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Cardiopulmonary Exercise Testing with Lactate and Ammonia Samples

The Influence of Age, Sex, Ketogenic Diet, and Neuromuscular Disease

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ACADEMIC DISSERTATION

The University of Helsinki, for public examination in the lecture room 2,
Haartman Institute of Helsinki University
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Tiivistelmä

Johdanto ja tavoitteet:

Kliininen rasituskoe hengityskaasuseurannassa (spiroergometria) antaa tietoa henkilön kardiorespiratorisesta suorituskyvystä. Tutkimusta voidaan käyttää myös metabolisten lihastautien, esimerkiksi mitokondriaalisten myopatioiden (MM) diagnostiikkaan, kun tutkimuksen yhteydessä kerätään myös laskimoveren maitohappo- (laktaatti), ammoniakki- ja verikaasunäytteitä sekä levossa että rasituksessa. Jokela-tyypin spinaalinen lihasatrofia (SMAJ) on harvinainen lihaksen motoneuronitauti, joka johtuu mitokondriaalista proteiinia koodaavan geenin geenivirheestä.

Tämän tutkimuksen tavoitteena oli tutkia iän, sukupuolen ja vähähiilihydraattisen ruokavalion vaikutuksia spiroergometriatuloksiin levossa, rasituskokeen aikana ja sen jälkeen palautumisvaiheessa kerättyihin laktaatti- ja ammoniakkiarvoihin terveillä henkilöillä. Lisäksi tavoitteena oli kuvata rasituskoetulosprofiilia molekyyligeneettisesti vahvistetuilla SMAJ-potilailla ja verrata tuloksia MM-potilaiden ja terveiden henkilöiden tuloksiin.

Menetelmät ja tutkittavat henkilöt:

Ensimmäisessä osatutkimuksessa arvioitiin iän ja sukupuolen vaikutusta rasituskokeen yhteydessä mitattavien laskimoveren laktaatti- ja ammoniakkipitoisuuksiin 73 terveellä osallistujalla (34 miestä, 39 naista). Laskimoverinäytteiden tuloksia vertailtiin sekä kolmessa eri ikäryhmässä (<35-vuotiaat, 35-50-vuotiaat, >50-vuotiaat), että ikä jatkuvana muuttujana. Tämän lisäksi analysoitiin rasituskokeen yhteydessä mitattuja verikaasunäytteitä levossa, rasituksen aikana ja palautumisvaiheessa.

Toisessa osatutkimuksessa arvioitiin 10:llä terveellä vapaaehtoisella modifioidun vähähiilihydraattisen Atkins-ruokavalion fysiologista vaikutusta analysoimalla rasitustestin tuloksia sekä ennen että jälkeen neljän viikon ruokavaliojakson.

Kolmannessa osatutkimuksessa verrattiin 11:sta SMAJ-potilaan, 26:n MM:aa sairastavan ja 28:n terveen verrokin rasituskoe- ja laktaatti- ja ammoniakkituloksia keskenään.

Tulokset:

Ensimmäisen osatutkimuksen tulokset tukivat ja laajensivat tietämystä muutoksista, jotka liittyvät normaaliin ikääntymiseen tai ovat riippuvaisia sukupuolesta: laktaatti- ja ammoniakkipitoisuudet rasituksen aikana ja sen jälkeisessä seurannassa olivat matalampia vanhemmissa ikäryhmissä ja tämän lisäksi iän vaikutus rasitukseen liittyviin laktaattiarvojen muutoksiin oli naisilla miehiä voimakkaampaa. Toisessa osatutkimuksessa tutkimushenkilöiden noudattama neljän viikon vähähiilihydraattinen ruokavalio vaikutti spiroergometrian tuloksiin johtaen rasituksen mekaanisen hyötysuhteen heikkenemiseen, minuuttiventilaation lisääntymiseen ja loppu-uloshengityksen hiilidioksiditason ($FetCO_2$) pienenemiseen, joista jälkimmäiset löydökset

sopivat hyperventilaatiotyyppisiin epäedullisiin muutoksiin hengityksessä. Kolmannen osatutkimuksen havainnot tukevat aiempaa tietämystä laktaatin käyttäytymisestä MM:ssa. Sitä vastoin SMAJ-potilaiden erot verrokkeihin olivat erilaisia kuin mitokondriotaudeissa. SMAJ-potilaiden suorituskyky ja maksimaalinen hapenkulutus olivat madaltuneet samalla tavoin kuin MM-potilailla verrattuna terveisiin henkilöihin. MM-potilaiden laskimoveren laktaattipitoisuus oli korkeampi kuin terveillä levossa, kevyessä rasituksessa ja 30 minuuttia rasituksen päättymisestä, ja heillä hapen hengitysekvivalentti ja $FetCO_2$ olivat korkeat terveisiin verrattuna maksimaalisessa rasituksessa. Vastaavia muutoksia hengityksessä ja laktaatissa ei todettu SMAJ-potilailla.

Johtopäätökset:

Tässä väitöskirjassa kuvataan spiroergometriatutkimuksen löydöksiä sekä terveillä että lihassairautta sairastavilla. Tutkimustulokset antavat lisätietoa iän ja sukupuolen vaikutuksesta laktaatti- sekä ammoniakkitasoihin rasituskokeen yhteydessä ja etenkin palautumisvaiheessa. Ketogeeninen ruokavalio heikensi mekaanista hyötysuhdetta ja aiheutti epäedullisia muutoksia hengitykseen. SMAJ-potilaiden suorituskyky ja maksimaalinen hapenkulutus (oksidatiivinen kapasiteetti) olivat alentuneet kuten MM-potilailla, mutta SMAJ-potilailla ei havaittu MM-potilaille rasituskokeessa tyypillistä ventilaation kasvua tai veren laktaattitason muutoksia. Nämä havainnot tarjoavat lisätietoa rasitustutkimusten tulosten tulkintaan kliinisessä työssä.

Abstract

Introduction and Objectives:

Cardiopulmonary exercise testing with lactate, ammonia, and blood gas samples is used as a part of the diagnostic palette for metabolic myopathies, a group of hereditary disorders of muscle metabolism, such as mitochondrial myopathies (MM). The aim of this study was to explore the impact of age, sex, and a low-carbohydrate diet on the results of the exercise test, as well as to present the result profile of Spinal Muscular Atrophy, Jokela type (SMAJ), a rare motor neuron disease common in the Finnish population with a mitochondrial component, and compare the findings to both healthy controls and subjects with genetically verified mitochondrial myopathy.

Subjects and methods:

The first study assessed the effects of age and sex on lactate and ammonia levels in 73 healthy subjects (34 male, 39 female) across three age groups (<35, 35-50, >50 years) and with age as a continuous variable, during a cardiopulmonary exercise test with blood gas, plasma lactate, and ammonia measurements taken at rest, during, and after exercise.

The second study evaluated the influence of a modified Atkins diet (mAD) on the physiology of 10 healthy volunteers by comparing the results of two cardiopulmonary exercise tests, one performed before and the other after four weeks on the diet.

The third study compared the cardiopulmonary oxidative capacity of 11 SMAJ subjects and 26 subjects with MM to 28 healthy controls using cardiopulmonary exercise testing with lactate and ammonia sampling.

Results:

In the first study, lactate and ammonia concentrations during exercise and recovery were lower in older age groups and lower in women compared to men, with the effect of age also being more prominent in women. In the second study, four weeks of mAD did influence cardiopulmonary exercise test results, causing mechanical efficiency to decrease and increasing ventilation at the same time as fractional end-tidal expired carbon dioxide (FetCO2) decreased, suggesting unbeneficial changes in ventilation. In the third study, the lactate results of MM subjects were similar to those of earlier studies, but the SMAJ subjects did not exhibit similar results. The SMAJ subjects exhibited a lower power output and maximal oxygen consumption compared to healthy controls, but similar to MM subjects. The MM subjects exhibited higher lactate levels than healthy controls at rest, during light exercise, and 30 minutes post-exercise, and had higher ventilatory equivalents for oxygen as well as lower $FetCO_2$ compared to healthy controls during maximal exercise. These changes in ventilation and lactate were absent in subjects with SMAJ.

Conclusions:

This dissertation presents findings for cardiopulmonary exercise testing results with lactate and ammonia samples, both for healthy subjects and subjects with muscle disease, providing more information about the effects of age on these results during exercise and especially recovery. The ketogenic diet had an unfavorable effect on work efficacy and ventilation. For SMAJ subjects, though they displayed reduced exercise capacity and oxidative capacity similar to those in MM, they did not exhibit changes in ventilation and lactate typical of MM during the exercise stress test. These findings provide important insights into the interpretation of cardiopulmonary exercise testing results in clinical contexts.

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I would like to thank my supervisors, Professor Päivi Piirilä and Docent Mari Auranen, for their valuable guidance and support throughout this thesis. Their feedback and expertise were essential to the progress and completion of this work.

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I am deeply grateful to all co-authors of the original publications for their valuable contributions. In more detail, I would like to thank Dr. Hanna Lantto for supervising parts of the data collection, recruiting control participants, and reviewing clinical statements for both Articles I and III; For article I, I am thankful for Dr. Emmi Rotgers and Dr. Veli-Pekka Kouri for contributing their expertise in clinical chemistry for the lactate and ammonia analyses; For article II, I would like to thank Lauri Saksa for his preliminary work on the subject in his undergraduate thesis, which was here expanded, recalculated, and presented in a more comprehensive form, Professor Kirsi Pietiläinen, who oversaw the dietary component of the study, and Professor Anu Suomalainen, who designed and led the implementation of the original dietary intervention study, that the present study is a substudy of. For article III, I would like to thank Edouard Palu, a fellow doctoral researcher, for contributing his expertise on electroneuromyography, Docent Emil Ylikallio, for contributing his expertise on Spinal muscular atrophy, Jokela type, and Professor Anu Suomalainen for allowing me to continue work with mitochondrial myopathy subjects originally recruited for studies of her design. I would also like to thank Ritva Luukkonen for her expert consultations for statistical analyses across all three original articles.

In addition, I would like to thank the pre-examiners of this thesis, Professor Mika Martikinen and Docent Hanna Mussalo, for their valuable time and constructive feedback. I would like to acknowledge the many members of hospital staff and other volunteers who participated as control subjects—your involvement was truly appreciated. I am also sincerely grateful to the staff of the Meilahti Clinical

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Finally, I would like to thank my family and friends for their encouragement and support during my studies—and especially my husband, Joonas, without whose unwavering encouragement, support, and excellent coffee-brewing service, this work would not have been finished.

List of Abbreviations

Acetyl-CoA = acetyl-Coenzyme A
Acyl-CoA = acyl-Coenzyme A
ADP = Adenosine diphosphate
ALS = Amyotrophic lateral sclerosis
AMP = Adenosine monophosphate
ANOVA = One-way analysis of variance
ATP = Adenosine triphosphate
BE = Base excess
BMI = Body mass index
Ca^{2+} = Calcium ion
CK = Creatine kinase
CO_2 = Carbon dioxide
CPEO = Chronic progressive external ophthalmoplegia
CHCHD2-protein = the coiled-coil-helix-coiled-coil-helix domain-containing protein 2
CHCHD10-protein = the coiled-coil-helix-coiled-coil-helix domain-containing protein 10
DNA2 = DNA replication helicase/nuclease 2
FADH2 = Flavin adenine dinucleotide
$FetCO_2$ = Fraction of end-tidal CO_2
FEV1 = Forced expiratory volume in one second
FVC = Forced vital capacity
GSD = Glycogen storage disease
ENMG = Electroneuromyography
ECG = Electrocardiogram
HR = Heart rate
IMP= Inosine monophosphate
IPAQ = International Physical Activity Questionnaire
LOSMoN = late-onset spinal motor neuronopathy
mAD = Modified Atkins diet
MELAS = Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes
MERRF = myoclonic epilepsy and ragged red fibres

MM = Mitochondrial myopathy
mtDNA = Mitochondrial deoxyribonucleic acid
MT-TL1 = Mitochondrial tRNA leucine 1
NAD+/NADH = Nicotinamide-adenine dinucleotide
NARP = Neuropathy, ataxia, and retinitis pigmentosa
nDNA = Nuclear deoxyribonucleic acid
ND_2 = NADH dehydrogenase 2
ND = Normalized isocaloric standard diet
OUES/BSA = Oxygen uptake efficiency slope per body surface area
O_2 = Oxygen
PCr = Phosphocreatine
PO_2 = Partial pressure of oxygen
POLG = Polymerase gamma
PYGM gene = Glycogen phosphorylase, muscle-associated gene
PET = Positron emission tomography
RER = Respiratory exchange rate ($V'CO_2/V'O_2$)
SD = Standard deviation
SE = Standard error
SMAJ = Spinal muscular atrophy, Jokela type
SpO_2 = Peripheral arterial oxygen saturation
TCA cycle = Tricarboxylic acid cycle (citric acid cycle)
TYMP = Thymidine phosphorylase
TWNK = twinkle mtDNA helicase
V'E = Minute ventilation
$V'E/V'O_2$ = Ventilatory equivalent to O_2
$V'E/V'CO_2$ = Ventilatory equivalent to CO_2
$V'O_{2max}$ = Maximal oxygen uptake during exercise
$V'O_{2max}$/kg (ml/min/kg) = Maximal oxygen uptake per kilogram during exercise$V'O_2$/HR = Oxygen pulse
W_{max}/3 min = Maximal power during the last three maximal minutes of exercise
$W_{max}/V'O_{2max}$ (%) = Mechanical work efficiency
W_{peak}= Peak power during the last 30 seconds of exercise

Table of Contents

List of Original Publications

This thesis is based on the following publications:

I Ratia N, Lantto H, Rotgers E, Kouri VP, Auranen M, Luukkonen R, Piirilä P. Lactate and ammonia measurements during cardiopulmonary exercise testing and its recovery phase-Consideration of age and sex in its interpretation. Clin Physiol Funct Imaging. 2023 Jul;43(4):278-290.

II Ratia N, Pietiläinen KH, Auranen M, Saksa L, Luukkonen R, Suomalainen A, Piirilä P. Modified Atkins diet modifies cardiopulmonary exercise characteristics and promotes hyperventilation in healthy subjects. Journal of Functional Foods. 2021 Jun;81; 104459

III Ratia N, Palu E, Lantto H, Ylikallio E, Luukkonen R, Suomalainen A, Auranen M, Piirilä P. Lowered oxidative capacity in spinal muscular atrophy, Jokela type; comparison with mitochondrial muscle disease. Front Neurol. 2023 Nov 8;14:1277944.

The publications are referred to in the text by their roman numerals.

1 Introduction

Metabolic myopathies are a diverse group of hereditary disorders caused by defects in the energy metabolism of the muscle. These conditions often manifest during or after physical activity with symptoms such as exercise intolerance, muscle fatigue, pain, and even muscle damage. In more severe cases, these symptoms can significantly impair the quality of life and contribute to disability. These disorders can be broadly classified into three categories based on the energy pathway affected: glycogen storage diseases, disorders of lipid metabolism, and mitochondrial myopathies (MM). (Ahmed et al., 2018; Chin et al., 2024) Besides the MM, gene defects affecting mitochondrial function are present in many other neuromuscular diseases, including Spinal Muscular Atrophy of Jokela type (SMAJ), a form of spinal muscular atrophy found in Finland. (Jokela et al., 2011; Penttilä et al., 2014, 2015)

The abnormalities in different metabolic pathways are often highlighted during exercise as the energy requirement in the muscle increases, making exercise tests an interesting diagnostic tool. Various forms of exercise testing have been employed, including ischemic and non-ischemic forearm tests, as well as cardiopulmonary exercise testing combined with lactate, ammonia, and blood gas samples, both as incremental maximal exercise tests and as submaximal constant-load tests. (Jeppesen et al., 2021; M. Tarnopolsky, 2012) The maximal form of cardiopulmonary exercise testing combined with lactate, ammonia, and blood gas samples has been in use at the Helsinki University Hospital.

This dissertation presents three studies focused on cardiopulmonary exercise testing combined with lactate, ammonia, and blood gas samples. In the first study, the exercise test results of 73 healthy control subjects were obtained using cardiopulmonary exercise testing. The second study investigated the impact of a modified Atkins diet (mAD) on exercise test results and metabolic markers in 10 healthy control subjects who underwent cardiopulmonary exercise testing before and after a 4-week dietary intervention. The third study focused on the exercise testing profiles of subjects with SMAJ and MM.

The primary aim of this research was to assess factors that affect cardiopulmonary exercise testing combined with lactate, ammonia, and blood gas samples in both healthy subjects and subjects with muscle disease. Specifically, this thesis sought to (1) examine the effects of age and sex on cardiopulmonary

exercise testing results over a 30-minute follow-up period, (2) investigate the impact of a modified Atkins diet on metabolic markers of breathing and venous lactate and ammonia samples in healthy subjects, and (3) to describe the lactate and ammonia profiles and oxidative capacity of subjects with SMAJ and MM in comparison with healthy subjects and to find out if signs of mitochondrial dysfunction would appear during exercise in SMAJ.

2 Literature Review

2.1 Normal Energy Metabolism in Muscle During Exercise

Energy metabolism in muscle cells involves a complex interplay of biochemical pathways to provide the adenosine triphosphate (ATP) necessary for muscle contraction and other cellular processes. The main pathways of energy metabolism are summarised in Figure 1. As muscle contraction is an energy-heavy process, the small amount of ATP immediately available in the muscle is quickly depleted during exercise, breaking into adenosine diphosphate (ADP) and phosphate, adenosine monophosphate (AMP), and finally into inosine, generating ammonia. The increased demand mandates increased ATP production through a variety of pathways. The fast, substrate-level phosphorylation pathways that do not require oxygen, the ATP-PCr (phosphocreatine) system and glycolysis, are predominant at the start of the exercise and during high-intensity activities lasting up to several minutes, such as sprinting or intense weightlifting. The ATP-PCr system provides rapid energy through the breakdown of PCr to create ATP from ADP. This reaction is regulated by the equilibrium of its substrates, driven by the rise in free ADP, and catalyzed by the creatine kinase enzyme. This system is highly effective for short bursts of intense activity lasting only a few seconds. (Egan & Zierath, 2013; Hargreaves & Spriet, 2020; van Loon et al., 2001)

In glycolysis, glucose derived from glycogen stored in muscle cells and glucose extracted from blood are broken into pyruvate, reducing nicotinamide adenine dinucleotide (NAD+ -> NADH), and releasing ATP. The excess pyruvate created in glycolysis is converted to lactate by the lactate dehydrogenase enzyme within the cytoplasm of muscle cells. This process allows for the regeneration of NAD+ from NADH, enabling glycolysis to continue in the absence of oxygen. This pathway is less efficient in terms of ATP production compared to complete oxidative phosphorylation of carbohydrates, but can provide energy rapidly without requiring oxygen. (Dashty, 2013; Hargreaves & Spriet, 2020)

For sustained activities requiring lower to moderate intensity and longer durations, and when oxygen is available, muscle cells rely on oxidative phosphorylation. Oxidative phosphorylation occurs in the mitochondria and involves the complete oxidation of substrates (pyruvate, fatty acids, and amino

acids) to produce ATP. For carbohydrates, the pyruvate from glycolysis enters the mitochondria and undergoes conversion to acetyl-coenzyme-A (acetyl-CoaA) through the pyruvate dehydrogenase complex. For fats, free fatty acids derived either from the intramuscular triglycerides or through the bloodstream from the adipose tissue are converted into acyl-CoA and transported into the mitochondria by acyltransferases such as Carnitine palmitoyl transferase I and II, and fat-transport proteins. In the mitochondria, they are further converted into acetyl-CoA through beta-oxidation. To a lesser extent, increased metabolic activity in skeletal muscle also leads to elevated breakdown of amino acids. Branched-chain amino acids break down products can also be converted to acetyl-CoA, which can further be oxidized in the mitochondria, releasing ammonia as a side product. (Hargreaves & Spriet, 2020; Houten et al., 2016; Lowenstein, 1972)

Acetyl-CoA derived from the above pathways enters the citric acid cycle, generating reducing equivalents (NADH and flavin adenine dinucleotide (FADH2) and guanine triphosphate (GTP), an energy-carrying equivalent to ATP. NADH and FADH2 produced in the citric acid cycle donate electrons to the electron transport chain located in the inner mitochondrial membrane. As electrons pass through the electron transport chain, they generate a proton gradient across the membrane, enabling ATP synthesis from AMP, ADP, and phosphate through oxidative phosphorylation in the mitochondria. This system is highly efficient in producing ATP but is dependent on the availability of oxygen. (Hargreaves & Spriet, 2020; van Loon et al., 2001)

The energy metabolism of muscle cells is tightly regulated to match energy supply with demand. Muscle contraction itself promotes increased carbohydrate metabolism by inducing Ca^{2+} release from the sarcolemma, and the increase in ADP, AMP, phosphate, and altered energy charge caused by ATP depletion drives ATP production to restore the equilibrium. Hormonal and neural signals, such as adrenaline through β-adrenergic activation of cyclic AMP and calcium release, also play critical roles in regulating metabolic pathways. Repeated exercise training drives muscle cell adaptation to the strain through partly the same mechanisms. ATP turnover, calcium flux, contraction-induced changes in mechanical strain, redox balance, the production and presence of reactive oxygen species, and intracellular oxygen pressure have all been implicated to be involved in the activation of signal transduction cascades regulating the plasticity of skeletal muscle. The adaptation mechanisms include increased mitochondrial density and improved oxidative capacity, enhancing ATP production. (Egan & Zierath, 2013; Greenhaff et al., 1994; Parolin et al., 1999; Richter et al., 1982; van Loon et al., 2001)

2.1.1 Ketogenic Diet

Diet can also affect energy metabolism: During a low-carbohydrate diet, limited carbohydrate availability results in the activation of lipolysis and fatty acid metabolism and ketone body (acetoacetate, β-hydroxybutyrate, and acetone) synthesis. Both fatty acids and ketone bodies are broken down by beta-oxidation to be used in energy metabolism, with ketone bodies and the glucose derived from gluconeogenesis in the liver being important fuel sources for the central nervous system. (Augustin et al., 2018) As a result of these adaptation mechanisms, low-carbohydrate diets have been proven effective in weight reduction, and they have been widely studied in sports medicine for potential benefits to exercise performance, though the results have been inconclusive. (Babij et al., 1983; Burke et al., 2002; Carr et al., 2018; Cipryan et al., 2018; Goedecke et al., 1999; Harvey et al., 2019; McSwiney et al., 2019; Rowlands & Hopkins, 2002; Zajac et al., 2014) In addition, the ketogenic shift in metabolism has been shown to be beneficial in treatment-resistant epilepsy in both children and adults, although the exact mechanisms of the effects are not quite clear. (Kossoff & Dorward, 2008; Liu et al., 2018; Meira et al., 2019; Park et al., 2018) The referrals to dieticians and initializations of specific low-carbohydrate diets as treatment methods for several rare metabolic muscle diseases are also in use, as specific treatment options are still lacking. (Heydemann, 2018; Ørngreen & Vissing, 2017)

Energy Metabolism in the Muscle Cell

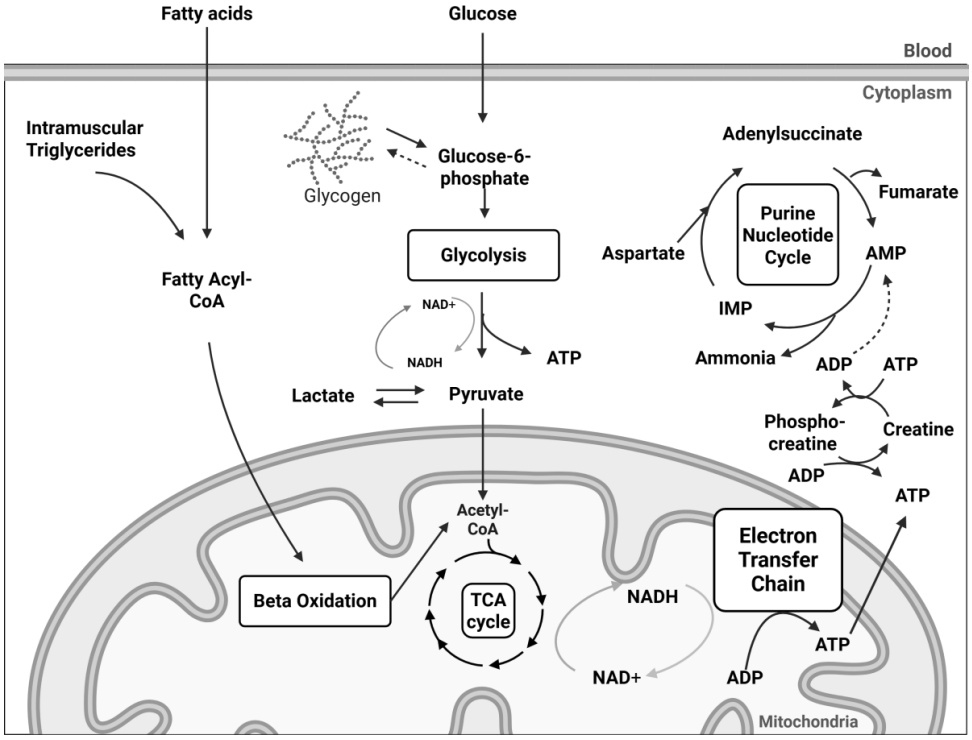

Figure 1 An overview of the primary energy pathways in muscle cells. Acetyl-CoA = acetyl coenzyme A, Acyl-CoA = acyl coenzyme A, ADP = adenosine diphosphate, AMP = adenosine monophosphate, ATP = adenosine triphosphate, IMP = inosine monophosphate. TCA cycle = the citric acid cycle. Created in BioRender.com.

2.2 Changes in Lactate and Ammonia during Exercise

2.2.1 Lactate

As the lactate dehydrogenase enzyme keeps the pyruvate created in glycolysis and its conversion product lactate in near equilibrium, the lactate level in cells is predominantly controlled by the rate of glycolysis. Thus, during exercise, as the muscle cell energy requirement causes glycolysis to increase, lactate levels also increase. Lactate accumulation during exercise increases significantly when oxygen demand surpasses oxygen delivery during heavy exercise, and the intracellular partial pressure of oxygen in skeletal muscle decreases. (Ferguson et al., 2018; Gladden, 2004)

As the rate of lactate production increases, lactate accumulates in the muscle cells, and consequently, its release into the blood also increases. Lactate produced during exercise can be taken up and oxidized by other tissues, including the brain, heart, liver, and oxidative skeletal muscles that are contracting in a submaximal steady state condition, to generate additional ATP, thus distributing substrates between different tissues and coordinating their redox state with little change in pH. Blood lactate levels increase when lactate production in active muscles exceeds the lactate removal by other tissues. Moreover, lactate serves as a gluconeogenic precursor in the liver, promoting glucose synthesis during and after exercise. This process, where lactate produced by muscles during anaerobic metabolism is transported to the liver, converted back into glucose, and then returned to the muscles for further energy use, contributing to the body's energy management, is called the Cori cycle. The traditional belief that lactate is solely a waste product of anaerobic metabolism has been disproven. Lactate is now considered to be an important signalling molecule and metabolic substrate. Lactic acid, which, within the physiological pH range of muscle and blood, is dissociated into lactate anions and protons, is one of the factors involved in the acid-base equilibrium, contributing to the strong ion gap and affecting pH both intracellularly and in the bloodstream. During hypoxic conditions in tissue due to e.g. septic shock or severe pulmonary diseases or in conditions where oxidative phosphorylation is impaired (e.g. mitochondrial disease, metformin use), especially when the lactate clearance in the liver or the kidneys is simultaneously impaired, this balance can be disrupted, leading to hyperlactatemia and, in extreme cases, lactate acidosis, a life-threatening condition. (Brooks, 1986; Gladden, 2004; Hui et al., 2017)

During the recovery period after exercise, lactate produced in the muscle due to exercise continues to be released into the bloodstream. Thus, the concentration of lactate in blood may continue to rise for up to 5 minutes after exercise before starting to decrease, though at a slower rate than during exercise. During this time, pyruvate levels are still high due to increased levels of glycolysis. Depending on the

strenuousness of the exercise, oxygen consumption remains over resting values for longer, even up to an hour. This difference, often called the "oxygen debt," depends on a multitude of factors, including residual catecholamine presence and higher temperature in tissue after exercise, which directly or indirectly influence mitochondrial oxygen consumption. During this time, excess lactate serves as a carbon source for both oxidative ATP production and for the resynthesis of glucose, glycogen, amino acids, and citric acid cycle intermediates depleted during exercise. (Gladden, 2004; Sietsema & Rossiter, 2023)

The rate of change in the increase in blood lactate concentration during exercise, often referred to as the lactate threshold, depends on factors such as exercise intensity and duration, muscle glycogen stores, muscle fiber type composition, and the aerobic fitness level of the individual. Though lactate is a product of carbohydrate metabolism, even during a low-carbohydrate diet, the blood lactate levels stay relatively stable. Physiological adaptations caused by endurance training enhance lactate clearance and utilization, improving exercise performance and raising the lactate threshold. These adaptations include increased mitochondrial density, improved oxidative capacity, enhanced lactate transport capacity, and upregulation of lactate clearance enzymes. (Brooks, 1986; Egan & Zierath, 2013)

2.2.2 Ammonia

During exercise, increased metabolic activity in skeletal muscle leads to elevated ammonia production through increased amino acid catabolism, particularly the metabolism of branched-chain amino acids and glutamine, as well as ATP hydrolysis and purine nucleotide degradation (also known as the purine nucleotide cycle), both of which also release ammonia as a byproduct. As with lactate, the ammonia concentration in blood continues to rise at the beginning of the recovery period, up to 5 minutes post-exercise. The magnitude of the increase in blood ammonia during exercise depends on various factors influencing both the production of ammonia and its clearance rate, including exercise intensity and duration, the muscle mass involved, and individual fitness level. High-intensity or prolonged exercise tends to elicit greater elevations in blood ammonia. Regular exercise can induce metabolic adaptations that enhance ammonia clearance and utilization, thereby mitigating the rise in blood ammonia during exercise. These adaptations include increased expression and activity of ammonia-detoxifying enzymes, improved mitochondrial function, and enhanced muscle buffering capacity, all of which contribute to maintaining metabolic homeostasis during exercise. (Graham & MacLean, 1992; Lowenstein, 1972; Van den Berghe et al., 1992)

The liver plays a crucial role in clearing ammonia from the bloodstream through urea synthesis via the urea cycle, which is then excreted by the kidneys in the urine. However, the hepatic clearance rate can be insufficient during intense exercise, causing transient increases in blood ammonia levels. Apart from the liver, skeletal muscle has a high capacity to uptake and utilize ammonia, both in the purine nucleotide cycle, creating AMP from IMP and, through the synthesis of glutamine, a non-toxic nitrogen carrier, from glutamate, catalyzed by the glutamine synthetase. This process not only serves to detoxify ammonia but also facilitates its transport to other tissues, mainly the liver and kidneys, for further metabolism and excretion. (Eriksson et al., 1985; Lowenstein, 1972; Yamato et al., 1995)

2.3 Overview of Metabolic Myopathies

2.3.1 Classification and Symptoms

Metabolic myopathies are a group of rare genetic disorders characterized by dysfunction of specific energy metabolism pathways. As the name suggests, they manifest with symptoms in skeletal muscle, but the clinical spectrum of metabolic myopathies is broad, ranging from mild exertional symptoms to severe, life-threatening metabolic crises. Depending on the disorder, the symptoms can present at any age, with the common symptom profiles varying by the age of onset of the disease: older children and adults often exhibit muscle weakness, exercise intolerance, and myoglobinuria, whereas infants and young children present with severe multisystem disorders. The symptoms can be continuous or limited only to exercise or other stress situations. Common manifestations include progressive muscle weakness, particularly during exertion and often accompanied by fatigue and exercise intolerance; recurrent muscle cramps, myalgia, and muscle stiffness; rhabdomyolysis and subsequent myoglobinuria arise due to rapid breakdown of muscle tissue during exercise or metabolic stress. Systemic involvement is common, affecting other energy-intensive tissues, manifesting as neurological deficits such as seizures and stroke-like episodes; cardiac abnormalities such as cardiomyopathy and arrhythmias; liver complications such as fibrosis and cirrhosis; ophthalmological manifestations including progressive external ophthalmoplegia and retinal degeneration. The metabolic myopathies are commonly categorized by the specific metabolic pathway affected. Broadly, these disorders can be classified into glycogen storage diseases (GSDs), fatty acid oxidation disorders, and mitochondrial myopathies. In addition, amino acid oxidation defects are sometimes included as a separate category, as amino acids are oxidized to a small extent by skeletal muscle, but instead of myopathies, these disorders typically manifest as severe multisystemic neurological symptoms in

childhood. (Angelini et al., 2020; Tarnopolsky, 2016; Tobon, 2013) These categories, along with the metabolic pathways affected, are summarised in Figure 2.

