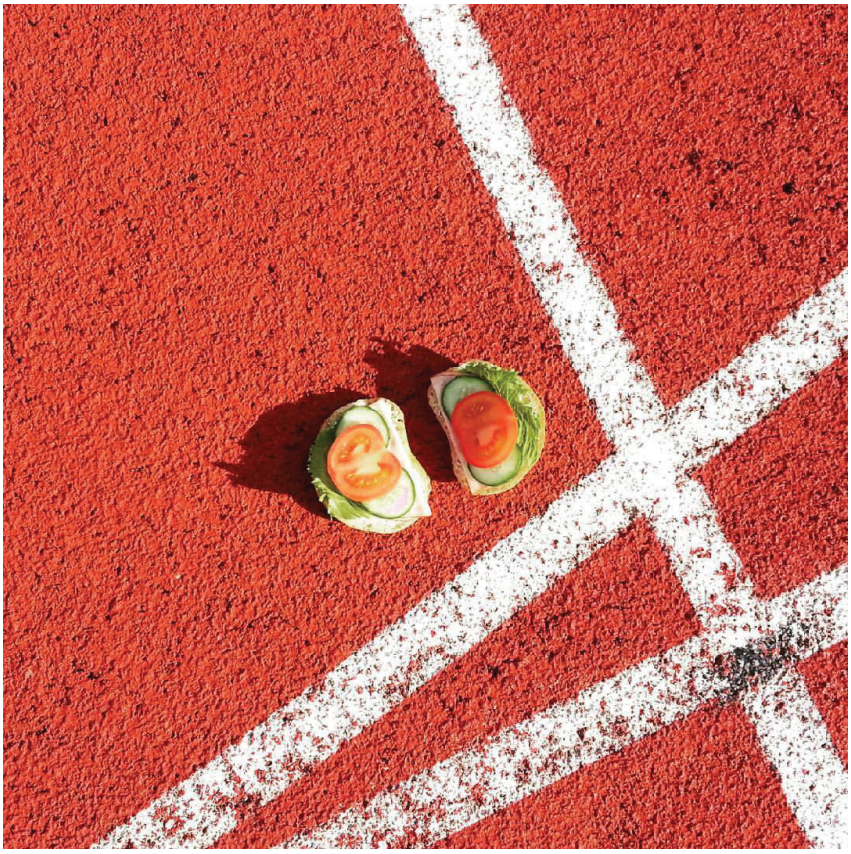
STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH
268

Enni-Maria Hietavala

Dietary Acid Load and Acid-Base Balance in Exercise and Health from Adolescence to Late Adulthood



JYVÄSKYLÄN YLIOPISTO

STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 268

Enni-Maria Hietavala

Dietary Acid Load and Acid-Base Balance in Exercise and Health from Adolescence to Late Adulthood

Esitetään Jyväskylän yliopiston liikuntatieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Liikunnan salissa L304
huhtikuun 21. päivänä 2018 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
in building Liikunta, hall L304, on April 21, 2018 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2018

Dietary Acid Load and Acid-Base Balance in Exercise and Health from Adolescence to Late Adulthood

STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 268

Enni-Maria Hietavala

Dietary Acid Load and Acid-Base Balance in Exercise and Health from Adolescence to Late Adulthood

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2018

Editors
Taija Juutinen
Faculty of Sport and Health Sciences, University of Jyväskylä
Sini Tuikka
Publishing Unit, University Library of Jyväskylä

Cover picture by Enni-Maria Hietavala.

Permanent link to this publication: http://urn.fi/URN:ISBN:978-951-39-7404-6

URN:ISBN:978-951-39-7404-6
ISBN 978-951-39-7404-6 (PDF)

ISBN 978-951-39-7403-9 (nid.)
ISSN 0356-1070

Jyväskylä University Printing House, Jyväskylä 2018

Look up at the stars and not down at your feet. Try to make sense of what you see, and wonder about what makes the universe exist. Be curious.

Stephen Hawking

ABSTRACT

Hietavala, Enni-Maria
Dietary acid load and acid-base balance in exercise and health from adolescence to late adulthood
Jyväskylä: University of Jyväskylä, 2018, 87 p.
(Studies in Sport, Physical Education and Health
ISSN 0356-1070; 268)
ISBN 978-951-39-7403-9 (nid.)
ISBN 978-951-39-7404-6 (PDF)

This thesis investigated whether dietary acid load has either short-term (4 to 7 days) or prolonged (12 weeks) effects on acid-base status at rest and during submaximal and maximal aerobic exercise; whether the effects of dietary acid load on acid-base status differ between adolescents, young adults and the elderly, and between men and women; and whether the changes in acid-base balance have a further effect on aerobic exercise performance. These questions were addressed in three different study settings in healthy and recreationally active men and women. In studies 1 and 2, which followed a crossover study design, participants were assigned in randomized order to follow a diet with a low or high acid load for 4 or 7 days. Study 3 was a 12-week longitudinal study in which participants were divided into two groups of lower and higher acid intake. Nine 18- to 30-year-old men participated in study 1. In study 2, 93 men and women were recruited from three age groups: 12 to 15 years, 25 to 35 years and 60 to 75 years. Forty-nine men and women aged 20 to 50 years participated in study 3. The main finding was that dietary acid load has acute and prolonged effects on blood and urine acid-base status and may also have effects on exercise performance. In young and elderly women, in particular, blood was more acidic at rest and during submaximal cycling after a 7-day high compared to low acid intake. In young women, maximal cardiorespiratory measures were lower and time to exhaustion shorter after high compared to low acid intake. During exercise, better renal function may be associated with higher bicarbonate ion availability in blood, which can diminish exercise-induced acidosis and delay fatigue. Lower kidney function in the elderly compared to younger participants, and in women compared to men may explain why the diet-induced changes in blood acid-base status were greater in the elderly participants and in women compared to younger participants and men. Moreover, even slightly acidogenic diets combined with regular training may be accompanied with increased acid load to the body and start to impair kidney function. These results emphasize the importance of an adequate intake of fruits and vegetables as a part of a healthy diet and a physically active lifestyle across the lifespan.

Keywords: dietary acid load, acid-base status, exercise performance, aging, kidney function

Author's address

Enni-Maria Hietavala, MSc
Biology of Physical Activity
Faculty of Sport and Health Sciences
University of Jyväskylä
P.O. Box 35
FI-40014 University of Jyväskylä, Finland
enni.hietavala@jyu.fi

Supervisors

Professor Antti A. Mero, PhD
Biology of Physical Activity
Faculty of Sport and Health Sciences
University of Jyväskylä
Jyväskylä, Finland

Professor Heikki Kainulainen, PhD
Biology of Physical Activity
Faculty of Sport and Health Sciences
University of Jyväskylä
Jyväskylä, Finland

Reviewers

Professor David Bishop, PhD
Institute of Sport, Exercise and Active Living
Victoria University
Melbourne, Australia

Professor Thomas Remer, PhD
Department of Nutrition and Food Science
University of Bonn
Dortmund, Germany

Opponent

Professor Lars McNaughton, PhD
The Department of Sport and Physical Activity
Edge Hill University
Ormskirk, United Kingdom

ACKNOWLEDGEMENTS

The work presented in this thesis was carried out at the Faculty of Sport and Health Sciences at the University of Jyväskylä. I would like to thank the faculty for providing me with all the facilities needed for conducting my research. This study has also been funded by Tekes and in collaboration with Tekes by the following Finnish companies: Honkatarhat Oy, Kyröntarhat Oy, Mykora Oy, Lihajaloste Korpela Oy, Laihian Mallas Oy, and KKK-Vihannes Oy / Lykobene. This study has also received funding from the Juho Vainio Foundation, Ellen and Artturi Nyyssönen Foundation, Finnish Cultural Foundation and Ministry of Education and Culture of Finland.

I sincerely wish to thank my supervisor Professor Antti A. Mero for his guidance and support along my PhD path. Our collaboration started back in 2005 when I began to work on my bachelor's thesis. Ever since, we have maintained our belief that this theme is worth researching. I am, consequently, grateful for having had the opportunity to contribute to research on this topic. I may have not been the easiest person to supervise - not least with all the maternity leaves I took during the project, but we have finally arrived! I hope we may continue to collaborate in the fields of acid-base balance and sports nutrition in the future. As you once said: "Let's keep up the good pH vibes!" I would also like to thank Professor Heikki Kainulainen, my second supervisor, whom I remember as an inspiring teacher from the days of my master's studies. You guided me, especially in the beginning of my PhD studies, and you have been an important lactic acid mentor to me.

I thank the official reviewers of my thesis, Professors David Bishop and Thomas Remer, for their valuable comments. I am very happy that, as top experts in the field, you both agreed to do the job within a rather tight schedule, and I am grateful for the time and effort you put into this. Your comments helped me to dot the i's and cross the t's in my final draft. I am also highly honored to have, as my opponent, Professor Lars McNaughton, who has a wide experience in the fields of acid-base balance and sports nutrition.

I wish to thank Professor Jeffrey Stout for good collaboration and for the opportunity to visit you and the University of Central Florida during my studies. I am also very grateful to Professor Lynda Frassetto. Your dedication to this whole field and to advising me in my work has been very encouraging and inspiring. I think I would not be where I am right now with this topic without your support. I feel honored to have met you at a conference in New Orleans last fall and I look forward to meeting you again in the International Acid-Base Symposium next summer.

I also feel honored to have had the best fellow PhD students, who, in addition to their scientific contribution to my work, have fostered my mental well-being during my studies. My young friends Tuuli Nissinen and Jaakko Hentilä, have especially made the last two years of my PhD studies a joy. The meaning of this time would be totally different without you. It has been an enormous pleasure spending lunch breaks with you discussing all kinds of health promo-

tion strategies and trying to figure out the deepest secrets of lactic acid. Also, thank you for making me stronger (at least physically but maybe also mentally). I look forward to further lunches, strength training, rock concerts, "egg" stuff and band rehearsals in Rokkiluola. And Tuuli, I won't ever forget the last week when I was finalizing my thesis, you were writing your article and we were working very late hours with snacks that almost, if not quite, conformed to the general dietary guidelines. Juulia Lautaoja and Anita Lampinen joined our Maca group a bit later – I am very happy with all the (unicorn) energies I've been getting from this group. I would also like to thank Johanna Ihalainen for all her help, support and understanding along the way - you even covered me when I was on one of my maternity leaves. Without you and your tips I doubt I would have gotten all the research and travel grants I received. But, most importantly, I may have come to your office door with some minor query and ended up talking with you for an hour or two. So, thank you for all those therapy sessions!

I am very happy to have had the opportunity to work as a member of the tremendous Libi team (Biology of Physical Activity) alongside all the fantastic people here. All the knowledge, laughter and twerking has made this a truly agreeable place to work. I wish to thank Katja Pylkkänen, Minna Herpola, Risto Puurtinen, Aila Ollikainen, Pirkko Puttonen and Mervi Matero for their help and contribution. Professor Vesa Linnamo, thank you for taking me under your wing - I am grateful for the opportunity I had to work in the Chinese project during the final stages of my PhD studies. Who knows how valuable this experience may eventually be. Juha Hulmi, you have always been my scientific idol (I'm not sure who my homeopathic idol is). Thank you, Simon Walker and Pedro Valadao, my room mates, for your company and all the coffee I've been given when I've most needed it. And, Katja Waller, thank you for collaboration on the nutrition course. I have enjoyed all the discussions we've had over all these years.

I would also like to thank all the co-authors, students and assistants who have been involved in my project - it would be impossible to complete a project on this scale without the help of other people. Among these, I would like to single out Tanja Laine, Hanna Minkkinen, Riikka Lamminen, Susanna Karvinen, Opri Jokelainen, and Elina Vaara. In addition, several students who have worked on their own studies and spent research training hours within the project also deserve recognition - thank you all! I also take this opportunity to thank everyone who volunteered to participate in the study. The English language of this thesis has been revised by Michael Freeman whom I also want to acknowledge.

I would like to express my deepest gratitude to my family. Mom and Dad, without your support and guidance I probably wouldn't be where I am right now. I may have gotten this apparently infinite desire to study and learn from my mother's milk, as I was present in some lectures with Mom already as a baby. I think that ever since I've been encouraged to be curious and fascinated about what is going on around us. Moreover, every Urheiluruutu and ice hock-

ey game I've watched with Dad and all the trips to cross-country skiing competitions have definitely influenced my continuing interest in sports. Thank you both for everything. My sisters, Mimmi, Saara, Riikka and Inkeri, thank you for your friendship and all the stupid jokes over the years. Your peer support and help during my maternity leaves and during the times when we have needed baby sitters have also been invaluable. I would like to thank Jopi and Riitta, my parents-in-law, who have also been such supportive companions all these years we've known each other. I also want to acknowledge Isoäiti and Eugen, and Mummu and Pappa. In addition, I have many important friends outside work and family. Some of you I have known since we were kids. To all my friends from Laukaa, Liikunta and Flamenco, thank you for being there.

I don't know what words would be adequate to express my gratitude to my husband Aki who has been my love and best friend for already half of my life. The final stages (especially) of my PhD project have been just crazy and your endless support and encouragement have carried me through on several occasions. You made it easier to push on to the finish line by enabling me to fully concentrate on this when needed. This may have been a huge effort for me, but the amount of work you have done to make this happen has been enormous and indispensable. Finally, I would also like to thank our amazing kids, Veikko, Arttu and Riina, for their understanding and stamina regarding mom's project. I can now finally say that there is not one page left to write. Imagine! Although there have been times when it seemed as if my thesis was the most important thing in the world, remember that it is not true. You are what mean most to me. You have helped me to put my work in perspective - also literally. As Veikko (6 years) once said: "Mom, you know a lot about humans, but listen, I know a lot about this entire universe."

Mää pakahun.

Jyväskylä 2.4.2018
Enni-Maria Hietavala

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following original articles, which are referred to in the text by their roman numerals. The papers are based on study 1 (I), study 2 (II, III) and study 3 (IV).

I Hietavala, E-M., Puurtinen, R., Kainulainen, H. & Mero, A.A. 2012. Low-protein vegetarian diet does not have a short-term effect on blood acid-base status but raises oxygen consumption during submaximal cycling. Journal of the International Society of Sports Nutrition 9, 50. doi: 10.1186/1550-2783-9-50

II Hietavala, E-M., Stout, J.R., Hulmi, J.J., Suominen, H., Pitkänen, H., Puurtinen, R., Selänne, H., Kainulainen, H. & Mero, A.A. 2015. Effect of diet composition on acid–base balance in adolescents, young adults and elderly at rest and during exercise. European Journal of Clinical Nutrition 69 (3), 399–404. doi:10.1038/ejcn.2014.245

III Hietavala, E-M., Stout, J.R., Frassetto, L.A., Puurtinen, R., Pitkänen, H., Selänne, H., Suominen, H. & Mero, A.A. 2017. Dietary acid load and renal function have varying effects on blood acid-base status and exercise performance across age and sex. Applied Physiology, Nutrition and Metabolism 42 (12), 1330–1340. doi:10.1139/apnm-2017-0279

IV Hietavala, E-M., Ihalainen, J.K., Frassetto, L.A., Schumann, M., Eklund, D., Häkkinen, K. & Mero, A.A. 2018. Effects of 12-week low or moderate dietary acid intake on acid–base status and kidney function at rest and during submaximal cycling. Nutrients 10 (3), 323. doi:10.3390/nu10030323

This thesis also includes some previously unpublished results.

ABBREVIATIONS

AD	Adolescents
A_{tot}	Total concentration of weak acids
CKD	Chronic kidney disease
CVD	Cardiovascular diseases
EL	The elderly
GFR	Glomerular filtration rate
H^+	Hydrogen ion
HCO_3^-	Bicarbonate ion
IFV	Intake of fruits and vegetables
LD	Diet with low acid load
LPVD	Low-protein vegetarian diet (diet with low acid load)
HD	Diet with high acid load
pCO_2	Partial pressure of carbon dioxide
NAE	Net acid excretion
ND	Normal diet
NEAP	Net endogenous acid production
PRAL	Potential renal acid load
SID	Strong ion difference
UCR	Urea-to-creatinine ratio
YA	Young adults

CONTENTS

1 INTRODUCTION

While the effects of diet composition on acid-base balance have not been very widely studied, increasing interest is currently being shown in understanding both the acute and long-term impacts of dietary acid intake on health. Acid-base balance is one of the most strongly regulated variables in human physiology. The normal functioning of the human body requires equilibrium between acids and alkali in body fluids (Adrogué & Adrogué 2001), since almost all enzyme and cell function is dependent on the acid-base balance (Vormann & Goedecke 2006). Many biochemical reactions in the human body generate and consume hydrogen ions (H^+). Under normal physiological conditions, dietary composition is the primary modifier of net endogenous acid production (NEAP) and may further affect the acid-base status of the body (Poupin et al. 2012).

Dietary acid load can be estimated by determining the renal net acid excretion caused by a foodstuff (Remer et al. 2003). A diet rich in animal proteins and cereal grains and deficient in vegetables and fruits – a typical diet in many Westernized cultures – leads to a net production of acids and may cause low-grade metabolic acidosis (Adeva & Souto 2011). This mild and subclinical but chronic acidosis can have adverse health consequences, especially with aging (Alexy et al. 2005; Dawson-Hughes et al. 2008; Souto et al. 2011). The kidneys regulate acid-base balance and play the predominant role in regulating the systemic bicarbonate concentration, which is the metabolic component of acid-base balance (Hamm et al. 2015). Aging-related decline in kidney function and the ability to regulate acid-base balance have been thought to lead to slowly increasing metabolic acidosis, especially if the dietary acid load is high (Frassetto & Sebastian 1996). The rate of renal diseases is also increasing along with the incidence of type 2 diabetes, blood pressure and coronary heart diseases.

In addition to health, dietary acid load may play a role in exercise. Increasing H^+ concentrations in muscle and blood during high-intensity exercise cause acidosis, which is considered one of the causes of fatigue (Lancha Junior et al. 2015; Robergs et al. 2004; Sutton et al. 1981). Pre-exercise acid-base

status is known to have the potential to affect physical performance (Carr et al. 2011; Siegler et al. 2016). Over the decades, many studies have reported positive effects of sodium bicarbonate supplementation and some other ergogenic aids on blood buffering capacity and exercise performance (Bishop et al. 2004; Krustrup et al. 2015; Mero et al. 2013; Wilkes et al. 1983), yet only a few studies have investigated the impact of diet composition on acid-base status, particularly during exercise performance (Greenhaff et al. 1987). Hence, the purpose of the present study was to examine whether dietary acid load has either acute or prolonged effects on the acid-base status of the body in healthy adolescents, young adults and the elderly, whether these effects have an impact on aerobic submaximal and maximal exercise performance and whether they could have further effects on health.

2 REVIEW OF THE LITERATURE

2.1 Acid-base balance and its regulation

Cellular metabolism involves many intermediates that are acids. Together, these constitute a significant part of the daily acid challenge to the body (Cockerill & Reed 2011, 18). In addition to cellular metabolism, acids enter the body via nutrition (Poupin et al. 2012). An acid is a molecule that can release hydrogen ions (H^+) in solutions, whereas a base is a molecule that can accept a H^+ ion. Acid-base balance refers to an equilibration between hydrogen ion intake and generation, and hydrogen ion removal from the body. Precise H^+ regulation is vital since almost all enzyme and cell function, and thus the whole body, is influenced by H^+ concentrations. Hydrogen ion concentrations of body fluids are extremely low, the normal value being about 0.00004 mEq/l (40 nEq/l), and are usually expressed by pH, which is a negative logarithm of the H^+ concentration ($[H^+]$):

$$pH = -\log [H^+]$$

About 80 mEq of hydrogen ions is ingested and generated daily by metabolism, but H^+ concentrations in body fluids are regulated within rather narrow limits. In arterial blood, normal pH is around 7.4 and is normally maintained strictly between 7.35-7.45 at rest, which means that variation in H^+ concentration is only about 3 to 5 nEq/l. An excess of hydrogen ions and arterial pH below 7.35 are considered acidosis, whereas loss of hydrogen ions and an increase in pH above 7.45 are considered alkalosis. The normal pH of venous blood and interstitial fluids is 7.35. In intracellular fluids, pH can vary between 6.0 and 7.4 and in urine between 4.5 and 8.0. However, under extreme conditions plasma pH can vary between 6.8 and 8.0 (and H^+ concentration between 10 and 160 nEq/l) without causing death (Hall 2011, 379-381). With maximal exercise plasma pH can fall from 7.4 to 6.9 (Cairns 2006) and muscle pH can decrease from 7.2 to as low as 6.6 (Hostrup & Bangsbo 2017).

However, acid-base balance cannot be seen only in terms of blood pH; rather, it is a result of several mechanisms that regulate systemic acid-base status. The net effect of ion transfers across the cell membranes and mechanisms involved in renal and gastrointestinal water and electrolyte regulation affect acid-base balance (Lindinger & Heigenhauser 2012). To prevent cellular dysfunction, the acid loads that the body confronts daily must be neutralized or eliminated, and hence the body will normally compensate rapidly for acute changes in acid-base balance (Cockerill & Reed 2011, 232; Kellum 2000). The most immediate step in the regulation of H^+ concentrations is the function of extra- and intracellular buffers that react within seconds to alterations in H^+ concentrations. The second step is the elimination of carbon dioxide (CO_2), and hence, carbonic acid (H_2CO_3), by the respiratory system, a process that is initiated within a few minutes. The slowest but the most efficient regulators are renal mechanisms, which also include the only way to excrete excess hydrogen ions from the body (Hall 2011, 380; Poupin et al. 2012). Excretion of H^+ by the kidneys is an important long-term mechanism that maintains and replenishes the buffer reserve of the body (McArdle et al. 2001, 302; Yucha 2004).

2.1.1 Extra- and intracellular buffering

A buffer is a molecule that can bind and release hydrogen ions and thus regulate H^+ concentrations and resist pH changes. Several such buffer systems exist in body fluids. They can be classified in general as carbonate (mainly bicarbonate) and non-carbonate buffers. A buffer is composed of a weak acid and a salt of that weak acid. The most important buffer systems in the body are:

- Bicarbonate-carbonic acid buffer system in plasma
- Proteins (mainly albumin) in plasma and in intracellular fluids
- Hemoglobin in red blood cells
- Phosphate buffer system in intracellular fluids and in renal tubular fluid (Cockerill & Reed 2011, 232; Hall 2011, 381-383).

Each buffer system has an equilibrium point (acid dissociation constant, pKa), which is a pH where 50% of the buffer members are in the acid form and 50% in the base form. It is at this specific pH that the buffer system has its highest buffering potential. The most efficient buffer system in extracellular fluids and blood plasma is the phosphate system, as it has a pKa closest to the normal physiological pH of extracellular fluids. However, the bicarbonate concentration in extracellular fluids is 4-fold, which makes the bicarbonate buffer system the most important of these systems in this fluid compartment, as bicarbonate has a larger effect on the total buffer pool than phosphate. If, owing to increased acid or alkali loads, change in pH is detected in the body fluids, the buffer systems will immediately respond to prevent minute-to-minute fluctuations in pH (Yucha 2004).

Extracellular buffering, which is mainly bicarbonate buffering, occurs almost immediately when H^+ is added to plasma (Yucha 2004). The bicarbonate buffer system consists of carbonic acid (H_2CO_3), which is a weak acid, and a bicarbonate salt such as $NaHCO_3$. The simplified chemical equation of the entire system is as follows:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

This buffer system is physiologically the most important because its quantitative capacity to buffer acid or alkali loads is prominent, and HCO_3^- and CO_2 can be regulated independently by the kidneys and lungs, respectively (Hamm et al. 2015). The phosphate system is an important buffer system in intracellular and renal tubular fluids. In addition, proteins are abundant in the cells and play a major role as intracellular buffers. Hemoglobin is an important buffer in red blood cells, and carnosine is an example of a protein that acts as a buffer in muscle cells. The vast majority of the chemical buffering in intracellular fluids is protein-driven. Protein concentrations in the cells are high and their pKa values close to intracellular pH. However, H^+ and HCO_3^- diffuse slowly through the cell membranes (except for red blood cells), and for that reason it may take several hours before intracellular proteins reach their maximal capacity to buffer against extracellular acid-base variations (Hall 2011, 383).

2.1.2 Respiratory regulation

Neurons in the medullary respiratory center respond to change in plasma pH by altering the rate and depth of respiration. When the excretion of CO_2 increases, plasma CO_2, which is a part of the bicarbonate buffer system, decreases. This drives the bicarbonate buffer reaction to the right and enables more H^+ to be bound with HCO_3^-, thereby increasing plasma pH. The respiratory response is identified as a separate response mechanism, and it uses the bicarbonate buffer system to provide CO_2, the regulated substrate (Yucha 2004). This renders the lungs an important organ in acid-base regulation, since they regulate parts of the hydrogen ion concentration by regulating the CO_2 content of the body. Respiratory regulation is a physiologic buffer system and it is up to two times more efficient than chemical buffers (Hall 2011, 385).

Carbon dioxide is produced constantly in cell metabolism from where it diffuses into the plasma and affects the partial pressure of CO_2 (pCO_2). Increased ventilation eliminates CO_2 from the body and decreases the H^+ concentration, whereas a decrease in ventilation increases the H^+ concentration. On the other hand, as the respiratory system is a negative feedback regulator of the H^+ concentration: the higher the H^+ concentration, the higher the ventilation. For example, when plasma pH decreases from 7.4 to 7.0, ventilation increases 4-5-fold (Hall 2011, 384-385). Alveolar ventilation eliminates 15 mol of the CO_2 per day that is produced in normal cellular oxidative metabolism and maintains pCO_2 around 40 mmHg (Hamm et al. 2015).

2.1.3 Renal regulation

The kidneys have many important functions – they are continuously filtering blood to remove metabolic waste products and foreign chemicals, regulating water and electrolyte balances and blood pressure, taking part in the secretion, metabolism and excretion of hormones, and performing gluconeogenesis. In addition, the kidneys play a fundamental role in the regulation of acid-base metabolism (Remer & Manz 1995). They play the predominant role in regulating the systemic bicarbonate concentration, which is the metabolic component of acid-base balance (Hamm et al. 2015).

2.1.3.1 Kidney function

Each kidney contains 800 000 - 1 000 000 nephrons, each of which is a functional, urine-forming unit of the kidneys. A nephron is composed of a renal tubule and a renal corpuscle, which consists of a tuft of capillaries called a glomerulus. Filtration, reabsorption, secretion, and excretion are the four mechanisms used to create and process the filtrate; to convert blood to urine. Filtration occurs in the glomerulus and is largely passive. About one-fifth of the plasma is filtered as the blood passes through the glomerular capillaries, the remaining four-fifths continuing into the peritubular capillaries. The glomerular filtration rate (GFR) is used to assess kidney function, which describes the rate of blood flow through the kidneys. It can be evaluated by measuring creatinine content in serum. The higher the blood creatinine level, the lower the estimated GFR and creatinine clearance. A high GFR allows the kidney to filter and process the body fluids many times a day and to rapidly remove from the body those waste products that depend mainly on glomerular filtration for their excretion, such as H^+ (Hall 2011, 305, 312).

2.1.3.2 HCO_3^- reabsorption and H^+ excretion

The kidneys contribute to acid-base regulation by excreting acids and by regulating the body fluid buffer stores (Passey 2017). The kidneys regulate extracellular fluid H^+ concentration through three fundamental mechanisms: reabsorption of filtered HCO_3^-, secretion of H^+, and production of new HCO_3^-. The hydrogen ion concentration is 0.00004 mEq/l at pH 7.4, but about 80 mEq of H^+ is either ingested or generated in the metabolism daily. These H^+ ions come from nonvolatile acids that are not H_2CO_3 and cannot, therefore, be excreted by the lungs. The primary mechanism for removal of these acids from the body is renal excretion. The kidneys are the only means of eliminating certain types of acids, such as sulfuric and phosphoric acids that are generated in the metabolism of proteins. The kidneys must also prevent the loss of HCO_3^- to urine, and to this end they filter 4320 mEq of bicarbonate daily. Both the reabsorption of bicarbonate and excretion of H^+ are accomplished through the process of H^+ secretion by the tubules. Because the bicarbonate must react with a secreted H^+ ion to form H_2CO_3 before it can be reabsorbed, 4 320 mEq of H^+ must be secret-

ed each day just to reabsorb the filtered bicarbonate (Hall 2011, 304, 385-386). The bicarbonate concentration in the filtrate entering the proximal tubule is equal to that in plasma. All bicarbonates filtered into the proximal tubule are reabsorbed and added to plasma, thereby conserving the primary buffer system of extracellular fluid. Approximately 80% of the filtered bicarbonate is reabsorbed in the proximal tubule, 15% in the thick ascending limb, and 5% in the collecting duct. There is no net consumption or production of H^+ at the point of bicarbonate reabsorption, since H^+ ions circulate between the intracellular and tubular fluid compartments (Yucha 2004). In alkalosis, bicarbonate ions are excreted and in acidosis they are reabsorbed back into extracellular fluids (Hall 2011, 385).

HCO_3^- generation in the kidney is coupled with the excretion of H^+ from the body. Reabsorption of HCO_3^- does not replenish the HCO_3^- ions that are consumed to titrate the nonvolatile acids produced by metabolism. The kidney needs to generate new ones through the non-bicarbonate buffer systems and by metabolizing glutamine. Generation occurs mainly in the collecting tubule, although some of it also occurs in earlier nephron segments. The major difference between HCO_3^- generation and absorption is that the secreted H^+ is excreted in the regeneration process but not in the reabsorption process. The balance between the acid and base forms of each buffer system affects acid-base homeostasis. The renal bicarbonate generation process is the primary mechanism responsible for maintaining the balance between acid and base forms of the bicarbonate buffer system. When this system buffers H^+, HCO_3^- is consumed and is effectively lost from the body via the respiratory system. When the kidney regenerates HCO_3^-, the base molecule of the buffer system is replenished and homeostasis reestablished (Yucha 2004). In addition to 4 320 mEq of H^+ that must be secreted each day just to reabsorb the filtered bicarbonate, 80 mEq of H^+ must then be secreted to rid the body of the nonvolatile acids produced each day, for the total of 4 400 mEq of H^+ secreted into the tubular fluid each day (Hall 2011, 385).

2.1.4 Traditional and modern acid-base approaches

The Henderson-Hasselbalch equation is the traditional model used to describe the changes in acid-base balance:

$$pH = pK_a + \log (HCO_3^- / H_2CO_3)$$

The equation provides insight into the physiological control of acid and base composition of the extracellular fluid and the relationship between concentrations of the acid component and the base component of any buffer system. The acid component (H_2CO_3) and the base component (HCO_3^-) are regulated by different organs. Bicarbonate is regulated by the kidney, whereas H_2CO_3 is regulated by the respiratory system and the lungs. The equation suggests that changes in blood pH are directly proportional to the bicarbonate and inversely related to the carbonic acid concentrations. The co-operation between respirato-

ry and renal regulation makes the bicarbonate buffer system the most powerful buffer system in the body, even though the concentrations of HCO_3^- and CO_2 in the body fluids are low (Cockerill & Reed 2011, 234; Hall 2011, 382-383). Although the lungs and kidneys can compensate for disturbances in one or other variable, normal homeostasis requires that both CO_2 and HCO_3^- are normal (Hamm et al. 2015).

Acid-base disturbances can be divided into metabolic and respiratory disturbances depending the primary reason for the change in pH. When the primary change in the extracellular fluids is due to changes in the HCO_3^- concentration, the state is considered as metabolic acidosis or alkalosis. Respiratory acidosis or alkalosis occurs when the primary reason for the disturbance is a change in CO_2. In metabolic alkalosis, bicarbonates cannot be reabsorbed and are excreted in urine. In metabolic acidosis, all the bicarbonates are reabsorbed and H^+ secreted to the renal tubulus, buffered, and excreted to urine as salts (Hall 2011, 382, 385).

The traditional Henderson-Hasselbalch equation has been criticized because it accords HCO_3^- an active role, even though it is not a factor that regulates the H^+ concentration (Jones 1990). Instead, according to the physicochemical acid-base approach of Stewart (2009), which was introduced in 1981, at least three independent variables determine the H^+ concentration and thereby pH in the body fluids: strong ion difference (SID), partial pressure of carbon dioxide (pCO_2), and total concentration of weak acids (A_{tot}). The approach suggests that the physical behavior of molecules in aqueous solutions is independent of the transport and buffering mechanisms, and that H^+ and HCO_3^- are dependent variables that do not cause acid-base alterations (Lindinger et al. 2005). SID is the difference between strongly dissociated positive (e.g. Na^+, K^+) and negative (e.g. Cl^-) ions in the body fluids. SID represents the metabolic component of the acid-base balance and is mainly regulated by the kidneys. The respiratory component of the acid-base balance is affected by pCO_2 and regulated by alveolar ventilation. The weak acids are mostly proteins and phosphates, and they contribute the third determinant of H^+ concentrations. SID can be calculated as follows:

SID (mEq/l) = $[Na^+] + [K^+] - [Cl^-] - [Lac^-]$ (Kellum 2000).

When SID increases, the H^+ concentration decreases according to the rule of electroneutrality. SID is usually slightly positive, but fluids of the body cannot be electrically charged. The necessary negative charge comes from pCO_2 and A_{tot}. When the production of CO_2 exceeds the removal of CO_2 in the metabolism of cells, pCO_2 increases and causes a rise in the H^+ concentration. A_{tot} mostly comprises proteins (mainly albumin) and phosphates and through these the rule of electroneutrality is fulfilled. If there is a change in one or more of the independent variables, the H^+ concentration, and thus pH also change (Lindinger 1995). The Stewart method has also been criticized on the grounds that it is mainly a mathematical model that has not been validated physiologi-

cally and that has not significantly improved the ability to understand, diagnose, and treat acid-base alterations, at least in the clinical context, where the principals of the Henderson-Hasselbalch equation are still routinely used (Masevicius & Dubin 2015). However, according to Lindinger et al. (2005), changes in the concentrations of strong ions contribute to the change in the H^+ concentration in contracting skeletal muscle, whereas pCO_2 and A_{tot} show lesser effects.

2.2 Dietary acid load and its estimation

Diet composition is known to influence net endogenous acid production (NEAP), which may further affect the acid-base status of the body. Many biochemical reactions either generate or consume hydrogen ions, and thus acids and bases. Under normal physiological conditions, diet is the main moderator of NEAP, and perturbations in it may have consequences for acid-base balance (Poupin et al. 2012). The magnitude of the metabolic acid load depends on the composition of the diet, consumes varying amounts of the base members of the buffer systems and places varying demands on the kidney regarding the amount of bicarbonate that must be generated daily (Yucha 2004) (FIGURE 1). Phosphorous and proteins are nutrients that release acid precursors into the bloodstream. Acidogenic proteins are mainly those with sulfur-containing amino acids such as cysteine and methionine. The nutrients that are the precursors of bases are potassium, magnesium, and calcium. Additionally, sodium chloride intake has been reported to be an independent predictor of plasma bicarbonate concentration (Carnauba et al. 2017).

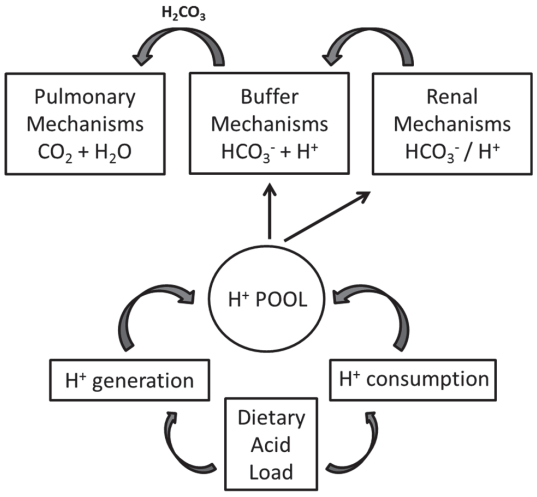


FIGURE 1 Dietary acid load and acid-base regulation (idea adopted from Poupin et al. 2012).

2.2.1 Net acid excretion (NAE)

In renal physiology, net acid excretion (NAE) is the net amount of acid excreted in urine per time unit. It is dependent on the urine flow rate, urine acid concentration, and the concentration of bicarbonate in urine. The bicarbonate lost in urine is physiologically equivalent to a gain in acid in the body. NAE can be measured in urine as follows:

$$NAE = TA + NH_4^+ - HCO_3^-$$

where TA is titratable acid.

Because the sum of the cations excreted in urine equals the sum of anions, NAE can also be estimated as follows:

$$NAE_{indirect}\ (mEq/d) = (Cl^- + P_i^{-1.8} + SO_4^{2-} + OA) - (Na^+ + K^+ + Ca^{2+} + Mg^{2+})$$

where SO_4^{2-}= 0.4875 * dietary protein intake (g)
OA= (BSA*41)/1.73
BSA= $[(\text{body mass (kg)} * \text{height (m)})/3600]^{½}$ (Remer et al. 2003; Remer & Manz 1995).

The main nutrients associated with the release of acids and bases are protein and potassium, and thus a simpler way to estimate net endogenous acid production would be the method developed by Frassetto et al. (1998), which takes the amounts of these two nutrients into account.

2.2.2 Potential renal acid load (PRAL)

Dietary acid load, i.e., the acid load of foods or diets, can be estimated by calculating the potential renal acid load (PRAL), which represents the renal net acid excretion caused by a foodstuff or the entire diet (Remer et al. 2003). PRAL estimates the production of endogenous acid that exceeds the production of alkali for a certain amounts of foods ingested daily. The concept of PRAL calculation is physiologically based, taking into account the different intestinal absorption rates of individual minerals and sulfur-containing protein, as well as the amount of sulfate produced from metabolized proteins. This model has been experimentally validated in healthy children, adolescents, and adults. Acid load can be reliably estimated from diet composition by calculating the PRAL value for 100 g of a given foodstuff as follows:

PRAL (mEq/100 g) = 0.49 x protein (g/100 g) + 0.037 x phosphorous (mg/100 g) - 0.021 x potassium (mg/100 g) - 0.026 x magnesium (mg/100 g) - 0.013 x calcium (mg/100 g) (Remer et al. 2003).

Foodstuffs with positive PRAL are acid-forming and those with negative PRAL alkali-forming. The digestion of meat, grain and some dairy products increases the acid load on the body. Fruits and vegetables, which contain organic anions (e.g. citrate, malate) that are metabolizable to bicarbonate, lower the net acid load. They are also abundant in potassium, which is a base-forming cation along with magnesium and calcium (Remer 2001). When sulfur-containing amino acids (R-SH) are oxidized, H^+ ions are generated in urea synthesis as follows:

$$R\text{-}SH \rightarrow CO_2 + \text{urea} + 2\,H^+ + SO_4^{2-}$$

On the other hand, when organic anions (AO^-) such as citrate, malate and lactate are oxidized, HCO_3^- ions are generated as follows:

$$AO^- + H_2CO_3 \rightarrow CO_2 + H_2O + HCO_3^-$$ (Poupin et al. 2012).

The Western diet typically contains large amounts of animal protein and grain products but only small amounts of vegetables and fruits, leading to a net production of acids in the body (Adeva & Souto 2011). The PRAL values of selected foods are presented in TABLE 1. However, instead of focusing solely on single foodstuffs, the composition of the diet in its entirety should be taken into account when assessing daily acid loads.

TABLE 1 PRAL values (potential renal acid load) per 100 g of a foodstuff.

Alkali-forming	**PRAL (mEq/100g)**	**Acid-forming**	**PRAL (mEq/100g)**
Raisin	-10.4	Hard cheese	17.8
Carrot	-7.2	Cottage cheese	11.5
Potato	-7.1	Egg	10.1
Banana	-7.0	Oatmeal	10.4
Avocado	-6.9	Salmon	9.0
Lettuce	-6.8	Cashew seed	8.7
Green bean	-5.8	Pork	8.6
Tomato	-5.1	Beef	8.5
Cucumber	-4.2	Chicken	7.5
Strawberry	-3.3	Quark	6.1
Lemon, orange	-3.0	Wheat flour	4.7
Pineapple	-2.4	Wheat roll	3.0
Apple	-2.3	Rye bread	2.4
Yoghurt	-0.5	Cornflakes	2.4
Margarine	-0.4	Lentil	1.9
Milk	-0.3	Spaghetti	1.3
Tofu	-0.3	Rice	1.2
Sugar	-0.1	Butter	1.1

2.3 Acid-base balance and dietary acid load during exercise

In addition to digestion of food, physical activity causes acute metabolic changes that may result in an increase in H^+ generation. Even moderate intensity physical activity causes metabolic changes, which affect the acid-base balance in skeletal muscles, blood and other tissues (Lindinger 1995). On the other hand, pre-exercise acid-base status is also known to have the potential to affect physical performance (Siegler et al. 2016).

2.3.1 Exercise-induced acidosis and muscle fatigue

Increasing hydrogen ion concentrations in muscle and blood during high-intensity exercise cause acidosis, which is known to be one of the causes of fatigue (Lancha Junior et al. 2015; Robergs et al. 2004; Sutton et al. 1981). Many exercise physiology and biochemistry texts contain descriptions of how lactic acid is produced during high-intensity exercise. Lactic acid is said to dissociate into lactate and H^+, which cause the increased acidity and muscle fatigue. However, this theory has been challenged and another version will be reviewed below.

2.3.1.1 Origins of H^+ during exercise

To fuel muscular contractions during physical activity, energy is needed in the form of adenosine triphosphate (ATP), known as high-energy phosphate. Different metabolic pathways and fuels for ATP regeneration are selected during times of intense exercise or times of modest physical activity (Tiidus et al. 2012, 77). Energy in food and macronutrients (carbohydrates, lipids, and protein) needs to be extracted and conserved within the bonds of ATP, and, to power biologic work, the chemical energy in ATP needs to be extracted and converted (McArdle et al. 2001, 132). That is, ATP hydrolysis liberates energy and enables muscles to contract. ATP hydrolysis is the reaction that is the major source of hydrogen ions in the organism and can be represented as follows:

$$ATP + H_2O \leftrightarrow ADP + P_i + H^+$$

Thus, ATP hydrolysis has an acidifying effect in a cell. However, under steady conditions hydrogen ions are continuously generated and consumed and do not accumulate in the body (Poupin et al. 2012). ATP stores in the body are small, and ATP needs to be continuously resynthesized. Three energy systems are responsible for maintaining ATP concentrations in skeletal and cardiac muscle. These are the phosphocreatine system, oxidative phosphorylation, and anaerobic glycolysis (Tiidus et al. 2012, 88).

At the onset of exercise and during very vigorous exercise, some energy for ATP resynthesis derives directly from the anaerobic splitting of phosphate from phosphocreatine (PCr), which is also a high-energy phosphate:

$$ADP + PCr + H^+ \leftrightarrow ATP + Cr$$

In this reaction, the hydrogen ion is consumed; this can be beneficial to the muscle during high-intensity exercise when high rates of ATP hydrolysis acidify the muscle (Tiidus et al. 2012, 92).

Oxidative phosphorylation refers to the aerobic energy system and cellular respiration. In this process the formation of ATP from ADP and P_i occurs in association with the transfer of electrons from fuel molecules to coenzymes and to oxygen (Tiidus et al. 2012, 88). When electrons are transferred, the molecules are oxidized and reduced. This cellular oxidation-reduction constitutes the underlying biochemical mechanism in energy metabolism (McArdle et al. 2001, 134). Electrons of the hydrogen ions are transferred from fuel molecules (such as glucose, fatty acids, and amino acids) to coenzyme NAD^+ (nicotinamide adenine dinucleotide) and FAD^+ (flavin adenine dinucleotide) that reduce to NADH and $FADH_2$. Eventually the electrons extracted from hydrogen are transferred to oxygen and H_2O is formed. In this way, oxidative phosphorylation synthesizes ATP in the respiratory chain by transferring electrons from NADH and $FADH_2$ to oxygen. Oxidation of hydrogen and subsequent phosphorylation occurs as follows:

$$NADH + H^+ + 3\ ADP + 3\ P_i + \tfrac{1}{2}\ O_2 \rightarrow NAD^+ + H_2O + 3\ ATP$$

During aerobic ATP resynthesis, oxygen serves as the final electron acceptor in the respiratory chain and combines with hydrogen to form water (McArdle et al. 2001, 135-138).

Glycolysis is a metabolic pathway where a sequence of enzyme-catalyzed reactions takes place to produce ATP from glucose or glycogen:

$$Glucose + 2\ NAD^+ \rightarrow 2\ pyruvate + 2\ NADH + 2\ H^+ + 2\ ATP$$

$$Glycogen + 2\ NAD^+ \rightarrow 2\ pyruvate + 2\ NADH + H^+ + 3\ ATP$$

NADH and H^+ formed in glycolysis can be utilized in the electron transport chain. The pyruvate molecules have two major fates. They can either be transported into a mitochondrion and oxidized in oxidative phosphorylation or they can be converted to lactate. The net production of ATP from glycolysis and PCr is known as substrate-level phosphorylation (in contrast to oxidative phosphorylation) (Tiidus et al. 2012, 90-91).

During high-intensity exercise, when the respiratory chain cannot process all the hydrogen joined to NADH, and NADH cannot be oxidized to NAD^+, nonoxidized hydrogens from NADH combine temporarily with pyruvate to form lactate:

$$Pyruvate + NADH + H^+ \leftrightarrow lactate + NAD^+$$

Thus, lactate production enables glycolysis to continue by ensuring that there is enough NAD^+ for the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) reaction of glycolysis. NAD^+ is then also able to accept the additional H^+ generated in glycolysis. Moreover, lactate production consumes H^+ and buffers against the increasing acidity (McArdle et al. 2001, 143-144; Tiidus et al. 2012, 90-91). However, when high-intensity exercise continues, and ATP is being hydrolyzed at higher rates than mitochondria are able to reproduce aerobically, H^+ release eventually increases, causing acidosis (Robergs et al. 2004). It has been suggested that, as pH decreases below 7.0, the rate of H^+ generation during glycolysis surpasses the rate of H^+ binding in lactate production (Robergs 2017). In view of the chemical reactions presented above it seems that lactic acid is not produced in the human muscle. Rather, during high-intensity exercise, hydrogen ions originate from glycolysis and ATP hydrolysis. To sum up, while the term lactic acidosis can be used to describe the increased acidity that accompanies increased lactate production, it should not be used to refer to lactic acid production in relation to human muscles.

Moreover, lactate is not solely a metabolic waste product of anaerobic energy production. Rather, it provides an important source of energy. When sufficient oxygen is again available, NAD^+ captures the hydrogen ions from the lactate for subsequent oxidation to form ATP. The re-formed pyruvate can be oxidized in the citric acid cycle or synthesized to glucose in the Cori cycle that takes place in the liver (gluconeogenesis) (McArdle et al. 2001, 144).

2.3.1.2 Acidosis and muscle fatigue

Skeletal muscle fatigue can be defined as inability to sustain the force level needed to meet the demand for a given exercise intensity (McKenna et al. 2008). The mechanisms behind muscle fatigue are complex and controversial (Hostrup & Bangsbo 2017) while the role of acidosis is also under debate (Fitts 2016; Westerblad 2016). The biochemical factors associated with skeletal muscle fatigue may be divided into three fundamental events: 1) impairment of metabolism and reduction in energy production, 2) alteration in sarcolemmal, transverse tubular and sarcoplasmic reticulum membrane properties, and 3) impaired function of the contractile proteins. Some of the biochemical factors along with increased acidosis that contribute to fatigue in exercising skeletal muscle are glycogen depletion, phosphocreatine depletion, ATP depletion, and increase in inorganic phosphate (Lindinger & Heigenhauser 1990). It is unlikely that only one of the many changes occurring during exercise, such as increased acidosis, would be the only causal cue for muscle fatigue. Rather, at the cellular level, fatigue results from changes in various metabolites and ions. For example, changes in the concentrations of H^+, K^+, Na^+, Ca^{2+}, Cl^-, HCO_3^-, Mg^{2+}, and Lac^- may impair excitation-contraction coupling, decrease mechanical efficiency and lead to muscle fatigue (Cairns & Lindinger 2008).

However, the increased H^+ concentrations in muscle and blood and subsequent acidosis are thought to be one of the causes of fatigue (Lancha Junior et al. 2015; Robergs et al. 2004; Sutton et al. 1981). During high-intensity exercise,

glycolysis is a major contributor to energy production, a factor which eventually leads to accumulation of H^+ as described above. With maximal exercise, plasma pH can fall from 7.4 to 6.9–7.0 (Cairns 2006) and during intense exhaustive exercise lasting a few minutes, muscle pH can decrease from 7.2 to below 6.6 (Hostrup & Bangsbo 2017). At the onset of exercise or muscle stimulation, pH may initially increase by 0.1 pH units due to the H^+ consumed during PCr breakdown. Conversely, as PCr is resynthesized after exercise ceases, pH can decrease a further 0.1 pH units (Allen et al. 2008). Changes in H^+ concentration during exercise may have wide-ranging effects on exercise metabolism from hormonal changes to rate-limiting enzyme changes in muscles and other tissues (Jones 1990). For example, acidosis has been reported to limit mitochondrial function and it may limit enzyme activities in glycolytic energy production (Hollidge-Horvat et al. 2000; Jubrias et al. 2003; Lindinger 1995). Hydrogen ion concentration has also been shown to adversely affect interstitial K^+ accumulation, which impairs muscle performance (Street et al. 2005). In addition, acidosis may contribute to the onset of fatigue by decreasing the buffering capacity of the muscle. This in turn could cause decreased Ca^{2+} sensitivity, impaired cross-bridge formation and result in decreased muscle contractile force (Dutka et al. 2012; Sahlin 2014). On the other hand, muscle preparations studies have indicated that decreased pH has a minor detrimental effect on fatigue development and could even enhance muscle fiber excitability with small direct effects on force production (Allen et al. 2008). However, studies in humans on the accumulation of H^+ have reported an adverse effect on performance during high-intensity exercise. Moreover, it has been shown that athletes have higher skeletal muscle buffering capacity compared to trained and untrained individuals (Hostrup & Bangsbo 2017).

2.3.2 Buffering capacity and dietary acid load

Many studies have examined the effects of blood pH on exercise performance, most showing deleterious effects of acidosis and beneficial effects of alkalosis (Cairns 2006). In extracellular fluids, HCO_3^- is considered the most important chemical H^+ buffer (McNaughton et al. 2008). Maintenance of high alkalinity and HCO_3^- concentration in extracellular fluids is considered to enable faster H^+ removal from the muscle cell, which delays muscle fatigue caused by increased acidosis (Bishop et al. 2004; Siegler et al. 2016). Both increased muscle buffer capacity and enhanced removal of lactate and hydrogen ions also increase the capacity of glycolytic ATP production (Sahlin 2014). In addition to exercise training, which enhances muscle buffer capacity (Edge et al. 2006), many studies have reported positive effects of sodium bicarbonate supplementation and some other ergogenic aids on blood buffering capacity (Krustrup et al. 2015, Wilkes et al. 1983). For example, sodium bicarbonate or sodium citrate supplementation has been reported to improve performance both in short (~1-2 min) (Mero et al. 2013, Van Montfoort et al. 2004) and longer (~20-60 min) bouts of exercise (Oöpik et al. 2003; Pottaiger et al. 1996). However, only a few studies have investigated the impact of diet composition on acid-base status and exer-

cise performance (e.g. Baguet et al. 2011; Caciano et al. 2015; Greenhaff et al. 1987).

Since diet composition is known to influence net endogenous acid production, which may further affect the acid-base status of the body (Poupin et al. 2012), a diet high in vegetables and fruits, and thus with a low acid intake, could have the potential to affect blood buffering capacity and physical performance by attenuating exercise-induced acidosis, as also reviewed by Applegate et al. (2017). Maughan et al. (1997) observed that a diet high in protein combined with a low intake of carbohydrate caused acidosis and had a negative influence on performance. Greenhaff et al. (1987) reported that a low-acid diet with low protein (9.4 ± 1.8%) and high carbohydrate (65.5 ± 9.8%) intake followed for 4 days resulted in higher plasma pH and HCO_3^- prior to the exercise test compared with a high-acid diet with high protein (25.3 ± 4.1%) and low carbohydrate (10.1 ± 6.8%) intake. The increased blood alkalinity resulted in longer time to exhaustion during cycling at 100% of VO_2max. Moreover, while pH and HCO_3^- were higher at rest, they decreased to the same or an even lower level after longer cycling time to exhaustion at 100 % of VO_2max.

