Modified Atkins diet modifies cardiopulmonary exercise characteristics and promotes hyperventilation in healthy subjects

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ABSTRACT

Background: Low-carbohydrate diets, including the modified Atkins diet (mAD), are commonly used to promote weight loss, improve exercise performance, and treat refractory epilepsy and inherited metabolism disorders. However, the effects of the high-fat-low-carbohydrate diet on the physiology of healthy subjects still need further study. We evaluated the physiological influence of mAD on cardiopulmonary exercise results in healthy adult subjects.

Materials and methods: Ten healthy volunteers followed mAD for four weeks with laboratory follow-up. Cardiopulmonary exercise tests were performed before, and at the end of mAD, and venous lactate, ammonia, and blood gases were collected before, during, and after exercise testing.

Results and conclusions: Four-week mAD decreased the subjects' mechanical efficiency in the cardiopulmonary exercise test and caused increased ventilation and decreased fraction of expired CO2 in maximal exercise. This evidence suggests that mAD can cause hyperventilation tendency at least in the short term, a possible adverse effect of the diet.

1. Introduction

Low-carbohydrate diets, including ketogenic diets and the modified Atkins diet (mAD), have become popular, especially for their weight-reducing effects. However, high-fat-low-carbohydrate diets have previously only been shown to be effective in short-term weight loss (Paoli, Rubini, Volek, & Grimaldi, 2013). As the side effects of the diets may include the increase of low-density lipoprotein (LDL), an increased risk for the ischaemic cardiac disease has been raised as a potential concern (Buono, Clancy, & Cook, 1984; Foster et al., 2003; Goday et al., 2016; Mahdi, 2006; O'Neill & Raggi, 2020). In addition to weight loss, ketogenic diets and mAD are used in treatment-resistant epilepsy in both children and adults (D'Andrea Meira et al., 2019; Kossoff & Dorward, 2008; Liu et al., 2018; Park, Lee, & Lee, 2018), although the exact mechanisms of the effect are not quite clear. Low-carbohydrate diets have also been studied as a possible treatment in other diseases, including mitochondrial myopathies lacking specific therapy (Ahola et al., 2016). Application of ketogenic diets in treatment, especially in metabolic muscle diseases (Finsterer, 2018) and dystrophic disorders (Heydemann, 2018), have been attempted. In McArdle's disease (GSDV) (Busch et al., 2005; Løkken et al., 2020) and phosphofructokinase deficiency (GSDVII or Tarui disease), there is a suggestion of a benefit to

a low carbohydrate diet on muscular symptoms and function (Similä, Auranen, & Piirilä, 2020; Swoboda et al., 1997).

Low carbohydrate intake has been associated with a compensatory increase in fat oxidation (Tiller et al., 2019; Zajac et al., 2014). One of the diet benefits might be the improvement of body composition (greater percent lean body mass) (Kaspar, Austin, Huecker, & Sarav, 2019; Zajac et al., 2014). Because of the metabolic benefits, there has been a lot of interest in the effects of low-carbohydrate diets on exercise performance, especially in athletes. The effects of low-carbohydrate diets on exercise performance of highly trained athletes in different, often long-duration sports, including ultramarathons and off-road cycling, have been extensively studied (Babij, Matthews, & Rennie, 1983; Burke et al., 2017; Carr et al., 2018; Cipryan, Plews, Ferretti, Maffetone, & Laursen, 2018; Goedecke et al., 1999; Harvey, Holcomb, & Kolwicz, Stephen C, Jr, 2019; McSwiney, Doyle, Plews, & Zinn, 2019; Rowlands & Hopkins, 2002; Zajac et al., 2014). However, the results of the benefits of low-carbohydrate diets on oxygen uptake and cardiorespiratory performance in sports have been inconclusive, and no significant improvement in exercise performance has been proven (Babij et al., 1983; Burke et al., 2017; Cipryan et al., 2018; Goedecke et al., 1999; Harvey, Holcomb, & Kolwicz, 2019; McSwiney et al., 2019; Rowlands & Hopkins, 2002; Zajac et al., 2014). These studies have usually utilized a combination of both the use of a low carbohydrate diet and exercise training (Cipryan et al., 2018; Kaspar et al., 2019; Zajac et al., 2014)

Because of the wide use of low carbohydrate diets, including mAD, it is important to know the physiological exercise responses to the diet. In most previous studies concentrating on the exercise response, the subjects have been highly trained athletes, or the studies have included a specific training-plan. The aim of the present study was to determine the normal physiological response of mAD on cardiopulmonary exercise testing with breath gas analysis as well as on venous blood gases and lactate and ammonia levels in healthy adult subjects.