Glycogen storage diseases result from defects in glycogenesis (glycogen synthesis), glycogenolysis (breakdown of glycogen to glucose via glucose-6-phosphate), or glycolysis (conversion of glucose into pyruvate), predominantly affecting tissues with large glycogen stores: the muscle tissue of the skeletal muscles and the heart, and the liver. Glycogen content in the affected tissues is markedly increased in many GSDs, except for glycogen synthase deficiency, where it is, on the contrary, decreased. As carbohydrate storage in energy-intensive tissues is impaired, carbohydrate utilization efficiency, especially at the start of exercise before slower-acting routes like fat mobilization have started, is decreased in these patients. The affected patients usually experience the "second wind" phenomenon, where the muscle weakness and exhaustion at the start of the exercise decrease as the exercise continues, when new energy metabolites have had time to be transported by blood to tissue, and for fat oxidation to have started, compensating for the impaired carbohydrate metabolism. (Gümüş & Özen, 2023) The phenomenon is typical for McArdle's disease (GSD type V), the most common disorder of glycogenolysis caused by mutations in the PYGM (Glycogen Phosphorylase, Muscle Associate) gene encoding myophosphorylase, also characterized by exercise intolerance with premature fatigue, cramps, myalgia, and a rise in serum creatine kinase. (Andreu et al., 2007; Gümüş & Özen, 2023) Another example of GSDs is Pompe disease (GSD type II), which results from the deficiency of acid alpha-glucosidase enzyme, disrupting glycogen breakdown and leading to glycogen buildup in lysosomes in multiple tissues. The disease has two forms: the more severe infantile-onset Pompe disease, hallmarked by muscle hypotonia, delayed motor development, and hypertrophic cardiomyopathy, and late-onset Pompe disease with myopathy and progressive muscle weakness leading to motor difficulties and respiratory failure over time. (Dasouki et al., 2014; Taverna et al., 2020)

Fatty acid oxidation disorders are caused by impairments in the transport or metabolism of fatty acids in the cell. The defect can exist in the proteins transporting fatty acids into the cytoplasm-mainly by the carnitine protein of the sarcoplasmic membrane; storage or breakdown of fatty acids stored in intracellular lipid droplets; transportation of fatty acids into the mitochondria by Carnitine Palmitoyl Transferase I, Mitochondrial Trifunctional Protein, Carnitine-Acylcarnitine Translocase or Carnitine Palmitoyl Transferase II; or in the fatty acid beta-oxidation in the mitochondria. Common symptoms include exercise intolerance, muscle symptoms that typically worsen during fasting or prolonged physical exertion when the need for fatty acid consumption is increased, as well as rhabdomyolysis episodes. Notable entities include carnitine palmitoyl transferase

deficiency, medium-chain acyl-CoA dehydrogenase deficiency, and very-long-chain acyl-CoA dehydrogenase deficiency. (Houten et al., 2016; Pennisi et al., 2018; Vengalil et al., 2017) This thesis examines the mitochondrial diseases that affect muscle metabolism. Mitochondrial diseases are a diverse group of disorders affecting the structure and function of the mitochondria that can manifest with symptoms in many different tissues. Primary MMs are mitochondrial disorders caused by a genetic defect (hereditary pathogenic variants or sporadic wild-type mutations) leading to dysfunction of oxidative phosphorylation. They typically manifest with muscle symptoms like fatigue, muscle weakness, and exercise intolerance, but as mitochondria are vital for the energy metabolism of all cells, the involvement of other energy-intensive tissues, including the heart, the nervous system, and the retinae, is also common. As mitochondrial function is under the control of both the mitochondrial genome (mtDNA) and the nuclear genome (nDNA), MM can originate from mutations in both the mtDNA of the mitochondria and the nDNA of the nucleus. In the case of disorders stemming from mutations of the mtDNA, due to heteroplasmy—or the presence of more than one type of mtDNA in an individual due to more than one mitochondrion being inherited from the mother—usually, only a portion of the patient's mitochondria carry the pathogenic variant of mtDNA. Furthermore, as the mitochondria are not identically distributed between cell divisions, the concentrations of the defective mitochondria differ between both individual cells and different tissues. This explains the varying severity in the symptoms of patients carrying the same mtDNA pathogenic variant, with the variability not explained by the heteroplasmy measured in blood, skeletal muscle, or other single tissue. Notable examples of primary MM include Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy and ragged red fibers (MERRF), neuropathy, ataxia, and retinitis pigmentosa (NARP), maternally inherited Leigh syndrome, and chronic progressive external ophthalmoplegia (CPEO). (Ahmed et al., 2018; Chin et al., 2024; McFarland et al., 2010; Taylor & Turnbull, 2005)

Of the MM types included in the current study, MELAS is one of the most well-known MM-associated clinical syndromes, typically caused by the maternally inherited m.3243A>G pathogenic variant in the mitochondrially encoded leucine 1 (*MT-TL1*) gene encoding the leucine (UUR) mitochondrial transfer RNA. (El-Hattab et al., 2015; Goto et al., 1990; Pavlakis et al., 1984) In addition to the symptoms included in its name, cardiomyopathy usually involving left ventricular hypertrophy, has been observed in around a third of the subjects with MELAS, though in its adult-onset form, it is often mild and non-progressive. (Bourke et al., 2022; Brambilla et al., 2019; Hsu et al., 2016) In contrast, CPEO, a syndrome characterized by bilateral symmetrical progressive ptosis and reduced ocular motility, retinal degradation, and heart block, is more genetically heterogeneous.

Around 50% of the cases are caused by sporadic deletion of the mtDNA, but it can also be caused by hereditary changes in the nDNA interfering with mtDNA synthesis or mitochondrial fusion. Prominent nuclear genes with pathogenic variants causing CPEO include the DNA polymerase gamma (*POLG)*, twinkle mtDNA helicase (*TWNK)*, DNA replication helicase/nuclease 2 (*DNA2),* and Thymidine phosphorylase (*TYMP).* (Ali et al., 2024; Hirano & Pitceathly, 2023; Rusecka et al., 2018)

Determining the prevalence of MMs remains challenging due to their clinical and genetic heterogeneity. Not all carriers of the pathogenic variants are symptomatic, and the phenotypes of the same pathogenic variant can vary, with the same variant being capable of causing multiple clinical syndromes depending on the amount and distribution of the affected mitochondria between tissues. For m.3243A>G, in addition to the classical MELAS phenotype, these include e.g, the more common and less severe Maternally Inherited Diabetes and Deafness syndrome, CPEO, and clinical features overlapping or not consistent with the classical syndromes associated with the m.3243A>G mutation. (Mancuso et al,. 2014; Nesbitt et al., 2013)

In Finland, Martikainen and Majamaa (2024) have recently published the prevalence for mitochondrial disease in Southwestern Finland, the minimum prevalence estimate of adult mtDNA-related MM in general to be 9.2/100,000, and the prevalence of adult mtDNA disease associated with m.3243A>G variant, including but not limited to MELAS, of 4.2/100,000. (Martikainen & Majamaa, 2024) Previously, slightly higher prevalences of 10-18/100,000 for m.3243A>G variants have been reported in other parts of Finland. (Majamaa et al., 1998; Uusimaa et al., 2007). These results are similar to results reported in the United Kingdom, with mitochondrial disease attributable to pathogenic gene variants having a prevalence of 9.2-12.5/100,000. (Gorman et al., 2015; Schaefer et al., 2008) Though in the general population, prevalences as high as 236/100,000 have been reported in Australia. (Manwaring et al., 2007)

In addition to traditional mitochondrial disease, mitochondrial abnormalities have been shown to play a role in common neurodegenerative disorders such as Alzheimer's disease (Bender et al., 2006; Gowda et al., 2022; Swerdlow, 2018; Wang et al., 2020) and Parkinson's disease (Borsche et al., 2021; Funayama et al., 2015; Grünewald et al., 2016; Harjuhaahto et al., 2020; Ye et al., 2023), both through single-gene hereditary forms and in idiopathic disease affecting multiple genes. One of the genes participating in mitochondrial function and involved in the pathogenesis of other neurological diseases is the coiled-coil-helix-coiled-coil-helix domain-containing 10 (*CHCHD10*) gene on chromosome 22. *CHCHD10* and coiled-coil-helix-coiled-coil-helix domain-containing protein 2 gene (*CHCHD2*) encode two homologous proteins that belong to the mitochondrial CHCH domain protein family, forming a complex in the mitochondrial intermembrane space and

cooperating to regulate mitochondrial function. (Burstein et al., 2018; Ruan et al., 2022) *CHCHCD10* has drawn significant attention in recent years due to its association with neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) (Ait-El-Mkadem Saadi et al., 1993; Bannwarth et al., 2014; Harjuhaahto et al., 2020), axonal Charcot Marie Tooth disease, and frontotemporal dementia, as well as some forms of autosomal dominantly hereditary mitochondrial disease (Ajroud-Driss et al., 2015; Auranen et al., 2015; Müller et al., 2014). CHCHD10 belongs to a family of mitochondrial proteins localized in the mitochondrial intermembrane space that have been shown to be involved in the regulation of mitochondrial function and oxidative phosphorylation. (Harjuhaahto et al., 2020; Ruan et al., 2022) Some pathogenic variants in CHCHD10 have been shown to impair mitochondrial regulation, particularly in stressful situations, and to cause ATP production to be less efficient due to increased proton leakage in mitochondria. (Straub et al., 2021) Nonetheless, the precise effects of CHCHD10 variants on mitochondrial function are not fully understood. (Shammas et al., 2022) Though the existence of CHCHD10-linked diseases points to a direct link between mitochondrial function and the pathogenesis of motor neuron degeneration, the global metabolic consequences of *CHCHD10* defects are less clear. (Habets et al., 2022)

While CHCHD10-linked hereditary diseases have been identified worldwide, they remain relatively rare. Of these diseases, Spinal muscular atrophy, Jokela type (SMAJ), also known as late-onset spinal motor neuronopathy (LOSMoN), is globally the most prevalent, primarily due to its higher occurrence in the Finnish population. (Penttilä et al., 2014) First reported by Jokela et al. in 2011, SMAJ is an autosomal dominantly inherited spinal muscular atrophy caused by Finnish founder mutation c.197G>T (Gly66Val) in the *CHCHD10* gene. Typical of a motor neuron disease, common symptoms of SMAJ include slowly progressive muscle weakness, fasciculation, and cramps, concentrating in the lower limbs. (Jokela et al., 2011; Pasanen et al., 2016; Penttilä et al., 2014) SMAJ manifests as a motor neuron disease with skeletal muscle damage indicated, e.g., by the rise in creatine kinase, hallmarking the disease. (Järvilehto et al., 2022; Jokela et al., 2011) Metabolic examinations in SMAJ patients have shown changes in pyruvate and succinate levels, suggesting some level of mitochondrial dysfunction, although mitochondrial pathology is typically not evident in muscle biopsy samples. (Järvilehto et al., 2022)

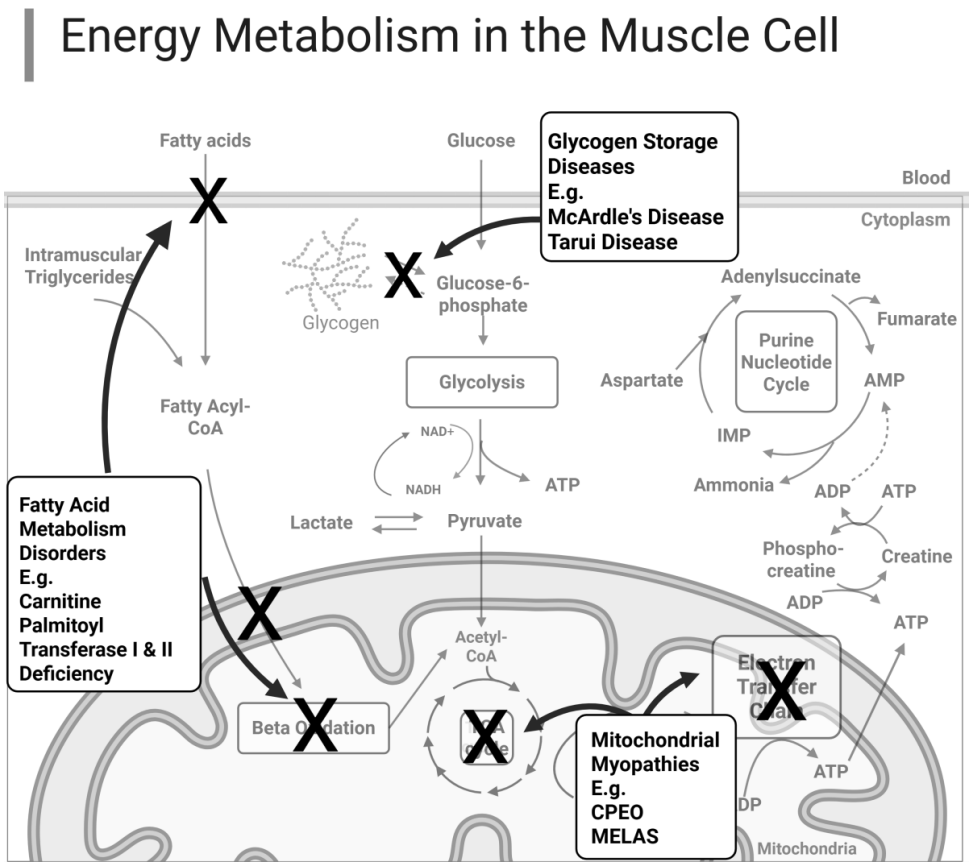

Figure 2 An overview of the energy pathways affected by the metabolic myopathies. Acetyl-CoA = acetyl coenzyme A, Acyl-CoA = acyl-coenzyme A, ADP = adenosine diphosphate, AMP = adenosine monophosphate, ATP = adenosine triphosphate, CPEO = Chronic progressive external ophthalmoplegia, IMP = inosine monophosphate, MELAS = Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes syndrome. TCA cycle = the citric acid cycle. Created in BioRender.com.

2.3.2 Diagnostic Approach and Treatment Modalities

As many of the symptoms caused by metabolic myopathies, such as exercise intolerance and myalgia, are nonspecific and common in the wider population, the diagnostics of metabolic myopathies necessitate a systematic approach integrating clinical evaluation focusing on symptomatology and family history, laboratory investigations, muscle biopsy, and specialized functional and neurophysiological studies as well as genetic testing that confirms the diagnosis. The diagnostic

pathway usually involves several procedures and the interpretation of results by different specialties. (Ahmed et al., 2018; Angelini et al., 2020; Tarnopolsky, 2016)

In the diagnostic process of metabolic myopathies, laboratory tests indicative of biochemical abnormalities in muscle disease in general or specific metabolic defects play a pivotal role. These measurements include serum creatine kinase (CK) levels indicating muscle damage, high resting lactate observed in MM, and the screening for disease-specific acylcarnitines accumulating in blood in fatty acid oxidation disorders. In MM, immunohistochemical studies and enzymatic analysis of the oxidative phosphorylation complexes of muscle tissue samples are also utilized. (Ahmed et al., 2018, Milone & Wong, 2013, M. A. Tarnopolsky, 2016) In addition, specialized functional studies, such as cardiopulmonary and forearm exercise testing and P-31 magnetic resonance spectroscopy, provide a dynamic assessment of muscle metabolism during exercise, helping to identify underlying metabolic derangements and distinguish between different metabolic myopathies. (Mattei et al., 2004; Milone & Wong, 2013)

Although the results of genetic analysis are the gold standard of the diagnosis, muscle tissue biopsy remains a valuable diagnostic tool, offering histological and biochemical insights into muscle pathology, including glycogen accumulation, lipid droplet deposition, mitochondrial abnormalities, and enzyme deficiencies, which aid in confirming the diagnosis and guiding further investigations. Still, the ability to detect pathogenic variants in suspected mitochondrial disease via genetic studies remains imperfect, with a reported diagnostic yield ranging from 25% to 75 % through next-generation sequencing and tissue mtDNA analysis, facilitating the identification of pathogenic variants in new genes implicated in metabolic pathways. (Parikh et al., 2019)

The specific genetic diagnosis provides the framework for the multidisciplinary care of the patients, allowing the possibility for precise genetic counseling and understanding the natural progression of the disease in question. For some of the disorders, specific pharmacological interventions targeting and augmenting the disorder-specific metabolic defects have also become available. These include enzyme replacement therapy, such as alglucosidase alfa and cipaglucosidase alfa for Pompe disease (Blair, 2023), and coenzyme Q10 administration in MM patients with coenzyme Q10 deficiency (Pirinen et al., 2020), aiming to restore energy metabolism and alleviate symptoms. Dietary modifications, including carbohydrate loading and avoidance of prolonged fasting in fatty acid oxidation disorders and ketogenic diets in glycogen storage diseases, can also help maintain euglycemia and prevent muscle damage. (Yakupova et al., 2022) Some patients with fatty acid oxidation disorders benefit from specialized diets low in long-chain fatty acids with medium-chain fatty acid supplementation. (Longo et al., 2016) All in all, the management of metabolic myopathies is multifaceted, encompassing

exercise management, symptomatic relief, monitoring, and genetic counseling in addition to dietary intervention and pharmacotherapy. (Ahmed et al., 2018)

2.4 Exercise Testing Methods in Metabolic Myopathies

Multiple modalities of exercise testing have been used in the diagnostic process of metabolic myopathies. Forearm tests have been tailored specifically to metabolic myopathies. Historically, Ischemic exercise of the forearm muscles was first used by McArdle to describe the absence of elevation of blood lactate during exercise in a patient with myophosphorylase deficiency. (McArdle, 1951) In it, the circulation in the exercising arms is cut off with pressure from, e.g., a blood pressure cuff. Due to pain and potential damage to the muscle, non-ischemic forms without the restriction from a cuff were later developed and are currently in use. In the forearm tests, the subject performs an isometric handgrip exercise for a set amount of time, during and after which venous blood samples of lactate, ammonia, and/or blood gases can be collected, allowing blood sampling for the exercising limb. (Hogrel et al., 2001; Jensen et al., 2002; Tarnopolsky, 2016)

On its own, maximal cardiopulmonary exercise testing offers information on both cardiac and pulmonary capacity, as well as data on different parameters associated with the performance of the subject during exercise. Even though the role of the traditional electrocardiogram (ECG)-based diagnosis of coronary artery disease has diminished in recent years as more sensitive and specific functional imagining techniques, including coronary artery computer tomography and functional positron-emission-tomography (PET) imagining of the heart, have become more widely available (Knuuti et al., 2020), cardiopulmonary exercise testing still offers differential diagnosis options for both cardiac and pulmonary exercise capacity.

The simultaneous breath gas collection allows for several parameters to be obtained: maximal oxygen uptake during exercise ($V'O_{2max}$)—a comprehensive measure of exercise capacity—and its ratio with the heart rate; the oxygen pulse, a measure of oxygen transport ($V'O_{2max}$/HR); the first and second ventilatory thresholds depicting changes in the rate of oxygen consumed and carbon dioxide produced; slope of ventilation divided by exhaled carbon dioxide relationship; and partial pressure of end-tidal carbon dioxide as a surrogate for alveolar ventilation are among the most relevant in the clinical setting. The breath gases collected also offer indirect information about energy metabolism during exercise, as the amount of oxygen spent in relation to carbon dioxide produced depends on the metabolic pathways currently active in the cells, reflected in the ventilatory thresholds and the respiratory exchange ratio (RER). (Guazzi et al., 2018; Mezzani, 2017)

In cases of suspected metabolic myopathies, the cardiopulmonary exercise testing can be combined with lactate and ammonia samples taken during and after

exercise, either in maximal exercise (Mouadil et al., 2012) or, specifically, to study the subjects during light exercise where oxidative phosphorolysis is the predominant energy provider, using a set exercise protocol of lighter strenuousness, where the exercise effort is designed to stay under the anaerobic threshold. These protocols have been suggested to be utilized especially in patients with suspected mitochondrial myopathy or in patients vulnerable to symptom aggravation during exercise. These protocols include both fixed constant load exercise tests and submaximal relative protocols, with lactate measured during and after exercise. In a fixed constant load exercise test, the subject pedals at a pre-determined fixed absolute workload, usually 30 W, for a set period of time. (Finsterer et al, 1998; Finsterer & Milvay, 2002; Hanisch, Eger, et al., 2006) The submaximal relative protocols are based on the individual subject's exercise capacity and include, e.g., sub-anaerobic threshold exercise where the constant exercise load is set to 90 % of the subject's predicted anaerobic threshold or at 65 % of the subject's measured maximal $V'O_2max$ for a set period of time. (Hammarén et al., 2004; Nashef & Lane, 1989)

2.4.1 Previous Research on Exercise Testing Results in Patients with Muscle Disease

In glycogen storage diseases, the decreased availability of carbohydrates in muscle predictably inhibits the typical increase in lactate during exercise and high ammonia due to excess purine degradation. (Hogrel et al., 2001) Most notably, the absence of lactate rise during and after exercise, accompanied by hyperammonemia, is typical in McArdle disease (GSD V) and observable both from the forearm test and cycling exercise test. (Delaney et al., 2017; J. Y. Hogrel et al., 2001; J. Y. Hogrel et al., 2017; McArdle, 1951; Mineo et al., 1985; Ørngreen et al., 2015). Similarly, in Tarui's disease (GSD VII), the rise in lactate during exercise is low, and ammonia is high, observed both in a maximal exercise test (Piirilä et al., 2016), submaximal exercise test (Ørngreen et al., 2015), and the recovery after a forearm test (Mineo et al., 1985). In contrast, myoadenylate deaminase deficiency, a disorder affecting purine metabolism, especially in muscle cells, is hallmarked by the absence of ammonia elevation during exercise. (Fishbein et al., 1978; Sabina et al., 1984)

In carnitine deficiency, a disorder of fatty acid metabolism, strenuous, continued exercise can lead to rhabdomyolysis and, in extreme cases, renal failure. However, in short-term exercise, such as an exercise test, carbohydrate metabolism predominates, likely lessening the symptoms. Exercise test data of these patients is rare, and cardiopulmonary exercise tests have been reported as near normal in some cases. (Haller et al., 1978) In a case report of a single patient, mild exercise of 15-30 watts for 15 minutes induced myalgia and marked elevation

in serum CK, lactate, and pyruvate, but lactate in an ischemic forearm test was normal. (Miyajima et al., 1989)

For the mitochondrial myopathies, the results of exercise testing are often less clear, and the strength of the findings in the different exercise test modalities depends heavily on the level of heteroplasmy in the muscle. During maximal cardiopulmonary exercise testing, low maximal oxygen uptake $V'O_{2max}$ is typical, though it depends on the level of the pathogenic variant present in the muscle, not universal. MM patients also commonly exhibit exaggerated ventilation relative to metabolic rate, sometimes to the point of hyperventilation, manifesting with increased minute ventilation, high $V'E/V'O_2$ in maximal exercise, a steeper slope of increase in $V'E/V'CO_2$ slope, and elevated peak respiratory exchange ratio (RER) as well as increased perceived breathing effort have been reported, though these are not specific findings to mitochondrial myopathies. (Dandurand et al., 1995; Heinicke et al., 2011; Jeppesen et al., 2021; Lindholm et al., 2004; Taivassalo et al., 2003). These are at least partially due to the defects in oxidative phosphorylation, increasing the rate of glycolysis, leading to an increase in lactate and a concurrent decrease in pH. These, in turn, drive a compensatory rise in ventilation, but the effect is not only dependent on the lactate and pH levels, which suggests that other factors, such as an exaggerated sympathetic neural response, contribute to the exaggerated ventilatory drive. (Heinicke et al., 2011) In addition, patients with MM show an exaggerated circulatory response to exercise, including increased ventilation and elevated cardiac output relative to $V'O_{2max}$. (Dandurand et al., 1995; Heinicke et al., 2011; Taivassalo et al., 2003), with near-arterial-level oxygen levels in venous blood of the active muscle, as well as elevated muscle blood flow using Doppler measurements (Jeppesen et al., 2012). All in all, the changes due to lowered oxidative capacity depend more on the mutation burden than the genotype. (Taivassalo et al., 2002)

Several studies have combined blood sampling with exercise testing. Resting lactate concentrations tend to be higher in subjects with mitochondrial disease than controls, with values over 1.8-2.0 mmol/l considered abnormal and over 3.0 mmol/l diagnostic for mitochondrial myopathies. (Parikh et al., 2014) During exercise and recovery, the results have been more varied with differing protocols. For maximal exercise tests, Dandurand et al (1995) found no difference in lactate levels during the incremental exercise protocol to exhaustion among 15 subjects compared to controls, but in 10 of the subjects, the type of mitochondrial disease was undefined. However, in other studies, there is evidence of higher lactate levels in some mitochondrial myopathy patients during the recovery period. In two studies by the same group, there was evidence of higher lactate in early recovery in patients, including both MELAS and CPEO patients, but without mention of the exact time of measurement. (Lindholm et al., 2004; Löfberg et al., 2001) In a study on three patients with Complex I deficiency, a form of MM manifesting during

childhood, lactate and pyruvate levels were significantly higher in patients than in controls during exercise. (Roef et al., 2002) In one study, including seven MM patients with CPEO, four with MELAS A3243G and one each with pathogenic variants T4409Ca, T8344A, A15579Ga, and one with NADH dehydrogenase 2 (ND2) gene deletion, the post-exercise recovery rate of plasma lactate was significantly slower in MM than in healthy subjects, but there was considerable overlap in the two groups and findings in myotonic dystrophy patients were virtually identical to those in MM patients, though some of the myotonic dystrophy patients also had high lactate at rest. (Dysgaard Jeppesen et al., 2003) Some studies have shown a statistically significant rise in lactate only when levels at peak exercise are calculated relative either to peak workload or to maximal $V'O_2$ (Taivassalo et al., 2003) or compared to pyruvate levels (Mouadil et al., 2012).

In submaximal tests including fixed absolute workload (Hanisch, Eger, et al., 2006) and with methods adjusted to subject's exercise tolerance (Dysgaard Jeppesen et al., 2003; Hammarén et al., 2004), lactate has been shown to be higher than in controls during exercise and especially in the recovery period, but the sensitivity has varied between 21–100% and specificity of these tests between 60–96%, respectively, with less specificity compared to subjects with other muscle diseases compared to healthy controls and of a similar level with a resting lactate test. (Jeppesen et al., 2021)

In mitochondrial myopathies, the forearm test results are the best for measurements of impaired extraction of available oxygen during exercise. This can be achieved either by measuring oxygen partial pressure (PO_2) or desaturation in venous blood (Hanisch, Eger, et al., 2006; Jensen et al., 2002; Taivassalo et al., 2002) or noninvasively by measuring deoxyhemoglobin using near-infrared spectroscopy (Celie et al., 2015). In both methods, mitochondrial myopathy patients exhibit higher oxygen saturation than healthy controls. From venous blood samples during the handgrip test, Taivassalo et al. (2002) reported a sensitivity of 77%, a specificity of 100%, and an accuracy of 88% for values over 30mmHg PO_2 threshold in patients with mixed-type MM, with the level of heteroplasmy in the mtDNA correlating to the strength of the findings. Plasma lactate concentrations during handgrip tests' exercise and recovery have also been studied. During a forearm test in MM subjects, lactate can be elevated during the test and its recovery, but the finding is less consistent than that of high oxygen saturation and is not diagnostic in MM. (Hanisch, Eger, et al., 2006; Hogrel et al., 2001)

3 Aims of the Study

The aims of the study were to further study the effects of age, sex, and dietary factors on the results of cardiopulmonary exercise test combined with lactate and ammonia samples in healthy subjects, as well as to study the exercise test profile in subjects with spinal muscular atrophy Jokela type (SMAJ) and mitochondrial myopathy.

In detail:

1. To study the effects of sex and age on the results of healthy subjects performing a cardiopulmonary exercise test combined with lactate and ammonia samples with a 30-minute follow-up during the recovery phase.

2. To study if the modified Atkins diet affects the cardiopulmonary exercise test results as well as lactate, ammonia, and breath gas levels during exercise in healthy subjects.