2.3.3 Kidney function and exercise

In the field of exercise physiology, the effects of exercise on kidney function have been studied less than those of other organs like the lungs and heart. The kidney has an essential role in the homeostasis of the body at rest, and many changes in renal function also occur with exercise. Under resting conditions, blood flow to the kidney is among the highest to any organ. This blood flow is needed more for the filtration function of the kidney than the oxygen demands of the renal tissue. Oxygen consumption is not increased in renal tissue during exercise, and blood flow is redistributed away from the kidney to skeletal muscles (Mueller et al. 1998). In general, exercise impairs renal function (Suzuki 2015). For example, temporary impairment of renal function was found after ultramarathon cycling (Neumayr et al. 2005). At exercise intensities above 50% of VO_2max, the decrease in renal blood flow (RBF) is proportional to the exercise intensity. This is due to elevation in renal vascular resistance, which helps to maintain the necessary total peripheral resistance and systemic blood pressure. With exercise loads up to 50% of VO_2max, GFR is slightly increased or unchanged, but at higher exercise intensities, GFR decreases at a higher rate than RBF. Sodium secretion also decreases with heavier workloads. Conservation of sodium and water is not a very important task for the kidney during exercise, but during the post-exercise recovery phase, lasting from several hours to several days, the renal response is important. It will adjust the amount and chemical composition of urine until fluid and electrolyte losses are corrected. Renal function may also be important in athletes during exercise in warm conditions (Suzuki 2015).

Morales et al. (2017) measured cardiorespiratory variables during cardiopulmonary exercise testing in soccer players who showed acute alterations in their glomerular filtration rate after the pre-season training protocol. They

found that players with reduced kidney function presented reduction in VO_2max and increase in heart rate during an incremental exercise test to voluntary exhaustion. These results suggest a possible association between exercise performance and renal functional capacity. The long-term effects of exercise training on kidney function have not been studied in healthy populations, but in patients with decreased kidney function an association between diminished kidney function and lower aerobic exercise capacity has been reported (Reinecke et al. 2014; Scrutinio et al. 2015).

2.4 Acid-base balance in aging and health

2.4.1 Aging kidney

As with the whole human body, the kidney undergoes age-related, imminent changes that lead to progressive decline in renal function. Renal aging is a process in which many factors such as sex, genetic background, chronic inflammation, oxidative stress, and impairment in kidney repair capacities play a significant role. Male gender enhances the age-related decline in renal function and GFR loss, which is androgen-dependent (Bolignano et al. 2014). Nephrons cannot be regenerated and therefore their amount decreases with normal aging as well as with renal injury or disease. After age 40, the number of functioning nephrons decreases by 10% every 10 years. Adaptive changes in the remaining nephrons ensure that the decrease in the number of nephrons is not life threatening (Hall 2011, 305). The kidneys have a huge reserve capacity and most people can lose 30 to 40 percent of their renal function without having significant problems. However, because kidney function naturally declines with age, it is important to act early to preserve all possible kidney function (Hall 2011, 312).

Impaired renal function is common in the elderly. In persons older than 70 years, the prevalence of renal dysfunction has been reported to be 15%, and 35% of the elderly population have stage 3 chronic kidney disease (CKD) (Bolignano et al. 2014). CKD is becoming one of the most important global health issues owing to its association with cardiovascular diseases and its rapidly increasing incidence and prevalence worldwide (So et al. 2016). Subjects with pre-existing cardiovascular disease or risk factors are prone to greater decline in renal function (Bolignano et al. 2014). For example, metabolic syndrome and insulin resistance have been reported to predict the risks of existing and incident CKD (Cheng et al. 2012). Hypertension and vascular stiffness are associated with typical changes in renal structure and function, in particular those associated with aging (Duarte et al. 2011). Moreover, elderly people may develop vitamin D deficiency due to the impaired capacity of the aging kidney to convert 25-hydroxy vitamin-D to its bioactive form 1.25-dihydroxy vitamin D (Gallagher et al. 2007).

2.4.2 Dietary acid load and chronic diseases

The Western-style diet, characterized by higher consumption of animal products and lower consumption of vegetables and fruits, has been found to associate with increased risk for many non-communicable diseases such as type 2 diabetes, hypertension and chronic kidney disease (CKD) (Carnauba et al. 2017; Odermatt 2011). A diet rich in animal proteins and cereal grains and deficient in vegetables and fruits can cause low-grade metabolic acidosis (Adeva & Souto 2011; Carnauba et al. 2017). Low-grade metabolic acidosis is a condition in which blood pH is closer to the lower limit (7.35) of the normal range (but still above it). Although diet composition is known to be one of the risk factors for the progression of CKD, its role in the development of the disease is not clear (So et al. 2016). Scialla et al. (2012) found that higher net endogenous acid production was significantly associated with a faster decline in GFR. Decreased dietary acid load has been reported to slow down the progression of kidney injury (Goraya et al. 2012) and to affect the risk for type 2 diabetes (Fagherrazzi et al. 2014). Nutritional supplements such as sodium bicarbonate and sodium citrate have also been reported to slow the progression of chronic kidney disease (de Brito-Arhurst et al. 2009, Phisitkul et al. 2010). Chronic kidney insufficiency may lead to the accumulation of acids and, via acidosis-induced insulin resistance, to increased CVD risk (Souto et al. 2011). Abnormalities in systemic acid-base status may also affect immune function and response, as many of the clinical acidoses are accompanied by immunodeficiency (Kellum et al. 2004, Lardner 2001).

Aging-related decline in renal function and the ability to excrete excessive hydrogen ions have also been proposed to lead to mild but slowly increasing metabolic acidosis, especially if the dietary acid load is high (Frassetto & Sebastian 1996). Aging-related decline in renal functional capacity diminishes the accuracy and speed of regulation of the volume and composition of the body fluids (Bolignano et al. 2014). Consequently, blood HCO_3^- concentration and pH may be regulated at lower levels (Frassetto & Sebastian 1996). Tabatabai et al. (2015) reported an association between lower plasma HCO_3^- and a higher rate of bone loss in 70-year-old participants. In addition, Dawson-Hughes et al. (2008) concluded that higher intake of fruits and vegetables that contain base-producing compounds and decrease net endogenous acid production favored the preservation of muscle mass in men and women over 65 years of age.

3 AIMS AND HYPOTHESIS

The purpose of the study was to examine the effects of dietary acid load on the acid-base status of the body in healthy adolescents, young adults and the elderly with special reference to aerobic submaximal and maximal exercise. The effects of lower and higher acid intake were studied in three different study settings that included both acute and prolonged interventions.

The specific aims were to study:

1. The acute (4 to 7 days) effects of dietary acid load on acid-base status at rest and during submaximal and maximal aerobic exercise.

   Hypothesis: Low dietary acid load decreases blood and urine acidity as compared to high dietary acid load. These differences can be seen at rest and during aerobic exercise.

   Diet composition is known to influence net endogenous acid production, which may further affect acid-base status of the body.

2. Whether the acute effects of dietary acid load on acid-base status differ between adolescents, young adults, and the elderly, and between men and women.

   Hypothesis: Aging affects the responses in acid-base parameters both at rest and during exercise. There is no difference between men and women in acid-base balance.

   Reduced functional capacity of the kidneys impairs the ability to excrete hydrogen ions, thereby rendering the elderly more susceptible to the effects of dietary acid load. Moreover, the effects of dietary acid load on acid-base status have not been reported during exercise in the elderly. No

literature exists on differences between men and women in acid-base balance.

3. The acute effects of dietary acid load and acid-base status on aerobic exercise performance.

   Hypothesis: Low dietary acid load improves exercise performance as compared to high dietary acid load.

   Many studies have reported positive effects of sodium bicarbonate supplementation and some other ergogenic aids on blood acid-base status and exercise performance. The impact of diet composition on acid-base status and exercise performance has been much less widely investigated. A diet with low acid intake could potentially improve blood buffering capacity and physical performance by attenuating exercise-induced acidosis, which is thought to be one of the causes of muscle fatigue.

4. The prolonged (12 weeks) effects of dietary acid-load on acid-base status and kidney function at rest and during submaximal and maximal exercise.

   Hypothesis: Lower acid intake induces less acidic blood acid–base status at rest and during submaximal exercise and preserves kidney function at rest.

   Prolonged effects of dietary acid intake on acid-base status and kidney function have not yet been investigated in an intervention study in healthy subjects. The long-term effects of exercise training on kidney function have also not been studied in healthy populations.

4 MATERIALS AND METHODS

The data for this thesis was collected in three different study settings. In studies 1 and 2 the acute effects of dietary acid load were studied over 4- and 7-day diet periods, whereas in study 3 the subjects completed a 12-week dietary and training intervention.

4.1 Subjects and ethical considerations

In total, 151 healthy and recreationally active men and women resident in the city of Jyväskylä, Finland volunteered to participate in the study. In study 1 (paper I), nine healthy, recreationally active men in the age range 18-30 volunteered for the study and completed the whole data collection. In study 2 (papers II and III), 93 men and women volunteered and were selected to participate in the study. Subjects were recruited from three age groups: 12- to 15-year-old adolescents (AD), 25- to 35-year-old young adults (YA) and 60- to 75-year-old elderly persons (EL). At study start, the AD group comprised 14 boys and 10 girls, the YA group 16 men and 19 women, and the EL group 17 men and 17 women. Altogether five subjects were unable to complete the study. The AD group was recruited from local sports clubs who were participating in ice hockey, figure skating, gymnastics and athletics. All the adult subjects were recreationally active (e.g., walking, jogging, cycling, resistance training 1-4 times per week) but were not engaging in any systematic endurance or strength training or training for competitive purposes. The YA group participants mainly consisted of students studying at the University of Jyväskylä. The community-dwelling EL participants were recruited from the Ageing Program of the University of Jyväskylä. In study 3 (paper IV), 49 healthy, 20- to 50-year-old men and women volunteered and were selected to participate in the study. These subjects were recruited through email lists of the University of Jyväskylä students and staff. Before inclusion in the study, potential participants were screened for their physical activity, which could include walking, cycling, team sports, or strength

training at a light-to-moderate intensity, at a frequency of 1–3 times per week, but no systematic engagement in any endurance or strength training. At the beginning of study 3, participants meeting the above criteria were randomly divided into two diet groups: low-PRAL and moderate-PRAL. Over the study period, for reasons unrelated to the intervention, three participants dropped out. In all three studies, with two exceptions, no medication was to be used by the participants during the study period. The exceptions were the use of contraceptive pills by women and medication for high blood pressure and high cholesterol by those in the elderly group. Persons whose body mass index were above 33 kg/m^2 or who had any relevant food allergy or were using other medications were excluded from the study. The baseline anthropometric characteristics of the participants who completed the data collection process are presented in TABLE 2.

TABLE 2 Baseline anthropometric characteristics of the participants (mean ± SD).

Paper	Group	Sex	N	Age (yr)	Body mass (kg)	Height (m)	BMI (kg/m^2)	Body fat (%)
I		M	9	23 ± 3.4	76.7 ± 7.4	1.79 ± 0.06	24.0 ± 1.8	15.6 ± 3.0
II, III	AD	M	13	13.4 ± 1.4	52.3 ± 10.9	1.61 ± 0.12	20.0 ± 2.4	13.0 ± 7.5
		F	9	13.0 ± 1.2	49.4 ± 7.7	1.58 ± 0.06	19.6 ± 2.1	18.5 ± 5.4
	YA	M	15	29.1 ± 2.7	79.5 ± 9.7	1.80 ± 0.06	24.5 ± 2.6	17.3 ± 4.6
		F	18	27.6 ± 3.4	58.3 ± 5.0	1.65 ± 0.06	21.6 ± 2.2	22.2 ± 5.1
	EL	M	17	67.1 ± 3.7	78.8 ± 10.0	1.75 ± 0.06	22.4 ± 6.4	25.5 ± 2.0
		F	16	65.4 ± 3.6	67.6 ± 11.0	1.64 ± 0.07	25.3 ± 3.9	34.1 ± 7.9
IV	Low-	M	9	32.0 ± 9.6	86.0 ± 9.2	1.78 ± 0.07	27.2 ± 3.1	23.9 ± 6.8
	PRAL	F	13	34.3 ± 6.9	64.2 ± 7.5	1.67 ± 0.07	23.0 ± 3.5	30.5 ± 6.9
	Mod-	M	12	31.3 ± 5.1	79.1 ± 10.2	1.77 ± 0.06	25.2 ± 2.1	25.3 ± 6.9
	PRAL	F	12	32.0 ± 5.9	65.6 ± 11.4	1.66 ± 0.06	23.7 ± 3.5	31.9 ± 9.7

AD, adolescents; YA, young adults; EL, the elderly; Mod-PRAL, moderate-PRAL.

Ethical approval for all three studies was obtained from the Ethical Committee of the University of Jyväskylä and the studies were conducted in accordance with the guidelines of the Helsinki Declaration. Before any data were collected, participants were informed of the purpose and methods of the study and they signed a written informed consent. All participants completed a health questionnaire and the elderly participants in study 2 also completed a health examination performed by a physician. In addition, the study 3 participants completed questionnaires about their diet and exercise background, and underwent a standardized resting electrocardiogram procedure.

4.2 Research design

Studies 1 and 2 utilized a cross-over study design in which participants were randomly assigned to follow a diet with either a low acid load or a high acid load for 4 days (study 1; I) or 7 days (study 2; II, III). After a wash-out period of approximately 2-4 weeks, participants were assigned to the other diet. Study 3 (IV) was a 12-week longitudinal study in which participants were divided into two groups, one group following a diet with low and the other a diet with moderate acid load.

4.2.1 Short-term interventions (Studies 1 and 2)

4.2.1.1 Baseline testing

Before embarking on the experimental cross-over design in studies 1 and 2, participants' VO_2max and maximal workload were measured (M1). In study 1, an incremental cycle ergometer test was performed on a mechanically-braked cycle ergometer (Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden) (I). The workload was initially 75 W and was increased stepwise by 25 W every 2 min until exhaustion. Pedaling frequency was sustained at 60 rpm throughout the test. Before the ergometer test, participants' height, body mass and body mass index (BMI) were determined. Body fat percentage was estimated using a 4-point skinfold method. Thicknesses of biceps, triceps, subscapular, and suprailiac skinfolds were measured and standard equations of Durnin & Womersley were used for the determination of fat percentage. Before M1, the subjects followed their normal diet and kept food diaries for 4 days. The eating and drinking habits of the subjects were checked for conformity with the general dietary guidelines.

Before any measurements were conducted in study 2, participants' height was measured and baseline body composition data obtained using an InBody720 Body Composition Analyzer (Biospace Co., Seoul, Korea). To determine participants' VO_2max and maximal workload at baseline (M1) an incremental cycle ergometer test was performed on a microprocessor-controlled, eddy current brake-equipped ergometer (Ergoline ergometrics 800, D-72475, Bitz, Germany) (II, III). For the AD group in study 2, the initial workload was 30 W, increasing at each stage by 20 W for boys and by 15 W for girls. For YA, the initial workload was 50 W, increasing at each stage by 25 W for men and by 20 W for women. For EL, the initial workload was 30 W, increasing at each stage by 25 W for men and 20 W for women. Participants were advised to select a comfortable pedaling cadence between 60 and 90 rpm and to maintain it for the duration of the test. In EL group, workload was increased every 2 min until 85% of the age-predicted HR_{max} was attained, and VO_2max was estimated submaximally with Aino FitWare Pro - physical performance testing software (Aino Health Management, Finland).

In both studies, workload was increased every 2 min until volitional exhaustion occurred (except in the EL group). VO_2max was determined as the highest 30-s VO_2 value produced during the test coincident with at least one of the following two criteria: a) respiratory exchange ratio > 1.1; and/or b) a plateau of oxygen uptake (less than 150 ml/min increase in VO_2 during the last 60 s of the test). VO_2max thus determined at the baseline was used in all participant groups in setting the workloads for the cycling tests performed during the experimental designs.

4.2.1.2 Experimental design

After the baseline testing, all participant groups were randomly divided into two subgroups to compare the effects of lower dietary acid intake vs. higher dietary acid intake. A cross-over study design was implemented in which participants were randomly assigned to follow either a low-protein vegetarian diet (LPVD) or a normal diet (ND) for 4 days (I), or a diet with either low (LD) or high (HD) acid load for 7 days (II, III). Approximately two to four weeks after finishing the first diet period, participants were assigned to the alternative diet. Thus, in both studies participants acted as their own dietary controls. The total numbers of participants in the diet groups were 9 (I) and 88 (II, III). For the female participants, the diet periods were scheduled to take place during the same phase of the menstrual cycle.

In study 1, participants began the experimental design by coming to the laboratory after an overnight fast for the drawing of fasting blood samples (PRE) from a fingertip capillary and an antecubital vein. Starting with the PRE sample, participants followed either the LPVD or ND and kept food diaries for 4 days. On the 5th day they completed the second measurement (M2). On the morning of M2, after an overnight fast, the second resting blood samples (POST) were drawn. A light breakfast, consistent with the assigned diet, was eaten thereafter. After a rest of 30 min, resting blood samples were drawn once more (REST). Participants started M2 by a 5-min warm-up followed by a 4-min break before actual test start. Participants cycled 3 x 10 min at 40, 60 and 80% of VO_2max. Finally, they cycled at 100% of VO_2max until exhaustion. All workloads were separated by 4-min rest periods, during which blood samples (CT40, CT60, CT80, CT100, respectively; CT= cycling test) were collected from a fingertip capillary and an antecubital vein.

In study 2 (FIGURE 2), participants began the experimental design by following their normal diet (ND) and keeping a food diary for 3 days. During the last 12 hours of the ND period, subjects had a 12-hour overnight fast and collected a 12-h urine sample. At a laboratory on the 4th morning, fasting blood samples (PRE) were drawn from a fingertip capillary and an antecubital vein. The last meal before the PRE sampling was consistent with the participants' normal diet. Starting from the PRE sample, participants followed either the LD or HD and kept food diaries for 7 days. During the last 12 h of the diet period, another urine sample was collected. On the 8th morning, after a 12-hour overnight fast and after collecting another 12-h urine sample, fasting blood samples

(POST) were drawn at the same time as the PRE sample. The last meal before the POST sample was consistent with the diet followed during the 7-day period (either LD or HD). A light breakfast, consistent with the assigned diet, was eaten thereafter. After 45 min of rest, resting blood samples were drawn once more (REST) before performance of a cycle ergometer test (M2 and M3). M2 and M3 started with a 5-min warm-up followed by a 4-min break. Thereafter, participants completed three 10-min trials at 35, 55 and 75% of the VO_2max obtained during M1. The AD and YA groups also completed a trial until volitional exhaustion at a workload equivalent to 100% of VO_2max. All workloads were separated by 4-min rest periods, during which blood samples (CT35, CT55, CT75, CT100, respectively) were collected from a fingertip capillary and an antecubital vein.

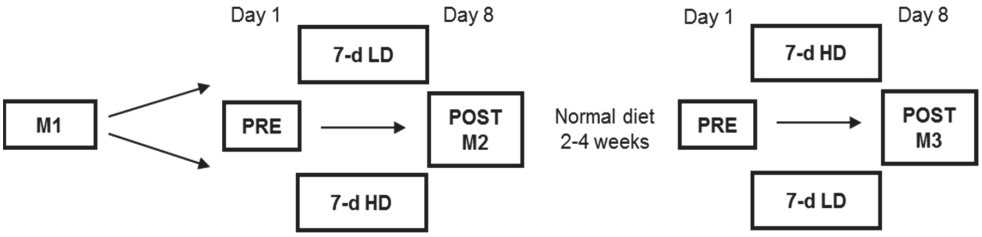


FIGURE 2 Experimental design in study 2.

In both studies, the breakfasts eaten before the cycling tests were standard and isoenergetic in all groups with subtle adjustments in the amounts of the foods. Maximal workload was continued until volitional exhaustion occurred or a participant was unable to continue pedaling over 60 rpm. After completion of M2, participants were permitted to eat again according to their normal dietary habits. Approximately 2-4 weeks after M2, participants were then assigned to the alternative diet and completed other 4- or 7-day diet period (M3). Participants were permitted to exercise moderately during the diet periods. During the last 24 hours before every fasting blood sample, participants were instructed to minimize their physical activity. The subjects were also instructed to report their physical activity along with the food diaries over the experimental diet periods.

4.2.2 Prolonged intervention (Study 3)

Study 3 period lasted 12 weeks, during which participants were assigned either to the low- or moderate-PRAL diet group for the entire duration of the study period. Participants trained twice a week and each training session consisted of endurance and strength training (45 min + 45 min each). The measurement sessions took place before the intervention period (PRE), during the 6- to 8- week mid-phase (MID) and after the intervention (POST). At PRE, MID, and POST, participants participated in measurement sessions in which a 12-hour urine sample and fasting blood samples were collected. In addition, participants rec-

orded their food intake in a 3-day food diary. At PRE and POST, participants also performed a submaximal cycle ergometer test during which blood samples were collected from a fingertip capillary and an antecubital vein.

One week before the start of the 12-week intervention, participants' VO_2max and maximal workload were measured (M1) by an incremental cycle ergometer test performed on a microprocessor-controlled, eddy current brake-equipped ergometer (Ergoline ergometrics 800, D-72475, Bitz, Germany). The initial workload was 50 W and increased stepwise by 25 W every 2 min until exhaustion. VO_2max was determined as the highest 30-s VO_2 value during the test. Gaseous exchange was measured using a Jaeger Oxycon Pro breath-by-breath gas analyzer (VIASYS Healthcare GmbH, Hoechburg, Germany). The device was calibrated for volume and gas analyzer before every measurement. VO_2max determined at the baseline (PRE) was used in all participant groups to set the workloads for the submaximal cycling tests. Three days after the VO_2max test, participants performed a submaximal cycling test, which is described below. VO_2max test was repeated at POST, again three days before the submaximal test.

During the last 12 hours (overnight) before the start of the dietary intervention, participants fasted and a 12-h urine sample was collected. The following morning, in a laboratory, fasting blood samples (PRE) were drawn from a fingertip capillary and an antecubital vein. Starting from PRE, participants followed either the low- or moderate-PRAL diet, and the same urine and blood sampling sessions were repeated at both MID and POST. At PRE and POST, after the fasting blood sampling, participants ate a light breakfast consistent with their assigned diet. After 45 min of rest, resting blood samples were drawn once more (REST) before completion of a submaximal cycle ergometer test that started with a 5-min warm-up followed by a 4-min break. Thereafter, participants completed three 8-min trials at 35, 55 and 75% of the VO_2max obtained in M1. All workloads were separated by 4-min rest periods, during which blood samples (CT35, CT55, CT75, respectively) were collected from a fingertip capillary and an antecubital vein. Before the measurements, participants' height was measured. Participants' body composition was assessed by Dual X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE Medical Systems, Madison, USA) at PRE, MID and POST. Total fat mass and total lean mass were automatically analyzed (Encore-software, version 14.10.022).

The training protocol has been described elsewhere (Schumann et al. 2014). Briefly, the endurance training was conducted on a cycle ergometer and the training program mostly consisted of steady-state cycling of low to moderate intensity (below and above the aerobic threshold). The duration of the endurance cycling training increased progressively from 30 to 50 min. During the last 4 weeks of the training period, the intensity of cycling also increased from the aerobic to the anaerobic threshold and then further, until participants were completing maximal aerobic workloads. The resistance training program included exercises for all major muscle groups with a focus on the lower extremities. During the first two weeks, training was performed as circuit training us-

ing low intensities. Thereafter, protocols aiming at muscle hypertrophy and maximal and explosive strength were performed. During the second half of the study, both training volume and frequency were increased. The overall duration of the strength training sessions was 30–50 min.

4.3 Dietary recording

All the experimental diets used in the studies 1-3 were planned by calculating the potential renal acid loads (PRAL) of the diets. The PRAL value of each foodstuff was calculated according to an equation that takes into account the contents of certain nutrients per 100 g of foodstuff, their intestinal absorption rates, grade of dissociation of phosphate at pH 7.4 and the ionic valence of magnesium and calcium. The equation is as follows:

> PRAL (mEq/100 g) = 0.49 x protein (g/100 g) + 0.037 x phosphorous (mg/100 g) - 0.021 x potassium (mg/100 g) - 0.026 x magnesium - 0.013 x calcium (mg/100 g) (Remer et al. 2003).

The nutrient contents of the foodstuffs were taken from the Finnish Food Composition Database (Fineli, Finnish National Institute of Health and Welfare). In studies 1 and 2, participants were given exact instructions on how to follow the experimental diets. Specific 1-day menus were designed for the LPVD, LD and HD, and every day during these diet periods participants repeated the meals and snacks assigned according to the diet menus. Vitamin and mineral supplements were not allowed during the study periods. LPVD (I) was a vegetable- and fruit-based diet with very limited use of grain and dairy products. Participants were not allowed to eat meat, cheese or bread at all during the 4 days. During ND (I), participants followed their habitual diet. LD (II, III) was based on a large intake of vegetables and fruits and a limited use of grain and dairy products. Participants were instructed not to eat red meat, eggs or cheese during the 7 days. However, the diet included chicken (2 g/kg/d) to ensure an adequate intake of protein. HD (II, III) was planned to include no vegetables and fruits at all. It mainly consisted of grain products, chicken, red meat and eggs. Participants were advised to eat according to their perceived energy needs during the first diet period. The dietary instructions for the second diet period were adjusted so that the diet periods were isoenergetic. Between the two diet periods, participants were permitted to eat according to their habitual diet.

In study 3, before the start of the study, participants followed their normal diet and kept food diaries for 3 days. Appropriate dietary counselling was given to both diet groups according to the baseline dietary analysis. Before the start of the 12-week intervention period, participants were given instructions on how to follow the low- and moderate-PRAL diets. Both diet groups ate according to the general dietary guidelines, but in the low-PRAL diet participants were advised to increase the consumption of fruits and vegetables up to 800-1000 g per

day. On the other hand, in the moderate-PRAL diet participants were advised to limit their intake of fruits and vegetables (200-300 g per day). The aim was that the low-PRAL diet would enhance alkali production in the body (PRAL<0), whereas the moderate-PRAL diet would slightly increase acid production (PRAL>0). Participants kept food diaries for 3 days during the PRE, MID, and POST periods and these dietary records were analyzed to compare differences in dietary intakes between and within the diet groups. In addition, participants recorded their food intake during weeks 1-4 to check that the assigned diet was being followed as per instructions.

During all the periods in which participants recorded their food and fluid intake, the amounts of foods and fluids eaten was to be reported as precisely as possible. The foods eaten were recorded in grams, whenever possible, or in household measures. In studies 2 and 3, participants were provided with a kitchen scale for the duration of the study periods. The food diaries were analyzed for energy, protein, carbohydrate, fat, phosphorous, potassium, magnesium and calcium intake using Nutrica 3.11 software (The Social Insurance Association of Finland 1999) (I), or Nutri-Flow software (Flow-Team Oy, Oulu, Finland) (II, III, IV). Mean daily PRAL during the experimental diets was calculated according to the relevant dietary intake data.

4.4 Urine sampling and analysis

In studies 2 and 3, participants collected 12-h urine samples before the PRE and POST blood samples. Each urine sample was collected in a sterile container and refrigerated until the donor brought the container to the laboratory. Upon receipt, samples were immediately analyzed for pH by dipping a pH strip into the urine sample (Combur-7 Test urinalysis test strips; Cobas, Roche, Germany). Urine electrolytes were analyzed by the direct ISE in vitro test (Ion Selective Microlyte Analyzer, Konelab 20 XTi; Kone Instruments, Espoo, Finland). Indirect NAE was calculated as follows:

$$NAE_{indirect}\ (mEq/d) = (Cl^- + P_i^{-1.8} + SO_4^{2-} + OA) - (Na^+ + K^+ + Ca^{2+} + Mg^{2+})$$

where SO_4^{2-}= 0.4875 * dietary protein intake (g)
OA= (BSA*41)/1.73
BSA= $[(\text{body mass (kg)} * \text{height (m)})/3600]^{½}$ (Remer et al. 2003; Remer & Manz 1995).

4.5 Blood sampling and analysis

In study 1, Li-heparinized whole blood samples (200 µl) from a fingertip capillary were analyzed immediately after sampling for pH, lactate, HCO_3^- and

pCO_2. For the determination of pH the direct ISE (ion selective electrolyte), an in vitro test was used. Lactate was analyzed quantitatively by the enzymatic and amperometric in vitro test. PCO_2 was analyzed by the membrane amperometric method. HCO_3^- was determined computationally (Nova Biomedical STAT Profile pHOX Plus L Blood Gas Analyzator, Nova Biomedical, Waltham, MA, USA). Whole blood samples (4 ml) from the antecubital vein were analyzed for sodium, potassium and chloride by the direct ISE in vitro test (Ion Selective Microlyte Analyzer, Kone Instruments, Espoo Finland) and whole protein content of plasma spectrophotometrically by the Biuret method (Shimadzu CL 720 Spectrophotometry, Shimadzu Co., Kyoto, Japan).

In studies 2 and 3, all capillary and antecubital vein blood samples were drawn at the same time in the morning during all diet periods. Li-heparinized whole blood samples (200 and 20 µl) from a fingertip capillary were analyzed immediately after sampling for pH, HCO_3^-, standard BE and lactate. The determination of pH was based on the principle of ion selective electrode whereas HCO_3^- and BE were determined computationally from pH and pCO_2 values (GEM Premier 3000, Instrumentation Laboratory, Lexington, MA, USA). Lactate was analyzed by the amperometric and enzymatic method (BIOSEN C_line, Sport, EKF Diagnostic, Magdeburg, Germany). Whole blood samples (4 ml) from the antecubital vein were drawn in Venosafe gel tubes and analyzed for sodium (Na^+), potassium (K^+) and chloride (Cl^-) by the direct ISE in vitro test (Ion Selective Microlyte Analyzer, Konelab 20 XTi; Kone Instruments, Espoo, Finland). Whole protein content of plasma (P_{tot}) was analyzed spectrophotometrically by the Biuret method (Ion Selective Microlyte Analyzer, Konelab 20 XTi; Kone Instruments). The blood samples from antecubital vein were also drawn in vacuum tubes, held at room temperature for 30 min and centrifuged for 10 min at 3500 rpm (2100g). The serum was separated and creatinine and urea analyzed with a KoneLab 20 XTi analyzator (Thermo Electron Corporation, Vantaa, Finland). To calculate the glomerular filtration rate (GFR), serum creatinine values were used in the CKD-EPI equation, which uses also age, race, gender and body size to estimate GFR (Levey et al. 2009).

SID and A_{tot} were calculated as follows:

$$SID\ (mEq/l) = ([Na^+] + [K^+]) - ([Cl^-] + [Lac^-])$$ (Miller et al. 2005)

$$A_{tot}\ (mEq/l) = 2.45 \times [P_{tot}]\ (g/dl)$$ (Putman et al. 2003).

4.6 Breath gas analysis

Gaseous exchange during cycling was measured using a Sensor Medics Breath Gas Analyzator (Vmax series 229, California, USA) (study 1) or Jaeger Oxycon Pro breath-by-breath gas analyzer (VIASYS Healthcare GmbH, Hoechburg, Germany) (studies 2 and 3). The devices were calibrated before every meas-

urement. Mean cardiorespiratory variables (VO_2, VCO_2, RQ and VE) were determined from the final 30 seconds of every workload. Heart rate was measured by a Polar heart rate monitor (Polar Electro Oy, Kempele, Finland).

4.7 Statistical analysis

In study 1, all the variables were analyzed by SPSS 14.0 for Windows software. The resting blood samples (PRE and POST), the cardiorespiratory variables, and the nutrient intake values were compared by paired t-test. Variables from the blood samples obtained during M2 and M3 were compared to the resting blood sample of the same day (POST) between the two groups (ND vs. LPVD) with repeated measures ANOVA (2 group × 5 time). If there was a difference between the groups the analysis was continued with a paired t-test.

In study 2, the differences in blood variables between the diet groups were tested with mixed models with random ID. Comparisons were made separately at rest (PRE and POST) and during the cycling test day (POST, REST, CT35, CT55, CT75 and CT100). When the main effect of diet composition, age, gender or time (between PRE and POST) was statistically significant, the comparison was continued with LSD pairwise comparisons. The effect of dietary acid load on cardiorespiratory variables and GFR was examined by two-way repeated measures analysis of variance (ANOVA), and if a statistically significant difference was observed, the comparison was continued by a paired t-test. The variables of dietary intake analysis and the difference in the duration of maximal workload were compared within each age and gender group with paired samples t-test. The correlations of GFR with acid-base parameters were analyzed by Pearson correlation analysis. Statistical analyses were performed with IBM SPSS Statistics 22.0 (SPSS, Inc., an IBM Company).

In study 3, the effect of the 12-week intervention period on blood and urine variables, and variables of dietary intake analysis were examined by a two-way repeated measures analysis of variance (ANOVA). If a statistically significant difference was observed within one of the diet groups, or between groups, the comparison was continued with a suitable t-test.

Data are presented as means ± SDs. The statistical difference was considered to be significant at the $p < 0.05$ level.

5 RESULTS

5.1 Body composition

No significant changes were observed in the body composition of participants in any of the studies. Participants' body composition data obtained by DXA in study 3 are presented in TABLE 3. The body mass of all participants during the experimental diets are presented in TABLE 4 along with the dietary intake data.

TABLE 3 Body composition before (PRE), and after (POST) the 12-week diet period in the low-PRAL and moderate-PRAL (Mod-PRAL) diet groups.

	Men				Women			
	Low-PRAL		Mod-PRAL		Low-PRAL		Mod-PRAL	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Body mass (kg)	85.5 ± 9.8	83.7 ± 9.5	79.2 ± 10.2	79.6 ± 9.8	64.3 ± 7.8	63.8 ± 7.9	67.0 ± 11.1	67.9 ± 11.5
Lean mass (kg)	61.5 ± 5.6	61.3 ± 5.2	56.1 ± 4.8	57.2 ± 5.8	41.2 ± 3.4	41.5 ± 2.6	40.9 ± 4.8	41.7 ± 4.9
Fat %	23.9 ± 7.4	22.0 ± 7.9	25.3 ± 6.9	23.8 ± 5.9	31.0 ± 7.0	30.4 ± 6.6	33.2 ± 9.3	33.8 ± 9.0

5.2 Dietary acid load and macronutrient intakes

Daily PRAL, intake of fruits and vegetables (IFV) and intake of protein are presented in TABLE 3. Intakes of fruits and vegetables and protein are presented here since they are the most important contributors to PRAL. The complete dietary intake data can be found in the original articles.

In study 1, daily PRAL ($P<0.001$) and IFV ($P<0.001$) were significantly higher during the LPVD compared to ND (TABLE 3). Energy intake and protein and fat intakes (% of total energy intake) were significantly lower ($P=0.033$,

P<0.001, p=0.015, respectively) during the LPVD compared to ND. The intake of carbohydrates (% of total energy intake) was significantly higher (P=0.003) during the LPVD compared to ND.

In study 2, PRAL was significantly lower (P<0.001 in all groups) and IFV significantly higher (P≤0.001) in the LD compared to HD in all groups. In the AD boys and EL women, energy intake did not differ between the diets; however, in the other groups it was significantly lower during the LD than HD (P≤0.022). Protein intake (g/kg/d) was significantly lower (P<0.001) during the LD than HD in all groups except the AD girls.

In study 3, no significant differences in energy or macronutrient intake was observed within or between the diet groups. PRAL was significantly lower and IFV higher at MID and POST in low-PRAL as compared to moderate-PRAL in both men and women (P<0.001 in all). No significant differences were observed in PRAL or IFV between MID and POST within the diet groups.

TABLE 4 Dietary intake data during all the experimental diets of the present study.

Paper	**Age**	**Sex**	**Diet**	**PRAL (mEq/d)**	**IFV (g/d)**	**Protein (g/kg/d)**	**Body mass (kg)**
I			LPVD	-117 ± 20***	1150 ± 200	0.80 ± 0.11***	75.6 ± 7.9
			ND	3.2 ± 19	350 ± 70	1.59 ± 0.28	76.2 ± 7.6
II, III	AD	M	LD	-47 ± 44***	830 ± 630**	1.3 ± 0.4***	52.6 ± 11
			HD	25 ± 11	40 ± 18	2.1 ± 0.5	53.0 ± 11
		F	LD	-43 ± 18***	890 ± 370***	1.0 ± 0.2	48.8 ± 6.6
			HD	15 ± 16	28 ± 16	1.5 ± 0.7	49.3 ± 6.9
	YA	M	LD	-68 ± 30***	1410 ± 460***	1.3 ± 0.4***	77.5 ± 9.1
			HD	61 ± 22	24 ± 11	2.1 ± 0.6	77.5 ± 9.1
		F	LD	-68 ± 17***	1400 ± 350***	1.2 ± 0.2***	57.0 ± 5.3
			HD	47 ± 8.3	22 ± 8	2.3 ± 0.3	57.3 ± 5.1
	EL	M	LD	-61 ± 17***	1270 ± 380***	1.1 ± 0.2***	77.7 ± 9.6
			HD	57 ± 12	20 ± 8	1.9 ± 0.4	77.7 ± 9.5
		F	LD	-63 ± 12***	1350 ± 290***	1.0 ± 0.2***	66.6 ± 10
			HD	49 ± 14	17 ± 6	1.9 ± 0.5	66.5 ± 10.9
IV		M	Low-PRAL	-37 ±24**	800 ± 380***	1.1 ± 0.2	83.7 ± 9.5
			Mod-PRAL	11 ± 17**	230 ± 100	1.4 ± 0.4	79.6 ± 9.8
		F	Low-PRAL	-56 ± 40**	1070 ± 630	1.1 ± 0.3	63.8 ± 7.9
			Mod-PRAL	-0.8 ± 17**	260 ± 270	1.1 ± 0.2	67.9 ± 11.5

AD, adolescents; YA, young adults; EL, the elderly; LPVD, low-protein vegetarian diet; ND, normal diet; LD, diet with low acid load; HD diet with high acid load; Low-PRAL, diet with low acid load; Mod-PRAL, diet with moderate acid load; PRAL, potential renal acid load; IFV, intake of fruits and vegetables; CHO, carbohydrates. *P<0.05, ** P<0.01, *** P<0.001, statistically significant difference between the diet groups paired or independent samples t-test). Values are mean ± SD.

5.3 Urine acid-base status

5.3.1 Net acid excretion (NAE)

NAE decreased over the 7-day LD in the YA men (P=0.022) and EL men (P=0.027) (FIGURE 3). NAE increased during the HD and was significantly lower after the LD than HD in the YA men (P=0.014, P=0.005, respectively), YA women (P<0.001 in both), EL men (P<0.001 in both) and EL women (P<0.001 in both). In the adolescents, no significant changes in NAE were observed.

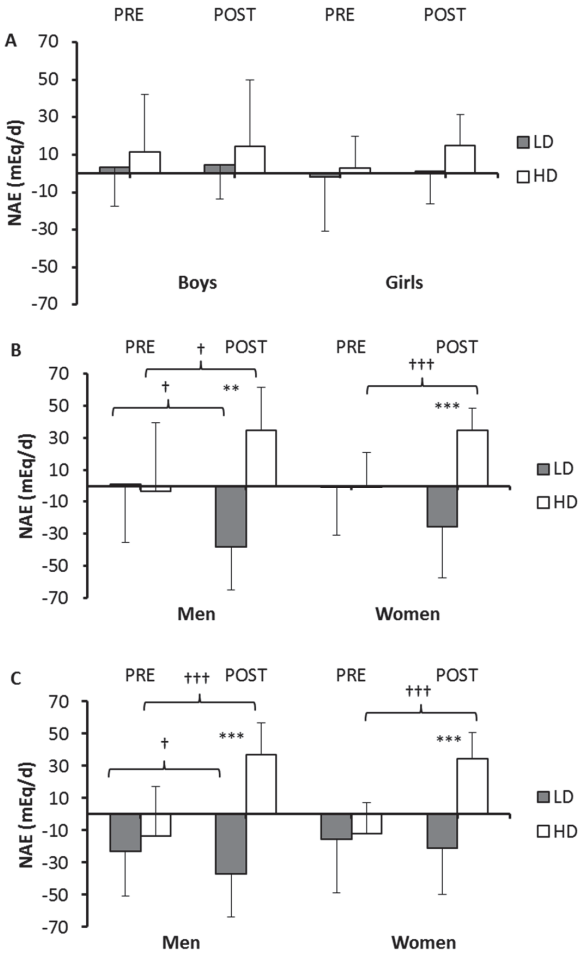


FIGURE 3 Net acid excretion (NAE) in adolescents (A), young adults (B) and the elderly (C) at the beginning (PRE) and end (POST) of the 7-d low acid load diet (LD) and high acid load diet (HD). ** P<0.01, *** P<0.001 statistically significant difference between LD and HD; † P<0.05, ††† P<0.001 statistically significant difference between PRE and POST during LD or HD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.

After the 12-week diet intervention, NAE was lower in the low- compared to moderate-PRAL group in both men (P=0.001) and women (P=0.047) (FIGURE 4).

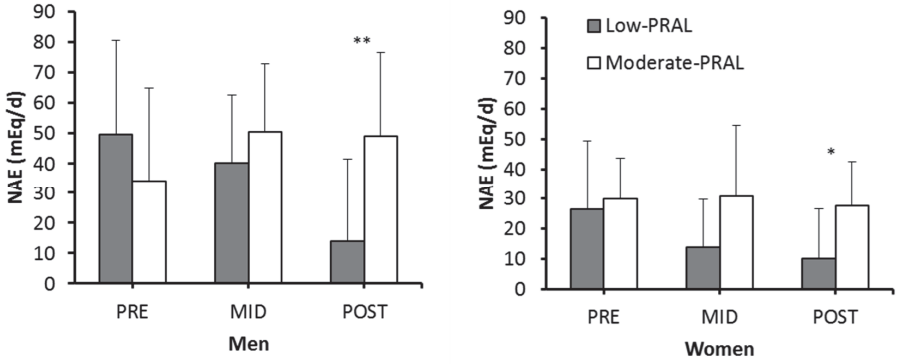


FIGURE 4 Net acid excretion (NAE) in the low-PRAL and moderate-PRAL diet groups at baseline (PRE), in the middle (MID) and after (POST) the 12-week diet period. * P<0.05, ** P<0.01 statistically significant difference between the diet groups at POST (two-way repeated measures ANOVA, an independent t-test). Values are mean ± SD.

5.3.2 Urine pH

Urine pH increased in all the adult groups over the 7-day LD (YA men, P=0.011; YA women, P=0.001; EL men, P<0.001; EL women, P<0.001) (FIGURE 5). Urine pH decreased during LD in the YA women, EL men, and EL women (P<0.001 in all). In the adolescents, no significant changes in urine pH were observed.

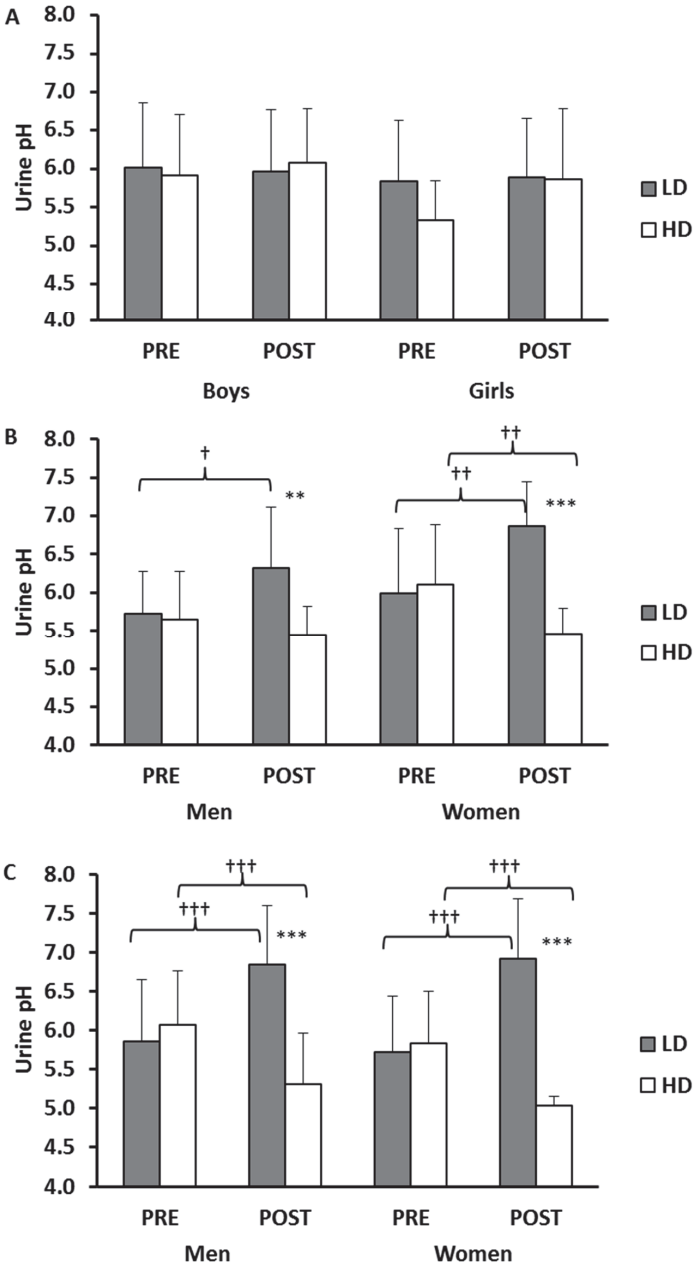


FIGURE 5 Urine pH in adolescents (A), young adults (B) and the elderly (C) at the beginning (PRE) and end (POST) of the 7-d low acid load diet (LD) and high acid load diet (HD). ** P<0.01, *** P<0.001 statistically significant difference between LD and HD; † P<0.05, †† P<0.01, ††† P<0.001 statistically significant difference between PRE and POST during LD or HD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.

Over the 12-week diet intervention, no significant changes were observed in urine pH in any of the subject groups (FIGURE 6).

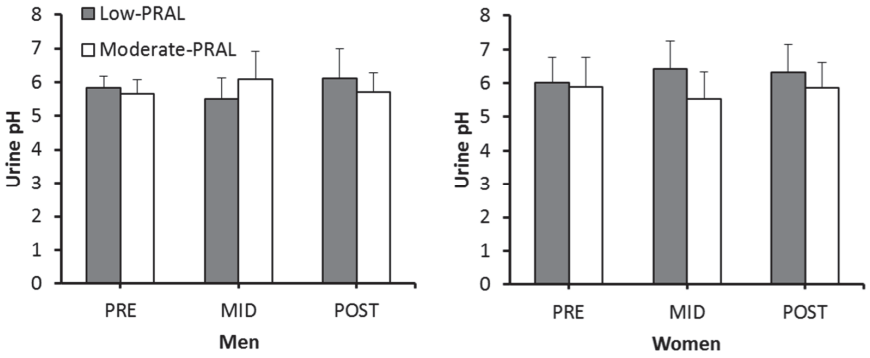


FIGURE 6 Urine pH in the low-PRAL and the moderate-PRAL diet groups at baseline (PRE), in the middle (MID) and after (POST) the 12-week diet period. Values are mean ± SD.

5.4 Capillary acid-base status and lactate

In study 1, no significant differences in capillary pH, HCO_3^- or lactate were observed between the diet groups (FIGURE 7 and 8).

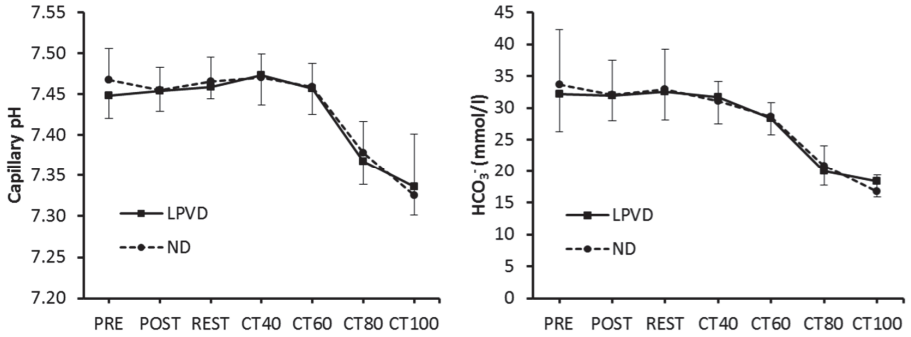


FIGURE 7 Capillary pH and HCO_3^- in young men at the beginning (PRE) and end (POST) of the 4-d low-protein vegetarian diet (LPVD) and normal diet (ND), after breakfast (REST) and during exercise in which cycling was performed for 10 min at 40%, 60% and 80% of VO_2max (CT40, CT60, CT80). In addition, participants cycled at 100% of VO_2max until exhaustion (CT100). Values are mean ± SD.

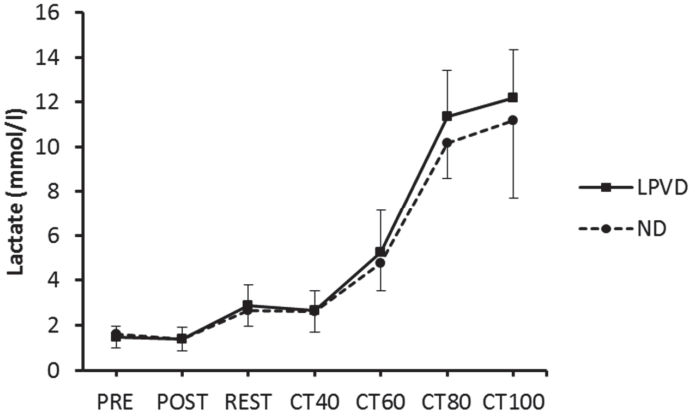


FIGURE 8 Lactate in young men at the beginning (PRE) and end (POST) of the 4-d low-protein vegetarian diet (LPVD) and normal diet (ND), after breakfast (REST) and during exercise in which cycling was performed for 10 min at 40%, 60% and 80% of VO_2max (CT40, CT60, CT80). In addition, participants cycled at 100% of VO_2max until exhaustion (CT100). Values are mean ± SD.

In study 2, capillary pH and HCO_3^- decreased over the 7-day HD period in the YA men (P=0.039, P=0.027, respectively), YA women (P=0.019, P=0.001), and EL women (P<0.001) (FIGURE 9 and 10). In girls, HCO_3^- increased (P=0.005) over the LD period. In the EL men, HCO_3^- decreased (P<0.001) over the HD period and increased (P=0.039) during the LD diet. In all the adult groups, pH was higher at POST after the LD compared to HD (YA men P=0.038, 95% confidence interval for the difference (95% CI) [0.010, 0.026], effect size (ES, Hedges' g) 0.38; YA women P<0.001, 95% CI [0.017, 0.040], ES 1.33; EL men P=0.021, 95% CI [0.020, 0.027], ES 0.40; EL women P<0.001, 95% CI [0.013, 0.038], ES 1.07). In all the adult groups, HCO_3^- was higher at POST after the LD compared to HD (YA men P=0.038, 95% CI [0.04, 1.81], ES 0.51; YA women P<0.001, 95% CI [1.27, 2.89], ES 0.94; EL men P<0.001, 95% CI [1.18, 2.89], ES 0.96; EL women P<0.001, 95% CI [1.80, 3.54], ES 1.38).

