The effects of acetazolamide on physiological variables among adolescents at high altitude

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Article Info	Abstract
	Background: High altitude environments can cause a range of physiological changes in the body, and adolescents may be particularly vulnerable to the effects of altitude sickness. Acetazolamide has been shown to be an effective treatment for altitude sickness, but further research is needed to determine its impact on physiological variables among adolescents at high altitude. **Aim:** The aim of this study was to investigate the effects of acetazolamide in adolescents over 17 days at high altitude. **Materials and Methods:** Twenty-five healthy adolescents volunteered for this study and were randomly separated into control group (CG, n=12) and acetazolamide group (AG, n=13). AG took 250mg of acetazolamide. Resting Heart Rate (RHR) and Acute Mountain Sickness (AMS) using the Lake Louise questionnaire were measured each morning and afternoon during field testing; Resting Blood Pressure (RBP) was measured each afternoon. Pre- and post-blood samples (haemoglobin (Hb) and haematocrit (Hct)) were taken at sea level (SL) one week before departure and within 40 hours on return to SL. **Results**: Significant differences between groups for RHR on days 2, 3 and 5; systolic blood pressures for day 4 and 13 ($P<0.05$). No significant difference between groups for diastolic blood pressure, pre- and post-altitude for Hb, Hct and PV. AMS scores did not differ from the two groups except on morning of day 12 ($P<0.05$). **Discussion and Conclusion**: Results revealed that acetazolamide did not significantly induce changes to adolescents' AMS scores and physiological changes. However, adolescents may consume acetazolamide to achieve lower RHR that may enable them to accommodate to HA, prolonging exercise in similar environments.

1. Introduction

High elevation or high altitude (HA) from 8,000 feet to 12,000 feet above sea level (SL) could start to bring about altitude sickness; very HA is from 12,000 feet to 18,000 feet; and extremely HA is 18,000 feet and above [1]. Acetazolamide is a sulphur-based drug, carbonic anhydrase inhibitor (CAI), approved by the Food and Drug Administration that has been scientifically proven to prevent or treat acute mountain sickness (AMS)/ altitude sickness that came to medical use in 1952 [2, 3, 4, 5]. HA travelling and trekking is common and trekkers want to shorten the duration of acclimatisation [6].

Carbonic anhydrase is an enzyme that catalyses the conversion of carbon dioxide (CO_2) and water (H_2O) to carbonic acid (H_2CO_3). Therefore, there is interference with CO_2 transport when acetazolamide is administered. Renal excretion of bicarbonate occurs, causing intracellular acidosis, which stimulates respiration [7, 8, 9, 10]. Mature infants have 4086 units•mL^{-1} packed cells of carbonic anhydrase activity and as adults, it increases to 23 809 units•mL^{-1} [11]. The carbonic anhydrase activity in infants and children increases with age [12].

Moreover, acetazolamide also stimulates ventilation, which brings greater volume of air into the lungs, therefore, this increases the amount of oxygen entering into the body and eases acclimatisation at HA. At HA, the mean oxygen saturation (SpO_2) increases during children's growth [13]. Acetazolamide also regulates blood pressure at HA and prevents increase in blood pressure in healthy individuals [14]. Furthermore, this causes CO_2 retention in the tissues and metabolic acidosis, which also causes a rise in cerebral extra-cellular fluid partial pressure of CO_2 ($PaCO_2$) and

(H)+. This also results in a stimulation of ventilation, presumably via both peripheral and central chemoreceptors, thus lowering $PaCO_2$ and increasing partial pressure of oxygen (PaO_2). Grissom et al. (1992) found a significant correlation between the change in the $PaCO_2 - PaO_2$ difference and AMS [15]. Furthermore, acetazolamide has been proven to reduce the production of cerebrospinal fluid and aqueous humor, therefore decreasing the creation of intracranial pressure and intraocular pressure [16].

A proper acclimatisation through gradual ascent could prevent AMS. To ease or speed up this process, the use of acetazolamide is also effective for the first 4-6 days at HA [7]. The effectiveness of acetazolamide in the prevention and treatment of AMS has been clearly established in adults in a number of well-controlled studies since the mid 1960's [15, 17, 18, 19, 20]. Although few clinical trials of acetazolamide have been conducted on adolescents, there may be no concluding evidence to suggest that its use would not be effective [10]. However, a recent study concluded that acetazolamide did not affect the children's emotions during a trek up a Taiwan mountain at altitude of 3886m [21]. Most scientific research at HA involved mostly male adults, therefore, there may be limited research on adolescents at HA.