3. To describe the cardiopulmonary exercise test result profile with lactate and ammonia samples in subjects with SMAJ, a disorder caused by a mitochondrial gene defect, and to see whether the lactate and ammonia would be comparable with normal subjects or with the functional changes in subjects with mitochondrial myopathy.

4 Methods

4.1 Participants

The basic characteristics of the subjects through the three studies are collected in Table 1.

Table 1 The participants in the partial studies on cardiopulmonary exercise testing with venous lactate, ammonia, and blood gas follow-up.

For age and body mass index (BMI), means and standard deviations (= SD, in parentheses) are presented. CPEO = Chronic progressive external ophthalmoplegia, MELAS = Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes syndrome, SMAJ = Spinal muscular atrophy, Jokela type.

	Study I: Effects of sex and age	Study II: Effects of a ketogenic diet	Study III: SMAJ and mitochondrial disease		
Population	Healthy subjects	Healthy subjects	SMAJ subjects	CPEO/ MELAS	Healthy controls
Sex male/ female	34 / 39	6 / 4	4 / 7	9 / 17	11 / 17
Age (years)	34.4 (14.0)	49.4 (8.7)	55.7(14.7)	44.3 (14.7)	50.0 (18.2)
BMI	24.3 (3.0)	24.9 (2.8)	24.6 (3.6)	23.5 (3.7)	24.3 (2.9)

4.1.1 Participants in Study I

In study I, we analyzed the venous lactate, ammonia, and acid-base balance during cardiopulmonary exercise testing in 73 healthy nonsmoking volunteers, 34 male and 39 female, whose ages ranged between 22 and 69 years. They were either personnel or medical students at the Helsinki University Hospital or their acquaintances who answered a call for healthy volunteers. Of these 73 subjects, ten (six males and four females) had originally acted as controls in a study on the therapeutic effects of modified Atkins on mitochondrial myopathy (Ahola et al., 2016). According to the International Physical Activity Questionnaire the subjects filled out (Craig et al., 2003), they were physically active, but none were professional athletes.

4.1.1.1 Exercise Questionnaire

All but one of the healthy controls filled in a questionnaire regarding their exercise habits based on the IPAQ long-form questionnaire (Craig et al., 2003) to account for the possible effects of different exercise habits. The subjects were asked to fill in, per week, times, types, and durations for any exercise; the amount walked both for commute and during free time separately, and any physical activity at work. In addition, smoking status and alcohol consumption were asked for. The subjects were assigned to three physical activity groups (Low, Moderate, or High) for analysis based on their answers. Metabolic energy expenditure estimates called MET-minutes (one MET minute equals the amount of energy expended during one minute at rest) were calculated for walking, moderate activity, and vigorous activity, respectively. To be classified in the high category, the subject had to report vigorous activity on three days for a cumulative exercise of 1500 MET-minutes or over 3000 MET-minutes/week of any intensity exercise on seven or more days. To be classified in the moderate category, the subject had to fill one of the following three criteria: three days of over 20 minutes of vigorous activity, five days of moderate-intensity activity or walking over 30 min/day, or five or more days of any cumulative activity over 600 MET-minutes without filling the criteria for high activity. If the subjects reported no activity or didn't meet the criteria for the higher categories, they were categorized in the low physical activity group.

4.1.2 Participants in Study II

The study II subjects were 10 healthy subjects, six males and four females, who underwent a four-week modified Atkins diet. The participants had previously been enrolled as controls in a study in which patients with mitochondrial diseases were treated with a four-week ketogenic diet, as reported earlier (Ahola et al., 2016).

They performed a cardiopulmonary exercise test with venous lactate, ammonia, and blood gas results before starting the diet as well as at the end of it.

4.1.2.1 Ketogenic Diet

Before the start of the ketogenic diet, the initial energy intake for each healthy participant was assessed using a food diary. A Normalized Isocaloric Standard Diet (ND) was implemented for all study participants over a two-week period, after which they were gradually transitioned to a mAD one meal per day at a time. Prior to starting mAD, subjects had a median energy intake of 1901 kcal (SD 429), with 43% (SD 8) from carbohydrates, 18% (SD 5) from proteins, and 34% (SD 7) from fat. During mAD, carbohydrate consumption was restricted to 3–9% of daily energy intake. In the final two weeks of mAD, the mean total energy intake was 2194 kcal (SD 564), comprising 4% (SD 1) carbohydrates, 26% (SD 3) proteins, and 69% (SD 4) fat. The subjects received diet-compliant meals from the hospital and adhered to the diet for four weeks.

To monitor adherence, ketosis was assessed via serum/plasma β-hydroxybutyrate testing, with a target level of 2.5–5 mmol/L, measured weekly from fasting blood samples during the diet. (Ahola et al., 2016) Additionally, plasma glucose, cholesterol levels, creatine kinase, alkaline phosphatase, alanine aminotransferase, glutamyl transferase, urea, bilirubin, and triglycerides were analyzed using standard laboratory methods before and after the diet.

4.1.3 Participants in Study III

In study III, the study subjects were 11 patients with genetically verified SMAJ (four men and seven women) with *CHCHD10* c.197G>T p. (Gly66Val) pathogenic variant, who had previously taken part in a biomarker study (Jokela et al., 2016). All patients exhibited muscle symptoms in their lower limbs, most commonly muscle weakness, pain, and fasciculations. 7 out of 11 subjects also exhibited upper limb symptoms as well as findings related to muscle disease (presented more thoroughly in Table 1 in Article III): The subjects' symptoms had started at the age of 30-55 years, 2-26 years before the exercise test. In electroneuromyography (ENMG), mild to severe motor neuropathy was present in all subjects, and mild to moderate sensory neuropathy in four subjects, with denervation atrophy findings in the available muscle biopsy (two subjects) and magnetic resonance imagining results (three subjects).

The results of SMAJ patients were compared with the results of 26 subjects with mitochondrial disease genetically verified from either blood or muscle samples (9 men and 17 women). Of the subjects with mitochondrial disease, 15 subjects were diagnosed with CPEO (multiple mitochondrial DNA deletions—five

of them due to Twinkle duplication mutation of p.352–364, two associated with POLG T955>C, and one with POLG N468>D/A1105>T—and seven with a single deletion) and 11 with MELAS (ten of them with pathogenic variant m.3243A>G and one with m.3302A>G).

The diagnosis, symptoms, and previous laboratory results of the subjects with SMAJ and mitochondrial disease were verified from the electronic patient report of the hospital. All patients with SMAJ and all mitochondrial disease patients except one with CPEO had exhibited skeletal muscle symptoms, but not severe enough to prevent an exercise test. The subjects with mitochondrial disease included all patients who had undergone the exercise test in Helsinki University Hospital's Department of Clinical Physiology between 2008 and 2022 as part of their diagnostic work-up or as the baseline test for two diet intervention studies (five subjects each) (Ahola et al 2016; Pirinen et al., 2020) and signed the consent form for this study also. One patient was excluded from the analysis due to a very low rise in heart rate. (51 % of age-predicted maximal heart rate)

Due to the diseases' rarity and to better match sex and age distributions among groups, and considering the strong age and sex dependencies of exercise parameters, we included both CPEO and MELAS patients in the mitochondrial disease control group. The age range was 32-76 years (mean 55.97, SD 14.7) for the SMAJ group, 16-71 years (mean 44.4, SD 14.7) for MELAS/CPEO, and 22-76 years (mean 50.0, SD 18.2) for the controls.

Due to changes in ammonia analysis methods of HUS laboratories, further discussed in Chapter 4.2, only the 13 healthy subjects from Study I whose ammonia results had been analyzed with the modified Siemens Atellica® platform were included as controls in Study III. In addition, 15 additional healthy controls were recruited later and were included only in the SMAJ study analysis to better match the sex and age distribution with the SMAJ patients. All in all, this part of the study included 28 healthy volunteers (11 men and 17 women).

All exercise tests were performed in the Helsinki University Hospital's Department of Clinical Physiology.

4.2 Exercise Testing Protocol

Work-conducted maximal cardiopulmonary exercise test with gas exchange analysis (spiroergometry) was conducted using established protocols (Balady et al., 2010; Ollila et al., 2017; Piirilä et al., 2016).

Respiratory gases were measured using a tightly secured facemask (Rudolph series 7910; Hans Rudolph Inc.), varying in dead space size (small, medium, or large) according to the manufacturer's instructions. After approximately 10 minutes of rest, subjects mounted the bicycle ergometer, and gas exchange recording commenced. Before the start of the exercise, one minute of resting

breathing was recorded while the subjects sat upright, utilizing a breath-by-breath gas exchange analysis system (Vmax Encore 29 C from 2008 to 2016, and Vyntus CPX from 2017 to 2021, both by SensorMedics). Throughout the exercise test, breath gas data were continuously recorded, with 30-second average values reported.

The exercise protocol utilized an electrically braked bicycle ergometer (Ergoselect 200P; Ergoline GmbH). Women started with a 40 W workload and men with 50 W, with workload increments of 40 or 50 W every 3 minutes, respectively. Exercise continued until subjects reached hard exertion (17–20/20 on the perceived exertion scale) and achieved a respiratory exchange ratio (RER) of at least 1.0. Post-exercise, subjects adhered to the hospital's standard protocol, reclining in a supine position for 30 minutes, with permission to drink a small glass of water.

Blood pressure was manually measured from the right arm using a stethoscope and sphygmomanometer (Erka) before exercise, during each exercise step as well as 3 and 6 minutes post-exercise. A continuous 12-lead ECG was monitored using digital equipment and recorded with CardioSoft software (versions V6-5 and later 7-7.0851; GE Medical Systems), employing Mason-Likar leads during exercise. Peripheral arterial oxygen saturation (SpO_2) was measured using pulse oximeters (Datex-Ohmeda 3900 and 3800 until 2017, then MySignS, EviteC), with sensors on the subject's earlobe and left middle finger providing reliable signals.

4.3 Laboratory Methods

Venous blood samples for blood gases, lactate, and ammonia analysis were collected via cannula from the left antecubital fossa. Sampling occurred at rest, during initial exercise stages, maximal exercise, and at 2, 4, 6, 10, 20, and 30 minutes post-exercise. Blood gas analysis utilized Radiometer ABL90 or ABL825 analyzers in the central laboratory (Radiometer Medical). Lactate and ammonia samples were collected in lithium-heparin tubes, with ammonia samples promptly cooled and centrifuged at 2000g for 10 minutes at +4°C upon arrival at the laboratory.

During the study period, three different automated clinical chemistry platforms were used at the Helsinki University Hospital's laboratory: Cobas Integra® 400 (Roche Modular, Roche Diagnostics) from 2008 to 2016, Abbott™ Architect™ c16000 or c8000 (Abbott Laboratories) from 2016 to 2020, and Atellica® Solution (SiemensHealthineers) from 2020 to 2022. The changes in equipment were due to the antitrust legislation in Finland requiring a competitive contract in purchasing medical devices for communal hospitals at set time intervals.

All the ketogenic diet participants' test results were analyzed using Cobas Integra® 400, and all the SMAJ patient test results using Siemens Atellica®

Solution Chemistry analyzer. However, of the healthy control subjects as a whole, the samples of a total of 40 participants were analyzed with Cobas Integra® 400 chemistry analyzer, the samples of 20 subjects with Abbott™ Architect™ c16000 or c8000 (Abbott Laboratories), and the samples of the last 13 of the healthy control participating in Study Is with Siemens Atellica® Solution Chemistry analyzer (Siemens Healthineers). 15 more healthy controls were later included with this method for Study III. The platform used also varied between subjects with MM.

At the laboratory, prior to changing the instrument and assays, the lactate and ammonia assays were verified for use, and the reproducibility of the assay was estimated using internal quality control materials and trueness with relation to the previous assay using patient samples (n = 44-56) and external quality control materials. The change from one chemistry platform to another did not significantly influence lactate levels. For ammonia levels higher than 50 µmol/l, the assays performed with an acceptable accuracy compared to each other. However, according to the patient sample comparisons from method verification studies, there was significant variation between assays in ammonia levels below 50 µmol/l, with Abbott Architect showing a median bias of +9 µmol/l (SD ±3 µmol/l) as compared with Cobas Integra, in routine method comparisons in the laboratory. The sensitivity of the routine ammonia assay (Atellica CH Amm) on the Siemens Atellica® platform was not sufficient for reliable analysis of plasma ammonia concentrations less than 30 µmol/l, leading to the laboratory modifying the routine assay to detect lower levels of ammonia. The modified assay was validated for clinical use by comparison to the Abbott Architect assay.

As plasma ammonia concentrations, especially within the low ranges typical for a healthy reference population, varied between the different analyses, in the sub-study of the healthy population, we concluded to only use the ammonia values of 40 subjects measured with the Roche Modular device in the healthy population ammonia level analysis. Likewise, for comparison with subjects with SMAJ, only the healthy controls whose results were measured with Siemens Atellica® Solution Chemistry analyzer were used.

4.4 Data Collection and Analysis

For all the sub-set studies, data were collected into Excel-sheets prior to transfer and analysis using IBM SPSS 27 Statistics program.

The statistical significance threshold for the p-value in all sub-sets was <0.05.

4.4.1 Study I

The normality of distribution was determined using Shapiro–Wilk's test. The significance of differences between subgroups in normally distributed variables was determined using one-way analysis of variance (ANOVA) with Tukey's post hoc test, and the non-normally distributed variables using the Kruskal–Wallis test with Dunn's post hoc test. A two-way repeated measures ANOVA was performed to compare the effect of age group and sex on lactate and ammonia in the recovery phase compared to maximal exercise.

A linear model was utilized to assess the effects of age and maximal RER on plasma lactate and ammonia concentrations as continuous variables, adjusting for each other as well as for the sex of the subjects. Regression coefficients (B), standard errors (SE), and partial correlation coefficients for the model have been presented. Finally, we estimated the linear model for the sexes separately.

4.4.2 Study II

Research data was analyzed using the SPSS 25 statistics program. Most of the variables were determined to be normally distributed using the Shapiro-Wilk´s test. The significance of changes in normally distributed variables was determined using the paired t-test, and in the non-normally distributed variables using the Wilcoxon signed-rank test. The statistical significance threshold for p-value was <0.05. Breath gas variables were normally distributed, but the number of workload steps completed differed between the subjects. Therefore, paired T-tests for breath gas variables were performed on every exercise step stepwise and at maximum exercise levels.

4.4.3 Study III

A linear model was utilized to assess the effects of the diagnosis groups on the cardiopulmonary exercise test results, plasma lactate, ammonia, and venous blood gas results, adjusted for age, sex, and BMI.
Repeated samples two-way ANOVA was also calculated for the lactate and ammonia samples from the resting state on.

In addition, a one-way analysis of variance (ANOVA) with Tukey's post hoc test was used for the lactate results between MELAS and CPEO subjects separately compared with each other and the SMAJ and control groups.

4.5 Ethical Considerations

Signed, informed consent was acquired from all participants, and if they have been taken part in another study, consent has been asked for the present study also.

The study was undertaken according to the Declaration of Helsinki. Helsinki University Hospital's medical ethics review board has approved the study: 314/13/03/01/2008, 199/13/3/01/2011, 108/29/04/2015, and HUS/2084/2020. The diet study by Professor Suomalainen/Wartiovaara was approved by the moment 33/13/03/01/2011 and 110/13/03/01/2014.

5 Results

5.1 Cardiopulmonary Exercise Results Across the Sub-Studies

5.1.1 Cardiopulmonary Exercise Results in Study I

The anthropometric data and the results of the cardiopulmonary exercise testing collected for the 73 healthy controls are given in Table 2. Only four of the subjects were obese (body mass index ≥ 30, with a maximum of 32 kg/m2). The spirometry results of the subjects were normal except for one with a slight restriction. The exercise test results showed higher results in men than in women in most measured values, as could be expected. All cardiopulmonary exercise tests were clinically maximal as considered for obtaining over 85 % of age-predicted maximal heart rate and RER of over 1.0 without a significant difference between the age groups or sexes.

Measured in MET-minutes, most of the subjects volunteering for an exercise test were physically active (Low 2, Moderate 22, High 48) (data not shown).

Table 2 The anthropometric data, lung function data, and the results associated with cardiopulmonary exercise testing in the healthy population.

Statistically significant p-values <0.05 in bold. FVC = Forced vital capacity, FEV1 = Forced expiratory volume, $V'O_2$= oxygen uptake at rest, RER = Respiratory exchange rate ($V'CO_2/V'O_2$), W_{peak} = peak power during last 30 seconds of exercise, W_{max}/3 min = Maximal power during last 3 maximal minutes, $V'O_{2max}$ = Maximal oxygen uptake, $V'O_{2max}$/kg = Maximal oxygen uptake per kilogram during exercise, Wmax/V'O2max = Mechanical work efficiency, $FETCO_2$ = Fraction of end-tidal CO_2, $V'O_2$/HR = Oxygen pulse, $V'E/V'O_2$ = Ventilatory equivalent to O_2, $V'E/V'CO_2$ = Ventilatory equivalent to CO_2).*Heart rate age maximum = 205 - age / 2. **Nordesjö et al.1975. Adapted with permission from Ratia N. et al. Lactate and ammonia measurements during cardiopulmonary exercise testing and its recovery phase - Consideration of age and sex in its interpretation. Clin Physiol Funct Imaging (2023).

	Men, N=34		**Women, N=39**		
	Mean	**SD**	**Mean**	**SD**	**p-values**
Age (years)	43.7	14.1	42.5	13.9	0.73
Height (cm)	180.8	7.1	165.3	5.4	**< 0.001**
Weight (kg)	81.7	12.7	64.7	8.6	**< 0.001**
BMI	24.9	2.8	23.7	3.0	0.08
EXERCISE, maximal values					
Systolic pressure (mmHg)	211.0	25.7	184.4	18.3	<**0.001**
Diastolic pressure (mmHg)	85.9	14.6	84.5	13.9	0.70
Heart rate (beats/min)	172.3	15.4	175.5	11.5	0.32
Heart rate (beats/age maximum*)	94.0	6.5	96.2	28.7	0.12
RER, max	1.14	0.067	1.16	0.081	0.30
Breathing frequency (1/min)	35.5	9.11	39.2	5.45	**0.04**
Anaerobic threshold (L/min)	1.94	0.61	1.36	0.38	**< 0.001**
W_{peak}(W)	266	62.4	182	50.6	**< 0.001**
W_{max}/3 min (W)	246	62.0	169	46.7	**< 0.001**
W_{max}/3 min (% of predicted value**)	115	21.2	121	26.2	0.34
$V'O_{2max}$ (L/min)	3.22	0.80	2.28	0.56	**< 0.001**
$V'O_{2max}$/kg (ml/min/kg)	40.1	10.3	35.2	8.5	0.56
$W_{max}/V'O_{2max}$ (%)	22.0	2.8	21.2	1.7	0.14
$V'O_2$/HR	18.6	4.00	12.9	9.1	**< 0.001**
Breathing reserve (%)	34	13	32	12	0.43
$V'E/V'O_2$	34.9	5.43	36.8	5.29	0.14
$V'E/V'CO_2$	30.7	4.06	31.5	3.5	0.36
$FETCO_2$ (%)	5.23	0.63	5.03	0.54	0.15
Tidal volume (L)	3.31	0.62	2.18	0.35	**< 0.001**

5.1.2 Cardiopulmonary Exercise Results in Study II

The cardiopulmonary exercise test results for the subset of 10 healthy subjects who adhered to mAD for four weeks and underwent two exercise tests are presented in Table 3. During the mAD period, these subjects experienced significant weight loss (on average, from 74.1 to 72.0 kg, p=0.007), despite maintaining an isocaloric diet with no intention for weight reduction. There were no observed differences in resting heart rate or blood pressure, but resting breathing frequency increased significantly by the end of the mAD period (p=0.024).

Comparing pre-mAD and post-mAD cardiopulmonary test results, mechanical efficiency, represented by $W_{max}/V'O_{2max}$, decreased significantly (p=0.049), although the changes in maximal workload in the last 3 minutes of exercise (W_{max}/3 min) and maximal oxygen uptake ($V'O_{2max}$) themselves were not statistically significant. During exercise, the maximum heart rate was higher at the end of the mAD compared to pre-mAD (p=0.001). The respiratory exchange ratio (RER), reflecting the ratio of carbon dioxide production to oxygen uptake ($V'CO_2/V'O_2$), decreased (p=0.015), indicative of increased lipid utilization as a fuel source (Table 2).

Minute ventilation during exercise testing increased significantly by the end of the mAD period, observed stepwise at both the 4th (p=0.003) and 5th (p=0.001) exercise loads (Figure 3), and during maximal exercise (p=0.040, Figure 4). The ventilatory equivalent for CO_2 ($V'E/V'CO_2$) increased at the 4th load (p=0.015, Figure 5) and during maximal exercise (p=0.002, Figure 6), while the fraction of end-tidal CO_2 (FetCO2) decreased at the 4th load (p=0.003) and during maximal exercise (p<0.001, Figure 5, Table 3. Moreover, at end-of-mAD, four out of ten subjects had $FetCO_2$ under 4.5% during maximal exercise, whereas pre-mAD, none did.

However, no significant change was observed in the ventilatory equivalent for O_2 ($V'E/V'O_2$). As this study focused on exercise and muscle function in healthy subjects, there was no systematic assessment of exercise-related respiratory symptoms. Typically, exercise cessation was due to leg fatigue; however, two subjects reported significant breathlessness at the end-of-mAD exercise test.

Table 3 The anthropometric values and the maximal results of the cardiopulmonary exercise before and at the end of the modified Atkins (mAD) diet.

The p-values of paired tests are given. Statistically significant p-values <0.05 in bold. SD = Standard deviation, FVC = forced vital capacity, FEV1 = Forced expiratory volume in one second, RER = Respiratory exchange rate, V'E = minute ventilation, W_{max} / 3 min = Maximal power during the last three maximal minutes of exercise, $V'O_{2max}$ = Maximal oxygen uptake, $V'O_{2max}$/kg = Maximal oxygen uptake per kilogram during exercise, Wmax/V'O2max (%) = Mechanical work efficiency, $V'O_2$/HR = Oxygen pulse, $V'E/V'O_2$ = Ventilatory equivalent to O_2, $V'E/V'CO_2$ = Ventilatory equivalent to CO_2, $FETCO_2$ = Fraction of end-tidal CO_2. *Heart rate age maximum = 205 - age / 2. **Nordesjö et al.1975. Adapted with permission from Ratia N. et al. Modified Atkins diet modifies cardiopulmonary exercise characteristics and promotes hyperventilation in healthy subjects. Journal of Functional Foods 2021).

	Normal Values	**Before the Start of mAD**		**After 4 Weeks of mAD**		
		Mean	**SD**	**Mean**	**SD**	**p-value**
Age (years)		**49.4**	8.7			
Height (cm)		**172.2**	9.81			
Weight (kg)		**74.1**	13.8	**72.0**	13.3	**0.007**
EXERCISE, **maximal values**						
Systolic pressure (mmHg)		**184.1**	23.2	**176.7**	27.2	0.238
Diastolic pressure (mmHg)		**84.0**	13.0	**87.1**	12.4	0.186
Heart rate (beats/min)		**169.8**	3.4	**178.0**	3.1	**0.001**
Heart rate (beats/ predicted age maximum*)	**>80**	**94.1**	5.0	**98.9**	4.4	**0.001**
RER	**>1**	**1.15**	0.08	**1.09**	0.05	**0.015**
Breathing frequency (1/min)	**>35**	**35.0**	5.6	**38.5**	7.04	0.062
V'E (L/min)		**87.3**	24.6	**94.9**	25.8	**0.040**
Anaerobic threshold (L/min)		**1.43**	0.48	**1.45**	0.45	0.402
W_{max}/3 min (W)		**175.2**	60.1	**171.8**	60.6	0.305
W_{max}/3min (% of predicted value**)	**≥80**	**101.1**	25.6	**100.1**	21.6	0.792
$V'O_{2max}$ (L/min)		**2.5**	0.8	**2.6**	9.9	0.286
$V'O_{2max}$/kg (ml/min/kg)		**33.3**	8.0	**35.6**	9.2	0.096
$W_{max}/V'O_{2max}$ (%)	**≥80**	**20.3**	1.6	**19.1**	0.86	**0.049**
$V'O_2$/HR	**≥85**	**14.6**	4.3	**14.4**	4.7	0.258
Breathing reserve (%)	**<40**	**38.4**	10.6	**31.9**	12.7	**0.038**
$V'E/V'O_2$		**34.8**	4.8	**36.3**	4.4	0.094
$V'E/V'CO_2$		**30.1**	2.7	**33.7**	3.8	**0.002**
$FETCO_2$ (%)	**4.5 - 6**	**5.4**	0.3	**4.8**	0.4	**< 0.001**
Tidal volume (L)		**2.5**	0.75	**2.59**	0.98	0.569

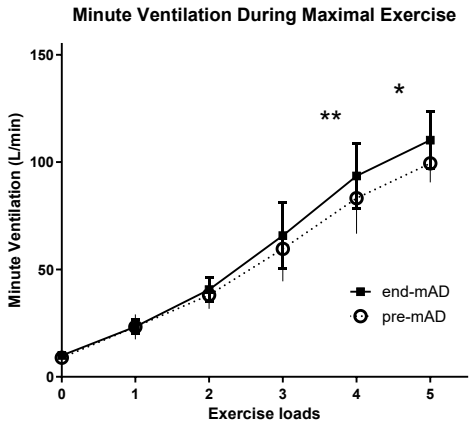

Figure 3 Minute ventilation (V'E) associated with cardiopulmonary exercise test at each exercise step in all participants before and at the end of mAD (modified Atkins diet). The significant differences (*p<0.05, **<0.01) are indicated with an asterisk. Reproduced with permission from Ratia N. et al. Modified Atkins diet modifies cardiopulmonary exercise characteristics and promotes hyperventilation in healthy subjects. Journal of Functional Foods (2021).

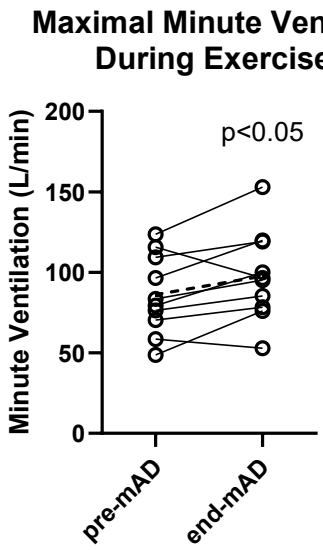

Figure 4 Minute ventilation (V'E) associated with cardiopulmonary exercise test at maximal exercise in all participants before and at the end of mAD (modified Atkins diet). The significant differences (*p<0.05, **<0.01) are indicated with an asterisk. Adapted with permission from Ratia N. et al. Modified Atkins diet modifies cardiopulmonary exercise characteristics and promotes hyperventilation in healthy subjects. Journal of Functional Foods (2021).

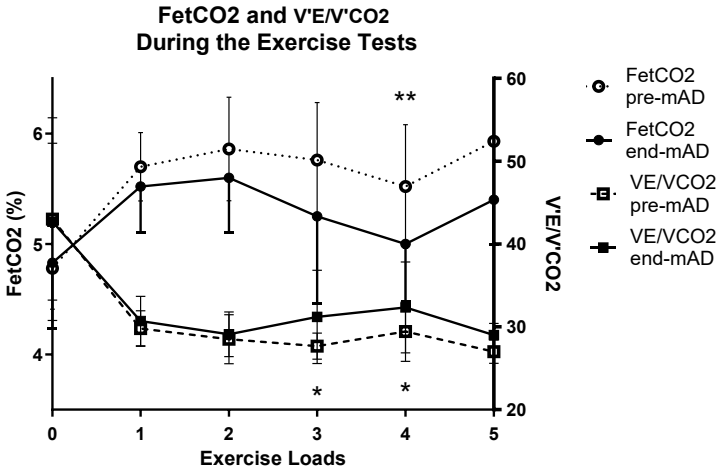

Figure 5 Ventilatory equivalent for CO_2 (Minute ventilation in relation to $V'CO_2 = V'E/V'CO_2$) and $FetCO_2$ (fraction of end-tidal CO_2) associated with cardiopulmonary exercise before and at the end of mAD (modified Atkins diet). The significant differences (* $p<0.05$, *$p<0.01$) are indicated with an asterisk.

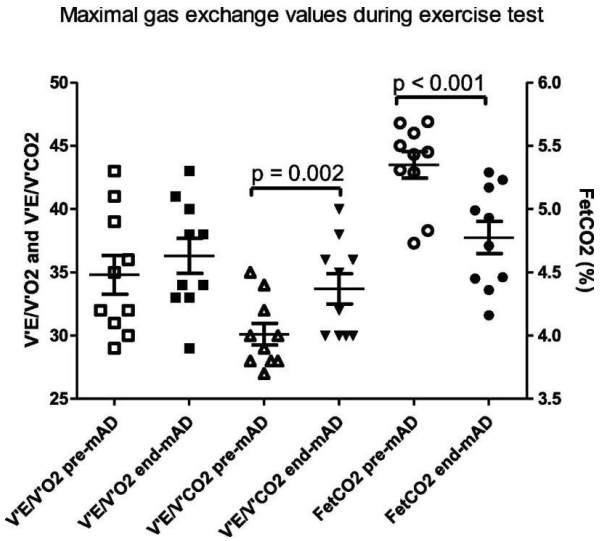

Figure 6 The gas exchange values during maximal exercise before and at the end of mAD (modified Atkins diet) (N=10). The mean (SD) maximal values of the ventilatory equivalent for CO_2 ($V'E/V'CO_2$) and O_2 ($V'E/V'O_2$) and a fraction of end-tidal CO_2 ($FetCO_2$). The individual maximal values are given as symbols, the mean values as short horizontal lines, and the standard deviations as error bars. Reproduced with permission from Ratia N. et al. Modified Atkins diet modifies cardiopulmonary exercise characteristics and promotes hyperventilation in healthy subjects. Journal of Functional Foods. (2021)

5.1.3 Cardiopulmonary Exercise Results in Study III

Exercise testing results per diagnosis group in SMAJ and mitochondrial myopathy patients, as well as their respective controls, and divided by sex, are presented in Table 4. Higher values in men compared to women were observed in most measured parameters, consistent with expectations. The SMAJ group tended to be slightly older than both the healthy controls and the MELAS/CPEO group.