HCO_3^- was higher at all submaximal workloads after the LD compared to HD in the YA women (P<0.020, ES 0.62; P=0.005, ES 0.72; P=0.022, ES 0.49; respectively) and EL women (P<0.008, ES 0.79; P<0.001, ES 1.04; P<0.001, ES 0.76; respectively). Moreover, pH was also higher at CT75 in the YA women (P=0.005, 95% CI [0.009, 0.049], ES 0.60) and EL women (P=0.002, 95% CI [0.013, 0.056], ES 0.72) after the LD than after HD. In the YA men, pH was higher at CT100 (P=0.034, 95% CI [0.002, 0.048], ES 0.33) after the LD than after HD. In the EL men, HCO_3^- was higher at CT75 (P=0.020, 95% CI [0.22, 2.58], ES 0.44) after the LD than after HD.

In the YA women, lactate was higher, at CT100, after the LD compared to HD (10.0 ± 1.8 vs. 8.6 ± 1.2 mmol/l, P=0.002, 95% CI [0.50, 2.12], ES 0.89). In the EL women, lactate was higher before the cycling test at REST after the LD than

HD (3.0 ± 0.5 vs. 2.0 ± 0.5 mmol/l, P=0.026). No other significant differences in lactate were observed between the diet periods in study 2.

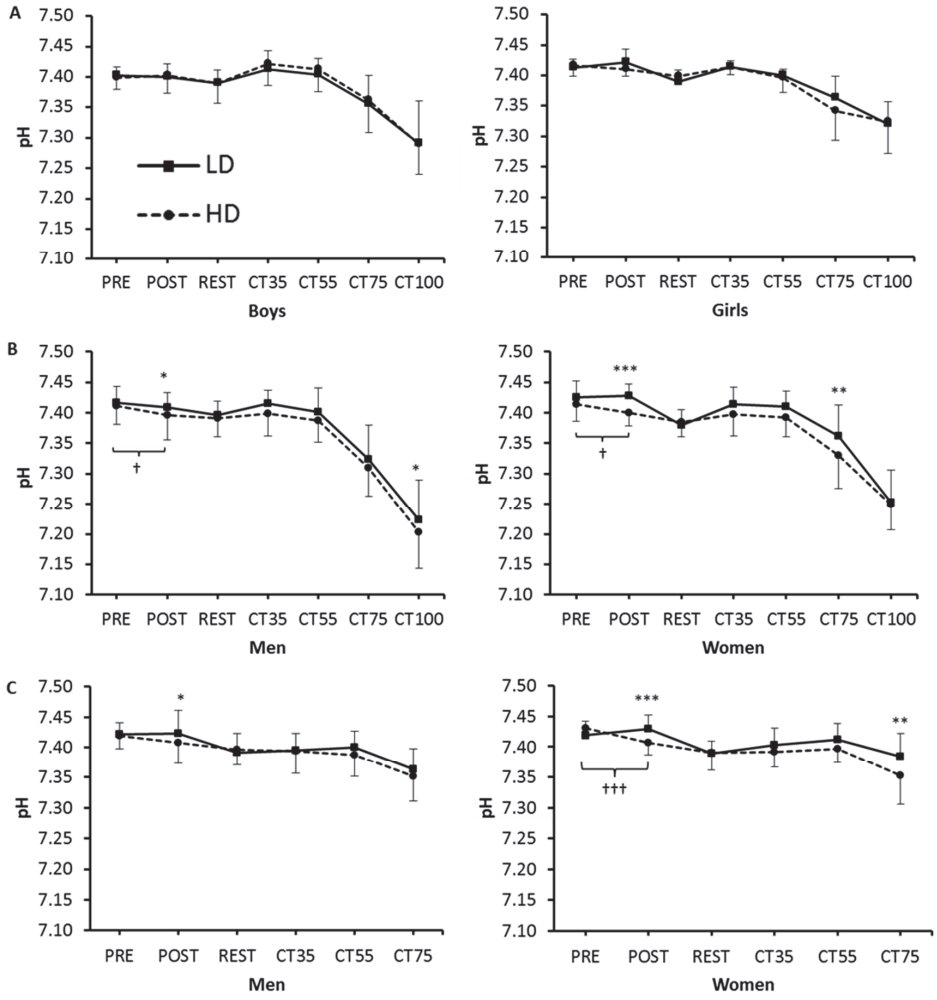


FIGURE 9 Capillary pH in adolescents (A), young adults (B) and elderly (C) men and women at the beginning (PRE) and end (POST) of the 7-d low acid load diet (LD) and high acid load diet (HD), after breakfast (REST) and during exercise in which cycling was performed for 10 min at 35%, 55% and 75% of VO_2max (CT35, CT55, CT75). Adolescents and young adults also cycled at 100% of VO_2max until exhaustion (CT100). * P<0.05, ** P<0.01, *** P<0.001 statistically significant difference between LD and HD; † P<0.05, ††† P<0.001 statistically significant difference between PRE and POST during HD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.

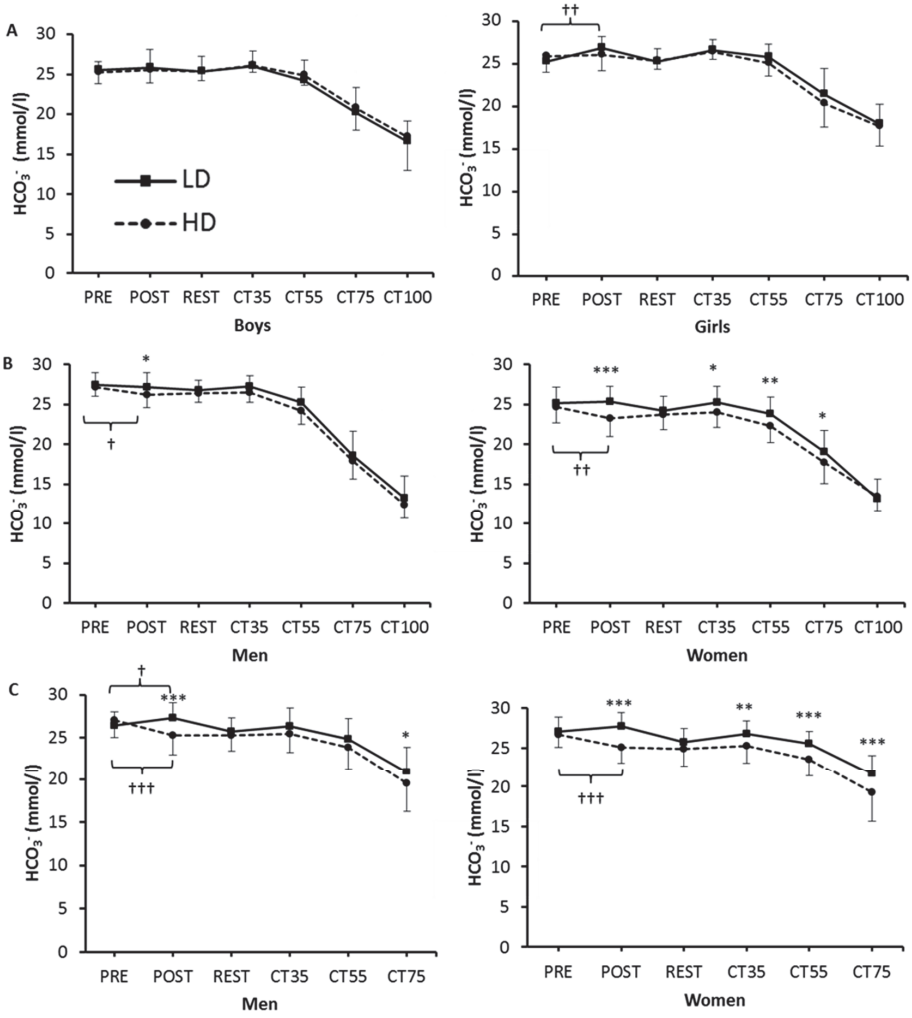


FIGURE 10 Bicarbonate (HCO_3^-) in adolescents (A), young adults (B) and elderly (C) men and women at the beginning (PRE) and end (POST) of the 7-d low acid load diet (LD) and high acid load diet (HD), after breakfast (REST) and during exercise in which cycling was performed for 10 min at 35%, 55% and 75% of VO_2max (CT35, CT55, CT75). Adolescents and young adults also cycled at 100% of VO_2max until exhaustion (CT100). * P<0.05, ** P<0.01, *** P<0.001, statistically significant difference between LD and HD; † P<0.05, †† P<0.01, ††† P<0.001 statistically significant difference between PRE and POST during LD or HD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.

In study 3, no significant differences were observed between the diet groups in capillary pH (FIGURE 11). Within both diet groups, some significant changes occurred in capillary pH. In the low-PRAL women, pH was significantly lower at 35% (P=0.014, 95% CI [0.002, 0.015], ES 0.39) and higher at 75% of VO_2max

(P=0.020, 95% CI [0.002, 0.040], ES 0.52) at POST compared to PRE. In the moderate-PRAL women, pH was significantly lower at 55% of VO_2max (P=0.033, 95% CI [0.002, 0.040], ES 0.52) at POST compared to PRE.

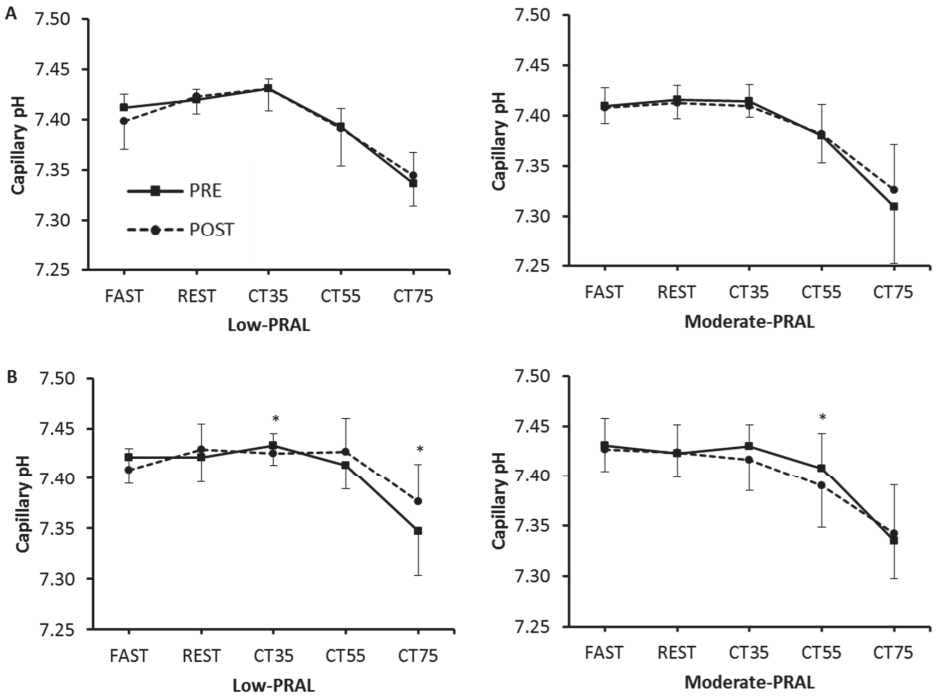


FIGURE 11 Capillary pH in men (A) and women (B) at baseline (PRE) and after (POST) the 12-week low-PRAL and moderate-PRAL diet period at rest (FAST, REST) and during submaximal cycling (CT35, CT55, CT75). * P<0.05, statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired t-test). Values are mean ± SD.

In study 3, in the low-PRAL women, HCO_3^- was higher at 75% of VO_2max (P=0.006, 95% CI [-2.85, -0.61], ES 0.67) at POST compared to PRE (FIGURE 12). In the moderate-PRAL men, HCO_3^- was higher at 75% of VO_2max (P=0.002, 95% ci [-2.97, -0.86], ES 0.75) after the training period compared to PRE. The only significant differences in HCO_3^- between the diet groups occurred in men, as HCO_3^- was higher at FAST, REST and CT35 in the low- compared to moderate-PRAL group (P=0.015, P=0.039, P=0.010, respectively).

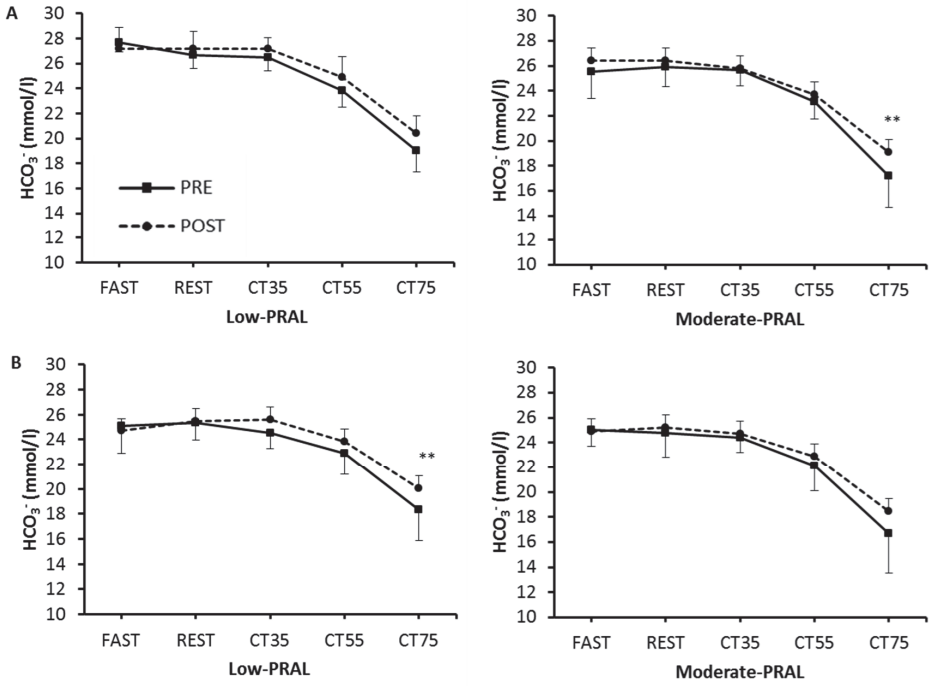


FIGURE 12 Capillary bicarbonate (HCO_3^-) in men (A) and women (B) at baseline (PRE) and after (POST) the 12-week low-PRAL and moderate-PRAL diet period at rest (FAST, REST) and during submaximal cycling (CT35, CT55, CT75). ** P<0.01, statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired t-test). Values are mean ± SD.

No statistically significant differences in lactate were observed between the low- and moderate-PRAL diet groups (FIGURE 13). In the moderate-PRAL men, lactate was lower at 55% (P=0.006, 95% CI [0.27, 1.23], ES 0.85) and 75% (P<0.001, 95% CI [0.88, 2.25], ES 0.66) of VO_2max at POST compared to PRE. In the moderate-PRAL women (P=0.044, 95% CI [0.02, 1.12], ES 0.68) and low-PRAL men (P=0.017, 95% CI [0.26, 1.85], ES 0.71), the difference between PRE and POST was significant at 55% of VO_2max. In the low-PRAL women lactate was significantly lower at CT75 (P=0.005, 95% CI [0.49, 2.16], ES 0.81) at PRE compared to POST.

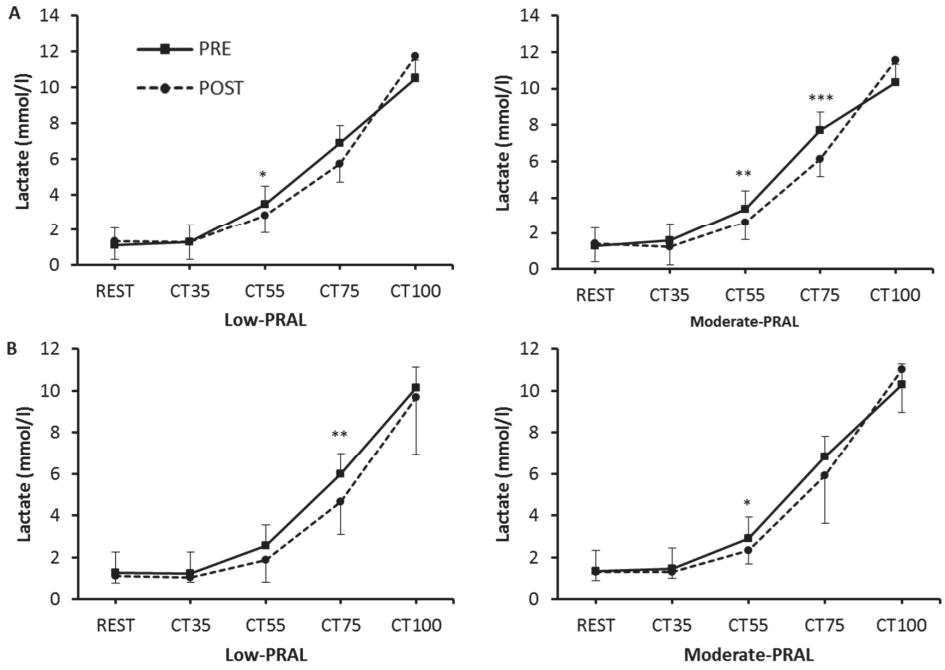


FIGURE 13 Capillary lactate in men (A) and women (B) at baseline (PRE) and after (POST) the 12-week low-PRAL and moderate-PRAL diet period at rest (FAST, REST) and during submaximal cycling (CT35, CT55, CT75). Lactate was also measured in a separate VO_2max test (CT100). * P<0.05, ** P<0.01, *** P<0.001 statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired t-test). Values are mean ± SD.

5.5 Exercise performance

5.5.1 Duration of maximal workload

In study 1, not all subjects were able to finish the 10-min workload cycled at 80% of VO_2max. The duration of CT80 was 8.84 ± 1.46 min after the LPVD and 8.56 ± 1.87 min after the ND. The duration of maximal workload, which was cycled until exhaustion was 1.81 ± 0.80 min after the LPVD and 2.89 ± 1.91 min after the ND. None of these differences were statistically significant.

In the YA women (study 2), the duration of maximal workload was shorter after the HD compared to LD (3.12 ± 1.01 vs. 3.84 ± 1.28 min, P=0.001, 95% CI [0.33, 1.12], ES 0.61). Men showed no differences in the duration of maximal workload (3.79 ± 1.48 vs. 3.67 ± 1.20 min, respectively) between HD and LD.

Four older women were unable to finish the CT75 after the HD period (study 2). One older man was not able to finish the CT75 after either the LD or HD. The duration of CT75 was shorter after the HD compared to LD in both

men (9.66 ± 1.39 vs. 9.81 ± 0.79 min) and women (9.23 ± 2.07 vs. 10.0 ± 0 min) but the differences were non-significant. In boys, the time to exhaustion at CT100 was 3.21 ± 1.74 min after the LD and 3.34 ± 1.58 min after the HD. In girls, the corresponding durations were 3.20 ± 0.78 min and 3.17 ± 0.88 min. The differences were not statistically significant.

In study 3, the duration of the separate VO_2max test was longer in the moderate-PRAL men at POST compared to PRE (19.8 ± 3.8 vs. 18.1 ± 3.9 min, P=0.014, 95% CI [-2.85, -0.41], ES 0.42) and in the moderate-PRAL women at POST compared to PRE (12.6 ± 1.7 vs. 11.1 ± 1.7, P<0.001, 95% CI [-1.95, -1.07], ES 0.86). The duration of the VO_2max test was longer also in the low-PRAL men and women at POST compared to PRE, but the differences were not statistically significant (men: 20.3 ± 3.8 vs. 19.3 ± 2.5 min; women: 13.8 ± 2.0 vs. 13.4 ± 1.8 min).

5.5.2 Oxygen consumption

In study 1, VO_2 was significantly higher at 40, 60 and 80% of VO_2max after the LPVD compared to ND (P=0.035, P<0.001, P<0.001; respectively) (FIGURE 14). VO_2max was also higher after the LPVD than ND, but the difference was not statistically significant (3.87 ± 0.90 vs. 3.65 ± 0.65 l/min).

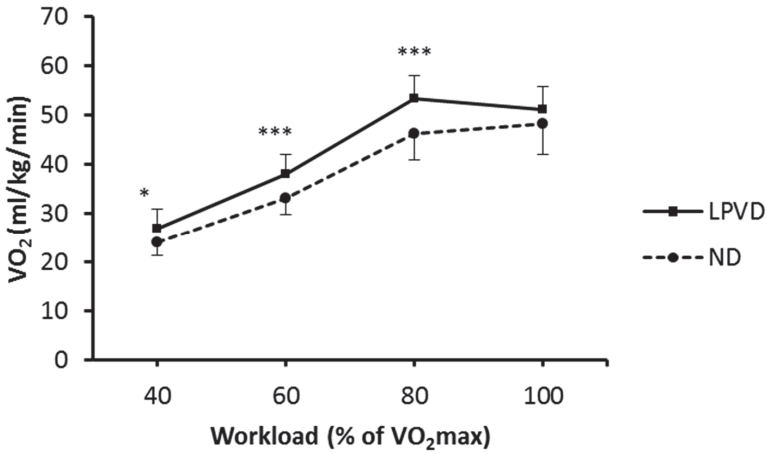


FIGURE 14 Oxygen consumption (VO_2) during the cycle ergometer tests after the 4-day low acid load diet (LPVD) and normal diet (ND). * P<0.05, *** P<0.001, statistically significant difference between LPVD and ND (two-way repeated measures ANOVA, a paired t-test). Values are mean ± SD.

In study 2, the only statistically significant difference in VO_2 was observed in the YA women, whose VO_2max was higher (P=0.006, 95% CI [0.05, 0.25], ES 0.39) after the LD than HD. VO_2 data for all the participant groups is presented in TABLE 5.

TABLE 5 Oxygen consumption (VO_2) during the cycle ergometer tests after the 7-day low acid load diet (LD) and high acid load diet (HD) in adolescents (AD), young adults (YA) and the elderly (EL).

	VO_2 (l/min)	**Men**		**Women**	
	Workload (% of VO_2max)	**LD**	**HD**	**LD**	**HD**
AD	35	1.10 ± 0.31	1.06 ± 0.30	0.90 ± 0.17	0.91 ± 0.18
	55	1.70 ± 0.50	1.71 ± 0.48	1.39 ± 0.26	1.40 ± 0.30
	75	2.44 ± 0.65	2.48 ± 0.65	1.94 ± 0.39	1.99 ± 0.36
	100	2.97 ± 0.97	3.05 ± 0.89	2.33 ± 0.35	2.35 ± 0.39
YA	35	1.51 ± 0.23	1.53 ± 0.19	1.06 ± 0.19	1.04 ± 0.16
	55	2.27 ± 0.42	2.35 ± 0.29	1.55 ± 0.22	1.53 ± 0.23
	75	3.32 ± 0.51	3.31 ± 0.43	2.17 ± 0.28	2.11 ± 0.31
	100	4.00 ± 0.47	4.05 ± 0.43	2.65 ± 0.35**	2.50 ± 0.40
EL	35	0.97 ± 0.11	1.00 ± 0.12	0. 81 ± 0.09	0.83 ± 0.09
	55	1.44 ± 0.18	1.46 ± 0.19	1.13 ± 0.13	1.15 ± 0.11
	75	2.02 ± 0.28	2.04 ± 0.29	1.53 ± 0.18	1.53 ± 0.19

After the 7-d low acid load diet (LD) and high acid load diet (HD), cycle ergometer tests were performed in which cycling was performed for 10 min at 35%, 55% and 75% of VO_2max (CT35, CT55, CT75). Adolescents (AD) and young adults (YA) also cycled at 100% of VO_2max until exhaustion (CT100). VO_2 was determined as a mean from the final 30 seconds of each workload. EL, the elderly. ** $P<0.01$, statistically significant difference between LD and HD within each participant group (two-way repeated measures analysis of variance (ANOVA), a paired samples-test). Values are mean ± SD.

In study 3, VO_2max was significantly higher ($P=0.024$) in the low-PRAL women than moderate-PRAL women at POST (TABLE 6). In the moderate-PRAL women, VO_2 was lower at 55% ($P=0.005$), 75% ($P=0.008$) and 100% ($P=0.003$) of VO_2max at POST compared with PRE. In the low-PRAL men, the differences were significant at 55% ($P=0.48$) and 75% ($P=0.039$) of VO_2max at POST compared to PRE.

TABLE 6 Oxygen consumption (VO_2) during separate submaximal and maximal cycling tests in the low-PRAL and moderate-PRAL (Mod-PRAL) groups at baseline (PRE) and after (POST) the 12-week intervention.

VO_2 (l/min)	**Men**				**Women**			
Workload	**Low-PRAL**		**Mod-PRAL**		**Low-PRAL**		**Mod-PRAL**	
(of VO_2max)	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
35%	1.27 ± 0.23	1.17 ± 0.20	1.16 ± 0.20	1.14 ± 0.20	0.90 ± 0.10	0.89 ± 0.08	0.84 ± 0.13	0.80 ± 0.13
55%	2.06 ± 0.34	1.89 ± 0.31†	1.86 ± 0.36	1.81 ± 0.34	1.37 ± 0.14	1.35 ± 0.11	1.21 ± 0.21††	1.12 ± 0.20
75%	2.85 ± 0.44	2.57 ± 0.31†	2.55 ± 0.43	2.46 ± 0.45	1.91 ± 0.23	1.88 ± 0.20	1.70 ± 0.26††	1.57 ± 0.26
100%	3.66 ± 0.38	3.69 ± 0.47	3.29 ± 0.63	3.42 ± 0.58	2.33 ± 0.31	2.37 ± 0.30*	2.01 ± 0.27††	2.21 ± 0.28

* P<0.05 statistically significant difference between the diet groups at POST (two-way repeated measures ANOVA, an independent t-test). † P<0.05, †† P<0.01 statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired t-test). Values are mean ± SD.

5.6 Kidney function

The GFR data are presented in FIGURE 15. In the young men, GFR decreased over the LD period (P=0.009) whereas in the elderly men and women it was higher after the HD than LD (P<0.001 and P=0.047, respectively). Age had a significant (P<0.001) effect on GFR. GFR was significantly lower (P<0.001) in the young adults than adolescents, in the elderly than young adults and in the elderly than adolescents throughout the study period. Gender also had a significant (P<0.001) effect on GFR. GFR was higher (P≤0.009) in boys than girls at all points. GFR was also higher (P≤0.030) in the YA men compared to YA women at all points except at POST after the LD. In the elderly, the gender differences in GFR were smaller and GFR was significantly higher (P=0.029) during the HD period in men than women only at PRE.

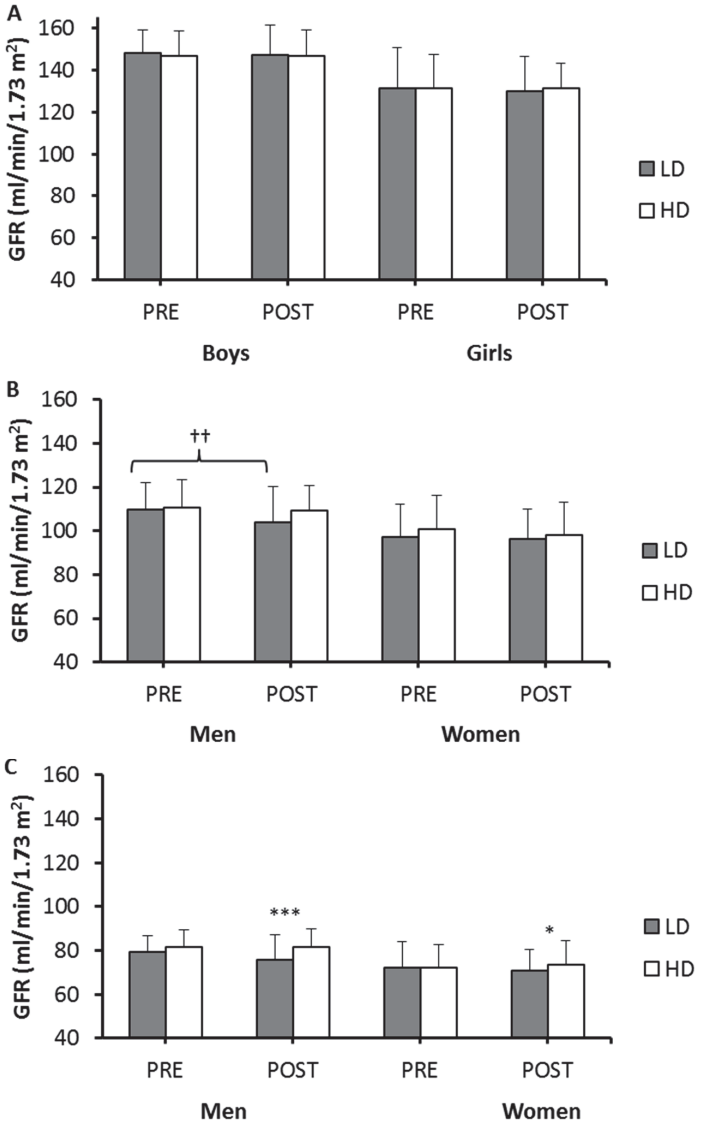


FIGURE 15 Glomerular filtration rate (GFR) in adolescents (A), young adults (B) and the elderly (C) at the beginning (PRE) and end (POST) of the 7-d low acid load diet (LD) and high acid load diet (HD). * P<0.05, *** P<0.001 statistically significant difference between LD and HD; †† P<0.01 statistically significant difference between PRE and POST during LD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.

In study 3, GFR decreased in the moderate-PRAL men (P=0.009, 95% CI [3.33, 18.8], ES 0.73) and women (P=0.036, 95% CI [0.97, 23.8], ES 0.62) over the diet intervention (FIGURE 16). No significant changes were observed in the low-PRAL groups over the study period.

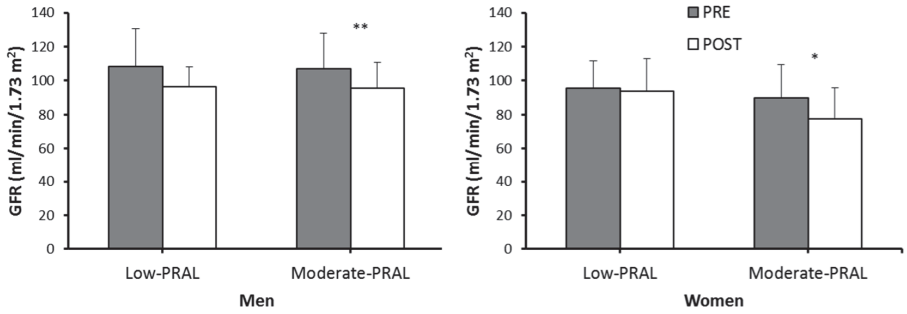


FIGURE 16 Glomerular filtration rate (GFR) in the low-PRAL and moderate-PRAL groups at baseline (PRE) and after (POST) the 12-week diet period. * P<0.05, ** P<0.01 statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired t-test). Values are mean ± SD.

Serum urea decreased significantly in the low-PRAL men (P=0.037, 95% CI [0.10, 2.43], ES 1.11) and women (P=0.013, 95% CI [0.27, 1.86], ES 0.97) (FIGURE 17). Also, the serum urea to creatinine ratio decreased over the low-PRAL diet period in both men (P=0.030, 95% CI [1.92, 28.9], ES 0.98) and women (P=0.016, 95% CI [3.23, 25.8], ES 0.86) (FIGURE 16). No significant changes were observed in the moderate-PRAL diet groups for either of the variables.

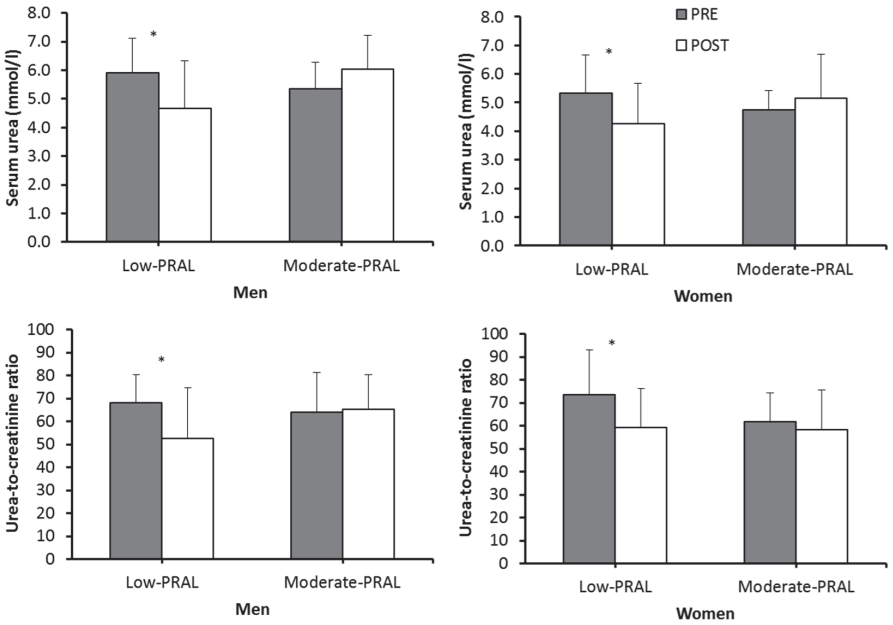


FIGURE 17 Serum urea and urea-to-creatinine ratio in the low-PRAL and moderate-PRAL groups at baseline (PRE) and after (POST) the 12-week diet period. * P<0.05 statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired t-test). Values are mean ± SD.

6 DISCUSSION

The purpose of this thesis was to study the effects of dietary acid load on acid-base status and exercise performance from adolescence through young adulthood to old adulthood. The acute effects of dietary acid load were studied over 4- and 7-day diet periods, whereas for the investigation of the longer-term effects participants completed a 12-week dietary and training intervention. The main finding was that dietary acid load has acute and prolonged effects on blood and urine acid-base status and may have further effects on exercise performance. These effects were observed to apply more to women than men. In young and elderly women in particular, blood was more acidic at rest and during submaximal cycling after high compared to low acid intake. In young women, these changes affected exercise performance, as their maximal cardiorespiratory measures were lower and time to exhaustion shorter after high acid intake. These results suggest that reduced aging-related kidney function may explain why the diet-induced changes in blood acid-base status were greater in the elderly than younger participants. Similarly, the differences between men and women may be explained by the lower renal functional capacity of women compared to men. Moreover, to the best of my knowledge, this study is the first to indicate that, during exercise, better renal function may be associated with higher blood bicarbonate availability, which can diminish exercise-induced acidosis. In addition to the elderly, who have reduced renal function, this association could be important for athletes. Although the diet-induced changes in systemic pH and acid-base balance are small and subclinical, they may nevertheless be important for physical performance and have effects on health over the long term.

6.1 Dietary acid load and acid-base status

The effects of dietary acid load on acid-base status were dependent on the magnitude and direction of the net acid excretion induced by a given diet. A major increase in net acid excretion (increased acidogenic potential) was observed to have a greater impact on acid-base status than a major decrease in net acid excretion. The present results also demonstrated that the effects were different between adolescents, young adults and the elderly, and between men and women. No significant differences in capillary blood acid-base status were observed in the young adult men when the slightly acidogenic, habitual diet was compared to the 4-day highly alkalogenic diet (I). On the other hand, when low acid intake was compared to high acid intake over the 7-day diet periods, clear effects were observed in both urine and blood acid-base status (II). More specifically, in the young and elderly participants capillary pH and HCO_3^- were significantly higher at rest after high consumption of vegetables and fruits and lower protein intake. The longer-term effects of dietary acid load were minor (IV). No significant changes in urine or capillary pH or capillary bicarbonate over the study period were found between the diet groups. Nevertheless, after the diet and training intervention, capillary pH was higher at the two highest submaximal exercise intensities in the low-PRAL women, whereas blood pH was more acidic at the two lowest exercise intensities in the moderate-PRAL women. These data contribute to the evidence that while diet-induced changes in acid-base status are small, they are feasible. Despite the large amounts of acids constantly produced in metabolism, pH in body fluids is rather stable (Adrogue & Adrogue 2001). In spite of the powerful regulatory mechanisms that ensure that large changes are not reflected in acid-base balance (Kellum 2000), it seems that these regulatory mechanisms do not maintain pH and HCO_3^- on a static level; instead, the daily diet- and exercise-induced acid load that the body confronts can induce some degree of variation in these values.

According to the daily PRAL analysis, the acid loads of the diets investigated in study 1 were low and neutral (I), whereas the dietary acid loads in study 2 were low and high (II, III). In study 3, the differences in PRAL between the low and moderate acid loads were smaller (IV). In general, a high intake of vegetables and fruits and a low intake of meat, grains, eggs and cheese during the low acid diets and the opposite dietary pattern during high acid diet regimens, explained the differences in dietary acid load. The present results strengthen previous findings that in addition to lower protein intake, fruits and vegetables play an important role in diminishing the acid load on the body (Frassetto et al. 1998; Remer 2001) and seem to be effective in a relatively short period of time. In the adult participants while the difference in dietary acid intake between the experimental 7-day diets was large, the acidity of capillary blood also increased when the high acid diet was compared to their normal diet (II, III). That is, when the daily intake of vegetables and fruits fell from approximately 400 g to almost zero and the intake of protein rose from 1.3 g/kg (~17%

of total energy intake) to 2.1 g/kg (~29%), a significant decrease in blood pH and HCO_3^- was observed. In study 1, the daily intake of vegetables and fruits was approximately 1 150 g during the LPVD as against 350 g during the ND. Protein intake, in turn, increased from 0.8 g/kg (10%) to 1.6 g/kg (18%). These results suggest that it is not necessary to increase the daily intake of fruits and vegetables to extremely high amounts, but if their intake is clearly below the minimum recommendation of 400 g (WHO), the diet may have an acidogenic effect. On the other hand, the results of the present study show that the dietary protein intake does not have to be low to reduce the acid load on the body; instead, protein intake can be sustained at a moderately higher level than the current recommended daily allowance (RDA) (0.8 g/kg/d), if the intake of fruits and vegetables is high enough. It is important to acknowledge the importance of adequate protein intake for health (Westerterp-Plantenga et al. 2012) and in maintaining or improving skeletal muscle size (Houston et al. 2008, Hulmi et al. 2009). For the elderly, to minimize the aging-related loss in muscle mass (sarcopenia), the recommended protein intake is 1.0-1.5 g/kg (Morley et al. 2010). For athletes, the recommended optimal protein intake is also distinctly higher than the RDA and varies between 1.4-2.0 g/kg (Jäger et al. 2017). For acid-base balance in athletes and the elderly, adequate intake of fruits and vegetables is thus of especial importance.

The excretion of acid in urine is important for the stability of the systemic acid-base balance, and hence urine pH is an indicator of diet-induced acid load and renal net acid excretion (Frassetto & Sebastian 1996; Remer & Manz 1995; Welch et al. 2008). As a result of this regulatory mechanism urine pH increased during the 7-day low acid intake period and was higher after the low compared to high acid intake period in the young adults and elderly (II). No significant changes in urine pH over the 12-week study period were observed between the diet groups (IV). In renal physiology, net acid excretion (NAE) is the net amount of acid excreted in the urine per unit of time. Its value depends on the urine flow rate, urine acid concentration, and the concentration of bicarbonate in the urine (Remer et al. 2003). NAE was significantly lower after 12 weeks of lower dietary acid intake compared to higher acid intake in both men and women. In study 2, larger differences in NAE were observed, and thus the effects of diet composition on urine pH were also larger. However, the present data support the idea that although NAE changes, these changes do not necessarily reflect changes in urine and blood pH. For example, Wesson et al. (2011) speculate that some of the increased diet-induced H^+ may predominantly be bound to intracellular body buffers. This so-called H^+ retention could take place in the kidney cortex and skeletal muscle, and it has been shown to occur in rats. H^+ retention has also been suggested to occur in humans, but the method used by Wesson et al. (2011) to study H^+ retention has not been validated by others. Wesson and Simoni (2009) compared rats with and without subtotal nephrectomy and found no differences in blood acid-base levels between the two groups. However, the rats with lower kidney mass were unable to excrete the same acid load in urine, while at the same time demonstrating higher renal tis-

sue acid levels. The differences between men and women in the effects of dietary acid load may lend some support to the H^+ retention theory. Their larger muscle mass may offer men a larger tissue buffering capacity compared to that of women. This may help to explain the finding that blood acid-base status was not affected by dietary acid load as much in men as it was in women.

Diet composition had different effects on capillary and urine pH between the age groups (II, III). While the young adults showed clear changes in urine pH, the elderly participants showed even greater changes. The adolescents, however, showed no changes at all. It has been suggested that the acidity of the body increases with aging as the functional capacity of the kidneys to excrete acids declines (Frassetto & Sebastian 1996, Goraya et al. 2012). Although the cross-sectional nature of the present study does not allow conclusions to be drawn on how the acid-base status of each individual changes from adulthood to old age, the present data show that despite the clear decline in GFR with aging, blood acidity was not higher in the elderly compared to younger participants. This might be explained by the lower dietary acid intake in the habitual diets of the elderly participants than that in the habitual diets of the younger participants. While the healthy elderly men and women showed an acute capacity to change urine pH in response to dietary changes, it was also observed that the kidneys in the elderly have to function at higher levels to prevent alteration in the acid-base status of the body. It is known that increasing protein intake increases the renal capacity to excrete acids (Remer et al. 2003). For a given net acid load, the capacity to excrete acid in the form of ammonium (NH_4^+) is improved with elevated protein intake. The underlying mechanism appears to be that an increase in protein intake stimulates the glomerular filtration rate (GFR), which also leads to an increased renal energy requirement (Remer 2001).

In the young adults and the elderly, pH and HCO_3^- decreased significantly over the high acid intake period (II, III). In contrast, the only significant change in the acid-base status of the adolescents occurred in girls, in whom HCO_3^- increased during the low acid intake period. Despite the fact that the difference in PRAL of the adolescents' diets was also large, their urine pH or NAE exhibited no changes. This conflicts with the results of Remer et al. (2003), who reported that the PRAL of the diet correlated highly with net acid excretion in children. In the present study, the difference in acid load between the experimental diet periods was smaller in the adolescents' diets than in those of the older subjects, a factor that may have had an impact on the adolescents' acid-base responses. However, the acid-base status of adolescents may not be as sensitive to alteration in diet composition as that of adults, and especially the elderly, since their renal functional capacity is also higher than that of older individuals. The specific stimulating effect of protein on renal ammoniagenesis is also true for children (Remer 2001). Apparently the acid loads in the present study were not high enough to cause differences in the acid-base status of the adolescents. Moreover, adolescents have higher oxidative capacity compared with adults at rest (Ratel et al. 2006), a factor which could enable a more efficient utilization of H^+ in the tricarboxylic acid cycle and explain why the acid load of the adoles-

cents was not as sensitive as it was in the adults to changes in diet composition. Growing adolescents also have higher needs for protein to ensure the growth and maturation of tissues, and hence efficacy in the use of nitrogen is increased (Giovannini et al. 2000). Thus, the acute potential of proteins to increase the acid load of the body might be lower in adolescents. Nevertheless, the acute data in the present study do not necessarily reflect the effect of diet composition on health in adolescents over a longer period of time (Krupp et al. 2012).

6.2 Dietary acid load and exercise performance

Increasing H^+ concentrations in myocytes and blood during high-intensity exercise eventually lead to acidosis, which is considered one of the causes of fatigue (Lancha Junior et al. 2015, Robergs et al. 2004). Although H^+ accumulation in myocytes is not solely responsible for muscle fatigue (Allen et al. 2008), acidosis has been reported to affect it in many ways. It may, for example, limit mitochondrial function and enzyme activities in glycolytic energy production (Hollidge-Horvat et al. 2000; Jubrias et al. 2003; Lindinger 1995). Hydrogen ion concentration has also been shown to adversely affect interstitial K^+ accumulation, which impairs muscle performance (Street et al. 2005). In extracellular fluids, HCO_3^- is considered the most important chemical H^+ buffer (McNaughton et al. 2008). Maintaining a high extracellular HCO_3^- concentration enables H^+ to be removed faster from the muscle cells and to be buffered at a higher rate (Lancha Junior et al. 2015). This may delay muscle fatigue and improve physical performance. Over the decades, many studies have reported positive effects of sodium bicarbonate supplementation and some other ergogenic aids on blood buffering capacity and exercise performance (Carr et al. 2011; Krustrup et al. 2015, Wilkes et al. 1983), although these effects are somewhat controversial and depend on the exercise type and intensity studied. For example, sodium bicarbonate supplementation has been reported to improve performance in both short (~1-2 min) (Mero et al. 2013; Van Montfoort et al. 2004) and longer (~20 min) exercise performances (Oöpik et al. 2003). One of the aims of the present study was to investigate whether diet and dietary acid load could also affect exercise performance via acid-base status, as recently reviewed by Applegate et al. (2017). The results of the present study strengthen the idea that it might be possible to improve performance via a decrease in dietary acid load. Particularly in the young and elderly women, blood was more acidic during submaximal cycling after high acid intake than low acid intake (III). These changes affected performance in the young women, as their maximal cardiorespiratory measures were lower and time to exhaustion shorter after high than low acid intake. However, a low-protein vegetarian diet followed for 4 days in study 1 had no acute effect on venous blood acid-base status during submaximal and maximal aerobic exercise in young recreationally active men when compared to the participants' normal diet. To the best of my knowledge, the effects of dietary acid load during exercise have not been reported in the elderly. Dietary acid load

could be an important issue for the health of the elderly also in relation to exercise, as the exercise-induced increase in acidosis was smaller after lower dietary acid load in both the elderly men and women. This would diminish the need of aging kidneys to secrete acids and could help in maintaining renal functional capacity (Goraya & Wesson 2012).

In the young women, the time to exhaustion at a workload equivalent to 100% of VO_2max was 19% shorter and their maximal VO_2 lower after high than low acid intake (III). The maximal aerobic capacity of young men in the present study did not differ between the diets in either of the short-term study settings. Interestingly, in the young women, blood HCO_3^- decreased to the same level during maximal workload after both diet periods, even though it was significantly higher at submaximal workloads after the low compared to high acid intake. In addition, pH decreased to the same level after both diets, suggesting that higher blood alkalinity during the submaximal workloads may have enabled the longer duration of the maximal workload. These results are in line with those of Greenhaff et al. (1987), who reported that pH, HCO_3^- and BE were higher at rest but decreased to the same or an even lower level, while cycling time to exhaustion at 100% of VO_2max was longer, after a low than high dietary acid intake. The present results support the idea that consuming a high acid load diet produces a more acidic environment in the body and results in diminished blood buffering capacity. This may play an important role in the occurrence of muscle fatigue during submaximal and maximal exercise intensities. The results of the present study are in line with those of Correia-Oliveira et al. (2017), who showed that ammonium chloride-induced acidosis affected anaerobic performance, whereas no effect on performance was found for sodium bicarbonate-induced alkalosis. In addition, it has been suggested that extracellular acidosis could be involved in the sensation of discomfort in fatigue (Westerblad et al. 2002), which could also contribute to impaired performance in the groups of young and elderly women. In the present elderly group, four women were unable to finish the CT75 after the high acid load period, which could also be due to the lower blood pH and HCO_3^- induced during this dietary period. A more acidic environment in the body may play an important role in the occurrence of muscle fatigue during submaximal and maximal exercise intensities, particularly in women, who have lower renal functional capacity compared to men. The young men and women had significantly lower pH and HCO_3^- at the two highest exercise intensities after adjusting for VO_2max compared to both the elderly men and women and the boys and girls. This and the positive correlations between GFR and HCO_3^- suggest that renal functional capacity may affect the level of acidity that it is possible to achieve via the availability of bases (III). The performance or exercise metabolism of adolescents, however, was not different between the diet groups, which suggests that there are also other factors that affect the high-intensity exercise capacity.

Young women had higher maximal blood lactate after the low than high acid intake (III). Men showed no differences in blood lactate in any of the study settings (I, III). The higher blood lactate observed in the young women is most

likely a consequence of the longer duration of the maximal workload exercise. Moreover, increased pre-exercise alkalosis could enhance high-intensity performance by contributing to enhanced glycolytic ATP production, and thus enhanced lactate production (Stephens et al. 2002). When the capacity of aerobic energy production is exceeded, lactate production ensures that NAD^+ is available for glycolysis to continue, and it also attenuates the increase in H^+ concentration (Baker et al. 2010; Robergs ym. 2004). Lactate can be oxidized back to pyruvate, which can be converted to acetyl-coenzyme A or oxaloacetate and used in the tricarboxylic acid cycle to produce ATP or in gluconeogenesis to produce glucose (Adeva-Andany et al. 2014). In the study by Hollidge-Horvat et al. (2000), sodium bicarbonate-induced alkalosis increased muscle glycogen use, lactate accumulation and production, and muscle H^+ concentration. Similarly, in the study by Oöpik et al. (2003), participants had 21% higher maximal blood lactate and completed a 5-km time trial 2.6% faster after a citrate supplementation compared to placebo. It can be speculated that the impaired maximal aerobic performance in the group of young women was due to lower carbohydrate intake during the high dietary acid load period. However, the difference in the intake of carbohydrates may not have been large enough for the difference in time to exhaustion to be explained by lower muscle glycogen availability. In the young women, the cycling tests performed during the study lasted approximately 33 minutes, of which 20 minutes were cycled at 35 and 55% of VO_2max, when fat is the most dominant source of energy (Knuiman et al. 2015). Participants were not engaging in high-intensity exercise during the study periods. Moreover, according to the body composition analysis, the weight of body fluids was not reduced over the high acid period, which strengthens the conjecture that the muscle glycogen stores were not depleted prior to the cycling tests. Each gram of glycogen is carried in 2.5-3.0 g of water, and hence loss of muscle glycogen would cause a decrease in body mass; this was not observed.

In study 1, during cycling, diet composition caused some differences in aerobic energy production. The vegetarian diet increased VO_2 during submaximal aerobic cycling, suggesting that the submaximal cycling economy was poorer after the LPVD than ND. However, this had no further effects on maximal aerobic performance. These results suggest that, contrary to what was hypothesized, a low-protein vegetarian diet cannot be recommended as a means to improve submaximal or maximal aerobic performance via acid-base balance. The results of study 2 also suggest that a low-acid diet might be advantageous in short bouts of high-intensity exercise but unfavorable in more prolonged bouts, during which it is important to conserve the body's glycogen stores. The results of the 12-week training period do not show the preeminence of lower acid intake for enhancing training effects during combined endurance and strength training. However, in the low-PRAL women, capillary pH and bicarbonate were significantly higher at 75% of VO_2max, whereas in the moderate-PRAL women, capillary pH was significantly lower at 55% of VO_2max after the study period compared to baseline. Although the changes in blood pH and HCO_3^- are smaller compared with those achieved with supplements, it is im-

portant to recognize that a habitual diet may also have a constant effect on the bicarbonate concentrations in the body. According to this study, women, especially, should pay attention to their dietary patterns prior to competitions and ensure adequate consumption of vegetables and fruits to maximize their performance during high-intensity exercise. Athletes can be recommended to reduce dietary acid intake if they continue to follow other sports nutrition recommendations.

6.3 Modern acid-base approach in exercise science

A physicochemical acid-base approach determines the independent acid-base variables affecting the hydrogen ion concentrations in body fluids and the role of physiological systems that are linked in the regulation of the plasma acid-base balance (Kellum 2000). The differences between the different diet groups in the independent acid-base variables (pCO_2, SID, A_{tot}) studied showed no particular pattern (the results are given in original paper II). Some differences, however, are worth mentioning. In the elderly group, pCO_2 was higher after low than high acid intake (II). This difference in the respiratory component of the acid-base balance shows that the differences in capillary blood acid-base status were genuine and not solely due to respiratory compensation, since metabolic acidosis may be accompanied by decreased pCO_2. In study 3, SID was significantly higher during submaximal cycling in the low-PRAL women than moderate-PRAL women after the diet and training intervention. In their study, Putman et al. (2003) reported that short-term training attenuated the increase in plasma H^+ concentration, which led to increased SID and decreased A_{tot} in arterial and venous plasma during submaximal cycling. Thus, the increase in SID indicates that some changes in acid-base status occurred over the 12-week study period.