2. Methods

2.1. Participants and the diet

The study subjects were ten healthy nonsmoking volunteers, six male and four female, who acted as controls in a study on the therapeutic effects of mAD on mitochondrial myopathy. The controls' physiological responses to mAD have not been reported earlier. All the subjects gave their written informed consent to participate in the study. The study was undertaken according to the Helsinki declaration. Helsinki University Hospital's ethics review board has approved the study (reference number 33/13/03/01/2011).

The subjects' mean age was 49.4 (range 35–61) years and the mean weight 74.1 (range 60–104) kg. Their spirometry results were normal (Table 1). Each subject's initial energy intake was determined with a food diary. Normalized isocaloric standard diet (ND) was introduced to all study subjects for two weeks, after which they were gradually switched to mAD one meal per day. Before mAD, the subjects' median energy intake was 1901 (SD 429) kcal, 43 (SD 8) % of which came from carbohydrates, 18 (SD 5) % from proteins, and 34 (SD 7) % from fat. During mAD, carbohydrate consumption was limited to 3–9% percent of daily energy intake. During the two last weeks of mAD, the total mean energy intake was 2194 (SD 5 6 4) kcal, of which 4 (SD 1)% were carbohydrates, 26 (SD 3)% proteins, and 69 (SD 4)% fat. The subjects received diet-conforming meals from the hospital and continued the diet for four weeks. To ensure adherence to the diet, ketosis was determined by serum/plasma β-hydroxybutyrate testing (target level 2.5–5 mmol/L) measured weekly from fasting blood samples during the diet(Ahola et al., 2016).

Table 1

The anthropometric values and the results of the cardiopulmonary exercise before and at the end of the modified Atkins (mAD) diet. The p-values of paired tests are given. (FVC = forced vital capacity, FEV1 = Forced expiratory volume in one second, RER = Respiratory exchange rate (V′CO2/V′O2), V′E = minute ventilation, Wmax3 = Maximal power during last three maximal minutes of exercise, V′O2max = Maximal oxygen uptake, FETCO2 = Fraction of end-tidal CO2, V′O2/HR = Oxygen pulse, V′E/V′O2 = Ventilatory equivalent to O2, V′E/V′CO2 = Ventilatory equivalent to CO2.

	normal values	Before mAD		At the end of 4 weeks on mAD		
		Mean	Standard deviation	Mean	Standard deviation	p-value
Age (years)		**49.4**	8.7			
Height (cm)		**172.2**	9.81			
Weight (kg)		**74.1**	13.8	**72.0**	13.3	**0.007**
FVC (% of pred.)	≥80	**100.5**	2.3			
FEV1 (% of pred.)	≥80	**97.8**	8.9			
REST						
Systolic blood pressure, rest (mmHg)		**121.5**	12.2	**118**	11.2	0.213
Diastolic blood pressure, rest (mmHg)		**75.8**	7.9	**75.6**	5.6	0.799
Heart rate (1/min)		**66.8**	8.3	**74.1**	11.2	0.085
Breathing frequency (1/min)		**12.3**	3.9	**15.1**	4.1	**0.024**
RER, rest		**0.75**	0.09	**0.73**	0.04	0.507
V′E, rest (L/min)		**8.87**	2,01	**9.91**	1.75	0.313
EXERCISE, maximal values						
Systolic pressure (mmHg)		**184.1**	23.2	**176.7**	27.2	0.238
Diastolic pressure (mmHg)		**84.0**	13.0	**87.1**	12.4	0.186
Heart rate (beat/min)		**169.8**	3.4	**178.0**	3.1	**0.001**
Heart rate (beat/age maximum)	>80	**94.1**	5.0	**98.9**	4.4	**0.001**
RER	>1	**1.15**	0.08	**1.09**	0.05	**0.015**
Breathing frequency, (1/min)	>35	**35.0**	5.6	**38.5**	7.04	0.062
V′E (L/min)		**87.3**	24.6	**94.9**	25.8	0.093
Anaerobic threshold (L/min)		**1.43**	0.48	**1.45**	0.45	0.402
Wmax/3 (W)		**175.2**	60,1	**171.8**	60,6	0.305
Wmax/3 min (% of predicted value)	≥80	**101.1**	25.6	**100.1**	21.6	0.792
V′O2max (L/min)		**2.5**	0.8	**2.6**	9.9	0.286
V′O2/kg (ml/min/kg)		**33.3**	8.0	**35.6**	9.2	0.096
Wmax/VO′2max (%)	≥80	**20.3**	1.6	**19.1**	0.86	**0.049**
V′O2/HR	≥85	**14.6**	4.3	**14.4**	4.7	0.258
V′E/V′CO2		**30.1**	2.7	**33.7**	3.8	**0.002**
FETCO2 (%)	4.5–6	**5.4**	0.3	**4.8**	0.4	

