolled hypertension, or pulmonary, liver, kidney, and untreated thyroid disease, or orthopedic, arthritis, or musculoskeletal problems that would have prevented exercise excluded prospective participants from enrolling in the study. Potential participants were also excluded if they had undergone surgery that had lasting effects on swallowing or digestion.

3.3.4 Power Analysis

We performed a simulation-based power analysis using the *Superpower* package in *R* [31,38]. Based on unpublished data collected in our lab in a representative sample, we expected the TT to take approximately 60 ± 6 min. The within-subjects correlation between repeated time trials in our pilot work was 0.98; high within-subjects correlations (r = 0.89) have been shown in the existing literature.[18] To employ a conservative approach, we elected to use the average of the within-subjects correlation in our pilot work and in Burke et al., resulting in r = 0.93 for our power analysis.[18] We analyzed finishing times from the past four years (2015-2018) of the Texas State Time Trial Championships to establish a practically meaningful effect size. In male and female athletes of age groups up to 55+ years old, the average finishing time of the top 10 riders was 61 ± 6 min. On average, an improvement of 1.5 min would have resulted in a rider moving up by one place in the final standings. Therefore, we decided on a meaningful difference of 90 seconds for our power analysis. All finishing times used in our analysis can be found at https://osf.io/ujx6e/.

At an alpha level of 0.05, our power analysis revealed that 30 participants would have yielded 90% power for the omnibus linear model for time to completion (TTC) of the 30-km TT. The syntax for the power analysis can be found at https://osf.io/ujx6e/. As discussed, we were unable to reach our desired sample size due to COVID-19 restrictions on in-person research. Therefore, we do not present any inferential statistics for our primary outcome measure.

3.3.5 Screening

3.3.5.1 Visit 1

Following a 12-hour overnight fast, participants reported the laboratory for Visit 1, which included completing informed consent and demographic, behavioral, and health questionnaires. Additionally, participants underwent anthropometric measurements (height, body mass, waist, and hip circumference) and blood pressure (BP) measurements. Further, we assessed participants' body composition using air displacement plethysmography (ADP) with measured thoracic lung volume (BOD POD, COSMED USA Inc., Concord, CA). Following body composition and anthropometric measurements, we assessed participants' resting metabolic rate (RMR) via indirect calorimetry using the ParvoMedics TrueOne® 2400 metabolic cart (ParvoMedics, Sandy, UT, USA) with a ventilated hood system. BP measurements were performed in triplicate, using an automated blood pressure monitor (Omron M6 Comfort IT, Omron, Milton Keyes, UK) as described by the American College of Cardiology/American Heart Association Task Force.[39]

3.3.5.2 Visit 2

At Visit 2, participants performed an incremental exercise test to task failure to determine $\dot{V}O_2max$ using a CompuTrainer® ergometer (RacerMate Inc., Seattle, WA). Participants were instructed to refrain from any exercise in the 24 hours leading up to $\dot{V}O_2max$ testing and to only perform light or moderate exercise 24-48 hours before testing.

3.3.6 Experimental Trials

Participants reported to the laboratory following a 12-hour overnight fast. Additionally, they performed only light to moderate exercise 24-48 hours prior to testing and refrained from all exercise in the 24 hours leading up to the experimental trials (ET). Upon arrival, participants underwent measurements of body mass, BP, and capillary beta-hydroxybutyrate (BHB) concentration, and an ultrasonic assessment of the right and left rectus femoris (RF). Following resting measures, participants consumed a liquid test meal approximately 180 min prior to the start of the TT. They were allowed 10 min to consume the test meal in its entirety; time to consume the meal was standardized between trials based on the time taken for consumption of the meal during the initial trial. Following 180 min of supine rest and postprandial measures described elsewhere, participants underwent RF ultrasound assessment and provided capillary samples for BHB measurement.[37] Then, they completed a 30-km simulated cycling TT. A diagram showing all measures performed during each experimental trial is presented in **Figure 3.3.**

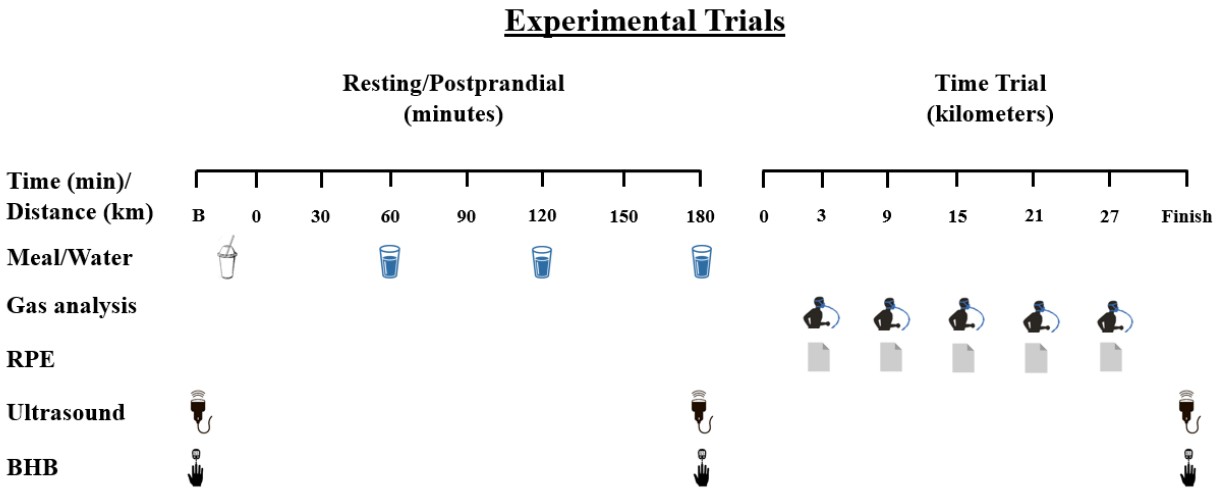

Figure 3.3. Experimental Trial Procedures. RPE = Rating of Perceived Exertion; BHB = beta hydroxybutyrate.

3.3.7 Dietary Interventions, Compliance, and Physical Activity

Dietary interventions, compliance measures, and experimental controls regarding physical activity are described in detail elsewhere.[37] Briefly, participants completed 3-day dietary records to quantify their HD before ET 1. Thereafter, they followed a KD (<10% CHO, 75-85% FAT, 15% PRO) and HC (>65% CHO, <20% FAT, 15% PRO) in randomized order. We considered participants to be compliant with the diet if they met CHO macronutrient percentages on at least 80% of days. Compliance with the diets was assessed by a registered dietitian (RD) via daily diet logging and daily check-ins using mobile applications (WhatsApp, WhatsApp Inc., Mountain View, CA; NutritIO, Bucharest, Romania). Further, participants provided capillary BHB samples at each ET and seven days into each diet, as well as daily images of urinary ketone body test strips (VALI, CA) to test for ketosis, i.e., urinary BHB concentration ≥ 0.5 mmol/L.[40] We instructed participants to attempt to maintain body mass throughout the study and considered weight maintenance as a body mass loss or gain of no more than 5%.

During experimental trials, participants consumed liquid test meals containing 60% of the participants' measured RMR (kcals/day). Test meal compositions corresponded to a standard American Diet for HD (31.4% FAT, 53.4% CHO, 15.2% PRO) and to the respective dietary interventions following HC (15.7% FAT, 69.1% CHO, 15.2% PRO) and KD (75.1% FAT, 9.5% CHO, 15.4% PRO); test meal volumes and caloric content were the same across conditions. Test meals were consumed in the same amount of time in each condition. Participants consumed standardized amounts of water during the postprandial period and were provided with and instructed to ingest the same volume of water during each TT.

We instructed participants to keep their training levels stable throughout the study and monitored physical activity using self-reported written training logs including distance covered,

time spent, and rating of perceived exertion for the session (RPE; 1-10). We calculated session RPE (sRPE) by multiplying the indicated RPE by the time elapsed during the session.

3.3.8 Measures

3.3.8.1 Exercise Equipment

To ensure familiarity with the exercise equipment and to avoid learning effects across trials, participants completed all testing on their personal bicycles mounted to a CompuTrainer® cycling ergometer (RacerMate Inc., Seattle, WA), which has previously been shown to be reliable in TT tasks similar to the present study.[41] The CompuTrainer® was calibrated according to manufacturer's recommendations, and tire pressure was standardized for each trial at 100 psi. Participants were asked to remove devices from their bicycles or deactivate any devices that could give them feedback on their exercise performance, such as power meters and cycle computers. The only data displayed to participants during the TT were distance and gradient of the road.

3.3.8.2 $\dot{V}O_2$max Testing

For the 24 hours leading up to testing, participants were asked to refrain from all exercise. For the initial incremental maximal exercise test, participants warmed up for 5 min at a self-selected intensity. Thereafter, participants began the incremental test at a load of 50-100 watts (W). Exercise intensity was increased by 25 W per minute until task failure. Oxygen uptake ($\dot{V}O_2$) was continuously monitored using a TrueOne 2400 metabolic cart (Parvo Medics, Sandy, UT, USA) and heart rate (HR) was collected throughout the test using a Polar H7 HR monitor (Polar Inc., Lake Success, NY). $\dot{V}O_2$max was defined as the highest 30-second $\dot{V}O2$ value obtained during the test. To ensure validity of the $\dot{V}O_2$max measurement, participants performed a validation bout at 110% of their peak power output (PPO) achieved in the initial test following at least 15 min rest as described by Poole & Jones.[42] PPO was calculated as described by Hawley & Noakes[43]:

$$PPO = P_{final} + \left(\frac{t}{60} \times 25\right),$$

where P_{final} is the highest work rate achieved and t is the time completed in the final stage.

Following a two-minute warmup at 100 W, participants performed a steady work rate test that achieved exhaustion within three to six min. If the greatest $\dot{V}O_2$ measured during this validation test did not exceed the $\dot{V}O_2$max measured during the incremental test, considering a possible ~3% measurement error based on the equipment used, the achievement of a $\dot{V}O_2$ plateau was accepted. When the $\dot{V}O_2$ achieved during validation exceeded that measured during the incremental test, a new incremental test was performed on a separate day.

3.3.8.3 Performance Assessment

Participants completed a simulated 30-km time trial (TT) 180 min following ingestion of the test meal. With their personal bicycle mounted to the CompuTrainer® and tire pressures standardized at 100 psi, participants performed a 10-minute warm up followed by calibration of the press-on force (POF) of the load generator per manufacturer's guidelines. Participants then completed the 30-km TT on a virtual course in the RacerMate One™ software (RacerMate Inc., Seattle, WA). A copy of the course file can be found at https://osf.io/ujx6e/. Participants were instructed to complete the TT as quickly as possible and were verbally encouraged throughout the trial. Participants' HR was monitored continuously using a Polar H7 HR sensor and chest strap (Polar Electro Oy, Kempele, Finland). Respiratory gas measurements and ratings of perceived exertion (RPE) on a 6-20 Borg Scale were collected at 3 km and every 6 km thereafter.

3.3.8.4 Respiratory Gas Analysis

Respiratory gas measurements were collected using an open circuit automated gas analysis system (TrueOne2400, Parvo Medics, Sandy, UT). Participants breathed through a two-way valve

(Hans Rudolph, Shawnee, KS) attached to a 7450 Series Silicone V2TM Oro-Nasal Mask (Hans Rudolph) for three min at each collection time point. Substrate oxidation was calculated using the following equations, which assume a non-protein RER[44]:

$$\text{CHO oxidation (g/min)} = 4.585 \times \dot{V}CO_2 - 3.226 \times \dot{V}O_2$$

$$\text{Fat oxidation (g/min)} = 1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2$$

3.3.8.5 Muscle Ultrasound

Session fuel percentile (SFP) was determined using ultrasonic assessment of the right and left rectus femoris (RF). SFP provides an estimate of the muscle content of glycogen and other constituents based on the mean pixel intensity of an ultrasound image. Ultrasonic imaging was performed with a diagnostic high-resolution GE LOGIQ-e (GE Healthcare, Milwaukee, WI) using a 9L transducer at 8 Hz. Images from both RF were taken in triplicate. Ultrasound images were uploaded via DICOM to a secure cloud-based web application (MuscleSound Inc, Denver, CO), which analyzes the echogenicity of the ultrasound image as an estimate of the content of muscle glycogen and other constituents. This method has been shown to correlate highly with glycogen content measured by muscle biopsy.[29,45] However, some studies have questioned the validity and utility of this technique.[46,47] In the present study, we investigated whether the MuscleSound® system was able to detect assumed changes in muscle glycogen content resulting from dietary interventions and a 30-km TT. Following recommendations in personal communications with the company, we used the SFP score, which was implemented after publication of the MuscleSound® position stand on the application of the system.[48]

3.3.8.6 Resting Metabolic Rate

RMR was measured by indirect calorimetry using the TrueOne® 2400 (ParvoMedics, Sandy, UT, USA) indirect calorimeter with a ventilated hood system following a 12-hour overnight fast from food, supplements, and medication and a 24-hour abstinence from exercise. The first ten min of the 30 min measurement period were used to allow the participants to achieve resting status; the final 15 min were used for analysis.

3.3.8.7 Air Displacement Plethysmography

Participants entered the BOD POD (COSMED USA Inc., Concord, CA) wearing a bathing suit or cycling kit with all hair collected into a swim cap. Thoracic lung volume were measured during the test using the BOD POD system.

3.3.9 Data Analysis

3.3.9.1 Time to Completion and Average Power Output

As described above, the study was powered based on a TTC analysis of finishing times at the Texas State Time Trial Championships. Thus, we deemed TTC for the present TT our primary outcome measure. However, following the completion of three participants, we identified an error in our protocol that caused assigned rider weights (RW) in the RacerMate One™ software to be incorrect for some participants/conditions. The software calculates the speed the avatar achieves on the virtual course using RW, bike weight, road gradient, and measured power output. Thus, several finishing times were incorrect. Therefore, we present the average power outputs during the TT as our measure of endurance performance below. Further, we discuss considerations regarding the calculations that produce speed output from power input in the RacerMate One™ software in the *Discussion* section. As detailed above, since we did not achieve the desired statistical power,

we only present means and standard deviations for these outcome measures; inferential statistics are not presented.

3.3.10 Statistical Analysis

All analyses were performed in the *R* statistical environment.[31] One participant with missing data for one TT (tire failure at 26 km) was removed from the analysis of average power output. All analysis scripts and data used in this manuscript can be found at https://osf.io/ujx6e/.

3.3.10.1 Exploratory Analyses

Missing data for exploratory analyses (e.g., SFP) were imputed using the *MICE* package in *R* [49] using the PAN method created by Schafer and Yucel.[49,50] Exploratory variables were analyzed using a linear mixed-effects model with a Holm-Bonferroni post *hoc* test using the *lme4* and *emmeans* packages in *R*.[51,52] Fixed effects for these models include diet (HD, KD, HC) and TT time points (3km, 9km, 15km, 21km, 27km). Participant intercept was treated as a random effect. While prior research would have allowed the generation of directional hypotheses regarding RER, substrate oxidation, and RPE, we treated these variables as exploratory, since we did not power the study to these variables. Alpha level was set at 0.05 for all exploratory analyses.

3.3.10.2 Control Variables

Dietary intake, body mass, physical activity, environmental conditions during the TT, and capillary BHB were treated as control variables. Potential mean differences in body mass by diet condition, dietary intake, and capillary BHB were analyzed using linear mixed-effects models as explained above. Differences in environmental conditions (humidity and fluid intake), were analyzed using standard linear models. We did not perform statistical analysis of lab temperature, since the temperature was 22.0 degrees during all but four trials, where the temperature was 21.0

degrees. Potential mean differences in physical activity (total distance and sRPE) between diet conditions were assessed using paired t-tests.

3.3.10.3 Assumption Checks

Visual inspection of residual plots confirmed that normality and homoscedasticity assumptions were met for all analyses.

3.4 Results

3.4.1 Cycling Performance

3.4.1.1 Average Power Output

Five participants completed all three TT (m = 1, f = 4). One additional participant completed the TT in the HD and HC conditions but had to abort the trial in the KD condition due to a tire failure at 26 km; he completed all other measures in the KD condition. Average power output was greatest in the HC condition (199.7 ± 92.2 W), followed by HD (188.0 ± 80.6 W) and KD (172.0 ± 93.2 W). A raincloud plot of average power outputs is presented in **Figure 3.4**.

3.4.2 Physiological Responses during the TT

3.4.2.1 Oxygen Consumption

$\dot{V}O_2$ during the TT was similar in all conditions across all time points. During the HD and HC condition, participants relative $\dot{V}O_2$ was 29.9 ± 7.1 ml/kg/min (63.8 ± 10.0% $\dot{V}O_2$max) and 29.9 ± 7.1 ml/kg/min (63.6 ± 6.9 % $\dot{V}O_2$max) respectively. In the KD condition, participants cycled at 58.6 ± 15.4 % of their $\dot{V}$O2max (27.8 ± 7.1 ml/kg/min). There were no main effects for condition, $F(2, 69) = 1.853$, $p = 0.165$, $\eta^2_p = 0.05$, or time, $F(4, 69) = 0.995$, $p = 0.416$, $\eta^2_p = 0.05$, and no time x condition interaction $F(8, 69) = 0.556$, $p = 0.810$, $\eta^2_p = 0.06$.

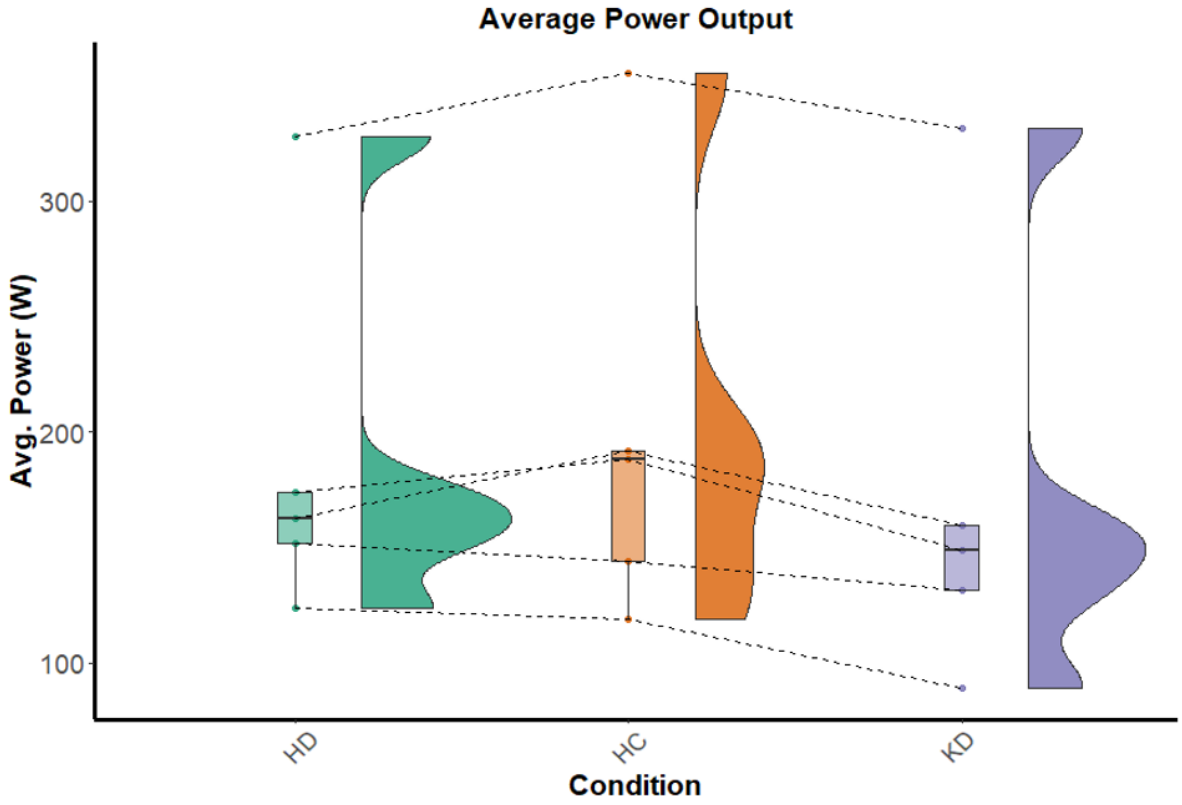

Figure 3.4. Average Power Output During the Time Trial. n = 5; HD = habitual diet; HC = high carbohydrate diet; KD = ketogenic diet.

3.4.2.2 Heart Rate

There was no main effect for condition, $F(2, 69) = 0.387$, $p = 0.680$, $\eta^2_p = 0.01$, and no time by condition interaction, $F(8, 69) = 0.270$, $p = 0.974$, $\eta^2_p = 0.03$, for HR during the TT. Participants' HR was 163 ± 17 beats/min, 161 ± 22 beats/min, and 162 ± 21 during HD, KD, and HC respectively. Mean HR rose throughout all trials (3km: 159 ± 17 beats/min; 27km: 167 ± 23 beats/min), but this increase was not statistically significant, $F(4, 69) = 2.439$, $p = 0.055$, $\eta^2_p = 0.12$.

3.4.2.3 Substrate Oxidation

There were main effects for condition ($F(2, 69) = 118.178$, $p < 0.001$, $\eta^2_p = 0.77$) and time ($F(4, 69) = 6.855$, $p < 0.001$, $\eta^2 p = 0.28$) for CHOox, but not time x condition interaction ($F(8, 69) = 1.177$, $p = 0.326$, $\eta^2_p = 0.12$). During KD, participants oxidized significantly less CHO compared with HD (Mean Difference [*MD*] = -1.11 g/min; 95% CI [*95CI*] = -1.37, -0.86; $t(69) = -10.856$; $p < 0.001$) and HC (*MD* = -1.53 g/min; 95CI = -1.78, -1.28; $t(69) = -14.9$; $p < 0.001$). Additionally, CHOox was significantly greater in the HC condition compared with HD (*MD* = 0.42 g/min; *95CI* = 0.06, 1.58; $t(69) = 3.41$; $p < 0.001$). Across all condition, CHOox decreased significantly following the 3km measurement (1.87 ± 0.75 g/min) with the lowest average CHOox measured at 21km (1.54 ± 0.76. g/min).

FATox opposed the pattern of CHOox: it was greatest in KD (0.62 ± 0.11 g/min), followed by HD (0.32 ± 0.11 g/min), and HC (0.14 ± 0.11 g/min), $F(2, 69) = 69.101$, $p < 0.001$, $\eta^2_p = 0.74$. Averaged across conditions, FATox was lowest at 3km (0.26 ± .12 g/min) and highest at 15km (0.41 ± 0.12 g/min); a main effect for time was observed, $F(4, 69) = 3.629$, $p = 0.010$, $\eta^2_p = 0.17$. There was no time x condition interaction for FATox, $F(8, 69) = 0.445$, $p = 0.890$, $\eta^2_p = 0.05$. Substrate oxidation during the TT is presented in **Figure 3.5**.

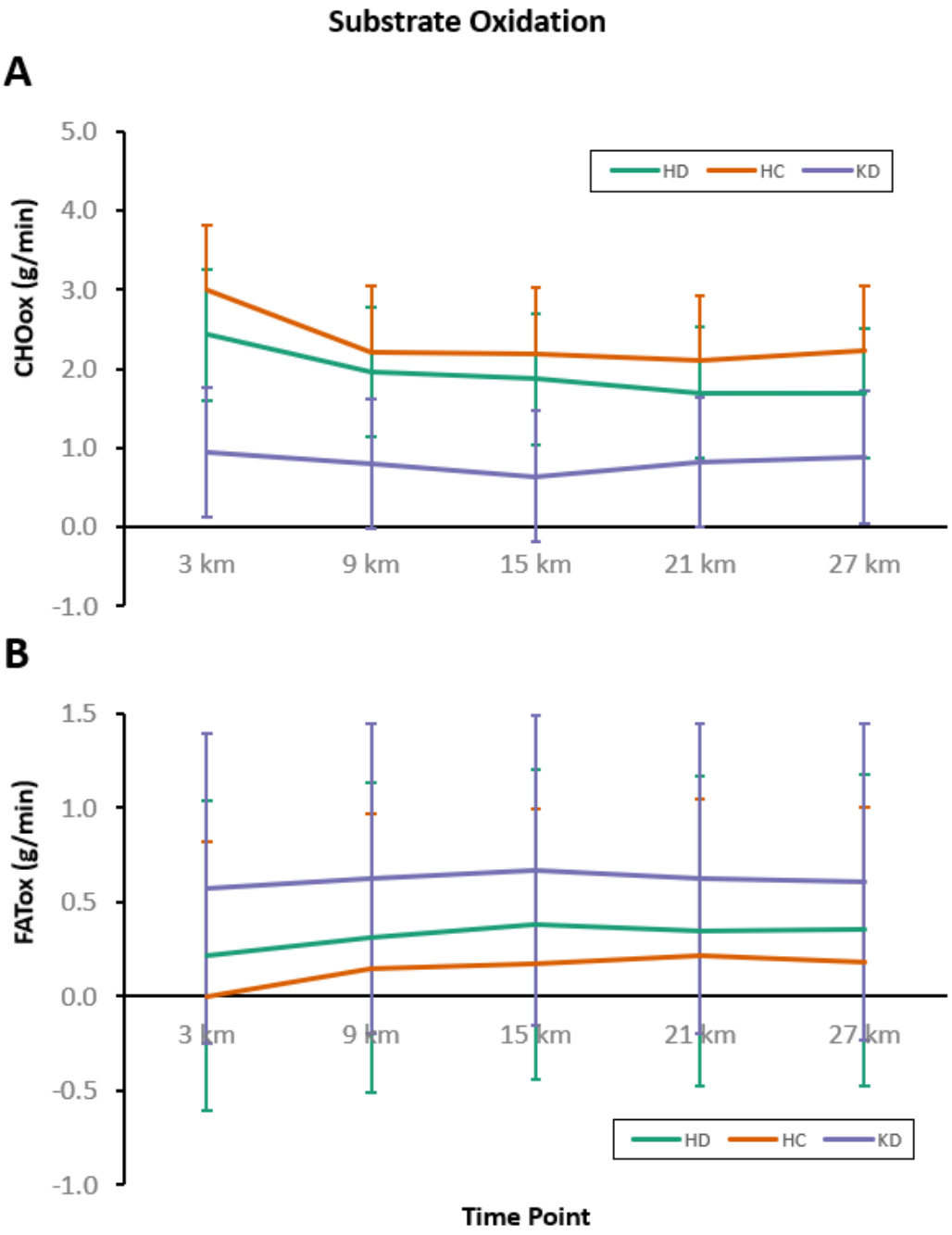

Figure 3.5. Substrate Oxidation During the Time Trial. n = 5; A = Carbohydrate oxidation (CHOox); B = Fat oxidation (FATox). HD = habitual diet; HC = high carbohydrate diet; KD = ketogenic diet.