The purpose of this study was to investigate the effects of a CAI, acetazolamide, on the following physiological responses; resting heart rate (RHR), resting blood pressure (RBP), systolic blood pressure (SBP), diastolic blood pressure (DBP), haemoglobin (Hb) and haematocrit (Hct) in 15-year-olds on a HA trek in Ladakh, India. The results will serve as an indication if acetazolamide will also prevent or treat AMS in adolescents at

HA as little research has revealed its effectiveness and side effects in children [22].

2. Methods

A total of 25 fifteen-year-old healthy students, 17 females and 8 males, residents in Singapore volunteered for this study. They exercised 2 to 3 times weekly and had no experience at HA. This study was ethically approved by the Ethical Review Board at the Physical Education and Sports Science, National Institute of Education, Nanyang Technological University (NTU), Singapore. Informed consent was sought from the participants and their parents or legal guardians and anthropometric measurements were taken. The participants were randomly assigned into a control group (CG) (n= 12, height: 1.72±8.7 m, weight: 62.6±7.8 kg, body mass index (BMI): 21.2±2.2 kg•m^{-2})) and acetazolamide group (AG) (n= 13, height:

1.62±6.1 m, weight: 58.3 ± 9.2 kg, BMI: 21.8 ± 2.5 2.2 kg•m^{-2}; Table 1). For safety and ethical reasons, the participants' parents were informed that acetazolamide is a sulphur-based drug and if any of the participants is sensitive or have any allergic reactions will be precluded from the study immediately or will be given immediate medical care if any complications occurred.

2.1. Days at high altitude

Both AG and CG were monitored for changes in RHR, RBP and AMS scores during the first 13 days at altitudes from 3,500 to 5,100m. Participants spent 17 days at HA; whereby the initial 3 days were spent at 3,500m for acclimatisation purposes, followed by a 10-day trek. The HA on Markha Valley ranges from 3000m to 5200m above SL with specific locations indicated during the 17 days (Figure 1; Table 2).

Table 1. Descriptive statistics of participants (n = 25)

	Control (n = 12)	Acetazolamide (n = 13)
Height (m)	1.72 ± 8.7	1.62 ± 6.1*
Weight (kg)	62.6 ± 7.8	58.3 ± 9.2
BMI (kg•m^{-2})	21.2 ± 2.2	21.8 ± 2.5
Sea level RHR (beats•min^{-1})	67 ± 7	72 ± 7
Sea level SBP (mmHg)	117 ± 10	113 ± 9
Sea level DBP (mmHg)	74 ± 9	72 ± 6
Pre-Hct (%)	40 ± 2.7	42.0 ± 2.2 (n = 12)
Post-Hct (%)	47.2 ± 3.9	46.9 ± 3.0 (n =12)
Pre-Hb (g/dl)	14.4 ± 1.3	14.7 ± 0.8
Post-Hb (g/dl)	15.4 ± 1.6**	15.6 ± 1.2**

Values are mean ± SD for height (meters), weight (kilograms), Body Mass Index (BMI) (kg•m^{-2}), Sea level Resting Heart Rate (RHR), beats per minute (beats•min^{-1}); Sea level Systolic Blood Pressure (SBP), millimetres of mercury (mmHg); Sea level Diastolic Blood Pressure (DBP) millimetres of mercury (mmHg); Pre-Hematocrit (Hct), percentage (%); Post-Hematocrit (Hct), percentage (%); Pre-Haemoglobin (Hb), grams per decilitre (g/dl); Post-Haemoglobin (Hb), grams per decilitre (g/dl).

* indicates significant difference ($P < 0.05$) bet control vs Acetazolamide for height, weight, BMI, RHR, SBP and DBP.