In the exercise test results, adjusted for sex, age, and BMI, the SMAJ group exhibited lower mean power output during the last three minutes of exercise (W_{max}/3 min) ($p<0.001$), $V'O_{2max}$ ($p=0.001$), maximal oxygen consumption per kilogram ($V'O_{2max}$/kg) ($p=0.007$) (Figure 7), oxygen pulse ($p<0.001$), ventilatory threshold ($p=0.045$), and mechanical efficiency ($W_{max}/V'O_{2max}$) ($p<0.001$) compared to the healthy controls. Similar differences were also observed between the MELAS/CPEO group and the healthy controls (Table 5). No significant differences were found between the SMAJ and MELAS/CPEO groups in these variables (data not shown).

The fraction of end-tidal CO_2 ($FetCO_2$) was significantly lower ($p=0.023$), and the ventilatory equivalent for oxygen ($VE/V'O_2$) was higher ($p=0.040$) in the MELAS/CPEO group compared to the healthy controls, a difference that, in contrast, was not observed between the SMAJ group and the healthy controls (Table 5).

To assess exercise maximality, all subjects achieved RER of at least 1.0. No statistically significant differences in RER or maximal heart rate were observed among the diagnosis groups after adjusting for sex, age, and BMI (Table 4).

Table 4 The anthropometric values and the maximal results of the cardiopulmonary exercise in subjects with spinal muscular atrophy, Jokela type (SMAJ), subjects with mitochondrial myopathy, and healthy controls.

SD = standard deviation, SMAJ = Spinal Muscular Atrophy, Jokela Type. MELAS/CPEO = mitochondrial disease group. RER = Respiratory exchange rate ($V'CO_2/V'O_2$), W_{max}/3 min = Maximal power during the last three maximal minutes of exercise. Pred = predicted maximum, Wmax/V'O2max = Mechanical work efficiency, $V'O_{2max}$/kg = Maximal oxygen uptake per kilogram during exercise, $V'O_2$/HR = Oxygen pulse, V'E = Minute ventilation, $V'E/V'O_2$ = Ventilatory equivalent to O_2, $V'E/V'CO_2$ = Ventilatory equivalent to CO_2, $V'O_{2max}$ = Maximal oxygen uptake, $FetCO_2$ = Fraction of end-tidal CO_2.. *Heart rate age maximum = 205 - age / 2. **Nordesjö et al.1975.1975. Adapted with permission from Ratia N. et al. Lowered oxidative capacity in spinal muscular atrophy, Jokela type; comparison with mitochondrial muscle disease. Front Neurol (2023).

	Men, Mean (SD)			**Women, Mean (SD)**		
	SMAJ n=4	**MELAS/ CPEO n=9**	**Healthy Controls n =11**	**SMAJ n=7**	**MELAS/ CPEO n=17**	**Healthy Controls n=17**
Age (years)	48.5 (17.1)	45.0 (12.7)	45.8 (18.2)	59.9 (12.6)	44.0 (16.1)	52.8 (18.2)
Height (cm)	174.6 (3.3)	172.7 (6.3)	183.5 (6.5)	163.2 (4.6)	164.4 (4.4)	161.6 (6.2)
Weight (kg)	77.1 (8.8)	74.1 (12.3)	73.4 (11.5)	64.3 (10.3)	61.8 (11.3)	64.3 (9.9)
BMI	25.3 (2.7)	24.7 (2.6)	24.0 (3.0)	24.2 (4.1)	22.9 (4.1)	24.5 (3.0)
EXERCISE, maximal values						
Syst. pressure (mmHg)	216.0 (28.9)	207.4 (29.0)	207.2 (9.4)	187.3 (23.6)	177.4 (18.8)	190.9 (24.4)
Diast. Pressure(mmHg)	92.0 (18.8)	99.4 (14.9)	82.0 (17.6)	99.3 (12.0)	86.1 (28.1)	89.7 (14.4)
Heart rate (bpm)	163.5 (25.3)	166.7 (17.0)	166.7 (16.9)	148.6 (32.4)	158.1 (18.2)	166.0 (14.8)
Heart rate (% of pred.)*	90.0 (11.3)	91.4 (9.0)	91.4 (7.8)	84.2 (15.9)	91.8 (24.2)	93.8 (5.6)
RER, max	1.18 (0.06)	1.22 (0.11)	1.16 (0.07)	1.14 (0.10)	1.21 (0.12)	1.18 (0.09)
Breathing frequency(1/min)	33.0 (4.1)	35.0 (7.1)	37.3 (7.8)	33.6 (8.1)	37.8 (8.9)	36.2 (5.8)
Ventilatory threshold(L/min)	1.12 (0.14)	1.20 (0.38)	1.70 (0.53)	0.91 (0.23)	1.04 (0.36)	0.91 (0.23)
W_{max}/3 min (W)	162.5 (43.7)	156.0 (37.1)	232.4 (51.6)	75.1 (47.7)	101.7 (41.3)	131.8 (39.8)
W_{max}/3 min (% of pred.)**	84.68 (11.1)	81.7 (20.7)	117.4 (13.7)	66.0 (37.8)	73.5 (25.4)	100.4 (18.6)
$W_{max}/V'O_{2max}$ (%)	20.5 (3.0)	20.2 (2.3)	22.4 (1.4)	15.4 (4.6)	18.6 (3.5)	20.6 (2.3)
$V'O_{2max}$ (L/min)	2.25 (0.34)	2.27 (0.60)	2.98 (0.64)	1.31 (0.46)	1.54 (0.51)	1.78 (0.45)
$V'O_{2max}$/kg (ml/min/kg)	29.1 (5.9)	30.2 (4.9)	37.4 (9.7)	20.9 (8.2)	25.6 (9.8)	27.2 (7.3)
$V'O_2$/HR	13.9 (0.9)	13.5 (3.2)	17.9 (3.1)	9.0 (1.5)	9.8 (3.0)	10.5 (2.3)
Breathing reserve (%)	30.5 (6.2)	30.0 (11.3)	32.0 (11.5)	34.9 (20.7)	37.7 (13.5)	32.1 (13.0)
$V'E/V'O_2$	40.5 (5.3)	40.7 (6.2)	38.1 (5.7)	40.3 (8.1)	43.3 (7.0)	39.2 (6.6)
$V'E/V'CO_2$	34.50 (6.1)	33.2 (2.8)	33.0 (4.1)	35.3 (4.7)	36.6 (4.7)	33.8 (4.0)
$FetCO_2$ (%)	4.70 (0.57)	4.84 (0.49)	4.90 (0.61)	4.70 (0.78)	4.39 (0.49)	4.90 (0.62)
Tidal volume (L)	2.90 (0.27)	2.66 (0.57)	3.40 (0.58)	1.74 (0.51)	1.91 (0.54)	*7.03* 0.33)

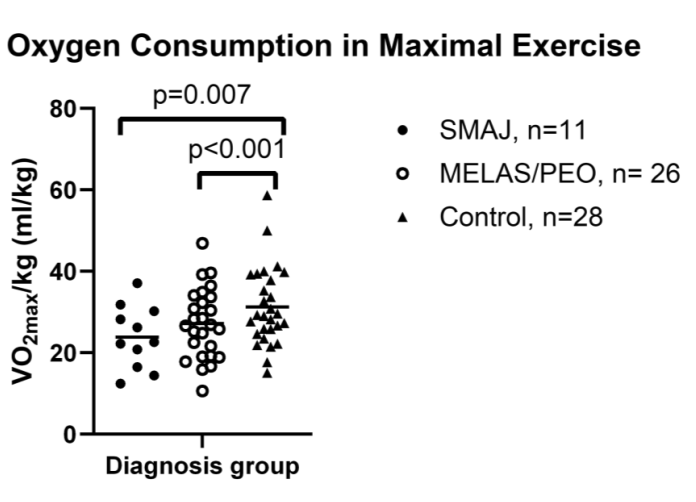

Figure 7 The maximal oxygen uptake (V'O2maz/kg) during maximal exercise between diagnosis groups. The mean is displayed as a diagonal line. SMAJ = spinal muscular atrophy, Jokela type; MELAS/PEO = mitochondrial disease group. Statistical significance is presented for linear model results adjusted for age, sex, and BMI. Reproduced with permission from Ratia N. et al. Lowered oxidative capacity in spinal muscular atrophy, Jokela type; comparison with mitochondrial muscle disease. Front Neurol (2023).

Table 5 Exercise test linear model results in subjects with SMAJ and mitochondrial myopathy compared to controls adjusted for age, sex, and BMI.

B = Estimated regression coefficient for a one-unit increase in continuous variables. SE=Standard error. SMAJ = Spinal Muscular Atrophy, Jokela Type. MELAS/CPEO = mitochondrial disease group. Statistically significant results ($p < 0.05$) are in bold. RER = Respiratory Exchange rate ($V'CO_2/V'O_2$), W_{max}/3 min = Maximal power during last three maximal minutes of exercise, $V'O_{2max}$ = Maximal oxygen uptake, $V'O_2$/HR = Oxygen pulse, Wmax/V'O2max = Mechanical work efficiency, $V'E/V'O_2$ = Ventilatory equivalent to O_2, $V'E/V'CO_2$ = Ventilatory equivalent to CO_2, $FETCO_2$ = Fraction of end tidal CO2,. Reproduced with modifications with permission from Ratia N. et al. Lowered oxidative capacity in spinal muscular atrophy, Jokela type; comparison with mitochondrial muscle disease. Front Neurol (2023).

		B(SE)	p-value			B(SE)	p-value
Maximal heart rate	Control	-	reference	**$V'O_2$/HR**	Control	-	reference
	SMAJ	-8.6 (5.7)	0.129		SMAJ	**-2.0 (0.9)**	**< 0.001**
	MELAS/CPEO	-8.6 (4.4)	0.050		MELAS/CPEO	**-2.4 (0.6)**	**< 0.001**
Ventilatory threshold	Control	-	reference	**W_{max}/ $V'O_{2max}$%**	Control	-	reference
	SMAJ	**-0.24 (0.12)**	**0.040**		SMAJ	**-3.60 (0.87)**	**< 0.001**
	MELAS/CPEO	**-0.32 (0.09)**	**< 0.001**		MELAS/CPEO	**-2.65 (0.67)**	**< 0.001**
RER	Control	-	reference	**$V'E/V'O_2$**	Control	-	reference
	SMAJ	-0.03 (0.03)	0.457		SMAJ	0.72 (2.19)	0.741
	MELAS/PEO	0.04 (0.03)	0.106		MELAS/CPEO	**3.46 (1.69)**	**0.041**
W_{max}/3 min	Control	-	reference	**$V'E/V'CO_2$**	Control	-	reference
	SMAJ	**-51.7 (10.9)**	**< 0.001**		SMAJ	1.22 (1.44)	0.398
	MELAS/CPEO	**-57.7 (8.4)**	**< 0.001**		MELAS/CPEO	1.95 (1.11)	0.079
$V'O_{2max}$	Control	-	reference	**$FetCO_2$**	Control	-	reference
	SMAJ	**-0.46 (0.13)**	**< 0.001**		SMAJ	-0.18 (0.20)	0.378
	MELAS/CPEO	**-0.52 (0.10)**	**< 0.001**		MELASC/PEO	**-0.35 (0.16)**	**0.024**
$V'O_{2max}$/kg	Control	-	reference	**Breathing frequency**	Control	-	reference
	SMAJ	**-5.3 (1.9)**	**0.006**		SMAJ	-3.04 (2.54)	0.230
	MELAS/CPEO	**-6.0 (1.5)**	**< 0.001**		MELAS/CPEO	-0.16 (1.96)	0.936

5.2 Lactate and Ammonia Results in the Sub-Studies

5.2.1 Lactate and Ammonia Results in Study I

In the 73 healthy controls, the mean lactate was at its highest 4-6 min into the recovery phase, after which it began to decline. The individual turning point varied between 2 and 10 min into the recovery. The mean of each subject's maximum lactate, irrespective of the exercise step, was 11.27 mmol/L (SD 2.91), and the lactate increased on average nine-fold (SD 3.41) compared to the resting level (Table 2 in Article I).

The lactate values in men were significantly higher than in women, 4 and 6 min ($p = 0.021–0.044$) into the recovery phase (Figure 8). In the group-wise comparison for age, there was a significant difference in lactate levels between the age groups at maximal exercise and 2–10 min into the recovery ($p \leq 0.001–0.012$) (Figure 9). In all these instances, the lactate levels were higher in the younger age groups, and there was a difference between age groups <35 and >50 years (Tukey adjustment $p \leq 0.001–0.040$). In addition, there was a statistically significant difference between groups <35 and 35–50 years in maximal exercise and 2 and 4 min into recovery ($p = 0.001–0.036$).

Repeated measures ANOVA for lactate over the exercise test and the following recovery showed that there were statistically significant differences in the two parameters: the lactate values over the different time points ($p < 0.0001$) and sexes ($p = 0.0341$). However, their interaction was not statistically significant ($p = 0.4653$). Between the age groups, there was a statistically significant difference both in relation to measurement step ($p < 0.001$) and the age groups ($p = 0.0064$) as well as their interaction ($p < 0.0001$), indicating that the difference in the rate of change in lactate was different between age groups but not between the sexes. The statistically significant difference in age groups was between age groups <35 and >50 years ($p < 0.001$) (Table 6). Similarly, in the linear model, significant changes related to age, adjusted for sex and RER, were observed in maximal exercise and all time points in the recovery phase ($p \leq 0.001–0.032$). The linear model also showed a difference in lactate values between the sexes, observed in maximal exercise and throughout recovery ($p \leq 0.001–0.020$). The regression coefficients corresponding to the change in lactate (mmol/L) for a 1-year increase in age varied between −0.033 and −0.119 mmol/L, with the largest change measured 2 min into the recovery phase. The influence of age was clearer in women than in men, with statistically significant changes present from maximal exercise to the end of recovery in women ($p \leq 0.001–0.006$) versus changes in maximal exercise and 2–10 min into recovery in men ($p = 0.005–0.046$). For both sexes, the largest yearly decrease was observed 2 min into recovery (women −0.131, men −0.099). (Table 7)

Table 6 Repeated measurement ANOVA results for lactate in healthy subjects

Df = degrees of freedom. Statistically significant results (p<0.05) are in bold.

Lactate			
	Df	Mean Square	p-value
Sex	1	4.5	0.034
Measurement step	6	713.1	**< 0.001**
Sex * Measurement step	6	5.6	0.466
Age group	2	10.1	0.006
Measurement step	6	824.5	**< 0.001**
Age group * Measurement step	12	41.4	**< 0.001**

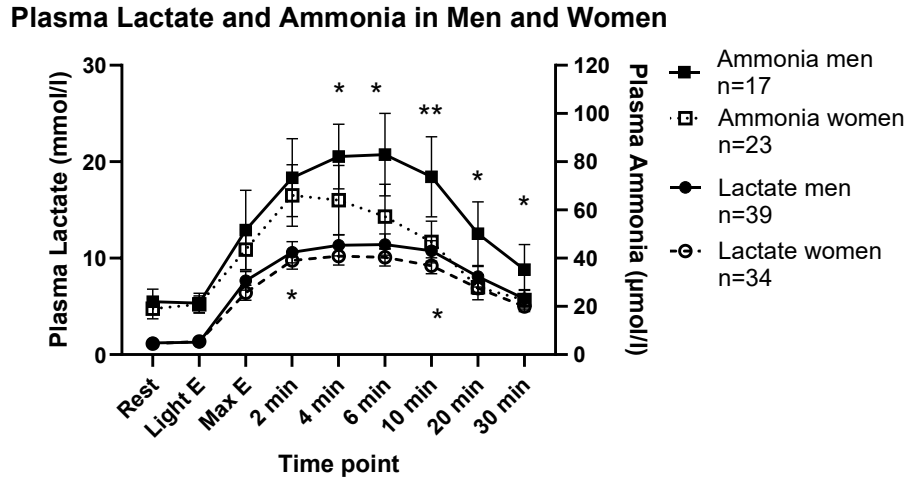

Figure 8 Plasma lactate and ammonia in men and women as measured during and after the cardiopulmonary exercise test in all participants. Means and 95% confidence intervals are shown. The significant differences are indicated with an asterisk (*p<0.05, **<0.01). Reproduced with permission from Ratia N. et al. Lactate and ammonia measurements during cardiopulmonary exercise testing and its recovery phase-Consideration of age and sex in its interpretation. Clin Physiol Funct Imaging (2023).

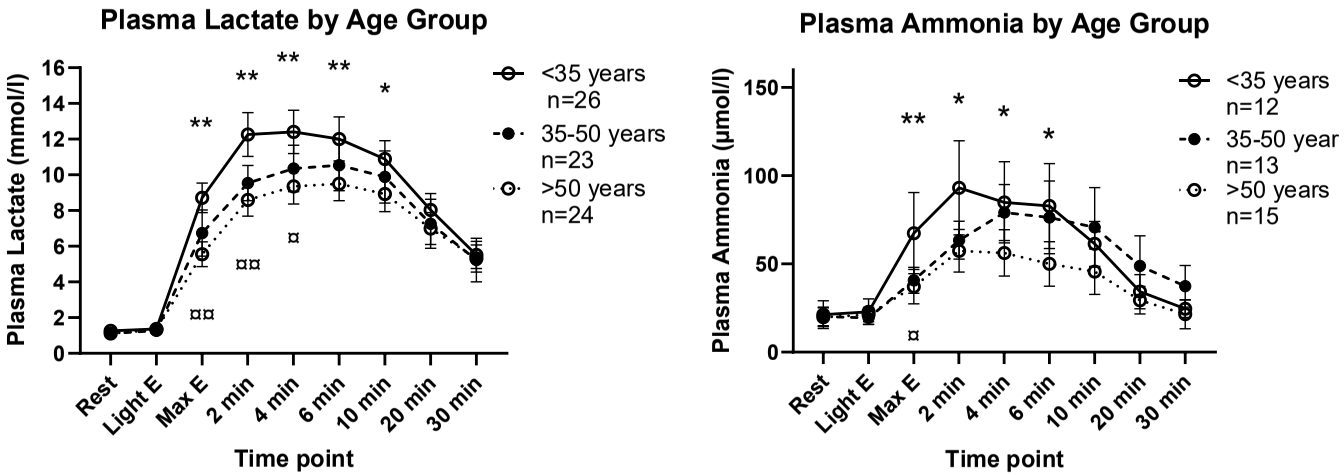

Figure 9 Plasma lactate and ammonia in three age groups (<35 years, 35-50 years, and >50 years) as measured during and after the cardiopulmonary exercise test in all participants at rest, during light exercise (Light E), maximal exercise (Max E) and during recovery (2-30 minutes). Means and 95% confidence intervals are shown. The significant differences are indicated with an asterisk for changes between age groups <35 years and >50 years (* $p<0.05$, ** <0.01) and with a symbol ¤ for changes between age groups <35 and 35-50 (¤ $p<0.05$, ¤¤ <0.01). Reproduced with permission from Ratia N. et al. Lactate and ammonia measurements during cardiopulmonary exercise testing and its recovery phase-Consideration of age and sex in its interpretation. Clin Physiol Funct Imaging (2023).

Table 7 Linear model results for plasma lactate in healthy subjects adjusted for other variables listed in the table.

B = Estimated regression coefficient for a one-unit increase in continuous variables, SE = Standard error. Partial corr. = adjusted correlations. Linear model results are age-adjusted for other variables in the table. Statistically significant results ($p<0.05$) are in bold. Reproduced with permission from Ratia N. et al. Lactate and ammonia measurements during cardiopulmonary exercise testing and its recovery phase-Consideration of age and sex in its interpretation. Clin Physiol Funct Imaging (2023).

All participants, N=73								**Men, N=34**		**Women, N=39**	
	Age			**RER**		**SEX**		**Age**			
	B(SE)	**Partial Corr.**	**p-value**	**Partial Corr.**	**p-value**	**Partial Corr.**	**p-value**	**B(SE)**	**p-value**	**B(SE)**	**p-value**
Rest	-0.006 (0.003)	-0.224	0.062	0.065	0.534	-0.130	0.275	-0.006 (0.005)	0.210	-0.006 (0.004)	0.182
Light exercise	-0.002 (0.003)	-0.073	0.549	0.228	0.057	0.049	0.684	0.002 (0.005)	0.727	-0.006 (0.004)	0.145
Max exercise	**-0.098 (0.019)**	**-0.522**	**<0.001**	**0.177**	**0.147**	**-0.306**	**0.011**	**-0.075 (0.031)**	**0.021**	**-0.114 (0.024)**	**<0.001**
2 min	**-0.119 (0.021)**	**-0.593**	**<0.001**	**0.385**	**0.002**	**-0.291**	**0.020**	**-0.099 (0.032)**	**0.005**	**-0.131 (0.028)**	**<0.001**
4 min	**-0.108 (0.021)**	**-0.538**	**<0.001**	**0.455**	**<0.001**	**-0.312**	**0.010**	**-0.089 (0.033)**	**0.012**	**-0.128 (0.028)**	**<0.001**
6 min	**-0.097 (0.021)**	**-0.484**	**<0.001**	**0.499**	**<0.001**	**-0.344**	**0.004**	**-0.071 (0.033)**	**0.039**	**-0.117 (0.029)**	**<0.001**
10 min	**-0.078 (0.020)**	**-0.428**	**<0.001**	**0.527**	**<0.001**	**-0.393**	**<0.001**	**-0.059 (0.028)**	**0.046**	**-0.083 (0.028)**	**0.006**
20 min	**-0.055 (0.017)**	**-0.359**	**0.002**	**0.617**	**<0.001**	**-0.385**	**0.001**	-0.024 (0.028)	0.390	**-0.069 (0.019)**	**0.001**
30 min	**-0.033 (0.015)**	**-0.285**	**0.032**	**0.635**	**<0.001**	**-0.333**	**0.011**	-0.007 (0.023)	0.761	**-0.044 (0.016)**	**<0.001**

In the ammonia samples taken during clinical cardiopulmonary exercise testing of the healthy controls, there were significant differences observed in ammonia levels analyzed using the three different analyzers at rest, light exercise, maximal exercise, and 30 minutes into the recovery (p-values <0.001-0.009). The measured values analyzed with the three analyzers at rest were significantly different (Bonferroni corrected p-values p <0.001-0.029) between Modular and Architect as well as between Architect and the modified Atellica assay at maximal exercise (p-values 0.001-0.020) and between Architect and Atellica assays also 30 minutes in the recovery (p-value 0.007). (Figure 10)

Because of the variation in the ammonia results between the different analyzers, only the results for the Modular group, which was the largest, were used for the statistical analysis. The mean ammonia concentration was at its highest at the beginning of the recovery phase. The turning point varied between maximal exercise and 2–10 min of recovery for individual subjects, with 4 min and 6 min of recovery being the most common. The maximum ammonia varied significantly among the subjects (37.0–163.0 μmol/L). The mean for the subjects' maximum ammonia during the follow-up, irrespective of the time point at which it was achieved, was 80.5 μmol/L (SD 32.1), and the increase was 4.3-fold (SD 2.47) compared to light exercise (Table 2 in article 1).

The ammonia levels in the recovery phase were lower in women compared to men, with significant differences at time points 4–30 min into the recovery ($p = 0.002–0.038$) (Figure 8). In the group-wise comparisons, there was a significant difference in ammonia between age groups in maximal exercise as well as 2–6 min into the recovery ($p = 0.002–0.028$), with significant differences between age groups <35 and >50 years ($p = 0.002–0.034$) in all timepoints and between <35 and 35–50 years at maximal exercise ($p = 0.045$), the values being higher in the younger groups (Figure 9).

Repeated measures ANOVA for ammonia showed that there were statistically significant differences both in time points ($p < 0.0001$) and sexes ($p = 0.058$). However, their interaction was not statistically significant ($p = 0.2442$). Between age groups, there was a statistically significant difference both in relation to measurement step ($p < 0.001$) and the age groups ($p = 0.0166$) as well as their interaction ($p = 0.0008$), indicating a difference in the rate of change in ammonia as well as level between age groups but not between the sexes. When comparing the age groups, the differences were statistically significant between the age groups <35 and 35–50 years, as well as <35 and >50 years ($p < 0.001$) (Table 8).

In agreement with these results, the linear model showed significant changes in ammonia levels related to age, adjusted for sex and RER, in maximal exercise and 2–20 min into the recovery phase ($p \leq 0.001–0.012$). The regression coefficients corresponding to the change in ammonia (μmol/L) for a 1-year increase in age varied between −0.834 and −1.514 μmol/L, the highest change seen

2 min into the recovery. There was also a difference in ammonia observed between sexes in 6–30 min of recovery (p = 0.001–0.017). When calculated separately for the sexes, the strongest influence of age was observed in men 2 min into recovery (–1.913) and the highest ammonia in women at 4 min into recovery (–1.324), but the changes between sexes were less consistent than those observed in lactate (Table 9).

There were no significant differences in ammonia or lactate levels between physical activity groups or BMI groups (data not shown).

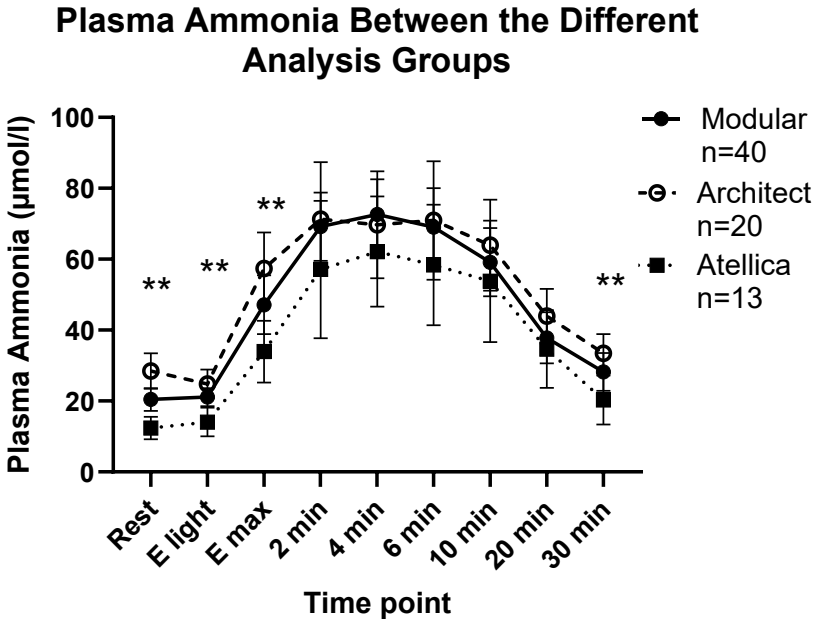

Figure 10 Plasma ammonia in the three laboratory analysis methods (Modular, Architect, and Atellica) during the cardiopulmonary exercise test in all participants. Means and 95% confidence intervals are shown. E light= first exercise step; E max = maximal exercise The significant differences are indicated with an asterisk (* p < 0.05). Reproduced with permission from Ratia N. et al. Lactate and ammonia measurements during cardiopulmonary exercise testing and its recovery phase-Consideration of age and sex in its interpretation. Clin Physiol Funct Imaging (2023).

Table 8 Repeated measurement ANOVA results for ammonia

Df = degrees of freedom. Statistically significant results ($p<0.05$) are in bold.

Ammonia			
	Df	Mean Square	p-value
Sex	1	7.6	**0.006**
Measurement step	6	154.3	**< 0.001**
Sex * Measurement step	6	7.9	0.244
Age group	2	8.2	**0.017**
Measurement step	6	174.0	**< 0.001**
Age group * Measurement step	12	33.6	**0.001**

Table 9 Linear model results for ammonium in healthy subjects adjusted for other variables listed in the table.

B = Estimated regression coefficient for a one-unit increase in continuous variables. SE = Standard error. Partial corr. = adjusted correlations. Linear model results are age-adjusted for other variables in the table. Bold figures indicate statistically significant results (p<0.05). Reproduced with permission from Ratia N. et al. Lactate and ammonia measurements during cardiopulmonary exercise testing and its recovery phase-Consideration of age and sex in its interpretation. Clin Physiol Funct Imaging (2023).

All participants N = 40								**Men, N=17**		**Women, N=23**	
	Age			**RER**		**Sex**		**Age**			
	B(SE)	**Partial Corr.**	**p-value**	**Partial Corr.**	**p-value**	**Partial Corr.**	**p-value**	**B(SE)**	**p-value**	**B(SE)**	**p-value**
Rest	-0.165 (0.143)	-0.201	0.255	0.207	0.241	-0.191	0.280	-0.370 (0.224)	0.120	0.012 (0.177)	0.984
Light exercise	-0.193 (0.113)	-0.290	0.096	0.3395	0.050	-0.074	0.679	**-0.446 (0.148)**	**0.009**	0.050 (0.147)	0.736
Max exercise	**-0.935 (0.212)**	**-0.609**	**<0.001**	0.2613	0.129	-0.047	0.790	**-1.204 (0.234)**	**<0.001**	**-0.754 (0.311)**	**0.026**
2 min	**-1.514 (0.348)**	**-0.604**	**<0.001**	**0.3543**	**0.037**	-0.152	0.384	**-1.913 (0.550)**	**0.004**	**-1.277 (0.444)**	**0.010**
4 min	**-1.255 (0.338)**	**-0.549**	**<0.001**	**0.5001**	**0.003**	**-0.397**	**0.020**	-1.090 (0.530)	0.059	**-1.324 (0.491)**	**0.016**
6 min	**-1.475 (0.334)**	**-0.609**	**<0.001**	**0.6222**	**<0.001**	**-0.529**	**0.001**	**-1.583 (0.596)**	**0.019**	**-1.266 (0.428)**	**0.009**
10 min	**-0.834 (0.312)**	**-0.422**	**0.012**	**0.5686**	**<0.001**	**-0.582**	**<0.001**	-0.834 (0.640)	0.214	**-0.682 (0.287)**	**0.030**
20 min	-0.420 (0.253)	-0.282	0.106	**0.5067**	**0.002**	**-0.619**	**<0.001**	-0.344 (0.492)	0.496	-0.250 (0.207)	0.243
30 min	-0.394 (0.209)	-0.380	0.074	**0.5166**	**0.012**	**-0.603**	**0.002**	-0.063 (0.412)	0.882	-0.222 (0.159)	0.191

5.2.2 Lactate and Ammonia Results in Study II

The lactate and ammonia levels in the mAD group before and during the diet are presented in Figure 11. Compared to pre-mAD, lactate concentration decreased significantly at the end of maximal exercise (4.4 vs. 6.2 mmol/L, p = 0.018). However, there were no other significant changes in lactate levels due to the mAD intervention. Ammonia levels tended to be higher at the end of the mAD period compared to baseline, although this difference did not reach statistical significance.