3.4.2.4 Perceived Exertion

RPE was similar across all three conditions, $F(2, 69) = 2.244$, $p = 0.114$, $\eta^2_p = 0.06$; participants reported RPEs of 14.5 ± 1.2 for HD, 14.9 ± 0.8 for KD, and 15.0 ± 1.1 for HC. Perceived exertion significantly increased throughout the trial from 13.1 ± 1.2 at 3km to 16.3 ± 1.0 at 27km (time main effect: $F(4, 69) = 23.655$ $p < 0.001$, $\eta^2_p = 0.58$). RPE throughout the TT is shown in **Figure 3.6**.

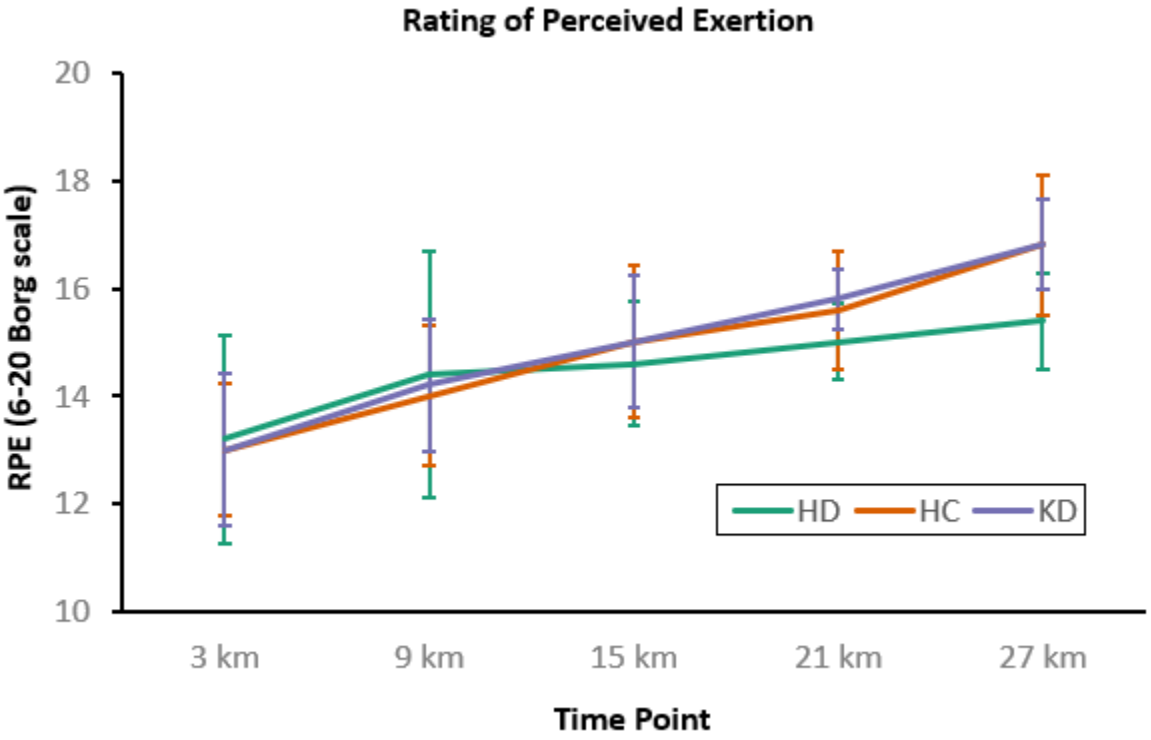

Figure 3.6. Rating of Perceived Exertion During the Time Trial. n = 6; HD = habitual diet; HC = high carbohydrate diet; KD = ketogenic diet. Data are presented as estimated marginal means ± SD.

3.4.2.5 Muscle Ultrasound

Figure 7 shows estimated mean differences in SFP by condition and time following 100 imputations of missing data using the *MICE* package with the PAN method, as described above. Pooled estimates across the 100 imputations were compatible with a lower SFP following two weeks of KD compared with HD, *MD* = -10.0, *95CI* [-21.0, 0.6], $p = 0.063$. Similarly, pooled estimates were compatible with lower SFP following the TT compared with baseline measures, *MD* = -8.8, *95CI* [-19.0, 1.3], $p = .0085$. SFP was similar between HD and HC, as well as between baseline and PRE-TT measures. There appeared to be no interactions between condition and time.

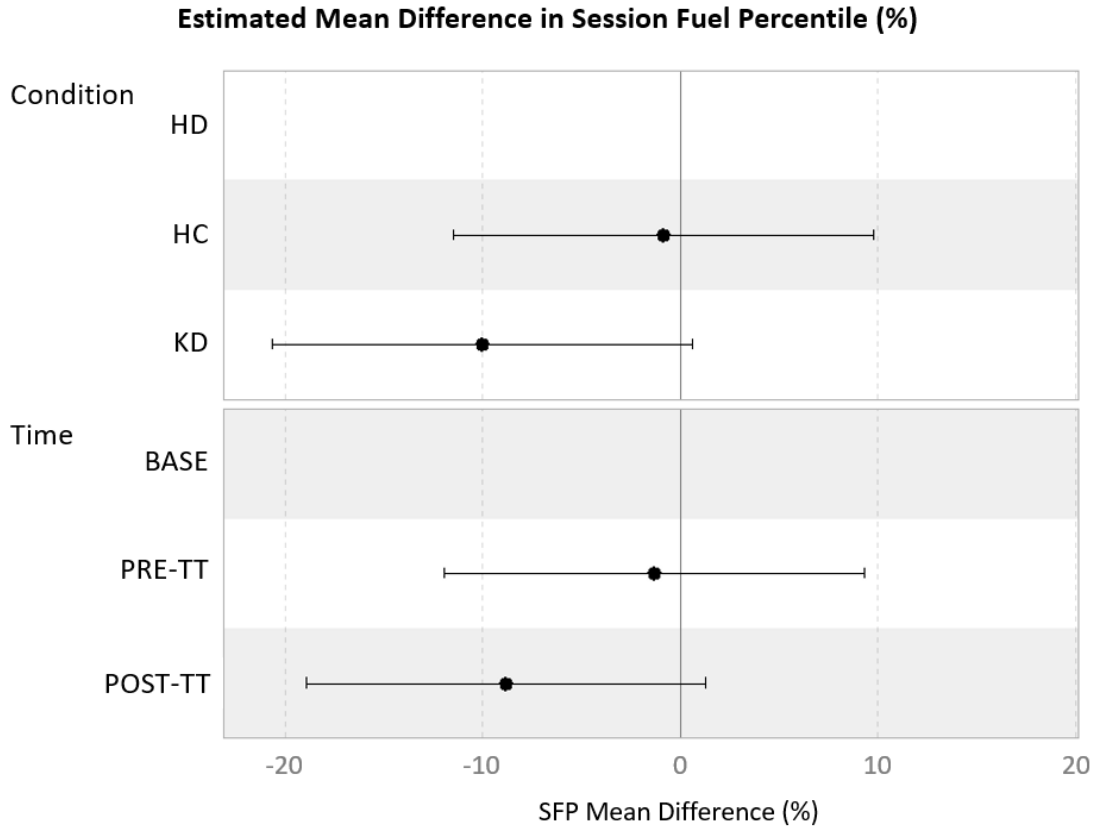

Figure 3.7. Estimated Mean Difference in Session Fuel Percentile. n = 6; based on 100 imputations of missing data. Error bars represent 95% Confidence Intervals. HD = habitual diet; HC = high carbohydrate diet; KD = ketogenic diet. BASE = fasted baseline measure; PRE-TT = 180 min following the test meal, immediately prior to the TT; POST-TT = immediately following the TT.

3.4.3 Control Variables

Means and standard deviations for all control variables are reported in **Table 3.2** and have been in part reported elsewhere.[37]

Table 3.2. Control Variables for the Three Diet Conditions.

	HD	KD	HC
Total Energy Intake (kcal)	2140 ± 555	2447 ± 509	2418 ± 652
Carbohydrate (% total energy)	45.8 ± 6.9	8.7 ± 2.9	63.3 ± 8.8
Fat (% total energy)	38.2 ± 7.8	64.1 ± 5.4	20.8 ± 7.6
Protein (% total energy)	16.5 ± 4.2	26.0 ± 2.9	14.4 ± 3.2
Body Mass (kg)	68.7 ± 17.5	66.4 ± 16.8	68.6 ± 17.3
Average Training sRPE (A.U.)	-	482 ± 225	579 ± 262
Total Training Volume (km)	-	339 ± 165	365 ± 188
Fluid Intake During TT (mL)	383 ± 74	352 ± 146	343 ± 100
Fasting BHB (mmol/L)	0.27 ± 14	0.99 ± 61	0.10 ± 18
Ambient Temperature (°C)	21.8 ± 0.4	21.7 ± 0.5	21.8 ± 0.4
Relative Humidity (%)	51.3 ± 6.0	36.8 ± 8.4	36.5 ± 12.2

n = 6; data are presented as means ± SD. HD = habitual diet; KD = ketogenic diet; HC = high-carbohydrate diet; sRPE = session RPE; TT = time trial; BHB = beta hydroxybutyrate.

3.4.3.1 Dietary Intake and BHB

Detailed dietary intake and BHB results are reported elsewhere [37]. Briefly, participants consumed similar amounts of total daily energy. Further, participants had the greatest protein intake during KD when compared with HD and HC. As intended, CHO consumption was greatest in HC and lowest in KD. Fat consumption was highest in KD and lowest in HC.

Capillary BHB was greater following KD compared with HC and HD, indicating successful compliance with the diet. This is further reflected in the daily urinary ketone measurements during the KD, which averaged 1.82 ± 0.52 mmol/L during the KD.

3.4.3.2 Body Mass

Detailed changes in boy mass during the interventions are reported elsewhere.[37] Briefly, participants weighed significantly less following the KD compared with HD and HC. There was no significant difference in body mass between HD and HC conditions. It is important to note that, while all participants lost weight during the KD, none of them surpassed our threshold of 5% body mass loss.

3.4.3.3 Training

As reported elsewhere, participants' training was similar between HC and KD.[37] There were no significant differences in total kilometers cycled or sRPE when comparing the two diet conditions.

3.4.3.4 Water Intake during the TT

Water intake during the TT was similar between conditions, $F(2, 15) = 0.214$, $p = 0.810$, $\eta^2_p = 0.028$. Participants consumed 383 ± 74 mL, 352 ± 146 mL, and 343 ± 100 mL of water during HD, KD, and HC respectively.

3.4.3.5 Environmental Conditions during the TT

Temperature in the lab was consistent across all trials averaging 21.8 ± 0.4 °C during HD, 21.7 ± 0.5 °C during KD, and 21.8 ± 0.4 °C during HC. There was a significant effect of condition on relative humidity during the TT, $F(2, 15) = 5.037$, $p = 0.021$, $\eta^2_p = 0.402$. Humidity was greatest during HD (51.3 ± 6.0 %); it was similar between KD (36.8 ± 8.4 %) and HC (36.5 ± 12.3 %).

3.5 Discussion

3.5.1 Methodological Insights and Considerations

3.5.1.1 Equipment and Outcome Measure Selection

3.5.1.1.1 Cycle Ergometer.

Based on participant feedback during previous studies and pilot work as well as to minimize learning effects, we chose to use the CompuTrainer® cycle ergometer as our testing device. This allowed participants to mount their own bicycle to the ergometer maximizing familiarity with the equipment. In prior work in our laboratory, some participants had voiced concerns that bicycle fit was suboptimal with other ergometers, such as the Velotron Pro (RacerMate Inc., Seattle, WA) and Monark Ergomedic 894e (Monark, Sweden). In a meta-analysis by Hopkins et al. cycle ergometers that allowed participants to use their own bicycles produced some of the smallest coefficients of variation (CV) in the study.[53] Participants in the present study expressed that they favored using their own equipment over using other ergometers, validating our choice of equipment.

However, certain challenges can come with the use of ergometers that allow participants to use their own bicycles. First, tire inflation pressure, and press-on force (POF) between the tire and the friction roller of the load generator must be standardized for each condition between conditions. The manufacturer's manual for the CompuTrainer® suggests inflating tires to the maximum rated tire pressure and provides a guide for setting the POF based on maximal road gradients or maximal expected power output during the exercise bout. We decided to standardize tire pressure at 100 psi unless the tires were rated for lower pressure. However, unbeknownst to the investigators present at the trial, one of our participants used an inner tube in a tubeless tire during one TT, causing over inflation and tire failure. This illuminates another challenge in

allowing participants to use their own bicycles: the need to ensure that participants do not make changes to their equipment between trials. One of our participants changed tires between conditions; the new tires were rated at a lower pressure than the ones he used in the initial trial. However, the participant had discarded the old tires, thus making it impossible to keep tire pressure constant across trials. Data for this participant are not included in this manuscript, since we had to terminate the study prior to his final ET due to COVID-19 regulations.

3.5.1.1.2 Performance Measure.

To maximize external validity, we decided to use a TT that was similar in length (time) to what our participants typically experience in competition. To align our statistical inference with this strategy, we powered our study to be able to detect a practical meaningful difference of 90 seconds between the HC and KD conditions, which, on average, reflected an improvement of one position in the final standings of the Texas State Time Trial Championships across the past four years. Thus, we selected time to completion (TTC) as our primary outcome measure. While we have used TTC successfully in previous work using the Velotron and Monark 894e, the use of this measure with the CompuTrainer® created additional challenges. As described above, an error in our protocol caused inconsistencies in the rider weight (RW) used during CompuTrainer® setup. While the RacerMate One™ software manual provides load curves for the ergometer, we were unable to determine the exact formula to translate power output (W) to speed (km/h); one factor influencing this is the built-in Drag Factor™ (DF) function, which allows users to set a percentage based "drag factor" equivalent to an estimated coefficient of aerodynamic drag multiplied by the frontal area of the rider (CdA). The default value for this and rolling resistance are unknown to the authors. Our initial strategy was to recalculate finishing times for each participant by using the speed achieved per watt measured during the initial TT (following their HD). We applied this

speed-per-watt factor to the measured power outputs for all other trials to recalculate finishing times (**Table 3.3**). Calculation scripts and speed-per-watt data for each rider by road gradient can be found at https://osf.io/ujx6e/.

Using the crude estimation of speed-per-watt employed for our recalculation of TTC, it appears that even when setting the RW and POF to nearly identical values a meaningful difference in speed and finishing time arises. Participant 17 completed the KD (RW: 68.0 kg; bike weight (BW): 10 kg; POF: 3.06 lbs.; DF: 100%) and HC (RW: 68.0 kg; BW: 10 kg; POF: 3.07; DF: 100%) with nearly identical settings but received meaningfully different speed-per-watt values. This is in part due to the increase in CdA with increasing speed, as the wind resistance experienced by a rider becomes greater at higher speed.

With the participant riding slower during KD, the software correctly generated greater speed-per-watt in this condition compared with HC. To control this factor and to further investigate the speed achieved for the power applied, we analyzed speed-per-watt at different power outputs across the two trials. Further, we compared these numbers to a model of overground road cycling, which allows manual entry of all parameters associated to cycling (**Figure 3.8**).[54]

Table 3.3. Recalculation of Time-to-Completion

ID	COND	RW (kg)	POF (lbs)	AVG POW (W)	AVG SPD (km/h)	TTC (min)	AVG SPD/W (km/h/W)	AVG SPD REC (km/h)	TTC REC (min)
08	HD	57.2	3.20	173.84	31.56	57.03	0.183	31.87	56.48
	HC	94.8	3.12	188.26	30.53	58.96	0.165	34.51	52.16
	KD	54.0	3.15	148.87	29.54	60.94	0.200	27.29	65.96
12	HD	83.0	4.67	328.31	37.38	48.15	0.115	37.81	47.60
	HC	83.9	4.67	355.31	39.45	45.63	0.112	40.92	43.99
	KD	83.9	4.43	331.63	38.54	46.70	0.117	38.20	47.13
14	HD	57.6	3.38	162.66	30.33	59.35	0.188	30.55	58.92
	HC	54.9	3.17	191.67	33.04	54.48	0.173	36.00	50.01
	KD	54.9	3.24	159.31	30.19	59.62	0.192	29.92	60.16
17	HD	68.9	3.01	151.95	29.37	61.29	0.196	29.71	60.59
	HC	68.0	3.07	144.29	28.38	63.41	0.199	28.21	63.81
	KD	68.0	3.06	131.31	27.35	65.82	0.208	25.67	70.12
28	HD	68.9	2.87	123.60	25.55	70.46	0.210	25.98	69.30
	HC	67.9	2.71	118.88	25.58	70.36	0.217	24.98	72.05
	KD	68.0	2.75	88.87	21.60	83.33	0.2443	18.68	96.38

RW = rider weight; POF = press-on force; AVG POW = average power output; AVG SPD = average speed; TTC = time-to-completion; AVG SPD/W = average speed-per-watt;
REC = recalculated based on AVG SPD/W achieved in HD.

We limited the analysis to flat stretches of the TT to eliminate the effect of road gradient and only included power outputs between 100 W and 200 W. It was apparent, that speed-per-watt values fluctuated greatly immediately following return from a descent to a flat stretch on the course After removing the 20 seconds following each descent and large outliers based on visual inspection of the graph, we fit a power function for all three analyses.

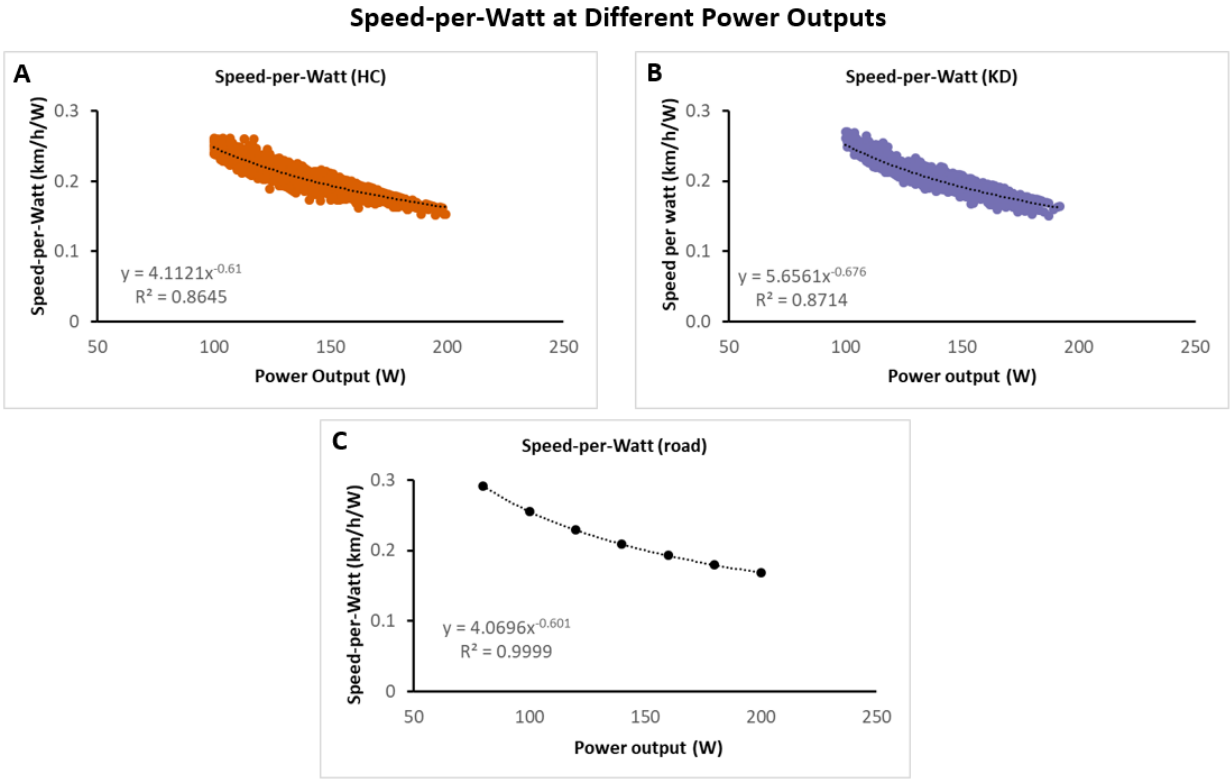

Figure 3.8. Speed-per-Watt at Different Power Outputs. HC = high carbohydrate diet; KD = ketogenic diet; road = speed-per-watt modeled using a road cycling model calculator.

As **Table 3.4** shows, even small differences in the speed-per-watts conversion, can have meaningful effects on finishing time during a simulated TT. At a fictitious power output of 150 in a flat TT, the conversion alone would lead to a difference of 44.4 seconds in TTC. These conversion calculations were highly sensitive to the inclusion/exclusion of individual datapoints

as the same power input can result in different instantaneous speed output. Actual differences might not be as large, as individual datapoints account for only one second of the speed achieved. However, in the HC trial shown above, power output was measured at 150W on flat road sections 41 times, with speed-per-watt ranging from 0.179 km/h/W (26.8 km/h) to 0.202 km/h/W (30.3 km/h). It is important to note, that despite these challenges, the CompuTrainer® very closely mirrors the time achieved in an overground road cycling TT.

Table 3.4. Speed-per-Watt Comparisons.

	POW (W)	Formula	SPD/W (km/h/W)	TTC CALC (min)
HC	150	$y = 4.1121x^{-0.61}$	0.193485	62.02
KD	150	$y = 5.6561x^{-0.676}$	0.1912	62.76
Road model	150	$y = 4.0696x^{-0.601}$	0.200318	59.90

HC = high-carbohydrate diet; KD = ketogenic diet; POW = power output; SPD/W = speed-per-watt; TTC CALC = calculated time-to-completion.

Despite some limitations regarding the conversion of power output to speed and the challenges of standardizing between conditions, we believe the CompuTrainer® is an effective tool for performance analysis. The familiarity of participants with their own equipment and the positive feedback regarding bicycle fit and feel may outweigh any challenges faced with implementing this performance assessment. Based on our experience in this project, we recommend using mean power output during a TT as the performance outcome variable rather than TTC. We also suggest extensive piloting of the TT course and protocols to ensure all important factors are kept constant between conditions. Further, we recommend giving participants written instructions to avoid any changes to their equipment and checking all aspects of the bicycle setup (including tires) on the day of the trial.

Additionally, we would recommend researchers employing a repeated measures design use participant's actual body mass on the day of each trial as RW. Since the RacerMate One™ software accurately models differences in RW, potential benefits from decreased body mass on cycling speed, especially during uphill sections of a course, should be captured by the performance assessment.

3.5.1.2 Nutrition Intervention

A multi-week nutrition intervention like the one applied in the present study requires considerable labor and time from the investigators as well as personal investment from participants. The following section discusses insights and considerations regarding the nutritional intervention.

3.5.1.2.1 Diet Tracking and Meal Planning.

Following dietary interventions like the ones employed in the present study requires careful tracking of nutrition intake and exercise energy expenditure. The participants in our study provided verbal feedback that tracking their dietary intake and finding foods to match the macronutrient requirements for each diet added a sizeable burden to their daily routines. With this in mind, it is unsurprising that less than 20% of recreational cyclists regularly track their nutritional intake (unpublished data from a survey study conducted in our laboratory). In fact, in our pre-study screening questionnaire, none of the participants in the present study reported tracking total energy intake or macronutrients nor following a specific diet. It stands to reason that keeping a record of dietary intake and planning meals to achieve certain nutritional goals might create a steep barrier for recreational athletes trying to follow HC or KD.

3.5.1.2.2 Diet Adherence.

Our three-day dietary records indicated that participants followed the intervention diets as prescribed, with the exception of higher-than-desired protein intake during the KD (Table 3). Yet, based on levels of BHB in urine and blood during the KD, participants met our requirement of being in a ketogenic state. Based on verbal and written feedback from our participants, even with the daily feedback they received from the RD, participants struggled to find high-fat foods that limited their intake of protein. However, it appears that the protein intake in our KD condition (26.0 ± 2.9% of total energy intake) was similar to what other studies have reported when participants were allowed to consume protein *ad libitum*.[55–57] Thus, allowing *ad libitum* intake of protein during the KD condition appears to be a practical way to reduce the burden on participants to find low-protein high-fat foods. To control for the effect of changes in fat-free body mass, which could have an impact on exercise performance, we suggest measuring body composition following each diet, if resources allow it. In the present study, equipment availability prohibited us from performing these measurements.

Similarly, participants reported struggling to consume the high percentage of CHO to fulfill the requirements of the HC without resorting to sugary drinks and foods. This could be one reason why our own findings and those of other researchers, that free-living recreational endurance athletes consume less CHO than what is recommended for optimizing performance.[1,58] The strongest experimental design regarding diet adherence would include supplying food for participants throughout the study. This would take the burden of diet tracking and meal planning off the participants. However, with a free-living cohort such as ours, this is difficult and costly.

3.5.1.2.3 Blinding.

Blinding of participants to the study condition is impossible in a study design like the present. Participants' effort during training and performance assessment could be influenced by preconceived opinions about the interventions employed. Recent research has shown that recreational endurance athletes are more aware of the effects of CHO intake before, during, and after events than the general public.[59] Thus, participants might have expected to perform worse during the KD. This became apparent in the present from verbal comments by the participants, who mentioned not looking forward to completing the KD condition. Additionally, during the KD, they reported feeling like they could not produce the same amount of power and fatiguing more quickly during training rides. One participant completed the TT approximately 13 min slower during the KD than during the HD and HC. This participant specifically expressed feeling fatigued during the KD. It is unclear whether a preconceived notion of the KD on endurance performance might have impacted the participant's effort during the TT or whether the participant truly experienced such strong effects of the diet.