(continued on next page)

Table 1 (*continued*)

	normal values	Before mAD		At the end of 4 weeks on mAD		
		Mean	Standard deviation	Mean	Standard deviation	p-value
Tidal volume (L)	2.5	**2.5**	0.75	**2.59**	0.98	**< 0.001** 0.569

2.2. Cardiopulmonary exercise testing with blood samples

Work-conducted maximal exercise test with gas exchange analysis (spiroergometry) was performed before the beginning of mAD (referred to as pre-mAD from here on) and at the end of the 4th week of mAD (referred to as end-of-mAD). The methods have been described previously (Ollila et al., 2017; Piirilä et al., 2016). For measurement of respiratory gases, a tightly secured facemask (Rudolph series 7910, Hans Rudolph) with a dead space of 185 ml was used. After about 10 min' rest, the subject sat on the bicycle, and the breath gas recording was started. One minute of rest breathing was recorded before cycling with the test subject sitting upright breathing through a breath-by-breath gas exchange analysis system (Vmax Encore, Sensormedics, Yorba Linda). Breathing gas recording continued through the exercise test, and 30-second mean values of breath gases were reported at the end of each exercise step. The exercise test was performed using an electrically braked bicycle ergometer (900 ERG Ergometer; Marquette Hellige, Marquette Medical Systems). The starting workload was 40 W for women and 50 W for men, and the load was increased on 3–minute intervals by 40 or 50 W, respectively. The exercise was continued until hard exertion (17–20/20 scale of perceived exertion) and the respiratory exchange rate (RER = V'CO2/V'O2) of at least 1.0 were reached.

Blood pressure was measured manually from the right arm using a stethoscope and a sphygmomanometer (Erka) before, during, and 3 and 6 min after exercise. A 12-lead ECG was continuously monitored and recorded during the exercise test using a digital ECG-device (Case12, Marquette). Mason-Likar leads were used during the exercise. Peripheral arterial oxygen saturation (SpO_2) was measured with two pulse oxymeters (Datex-Ohmeda 3900 and Datex-Ohmeda 3800; Datex-Ohmeda). The sensors were attached to the subject's earlobe giving a reliable signal and the left middle finger.

A cannula was inserted in a vein in the subject's left antecubital fossa. Through this, samples of venous blood gases (including HbO_2 and pCO_2), electrolytes (Na, K, Ca-Ion), lactate, and ammonia (NH4 +) were obtained before exercise, during light and maximal exercise and 2, 4, 6, 10, 20 and 30 min after exercise. Blood gas and electrolyte samples were analyzed using a Radiometer ABL800 analyzer (Radiometer Medical). Lactate and ammonia samples were centrifuged and analyzed with Cobas Integra 400 analyzer (Roche Diagnostics).

Plasma glucose, cholesterols, creatine kinase, alkaline phosphatase, alanine aminotransferase, glutamyl transferase, urea, bilirubin, glucose, β-hydroxybutyrate, and triglycerides were studied from fasting blood samples by standard laboratory methods.

2.3. Statistical analysis

Research data was analyzed using SPSS 25 statistics program. Most of the variables were determined to be normally distributed using the Shapiro-Wilk test. The significance of changes in normally distributed variables was determined using the paired *t*-test and in the non-normally distributed variables (mainly laboratory results in Table 2) using the Wilcoxon signed-rank test. The significance threshold for p-value was < 0.05. Breath gas variables were normally distributed, but the number of workload steps differed between patients. Therefore, paired t-tests for breath gas variables were performed on every exercise load

Table 2

The laboratory values before and at the end of mAD at 4 weeks.