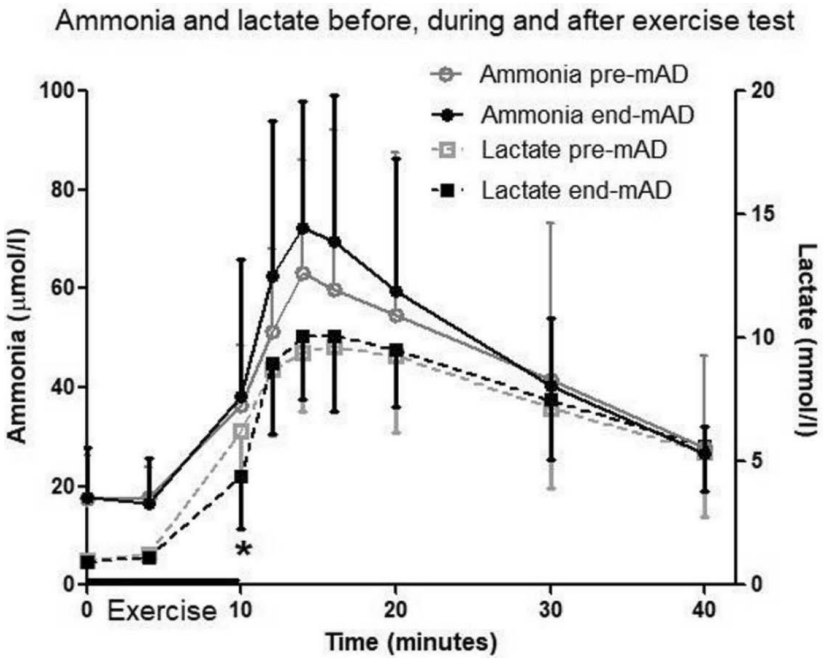

Figure 11 The lactate and ammonia levels before and at the end of mAD (modified Atkins diet) (N=10). The mean values before, during, and 2, 4, 6, 10, 20, and 30 minutes after exercise are given as lines and the standard deviations as error bars. The significant differences ($p<0.05$) are indicated with an asterisk. Reproduced with permission from Ratia N. et al. Modified Atkins diet modifies cardiopulmonary exercise characteristics and promotes hyperventilation in healthy subjects. Journal of Functional Foods (2021).

5.2.3 Lactate and Ammonia Results in Study III

The linear model results with SMAJ and the combined MELAS/CPEO group compared to the healthy controls are presented in Table 10. There was a statistically significant difference in plasma lactate concentration between the healthy controls and the MELAS/CPEO group at rest and during light exercise ($p < 0.001$), as well as 30 minutes into the recovery period ($p = 0.020$). Similarly, there was a statistically significant difference between the SMAJ and MELAS/CPEO groups at rest and during light exercise ($p < 0.001$) (data not shown). Between 2 and 10 minutes into the recovery phase, lactate levels in the MELAS/CPEO and control groups were comparable and higher compared to the SMAJ group. In repeated samples ANOVA, there was a significant difference in lactate values in relation to time (Wilks' Lambda $p < 0.001$) and between diagnosis groups in relation to time (Wilks' Lambda $p=0.018$).

In Figure 12, plasma lactate results are presented for subjects with SMAJ, MELAS, and CPEO separately, and the healthy controls. (The results for MELAS and CPEO together are in Figure 2 in Article III). In one-way ANOVA (Figure 12), when the CPEO and MELAS groups were separated, the lactate values were significantly higher in the MELAS group compared to both the healthy controls and the SMAJ group at rest and at light exercise ($p<0.001$-0.035 with Tukey's adjustment). The values in the CPEO group were also higher than the healthy controls at rest and at light exercise ($p<0.001$-0.003) and also higher than the SMAJ group at light exercise ($p=0.024$). There was also a slight statistically significant difference ($p=0.043$) between the CPEO and SMAJ groups 4 minutes into recovery, but this is likely due to the fluctuation in lactate values in the smaller-sized SMAJ group at this particular time point. The differences between the MELAS and CPEO groups did not reach statistical significance at any time point, but the lactate values tended to be lower in the MELAS group.

When the subjects with MELAS and CPEO were analyzed separately for the linear model (unpublished results, Ratia et al.), the lactate values were significantly higher in both MELAS and CPEO groups at rest and light exercise compared to healthy controls, but the statistically significant change 30 minutes into recovery was only observed in the CPEO group ($p= 0.033$), the larger group of the two. (Table 11) Ammonia results per diagnosis group are presented in Figure 13, and the linear model results adjusted for sex, age, and BMI in Table 12, with the exception of the ammonia results for the MELAS/CPEO group that were left out due to the different analysis arrays in use during the testing of these patients. For ammonia, there was no statistically significant difference between the SMAJ group and the healthy volunteers in the linear model or between the diagnosis groups in relation to time in the repeated samples ANOVA.

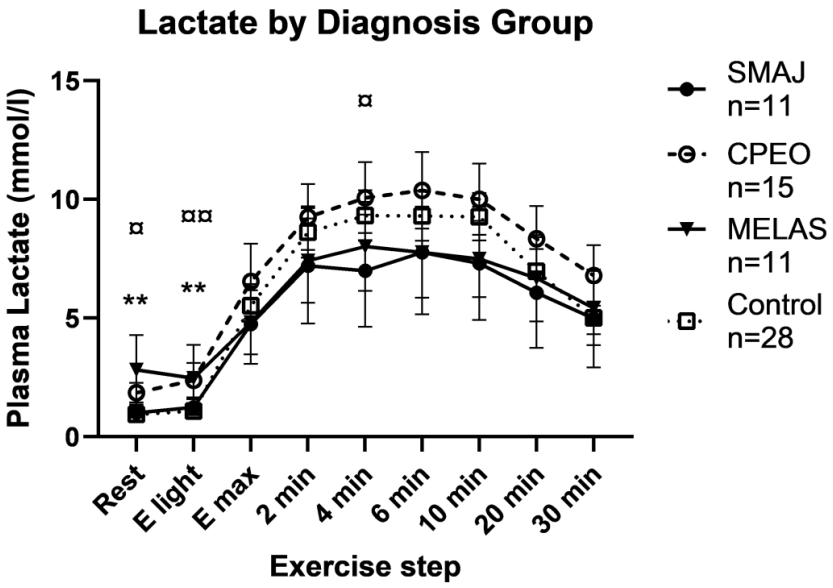

Figure 12 The lactate levels at rest, during exercise, and during the recovery phase associated with cardiopulmonary exercise testing. Means and 95% confidence intervals for plasma lactate in the diagnosis groups are given. SMAJ = spinal muscular atrophy, Jokela type; MELAS/PEO = mitochondrial disease group; E light= first exercise step; E max = maximal exercise; Statistical significance for one-way ANOVA with Tukey's adjustment between diagnosis groups is presented with an asterisk (* for $p < 0.05$ and ** for $p < 0.01$) for changes associated with the MELAS group and with the symbol "¤" for CPEO group (¤ for $p < 0.05$ and ¤¤ for $p < 0.01$). Adapted with permission from Ratia N. et al. Lowered oxidative capacity in spinal muscular atrophy, Jokela type; comparison with mitochondrial muscle disease. Front Neurol (2023).

Table 10 Linear model results for plasma lactate in subjects with SMAJ and the combined mitochondrial myopathy groups compared to healthy controls.

Linear model results are adjusted for sex, age, and BMI. B = Estimated regression coefficient for a one-unit increase in continuous variables. SE = Standard error. SMAJ = Spinal Muscular Atrophy, Jokela Type. MELAS/CPEO = mitochondrial disease group. Statistically significant results ($p < 0.05$) are in bold. Reproduced with permission from Ratia N. et al. Lowered oxidative capacity in spinal muscular atrophy, Jokela type; comparison with mitochondrial muscle disease. Front Neurol (2023).

		B(SE)	**p-value**			**B(SE)**	**p-value**
Rest	Control	0	reference	**6 min**	Control	0	reference
	SMAJ	0.072 0.35)	0.83		SMAJ	-1.22 (0.92)	0.19
	MELAS/CPEO	**1.30 (0.27)**	**<0.001**		MELAS/CPEO	-0.26 (0.71)	0.71
Light Exercise	Control	0	reference	**10 min**	Control	0	reference
	SMAJ	0.16 (0.34)	0.63		SMAJ	-1.73 (0.89)	0.051
	MELAS/CPEO	**1.33 (0.27)**	**<0.001**		MELAS/CPEO	-0.48 (0.69)	0.49
Maximal Exercise	Control	0	reference	**20 min**	Control	0	reference
	SMAJ	-0.52 (0.77)	0.50		SMAJ	-0.78 (0.86)	0.37
	MELAS/CPEO	0.092 0.60)	0.88		MELAS/CPEO	0.57 (0.68)	0.40
2 min into Recovery	Control	0	reference	**30 min**	Control	0	reference
	SMAJ	-1.09 (0.84)	0.20		SMAJ	-0.0004(0.71)	1.00
	MELAS/CPEO	-0.61 (0.68)	0.37		**MELAS/CPEO**	**1.17 (0.57)**	**0.041**
4 min	Control	0	reference				
	SMAJ	-1.66 (0.87)	0.058				
	MELAS/CPEO	-0.49 (0.66)	0.45				

Table 11 Linear model results for plasma lactate in subjects with SMAJ as well as MELAS and CPEO presented separately compared to healthy controls.

Linear model results are adjusted for sex, age, and BMI. B = Estimated regression coefficient for a one-unit increase in continuous variables. SE = Standard error. SMAJ = Spinal Muscular Atrophy, Jokela Type. MELAS = Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes, CPEO = Chronic progressive external ophthalmoplegia. Statistically significant results (p <0.05) are in bold.

		B(SE)	**p-value**	**Mean (SD)**			**B(SE)**	**p-value**	**Mean (SD)**
Rest	Control	0	reference	0.94 (0.23)	**6 min**	Control	0	reference	9.31 (2.65)
	SMAJ	0.06 (0.36)	0.874	1.01 (0.30)		SMAJ	-1.19 (0.96)	0.217	7.76 (3.87)
	CPEO	**0.93 (0.32)**	**0.006**	**1.86 (0.74)**		CPEO	0.48 (0.86)	0.582	10.38 (2.91)
	MELAS	**1.87 (0.38)**	**<0.001**	**2.82 (2.18)**		MELAS	-1.41 (1.01)	0.169	7.77 (2.85)
Light Exercise	Control	0	reference	1.09 (0.31)	**10 min**	Control	0	reference	9.27 (2.58)
	SMAJ	0.16 (0.36)	0.651	1.25 (0.51)		SMAJ	-1.71 (0.92)	0.068	7.31 (3.55)
	CPEO	**1.33 (0.32)**	**<0.001**	**2.38 (1.32)**		CPEO	0.27 (0.83)	0.750	10.01 (2.72)
	MELAS	**1.33 (0.40)**	**0.002**	**2.47 (1.82)**		MELAS	-1.68 (0.99)	0.096	7.49 (2.25)
Maximal Exercise	Control	0	reference	5.52 (2.02)	**20 min**	Control	0	reference	6.96 (2.44)
	SMAJ	-0.50 (0.88)	0.532	4.75 (2.50)		SMAJ	-0.76 (0.91)	0.405	6.07 (3.47)
	CPEO	0.71 (0.72)	0.333	6.55 (2.85)		CPEO	1.02 (0.82)	0.221	8.35 (2.49)
	MELAS	-0.88 (0.85)	0.304	4.82 (2.00)		MELAS	-0.21 (1.01)	0.833	6.68 (2.37)
2 min into Recovery	Control	0	reference	8.62 (2.68)	**30 min**	Control	0	reference	5.00 (1.78)
	SMAJ	-1.06 (0.88)	0.229	7.20 (3.62)		SMAJ	0.02 (0.75)	0.978	4.98 (3.08)
	CPEO	0.02 (0.80)	0.976	9.25 (2.50)		**CPEO**	**1.57 (0.72)**	**0.033**	**6.79 (2.11)**
	MELAS	-1.80 (1.00)	0.076	7.42 (2.31)		MELAS	0.56 (0.84)	0.511	5.42 (2.03)
4 min	Control	0	reference	8.62 (2.70)					
	SMAJ	-1.66 (0.90)	0.071	7.20 (3.30)					
	CPEO	0.22 (0.79)	0.782	9.25 (2.69)					
	MELAS	-1.67 (0.94)	0.083	7.42 (2.62)					

Table 12 Linear model results for plasma ammonia in subjects with SMAJ compared to healthy controls.

Linear model results are adjusted for sex, age, and BMI. B = Estimated regression coefficient for a one-unit increase in continuous variables. SE = Standard error. SMAJ = Spinal Muscular Atrophy, Jokela Type. Statistically significant results ($p < 0.05$) are in bold. Adapted with permission from Ratia N. et al. Lowered oxidative capacity in spinal muscular atrophy, Jokela type; comparison with mitochondrial muscle disease. Front Neurol (2023).

		B(SE)	p-value		B(SE)	p-value	
Rest	Control	0	reference	**6 min**	Control	0	reference
	SMAJ	1.14 (2.36)	0.63		SMAJ	3.87 (9.16)	0.67
Light Exercise	Control	0	reference	**10 min**	Control	0	reference
	SMAJ	-1.43 (2.78)	0.61		SMAJ	-0.80 (9.63)	0.93
Maximal Exercise	Control	0	reference	**20 min**	Control	0	reference
	SMAJ	-1.98 (4.30)	0.65		SMAJ	-0.13 (5.5)	0.98
2 min into Recovery	Control	0	reference	**30 min**	Control	0	reference
	SMAJ	1.55 (8.63)	0.86		SMAJ	2.5 (3.83)	0.46
4 min	Control	0	reference				
	SMAJ	-2.42 (8.00)	0.76				

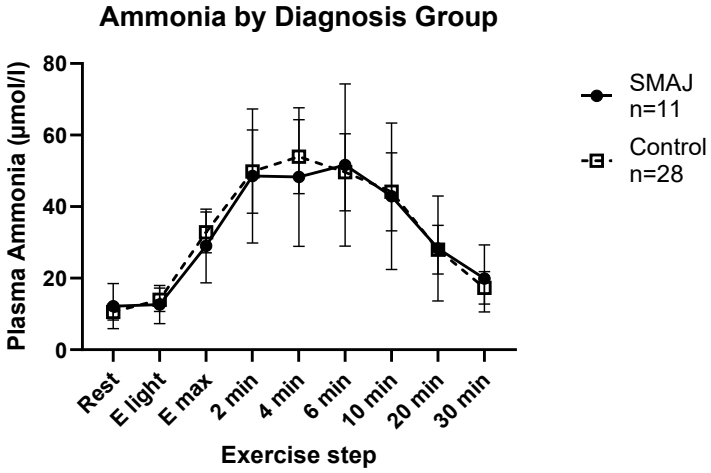

Figure 13 The ammonia levels of the patients with SMAJ and healthy controls at rest, during exercise, and at the recovery phase associated with cardiopulmonary exercise testing. SMAJ, spinal muscular atrophy, Jokela type. Statistical significance is presented for linear model results adjusted for age, sex, and BMI with an asterisk (*). Reproduced with permission from Ratia N. et al. Lowered oxidative capacity in spinal muscular atrophy, Jokela type; comparison with mitochondrial muscle disease. Front Neurol (2023).

5.3 Blood Gases and pH

5.3.1 Blood Gases and pH in Study I

Concomitantly with increasing lactate, pH decreased during exercise and at the beginning of the recovery phase, being at its lowest 2 min into the recovery (mean 7.25, SD 0.05). There was no difference in pH values between sexes (Figure 14) or age groups (Figure 15). Similarly, base excess (BE) decreased during exercise and early recovery, being at its lowest 6 min into recovery (−8.59 mmol/L, SD 3.80). A significant difference in BE between the sexes was observed only at rest and in light exercise ($p \leq 0.001$) (Figure 14), with BE being lower in women. Between the age groups, there were differences in BE values in maximal exercise and 2, 4, 6, 10, and 20 min into recovery ($p \leq 0.001–0.008$) between the age groups <35-year-olds and > 50-year-olds. (Figure 14)

Figure 14 Venous pH and base excess (BE) in men and women as measured during and after the cardiopulmonary exercise test in all participants. Means and 95% confidence intervals are shown. E light = first exercise step; E max = maximal exercise. The significant differences are indicated with an asterisk (*p<0.05, **<0.01).

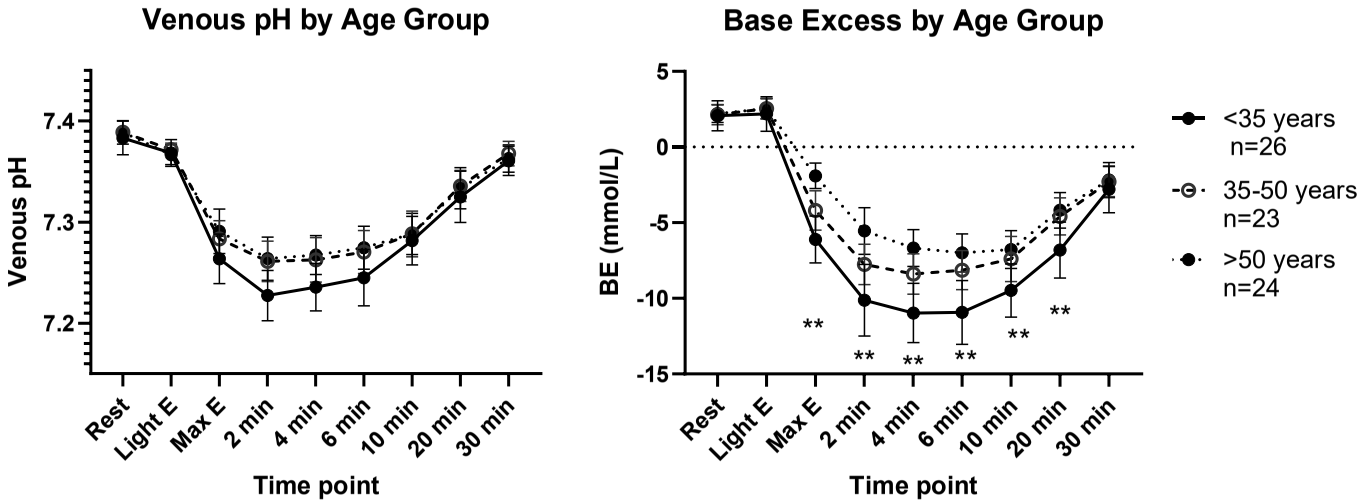

Figure 15 Venous pH and base excess (BE) in the three age groups as measured during and after the cardiopulmonary exercise test in all participants. Means and 95% confidence intervals are shown. E light = first exercise step; E max = maximal exercise. The significant differences are indicated with an asterisk (*p<0.05, **<0.01).

5.3.1 Blood Gases and pH in Study II

In the mAD group, at rest and during light exercise, pH decreased end-of-mAD versus pre-mAD (p-values 0.04-0.006). (Figure 16) Base excess (BE) decreased end-of-mAD at 6 and 10 minutes after exercise compared to pre-mAD (– 9.0 vs -6.3, p = 0.038 and -8.1 vs -5.53 mmol/L, p =0.037, respectively), and bicarbonate decreased at 6 minutes after exercise (17.1 vs 19 mmol/L, p = 0.046). (Figure 16) Changes in the percent of oxygenated Hb (HbO2) and other blood gas values in venous blood samples were not significant.

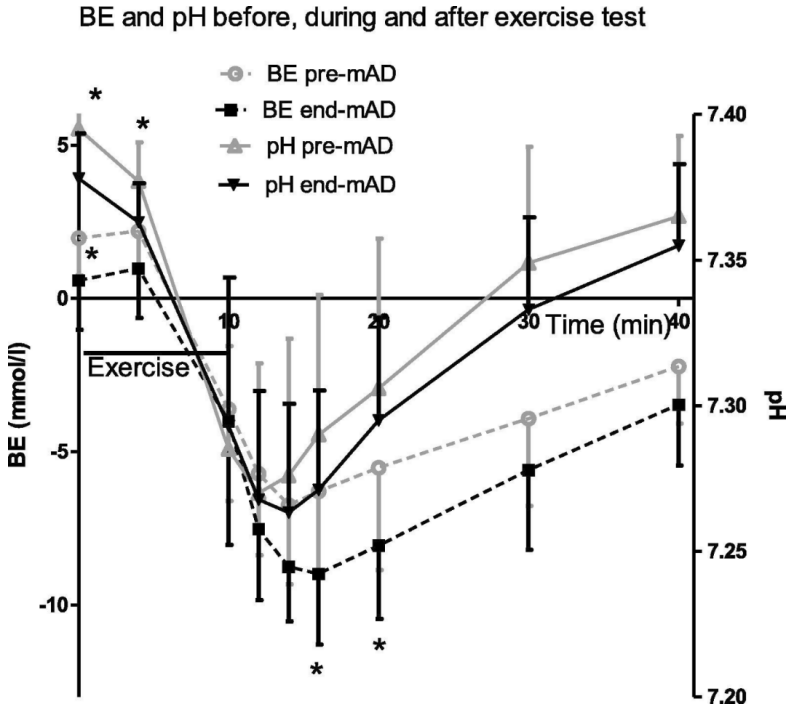

Figure 16 The base excess (BE) and pH values before (pre-mAD) and at the end of mAD modified Atkins diet (end-mAD) (N=10). The mean (SD) values before, during, and 2, 4, 6, 10, 20, and 30 minutes after exercise are given as lines and the standard deviations as error bars. The significant differences ($p<0.05$) are indicated with an asterisk. Reproduced with permission from Ratia N. et al. Modified Atkins diet modifies cardiopulmonary exercise characteristics and promotes hyperventilation in healthy subjects. Journal of Functional Foods (2021).

5.3.2 Blood Gases and pH in Study III

In the linear model, adjusted for sex, age, and BMI, pH differed 6 min into recovery between the SMAJ group and the healthy controls (p= 0.026) and similarly between MELAS/CPEO and the healthy controls (p= 0.022). (Figure 17)

In base excess (BE), there was a significant difference between the MELAS/CPEO group and the healthy controls at rest (p= 0.028) and light exercise (p< 0.001), but no statistically significant changes between the SMAJ group and the healthy volunteers. (Figure 17)

As oxygen saturation differences are used in mitochondrial disease diagnostics in the forearm exercise tests, venous blood oxygen saturation was also measured from the subjects (Table 13). In a linear model, adjusted for age, sex, and BMI, this showed higher values only in the CPEO group compared to the healthy controls during light exercise (p= 0.015). In contrast, in the MELAS group, the oxygen saturation was slower to recover than in the healthy controls, being lower than the other groups 2 minutes into the recovery (p= 0.023). At other time points, there were no significant differences. (Ratia et al. Unpublished data)

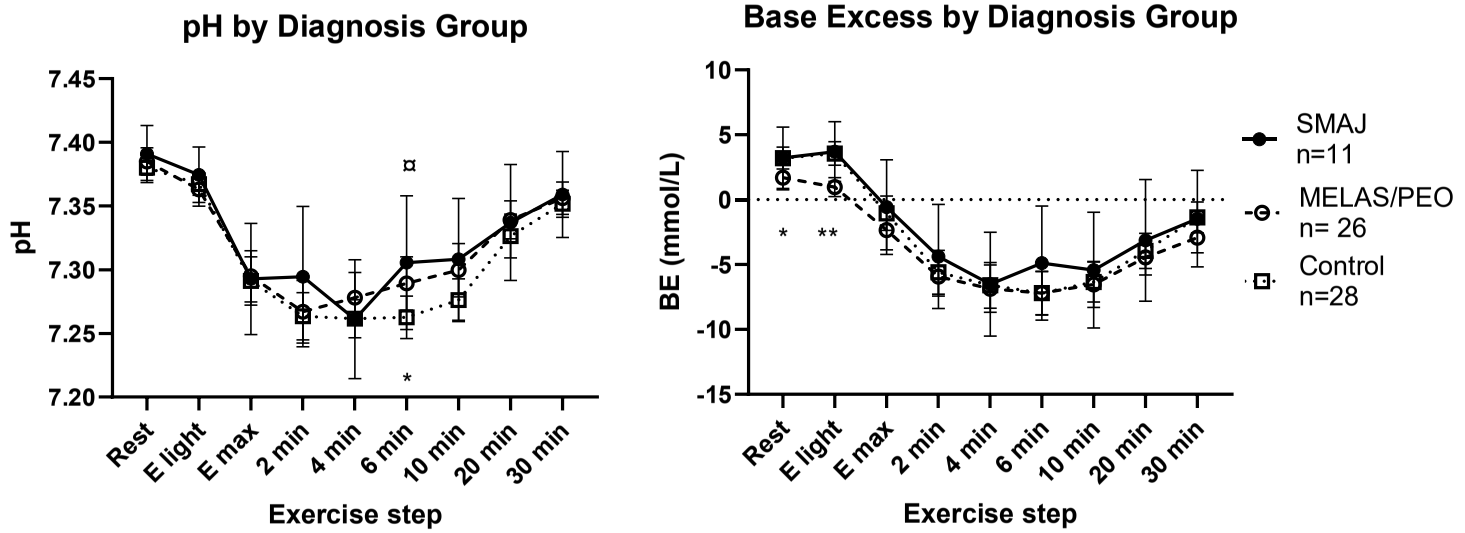

Figure 17 pH and base excess (BE) levels at rest, during exercise, and during the recovery phase associated with cardiopulmonary exercise testing. Mean and 95% confidence interval for plasma lactate in the diagnosis groups are given. SMAJ= spinal muscular atrophy, Jokela type; MELAS/PEO = mitochondrial disease group; E light= first exercise step; E max= maximal exercise; Statistical significance in the linear model ($p < 0.05$) is presented for linear model results adjusted for age, sex, and BMI between MELAS/PEO group and the controls is presented with an asterisk (* for $p < 0.05$) and with the symbol "¤" between the SMAJ group and the healthy controls (¤ for $p < 0.05$ and ¤¤ for $p < 0.01$).

Table 13 Linear model results for venous oxygen saturation (%) for subjects with SMAJ as well as MELAS and CPEO separately compared to controls.

Linear model results are adjusted for age, sex, and BMI (body mass index). B = Estimated regression coefficient for a one-unit increase in continuous variables. SE = Standard error. SMAJ = Spinal Muscular Atrophy, Jokela Type. MELAS = Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, CPEO = Chronic progressive external ophthalmoplegia. Statistically significant results ($p < 0.05$) are in bold.

		B(SE)	**p-value**	**Mean (SD)**			**B(SE)**	**p-value**	**Mean (SD)**
Rest	Control	0	reference	64.07 (15.86)	**6 minutes**	Control	0	reference	75.37 (16.22)
	SMAJ	1.13 (5.07)	0.824	66.21 (12.51)		SMAJ	-1.28 (5.30)	0.810	73.95 (19.38)
	CPEO	0.74 (4.58)	0.872	66.40 (15.81)		CPEO	-0.42 (4.85)	0.931	77.83 (11.75)
	MELAS	4.14 (5.35)	0.443	61.74 (15.08)		MELAS	4.27 (5.67)	0.454	76.72 (12.65)
Light Exercise	Control	0	reference	48.55 (17.66)	**10 minutes**	Control	0	reference	71.85 (16.12)
	SMAJ	0.23 (5.25)	0.965	49.09 (16.30)		SMAJ	7.94 (4.88)	0.109	79.53 (13.68)
	CPEO	**11.93 (4.75)**	**0.015**	**59.39 (10.14)**		CPEO	4.57 (4,41)	0.305	78.52 (10.47)
	MELAS	-1.19 (5.93)	0.842	47.07 (4.63)		MELAS	0.0006 (5.44)	1.000	70.10 (13.98)
Maximal Exercise	Control	0	reference	53.61 (22.81)	**20 minutes**	Control	0	reference	73.05 (14.43)
	SMAJ	-9.12 (7.39)	0.223	43.64 (22.54)		SMAJ	-0.58 (4.95)	0.907	72.56 (21.12)
	PEO	-10.06 (6.84)	0.147	46.06 (16.53)		CPEO	-1.63 (4.31)	0.706	74.03 (10.60)
	MELAS	-2.37 (7.89)	0.765	47.11 (20.03)		MELAS	-0.52 (5.31)	0.922	70.03 (10.56)
2 minutes into Recovery	Control	0	reference	72.73 (19.05)	**30 minutes**	Control	0	reference	67.09 (18.69)
	SMAJ	0.16 (5.99)	0.979	71.35 (18.00)		SMAJ	1.89 (5.54)	0.734	69.79 (22.58)
	CPEO	-6.81 (5.45)	0.217	69.25 (14.61)		CPEO	0.11 (5.12)	0.982	69.28 (10.40)
	MELAS	**-16.52 (7.03)**	**0.023**	**55.86 (19.56)**		MELAS	5.69 (6.18)	0.362	67.50 (7.40)
4 minutes	Control	0	reference	74.42 (19.91)					
	SMAJ	-3.86 (6.73)	0.569	70.23 (19.64)					
	CPEO	-2.29 (5.70)	0.690	74.87 (11.92)					
	MELAS	-6.16 (6.86)	0.373	67.69 (21.90)					

6 Discussion

6.1 Study I

In healthy subjects, exercise capacity and many exercise testing parameters such as maximal oxygen uptake ($V'O_{2max}$) and maximal exercise workload are heavily dependent on the sex and age of the subject as well as their prior history of exercise training. The differences in exercise capacity between the sexes are driven by sex hormones during and after puberty. Muscle mass and muscle power are generally higher in men, with the muscle strength and power of limb skeletal muscles in women being 50–70% in comparison to males. This difference is more pronounced in the upper limb. (Alcazar et al., 2020; Hunter & Senefeld, 2024)

As we age, skeletal muscle mass, muscle quality, and strength start to decline. This phenomenon is known as sarcopenia. Numerous changes affecting the muscle that occur due to aging have been identified, including signaling pathway disruptions, changes in the muscle fibres and extracellular matrix composition, a decrease in mitochondrial function, and changes in systemic responses to inflammation and neurological inputs, but the precise etiology of sarcopenia is still unclear. In healthy subjects, these changes become more prominent after the age of 75, but they can be expedited by different diseases. (Argilés et al., 2015; Grima-Terrén et al., 2024)

Repeated exercise training drives adaptation in both the cardiovascular and musculoskeletal systems, causing an overall improvement in exercise capacity and performance. These adaptations include changes in skeletal muscle, such as increased mitochondrial biogenesis and capillary density, and in the cardiovascular system, such as an increase in cardiac and blood volumes as well as improved contractility of the cardiac muscle. (Hellsten, 2016; Hughes et al., 2018)

Although the effects of age, sex, and training on exercise capacity or muscle function have been extensively studied, their influence on the level of lactate and especially ammonia levels during clinical exercise testing are less studied. This can cause difficulties in the interpretation of clinical exercise test results with these metabolic indices and provides the motivation for the present examinations.