The contribution of each independent acid-base variable to capillary pH was studied with linear regression analysis, which showed that pCO_2, SID and A_{tot} variously explained capillary pH and their association with capillary pH became less significant as exercise intensity increased (II). In general, the most powerful factor explaining the variation in blood pH was pCO_2, although its impact was observed to decrease with increased exercise intensity. Weinstein et al. (1991) also reported that most of variance in H^+ concentration at rest was explained by pCO_2. Putman et al. (2003) reported that the exercise-induced increase in venous plasma H^+ concentration was mainly due to CO_2 accumulation in the venous plasma. During exercise, pCO_2 decreases with the removal of CO_2 by the lungs, which helps to attenuate the decrease in pH. In turn, the decrease in SID and increase in A_{tot} have the opposite effect. The lowest correlation coefficients were observed in general at the two highest exercise workloads, suggesting that during high-intensity exercise the importance of some other factors increased and had a larger effect on capillary pH than those that were included in the calculations. For example, increasing amounts of phosphocreatine, inor-

ganic phosphate and ADP could affect SID and A_{tot} (Lindinger 1995). However, the suitability of the Stewart approach to the study of exercise-induced acid-base changes remains slightly unclear. Differences in the independent acid-base variables were very few, despite the differences observed in blood pH and HCO_3^-.

6.4 Dietary acid load and health

At present, the very long-term effects of high acid load in healthy populations are not known, but high dietary acid intake has been reported to associate with faster decline in kidney function in kidney patients, as it reduces the capacity of the kidneys to cope with acids and may further increase the acidity of the body (Goraya & Wesson 2012). The present results suggest that a slightly acidogenic diet and regular training combined may increase the acid load on the body and start to impair kidney function in recreationally active participants (IV). According to the estimated GFR in the 12-week intervention, kidney function decreased slightly over the study period in the moderate-PRAL men and women. Over the 12-week intervention, no changes in serum urea or UCR were observed in the moderate-PRAL groups, whereas both decreased in the low-PRAL groups. There has been some debate in the literature on whether diet composition could affect renal function over a longer period. A high intake of protein, in particular, has been suggested to potentially impair kidney function. In individuals with moderate to severe renal insufficiency, low protein intake may slow the decline in renal function (Knight et al. 2003; Rhee et al. 2017). On the other hand, a recent paper by Møller et al. (2018) reported no association between higher protein intake and decreased kidney function in pre-diabetic older adults during a one-year intervention. In the study by Antonio et al. (2016), high protein intake (2.5 g/kg/d) was compared to higher protein intake (~3.3 g/kg/d). The results showed that these high protein diets were not deleterious for kidney function in body builders over a one-year crossover study period. However, the ap-plicability of these results to individuals with lower muscle mass remains unclear, since some of the increased H^+ is bound to body buffers in muscle cells (Wesson et al. 2011). In the present study (IV), protein intake did not differ between the diet groups, suggesting that not only protein intake but also the total dietary acid load should be considered as a factor that may affect renal function. So et al. (2016) also proposed that dietary acid load could be a better indicator of changes in renal function associated with the habitual dietary pattern than merely the total amount of dietary protein. Reducing dietary acid load with increased intake of vegetables and fruits or bicarbonate supplementation has been reported to decrease the risk factors for chronic kidney disease (Banerjee et al. 2014, de Brito-Ashurst et al. 2009). Ko et al. (2017) reported that potassium intake was negatively associated with CKD, whereas protein intake showed no association.

Diets that have low acid load and promote alkalinity of the body may be beneficial for the health of elderly populations, who have ageing-related decrease in renal functional capacity to excrete acids. In the present study, the glomerular filtration rate of the elderly participants was, as expected, significantly lower than that of the younger participants groups. Aging persons are likely to be more sensitive to increased acid loads, and thus it might be beneficial for health to maintain a lower acid load across the lifespan. In elderly people, structural and functional changes in the kidneys may decrease their ability to excrete acids, possibly leading to chronic metabolic acidosis, especially if the diet does not include enough vegetables and fruits (Berkemeyer et al. 2008; Dawson-Hughes et al. 2008: Tareen et al. 2004). Reducing dietary acid load with increased intake of vegetables and fruits may help in the preservation of muscle mass (Dawson-Hughes et al. 2008, Welch et al. 2013), and lower serum bicarbonate has been reported to be associated with reduced muscle strength, and greater risk for incident and functional limitations (Abramowitz et al. 2011, Yencheck et al. 2014). At the present study, it was observed that elderly persons who had lower muscle mass, and thus lower tissue buffering capacity, had to excrete more acids or alkali into urine to maintain favorable blood acid-base status. For those who have intact renal functional capacity, constant alkali intake might also be beneficial by providing a greater reserve to buffer against high acid loads; however, more long-term studies are needed to confirm this.

6.5 Methodological strengths and limitations

The effects of dietary acid load on acid-base status and exercise performance have not been studied very intensively. Intervention studies are particularly scarce in this research area. The present study had several strengths as it investigated both short-term and prolonged effects of different dietary acid loads in three different study settings and in three different age groups. In the two short-term studies, the effects of dietary acid load were studied in randomized crossover settings, where all the participants acted as their own controls. In the 12-week longitudinal study two separate groups were compared.

However, some shortcomings in the methodology must be acknowledged. The diets during short-term dietary interventions were meticulously planned with the help of Finnish dietary guidelines and PRAL calculations. Participants were instructed to eat according to their perceived energy needs during the first diet period. The recommendations for the second diet period were planned according to the energy intake of the first diet period. Nevertheless, energy intake during the low acid load diets tended to be lower in all studies. It may be that high intake of dietary fiber, which has a satiating effect, may have made it difficult to maintain energy intake at same level during both diet periods. On the other hand, the foods consumed during the higher acid load diets were denser in energy than fruits and vegetables. In future studies, the energy intake, particularly carbohydrate intake, of the diets should be more accurately controlled

to minimize the effect of difference in caloric and macronutrient intakes on performance. The size of the subject groups was in general adequate. With the expected effect size (Cohen's d) of 1.0 for capillary pH the statistical power of 0.8 and a P-level of 0.05 are achieved when the a-priori sample size of each group (diet/sex) is 17. This was mainly the case in the adult groups in study 2, whereas in the other groups the number of participants was lower.

6.6 Future directions

To validate the results of the present study, the interventions presented should be repeated with different participants. In addition to dietary acid loads, sodium bicarbonate-induced alkalosis could also be compared to observe how diet- and supplement-induced variations in acid-base status differ. This comparison would also help to identify whether possible variations in exercise performance are due to variations in acid-base status or due to some other factors in diet (such as nitrates, antioxidants etc.).

The effects of dietary acid load on health should be studied further, especially in patients who have impaired kidney function or increased risk for kidney injury. However, the effects of dietary acid load on populations with intact kidney function should also be researched, since the very long-term effects are not known. Because kidney function naturally declines with age, and because decreased GFR has been reported to associate with increased acidity of the body, insulin resistance and risk for chronic kidney diseases – which in turn are risk factors for type 2 diabetes and cardiovascular diseases – it would be important to know how to act to preserve all possible kidney function.

Many health claims have been made about diet and acid-base balance in the internet and social media. The results of this thesis indicate that some of those claims can be debunked and that many of them remain to be confirmed. I hope, nevertheless, that this thesis has shown that the effects of dietary acid load merit additional high-quality research.

7 MAIN FINDINGS AND CONCLUSIONS

The main results and conclusions of this thesis can be summarized as follows:

1. Diet composition acutely affects blood and urine acid-base status. A diet deficient in fruits and vegetables and rich in meat and grains leads to a high dietary acid load, and thus may induce more acidic blood and urine acid-base status than a diet with a lower acid intake. These effects can be seen at rest and during aerobic exercise in young and elderly adults.
2. Increased acute acidity of the blood induced by high dietary acid intake may impair maximal aerobic performance, such as occurred in the young women in this study. A diet high in fruits and vegetables maintains a higher extracellular HCO_3^- concentration, which enables hydrogen ions to be removed faster from the muscle cells and buffered at a higher rate. This may delay muscle fatigue and improve physical performance. Better renal function may be associated with higher HCO_3^- availability in blood during exercise.
3. The acute effects of dietary acid load are greater in the elderly than in younger adults and in women than in men, a phenomenon that is likely explained by age and gender differences in renal function capacity. The acid-base status of adolescents may not be as sensitive to alteration in diet composition as that of adults and the elderly, since their renal functional capacity is also higher compared to that of older individuals.
4. Aging-related decline in renal function and the ability to excrete excessive hydrogen ions may lead to mild but slowly increasing metabolic acidosis, especially if the dietary acid load is high. Maintaining better renal function with aging may be associated with higher availability of bicarbonate ions in blood. This, in turn, may diminish exercise-induced acidosis and decrease the need of aging kidneys to excrete acids.
5. Prolonged low dietary acid intake decreases net acid excretion and has some effects on blood acid-base status that are more apparent in women than in men. However, a slightly acidogenic diet and regular training combined may start to impair kidney function in both men and women.

6. Acid-base status can be impaired if the daily intake of vegetables and fruits is extremely low. The recommendation of a minimum daily intake of 400 g of vegetables and fruits (excluding potatoes and other starchy tubers) by the World Health Organization can also be applied as a minimum guideline to decrease the acidogenic effect of a diet moderate in protein intake.

This study shows that diet composition along with renal functional capacity affects acid-base status of the body at rest and during exercise. Dietary acid intake should be studied further as a potential factor when assessing the overall effects of diet on exercise and health, especially in athletes, elderly populations and patients with kidney disease.

YHTEENVETO (FINNISH SUMMARY)

Elimistön happo-emästasapaino on yhteydessä kehon nesteiden vetyionikonsentraatioihin. Vetyioneja (H^+) syntyy elimistössä päivittäin erilaisissa aineenvaihduntareaktioissa ja niitä tulee elimistöön myös ravinnon mukana. Happo-emästasapainolla tarkoitetaan elimistön tilaa, jossa vetyionien tuotto ja sisäänotto ovat tasapainossa vetyionien poiston kanssa. Vetyionikonsentraation tarkka säätely on elintärkeää, koska lähes kaikkien elimistön entsyymien ja siten myös solujen toiminta on siitä riippuvaista. Vetyioni on protoni, joka jää jäljelle, kun vetyatomi luovuttaa ainoan elektroninsa. Hapot ovat molekyylejä, jotka voivat luovuttaa vetyioneja ja lisätä nesteen vetyionikonsentraatiota. Emäkset puolestaan ovat molekyylejä, jotka voivat vastaanottaa vetyioneja ja pienentää niiden pitoisuutta. Elimistön vetyionikonsentraatiot ovat äärimmäisen pieniä. Esimerkiksi valtimoveressä konsentraatio on normaalisti noin 40 nEq/l. Yleensä käytetäänkin pH-lukua, joka on vetyionikonsentraation negatiivinen logaritmi:

$$pH = -\log [H^+]$$

Happamuuden kasvaessa elimistön nesteiden vetyionikonsentraatio kasvaa ja pH laskee, kun taas emäksisyyden kasvaessa vetyionikonsentraatio laskee ja pH nousee. Valtimoveren pH:ssa ei tapahdu normaalisti suuria muutoksia, vaan se pyritään pitämään välillä 7,35–7,45. Äärimmäisissä tapauksissa se voi kuitenkin vaihdella välillä 6,8–8,0, jolloin vetyionipitoisuus vaihtelee vastaavasti välillä 10–160 nEq/l. Laskimoissa ja soluvälitilassa normaali pH on 7,35, ja myös solunsisäinen pH on hieman valtimoveren pH:ta matalampi, koska solujen aineenvaihdunnassa syntyy happoja. Virtsan pH voi vaihdella välillä 4,5-8. Raskaan liikunnan aikana veren pH voi laskea 7,4:stä 6,9:ään, ja lihassoluissa pH voi laskea 7,2:sta jopa 6,6:een.

Vaikka aineenvaihdunnassa tuotetaan ja käsitellään päivittäin suuri määrä vetyioneja (noin 80 mEq), elimistön vetyionikonsentraatiot pysyvät lähellä normaalia (noin 0,00004 mEq/l). Happo-emästasapaino onkin yksi voimakkaimmin säädellyistä muuttujista ihmisen fysiologiassa. Solunsisäiset ja -ulkoiset kemialliset puskurit, hengityselimistö ja munuaiset säätelevät elimistön vetyionikonsentraatioita jatkuvasti. Vetyionikonsentraation säätelyn ensimmäinen ja nopein vaihe on kehon nesteiden kemiallisten happo-emäspuskureiden toiminta. Puskuri on molekyyli, joka voi sitoa ja edelleen luovuttaa vetyioneja. Puskuroinnin avulla elimistö sitoo ylimääräisiä vetyioneja tai vapauttaa niitä verenkiertoon ja minimoi siten happojen ja emästen aiheuttamia muutoksia kehon nesteiden pH:ssa. Solunulkoisen nesteen tärkein puskuri on bikarbonaattisysteemi, joka koostuu heikosta haposta (hiilihappo H_2CO_3) sekä bikarbonaattisuolasta kuten natriumbikarbonaatista ($NaHCO_3$). Solunsisäisiä puskureita ovat esimerkiksi proteiinit kuten punasolujen hemoglobiini ja lihassolujen karnosiini. Säätelyn toisessa vaiheessa hengityselimistö osallistuu vetyionien puskurointiin säätelemällä hiilidioksidin määrää elimistössä. Kun bikarbonaatti-ionit (HCO_3^-)

sitovat vetyioneja itseensä, muodostuu hiilihappoa (H_2CO_3), joka hajoaa edelleen hiilidioksidiksi ja vedeksi:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

Muodostunut hiilidioksidi poistuu elimistöstä keuhkojen kautta hengityksen mukana. Ventilaation kasvu lisää hiilidioksidin poistumista elimistöstä, mikä puolestaan johtaa vetyionikonsentraation laskuun elimistössä, kun taas ventilaation lasku nostaa konsentraatiota. Hitain mutta tehokkain vetyionipitoisuuden säätelijä ovat munuaiset, jotka ylläpitävät happo-emästasapainoa reabsorboimalla bikarbonaatti-ioneja takaisin verenkiertoon munuaisten tubuluksista, erittämällä vetyioneja virtsaan sekä tuottamalla uusia bikarbonaatti-ioneja verenkiertoon. Mitä enemmän munuaisten täytyy erittää vetyioneja elimistöstä, sitä matalampi on myös virtsan pH.

Raskaan liikunnan aikana veren ja lihassolujen sisäinen happamuus lisääntyvät, kun vetyionien tuotto ylittää sen määrän, mitä voidaan hyödyntää tai puskuroida muissa aineenvaihdunnan reaktioissa. Lihassolut tarvitsevat ATP-molekyylien pilkkomisesta vapautuvaa energiaa, jotta ne voisivat supistua. Uutta ATP:tä tuotetaan aineenvaihdunnassa jatkuvasti energia-aineenvaihdunnan tarpeisiin. Sitä voidaan tuottaa hiilihydraatista, rasvasta tai aminohapoista aerobisesti (hapen avulla) mitokondrioissa tapahtuvassa sitruunahappokierrossa tai hiilihydraatista glykolyysissä. Glykolyysin lopputuotteena syntyvät pyruvaattimolekyylit ja vetyionit voidaan edelleen hyödyntää aerobisessa energiantuotannossa mitokondrioissa. Sen sijaan kovatehoisen liikunnan aikana, kun happea ei ole tarpeeksi saatavilla eikä aerobisen energiantuoton teho riitä, pyruvaatista tuotetaan laktaattia, jota voidaan pitää anaerobisen glykolyysin lopputuotteena. Tässä reaktiossa myös sidotaan vetyioni ja samalla syntyy NAD^+-molekyyli, mikä on elinehto glykolyysin jatkumiselle. Laktaatin muodostaminen siis puskuroi vetyionikonsentraation nousua ja mahdollistaa glykolyysin jatkumisen. Kun ATP:tä pilkotaan lihassoluissa nopeammin kuin mitokondriot ehtivät tuottaa sitä hapen avulla lisää, täytyy ATP:tä tuottaa yhä enemmän anaerobisesti eli ilman happea, jolloin myös ATP:n pilkkomisesta vapautuvien sekä glykolyysissä syntyvien vetyionien määrä kasvaa. Yhden ATP-molekyylin hajoamisessa vapautuu vetyioni. Anaerobisen liikunnan aikana ei siis tuoteta maitohappoa vaan laktaattia ja vetyioneja, joista vetyionit aiheuttavat happamuuden lisääntymisen. Laktaattia voidaan edelleen hyödyntää energiantuotannossa lihaksissa, sydämessä, maksassa, aivoissa ja munuaisissa. Se voidaan hapettaa sitruunahappokierrossa tai siitä voidaan muodostaa maksassa glukoosia glukoneogeneesissä.

Liikunnan aikana kasvavaa happamuutta pidetään yhtenä lihasväsymyksen syynä. Happamuuden rooli väsymyksen synnyssä ei ole yksiselitteinen, mutta sen on raportoitu rajoittavan mm. mitokondrioiden toimintaa sekä glykolyyttisten entsyymien aktiivisuutta. Tutkimuksissa on havaittu, että suurempi solunsisäinen tai -ulkoinen puskurikapasiteetti saattaa viivästyttää happamuuden lisääntymistä ja parantaa suorituskykyä, koska lihassolujen sisällä vapau-

tuvat vetyionit voidaan silloin kuljettaa lihassolusta verenkiertoon ja puskuroida tehokkaammin. Esimerkiksi natriumbikarbonaatilla (ruokasooda) ja beetaalaniinilla on havaittu olevan suorituskykyä parantavia vaikutuksia.

Eri ruoka-aineet vaikuttavat siihen, kuinka paljon munuaisten täytyy erittää happoja elimistöstä. Ravinnon elimistölle aiheuttamaa happokuormaa voidaan arvioida laskemalla ruoka-aineille PRAL-arvo (*potential renal acid load*), joka kuvaa sitä, kuinka paljon ruoka-aineen sulattaminen joko lisää tai vähentää elimistön happokuormaa. Yleisesti ottaen esimerkiksi liha- ja viljatuotteet sekä juustot ja kananmunat lisäävät happojen tuottoa elimistössä, kun taas vihannekset ja hedelmät pienentävät happokuormaa. Erityisesti rikkiä sisältävät aminohapot eli metioniini ja kysteiini lisäävät happojen eritystä virtsaan. Hedelmät ja vihannekset sen sijaan sisältävät muun muassa sitraattia ja malaattia, joista voidaan muodostaa bikarbonaatti-ioneja eli niillä on elimistössä emäksisyyttä lisäävä vaikutus.

Tämän väitöskirjan tarkoituksena oli tutkia, onko ravinnon happokuormalla lyhyt- tai pitkäaikaisia vaikutuksia happo-emästasapainoon levossa ja liikunnan aikana, ovatko mahdolliset vaikutukset erilaisia lapsilla, nuorilla aikuisilla sekä ikääntyvillä ja vaikuttavatko mahdolliset muutokset aerobiseen kestävyyssuorituskykyyn. Erilaisten ruokavalioiden vaikutusta tutkittiin 4 ja 7 päivän mittaisten jaksojen sekä 12 viikon jakson aikana terveillä, liikuntaa harrastavilla miehillä ja naisilla. Tutkimukseen 1 osallistui yhdeksän 18–30 -vuotiasta miestä. Tutkimukseen 2 osallistui 88 miestä ja naista kolmesta ikäryhmästä: lasten ryhmässä tutkittavat olivat keskimäärin 13-vuotiaita, nuorten aikuisten ryhmässä 28-vuotiaita ja ikääntyvien ryhmässä 66-vuotiaita. Tutkimukseen 3 osallistui 46 miestä ja naista, jotka olivat 20–50 -vuotiaita. Tutkimukset 1 ja 2 olivat ristikkäistutkimuksia, joissa kaikki koehenkilöt noudattivat kahta ruokavaliota neljän (tutkimus 1) tai seitsemän (tutkimus 2) vuorokauden ajan satunnaistetussa järjestyksessä. Tutkimus 3 oli pitkittäistutkimus, jossa kaksi erillistä ryhmää noudattivat erilaisia ruokavalioita. Ryhmät osallistuivat samanlaiseen yhdistettyyn kestävyys- ja voimaharjoitteluun koko jakson ajan.

Jokaista mittausasetelmaa varten suunniteltiin kaksi ruokavaliota, joista toinen oli elimistön happokuormaa pienentävä ja toinen happokuormaa lisäävä. Happokuormaa pyrittiin pienentämään kasvispainotteisella ruokavaliolla, joka sisälsi myös hieman vilja- ja lihatuotteita. Happokuormaa kasvattava ruokavalio koostui enimmäkseen liha- ja viljatuotteista sekä juustosta ja kananmunista, ja sisälsi vaihtelevan määrän kasviksia tutkimusasetelmasta riippuen.

Tutkimusten alussa mitattiin koehenkilöiden maksimaalinen hapenottokyky (VO_2max) polkupyöräergometrilla. Sen jälkeen koehenkilöt jaettiin iän ja sukupuolen mukaan jaettujen ryhmien sisällä edelleen kahtia. Puolet ryhmästä noudattivat neljän tai seitsemän vuorokauden ajan emäsruokavaliota ja puolet happoruokavaliota. Tutkimuksessa 3 osallistujat noudattivat heille arvottua ruokavaliota koko 12 viikon jakson ajan. Ravintojaksojen päätteeksi suoritettiin kuormitus polkupyöräergometrilla. Kuormituksessa poljettiin kolme 8-10 minuutin mittaista kuormaa, jotka olivat tehoiltaan 35–40 %, 55–60 %, ja 75–80 % kunkin koehenkilön VO_2max:sta. Lopuksi lapset ja nuoret aikuiset polkivat vie-

lä maksimaalisella aerobisella teholla uupumukseen asti. Muutaman viikon tauon jälkeen koehenkilöt suorittivat ravintojakson ja polkupyöräergometritestin uudestaan toista ruokavaliota noudattaen (tutkimukset 1 ja 2). Tutkimuksessa 3 submaksimaalinen pyörätesti poljettiin sekä tutkimusjakson alussa että sen jälkeen. Lisäksi myös tutkimusjakson päätteeksi poljettiin erillinen VO_2max-testi. Ruokavaliojaksojen alussa ja lopussa tutkittavilta otettiin verinäytteitä, joista analysoitiin mm. pH ja HCO_3^-. Munuaisten glomerulussuodosnopeus (GFR) laskettiin CKD-EPI -kaavalla, joka huomioi tutkittavan iän, sukupuolen ja plasman kreatiniinipitoisuuden.

Tämän tutkimuksen päätulos oli, että ravinnon happokuormalla on sekä akuutteja että pidempiaikaisia vaikutuksia veren ja virtsan happo-emästasapainoon, ja se voi vaikuttaa myös fyysiseen suorituskykyyn. Elimistön happokuormaa lisääväksi suunniteltu, seitsemän vuorokauden ajan nautittu ruokavalio lisäsi veren happamuutta nuorilla aikuisilla sekä ikääntyvillä, mutta ei lapsilla. Happojen tuottoa lisäävän ruokavalion jälkeen veren pH ja HCO_3^--konsentraatio olivat matalampia myös submaksimaalisen liikunnan aikana erityisesti nuorilla ja ikääntyvillä naisilla verrattuna kasvispainotteiseen, happokuormaa pienentävään ruokavalioon. Nuorten naisten maksimaalinen aerobinen kestävyyssuorituskyky heikentyi happokuormaa lisäävän ruokavalion jälkeen. Maksimaalisen kuorman kesto sekä maksimaalinen hapenkulutus, laktaatti ja syke olivat matalampia happokuormaa lisäävän ruokavalion jälkeen verrattuna happokuormaa pienentävään ruokavalioon. Lapsilla ruokavalion koostumus ei näyttänyt aiheuttavan eroja happo-emästasapainossa tai fyysisessä suorituskyvyssä. Munuaisten toimintakapasiteettia kuvaava GFR oli matalampi vanhemmilla koehenkilöryhmillä nuorempiin verrattuna ja naisilla verrattuna miehiin. Näyttäisi siltä, että erot munuaisten toimintakapasiteetissa voivat selittää sitä, miksi ravinnon happokuorma vaikutti enemmän vanhempiin kuin nuorempiin tutkittaviin ja enemmän naisiin kuin miehiin. Tulosten perusteella voi myös pohtia, onko suurempi munuaisten toimintakapasiteetti yhteydessä suurempaan emästen saatavuuteen verenkierrossa, mikä voi hidastaa liikunnan aiheuttamaa happamuuden lisääntymistä ja parantaa suorituskykyä. Näyttää myös siltä, että vain hieman happojen muodostusta elimistössä lisäävä ruokavalio yhdistettynä säännölliseen kestävyys- ja voimaharjoitteluun saattaa heikentää munuaisten toimintaa.

Ravinnon happokuorman pienentäminen saattaa olla yksi monista hyvistä syistä huolehtia riittävästä vihannesten ja hedelmien syömisestä. Väitöskirjani tulokset tukevat ajatusta siitä, että ravinnon pienempi happokuorma ja sen myötä lisääntyvä happojen käsittelykapasiteetti ja vähentynyt liikunnanaikainen happamuus voisivat olla hyödyllisiä esimerkiksi urheilijoille ja liikunnan harrastajille. Elimistön pienemmästä happokuormasta voisi olla hyötyä myös ikääntyville, joiden happojen erityskyky joka tapauksessa heikkenee, kun munuaisten toimintakapasiteetti laskee ikääntymisen myötä. Lisääntynyt happojen tuotto voi entisestään kiihdyttää munuaisten toiminnan heikkenemistä ja se voi kiihdyttää myös lihasmassan vähenemistä ikääntyvillä. Elimistön pienemmästä

happokuormasta hyötyvät todennäköisesti myös esimerkiksi kroonista munuaistautia sairastavat.

Ruokavalion happokuorman pienentämiseksi ei tarvitse jättää yksittäisiä ruoka-aineita tai ruoka-aineryhmiä syömättä eikä käyttää mitään erikoisia valmisteita, vaan ruokavalion kokonaisuus ratkaisee. Monipuolinen ruokavalio, joka sisältää vihanneksia ja hedelmiä vähintään suositellun puoli kiloa päivässä sekä perunaa lisukkeena riittää pitämään ruokavalion happokuorman lähellä neutraalia tai hieman emäksisen puolella. Tämän väitöskirjan tulokset korostavat vihannesten ja hedelmien riittävän syömisen tärkeyttä osana terveellistä ruokavaliota ja liikunnallisesti aktiivista elämäntapaa koko elämänkaaren aikana.

REFERENCES

Abramowitz, M. K., Hostetter, T. H. & Melamed, M. L. 2011. Association of serum bicarbonate levels with gait speed and quadriceps strength in older adults. American Journal of Kidney Disease 58 (1), 29-38.

Adeva, M. M. & Souto, G. 2011. Diet-induced metabolic acidosis. Clinical Nutrition 30 (4), 416-421.

Adeva-Andany, M., López-Ojén, M., Funcasta-Calderón, R., Ameneiros-Rodríguez, E., Donapetry-García, C., Vila-Altesor, M. & Rodríguez-Seijas, J. 2014. Comprehensive review on lactate metabolism in human health. Mitochondrion 17, 76-100.

Adrogué, H. E. & Adrogué, H. J. 2001. Acid-base physiology. Respiratory Care 46 (4), 328-341.

Alexy, U., Kersting, M. & Remer, T. 2008. Potential renal acid load in the diet of children and adolescents: impact of food groups, age and time trends. Public Health Nutrition 11 (3), 300-306.

Allen, D. G., Lamb, G. D. & Westerblad, H. 2008. Skeletal muscle fatigue: cellular mechanisms. Physiological Reviews 88 (1), 287-332.

Antonio, J., Ellerbroek, A., Silver, T., Vargas, L., Tamayo, A., Buehn, R. & Peacock, C. A. 2016. A high protein diet has no harmful effects: a one-year crossover study in resistance-trained males. Journal of Nutrition and Metabolism 2016 (Article ID 9104792).

Applegate, C., Mueller, M. & Zuniga, K. E. 2017. Influence of dietary acid load on exercise performance. International Journal of Sport Nutrition and Exercise Metabolism 27 (3), 213-219.

Baguet, A., Everaert, I., De Naeyer, H., Reyngoudt, H., Stegen, S., Beeckman, S., Achten, E., Vanhee, L., Volkaert, A., Petrovic, M., Taes, Y. & Derave W. 2011. Effects of sprint training combined with vegetarian or mixed diet on muscle carnosine content and buffering capacity. European Journal of Applied Physiology 111, 2571-2580.

Baker, J. S., McCormick, M. C. & Robergs, R. A. 2010. Interaction among skeletal muscle metabolic energy systems during intense exercise. Journal of Nutrition and Metabolism 2010 (Article ID 905612).

Banerjee, T., Crews, D. C., Wesson, D. E., Tilea, A., Saran, R., Rios Burrows, N., Williams, D. E. & Powe, N. R.; Centers for Disease Control and Prevention Chronic Kidney Disease Surveillance Team. 2014. Dietary acid load and chronic kidney disease among adults in the United States. BMC Nephrology 15 (1), 137.

Berkemeyer, S. 2009. Acid–base balance and weight gain: Are there crucial links via protein and organic acids in understanding obesity? Medical Hypotheses 73 (3), 347-356.

Bishop, D., Edge, J., Davis, C. & Goodman, C. 2004. Induced metabolic alkalosis affects muscle metabolism and repeated-sprint ability. Medicine and Science in Sports and Exercise 36 (5), 807-813.

Bolignano, D., Mattace-Raso, F., Sijbrands, E. J. & Zoccali, C. 2014. The aging kidney revisited: a systematic review. Ageing Research Reviews 14, 65-80.

Caciano, S. L., Inman, C. L., Gockel-Blessing, E. E. & Weiss, E. P. 2015. Effects of dietary acid load on exercise metabolism and anaerobic exercise performance. Journal of Sports Science & Medicine 14 (2), 364-371.

Cairns, S. P. 2006. Lactic acid and exercise performance - culprit or friend? Sports Medicine 36 (4), 279-291.

Cairns, S. P. & Lindinger, M. I. 2008. Do multiple ionic interactions contribute to skeletal muscle fatigue? The Journal of Physiology 586 (17), 4039-4054.

Carnauba, R. A., Baptistella, A. B., Paschoal, V. & Hbscher, G. H. 2017. Diet-induced low-grade metabolic acidosis and clinical outcomes: a review. Nutrients 9 (6), 538.

Carr, A. J., Hopkins, W. G. & Gore, C. J. 2011. Effects of acute alkalosis and acidosis on performance. Sports Medicine 41 (10), 801-814.

Cheng, H., Huang, J., Chiang, C., Yen, C., Hung, K., et al. 2012. Metabolic syndrome and insulin resistance as risk factors for development of chronic kidney disease and rapid decline in renal function in elderly. The Journal of Clinical Endocrinology & Metabolism 97 (4), 1268-1276.

Cockerill, G. & Reed, S. 2011. Essential fluid, electrolyte and pH homeostasis. Wiley.

Correia-Oliveira, C. R., Lopes-Silva, J. P., Bertuzzi, R., Mcconell, G. K., Bishop, D. J., Lima-Silva, A. E. & Kiss, M. A. P. D. 2017. Acidosis, but not alkalosis, affects anaerobic metabolism and performance in a 4-km time trial. Medicine and Science in Sports and Exercise 49 (9), 1899-1910.

Dawson-Hughes, B., Harris, S. S. & Ceglia, L. 2008. Alkaline diets favor lean tissue mass in older adults. The American Journal of Clinical Nutrition 87 (3), 662-665.

De Brito-Ashurst, I., Varagunam, M., Raftery, M. J. & Yaqoob, M. M. 2009. Bicarbonate supplementation slows progression of CKD and improves nutritional status. Journal of the American Society of Nephrology 20 (9), 2075-2084.

Duarte, D., Santos-Araújo, C. & Leite-Moreira, A. F. 2011. Hypertension and angiogenesis in the aging kidney: A review. Archives of Gerontology and Geriatrics 52 (3), e93-e102.

Dutka, T. L., Lamboley, C. R., McKenna, M. J., Murphy, R. M. & Lamb, G. D. 2011. Effects of carnosine on contractile apparatus Ca^{2+} sensitivity and sarcoplasmic reticulum Ca^{2+} release in human skeletal muscle fibers. Journal of Applied Physiology 112 (5), 728-736.

Edge, J., Bishop, D. & Goodman, C. 2006. Effects of chronic $NaHCO_3$ ingestion during interval training on changes to muscle buffer capacity, metabolism, and short-term endurance performance. Journal of Applied Physiology 101 (3), 918-925.

Fagherazzi, G., Vilier, A., Bonnet, F., Lajous, M., Balkau, B., Boutron-Rualt, M. C. & Clavel-Chapelon, F. 2014. Dietary acid load and risk of type 2 diabetes: the E3N-EPIC cohort study. Diabetologia 57 (2), 313-320.

Fitts, R. 2016. The role of acidosis in fatigue: pro perspective. Medicine & Science in Sports & Exercise 48 (11), 2335-2338.

Frassetto, L. A., Todd, K. M., Morris, R. C. & Sebastian, A. 1998. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. The American Journal of Clinical Nutrition 68 (3), 576-583.

Frassetto, L. & Sebastian, A. 1996. Age and systemic acid-base equilibrium: analysis of published data. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 51 (1), B91-B99.

Gallagher, J. C., Rapuri, P. & Smith, L. 2007. Falls are associated with decreased renal function and insufficient calcitriol production by the kidney. The Journal of Steroid Biochemistry and Molecular Biology 103 (3), 610-613.

Giovannini, M., Agostoni, C., Gianni, M., Bernardo, L. & Riva, E. 2000. Adolescence: macronutrient needs. European Journal of Clinical Nutrition 54 (3).

Goraya, N., Simoni, J., Jo, C. & Wesson, D. E. 2012. Dietary acid reduction with fruits and vegetables or bicarbonate attenuates kidney injury in patients with a moderately reduced glomerular filtration rate due to hypertensive nephropathy. Kidney International 81 (1), 86-93.

Goraya, N. & Wesson, D. E. 2012. Acid–base status and progression of chronic kidney disease. Current Opinion in Nephrology and Hypertension 21 (5), 552-556.

Greenhaff, P. L., Gleeson, M. & Maughan, R. J. 1987. The effects of dietary manipulation on blood acid-base status and the performance of high intensity exercise. European Journal of Applied Physiology 56, 331-337.

Hall, J.E. 2011. Guyton and Hall - Textbook of medical physiology. Saunders.

Hamm, L. L., Nakhoul, N. & Hering-Smith, K. S. 2015. Acid-base homeostasis. Clinical Journal of the American Society of Nephrology 10 (12), 2232-2242.

Hollidge-Horvat, M. G., Parolin, M. L., Wong, D., Jones, N. L. & Heigenhauser, G. J. F. 2000. Effect of induced metabolic alkalosis on human skeletal muscle metabolism during exercise. American Journal of Physiology – Endocrinology and Metabolism 278, E316-E329.

Hostrup, M. & Bangsbo, J. 2017. Limitations in intense exercise performance of athletes–effect of speed endurance training on ion handling and fatigue development. The Journal of Physiology 595 (9), 2897-2913.

Houston, D. K., Nicklas, B. J., Ding, J., Harris, T. B., Tylavsky, F. A., et al. 2008. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. The American Journal of Clinical Nutrition 87 (1), 150-155.

Hulmi, J. J., Kovanen, V., Selänne, H., Kraemer, W. J., Häkkinen, K., et al. 2009. Acute and long-term effects of resistance exercise with or without protein ingestion on muscle hypertrophy and gene expression. Amino Acids 37 (2), 297-308.

Jones, N. L. 1990. [H^+] control in exercise: Concepts and controversies. In: Taylor, A. W., Gollnick, P. D., Green, H. J., Ianuzzo, C. D., Noble, E.G., Métiv-

ier, G. & Sutton, J. R. Biochemistry of exercise VII. International Series on Sport Sciences. Volume 21: 333-328.

Jubrias, S. A., Crowther, G. J., Shankland, E. G., Gronka, R. K. & Conley, K. E. 2003. Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle in vivo. The Journal of Physiology 553, 589-599.

Jäger, R., Kerksick, C. M., Campbell, B. I., Cribb, P. J., Wells, S. D., Skwiat, T. M., Purpura, M., Ziegenfuss, T. N., Ferrando, A. A., Arent, S. M., Smith-Ryan, A. E., Stout, J. R., Arciero, P. J., Ormsbee, M. J., Taylor, L. W., Wilborn, C. D., Kalman, D. S., Kreider, R. B., Willoughby, D. S., Hoffman, J. R., Krzykowski, J. L. & Antonio, J. 2017. International Society of Sports Nutrition position stand: protein and exercise. Journal of the International Society of Sports Nutrition 14 (1), 20.

Kellum, J. A. 2000. Determinants of blood pH in health and disease. Critical Care 4 (1), 6-14.

Kellum, J. A., Song, M. & Li, J. 2004. Science review: extracellular acidosis and the immune response: clinical and physiologic implications. Critical Care 8 (5), 331-336.

Knight, E. L., Stampfer, M. J., Hankinson, S. E., Spiegelman, D. & Curhan, G. C. 2003. The impact of protein intake on renal function decline in women with normal renal function or mild renal insufficiency. Annals of Internal Medicine 138 (6), 460-467.

Knuiman, P., Hopman, M. T. & Mensink, M. 2015. Glycogen availability and skeletal muscle adaptations with endurance and resistance exercise. Nutrition & Metabolism 12 (1), 59.

Ko, B. J., Chang, Y., Ryu, S., Kim, E. M., Lee, M. Y., Hyun, Y.Y. & Lee, K. B. 2017. Dietary acid load and chronic kidney disease in elderly adults: Protein and potassium intake. PLoS One 12 (9), e0185069.

Krupp, D., Shi, L. & Remer, T. 2014. Longitudinal relationships between diet-dependent renal acid load and blood pressure development in healthy children. Kidney International 85 (1), 204-210.

Krustrup, P., Ermidis, G. & Mohr, M. 2015. Sodium bicarbonate intake improves high-intensity intermittent exercise performance in trained young men. Journal of the International Society of Sports Nutrition 12 (1), 25.

Lancha Junior, A. H., de Salles Painelli, V., Saunders, B. & Artioli, G. G. 2015. Nutritional strategies to modulate intracellular and extracellular buffering capacity during high-intensity exercise. Sports Medicine 45 (1), 71-81.

Lardner, A. 2001. The effects of extracellular pH on immune function. Journal of Leukocyte Biology 69 (4), 522-530.

Levey, A. S., Stevens, L. A., Schmid, C. H., Zhang, Y. L., Castro, A. F., Feldman, H. I., Kusek, J. W., Eggers, P., Van Lente, F., Greene, T. & Coresh, J.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). 2009. A new equation to estimate glomerular filtration rate. Annals of Internal Medicine 150 (9), 604-612.

Lindinger, M. I. 1995. Origins of [H^+] changes in exercising skeletal muscle. Canadian Journal of Applied Physiology 20 (3), 357-368.

Lindinger, M. I. & Heigenhauser, G. J. 2012. Effects of gas exchange on acid-base balance. Comprehensive Physiology 2 (3), 2203-2254.

Lindinger, M. I. & Heigenhauser, G. J. 1990. Acid-base systems in skeletal muscle and their response to exercise. In: Taylor, A. W., Gollnick, P. D., Green, H. J., Ianuzzo, C. D., Noble, E.G., Métivier, G. & Sutton, J. R. Biochemistry of exercise VII. International Series on Sport Sciences. Volume 21: 341-358.

Lindinger, M. I., Kowalchuk, J. M. & Heigenhauser, G. J. 2005. Applying physicochemical principles to skeletal muscle acid-base status. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 289 (3), R891-R894.

Maughan, R. J., Greenhaff, P. L., Leiper, J. B., Ball, D., Lambert, C. P. & Gleeson, M. 1997. Diet composition and the performance of high-intensity exercise. Journal of Sports Sciences 15 (3), 265-275.

Masevicius, F. D. & Dubin, A. 2015. Has Stewart approach improved our ability to diagnose acid-base disorders in critically ill patients? World Journal of Critical Care Medicine 4 (1), 62.

McArdle, W. D., Katch, F. I. & Katch, V. L. 2001. Exercise physiology. Lippincott Williams and Wilkins.

McKenna, M. J., Bangsbo, J. & Renaud, J. 2008. Muscle K , Na , and Cl− disturbances and Na -K pump inactivation: implications for fatigue. Journal of Applied Physiology 104 (1), 288-295.

McNaughton, L. R., Siegler, J. & Midgley, A. 2008. Ergogenic effects of sodium bicarbonate. Current Sports Medicine Reports 7 (4), 230-236.

Mero, A., Hirvonen, P., Saarela, J., Hulmi, J., Hoffman, J. & Stout, J. R. 2013. Effect of sodium bicarbonate and beta-alanine supplementation on maximal sprint swimming. Journal of the International Society of Sports Nutrition 10 (1), 52.

Miller, B. F., Lindinger, M. I., Fattor, J. A., Jacobs, K. A., LeBlanc, P. J., Duong, M., Heigenhauser, G. J. & Brooks, G. A. 2005. Hematological and acid-base changes in men during prolonged exercise with and without sodium-lactate infusion. Journal of Applied Physiology 98 (3), 856-865.

Møller, G., Rikardt Andersen, J., Ritz, C., P Silvestre, M., Navas-Carretero, S., Jalo, E., Christensen, P., Simpson, E., Taylor, M., Martinez, J. A., Macdonald, I., Swindell, N., Mackintosh, K. A., Stratton, G., Fogelholm, M., Larsen, T. M., Poppitt, S. D., Dragsted, L. O.& Raben, A. 2018. Higher protein intake is not associated with decreased kidney function in pre-diabetic older adults following a one-year intervention—a preview sub-study. Nutrients 10 (1), 54.

Morales, A. P., Sampaio-Jorge, F., da Cruz Rangel, Luiz Felipe, de Souza Menezes, J., Leite, T. C. & Ribeiro, B. G. 2017. Cardiopulmonary performance during maximal exercise in soccer players with alterations in renal function. Journal of Human Kinetics 57 (1), 107-115.

Morley, J. E., Argiles, J. M., Evans, W. J., Bhasin, S., Cella, D., Deutz, N. E., Doehner, W., Fearon, K. C., Ferrucci, L., Hellerstein, M. K., Kalantar-Zadeh, K., Lochs, H., MacDonald, N., Mulligan, K., Muscaritoli, M., Poni-

kowski, P., Posthauer, M. E., Rossi Fanelli, F., Schambelan, M., Schols, A. M., Schuster, M. W. & Anker, S. D.; Society for Sarcopenia, Cachexia, and Wasting Disease. 2010. Nutritional recommendations for the management of sarcopenia. Journal of the American Medical Directors Association 11 (6), 391-396.

Mueller, P. J., O'Hagan, K. P., Skogg, K. A., Buckwalter, J. B. & Clifford, P. S. 1998. Renal hemodynamic responses to dynamic exercise in rabbits. Journal of Applied Physiology 85 (5), 1605-1614.

Neumayr, G., Pfister, R., Hoertnagl, H., Mitterbauer, G., Prokop, W. & Joannidis, M. 2005. Renal function and plasma volume following ultramarathon cycling. International Journal of Sports Medicine 26 (01), 2-8.

Odermatt, A. 2011. The Western-style diet: a major risk factor for impaired kidney function and chronic kidney disease. American Journal of Physiology-Renal Physiology 301 (5), F919-F931.

Oöpik, V., Saaremets, I., Medijainen, L., Karelson, K., Janson, T. & Timpmann, S. 2003. Effects of sodium citrate ingestion before exercise on endurance performance in well trained college runners. British Journal of Sports Medicine 37 (6), 485-489.

Passey, C. 2017. Reducing the dietary acid load: How a more alkaline diet benefits patients with chronic kidney disease. Journal of Renal Nutrition 27 (3), 151-160.

Phisitkul, S., Khanna, A., Simoni, J., Broglio, K., Sheather, S., Rajab, M. H. & Wesson, D. E. 2010. Amelioration of metabolic acidosis in patients with low GFR reduced kidney endothelin production and kidney injury, and better preserved GFR. Kidney International 77 (7), 617-623.

Potteiger, J. A., Nickel, G. L., Webster, M. J., Haub, M. D. & Palmer, R. J. 1996. Sodium citrate ingestion enhances 30 km cycling performance. International Journal of Sports Medicine 17 (1), 7-11.

Poupin, N., Calvez, J. F., Lassale, C. F., Chesneau, C. F. & Tome, D. 2012. Impact of the diet on net endogenous acid production and acid-base balance. Clinical Nutrition 31 (3), 313-321.

Putman, C. T., Jones, N. L. & Heigenhauser, G. J. 2003. Effects of short-term training on plasma acid-base balance during incremental exercise in man. The Journal of Physiology 550 (2), 585-603.

Ratel, S., Duche, P., Hennegrave, A., Van Praagh, E. & Bedu, M. 2002. Acid-base balance during repeated cycling sprints in boys and men. Journal of Applied Physiology 92 (2), 479-485.

Reinecke, N. L., Cunha, T. M., Heilberg, I. P., Higa, E. M. S., Nishiura, J. L., Neder, J. A., Almeida, W. S. & Schor, N. 2014. Exercise capacity in polycystic kidney disease. American Journal of Kidney Diseases 64 (2), 239-246.

Remer, T. 2001. Influence of nutrition on acid-base balance – metabolic aspects. European Journal of Nutrition 40 (5), 214-220.

Remer, T., Dimitriou, T. & Manz, F. 2003. Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. The American Journal of Clinical Nutrition 77 (5), 1255-1260.

Remer, T. & Manz, F. 1995. Potential renal acid load of foods and its influence on urine pH. Journal of the American Dietetic Association 95 (7), 791-797.

Robergs, R. A., Ghiasvand, F. F. & Parker, D. 2004. Biochemistry of exercise-induced metabolic acidosis. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 287, R502-R516.

Robergs, R. A. 2017. Competitive cation binding computations of proton balance for reactions of the phosphagen and glycolytic energy systems within skeletal muscle. PloS one 12 (12), e0189822.

Sahlin, K. 2014. Muscle energetics during explosive activities and potential effects of nutrition and training. Sports Medicine 44 (2), 167-173.

Schumann, M., Küüsmaa, M., Newton, R. U., Sirparanta, A., Syväoja, H., Häkkinen, A. & Häkkinen, K. 2014. Fitness and lean mass increases during combined training independent of loading order. Medicine and Science in Sports and Exercise 46 (9), 1758-1768.

Scialla, J. J., Appel, L. J., Astor, B. C., Miller, E. R., Beddhu, S., Woodward, M., Parekh, R. S. & Anderson, C. A.; African American Study of Kidney Disease and Hypertension Study Group. 2012. Net endogenous acid production is associated with a faster decline in GFR in African Americans. Kidney International 82 (1), 106-112.

Scrutinio, D., Agostoni, P., Gesualdo, L., Corrà, U., Mezzani, A., Piepoli, M., Di Lenarda, A., Iorio, A., Passino, C., Magrì, D., Masarone, D., Battaia, E., Girola, D., Re, F., Cattadori, G., Parati, G., Sinagra, G., Villani, G. Q., Limongelli, G., Pacileo, G., Guazzi, M., Metra, M., Frigerio, M., Cicoira, M., Minà, C., Malfatto, G., Caravita, S., Bussotti, M., Salvioni, E., Veglia, F., Correale, M., Scardovi, A. B., Emdin, M., Giannuzzi, P., Gargiulo, P., Giovannardi, M., Perrone-Filardi, P., Raimondo, R., Ricci, R., Paolillo, S., Farina, S., Belardinelli, R., Passantino, A. & La Gioia, R.; Metabolic Exercise test data combined with Cardiac and Kidney Indexes (MECKI) Score Research Group. 2015. Renal function and peak exercise oxygen consumption in chronic heart failure with reduced left ventricular ejection fraction. Circulation Journal 79 (3), 583-591.

Siegler, J. C., Marshall, P. W., Bishop, D., Shaw, G. & Green, S. 2016. Mechanistic insights into the efficacy of sodium bicarbonate supplementation to improve athletic performance. Sports Medicine - Open 2 (1), 41.

So, R., Song, S., Lee, J. E. & Yoon, H. 2016. The association between renal hyperfiltration and the sources of habitual protein intake and dietary acid load in a general population with preserved renal function: The KoGES Study. PloS one 11 (11), e0166495.

Souto, G., Donapetry, C., Calvino, J. & Adeva, M. M. 2011. Metabolic acidosis-induced insulin resistance and cardiovascular risk. Metabolic Syndrome and Related Disorders 9 (4), 247-253.

Stephens, T. J., McKenna, M. J., Canny, B. J., Snow, R. J. & McConell, G. K. 2002. Effect of sodium bicarbonate on muscle metabolism during intense endurance cycling. Medicine and Science in Sports and Exercise 34 (4), 614-621.

Street, D., Nielsen, J., Bangsbo, J. & Juel, C. 2005. Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle interstitium. Journal of Physiology 566 (2), 481-489.

Sutton, J. R., Jones, N. L. & Toews, C. J. 1981. Effect of pH on muscle glycolysis during exercise. Clinical Science 61 (3), 331-338.

Suzuki, M. 2015. Physical exercise and renal function. The Journal of Physical Fitness and Sports Medicine 4 (1), 17-29.

Tabatabai, L. S., Cummings, S. R., Tylavsky, F. A., Bauer, D. C., Cauley, J. A., et al. 2015. Arterialized venous bicarbonate is associated with lower bone mineral density and an increased rate of bone loss in older men and women. The Journal of Clinical Endocrinology & Metabolism 100 (4), 1343-1349.

Tareen, N., Zadshir, A., Martins, D., Nagami, G., Levine, B. & Norris, K. C. 2004. Alterations in acid-base homeostasis with aging. Journal of the National Medical Association 96 (7), 921-925.

Tiidus, P., Tupling, A. R. & Houston, M. 2012. Biochemistry primer for exercise science. Human Kinetics.

Van Montfoort, M. C., Van Dieren, L., Hopkins, W. G. & Shearman, J. P. 2004. Effects of ingestion of bicarbonate, citrate, lactate, and chloride on sprint running. Medicine and Science in Sports and Exercise 36 (7), 1239-1243.

Vormann, J. & Goedecke, T. 2006. Acid-base homeostasis: Latent acidosis as a cause of chronic diseases. Schweizerische Zeitschrift fur Ganzheits Medizin 18 (5), 255.

Weinstein, Y., Magazanik, A., Grodjinovsky, A., Inbar, O., Dlin, R. A. & Stewart, P. A. 1991. Reexamination of Stewart's quantitative analysis of acid-base status. Medicine and Science in Sports and Exercise 23 (11), 1270-1275.

Welch, A. A., MacGregor, A. J., Skinner, J., Spector, T. D., Moayyeri, A. & Cassidy, A. 2013. A higher alkaline dietary load is associated with greater indexes of skeletal muscle mass in women. Osteoporosis International 24 (6), 1899-1908.

Wesson, D. E. & Simoni, J. 2009. Increased tissue acid mediates a progressive decline in the glomerular filtration rate of animals with reduced nephron mass. Kidney International 75 (9), 929-935.

Wesson, D. E., Simoni, J., Broglio, K. & Sheather, S. 2011. Acid retention accompanies reduced GFR in humans and increases plasma levels of endothelin and aldosterone. American Journal of Physiology - Renal Physiology 300 (4), F830-F837.

Westerblad, H. 2016. Acidosis is not a significant cause of skeletal muscle fatigue. Medicine and Science in Sports and Exercise 48 (11), 2339-2342.

Westerblad, H., Allen, D. G. & Lännergren, J. 2002. Muscle fatigue: lactic acid or inorganic phosphate the major cause? News in Physiological Sciences 17, 17-21.