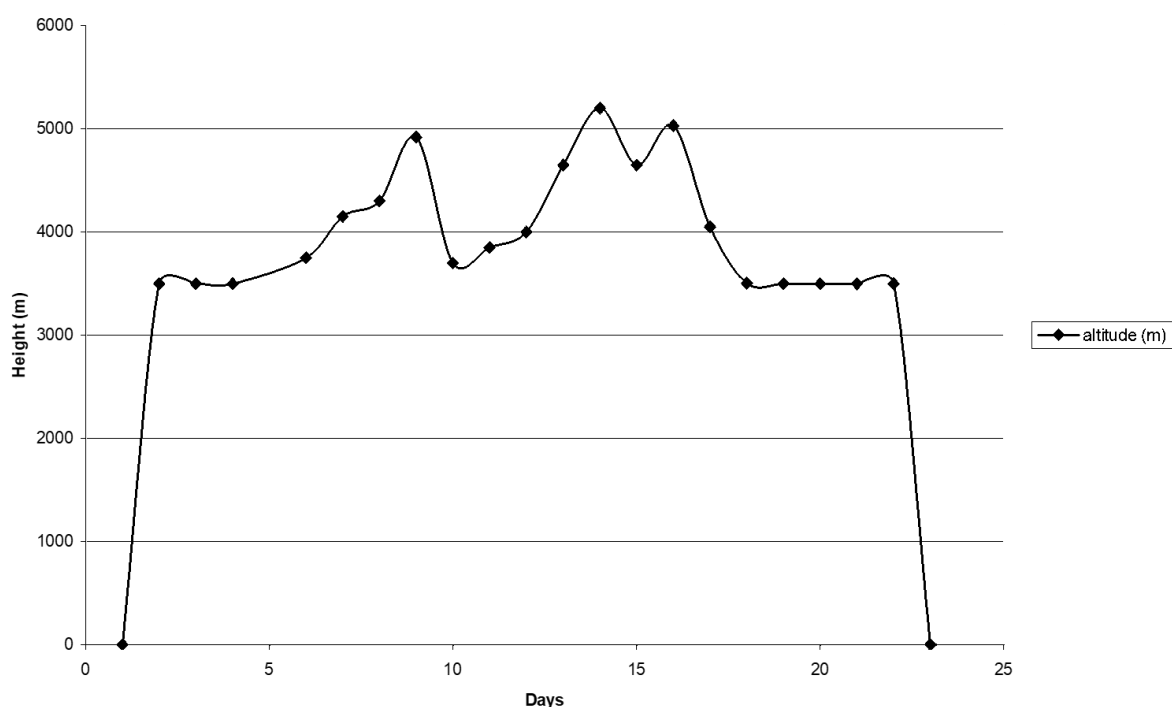** indicates significance differences bet pre- vs post-, control vs acetazolamide ($P < 0.0$).

Figure 1. Altitude during Markha Valley Trek

Table 2. Altitude, location and time during the 17-day Markha Valley Trek

1(am)	3500	Leh	8(pm)	3850	Markha
1(pm)	3500	Leh	9(am)	3850	Markha
2(am)	3500	Leh	9(pm)	4000	Hankar
2(pm)	3500	Leh	10(am)	4000	Hankar
3(am)	3500	Leh	10(pm)	4650	Nimaling
3(pm)	3500	Leh	11(am)	5200	Kangyatze
4(am)	3500	Leh	11(pm)	4650	Nimaling
4(pm)	3750	Rumbak	12(am)	4650	Nimaling
5(am)	3750	Rumbak	12	5030	Kongmaru La
5(pm)	4150	Yurutse	12(pm)	4050	Chogdo
6(am)	4150	Yurutse	13(am)	4050	Chogdo
6(pm)	4300	High camp	13(pm)	3505	Hemis
7(am)	4300	High camp	14	3500	Leh
7	4920	Ganda La	15	3500	Leh
7(pm)	3700	Skiu	16	3500	Leh
8(am)	3700	Skiu	17	3500	Leh
			18	0	Delhi

pm – afternoon, am – morning

2.2. Acetazolamide
AG consumed 125mg of acetazolamide (recommended by J. B. West and J. Dallimore via email communication [22]; twice daily (6-7 am and 6-7 pm) from day 1 to the morning of day 7. These time frames were selected as on day 8 at HA,

participants would ascend to a HA of 4950m followed by a descent to 3500m, which was the same altitude that the participants started on day 1. Therefore, both groups would have achieved similar levels of acclimatisation by this stage (Table 2, Figure 1). Other authors also

recommended 250-500mg doses for short-term use in adults [7, 10]. Pollard & Murdoch (1997) recommended a consumption of 5mg/kg body weight every 8-12 hours for children [10] whereas Harris et al. (1998) suggested a dosage ranging from 250mg-1000mg per day starting 12-24 hours before climb and to continue for 4-5 days [23]. In this study, the time period of 6 and a half days coincided with the 3-day acclimatisation period at 3500m, and the highest altitude gained was on day 7 (4950m) followed by a descent to 3500m.