6.1.1 Lactate

Lactate measurements during exercise have been a key tool in sports medicine where they have been used to optimize training, often focusing on the lactate threshold—the exercise intensity at which lactate starts to accumulate in the bloodstream at a faster rate than it can be cleared by the body, marking the transition from predominantly aerobic metabolism to anaerobic metabolism resulting in an increase in lactate production—and changes in lactate levels alone or in relation to $V'O_2$ over time to help monitor the subject's fitness and progress. (Hall et al., 2016) Studies including both lactate and ammonia values, and especially those during a clinical exercise test, are fewer. Earlier Mouadil et al. (2012) have published lactate and ammonia values associated with maximal incremental cardiopulmonary exercise testing in healthy sedentary subjects (N=48). In Study I, the aim was to include a larger population (N= 73) with a wider age range of subjects with exercise habits close to a healthy general population. Even so, the subjects volunteering for an exercise test tended to be quite physically active. However, the results of the present study did not markedly differ from those of Mouadil et al., where the subjects were described as sedentary without further details to classify their exercise habits.

During exercise, lactate increases in relation to the increase in glycolysis. Typically, during incremental exercise, lactate increases gradually at the beginning until the lactate threshold is reached, after which the levels increase rapidly. Lactate continues to rise until up to 1-10 minutes after exercise before starting to decline. Maximum lactate values of 8-10 mmol/l have formerly been observed 1-10 min after maximal exercise, similar to the maximal values of 11 mmol/l 4-8 min post-exercise observed in this study, with the values declining slowly up until an hour after exercise has ended. (Goodwin et al., 2007; Mouadil et al., 2012; Sjödin & Jacobs, 1981)

Previously, the decrease in maximal exercise lactate level for a one-year rise in age has been shown to be as high as -0.16 mmol/l per year. (Massé-Biron et al., 1992) In our study, the yearly decrease of lactate was slightly lower (coefficient -0.119 mmol/l), with the decrease being more pronounced in women compared to men. This age-related decrease in lactate is likely caused by multiple factors contributing to sarcopenia: both oxidative and glycolytic capacity in cells have been found to decrease independent of physical activity. (Hunter et al., 2002) As the rise in lactate is dependent on the amount of muscle tissue engaged, a decrease in muscle mass has been considered as one of the factors contributing to the decrease in lactate due to age. In women, estrogen deficiency is involved in the earlier and faster development of sarcopenia compared to men, though the exact mechanism of this is still unclear. This effect becomes more pronounced after menopause as estrogen levels fall rapidly compared to the more gradual change in testosterone levels in men. (Maltais et al., 2009; Pellegrino et al., 2022) This more

rapid decrease in muscle mass could be one factor explaining the difference in the age-related decrease in lactate between the sexes observed in our study.

6.1.2 Ammonia

Our results on ammonia measurements are in line with the earlier study (Mouadil et al. 2012), which used the same Roche Diagnostics analyzer for ammonia. The maximal values were achieved in early recovery. Similar to lactate, the maximal ammonia levels decreased with increasing age. The estimate for the decline of ammonia with age was -1.51 µmol/l per year. As the amount of ammonia production is strongly related to muscle mass (Graham & MacLean, 1992), and as lean muscle mass decreases with age, starting from 40-50 years in some studies (Alcazar et al., 2020), these changes in ammonia values are likely largely related to age-related changes in muscle mass, though changes also occur in the activity of, e.g., AMP deaminase and glutamate dehydrogenase enzymes involved in ammonia production (Mohan et al., 1987).

Similarly, there was also a significant difference in the ammonia values between sexes associated with exercise, with ammonia tending to be higher in men, as has been reported in earlier studies. (Itoh & Ohkuwa, 1993; Mouadil et al.. 2012) As with aging, the difference in lean muscle mass has been attributed as a factor in explaining the differences in ammonia values between sexes. (Itoh & Ohkuwa, 1993; Yuan & Chan, 2000) When examining the effect of age separately for both sexes, the maximal decrease of ammonia values was higher in men (highest values two minutes after exercise) compared to women (maximal value at four minutes after exercise), but unlike in lactate, the direction and strength of the decrease varied slightly. This is likely due to the smaller sample size and the large variation in the plasma ammonia levels between patients, both at rest and during exercise. Variance in muscle mass and the maximality of exercise could explain some of the variation. Endurance training has been shown to reduce ammonia levels in submaximal exercise while not affecting the maximum value (Lo & Dudley, 1987; Yuan & Chan, 2000), but in these current data, prior physical activity did not statistically significantly affect the results.

The chemistry analyzer used also significantly affected ammonia results. When analyzing the ammonia results of the three different chemistry platforms, there was no significant difference in lactate levels, but there was a significant variation between assays in ammonia levels below 50 µmol/l. Plasma ammonia is a challenging measure due to both preanalytical and analytical issues. Hemolysis due to handling of the samples, nonoptimal cooling, and delays in analysis caused by the transportation of the samples can cause an increase in ammonia levels of up to 7-30 % in an hour, as more ammonia is released from the blood cells. (Goldstein et al., 2017; Gifford et al., 2018; Nikolac et al., 2014) Furthermore, the

routine clinical use of ammonia assays on automated clinical chemistry platforms is to rule out significant hyperammonaemia related to, e.g., inborn errors in metabolism or liver failure. As these disorders present with ammonia levels above 50 μmol/l and up to 400–500 μ μmol/l range (Häberle, 2011), the analyzers are not optimized for precise detection of low levels of ammonia. Considering this, it is understandable that the standardization and precision of the routine ammonia methods at levels below 50 μmol/l, which are considered normal, are relatively poor. In fact, we showed in this study that different assays used resulted in significant changes in plasma ammonia, especially at rest and late recovery when the ammonia values were at their lowest. The assay-dependent variation and imprecision at low concentration levels should be considered when interpreting plasma ammonia results in the context of exercise testing.

Considering all the above points, it is difficult to establish the range of normal values, especially for ammonia, due to the variation between the subjects as well as a marked difference between analyzers.

6.2 Study II

The four-week modified mAD had a slight negative impact on the ten subjects' cardiopulmonary exercise capacity: $W_{max}/3$ min decreased while $V'O_{2max}$ increased, and while the changes in them were not statistically significant, their relation (mechanical efficiency) decreased significantly, indicating that to create the same work output as pre-mAD, a greater level of oxygen consumption was necessary end of maD, suggesting that some unfavorable effects of the low carbohydrate diet on the exercise capacity persist at least four weeks from the start of mAD. In previous studies, a temporary decrease in exercise performance during the first days of a ketogenic diet is typical, but metabolic adaptation mechanisms, including enhanced fat oxidation, later compensate for these early changes. (Spriet & Peters, 1998) All in all, the effects of low-carbohydrate diets on oxygen uptake and cardiorespiratory performance have been inconclusive, with no significant improvement in exercise performance having been proven, with some of the studies showing a slight improvement and some a negative impact. (Babij et al., 1983; Burke et al., 2002; Cipryan et al., 2018; Goedecke et al., 1999; Harvey et al., 2019; McSwiney et al., 2019; Rowlands & Hopkins, 2002; Zajac et al., 2014)

As using fat as a fuel consumes less oxygen than carbohydrates per ATP produced, increased fatty acid oxidation caused by the low-carbohydrate diet produces less CO2 per amount of oxygen consumed compared to a carbohydrate-rich diet. During exercise testing, in earlier studies, this has been associated with a decrease in RER (Burke et al., 2002; Spriet & Peters, 1998; Zajac et al., 2014), and we observed a similar decrease here. The decrease in CO2 production has been thought to potentially be beneficial in hypoventilation syndrome, where the build-

up of CO2 in blood can be life-threatening. (Gangitano et al., 2021; Rubini et al., 2015) In our study, the subjects' minute ventilation increased during maximal exercise. This rise in ventilation is likely explained by acidic ketone bodies (Greenhaff et al., 1987; Yancy et al., 2007), causing a statistically significant decrease in pH in our subjects' end-of-mAD both at rest and during light exercise. Together with the decreased bicarbonate and increased ionized calcium observed in our study subjects, the increase in ventilation would fit to be respiratory compensation for the increase in metabolic acidity. (Kraut & Madias, 2010)

The increase in ventilation was accompanied by a rise in the carbon dioxide equivalent $V'E/V'CO_2$, and simultaneously, a decrease in $FetCO_2$. Both of these changes are observed when ventilation is exaggerated in relation to the amount of CO_2 produced and have been linked to hyperventilation at rest and during exercise. (Gardner et al., 1986; Ionescu et al., 2021; Kinnula & Sovijärvi, 1993; Malmberg et al., 2000; Vansteenkiste et al., 1991). The decrease in the production of CO_2 due to the diet likely explains some of this decrease, but the paradoxical, simultaneous rise in ventilation suggests it not to be the only factor. The rise of arterial CO_2 level, promoting ventilation through chemoreceptors of the carotid arteries, is a central form of respiratory regulation. (Carr 2021) The reduced CO_2 production alone should, therefore, lead to a decrease in ventilation, the opposite suggesting that the changes in ventilation, at least in all subjects, are not necessarily beneficial. In studies about ventilation during a ketogenic diet done at rest, ventilation has not been shown to increase. Instead, in patients with chronic obstructive pulmonary disease, it has even been shown to decrease (Angelillo et al., 1985). However, many of these earlier studies have included patients with hypoventilation syndrome associated with changes in the regulation of the respiratory system or have not followed up on compliance with ketosis. (Gangitano et al., 2021; Rubini et al., 2015)

Earlier studies suggest that the body's compensation mechanisms will gradually diminish the ketosis and pH changes associated with low-carbohydrate ingestion during prolonged diets. (Brehm et al., 2003; Volek et al., 2002) Our results show that mAD-induced changes in pH-related increased ventilation, the changes persisting for at least four weeks on a strict ketogenic diet. Increased risk of hyperventilation, especially in patients already having the tendency, could potentially be an overlooked side effect of mAD. Hyperventilation tendency is often underdiagnosed as a cause of respiratory symptoms. (Tavel, 2021) However, further research is needed to determine the long-term clinical impact of these findings. In hypoventilation patients, the rise in ventilation might even be beneficial.

As for how the ketogenic diet affected lactate and ammonia samples taken during a clinical exercise test, there were slight changes in lactate but not ammonia: end-of-mAD lactate levels were significantly lower during maximal

exercise compared to pre-mAD, but this difference did not persist during the recovery phase. As lactate is a byproduct of glycolysis, a metabolic pathway primarily for carbohydrates, the lower level of lactate during high energy demand, such as exercise, is a logical result of low glucose availability. (Gladden, 2004) In an earlier study (Ahola et al.2016) that included the same subjects as healthy controls for CPEO patients, resting lactate levels did not change during the diet. Healthy subjects can likely oxidize non-glucose carbon sources such as branched-chain amino acids through increased gluconeogenesis, allowing average amounts of lactate production when the subjects are at rest, even during low intake of carbohydrates. However, despite the increase in protein ingested during mAD, there was no significant difference in ammonia levels during the exercise test.

6.3 Study III

6.3.1 SMAJ

During the cardiopulmonary exercise test, the SMAJ group presented with reduced maximal oxygen consumption, indicating decreased oxidative capacity. The maximal power output measured in the last three minutes of exercise was also statistically significantly lower than in the healthy controls, but similar to that observed in the MELAS/CPEO group. Maximal oxygen consumption derived from the cardiopulmonary exercise test is the single most comprehensive measure of aerobic or oxidative capacity. It is sex and age-dependent and can decrease due to a multitude of causes, including diseases limiting cardiac output, pulmonary function, or muscle work, as well as, more mildly, in association with poor aerobic fitness. (Guazzi et al., 2018; Ward, 2018) In other neuromuscular diseases, a decrease in maximal oxygen consumption has also been observed in ALS and other types of spinal muscular atrophy and has been shown to relate to the severity and prognosis of the disease. (Braga et al., 2018; He et al., 2022; Montes et al., 2021) Of other measurements related to aerobic capacity and cardiac output, the oxygen pulse (V'O2 per heart rate in maximal exercise) that offers a surrogate for stroke volume was also similarly lower compared to the healthy controls both in the SMAJ group and MELAS/CPEO group, and corresponding to conditions limiting O_2 delivery (Dandurand et al., 1995; Haller et al., 1978; Jeppesen et al., 2021), and possible training effect in the healthy controls (Poole et al., 2021; Wasserman, 1984).

In a recent study on biomarkers of SMAJ by Järvilehto et al., (2022), there was no increase in lactate in SMAJ subjects' resting blood samples. However, changes in pyruvate and succinate levels indicated that some metabolic changes related to mitochondrial dysfunction were present. Previously, mitochondrial myopathy findings, such as COX-negative fibers, have also been shown to be scarce in muscle

biopsies of individuals with SMAJ. (Jokela et al., 2011) During the exercise test in the SMAJ group here, the resting lactate was similarly within normal values, and there was no abnormal rise in lactate in the recovery phase after exercise testing. In fact, lactate levels were even lower than in healthy controls, with a significant difference in repeated measurement tests. Combined with the lack of changes in ventilation found in MELAS and CPEO subjects, these results provide further confirmation that features typical of mitochondrial myopathy in SMAJ subjects are minimal, even under conditions of strenuous exercise. This discovery emphasizes the distinctions between disease-associated variants of CHCHD10.

Some pathogenic variants in *CHCHD10*, the gene causing SMAJ, have been shown to affect the stability of mitochondrial structure and function, particularly under stress, causing ATP production to be less efficient due to increased proton leakage in mitochondria. (Genin et al., 2016; Ruan et al., 2022) Some of the mitochondrial effects of other pathogenic variants of *CHCHD10* (e.g., S59L and R15L) happen through the impairment of the MICOS complex, an important multi-subunit complex present in the inner mitochondrial membrane that takes part in multiple mitochondrial processes. (Bannwarth et al., 2014; Genin et al., 2016; Straub et al., 2021). Notably, the p.Gly58Arg variant is associated with isolated mitochondrial myopathy, and some affected patients also exhibit elevated lactate levels (Ajroud-Driss et al., 2015; Heiman-Patterson et al., 1997; Shammas et al., 2022), and mitochondrial myopathy has also been demonstrated in patients with the Ser59Leu variant (Bannwarth et al., 2014). Recently, Alici et al.(2023) have shown that the G66V pathogenic variant causing SMAJ causes the CHCHD10-G66V protein to be more flexible and more prone to degradation, but it does not seem to impair the MICOS complex, which is likely why the impact of the G66V pathogenic variant on the mitochondrial function is less severe.

6.3.2 Mitochondrial Myopathies

As stated above, we observed higher $V'E/V'O_2$ and significantly lower $FetCO_2$ in subjects with MELAS/CPEO but not in the patients with SMAJ. These results are in line with earlier literature where low $V'O_{2max}$ in combination with exaggerated ventilation relative to metabolic rate, manifesting as increased minute ventilation, high $V'E/V'O_2$ in maximal exercise, a steeper slope of increase in $V'E/V'CO_2$ slope, and increased perceived breathing effort are typical in MM subjects during maximal cardiopulmonary exercise testing. (Dandurand et al., 1995; Heinicke et al., 2011; Jeppesen et al., 2021; Lindholm et al., 2004; Taivassalo et al., 2003). The ratio between $V'CO_2$ and $V'O_2$ (RER), a value commonly used to indicate the level of exercise maximality (Wasserman 2012), can also be exceptionally high in MM. (Heinicke et al., 2011; Taivassalo et al., 2003) In patients with severe mitochondrial disease, it has been proposed that skeletal muscle's decreased

capacity to increase V′O_2 consumption is due to the impairment of oxidative phosphorylation. Meanwhile, the increase in lactate production leads to a relative increase in V′CO_2, the rise of CO_2 in arterial blood, and, in turn, increased ventilation. (Heinicke et al., 2011) Apart from mitochondrial disease, these findings in ventilation have also been shown in relation to hyperventilation and indicate an increase in ventilation in mitochondrial disease to be excessive (Gardner et al., 1986; Kinnula & Sovijärvi, 1993), though this might pose a challenge in differential diagnosis in some cases, especially in routine cardiopulmonary tests when MM is not suspected beforehand.

For other parameters obtainable from a cardiopulmonary exercise test, Bhatia et al., (2021) have suggested an algorithm based on the abnormalities of oxygen uptake measured with maximal volume of oxygen consumed in peak exercise (V'O_{2peak}) percent of predicted maximum, V'O_2/work slope, and oxygen uptake efficiency slope per body surface area (OUES/BSA). In this algorithm, V'O_{2peak} predicted ≤62%, V'O_2/work slope ≤10 mL/min per watt, and OUES/BSA ≤630.6 mL/L per m2 in combination, suggesting the likelihood of mitochondrial myopathy when the patient's medical history fits the diagnosis. Similarly, V'$O2_{peak}$ ≥96% predicted, V'O_2/work slope ≥10.9 mL/min per watt, and OUES/BSA ≥1032 mL/L per m2 would strongly suggest the absence of mitochondrial dysfunction. Unfortunately, due to our cardiopulmonary exercise test results being collected with multiple devices during the long duration of data collection, it was no longer possible to obtain the data to calculate V'O_2/work slope and OUES/BSA for all patients to test this algorithm properly. For what could be calculated in our material, in the MELAS/CPEO group, the mean V'O_{2peak} of the predicted maximum was 82 %, with 4 out of 28 patients under 62 % and 8 out of 28 over 96 %. Though the reference values used (Seliger et al., 1978) are not the same as the ones used by Bhatia et al., this highlights that there is a large grey area in maximal V'O_2 capacity during exercise in mitochondrial myopathy patients, especially as it has been shown to be dependent on the heteroplasmy level of mtDNA. (Jeppesen et al., 2003)

For lactate in the MM subjects, there were statistically significant changes in lactate at rest and in light exercise, both with a combined MELAS/CPEO group and when they were analyzed separately, as well as at 30 minutes after exercise when analyzed together but only for the CPEO subgroup when MELAS and CPEO when analyzed separately. The groups were originally combined due to the small sample size. The MELAS patients were overwhelmingly female, with only one male patient, which is a factor associated with lower lactate, as discussed above in 6.1, and the group was smaller compared to the CPEO group, which could affect the findings despite the calculations being adjusted for sex, weight, and age. Still, the slower decrease in lactate in late recovery might be dependent on the type of mitochondrial disease, though with MELAS patients in general having more

widespread muscle symptoms and systemic involvement, this seems like an unlikely direction. The 30-minute follow-up after exercise used was longer than in some of the previous studies. (Dysgaard Jeppesen et al., 2003; Lindholm et al., 2004; Löfberg et al., 2001) A previous study in our hospital reported a late increase in lactate about three times the base level was seen 10-30 min after exercise in two patients with Tarui disease (Piirilä et al., 2016), highlighting the need for an adequately long follow-up, and in light of our findings here might also apply to the slower decrease in lactate in late recovery for MM-patients.

However, the previous lactate value findings in MM patients in maximal incremental exercise tests have been varied: Dandurand et al. (1995) found no significant difference in lactate despite an hour-long follow-up, but. In two studies by the same group, there was evidence of higher lactate already in early recovery without mention of the exact time of measurement (Lindholm et al., 2004; Löfberg et al., 2001). Dysgaard Jeppesen et al. (2003) found that the post-exercise recovery rate of plasma lactate was significantly slower in MM than in healthy subjects, but there was considerable overlap in the two groups, and findings in myotonic dystrophy patients were virtually identical to those in MM patients. However, the follow-up was shorter at 20 minutes. At maximal exercise, the lactate levels are remarkably similar to healthy subjects. Jeppesen et al., (2003) showed with simultaneous arterial and venous samples from the exercising lower limb during a 30-minute constant load test at 65% of the patients' $V'O_{2max,}$ that even though lactate concentration and production rates were several-fold higher in MM patients, during prolonged exercise, lactate was oxidized in muscle to the same extent as in healthy control subjects. This was likely due to the variability and adaptability of oxidative capacity among muscle fibers. However, there was a short period in the early exercise where oxidation was slower in MM subjects than in controls, and the study did not include the recovery period, so it is unclear whether a similar mismatch happens there. (Jeppesen et al., 2013) All in all, while the current study shows higher, more slowly decreasing lactate in the recovery period, and there is some prior clinical evidence of this phenomenon combined with low $V'O_{2max}$ in MM patients (M. Tarnopolsky, 2012), it is not universal, and there is considerable overlap with the healthy population.

Though not the main objective of the study, oxygen saturation results for the MM groups were also analyzed, keeping in mind the typical findings of high PO2 and saturation during exercise in forearm tests. (Taivassalo et al., 2002) As our samples were collected from the arm, they reflect oxygen consumption in the arm, not the legs, which are mainly exerted in an ergometer test. Our results showed higher oxygen saturation compared to the healthy controls only in the CPEO group during light exercise and lower than the controls in recovery—in opposition to findings in forearm tests, where oxygen saturation has been shown to be higher in mitochondrial myopathy patients during exercise and higher or the same level as

in controls in recovery. (Jensen et al., 2002; Taivassalo et al., 2002) However, as the exercise load is standardized for the legs, the strenuousness of the exercise level in the upper body is not the same between different subjects. In the CPEO-subjects, muscle symptoms tend to be most pronounced in the ocular muscles, whereas the MELAS subjects were more broadly symptomatic, which likely led to the using their arms more to help with the cycling, putting more strain on them, increasing the oxygen consumption, and thus decreasing the oxygen saturation and increasing the variability of these results. Thus, comparing the results to the healthy subjects is difficult, and this method of sampling is better suited for the forearm test.

6.3.3 Exercise Testing Methods

Multiple protocols of exercise testing with lactate, ammonia, and venous blood samples are still in use for the diagnostic process of mitochondrial myopathies, including the non-ischemic forearm test, different forms of submaximal ergometric exercise tests, and the incremental maximal cardiopulmonary exercise test. For MM, specifically, UptoDate has included the forearm test with venous saturation samples as part of the recommended diagnostic route (Darras, 2020), and it has become the foremost mode of exercise testing for metabolic myopathies as a whole. However, the other exercise methods have still been included as an option in some recent reviews, especially in cases where the forearm test is inconclusive. (Tarnopolsky, 2016, 2022)

There are few studies directly comparing these methods. (Hanisch, Müller, et al., (2006) studied 27 patients with genetically defined mitochondrial disorders, of which 16 performed both an intermittent isometric handgrip exercise with oxygen saturation and partial pressure in cubital venous blood measured from the exercising arm and fixed-load cycle ergometry at 30 W for 15 minutes with venous lactate measured, with both tests showing similar specificity (92-96%), but the forearm test showing better sensitivity (33-67 % vs. 21-26 %), and the combination of the tests improving specificity further to 87%. Furthermore, (Dysgaard Jeppesen et al., 2003) compared an incremental workload and a constant relative workload of 65% of $V'O_{2max}$ protocol for 20 minutes in 15 patients with mixed mitochondrial myopathies compared with healthy subjects and subjects with myotonic dystrophy, with the constant relative workload test being more sensitive, though still around as sensitive as resting lactate, though this study has been criticized for the small sample size including mostly CPEO patients without a description of the severity of their muscle symptoms, and including controls with high lactate values. (Finsterer, 2005) To our knowledge, there is no study directly comparing the maximal exercise test with lactate and ammonia samples and the

forearm oxygen saturation test, or especially their combination. In the future, this might be one possible course of study.

In the maximal exercise test with lactate and ammonia samples, even though there are differences in lactate values between diagnosis groups, there is great variation between individuals and the many factors contributing to it, including age and sex, as well as other factors, e.g., possible variations in exercise habits or nutritional aspects. This can make it hard to draw a line between normal and abnormal results on an individual level, especially in patients with less severe disease–an important distinction in clinical practice. For ammonia, there is also the question of the variability of the results between different commercially available analyzers that are configured for higher ammonia levels, more commonly needed in clinical practice. This could likely also affect the forms of forearm tests that include ammonia measurements, especially in tests for patients with suspected GSD, as the levels of ammonia recorded are also low in that method.

In the case of scientific follow-up studies where exercise tolerance is of interest, maximal exercise testing with lactate and ammonia sampling can be useful, which has also been suggested before. (Dysgaard Jeppesen et al., 2003,; Jeppesen et al., 2021) In our hospital, this has been used as a control for patients with phosphofructokinase deficiency treated with a modified Atkins diet (Similä et al., 2020) as well as in mitochondrial diseases like CPEO, where niacin has been found to have a favorable effect on the disease (Pirinen et al., 2020). In repeated exercise tests on the same subject, the differences in individual factors are mitigated.

6.4 Limitations and Challenges

The number of healthy participants for the present study is larger than in many previous clinical studies about the cardiopulmonary exercise test with lactate and ammonia measurements (Babij et al., 1983; Buono et al., 1984; Freund & Gendry, 1978; Mouadil et al., 2012; ; Withers et al., 1991), although the current sample size could still be larger. However, as a cardiopulmonary exercise test with ammonia, lactate, and blood gas follow-up is time-consuming and expensive, a larger sample size is difficult to obtain. This is also exacerbated by the fact that there is a lot of variation in lactate and especially the ammonia levels during exercise testing between the subjects, depending on the individual factors, even in the healthy subjects, including muscle mass, sex, and age, making the normal values difficult to ascertain. The variance in the timepoint reaching the peak lactate and ammonia values. Additionally, three different models of chemistry analysers were in use for measuring lactate and ammonia in the subjects. The change of the analysis device did not influence the lactate values, but the ammonia values varied between the models, and therefore, only those ammonia values analyzed with the same

analyzer model were used for the sub-studies. However, this reduced the number of ammonia samples available for study.

One of the study's limitations includes the variation in RER between subjects. The absolute minimum for RER to be included in the studies was set at 1.0, which is lower than some recommendations for maximal exercise tests. However, a recent large-scale study of 22,379 exercise tests by Kaminsky et al. (2022) found little difference in peak oxygen consumption with the RER criterion of greater than or equal to 1.0 compared to RER greater than or equal to 1.1 across the tests. In clinical practice, it is difficult to reach the same maximality in cardiopulmonary exercise tests both for healthy subjects and subjects with muscle disease, with different exercise capacities. In a recent study, only 31% of 86 patients with various muscle diseases reached RER ≥1.10. (Veneman et al., 2024) Factors other than exercise maximality can also influence RER. In association with suspected or verified muscle metabolic diseases, it is important to note that the RER levels can be exceptionally high in muscle mitochondrial disease (Taivassalo et al., 2003) while hardly rising during exercise in glycogen storage diseases that limit carbohydrate utilization and thus lactate production (Piirilä et al., 2016). In addition, in maximal lactate steady-state, RER has been found to be lower in women, possibly due to metabolic gender-related factors (Hafen & Vehrs, 2018), and in trained individuals, lower RER values have been reported due to increased utilization of fat during exercise caused by increased lipolysis of muscle triglycerides (Hurley et al., 1986; Meijer et al., 2000). All these factors impact RER as an indicator of exercise maximality, especially in patients with muscle metabolic diseases.

Specifically concerning Study III, as the metabolic myopathies and other muscle diseases affect the subjects' muscle function to a different degree, depending on the disorder and individual factors, their ability to cycle can vary between individuals. Furthermore, for the MELAS and CPEO patients, the information available about their muscle symptoms was less detailed than that of the SMAJ patients, though the subjects' maximal exercise capacity in all these groups was similarly reduced. Especially when measuring blood gas levels from the arm, the differences in symptoms might affect the measured results, as the subjects might compensate for lower limb weakness with their upper body. However, for lactate and ammonia values, dependent on the larger working muscle groups of the lower limbs, this should be of less influence.

7 Conclusions

This dissertation presents findings for cardiopulmonary exercise testing results with lactate and ammonia samples, both for healthy subjects and subjects with muscle disease.

Study I on healthy participants provides more information about the normal effects of aging on these results during exercise and especially recovery, presenting approximations of the decrease in lactate according to age, and the results show the yearly decrease in lactate to be more apparent in women than in men. However, for ammonia, the sex-specific changes related to age were less clear, and it is important to note that there is considerable variation in the ammonia results partly based on the chemistry analyzer.

Study II explored the effects of the modified Atkins diet on cardiopulmonary exercise test results of healthy participants, showing an unbeneficial effect on work efficacy and an increase in ventilation during exercise.

In study III, though SMAJ patients displayed reduced exercise capacity and oxidative capacity during the exercise stress test, similar to those with mitochondrial myopathy, they did not exhibit changes in ventilation or lactate accumulation as seen in mitochondrial myopathy. This further supports the conclusion that mitochondrial function is not compromised in this disease, even under exercise conditions.

Internationally, forearm tests have become widely used among neurologists, especially for suspected mitochondrial diseases as well as in glycogen storage diseases. For mitochondrial myopathies, the UptoDate database has included the forearm test with venous saturation samples as part of the recommended diagnostic pathway. (Darras, 2020) These tests allow for sampling from the exercising limb, are quicker to perform, and can also be more easily performed in regular hospital departments. They have become the foremost mode of exercise testing for metabolic myopathies. However, other exercise methods have still been included as an option, especially in cases where the forearm test is inconclusive.

Although new diagnostic findings for muscle metabolic diseases based on cardiopulmonary exercise testing are rare, the tests have been routinely used in diagnostics and assessment of cardiopulmonary conditions of patients with suspected or diagnosed muscle metabolic diseases. The current study presents more information about the possibilities and limitations of cardiopulmonary

exercise testing in the present practice of our hospital, and despite not being able to give exact reference values, it has given practical insight for everyday clinical analysis work for the lactate, ammonia, and breath gas results of the test.

A maximal exercise test can give information about the subject's exercise capacity and physical performance, which can be valuable in planning treatment and rehabilitation of patients with muscle metabolic diseases, or help in differential diagnosis. Especially in the case of scientific follow-up studies or clinical follow-up of e.g., dietary interventions where exercise tolerance is of interest, maximal exercise testing with lactate and ammonia sampling will continue to be useful. In these cases, with repeated exercise tests on the same subject, the differences in subject-dependent factors are mitigated.