3.5.1.3 Statistical Analysis

3.5.1.3.1 Sample Heterogeneity and Statistical Power.

Our goal for the present study was to collect data from men and women across a wider age-range than previously reported in the literature. However, this has important implications on statistical power. Based on our analysis of the Texas State Time Trial Championships, finishing times and standard deviations of the top 10 athletes in male and female age groups up to 55+ years old (61 ± 6 min) was similar to pilot work on the CompuTrainer® course in our own lab (60 ± 6). However, our final sample comprised athletes with much greater heterogeneity in the main

performance outcome. This sample heterogeneity has a drastic impact on statistical power in a frequentist framework.[60,61]

We attempted to limit sample heterogeneity by requiring minimum training experience and distance along with a $\dot{V}O_2$max criterion for enrollment in the study. Average TTC was similar to what we expected, but standard deviations in our sample ranged from 8.0 min (HD) to 13.2 min (KD). Simply raising the standard deviation in our power analysis from 6.0 to 10.2 (average of our observed standard deviations), while leaving all other parameters the same would decrease statistical power for the omnibus test with 30 participants from 90% to 45%. One avenue to further limit this heterogeneity and increase statistical power, would be employing a TT as part of the screening process to ensure participants can complete the course in a predetermined maximal time or at a predetermined minimal average power output. This trial could also serve as a familiarization trial for participants to become accustomed to the laboratory and the bike setup.

3.5.1.3.2 Analysis Options.

A common strategy to analyze data like the present is to employ repeated measures analysis of variance (RM-ANOVA). However, other fields including psychology, biology, and medicine, have transitioned to using linear mixed-effects models (LMM) for designs similar to ours.[62] In the following section we present different analysis options for our primary outcome (TTC) and for one example of a secondary outcomes (CHOox). To avoid reporting inferential statistics based on observed data of our primary outcome, we used simulated data to show the different analysis options. All simulations and analysis scripts can be found here: https://osf.io/ujx6e/ .

We investigated the outcome of three statistical methods to analyze our primary outcome (TTC) with simulated data based on the following parameters using the *faux* package in R[63]:

$$n = 18$$

$$HD: \mu = 61.0 \text{ min}; \sigma = 8.0 \text{ min}$$

$$HC: \mu = 60.0 \text{ min}; \sigma = 9.0 \text{ min}$$

$$KD: \mu = 62.5 \text{ min}; \sigma = 10.5 \text{ min}$$

These parameters are loosely based on our actual data in combination with the practically meaningful effect size of 90 seconds discussed above. The three methods investigated were: 1) LMM using the *lme4* package, 2) standard RM-ANOVA using the *afex* package, and 3) analysis of covariance (ANCOVA), as recommended by Senn [64] using the *rstatix* package.[64,65] As an example of the secondary outcome analysis, we chose observed data for CHOox and analyzed them using 1) LMM and 2) condition x time RM-ANOVA. Inferential statistics for all analyses are shown in **Table 3.5**.

Table 3.5. Inferential Statistics for Different Analysis Options.

Outcome and model	*NumDF*	*DenDF*	*F*	*p*
TTC				
LMM	2	34	6.06	**0.006**
RM-ANOVA	2	34	6.06	**0.006**
ANCOVA (BASE)	1	33	533.29	**<0.001**
ANCOVA (COND)	1	33	8.12	**0.007**
CHOox				
LMM				
COND	2	69	118.18	**<0.001**
TIME	4	69	6.86	**<0.001**
COND X TIME	8	69	1.18	0.326
RM-ANOVA				
COND	2	8	100.76	**<0.001**
TIME	4	16	4.02	**0.019**
COND X TIME	8	32	1.54	0.184

NumDF = numerator degrees of freedom; DenDF = denominator degrees of freedom; LMM = Linear mixed-effects model; RM-ANOVA = repeated measures analysis of variance; ANCOVA = analysis of covariance; BASE = baseline time from HD trial; COND = condition; TTC = time to completion; CHOox = carbohydrate oxidation

To further analyze statistical outcomes of these strategies, we investigated pairwise comparisons of the estimated marginal mean differences using the *emmeans* and *statix* packages. Results for TTC are shown in **Table 3.6**. We used a Holm correction for multiple comparisons and a Bonferroni correction for the 95% confidence intervals reported.

Table 3.6. Estimated Mean Differences for Time-to-Completion Between Conditions

Comparison and model	*DF*	*t*	*EMD*	*95%CI*	*p*
HD – HC					
LMM	34	1.99	1.28	-0.34, 2.90	0.109
RM-ANVOA	17	3.07	1.28	0.18, 2.39	**0.021**
ANCOVA	-	-	-		-
HD – KD					
LMM	34	-1.48	-0.95	-2.57, 0.67	0.149
RM-ANOVA	17	-1.37	-0.95	-2.79, 0.89	0.187
ANCOVA	-	-	-	-	-
HC - KD					
LMM	34	-3.47	-2.23	-3.86, -0.61	**0.004**
RM-ANOVA	17	-2.90	-2.23	-4.28, -0.19	**0.021**
ANCOVA	33	2.85	-2.23	-3.83, -0.64	**0.007**

DF = degrees of freedom; t = t ratio; EMD = estimated mean difference; 95%CI = 95% confidence limits; LMM = Linear mixed-effects model; RM-ANOVA = repeated measures analysis of variance; ANCOVA = analysis of covariance;

Results for the pairwise comparisons and estimated mean differences between time points is shown in Table 7. For pairwise comparisons by time point, we have limited the table to those that were statistically significant in at least one analysis strategy. Full results can be found using the analysis script at https://osf.io/ujx6e/.

Table 3.7. Estimated Mean Differences for Carbohydrate Oxidation Between Conditions and Time Points.

Comparison and model	*DF*	*t*	*EMD*	*95%CI*	*p*
CONDITION					
HD - HC					
LMM	69	-4.09	-0.42	-0.66, -0.17	**<0.001**
RM-ANVOA	4	-3.41	-0.39	-0.83, 0.06	**0.027**
HD – KD					
LMM	69	10.86	1.11	0.86, 1.37	**<0.001**
RM-ANOVA	4	10.15	1.15	0.70, 1.60	**0.001**
HC - KD					
LMM	69	14.90	1.53	1.28, 1.78	**<0.001**
RM-ANOVA	4	13.78	1.54	1.10, 1.98	**0.001**
TIME					
3km – 9km					
LMM	69	3.57	0.47	0.09, 0.85	**0.005**
RM-ANOVA	4	2.63	0.45	-0.51, 1.41	0.525
3km – 15km					
LMM	69	4.25	0.56	0.18, 0.94	**0.001**
RM-ANOVA	4	2.61	0.47	-0.54, 1.47	0.525
3km – 21km					
LMM	69	4.45	0.58	0.20, 0.96	**<0.001**
RM-ANOVA	4	2.32	0.48	-0.68, 1.64	0.570
3km – 27km					
LMM	69	3.98	0.53	0.15, 0.92	**0.001**
RM-ANOVA	4	3.25	0.39	-0.82, 1.06	0.314

DF = degrees of freedom; t = t ratio; EMD = estimated mean difference; 95%CI = 95% confidence limits; LMM = Linear mixed-effects model; RM-ANOVA = repeated measures analysis of variance; ANCOVA = analysis of covariance.

All three strategies result in similar omnibus test for TTC leading to the same inferential interpretation. As expected, the results for TTC were nearly identical between models. Interestingly, there were important differences in the comparisons for estimated marginal mean differences. While the point estimates for mean differences between conditions were exactly the same for LMM and RM-ANOVA, the 95% CI differed considerably, leading to a different inferential interpretation (see **Table 3.6**) The RM-ANOVA yielded a statistically significant difference between HD and HC, whereas the LMM did not. Confidence intervals were wider in the LMM for the HD and HC comparison only, but narrower for the other comparisons. One downside to the ANCOVA approach is that it only allowed for pairwise comparison between HC and KD, since the TTC HD trial was used as a covariate.

When analyzing CHOox, the omnibus tests for both models indicated main effects for condition and time without an interaction. However, only the LMM showed significant differences in the follow-up pairwise comparisons. The results for *post hoc* comparison of estimated marginal means in the LMM indicated significant difference when comparing CHOox at the 3km mark in the TT compared with all other time points. Interestingly, while the omnibus test for the RM-ANOVA did indicate a main effect for time, none of the follow-up pairwise comparisons were statistically significant.

Based on this analysis, we suggest researchers explore the option of using an LMM in similar designs. The LMM as applied here allows for a random intercept for each participant; further benefits of LMM allow the specification of additional random effects (e.g., participant-level slopes) and using multiple imputation to handle missing data as employed in our analysis of the muscle ultrasound data.[66] When deciding between an RM-ANOVA and an ANCOVA, researchers should consider the study design and research questions. In the present study, we chose

the LMM over ANCOVA to allow for the pairwise comparison of all three conditions. It could be argued, that an ANCOVA approach would have been prudent, since we did not control diet in the HD condition; thus, the HD condition would have lent itself as a true baseline test used as a covariate in the comparison of HC and KD. However, we believe that this also allowed a true comparison of a truly habitual condition compared to two controlled conditions.

3.5.2 Performance

To avoid any inferential interpretation of our TTC data, we will discuss our results in directional terms only. Our data suggests similar trends to the studies of Burke et al. in elite racewalkers.[19,20] Those studies showed improvements in 10-km race walk finishing times in HC conditions with decrements in performance in the KD condition; those performance trials were approximately 15 min shorter than ours and likely completed at a similar or higher relative intensity. In contrast, McSwiney et al. showed a greater improvement in a 100 km TT in the KD group compared with the HC group following a 12-week nutritional intervention.[22] Similarly, in a crossover study, Lambert et al. reported greater TTE in a moderate-intensity cycling task (50% of PPO) following two weeks of KD compared with two weeks of HC.[7] In the same study TTE in a high-intensity cycling task (85% of PPO) was greater following HC compared with KD. In the present study, participants cycled at 65.7 ± 10.9 %, 59.7 ± 15.0%, and 69.0 ± 12.2% PPO in the HD, KD, and HC conditions respectively. Thus, it appears that endurance athletes might benefit or see no decrements from a KD during longer, lower-intensity events; during shorter, higher-intensity tasks, exercise performance appears to be impaired secondary to decreased economy/efficiency.[19,26] A recent review by McSwiney et al. details the effect of KD on a variety of exercise tasks across different populations.[67]

3.5.3 Physiological Responses

3.5.3.1 Oxygen Consumption

While elite cyclists can maintain relative intensities of > 90% of $\dot{V}O_2$max, we expected our participants to perform at intensities > 70% $\dot{V}O_2$max during our TT. The lower-than-expected relative intensities achieved during the TT, especially during KD, was in part driven by a single participant, who completed the KD TT at < 30% $\dot{V}O_2$max.[68] After removal of this participant's data, average $\dot{V}O_2$ was 66.2 ± 8.9 %, 64.4 ± 6.7 %, and 65.9 ± 4.4 % during the HD, KD, and HC conditions respectively. This was still lower than the relative exercise intensity achieved during a similar TT in a study by Coyle et al.; however, their "good state" cyclists were more highly trained than our cohort.[68]

3.5.3.2 Substrate Utilization

CHOox in our sample was greatest during the HC condition and lowest during the KD condition with the opposite pattern emerging for FATox. This is similar to what has been reported in other investigations.[6,16–24] FATox rates during the KD in the present study were lower (0.60 ± 0.15 g/min) compared with data from Carey et al., who reported FATox rates of 1.06 ± 0.29 g/min to 1.16 ± 0.32 g/min during the first 60 min of a 4-hour cycling task at similar intensities to our TT (65% $\dot{V}O_2$max).[6] Participants in that study ate a breakfast containing 3 g/kg BM of CHO and ingested a glucose solution every 30 min during exercise. It is important to consider that participants in the study by Carey et al. performed exercise at a constant load/intensity, whereas participants in the present study attempted to complete the TT as quickly as possible. FATox during KD in our study was similar to that reported by Prins et al., who also employed a TT task, albeit using a different mode of exercise (running) for a shorter duration (5 km; ~20 min) at higher relative intensities (84.2 ± 8.0% $\dot{V}O_2$max); during the TT performed on day 14 of their study, Prins

et al. reported FATox rates of 0.71 ± 0.23 g/min.[23] Removing our participant, who worked at a noticeably lower relative intensity during KD and thus expended less total energy, FATox rates in the present study averaged 0.68 ± 0.12 g/min. FATox rates dropped to 0.14 ± 0.05 g/min during HC, similar to what was reported by Prins et al.[23]

3.5.3.3 Perceived Exertion

RPE during the TT was similar in all conditions in the present study, and increased steadily throughout the performance tests. Thus, participants perceived the same amount of exertion while working at a lower power output during the KD compared with the HD and HC. This was in accordance with verbal feedback provided by our participants, who reported feeling fatigued and unable to produce their usual power outputs during the TT as well as during their training sessions outside the lab. Stepto et al. similarly reported higher RPE throughout nonlaboratory training in their KD condition and during laboratory testing on Day 4 of the KD.[24]

3.5.3.4 Muscle Ultrasound

Despite initial validation studies showing a strong correlation between MuscleSound® estimates of muscle glycogen content and direct measurements via muscle biopsy, some researchers have questioned the utility of this technique.[29,45,47] Routledge et al. were unable to detect changes in MuscleSound® score in response to an 80-minute competitive rugby league game (Study 1) nor in response to glycogen-depleting cycling protocol followed by 36 hours of low compared with high CHO intake (Study 2), while glycogen content measured by biopsy decreased significantly in both studies.[47] It is unclear, which MuscleSound® measure Routledge et al. employed and whether SFP was available as an analysis option in the MuscleSound® cloud application at the time of that study. While we did not measure muscle glycogen content directly, and thus cannot speak to the relationship between SFP and muscle glycogen directly, we believe

that SFP is a measure that is sensitive enough to detect changes induced by exercise and diet. Due to its non-invasive nature and ease of application, this ultrasonic technique appears to be a valuable tool that allows athletes and practitioners to estimate muscle "fuel" changes in response to dietary and exercise interventions.

3.5.4 Conclusions

We found that participants completed a simulated 30-km TT at the lowest mean power output following two weeks of the KD. We also showed that FATox was greatest during the TT following KD and lowest following HC. Further, MuscleSound® SFP, an estimate of muscle "fuel" was lower following KD compared to HD; additionally, SFP was lower following the TT compared to fasted baseline measures and 3-hour post-meal measures. In summary, while this study did not achieve the desired sample size to make inferential claims about the effect of the KD and HC on endurance exercise performance, we believe that the insights gained from our work could be valuable to other researchers, athletes, and practitioners. We argue that allowing participants to use their own bicycles for studies like this on a cycle ergometer such as the CompuTrainer® reduces learning effects and minimizes the need for familiarization; further, it provides a valid measurement of endurance exercise performance, as long as standardization protocols are followed and appropriate outcome measures (e.g., mean power output during a TT) are selected. Further, we contend that employing LMM should be the preferred analysis technique for repeated measures design in a frequentist framework. LMM offer the option to include random intercepts at the participant level, which allows modeling of inter-individual response differences better than using a fixed intercept. Further, LMM allow multiple imputation of missing data, providing a route for researchers to use partial data for participants rather than being forced to delete data listwise, as is typically done using RM-ANOVA. Depending on the study design and

research question, ANCOVA with baseline performance as the covariate also offers a valid analysis strategy. Finally, we believe that using muscle ultrasound for a determination of muscle "fuel" using the MuscleSound® SFP offers a valuable and easy-to-use tool for practitioners and athletes.

3.5.5 Practical Applications

From a practical perspective, following strict diets in the long-term adds considerable burdens to recreational athletes' lives. Thus, a more reasonable approach might be to "fuel for the work required", as proposed by Impey et al.[69] In this paradigm, athletes base their CHO requirements on the work anticipated and/or performed on a given day. Often, recreational cyclists will complete longer training sessions (five to six hours) on weekends and more intense sessions on one or two days during the week. To minimize the added labor and stress of daily macronutrient and energy tracking, athletes could increase CHO intake on the day prior to and during longer and/or more intense training sessions, while eating entirely *ad libitum* on days with easier rides. Recreational athletes using power meters, could calculate energy expenditure based on the average power produced during a ride. In fact, most exercise tracking applications, which are popular among this population, already provide energy expenditure measures based on actual work performed when power meter data are included. Those who do not use power meters, could use heart rate and/or the talk test to estimate energy expenditure and exercise intensity.[70,71] These calculations would allow recreational athletes to fuel longer and harder sessions adequately, while not needing to invest the time and energy to plan and track dietary intake on shorter and easier days.

Single-session CHO restriction for certain low to moderate intensity workouts, i.e., "training low", has been shown to be effective in augmenting gene expression, cell signaling, and

oxidative enzyme activity related with improved endurance performance.[69,72] These strategies might be more feasible and sensible for elite athletes, who typically work with nutrition professionals and often have already optimized all other aspects of their training and racing. However, recreational cyclists looking to use this strategy could implement a higher intensity training session in the morning followed by CHO restriction and a lower intensity training session in the evening.[72]

In summary, recreational athletes looking to improve their cycling performance using nutrition interventions might be better served by focusing on "fueling for the work required" and interspersing occasional training session with low CHO availability than by trying to implement a daily diet designed to restrict or enhance the intake of CHO.[69]

Contributions

Contributed to conception and design: AK, AJG, PPR, JLW, MS

Contributed to acquisition of data: AK, AJG, PPR, KM, GRA

Contributed to analysis and interpretation of data: AK, AJG, MS

Drafted and/or revised the article: AK, AJG, PPR, KM, GRA, JLW, RB-T, MS

Approved the submitted version for publication: AK, AJG, PPR, KM, GRA, JLW, RB-T, MS

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Data and Supplementary Material Accessibility

All data and analysis code used for this manuscript are available at https://osf.io/ujx6e/

3.6 References

1. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Med Sci Sports Exerc*. 2016;48(3):543-568. doi:10/ggnn5v

2. Jeukendrup AE. Carbohydrate Intake During Exercise and Performance. *Nutrition*. 2004;20(7-8):669-677. doi:10/bnpn7g

3. Jeukendrup AE. Nutrition for Endurance Sports: Marathon, Triathlon, and Road Cycling. *J Sports Sci*. 2011;29 Suppl 1:S91-99. doi:10/b52hvj

4. Burke L. Low Carb High Fat (LCHF) Diets for Athletes – Third Time Lucky? *J Sci Med Sport*. 2017;20 Suppl 1:S1. doi:10/ghp7b8

5. Feinman RD, Pogozelski WK, Astrup A, et al. Dietary Carbohydrate Restriction As the First Approach in Diabetes Management: Critical Review and Evidence Base. *Nutrition*. 2015;31(1):1-13. doi:10.1016/j.nut.2014.06.011

6. Carey AL, Staudacher HM, Cummings NK, et al. Effects of Fat Adaptation and Carbohydrate Restoration on Prolonged Endurance Exercise. *J Appl Physiol (1985)*. 2001;91(1):115-122. doi:10.1152/jappl.2001.91.1.115

7. Lambert EV, Speechly DP, Dennis SC, Noakes TD. Enhanced Endurance in Trained Cyclists During Moderate Intensity Exercise Following 2 Weeks Adaptation to a High Fat Diet. *Eur J Appl Physiol Occup Physiol*. 1994;69(4):287-293. doi:10.1007/bf00392032

8. Lambert EV, Hawley JA, Goedecke J, Noakes TD, Dennis SC. Nutritional Strategies for Promoting Fat Utilization and Delaying the Onset of Fatigue During Prolonged Exercise. *J Sports Sci*. 1997;15(3):315-324. doi:10.1080/026404197367326

9. Kenney WL, Wilmore JH, Costill DL. *Physiology of Sport and Exercise*. 7th ed. Human Kinetics; 2020.

10. Burke LM, Castell LM, Casa DJ, et al. International Association of Athletics Federations Consensus Statement 2019: Nutrition for Athletics. *Int J Sport Nutr Exerc Metab*. 2019;29(2):73-84. doi:10/gjbrp2

11. Kerksick CM, Arent S, Schoenfeld BJ, et al. International Society of Sports Nutrition Position Stand: Nutrient Timing. *J Int Soc Sports Nutr*. 2017;14(1):33. doi:10/gg2nh6

12. Galbo H, Holst JJ, Christensen NJ. The Effect of Different Diets and of Insulin on the Hormonal Response to Prolonged Exercise. *Acta Physiol Scand*. 1979;107(1):19-32. doi:10.1111/j.1748-1716.1979.tb06438.x

13. Pitsiladis YP, Maughan RJ. The Effects of Exercise and Diet Manipulation on the Capacity to Perform Prolonged Exercise in the Heat and in the Cold in Trained Humans. *J Physiol*. 1999;517(Pt 3):919-930. doi:10.1111/j.1469-7793.1999.0919s.x

14. Goedecke JH, Christie C, Wilson G, et al. Metabolic Adaptations to a High-Fat Diet in Endurance Cyclists. *Metab Clin Exp*. 1999;48(12):1509-1517. doi:10.1016/s0026-0495(99)90238-x

15. Burke LM, Hawley JA. Effects of Short-Term Fat Adaptation on Metabolism and Performance of Prolonged Exercise. *Med Sci Sports Exerc*. 2002;34(9):1492-1498. doi:10.1097/00005768-200209000-00015

16. Burke LM, Whitfield J, Heikura IA, et al. Adaptation to a Low Carbohydrate High Fat Diet Is Rapid but Impairs Endurance Exercise Metabolism and Performance Despite Enhanced Glycogen Availability. *J Physiol*. 2021;599(3):771-790. doi:10/ghvh2b

17. Volek JS, Freidenreich DJ, Saenz C, et al. Metabolic Characteristics of Keto-Adapted Ultra-Endurance Runners. *Metabolism*. 2016;65(3):100-110. doi:10.1016/j.metabol.2015.10.028

18. Burke LM, Angus DJ, Cox GR, et al. Effect of Fat Adaptation and Carbohydrate Restoration on Metabolism and Performance During Prolonged Cycling. *J Appl Physiol (1985)*. 2000;89(6):2413-2421. doi:10.1152/jappl.2000.89.6.2413

19. Burke LM, Ross ML, Garvican-Lewis LA, et al. Low Carbohydrate, High Fat Diet Impairs Exercise Economy and Negates the Performance Benefit From Intensified Training in Elite Race Walkers. *J Physiol*. 2017;595(9):2785-2807. doi:10.1113/JP273230

20. Burke LM, Sharma AP, Heikura IA, et al. Crisis of Confidence Averted: Impairment of Exercise Economy and Performance in Elite Race Walkers by Ketogenic Low Carbohydrate, High Fat (LCHF) Diet Is Reproducible. *PLoS One*. 2020;15(6):e0234027. doi:10/gg23h5

21. Durkalec-Michalski K, Nowaczyk PM, Siedzik K. Effect of a Four-Week Ketogenic Diet on Exercise Metabolism in Crossfit-Trained Athletes. *J Int Soc Sports Nutr*. 2019;16(1):16. doi:10.1186/s12970-019-0284-9

22. McSwiney FT, Wardrop B, Hyde PN, Lafountain RA, Volek JS, Doyle L. Keto-Adaptation Enhances Exercise Performance and Body Composition Responses to Training in Endurance Athletes. *Metabolism*. 2018;81:25-34. doi:10.1016/j.metabol.2017.10.010

23. Prins PJ, Noakes TD, Welton GL, et al. High Rates of Fat Oxidation Induced by a Low-Carbohydrate, High-Fat Diet, Do Not Impair 5-km Running Performance in Competitive Recreational Athletes. *J Sports Sci Med*. 2019;18(4):738-750.

24. Stepto NK, Carey AL, Staudacher HM, Cummings NK, Burke LM, Hawley JA. Effect of Short-Term Fat Adaptation on High-Intensity Training. *Med Sci Sports Exerc*. 2002;34(3):449-455. doi:10.1097/00005768-200203000-00011

25. Zinn C, Wood M, Williden M, Chatterton S, Maunder E. Ketogenic Diet Benefits Body Composition and Well-Being but Not Performance in a Pilot Case Study of New Zealand Endurance Athletes. *J Int Soc Sports Nutr*. 2017;14(22). doi:10.1186/s12970-017-0180-0

26. Shaw DM, Merien F, Braakhuis A, Maunder ED, Dulson DK. Effect of a Ketogenic Diet on Submaximal Exercise Capacity and Efficiency in Runners. *Med Sci Sports Exerc*. 2019;51(10):2135-2146. doi:10.1249/MSS.0000000000002008

27. Bergström J, Hultman E. A Study of the Glycogen Metabolism During Exercise in Man. *Scand J Clin Lab*. 1967;19(3):218-228. doi:10.3109/00365516709090629

28. Phinney SD, Bistrian BR, Evans WJ, Gervino E, Blackburn GL. The Human Metabolic Response to Chronic Ketosis Without Caloric Restriction: Preservation of Submaximal Exercise Capability With Reduced Carbohydrate Oxidation. *Metab Clin Exp*. 1983;32(8):769-776. doi:10.1016/0026-0495(83)90106-3

29. Hill JC, San Millán I. Validation of Musculoskeletal Ultrasound to Assess and Quantify Muscle Glycogen Content. A Novel Approach. *Physician Sportsmed*. 2014;42(3):45-52. doi:10.3810/psm.2014.09.2075

30. Snow G. *Blockrand: Randomization for Block Random Clinical Trials*.; 2013. Accessed October 26, 2019. https://CRAN.R-project.org/package=blockrand

31. R Core Team. *R: A Language and Environment for Statistical Computing*.; 2021. https://www.R-project.org/

32. Shan Z, Rehm CD, Rogers G, et al. Trends in Dietary Carbohydrate, Protein, and Fat Intake and Diet Quality Among US Adults, 1999-2016. *JAMA*. 2019;322(12):1178-1187. doi:10/ggdhbs

33. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*. 10th ed. (Riebe D, ed.). Wolters Kluwer Health; 2018.