Parameter	Baseline	SD	During mAD	SD	p-value
Blood leukocytes (E^9/L)	4.8	1.24	4.39	0.91	0.258
Blood erytrocytes (E^{12}/L)	4.5	0.39	4.39	0.31	0.079
Haemoglobin (g/L)	136.5	13.26	131.7	10.79	**0.024**
Haematokrite (%)	39.6	3.2	38.6	2.84	0.085
Blood trombocytes (E^9/L)	200.1	54.5	203.3	45.55	0.761
Serum alanine amino transpherase (U/L)	24.9	7.39	27.8	9.51	0.210
Serum asparatate amino transpherase (U/L)	27	5.01	28.2	6.63	0.367
Serum glutamyl transpherase (mmol/L)	24.1	16.8	20.4	12.71	**0.035**
Plasma cholesterol (mmol/L)	4.69	0.91	5.12	1.03	**0.003**
LDL cholesterol (mmol/L)	3.02	0.82	3.29	0.8	0.066
HDL cholesterol (mmol/L)	1.47	0.44	1.84	0.62	**0.009**
Serum triglycerides (mmol/L)	1.06	0.53	0.55	0.17	**0.006**
Serum creatine kinase (U/L)	120.4	52.6	116.9	43.75	0.796
Serum creatinine (μmol/L)	73.2	7.02	78.9	8.71	**0.024**
Serum urea (mmol/L)	5.62	1.32	8.01	1.17	0.000
Serum sodium (mmol/L)	140.9	2.18	140.5	2.8	0.657
Serum amylase (U/L)	60.3	15.85	56.3	18.58	0.301
Serum potassium (mmol/L)	3.84	0.18	4.1	0.24	**0.007**
Serum albumin bound calcium (mmol/L)	2.28	0.09	2.31	0.07	0.381
Serum calcium (mmol/L)	2.22	0.1	2.27	0.07	0.107
Albumin (g/L)	37.76	3.87	39.37	3.17	0.051
Lactate (mmol/L)	0.89	0.24	0.8	0.12	0.215
Pyruvate (μmol/L)	89.4	18.38	85.9	10.34	0.665
Plasma glucose (mmol/L)	5.54	0.55	5.23	0.32	**0.017**
Serum insulin (mU/L)	5.42	2.74	2.76	1.13	**0.005**
Plasma β-hydroxy butyrate (mmol/L)	0.125	0.036	0.4	0.14	**0.000**
Carnitine (μmol/L)	40.7	6.53	43.4	5.46	0.219

independently and at maximum exercise load.

3. Results

The subjects lost weight significantly during mAD (from 74.1 to 72.0 kg, p = 0.007), despite the isocaloric diet and no aim for weight-loss. There were no differences in resting heart rate or blood pressure, but breathing frequency at rest increased significantly end-of-mAD (p = 0.024) (Table 1).

3.1. Results of cardiopulmonary exercise testing

Table 1 summarizes the main results of cardiopulmonary exercise testing comparing pre-mAD and end-of-mAD results. The mechanical efficiency, Wmax/V'O2max, decreased (p = 0.049), although the decrease in maximal workload (Wmax3) and the increase in maximal oxygen uptake (V'O2max) themselves were not significant. During exercise, maximum heart rate was higher end-of-mAD compared to pre-mAD (p = 0.001). Respiratory exchange rate (RER), which is the ratio between the amount of carbon dioxide produced in metabolism and oxygen uptake (V'CO2/V'O2), decreased (p = 0.015), consistent with lipids being increasingly used as a fuel (Table 1).

Minute ventilation associated with exercise testing increased end-of-mAD compared to pre-mAD at both 4th and 5th exercise load (4th load p = 0.003 and 5th p = 0.001, Fig. 1) and also as tested only at maximal exercise (p = 0.040, Supplementary Fig. 1). The ventilatory equivalent for CO2 (V'E/V'CO2) increased at 4th load (p = 0.015, Fig. 2) and at maximal exercise (p = 0.002, Fig. 3), and the fraction of end-tidal CO2 (FetCO2) decreased at 4th load (p = 0.003, Fig. 4) and during maximal exercise (p < 0.001, Fig. 3). However, we found no significant change in ventilatory equivalent for O2 (VE/V'O2).

As the study was performed to examine exercise and muscle function

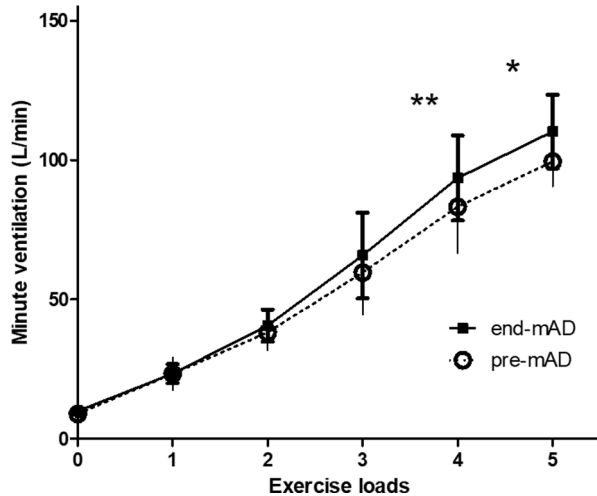**Minute ventilation during exercise test**