References

Ahmed, S. T., Craven, L., Russell, O. M., Turnbull, D. M., & Vincent, A. E. (2018). Diagnosis and Treatment of Mitochondrial Myopathies. *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics*, *15*(4), 943–953. https://doi.org/10.1007/s13311-018-00674-4

Ahola, S., Auranen, M., Isohanni, P., Niemisalo, S., Urho, N., Buzkova, J., Velagapudi, V., Lundbom, N., Hakkarainen, A., Muurinen, T., Piirilä, P., H, K. P., & Suomalainen, A. (2016). Modified Atkins diet induces subacute selective ragged-red-fiber lysis in mitochondrial myopathy patients. *EMBO Molecular Medicine,* 8(11), 1234–1247. https://doi.org/10.15252/emmm.201606592

Ait-El-Mkadem Saadi, S., Chaussenot, A., Bannwarth, S., Rouzier, C., & Paquis-Flucklinger, V. (1993). CHCHD10-Related Disorders. In M. P. Adam, D. B. Everman, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, L. J. Bean, K. W. Gripp, & A. Amemiya (Eds.), *GeneReviews®*. University of Washington, Seattle. http://www.ncbi.nlm.nih.gov/books/NBK304142/

Ajroud-Driss, S., Fecto, F., Ajroud, K., Lalani, I., Calvo, S. E., Mootha, V. K., Deng, H.-X., Siddique, N., Tahmoush, A. J., Heiman-Patterson, T. D., & Siddique, T. (2015). Mutation in the novel nuclear-encoded mitochondrial protein CHCHD10 in a family with autosomal dominant mitochondrial myopathy. *Neurogenetics*, *16*(1), 1–9. https://doi.org/10.1007/s10048-014-0421-1

Alcazar, J., Aagaard, P., Haddock, B., Kamper, R. S., Hansen, S. K., Prescott, E., Alegre, L. M., Frandsen, U., & Suetta, C. (2020). Age- and Sex-Specific Changes in Lower-Limb Muscle Power Throughout the Lifespan. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *75*(7), 1369–1378. https://doi.org/10.1093/gerona/glaa013

Ali, A., Esmaeil, A., & Behbehani, R. (2024). Mitochondrial Chronic Progressive External Ophthalmoplegia. *Brain Sciences*, 14(2), 135. https://doi.org/10.3390/brainsci14020135

Alici, H., Uversky, V. N., Kang, D. E., Woo, J. A., & Coskuner-Weber, O. (2023). Effects of the Jokela type of spinal muscular atrophy-related G66V mutation on the structural ensemble characteristics of CHCHD10. *Proteins*, *91*(6), 739–749. https://doi.org/10.1002/prot.26463

Andreu, A. L., Nogales-Gadea, G., Cassandrini, D., Arenas, J., & Bruno, C. (2007). McArdle disease: Molecular genetic update. *Acta Myologica: Myopathies and Cardiomyopathies: Official Journal of the Mediterranean Society of Myology*, *26*(1), 53–57.

Angelillo, V. A., Bedi, S., Durfee, D., Dahl, J., Patterson, A. J., & O'Donohue, W. J. (1985). Effects of low and high carbohydrate feedings in ambulatory patients with chronic obstructive pulmonary disease and chronic

hypercapnia. *Annals of Internal Medicine*, *103*(6 (Pt 1)), 883–885. https://doi.org/10.7326/0003-4819-103-6-883

Angelini, C., Marozzo, R., Pegoraro, V., & Sacconi, S. (2020). Diagnostic challenges in metabolic myopathies. *Expert Review of Neurotherapeutics,* 20(12), 1287–1298. https://doi.org/10.1080/14737175.2020.1825943

Argilés, J. M., Busquets, S., Stemmler, B., & López-Soriano, F. J. (2015). Cachexia and sarcopenia: Mechanisms and potential targets for intervention. *Current Opinion in Pharmacology*, *22*, 100–106. https://doi.org/10.1016/j.coph.2015.04.003

Augustin, K., Khabbush, A., Williams, S., Eaton, S., Orford, M., Cross, J. H., Heales, S. J. R., Walker, M. C., & Williams, R. S. B. (2018). Mechanisms of action for the medium-chain triglyceride ketogenic diet in neurological and metabolic disorders. *The Lancet. Neurology,* 17(1), 84–93. https://doi.org/10.1016/S1474-4422(17)30408-8

Auranen, M., Ylikallio, E., Shcherbii, M., Paetau, A., Kiuru-Enari, S., Toppila, J. P., & Tyynismaa, H. (2015). CHCHD10 variant p.(Gly66Val) causes axonal Charcot-Marie-Tooth disease. *Neurology. Genetics*, *1*(1), e1. https://doi.org/10.1212/NXG.0000000000000003

Babij, P., Matthews, S. M., & Rennie, M. J. (1983). Changes in blood ammonia, lactate and amino acids in relation to workload during bicycle ergometer exercise in man. *European Journal of Applied Physiology and Occupational Physiology*, *50*(3), 405–411. https://doi.org/10.1007/BF00423246

Balady, G. J., Arena, R., Sietsema, K., Myers, J., Coke, L., Fletcher, G. F., Forman, D., Franklin, B., Guazzi, M., Gulati, M., Keteyian, S. J., Lavie, C. J., Macko, R., Mancini, D., Milani, R. V., American Heart Association Exercise, Cardiac Rehabilitation, and Prevention Committee of the Council on Clinical Cardiology, Council on Epidemiology and Prevention, Council on Peripheral Vascular Disease, & Interdisciplinary Council on Quality of Care and Outcomes Research. (2010). Clinician's Guide to cardiopulmonary exercise testing in adults: A scientific statement from the American Heart Association. *Circulation*, *122*(2), 191–225. https://doi.org/10.1161/CIR.0b013e3181e52e69

Bannwarth, S., Ait-El-Mkadem, S., Chaussenot, A., Genin, E. C., Lacas-Gervais, S., Fragaki, K., Berg-Alonso, L., Kageyama, Y., Serre, V., Moore, D. G., Verschueren, A., Rouzier, C., Le Ber, I., Augé, G., Cochaud, C., Lespinasse, F., N'Guyen, K., de Septenville, A., Brice, A., … Paquis-Flucklinger, V. (2014). A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through CHCHD10 involvement. *Brain: A Journal of Neurology*, *137*(Pt 8), 2329–2345. https://doi.org/10.1093/brain/awu138

Bender, A., Krishnan, K. J., Morris, C. M., Taylor, G. A., Reeve, A. K., Perry, R. H., Jaros, E., Hersheson, J. S., Betts, J., Klopstock, T., Taylor, R. W., & Turnbull, D. M. (2006). High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nature Genetics, 38(5), 515–517. https://doi.org/10.1038/ng1769

Bhatia, R., Cohen, B. H., & L McNinch, N. (2021). A novel exercise testing algorithm to diagnose mitochondrial myopathy. *Muscle & Nerve*, *63*(5), 715–723. https://doi.org/10.1002/mus.27191

Blair, H. A. (2023). Cipaglucosidase Alfa: First Approval. *Drugs*, *83*(8), 739–745. https://doi.org/10.1007/s40265-023-01886-5

Borsche, M., Pereira, S. L., Klein, C., & Grünewald, A. (2021). Mitochondria and Parkinson's Disease: Clinical, Molecular, and Translational Aspects. *Journal of Parkinson's Disease*, *11*(1), 45–60. https://doi.org/10.3233/JPD-201981

Bourke, J. P., Ng, Y. S., Tynan, M., Bates, M. G. D., Mohiddin, S., Turnbull, D., & Gorman, G. S. (2022). Arrhythmia prevalence and sudden death risk in adults with the m.3243A>G mitochondrial disorder. Open Heart, 9(1), e001819. https://doi.org/10.1136/openhrt-2021-001819Braga, A. C. M., Pinto, A., Pinto, S., & de Carvalho, M. (2018). The Role of Moderate Aerobic Exercise as Determined by Cardiopulmonary Exercise Testing in ALS. *Neurology Research International*, *2018*, 8218697. https://doi.org/10.1155/2018/8218697

Brambilla, A., Favilli, S., Olivotto, I., Calabri, G. B., Porcedda, G., De Simone, L., Procopio, E., Pasquini, E., & Donati, M. A. (2019). Clinical profile and outcome of cardiac involvement in MELAS syndrome. International Journal of Cardiology, 276, 14–19. https://doi.org/10.1016/j.ijcard.2018.10.051

Brehm, B. J., Seeley, R. J., Daniels, S. R., & D'Alessio, D. A. (2003). 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. *The Journal of Clinical Endocrinology and Metabolism*, *88*(4), 1617–1623. https://doi.org/10.1210/jc.2002-021480 [doi]

Brooks, G. A. (1986). The lactate shuttle during exercise and recovery. *Medicine & Science in Sports & Exercise*, *18*(3), 360.

Buono, M. J., Clancy, T. R., & Cook, J. R. (1984). Blood lactate and ammonium ion accumulation during graded exercise in humans. *Journal of Applied Physiology*, *57*(1), 135–139. https://doi.org/10.1152/jappl.1984.57.1.135

Burke, L., Hawley, J., Angus, D., Cox, G., Clark, S., Cummings, N., Desbrow, B., & Hargreaves, M. (2002). Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability. *Medicine and Science in Sports and Exercise*, *34*, 83–91. https://doi.org/10.1097/00005768-200201000-00014

Burstein, S. R., Valsecchi, F., Kawamata, H., Bourens, M., Zeng, R., Zuberi, A., Milner, T. A., Cloonan, S. M., Lutz, C., Barrientos, A., & Manfredi, G. (2018). In vitro and in vivo studies of the ALS-FTLD protein CHCHD10 reveal novel mitochondrial topology and protein interactions. *Human Molecular Genetics*, *27*(1), 160–177. https://doi.org/10.1093/hmg/ddx397

Carr, A. J., Sharma, A. P., Ross, M. L., Welvaert, M., Slater, G. J., & Burke, L. M. (2018). Chronic Ketogenic Low Carbohydrate High Fat Diet Has Minimal Effects on Acid-Base Status in Elite Athletes. *Nutrients*, *10*(2), 236. https://doi.org/10.3390/nu10020236

Celie, B. M., Boone, J., Smet, J. E., Vanlander, A. V., De Bleecker, J. L., Van Coster, R. N., & Bourgois, J. G. (2015). Forearm deoxyhemoglobin and deoxymyoglobin (deoxy[Hb + Mb]) measured by near-infrared spectroscopy (NIRS) using a handgrip test in mitochondrial myopathy. *Applied Spectroscopy*, *69*(3), 342–347. https://doi.org/10.1366/14-07604

Chin, H.-L., Lai, P. S., & Tay, S. K. H. (2024). A clinical approach to diagnosis and management of mitochondrial myopathies. *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics*, *21*(1), e00304. https://doi.org/10.1016/j.neurot.2023.11.001

Cipryan, L., Plews, D. J., Ferretti, A., Maffetone, P. B., & Laursen, P. B. (2018). Effects of a 4-Week Very Low-Carbohydrate Diet on High-Intensity Interval Training Responses. *Journal of Sports Science & Medicine*, *17*(2), 259–268.

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F., & Oja, P. (2003). International physical activity questionnaire: 12-country reliability and validity. *Medicine and Science in Sports and Exercise*, *35*(8), 1381–1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FB

Dandurand, R. J., Matthews, P. M., Arnold, D. L., & Eidelman, D. H. (1995). Mitochondrial disease. Pulmonary function, exercise performance, and blood lactate levels. *Chest*, *108*(1), 182–189. https://doi.org/S0012-3692(16)38614-7 [pii]

Darras, B. T. (2020). Approach to the metabolic myopathies. In M. C. Patterson (Ed.), *UpToDate*. Wolters Kluwer. https://www.uptodate.com/contents/approach-to-the-metabolic-myopathies

Dashty, M. (2013). A quick look at biochemistry: Carbohydrate metabolism. In *Clinical Biochemistry* (Vol. 46, Issue 15, pp. 1339–1352). https://doi.org/10.1016/j.clinbiochem.2013.04.027

Dasouki, M., Jawdat, O., Almadhoun, O., Pasnoor, M., McVey, A. L., Abuzinadah, A., Herbelin, L., Barohn, R. J., & Dimachkie, M. M. (2014). Pompe disease: Literature review and case series. *Neurologic Clinics*, *32*(3), 751–776, ix. https://doi.org/10.1016/j.ncl.2014.04.010

Delaney, N. F., Sharma, R., Tadvalkar, L., Clish, C. B., Haller, R. G., & Mootha, V. K. (2017). Metabolic profiles of exercise in patients with McArdle disease or mitochondrial myopathy. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(31), 8402–8407. https://doi.org/10.1073/pnas.1703338114

Dysgaard Jeppesen, T., Olsen, D., & Vissing, J. (2003). Cycle ergometry is not a sensitive diagnostic test for mitochondrial myopathy. *Journal of Neurology*, *250*(3), 293–299. https://doi.org/10.1007/s00415-003-0993-4

Egan, B., & Zierath, J. R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metabolism*, *17*(2), 162–184. https://doi.org/10.1016/j.cmet.2012.12.012

El-Hattab, A. W., Adesina, A. M., Jones, J., & Scaglia, F. (2015). MELAS syndrome: Clinical manifestations, pathogenesis, and treatment options. *Molecular Genetics and Metabolism*, *116*(1–2), 4–12. https://doi.org/S1096-7192(15)30024-X [pii]

Eriksson, L. S., Broberg, S., Björkman, O., & Wahren, J. (1985). Ammonia metabolism during exercise in man. *Clinical Physiology*, *5*(4), 325–336. https://doi.org/10.1111/j.1475-097X.1985.tb00753.x

Ferguson, B. S., Rogatzki, M. J., Goodwin, M. L., Kane, D. A., Rightmire, Z., & Gladden, L. B. (2018). Lactate metabolism: Historical context, prior misinterpretations, and current understanding. *European Journal of Applied Physiology*, *118*(4), 691–728. https://doi.org/10.1007/s00421-017-3795-6

Finsterer, J. (2005). The usefulness of lactate stress testing in the diagnosis of mitochondrial myopathy. *Journal of Neurology*, *252*(7), 857–858. https://doi.org/10.1007/s00415-005-0772-5

Finsterer, J., & Milvay, E. (2002). Lactate stress testing in 155 patients with mitochondriopathy. *The Canadian Journal of Neurological Sciences.Le Journal Canadien Des Sciences Neurologiques*, *29*(1), 49–53. https://doi.org/10.1017/s0317167100001712 [doi]

Finsterer, J., Shorny, S., Capek, J., Cerny-Zacharias, C., Pelzl, B., Messner, R., Bittner, R. E., & Mamoli, B. (1998). Lactate stress test in the diagnosis of mitochondrial myopathy. *Journal of the Neurological Sciences*, *159*(2), 176–180. https://doi.org/S0022510X98001701 [pii]

Fishbein, W. N., Armbrustmacher, V. W., & Griffin, J. L. (1978). Myoadenylate deaminase deficiency: A new disease of muscle. *Science (New York, N.Y.)*, *200*(4341), 545–548. https://doi.org/10.1126/science.644316

Freund, H., & Gendry, P. (1978). Lactate kinetics after short strenuous exercise in man. *European Journal of Applied Physiology and Occupational Physiology*, *39*(2), 123–135. https://doi.org/10.1007/BF00421717

Funayama, M., Ohe, K., Amo, T., Furuya, N., Yamaguchi, J., Saiki, S., Li, Y., Ogaki, K., Ando, M., Yoshino, H., Tomiyama, H., Nishioka, K., Hasegawa, K., Saiki, H., Satake, W., Mogushi, K., Sasaki, R., Kokubo, Y., Kuzuhara, S., … Hattori, N. (2015). *CHCHD2* mutations in autosomal dominant late-onset Parkinson's disease: A genome-wide linkage and sequencing study. *The Lancet Neurology*, *14*(3), 274–282. https://doi.org/10.1016/S1474-4422(14)70266-2

Gangitano, E., Tozzi, R., Mariani, S., Lenzi, A., Gnessi, L., & Lubrano, C. (2021). Ketogenic Diet for Obese COVID-19 Patients: Is Respiratory Disease a Contraindication? A Narrative Review of the Literature on Ketogenic Diet and Respiratory Function. *Frontiers in Nutrition*, *8*, 771047. https://doi.org/10.3389/fnut.2021.771047

Gardner, W. N., Meah, M. S., & Bass, C. (1986). Controlled Study of Respiratory Responses During Prolonged Measurement in Patients with Chronic Hyperventilation. *The Lancet*, *328*(8511), 826–830. https://doi.org/10.1016/S0140-6736(86)92867-9

Genin, E. C., Plutino, M., Bannwarth, S., Villa, E., Cisneros-Barroso, E., Roy, M., Ortega-Vila, B., Fragaki, K., Lespinasse, F., Pinero-Martos, E., Augé, G., Moore, D., Burté, F., Lacas-Gervais, S., Kageyama, Y., Itoh, K., Yu-Wai-Man, P., Sesaki, H., Ricci, J.-E., … Paquis-Flucklinger, V. (2016). CHCHD10 mutations promote loss of mitochondrial cristae junctions with impaired mitochondrial genome maintenance and inhibition of apoptosis. *EMBO Molecular Medicine*, *8*(1), 58–72. https://doi.org/10.15252/emmm.201505496

Gifford JL, Nguyen WNT, de Koning L, Seiden-Long I. (2018) Stabilizing specimens for routine ammonia testing in the clinical laboratory. *Clinica Chimica Acta; International Journal of Clinical Chemistry*. 478,37-43. doi: 10.1016/j.cca.2017.12.022. Epub 2017 Dec 16. PMID: 29258744.

Gladden, L. B. (2004). Lactate metabolism: A new paradigm for the third millennium. In *The Journal of physiology* (Vol. 558, pp. 5–30). https://doi.org/10.1113/jphysiol.2003.058701

Goedecke, J. H., Christie, C., Wilson, G., Dennis, S. C., Noakes, T. D., Hopkins, W. G., & Lambert, E. V. (1999). Metabolic adaptations to a high-fat diet in

endurance cyclists. In *Metabolism* (Vol. 48, Issue 12, pp. 1509–1517). https://doi.org///doi.org/10.1016/S0026-0495(99)90238-X

Goldstein, B. N., Wesler, J., Nowacki, A. S., Reineks, E., & Natowicz, M. R. (2017). Investigations of blood ammonia analysis: Test matrices, storage, and stability. *Clinical Biochemistry*, *50*(9), 537–539. https://doi.org/10.1016/j.clinbiochem.2017.01.002

Goodwin, M. L., Harris, J. E., Hernández, A., & Gladden, L. B. (2007). Blood Lactate Measurements and Analysis during Exercise: A Guide for Clinicians. *Journal of Diabetes Science and Technology (Online)*, *1*(4), 558–569.

Gorman, G. S., Schaefer, A. M., Ng, Y., Gomez, N., Blakely, E. L., Alston, C. L., Feeney, C., Horvath, R., Yu-Wai-Man, P., Chinnery, P. F., Taylor, R. W., Turnbull, D. M., & McFarland, R. (2015). Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. Annals of Neurology, 77(5), 753–759. https://doi.org/10.1002/ana.24362

Goto, Y., Nonaka, I., & Horai, S. (1990). A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature, 348(6302), 651–653. https://doi.org/10.1038/348651a0

Gowda, P., Reddy, P. H., & Kumar, S. (2022). Deregulated mitochondrial microRNAs in Alzheimer's disease: Focus on synapse and mitochondria. Ageing Research Reviews, 73, 101529. https://doi.org/10.1016/j.arr.2021.101529

Graham, T. E., & MacLean, D. A. (1992). Ammonia and amino acid metabolism in human skeletal muscle during exercise. *Canadian Journal of Physiology and Pharmacology*, *70*(1), 132–141. https://doi.org/10.1139/y92-020

Greenhaff, P. L., Nevill, M. E., Soderlund, K., Bodin, K., Boobis, L. H., Williams, C., & Hultman, E. (1994). The metabolic responses of human type I and II muscle fibres during maximal treadmill sprinting. *The Journal of Physiology*, *478 (Pt 1)*(Pt 1), 149–155. https://doi.org/10.1113/jphysiol.1994.sp020238

Grima-Terrén, M., Campanario, S., Ramírez-Pardo, I., Cisneros, A., Hong, X., Perdiguero, E., Serrano, A. L., Isern, J., & Muñoz-Cánoves, P. (2024). Muscle aging and sarcopenia: The pathology, etiology, and most promising therapeutic targets. *Molecular Aspects of Medicine*, *100*, 101319. https://doi.org/10.1016/j.mam.2024.101319

Grünewald, A., Rygiel, K. A., Hepplewhite, P. D., Morris, C. M., Picard, M., & Turnbull, D. M. (2016). Mitochondrial DNA Depletion in Respiratory Chain-Deficient Parkinson Disease Neurons. Annals of Neurology, 79(3), 366–378. https://doi.org/10.1002/ana.24571

Guazzi, M., Arena, R., Halle, M., Piepoli, M. F., Myers, J., & Lavie, C. J. (2018). 2016 focused update: Clinical recommendations for cardiopulmonary exercise testing data assessment in specific patient populations. *European Heart Journal*, *39*(14), 1144–1161. https://doi.org/10.1093/eurheartj/ehw180

Gümüş, E., & Özen, H. (2023). Glycogen storage diseases: An update. *World Journal of Gastroenterology*, *29*(25), 3932–3963. https://doi.org/10.3748/wjg.v29.i25.3932

Häberle, J. (2011). Clinical practice: The management of hyperammonemia. *European Journal of Pediatrics*, *170*(1), 21–34. https://doi.org/10.1007/s00431-010-1369-2

Habets, L. E., Bartels, B., Asselman, F.-L., Hooijmans, M. T., van den Berg, S., Nederveen, A. J., van der Pol, W. L., & Jeneson, J. A. L. (2022). Magnetic resonance reveals mitochondrial dysfunction and muscle remodelling in spinal muscular atrophy. *Brain: A Journal of Neurology*, *145*(4), 1422–1435. https://doi.org/10.1093/brain/awab411

Hafen, P. S., & Vehrs, P. R. (2018). Sex-Related Differences in the Maximal Lactate Steady State. *Sports (Basel, Switzerland)*, *6*(4), E154. https://doi.org/10.3390/sports6040154

Hall, M. M., Rajasekaran, S., Thomsen, T. W., & Peterson, A. R. (2016). Lactate: Friend or Foe. *PM&R*, *8*(3, Supplement), S8–S15. https://doi.org/10.1016/j.pmrj.2015.10.018

Haller, R. G., Mukherjee, A., Gaffney, F. A., & Blomquist, C. G. (1978). Mitochondrial myopathy presenting as exercise intolerance. *Transactions of the American Neurological Association*, *103*, 6–10.

Hammarén, E., Rafsten, L., Kreuter, M., & Lindberg, C. (2004). Modified exercise test in screening for mitochondrial myopathies—Adjustment of workload in relation to muscle strength. *European Neurology*, *51*(1), 38–41. https://doi.org/10.1159/000074981

Hanisch, F., Eger, K., Bork, S., Lehnich, H., Deschauer, M., & Zierz, S. (2006). Lactate production upon short-term non-ischemic forearm exercise in mitochondrial disorders and other myopathies. *Journal of Neurology*, *253*(6), 735–740. https://doi.org/10.1007/s00415-006-0101-7 [doi]

Hanisch, F., Müller, T., Muser, A., Deschauer, M., & Zierz, S. (2006). Lactate increase and oxygen desaturation in mitochondrial disorders–evaluation of two diagnostic screening protocols. *Journal of Neurology*, *253*(4), 417–423. https://doi.org/10.1007/s00415-006-0987-0 [doi]

Hargreaves, M., & Spriet, L. L. (2020). Skeletal muscle energy metabolism during exercise. *Nature Metabolism*, *2*(9), 817–828. https://doi.org/10.1038/s42255-020-0251-4

Harjuhaahto, S., Rasila, T. S., Molchanova, S. M., Woldegebriel, R., Kvist, J., Konovalova, S., Sainio, M. T., Pennonen, J., Torregrosa-Muñumer, R., Ibrahim, H., Otonkoski, T., Taira, T., Ylikallio, E., & Tyynismaa, H. (2020). ALS and Parkinson's disease genes CHCHD10 and CHCHD2 modify synaptic transcriptomes in human iPSC-derived motor neurons. *Neurobiology of Disease*, *141*, 104940. https://doi.org/10.1016/j.nbd.2020.104940

Harvey, K. L., Holcomb, L. E., & Kolwicz, J., Stephen C. ,. (2019). Ketogenic Diets and Exercise Performance. *Nutrients*, *11*(10), 2296. https://doi.org/10.3390/nu11102296

He, J., Fu, J., Zhao, W., Ren, C., Liu, P., Chen, L., Li, D., Zhou, L., Tang, L., Liu, X., Ye, S., Liu, X., Ma, Y., Zhang, Y., Ma, X., Zhang, L., Zhang, G., Li, N., & Fan, D. (2022). Exercise Physiology Impairments of Patients With Amyotrophic Lateral Sclerosis: Cardiopulmonary Exercise Testing Findings. *Frontiers in Physiology*, *13*, 792660. https://doi.org/10.3389/fphys.2022.792660

Heiman-Patterson, T. D., Argov, Z., Chavin, J. M., Kalman, B., Alder, H., DiMauro, S., Bank, W., & Tahmoush, A. J. (1997). Biochemical and genetic

studies in a family with mitochondrial myopathy. *Muscle & Nerve*, *20*(10), 1219–1224. https://doi.org/10.1002/(sici)1097-4598(199710)20:10<1219::aid-mus2>3.0.co;2-f

Heinicke, K., Taivassalo, T., Wyrick, P., Wood, H., Babb, T. G., & Haller, R. G. (2011). Exertional dyspnea in mitochondrial myopathy: Clinical features and physiological mechanisms. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology*, *301*(4), 873. https://doi.org/10.1152/ajpregu.00001.2011 [doi]

Hellsten, Y. (2016). Limitations of skeletal muscle oxygen supply in ageing. *The Journal of Physiology*, *594*(8), 2259–2260. https://doi.org/10.1113/JP272062

Heydemann, A. (2018). Skeletal Muscle Metabolism in Duchenne and Becker Muscular Dystrophy-Implications for Therapies. *Nutrients*, *10*(6), 796. https://doi.org/10.3390/nu10060796

Hirano, M., & Pitceathly, R. D. S. (2023). Progressive external ophthalmoplegia. *Handbook of Clinical Neurology*, *194*, 9–21. https://doi.org/10.1016/B978-0-12-821751-1.00018-X

Hogrel, J. Y., Laforêt, P., Ben Yaou, R., Chevrot, M., Eymard, B., & Lombès, A. (2001). A non-ischemic forearm exercise test for the screening of patients with exercise intolerance. *Neurology*, *56*(12), 1733–1738. https://doi.org/10.1212/wnl.56.12.1733

Hogrel, J.-Y., Janssen, J. B. E., Ledoux, I., Ollivier, G., Béhin, A., Stojkovic, T., Eymard, B., Voermans, N. C., & Laforet, P. (2017). The diagnostic value of hyperammonaemia induced by the non-ischaemic forearm exercise test. *Journal of Clinical Pathology*, *70*(10), 896–898. https://doi.org/10.1136/jclinpath-2017-204324

Houten, S. M., Violante, S., Ventura, F. V., & Wanders, R. J. A. (2016). The Biochemistry and Physiology of Mitochondrial Fatty Acid β-Oxidation and Its Genetic Disorders. *Annual Review of Physiology*, *78*, 23–44. https://doi.org/10.1146/annurev-physiol-021115-105045

Hsu, Y.-H. R., Yogasundaram, H., Parajuli, N., Valtuille, L., Sergi, C., & Oudit, G. Y. (2016). MELAS syndrome and cardiomyopathy: Linking mitochondrial function to heart failure pathogenesis. *Heart Failure Reviews*, *21*(1), 103–116. https://doi.org/10.1007/s10741-015-9524-5

Hughes, D. C., Ellefsen, S., & Baar, K. (2018). Adaptations to Endurance and Strength Training. *Cold Spring Harbor Perspectives in Medicine*, *8*(6), a029769. https://doi.org/10.1101/cshperspect.a029769

Hui, S., Ghergurovich, J. M., Morscher, R. J., Jang, C., Teng, X., Lu, W., Esparza, L. A., Reya, T., Le Zhan, null, Yanxiang Guo, J., White, E., & Rabinowitz, J. D. (2017). Glucose feeds the TCA cycle via circulating lactate. *Nature*, *551*(7678), 115–118. https://doi.org/10.1038/nature24057

Hunter, G. R., Newcomer, B. R., Weinsier, R. L., Karapondo, D. L., Larson-Meyer, D. E., Joanisse, D. R., & Bamman, M. M. (2002). Age is independently related to muscle metabolic capacity in premenopausal women. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *93*(1), 70–76. https://doi.org/10.1152/japplphysiol.01239.2001

Hunter, S. K., & Senefeld, J. W. (2024). Sex differences in human performance. *The Journal of Physiology*, *602*(17), 4129–4156. https://doi.org/10.1113/JP284198

Hurley, B. F., Nemeth, P. M., Martin, W. H., Hagberg, J. M., Dalsky, G. P., & Holloszy, J. O. (1986). Muscle triglyceride utilization during exercise: Effect of training. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *60*(2), 562–567. https://doi.org/10.1152/jappl.1986.60.2.562

Ionescu, M. F., Mani-Babu, S., Degani-Costa, L. H., Johnson, M., Paramasivan, C., Sylvester, K., & Fuld, J. (2021). Cardiopulmonary Exercise Testing in the Assessment of Dysfunctional Breathing. *Frontiers in Physiology*, *11*, 620955. https://doi.org/10.3389/fphys.2020.620955 [doi]

Itoh, H., & Ohkuwa, T. (1993). Blood ammonia concentration after supramaximal treadmill running in males and females. *The Journal of Sports Medicine and Physical Fitness*, *33*(3), 239–245.