34. Basset FA, Boulay MR. Specificity of Treadmill and Cycle Ergometer Tests in Triathletes, Runners and Cyclists. *Eur J Appl Physiol*. 2000;81(3):214-221. doi:10.1007/s004210050033

35. De Pauw K, Roelands B, Cheung SS, de Geus B, Rietjens G, Meeusen R. Guidelines to Classify Subject Groups in Sport-Science Research. *Int J Sports Physiol Perform*. 2013;8(2):111-122. doi:10/f4nt76

36. Decroix L, De Pauw K, Foster C, Meeusen R. Guidelines to Classify Female Subject Groups in Sport-Science Research. *Int J Sports Physiol Perform*. 2016;11(2):204-213. doi:10/ggcfjx

37. Graybeal AJ, Kreutzer A, Rack P, et al. Perceptions of Appetite Do Not Match Hormonal Measures of Appetite in Trained Competitive Cyclists and Triathletes Following a Ketogenic Diet Compared to a High-Carbohydrate or Habitual Diet: A Randomized Crossover Trial. *Nutr Res*. 2021;93:111-123. doi:10/gmmtch

38. Lakens D, Caldwell AR. Simulation-Based Power Analysis for Factorial Analysis of Variance Designs. *AMPPS*. 2021;4(1):2515245920951503. doi:10/gj2hw8

39. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2018;71(19):e127-e248. doi:10/gc8qvn

40. Misra S, Oliver NS. Utility of Ketone Measurement in the Prevention, Diagnosis and Management of Diabetic Ketoacidosis. *Diabet Med*. 2015;32(1):14-23. doi:10.1111/dme.12604

41. Sparks AS, Williams EL, Jones HJ, Bridge CA, Marchant D, McNaughton L. Test-Retest Reliability of a 16.1 km Time Trial in Trained Cyclists Using the CompuTrainer Ergometer. *J Sci Cycl*. 2016;5(3):35-41. doi:10.28985/jsc.v5i3.272

42. Poole DC, Jones AM. Measurement of the Maximum Oxygen Uptake V̇o2max: V̇o2peak Is No Longer Acceptable. *J Appl Physiol (1985)*. 2017;122(4):997-1002. doi:10.1152/japplphysiol.01063.2016

43. Hawley JA, Noakes TD. Peak Power Output Predicts Maximal Oxygen Uptake and Performance Time in Trained Cyclists. *Eur J Appl Physiol Occup Physiol*. 1992;65(1):79-83. doi:10.1007/bf01466278

44. Péronnet F, Massicotte D. Table of Nonprotein Respiratory Quotient: An Update. *J Canad Sci Sport*. 1991;16(1):23-29.

45. Nieman DC, Shanely RA, Zwetsloot KA, Meaney MP, Farris GE. Ultrasonic Assessment of Exercise-Induced Change in Skeletal Muscle Glycogen Content. *BMC Sports Sci Med Rehabil*. 2015;7:9. doi:10/gb3pw8

46. Bone JL, Ross ML, Tomcik KA, Jeacocke NA, McKay AKA, Burke LM. The Validity of Ultrasound Technology in Providing an Indirect Estimate of Muscle Glycogen Concentrations Is Equivocal. *Nutrients*. 2021;13(7):2371. doi:10/gmmtcg

47. Routledge HE, Bradley WJ, Shepherd SO, et al. Ultrasound Does Not Detect Acute Changes in Glycogen in Vastus Lateralis of Man. *Med Sci Sports Exerc*. 2019;51(11):2286-2293. doi:10/gmmtcf

48. The MuscleHealth Company. *Position Stand. Science and Application*.; 2018.

49. van Buuren S, Groothuis-Oudshoorn K. MICE: Multivariate Imputation by Chained Equations in R. *J Stat Softw*. 2011;45(3):1-67. doi:https://doi.org/10.18637/jss.v045.i03

50. Schafer JL, Yucel RM. Computational Strategies for Multivariate Linear Mixed-Effects Models With Missing Values. *J Comput Graph Stat*. 2012;11(2):437-457. doi:10/b4r25x

51. Bates D, Maechler M, Bolker B, et al. *Lme4: Linear Mixed-Effects Models Using "Eigen" and S4*.; 2019. Accessed October 25, 2019. https://CRAN.R-project.org/package=lme4

52. Lenth R, Singmann H, Love J, Buerkner P, Herve M. *Emmeans: Estimated Marginal Means, Aka Least-Squares Means*.; 2019. Accessed October 25, 2019. https://CRAN.R-project.org/package=emmeans

53. Hopkins WG, Schabort EJ, Hawley JA. Reliability of Power in Physical Performance Tests. *Sports Med*. 2001;31(3):211-234. doi:10/c2mfh9

54. Gribble S. An Interactive Model-Based Calculator of Cycling Power vs. Speed. The Computational Cyclist. Accessed September 5, 2021. https://www.gribble.org/cycling/power_v_speed.html

55. Brehm BJ, Seeley RJ, Daniels SR, D'Alessio DA. A Randomized Trial Comparing a Very Low Carbohydrate Diet and a Calorie-Restricted Low Fat Diet on Body Weight and Cardiovascular Risk Factors in Healthy Women. *J Clin Endocrinol Metab*. 2003;88(4):1617-1623. doi:10.1210/jc.2002-021480

56. Brehm BJ, Spang SE, Lattin BL, Seeley RJ, Daniels SR, D'Alessio DA. The Role of Energy Expenditure in the Differential Weight Loss in Obese Women on Low-Fat and Low-Carbohydrate Diets. *J Clin Endocrinol Metab*. 2005;90(3):1475-1482. doi:10.1210/jc.2004-1540

57. Yancy WS, Olsen MK, Guyton JR, Bakst RP, Westman EC. A Low-Carbohydrate, Ketogenic Diet Versus a Low-Fat Diet to Treat Obesity and Hyperlipidemia. *Ann Intern Med*. 2004;140(10):769-777. doi:10/gf9nd9

58. Worme JD, Doubt TJ, Singh A, Ryan CJ, Moses FM, Deuster PA. Dietary Patterns, Gastrointestinal Complaints, and Nutrition Knowledge of Recreational Triathletes. *Am J Clin Nutr*. 1990;51(4):690-697. doi:10.1093/ajcn/51.4.690

59. Sampson G, Pugh JN, Morton JP, Areta JL. Carbohydrate for Endurance Athletes in Competition Questionnaire (CEAC-Q): Validation of a Practical and Time-Efficient Tool for Knowledge Assessment. *Sport Sci Health*. 2021;17(3). doi:10/gmq2rx

60. Heidel RE. Causality in Statistical Power: Isomorphic Properties of Measurement, Research Design, Effect Size, and Sample Size. *Scientifica (Cairo)*. 2016;2016:8920418. doi:10/gk8w8p

61. Jiang D, Pepler D, Yao H. The Effect of Population Heterogeneity on Statistical Power in the Design and Evaluation of Interventions. *Int J Behav Dev*. 2010;34(5):473-480. doi:10/b9rbvf

62. Boisgontier MP, Cheval B. The ANOVA to Mixed Model Transition. *Neurosci Biobehav Rev*. 2016;68:1004-1005. doi:10/f83jzs

63. DeBruine L, Krystalli A, Heiss A. *Faux: Simulation for Factorial Designs*.; 2021. Accessed September 8, 2021. https://CRAN.R-project.org/package=faux

64. Senn S. Change From Baseline and Analysis of Covariance Revisited. *Stat Med*. 2006;25(24):4334-4344. doi:10/fdmg6x

65. Kassambara A. *Rstatix: Pipe-Friendly Framework for Basic Statistical Tests*.; 2021. Accessed September 8, 2021. https://CRAN.R-project.org/package=rstatix

66. Harrison XA, Donaldson L, Correa-Cano ME, et al. A Brief Introduction to Mixed Effects Modelling and Multi-Model Inference in Ecology. *PeerJ*. 2018;6:e4794. doi:10/gdh936

67. McSwiney FT, Doyle L, Plews DJ, Zinn C. Impact Of Ketogenic Diet On Athletes: Current Insights. *Open Access J Sports Med*. 2019;10:171-183. doi:10/ggp3jm

68. Coyle EF, Feltner ME, Kautz SA, et al. Physiological and Biomechanical Factors Associated With Elite Endurance Cycling Performance. *Med Sci Sports Exerc*. 1991;23(1):93-107.

69. Impey SG, Hearris MA, Hammond KM, et al. Fuel for the Work Required: A Theoretical Framework for Carbohydrate Periodization and the Glycogen Threshold Hypothesis. *Sports Med*. 2018;48(5):1031-1048. doi:10/gdwk98

70. Hiilloskorpi HK, Pasanen ME, Fogelholm MG, Laukkanen RM, Mänttäri AT. Use of Heart Rate to Predict Energy Expenditure From Low to High Activity Levels. *Int J Sports Med*. 2003;24(5):332-336. doi:10/fbbg7z

71. Quinn TJ, Coons BA. The Talk Test and Its Relationship With the Ventilatory and Lactate Thresholds. *J Sports Sci*. 2011;29(11):1175-1182. doi:10/bgs275

72. Impey SG, Hammond KM, Shepherd SO, et al. Fuel for the Work Required: A Practical Approach to Amalgamating Train-Low Paradigms for Endurance Athletes. *Physiol Rep*. 2016;4(10):e12803. doi:10.14814/phy2.12803

CHAPTER IV: Effects of Training Characteristics on Cycling Performance in Competitive Recreational Cyclists and Triathletes

Authors:

Andreas Kreutzer[1], Julie A. Swets[2], Cathy R. Cox[2], Meena Shah[1]

Author Affiliation:

[1]Exercise Physiology Lab, Department of Kinesiology, Harris College of Nursing & Health Sciences, Texas Christian University, Fort Worth, TX 76129, USA.

[2]Existential Social Psychology Research Lab, Department of Psychology, College of Science & Engineering, Texas Christian University, Fort Worth, TX 76129, USA

Corresponding Author:

Andreas Kreutzer, M.S., Department of Kinesiology, Texas Christian University, Fort Worth, TX 76129, USA. Email: a.kreutzer@tcu.edu. Tel: 817-257-6859

Meena Shah, Department of Kinesiology, Texas Christian University, Fort Worth, TX 76129, USA. Email: m.shah@tcu.edu. Tel: 817-257-6871

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4.1 Abstract

There is active scientific debate about whether a polarized training intensity distribution (TID; time in intensity Zone 1 > Zone 3 > Zone 2) is the optimal strategy for endurance performance. The training characteristics among recreational athletes and their impact on performance are unclear. Thus, the purpose of this study was to analyze the training characteristics among recreational cyclists and triathletes and to estimate the associations of these characteristics with endurance performance. We analyzed raw training and race data from 232 recreational athletes (age = 41.8 ± 11.0 years; body mass = 71.1 ± 8.2 kg, relative Critical Power [CPrel] = 4.3 ± 0.6 $W \cdot kg^{-1}$) using R statistical language. We investigated the associations of volume ($hours \cdot week^{-1}$), intensity (mean heart rate [HR] as a percent of maximal HR [HRmax]), frequency ($sessions \cdot week^{-1}$), and polarization (polarization index [PI]) over 20 training weeks with CPrel calculated from maximal mean power (MMP) outputs over 2, 5, and 12 minutes. Only 17 participants employed a polarized training approach as defined by a PI > 2.0. Time spent below 80% of HRmax was considered Zone 1 (Z1) training. Z2 spanned from 80-87% HRmax with Z3 including time spent above 87% HRmax. Athletes completed 70.6 ± 11.5% of their training in Z1, 17.4 ± 6.5% in Z2, and 12.1 ± 7.3% in Z3. They trained 9.4 ± 3.2 hours per week over 5.9 ± 2.3 training sessions and amassed 233 ± 82 km per week. When controlling for age, volume exhibited the most consistent positive association with CPrel (*b* (*SE*) = .052 (.012); 90% compatibility intervals (90CI) = [.032; .071]). Training polarization was also positively associated with CPrel, albeit with considerable uncertainty (*b* (SE) = 0.184 (.134); 90CI = [-.037; .404]. Our model explained only 21.5% of the variance in CPrel; similarly, out-of-sample performance was unsatisfactory (RMSE = .53 $W \cdot kg^{-1}$). In summary, few recreational athletes employ a polarized TID despite potential benefits. However, training volume appears to be the most important factor to optimize performance in this population.

4.2 Introduction

Cycling and triathlon remain popular among competitive recreational endurance athletes in the United States; according to the 2021 Outdoor Foundation participation report[1], approximately 48.6 million Americans participated in cycling activities (Road/Mountain Bike/Bicycle Motocross) and 3.6 million Americans in triathlon (Road/Off-Road) in 2020. According to the Outdoor Industry Association, Americans spend close to $14 billion per year on cycling gear and almost $83 billion on cycling-related travel.[2] Competitive recreational cyclists train on average 12.04 hours per week across 5.3 days to improve their performance.[3]

Professional and recreational cyclists manipulate a variety of training variables to improve performance.[4] Training characteristics include frequency (number of sessions per week), volume (distance or time spent cycling), and intensity (power output or speed during a given training session); all are collectively used to establish a training load. However, an imbalance between training load and recovery can cause overtraining, which can lead to a decrease in physiological adaptation to training and performance, as well as potential injury or illness.[5] Thus, recent research has focused on the distribution of intensity across sessions.[6,7] This training intensity distribution (TID) is often measured using time spent in training zones based on physiological thresholds. Scientific analyses of TID frequently employ a three-zone model, with Zone 1 (Z1) corresponding to intensities below the first ventilatory/lactate threshold (VT1/LT1), Z2 corresponding to intensities between VT1/LT1 and the second ventilatory/lactate threshold (VT2/LT2), and Z3 corresponding to intensities above VT2/VT3.[6–9] These zones then roughly correspond to exercise in the moderate, heavy, and severe intensity domains established using VT1/LT1 and critical power (CP).[10]

While some research has suggested that a polarized TID (time in Z1 > Z3 > Z2) might be beneficial for endurance performance and success in endurance sport,[6–8,11–18] there is active scientific debate whether TID was misclassified in some of these studies and whether training polarization is truly an optimal strategy.[19,20]

Competitive recreational athletes often rely on planning and monitoring their own training. They frequently use field tests to structure their training sessions and track progress, which, if not properly standardized, can introduce considerable error.[21] Additionally, the TID among competitive recreational cyclists and triathletes and their association with cycling performance are unknown. The recent success of workout tracking and analysis apps and websites such as Strava© (Strava, Inc., San Francisco, CA), has made abundant data from this population publicly accessible. The use of this kind of data for scientific analysis has become more commonplace and allows the analysis of actual training patterns and their association with performance in a large cohort of recreational athletes.[18,22,23]

Therefore, the purpose of the present study was to use raw longitudinal training and racing data to investigate the TID among recreational cyclists and triathletes. Further, the study assessed the effects of training characteristics (volume, intensity, and TID) on cycling performance as measured by relative critical power (CPrel; $W \cdot kg^{-1}$) while controlling for age, as it is known to be negatively associated with endurance performance.[24] We expected the training polarization among this group to be low. Further, we hypothesized that, when adjusting for age, greater total volume, greater average intensity, and a polarized TID would have positive associations with cycling performance.

4.3 Method

4.3.1 Study Design

We analyzed raw cycling activity data recorded by participants with their own devices to find the highest estimated CPrel achieved over the course of a single week ("performance week"). We then investigated the association of training volume (hours·week^{-1}), average training intensity (% of HRmax), and TID (% of time in HR-based zones) during the 20 weeks leading up to the performance week with CPrel while controlling for participant age.

4.3.2 Participants and Data Inclusion

We combined data from a large publicly available dataset of raw activity data for cycling, running, swimming, and other exercise (GoldenCheetah OpenData; GCOD; http://goldencheetah-opendata.s3-website-us-east-1.amazonaws.com/), with data (https://osf.io/ez6x5/) collected under a protocol approved by the Texas Christian University Institutional Review Board (Protocol ID #1810-031-1810). For the purpose of this document, we consider the latter our "local data". The participants providing local data (n = 65) signed an IRB-approved informed consent, and provided raw activity files, which they had downloaded from their Strava© accounts. GCOD was accessed on Feb. 14, 2022, and included 2,398,134 activity files from 6,043 athletes.

To ensure sufficient data availability for power and heart rate (HR) analysis, we limited inclusion of participants to those athletes who had provided at least 500 cycling files and for whom power and HR data was available. Among our local participants, 20 (m = 16, f = 4) athletes fulfilled these criteria. The GCOD contained 1,014 athletes (m = 990, f = 24) meeting these criteria. We further cleaned the dataset by removing participants who had not provided their age or had reported their age as <18or >100 years. We then removed athletes with unrealistic power output values as follows: 1) 1-second power > 2,500 W; 2) 1-second power exactly 1,000W or 2,000 W, which

indicates a virtual power algorithm rather than measured power; 3) 4-minute power ≥ 500 W (greater than the current track-cycling pursuit world record); 4) 5-minute relative power ≥ 7.5 W/kg and 1-minute relative power ≥ 11.5 W/kg (greater than maximal power outputs reported for professional international cyclists).[25,26] The final sample from the GCOD included 695 men and 23 women. Due to this unfortunate large difference in data availability, we limited our analysis to male participants.

During individual data analysis and based on pilot work, we further removed athletes who did not have power and heart rate data for at least 30% of their data points and those who did not have HR measurements during the time period in which we analyzed their training and performance. We also removed those whose performance occurred during a time when they were younger than 18 years old, those whose CP-model exhibited a poor fit ($R^2 < 0.9$), and those whose power profile did not resemble the typical curve described by the omni-domain power-duration model.[27] We additionally removed participants whose training volume appeared too low (< 5 hours·week^{-1} or < 100 km·week^{-1}), as this might indicate that they did not record or share all of their activities. **Figure 4.1** presents a CONSORT diagram for the study. Our final sample, combining local data and GCOD, included 232 participants with a total of 270,070 activity files and 224,262 cycling files. An additional 273 were left to be analyzed at the time of submission of this document.

4.3.3 Data Handling

GCOD data were provided as individual comma-separated values files (CSV) for each activity with one data point per second. Participants submitted local data in sport activity file formats including ".gpx", ".tcx", and ".fit". We imported these files into Golden Cheetah V3.5 and converted them to CSV files with the same properties as those downloaded from the GCOD. We

then imported all CSV files for each participant into *R* statistical software V4.1.2[28]. We used the *data.table* V1.14.2[29], *tidyverse* V1.3.1[30], and *lubridate* V1.8.0[31] packages for training and performance data analysis.

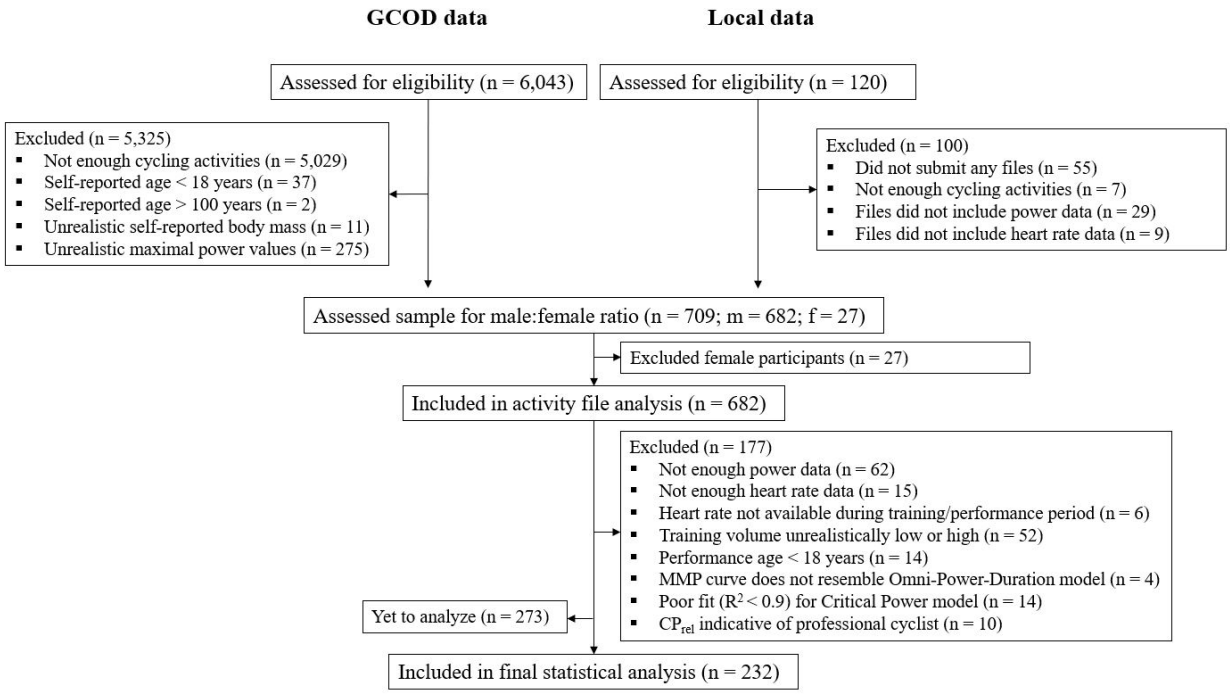

Figure 4.1. CONSORT Diagram

4.3.4 Power Profiling and Critical Power

We collected absolute and relative maximal mean power outputs (MMP) for the following durations for all participants: 5 seconds, 10 seconds, 30 seconds, 1 minute, 2 minutes, 5 minutes, 6 minutes, 10 minutes, 12 minutes, and 20 minutes. Using the 2-, 5-, and 12-minute MMP values, we calculated weekly absolute and relative CP and work capacity above CP (W') using a linear regression of power vs. the inverse of time, where the y-intercept equals CP and the slope of the regression line equals W'.[32] We further investigated CPrel values that were more than 2.5 standard deviations (DV) greater than the participant's mean CPrel across all of their data and removed those who appeared to be caused by power output spikes or unrealistically high MMP values. We

deemed the highest CPrel achieved over a single week our performance measure. When CP-model fit during that week was poor ($R^2 < 0.9$), we evaluated the weeks with CPrel values that fell within 0.2 $W \cdot kg^{-1}$ of the highest CPrel measured. Out of those additional measurements, we selected the highest CPrel with acceptable model fit ($R^2 \geq 0.9$). If no CPrel values within 0.2 $W \cdot kg^{-1}$ of the highest CPrel exhibited acceptable model fit, we removed the participant from the final analysis as indicated above.

4.3.5 Body Mass

Participants reported body mass at the time of file submission. Some participants provided up-to-date body mass measurements throughout the time of their data availability. Others had the same body mass associated with all of their files. When available, we used body mass reported during the performance week for relative CP and MMP calculations.

4.3.6 Performance Age

For participants in the GCOD, only year of birth was available. Thus, we calculated their "performance age" based on the year of each individual's performance week. Local participants reported exact dates of birth, which allowed us to calculate exact performance age based on their date of birth and the final day of the performance week.

4.3.7 Maximal Heart Rate Determination

We determined maximal HR (HRmax) by visually analyzing HR and power output plots from all sessions that contained recorded HR above age-predicted HRmax based on the Tanaka formula (208-0.7*age) using participants' performance age.[33] We disregarded all sessions with obvious HR spikes and HR values not matching power output measures. We considered the highest HR achieved during one or multiple training sessions with a clean HR curve indicative of maximal testing or high-intensity intervals as HRmax for the individual. If a participant did not record any

HR > than age-predicted, we visually inspected sessions with the highest ten HR achieved by the individual and applied the aforementioned criteria to set HRmax.

4.3.8 Analysis of Training Characteristics

We considered the 20 weeks leading up to the performance week the training period for each participant.

4.3.8.1 Training Frequency

We determined the number of training sessions over the course of the training period based on the number of individual workout files submitted by each participant. Since our dataset included a mix of cyclists and triathletes, we included all types of activities in the training frequency count. We calculated the number of sessions per week based on these data.

4.3.8.2 Training Volume

We extracted training volume as hours spent exercising and the distance covered over the course of the training period. While volume based on time and distance were strongly correlated ($r = 0.87$), we chose to use only training hours for statistical analyses, since distance achieved over a certain time varies markedly by type of activity (cycling, running, swimming) and athlete ability.

4.3.8.3 Training Intensity

We estimated training intensity as the average training HR as a percentage of HRmax over the course of the training period. We removed any HR data above HRmax and below 50 bpm from the analysis to eliminate HR spikes and drop-outs. We considered 50 bpm the lower bound for measured HR to include lower HR potentially achieved during downhill coasting, while eliminating any HR that would be indicative of measurement errors.

4.3.8.4 Training Intensity Distribution

We determined TID based on a 3-zone model using HR data to calculate time-in-zone. We established training zones based on %HRmax thresholds suggested by Sylta et al. and Seiler:[34,35]

Zone 1: <80% HRmax

Zone 2: 80-87% HRmax

Zone 3: >87% HRmax

Additionally, we established power-based training zones based on %CP as adapted from a 6-zone model proposed by Skiba:[36]

Zone 1: ≤75% CP

Zone 2: 76-100% CP

Zone 3: >100% CP

Further, we calculated a polarization-index (PI) as described by Treff et al. based on time spent in the three timing zones.[37] A PI > 2 indicates polarized training, whereas a PI ≤ 2 indicates non-polarized training. We used HR-based values for statistical analyses to allow the inclusion of HR recordings from cycling sessions that did not contain power data and from activities performed in other sports. Additionally, we compared TID between HR and power-based models for athletes with ≥ 90% of HR and power availability in their data to gain a better understanding of how they correspond in real world data.

4.3.9 Statistical Analysis

We performed all statistical analyses in *R* statistical language. We analyzed the association of training intensity (%HRmax), volume (hours·week^{-1}), and TID with CPrel while controlling for age using ordinary least squares (OLS) multiple linear regression. Further, we performed simple linear regression to estimate the effect of age on CPrel.

Based on the well-established effects of age and training on performance, applying a statistical model under the assumption of a true null hypothesis appeared nonsensical. Further, overemphasizing null-hypothesis significance testing and making inferences about the dichotomized existence of an effect has been strongly criticized.[38] Therefore, as suggested by Gardner & Altman and the American Statistical Association, we focused on the magnitude and uncertainty (90% compatibility intervals; CIs) of the association of each explanatory variable on cycling performance.[39–42] Thus, when interpreting results, we considered implications of all results that are compatible with the present data, from the lower limit to the upper limit of the CI, with the greatest emphasis placed on the point estimate.

We visually inspected residual and Q-Q plots to confirm that the assumptions of normality and heteroskedasticity were met for the linear model. Further, we found no multicollinearity in our final model using bivariate correlations of all predictors and the variance inflation factor (VIF). We employed leave-one-out cross-validation (LOOCV) using the *caret* package to investigate the out-of-sample performance of our model.[43] We report root mean squared error (RMSE), mean absolute error (MAE), and LOOCV R^2 as out-of-sample performance metrics.

Since it has been reported that CP estimated exclusively from training data is less reliable than that estimated from data including races and formal testing, we performed a sensitivity analysis to ensure that potential CP estimation errors did not have undue effects on our model coefficients.[44]

To achieve this, we also employed linear regressions in the full data set with the same explanatory variables using individual MMP values (1-minute, 2-minute, 5-minute, 6-minute, 10-minute, 12-minute, 20-minute) extracted over the entire length of the training and performance period as the response variables.