Fig. 1. Minute ventilation (V′E) associated with cardiopulmonary exercise test in all participants before and at the end of mAD (modified Atkins diet). The significant differences (*p < 0.05, **<0.01) are indicated with an asterisk.
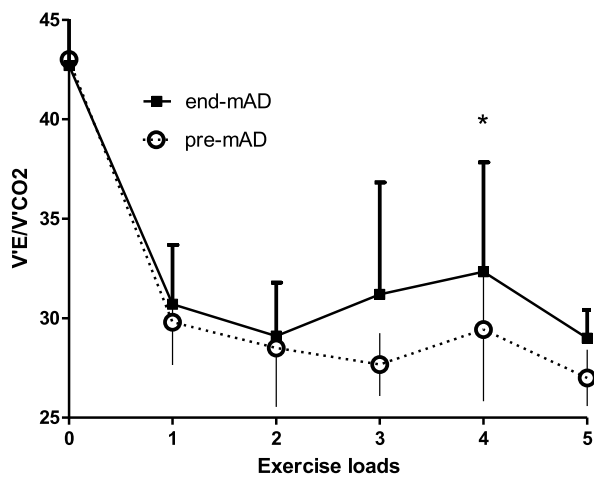
Ventilatory equvalent for CO2 during exercise test

Fig. 2. Minute ventilation related to V′CO2 (VE/V′CO2) associated with cardiopulmonary exercise before and at the end of mAD (modified Atkins diet). The significant differences (p < 0.05) are indicated with an asterisk.

in healthy subjects, we did not systematically follow-up the subjects' respiratory symptoms associated with exercise. Usually, the reason to stop the exercise test was leg fatigue; however, end-of-MAD two subjects also reported strong breathlessness.

3.2. Laboratory results, at rest, exercise, and recovery phases

At rest and during light exercise, pH decreased end-of-mAD versus pre-mAD (p-values varying from 0.04 −0.006) (Fig. 5).

Base excess (BE) decreased end-of-mAD at 6 and 10 min after exercise compared to pre-mAD, (– 9.0 vs −6.3, p = 0.038 and −8.1 vs −5.53 mmol/L, p = 0.037, respectively) (Fig. 5), and bicarbonate decreased at 6 min after exercise (17.1 vs 19 mmol/L, p = 0.046Supplementary Fig. 2) . There were no significant differences in the values of venous sodium or potassium or to pH normalized calcium values associated with the exercise tests end of-mAD. Actual ionized calcium levels increased at the end-of-mAD 30 min after exercise (1.217 vs. 1.194 mmol/l, p = 0.032) but at other time points, the changes were not
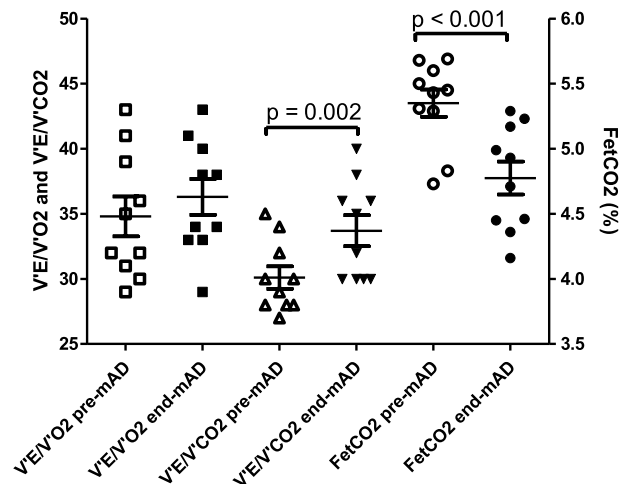
Maximal gas exchange values during exercise test

Fig. 3. The gas exchange values during maximal exercise before and at the end of mAD (modified Atkins diet) (N = 10). The mean (SD) maximal values of the ventilatory equivalent for CO_2 (V′E/V′CO_2) and for O_2 (V′E/V′O_2) and fraction of end-tidal CO_2 (FetCO2) The individual maximal values are given as symbols and the mean values as short lines and the standard deviations as error bars.
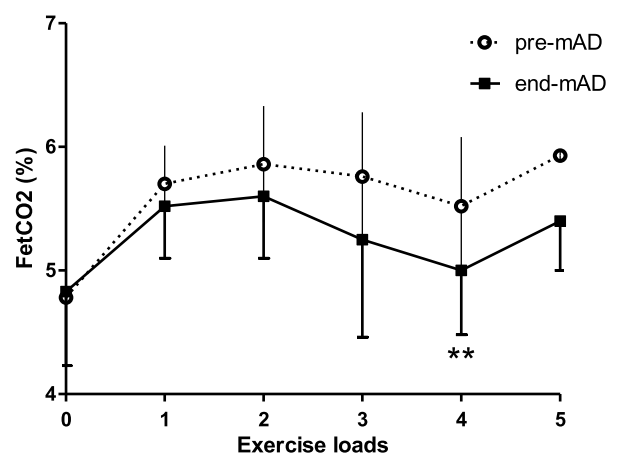
FetCO2 during exercise test

Fig. 4. FetCO2 (fraction of end-tidal CO_2) associated with cardiopulmonary exercise test before and at the end of mAD (the modified Atkins diet). The significant differences (*p < 0.05, **<0.01) are indicated with an asterisk.

significant (Supplementary Fig. 3).