Järvilehto, J., Harjuhaahto, S., Palu, E., Auranen, M., Kvist, J., Zetterberg, H., Koskivuori, J., Lehtonen, M., Saukkonen, A. M., Jokela, M., Ylikallio, E., & Tyynismaa, H. (2022). Serum Creatine, Not Neurofilament Light, Is Elevated in CHCHD10-Linked Spinal Muscular Atrophy. *Frontiers in Neurology*, *13*, 793937. https://doi.org/10.3389/fneur.2022.793937

Jensen, T. D., Kazemi-Esfarjani, P., Skomorowska, E., & Vissing, J. (2002). A forearm exercise screening test for mitochondrial myopathy. *Neurology*, *58*(10), 1533–1538. https://doi.org/10.1212/wnl.58.10.1533

Jeppesen, T. D., Madsen, K. L., Poulsen, N. S., Løkken, N., & Vissing, J. (2021). finstere *Journal of Clinical Medicine,* 10(8), 1796. https://doi.org/10.3390/jcm10081796

Jeppesen, T. D., Schwartz, M., Olsen, D. B., & Vissing, J. (2003). Oxidative capacity correlates with muscle mutation load in mitochondrial myopathy. *Annals of Neurology*, *54*(1), 86–92. https://doi.org/10.1002/ana.10594

Jeppesen, T. D., Vissing, J., & González-Alonso, J. (2012). Influence of erythrocyte oxygenation and intravascular ATP on resting and exercising skeletal muscle blood flow in humans with mitochondrial myopathy. *Mitochondrion*, 12(3), 414–422. https://doi.org/10.1016/j.mito.2011.11.003

Jokela, M., Penttilä, S., Huovinen, S., Hackman, P., Saukkonen, A. M., Toivanen, J., & Udd, B. (2011). Late-onset lower motor neuronopathy: A new autosomal dominant disorder. *Neurology*, *77*(4), 334–340. https://doi.org/10.1212/WNL.0b013e3182267b71

Kaminsky, L. A., Arena, R., Myers, J., Peterman, J. E., Bonikowske, A. R., Harber, M. P., Medina Inojosa, J. R., Lavie, C. J., & Squires, R. W. (2022). Updated Reference Standards for Cardiorespiratory Fitness Measured with Cardiopulmonary Exercise Testing: Data from the Fitness Registry and the Importance of Exercise National Database (FRIEND). *Mayo Clinic Proceedings*, *97*(2), 285–293. https://doi.org/10.1016/j.mayocp.2021.08.020

Kinnula, V. L., Sovijärvi, A. R. A. (1993). Elevated Ventilatory Equivalents during Exercise in Patients with Hyperventilation Syndrome. In *Respiration* (Vol. 60, Issue 5, pp. 273–278). https://doi.org/10.1159/000196215

Knuuti, J., Wijns, W., Saraste, A., Capodanno, D., Barbato, E., Funck-Brentano, C., Prescott, E., Storey, R. F., Deaton, C., Cuisset, T., Agewall, S., Dickstein, K., Edvardsen, T., Escaned, J., Gersh, B. J., Svitil, P., Gilard, M., Hasdai, D., Hatala, R., … ESC Scientific Document Group. (2020). 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *European Heart Journal*, *41*(3), 407–477. https://doi.org/10.1093/eurheartj/ehz425

Kossoff, E. H., & Dorward, J. L. (2008). The Modified Atkins Diet. *Epilepsia*, *49*, 37–41. https://doi.org/10.1111/j.1528-1167.2008.01831.x

Kraut, J. A., & Madias, N. E. (2010). Metabolic acidosis: Pathophysiology, diagnosis and management. *Nature Reviews. Nephrology*, *6*(5), 274–285. https://doi.org/10.1038/nrneph.2010.33

Lindholm, H., Löfberg, M., Somer, H., Näveri, H., & Sovijärvi, A. (2004). Abnormal blood lactate accumulation after exercise in patients with multiple mitochondrial DNA deletions and minor muscular symptoms. *Clinical Physiology and Functional Imaging*, *24*(2), 109–115. https://doi.org/10.1111/j.1475-097X.2004.00531.x

Liu, H., Yang, Y., Wang, Y., Tang, H., Zhang, F., Zhang, Y., & Zhao, Y. (2018). Ketogenic diet for treatment of intractable epilepsy in adults: A meta-analysis of observational studies. *Epilepsia Open*, *3*(1), 9–17. https://doi.org/10.1002/epi4.12098

Lo, P. Y., & Dudley, G. A. (1987). Endurance training reduces the magnitude of exercise-induced hyperammonemia in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *62*(3), 1227–1230. https://doi.org/10.1152/jappl.1987.62.3.1227

Löfberg, M., Lindholm, H., Näveri, H., Majander, A., Suomalainen, A., Paetau, A., Sovijärvi, A., Härkönen, M., & Somer, H. (2001). ATP, phosphocreatine and lactate in exercising muscle in mitochondrial disease and McArdle's disease. *Neuromuscular Disorders*, *11*(4), 370–375. https://doi.org/10.1016/S0960-8966(00)00205-4

Longo, N., Frigeni, M., & Pasquali, M. (2016). Carnitine transport and fatty acid oxidation. *Biochimica Et Biophysica Acta*, *1863*(10), 2422–2435. https://doi.org/10.1016/j.bbamcr.2016.01.023

Lowenstein, J. M. (1972). Ammonia production in muscle and other tissues: The purine nucleotide cycle. *Physiological Reviews*, *52*(2), 382–414. https://doi.org/10.1152/physrev.1972.52.2.382

Majamaa, K., Moilanen, J. S., Uimonen, S., Remes, A. M., Salmela, P. I., Kärppä, M., Majamaa-Voltti, K. A., Rusanen, H., Sorri, M., Peuhkurinen, K. J., & Hassinen, I. E. (1998). Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: Prevalence of the mutation in an adult population. *American Journal of Human Genetics*, *63*(2), 447–454. https://doi.org/10.1086/301959

Malmberg, L. P., Tamminen, K., & R.A, A. S. (2000). Orthostatic increase of respiratory gas exchange in hyperventilation syndrome. *Thorax*, *55*(4), 295. https://doi.org/10.1136/thorax.55.4.295

Maltais, M. L., Desroches, J., & Dionne, I. J. (2009). Changes in muscle mass and strength after menopause. *Journal of Musculoskeletal & Neuronal Interactions*, *9*(4), 186–197.

Mancuso, M., Orsucci, D., Angelini, C., Bertini, E., Carelli, V., Comi, G. P., Donati, A., Minetti, C., Moggio, M., Mongini, T., Servidei, S., Tonin, P., Toscano, A., Uziel, G., Bruno, C., Ienco, E. C., Filosto, M., Lamperti, C., Catteruccia, M., Moroni, I., … Siciliano, G. (2014). The m.3243A>G mitochondrial DNA mutation and related phenotypes. A matter of gender?. *Journal of Neurology,* 261(3), 504–510. https://doi.org/10.1007/s00415-013-7225-3

Manwaring, N., Jones, M. M., Wang, J. J., Rochtchina, E., Howard, C., Mitchell, P., & Sue, C. M. (2007). Population prevalence of the MELAS A3243G mutation. *Mitochondrion,* 7(3), 230–233. https://doi.org/10.1016/j.mito.2006.12.004

Martikainen, M. H., & Majamaa, K. (2024). Incidence and prevalence of mtDNA-related adult mitochondrial disease in Southwest Finland, 2009-2022: An observational, population-based study. *BMJ Neurology Open,* 6(1), e000546. https://doi.org/10.1136/bmjno-2023-000546

Massé-Biron, J., Mercier, J., Collomp, K., Hardy, J. M., & Préfaut, C. (1992). Age and training effects on the lactate kinetics of master athletes during maximal exercise. *European Journal of Applied Physiology and Occupational Physiology,* 65(4), 311–315. https://doi.org/10.1007/BF00868133

Mattei, J. P., Bendahan, D., & Cozzone, P. (2004). P-31 magnetic resonance spectroscopy. A tool for diagnostic purposes and pathophysiological insights in muscle diseases. *Reumatismo*, 56(1), 9–14. https://doi.org/10.4081/reumatismo.2004.9

McArdle, B. (1951). Myopathy due to a defect in muscle glycogen breakdown. *Clinical Science*, *10*(1), 13–35.

McFarland, R., Taylor, R. W., & Turnbull, D. M. (2010). A neurological perspective on mitochondrial disease. *The Lancet. Neurology*, 9(8), 829–840. https://doi.org/10.1016/S1474-4422(10)70116-2

McSwiney, F. T., Doyle, L., Plews, D. J., & Zinn, C. (2019). Impact Of Ketogenic Diet On Athletes: Current Insights. *Open Access Journal of Sports Medicine*, *10*, 171–183. https://doi.org/10.2147/OAJSM.S180409

Meijer, E. P., Westerterp, K. R., & Verstappen, F. T. (2000). Effect of exercise training on physical activity and substrate utilization in the elderly. *International Journal of Sports Medicine*, *21*(7), 499–504. https://doi.org/10.1055/s-2000-7419

Meira, I. D., Romão, T. T., Pires, do P., Krüger, L. T., Pires, M. E. P., & Conceição, P. O. da. (2019). Ketogenic Diet and Epilepsy: What We Know So Far. *Frontiers in Neuroscience*, *13*, 5. https://doi.org/10.3389/fnins.2019.00005

Mezzani, A. (2017). Cardiopulmonary Exercise Testing: Basics of Methodology and Measurements. *Annals of the American Thoracic Society*, *14*(Supplement_1), S3–S11. https://doi.org/10.1513/AnnalsATS.201612-997FR

Milone, M., & Wong, L.-J. (2013). Diagnosis of mitochondrial myopathies. *Molecular Genetics and Metabolism*, 110(1), 35–41. https://doi-org.libproxy.helsinki.fi/10.1016/j.ymgme.2013.07.007

Mineo, I., Kono, N., Shimizu, T., Hara, N., Yamada, Y., Sumi, S., Nonaka, K., & Tarui, S. (1985). Excess purine degradation in exercising muscles of patients with glycogen storage disease types V and VII. *The Journal of Clinical Investigation*, *76*(2), 556–560. https://doi.org/10.1172/JCI112006

Miyajima, H., Sakamoto, M., Takahashi, Y., Mizoguchi, K., & Nishimura, Y. (1989). [Muscle carnitine deficiency associated with myalgia and rhabdomyolysis following exercise]. *Rinsho Shinkeigaku = Clinical Neurology*, *29*(1), 93–97.

Mohan, P. K., Reddy, L. V., Satyanarayana, N., & Indira, K. (1987). Age-related changes in muscle ammonia detoxification potential in exhausted rats. *Archives Internationales De Physiologie Et De Biochimie*, *95*(1), 37–42. https://doi.org/10.3109/13813458709075023

Montes, J., Goodwin, A. M., McDermott, M. P., Uher, D., Hernandez, F. M., Coutts, K., Cocchi, J., Hauschildt, M., Cornett, K. M., Rao, A. K., Monani, U. R., Ewing Garber, C., & De Vivo, D. C. (2021). Diminished muscle oxygen uptake and fatigue in spinal muscular atrophy. *Annals of Clinical and Translational Neurology*, *8*(5), 1086–1095. https://doi.org/10.1002/acn3.51353

Mouadil, A., Debout, C., Read, M. H., Morello, R., Allouche, S., & Chapon, F. (2012). Blood metabolite data in response to maximal exercise in healthy subjects. *Clinical Physiology and Functional Imaging*, *32*(4), 274–281. https://doi.org/10.1111/j.1475-097X.2012.01122.x [doi]

Müller, K., Andersen, P. M., Hübers, A., Marroquin, N., Volk, A. E., Danzer, K. M., Meitinger, T., Ludolph, A. C., Strom, T. M., & Weishaupt, J. H. (2014). Two novel mutations in conserved codons indicate that CHCHD10 is a gene associated with motor neuron disease. *Brain: A Journal of Neurology*, *137*(Pt 12), e309. https://doi.org/10.1093/brain/awu227

Nashef, L., & Lane, R. J. (1989). Screening for mitochondrial cytopathies: The sub-anaerobic threshold exercise test (SATET). *Journal of Neurology, Neurosurgery, and Psychiatry*, *52*(9), 1090–1094. https://doi.org/10.1136/jnnp.52.9.1090

Nesbitt, V., Pitceathly, R. D. S., Turnbull, D. M., Taylor, R. W., Sweeney, M. G., Mudanohwo, E. E., Rahman, S., Hanna, M. G., & McFarland, R. (2013). The UK MRC Mitochondrial Disease Patient Cohort Study: Clinical phenotypes associated with the m.3243A>G mutation--implications for diagnosis and management. *Journal of Neurology, Neurosurgery, and Psychiatry,* 84(8), 936–938. https://doi.org/10.1136/jnnp-2012-303528

Nikolac, N., Omazic, J., & Simundic, A.-M. (2014). The evidence based practice for optimal sample quality for ammonia measurement. *Clinical Biochemistry*, *47*(12), 991–995. https://doi.org/10.1016/j.clinbiochem.2014.05.068

Nordesjö L-O, Landelius J. Clinical evaluation of physical work capacity. Scand J Clin Lab Invest 1975;35:64

Ollila, L., Heliö, T., Sovijärvi, A., Jalanko, M., Kaartinen, M., Kuusisto, J., Kärkkäinen, S., Jurkko, R., Reissell, E., Palojoki, E., & Piirilä, P. (2017). Increased ventilatory response to exercise in symptomatic and asymptomatic LMNA mutation carriers: A follow-up study. *Clinical Physiology and Functional Imaging*, *37*(1), 8–16. https://doi.org/10.1111/cpf.12260

Ørngreen, M. C., Jeppesen, T. D., Taivassalo, T., Hauerslev, S., Preisler, N., Heinicke, K., Haller, R. G., Vissing, J., & van Hall, G. (2015). Lactate and Energy Metabolism During Exercise in Patients With Blocked Glycogenolysis (McArdle Disease). *The Journal of Clinical Endocrinology and Metabolism*, *100*(8), E1096-1104. https://doi.org/10.1210/jc.2015-1339

Ørngreen, M. C., & Vissing, J. (2017). Treatment Opportunities in Patients With Metabolic Myopathies. *Current Treatment Options in Neurology*, *19*(11), 37. https://doi.org/10.1007/s11940-017-0473-2

Parikh, S., Goldstein, A., Koenig, M. K., Scaglia, F., Enns, G. M., Saneto, R., Anselm, I., Cohen, B. H., Falk, M. J., Greene, C., Gropman, A. L., Haas, R., Hirano, M., Morgan, P., Sims, K., Tarnopolsky, M., Van Hove, J. L., Wolfe, L., & DiMauro, S. (2015). Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. *Genetics in medicine: official journal of the American College of Medical Genetics,* 17(9), 689–701. https://doi.org/10.1038/gim.2014.177

Parikh, S., Karaa, A., Goldstein, A., Bertini, E. S., Chinnery, P. F., Christodoulou, J., Cohen, B. H., Davis, R. L., Falk, M. J., Fratter, C., Horvath, R., Koenig, M. K., Mancuso, M., McCormack, S., McCormick, E. M., McFarland, R., Nesbitt, V., Schiff, M., Steele, H., … Rahman, S. (2019). Diagnosis of "possible" mitochondrial disease: An existential crisis. *Journal of Medical Genetics*, *56*(3), 123–130. https://doi.org/10.1136/jmedgenet-2018-105800

Park, E. G., Lee, J., & Lee, J. (2018). Use of the Modified Atkins Diet in Intractable Pediatric Epilepsy. *Journal of Epilepsy Research*, *8*(1), 20–26. https://doi.org/10.14581/jer.18004

Parolin, M. L., Chesley, A., Matsos, M. P., Spriet, L. L., Jones, N. L., & Heigenhauser, G. J. (1999). Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. *The American Journal of Physiology*, *277*(5), E890-900. https://doi.org/10.1152/ajpendo.1999.277.5.E890

Pasanen, P., Myllykangas, L., Pöyhönen, M., Kiuru-Enari, S., Tienari, P. J., Laaksovirta, H., Toppila, J., Ylikallio, E., Tyynismaa, H., & Auranen, M. (2016). Intrafamilial clinical variability in individuals carrying the CHCHD10 mutation Gly66Val. *Acta Neurologica Scandinavica*, *133*(5), 361–366. https://doi.org/10.1111/ane.12470

Pavlakis, S. G., Phillips, P. C., DiMauro, S., De Vivo, D. C., & Rowland, L. P. (1984). Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: A distinctive clinical syndrome. *Annals of Neurology,* 16(4), 481–488. https://doi.org/10.1002/ana.410160409

Pellegrino, A., Tiidus, P. M., & Vandenboom, R. (2022). Mechanisms of Estrogen Influence on Skeletal Muscle: Mass, Regeneration, and Mitochondrial Function. *Sports Medicine (Auckland, N.Z.)*, *52*(12), 2853–2869. https://doi.org/10.1007/s40279-022-01733-9

Pennisi, E. M., Garibaldi, M., & Antonini, G. (2018). Lipid Myopathies. *Journal of Clinical Medicine*, *7*(12), 472. https://doi.org/10.3390/jcm7120472

Penttilä, S., Jokela, M., Bouquin, H., Saukkonen, A. M., Toivanen, J., & Udd, B. (2015). Late onset spinal motor neuronopathy is caused by mutation in CHCHD10. *Annals of Neurology*, *77*(1), 163–172. https://doi.org/10.1002/ana.24319

Penttilä, S., Jokela, M., Huovinen, S., Saukkonen, A. M., Toivanen, J., Lindberg, C., Baumann, P., & Udd, B. (2014). Late-onset spinal motor neuronopathy—A common form of dominant SMA. *Neuromuscular Disorders: NMD*, *24*(3), 259–268. https://doi.org/10.1016/j.nmd.2013.11.010

Piirilä, P., E, M. S., Palmio, J., Wuorimaa, T., Ylikallio, E., Sandell, S., Haapalahti, P., Uotila, L., Tyynismaa, H., Udd, B., & Auranen, M. (2016). Unique Exercise Lactate Profile in Muscle Phosphofructokinase Deficiency (Tarui Disease); Difference Compared with McArdle Disease. *Frontiers in Neurology*, *7*, 82. https://doi.org/10.3389/fneur.2016.00082

Pirinen, E., Auranen, M., Khan, N. A., Brilhante, V., Urho, N., Pessia, A., Hakkarainen, A., Kuula, J., Heinonen, U., Schmidt, M. S., Haimilahti, K., Piirilä, P., Lundbom, N., Taskinen, M.-R., Brenner, C., Velagapudi, V., Pietiläinen, K. H., & Suomalainen, A. (2020). Niacin Cures Systemic NAD+ Deficiency and Improves Muscle Performance in Adult-Onset Mitochondrial Myopathy. *Cell Metabolism*, *31*(6), 1078-1090.e5. https://doi.org/10.1016/j.cmet.2020.04.008

Richter, E. A., Ruderman, N. B., Gavras, H., Belur, E. R., & Galbo, H. (1982). Muscle glycogenolysis during exercise: Dual control by epinephrine and contractions. *The American Journal of Physiology*, *242*(1), E25-32. https://doi.org/10.1152/ajpendo.1982.242.1.E25

Roef, M. J., Kalhan, S. C., Reijngoud, D.-J., De Meer, K., & Berger, R. (2002). Lactate Disposal via Gluconeogenesis Is Increased During Exercise in Patients with Mitochondrial Myopathy Due to Complex I Deficiency. *Pediatric Research*, *51*(5), 592–597. https://doi.org/10.1203/00006450-200205000-00008

Rowlands, D. S., & Hopkins, W. G. (2002). Effects of high-fat and high-carbohydrate diets on metabolism and performance in cycling. In *Metabolism* (Vol. 51, Issue 6, pp. 678–690). https://doi.org///doi.org/10.1053/meta.2002.32723

Ruan, Y., Hu, J., Che, Y., Liu, Y., Luo, Z., Cheng, J., Han, Q., He, H., & Zhou, Q. (2022). CHCHD2 and CHCHD10 regulate mitochondrial dynamics and integrated stress response. *Cell Death & Disease*, *13*(2), 156. https://doi.org/10.1038/s41419-022-04602-5

Rubini, A., Bosco, G., Lodi, A., Cenci, L., Parmagnani, A., Grimaldi, K., Zhongjin, Y., & Paoli, A. (2015). Effects of Twenty Days of the Ketogenic Diet on Metabolic and Respiratory Parameters in Healthy Subjects. *Lung*, *193*(6), 939–945. https://doi.org/10.1007/s00408-015-9806-7

Rusecka, J., Kaliszewska, M., Bartnik, E., & Tońska, K. (2018). Nuclear genes involved in mitochondrial diseases caused by instability of mitochondrial DNA. *Journal of Applied Genetics,* 59(1), 43–57. https://doi.org/10.1007/s13353-017-0424-3

Sabina, R. L., Swain, J. L., Olanow, C. W., Bradley, W. G., Fishbein, W. N., DiMauro, S., & Holmes, E. W. (1984). Myoadenylate deaminase deficiency. Functional and metabolic abnormalities associated with disruption of the purine nucleotide cycle. *The Journal of Clinical Investigation*, *73*(3), 720–730. https://doi.org/10.1172/JCI111265

Schaefer, A. M., McFarland, R., Blakely, E. L., He, L., Whittaker, R. G., Taylor, R. W., Chinnery, P. F., & Turnbull, D. M. (2008). Prevalence of mitochondrial DNA disease in adults. *Annals of Neurology,* 63(1), 35–39. https://doi.org/10.1002/ana.21217

Seliger, V., Mácek, M., Skranc, O., Horák, J., Piric, J., Handzo, P., Rous, J., & Jirka, Z. (1978). Work capacity of the Czechoslovakian population. *European Journal of Applied Physiology and Occupational Physiology*, *39*(3), 155–164. https://doi.org/10.1007/BF00421342

Shammas, M. K., Huang, X., Wu, B. P., Fessler, E., Song, I. Y., Randolph, N. P., Li, Y., Bleck, C. K., Springer, D. A., Fratter, C., Barbosa, I. A., Powers, A. F., Quirós, P. M., Lopez-Otin, C., Jae, L. T., Poulton, J., & Narendra, D. P. (2022). OMA1 mediates local and global stress responses against protein misfolding in CHCHD10 mitochondrial myopathy. *The Journal of Clinical Investigation*, *132*(14), e157504. https://doi.org/10.1172/JCI157504

Sietsema, K. E., & Rossiter, H. B. (2023). Exercise Physiology and Cardiopulmonary Exercise Testing. *Seminars in Respiratory and Critical Care Medicine*, *44*(5), 661–680. https://doi.org/10.1055/s-0043-1770362

Similä, M. E., Auranen, M., & Piirilä, P. L. (2020). Beneficial Effects of Ketogenic Diet on Phosphofructokinase Deficiency (Glycogen Storage Disease Type VII). *Frontiers in Neurology*, *11*, 57. https://doi.org/10.3389/fneur.2020.00057

Sjödin, B., & Jacobs, I. (1981). Onset of blood lactate accumulation and marathon running performance. *International Journal of Sports Medicine*, *2*(1), 23–26. https://doi.org/10.1055/s-2008-1034579

Spriet, L. L., & Peters, S. J. (1998). Influence of diet on the metabolic responses to exercise. *The Proceedings of the Nutrition Society*, *57*(1), 25–33. https://doi.org/10.1079/pns19980006

Straub, I. R., Weraarpachai, W., & Shoubridge, E. A. (2021). Multi-OMICS study of a CHCHD10 variant causing ALS demonstrates metabolic rewiring and activation of endoplasmic reticulum and mitochondrial unfolded protein responses. *Human Molecular Genetics*, *30*(8), 687–705. https://doi.org/10.1093/hmg/ddab078

Swerdlow, R. H. (2018). Mitochondria and Mitochondrial Cascades in Alzheimer's Disease. *Journal of Alzheimer's Disease: JAD*, *62*(3), 1403–1416. https://doi.org/10.3233/JAD-170585

Taivassalo, T., Abbott, A., Wyrick, P., & Haller, R. G. (2002). Venous oxygen levels during aerobic forearm exercise: An index of impaired oxidative metabolism in mitochondrial myopathy. *Annals of Neurology*, *51*(1), 38–44. https://doi.org/10.1002/ana.10027 [pii]

Taivassalo, T., Jensen, T. D., Kennaway, N., DiMauro, S., Vissing, J., & Haller, R. G. (2003). The spectrum of exercise tolerance in mitochondrial myopathies: A study of 40 patients. *Brain : A Journal of Neurology*, *126*(Pt 2), 413–423. https://doi.org/10.1093/brain/awg028 [doi]

Tarnopolsky, M. (2012). Exercise testing in metabolic myopathies. *Physical Medicine and Rehabilitation Clinics of North America*, 23(1), 173–186, xii. https://doi.org/10.1016/j.pmr.2011.11.011

Tarnopolsky, M. A. (2016). Metabolic Myopathies. *CONTINUUM: Lifelong Learning in Neurology*, *22*(6). https://journals.lww.com/continuum/Fulltext/2016/12000/Metabolic_Myopathies.9.aspx

Tarnopolsky, M. A. (2022). Metabolic Myopathies. *Continuum (Minneapolis, Minn.)*, *28*(6), 1752–1777. https://doi.org/10.1212/CON.0000000000001182

Tavel, M. E. (2021). Hyperventilation Syndrome: Why Is It Regularly Overlooked? *The American Journal of Medicine*, *134*(1), 13–15. https://doi.org/S0002-9343(20)30678-1 [pii]

Taverna, S., Cammarata, G., Colomba, P., Sciarrino, S., Zizzo, C., Francofonte, D., Zora, M., Scalia, S., Brando, C., Curto, A. L., Marsana, E. M., Olivieri, R., Vitale, S., & Duro, G. (2020). Pompe disease: Pathogenesis, molecular genetics and diagnosis. *Aging*, *12*(15), 15856–15874. https://doi.org/10.18632/aging.103794

Taylor, R. W., & Turnbull, D. M. (2005). Mitochondrial DNA mutations in human disease. *Nature Reviews. Genetics,* 6(5), 389–402. https://doi.org/10.1038/nrg1606

Tobon, A. (2013). Metabolic myopathies. *Continuum (Minneapolis, Minn.)*, *19*(6 Muscle Disease), 1571–1597. https://doi.org/10.1212/01.CON.0000440660.41675.06

Uusimaa, J., Moilanen, J. S., Vainionpää, L., Tapanainen, P., Lindholm, P., Nuutinen, M., Löppönen, T., Mäki-Torkko, E., Rantala, H., & Majamaa, K. (2007). Prevalence, segregation, and phenotype of the mitochondrial DNA 3243A>G mutation in children. *Annals of Neurology*, *62*(3), 278–287. https://doi.org/10.1002/ana.21196

Van den Berghe, G., Bontemps, F., Vincent, M. F., & Van den Bergh, F. (1992). The purine nucleotide cycle and its molecular defects. *Progress in Neurobiology*, *39*(5), 547–561. https://doi.org/10.1016/0301-0082(92)90006-z

van Loon, L. J., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H., & Wagenmakers, A. J. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of Physiology*, *536*(Pt 1), 295–304. https://doi.org/10.1111/j.1469-7793.2001.00295.x

Vansteenkiste, J., Rochette, F., & Demedts, M. (1991). Diagnostic tests of hyperventilation syndrome. *The European Respiratory Journal*, *4*(4), 393–399.

Veneman, T., Koopman, F. S., Oorschot, S., de Koning, J. J., Bongers, B. C., Nollet, F., & Voorn, E. L. (2024). Validity of Cardiopulmonary Exercise Testing for Assessing Aerobic Capacity in Neuromuscular Diseases. *Archives of Physical Medicine and Rehabilitation*, *105*(10), 1846–1853. https://doi.org/10.1016/j.apmr.2024.07.006

Vengalil, S., Preethish-Kumar, V., Polavarapu, K., Christopher, R., Gayathri, N., Natarajan, A., Manjunath, M., Nashi, S., Prasad, C., & Nalini, A. (2017). Fatty acid oxidation defects presenting as primary myopathy and prominent dropped head syndrome. *Neuromuscular Disorders: NMD*, *27*(11), 986–996. https://doi.org/10.1016/j.nmd.2017.08.004

Volek, J. S., Sharman, M. J., Love, D. M., Avery, N. G., G[oacute]mez, A. L., Scheett, T. P., & Kraemer, W. J. (2002). Body composition and hormonal responses to a carbohydrate-restricted diet. *Metabolism*, *51*(7), 864–870. https://doi-org.libproxy.helsinki.fi/10.1053/meta.2002.32037

Wang, W., Zhao, F., Ma, X., Perry, G., & Zhu, X. (2020). Mitochondria dysfunction in the pathogenesis of Alzheimer's disease: Recent advances. *Molecular Neurodegeneration*, *15*, 30. https://doi.org/10.1186/s13024-020-00376-6

Ward, S. A. (2018). Determinants of the physiological systems responses to muscular exercise in healthy subjects. In P. Palange, P. Laveneziana, J. A. Neder, & S. A. Ward (Eds.), *Clinical Exercise Testing* (pp. 1–33). European Respiratory Society. https://doi.org/10.1183/2312508X.10010917

Withers, R. T., Sherman, W. M., Clark, D. G., Esselbach, P. C., Nolan, S. R., Mackay, M. H., & Brinkman, M. (1991). Muscle metabolism during 30, 60 and 90 s of maximal cycling on an air-braked ergometer. *European Journal of Applied Physiology and Occupational Physiology*, *63*(5), 354–362. https://doi.org/10.1007/BF00364462

Yamato, M., Muto, Y., Yoshida, T., Kato, M., & Moriwaki, H. (1995). Clearance rate of plasma branched-chain amino acids correlates significantly with blood ammonia level in patients with liver cirrhosis. *International*

Hepatology Communications, *3*(2), 91–96. https://doi.org/10.1016/0928-4346(94)00159-3

Yancy, W. S., Olsen, M. K., Dudley, T., & Westman, E. C. (2007). Acid-base analysis of *individuals* following two weight loss diets. European Journal of Clinical Nutrition, 61(12), 1416–1422. https://doi.org/10.1038/sj.ejcn.1602661

Yakupova, E. I., Bocharnikov, A. D., & Plotnikov, E. Y. (2022). Effects of Ketogenic Diet on Muscle Metabolism in Health and Disease. *Nutrients,* 14(18), 3842. https://doi.org/10.3390/nu14183842

Ye, H., Robak, L. A., Yu, M., Cykowski, M., & Shulman, J. M. (2023). Genetics and Pathogenesis of Parkinson's Syndrome. *Annual Review of Pathology*, *18*, 95–121. https://doi.org/10.1146/annurev-pathmechdis-031521-034145

Yuan, Y., & Chan, K. M. (2000). A review of the literature on the application of blood ammonia measurement in sports science. *Research Quarterly for Exercise and Sport*, *71*(2), 145–151. https://doi.org/10.1080/02701367.2000.10608892

Zajac, A., Poprzecki, S., Maszczyk, A., Czuba, M., Michalczyk, M., & Zydek, G. (2014). The effects of a ketogenic diet on exercise metabolism and physical performance in off-road cyclists. *Nutrients*, *6*(7), 2493–2508. https://doi.org/10.3390/nu6072493

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