Additionally, we compared TID established using HR data compared with that using power data. We employed an estimation approach to investigate mean differences between methods and present data in a Cumming estimation plot.[45]

All GCOD data are already publicly available at http://goldencheetah-opendata.s3-website-us-east-1.amazonaws.com/. Local data and GCOD IDs for all participants included in the study as well as all analysis code are available at https://osf.io/ez6x5/.

4.4 Results

We included 232 participants in the final analysis. Participant characteristics are presented in **Table 4.1**.

Table 4.1. Participant Characteristics (n = 232)

	Mean (SD)
Age (years)	43.7 (11.1)
Age at performance measure (years)	41.8 (11.0)
Body mass (kg)	71.1 (8.2)
Number of raw activities submitted	1,164 (678)
Number of cycling sessions	967 (553)
Availability of heart rate data (% of total data)	90.4 (11.9)
Availability of power data (% of total data)	76.7 (20.8)

4.4.1 Training and Performance Characteristics

Participants completed on average 5.9 ± 2.3 sessions per week over the 20-week training period. They trained for 9.4 ± 3.2 hours and covered 233 ± 82 km each week. Participants spent 70.6 ± 11.5 % of their training time in heart rate Z1, 17.4 ± 6.5% in Z2, and 12.1 ± 7.0% in Z3, which is consistent with a pyramidal TID. Only 17 participants (7.3%) followed a polarized TID as defined by a PI > 2. Average absolute CP in our final sample was 302 ± 41 W and average relative CP was 4.3 ± 0.6. **Table 4.2** presents training and performance characteristics.

Table 4.2. Training & Performance Characteristics (n = 232)

Training characteristics	**Mean (SD)**
Training frequency (sessions·week^{-1})	5.9 (2.3)
Training volume (hours·week^{-1})	9.4 (3.2)
Training distance (km·week^{-1})	233 (82)
Time in HR-Zone 1 (% of total)	70.6 (11.5)
Time in HR-Zone 2 (% of total)	17.4 (6.5)
Time in HR-Zone 3 (% of total)	12.1 (7.0)
Performance characteristics	
Absolute critical power (W)	302 (41)
Relative critical power (W·kg^{-1})	4.28 (0.59)
Critical power model R^2	0.97 (0.03)

4.4.2 Association of Training Characteristics with Performance

An ordinary least-squares multiple linear regression evaluated the association of training volume (hours·week^{-1}), training intensity (% of HRmax), training polarization (Yes/No) with estimated relative critical power (W·kg^{-1}) while controlling for age. The results of the regression revealed a point estimate of 0.052 [0.032; 0.071] for training volume. Thus, our results are compatible with a 0.032 – 0.071 W·kg^{-1} increase in CPrel for each additional hour of training per week. The point estimate for the coefficient of training intensity suggests no association of average

intensity with CPrel, while the 90% CIs are compatible with a 0.016 W·kg^{-1} decrease to a 0.016 W·kg^{-1} increase in CPrel for each one percent increase in average training intensity. Training polarization exhibited the largest uncertainty of any of the variables included in the model. The 90% CI for changing from a non-polarized to a polarized TID was compatible with a decrease of 0.037 W·kg^{-1} to an increase of 0.404 W·kg^{-1}. While there is considerable uncertainty in the association of TID with cycling performance, the point estimate (0.184 W·kg^{-1}) and 90% CI suggest a potential beneficial effect of changing from a non-polarized to a polarized-approach. **Table 4.3** presents unstandardized regression coefficient estimates, 90% CIs, and VIF for the multiple regression. In both the multiple and simple linear regressions, the coefficient for age indicated that CPrel decreases by .020 [.015; .025] W·kg^{-1} per year.

Table 4.3. Multiple Linear Regression on Relative Critical Power (n = 232)

	b (SE)	***5%***	***95%***	***B****	***R^2***	***VIF***
Age (years)	-.020 (.003)	-.025	-.015	-.218	.137	1.012
Training Intensity (% HRmax)	.000 (.010)	-.016	.016	-.001	.000	1.195
Training Volume (hours·week^{-1})	.052 (.012)	.032	.071	.162	.064	1.190
Training Polarization (No → Yes)	.184 (.134)	-.037	.404	.184	.006	1.028
Model Fit						
R^2 = .215	Adj. R^2 = .202	*AIC* = 364.9		*BIC* = 385.6		
Out-of-Sample Performance						
RMSE = .53	*LOOCV* R^2 = .18			*MAE* = .42		

b = unstandardized regression coefficient; SE = standard error; B = standardized regression coefficient (*Note: Training Polarization is a binary variable and could not be standardized); AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; VIF = Variance Inflation Factor; RMSE = Root Mean Squared Error; LOOCV = Leave-One-Out Cross-Validation; MAE = Mean absolute error

4.4.3 Explanatory and Predictive Performance of the Model

The multiple linear regression model including age, training intensity, training volume, and TID was able to explain 21.5% of the variance in CPrel among participants. LOOCV revealed an RMSE of 0.53 $W \cdot kg^{-1}$ and a MAE of 0.42 $W \cdot kg^{-1}$. The LOOCV R^2 was 0.18.

4.4.4 Sensitivity Analysis

To assess the sensitivity of our analysis to estimation errors in CPrel and to using MMP values of different lengths as markers of performance, we compared regression coefficients and 90% CIs between models with the same explanatory variables while using CPrel and MMP values between one and 20 minutes extracted over the entire length of the training and performance period as the response variables. All models produced similar point estimates for all explanatory variables. The 90% CIs were similar for all models except the 1-min MMP model, which exhibited wider CIs across all explanatory variables (see **Figure 4.2**).

4.4.5 Time-in-Zone Comparison

We compared time-in-zone using HR-based analysis and power-based determination in 36 participants who had at least 90% availability of power and HR data, and whose power and HR availabilities differed by less than 2% to ensure comparability between the two approaches. When using HR, these participants spent 72.3 ± 8.8% of their training time in Z1, 15.9 ± 4.6% in Z2, and 11.8 ± 6.5% in Z3. Using the power-based approach, participants spent 76.1 ± 7.7% in Z1, 17.4 ± 5.8% in Z2, and 6.5 ± 3.3% in Z3.

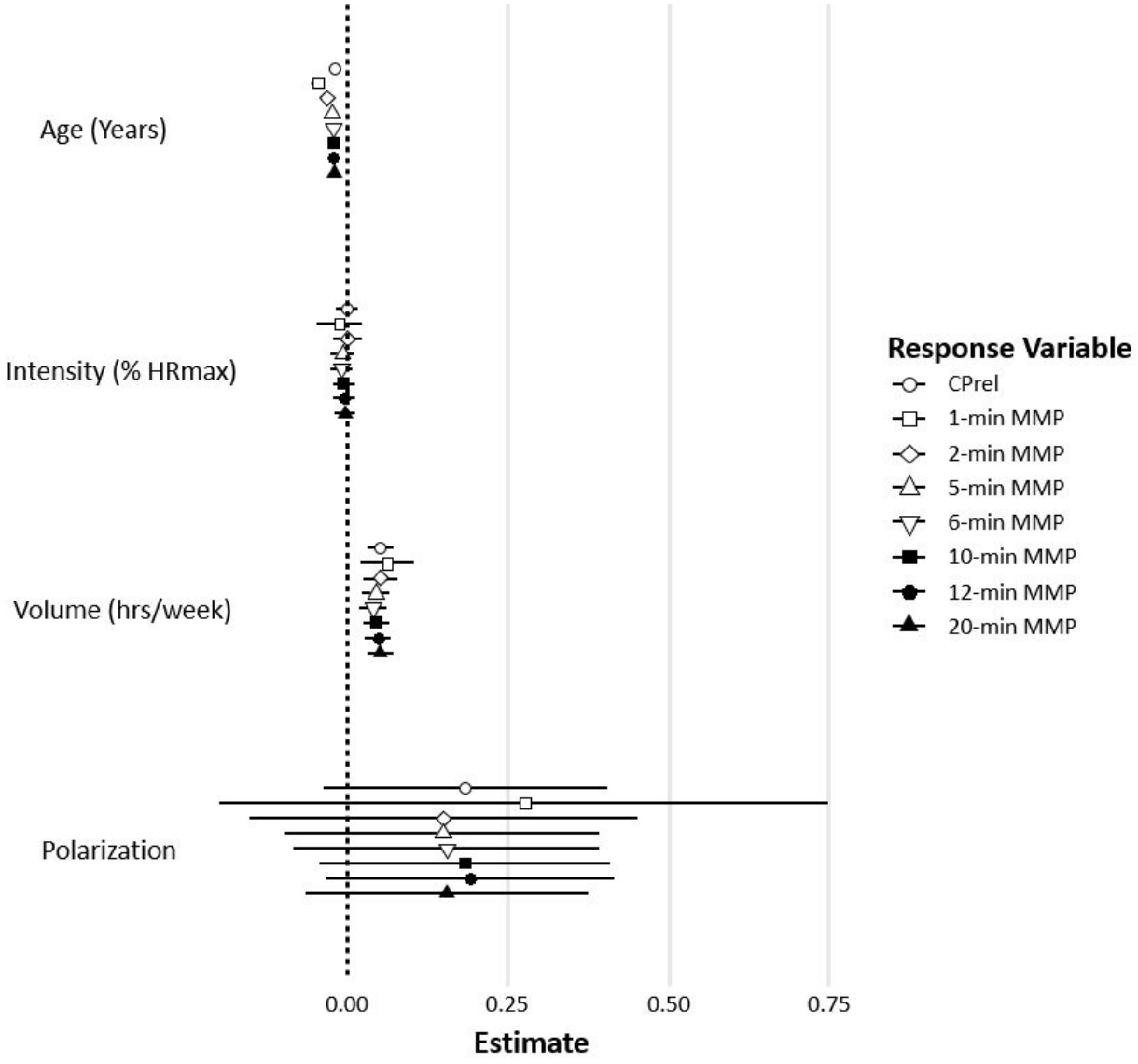

Figure 4.2. Sensitivity Analysis. CPrel = relative critical power; MMP = maximal mean power

The Cumming estimation plot in **Figure 3** shows estimated mean differences with 90% CIs between the two approaches. In our sample, the power-based approach led to more time recorded in Z1 (*MD* = 3.79% [1.96; 5.70]) and Z2 (*MD* = 1.47% [0.10; 2.83]), and less time recorded in Z3 (-5.3% [-6.87; -3.88]).

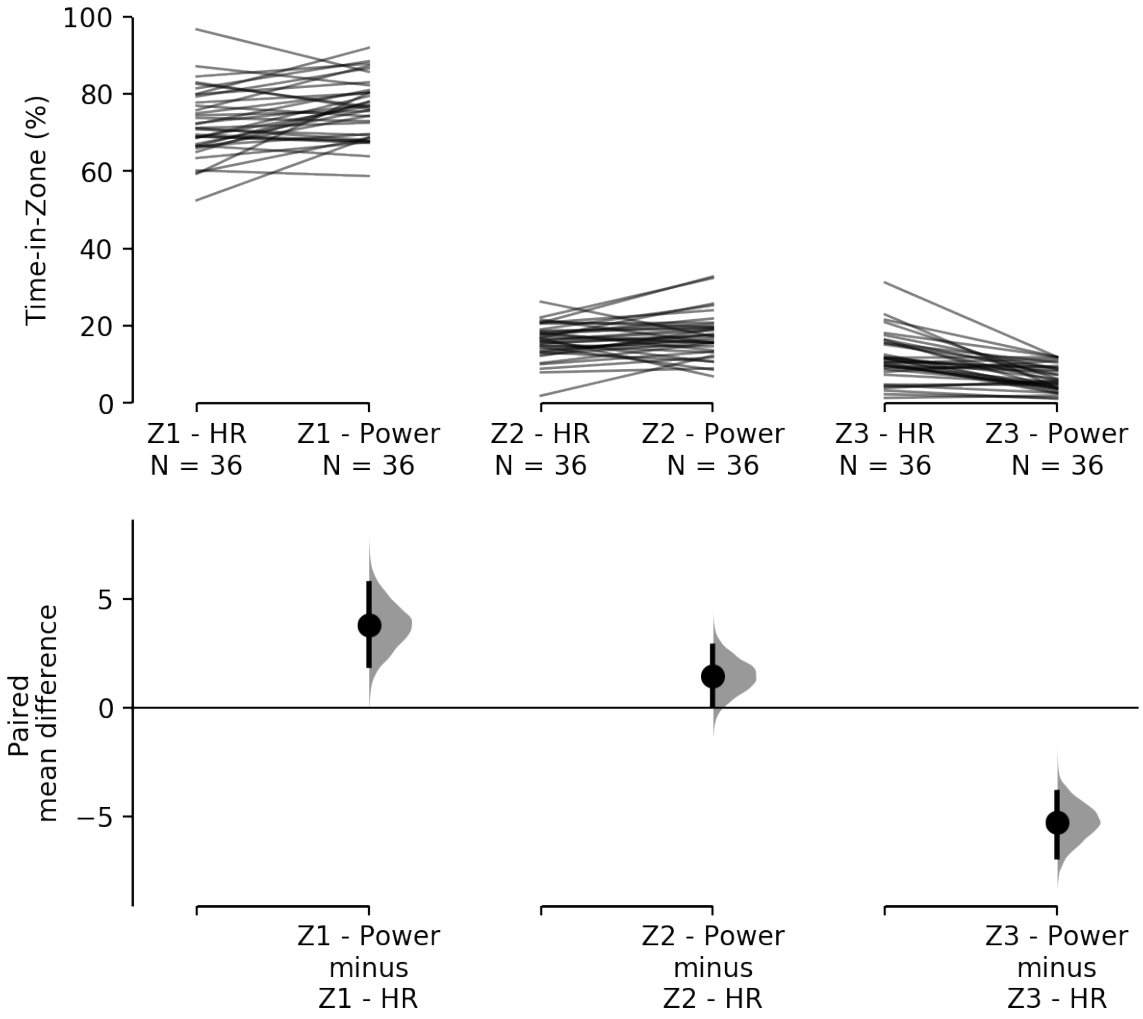

Figure 4.3. Time-in-Zone Measured Using Heart Rate vs. Power

4.5 Discussion

To our knowledge, this is the first study to investigate the training characteristics among a large group of recreational athletes and their effects on cycling performance using raw activity data. We showed that few recreational athletes employ a polarized TID. Further, training volume and a polarized TID were positively associated with cycling performance, as measured by CPrel. However, there was a large amount of uncertainty in the association of training polarization with performance, as evidenced by a large 90% CI. Yet, the CI, spanning from -0.037 to 0.404 and the

point estimate at 0.184 are compatible with data suggesting only a small potential decrease, but a large potential increase in CPrel when switching to a more polarized TID. Interestingly, there did not appear to be an association of training intensity with CPrel.

4.5.1 Association of Training Characteristics with CP

Our results are congruent with laboratory and field studies investigating the effect of TID on performance and durability.[6,7,12–14,16–18] Esteve-Lanao et al. investigated the effect of five months of polarized vs. threshold training in runners.[17] While the group that had been prescribed polarized training in fact arrived at a pyramidal TID (Z1: 80.5 ± 1.8%; Z2: 11.8 ± 2.0%; Z3: 8.3 ± 0.7%), the performance improvement in that group was greater than in the second group, which spent twice the amount of time in Z2 (Z1: 66.8 ± 1.1%; Z2: 24.7 ± 1.5%; Z3: 8.5 ± 1.0%). Similarly, Neal et al. found greater improvements in mean power output during a 40-km cycling time trial and submaximal markers of endurance performance following six weeks of polarized training compared with six weeks of threshold training.[13] Correspondingly, Röhrken et al. reported consistent improvements in running velocity at LT2 following six weeks of polarized training compared with a more varied response following six weeks of threshold training.[6] Further, Muñoz et al. found greater improvement in 10-km running time in a group performing ten weeks of polarized training compared with a threshold training group.[12]

In a 16-week training study, Filipas et al. investigated the effects of polarized training alone, pyramidal training alone, and a combination of the two TIDs in opposing sequences (8 weeks polarized → 8 weeks pyramidal; 8 weeks pyramidal → 8 weeks polarized) on 5-km running time $\dot{V}O_2$peak and running velocity at 2 and 4 $mmol \cdot L^{-1}$ blood lactate concentration.[7] The authors reported the greatest improvements in 5-km running time and velocity at blood lactate thresholds from baseline to the end of the study in the pyramidal → polarized group, followed by the polarized

only group. Interestingly, in the combined TIDs, they reported the greatest improvement following the polarized portion of the training. Further, Stöggl & Sperlich showed improvements in $\dot{V}O_2$peak and velocity/power at 4 mmol·L^{-1} blood lactate following nine weeks of polarized training and high-intensity interval training, but not high-volume or threshold training.[14] While not statistically significant for all comparisons, the improvements in the polarized training group were greater than those in the high-intensity interval group. In a recent study of professional cyclists, Spragg et al. found that a shift toward a more polarized TID was positively correlated with 12-min MMP in unfatigued and fatigued states and CP in a fatigued state.[16] In an analysis of raw running data, Altini & Amft demonstrated a positive association of training polarization with running performance.[18] These findings are consistent with our finding that a change to a polarized TID might be positively associated with CPrel.

The benefits of employing a polarized TID have been suggested to be two-fold: 1) improved recovery status and 2) more time spent at power outputs/speeds that elicit a high percentage of $\dot{V}O_2$max. Recovery of the autonomic nervous system, as measured by heart rate variability is delayed similarly following all training above VT1, i.e., in Z2 or Z3 when compared with Z1 training.[46] The greatest improvements in $\dot{V}O_2$max can be achieved by maximizing the time spent training at or near $\dot{V}O_2$max.[47–49] Employing a polarized TID might allow athletes to maximize recovery while also maximizing the time spent at or near $\dot{V}O_2$max. In fact, the participants who employed a polarized TID in our study spent more time in Z1 (72.4 ± 12.2%) and Z3 (17.1 ± 8.0%) compared with those employing a pyramidal TID (Z1: 70.4 ± 11.5%; Z3: 11.7 ± 6.8%). Thus, participants with a non-polarized approach spent more time above VT1 (29.6 ± 11.5%) than those following a polarized TID (27.6 ± 12.2%), potentially requiring more recovery time or possibly blunting adaptations from training performed in an under-recovered state.

Additionally, they spent less time near or at $\dot{V}O_2$max compared with those following a polarized TID, potentially leading to lesser cardiovascular adaptations.

Further, our finding that increased training volume is positively associated with endurance performance has been consistently reported in the literature. Milesis et al. were among the first to show an improvement in $\dot{V}O_2$max and time to task failure in a treadmill test that was proportional to increased training volume.[50] Similarly, Wenger & Bell suggested increasing improvements in $\dot{V}O_2$max with increasing exercise volume.[51] Applying the opposite approach, Hickson et al. reported a proportional negative effect of reduced training volume on cycling time to task failure.[52] Altini & Amft found a positive association of training volume with running performance.[18] These findings are in line with our finding that an increase in weekly training volume is positively associated with CPrel.

The positive association of increased volume we detected by analyzing raw training data matches results from mechanistic studies investigating the effect of training volume on skeletal muscle adaptations.[53] Granata et al.[54] demonstrated that skeletal muscle mitochondrial content increased following a switch from normal-volume to high-volume training, an adaptation that was quickly reversed when participants reduced their training volume below normal-volume levels. In contrast, an increase in exercise intensity does not appear to elicit the same mitochondrial adaptations.[53] The increase in mitochondrial content seen with greater training volume allows individuals to rely predominantly on oxidative phosphorylation for energy production during exercise at higher intensities, i.e., increase their maximal metabolic steady state (MMSS).[55] It has been argued that CP, our performance outcome, is the gold standard measure of MMSS. In our sample of recreational athletes, the increase in volume might be more important than a focus on exact TID or an overall increase in average intensity.

Interestingly, we were unable to detect an association of training intensity with CPrel. To test the sensitivity of our model to estimation errors in average intensity as a percentage of HRmax, we also included intensity as a percentage of 6-minute MMP. This did not change the magnitude or uncertainty of any of the model coefficients in a meaningful way (see supplementary material). This is in contrast to a finding by Wenger & Bell, who suggested that, when holding frequency and volume constant, an increase in exercise intensity is related to an increase in $\dot{V}O_2max$.[51] The discrepancy in our finding could be explained by the nature of the outcome measure used in the present investigation and by Wenger & Bell. The latter used $\dot{V}O_2max$, a maximal measure of oxygen consumption, whereas we investigated CP, a submaximal measure. Thus, increasing average training intensity might have a greater impact on maximal measures than submaximal measures. Additionally, our measure of average intensity across all training does not necessarily capture the relative amount of time spent at high intensities or the intensities achieved during those high-intensity training sessions. Therefore, our measure of TID and time spent in Z3 might be stronger indicators of the actual amount of high-intensity training performed than average intensity, and thus have a stronger association with improved performance.

4.5.2 Explanatory and Out-of-Sample Performance of Our Model

Our model including age and basic training characteristics explained only approximately 21.5% of the variance in CPrel. This confirms that the factors influencing endurance performance are multi-faceted. Specific training characteristics play only a small part in the bigger picture of performance improvement, with genetics and nutrition playing additional roles. $\dot{V}O_2max$ remains an important factor in endurance performance, especially in heterogenous samples like the present.[56,57] Heritability of $\dot{V}O_2max$ has been estimated to be 66% after adjusting for

anthropometrics and weekly hours of sport participation.[58] Thus, a large portion of the variance unaccounted for by our model could be explained by genetics.

Further our model's out-of-sample performance was suboptimal, with relatively large prediction errors (RMSE = 0.53 $W \cdot kg^{-1}$). Thus, it appears that knowledge of an individual's age and training characteristics is not enough to predict cycling performance. This is similar to findings by Altini & Amft, who reported that the RMSE for models predicting 10-km running time improved as more information was included.[18] Their model including only anthropometrics (BMI, age, sex) produced an RMSE of 6.27 minutes. Adding resting physiology (resting HR and HR variability), training volume and intensity, training physiology, and training polarization incrementally improved predictive accuracy, but still produced a final RMSE of 3.64 min. Only when adding a previous 10-km running performance, a variable that was determined by all pertinent factors (genetics, training, nutrition, motivation, environment, etc.), did the model's prediction error drop below three minutes. Similarly, Smyth & Muniz-Pumares estimated critical speed (CS) from training data of >25,000 runners and used it to predict race time in big city marathons.[23] Their best-performing model predicted marathon time with an error of approximately 8%. At an average marathon time of approximately 233 minutes among their sample, this amounts to a misprediction by approximately 18.6 minutes. Thus, performance prediction using only data available from training does not appear to be practically meaningful. However, including parameters from training and previous races to predict future performance appears to be a more promising tool, as shown by Emig & Peltonen, who were able to predict performance to within 2%.[22]

4.5.3 TID Measurement: HR vs. Power

We showed that TID measured using power time-in-zone and HR time-in-zone produce similar results when zone transitions are based on 75% and 100% CP and 80% and 87% HRmax respectively. Interestingly, in contrast to previous findings, among our participants time in Z1 and Z2 was greater when using power compared to HR.[9,59] Respectively, time in Z3 was greater when using HR to delineate zone transitions. The discrepancy might be due to the fact that the previous studies used power/running speed and HR determined during incremental exercise tests. Thus, the zone transition criteria for HR and power/speed were based on the same laboratory measurement, rather than on the estimated thresholds used in our study. Interestingly, this difference caused a meaningful change for classifying training as polarized or non-polarized based on the PI. When using power, none of our participants would have been classified as employing a polarized TID. However, it stands to reason that using HR as the zone-delineation criterion is the preferred method, as this reflects the physiological state of the body at a given moment, i.e. an internal load, rather than an external load placed on the body.[60]

One potential limitation of using HR to establish time in zones lies in the discrepancy between work rate changes and HR kinetics, i.e., the lag of heart rate response to increased or decreased workloads.[61] Thus, a training session structured to focus on Z3 work with rest periods in Z1, i.e., high-intensity interval training, might lead to a HR profile that includes extended periods in Z2: HR drifts through Z2 and often remains in Z2 for extended periods of time, while the actual work performed based on power output is completed in Z1 or Z3. Thus, a session-goal approach, as originally suggested and employed by Seiler, appears to be more practical for training prescription.[62] However, as in the present investigation, this approach might be near impossible to

use in studies analyzing raw training data without access to training diaries or communication with participants, who would need to provide their goal for each individual training session.

4.5.4 Limitations

One limitation to our study was the lack of a controlled performance measure. The accuracy of CPrel estimated from raw MMP data appears to depend on whether only training or training and racing data are included in the calculations.[44] We attempted to control for this by eliminating unrealistic MMP values and participants with poor model fit for CPrel estimation. Additionally, we performed a sensitivity analysis by comparing regression coefficients and CIs between models using CPrel and individual MMP values as the outcome measure. We were able to show that coefficients and CIs were not sensitive to the outcome measure used; thus, we believe our model presents accurate estimates of the influence of training characteristics on CPrel. However, we did not investigate performance in a particular event, where training specificity might become an important factor in performance. While an increase in the maximal metabolic steady state could be considered beneficial for performance in most events, Z2 training might be more important for those events that are predominantly performed in the heavy exercise intensity domain, as suggested by Burnley et al.[19]

Additionally, the thresholds we used to delineate our training zones were not based on laboratory tests. Thus, some of our participants' times in zones might have been misclassified. However, we used 80 and 87 % of HRmax as our zone-transitions based on previous literature and recommendations by physiologists and coaches.[34,35] An added limitation for our study was that HRmax was not measured during a standardized test, but rather determined based on maximal heart rates achieved during training. However, we carefully visually evaluated heart rate and power during individual training sessions to find sessions that either indicated high-intensity interval

training, maximal efforts, or maximal testing for HRmax determination. We ensured that that HR spikes were excluded and that power output was sustained above estimated CPrel for HRmax determination.

Further, we were unable to confirm what particular devices were used by participants to measure HR and power output and whether these devices were appropriately calibrated. Additionally, we did not have a direct way of evaluating whether power output values were true measurements or created by a power estimation algorithm. However, we believe that our data cleaning procedures, i.e., removing spikes, unrealistic values, and one-second MMPs indicative of power algorithms, sufficiently controlled for the potential confounding effects of these limitations.

We also did not have access to activity data outside of training for our participants. Treff et al. have suggested that TID calculations should integrate measures from activities of daily living and work.[63] They showed that off-training activities, which can raise HR above resting levels and sometimes into Z3 for non-trivial amounts of time, can alter estimated TID, volume, and training impulse.