Lactate concentration decreased at maximal exercise end-of-mAD vs. pre-mAD (4.4 vs. 6.2 mmol/L, p = 0.018), but otherwise, lactate was not affected by mAD (Fig. 6). Ammonia tended to be higher end-of-mAD than at baseline, but this difference was not significant (Fig. 6). Changes in the percent of oxygenated Hb (HbO$_2$) and other blood gas values in venous blood samples were not significant (Supplementary Fig. 4).

3.3. Laboratory follow-up of mAD

Routine blood tests showed an increase in total plasma cholesterol (p = 0.003) and HDL-cholesterol (p = 0.009), creatinine (p < 0.05), urea (p < 0.001), potassium (p = 0.007), β-hydroxybutyrate (p < 0.0001) and a small decrease in hemoglobin, glucose and gamma glutamyl transpherase (p < 0.05), triglycerides (p = 0.006), and insulin (p = 0.005) (Table 2). The pre-maD and end-of-mAD values of β-hydroxybutyrate
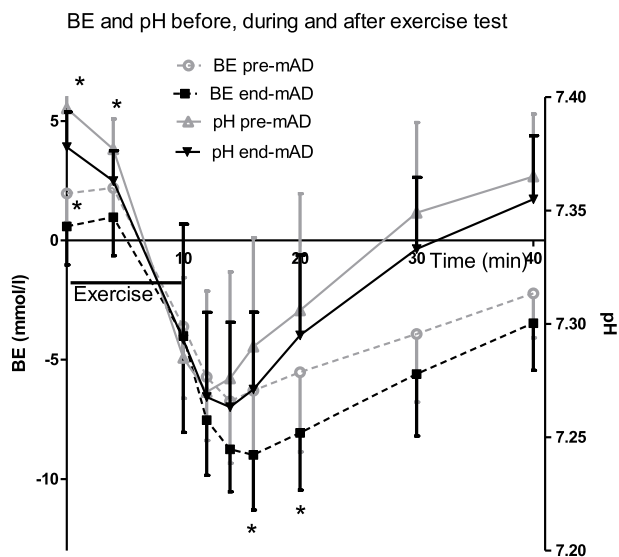
Fig. 5. The base excess (BE) and pH values before and at the end of mAD (modified Atkins diet) (N = 10). The mean (SD) values before, during, and 2, 4, 6, 10, 20, and 30 min after exercise are given as lines and the standard deviations as error bars. The significant differences (p < 0.05) are indicated with an asterisk.

Fig. 6. The lactate and ammonia levels before and at the end of mAD (modified Atkins diet) (N = 10). The mean values before, during, and 2, 4, 6, 10, 20, and 30 min after exercise are given as lines and the standard deviations as error bars. The significant differences (p < 0.05) are indicated with an asterisk.
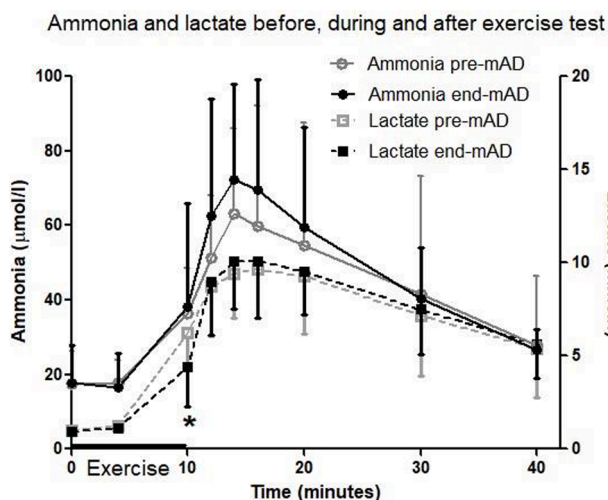
are given in Table 2, and in addition to this β-hydroxybutyrate values were 0.331 (mmol/L) (SD 0.20) in the first week, 0.49 (mmol/L) (SD 0.12) in the second week and 0.52 (mmol/L) (SD 0.27) in the third week.