Westerterp-Plantenga, M. S., Lemmens, S. G. & Westerterp, K. R. 2012. Dietary protein – its role in satiety, energetics, weight loss and health. British Journal of Nutrition 108 (S2), S105-S112.

Wilkes, D., Gledhill, N. & Smyth, R. 1983. Effect of acute induced metabolic alkalosis on 800-m racing time. Medicine and Science in Sports and Exercise 15 (4), 277-280.

Yenchek, R., Ix, J. H., Rifkin, D. E., Shlipak, M. G., Sarnak, M. J., Garcia, M., Patel, K. V., Satterfield, S., Harris, T. B., Newman, A. B. & Fried, L. F.; Health, Aging, and Body Composition Study. 2014. Association of serum bicarbonate with incident functional limitation in older adults. Clinical Journal of the American Society of Nephrology : CJASN 9 (12), 2111-2116.

Yucha, C. 2004. Renal regulation of acid-base balance. Nephrology Nursing Journal 31 (2), 201-206.

ORIGINAL PAPERS

I

LOW-PROTEIN VEGETARIAN DIET DOES NOT HAVE A SHORT-TERM EFFECT ON BLOOD ACID-BASE STATUS BUT RAISES OXYGEN CONSUMPTION DURING SUBMAXIMAL CYCLING

by

Hietavala E-M, Puurtinen R, Kainulainen H & Mero Antti A. 2012.

Journal of the International Society of Sports Nutrition, vol 9, 50.

Hietavala et al. Journal of the International Society of Sports Nutrition 2012, 9:50
http://www.jissn.com/content/9/1/50

jissn
journal of the international society of sports nutrition

RESEARCH ARTICLE Open Access

Low-protein vegetarian diet does not have a short-term effect on blood acid–base status but raises oxygen consumption during submaximal cycling

Enni-Maria Hietavala*, Risto Puurtinen, Heikki Kainulainen and Antti A Mero

Abstract

Background: Acid–base balance refers to the equilibrium between acids and bases in the human body. Nutrition may affect acid–base balance and further physical performance. With the help of PRAL (potential renal acid load), a low-protein vegetarian diet (LPVD) was designed to enhance the production of bases in body. The aim of this study was to investigate if LPVD has an effect on blood acid–base status and performance during submaximal and maximal aerobic cycling.

Methods: Nine healthy, recreationally active men (age 23.5 ± 3.4 yr) participated in the study and were randomly divided into two groups in a cross-over study design. Group 1 followed LPVD for 4 days and group 2 ate normally (ND) before performing a cycle ergometer test. The test included three 10-min stages at 40, 60 and 80% of VO_2max. The fourth stage was performed at 100% of VO_2max until exhaustion. After 10–16 days, the groups started a second 4-day diet, and at the end performed the similar ergometer test. Venous blood samples were collected at the beginning and at the end of both diet periods and after every stage cycled.

Results: Diet caused no significant difference in venous blood pH, strong ion difference (SID), total concentration of weak acids (A_{tot}), partial pressure of CO_2 (pCO_2) or HCO_3^- at rest or during cycling between LPVD and ND. In the LPVD group, at rest SID significantly increased over the diet period (38.6 ± 1.8 vs. 39.8 ± 0.9, p=0.009). Diet had no significant effect on exercise time to exhaustion, but VO_2 was significantly higher at 40, 60 and 80% of VO_2max after LPVD compared to ND (2.03 ± 0.25 vs. 1.82 ± 0.21 l/min, p=0.035; 2.86 ± 0.36 vs. 2.52 ± 0.33 l/min, p<0.001 and 4.03 ± 0.50 vs. 3.54 ± 0.58 l/min, p<0.001; respectively).

Conclusion: There was no difference in venous blood acid–base status between a 4-day LPVD and ND. VO_2 was increased during submaximal cycling after LPVD suggesting that the exercise economy was poorer. This had no further effect on maximal aerobic performance. More studies are needed to define how nutrition affects acid–base balance and performance.

Keywords: Nutrition, Acid–base balance, Aerobic performance

Background

For normal functioning of the human body, there must be equilibrium between acids and alkali in body fluids [1]. Almost all function of enzymes and cells is dependent on the acid–base balance [2]. The acidity or alkalinity of body fluids is usually expressed by pH, which is affected by hydrogen ion concentration ($[H^+]$). In arteries, normal pH is 7.4. During acidosis there is an excess of hydrogen ions and pH is below 7.4, whereas during alkalosis hydrogen ions are lost and pH is above 7.4. Regulation mechanisms of the acid–base balance try to maintain pH in body fluids strictly between 7.37 and 7.43 [2]. According to the physicochemical approach of Peter Stewart, there are three independent variables that determine the hydrogen ion concentration and, thus, pH

* Correspondence: enni.hietavala@jyu.fi
Department of Biology of Physical Activity, University of Jyväskylä, P.O. Box 35 (VIV), Jyväskylä FIN-40014, Finland

of body fluids: strong ion difference (SID), total concentration of weak acids (A_{tot}) and partial pressure of carbon dioxide (pCO_2) [3]. The approach of Stewart is a more versatile way to explore the acid–base balance than the traditional, CO_2-centered Henderson-Hasselbalch equation [4].

SID is the difference between strong cations and anions and can be calculated as: SID (mEq/l) = ($[Na^+]$ + $[K^+]$) - ($[Cl^-]$ + $[Lac^-]$) [5]. When SID increases, $[H^+]$ decreases according to the rule of electroneutrality. SID is usually slightly positive, but fluids of the body cannot be electrically charged. The necessary negative charge comes from pCO_2 and A_{tot}. When the production of CO_2 exceeds the removal of CO_2 in the metabolism of cells, pCO_2 increases and causes a rise in $[H^+]$. A_{tot} is mainly proteins (mainly albumin) and phosphates and through them the rule of electroneutrality is fulfilled. If there is a change in one or more independent variable, $[H^+]$ changes as a consequence [3].

It is known that nutrition has an effect on acid–base balance, that is, acid load of the human body can be changed via nutrition [6]. It can be evaluated via PRAL (potential renal acid load) whether a certain foodstuff increases the production of acids or alkali in the body [6,7]. PRAL can be calculated for 100 g of foodstuff as: PRAL (mEq/100 g) = 0.49 × protein (g/100 g) + 0.037 × phosphorous (mg/ 100 g) - 0.021 x potassium (mg/100 g) - 0.026 × magnesium (mg/100 g) - 0.013 × calcium (mg/100 g) [7]. A foodstuff with negative PRAL is more alkali than acid forming. For example, fruits and vegetables contain lots of potassium that is a base-forming cation along with magnesium and calcium. Conversely, meat, cheese and cereal products have a positive PRAL and they enhance the production of acids. All protein-rich foodstuffs contain amino acids methionine and cysteine that are acid forming, so nutrition rich in protein and poor in alkali-forming foodstuff increases the acid load of the body [6].

The acid–base balance has an effect on physical performance [8]. Even physical activity of moderate intensity causes metabolic changes, which affect the acid–base balance both in skeletal muscles and other tissues [3]. Maintenance of high alkalinity in extracellular fluids enables faster H^+ removal from the muscle cell and muscle fatigue caused by increased acidosis is delayed [8]. Enhanced acid buffering capacity seems to improve both high-intensity anaerobic [9,10] and aerobic [11] capacity. $NaHCO_3$ is a useful ergogenic aid to increase the $[HCO_3^-]$ and buffering capacity of the blood [12], but performance can be improved by dietary means as well [13,14]. It has been observed that protein-rich nutrition combined with a low intake of carbohydrate may cause acidosis and have a negative influence on performance [13]. In one study, for example, low-protein (9.4 ± 1.8%) and high-carbohydrate (65.5 ± 9.8%) diet obeyed for 4 days resulted in higher plasma pH and $[HCO_3^-]$ prior to the exercise test compared to high-protein (25.3 ± 4.1%) and low-carbohydrate (10.1 ± 6.8%) diet and resulted in a longer time to exhaustion during cycling at 100% of VO_2max (345 ± 187 s vs. 221 ± 58 s) [14]. In another study, the use of a plant-based nutrient supplement for 14 days increased the pH of urine, which indicates that the acid load of the body was decreased [15]. These findings provide rationale to study the effects of a low-protein vegetarian diet on acid–base balance and physical performance.

According to our knowledge, there are no previous studies where the PRAL method is used to evaluate the quality of food for the investigation of the effect of nutrition on aerobic performance in humans. Thus, the purpose of this study was to explore if a low-protein vegetarian diet, which was designed with the help of PRAL to enhance the production of bases, has an effect on acid–base balance in men. Moreover, the study was planned to determine whether the possible changes in venous blood acid–base status influence performance or fuel selection during submaximal and maximal cycling. It was hypothesized that a diet low in protein and rich in alkali-producing vegetables and fruits may have the potential to alter the blood acid–base status and, thus, enable higher aerobic capacity and influence fuel selection during exercise.

Methods

Subjects

Nine healthy, recreationally active men volunteered for the study and signed an informed consent. Subjects were students of University of Jyväskylä and were exercising recreationally (e.g. walking, jogging, cycling, resistance training). Subjects who were obese (body mass index above 30), were training for competitive purposes, were using any medication or had any food allergy were excluded from the study. Ethical approval for the study was obtained from the University's Ethics Committee and the study followed the declaration of Helsinki.

Pre-testing

Before the actual experimental cross-over design, VO_2max and maximal workload of the subjects were measured (measurement 1, M1). Before M1 the subjects followed their normal diet and kept food diaries for 4 days, thus, the eating and drinking habits of the subjects were checked to be in accordance with general dietary guidelines. On the fifth day, the subjects performed M1, which was an incremental VO_2max test performed on a mechanically braked cycle ergometer (Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden). The workload was initially 75 W and was increased by 25 W every

2 min until exhaustion. The pedaling frequency was sustained at 60 rpm throughout the test. Before the ergometer test, height, weight and body mass index (BMI) of the subjects were determined. For the estimation of body fat percentage, a 4-point skinfold method was used. Thicknesses of biceps, triceps, subscapular and suprailiac skinfolds were measured and standard equations of Durnin & Womersley [16] were used for the determination of fat percentage.

Experimental design

The study design is presented in Figure 1. After M1, subjects were randomly divided into two groups. Group 1 (n=5) followed a normal diet (ND) first and then a low-protein vegetarian diet (LPVD). Group 2 (n=4) followed LPVD first and then ND. 10–16 days after M1, subjects came to the laboratory at 8 or 10 am after a 12-hour overnight fast and fasting blood samples (PREdiet) from a fingertip capillary and an antecubital vein were drawn. The last meal before PREdiet was consistent with the normal diet of the subjects. Starting from the PREdiet sample, the subjects followed either LPVD or ND and kept food diaries for 4 days. On the 5th day they completed the second measurement (M2). On the morning of M2, after a 12-hour overnight fast, fasting blood samples (POSTdiet) were drawn at the same time as PREdiet. The last meal before POSTdiet was consistent with the diet followed during the 4 days (either LPVD or ND). A light breakfast, which was consistent with the assigned diet, was eaten thereafter. After a rest of 30 min, resting blood samples were drawn once more (PREtest). The subjects started M2 by a 5-min warm-up followed by a 4-min break before the actual test started. According to the results of M1, workloads for M2 and M3 (measurement 3) were determined. In M2 and M3, the subjects cycled 3 × 10 min at 40, 60 and 80% of VO_2max and finally at 100% of VO_2max until exhaustion. For every subject the workload was increased by 50 or 75 W in every stage. There were 4-min breaks after each 10-min cycling stage during which blood samples were collected (Stage 1–4).

After M2 was completed, the subjects were allowed to eat according to their normal dietary habits without keeping a food diary. 10–16 days after M2, the subjects started the second 4-day diet and on the 5th day completed M3. M3 was similar to M2, but before M3 the groups changed the diets. All the blood samples were drawn at the same time in the morning as during the first diet period.

The subjects were allowed to exercise moderately during the diet periods. However, during the last 24 hours before every fasting blood sample the subjects were advised to minimize their physical activity and strenuous exercise was not allowed. The subjects reported their physical activity during both diet periods along with food diaries. Thus, it was controlled that the instructions concerning physical activity were obeyed.

PRAL and the diets

LPVD was designed with the help of PRAL to enhance the production of alkali in the body. A PRAL value of every foodstuff used in LPVD was calculated according to an equation that takes into account the contents of certain nutrients per 100 g of foodstuff, their intestinal absorption rates, grade of dissociation of phosphate at pH 7.4 and the ionic valence of magnesium and calcium. The equation is as follows: PRAL (mEq/100 g) = 0.49 × protein (g/100 g) + 0.037 × phosphorous (mg/100 g) - 0.021 x potassium (mg/100 g) - 0.026 x magnesium (mg/100 g) - 0.013 × calcium (mg/100 g) [7]. The PRAL values were calculated according to the nutrient contents that were taken from the Finnish Food Composition Database (Fineli, Finnish National Institute of Health and Welfare). When the PRAL value is below 0 the foodstuff is assumed to enhance the production of alkali in the body, and when it is above 0 the foodstuff increases the production of acids. Foodstuffs used during LPVD were chosen according to their PRAL value so

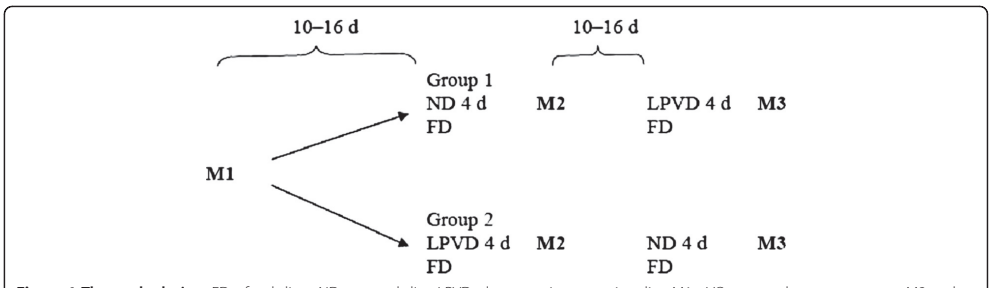


Figure 1 The study design. FD= food diary, ND= normal diet, LPVD= low-protein vegetarian diet, M1= VO_2max cycle ergometer test, M2 and M3= Cycle ergometer tests after the LPVD and ND.

that the diet would enhance the alkali production as much as possible. However, the general dietary guidelines were taken into account as well.

The subjects were given exact instructions how to realize LPVD. All the days during the vegetarian diet were similar and the diet mainly contained vegetables and fruits. The use of grain and dairy products was very limited. The subjects were not allowed to eat e.g. meat, cheese, eggs or bread at all during the 4 days. During both LPVD and ND the subjects were instructed to eat according to their energy needs and they reported the amount of foods eaten in a food diary.

Blood sampling and analysis

For the analysis of acid–base balance, Li-heparinized whole blood samples (200 μl) from a fingertip capillary were analyzed immediately after sampling for pH, lactate, HCO_3^- and pCO_2. For the determination of pH the direct ISE (ion selective electrolyte) in vitro test was used. Lactate was analyzed quantitatively by the enzymatic and amperometric in vitro test. PCO_2 was analyzed by the membrane amperometric method. HCO_3^- was determined computationally (Nova Biomedical STAT Profile pHOX Plus L Blood Gas Analyzator, Nova Biomedical, Waltham, MA, USA). Whole blood samples (4 ml) from the antecubital vein were collected to Venosafe gel tubes and analyzed for sodium, potassium and chloride by the direct ISE in vitro test (Ion Selective Microlyte Analyzer, Kone Instruments, Espoo Finland). Whole protein content of plasma and serum albumin were analyzed spectrophotometrically by the Biuret method (Shimadzu CL 720 Micro-Flow Spectrophotometry, Shimadzu Co., Kyoto, Japan).

Glucose was determined from the Li-heparinized fingertip samples (200 μl) quantitatively by the enzymatic and amperometric in vitro test (Nova Biomedical STAT Profile pHOX Plus L Blood Gas Analyzer). Non-esterified free fatty acids (FFA) and triglycerides (TG) were analyzed from the antecubital whole blood sample (4 ml). The blood samples were drawn in vacuum tubes and were centrifuged for 10 min at 3500 rpm. The serum was separated and FFA and TG were then analyzed by the spectrophotometric and enzymatic method. For the determination of FFA, NEFA C-kit was used (Shimadzu CL 720 Micro-Flow Spectrophotometry).

During cycling, the gaseous exchange was measured using Sensor Medics Breath Gas Analyzator (Vmax series 229, California, USA). The device was calibrated before every measurement. VO_2, VCO_2, RQ and VE were determined as a mean from the final 30 seconds of every stage. Heart rate was measured by a Polar heart rate monitor (Polar Electro Oy, Kempele, Finland).

SID and A_{tot} were calculated as follows: SID (mEq/l) = $([Na^+] + [K^+]) - ([Cl^-] + [Lac^-])$ [3], A_{tot} (mEq/l) = 2.43 × $[P_{tot}]$ (g/dl) [17].

Food diaries were analyzed for energy, protein, carbohydrate, fat, phosphorous, potassium, magnesium and calcium intake by the Nutri-Flow software (Flow-Team Oy, Oulu, Finland, 2012). The daily PRAL during LPVD and ND were calculated as the overall PRAL per one day according to the actual intake of relevant nutrients.

Statistical analysis

All the variables were analyzed by SPSS 14.0 for Windows software. The resting blood samples (PREdiet and POSTdiet), the gaseous values, and the nutrient intake values were compared by paired t-test. Variables from the blood samples of M2 and M3 (Stage1–4) were compared to the resting blood sample of the same day (POSTdiet) between the two groups (ND vs. LPVD) with repeated measures ANOVA (2 group × 5 time). If there was a difference between the groups the analysis was continued with paired t-test.

Results

Subjects

All nine subjects completed the study design. Subjects were 23.5 ± 3.4 years old (mean ± SD). Their weight measured during pre-testing was 76.7 ± 7.4 kg and height 1.79 ± 0.06 m. BMI of the subjects was 24.0 ± 1.8 and the body fat percentage was 15.6 ± 3.0%. In the incremental VO_2max test (M1) the exhaustion occurred at 25 ± 2.7 min and VO_2max of the subjects was 4.10 ± 0.44 l/min.

Diets

There was a significant difference between the daily PRAL during LPVD and ND (−117 ± 20 vs. 3.2 ± 19, p<0.000). During LPVD subjects consumed 1151 ± 202 g fruits and vegetables whereas during ND the intake of fruits and vegetables was 354 ± 72 g (p<0.000). Energy and nutrient contents of LPVD and ND are presented in Table 1. Energy intake was significantly lower during LPVD compared to ND (2400 ± 338 kcal vs. 2793 ± 554 kcal, p=0.033). During LPVD, the intake of protein was 10.1 ± 0.26% and during ND 17.6 ± 3.0% of the total energy intake (p=0.000). The intake of carbohydrates was significantly higher during LPVD compared to ND (58.7 ± 2.4% vs. 49.8 ± 5.4%, p=0.003). As well, the amount of fat differed between LPVD and ND (24.7 ± 2.3% vs. 28.1 ± 3.1%, p=0.015). In spite of lower energy intake during LPVD there was no difference in the weight of the subjects compared to ND (75.6 ± 7.9 kg vs. 76.2 ± 7.6 kg).

Acid–base balance

Diet had no significant effect on venous blood pH (Table 2). There were no significant differences between the diets in SID, A_{tot}, pCO_2 or HCO_3^-at rest or during exercise (Tables 2 and 3). The only significant change

Table 1 Energy and nutrient content of normal diet (ND) and low-protein vegetarian diet (LPVD)

		ND	LPVD
PRAL (mEq/d)		3.2 ± 19	−117 ± 20***
Energy (kcal/d)		2792 ± 554	2400 ± 338*
Protein	**(g/d)**	122 ± 29	61 ± 8.9***
	(g/kg/d)	1.59 ± 0.28	0.80 ± 0.11***
	(%)	17.6 ± 3.0	10.1 ± 0.26***
CHO	**(g/d)**	348 ± 80	349 ± 51
	(g/kg/d)	4.58 ± 0.93	4.63 ± 0.61
	(%)	49.8 ± 5.4	58.7 ± 2.4**
Fat	**(g/d)**	87 ± 20	66 ± 11**
	(g/kg/d)	1.14 ± 0.20	0.88 ± 0.13**
	(%)	28.1 ± 3.1	24.7 ± 2.3*

*= p<0.05; **= p<0.01; ***= p<0.001.

caused by nutrition was that SID was significantly higher after LPVD compared to before the diet (PREdiet vs. POSTdiet: 38.6 ± 1.8 mEq/l vs. 39.8 ± 0.9 mEq/l, p=0.009).

Within each diet group, cycling did cause some statistically significant alterations in the variables of acid–base balance, which are presented in Table 2 and 3. These acute responses were similar between both diets.

Workload and VO_2

Workload, heart rate and duration of each stage of M2 and M3 are presented in Table 4. Some subjects were not able to finish the 10-min stage of 80% of VO_2max. In the LPVD group the duration of the stage was 8.84 ± 1.46 min whereas in ND group it was 8.56 ± 1.87 min. The maximal stage (100% of VO_2max) which was cycled until exhaustion lasted 1.81 ± 0.80 min in the LPVD group and 2.89 ± 1.91 min in the ND group. However, differences in the durations of these stages were not significant. There were no differences in heart rates between the diet groups.

The values of VO_2, VCO_2, VE and RQ are presented in Table 5. After LPVD, VO_2 was significantly higher at 40, 60 and 80% of VO_2max (2.03 ± 0.25 vs. 1.82 ± 0.21 l/min, p=0.035; 2.86 ± 0.36 vs. 2.52 ± 0.33 l/min, p<0.001 and 4.03 ± 0.50 vs. 3.54 ± 0.58 l/min, p<0.001; respectively), but not at 100% of VO_2max, compared to ND (Figure 2). Also, VCO_2 differed significantly at all submaximal stages, being higher after LPVD (p=0.011. p=0.009, p=0.010, respectively). VE tended to be higher at all stages after LPVD, but the difference was significant (p=0.009) only at Stage 2. RQ was not different between the diet groups at any point of the cycling.

VO_2max measured in the first cycle test (M1) was 4.10 ± 0.44 l/min. After LPVD, the highest VO_2 achieved during Stage 4 was 3.87 ± 0.90, whereas after ND it was 3.65 ± 0.65 l/min. However, none of the VO_2max values differed significantly from each other.

Blood carbohydrate and fat metabolites and serum albumin

There were no differences in venous blood lactate, glucose, FFA or TG between the two diet groups at rest or during cycling. At rest, TG decreased significantly (p=0.021) during LPVD (PREdiet vs. POSTdiet). During cycling there were, within each diet group, some statistically significant changes that are presented in Table 6.

There were no differences in serum albumin between the diet groups at rest or during cycling. Within LPVD group, albumin increased from 39.4 ± 3.1 g/l (PREdiet) to 41.7 ± 2.0 g/l (POSTdiet) (p=0.032). Within each diet group, cycling caused some statistically significant changes, which are presented in Table 6.

Table 2 Plasma pH and [HCO_3^-] at rest and during cycle ergometer tests

Sample	pH		HCO_3 (mmol/l)	
	ND	LPVD	ND	LPVD
PREdiet	7.467 ± 0.039	7.448 ± 0.028	33.6 ± 8.7	32.2 ± 6.0
POSTdiet	7.455 ± 0.028	7.454 ± 0.025	32.0 ± 5.5	31.9 ± 3.9
PREtest	7.466 ± 0.030	7.459 ± 0.015	32.9 ± 6.3	32.6 ± 4.5
Stage1	7.470 ± 0.029	7.473 ± 0.036	31.0 ± 3.1	31.7 ± 4.2
Stage2	7.459 ± 0.028	7.457 ± 0.031	28.6 ± 2.3	20.8 ± 3.3
Stage3	7.378 ± 0.039*	7.368 ± 0.029**	20.8 ± 3.3**	19.9 ± 2.2***
Stage4	7.326 ± 0.076*	7.336 ± 0.03***	16.7 ± 2.5**	18.4 ± 2.4***

ND= normal diet.
LPVD= low-protein vegetarian diet.
PREdiet= a fasting blood sample taken in the morning before the start of ND or LPVD (day 1).
POSTdiet= a fasting blood sample taken in the morning after a 4-day ND or LPVD (day 5).
PREtest= a resting blood sample taken 30 min after a breakfast, before the cycle ergometer test (day 5).
Stage1–4= blood samples taken after 10-min cycling at 40, 60 and 80% of VO_2max and after the maximal stage (at 100% of VO_2max until exhaustion).
POSTdiet vs. Stage1–4 *= p<0.05; **= p<0.01; ***= p<0.001.

Table 3 Independent variables of acid–base balance at rest and during cycle ergometer tests

Sample	SID (mEq/l)		A_{tot} (mEq/l)		pCO_2 (mmHg)	
	ND	LPVD	ND	LPVD	ND	LPVD
PREdiet	38.6 ± 1.8	38.6 ± 1.8	18.5 ± 0.8	18.3 ± 0.6	6.07 ± 1.29	6.13 ± 1.09
POSTdiet	39.4 ± 1.2	39.8 ± 0.9#	18.1 ± 1.0	18.1 ± 1.0	6.05 ± 0.82	5.98 ± 0.64
PREtest	38.8 ± 1.5	38.5 ± 1.2*	18.1 ± 0.8	18.1 ± 1.0	5.98 ± 0.95	6.05 ± 0.89
Stage1	38.0 ± 1.1	37.9 ± 0.6**	18.8 ± 0.9	18.9 ± 0.5	5.60 ± 0.38	5.72 ± 0.97
Stage2	35.7 ± 1.0*	35.3 ± 1.7**	19.3 ± 0.8**	19.1 ± 0.8**	5.30 ± 0.28	5.27 ± 0.57
Stage3	30.6 ± 1.6**	29.5 ± 2.2***	20.2 ± 1.0***	20.1 ± 1.0**	4.61 ± 0.38*	4.55 ± 0.41**
Stage4	29.6 ± 3.5**	29.1 ± 2.8***	20.4 ± 1.5**	20.2 ± 1.0***	4.23 ± 0.66*	4.51 ± 0.56**

ND= normal diet.
LPVD= low-protein vegetarian diet.
PREdiet= a fasting blood sample taken in the morning before the start of ND or LPVD (day 1).
POSTdiet= a fasting blood sample taken in the morning after a 4-day ND or LPVD (day 5).
PREtest= a resting blood sample taken 30 min after a breakfast, before the cycle ergometre test (day 5).
Stage1–4= blood samples taken after 10-min cycling at 40, 60 and 80% of VO_2max and after the maximal stage (at 100% of VO_2max until exhaustion).
PREdiet compared to POSTdiet #= p<0.05.
POSTdiet vs. Stage1–4 *= p<0.05; **= p<0.01; ***= p<0.001.

Discussion

Main results

The main result of this study was that there was no difference in venous blood acid–base status and its independent or dependent variables between a 4-day LPVD and ND. However, one statistically significant change in acid–base status did occur in the LPVD group, as SID increased by 3.1% over the 4-day diet period. During cycling, the diet composition caused some differences in aerobic energy production, which could be seen in significantly higher VO_2 and VCO_2 at every submaximal workload after LPVD compared to ND. This finding had no further effect on maximal aerobic performance.

Acid–base balance and diets

LPVD did not affect the venous blood acid–base status at rest or during submaximal or maximal cycling compared to ND. The higher protein content of food increases acid production in the body [6], therefore, we hypothesized that lower protein content combined with plentiful consumption of alkalinizing fruits and vegetables would shift the acid–base balance to a more alkaline direction. The PRAL value of every foodstuff consumed in LPVD was under 0, so the diet was clearly designed to enhance the production of alkali in the body. However, during ND subjects ate according to their normal eating habits and PRAL varied from −18.8 to 32.9 mEq/d. Thus, the acid load of ND varied remarkably on an individual level. Changes in blood acid–base status caused by nutrition are generally small, and the large inter-subject variation in PRAL during ND may have masked the possible effects of LPVD on acid–base balance. Moreover, the large variability during ND combined with the small subject group may have made the possible influence of nutrition difficult to detect.

In the present study ND, 17.6 ± 3.0% of the total energy intake (1.59 ± 0.28 g/kg) contained protein and LPVD contained 10.1 ± 0.26% (0.80 ± 0.11 g/kg) protein. The difference was statistically significant, but was not enough to cause changes in acid–base balance. In other studies, the difference has been greater; e.g. there are studies where the protein intakes during high- and low-protein diets have been 25.3 ± 4.1% vs. 9.4 ± 1.8%; 29 ± 4% vs. 10 ± 2% and 33 ± 6% vs. 10 ± 1% [14, 18, 19 respectively]. According to the present and other studies, and in the light of the fact that the protein intake increases the renal capacity to excrete acids [7], it seems that the difference in protein content of the diet must be remarkable to cause differences in acid–base status. Furthermore, the body will normally compensate rapidly for

Table 4 Workload, duration and heart rate of every stage during cycle ergometer tests

Workload (% of VO_2max)	Workload (W)	Duration (min)		Heart rate (bpm)	
		ND	LPVD	ND	LPVD
40	140 ± 10	10	10	128 ± 15	131 ± 12
60	210 ± 20	10	10	156 ± 16	161 ± 10
80	275 ± 30	8.56 ± 1.87	8.84 ± 1.46	180 ± 15	184 ± 10
100	338 ± 35	2.89 ± 1.91	1.81 ± 0.80	183 ± 11	182 ± 12

ND= normal diet.
LPVD= low-protein vegetarian diet.

Table 5 VO_2, VCO_2, VE and RQ during cycle ergometer tests

Work load (% of VO_2max)	VO_2 (l/min)		VCO_2 (l/min)		VE (l/min)		RQ	
	ND	LPVD	ND	LPVD	ND	LPVD	ND	LPVD
40	1.82 ± 0.21	2.03 ± 0.25*	1.60 ± 0.2	1.80 ± 0.2**	43.7 ± 5.2	47.7 ± 4.3	0.88 ± 0.03	0.89 ± 0.02
60	2.52 ± 0.33	2.86 ± 0.36***	2.29 ± 0.3	2.59 ± 0.3***	62.9 ± 10	70.7 ± 7.1**	0.91 ± 0.02	0.91 ± 0.03
80	3.54 ± 0.58	4.03 ± 0.50***	3.48 ± 0.7	3.91 ± 0.3**	113 ± 30	130 ± 13	0.98 ± 0.05	0.98 ± 0.04
100	3.65 ± 0.65	3.87 ± 0.90	3.56 ± 0.8	3.62 ± 1.0	131 ± 27	130 ± 40	0.97 ± 0.1	0.95 ± 0.1

ND= normal diet.
LPVD= low-protein vegetarian diet.
*= p<0.05; **= p<0.01; ***= p<0.001.

acute changes in acid–base balance to sustain $[H^+]$ at the optimal level [5]. In the above mentioned studies [14,18,19], for example, pCO_2 compensated the changes in venous blood pH. As is generally known, pH in body fluids is quite stable, although there are large amount of acids produced constantly in metabolism [1]. It may be that changing diet for only 4 days is not enough to shift acid–base balance to any direction so remarkably that it could be seen in venous blood samples. Since blood pH is strictly regulated, it would be reasonable to also measure urine pH to see if acid load of the body has changed [15].

In the present study we wanted to explore if changing diet from neutral to clearly alkali-producing (instead of two extremes) affects acid–base balance and performance. SID increased by 3.1% during LPVD, which is an encouraging result, but this change was not large enough to cause a detectable change in dependent variables like H^+ or HCO_3^-. Moreover, SID remained at a normal level and did not rise above 40 mmol/l, which can be considered as the lower limit of alkalosis [20]. Nonetheless, our results show that the 4-day diets we compared in this study did not cause a measurable difference in venous blood acid–base status.

Oxygen consumption and fuel selection during cycling

Nutrition had a statistically significant impact on O_2 consumption and CO_2 production during aerobic cycling. After LPVD, both O_2 and CO_2 were approximately 13% higher at every submaximal stage of the cycle ergometer test compared to ND. There were no differences in heart rates between the two cycling tests, so the loading for the cardiovascular system and the workload were similar during both tests. When exercising at a constant workload, higher oxygen consumption is usually connected to an increased level of FFA in plasma or increased oxidation of lipids [21]. However, there were no differences in RQ or plasma FFA or TG between the

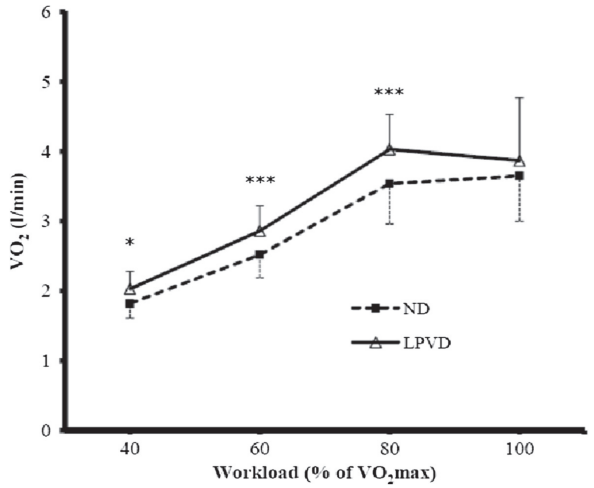


Figure 2 Oxygen consumption during cycle ergometer tests after normal diet (ND) and low-protein vegetarian diet (LPVD). *= p<0.05; ***= p<0.001.

Table 6 Carbohydrate and fat metabolites and albumin in blood at rest and during cycle ergometer tests

Sample	Lactate (mmol/l)		Glucose (mmol/l)		FFA (mmol/l)		TG (mmol/l)		Albumin (g/l)	
	ND	LPVD	ND	LPVD	ND	LPVD	ND	LPVD	ND	LPVD
PREdiet	1.6 ± 0.6	1.5 ± 0.5	4.80 ± 0.39	4.83 ± 0.27	0.34 ± 0.21	0.31 ± 0.06	1.18 ± 0.77	1.07 ± 0.30	40.3 ± 2.2	39.4 ± 3.1
POSTdiet	1.4 ± 0.5	1.4 ± 0.6	4.95 ± 0.42	4.81 ± 0.21	0.28 ± 0.17	0.35 ± 0.15	0.90 ± 0.23	0.85 ± 0.19$^{\#}$	39.1 ± 3.3	41.7 ± 2.0$^{\#}$
PREtest	2.6 ± 0.7	2.9 ± 1.0	5.16 ± 1.00	6.18 ± 1.28	0.15 ± 0.07	0.22 ± 0.09	0.91 ± 0.23	0.79 ± 0.23	40.3 ± 1.8	39.8 ± 2.9
Stage1	2.6 ± 0.9*	2.7 ± 0.9**	4.12 ± 0.44	3.88 ± 0.69	0.13 ± 0.04	0.13 ± 0.05	1.02 ± 0.25	0.82 ± 0.23	40.7 ± 2.4**	41.7 ± 2.8
Stage2	4.8 ± 1.2*	5.2 ± 1.9**	4.64 ± 0.63	4.38 ± 0.66	0.18 ± 0.08	0.19 ± 0.07	1.05 ± 0.22	0.89 ± 0.26	43.0 ± 2.5**	42.6 ± 1.2
Stage3	10.2 ± 1.6***	11.3 ± 2.1***	5.54 ± 0.79	5.66 ± 0.97	0.22 ± 0.10	0.22 ± 0.06	1.12 ± 0.26*	0.92 ± 0.28	44.8 ± 2.2**	44.7 ± 2.0*
Stage4	11.2 ± 3.4**	12.2 ± 2.1***	5.81 ± 0.99	5.21 ± 0.80	0.20 ± 0.10	0.20 ± 0.05	1.16 ± 0.29*	0.93 ± 0.28	44.3 ± 2.7**	44.3 ± 2.7*

ND= normal diet.
LPVD= low-protein vegetarian diet.
PREdiet= a fasting blood sample taken in the morning before the start of ND or LPVD (day 1).
POSTdiet= a fasting blood sample taken in the morning after a 4-day ND or LPVD (day 5).
PREtest= a resting blood sample taken 30 min after a breakfast, before the cycle ergometre test (day 5).
Stage1–4= blood samples taken after 10-min cycling at 40, 60 and 80% of VO_2max and after the maximal stage (at 100% of VO_2max until exhaustion).
PREdiet compared to POSTdiet $^{\#}$= p<0.05.
POSTdiet vs. Stage 1–4 *= p<0.05; **= p<0.01; ***= p<0.001.

dietary groups. Neither lactate nor glucose contents of plasma were different between the groups, so it is not possible to discuss the changes in the use of substrates in energy production, which could explain the differences in oxygen consumption. On the other hand, in the present study, serum albumin increased during LPVD by 5.8%. This could partially explain the higher oxygen consumption because serum albumin enables a higher rate of FFA transportation to muscle cells [22]. Metabolic acidosis inhibits albumin synthesis [23], so serum albumin content and SID, which both increased during LPVD, refer together to decreased acidosis. More controlled diet interventions should be used in the future to clarify this finding.

In an earlier study by Galloway and Maughan [21], oxygen consumption increased because of alkalosis, when the subjects exercised at 70% of VO_2max, but there was no difference in RQ. It was discussed that alkalosis would have caused a slight change in the use of substrates, which increased the oxygen consumption, but the change was so small that it could not be seen in RQ. In another study [24], metabolic alkalosis induced by $NaHCO_3$ accelerated the increase of VO_2 at the onset of high-intensity exercise (87% of VO_2max). However, at a lower intensity (40% of VO_2max), the alkalosis had no effect on the kinetics of breathing and oxygen consumption. Acidosis may, in turn, reduce the capacity of hemoglobin to bind oxygen and may reduce the oxygen content of the blood [25]. After LPVD, the subjects may have had an increased capacity to transport oxygen in the blood, but because of the lack of measurable change in acid–base status besides the minor change in SID, this is speculation.

It may also be that LPVD increased the need for oxygen, and as a consequence, oxidation of all substrates increased during submaximal cycling, which could explain the lack of changes in RQ. These results suggest that the energy expenditure was greater and cycling economy poorer after LPVD. In the present study insulin-like growth factor 1 (IGF-1) was not measured but according to our recently collected and unpublished data, serum IGF-1 increased during a 7 d high-protein diet and decreased during a 7 d low-protein vegetarian diet. The difference in IGF-1 could be one reason for the difference in oxygen consumption, since lower serum IGF-1 levels may result in poorer exercise economy [26].

In future studies it would be reasonable to control the energy intake of the diets to minimize the effect of difference in caloric intake on performance. However, the subjects were instructed to eat according to their perceived energy needs and they were free to make their own nutritional choices within the given instructions. Although the energy intake was approximately 390 kcal less during LPVD compared to ND, in our opinion, this was not a factor that would cause the difference in VO_2 between the two diet groups. Furthermore, there was no significant difference in the absolute carbohydrate intake between the diets, so e.g. muscle glycogen content should not have been lower after LPVD. Nonetheless, it seems that the vegetarian diet altered the need for oxygen during submaximal cycling. Since there were no differences in VO_2max or time until exhaustion between the diet groups the implications of the higher oxygen consumption at submaximal stages for maximal aerobic performance remains unclear.

Conclusions

A low-protein vegetarian diet followed for 4 days had no acute effect on venous blood acid–base status in young recreationally active men when compared to the normal diet of the subjects. The vegetarian diet increased VO_2 during submaximal aerobic cycling suggesting that the

submaximal cycling economy was poorer after LPVD compared to ND. However, this had no further effect on maximal aerobic performance. According to these results, a low-protein vegetarian diet cannot be recommended as a means to improve submaximal or maximal aerobic performance via acid–base balance as opposed to what was hypothesized. More studies are needed to define how nutrition, its comprehensive composition, and the duration of the diet period affect acid–base balance and performance. More specific measurements should also be used to determine the underlying mechanisms for higher VO_2 after the low-protein vegetarian diet.

Competing interests
This study project was funded by University of Jyväskylä, Department of Biology of Physical Activity. The authors declare that they have no competing interests.

Authors' contributions
EH (corresponding author) was responsible for the study design, the execution of the measurements, the statistical analysis and the preparation of the manuscript. RP participated in the study design and carried out all the blood sampling and analysis. HK helped in interpretation of data and revised the manuscript. AM supervised the study design, the implementation of the measurements and the drafting and revising the manuscript. All authors read and approved the final manuscript.

Acknowledgements
The authors would like to thank Rebekka Turkki for analyzing all the food diaries and Simon Walker for writing assistance.

Received: 26 June 2012 Accepted: 14 November 2012
Published: 26 November 2012

References

1. Adrogué HE, Adrogué HJ: **Acid–base physiology.** *Respir Care* 2001, **46**(4):328–341.
2. Vormann J, Goedecke T: **Acid–base homeostasis: Latent acidosis as a cause of chronic diseases.** *Ganzheits Medizin* 2006, **18:**255–266.
3. Lindinger MI: **Origins of [H^+] changes in exercising skeletal muscle.** *Can J Appl Phys* 1995, **20**(3):357–368.
4. Weinstein Y, Magazanik A, Grodjinovsky A, Inbar O, Dlin RA, Stewart PA: **Reexamination of Stewart's quantitative analysis of acid–base status.** *Med Sci Sports Exerc* 1991, **23**(11):1270–1275.
5. Kellum JA: **Determinants of blood pH in health and disease.** *Crit Care* 2000, **4**(1):6–14.
6. Remer T: **Influence of nutrition on acid–base balance – metabolic aspects.** *Eur J Nutr* 2001, **40:**214–220.
7. Remer T, Dimitriou T, Manz F: **Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents.** *Am J Clin Nutr* 2003, **77:**1255–1260.
8. Robergs RA, Ghiasvand F, Parker D: **Biochemistry of exercise-induced metabolic acidosis.** *Am J Phys – Reg I* 2004, **287:**R502–R516.
9. Mero AA, Keskinen KL, Malvela MT, Sallinen JM: **Combined creatine and sodium bicarbonate supplementation enhances interval swimming.** *J Strength Cond Res* 2004, **18:**306–310.
10. Wilkes D, Gledhill N, Smyth R: **Effect of acute induced metabolic alkalosis on 800-m racing time.** *Med Sci Sport Exer* 1983, **15:**277–280.
11. Ööpik V, Saaremets I, Medijainen L, Karelson K, Janson T, Timpmann S: **Effects of sodium citrate ingestion before exercise on endurance performance in well trained college runners.** *Brit J Sport Med* 2003, **37:**485–489.
12. McNaughton L, Thompson D: **Acute versus chronic sodium bicarbonate ingestion and anaerobic work and power output.** *J Sport Med Phys Fit* 2001, **41**(4):456–462.
13. Maughan RJ, Greenhaff PL, Leiper JB, Ball D, Lambert CP, Gleeson M: **Diet composition and the performance of high-intensity exercise.** *J Sport Sci* 1997, **15:**265–275.
14. Greenhaff PL, Gleeson M, Maughan RJ: **The effects of dietary manipulation on blood acid–base status and the performance of high intensity exercise.** *Eur J Appl Physiol O* 1987, **56:**331–337.
15. Berardi JM, Logan AC, Venket Rao A: **Plant based dietary supplement increases urinary pH.** *J Int Soc Sports Nutr* 2008, **5:**20. http://www.jissn.com/content/5/1/20.
16. Durnin JVGA, Womersley J: **Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 yr.** *Br J Nutr* 1974, **32:**77–97.
17. Constable PD: **Total weak acid concentration and effective dissociation constant of nonvolatile buffers in human plasma.** *J Appl Physiol* 2001, **91**(3):1364–1371.
18. Greenhaff PL, Gleeson M, Whiting PH, Maughan RJ: **Dietary composition and acid–base status: limiting factors in the performance of maximal exercise in man?** *Eur J Appl Physiol O* 1987, **56:**444–450.
19. Greenhaff PL, Gleeson M, Maughan RJ: **The effects of a glycogen loading regimen on acid–base status and blood lactate concentration before and after a fixed period of high intensity exercise in man.** *Eur J Appl Physiol O* 1988, **57:**254–259.
20. Schück O, Matoušovic K: **Relation between pH and the strong ion difference (SID) in body fluids.** *Biom Pap* 2005, **149**(1):69–73.
21. Galloway SDR, Maughan RJ: **The effects of induced alkalosis on the metabolic response to prolonged exercise in humans.** *Eur J Appl Physiol* 1996, **74:**384–389.
22. Van der Vusse GJ: **Albumin as fatty acid transporter.** *Drug Metab Pharmacokinet* 2009, **24**(4):300–307.
23. Ballmer PE, McNurlan MA, Hulter HN, Anderson SE, Garlick PJ, Krapf R: **Chronic metabolic acidosis decreases albumin synthesis and induces negative nitrogen balance in humans.** *J Clin Invest* 1995, **95:**39–45.
24. Zoladz JA, Szkutnik Z, Krzysztof D, Majerczak J, Korzeniewski B: **Preexercise metabolic alkalosis induced via bicarbonate ingestion accelerates VO_2 kinetics at the onset of a high-power-output exercise in humans.** *J Appl Phys* 2005, **98:**895–904.
25. Dersjant-Li Y, Verstegen MWA, Jansman A, Schulze H, Schrama JW, Verreth JA: **Changes in oxygen content and acid–base balance in arterial and portal blood in response to the dietary electrolyte balance in pigs during a 9-h period after a meal.** *J Anim Sci* 2002, **80:**1233–1239.
26. Irving BA, Patrie JT, Anderson SM, Watson-Winfield DD, Frick KI, Evans WS, Veldhuis JD, Weltman A: **The effects of time following acute growth hormone administration on metabolic and power output measures during acute exercise.** *J Clin Endocrinol Metab* 2004, **89**(9):4298–4305.

doi:10.1186/1550-2783-9-50
Cite this article as: Hietavala *et al.*: Low-protein vegetarian diet does not have a short-term effect on blood acid–base status but raises oxygen consumption during submaximal cycling. *Journal of the International Society of Sports Nutrition* 2012 **9**:50.

II

EFFECT OF DIET COMPOSITION ON ACID-BASE BALANCE IN ADOLESCENTS, YOUNG ADULTS AND ELDERLY AT REST AND DURING EXERCISE

by

Hietavala E-M, Stout JR, Hulmi JJ, Suominen H, Pitkänen H, Puurtinen R, Selänne H, Kainulainen H & Mero Antti A. 2015.

European Journal of Clinical Nutrition vol 69 (3), 399-404

European Journal of Clinical Nutrition (2015) **69,** 399–404

www.nature.com/ejcn

ORIGINAL ARTICLE

Effect of diet composition on acid–base balance in adolescents, young adults and elderly at rest and during exercise

E-M Hietavala[1], JR Stout[2], JJ Hulmi[1], H Suominen[3], H Pitkänen[4], R Puurtinen[1], H Selänne[5], H Kainulainen[1] and AA Mero[1]

BACKGROUND: Diets rich in animal protein and cereal grains and deficient in vegetables and fruits may cause low-grade metabolic acidosis, which may impact exercise and health. We hypothesized that (1) a normal-protein diet with high amount of vegetables and fruits (HV) induces more alkaline acid–base balance compared with a high-protein diet with no vegetables and fruits (HP) and (2) diet composition has a greater impact on acid–base balance in the elderly (ELD).

SUBJECTS/METHODS: In all, 12–15 (adolescents (ADO)), 25–35 (young adults (YAD)) and 60–75 (ELD)-year-old male and female subjects ($n = 88$) followed a 7-day HV and a 7-day HP in a randomized order and at the end performed incremental cycle ergometer tests. We investigated the effect of diet composition and age on capillary (c-pH) and urine pH (u-pH), strong ion difference (SID), partial pressure of carbon dioxide (pCO_2) and total concentration of weak acids (A_{tot}). Linear regression analysis was used to examine the contribution of SID, pCO_2 and A_{tot} to c-pH.

RESULTS: In YAD and ELD, c-pH ($P \leqslant 0.038$) and u-pH ($P < 0.001$) were higher at rest after HV compared with HP. During cycling, c-pH was higher ($P \leqslant 0.034$) after HV compared with HP at submaximal workloads in YAD and at 75% of VO_2max (maximal oxygen consumption) in ELD. The contribution of SID, pCO_2 and A_{tot} to c-pH varied widely. Gender effects or changes in acid–base balance of ADO were not detected.

CONCLUSIONS: A high intake of vegetables and fruits increases blood and u-pH in YAD and ELD. ELD compared with younger persons may be more sensitive for the diet-induced acid–base changes.

European Journal of Clinical Nutrition (2015) **69,** 399–404; doi:10.1038/ejcn.2014.245; published online 10 December 2014

INTRODUCTION

A diet containing plenty of animal proteins and cereal grains and deficient in vegetables and fruits–a typical diet for many Westernized cultures—may cause low-grade metabolic acidosis.[1,2] This chronic acidosis has adverse health consequences, which may be especially true as we age.[3–6] Aging-related decline in renal function and the ability to excrete the excessive hydrogen ions (H^+) have been thought to lead to mild but slowly increasing metabolic acidosis, especially if the dietary acid load is high.[4,7] Therefore, alkaline diets may help in the preservation of muscle mass and delay sarcopenia in older men and women.[4,8] In addition to digestion of food, physical activity causes acute metabolic changes that may result in an increase in H^+ production, which could affect the acid–base balance in skeletal muscles, blood and other tissues.[9]

Hydrogen ion concentration ($[H^+]$) in body fluids is regulated to remain in between rather narrow pH limits.[10,11] According to a physicochemical acid–base approach of Stewart,[11] there are at least three independent variables that determine $[H^+]$ and thereby pH in the body fluids: partial pressure of carbon dioxide (pCO_2), strong ion difference (SID) and total concentration of weak acids (A_{tot}).[9,11–13] The respiratory component of acid–base balance is affected by pCO_2 and regulated by alveolar ventilation. SID is the difference between strongly dissociated positive (e.g. Na^+, K^+) and negative (e.g. Cl^-) ions in body fluids. It represents the metabolic component of the acid–base balance and is mainly regulated by the kidney. The weak acids are mostly proteins and phosphates, and they contribute the third determinant of $[H^+]$.

To our knowledge, there are no previous studies that have examined the combined effects of diet and exercise on the acid–base balance and its independent variables in humans. Therefore, the purpose of the present study was to compare differences in acid–base balance from a 7-day normal-protein diet with high amount of vegetables and fruits (HV) and a 7-day high-protein diet with no vegetables and fruits (HP) at rest and during aerobic exercise in adolescents (ADO), young adults (YAD) and elderly (ELD). In addition, we wanted to determine the contributions of pCO_2, SID and A_{tot} on capillary pH (c-pH) at rest and during aerobic exercise, to possibly ascertain the physicochemical reasons for the changes in $[H^+]$.

MATERIALS AND METHODS

Subjects

The subjects of the present study were recruited for the intervention by advertising in newspapers and through email lists. In total, 88 voluntary and suitable men and women from three age groups were selected to participate in the study. In the group of ADO (12–15 years), there were 13 boys and 9 girls; in the group of YAD (25–35 years), there were 15 men and 18 women; and in the group of ELD (60–75 years), there were 17 men and 16 women. The ADO group was recruited from local sports' clubs who were participating in ice hockey, figure skating, gymnastics and track. The subjects of YAD group were mainly students in the local university, and the ELD subjects were recruited from the Aging Program of University of Jyväskylä. All subjects of YAD and ELD groups were recreationally active

[1]Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland; [2]Institute of Exercise Physiology and Wellness, University of Central Florida, Orlando, FL, USA; [3]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland; [4]Honka Holding, Honkajoki, Finland and [5]LIKES Research Center for Sport and Health Sciences, Jyväskylä, Finland. Correspondence: H Enni-Maria, Department of Biology of Physical Activity, University of Jyväskylä, PO Box 35 (VIV), Jyväskylä 40014, Finland.
E-mail: enni.hietavala@jyu.fi
Received 19 June 2014; revised 15 August 2014; accepted 7 September 2014; published online 10 December 2014

(e.g. walking, jogging, cycling, resistance training) and were not training for competitive purposes. All subjects were healthy and did not use any medication during the study period aside from two exceptions: women of YAD group were allowed to use contraceptive pills and in the ELD groups medications for high blood pressure and high cholesterol were acceptable. Subjects whose body mass index was above 33 kg/m^2 or who had any relevant food allergy were excluded from the study. Before the measurements, the subjects were informed of the purpose and the methods of the study and they signed a written informed consent. In addition, the subjects completed a health questionnaire, and the ELD subjects also completed a health examination that was performed by a physician. Ethical approval for the study was obtained from the Ethics Committee of University of Jyväskylä, and the study was in accordance with the Declaration of Helsinki.