An additional limitation was the uncertainty about self-reported data in the GCOD: body mass and age might have been misreported. Additionally, we did not have access to information regarding GCOD participants' training status, training age, or sport affiliation. Further, we do not know how many sessions were not recorded or included in the dataset; thus, it is possible that training time and frequency were underestimated for some participants. We addressed all of these concerns by thoroughly cleaning the data and removing participants with unrealistically low training volumes or distance, unrealistically high body mass, and unrealistic ages (e.g., >100 years). While we cannot guarantee that none of our participants are in fact professional athletes, we classified our athletes as recreationally competitive based on their power profile and training

volumes. We excluded participants whose CPrel was > 5.5, which might indicate domestic or international professional cyclist status.[25] Among our remaining athletes, only than 1.3% trained for more than 20 hours per week and only 3.9% trained for more than 15 hours per week, which could indicate professional or semi-professional athlete status. We believe that our thorough vetting of the data has produced results that are generalizable to male recreational athletes.

Regrettably, our biggest limitation is the lack of data from female athletes. Since we only had data for 27 women across both databases, we excluded female participants from the analysis. Thus, our results are not generalizable to female recreational athletes.

4.5.5 Conclusions

Despite some of the limitations of our study, we believe our large dataset, thorough data cleaning, and sensitivity analyses makes this the first study to use raw training and racing data to provide a strong indication of the association of individual training characteristics with cycling performance. It appears that among recreational athletes, training volume is the most consistently associated with cycling performance. While in elite athletes, who necessarily include a large amount of volume in their training, average intensity and a specific TID might be important factors to induce additional adaptations and performance improvements, there is considerable uncertainty in the role of these variables among recreational athletes. While there was some indication in our study that a polarized TID is positively associated with cycling performance, the uncertainty surrounding this effect was also the largest among all training characteristics examined. Our study confirmed a decline in CP as individuals age. Further, we showed that TID estimated using commonly recommended thresholds based on HR and estimated CPrel provide similar results; however, the times-in-zone were sufficiently different to cause differences in the binary polarized vs. non-polarized classification achieved by using the PI.

Future studies should attempt to combine large-scale raw training and racing data from recreationally competitive participants with standardized performance tests and threshold determination. This could be achieved by interacting virtually with participants who own a smart-trainer and could perform performance tests and threshold determination employing a standardized protocol while recording or live-streaming their test efforts on video-communication platforms. Most importantly, this research should be expanded to include female participants. With the recent increase in the popularity of women's road cycling and the fast growth in NCAA-sanctioned collegiate women's triathlon, it is important to analyze training characteristics and their effects on cycling performance among women, who are notoriously understudied in the sport and exercise science literature.

4.5.6 Practical Applications

Given our findings that raining volume is most reliably associated with increased performance, recreational endurance athletes should emphasize consistency in their training, which will help them achieve adequate training volumes to improve performance. Those already achieving relatively high training volumes might then include a polarized TID to potentially achieve additional training benefits. However, it is important to note that a polarized TID might not be the ideal prescription across an entire year or season; rather, a periodized training approach including separate training blocks that focus on high-volume-low-intensity, moderate intensity, and high intensity respectively, as often employed by endurance athletes, appears to remain the preferred training strategy.[64] A polarized TID can then be incorporated as appropriate in high-volume and high-intensity training blocks.

4.6 References

1. Outdoor Foundation. *2021 Outdoor Participation Trends Report*.; 2021:1-27. https://ip0o6y1ji424m0641msgjlfy-wpengine.netdna-ssl.com/wp-content/uploads/2015/03/2021-Outdoor-Participation-Trends-Report.pdf

2. Outdoor Industry Association. *The Outdoor Recreation Economy*.; 2017. Accessed November 21, 2020. https://outdoorindustry.org/wp-content/uploads/2017/04/OIA_RecEconomy_FINAL_Single.pdf

3. Frederick-Recascino CM, Schuster-Smith H. Competition and Intrinsic Motivation in Physical Activity: A Comparison of Two Groups. *J Sport Behav*. 2003;26(3):240-254.

4. Faria EW, Parker DL, Faria IE. The Science of Cycling: Physiology and Training – Part 1. *Sports Med*. 2005;35(4):285-312. doi:10/b7kk4r

5. Lehmann M, Foster C, Keul J. Overtraining in Endurance Athletes: A Brief Review. *Med Sci Sports Exerc*. 1993;25(7):854-862. doi:10/cfw52f

6. Röhrken G, Held S, Donath L. Six Weeks of Polarized Versus Moderate Intensity Distribution: A Pilot Intervention Study. *Front Physiol*. 2020;11:534688. doi:10.3389/fphys.2020.534688

7. Filipas L, Bonato M, Gallo G, Codella R. Effects of 16 Weeks of Pyramidal and Polarized Training Intensity Distributions in Well-Trained Endurance Runners. *Scand J Med Sci Sports*. 2022;32(3):498-511. doi:10.1111/sms.14101

8. Seiler KS, Kjerland GØ. Quantifying Training Intensity Distribution in Elite Endurance Athletes: Is There Evidence for an "Optimal" Distribution? *Scand J Med Sci Sports*. 2006;16(1):49-56. doi:10/btm8mw

9. Bellinger P, Arnold B, Minahan C. Quantifying the Training-Intensity Distribution in Middle-Distance Runners: The Influence of Different Methods of Training-Intensity Quantification. *Int J Sports Physiol Perform*. 2019;15(3):1-5. doi:10.1123/ijspp.2019-0298

10. Poole DC, Burnley M, Vanhatalo A, Rossiter HB, Jones AM. Critical Power: An Important Fatigue Threshold in Exercise Physiology. *Med Sci Sports Exerc*. 2016;48(11):2320-2334. doi:10/f9bcwp

11. Billat VL, Demarle A, Slawinski J, Paiva M, Koralsztein JP. Physical and Training Characteristics of Top-Class Marathon Runners. *Med Sci Sports Exerc*. 2001;33(12):2089-2097. doi:10.1097/00005768-200112000-00018

12. Muñoz I, Seiler KS, Bautista J, España J, Larumbe E, Esteve-Lanao J. Does Polarized Training Improve Performance in Recreational Runners? *Int J Sports Physiol Perform*. 2014;9(2):265-272. doi:10.1123/ijspp.2012-0350

13. Neal CM, Hunter AM, Brennan L, et al. Six Weeks of a Polarized Training-Intensity Distribution Leads to Greater Physiological and Performance Adaptations Than a Threshold Model in Trained Cyclists. *J Appl Physiol (1985)*. 2012;114(4):461-471. doi:10/gfvw42

14. Stöggl T, Sperlich B. Polarized Training Has Greater Impact on Key Endurance Variables Than Threshold, High Intensity, or High Volume Training. *Front Physiol*. 2014;5:33. doi:10.3389/fphys.2014.00033

15. Yu H, Chen X, Zhu W, Cao C. A Quasi-experimental Study of Chinese Top-Level Speed Skaters' Training Load: Threshold Versus Polarized Model. *Int J Sports Physiol Perform*. 2012;7(2):103-112. doi:10/ggkgmb

16. Spragg J, Leo P, Swart J. The Relationship Between Training Characteristics and Durability in Professional Cyclists Across a Competitive Season. *Eur J Sport Sci*. Published online March 3, 2022:1-17. doi:10.1080/17461391.2022.2049886

17. Esteve-Lanao J, Foster C, Seiler S, Lucia A. Impact of Training Intensity Distribution on Performance in Endurance Athletes. *J Strength Cond Res*. 2007;21(3):943-949. doi:10/bkd6qs

18. Altini M, Amft O. Estimating Running Performance Combining Non-invasive Physiological Measurements and Training Patterns in Free-living. *Annu Int Conf IEEE Eng Med Biol Soc* . Published online July 2018:2845-2848. doi:10.1109/EMBC.2018.8512924

19. Burnley M, Bearden SE, Jones AM. Polarized Training is Not Optimal for Endurance Athletes. *Med Sci Sports Exerc*. Published online February 9, 2022. doi:10.1249/MSS.0000000000002869

20. Foster C, Casado A, Esteve-Lanao J, Haugen T, Seiler S. Polarized Training is Optimal for Endurance Athletes. *Med Sci Sports Exerc*. Published online February 9, 2022. doi:10.1249/MSS.0000000000002871

21. Paton CD, Hopkins WG. Tests of Cycling Performance. *Sports Med*. 2001;31(7):489-496. doi:10/c2ccmn

22. Emig T, Peltonen J. Human Running Performance From Real-world Big Data. *Nat Commun*. 2020;11(1):4936. doi:10.1038/s41467-020-18737-6

23. Smyth B, Muniz-Pumares D. Calculation of Critical Speed from Raw Training Data in Recreational Marathon Runners. *Med Sci Sports Exerc*. 2020;52(12):2637-2645. doi:10.1249/MSS.0000000000002412

24. Tanaka H, Seals DR. Endurance Exercise Performance in Masters Athletes: Age-Associated Changes and Underlying Physiological Mechanisms. *J Physiol*. 2008;586(1):55-63. doi:10.1113/jphysiol.2007.141879

25. Allen H, Coggan AR, McGregor S. *Training and Racing With a Power Meter*. VeloPress; 2019.

26. Leo P, Spragg J, Mujika I, Menz V, Lawley JS. Power Profiling in U23 Professional Cyclists During a Competitive Season. *Int J Sports Physiol Perform*. 2021;16(6):881-889. doi:10.1123/ijspp.2020-0200

27. Puchowicz MJ, Baker J, Clarke DC. Development and Field Validation of an Omni-Domain Power-Duration Model. *J Sports Sci*. 2020;38(7):801-813. doi:10.1080/02640414.2020.1735609

28. R Core Team. *R: A Language and Environment for Statistical Computing*.; 2021. https://www.R-project.org/

29. Dowle M, Srinivasan A, Gorecki J, et al. *Data.Table: Extension of "Data.Frame."*; 2021. Accessed March 3, 2022. https://CRAN.R-project.org/package=data.table

30. Wickham H, RStudio. *Tidyverse: Easily Install and Load the "Tidyverse."*; 2021. Accessed March 3, 2022. https://CRAN.R-project.org/package=tidyverse

31. Spinu V, Grolemund G, Wickham H, et al. *Lubridate: Make Dealing with Dates a Little Easier*.; 2021. Accessed March 3, 2022. https://CRAN.R-project.org/package=lubridate

32. Whipp BJ, Huntsman DJ, Stoner D, Lamarra N, Wassermann K. A constant which determines the duration of tolerance to high intensity work. *Fed Proc*. 1982;41:1591.

33. Tanaka H, Monahan KD, Seals DR. Age-Predicted Maximal Heart Rate Revisited. *J Am Coll Cardiol*. 2001;37(1):153-156. doi:10.1016/S0735-1097(00)01054-8

34. Sylta Ø, Tønnessen E, Seiler S. From Heart-Rate Data to Training Quantification: A Comparison of 3 Methods of Training-Intensity Analysis. *Int J Sports Physiol Perform*. 2014;9(1):100-107. doi:10.1123/ijspp.2013-0298

35. Seiler KS. *Training Intensity Zones: General Rules and Importance of Individual Testing*.; 2020. Accessed March 3, 2022. https://www.youtube.com/watch?v=NPwyk9B0j-s

36. Skiba PF. *Scientific Training for Endurance Athletes*. 1st ed. PhysFarm Training Systems LLC; 2021.

37. Treff G, Winkert K, Sareban M, Steinacker JM, Sperlich B. The Polarization-Index: A Simple Calculation to Distinguish Polarized From Non-polarized Training Intensity Distributions. *Front Physiol*. 2019;10. doi:10.3389/fphys.2019.00707

38. McShane BB, Gal D, Gelman A, Robert C, Tackett JL. Abandon Statistical Significance. *Am Stat*. 2019;73(sup1):235-245. doi:10.1080/00031305.2018.1527253

39. Gardner MJ, Altman DG. Confidence Intervals Rather Than P Values: Estimation Rather Than Hypothesis Testing. *Br Med J (Clin Res Ed)*. 1986;292(6522):746-750. doi:10.1136/bmj.292.6522.746

40. Wasserstein RL, Lazar NA. The ASA Statement on p-Values: Context, Process, and Purpose. *Am Stat*. 2016;70(2):129-133. doi:10.1080/00031305.2016.1154108

41. Wasserstein RL, Schirm AL, Lazar NA. Moving to a World Beyond “p < 0.05.” *Am Stat*. 2019;73(sup1):1-19. doi:10.1080/00031305.2019.1583913

42. Rafi Z, Greenland S. Semantic and Cognitive Tools to Aid Statistical Science: Replace Confidence and Significance by Compatibility and Surprise. *BMC Med Res Methodol*. 2020;20(1):244. doi:10.1186/s12874-020-01105-9

43. Kuhn M, Wing J, Weston S, et al. *Caret: Classification and Regression Training*.; 2022. Accessed March 29, 2022. https://CRAN.R-project.org/package=caret

44. Spragg J, Leo P. Can Critical Power be Estimated from Training and Racing Data using Mean Maximal Power Outputs? *J Sci Cycl*. 2020;9(2):7-10.

45. Ho J, Tumkaya T, Aryal S, Choi H, Claridge-Chang A. Moving Beyond P Values: Data Analysis With Estimation Graphics. *Nat Methods*. 2019;16(7):565-566. doi:10.1038/s41592-019-0470-3

46. Seiler S, Haugen O, Kuffel E. Autonomic Recovery After Exercise in Trained Athletes: Intensity and Duration Effects. *Med Sci Sports Exerc*. 2007;39(8):1366-1373. doi:10.1249/mss.0b013e318060f17d

47. Laursen PB, Jenkins DG. The Scientific Basis for High-Intensity Interval Training: Optimising Training Programmes and Maximising Performance in Highly Trained Endurance Athletes. *Sports Med*. 2002;32(1):53-73. doi:10.2165/00007256-200232010-00003

48. Midgley AW, McNaughton LR, Wilkinson M. Is There an Optimal Training Intensity for Enhancing the Maximal Oxygen Uptake of Distance Runners?: Empirical Research Findings, Current Opinions, Physiological Rationale and Practical Recommendations. *Sports Med*. 2006;36(2):117-132. doi:10.2165/00007256-200636020-00003

49. Billat VL, Flechet B, Petit B, Muriaux G, Koralsztein JP. Interval Training at VO2max: Effects on Aerobic Performance and Overtraining Markers. *Med Sci Sports Exerc*. 1999;31(1):156-163. doi:10.1097/00005768-199901000-00024

50. Milesis CA, Pollock ML, Bah MD, Ayres JJ, Ward A, Linnerud AC. Effects of Different Durations of Physical Training on Cardiorespiratory Function, Body Composition, and Serum Lipids. *Res Q*. 1976;47(4):716-725. doi:10/gj7hm8

51. Wenger HA, Bell GJ. The Interactions of Intensity, Frequency and Duration of Exercise Training in Altering Cardiorespiratory Fitness. *Sports Med*. 1986;3(5):346-356. doi:10/fbqzcf

52. Hickson RC, Kanakis C, Davis JR, Moore AM, Rich S. Reduced Training Duration Effects on Aerobic Power, Endurance, and Cardiac Growth. *J Appl Physiol Respir Environ Exerc Physiol*. 1982;53(1):225-229. doi:10/gj7hnb

53. Granata C, Jamnick NA, Bishop DJ. Training-Induced Changes in Mitochondrial Content and Respiratory Function in Human Skeletal Muscle. *Sports Med*. 2018;48(8):1809-1828. doi:10.1007/s40279-018-0936-y

54. Granata C, Oliveira RSF, Little JP, Renner K, Bishop DJ. Mitochondrial Adaptations to High-Volume Exercise Training Are Rapidly Reversed After a Reduction in Training Volume in Human Skeletal Muscle. *FASEB J*. 2016;30(10):3413-3423. doi:10.1096/fj.201500100R

55. Jones AM, Burnley M, Black MI, Poole DC, Vanhatalo A. The Maximal Metabolic Steady State: Redefining the 'Gold Standard.' *Physiol Rep*. 2019;7(10):e14098. doi:10/ggx7x3

56. Bergh U, Ekblom B, Astrand PO. Maximal Oxygen Uptake "Classical" Versus "Contemporary" Viewpoints. *Med Sci Sports Exerc*. 2000;32(1):85-88. doi:10/dztrx8

57. di Prampero PE. Factors Limiting Maximal Performance in Humans. *Eur J Appl Physiol*. 2003;90(3-4):420-429. doi:10/dm3gwx

58. Fagard R, Bielen E, Amery A. Heritability of Aerobic Power and Anaerobic Energy Generation During Exercise. *J Appl Physiol (1985)*. 1991;70(1):357-362. doi:10/gntvrx

59. Sanders D, Myers T, Akubat I. Training-Intensity Distribution in Road Cyclists: Objective Versus Subjective Measures. *Int J Sports Physiol Perform*. 2017;12(9):1232-1237. doi:10.1123/ijspp.2016-0523

60. Halson SL. Monitoring Training Load to Understand Fatigue in Athletes. *Sports Med*. 2014;44(Suppl 2):139-147. doi:10.1007/s40279-014-0253-z

61. Zakynthinaki MS. Modelling Heart Rate Kinetics. *PLoS One*. 2015;10(4):e0118263. doi:10.1371/journal.pone.0118263

62. Seiler KS. What Is Best Practice for Training Intensity and Duration Distribution in Endurance Athletes? *Int J Sports Physiol Perform*. 2010;5(3):276-291. doi:10/ggkgpc

63. Treff G, Leppich R, Winkert K, Steinacker JM, Mayer B, Sperlich B. The Integration of Training and Off-Training Activities Substantially Alters Training Volume and Load Analysis in Elite Rowers. *Sci Rep*. 2021;11(1):17218. doi:10.1038/s41598-021-96569-0

64. Mølmen KS, Øfsteng SJ, Rønnestad BR. Block Periodization of Endurance Training – A Systematic Review and Meta-analysis. *Open Access J Sports Med*. 2019;10:145-160. doi:10.2147/OAJSM.S180408

Chapter V: Discussion

5.1 Summary

In this dissertation, I examined the effect of genetics, nutrition, and training on endurance performance in recreationally active and competitive runners, triathletes and cyclists.

In our first study, we demonstrated that there is no strong association of *ACTN3* genotype with endurance performance in recreationally active runners. While women with the *ACTN3* XX genotype reported faster 1-mile running personal records (PRs) than those with the *ACTN3* RR or RX genotypes, a similar observation could not be made for men. Additionally, there did not appear to be any influence of *ACTN3* genotype on 5-km running time in either men or women.

In the second study, recreationally competitive cyclists and triathletes completed three simulated 30-km time trials (TT) following their habitual diet (HD) and a two-week high-carbohydrate diet (HC) and a two-week ketogenic diet (KD). Participants exhibited higher fat oxidation during the TT following the KD when compared with the HD and the HC. However, this did not translate into improved performance following the KD. In fact, participants; average power output during the TT was highest after the HC and lowest after the KD.

In our third study, we analyzed raw training and racing data of a large number of recreational cyclists and triathletes. We showed that, when controlling for age, training volume exhibited the most consistent positive association with endurance performance as measured by estimated relative critical power (CPrel; $W \cdot kg^{-1}$). A more polarized training intensity distribution (TID) appeared to be positively associated with CPrel, but there was considerable uncertainty in this estimate. Despite the potential benefit of training polarization, few recreational cyclists and triathletes employ this approach.

5.2 Contribution to Knowledge Base, Gaps, and Future Research

5.2.1 Genetics

A number of studies have investigated the effects of single-nucleotide polymorphisms (SNPs), a DNA sequence variation in a single nucleotide, on physical performance, as reviewed recently by Appel et al.[1] Early studies of one such SNP, a cytosine to thymine transition in the *ACTN3* gene that converts arginine (R) to a stop codon (X), demonstrated that homozygosity for the 577X allele (XX genotype) results in complete deficiency of a structural protein found in Type II muscle fibers, α-actinin-3.[2] Human association studies have consistently shown an underrepresentation of the 577X allele in athletes participating in sprint/power sports.[3–6] While rodent studies indicated a potential benefit of the XX genotype for endurance performance,[7,8] human association studies produced mixed results,[3,9–14] Few studies have directly investigated an effect of *ACTN3* genotype on quantitative measures of endurance performance.[14–18] To our knowledge, our study remains the first and only investigation of the association of *ACTN3* genotype with middle-distance and distance running times in a diverse sample of young, recreationally active men and women. We showed a positive association of the *ACTN3* XX genotype with running performance only for one-mile running times in female participants. We did not detect an effect of ACTN3 genotype on one-mile times in male participants nor on 5-kilometer running times in men or women.

Our findings align with the observations of Papadimitriou et al., who found no association of the 577X allele with running times from 1,500m to the marathon in male and female Caucasian endurance athletes.[15] Similarly, Saunders et al. found no association of *ACTN3* genotype with long-distance triathlon performance in a sample of male Caucasian athletes.[14] Studies investigating the association of *ACTN3* genotype with proxy measures of endurance performance have shown

no associations of ACTN3 genotype with $\dot{V}O_2$peak,[17,18] ventilatory threshold (VT),[18] or energy cost of running.[16]

While there is some evidence of greater 577X allele frequency among female endurance athletes compared with female controls,[11,19] our study remains the only investigation to show a potential association using a direct marker of endurance performance (running times). However, one limitation of our study is that we used self-reported running times for competitions with relatively short distances. Thus, future research should investigate directly measured endurance performance, e.g., TT performance, across a wide range of distances and in diverse samples. Further, studies examining individual SNPs in other genes, e.g., *ACE*, *PPARGC1A, TFAM*, *ACVR1B*, and *NRF*, have shown no or only modest associations with endurance performance.[1] Thus, it appears that the effect of genetics on endurance performance appears to be conferred in a polygenic fashion. However, studies into these associations have so far failed to establish a clear polygenic profile that could predict endurance performance.[20–22] Therefore, additional genetic markers should be investigated and incorporated into polygenic profiles to test their association with endurance performance.

5.2.2 Nutrition

Nutrition plays an important role in optimizing endurance performance.[23] Traditionally, endurance athletes have employed strategies to maximize carbohydrate (CHO) availability to the exercising muscle during competition, which has been shown to delay fatigue and improve performance.[23,24] However, an opposing strategy is to employ low-CHO or ketogenic diets (KD) to induce fat adaptation, i.e., to increase the ability of the body to oxidize fat at intensities which typically elicit high CHO oxidation rates.[25,26] Researchers have proposed that this would unlock essentially unlimited energy resources in the form of large stores of subcutaneous, visceral, and

intramuscular fat.[27] While studies of low-CHO and KD have consistently demonstrated improved fat oxidation capabilities following adaptation, the findings regarding the diet's effects on performance are equivocal.[25,28–37] Additionally, studies investigating the effects of high-CHO diets (HC) and KD in women are rare.[34] Further, to our knowledge, no studies have compared the effects of participants' habitual diet (HD), HC, and KD in a within-participant design.

Therefore, our study aimed to address some of the gaps in the literature by aiming for a large sample including male and female participants across a wide age range and by including a performance measurement following participants' HD. While research-restrictions due to the COVID-19 pandemic did not allow us to reach our proposed sample size, we believe that this study is the first to assess metabolic and performance responses to a HD, KD, and HC in recreationally competitive female and male cyclists and triathletes. Additionally, we maximized external validity of the study by employing a performance measure that was modeled closely after real-world competitions that our population frequently competes in, while maintaining a high level of experimental control. We showed that power output during a 30-km simulated cycling TT was lowest following the KD despite fat oxidation being greatest. This is in line with the findings of Burke et al., who showed improved fat oxidation but reduced performance in race walkers following a KD.[32,33] A potential reason for this performance decrement or failure to improve performance appears to be impaired exercise economy.[32,33,38]

One important factor moderating the effect of a KD on endurance performance appears to be the duration, and thus the intensity, of the performance bout.[39] The KD appears to be detrimental for shorter, higher-intensity performance, but appears to preserve or potentially improve exercise performance in longer, lower-duration bouts.[32,33,35,38,40] However, these findings have been established across multiple studies which employed differing diet interventions and heterogenous

performance measures. Therefore, further studies comparing the effects of KD on exercise performance at different durations and intensities should be performed using consistent diet interventions and testing protocols.

An additional factor in the effectiveness of the KD is the duration of the diet intervention. While fat-adaptation in the form of increased fat oxidation rates can be achieved in as little as five days, some athletes and practitioners argue from anecdotal evidence, that endurance benefits might not materialize for months or even years after starting a KD.[29,41,42] In practice, these long-term interventions are essentially impossible to implement for professional athletes, whose livelihood depends on being competitive every year. However, longer-term diet interventions could be a potential tool for performance enhancement in recreational athletes. To our knowledge, the longest intervention used to test the effects of a KD on endurance performance was a 90-day diet employed by McSwiney et al, [35] who reported similar 100-km TT results following a HC and a KD. While difficult to perform and control, future studies should investigate long-term diet interventions to further elucidate the effect of KD on endurance performance.

Conversely, an idea emerging in the scientific and lay literature is that short-term dietary interventions such as the KD, fasting, and time-restricted eating have the potential to flip a so-called metabolic switch, i.e., increase fat oxidation and decrease reliance on CHO, which could persist even after HD is resumed.[43,44] Thus, some endurance athletes and coaches have begun employing short-term KD to increase fat oxidation rates with the hope of preserving this metabolic advantage even after resuming their HD. Burke et al. investigated the effects of short-term CHO restoration (1-day) following five days of KD.[25,31] They reported similar pre-exercise muscle glycogen levels between KD and HC diets following CHO restoration, but lower glycogen utilization in the KD condition during two hours of steady state cycling at 70% of $\dot{V}O_2max$. This

suggests that fat adaptation was maintained despite adequate glycogen availability for the exercise bout. However, the CHO restoration period was limited to a single day. Thus, future research should investigate the effects of prolonged CHO restoration or resumption of HD following short-term KD to examine how long the effects of fat adaptation can be preserved.

5.2.3 Training

Several studies have investigated the effect of training characteristics and specifically training intensity distribution (TID) on endurance performance in elite athletes.[45–55] Several of these studies have indicated a beneficial effect of so-called polarized training, where athletes spend most of their training time in Zone 1 (Z1) and most of the remainder in Z3, i.e., time in Z1 > Z3 > Z2. However, there is a strong debate whether TID was misclassified in some of these studies and whether training polarization is in fact an optimal strategy for endurance athletes.[56,57] Little is known about the TID employed by recreationally competitive athletes and its impact on endurance performance.