4. Discussion

In the present study, ten healthy subjects followed a low-carbohydrate diet for four weeks. Their condition was carefully followed up with regular laboratory testing and a cardiopulmonary exercise test at pre-mAD and end-of-mAD. The subjects were otherwise living their everyday lives without any training protocols. The mAD was well tolerated by the subjects; their weight decreased significantly, as reported in previous studies (Brinkworth, Noakes, Clifton, & Buckley, 2009; Harvey et al., 2019). Our results show that mAD impacted the subjects' cardiopulmonary exercise capacity. As work power decreased

slightly and oxygen uptake increased, their relation (mechanical efficiency) decreased, indicating that to create the same work output as pre-mAD, a greater level of oxygen consumption was necessary, thus impairing the exercise economy. Interestingly, the gas exchange findings (increased V'E, V'E/V'CO2, decreased FetCO2) suggest that mAD promotes hyperventilation tendency.

As a sign of increased fatty acid oxidation caused by the low-carbohydrate diet, RER (V'CO$_2$/V'O$_2$) decreased end-of-mAD (Burke et al., 2002; Spriet & Peters, 1998; Zajac et al., 2014). During a low-carbohydrate diet, limited carbohydrate availability results in activation of lipolysis and fatty acid metabolism and ketone body (acetoacetate, β-hydroxybutyrate, and acetone) synthesis (Kossoff & Dorward, 2008). Both fatty acids and ketone bodies are broken down by beta-oxidation to be used in energy metabolism (Spriet & Peters, 1998). This metabolic shift affects performance: during the first three days of a low-carbohydrate, high-fat diet, such as mAD, glycogen stores in the muscles and liver are depleted, associated with a decrease in exercise performance (Spriet & Peters, 1998). During extended diet (>5–7 days), metabolic adaptation starts to compensate for these early changes in exercise performance. However, the results on the effects of low-carbohydrate diets on long-term exercise performance and V'O2 max have been varied, possibly because of different exercise modalities (e.g., bicycle vs. treadmill), different ramp protocols, or varying associated exercise training protocols combined with the diet (Brinkworth et al., 2009; Burke et al., 2017; Carr et al., 2018; Goedecke et al., 1999; Harvey et al., 2019; McSwiney et al., 2019; Rowlands & Hopkins, 2002; Zajac et al., 2014). Our data of increased oxygen requirement in bicycle exercise suggests that some unfavorable effects of the low carbohydrate diet on the exercise capacity persist after four weeks of mAD.

The respiratory changes we observed end-of-mAD(increased ventilation, decreased breathing reserve, high minute ventilation and V'E/V'CO2, low FetCO2) are consistent with changes found in patients with hyperventilation tendency or hyperventilation syndrome during exercise (Gardner, Meah, & Bass, 1986; Kinnula & Sovijärvi, 1993; Kinnula & Sovijärvi, 1996; Malmberg, Tamminen, & Sovijärvi, 2000; Vansteenkiste, Rochette, & Demedts, 1991, Ionescu et al., 2021). These changes may be explained by acidic ketone bodies (Greenhaff, Gleeson, Whiting, & Maughan, 1987; Yancy, Olsen, Dudley, & Westman, 2007), causing an emphasized decrease in pH end-of-mAD, which we found to occur both at rest and during light exercise. Together with decreased bicarbonate and increased ionized calcium in our study subjects, the increase in ventilation would fit to be respiratory compensation for metabolic acidosis.

Earlier studies suggest that the body's compensation mechanisms would gradually diminish the ketosis and metabolic acidosis associated with low-carbohydrate ingestion during prolonged diets (Brehm, Seeley, Daniels, & D'Alessio, 2003; Volek et al., 2002; Yancy et al., 2007). The decrease in ketosis could be caused by the body's increased efficiency to metabolize ketone bodies or a decreased production of ketone bodies due to increased carbohydrate ingestion (Yancy et al., 2007). Previous reports suggest that keeping ketosis levels optimal can be challenging, leading to poor diet compliance (Brehm et al., 2003, Pilis et al., 2018). However, both mechanisms may play a role in prolonged diets, and the present findings may be most important at the beginning of mAD. However, the metabolic capacity may vary between the subjects, and the ketosis may persist in some but not all subjects, as also reported during a prolonged diet (Yancy et al., 2007). Also, the subjects may be differently sensitive to the effects of acidosis. Hypothetically, patients with prior hyperventilation tendency could be more affected. As hyperventilation tendency and hyperventilation syndrome are often underdiagnosed as causes for respiratory symptoms (Ionescu et al., 2021; Tavel, 2021), increased hyperventilation tendency could be possibly an overlooked side effect of mAD. The present study indicates that mAD induced changes in pH and increased ventilation persist at least four weeks during a strictly followed mAD. At least in the short term, these results may be important to patients' adherence to mAD. However, further

research is needed to determine the long-term clinical impact of these findings.