Pretesting

An incremental cycle ergometer test (Ergoline ergometrics 800 (Ergoline GmbH, Bitz, Germany); Jaeger Oxycon Pro breath-by-breath gas analyzer (VIASYS Health-care GmbH, Hoechburg, Germany)) was used to determine VO_2max (maximal oxygen consumption) and maximal workload at baseline (TEST1) for all subjects. For the ADO group, the initial workload was 30 W, and at each stage it was increased by 20 W for boys and by 15 W for girls. For the YAD group, the initial workload was 50 W, and at each stage it was increased by 25 W for men and by 20 W for women. For the ELD group, the initial workload was 30 W, and at each stage it was increased by 25 W for men and 20 W for women. In the ADO and YAD groups, the workload was increased every 2 min until volitional exhaustion occurred or the subject was unable to continue pedaling over 60 r.p.m. In ELD subjects, the workload was increased every 2 min until 85% of the age-predicted maximal heart rate was achieved, and VO_2max was estimated submaximally to prevent possible complications. Subjects were advised to select a comfortable pedaling cadence between 60 and 90 r.p.m. and to maintain it for the duration of the test. The cycle ergometer was equipped with a microprocessor-controlled eddy current brake; thus, the workload of the ergometer was speed independent. Before the ergometer test, the height of the subjects was measured, and the baseline body composition data were obtained by InBody720 Body Composition Analyzer (Biospace Co., Seoul, Korea).

Experimental design

The study design is presented in Figure 1. After pretesting, each age group was randomly divided into two subgroups. The subjects went through a cross-over study design during which they were randomly assigned to follow either an HV or an HP diet for 7 days in an attempt to increase the production of either alkali or acids in the body, respectively. After 2–4 weeks, subjects were then assigned to the alternate diet. Thus, in both diet groups, the total number of subjects was 88 and subjects acted as their own controls. For the female subjects, the diet periods were scheduled in the same phase of their menstrual cycles.

Subjects began the experimental design by following their normal diet (ND) and by keeping a food diary for 3 days. During the last 12 h of ND period, subjects had a 12-h overnight fast and collected a 12-h urine sample (in the beginning (PRE)). In a laboratory on the fourth morning, fasting blood samples (PRE) from a fingertip capillary and an antecubital vein were drawn. The last meal before PRE samples was consistent with the ND of the subjects. Starting from the PRE sample, the subjects followed either HV or HP and kept food diaries for 7 days. During the last 12 h of the diet period, subjects collected another urine sample (at the end (POST)). On the morning of the eighth day, after a 12-h overnight fast, fasting blood samples (POST) were drawn at the same time as the PRE sample. The last meal before the POST sample was consistent with the diet followed during the 7-day period (either HV or HP). A body composition of the subjects was measured by InBody720 Body Composition Analyzer (Biospace Co.). A light breakfast, which was consistent to the assigned diet, was eaten thereafter. After 45 min of rest, resting blood samples were drawn once more (after breakfast (REST)) before completing a cycle ergometer test (TEST2/3). TEST2/3 started with a 5-min warm-up followed by a 4-min break. Thereafter, subjects completed three 10 min trials at 35, 55 and 75% of the VO_2max obtained during TEST1. The ADO and YAD groups also completed a trial at 100% of VO_2max until volitional exhaustion. Workloads were separated by 4-min rest periods, during which venous blood samples (CT35, CT55, CT75 and CT100, respectively; CT = cycling test) were collected from a fingertip capillary and an antecubital vein. Blood draws from the ADO group were only drawn from a fingertip capillary.

The subjects were allowed to exercise moderately during the diet periods. During the last 24 h before every fasting blood sample, the subjects were instructed to minimize their physical activity and strenuous exercise was not allowed. It was controlled that the instructions concerning physical activity were obeyed by asking the subjects to report their physical activity along with food diaries. Between the two diet periods, subjects were allowed to eat according to their normal dietary habits without keeping any food diaries.

Diets and analysis

The diets used in the present study were designed with the help of PRAL (potential renal acid load), which is a value that can be calculated for any foodstuff according to its nutrient content. HV was designed to enhance the production of alkali in the body, whereas HP was designed to increase the production of acids. However, the general dietary guidelines were taken into account as well. A PRAL value of every foodstuff used during diets was calculated using an equation: PRAL (mEq/100 g) = 0.49 × protein (g/100 g)+0.037 × phosphorous (mg/100 g) − 0.021 × potassium (mg/100 g) − 0.026 × magnesium (mg/100 g) − 0.013 × calcium (mg/100 g).[14] The PRAL values were calculated according to the nutrient contents that were taken from the Finnish Food Composition Database (Fineli, Finnish National Institute of Health and Welfare, Finland). When the PRAL value is below 0, the foodstuff enhances the production of alkali in the body, and when it is above 0, the foodstuff increases the production of acids.

The subjects were given exact instructions on how to follow the diets. Everyday during the diet periods subjects ate similar foods and noted down the amount of foods eaten in grams after weighing each foodstuff. HV was based on the large intake of vegetables and fruits, which were mainly tomatoes, potatoes, cucumber, lettuce, apples, citrus and bananas, whereas the use of grain and dairy products was very limited. The subjects were instructed not to eat red meat, eggs or cheese at all during the 7 days. However, the diet included chicken (2 g/body weight kg per day), rice and bread to ensure the adequate intake of protein (~1.0–1.3 g/kg per day) and carbohydrates. HP was planned to include no vegetables and fruits at all. It mainly consisted of grain products, chicken, red meat and eggs. Vitamin and mineral supplements were not allowed during the study period. It was recommended that, within the given instructions, subjects should eat according to their perceived energy needs during the first diet period. The first food diary was analyzed and the recommendations for the second diet period were determined, so that the energy intake would be similar during both diet periods.

Food diaries were analyzed for energy, protein, carbohydrate, fat, phosphorous, potassium, magnesium and calcium intake by the Nutri-Flow software (Flow-Team Oy, Oulu, Finland). The daily PRAL during HV and HP was calculated as the overall PRAL value per one day according to the actual intake of relevant nutrients.

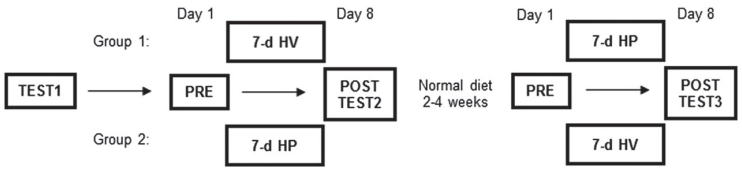


Figure 1. The study design. After the cycle ergometer test (VO_2max) at baseline (TEST1), the subjects were divided into two groups who followed both HV and HP. Blood and 12-h urine samples were collected PRE and POST the diet periods. Cycle ergometer tests (TEST2, TEST3; 3 × 10 min at 35, 55, 75% and finally until exhaustion at 100% of VO_2max) were completed at POST.

Blood sampling and analysis

All venous blood samples were drawn at the same time in the morning during both diet periods. Li-heparinized whole blood samples (200 and 20 µl) from a fingertip capillary were analyzed immediately after sampling for pH, pCO_2 and lactate (Lac^-). The determination of pH was based on the principle of ion-selective electrode; pCO_2 was analyzed by the potentiometric membrane method (GEM Premier 3000; Instrumentation Laboratory, Lexington, MA, USA); and lactate was analyzed by the amperometric and enzymatic method (BIOSEN C_line, Sport; EKF Diagnostic, Magdeburg, Germany). Whole blood samples (4 ml) from the antecubital vein were collected to Venosafe gel tubes and analyzed for sodium (Na^+), potassium (K^+) and chloride (Cl^-) by the direct ISE *in vitro* test (Ion Selective Microlyte Analyzer, Konelab 20 XTi; Kone Instruments, Espoo, Finland). Whole protein content of plasma (P_{tot}) was analyzed spectrophotometrically by the Biuret method (Ion Selective Microlyte Analyzer, Konelab 20 XTi; Kone Instruments).

SID and A_{tot} were calculated as follows: SID (mEq/l) = ($[Na^+]+[K^+]$) − ($[Cl^-]+[Lac^-]$), A_{tot} (mEq/l) = 2.45 × $[P_{tot}]$ (g/dl).[15–17]

Urine sampling and analysis

The subjects collected 12-h urine samples before the PRE and POST blood samples.

Each urine sample was collected in a sterile container and refrigerated until subjects came to the laboratory and brought the container with them. Upon receipt, samples were immediately analyzed for pH by dipping a pH strip into urine (Combur-7 Test urinalysis test strips; Cobas, Roche, Germany).

Statistical analysis

The main purpose of the present study was to determine the effect of diet composition on the primary outcome variable acid–base balance in ADO, YAD and ELD. c-pH, urine pH (u-pH) and independent acid–base variables (pCO_2, SID and A_{tot}) were analyzed to identify the possible differences in acid–base balance between the diet groups. In blood variables, differences between the diet groups were tested with mixed models with random ID. Comparisons were made separately at rest (PRE and POST) and during the cycling test day (POST, REST, CT35, CT55, CT75 and CT100). When the main effect of diet composition, age or time (between PRE to POST) was statistically significant, the comparison was continued with least significant difference pairwise comparisons. The effect of diet composition and time (between PRE to POST) on u-pH was examined by one-way analysis of variance, and if a statistically significant difference was observed, the paired comparison was continued by a paired *t*-test. Parameters of dietary intake data were compared inside each age group with a paired sample *t*-test.

A linear regression analysis was used to examine the contribution of independent acid–base variables (SID, A_{tot} and pCO_2) to c-pH. Multicollinearity of predictors was checked and it was ruled out when variance inflation was < 5 for all explanatory variables.

Statistical analyses were performed with IBM SPSS Statistics 19.0 (SPSS Inc., an IBM Company, Chicago, IL, USA). Data are presented as means ± s.d.'s. Statistical significance was set at $P < 0.05$.

RESULTS

All 88 subjects completed the study design and followed both experimental diets (HV and HP) for 7 days in a randomized order. The baseline anthropometric characteristics of the subjects and the dietary intake data of the ND of the subjects are presented in Table 1. As major gender effects were not detected in the present study, the results of male and female subjects inside each age group (ADO, YAD and ELD) were combined.

Table 1. Baseline anthropometric characteristics and dietary intake data of the subjects

	ADO		*YAD*		*ELD*	
	Boys	*Girls*	*Men*	*Women*	*Men*	*Women*
N	13	9	15	18	17	16
Age (years)	13.4 ± 1.4	13.0 ± 1.2	29.1 ± 2.7	27.6 ± 3.4	67.1 ± 3.7	65.4 ± 3.6
Weight (kg)	52.3 ± 10.9	49.4 ± 7.7	79.5 ± 9.7	58.3 ± 5.0	78.8 ± 10.0	67.6 ± 11.0
Height (cm)	161.0 ± 11.8	158.3 ± 5.8	180.0 ± 5.6	164.6 ± 6.1	183 ± 31	163.6 ± 7.4
Body fat (%)	13.0 ± 7.5	18.5 ± 5.4	17.3 ± 4.6	22.2 ± 5.1	22.4 ± 6.4	34.1 ± 7.9
BMI (kg/m^2)	20.0 ± 2.4	19.6 ± 2.1	24.5 ± 2.6	21.6 ± 2.2	24.6 ± 4.9	25.3 ± 3.9
PRAL (mEq per day)	4.4 ± 16.0	− 5.7 ± 23.3	1.7 ± 17.0	− 12.7 ± 16.7	− 4.4 ± 9.5	− 14.2 ± 11.5
IVF (g per day)	121 ± 67	190 ± 145	390 ± 285	491 ± 207	359 ± 169	460 ± 133
Protein (g/kg per day)	1.84 ± 0.56	1.55 ± 0.45	1.10 ± 0.21	1.48 ± 0.48	1.51 ± 0.45	1.05 ± 0.22
Energy (kcal per day)	2153 ± 720	1781 ± 243	2661 ± 501	1957 ± 353	1878 ± 363	1654 ± 237

Abbreviations: ADO, adolescents; BMI, body mass index; ELD, elderly; IVF, intake of vegetables and fruits; PRAL, potential renal acid load; YAD, young adults. The dietary intake data of the normal diet of the subjects preceding both 7-day HV and 7-day HP. Values are mean ± s.d.

Table 2. Experimental dietary intake data during a 7-day HV and a 7-day HP in all age groups

	ADO		*YAD*		*ELD*	
	HV	*HP*	*HV*	*HP*	*HV*	*HP*
PRAL (mEq per day)	− 47.1 ± 34.8***	22.8 ± 13.5	− 68.1 ± 23.0***	53.3 ± 16.8	− 61.8 ± 14.5***	53.5 ± 13.7
IVF (g per day)	861 ± 538***	35 ± 17	1407 ± 396***	23 ± 10	1310 ± 342***	19 ± 7
Protein (g/kg per day)	1.22 ± 0.33***	1.81 ± 0.28	1.25 ± 0.26***	2.20 ± 0.45	1.06 ± 0.19***	1.92 ± 0.43
CHO (g/kg per day)	4.91 ± 1.30	4.13 ± 1.79	4.06 ± 1.00***	3.15 ± 0.80	3.38 ± 0.86***	2.74 ± 0.81
Fat (g/kg per day)	0.91 ± 0.36***	1.26 ± 0.38	0.72 ± 0.24***	1.02 ± 0.29	0.78 ± 0.22	0.85 ± 0.20
Energy (kcal per day)	1668 ± 527**	1893 ± 543	1841 ± 477***	2023 ± 557	1817 ± 339	1893 ± 383

Abbreviations: ADO, adolescents; CHO, carbohydrates; ELD, elderly; HP, high-protein diet with no vegetables and fruits; HV, normal-protein diet with high amount of vegetables and fruits; IVF, intake of vegetables and fruits; PRAL, potential renal acid load; YAD, young adults. Dietary intake data during a 7-day HV and a 7-day HP in ADO, YAD and ELD. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ indicate statistically significant differences between HV and HP inside each age group (a paired sample *t*-test). Values are mean ± s.d.

Diets

Dietary intake data are presented in Table 2. In all age groups, PRAL and protein intake were significantly lower ($P \leqslant 0.001$) and intake of vegetables and fruits was significantly higher ($P \leqslant 0.001$) in HV compared with HP. HV contained from 861 ± 538 to 1407 ± 396 g vegetables and fruits, which were mainly tomatoes, potatoes, cucumber, lettuce, apples, citrus and bananas. HP included almost no vegetables and fruits (from 19 ± 7 to 35 ± 17 g) and consisted mainly of grain, meat and dairy products.

Effect of diet composition on c-pH

All c-pH data are presented in Figure 2. In YAD, c-pH was significantly higher at POST after HV compared with HP ($P < 0.001$). During cycling, c-pH was also higher after HV compared with HP, with a significant difference ($P < 0.034$) at all three submaximal workloads. In ELD, c-pH was significantly higher ($P < 0.001$) at POST after HV compared with HP. During cycling, c-pH was higher after HV compared with HP and the difference was significant at CT75 ($P = 0.003$). In ADO, diet composition did not cause any significant differences in c-pH at rest or in exercise.

Effect of diet composition on u-pH

All u-pH data are presented in Figure 3. In YAD and ELD, u-pH at POST was significantly higher ($P < 0.001$) after HV compared with HP. Moreover, u-pH increased ($P < 0.001$) during HV and decreased ($P \leqslant 0.003$) during HP in YAD and ELD. In ADO, u-pH was similar during HV and HP.

Effect of age on acid–base balance

Age had no effect on u-pH or c-pH at rest, but age × diet composition effects were significant ($P = 0.003$ and $P = 0.048$, respectively). During exercise, YAD had lower c-pH compared with ELD at CT75 ($P < 0.001$) and compared with ADO at CT100 ($P < 0.001$) after HV. After HP, ADO had higher c-pH at 35% compared with YAD ($P = 0.027$) and ELD ($P = 0.008$). YAD had lower c-pH at CT75 compared with ADO ($P = 0.001$ and ELD ($P < 0.001$) and compared with ADO at CT100 ($P < 0.001$).

At rest, YAD had significantly higher A_{tot} at PRE and POST compared with both ADO ($P < 0.001$) and ELD ($P \leqslant 0.014$) after both diet periods. During exercise, YAD had significantly higher ($P \leqslant 0.025$) A_{tot} at all submaximal workloads compared with ELD after both diet periods. Age had no effect on pCO_2 or SID.

Contributions of independent acid–base variables to c-pH

For all subject groups, the coefficient of determinations (R^2) of pCO_2, SID and A_{tot} for c-pH are presented in Table 3. Also, standardized βs of single variables (pCO_2, SID and A_{tot}) are presented. Take together, pCO_2, SID and A_{tot} explained 9.9–60.2% of the variation in c-pH. In general, the contribution of independent acid–base variables to c-pH was decreased as the exercise intensity increased. The data of each independent acid–base variable are presented in Supplementary Tables 1–3. The diet-induced changes were few.

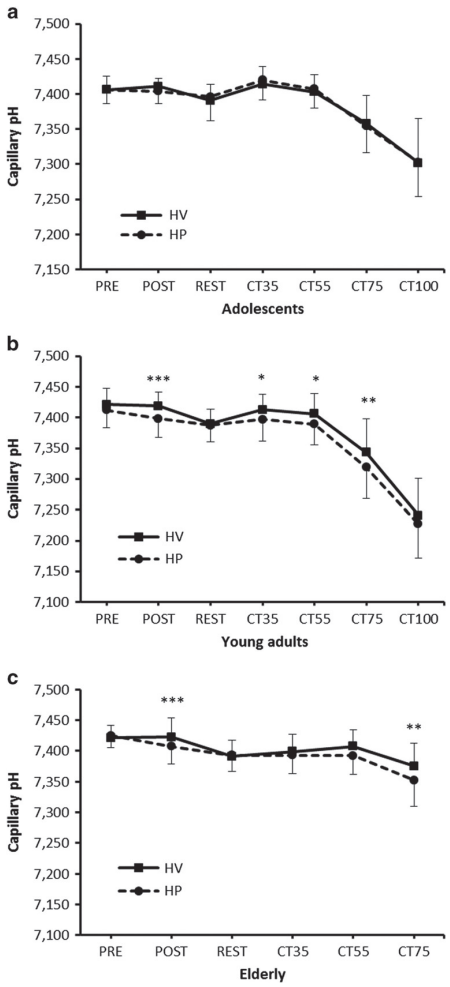


Figure 2. Capillary pH. C-pH in ADO (**a**), YAD (**b**) and ELD (**c**) PRE and POST the 7-day HV and the 7-day HP, REST and during exercise, wherein 10 min at 35, 55 and 75% of VO_2max were cycled (CT35, CT55 and CT75). ADO and YAD cycled additionally at 100% of VO_2max until exhaustion (CT100). $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate statistically significant differences between HV and HP inside each age group (mixed models with random ID, least significant difference (LSD) pairwise comparison).

DISCUSSION

During the present study, ADO, YAD and ELD followed both HV and HP to compare diet-induced differences in acid–base balance. We found that in healthy, recreationally active YAD and ELD subjects, blood pH was higher after HV compared with HP at rest and, especially in YAD, also during high-intensity cycling. In addition, u-pH was significantly higher after a short-term HV compared with a HP. In contrast, diet composition had no effect on acid–base balance in ADO. Moreover, our findings show that the independent acid–base variables (pCO_2, SID and A_{tot}) explained c-pH with a very wide range and their association was less significant as the exercise intensity increased.

The diets used in the present study were designed with the help of PRAL, which represents the renal net acid excretion caused by a foodstuff.[14,18] The nutrients included in the calculation of PRAL are potassium, magnesium and calcium, which decrease the dietary acid load, and protein and phosphorous, which increase the dietary acid load. The most important foods causing the difference in the intake of these nutrients and PRAL between the diets were higher intake of vegetables and fruits

and lower intake of meat, eggs, dairy products and grain products during HV compared with HP. The diets used during the present study gave us the possibility to compare the differences in acid–base balance after highly alkaline and highly acidogenic diets, which HV and HP were, respectively.

After high consumption of vegetables and fruits and lower protein intake, c-pH was significantly higher at rest in YAD and ELD subjects. In YAD, this difference could also be seen during submaximal cycling. The results of this study indicate that even though pH in bodily fluids is tightly regulated and acute changes in blood pH turn on powerful regulatory mechanisms,[13] within the vital limits variations can occur. Excretion of acid in urine is important for the stability of systemic acid–base balance.[7] As a result of this regulation, u-pH increased during the HV period and was higher after HV compared with HP in YAD and ELD. Even though the values of u-pH do not necessarily represent a clinically significant metabolic acidosis or alkalosis,[19] which were not detected in this study, u-pH is an indicator of the diet-induced acid load and renal net acid excretion.[1,18,20,21] Our results strengthen previous findings that, in addition to lower protein intake, vegetables and fruits have an important role in diminishing the acid load of the body[2,22] and they seem to be effective in a short period of time. Albeit the diet-induced changes in systemic pH and acid–base balance are small and subclinical, they may have certain health effects over a longer period of time[23] and be important for physical performance. Our data indicate that the smaller dietary acid load may be in connection to healthier blood lipid profile, higher buffering capacity, improved aerobic performance and reduced exercise-induced inflammation (manuscripts in preparation). As large amounts of acids are produced during metabolism anyway,[10,11] it is not reasonable to increase the dietary acid load by not consuming vegetables and fruits.

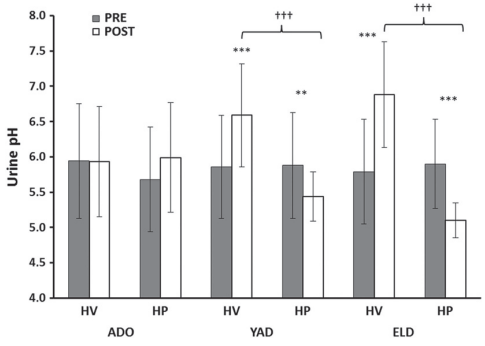


Figure 3. Urine pH. U-pH in ADO, YAD and ELD PRE and POST the 7-day HV and the 7-day HP. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate statistically significant differences between HV and HP inside each age group (one-way analysis of variance (ANOVA), a paired sample *t*-test); $^{\dagger\dagger\dagger}P < 0.001$ indicates statistically significant difference between PRE and POST inside each age group (one-way ANOVA, a paired sample *t*-test).

Diet composition had different effects on c-pH and u-pH between the age groups. In YAD, the changes in u-pH were clear and even greater differences occurred in the group of ELD subjects, whereas in ADO there were no changes at all. It has been reported that in ELD people, the structural and functional changes of the kidneys may decrease the ability to excrete acids, which may result in chronic metabolic acidosis, especially if the diet does not include enough vegetables and fruits.[4,24,25] Even though our results suggest that healthy ELD men and women can still have an acute capacity to change the u-pH in response to dietary changes, it may be that the kidneys of ELD have to function at higher levels

Table 3. Regression analysis of capillary pH in all subject groups

	Regression R^2		pCO_2		*SID*		A_{tot}	
	HV	*HP*	*HV*	*HP*	*HV*	*HP*	*HV*	*HP*
ADO								
PRE	−0.612**	0.385	−0.710††	−0.247	−0.101	−0.593†	−0.197	0.231
POST	0.179	0.201	−0.348	−0.293	−0.340	−0.226	0.333	0.175
YAD								
PRE	0.467**	0.425**	−0.703†††	−0.652††	0.005	0.044	0.196	0.244
POST	0.393**	0.434**	−0.468†	−0.676††	−0.203	0.050	−0.070	0.291
REST	0.317*	0.309*	−0.375	−0.568††	−0.246	0.054	−0.023	0.152
CT35	0.480**	0.292*	−0.505†	−0.310	−0.262	−0.355	−0.025	0.017
CT55	0.305*	0.302*	−0.674††	−0.594††	0.310	0.083	0.002	−0.081
CT75	0.247	0.281*	−0.285	−0.486††	0.429†	0.513†	−0.157	0.413
CT100	0.099	0.122	−0.155	−0.303	−0.042	0.236	−0.289	0.013
ELD								
PRE	0.439**	0.421**	−0.657†††	−0.606†††	0.487††	0.133	−0.014	0.294
POST	0.602***	0.471**	−0.850†††	−0.632†††	0.269	0.172	−0.055	0.270
REST	0.518***	0.346*	−0.739†††	−0.592†††	0.213	0.206	0.045	0.046
CT35	0.586***	0.493**	−0.854†††	−0.701†††	0.222	0.025	−0.029	0.073
CT55	0.402**	0.137	−0.712††	−0.413	0.441†	−0.147	−0.128	−0.123
CT75	0.157	0.121	−0.308	−0.005	0.166	0.228	−0.345	−0.227

Abbreviations: ADO, adolescents; A_{tot}, total concentration of weak acids; ANOVA, analysis of variance; CHO, carbohydrates; ELD, elderly; HP, high-protein diet with no vegetables and fruits; HV, normal-protein diet with high amount of vegetables and fruits; IVF, intake of vegetables and fruits; pCO_2, partial pressure of carbon dioxide; POST, at the end; PRAL, potential renal acid load; PRE, in the beginning; SID, strong ion difference; VO_2max, maximal oxygen consumption; YAD, young adults. Coefficient of determinations (R^2) for capillary pH in ADO PRE and POST the 7-day HV and the 7-day HP. Coefficient of determinations (R^2) for capillary pH in YAD and ELD PRE and POST the 7-day HV and the 7-day HP, and REST and during exercise, wherein 10 min at 35, 55 and 75% of VO_2max and at 100% of VO_2max until exhaustion were cycled (CT35, CT55, CT75 and CT100). Also, β coefficients of each independent acid–base variable (pCO_2, SID and A_{tot}) included in regression analysis are shown. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, statistical significance of linear regression (ANOVA); $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$, statistical significance of β coefficient in regression.

to prevent disadvantageous alterations in the body's acid–base status. In addition, only in the group of ELD there was a difference in the respiratory component of the acid–base balance, as pCO_2 was higher after HV compared with HP. On the contrary, despite the significant and large difference between the PRAL of the diets also in ADO, there were no changes in their u-pH. This is controversial to the results of Remer *et al.*[14] who reported that PRAL of the diet was highly correlated with the net acid excretion in children. However, children may have a lower glycolytic enzyme activity and higher oxidative capacity compared with adults at rest,[26] which could enable the higher utilization of H^+ in energy production and explain why the acid load of the ADO was not as sensitive to changes in diet composition as it was in adults. Nevertheless, the acute data in the current study do not necessarily reflect the effect that diet composition would have on health in ADO over a longer period of time.[27] It may also be that the small group size of the ADO may have masked the possible effects of diet composition or there were errors in dietary self-reporting, which could cause bias in the dietary data of the ADO. However, urinary urea (data not shown) was significantly higher in all age groups after HP compared with HV, which might indicate that there has been a real difference in protein intake also in the group of ADO.[28]

The current study used an approach developed by Stewart[11] that determines the independent acid–base variables affecting the hydrogen ion concentration in the bodily fluids and the role of linked physiological systems in the regulation of plasma acid–base balance.[13] The contribution of independent acid–base variables for c-pH varied widely. In general, the most powerful factor explaining the variation in blood pH was pCO_2, although its impact seemed to decrease while the exercise intensity increased. The lowest coefficients of correlation were observed in general at two highest exercise workloads, suggesting that during high-intensity exercise the importance of some other factors increased and had a larger effect on c-pH beside those that were included in the calculations. For example, increasing amounts of phosphocreatine, inorganic phosphate and ADP could affect SID and A_{tot}.[9]

In conclusion, an HV induces more alkaline systemic acid–base balance and decreases the acid load of the body at rest and during exercise compared with an HP. This can be seen in blood and urine pH of YAD and ELD, even after short periods of time. Furthermore, our results suggest that the ELD may be more sensitive to diet-induced changes in acid–base balance as compared with younger groups in the current study.

CONFLICT OF INTEREST

HP has a commercial association with Honkatarhat Ltd, Kyröntarhat Ltd and Mykora Ltd. He accepts full responsibility for the implementation and publication of this study. He also had full access to all the data. The remaining authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank TEKES (Finnish Funding Agency for Technology and Innovation), Honkatarhat Ltd, Kyröntarhat Ltd, Mykora Ltd, Lihajaloste Korpela Ltd, Laihian Mallas Ltd and KKK-Vihannes Ltd/Lykobene for providing financial support to the study. We also thank Tanja Laine, Johanna Stenholm, Elina Vaara, Hanna Minkkinen, Susanna Karvinen and Riikka Lamminen for all the assistance in establishing this study.

REFERENCES

1 Adeva MM, Souto G. Diet-induced metabolic acidosis. *Clin Nutr* 2011; **30**: 416–421.
2 Frassetto LA, Todd KM, Morris RC Jr, Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *Am J Clin Nutr* 1998; **68**: 576–583.
3 Alexy U, Kersting M, Remer T. Potential renal acid load in the diet of children and adolescents: impact of food groups, age and time trends. *Public Health Nutr* 2005; **11**: 300–306.
4 Dawson-Hughes B, Harris SS, Ceglia L. Alkaline diets favour lean tissue mass in older adults. *Am J Clin Nutr* 2008; **87**: 662–665.
5 Maurer M, Riesen W, Muser J, Hulter HN, Krapf R. Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. *Am J Physiol* 2003; **284**: F32–F40.
6 Souto G, Donapetry C, Calviño J, Adeva MM. Metabolic acidosis-induced insulin resistance and cardiovascular risk. *Metab Syndr Relat Disord* 2011; **9**: 247–253.
7 Frassetto L, Sebastian A. Age and systemic acid-base equilibrium: analysis of published data. *J Gerontol A* 1996; **51**: B91–B99.
8 Welch AA, Macgregor AJ, Skinner J, Spector TD, Moayyeri A, Cassidy A. A higher alkaline dietary load is associated with greater indexes of skeletal muscle mass in women. *Osteoporos Int* 2013; **24**: 1899–1908.
9 Lindinger MI. Origins of $[H^+]$ changes in exercising skeletal muscle. *Can J Appl Phys* 1995; **20**: 357–368.
10 Adrogué HE, Adrogué HJ. Acid–base physiology. *Respir Care* 2001; **46**: 328–341.
11 Stewart PA. How to understand acid–base. A quantitative acid–base primer for biology and medicine. Kellum JA, Elbers PWG (eds). *Stewart's Textbook of Acid–Base*. AcidBase.org/Paul WG Elbers: Amsterdam, The Netherlands, 2009.
12 Weinstein Y, Magazanik A, Grodjinovsky A, Inbar O, Dlin RA, Stewart PA. Reexamination of Stewart's quantitative analysis of acid-base status. *Med Sci Sports Exerc* 1991; **23**: 1270–1275.
13 Kellum JA. Determinants of blood pH in health and disease. *Crit Care* 2000; **4**: 6–14.
14 Remer T, Dimitriou T, Manz F. Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. *Am J Clin Nutr* 2003; **77**: 1255–1260.
15 Putman CT, Jones NL, Heigenhauser GJF. Effects of short-term training on plasma acid–base balance during incremental exercise in man. *J Phys* 2003; **550**: 585–603.
16 Lindinger MI, Franklin TW, Lands LC, Pedersen PK, Welsh DG, Heigenhauser GJ. Role of skeletal muscle in plasma ion and acid-base regulation after $NaHCO_3$ and $KHCO_3$ loading in humans. *Am J Physiol* 1999; **276**: R32–R43.
17 Miller BF, Lindinger MI, Fattor JA, Jacobs KA, Leblanc PJ, Duong M *et al.* Hematological and acid-base changes in men during prolonged exercise with and without sodium-lactate infusion. *J Appl Physiol* 2005; **98**: 856–865.
18 Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. *J Am Diet Assoc* 1995; **95**: 791–797.
19 Wagner EA, Falciglia GA, Amlal H, Levin L, Soleimani M. Short-term exposure to a high-protein diet differentially affects glomerular filtration rate but not acid-base balance in older compared to younger adults. *J Am Diet Assoc* 2007; **107**: 1404–1408.
20 Remer T, Manz F. Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. *Am J Clin Nutr* 1994; **59**: 1356–1361.
21 Welch AA, Mulligan A, Bingham SA, Khaw KT. Urine pH is an indicator of dietary acid–base load, fruit and vegetables and meat intakes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study. *Br J Nutr* 2008; **99**: 1335–1343.
22 Remer T. Influence of nutrition on acid-base balance – metabolic aspects. *Eur J Nutr* 2001; **40**: 214–220.
23 Krupp D, Shi L, Remer T. Longitudinal relationships between diet-dependent renal acid load and blood pressure development in healthy children. *Kidney Int* 2014; 204–210.
24 Berkemeyer S, Vormann J, Günther ALB, Rylander R, Frassetto LA, Remer T. Renal net acid excretion capacity is comparable in prepubescence, adolescence, and young adulthood but falls with aging. *J Am Geriatr Soc* 2008; **56**: 1442–1448.
25 Tareen N, Zadshir A, Martins D, Nagami G, Levine B, Norris KC. Alterations in acid–base homeostasis with aging. *J Natl Med Assoc* 2004; **96**: 921–925.
26 Ratel S, Duché P, Williams CA. Muscle fatigue during high-intensity exercise in children. *Sports Med* 2006; **36**: 1031–1065.
27 Krupp D, Johner SA, Kalhoff H, Buyken AE, Remer T. Long-term dietary potential renal acid load during adolescence is prospectively associated with indices of nonalcoholic fatty liver disease in young women. *J Nutr* 2012; **142**: 313–319.
28 Frank H, Graf J, Amann-Gassner U, Bratke R, Daniel H, Heemann U *et al.* Effect of short-term high-protein compared with normal-protein diets on renal hemodynamics and associated variables in healthy young men. *Am J Clin Nutr* 2009; **90**: 1509–1516.

Supplementary Information accompanies this paper on European Journal of Clinical Nutrition website (http://www.nature.com/ejcn)

III

DIETARY ACID LOAD AND RENAL FUNCTION HAVE VARYING EFFECTS ON BLOOD ACID-BASE STATUS AND EXERCISE PERFORMANCE ACROSS AGE AND SEX

by

Hietavala E-M, Stout JR, Frassetto LA, Puurtinen R, Pitkänen H, Selänne H, Suominen H & Mero AA. 2017.

Applied Physiology, Nutrition and Metabolism 42(12), 1330-1340

Dietary acid load and renal function have varying effects on blood acid-base status and exercise performance across age and gender

Enni-Maria Hietavala[1], Jeffrey R. Stout[2], Lynda A. Frassetto[3], Risto Puurtinen[1], Hannu Pitkänen[4], Harri Selänne[5], Harri Suominen[6], Antti A. Mero[1]

Corresponding author:

Enni-Maria Hietavala

E-mail address: enni.hietavala@jyu.fi

Mailing address: P.O. Box 35, 40014 University of Jyväskylä, Finland

[1]Biology of Physical Activity, Faculty of Sport and Health Sciences, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland (risto.j.puurtinen@jyu.fi, antti.a.mero@jyu.fi)

[2]Institute of Exercise Physiology and Wellness, University of Central Florida, P.O. Box 161250, FL 32816-1250, Orlando, Florida, USA (jeffrey.stout@ucf.edu)

[3]General Clinical Research Center, University of California San Francisco, 505 Parnassus Avenue, San Francisco CA 94117, California, USA (lynda.frassetto@ucsf.edu)

[4]Honka Holding, c/o Honkatarhat Oy, Kirkkokallio 20, 38950 Honkajoki, Finland (hannu.pitkanen@mykora.com)

[5]Department of Psychology, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland (harri.p.selanne@jyu.fi)

[6]Health Sciences, Faculty of Sport and Health Sciences, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland (harri.k.suominen@jyu.fi)

Abstract

Purpose: Diet composition influences acid-base status of the body. This may become more relevant as renal functional capacity declines with aging. We examined the effects of low (LD) versus high dietary acid load (HD) on blood acid-base status and exercise performance.

Methods: Participants included 22 adolescents (AD), 33 young adults (YA) and 33 elderly (EL), who followed a 7-day LD and HD in a randomized order. At the end of both diet periods the subjects performed a cycle ergometer test (3x10 min at 35%, 55%, 75%, and (except EL) until exhaustion at 100% of VO_2max). At the beginning of, and after the diet periods, blood samples were collected at rest and after all workloads. VO_2, RER and HR were monitored during cycling.

Results: In YA and EL, bicarbonate (HCO_3^-) and base excess (BE) decreased over the HD period, and HCO_3^-, BE and pH were lower at rest after HD compared to LD. In YA and EL women, HCO_3^- and BE were lower at submaximal workloads after HD compared to LD. In YA women, the maximal workload was 19 % shorter and maximal VO_2, RER and HR lower after HD compared to LD.

Conclusions: Our data uniquely suggests that better renal function is associated with higher availability of bases, which may diminish exercise-induced acidosis and improve maximal aerobic performance. Differences in glomerular filtration rate between the subject groups likely explains the larger effects of dietary acid load in the elderly compared to younger subjects and in women compared to men.

Key words: Potential renal acid load, dietary acid load, acid-base status, glomerular filtration rate, aging, alkalinity, aerobic exercise

Introduction

Diet composition is known to influence net endogenous acid production, which may further affect acid-base status of the body (Poupin et al. 2012). Dietary acid load can be estimated by calculating the potential renal acid load (PRAL) of foods, which represents the renal net acid excretion caused by a foodstuff (Remer et al. 2003). The digestion of meat, grain and some dairy products increases the acid load of the body. Fruits and vegetables, which contain organic anions (e.g. citrate, malate) metabolizable to bicarbonate, lower net acid load. Western diet typically contains large amounts of animal protein and grain products but only small amounts of vegetables and fruits, and leads to a net production of acids in the body (Adeva and Souto 2011). For elderly populations, the dietary acid load may be a particularly important issue as aging is associated with a decline in the renal functional capacity, which diminishes the accuracy and speed of the regulation of volume and composition of the body fluids (Bolignano et al. 2014). Glomerular filtration rate (GFR) decreases with aging and consequently, blood HCO_3^- concentration and pH are regulated at lower levels (Frassetto and Sebastian 1996). Increased acidity of the body has been reported to associate negatively with kidney function, bone health and muscle mass. For example, in the study of Scialla et al. (2012), higher net endogenous acid production was significantly associated with a faster decline in GFR. Tabatabai et al. (2015) found out that lower plasma HCO_3^- was associated with higher rate of bone loss in 70-year-old participants. In addition, Dawson-Hughes et al. (2008) came to the conclusion that higher intake of fruits and vegetables, which contain base-producing compounds and decrease net endogenous

acid production, favored the preservation of muscle mass in men and women over 65 years of age.

In addition to the health issues, dietary acid load may play a role in exercise. Over the decades, many studies have reported the positive effects of sodium bicarbonate supplementation and some other ergogenic aids on blood buffering capacity and exercise performance (Krustrup et al. 2015, Wilkes et al. 1983). Surprisingly, there are only a few studies that have investigated the impact of diet composition on acid-base status and exercise performance (e.g. Baguet et al. 2011, Caciano et al. 2015, Greenhaff et al. 1987; for review see Applegate et al. 2017). Moreover, to the best of our knowledge, the effects of dietary acid load on acid-base status of the elderly have not been reported during exercise. Increasing hydrogen ion (H^+) concentrations in blood and muscle during high-intensity exercise cause acidosis, which is thought to be one of the causes of fatigue (Lancha Junior et al. 2015, Robergs et al. 2004). A diet high in vegetables and fruits could have the potential to affect blood bicarbonate buffering capacity and physical performance by attenuating the exercise-induced acidosis. For example, sodium bicarbonate supplementation has been reported to improve performance both in short (~1-2 min) (Mero et al. 2004, Van Montfoort et al. 2004) and longer (~20 min) exercise performances (Oöpik et al. 2003). The main purpose of this paper was to find out if low dietary acid load (LD) and high dietary acid load (HD) have effects on blood acid-base status and exercise performance during submaximal and maximal aerobic cycling in adolescents, young adults, and the elderly. We hypothesized that a diet with high acid load increases acidity of the blood and age-related decline in renal function affects the responses in acid-base parameters both at rest and in exercise.

Materials and methods

Participants

In total, 93 men and women were selected to participate in the present study. Subjects were recruited from three age groups: 12-15-year-old adolescents (AD), 25-35-year-old young adults (YA) and 60-75-year-old elderly (EL). To recruit the AD group, we visited the local sports clubs who were participating in ice hockey, figure skating, gymnastics and athletics. The YA and EL groups were recruited via e-mail lists. The subjects of YA group were mainly students in the local University and the community-dwelling EL subjects were recruited from the Ageing Program of the local University. All subjects of YA and EL were recreationally active (e.g. walking, jogging, cycling, resistance training 2-4 times per week) but they were not training for competitive purposes. In the beginning of the study, there were 14 boys and 10 girls in the AD group, 16 men and 19 women in the YA group and 17 men and 17 women in the EL group. Altogether five subjects were not able to finish the study. Baseline anthropometric characteristics of the subjects who completed the whole data collection are presented in Table 1. The subjects did not use any medication during the study period aside from two exceptions: women of YA were allowed to use contraceptive pills and in the EL groups medications for high blood pressure and high cholesterol were acceptable. Subjects whose body mass index was above 33 kg/m^2 or who had other medications were excluded from the study. The experimental diets were planned beforehand and volunteers who had any relevant food allergy were not able to participate. Ethical approval for the study was obtained from the Ethical Committee of the local University and the study was in accordance with the Helsinki Declaration. Before any data

collection the subjects were informed of the purpose and the methods of the study and they signed a written informed consent. Additionally, the subjects completed a health questionnaire and the elderly subjects also completed a health examination that was performed by a physician.

Baseline testing

To determine the subjects' VO_2max and maximal workload at baseline (Test 1) an incremental cycle ergometer test was cycled with a microprocessor controlled, eddy current brake equipped ergometer (Ergoline ergometrics 800, D-72475, Bitz, Germany). For the AD group, the initial workload was 30 W and at each stage it was increased by 20 W for boys and by 15 W for girls. For YA, the initial workload was 50 W and at each stage it was increased by 25 W for men and by 20 W for women. For EL, the initial workload was 30 W and at each stage it was increased by 25 W for men and 20 W for women. The subjects were advised to select a comfortable pedaling cadence between 60 and 90 rpm and to maintain it for the duration of the test. In the AD and YA groups, the workload was increased every 2 min until volitional exhaustion occurred. VO_2max was determined to be the highest 30-s VO_2 value during the test and coincided with at least two of the following three criteria: a) 90% of age-predicted maximum heart rate; b) respiratory exchange ratio > 1.1; and/or (c) a plateau of oxygen uptake (less than 150 ml/min increase in VO_2 during the last 60 s of the test). In EL subjects, the workload was increased every 2 min until 85 % of the age-predicted HR_{max} was achieved and VO_2max was estimated submaximally with Aino FitWare Pro - physical performance testing software (Aino Health Management, Finland). VO_2max determined at the baseline was used in all subject groups to set the workloads for cycling tests performed during the experimental design.

Experimental design

After the baseline testing, each age and gender group was randomly divided into two subgroups for the experimental design. The subjects went through a cross-over study design during which they were randomly assigned to follow either a diet with low acid load (LD) or high acid load (HD) for 7 days. Two to four weeks after finishing the first diet period, the subjects were assigned to the alternate diet. Thus, in both diet groups the total number of subjects was 88 and the subjects acted as their own controls. For the female subjects the diet periods were scheduled in the same phase of their menstrual cycles.

Before both experimental diet periods the subjects followed their normal diet (ND) and kept a food diary for 3 days. During the last 12 hours of ND period, subjects had a 12-hour overnight fast and on the 4^{th} morning, fasting blood samples (PRE) were drawn from a fingertip capillary and an antecubital vein in a laboratory. The last meal before PRE samples was consistent with the normal diet of the subjects. Starting from the PRE sample, the subjects followed either LD or HD and kept food diaries for 7 days. On the 8^{th} morning, after a 12-hour overnight fast, fasting blood samples (POST) were drawn at the same time as the PRE sample. The last meal before the POST sample was consistent with the diet followed during the 7-day period (either LD or HD). A light breakfast, which was consistent with the assigned diet, was eaten thereafter. After 45 min of rest, resting blood samples were drawn once more (REST) before completing a cycle ergometer test (Test 2 and 3). Test 2 and 3 started with a 5-min warm-up followed by a 4-min break. Thereafter, the subjects completed three 10 min trials at 35, 55 and 75 % of the VO_2max.

The AD and YA groups also completed a trial until volitional exhaustion at a workload equivalent to 100 % of VO_2max. The maximal workload was continued until volitional exhaustion occurred or a subject was unable to continue pedaling over 60 rpm. The workloads for Test 2 and 3 were determined individually by calculating each subject's VO_2 at 35, 55, 75 and 100 % of VO_2max obtained during Test 1 and choosing the workload corresponding to each VO_2. The workloads were identical in Test 2 and 3. All workloads were separated by 4-min rest periods, during which blood samples (CT35, CT55, CT75, CT100, respectively; CT= cycling test) were collected from a fingertip capillary.

The subjects were allowed to exercise moderately during the diet periods. During the last 24 hours before every fasting blood sample the subjects were instructed to minimize their physical activity. The subjects were asked to report their physical activity along with the food diaries.

Diet periods and analysis

The diets used in the present study were designed with the help of PRAL calculations to have low and high acid loads. The PRAL values were calculated as follows: PRAL (mEq/100 g) = 0.49 x protein (g/100 g) + 0.037 x phosphorous (mg/100 g) - 0.021 x potassium (mg/100 g) - 0.026 x magnesium (mg/100 g) - 0.013 x calcium (mg/100 g) (Remer et al. 2003). The nutrient contents of the foodstuffs were taken from the Finnish Food Composition Database (Fineli, Finnish National Institute of Health and Welfare).

The subjects were given exact instructions how to follow the diets. Specific 1-day menus were designed for both diet periods, and every day during the diet periods subjects repeated the meals and snacks according to the menus. The subjects noted down the amount of foods eaten in grams after weighing each foodstuff. LD was based on a large intake of vegetables and fruits and a limited use of grain and dairy products. The subjects were instructed not to eat red meat, eggs or cheese during the 7 days. However, the diet included chicken (2 g/kg/d) to ensure the adequate intake of protein. HD was planned to include no vegetables and fruits at all. It mainly consisted of grain products, chicken, red meat and eggs. Vitamin and mineral supplements were not allowed during the study periods. Within the given instructions, the subjects were advised to eat according to their perceived energy needs during the first diet period. Thus, they were allowed to adjust the amounts of foods in the menus according to their appetite without leaving anything out or adding anything. The dietary instructions for the second diet period were adjusted so that the diet periods were isoenergetic. Between the two diet periods, the subjects were advised to eat according to their habitual diet. During both experimental diet periods, the subjects were compensated with some chicken, grain products, tomato, cucumber, lettuce and mushrooms, which were received from the companies who partially funded this study. The breakfasts before the cycling tests were standard and isoenergetic in all groups with subtle adjustments in the amount of the foods. During LD, the breakfast contained approximately 370 kcal of energy, 63 g of carbohydrates, 16 g of protein, 7.2 g of fat and its PRAL was -12.4 mEq. During HD, the breakfast contained 370 kcal of energy, 33 g of carbohydrates, 21 g of protein, 16 g of fat and its PRAL was 7.4 mEq.

The food diaries were analyzed for energy, protein, carbohydrate, fat, phosphorous, potassium, magnesium and calcium intake using Nutri-Flow software (Flow-Team Oy, Oulu, Finland). The average daily PRAL during LD and HD were calculated according to the relevant dietary intake data.

Blood sampling and analysis

All capillary and antecubital vein blood samples were drawn at the same time in the morning during both diet periods. Li-heparinized whole blood samples (200 and 20 µl) from a fingertip capillary were analyzed immediately after sampling for pH, HCO_3^-, standard BE and lactate. The determination of pH was based on the principle of ion selective electrode whereas HCO_3^- and BE were determined computationally from pH and pCO_2 values (GEM Premier 3000, Instrumentation Laboratory, Lexington, MA, USA). Lactate was analyzed by the amperometric and enzymatic method (BIOSEN C_line, Sport, EKF Diagnostic, Magdeburg, Germany). The blood samples from antecubital vein were drawn in vacuum tubes and centrifuged for 10 min at 3500 rpm. The serum was separated and creatinine was analyzed by KoneLab 20 XTi analyzator (Thermo Electron Corporation, Vantaa, Finland). Serum creatinine values were used to calculate the glomerular filtration rate (GFR) with the CKD-EPI equation, which uses also age, race, gender and body size to estimate GFR (Levey et al. 2009).

Breath gas analysis

In all three cycle ergometer tests, the gaseous exchange was measured using Jaeger Oxycon Pro breath-by-breath gas analyzer (VIASYS Healthcare GmbH, Hoechburg, Germany). The device was calibrated for volume and gas analyzer before every measurement. Cardiorespiratory variables (VO_2, RER) were determined as a mean from the final 30 seconds of every workload.

Statistical analysis

Differences in blood variables between the diet groups were tested with mixed models with random ID. Comparisons were made separately at rest (PRE and POST) and during the cycling test day (POST, REST, CT35, CT55, CT75 and CT100). When the main effect of diet composition, age, gender or time (between PRE and POST) was statistically significant the comparison was continued with LSD pairwise comparisons. The effect of dietary acid load on cardiorespiratory variables and GFR was examined by two-way repeated measures analysis of variance (ANOVA), and if a statistically significant difference was observed, the comparison was continued by a paired t-test. The variables of dietary intake analysis and the difference in the duration of maximal workload were compared inside each age and gender group with paired samples t-test. The correlations of GFR with acid-base parameters were analyzed by Pearson correlation analysis. Statistical analyses were performed with IBM SPSS Statistics 22.0 (SPSS, Inc., an IBM Company). Data are presented as means ± SDs. The statistical difference was significant at the $P<0.05$ level.

Results

Altogether 88 subjects of 93 completed the study design and followed both LD and HD for 7 days in a randomized order. One subject of every age and gender group except EL men dropped out before the second diet period. Those subjects were excluded from the statistical analysis.

Dietary intake

The PRAL and macronutrient contents were not different between the ND periods that preceded both LD and HD in any of the subject groups. The PRAL values presented in Table 1 are means from these two 3-day normal diet periods.

Experimental dietary intake data are presented in Table 2. PRAL was significantly lower ($P<0.001$ in all groups) and intake of vegetables and fruits was significantly higher ($P\leq0.001$) in LD compared to HD in all groups. In AD boys and EL women the energy intake did not differ between the diets, but in other groups it was significantly lower during LD compared to HD ($P\leq0.022$). The protein intake (g/kg/d) was significantly lower ($P<0.001$) during LD compared to HD in all groups except in AD girls.

Glomerular filtration rate

The GFR data are presented in Figure 1. In young men, GFR decreased over the LD period ($P=0.009$) and in the elderly men and women it was higher after HD compared to LD ($P<0.001$ and $P=0.047$, respectively). Age had a significant ($P<0.001$) effect on GFR. GFR was significantly lower ($P<0.001$) in young adults compared to the adolescents, in the elderly

compared to young adults and in the elderly compared to adolescents throughout the study period. Gender also had a significant ($P<0.001$) effect on GFR. GFR was higher ($P\leq0.009$) in boys compared to girls at all points. GFR was also higher ($P\leq0.030$) in YA men compared to YA women at all points except at POST after LD. In the elderly, the gender differences in GFR were smaller and it was significantly higher ($P=0.029$) in men compared to women only at PRE during HD.