2.3. Resting heart rate, resting blood pressure and AMS

RHR and AMS were recorded twice daily during field testing, once in the morning before rising (5.30-7.00am) and once in the afternoon (5-6 pm). An Omron automatic digital blood pressure monitor HEM-703C (Omron Asia Pacific Pte Ltd., 83 Clemenceau Ave. Singapore 239920) was used to measure RBP in the afternoon. Polar Accurex Plus heart rate monitors (Polar Electro Inc: 99, Seaview Boulevard, Port Washington, NY11050) was used to measure RHR. The Lake Louise AMS questionnaire was used to measure AMS symptoms every morning and evening [2, 24]. Participants had an hour of complete rest prior to data collection.

2.4. Blood samples

A Hemoglobin Meter Hb-202 (Optima INC, Japan) measured blood samples that were taken one week prior to departure and within 40 hours on return to SL. The 10ml-blood sample was taken by a clinically trained staff. A Micro-hematocrit centrifuge (Hawksley, England) was used to measure Hct. PV changes were calculated based on Dill and Costill's (1974) PV correction formula [25].

2.5. Statistical analyses

An independent t test was used to calculate the differences between AG and CG for RHR and RB. AMS scores were analysed by non-parametric Mann-Whitney U test and Bonferroni adjustment was utilised for multiple comparisons. A paired dependent t test was used to analyse AG and CG results for significant differences between pre- and post-HA for Hb, Hct and PV. Pearson product moment coefficient of correlation (r) was used to analyse correlations between AMS vs HA, RHR vs HA and RBP vs HA. All data analysis was performed using Microsoft Excel. The level of significance for all statistical analysis was set at $P< 0.05$.

3. Results

Table 3 indicates the SL values for RHR, SBP and DBP for AG and CG. RHR was always significantly different from SL ($P<0.05$) except for the morning of day 4 (AG) and the morning of day 10 (CG) (Table 4).

RHR was significantly higher in CG on days 2, 3 and 5 during drug administration. SBP was always significantly greater at HA for both groups ($P<0.05$ or less) with the exception of day 10 (CG and AG) and day 11 (CG) (Table 5).

Table 3. Groups' sea level values for resting heart rate, systolic blood pressure and diastolic blood pressure

	RHR (beats•min⁻¹)	SBP (mmHg)	DBP (mmHg)
CG (n=12)	67 ± 7	117 ± 10	74 ± 9
AG (n=13)	72 ± 7	113 ± 9	72 ± 6

Values are means ± SD for Resting Heart Rate (RHR), beats per minute (beats•min⁻¹); Systolic Blood Pressure (SBP), millimetres of mercury (mmHg); Diastolic Blood Pressure (DBP), millimetres of mercury (mmHg). Control Group (CG), Acetazolamide Group (AG).
* indicates significant difference ($P < 0.05$).