We found that end-of-mAD lactate levels were significantly lower during maximal exercise than pre-mAD, rising to the before-diet levels after the exercise and being at a normal level during the recovery phase. The lower level of lactate is a logical result of low-glucose availability and promoted oxidative metabolism with lipids used as the primary fuel, as lactate is a byproduct of glycolysis (Gladden, 2004). Typically, during exercise, lactate increases gradually at the beginning of progressive exercise, reaching a threshold, after which the levels increase fast and continue to rise until up to 3–8 min after exercise (Goodwin, Harris, Hernández, & Gladden, 2007; Sjödin & Jacobs, 1981). Blood ammonia levels, mainly a product of AMP deamination, rise in tandem with lactate levels during exercise (Babij et al., 1983) and are also at their highest levels in the recovery phase (Mouadil, Debout, Read, Morello et al., 2012). mAD did not influence ammonia levels despite the increase in ingested protein levels. In an earlier study (Ahola et al., 2016), we found that end-of-mAD both in patients with metabolic muscle disease and healthy controls, the energy metabolism was modified. Alternative energy sources such as branched-chain amino acids (BCAAs) were utilized in oxidative phosphorylation. Healthy subjects can oxidize these non-glucose carbon sources during low intake of carbohydrates, and average amounts of lactate production are seen. Furthermore, ammonia level remains normal even when part of the proteins or their derivatives may be used in glycolysis. The creatinine increase observed might involve high-energy phosphate synthesis (Ahola et al., 2016). The normal lactate levels at the end-of-mAD seen in our study suggest that the normal glycolytic metabolism pertains despite a low amount of ingested carbohydrates in healthy subjects. This is possibly due to the increased utilization of increasingly ingested proteins in gluconeogenesis resulting from changes in the metabolic processes.

In addition to the laboratory values measured associated with exercise testing, broader fasting laboratory testing was used to follow up the health of the subjects. In addition to ketosis, insulin levels decreased by lowered carbohydrate consumption and lower plasma glucose level during the diet. There were some changes in potassium values during mAD probably associated with acid buffering. There was no suggestion of negative effects of mAD on the liver. Although there was an increase in plasma cholesterol, the LDL cholesterol did not increase significantly. In contrast, the HDL cholesterol increased, and triglycerides decreased during mAD. In this small healthy population with a relatively short mAD duration, marked negative effects on cholesterol values did not develop. However, cholesterol values in relation to ketogenic diets have been previously studied extensively, and an unfavorable increase in LDL has been shown in some longer studies (Buono et al., 1984; Foster et al., 2003; Goday et al., 2016; Mahdi, 2006; O'Neill & Raggi, 2020). It is important to follow up cholesterol values to prevent negative cardiovascular side effects if long-duration mAD diets are used.

4.1. Strengths and limitations

A strength of the present study is the study setup, enabling dissection of the low-carbohydrate diet's physiological effects without predetermined training programs. The subjects' compliance to the diet and follow-up testing was excellent, and the subjects' diets were standardized and prepared by the hospital kitchen. Secondly, the duration of the laboratory follow-up associated with the recovery phase of exercise testing was long enough, as the metabolic response of exercise, especially metabolic acidosis, an increase of lactate and ammonia levels are best seen in the recovery phase (Mouadil et al., 2012; Piirilä et al., 2016). Thirdly, despite the relatively short duration of the diet of four weeks, we were able to show significant physiological consequences to a low-carbohydrate diet.

The most significant limitation of this study is a relatively small number of participants, which hindered, e.g., analysis of sex-specific differences, which would be of interest as some of the metabolic myopathies, e.g., glycogen-storage diseases, show gender-specificity (Spriet & Peters, 1998). Another limitation of the study is the relatively short duration (4 weeks) of the mAD as the accentuated metabolic acidosis induced by the diet has been previously shown to decrease as the diet continues longer, up to 24 weeks. However, several factors influence the persistence of ketosis, as previously discussed.

5. Conclusions

Here, we show that four weeks of mAD has consequences for cardiorespiratory exercise physiology. mAD affected oxygen uptake only slightly but significantly lowered mechanical efficiency in cardiorespiratory exercise tests and increased ventilation. Interestingly, healthy subjects on mAD developed signs of hyperventilation during a maximal exercise test and at rest. At least in the short term, hyperventilation tendency should be acknowledged as a possible side effect of mAD.

CRediT authorship contribution statement

Nadja Ratia: Writing - original draft, Formal analysis, Visualization. **Kirsi H. Pietiläinen:** Investigation, Writing - review & editing. **Mari Auranen:** Investigation, Writing - review & editing. **Lauri Saksa:** Formal analysis. **Ritva Luukkonen:** Methodology, Formal analysis. **Anu Suomalainen:** Resources, Conceptualization, Writing - review & editing. **Päivi Piirilä:** Writing - review & editing, Investigation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104459.

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