Therefore, our final study used a large set of raw activity data from recreational athletes to investigate the training characteristics in this population and its effects on CPrel. When controlling for age, we found that training volume had the most consistent positive association with CPrel. While there was considerable uncertainty surrounding the estimate, our results are compatible with a positive association of a polarized TID with CPrel. This is in accordance with findings from laboratory and field studies suggesting a beneficial effect of increased training volume[58–63] and polarized training[55,58,64–68]. To our knowledge, we were the first to analyze a large amount of raw training and racing data to establish the TID employed by recreational athletes and its impact on their cycling performance.

While we attempted to tightly control for potential errors introduced by the nature of our data, there remain certain limitations to our findings that should be addressed with future studies. We performed a sensitivity analysis that showed that our results are robust to errors in the estimation of CPrel from raw activity data.[69] Additionally, we were unable to establish individual training zone thresholds based on physiological responses in a standardized test. Therefore, future studies should combine the use of crowdsourced raw data to analyze actual training patterns with standardized performance and threshold testing. A potential means of obtaining large-scale data without the need for laboratory testing would be to devise standardized testing protocols that participants could perform on their home-trainer with supervision using video recordings or live-streams using video-communication platforms. This would still allow to analyze naturally occurring training characteristics in a "free-living" environment while improving the validity of performance measures and zone threshold determination by employing remote standardized testing.[58]

Most importantly, future research of the training characteristics and performance of recreational athletes should focus on the inclusion of female participants. While women make up a substantial fraction of recreational athletes, few data are available on their training and performance.[70] Thus, recruitment for future studies should focus on obtaining data from female recreational athletes to investigate whether patterns detected among male athletes are reproducible among women.

5.3 Implications for Recreational Endurance Athletes

The results of our studies have implications for recreational endurance athletes. We confirmed that *ACTN3* genotype does not appear to have a strong association with endurance performance. In fact, when combining our results with findings from other studies, it appears that

there are no evidence-based indications for recreational athletes to employ direct-to-consumer genetic testing in hopes to match their purported genetic potential to a specific sport. The effect of genetics on endurance performance appears to be polygenic. However, even studies investigating the effect of polygenic scores have not been able to definitively establish a genetic profile that is beneficial for endurance performance. Thus, direct-to-consumer genetic tests are currently not able to accurately predict endurance performance and should not be employed for talent identification or sport selection.

While the KD has become a popular strategy for performance improvement in recreational and professional endurance athletes, the evidence produced by our study and other investigations does not support this approach. The KD has been shown to improve fat oxidation capability even after short-term adaptation, but this does not appear to translate to improved endurance performance. Further, following strict long-term diets can be disruptive for recreational athletes' personal and professional lives. Therefore, a better option for recreational athletes might be to employ the approach to "fuel for the work required".[71] This would allow individuals to eat *ad libitum* for most days of the week, and target high CHO and occasional low CHO days to specific workouts. We argue that recreational athletes already use tools, such as exercise tracking apps, heart rate monitors, and power meters, that would allow them to calculate their energy and CHO requirements and plan their food intake based on their training plan.

Our study investigating the association of training characteristics among recreational athletes and their association with cycling performance suggests that training volume is the most important parameter for recreational athletes. Once these athletes consistently achieve a sufficient weekly training volume, additional factors, such as TID might become more important. Further, race specific training remains important and might require increased training time in Z2, i.e.,

"threshold training". This appears to be best achieved by employing a periodization strategy to structure athletes' annual training programs based on their most important competitions.[72] In training phases focusing on high-volume Z1 training, i.e., "base building", and those focusing on the improvement of maximal aerobic capacity ($\dot{V}O_2max$), which requires maximizing training time at or near $\dot{V}O_2max$ (i.e., in Z3), a polarized TID could help athletes balance training load and recovery. Thus, a polarized training approach should be employed in a targeted fashion, rather than as a year-long strategy.

5.4 Conclusions

In conclusion, a multitude of factors, including genetics, nutrition, and training, impact endurance performance in recreational athletes. While consistent and well-structured training is clearly important to optimize performance, our statistical model based on raw training and racing data from recreationally competitive cyclists and triathletes could only explain approximately 30% of the variance in performance. Thus, a large portion of the differences in performance in this population must be attributed to other factors, with genetics potentially playing the biggest role. In a heterogeneous population such as this, $\dot{V}O_2max$ is an important factor in determining success in competitions. Studies suggest that this measure of cardiorespiratory fitness is determined to a substantial degree by genetics. A large number of genes have been associated with differences in $\dot{V}O_2max$, but a definite polygenic profile that elicits high $\dot{V}O_2max$ values has not been established. Further, the associations of SNPs in individual genes such as *ACTN3*, *ACE*, and *PPARGC1A* are modest at best. We showed that *ACTN3* genotype was not strongly associated with endurance performance in recreationally active individuals.

While genetics undoubtedly play an important role in endurance performance, scientific inquiries have so far failed to establish definite markers to predict success in endurance sports.

This suggests that there is a large number of genes that may have small effects on endurance performance, and that the exact combination of genes might depend on the way performance is measured. Since very few individuals are likely to have optimal or entirely detrimental combinations of these genetic variants, it stands to reason that genetic differences are not an absolute limiting factor for endurance performance.

Thus, training and nutrition continue to play an important role for success in endurance sports. We showed that optimizing training volume and potentially including a polarized TID are associated with better endurance performance. We further demonstrated that the long-standing strategy of maximizing CHO availability before endurance events remains the most effective approach to improve performance, and that a low-carbohydrate ketogenic approach might be detrimental.

In summary, recreational athletes should not rely on direct-to-consumer genetic tests for talent identification or sport selection. Those competing in races, should focus on maximizing training volume while ensuring adequate recovery; a polarized TID appears to be a valuable option to achieve this in certain phases of an athlete's training cycle. Lastly, recreational endurance athletes should attempt to maximize CHO availability before and during races to improve performance. Additionally, "fueling for the work required", i.e., basing caloric and CHO intake on anticipated or completed training loads, appears to be a sensible approach for this population. This strategy would allow recreational athletes to vary their diet based on personal and social requirements while still optimizing adaptations to training.

5.5 References

1. Appel M, Zentgraf K, Krüger K, Alack K. Effects of Genetic Variation on Endurance Performance, Muscle Strength, and Injury Susceptibility in Sports: A Systematic Review. *Front Physiol*. 2021;12:991. doi:10/gntvrq

2. North KN, Yang N, Wattanasirichaigoon D, Mills M, Easteal S, Beggs AH. A Common Nonsense Mutation Results in Alpha-Actinin-3 Deficiency in the General Population. *Nat Genet*. 1999;21(4):353-354. doi:10.1038/7675

3. Eynon N, Duarte JA, Oliveira J, et al. ACTN3 R577X Polymorphism and Israeli Top-Level Athletes. *Int J Sports Med*. 2009;30(9):695-698. doi:10.1055/s-0029-1220731

4. Papadimitriou ID, Papadopoulos C, Kouvatsi A, Triantaphyllidis C. The ACTN3 Gene in Elite Greek Track and Field Athletes. *Int J Sports Med*. 2008;29(4):352-355. doi:10.1055/s-2007-965339

5. Roth SM, Walsh S, Liu D, Metter EJ, Ferrucci L, Hurley BF. The ACTN3 R577X Nonsense Allele Is Under-Represented in Elite-Level Strength Athletes. *Eur J Hum Genet*. 2008;16(3):391-394. doi:10.1038/sj.ejhg.5201964

6. Yang N, MacArthur DG, Wolde B, et al. The ACTN3 R577X Polymorphism in East and West African Athletes. *Med Sci Sports Exerc*. 2007;39(11):1985-1988. doi:10.1249/mss.0b013e31814844c9

7. Hogarth MW, Garton FC, Houweling PJ, et al. Analysis of the ACTN3 Heterozygous Genotype Suggests That α-Actinin-3 Controls Sarcomeric Composition and Muscle Function in a Dose-Dependent Fashion. *Hum Mol Genet*. 2016;25(5):866-877. doi:10.1093/hmg/ddv613

8. MacArthur DG, Seto JT, Raftery JM, et al. Loss of ACTN3 Gene Function Alters Mouse Muscle Metabolism and Shows Evidence of Positive Selection in Humans. *Nat Genet*. 2007;39(10):1261-1265. doi:10.1038/ng2122

9. Ben-Zaken S, Eliakim A, Nemet D, Rabinovich M, Kassem E, Meckel Y. ACTN3 Polymorphism: Comparison Between Elite Swimmers and Runners. *Sports Med Open*. 2015;1(1):13. doi:10.1186/s40798-015-0023-y

10. Niemi AK, Majamaa K. Mitochondrial DNA and ACTN3 Genotypes in Finnish Elite Endurance and Sprint Athletes. *Eur J Hum Genet*. 2005;13(8):965-969. doi:10.1038/sj.ejhg.5201438

11. Yang N, MacArthur DG, Gulbin JP, et al. ACTN3 Genotype Is Associated With Human Elite Athletic Performance. *Am J Hum Genet*. 2003;73(3):627-631. doi:10.1086/377590

12. Döring FE, Onur S, Geisen U, et al. ACTN3 R577X and Other Polymorphisms Are Not Associated With Elite Endurance Athlete Status in the Genathlete Study. *J Sports Sci*. 2010;28(12):1355-1359. doi:10.1080/02640414.2010.507675

13. Muniesa CA, González-Freire M, Santiago C, et al. World-Class Performance in Lightweight Rowing: Is It Genetically Influenced? A Comparison With Cyclists, Runners and Non-athletes. *Br J Sports Med*. 2010;44(12):898-901. doi:10.1136/bjsm.2008.051680

14. Saunders CJ, September AV, Xenophontos SL, et al. No Association of the ACTN3 Gene R577X Polymorphism With Endurance Performance in Ironman Triathlons. *Ann Hum Genet*. 2007;71(6):777-781. doi:10.1111/j.1469-1809.2006.00385.x

15. Papadimitriou ID, Lockey SJ, Voisin S, et al. No Association Between ACTN3 R577X and ACE I/D Polymorphisms and Endurance Running Times in 698 Caucasian Athletes. *BMC Genomics*. 2018;19(1):13. doi:10.1186/s12864-017-4412-0

16. Del Coso J, Moreno V, Gutiérrez-Hellín J, et al. ACTN3 R577X Genotype and Exercise Phenotypes in Recreational Marathon Runners. *Genes (Basel)*. 2019;10(6):413. doi:10.3390/genes10060413

17. Candrawati S, Gumilas NSA, Rujito L, Ardiansyah IR. The Relationship Between ACTN3 Gene Polymorphism With VO2 Max and Flexibility. *J Phys: Conf Ser*. 2019;1246(1):012007. doi:10.1088/1742-6596/1246/1/012007

18. Lucia A, Gómez-Gallego F, Santiago C, et al. ACTN3 Genotype in Professional Endurance Cyclists. *Int J Sports Med*. 2006;27(11):880-884. doi:10.1055/s-2006-923862

19. Shang X, Huang C, Chang Q, Zhang L, Huang T. Association Between the ACTN3 R577X Polymorphism and Female Endurance Athletes in China. *Int J Sports Med*. 2010;31(12):913-916. doi:10.1055/s-0030-1265176

20. Ruiz JR, Gómez-Gallego F, Santiago C, et al. Is There an Optimum Endurance Polygenic Profile? *J Physiol*. 2009;587(Pt 7):1527-1534. doi:10.1113/jphysiol.2008.166645

21. Malhotra S, Preet K, Tomar A, et al. Polygenic Study of Endurance-Associated Genetic Markers ACE I/D, ACTN3 Arg(R)577Ter(X), CKMM A/G NcoI and eNOS Glu(G)298Asp(T) in Male Gorkha Soldiers. *Sports Med Open*. 2017;3(1):17. doi:10.1186/s40798-017-0085-0

22. Pranckeviciene E, Gineviciene V, Jakaitiene A, Januska L, Utkus A. Total Genotype Score Modelling of Polygenic Endurance-Power Profiles in Lithuanian Elite Athletes. *Genes (Basel)*. 2021;12(7):1067. doi:10.3390/genes12071067

23. Jeukendrup AE. Carbohydrate Intake During Exercise and Performance. *Nutrition*. 2004;20(7-8):669-677. doi:10/bnpn7g

24. Jeukendrup AE. Nutrition for Endurance Sports: Marathon, Triathlon, and Road Cycling. *J Sports Sci*. 2011;29 Suppl 1:S91-99. doi:10/b52hvj

25. Carey AL, Staudacher HM, Cummings NK, et al. Effects of Fat Adaptation and Carbohydrate Restoration on Prolonged Endurance Exercise. *J Appl Physiol (1985)*. 2001;91(1):115-122. doi:10.1152/jappl.2001.91.1.115

26. Lambert EV, Hawley JA, Goedecke J, Noakes TD, Dennis SC. Nutritional Strategies for Promoting Fat Utilization and Delaying the Onset of Fatigue During Prolonged Exercise. *J Sports Sci*. 1997;15(3):315-324. doi:10.1080/026404197367326

27. Kenney WL, Wilmore JH, Costill DL. *Physiology of Sport and Exercise*. 7th ed. Human Kinetics; 2020.

28. Lambert EV, Speechly DP, Dennis SC, Noakes TD. Enhanced Endurance in Trained Cyclists During Moderate Intensity Exercise Following 2 Weeks Adaptation to a High Fat Diet. *Eur J Appl Physiol Occup Physiol*. 1994;69(4):287-293. doi:10.1007/bf00392032

29. Burke LM, Whitfield J, Heikura IA, et al. Adaptation to a Low Carbohydrate High Fat Diet Is Rapid but Impairs Endurance Exercise Metabolism and Performance Despite Enhanced Glycogen Availability. *J Physiol*. 2021;599(3):771-790. doi:10/ghvh2b

30. Volek JS, Freidenreich DJ, Saenz C, et al. Metabolic Characteristics of Keto-Adapted Ultra-Endurance Runners. *Metabolism*. 2016;65(3):100-110. doi:10.1016/j.metabol.2015.10.028

31. Burke LM, Angus DJ, Cox GR, et al. Effect of Fat Adaptation and Carbohydrate Restoration on Metabolism and Performance During Prolonged Cycling. *J Appl Physiol (1985)*. 2000;89(6):2413-2421. doi:10.1152/jappl.2000.89.6.2413

32. Burke LM, Ross ML, Garvican-Lewis LA, et al. Low Carbohydrate, High Fat Diet Impairs Exercise Economy and Negates the Performance Benefit From Intensified Training in Elite Race Walkers. *J Physiol*. 2017;595(9):2785-2807. doi:10.1113/JP273230

33. Burke LM, Sharma AP, Heikura IA, et al. Crisis of Confidence Averted: Impairment of Exercise Economy and Performance in Elite Race Walkers by Ketogenic Low Carbohydrate, High Fat (LCHF) Diet Is Reproducible. *PLoS One*. 2020;15(6):e0234027. doi:10/gg23h5

34. Durkalec-Michalski K, Nowaczyk PM, Siedzik K. Effect of a Four-Week Ketogenic Diet on Exercise Metabolism in Crossfit-Trained Athletes. *J Int Soc Sports Nutr*. 2019;16(1):16. doi:10.1186/s12970-019-0284-9

35. McSwiney FT, Wardrop B, Hyde PN, Lafountain RA, Volek JS, Doyle L. Keto-Adaptation Enhances Exercise Performance and Body Composition Responses to Training in Endurance Athletes. *Metabolism*. 2018;81:25-34. doi:10.1016/j.metabol.2017.10.010

36. Prins PJ, Noakes TD, Welton GL, et al. High Rates of Fat Oxidation Induced by a Low-Carbohydrate, High-Fat Diet, Do Not Impair 5-km Running Performance in Competitive Recreational Athletes. *J Sports Sci Med*. 2019;18(4):738-750.

37. Stepto NK, Carey AL, Staudacher HM, Cummings NK, Burke LM, Hawley JA. Effect of Short-Term Fat Adaptation on High-Intensity Training. *Med Sci Sports Exerc*. 2002;34(3):449-455. doi:10.1097/00005768-200203000-00011

38. Shaw DM, Merien F, Braakhuis A, Maunder ED, Dulson DK. Effect of a Ketogenic Diet on Submaximal Exercise Capacity and Efficiency in Runners. *Med Sci Sports Exerc*. 2019;51(10):2135-2146. doi:10.1249/MSS.0000000000002008

39. McSwiney FT, Doyle L, Plews DJ, Zinn C. Impact Of Ketogenic Diet On Athletes: Current Insights. *Open Access J Sports Med*. 2019;10:171-183. doi:10/ggp3jm

40. Phinney SD, Bistrian BR, Evans WJ, Gervino E, Blackburn GL. The Human Metabolic Response to Chronic Ketosis Without Caloric Restriction: Preservation of Submaximal Exercise Capability With Reduced Carbohydrate Oxidation. *Metab Clin Exp*. 1983;32(8):769-776. doi:10.1016/0026-0495(83)90106-3

41. Goedecke JH, Christie C, Wilson G, et al. Metabolic Adaptations to a High-Fat Diet in Endurance Cyclists. *Metab Clin Exp*. 1999;48(12):1509-1517. doi:10.1016/s0026-0495(99)90238-x

42. Burke LM, Hawley JA. Effects of Short-Term Fat Adaptation on Metabolism and Performance of Prolonged Exercise. *Med Sci Sports Exerc*. 2002;34(9):1492-1498. doi:10.1097/00005768-200209000-00015

43. Anton SD, Moehl K, Donahoo WT, et al. Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting. *Obesity (Silver Spring)*. 2018;26(2):254-268. doi:10.1002/oby.22065

44. Clement JW. *The Switch: Ignite Your Metabolism with Intermittent Fasting, Protein Cycling, and Keto*. 1st ed. Gallery Books; 2019.

45. Casado A, Hanley B, Santos-Concejero J, Ruiz-Pérez LM. World-Class Long-Distance Running Performances Are Best Predicted by Volume of Easy Runs and Deliberate Practice of Short-Interval and Tempo Runs. *J Strength Cond Res*. 2021;35(9):2525-2531. doi:10.1519/JSC.0000000000003176

46. Seiler KS, Kjerland GØ. Quantifying Training Intensity Distribution in Elite Endurance Athletes: Is There Evidence for an "Optimal" Distribution? *Scand J Med Sci Sports*. 2006;16(1):49-56. doi:10/btm8mw

47. Billat VL, Demarle A, Slawinski J, Paiva M, Koralsztein JP. Physical and Training Characteristics of Top-Class Marathon Runners. *Med Sci Sports Exerc*. 2001;33(12):2089-2097. doi:10.1097/00005768-200112000-00018

48. Steinacker JM, Lormes W, Lehmann M, Altenburg D. Training of Rowers Before World Championships. *Med Sci Sports Exerc*. 1998;30(7):1158-1163. doi:10.1097/00005768-199807000-00022

49. Orie J, Hofman N, de Koning JJ, Foster C. Thirty-Eight Years of Training Distribution in Olympic Speed Skaters. *Int J Sports Physiol Perform*. 2014;9(1):93-99. doi:10.1123/IJSPP.2013-0427

50. Bellinger P, Arnold B, Minahan C. Quantifying the Training-Intensity Distribution in Middle-Distance Runners: The Influence of Different Methods of Training-Intensity Quantification. *Int J Sports Physiol Perform*. 2019;15(3):1-5. doi:10.1123/ijspp.2019-0298

51. Esteve-Lanao J, Juan AFS, Earnest CP, Foster C, Lucia A. How Do Endurance Runners Actually Train? Relationship With Competition Performance. *Med Sci Sports Exerc*. 2005;37(3):496-504. doi:10.1249/01.mss.0000155393.78744.86

52. Treff G, Winkert K, Sareban M, Steinacker JM, Becker M, Sperlich B. Eleven-Week Preparation Involving Polarized Intensity Distribution Is Not Superior to Pyramidal Distribution in National Elite Rowers. *Front Physiol*. 2017;8:515. doi:10.3389/fphys.2017.00515

53. Yu H, Chen X, Zhu W, Cao C. A Quasi-experimental Study of Chinese Top-Level Speed Skaters' Training Load: Threshold Versus Polarized Model. *Int J Sports Physiol Perform*. 2012;7(2):103-112. doi:10/ggkgmb

54. Guellich A, Seiler KS, Emrich E. Training Methods and Intensity Distribution of Young World-Class Rowers. *Int J Sports Physiol Perform*. 2009;4(4):448-460. doi:10/ghk2ct

55. Spragg J, Leo P, Swart J. The Relationship Between Training Characteristics and Durability in Professional Cyclists Across a Competitive Season. *Eur J Sport Sci*. Published online March 3, 2022:1-17. doi:10.1080/17461391.2022.2049886

56. Burnley M, Bearden SE, Jones AM. Polarized Training is Not Optimal for Endurance Athletes. *Med Sci Sports Exerc*. Published online February 9, 2022. doi:10.1249/MSS.0000000000002869

57. Foster C, Casado A, Esteve-Lanao J, Haugen T, Seiler S. Polarized Training is Optimal for Endurance Athletes. *Med Sci Sports Exerc*. Published online February 9, 2022. doi:10.1249/MSS.0000000000002871

58. Altini M, Amft O. Estimating Running Performance Combining Non-invasive Physiological Measurements and Training Patterns in Free-living. *Annu Int Conf IEEE Eng Med Biol Soc* . Published online July 2018:2845-2848. doi:10.1109/EMBC.2018.8512924

59. Milesis CA, Pollock ML, Bah MD, Ayres JJ, Ward A, Linnerud AC. Effects of Different Durations of Physical Training on Cardiorespiratory Function, Body Composition, and Serum Lipids. *Res Q*. 1976;47(4):716-725. doi:10/gj7hm8

60. Wenger HA, Bell GJ. The Interactions of Intensity, Frequency and Duration of Exercise Training in Altering Cardiorespiratory Fitness. *Sports Med*. 1986;3(5):346-356. doi:10/fbqzcf

61. Hickson RC, Kanakis C, Davis JR, Moore AM, Rich S. Reduced Training Duration Effects on Aerobic Power, Endurance, and Cardiac Growth. *J Appl Physiol Respir Environ Exerc Physiol*. 1982;53(1):225-229. doi:10/gj7hnb

62. Granata C, Oliveira RSF, Little JP, Renner K, Bishop DJ. Mitochondrial Adaptations to High-Volume Exercise Training Are Rapidly Reversed After a Reduction in Training Volume in Human Skeletal Muscle. *FASEB J*. 2016;30(10):3413-3423. doi:10.1096/fj.201500100R

63. Granata C, Jamnick NA, Bishop DJ. Training-Induced Changes in Mitochondrial Content and Respiratory Function in Human Skeletal Muscle. *Sports Med*. 2018;48(8):1809-1828. doi:10.1007/s40279-018-0936-y

64. Neal CM, Hunter AM, Brennan L, et al. Six Weeks of a Polarized Training-Intensity Distribution Leads to Greater Physiological and Performance Adaptations Than a Threshold Model in Trained Cyclists. *J Appl Physiol (1985)*. 2012;114(4):461-471. doi:10/gfvw42

65. Röhrken G, Held S, Donath L. Six Weeks of Polarized Versus Moderate Intensity Distribution: A Pilot Intervention Study. *Front Physiol*. 2020;11:534688. doi:10.3389/fphys.2020.534688

66. Muñoz I, Seiler KS, Bautista J, España J, Larumbe E, Esteve-Lanao J. Does Polarized Training Improve Performance in Recreational Runners? *Int J Sports Physiol Perform*. 2014;9(2):265-272. doi:10.1123/ijspp.2012-0350

67. Filipas L, Bonato M, Gallo G, Codella R. Effects of 16 Weeks of Pyramidal and Polarized Training Intensity Distributions in Well-Trained Endurance Runners. *Scand J Med Sci Sports*. 2022;32(3):498-511. doi:10.1111/sms.14101

68. Stöggl T, Sperlich B. Polarized Training Has Greater Impact on Key Endurance Variables Than Threshold, High Intensity, or High Volume Training. *Front Physiol*. 2014;5:33. doi:10.3389/fphys.2014.00033

69. Spragg J, Leo P. Can Critical Power be Estimated from Training and Racing Data using Mean Maximal Power Outputs? *J Sci Cycl*. 2020;9(2):7-10.