In all male subjects together, GFR correlated negatively with HCO_3^- ($r=-0.42$, $P=0.007$) and BE ($r=-0.43$, $P=0.006$) at rest at the beginning of the HD period. After HD, GFR correlated with HCO_3^- ($r=0.57$, $P=0.003$) and BE ($r=0.60$, $P=0.001$) at CT100. Moreover, GFR correlated with BE at CT35 and CT55 ($r=0.32$, $P=0.039$; $r=0.31$, $P=0.045$, respectively). In all female subjects together, GFR correlated with HCO_3^- and BE at CT100 after both diet periods (LD: $r=0.57$, $P=0.003$; $r=0.57$, $P=0.004$, respectively. HD: $r=0.53$, $P=0.013$; $r=0.58$, $P=0.006$, respectively).

Blood acid-base status and lactate

For HCO_3^- and BE, the time x age effects were significant ($P<0.001$) at rest and in exercise. During exercise, diet x age and time x gender effects were significant for HCO_3^- ($P=0.026$ and $P=0.005$, respectively) and BE ($P=0.028$ and $P=0.002$, respectively). For pH, the time x age ($P<0.001$) and time x gender ($P=0.002$) effects were significant during exercise. In general, the changes in HCO_3^- and BE between the diet periods were larger in the elderly compared to younger subject groups at rest and during exercise. During exercise, the decrease in acid-base parameters within each of the diet periods was larger in young adults compared to adolescents

and the elderly and in men compared to women. In addition, in lactate, time x age and time x gender effects were significant ($P<0.001$) during exercise.

Elderly women. Capillary pH, HCO_3^- and BE decreased ($P<0.001$) over the HD period and were higher ($P<0.001$) at POST after LD compared to HD (Figure 2, Figure 3, Figure 4, respectively). BE increased ($P=0.048$) during LD. Lactate was higher before the cycling test at REST after LD compared to HD (3.0 ± 0.5 vs. 2.0 ± 0.5 mmol/l, $P=0.026$) (Figure 5). After LD compared to HD, both HCO_3^- ($P\leq0.020$) and BE ($P\leq0.011$) sustained at significantly higher level throughout the cycling test. Moreover, pH was higher also at CT75 ($P=0.002$) after LD compared to HD.

Elderly men. HCO_3^- and BE decreased significantly ($P<0.001$) over the HD period. HCO_3^- increased ($P=0.039$) during the LD diet. After LD compared to HD, pH ($P=0.021$) HCO_3^- ($P<0.001$) and BE ($P<0.001$) were higher at POST. BE was higher also at CT75 ($P=0.021$) after LD compared to HD. There were no differences in lactate concentrations between the diet periods at rest or during exercise.

Young women. Capillary pH ($P=0.019$), HCO_3^- ($P=0.001$) and BE ($P<0.001$) decreased over the HD period. During LD compared to HD, BE ($P=0.039$) was higher at PRE and pH, HCO_3^- and BE were higher at POST ($P<0.001$). Moreover, HCO_3^- ($P\leq0.022$) and BE ($P\leq0.015$) were higher at all submaximal stages and pH at CT75 ($P=0.005$). Also, lactate was higher at CT100 after LD compared to HD (10.0 ± 1.8 vs. 8.6 ± 1.2 mmol/l, $P=0.002$).

Young men. Capillary pH (P=0.039), HCO_3^- (P=0.027) and BE (P=0.012) decreased over the HD period. After LD compared to HD, pH (P=0.038), HCO_3^- (P=0.042) and BE (P=0.011) were higher at POST. Moreover, pH was higher at CT100 (P=0.034). There were no significant differences in lactate between the diet periods.

Adolescents. In the group of AD the only diet-induced change in the blood variables was the significant increase of HCO_3^- (P=0.005) and BE (P=0.003) during the LD diet period in girls.

Exercise performance and cardiorespiratory responses

The elderly. Four older women were not able to finish the CT75 after the HD period. One older man was not able to finish the CT75 after either LD or HD. The duration of CT75 was shorter after HD compared to LD both in men (9.66 ± 1.39 vs. 9.81 ± 0.79 min) and women (9.23 ± 2.07 vs. 10.0 ± 0 min) but the differences were not significant. In men, RER was lower (P≤0.009) at all submaximal workloads after HD compared to LD (Table 3). In women, there were no differences in cardiorespiratory responses between the diet periods.

Young adults. In women the duration of the maximal workload was shorter after HD compared to LD (3.12 ± 1.01 vs. 3.84 ± 1.28 min, P=0.001). VO_2 (P=0.006), RER (P=0.029) and HR (P=0.004) were lower at CT100 after HD compared to LD. RER (P=0.023) and HR (P=0.026) were lower also at CT35 after HD compared to LD. In men there were no differences in the duration of maximal workload (3.79 ± 1.48 vs. 3.67 ± 1.20 min, respectively) or in cardiorespiratory responses between HD and LD.

Adolescents. There were no significant differences in exercise performance or cardiorespiratory measures between the diet groups of adolescents. In boys, the time until exhaustion at CT100 was 3.21 ± 1.74 min after LD and 3.34 ± 1.58 min after HD. In girls, the durations were 3.20 ± 0.78 min and 3.17 ± 0.88 min, respectively.

There was no period effect in cardiorespiratory measures between the experimental diet periods. Highest VO_2 obtained during the cycling test and the duration of the last workload were not different between the first and second experimental diet period regardless of the sequence of the diets followed.

Discussion

During the present study adolescents, young adults and the elderly followed one diet with low (LD) and one with high acid load (HD) for seven days to examine the effects associated with diet composition on blood acid-base status and exercise performance. To the best of our knowledge, our study is the first to suggest that better renal function may be associated with higher base availability, which can diminish exercise-induced acidosis. This could be particularly important for the elderly who have diminished renal function. Our results show that in healthy, recreationally active men and women, pH, HCO_3^- and BE were lower after a 7-day HD compared to a 7-day LD, which is an indication of more acidic blood acid-base status. Particularly in young and elderly women, the blood was more acidic also during submaximal cycling. These changes affected the performance in young women, as their maximal

cardiorespiratory measures were lower and the time of exhaustion shorter after HD compared to LD. Our data is in accordance with the literature as we found a clear decrease in GFR with aging. This likely explains why the diet-induced changes in blood acid-base status were greater in elderly subjects compared to younger groups. Moreover, women had lower GFR compared to men and consequently were more sensitive to changes in dietary acid load.

It has been suggested that the acidity of the body increases with aging as the functional capacity of the kidneys declines (Frassetto and Sebastian 1996, Goraya et al. 2012). Our cross-sectional data does not allow us making a conclusion how acid-base status of a certain individual has changed from adulthood to elderly, but in our data, despite the clear decline in GFR with aging, the acidity of the blood was not higher in the elderly compared to younger subjects. This might be affected by the fact that the elderly subjects had lower dietary acid intake during their habitual diets compared to younger subjects. Moreover, Wesson and Simoni (2009) comparing rats with and without subtotal nephrectomy, demonstrated no differences in blood acid-base levels between the two groups. However, the rats with lower kidney mass were not able to excrete the same acid load in the urine, while at the same time demonstrated higher renal tissue acid levels. We suggest a similar effect might be occurring in the relatively reduced functional renal tissue in the elderly subjects. According to the analyzed daily PRAL, the experimental diets used in the present study had low and high dietary acid loads. A high intake of vegetables and fruits and a low intake of meat, grains, eggs and cheese during LD and the opposite dietary pattern during HD caused the difference in dietary acid loads. Diets that have low acid load and promote alkalinity of the body may be beneficial for the health of elderly populations, who have decreased renal functional capacity to excrete acids. Reducing dietary acid load with increased

intake of vegetables and fruits or bicarbonate supplementation has been reported to decrease the risk factors for chronic kidney disease (Banerjee et al. 2014, de Brito-Ashurst et al. 2009). These diets may also help in the preservation of muscle mass (Dawson-Hughes et al. 2008, Welch et al. 2013), and lower serum bicarbonate has been reported to associate with reduced muscle strength, and greater risk of incident and functional limitations (Abramowitz et al. 2011, Yencheck et al. 2014). It is important to acknowledge the significance of adequate protein intake for health (Westerterp-Plantenga et al. 2012) and in maintaining or improving skeletal muscle size (Houston et al. 2008, Hulmi et al. 2009). Our results show that to reduce the body's acid load the dietary protein intake does not have to be low; instead, it can be sustained at a moderately higher level than the current recommended daily allowance (0.8 g/kg/d) if the intake of fruits and vegetables is high enough.

Similar to the elderly subjects, in young men and women, pH, HCO_3^- and BE decreased significantly over the HD period. In contrast, the only significant change in acid-base status of adolescents occurred in girls, as HCO_3^- and BE increased during the period of LD. The difference in the acid loads between the experimental diet periods was smaller in adolescents compared to the older subjects, which may have impacted the acid-base responses in adolescents. However, adolescents' acid-base status may not be as sensitive to altered diet composition as is the acid-base status of adults and the elderly, since their renal functional capacity is also higher compared with older subjects. Moreover, adolescents have higher oxidative capacity compared with adults at rest (Ratel et al. 2006), which could enable a more efficient utilization of H^+ in tricarboxylic acid cycle. Growing adolescents also have higher needs for protein to ensure the growing and maturation of the tissues and the efficacy to use nitrogen is increased (Giovannini et

al. 2000). Thus, at least the acute potential of proteins to increase the acid load of the body might be lower in adolescents.

To the best of our knowledge, the effects of dietary acid load have not been reported during exercise in the elderly. In elderly women, HCO_3^- and pH were significantly lower during submaximal cycling after HD compared to LD. In addition, pH was significantly lower at 75 % of VO_2max after HD compared to LD in elderly men. Dietary acid load could be an important issue for the health of the elderly also in relation to exercise, as exercise-induced increase in acidosis was smaller after lower dietary acid load. This would diminish the need of aging kidneys to secrete acids. It could also be hypothesized that the more alkaline body prior to exercise would favor greater lipid oxidation (Sahlin and Harris 2008). That did not get support from our results as respiratory exchange ratio (RER; CO_2 production/O_2 uptake) of elderly men was significantly higher at all submaximal workloads after LD compared to HD. However, the higher carbohydrate intake during LD compared to HD has likely affected the RER values. Yet there were no differences in elderly women's cardiorespiratory measures between the diet periods.

In YA women, the time to exhaustion at a workload equivalent to 100 % of VO_2max was 19 % shorter and their maximal VO_2, RER and HR were lower after HD compared to LD. The maximal aerobic capacity of young men was not different between the diets. Interestingly, young women in this study depleted blood HCO_3^- during the maximal workload to the same level after both diet periods even though it was significantly higher at submaximal workloads after LD compared to HD. In addition, BE and pH decreased to the same level after both diets. These

results are in accordance with the results of Greenhaff et al. (1987), who reported that pH, HCO_3^- and BE were higher at rest but decreased to the same or even lower level while the cycling time to exhaustion at 100 % of VO_2max was longer after a low compared to a high dietary acid intake. Our results support the idea that consuming an LD diet produces a more alkaline environment within the body and results in a greater blood buffering capacity. This may play an important role in delaying the fatigue during submaximal and maximal exercise intensities particularly in women, who have lower renal functional capacity compared to men. Moreover, young men and women had significantly lower pH, HCO_3^- and BE compared to both elderly men and women and boys and girls at two highest exercise intensities after adjusting for VO_2max. This and the positive correlations between GFR and acid-base parameters suggest that the renal functional capacity may affect the level of acidity that is possible to achieve during high-intensity exercise in young and elderly adults. The performance or exercise metabolism of adolescents, however, was not different between the diet groups. It is important to recognize that not only ergogenic aids but also a habitual diet may have a constant effect on bicarbonate concentrations of the body. According to this study, particularly women should pay attention to adequate consumption of vegetables and fruits to maximize their performance during high-intensity exercise.

Young women had higher maximal blood lactate after LD compared to HD. This is in accordance with a hypothesis that increased pre-exercise alkalosis might enhance high-intensity performance by contributing to enhanced glycolytic ATP production (Stephens et al. 2002). For example, in the study of Hollidge-Horvat et al. (2000), sodium bicarbonate -induced alkalosis increased muscle glycogen use, lactate accumulation and production, and muscle H^+ concentration. Similarly, in the study of Oöpik et al. (2003), subjects had 21 % higher maximal

blood lactate and completed a 5-km time trial 2.6 % faster after a citrate supplementation compared to placebo. Moreover, RER of the young women was at higher level during cycling after LD compared to HD with a significant difference at 35 % and 100 % of VO_2max indicating that carbohydrate oxidation was increased after low dietary acid load. In contrast, in a study of Caciano et al. (2015), submaximal and maximal RER were lower after a 7-day low-PRAL diet and there were only trends towards a longer time to exhaustion and greater maximal VO_2 during a graded treadmill exercise test. The blood acid-base parameters were not measured during that study. In our study, the difference in carbohydrate intakes between the diet groups is one of the limitations of this study, and lower carbohydrate intake may have influenced the RER values and exercise capacity during HD. However, we think the difference in the intake of carbohydrates was not large enough to solely explain the difference in time to exhaustion by lower muscle glycogen availability. In the group of young women, the cycling tests performed during the study lasted approximately 33 minutes. Of that, 20 minutes were cycled at 35 and 55 % of VO_2max, when fat is the most dominant source of energy (Knuiman et al. 2015). Subjects were not engaging in high-intensity exercise during the study periods. Moreover, according to the body composition analysis, the weight of body fluids was not reduced over the HD period, which strengthens the postulation that the muscle glycogen stores were not depleted prior the cycling tests during the HD period.

To conclude, a diet deficient in vegetables and fruits and rich in meat and grains, leads to a high dietary acid load and may induce a more acidic blood acid-base status - that is, lower pH, lower bicarbonate and lower base excess. The effects of dietary acid load are larger in the elderly compared to younger persons and in women compared to men, which is likely explained by the

differences in GFR. Maintaining better renal function with aging may be associated with higher availability of bases, which also may diminish exercise-induced acidosis and decrease the need of aging kidneys to excrete acids. Decreased alkalinity of the blood may also impair maximal aerobic performance, such as occurred in the young women in this study. We conclude that the diet composition along with renal functional capacity affects acid-base status of the body at rest and in exercise. The significance of the dietary acid load as a part of a healthy diet needs to be studied further.

Acknowledgements

The authors would like to thank the participants of this study, and Tanja Laine, Johanna Ihalainen, Elina Vaara, Hanna Minkkinen, Susanna Karvinen, Riikka Lamminen and Juha Hulmi for all the assistance in establishing this study.

Conflict of interest and source of funding

This study was funded by TEKES (Finnish Funding Agency for Technology and Innovation) and in collaboration with TEKES by following Finnish companies: Honkatarhat Ltd, Honkajoki; Kyröntarhat Ltd, Honkajoki; Mykora Ltd, Honkajoki; Lihajaloste Korpela Ltd, Huittinen; Laihian Mallas Ltd, Laihia; KKK-Vihannes Ltd / LykoBEne, Honkajoki.

Hannu Pitkänen has a commercial association with Honkatarhat LTd, Kyröntarhat Ltd and Mykora Ltd. He accepts full responsibility for the implementation of this study. Other authors declare that they have no conflict of interest.

References

Abramowitz, M.K., Hostetter, T.H., and Melamed, M.L. 2011. Association of serum bicarbonate levels with gait speed and quadriceps strength in older adults. Am. J. Kidney Dis. **58**(1): 29–38. doi: 10.1053/j.ajkd.2010.12.021.

Adeva, M.M., and Souto, G. 2011. Diet-induced metabolic acidosis. Clin. Nutr. **30**(4): 416–421. doi: 10.1016/j.clnu.2011.03.008.

Applegate, C., Mueller, M., and Zuniga, K.E. 2017. Influence of dietary acid load on exercise performance. Int. J. Sport Nutr. Exerc. Metab. **27**(3): 213–219. doi: 10.1123/ijsnem.2016-0186.

Baguet, A., Everaert, I., De Naeyer, H., Reyngoudt, H., Stegen, S., Beeckman, S., Achten, E., Vanhee, L., Volkaert, A., Petrovic, M., Taes, Y., and Derave, W. 2011. Effects of sprint training combined with vegetarian or mixed diet on muscle carnosine content and buffering capacity. Eur. J. Appl. Phys. **111**: 2571–2580. doi: 10.1007/s00421-011-1877-4.

Banerjee, T., Crews, D.C., Wesson, D.E., Tilea, A., Saran, R., Rios Burrows, N., Williams, D.E., and Powe, N.R.; Centers for Disease Control and Prevention Chronic Kidney Disease Surveillance Team. 2014. Dietary acid load and chronic kidney disease among adults in the United States. BMC Nephrol. **15**: 137. doi: 10.1186/1471-2369-15-137.

Bolignano, D., Mattace-Raso, F., Sijbrands, E.J., and Zoccali, C. 2014. The aging kidney revisited: a systematic review. Ageing Res. Rev. **14**: 65-80. doi: 10.1016/j.arr.2014.02.003.

Caciano, S.L., Inman, C.L., Gockel-Blessing, E.E., and Weiss, E.P. 2015. Effects of dietary acid load on exercise metabolism and anaerobic exercise performance. J. Sports Sci. Med. **14**(2): 364-371.

Dawson-Hughes, B., Harris, S.S., and Ceglia, L. 2008. Alkaline diets favor lean tissue mass in older adults. Am. J. Clin. Nutr. **87**: 662−665.

De Brito-Ashurst, I., Varagunam, M., Raftery, M.J., and Yaqoob, M.M. 2009. Bicarbonate supplementation slows progression of CKD and improves nutritional status. J. Am. Soc. Nephrol. **20**(9): 2075-2084. doi: 10.1681/ASN.2008111205.

Frassetto, L., and Sebastian, A. 1996. Age and systemic acid-base equilibrium: analysis of published data. J. Gerontol. A. Biol. Sci. Med. Sci. **51**(1): B91−99.

Giovannini, M., Agostoni, C., Gianni, M., Bernardo, L., and Riva, E. 2000. Adolescence: macronutrient needs. Eur. J. Clin. Nutr. **54**(Suppl1): S7−10.

Goraya, N., Simoni, J., Jo, C., and Wesson, D.E. 2012. Dietary acid reduction with fruits and vegetables or bicarbonate attenuates kidney injury in patients with a moderately reduced glomerular filtration rate due to hypertensive nephropathy. Kidney Int. **81**(1): 86-93. doi: 10.1038/ki.2011.313.

Greenhaff, P.L., Gleeson, M., & Maughan, R.J. 1987. The effects of dietary manipulation on blood acid-base status and the performance of high intensity exercise. Eur. J. Appl. Physiol. **56**: 331−337.

Hollidge-Horvat, M.G., Parolin, M.L., Wong, D., Jones, N.L., and Heigenhauser, G.J.F. 2000. Effect of induced metabolic alkalosis on human skeletal muscle metabolism during exercise. Am J. Physiol. Endocrinol. Metab. **278**: E316−329.

Houston, D.K., Nicklas, B.J., Ding, J., Harris, T.B., Tylavsky, F.A., Newman, A.B., Lee, J.S., Sahyoun, N.R., Visser, M., and Kritchevsky, S.B.; Health ABC Study. 2008. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am. J. Clin. Nutr. **87**(1): 150–155.

Hulmi, J.J., Kovanen, V., Selänne, H., Kraemer, W.J., Häkkinen, K., and Mero, A.A. 2009. Acute and long-term effects of resistance exercise with or without protein ingestion on muscle hypertrophy and gene expression. Amino Acids. **37**(2): 297–308. doi: 10.1007/s00726-008-0150-6.

Knuiman, P., Hopman, M.T.E., and Mensink, M. 2015. Glycogen availability and skeletal muscle adaptations with endurance and resistance exercise. Nutr. Met. **12**: 59. doi: 10.1186/s12986-015-0055-9

Krustrup, P., Ermidis, G., and Mohr, M. 2015. Sodium bicarbonate intake improves high-intensity intermittent exercise performance in trained young men. J. Int. Soc. Sports Nutr. **12**: 25. doi: 10.1186/s12970-015-0087-6.

Lancha Junior, A.H., de Salles Painelli, V., Saunders, B., and Artioli, G.G. 2015. Nutritional strategies to modulate intracellular and extracellular buffering capacity during high-intensity exercise. Sports Medicine. **45**: 71–81. doi:10.1007/s40279-015-0397-5.

Levey, A.S., Stevens, L.A., Schmid, C.H., Zhang, Y., Castro, A.F. III, Feldman, H.I., Kusek, J.W., Eggers, P., Van Lente, F., Greene, T., and Coresh, J; for the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI). A new equation to estimate glomerular filtration rate. Ann. Intern. Med. **150**(9): 604–612, 2009.

Mero, A., Hirvonen, P., Saarela, J., Hulmi, J., Hoffman, J., and Stout, J. 2013. Effect of sodium bicarbonate and beta-alanine supplementation on maximal sprint swimming. J. Int. Soc. Sports Nutr. **10**(1): 52. doi:10.1186/1550-2783-10-52.

Oöpik, V., Saaremets, I., Medijainen, L., Karelson, K., Janson, T., and Timpmann, S. 2003. Effects of sodium citrate ingestion before exercise on endurance performance in well trained college runners. Br. J. Sports Med. **37**(6): 485–489. doi:10.1136/bjsm.37.6.485.

Poupin, N., Calvez, J. F., Lassale, C. F., Chesneau, C. F., and Tome, D. 2012. Impact of the diet on net endogenous acid production and acid-base balance. Clin. Nutr. **31**(3): 313–21. doi:10.1016/j.clnu.2012.01.006.

Ratel, S., Duché, P., and Williams, C.A. 2006. Muscle fatigue during high-intensity exercise in children. Sports. Med. **36**(12): 1031-1065.

Remer, T., Dimitriou, T., and Manz, F. 2003. Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. Am. J. Clin. Nutr. **77**: 1255–1260.

Robergs, R.A., Ghiasvand, F.F., & Parker, D. 2004. Biochemistry of exercise-induced metabolic acidosis. Am. J. Physiol. Regul. Integr. Comp. Physiol. **287**:R502–516. doi:10.1152/ajpregu.00114.2004.

Sahlin, K, and Harris, R.C. 2008. Control of lipid oxidation during exercise: role of energy state and mitochondrial factors. Acta Physiol. (Oxf). **194**(4): 283–291. doi: 10.1111/j.1748-1716.2008.01879.x.

Scialla, J.J., Appel, L.J., Astor, B.C., Miller, E.R. 3rd, Beddhu, S., Woodward, M., Parekh, R.S., and Anderson CA; African American Study of Kidney Disease and Hypertension Study Group. 2012. Net endogenous acid production is associated with a faster decline in GFR in African Americans. Kidney Int. **82**(1): 106-112. doi: 10.1038/ki.2012.82.

Stephens, T.J., McKenna, M.J., Canny, B.J., Snow, R.J., and McConell, G.K. 2002. Effect of sodium bicarbonate on muscle metabolism during intense endurance cycling. Med. Sci. Sports Exerc. **34**(4): 614–621.

Tabatabai, L.S., Cummings, S.R., Tylavsky, F.A., Bauer, D.C., Cauley, J.A., Kritchevsky, S.B., Newman, A., Simonsick, E.M., Harris, T.B., Sebastian, A., and Sellmeyer, D.E. 2015. Arterialized venous bicarbonate is associated with lower bone mineral density and an increased rate of bone loss in older men and women. J. Clin. Endocrinol. Metab. **100**(4): 1343–1349. doi: 10.1210/jc.2014-4166.

Van Montfoort, M.C., Van Dieren, L., Hopkins, W.G., and Shearman, J.P. 2004. Effects of ingestion of bicarbonate, citrate, lactate, and chloride on sprint running. Med. Sci. Sports Exerc. **36**(7): 1239–1243.

Welch, A.A., MacGregor, A.J., Skinner, J., Spector, T.D., Moayyeri, A., and Cassidy, A. 2013. A higher alkaline dietary load is associated with greater indexes of skeletal muscle mass in women. Osteoporos. Int. **24**(6): 1899–1908. doi: 10.1007/s00198-012-2203-7.

Wesson, D.E., and Simoni, J. 2009 Increased tissue acid mediates a progressive decline in the glomerular filtration rate of animals with reduced nephron mass. Kidney Int. **75**(9): 929–935. doi: 10.1038/ki.2009.6

Westerterp-Plantenga, M.S., Lemmens, S.G., and Westerterp, K.R. 2012. Dietary protein - its role in satiety, energetics, weight loss and health. Br. J. Nutr. **108**(Suppl2): S105–112. doi: 10.1017/S0007114512002589.

Wilkes, D., Gledhill, N., and Smyth R. 1983. Effect of acute induced metabolic alkalosis on 800-m racing time. Med. Sci. Sports Exerc. **15**(4): 277-280.

Yenchek, R., Ix, J.H., Rifkin, D.E., Shlipak, M.G., Sarnak, M.J., Garcia, M., Patel, K.V., Satterfield, S., Harris, T.B., Newman, A.B., and Fried, L.F.; Health ABC Study. 2014. Association of serum bicarbonate with incident functional limitation in older adults. Clin. J. Am. Soc. Nephrol. **9**(12): 2111-2116. doi: 10.2215/CJN.05480614.

Table 1. Baseline anthropometric characteristics, PRAL of habitual diets and cardiorespiratory measures at Test 1 (mean + SD).

	Adolescents		Young adults		Elderly	
	Boys	**Girls**	**Men**	**Women**	**Men**	**Women**
N	13	9	15	18	17	16
Age (yr)	13.4 ± 1.4	13.0 ± 1.2	29.1 ± 2.7	27.6 ± 3.4	67.1 ± 3.7	65.4 ± 3.6
Weight (kg)	52.3 ± 10.9	49.4 ± 7.7	79.5 ± 9.7	58.3 ± 5.0	78.8 ± 10.0	67.6 ± 11.0
Height (cm)	161 ± 12	158 ± 6	180 ± 6	165 ± 6	175 ± 6	164 ± 7
Body fat (%)	13.0 ± 7.5	18.5 ± 5.4	17.3 ± 4.6	22.2 ± 5.1	22.4 ± 6.4	34.1 ± 7.9
BMI (kg/m^2)	20.0 ± 2.4	19.6 ± 2.1	24.5 ± 2.6	21.6 ± 2.2	25.5 ± 2.0	25.3 ± 3.9
PRAL (mEq/d)	4.4 ± 16.0	-5.7 ± 23.3	1.7 ± 17.0	-13 ± 17	-4.4 ± 9.5	-14 ± 12
VO_2max (l/min)	2.9 ± 0.9	2.3 ± 0.5	4.2 ± 0.5	2.7 ± 0.5	2.5 ± 0.3	1.9 ± 0.2
RER	0.97 ± 0.04	1.01 ± 0.04	1.05 ± 0.06	1.04 ± 0.07	-	-
Heart rate (bpm)	186 ± 13	190 ± 7	189 ± 9	187 ± 8	161 ± 3	162 ± 2

Table 2. Dietary intake data during LD and HD in all subject groups.

	Adolescents				Young adults				Elderly			
	Boys		Girls		Men		Women		Men		Women	
	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
PRAL (mEq/d)	-47 ± 44***	25 ± 11	-43 ± 18***	15 ± 16	-68 ± 30***	61 ± 22	-68 ± 17***	47 ± 8.3	-61 ± 17***	57 ± 12	-63 ± 12***	49 ± 14
IVF (g/d)	830 ± 630**	40 ± 18	890 ± 370***	28 ± 16	1410 ± 460***	24 ± 11	1400 ± 350***	22 ± 8	1270 ± 380***	20 ± 8	1350 ± 290***	17 ± 6
Energy (kcal/d)	1790 ± 570	1980 ± 610	1360 ± 200*	1610 ± 300	2090 ± 600**	2330 ± 670	1650 ± 230*	1780 ± 300	1930 ± 350*	2070 ± 350	1690 ± 290	1690 ± 330
Protein (g/kg/d)	1.3 ± 0.4***	2.1 ± 0.5	1.0 ± 0.2	1.5 ± 0.7	1.3 ± 0.4***	2.1 ± 0.6	1.2 ± 0.2***	2.3 ± 0.3	1.1 ± 0.2***	1.9 ± 0.4	1.0 ± 0.2***	1.9 ± 0.5
Protein (%)	16 ± 3.4**	22 ± 3.7	13 ± 3.0***	22 ± 4.7	19 ± 3.6***	27 ± 2.2	17 ± 2.5***	30 ± 3.9	17 ± 1.7***	29 ± 2.9***	16 ± 2.8	29 ± 2.5
CHO (g/kg/d)	4.9 ± 1.6	4.5 ± 1.8	4.4 ± 0.6	3.3 ± 1.4	3.9 ± 1.2***	3.1 ± 1.0	4.2 ± 0.9***	3.2 ± 0.7	3.4 ± 0.9***	2.8 ± 0.9	3.4 ± 0.8***	2.7 ± 0.8
CHO (%)	57 ± 5.5**	46 ± 8.1	61 ± 5.4***	46 ± 5.9	56 ± 6.5***	39 ± 3.4	57 ± 5.0***	41 ± 4.3	54 ± 3.9***	41 ± 3.9***	52 ± 4.4***	41 ± 4.1
Fat (g/kg/d)	1.0 ± 0.3***	1.3 ± 0.4	0.8 ± 0.3***	1.2 ± 0.3	0.7 ± 0.3***	1.1 ± 0.3	0.7 ± 0.2***	1.0 ± 0.3	0.7 ± 0.2**	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.2
Fat (%)	25 ± 4.2***	31 ± 4.8	26 ± 8.1	32 ± 3.1	22 ± 5.8***	31 ± 4.6	23 ± 4.9***	28 ± 4.6	26 ± 3.9**	29 ± 3.7	29 ± 4.4	28 ± 4.4

LD, diet with low acid load; HD diet with high acid load; PRAL, potential renal acid load; IVF, intake of vegetables and fruits; CHO, carbohydrates.

*P<0.05, ** P<0.01, *** P<0.001, statistically significant difference between LD and HD inside each subject group (Paired samples t-test). Values are mean ± SD.

Table 3. Cardiorespiratory variables during the cycle ergometer tests after LD and HD in all subject groups.

	Workload	Men						Women					
		VO_2 (l/min)		RER		HR		VO_2 (l/min)		RER		HR	
	(% of VO_2max)	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
AD	35	1.10 ± 0.31	1.06 ± 0.30	0.83 ± 0.04	0.80 ± 0.05	116 ± 12	113 ± 14	0.90 ± 0.17	0.91 ± 0.18	0.84 ± 0.02	0.81 ± 0.03	121 ± 9	121 ± 10
	55	1.70 ± 0.50	1.71 ± 0.48	0.84 ± 0.04	0.83 ± 0.02	149 ± 13	146 ± 16	1.39 ± 0.26	1.40 ± 0.30	0.86 ± 0.03	0.84 ± 0.02	149 ± 10	152 ± 12
	75	2.44 ± 0.65	2.48 ± 0.65	0.88 ± 0.05	0.87 ± 0.03	179 ± 10	178 ± 13	1.94 ± 0.39	1.99 ± 0.36	0.90 ± 0.02	0.89 ± 0.03	176 ± 8	180 ± 10
	100	2.97 ± 0.97	3.05 ± 0.89	0.92 ± 0.1	0.92 ± 0.09	194 ± 9	192 ± 12	2.33 ± 0.35	2.35 ± 0.39	0.97 ± 0.05	0.93 ± 0.06	187 ± 5	188 ± 5
YA	35	1.51 ± 0.23	1.53 ± 0.19	0.83 ± 0.03	0.83 ± 0.04	108 ± 9	108 ± 11	1.06 ± 0.19	1.04 ± 0.16	0.84 ± 0.04*	0.82 ± 0.03	116 ± 13*	111 ± 12
	55	2.27 ± 0.42	2.35 ± 0.29	0.87 ± 0.04	0.87 ± 0.04	140 ± 11	142 ± 14	1.55 ± 0.22	1.53 ± 0.23	0.86 ± 0.04	0.85 ± 0.03	147 ± 9	143 ± 13
	75	3.32 ± 0.51	3.31 ± 0.43	0.94 ± 0.05	0.92 ± 0.04	172 ± 9	171 ± 11	2.17 ± 0.28	2.11 ± 0.31	0.92 ± 0.03	0.90 ± 0.04	174 ± 8	171 ± 9
	100	4.00 ± 0.47	4.05 ± 0.43	1.02 ± 0.09	1.02 ± 0.10	187 ± 10	187 ± 9	2.65 ± 0.35**	2.50 ± 0.40	1.01 ± 0.09*	0.99 ± 0.10	187 ± 9**	184 ± 9
EL	35	0.97 ± 0.11	1.00 ± 0.12	0.85 ± 0.03**	0.82 ± 0.03	87 ± 9	86 ± 11	0. 81 ± 0.09	0.83 ± 0.09	0.84 ± 0.03	0.83 ± 0.03	98 ± 13	100 ± 16
	55	1.44 ± 0.18	1.46 ± 0.19	0.90 ± 0.04**	0.87 ± 0.03	106 ± 12	106 ± 17	1.13 ± 0.13	1.15 ± 0.11	0.87 ± 0.03	0.87 ± 0.03	120 ± 16	123 ± 18
	75	2.02 ± 0.28	2.04 ± 0.29	0.95 ± 0.05**	0.93 ± 0.04	135 ± 17	136 ± 16	1.53 ± 0.18	1.53 ± 0.19	0.93 ± 0.04	0.92 ± 0.05	151 ± 16	152 ± 15

After a 7-d LD and a 7-d HD, experimental cycle ergometer tests were performed, where 10 min at 35%, 55% and 75% of VO_2max were cycled (CT35, CT55, CT75). AD and YA cycled additionally at 100% of VO_2max until exhaustion (CT100). Cardiorespiratory variables were determined as a mean from the final 30 seconds of every workload. LD, diet with low acid load; HD, diet with high acid load; HR, heart rate; AD, adolescents; YA, young adults; EL, elderly. *P<0.05, ** P<0.01, statistically significant difference between LD and HD inside each subject group (two-way repeated measures analysis of variance (ANOVA), a paired samples-test). Values are mean ± SD.

Figure Captions

Figure 1. Glomerular filtration rate in adolescents (A), young adults (B) and the elderly (C) in the beginning (PRE) and at the end (POST) of the 7-d LD and the 7-d HD. LD, diet with low acid load; HD, diet with high acid load. * P<0.05, *** P<0.001 statistically significant difference between LD and HD; † P<0.05 statistically significant difference between PRE and POST during HD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.

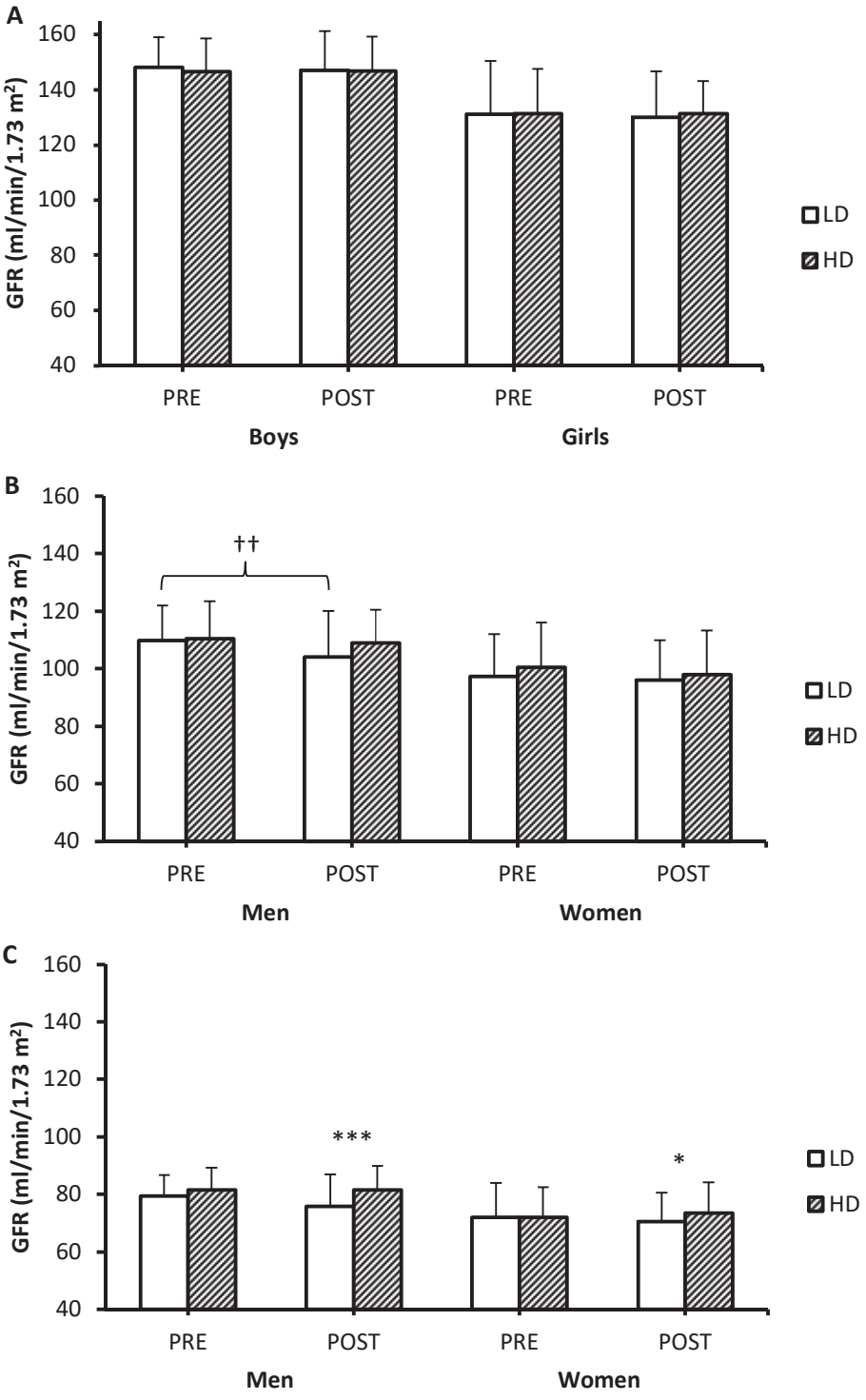
Figure 2. Capillary pH in adolescents (A), young adults (B) and elderly (C) men and women in the beginning (PRE) and at the end (POST) of the 7-d LD and the 7-d HD, after the breakfast (REST) and during exercise where 10 min at 35%, 55% and 75% of VO_2max (CT35, CT55, CT75) were cycled. Adolescents and young adults cycled additionally at 100% of VO_2max until exhaustion (CT100). LD, diet with low acid load; HD, diet with high acid load. * P<0.05, ** P<0.01, *** P<0.001 statistically significant difference between LD and HD; † P<0.05, ††† P<0.001 statistically significant difference between PRE and POST during HD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.

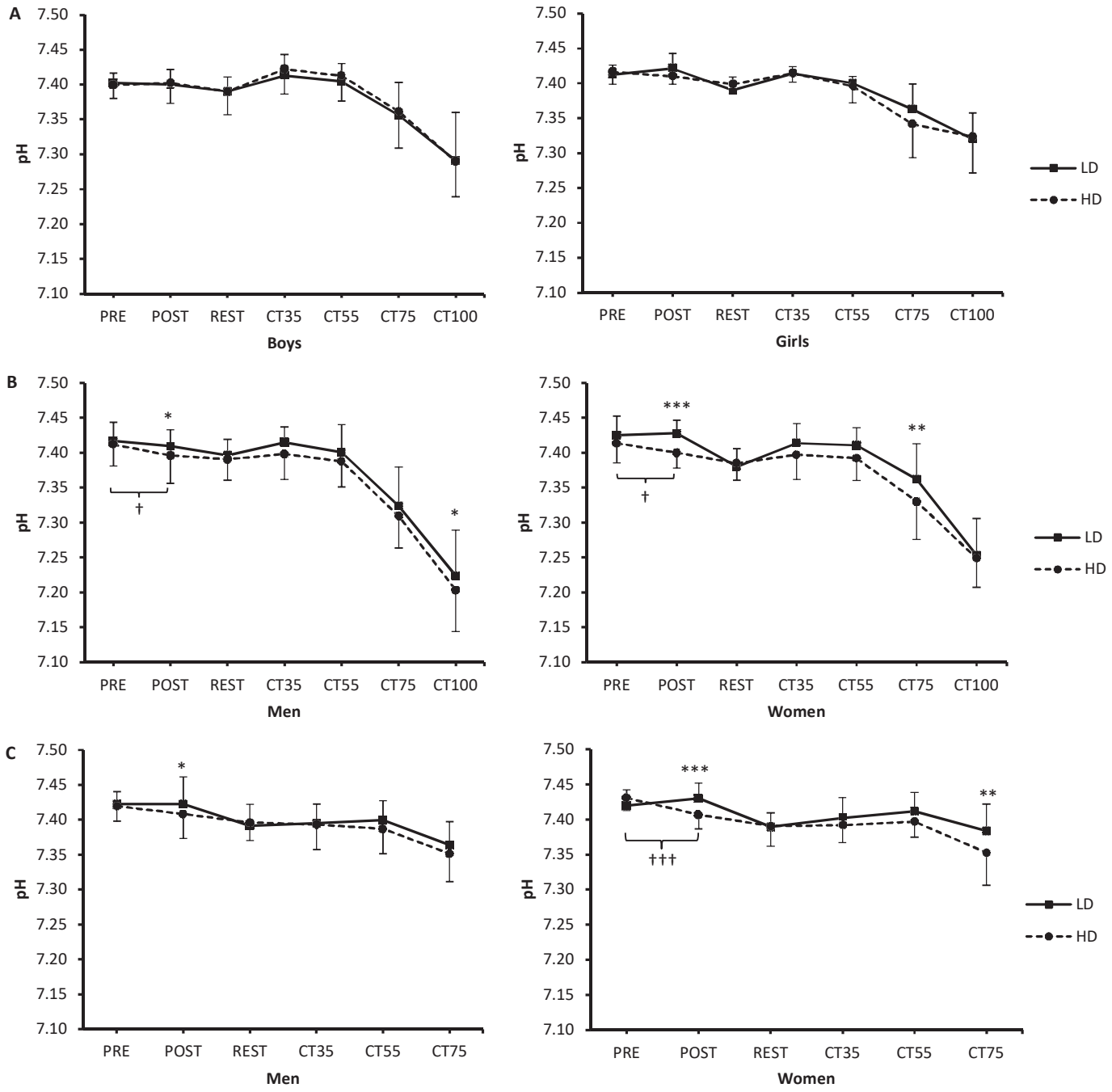
Figure 3. Bicarbonate (HCO_3^-) in adolescents (A), young adults (B) and elderly (C) men and women in the beginning (PRE) and at the end (POST) of the 7-d LD and the 7-d HD, after the breakfast (REST) and during exercise where 10 min at 35%, 55% and 75% of VO_2max (CT35, CT55, CT75) were cycled. Adolescents and young adults cycled additionally at 100% of VO_2max until exhaustion (CT100). LD, diet with low acid load; HD, diet with high acid load. * P<0.05, ** P<0.01, *** P<0.001, statistically significant difference between LD and HD; † P<0.05, †† P<0.01, ††† P<0.001 statistically significant difference between PRE and POST

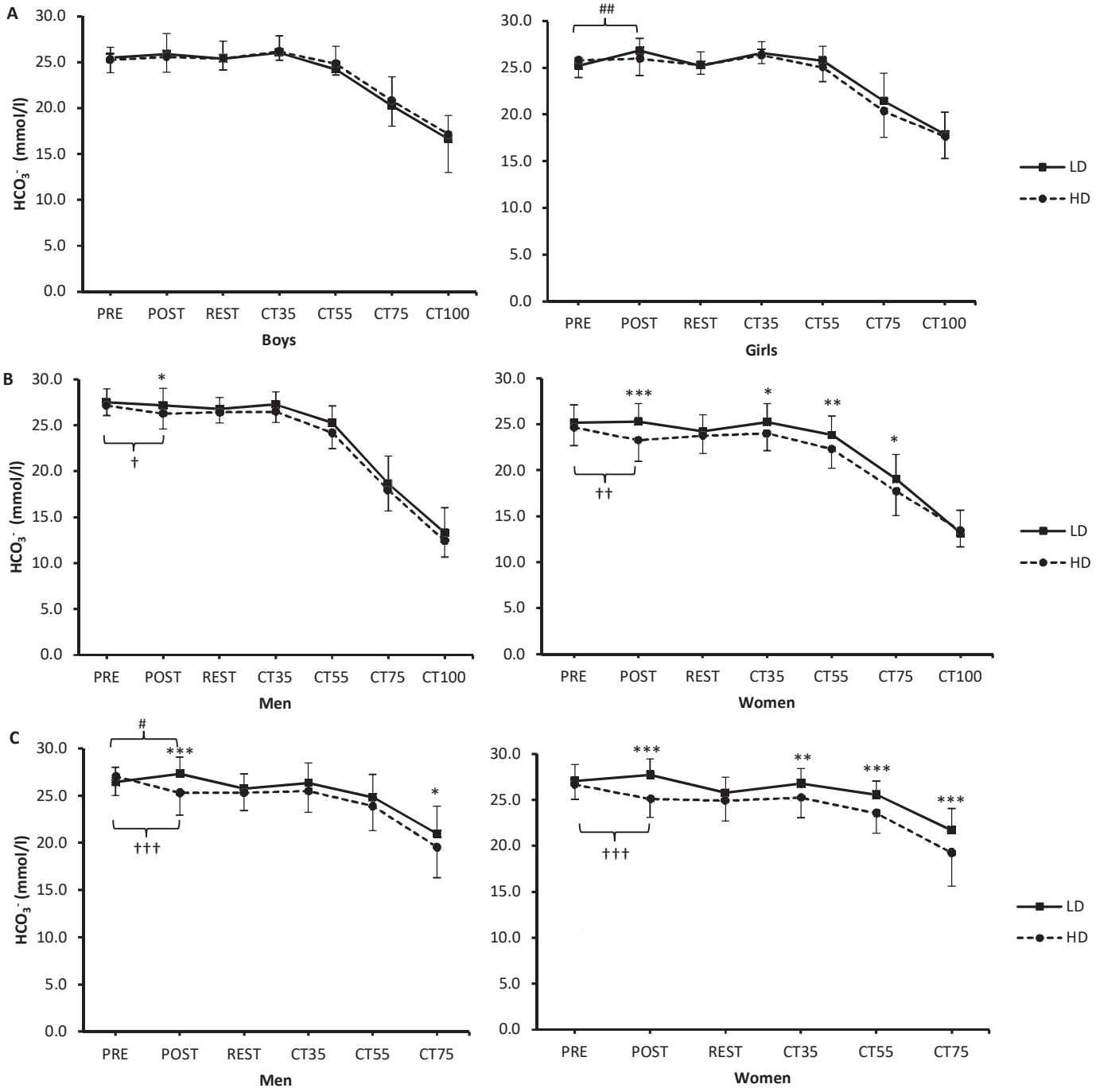
during HD; # P<0.05, ## P<0.01 statistically significant difference between PRE and POST during LD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.

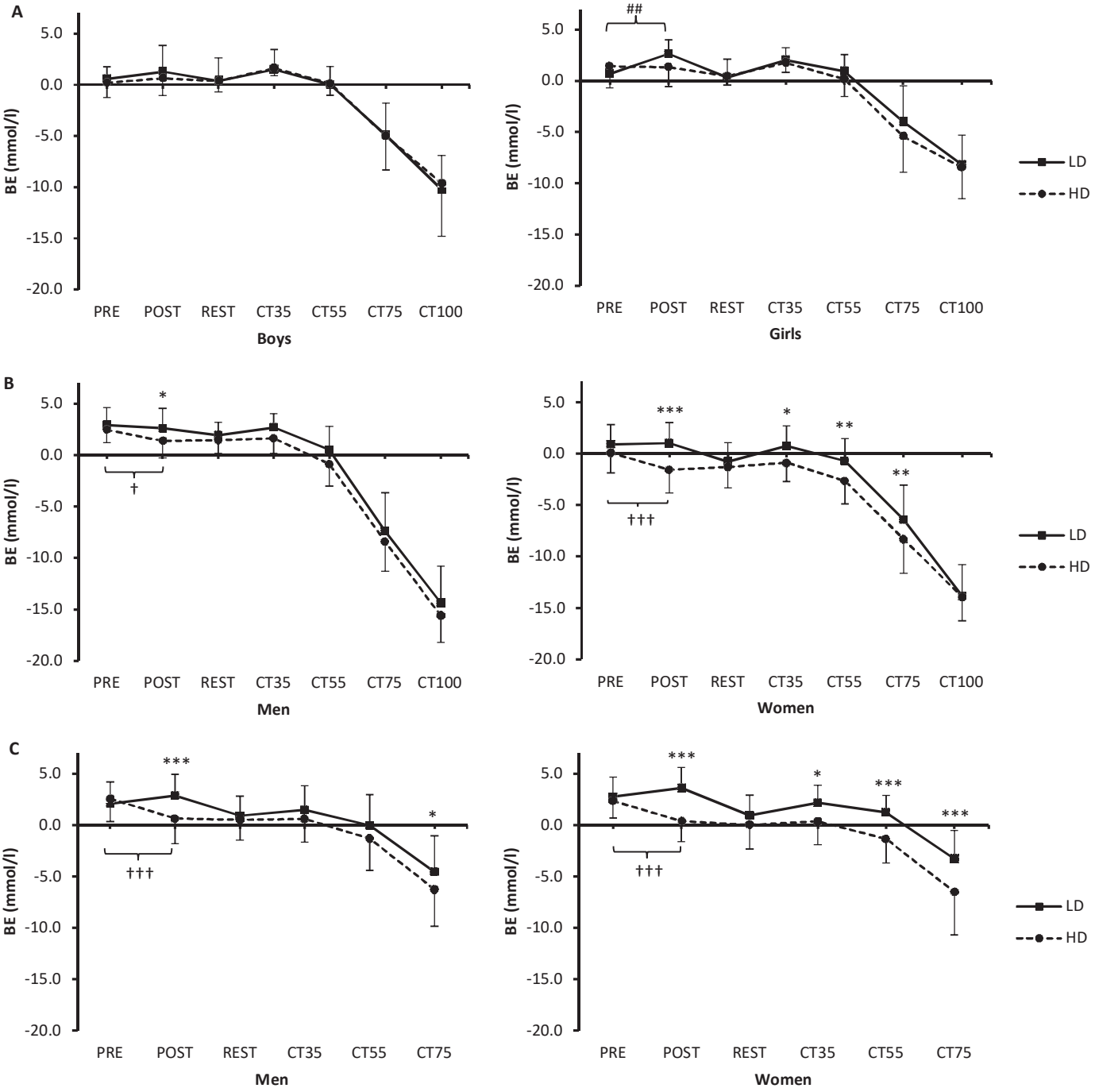
Figure 4. Base excess (BE) in adolescents (A), young adults (B) and elderly (C) men and women in the beginning (PRE) and at the end (POST) of the 7-d LD and the 7-d HD, after the breakfast (REST) and during exercise where 10 min at 35%, 55% and 75% of VO_2max (CT35, CT55, CT75) were cycled. Adolescents and young adults cycled additionally at 100% of VO_2max until exhaustion (CT100). LD, diet with low acid load; HD, diet with high acid load. * P<0.05, ** P<0.01, *** P<0.001, statistically significant difference between LD and HD; † P<0.05, ††† P<0.001 statistically significant difference between PRE and POST during HD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.

