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ORIGINAL ARTICLE

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

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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.

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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

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(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.

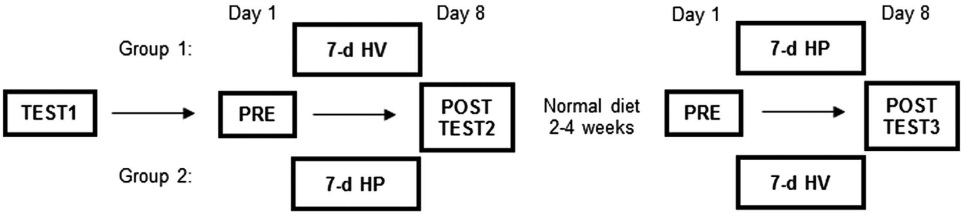

Figure 1. The study design. After the cycle ergometer test (VO_2max) at baseline (TEST1), the subjects were divided into two groups who followed both HV and HP. Blood and 12-h urine samples were collected PRE and POST the diet periods. Cycle ergometer tests (TEST2, TEST3; 3 × 10 min at 35, 55, 75% and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

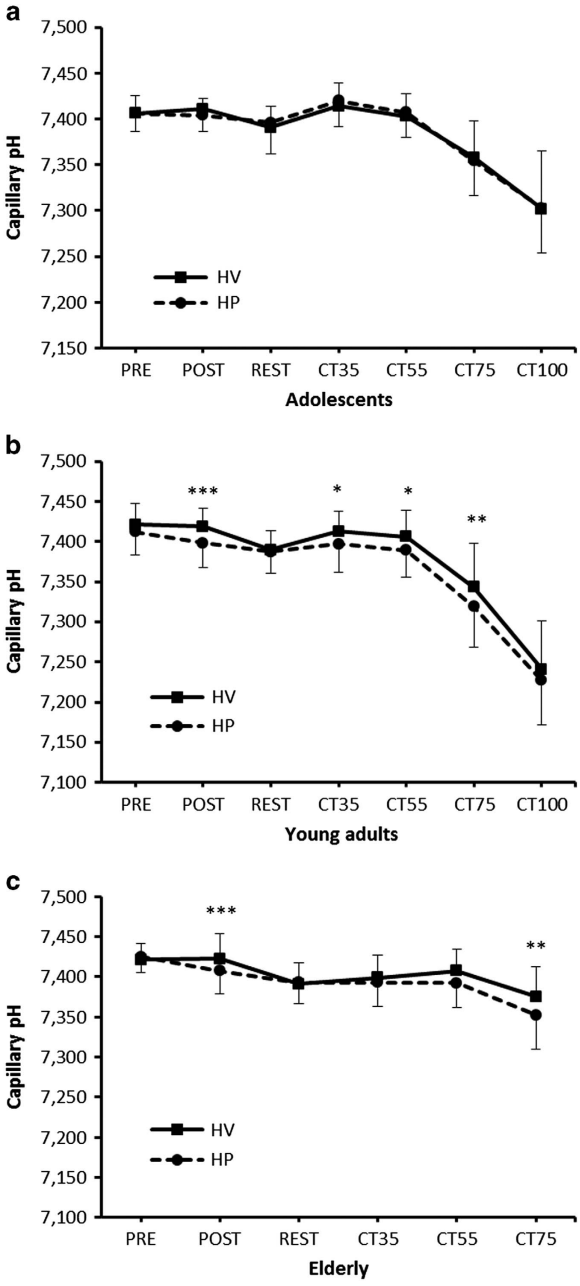

Figure 2. Capillary pH. C-pH in ADO (**a**), YAD (**b**) and ELD (**c**) PRE and POST the 7-day HV and the 7-day HP, REST and during exercise, wherein 10 min at 35, 55 and 75% of VO_2max were cycled (CT35, CT55 and CT75). ADO and YAD cycled additionally at 100% of VO_2max until exhaustion (CT100). $^*P < 0.05$, $^{**}P < 0.01$ 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.

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

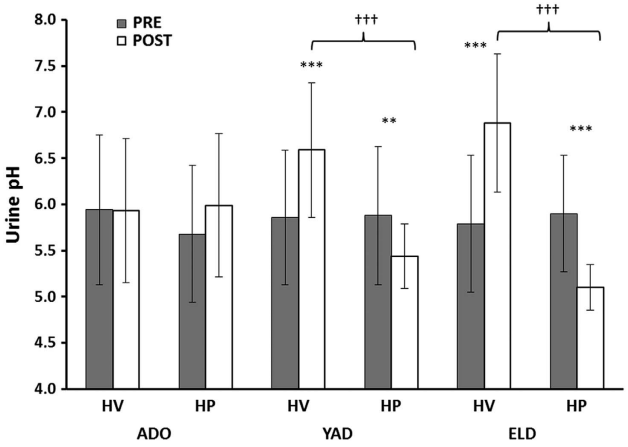

Figure 3. Urine pH. U-pH in ADO, YAD and ELD PRE and POST the 7-day HV and the 7-day HP. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate statistically significant differences between HV and HP inside each age group (one-way analysis of variance (ANOVA), a paired sample *t*-test); $^{\dagger\dagger\dagger}P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

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.

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