70. Outdoor Foundation. *2021 Outdoor Participation Trends Report*.; 2021:1-27. https://ip0o6y1ji424m0641msgjlfy-wpengine.netdna-ssl.com/wp-content/uploads/2015/03/2021-Outdoor-Participation-Trends-Report.pdf

71. Impey SG, Hearris MA, Hammond KM, et al. Fuel for the Work Required: A Theoretical Framework for Carbohydrate Periodization and the Glycogen Threshold Hypothesis. *Sports Med*. 2018;48(5):1031-1048. doi:10/gdwk98

72. Mølmen KS, Øfsteng SJ, Rønnestad BR. Block Periodization of Endurance Training – A Systematic Review and Meta-analysis. *Open Access J Sports Med*. 2019;10:145-160. doi:10.2147/OAJSM.S180408

Curriculum Vitae

Andreas Kreutzer

Haslet, TX
817.966.9890
akreutzer82@gmail.com

Education

Texas Christian University — Since Fall 2018
Fort Worth, TX
- Ph.D. Health Sciences – Kinesiology (Exercise Physiology)
- Expected graduation date: May 7, 2022

Texas Christian University — Fall 2012 – Spring 2014
Fort Worth, TX
- M.S. Kinesiology – Exercise Physiology
- Graduation date: May 10, 2014

Boise State University — Fall 2006 – Spring 2010
Boise, ID
- B.A. English – Writing
- Graduation date: May 15, 2010

Saarland University — Winter 2003 – Winter 2008
Saarbrücken, Germany
- Majors: English & Kinesiology
- Transferred to Boise State University

Selected Work Experience

Instructional Lab Coordinator — Since Aug 2015
Texas Christian University – Fort Worth, TX
- Taught undergraduate and graduate classes
- Mentored undergraduate and graduate students in study methodology
- Instructed students and junior faculty on laboratory techniques
- Supervised student lab workers
- Established and implemented biosafety guidelines for Kinesiology laboratories
- Established and implemented standard operating procedures and proficiency checklists for laboratory equipment
- Assisted with planning and execution of undergraduate, graduate, and faculty research
- Prepared documents for submission to IRB
- Reviewed undergraduate and graduate DRB protocols
- Maintained and repaired laboratory equipment
- Co-managed lab budget, inventory, and equipment acquisition, service, and repair

Research Assistant/Associate — Aug 2014 – Aug 2015
Institute for Exercise and Environmental Medicine – Dallas, TX
- Conducted Pulmonary Function Testing, Exercise Research, and Clinical Cardiopulmonary Exercise Testing
- Analyzed and prepared data for grants, manuscripts, and presentations
- Managed databases for a variety of studies
- Maintained and repaired laboratory equipment
- Calibrated and performed quality control on all equipment
- Managed laboratory inventory and ordered supplies
- Managed Pulmonary Laboratory webpage and social media efforts

Graduate Assistant – Exercise Physiology — Aug 2012 – May 2014
Texas Christian University – Fort Worth, TX
- Fulfilled teaching assistant duties for a variety of classes
- Performed calibration and maintenance of laboratory equipment
- Conducted and assisted with exercise physiology research
- Co-managed laboratory inventory and ordered supplies

Publications & Abstracts

Dissertation:

- Nature and Nurture: The Effects of Genetics, Dietary Composition, and Training on Endurance Performance

Master's Thesis:

- Acute kinematic, kinetic, and hormonal responses to cluster sets in parallel back squat exercise in trained and untrained young men utilizing hypertrophic intensities

Peer Reviewed Publications

- Kreutzer A, Graybeal AJ, Rack PP, Moss K, Augsburger GR, Willis JL, Braun-Trocchio, R, Shah M. Ketogenic and High-Carbohydrate Diets in Cyclists and Triathletes: Performance Indicators and Methodological Considerations From a Pilot Study. *SportRxiv*. 2022. (accepted pending minor revisions at *Communications in Kinesiology*)
- Kreutzer A, Graybeal AJ, Moss K, Braun-Trocchio R, Shah M. Caffeine Supplementation Strategies Among Endurance Athletes. *Front Sports Act Living* [Internet]. 2022;4 Available from: https://www.frontiersin.org/article/10.3389/fspor.2022.821750.
- Graybeal AJ, Kreutzer A, Willis JL, Moss K, Braun-Trocchio R, Shah M. Age Drives the Differences in Dietary Supplement Use in Endurance Athletes: A Cross-Sectional Analysis of Cyclists, Runners, and Triathletes. *J Diet Suppl*. 2022;1–19.
- Moss K, Zhang Y, Kreutzer A, et al. The Relationship between Dietary Intake and Sleep Quality in Endurance Athletes. *Front. Sports Act. Living*. 2022;4:810402.
- Braun-Trocchio R, Graybeal AJ, Kreutzer A, Warfield E, Renteria J, Harrison K, Williams A, Moss K, Shah M. Recovery Strategies in Endurance Athletes. *J. Funct. Morphol. Kinesiol.* 2022;7(1).
- Graybeal AJ, Kreutzer A, Rack PP, Moss K, Augsburger GR, Willis JL, Braun-Trocchio, R, Shah M. Perceptions of appetite do not match hormonal measures of appetite in trained competitive cyclists and triathletes following a ketogenic diet compared to a high-carbohydrate or habitual diet: A randomized crossover trial. *Nutr Res*. 2021;93:111–23.
- Shah M, Gloeckner A, Bailey S, Adams-Huet B, Kreutzer A, Cheek DJ, Willis, JL, Mitchell JB. Effect of a late afternoon/early evening bout of aerobic exercise on postprandial lipid and lipoprotein particle responses to a high-sugar meal breakfast the following day in postmenopausal women: a randomized cross-over study. *J Sports Sci*. 2021;0(0):1–10.
- Askow AT, Lobato AL, Arndts DJ, Jennings W, Kreutzer A, Erickson, JL, Esposito PE, Oliver JM, Foster C, Jagim AR. Session Rating of Perceived Exertion (sRPE) Load and Training Impulse Are Strongly Correlated to GPS-Derived Measures of External Load in NCAA Division I Women's Soccer Athletes. *J Funct Morphol Kinesiol*. 2021;6(4):90.
- Caldwell AR, Vigotsky AD, Tenan MS, Radel R, Mellor DT, Kreutzer A, Lahart IM, Mills JP, Boisgontier MP, Boardley I, Bouza B, Cheval B, Chow ZR, Contreras B, Dieter B, Halperin I, Haun C, Knudson D, Lahti J, … Consortium for Transparency in Exercise Science (COTES) Collaborators. Moving Sport and Exercise Science Forward: A Call for the Adoption of More Transparent Research Practices. *Sports Med*. 2020;50(3):449–459.
- Kreutzer A, Martinez CA, Kreutzer M, Stone JD, Mitchell JB, Oliver JM. Effect of ACTN3 polymorphism on self-reported running times. *J Strength Cond Res*. 2019;33(1):80-88.
- Gassen J, Prokosch ML, Eimerbrink MJ, Leyva RPP, White JD, Peterman JL, Burgess A, Cheek DJ, Kreutzer A, Nicolas SC, Boehm GW. Inflammation predicts decision-making characterized by impulsivity, present focus, and an inability to delay gratification. *Sci Rep*. 2019;9(1):1-10.
- Shah M, Bailey S, Gloeckner A, Kreutzer A, Adams-Huet B, Cheek DJ, Mitchell JB. Effect of acute exercise on postprandial endothelial function in postmenopausal women: a randomized cross-over study. *J Investig Med*, 2019;67(6):964-970.
- Tufano JJ, Conlon JA, Nimphius S, Oliver JM, Kreutzer A, Haff GG. Different Cluster Sets Result In Similar Metabolic, Endocrine, And Perceptual Responses In Trained Men. *J Strength Cond Res*. 2019;33(2):346-354.
- Stone JD, Kreutzer A, Mata JD et al. Changes in Creatine Kinase and Hormones over the Course of an American Football Season. *J Strength Cond Res*. 2019;33(9):2481-2487.
- Carbuhn A, Reynolds S, Campbell C, Bradford L, Deckert J, Kreutzer A, Fry, A. Effects of Probiotic (Bifidobacterium longum 35624) Supplementation on Exercise Performance, Immune Modulation, and Cognitive Outlook in Division I Female Swimmers. *Sports*. 2018;6(4):116.
- Oliver JM, Jenke SC, Kreutzer A, Mata JD, Jones MT. Acute effect of cluster set and traditional set configurations on myokines associated with hypertrophy. *Int J of Sports Med*. 2016;37(13):1019-24.

- Oliver JM, Stoner L, Rowlands DS, Cladwell AR, Sanders E, Kreutzer A, Mitchell JB, Purpura M, Jaeger, R. Novel Form of Curcumin Improves Endothelial Function in Young, Healthy Individuals: A Double-Blind Placebo Controlled Study. *J Nutr Metab. 2016;2016:1089653.*
- Oliver JM, Kreutzer A, Jenke SC, Phillips MD, Mitchell JB, Jones MT. Velocity Drives Greater Power Observed During Back Squat Using Cluster Sets. *J Strength Cond Res.* 2016;30(1):235-43.
- Mitchell JB, Goldston KR, Adams AN, Crisp KM, Franklin BB, Kreutzer A, Montalvo DX, Turner MG, Phillips MD. Temperature Measurement Inside Protective Headgear: Comparison With Core Temperatures and Indicators of Physiological Strain During Exercise in a Hot Environment. *J Occup Environ Hyg.* 2015;12(12):866-74.
- Oliver JM, Kreutzer A, Jenke S, Phillips MD, Mitchell JB, Jones MT. Acute response to cluster sets in trained and untrained men. *Eur J Appl Physiol.* 2015;115(11):2383-93.

In Preparation

- Kreutzer A, Carr JC, Porter R, Cheek DJ, Shah M. The Effect of Aerobic Exercise on Circulating microRNA Expression: A Systematic Review and Meta-Analysis. 2022; (in preparation)
- Kreutzer A, Swets J, Cox C, Shah M. Effects of Training Characteristics on Cycling Performance in Competitive Recreational Cyclists and Triathletes: Analysis of Raw Crowdsourced Data. 2022; (in preparation)
- Kreutzer A, Cheek DJ, Graybeal AJ, Rack PP, Moss K, Shah M. Effects of Ketogenic and High-Carbohydrate Diets on circulating microRNA. 2022; (in preparation)

Poster Presentations & Abstracts

- Kreutzer A, Swets JA, Cox, CR, Shah, M. Association of Training Characteristics with Critical Power in Competitive Recreational Cyclists and Triathletes. *Int J Exer Sci: Conf Proc.* 2022;2(14). *Texas ACSM 2022.*
- Kreutzer A, Graybeal AJ, Moss K, Braun-Trocchio R, Shah M. Caffeine Supplementation Strategies Among Endurance Athletes. *Int J Exer Sci: Conf Proc.* 2021;2(13). *Texas ACSM 2021. Virtual.*
- Graybeal AJ, Kreutzer A, Rack PP, Moss K, Augsburger GR, Willis JL, Braun-Trocchio R, Shah M. Appetite Alterations in Endurance Athletes Following the Ketogenic Diet. *Int J Exer Sci: Conf Proc.* 2021;2(13). *Texas ACSM 2021. Virtual.*
- Moss K, Kreutzer A, Graybeal AJ, Zhang Y, Braun-Trocchio R, Porter RR, Shah M. The Relationship between Dietary Intake and Sleep Quality in Endurance Athletes. *Int J Exer Sci: Conf Proc.* 2021;2(13). *Texas ACSM 2021. Virtual.*
- Renteria J, Warfield E, Kreutzer A, Graybeal AJ, Moss K, Williams A, Harrison K, Shah M, Braun-Trocchio R. Recovery Strategies in Endurance Athletes. *Int J Exer Sci: Conf Proc.* 2021;2(13). *Texas ACSM 2021. Virtual.*
- Graybeal A, Willis J, Kreutzer A, Moss K, Braun-Trocchio R, Shah M. Nutrition Beliefs and Practices Among Endurance Athletes. *J Acad Nutr Diet.* 2021;121(9):A27.
- Braun-Trocchio R, Kreutzer A, Graybeal AJ, Rack PP, Harrison K, Williams A. The Effect of Diet Composition on Mood in Highly Trained Cyclists: A Pilot Study. J Sport Exer Psych. 2021;43:S56–7. *NASPSA Conference 2021. Virtual.*
- Kreutzer, A, Zaragoza, JA, Oliver, JM, Anzalone, AJ, Como, T, Juovich, D, Hall, J, O'Bryant, S, Urbina, SL, Taylor, LW. Head Trauma Biomarkers in NCAA Men's Soccer Athletes Over The Course Of A Season. *Med Sci Sport Ex.* 2019;51(5):Supplement, S790. *ACSM Annual Meeting* 2019. *Orlando, FL.*
- Cook, CA, Kreutzer, A, Levitt, MM, Cardenas, MA, Teagle, GM, Thames, KA, Phillips, MD, & Cheek, DJ. The Effects of Combined Aerobic and Resistance Exercise Training on Flow-Mediated Dilation in Overweight, Postmenopausal Women. *FASEB J.* 2019;33(1):Supplement 695.10. *Experimental Biology 2019. Orlando, FL.*
- Levitt, MM, Cardenas, MA, Cook, CA, O'Connor, JB, Orr, KL, Richie, B, Kreutzer, A, Haynes, J, Cheek, DJ, Phillips, MD. Combined Aerobic and Resistance Exercise Training Reduces Acute Exercise-Induced Platelet-Monocyte Complex Formation in Overweight, Postmenopausal Women. *FASEB J.* 2019;33(1):Supplement 695.10. *Experimental Biology 2019. Orlando, FL.*
- O'Connor, J, Kreutzer, A, Levitt, MM, Cardenas, MA, Cook, CA, Souder, E, Orr, KL, Huber, S, Teagle, GM, Thames, KA, Erickson, E, Wichman, EK, Phillips, MD. The Effects of Twelve Weeks of Combined Resistance and Aerobic Training on Arm Lean Mass in Post-Menopausal, Obese Women. *Int J Ex Sci: Conf Proceed.* 2019;2(11). *Texas ACSM Annual Meeting 2019. Fort Worth, TX.*
- Lobato AL, Kreutzer A, Stone JD, Anzalone AJ, Martinez, CA, Kreutzer M, Arndts DA, Jones MT, Fields JB, Merrigan JM, Talor LW, Urbina SL, Villa KB, Zaragoza JA, Wilborn CD, Oliver JM. ACTN3 RR genotype is overrepresented in NCAA Division I and Division III collegiate athletics. 2018. *NSCA International Conference, Madrid, Spain.*
- Askow AT, Stone JD, Kreutzer A, Martinez CA, Kreutzer M, Mitchell JB, Oliver JM. ACTN3 Genotype Frequencies among Recreationally Trained Men and Women in a Major U.S. City. *NSCA Annual Meeting 2017. Las Vegas, NV.*

- Martinez CA, Kreutzer A, Kreutzer M, Stone JD, Mitchell JB, Oliver JM. The Effect of ACTN3 Genotype on Self-Reported One-mile Running Time in Young, Recreationally Active Women. *ACSM Annual Meeting 2017*. Denver, CO.
- Kreutzer A, Jenke SC, Stone JD, Mata JD, Jagim A, Jones MT, Mitchell JB, Oliver JM. Cluster and Traditional Set Configurations Elicit Similar Myokine Responses. *NSCA Annual Meeting 2016*. New Orleans, LA.
- Stone JD, Kreutzer A, Mata, JD, Jagim A, Jones MT, Oliver JM. Off-season and in-season plasma cortisol responses in National Collegiate Athletic Association Division-I football players. *NSCA Annual Meeting 2016*. New Orleans, LA.
- Bhammar DM, Stickford JL, Bernhardt V, Marines-Price R, Bassett T, Kreutzer A, Roman MC, Babb TG. Dyspnea Intensity, Descriptors, And Negative Symptoms During Exercise In Obese And Nonobese Children. *Med Sci Sports Exerc*. 2016;48(5 Suppl 1):455.
- Kreutzer A, Zavala P, Fleming S, Jones MT, Oliver JM, Jagim A. The effect of 8 weeks of colostrum and bio-active peptide supplementation on body composition in recreational male weight lifters. *ISSN Annual Conference 2016*. Clearwater Beach, FL.
- Stone JD, Kreutzer A, Oliver JM, Jones MT, Kisiolek J, Jagim AR. Comparison of Prediction Equations to Indirect Calorimetry in Men and Women Athletes. *ISSN Annual Conference 2016*. Clearwater Beach, FL.
- Kreutzer A, Jenke SC, Jones MT, Phillips MD, Mitchell JB, Oliver, JM. Velocity drives power output during the back squat using cluster set and traditional configurations. NSCA Annual Meeting. *J Strength Cond Res*. 2014;28:1-130.
- Jenke SC, Kreutzer A, Jones MT, Phillips MD, Oliver, JM. Differences in time under tension during the back squat using traditional versus cluster set configurations. NSCA Annual Meeting. *J Strength Cond Res*. 2014;28:1-130

Projects with Significant Mentorship Roles

Role: Co-Principal Investigator with Meena Shah, PhD — Nov 2016 – Apr 2018
Project: "The effect of diet composition on performance, energy expenditure, and blood lipids in highly trained cyclists"
Project Type: Master's Thesis, PhD project
Involvement: Research mentor (literature review, study design, laboratory procedures, data analysis, abstract and manuscript writing) for four graduate students
Output: One peer-reviewed publication, one preprint (under peer-review), multiple additional manuscripts in preparation, two TACSM conference poster presentations, one NASPSA conference poster presentation

Role: Co-Investigator — Oct 2017 – Apr 2018
Project: "The Effects of a Carbohydrate Mouth Rinse on Cycling Performance in Depleted and Non-Depleted Glucose States"
Project Type: Honors Thesis
Involvement: Research mentor (literature review, study design, laboratory procedures, data analysis, abstract writing, presentation) for two undergraduate honors students
Output: Honors thesis, Boller Presentation award, Student Research Symposium poster presentation

Role: Co-Investigator — Nov 2016 – May 2018
Project: "Genetic influences on sport performance and athlete health: ACTN3 R577X and APOE polymorphisms in highly trained NCAA athletes"
Project Type: Multi-site faculty research
Involvement: Research mentor (laboratory procedures, data analysis, abstract writing) for three graduate students
Output: NSCA International Conference poster presentation

Role: Co-Investigator — Jan 2017 – Apr 2017
Project: "The Effect of ACTN3 Genotype on Power Output During Repeated Wingate Anaerobic Tests"
Project Type: Senior Research
Involvement: Research mentor (literature review, study design, laboratory procedures, data analysis, abstract writing) for four undergraduate students
Output: Student Research Symposium poster presentation

Role: Principal Investigator **Project: "The effect of ACTN3 genotype on microRNA expression following high-intensity interval and steady state endurance exercise"** **Project Type: Master's Thesis** **Involvement: Research mentor (literature review, study design, laboratory procedures, data analysis, abstract writing) for one graduate student** **Output: NSCA conference poster presentation, TACSM conference poster presentation**	**Oct 2016 – Apr 2017**

Grants

Grant: TCU Graduate Student Travel Grant **Project: "Effects of Training Characteristics on Cycling Performance in Competitive Recreational Cyclists and Triathletes: Analysis of Raw Crowdsourced Data"** **Amount: $380**	**Jan 2022**
Grant: TCU Graduate Student Research Grant **Project: "The acute and chronic effects of habitual, and high carbohydrate diets on circulating microRNAs in endurance-trained male and female cyclists"** **Amount: $500**	**Feb 2021**
Grant: TCU Graduate Student Research Grant **Project: "The effect of diet composition on performance in highly trained Cyclists"** **Amount: $496**	**Oct 2019**
Grant: TCU Graduate Student Travel Grant **Project: "Head trauma biomarkers in NCAA men's soccer athletes over the course of a season"** **Amount: $1,836**	**Mar 2019**
Grant: Texas ACSM Student Research Development Award **Project: "Acute kinematic, kinetic, and hormonal responses to cluster sets in hypertrophic exercise in trained and untrained young and old men"** **Amount: $500**	**Feb 2014**

Teaching Experience

KINE 10101 - Introduction to Kinesiology	**Spring 2021**
KINE 40103 - Senior Seminar	**Since Fall 2020**
KINE 40903 - Senior Internship	**Since Spring 2020**
KINE 60613 - Graduate Exercise Physiology	**Fall 2019**
KINE 30634 - Undergraduate Exercise Physiology	**Since Spring 2019**
KINE 30523 - Exercise Assessment & Prescription	**Since Spring 2016**
KINE 10603 - Anatomical Kinesiology	**Since Fall 2015**
KINE 30343 - Theory of Coaching (Teaching Assistant)	**Spring 2014**
KINE 40623 - Physical Education for the Secondary Youth (Teaching Assistant)	**Spring 2014**
KINE 40313 - Individual & Dual Sports (Teaching Assistant)	**Fall 2012 & 2013**
KINE 30733 - Exercise Psychology (Teaching Assistant)	**Fall 2013**
KINE 30634 - Exercise Physiology Lab (Teaching Assistant)	**Spring 2013**

Skills

Laboratory skills

- Phlebotomy
- Maximal and submaximal exercise testing
- Metabolic testing
- Strength and power testing
- Sweat testing
- Kinematic and kinetic testing
- Pulmonary function testing
- Ultrasound assessment of flow-mediated dilation
- Musculoskeletal ultrasound
- Refractometer determination of urine specific gravity
- Freezing-point depression determination of osmolaltiy
- Body composition (skinfolds, Bod Pod, DXA)
- Magnetic Bead-Based (MagPix), Spectrophotometric, Radioimmuno- (RIA), and Enzyme-linked immunosorbent (ELISA) assays
- Polymerase Chain Reaction (PCR) and gel electrophoresis for genotyping
- Reverse transcription (RT)-PCR for determination of RNA expression

Computer skills

- General software and hardware skills
- Microsoft Office Suite and Adobe CS MS

Programming languages and statistical software

- R
- Python
- SPSS
- Jamovi
- JASP

Interpersonal skills

- Strong verbal & written communication skills
- Management skills
- Leadership skills
- Problem solving
- Decision making

Languages

- Bi-lingual German/English

Certifications

DEXA Solutions – Dual-Energy X-Ray Absorptiometry (16-hr CE)	Oct 2017
Texas Christian University – Radiation Safety Certification	Sep 2017
AHA – Basic Life Support (CPR, AED and First Aid)	Mar 2017
Radcom – Hazardous & Radioactive Material Handling and Shipping	Mar 2017
NSCA – Certified Strength and Conditioning Specialist	Jun 2014
GE Healthcare – Dual-Energy X-Ray Absorptiometry (Initial Operator Training)	Jan 2013

Professional Certificates

Computing in Python – Georgia Tech University	Jul 2021 – Dec 2021
Medical Statistics – Stanford University	Jun 2020 – Aug 2020
Improving your statistical Inferences – Eindhoven University of Technology	Jun 2018
Comprehensive Systematic Review Training – Joanna Briggs Institute	May 2018

Awards and Honors

Texas Christian University Outstanding Dissertation Award	Spring 2022
Certificate of Excellence for Outstanding Academic Achievement	Fall 2021 – Spring 2022
Certificate of Excellence for Outstanding Academic Achievement	Fall 2013 – Spring 2014
Harris College Student Research Symposium Poster Award ($100)	Apr 2014
Student Research Development Award – TACSM	Feb 2014

Memberships

Society for Transparency, Openness, and Replication in Kinesiology (STORK) – founding member	Since Dec 2018
American Physiological Society (APS)	Since Nov 2018
National Strength and Conditioning Association (NSCA)	Since Feb 2013
American College of Sports Medicine (ACSM)	Since Feb 2013
American College of Sports Medicine – Texas Chapter (TACSM)	Since Nov 2012

Service	
Graduate Student Senator (member: DEI committee, Student Parent & Childcare committee)	Apr 2020 – May 2021
Chief Review Editor & Preprint Server Director at SportRxiv	Since Mar 2020
STORK journals steering board member	Since Mar 2020
Review Editor at SportRxiv	Feb 2019 – Mar 2020
Reviewer for MDPI journals, PLOSone, Sports Medicine	Since Jan 2019
Publication Committee member at STORK	Since Jan 2019
Radiation Safety Committee member at TCU	Since Apr 2018
Departmental Review Board Reviewer at TCU	Feb 2018 – Dec 2020

Invited Presentations & Guest Lectures	
"Statistical Power Analysis using Simulation" (Lunch & Learn at TCU Institute of Behavioral Research)	Feb 2020
"Nutrition: Fitness & Sport" (Guest Lecture in TCU KINE 60673)	Jan 2020
"Research Ethics" (Presentation at EA Young Academy, North Richland Hills, TX)	Oct 2019
"Acute kinematic, kinetic, and hormonal responses to cluster sets in hypertrophic exercise in trained and untrained young and old men" (Podium Presentation at Texas ACSM)	Mar 2014

Abstract

Endurance sports, including running, cycling, and swimming, remain popular among recreational athletes in the U.S. and across the world. Many of these athletes compete in local, regional, and national races throughout the year. Their performance in competitions is affected by many factors, including genetics, nutrition, and training. Often, recreational athletes receive conflicting recommendations regarding strategies to optimize performance. Direct-to-consumer genetic testing companies promise to give insights into supposed genetic markers of endurance ability; blogs and social media tout the latest diet strategies to lose weight and perform better; virtual coaches and performance-improvement websites sell training plans with sometimes contradictory training strategies. It remains unclear which of these strategies actually benefit endurance athletes. Therefore, this dissertation examined the effects of genetics, diet composition, and training characteristics on endurance performance in recreational athletes, who often spend a considerable percentage of their disposable income and their time on improving race outcomes.

Single-nucleotide polymorphisms (SNPs), variations in a single base pair of a gene, have been proposed to affect physical performance. A SNP in the ACTN3 gene (XX genotype), results in deficiency of α-actinin-3, a structural muscle protein that appears important for explosive movements. Studies in rodents suggest that this deficiency could be beneficial for endurance performance. Yet, few studies in humans have directly assessed the effects of ACTN3 genotype on endurance performance. In our first study, we compared self-reported 1-mile and 5-km running personal records (PR) between participants expressing the three different ACTN3 genotypes. Among women, those with the ACTN3 XX genotype reported faster 1-mile PRs compared to those with the RR and RX genotype. We found no differences between genotypes for 1-mile PRs among men or 5-km PRs among either sex.

A long-standing strategy to improve endurance performance is to increase carbohydrate (CHO) availability before and during competition to slow the fatigue process. An opposing approach introduced by researchers, coaches, and athletes, is to increase fat oxidation (FATox) capacities by employing a low-CHO or ketogenic diet (KD). This improved ability for FATox at typical race-intensities would open access to an essentially limitless supply of energy substrates stored in the body as fat. However, studies

investigating the effects of KD and high-CHO diets (HC) on endurance performance have found conflicting results. In our second study, participants followed an HC and a KD for two weeks each in a random order. They performed a simulated 30-km cycling time trial (TT) at baseline and following each intervention. Participants' average power output or endurance performance during the TT was substantially lower following the KD when compared with the HC.

Endurance training is the main mechanism to improve general cardiorespiratory fitness and performance in races. Training characteristics include frequency, volume, intensity, and training intensity distribution (TID). TID can be determined by the time spent in the easy (Zone 1), moderate (Zone 2), and hard (Zone 3) training zones. Recently, a polarized TID (time in Z1 > Z3 > Z2) has gained popularity, after research suggested that many elite endurance athletes appear to follow this approach. However, the TID employed by recreational athletes and its association with performance is unknown. In our third study, we analyzed the training characteristics of recreational cyclists and triathletes. We investigated their association with endurance performance as measured by estimated relative critical power (CPrel). In our sample, very few recreational athletes followed a polarized TID. When controlling for age, we found that increased training volume and polarization were positively associated with CPrel.

In conclusion, *ACTN3* genotype does not appear to have a strong effect on endurance performance. Our results, along with findings from other studies investigating so-called endurance SNPs, suggest that individual genetic markers are not good indicators of endurance performance ability; thus, athletes and coaches should not rely upon direct-to-consumer genetic testing for talent identification and sport selection. Further, based on the results of our second study, the KD appears to decrease endurance performance. While additional research using longer nutrition interventions and different performance measures is needed, it appears that maximizing CHO availability remains the best strategy to improve endurance performance. Finally, we found that few recreational athletes follow a polarized TID despite its potentially beneficial effect on performance.