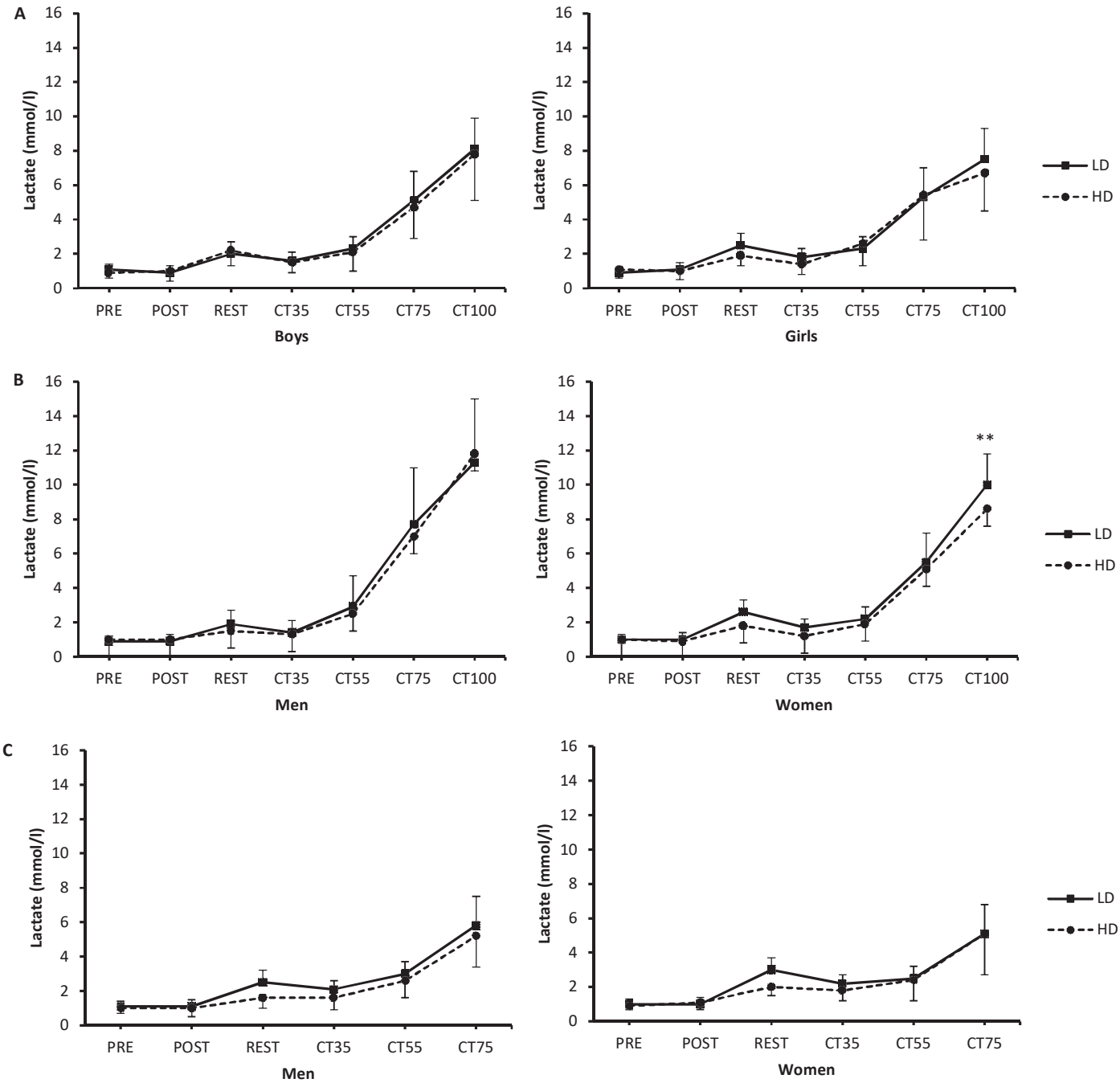
Figure 5. Lactate in adolescents (A), young adults (B) and elderly (C) men and women in the beginning (PRE) and at the end (POST) of the 7-d LD and the 7-d HD, after the breakfast (REST) and during exercise where 10 min at 35%, 55% and 75% of VO_2max (CT35, CT55, CT75) were cycled. Adolescents and young adults cycled additionally at 100% of VO_2max until exhaustion (CT100). LD, diet with low acid load; HD, diet with high acid load. ** P<0.01, statistically significant difference between LD and HD (mixed models with random ID, LSD pairwise comparison). Values are mean ± SD.


A
GFR (ml/min/1.73 m²)
160
140
120
100
80
60
40
PRE
POST
PRE
POST
Boys
Girls
LD
HD
B
GFR (ml/min/1.73 m²)
††
PRE
POST
PRE
POST
Men
Women
LD
HD
C
GFR (ml/min/1.73 m²)
***
*
PRE
POST
PRE
POST
Men
Women
LD
HD


A
B
C
pH
7.50
7.45
7.40
7.35
7.30
7.25
7.20
7.15
7.10
PRE
POST
REST
CT35
CT55
CT75
CT100
Boys
Girls
Men
Women
LD
HD
*
***
**
†
†††


A
B
C
HCO_3^- (mmol/l)
PRE
POST
REST
CT35
CT55
CT75
CT100
Boys
Girls
Men
Women
LD
HD
##
*
***
**
†
††
†††
#


A
B
C
BE (mmol/l)
5.0
0.0
-5.0
-10.0
-15.0
-20.0
PRE
POST
REST
CT35
CT55
CT75
CT100
Boys
Girls
Men
Women
LD
HD
##
*
***
**
†
†††


A
Lactate (mmol/l)
0
2
4
6
8
10
12
14
16
PRE
POST
REST
CT35
CT55
CT75
CT100
Boys
Girls
LD
HD
B
Men
Women
**
C

IV

EFFECTS OF 12-WEEK LOW OR MODERATE DIETARY ACID INTAKE ON ACID–BASE STATUS AND KIDNEY FUNCTION AT REST AND DURING SUBMAXIMAL CYCLING.

by

Hietavala, E-M., Ihalainen, J.K., Frassetto, L.A., Schumann, M., Eklund, D., Häkkinen, K. & Mero, A.A. 2018.

Nutrients 10 (3), 323

nutrients

MDPI

Article

Effects of 12-Week Low or Moderate Dietary Acid Intake on Acid–Base Status and Kidney Function at Rest and during Submaximal Cycling

Enni-Maria Hietavala [1,*], Johanna K. Ihalainen [1], Lynda A. Frassetto [2], Moritz Schumann [3], Daniela Eklund [1], Hannu Pitkänen [4], Keijo Häkkinen [1] and Antti A. Mero [1]

1 Biology of Physical Activity, Faculty of Sport and Health Sciences, University of Jyväskylä, P.O. Box 35 (VIV), 40014 Jyväskylä, Finland; johanna.k.ihalainen@jyu.fi (J.K.I.); daniela.eklund@gmail.com (D.E.); keijo.hakkinen@jyu.fi (K.H.); antti.a.mero@jyu.fi (A.A.M.)

2 General Clinical Research Center, University of California San Francisco, 505 Parnassus Avenue, San Francisco, CA 94117, USA; Lynda.Frassetto@ucsf.edu

3 Department of Molecular and Cellular Sports Medicine, German Sport University, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany; m.schumann@dshs-koeln.de

4 Honka Holding, c/o Honkatarhat Oy, Kirkkokallio 20, 38950 Honkajoki, Finland; hannu.pitkanen@mykora.com

* Correspondence: enni.hietavala@jyu.fi; Tel.: +358-40-805-4733

Received: 28 January 2018; Accepted: 5 March 2018; Published: 8 March 2018

Abstract: Prolonged effects of dietary acid intake on acid–base status and kidney function have not yet been studied in an intervention study in healthy subjects. Dietary acid load can be estimated by calculating the potential renal acid load (PRAL) of foods. Effects of low-PRAL and moderate-PRAL diets on acid–base status and kidney function were investigated during a 12-week exercise training period. Healthy, 20–50-year-old men (n = 21) and women (n = 25) participated in the study and were randomly divided into low-PRAL and moderate-PRAL groups. Before (PRE), mid-phase (MID) and after the intervention (POST), the subjects participated in measurement sessions, where a 12-h urine sample and fasting blood samples were collected, and a submaximal cycle ergometer test was performed. Net acid excretion was significantly lower after 12 weeks of the low-PRAL diet as compared to the moderate-PRAL diet, both in men and women. In low-PRAL females, capillary pH and bicarbonate were significantly higher at 75% of VO_{2max} at POST as compared to PRE. Glomerular filtration rate decreased over the study period in moderate-PRAL men and women. The results of the present study suggest that an acidogenic diet and regularly training together may increase the acidic load of the body and start to impair the kidney function in recreationally active subjects.

Keywords: dietary acid load; acid–base status; net acid excretion; exercise training; kidney function

1. Introduction

Many biochemical reactions release and bind hydrogen ions (H^+) in the human body. Under normal physiological conditions, diet composition is the primary modifier of net endogenous acid production (NEAP) and it may further affect the acid–base status of the body [1]. Dietary acid load can be estimated by calculating the potential renal acid load (PRAL) of foods, which estimates the acidic potential of foodstuffs [2]. Digestion of large amounts of animal protein and grain products, but only small amounts of vegetables and fruits, leads to a net production of acids in the body [3]. H^+ concentrations in body fluids are regulated to remain in between rather narrow pH limits and thus, only minor changes occur in blood pH. In arterial blood at rest, pH is normally maintained strictly between 7.35–7.45 and extracellular buffering (i.e., mainly bicarbonate buffering) occurs concomitantly

with any changes in plasma H^+ concentration. However, it has been shown that diet composition may cause acute changes inside the optimal blood pH range [4]. Urine pH can vary between 4.5 and 8.0, according to the amount of H^+ that needs to be excreted from the body by the kidneys. The systemic bicarbonate (HCO_3^-) concentration represents the metabolic component of acid–base balance, and the kidneys have a prevalent role in regulating it [5].

In the field of exercise physiology, effects of exercise on kidney function have not been studied very intensively. The kidney has an essential role in the homeostasis of the body at rest, but changes in renal function also occur with exercise. Under resting conditions, blood flow to the kidneys is among the highest to any organ. However, oxygen consumption is not increased in renal tissue during exercise, and blood flow is redistributed away from the kidney to skeletal muscles [6]. The glomerular filtration rate (GFR) is a measure of the amount of fluid filtered through the glomerular basement membrane in the kidneys, and it is considered to be the best overall assessment of kidney function [7]. With exercise loads up to 50% of VO_{2max}, GFR is slightly increased or unchanged, and at higher exercise intensities, GFR decreases at higher rates than renal blood flow [8]. The long-term effects of exercise training on kidney function have not been studied in healthy populations, but in patients suffering decreased kidney function there is evidence for an association between kidney function and exercise performance [9]. Moreover, in a study by Morales et al. [10], kidney function affected the physical performance of athletes, as VO_{2max} was lower and heart rate higher in a group of athletes with smaller GFR, compared to athletes with higher GFR. It was recently shown that dietary acid load may play a role in delaying fatigue during exercise as the effects of dietary acid load on acid–base status and physical performance were investigated during a 7-day diet period [11]. The data suggested that lower acid intake could help the kidneys to increase exercise capacity by maintaining a higher extracellular HCO_3^- concentration, which could delay the onset of fatigue caused by exercise-induced acidosis.

To the best of our knowledge, the prolonged effects of dietary acid intake on acid–base status and kidney function, with or without exercise training, have not yet been studied in an intervention study in healthy subjects. The aim of the present research was to study how dietary acid load affects the acid–base status of the body during a 12-week combined endurance and strength training period at rest and during submaximal cycling. In addition, the effects of a diet and training intervention on kidney function were investigated at rest. The kidney function was assessed with GFR, serum urea-to-creatinine ratio (UCR) and serum urea. It was hypothesized that the lower acid intake would induce a less acidic blood acid–base status—that is, higher pH and HCO_3^-—at rest and during submaximal exercise and would preserve kidney function at rest.

2. Materials and Methods

2.1. Subjects

In total, 49 healthy men and women volunteered and were selected to participate in the study. The study participants were required to be 20–50 years old and recreationally physically active. Before inclusion into the study, the physical activity of the subjects was characterized by walking, cycling, team sports, or strength training at a light-to-moderate intensity, at a frequency of 1–3 times per week, but a lack of systematic engagement in any endurance or strength training. The female participants were allowed to use contraceptive pills during the study period, but any other medication was considered to be exclusion criteria. Also subjects whose body mass indexes were above 33 kg/m^2 or who had any relevant food allergy were excluded from the study. Ethical approval was obtained from the Ethical Committee of the University of Jyväskylä, and the study was in accordance with the Helsinki Declaration. Prior to the first testing, subjects were informed of the purpose and the methods of the study, and they signed a written informed consent. Additionally, the subjects completed questionnaires about their health, diet, and exercise background, and underwent a standardized resting electrocardiogram procedure, which was reviewed by a cardiologist. At the beginning of the study, the subjects were randomly divided into the low-PRAL and the moderate-PRAL diet groups and

ate accordingly for the entire duration of the study period. Over the study period, there were three drop-outs, for reasons unrelated to the intervention. Baseline anthropometric characteristics of the subjects who completed the entire data collection are presented in Table 1.

Table 1. Baseline anthropometric characteristics of the subjects in the low-potential renal acid load (PRAL) and moderate-PRAL diet groups.

Parameters	Women		Men	
	Low-PRAL	Mod-PRAL	Low-PRAL	Mod-PRAL
N	13	12	9	12
Age (years)	34.3 ± 6.9	32.0 ± 5.9	32.0 ± 9.6	31.3 ± 5.1
Height (m)	1.67 ± 0.07	1.66 ± 0.06	1.78 ± 0.07	1.77 ± 0.06
Body mass (kg)	64.2 ± 7.5	65.6 ± 11.4	86.0 ± 9.2	79.1 ± 10.2
Body mass index (kg/m^2)	23.0 ± 3.5	23.7 ± 3.5	27.2 ± 3.1	25.2 ± 2.1

2.2. Study Design

The study period lasted for 12 weeks. The subjects trained twice a week, and every training session consisted of both endurance and strength training (approximately 45 min + 45 min each). The measurement sessions took place before the intervention period (PRE), at the mid-phase during weeks 6–8 (MID) and after the intervention (POST). During each testing session a 12-h urine sample and fasting blood samples were collected. In addition, the subjects recorded their food intake via a 3-day food diary. At PRE and POST, the subjects also performed a submaximal cycle ergometer test during which the blood samples were obtained.

One week before the start of the 12-week intervention, the VO_{2max} and maximal workloads of the subjects were measured via an incremental cycle ergometer test that was performed on a microprocessorcontrolled, eddy current brake equipped ergometer (Ergoline ergometrics 800, D-72475, Bitz, Germany). The initial workload was 50 W and it was increased by 25 W every 2 min until volitional exhaustion occurred. VO_{2max} was determined to be the highest 30-s VO_2 value during the test and coincided with at least one of the following two criteria: (a) respiratory exchange ratio >1.1; and/or (b) a plateau of oxygen uptake (less than 150 mL/min increase in VO_2 during the last 60 s of the test). Gaseous exchange was measured using a Jaeger Oxycon Pro breath-by-breath gas analyzer (VIASYS Healthcare GmbH, Hoechburg, Germany). The device was calibrated for volume and gas before every measurement. The VO_{2max} determined at baseline (PRE) was used in all subject groups to set the workloads for the submaximal cycling tests. Three days after the VO_{2max} test, the subjects performed a submaximal cycling test (PRE) that started with a 5-min warm-up, followed by a 4-min break. Thereafter, the subjects completed three 8 min trials at 35%, 55% and 75% of their VO_{2max}. All workloads were separated by 4-min rest periods, during which blood samples (CT35, CT55, CT75, respectively; CT = cycling test) were collected from a fingertip capillary and an antecubital vein.

During the last 12 h before the start of the dietary intervention, subjects had a 12-h overnight fast and, at the same time, collected a 12-h urine sample. The next morning, in a laboratory, fasting blood samples (FAST) were drawn from a fingertip capillary and an antecubital vein. The blood samples were obtained at 7–10 a.m. and kept similar throughout the study. The 12-h urine collection commenced 12 h before FAST. Starting from PRE, the subjects followed either the low-PRAL or the moderate-PRAL diet, and the same urine and blood sampling sessions were repeated at MID and at POST. At PRE and POST, after the blood sampling, the body composition of the subjects was assessed by dual X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE Medical Systems, Madison, WI, USA). Total fat mass and total lean mass were automatically analyzed (enCORE software, version 14.10.022, GE Medical Systems, Madison, WI, USA). Thereafter, the subjects ate a light breakfast, which was consistent with their assigned diet. Resting blood samples were drawn once more (REST) before a submaximal cycle ergometer test was completed.

2.3. Diets

Dietary acid load can be estimated by calculating the potential renal acid load (PRAL) of foods, which represents the renal net acid excretion caused by a foodstuff [2]. The diets used in the present study were designed with the PRAL calculations to have low and moderate acid loads. The aim was that the low-PRAL diet would enhance the production of alkaline compounds in the body (PRAL < 0), whereas the moderate-PRAL diet was aimed to slightly increase the production of acid compounds (PRAL > 0). The PRAL values were calculated as follows: PRAL (mEq/100 g) = 0.49 × protein (g/100 g) + 0.037 × phosphorous (mg/100 g) − 0.021 × potassium (mg/100 g) − 0.026 × magnesium (mg/100 g) − 0.013 × calcium (mg/100 g) [2]. The nutrient contents of the food were taken from the Finnish Food Composition Database (Fineli, Finnish National Institute of Health and Welfare). Before the start of the 12-week intervention period, the subjects followed their normal diet and kept food diaries for 3 days. Appropriate dietary counselling was given for both diet groups based on the baseline dietary analysis, and the subjects were given instructions on how to follow the low- and moderate-PRAL diets. Both groups controlled nutritional intake according to the general dietary guidelines, but in the low-PRAL group, the subjects were advised to increase the consumption of fruits and vegetables up to 800–1000 g. On the other hand, in the moderate-PRAL group, the subjects were advised to limit their intake of fruits and vegetables to 200–300 g.

The subjects kept food diaries for 3 days at PRE, MID, and POST. In addition, the subjects recorded their food intake during weeks 1–4 via a 3-day food diary, in order to check if the diet that the subjects were assigned was followed according to the instructions. The food diaries were analyzed for energy, protein, carbohydrate, fat, phosphorous, potassium, magnesium and calcium intake using Nutri-Flow software (Flow-Team Oy, Oulu, Finland). The average daily PRALs during the experimental diets were calculated according to the relevant dietary intake data.

2.4. Urine Sampling and Analysis

The subjects collected 12-h urine samples at PRE, MID and POST. Each urine sample was collected in a sterile container and refrigerated until subjects came to the laboratory and brought the container with them. Upon receipt, urine pH was determined by dipping a pH strip into the urine (Combur-7 Test urinalysis test strips; Cobas, Roche, Germany).

Urine electrolytes were analyzed by the direct ISE in vitro test (Ion Selective Microlyte Analyzer, Konelab 20 XTi; Kone Instruments, Espoo, Finland). Indirect Net acid excretion (NAE) was calculated as follows:

$$\text{NAE (mEq/day)} = (Cl^- + P_i + SO_4{}^- + \text{OA}) - (Na^+ + K^+ + Ca^{2+} + Mg^{2+}),$$

where $SO_4{}^- = 0.4875 \times$ dietary protein intake (g)

$$\text{OA (organic acids)} = (\text{BSA} \times 41)/1.73$$

where BSA (body surface area) = $[(\text{weight (kg)} \times \text{height (m)})/3600]^{\frac{1}{2}}$ [12].

2.5. Blood Sampling and Analysis

All fasting capillary and antecubital vein blood samples were drawn at the same time, in the morning, at all three sampling points. Li-heparinized whole blood samples (200 and 20 µL) from a fingertip capillary were analyzed immediately after sampling for pH, and $HCO_3{}^-$. The determination of pH was based on the principle of the ion selective electrode, whereas $HCO_3{}^-$ was determined computationally from pH and pCO_2 values (GEM Premier 3000, Instrumentation Laboratory, Lexington, MA, USA). The blood samples from the antecubital vein were drawn in vacuum tubes, stored at room temperature for 30 min and centrifuged for 10 min at 3500 rpm (2100× *g*). The serum was separated, and creatinine and urea were analyzed by a KoneLab 20 XTi analyzer

(Thermo Electron Corporation, Vantaa, Finland). Serum creatinine values were used to calculate the glomerular filtration rate (GFR) with the CKD-EPI equation [13]. Also, the serum urea to creatinine ratio (UCR) was calculated.

2.6. Training

The training protocol has been described elsewhere [14]. A combination of aerobic and resistance training has been proposed to be the most effective strategy for maintaining and/or improving physical fitness and health [15]. Briefly, the endurance training was conducted on a cycle ergometer and the training program included mostly steady-state cycling of low to moderate intensity (below and above the aerobic threshold). The duration of endurance cycling increased progressively from 30 to 50 min. During the last 4 weeks of the training period, the intensity of cycling also increased from the aerobic to the anaerobic threshold and then further, until subjects were completing maximal aerobic workloads. The resistance training program included exercises for all major muscle groups with a focus on the lower extremities. During the first two weeks, training was performed as a circuit using low intensities. Thereafter, protocols aiming for muscle hypertrophy and maximal and explosive strength were performed. During the second half of the study, both training volume and frequency were increased. The overall duration of the strength training sessions was 30–50 min.

2.7. Statistical Analysis

The main purpose of the present study was to determine if dietary acid intake has an effect on the primary outcome variable: acid–base status. NAE, capillary pH and capillary HCO_3^- were analyzed to identify the possible differences in acid–base status. The secondary outcome of the study was to assess kidney function, measured by GFR, the serum urea-to-creatinine ratio (UCR) and serum urea. The effect of a 12-week intervention period on blood and urine variables, and variables of dietary intake analysis were examined by a two-way repeated measures analysis of variance (ANOVA). If a statistically significant difference was observed within one of the diet groups, or between groups, the comparison was continued with a suitable *t*-test. Data are presented as means ± SDs. The statistical difference was considered to be significant at the $p < 0.05$ level.

3. Results

3.1. Diets

Dietary intake data are presented in Tables 2 and 3. There were no significant differences in energy or macronutrient intakes within or between the diet groups, except in moderate-PRAL men, as their energy intake was significantly decreased from PRE to MID ($p = 0.027$). In both men and women, PRAL was significantly lower ($p \leq 0.001$ in all) and the intake of fruits and vegetables (IFV) were higher ($p \leq 0.017$ in all) in low-PRAL compared to moderate-PRAL at MID and POST. In low-PRAL men and women, PRAL was significantly lower ($p \leq 0.005$ in all) and IFV higher ($p \leq 0.06$ in all) at MID and POST, compared to PRE. There were no significant differences in PRAL and IFV between MID and POST in any of the groups.

Table 2. Dietary intake data in men before (PRE), in the middle (MID) and after (POST) the 12-week diet period.

Parameters	Low-PRAL			Moderate-PRAL		
	PRE	MID	POST	PRE	MID	POST
PRAL (mEq/day)	23 ± 32	−41 ± 24 †††	−37 ± 24 ††	8.1 ± 16	10 ± 12 ***	11 ± 17 **
IFV (g/day)	250 ± 140	900 ± 300	800 ± 380	300 ± 250	250 ± 250 ***	230 ± 100 *
Energy(kcal/day)	2670 ± 910	1930 ± 570 †	1930 ± 520	2220 ± 630	2210 ± 650	2180 ± 690
Protein (g/kg/day)	1.5 ± 0.8	1.0 ± 0.3	1.1 ± 0.2	1.3 ± 0.5	1.4 ± 0.5	1.4 ± 0.4
CHO (g/kg/day)	3.4 ± 1.6	2.2 ± 1.0	2.5 ± 1.7	2.9 ± 0.9	3.2 ± 0.9	3.1 ± 0.9
Fat (g/kg/day)	1.2 ± 0.5	0.8 ± 0.3	0.8 ± 0.3	1.2 ± 0.4	1.2 ± 0.4	0.9 ± 0.4

CHO, carbohydrates; IFV, intake of fruits and vegetables. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant difference between low- and moderate-PRAL at POST. † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ statistically significant difference between PRE and MID or PRE and POST in low-PRAL (two-way repeated measures ANOVA, a paired or independent *t*-test).

Table 3. Dietary intake data in women before (PRE), in the middle (MID) and after (POST) the 12-week diet period.

Parameters	Low-PRAL			Moderate-PRAL		
	PRE	MID	POST	PRE	MID	POST
PRAL (mEq/day)	−7.2 ± 18	−51 ± 19 †††	−56 ± 40 ††	−2.9 ± 11	3.6 ± 11 ***	−0.8 ± 17 ***
IVF (g/day)	400 ± 200	930 ± 310	1070 ± 630	250 ± 80	210 ± 160 ***	260 ± 270 ***
Energy (kcal/day)	2010 ± 380	1880 ± 360	1860 ± 500	1900 ± 280	1990 ± 580	1870 ± 340
Protein (g/kg/day)	1.3 ± 0.5	1.1 ± 0.2	1.1 ± 0.3	1.2 ± 0.2	1.4 ± 0.4	1.1 ± 0.2
CHO (g/kg/day)	3.6 ± 0.8	3.6 ± 0.8	3.8 ± 1.3	3.6 ± 0.7	3.8 ± 1.6	3.2 ± 0.8
Fat (g/kg/day)	1.3 ± 0.3	1.1 ± 0.4	1.0 ± 0.8	1.1 ± 0.4	1.2 ± 0.5	1.0 ± 0.2

CHO, carbohydrates; IFV, intake of fruits and vegetables. *** $p < 0.001$ statistically significant difference between low- and moderate-PRAL at POST. †† $p < 0.01$, ††† $p < 0.001$ statistically significant difference between PRE and MID or PRE and POST in low-PRAL (two-way repeated measures ANOVA, a paired or independent *t*-test).

3.2. Body Composition of the Subjects

There were no significant changes in body mass, total lean mass or fat% in either of the subject groups over the 12-week study period (Table 4).

Table 4. Body composition before (PRE), and after (POST) the 12-week diet period in low-PRAL and moderate-PRAL diet groups.

Parameters	Men				Women			
	Low-PRAL		Mod-PRAL		Low-PRAL		Mod-PRAL	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Body mass (kg)	85.5 ± 9.8	83.7 ± 9.5	79.2 ± 10.2	79.6 ± 9.8	64.3 ± 7.8	63.8 ± 7.9	67.0 ± 11.1	67.9 ± 11.5
Lean mass (kg)	61.5 ± 5.6	61.3 ± 5.2	56.1 ± 4.8	57.2 ± 5.8	41.2 ± 3.4	41.5 ± 2.6	40.9 ± 4.8	41.7 ± 4.9
Fat %	23.9 ± 7.4	22.0 ± 7.9	25.3 ± 6.9	23.8 ± 5.9	31.0 ± 7.0	30.4 ± 6.6	33.2 ± 9.3	33.8 ± 9.0

3.3. Urine and Blood Acid-Base Status

NAE was lower in low-PRAL compared to moderate-PRAL at POST in both men ($p = 0.001$) and women ($p = 0.047$) (Figure 1). There were no statistically significant changes in urine pH, which was estimated with the pH test strips over the study period, in either of the subject groups. The urine strip results are not presented.

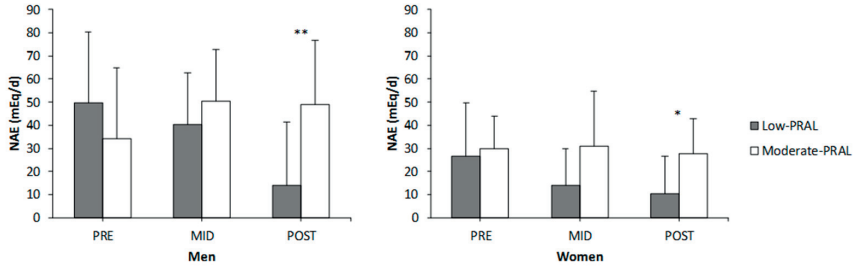


Figure 1. Net acid excretion (NAE) in the low-PRAL and the moderate-PRAL diet groups before (PRE), in the middle (MID) and after (POST) the 12-week diet period. * $p < 0.05$, ** $p < 0.01$ statistically significant difference between the diet groups at POST (two-way repeated measures ANOVA, an independent *t*-test).

For capillary pH, there were no significant differences between the diet groups (Figure 2). In low-PRAL women, pH was significantly lower ($p = 0.014$) at 35% and higher ($p = 0.020$) at 75% of VO_{2max} at POST compared to PRE. In moderate-PRAL women, pH was significantly lower at 55% ($p = 0.033$) of VO_{2max} at POST compared to PRE.

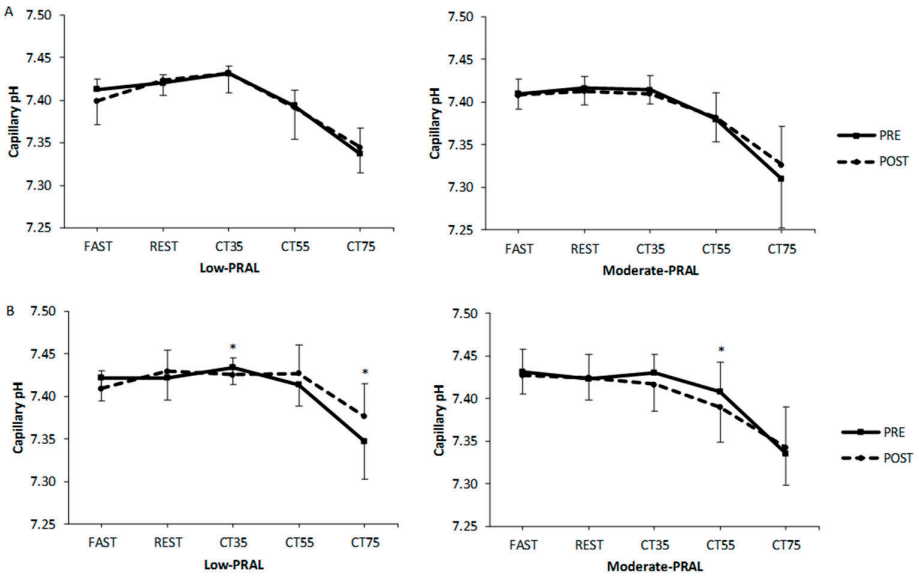


Figure 2. Capillary pH in men (**A**) and women (**B**) before (PRE) and after (POST) the 12-week diet period at rest (FAST, REST) and during submaximal cycling (CT35, CT55, CT75; CT = cycling test). * $p < 0.05$, statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired *t*-test). Values are mean ± SD.

In low-PRAL women, HCO_3^- was higher ($p = 0.006$) at 75% of VO_{2max} at POST compared to PRE (Figure 3). In moderate-PRAL men, HCO_3^- was higher ($p = 0.002$) at 75% of VO_{2max} after the training period compared to PRE. The only significant differences in HCO_3^- between the diet groups occurred in men, as HCO_3^- was higher at FAST, REST and during cycling at 35% of VO_{2max} in low-PRAL compared to moderate-PRAL ($p = 0.015$, $p = 0.039$, $p = 0.010$, respectively).

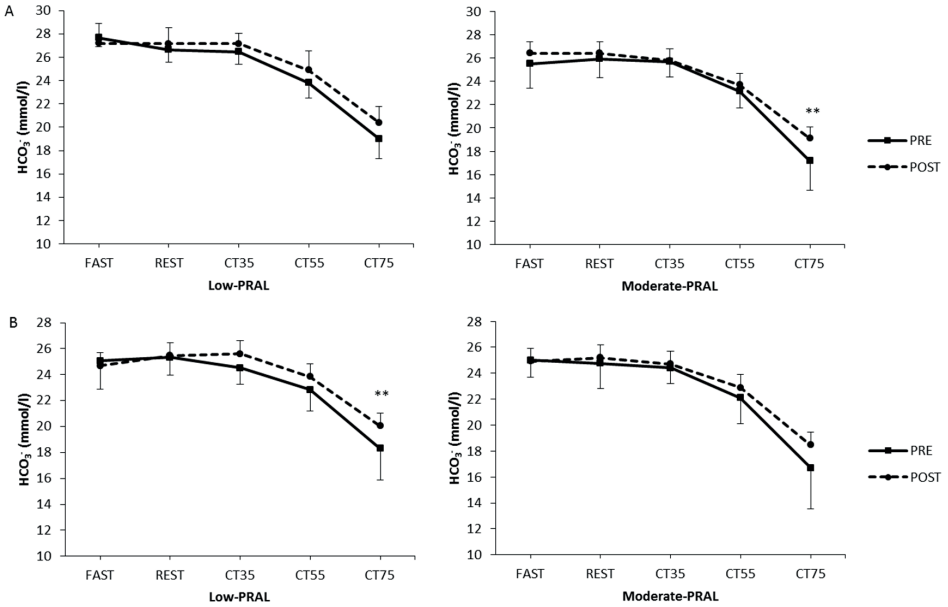


Figure 3. Capillary bicarbonate in men (**A**) and women (**B**) before (PRE) and after (POST) the 12-week diet period at rest (FAST, REST) and during submaximal cycling (CT35, CT55, CT75). ** $p < 0.01$, statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired *t*-test).

3.4. Renal Function

GFR decreased in the moderate-PRAL men ($p = 0.009$) and women ($p = 0.036$) over the dietary intervention (Figure 4). There were no significant changes in the low-PRAL groups over the study period.

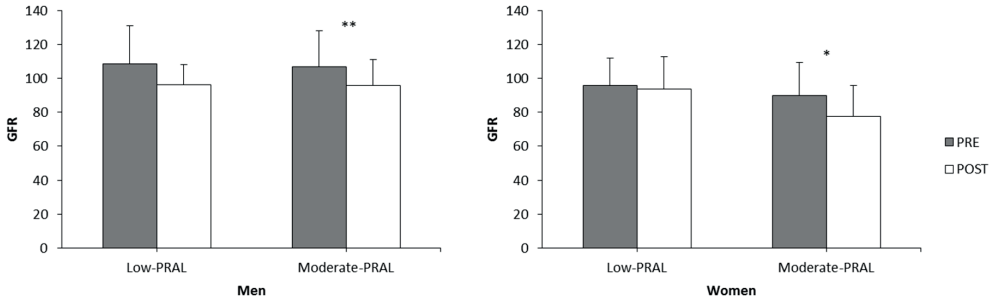


Figure 4. Glomerular filtration rate (GFR) in the low-PRAL and moderate-PRAL groups before (PRE) and after (POST) the 12-week diet period. * $p < 0.05$, ** $p < 0.01$ statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired *t*-test).

Serum urea decreased significantly in the low-PRAL men ($p = 0.037$) and women ($p = 0.013$) (Figure 5). Also, the serum urea to creatinine ratio decreased over the low-PRAL diet period in both men ($p = 0.030$) and women ($p = 0.016$) (Figure 5). No significant changes were observed in the moderate-PRAL diet groups for either of the variables.

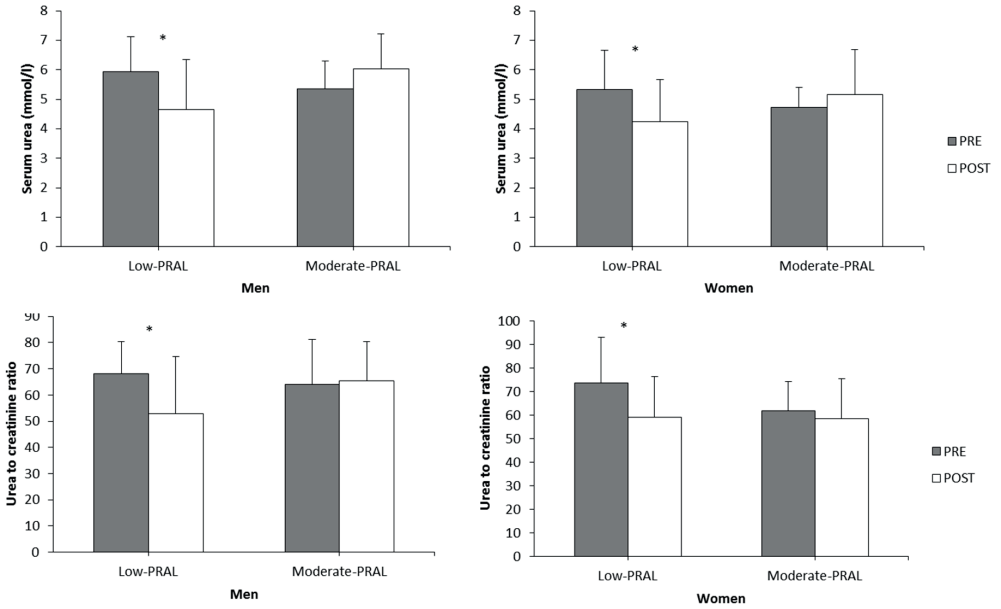


Figure 5. Serum urea and urea to creatinine ratio in the low-PRAL and moderate-PRAL groups before (PRE) and after (POST) the 12-week diet period. * $p < 0.05$ statistically significant difference between PRE and POST within a diet group (two-way repeated measures ANOVA, a paired *t*-test).

4. Discussion

In the present study, recreationally active, healthy men and women followed either a low-PRAL or a moderate-PRAL diet for 12 weeks and participated in same session of combined endurance and strength training twice a week. Net acid excretion (NAE) was significantly lower after 12 weeks of lower dietary acid intake compared to higher acid intake, both in men and women. There were no significant differences in urine or capillary pH or capillary bicarbonate between the diet groups after the study period. However, in low-PRAL women, capillary pH and bicarbonate were significantly higher at 75% of VO_{2max}, whereas in moderate-PRAL women, capillary pH was significantly lower at 55% of VO_{2max} after the study period compared to at baseline. According to the estimated glomerular filtration rate (GFR), kidney function was significantly decreased over the study period in both moderate-PRAL men and women. There was no change in serum urea or the urea-to-creatinine ratio (UCR) in the moderate-PRAL participants, whereas in the low-PRAL groups, these blood variables decreased over the 12-week intervention. These results suggest that even slightly acidogenic diets and regular training together may lead to an increased acid load to the body and start to impair kidney function in recreationally active subjects. However, these results should be interpreted with caution since there was some variation in energy intake and in the body composition, at least in the males, during the study period.

The only significant difference in the diet composition between the low-PRAL and moderate-PRAL groups was in the consumption of fruits and vegetables. This strengthens the fact that intake of fruits and vegetables is an important factor affecting dietary acid load and net acid excretion of the body. There were clear differences in NAE between the diet groups in both men and women. Urine pH was at higher level in low-PRAL as compared to moderate-PRAL, in both men and women, after the diet intervention, but the differences were not statistically significant. However, urine pH was not measured; rather, it was determined with test strips which provide only rough estimates of pH. It has been previously shown that urine and capillary pH can change acutely due to the dietary acid intake that has been estimated with PRAL [4]. There were no differences in capillary pH between the

diet groups, but inside both female groups there were some differences during submaximal exercise between PRE and POST. It was observed that in low-PRAL women, capillary pH was higher at the two highest exercise intensities after the diet and training intervention, whereas in moderate-PRAL women, the blood pH was more acidic at the two lowest exercise intensities after the study period. These data increase the body of evidence showing that diet-induced changes in acid–base status are small yet viable. In spite of powerful regulatory mechanisms, which ensure that there do not appear to be large changes in acid–base balance, it seems that these regulatory mechanisms do not keep pH and HCO_3^- at a static level; rather, some variations can occur, based on the daily diet- and exercise-induced acid loads that the body confronts. On the other hand, in the study of Wesson and Simoni [16] it was demonstrated that rats with lower kidney masses were not able to excrete the same acid load in their urine, while, at the same time, renal tissue acid levels were higher, with no differences in blood acid–base levels between the two groups. Our data supports the idea that even though NAE changes, it does not necessarily reflect the changes in urine and blood pH. It has been speculated that some of the increased H^+ is bound predominantly to intracellular body buffers [17]. This would also contribute to the fact that women seem to be more sensitive to the changes in dietary acid load than men, who have larger muscle masses and thus, larger tissue buffering capacities. However, for those who have an intact renal functional capacity, constant intake of alkaline products might be beneficial by providing a greater reserve to buffer high acid loads. This might be important, for example, for exercise performance and, in particular, for the elderly, who have diminished renal functional capacities [11,18].

The moderate-PRAL diet of the present study represented a typical diet for many Westernized cultures; it contained animal protein and cereal grains and was quite deficient in vegetables and fruits—a type of a diet that may increase the acid load of the body [3]. Also exercise training acutely impacts the acid–base status and after the capacity of chemical buffers and ventilation is surpassed, the kidneys need to excrete acids, neutralize acids and/or excrete anions to maintain the acid–base balance [19]. In the present study, GFR decreased in the moderate-PRAL groups of both men and women over the 12-week intervention, which could be due to the increased acid load from both the diet and exercise training. There has been some debate about whether diet composition could affect renal function over a longer period of time. In particular, a high intake of protein has been suggested to potentially impair kidney function. In individuals with moderate to severe renal insufficiency, a low protein intake may slow renal function decline, but the long-term impact of protein intake on kidney function in individuals with normal kidney function or mild renal insufficiency is unknown [20]. A recent paper by Møller et al. [21] reported no association between ahigher protein intake and decreased kidney function in pre-diabetic older adults during a one-year intervention. In a study by Antonio et al. [22], high protein intake (2.5 g/kg/day) was compared to higher intake (~3.3 g/kg/day), and it was reported that these high protein diets were not deleterious for kidney function over a one-year crossover study period. However, in the present study, protein intake did not differ between the diet groups, suggesting that not only the protein intake, but also the dietary acid load, should be considered as factors that may affect renal function. This was also proposed by So et al. [23], who showed that dietary acid load might be a better indicator of the changes in renal function associated with the habitual dietary pattern than merely the total amount of dietary protein. In addition to the effects of dietary acid load on blood and urine pH, a high dietary acid intake has been reported to be associated with faster decline in kidney function in kidney patients, which deteriorates the capacity of the kidneys to handle acids and may further increase the acidity of the body [24]. The aging population is likely more prone to increased dietary acid loads, but the results of the present study suggest that reducing the acid load in younger, physically active populations could be beneficial for kidney function. At present, the very long-term effects of high acid load in healthy populations are not known.

When assessing kidney function via GFR, it has to be kept in mind that serum creatinine can be affected by dietary protein intake and the lean mass of the body [8]. One of the limitations of the present study is that the CKD-EPI equation used to evaluate GFR does not take into account the effect of muscle mass on serum creatinine, which is a breakdown product of creatine phosphate in the muscle.

In the moderate-PRAL group, it seems that the lean mass was slightly increased over the 12-week intervention, which may have had an impact on serum creatinine and thus, on GFR. However, these changes were not significant and, furthermore, there was no correlation between serum creatinine and lean muscle mass in either of the subject groups. Nonetheless, serum urea and UCR were decreased in the low-PRAL group, and they remained unchanged in the moderate-PRAL group, both in men and women, even though there were no changes in protein intake within or between the diet groups. This supports the idea that the changes observed in GFR were not solely due to the changes in the lean mass of the subjects. On the other hand, it shows that markers that have been considered to be markers of protein intake and metabolism can change while the protein intake remains stable and highlights the importance of dietary acid load for kidney function. However, cystatin C, a more stable marker that is less affected by muscle mass, should be considered as a potential replacement for serum creatinine for assessing GFR in future studies [8]. Moreover, to confirm the results of the present study, the long-term effects of dietary acid load should be studied with larger sample sizes and with a longer duration, since 12 weeks is a short period of time to investigate changes in kidney function. In addition, one of the limitations of the present study was the decreased energy intake in the group of low-PRAL men over the study period.

In conclusion, 12-week low or moderate dietary acid intakes showed some varying effects on urine and blood acid–base status and kidney function in healthy, recreationally active men and women. NAE was significantly lower after 12 weeks of low dietary acid intake compared to moderate acid intake both in men and women, but the effect of a low dietary acid intake on blood acid–base status was apparent only for women. GFR decreased in the moderate-PRAL groups over the 12-week intervention, which suggests that a slightly acidogenic diet and regularly training together may increase the acid load of the body and start to impair kidney function. These results emphasize the importance of adequate intake of fruits and vegetables as a part of a healthy diet and a physically active lifestyle. In future studies, dietary acid intake should be taken into account when investigating the combined effects of diet and exercise on health.

Acknowledgments: This study was funded by TEKES (Finnish Funding Agency for Technology and Innovation) and in collaboration with TEKES by following Finnish companies: Honkatarhat Ltd., Honkajoki; Kyröntarhat Ltd., Honkajoki; Mykora Ltd., Honkajoki; Lihajaloste Korpela Ltd., Huittinen; Laihian Mallas Ltd., Laihia; KKK-Vihannes Ltd./LykoBEne, Honkajoki. This study was also funded by Ministry of Education and Culture of Finland.

Author Contributions: E.-M.H., M.S., H.P., K.H. and A.A.M. designed the experiments; J.K.I., M.S. and D.E. performed the experiments; E.-M.H. analyzed the data; E.-M.H., L.A.F. and A.A.M. participated in the interpretation of data; E.-M.H., J.K.I., L.A.F. and M.S. wrote the paper. All authors revised the manuscript and approved the submitted version.

Conflicts of Interest: Hannu Pitkänen has a commercial association with Honkatarhat Ltd., Kyröntarhat Ltd. and Mykora Ltd. He accepts full responsibility for the implementation of this study. Other authors declare that they have no conflict of interest.

References

1. Poupin, N.; Calvez, J.F.; Lassale, C.F.; Chesneau, C.F.; Tome, D. Impact of the diet on net endogenous acid production and acid-base balance. *Clin. Nutr.* **2012**, *31*, 313–321. [CrossRef] [PubMed]
2. Remer, T.; Dimitriou, T.; Manz, F. Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. *Am. J. Clin. Nutr.* **2003**, *77*, 1255–1260. [CrossRef] [PubMed]
3. Adeva, M.M.; Souto, G. Diet-induced metabolic acidosis. *Clin. Nutr.* **2011**, *30*, 416–421. [CrossRef] [PubMed]
4. Hietavala, E.M.; Stout, J.R.; Hulmi, J.J.; Suominen, H.; Pitkänen, H.; Puurtinen, R.; Selänne, H.; Kainulainen, H.; Mero, A.A. Effect of diet composition on acid-base balance in adolescents, young adults and elderly at rest and during exercise. *Eur. J. Clin. Nutr.* **2015**, *69*, 399–404. [CrossRef] [PubMed]
5. Hamm, L.L.; Nakhoul, N.; Hering-Smith, K.S. Acid-base homeostasis. *Clin. J. Am. Soc. Nephrol.* **2015**, *10*, 2232–2242. [CrossRef] [PubMed]
6. Mueller, P.J.; O'Hagan, K.P.; Skogg, K.A.; Buckwalter, J.B.; Clifford, P.S. Renal hemodynamic responses to dynamic exercise in rabbits. *J. Appl. Physiol.* **1998**, *85*, 1605–1614. [CrossRef] [PubMed]

7. Baxmann, A.C.; Ahmed, M.S.; Marques, N.C.; Menon, V.B.; Pereira, A.B.; Kirsztajn, G.M.; Heilberg, I.P. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin. J. Am. Soc. Nephrol.* **2008**, *3*, 348–354. [CrossRef] [PubMed]
8. Suzuki, M. Physical exercise and renal function. *J. Phys. Fit. Sports Med.* **2015**, *4*, 17–29. [CrossRef]
9. Scrutinio, D.; Agostoni, P.; Gesualdo, L.; Corrà, U.; Mezzani, A.; Piepoli, M.; Di Lenarda, A.; Iorio, A.; Passino, C.; Magrì, D. Renal function and peak exercise oxygen consumption in chronic heart failure with reduced left ventricular ejection fraction. *Circ. J.* **2015**, *79*, 583–591. [CrossRef] [PubMed]
10. Morales, A.P.; Sampaio-Jorge, F.; da Cruz Rangel, L.F.; de Souza Menezes, J.; Leite, T.C.; Ribeiro, B.G. Cardiopulmonary Performance During Maximal Exercise in Soccer Players with Alterations in Renal Function. *J. Hum. Kinet.* **2017**, *57*, 131–140. [CrossRef] [PubMed]
11. Hietavala, E.; Stout, J.R.; Frassetto, L.A.; Puurtinen, R.; Pitkänen, H.; Selänne, H.; Suominen, H.; Mero, A.A. Dietary acid load and renal function have varying effects on blood acid-base status and exercise performance across age and sex. *Appl. Physiol. Nutr. Metab.* **2017**, *42*, 1330–1340. [CrossRef] [PubMed]
12. Remer, T.; Manz, F. Potential renal acid load of foods and its influence on urine pH. *J. Am. Diet. Assoc.* **1995**, *95*, 791–797. [CrossRef]
13. Levey, A.S.; Stevens, L.A.; Schmid, C.H.; Zhang, Y.L.; Castro, A.F.; Feldman, H.I.; Kusek, J.W.; Eggers, P.; Van Lente, F.; Greene, T. A new equation to estimate glomerular filtration rate. *Ann. Int. Med.* **2009**, *150*, 604–612. [CrossRef] [PubMed]
14. Schumann, M.; Küüsmaa, M.; Newton, R.U.; Sirparanta, A.; Syväoja, H.; Häkkinen, A.; Häkkinen, K. Fitness and lean mass increases during combined training independent of loading order. *Med. Sci. Sports Exerc.* **2014**, *46*, 1758–1768. [CrossRef] [PubMed]
15. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2013.
16. Wesson, D.E.; Simoni, J. Increased tissue acid mediates a progressive decline in the glomerular filtration rate of animals with reduced nephron mass. *Kidney Int.* **2009**, *75*, 929–935. [CrossRef] [PubMed]
17. Wesson, D.E.; Simoni, J.; Broglio, K.; Sheather, S. Acid retention accompanies reduced GFR in humans and increases plasma levels of endothelin and aldosterone. *Am. J. Physiol. Ren. Physiol.* **2011**, *300*, F830–F837. [CrossRef] [PubMed]
18. Bolignano, D.; Mattace-Raso, F.; Sijbrands, E.J.; Zoccali, C. The aging kidney revisited: A systematic review. *Ageing Res. Rev.* **2014**, *14*, 65–80. [CrossRef] [PubMed]
19. Passey, C. Reducing the dietary acid load: How a more alkaline diet benefits patients with chronic kidney disease. *J. Ren. Nutr.* **2017**, *27*, 151–160. [CrossRef] [PubMed]
20. Knight, E.L.; Stampfer, M.J.; Hankinson, S.E.; Spiegelman, D.; Curhan, G.C. The impact of protein intake on renal function decline in women with normal renal function or mild renal insufficiency. *Ann. Int. Med.* **2003**, *138*, 460–467. [CrossRef] [PubMed]
21. Møller, G.; Rikardt Andersen, J.; Ritz, C.; P Silvestre, M.; Navas-Carretero, S.; Jalo, E.; Christensen, P.; Simpson, E.; Taylor, M.; Martinez, J.A. Higher Protein Intake Is Not Associated with Decreased Kidney Function in Pre-Diabetic Older Adults Following a One-Year Intervention—A Preview Sub-Study. *Nutrients* **2018**, *10*, 54. [CrossRef] [PubMed]
22. Antonio, J.; Ellerbroek, A.; Silver, T.; Vargas, L.; Tamayo, A.; Buehn, R.; Peacock, C.A. A high protein diet has no harmful effects: A one-year crossover study in resistance-trained males. *J. Nutr. Metab.* **2016**, *2016*, 9104792. [CrossRef] [PubMed]
23. So, R.; Song, S.; Lee, J.E.; Yoon, H. The Association between Renal Hyperfiltration and the Sources of Habitual Protein Intake and Dietary Acid Load in a General Population with Preserved Renal Function: The KoGES Study. *PLoS ONE* **2016**, *11*, e0166495. [CrossRef] [PubMed]
24. Goraya, N.; Wesson, D.E. Acid–base status and progression of chronic kidney disease. *Curr. Opin. Nephrol. Hypertens.* **2012**, *21*, 552–556. [CrossRef] [PubMed]