Table 4. Resting heart rate data at high altitude

Day	am/ pm	Altitude (m)	CG (beats•min⁻¹)	CG (beats•min⁻¹>SL)	RHR AG (beats•min⁻¹)	AG (beats•min⁻¹>SL)	CG v AG
1	pm	3500	77.2 ± 10.2	10.1 ± 9.7*	79.2 ± 10.7	7.0 ± 11.4*	
2	am	3500	78.6 ± 7.2	11.5 ± 10.6*	76.2 ± 14.2	3.3 ± 11.1*	**
2	pm	3500	92.3 ± 14.2*	25.3 ± 13.9	94.4 ± 11.0	21.5 ± 10.6*	
3	am	3500	82.2 ± 12.4	15.1 ± 12.7*	77.8 ± 10.6	4.9 ± 9.7*	**
3	pm	3500	87.2 ± 11.0	20.1 ± 16.0*	89.2 ± 12.0	16.3 ± 13.9*	
4	am	3500	77.3 ± 11.4	10.3 ± 14.5*	76.5 ± 12.8	3.7 ± 11.2	
4	pm	3500	104.4 ± 13.8	37.3 ± 14.5*	102.0 ± 14.7	29.2 ± 10.8*	
5	am	3500	78.1 ± 9.9	11.0 ± 12.2*	83.0 ± 12.1	10.2 ± 12.8*	
5	pm	3940	102.8 ± 8.8	35.8 ± 11.8*	95.0 ± 11.9	22.2 ± 12.1*	**
6	am	3940	78.1 ± 12.4	10.9 ± 12.6*	81.6 ± 11.1	8.8 ± 12.4*	
6	pm	4600	90.0 ± 17.1	22.9 ± 17.6*	92.6 ± 11.9	19.8 ± 11.9*	
7	am	4600	85.8 ± 14.3	18.6 ± 16.9*	83.7 ± 10.8	10.9 ± 11.7*	
7	pm	4600	99.4 ± 10.9	32.3 ± 10.7*	102.3 ± 13.2	29.5 ± 13.5*	
8	am	4600	78.6 ± 12.1	11.5 ± 13.2*	83.0 ± 9.6	10.2 ± 11.9*	
8	pm	3500	90.4 ± 13.9	23.3 ± 12.4*	93.3 ± 12.3	20.5 ± 10.5*	
9	am	3500	72.9 ± 9.2	5.8 ± 11.8*	78.9 ± 7.6	6.1 ± 7.8*	
9	pm	3800	84.0 ± 12.2	16.9 ± 14.7	87.6 ± 12.6	14.8 ± 11.7*	
10	am	3800	71.6 ± 11.4	4.5 ± 14.1*	75.4 ± 7.0	2.5 ± 6.0*	
10	pm	4000	88.8 ± 16.4	21.7 ± 16.3	98.8 ± 10.7	25.9 ± 11.9	
11	am	4000	72.8 ± 9.2	5.8 ± 11.8*	78.7 ± 6.6	5.9 ± 8.2*	
11	pm	4800	86.3 ± 14.7	19.3 ± 15.4	94.6 ± 15.0	21.8 ± 14.7*	
12	am	4800	76.2 ± 11.3	9.1 ± 15.3*	80.6 ± 9.2	7.8 ± 10.5*	
12	pm	4800	90.5 ± 12.3	23.4 ± 11.4*	90.7 ± 12.2	17.9 ± 13.0*	
13	am	4800	74.6 ± 7.25	7.5 ± 11.9*	78.9 ± 5.6	6.1 ± 9.9*	
13	pm	3700	95.5 ± 14.6	28.4 ± 16.4*	95.2 ± 14.9	22.4 ± 13.0*	

Values are mean ± SD for Resting Heart Rate (RHR), beats per minute (beats•min⁻¹); Control Group (CG); Acetazolamide Group (AG); Sea Level (SL); Afternoon (PM); Morning (AM).

* Indicates significant differences above sea level ($P<0.05$).

**Indicates Control Resting Heart Rate (RHR) significantly greater than act ($P<0.05$).

Table 5. Systolic blood pressure at high altitude

Day	am/ pm	Altitude (m)	SBP CG (mmHg)	SBP CG (mmHg > SL)	SBP AG (mmHg)	SBP AG (mmHg > SL)	CG v AG
1	pm	3500	120 ± 11.8	3.2 ± 10.3*	120.8 ± 10.6	8.0 ± 16.0*	-
2	pm	3500	116.1 ± 11.5	-1.2 ± '7.2	117.3 ± 14.6	3.4 ± 17.2*	-
3	pm	3500	124.7 ± 11.2	7.5 ± 8.0*	120.6 ± 7.8	7.9 ± 7.7*	-
4	pm	3500	127.2 ± 13.8	10.0 ± 8.9*	116.4 ± 8.5	2.6 ± 6.6*	CG>AG**
5	pm	3940	129.0 ± 12.2	11.8 ±12.9*	119.9 ± 9.4	5.9 ± 9.6*	-
6	pm	4600	126.9 ± 16.9	9.7 ± 17.7*	124.6 ± 11.3	11.0 ± 13.1*	-
7	pm	4600	130.1 ± 15.7	12.8 ± 9.8*	120.8 ± 8.6	7.1 ± 11.6*	-
8	pm	3500	127.8 ± 14.2	10.6 ± 11.3*	119.4 ± 8.3	5.2 ± 10.3*	-
9	pm	3800	130.7 ± 15.8	13.4 ± 11.3*	121.4 ± 10.0	7.6 ± 6.8*	-
10	pm	4000	116.1 ± 11.7	-1.2 ± 10.3	118.6 ± 7.3	5.0 ± 10.6	-
11	pm	4800	127.6 ± 18.1	13.5 ± 9.7	128.1 ± 9.8	14.5 ± 10.8*	-
12	pm	4800	127.6 ± 18.1	10.3 ± 13.1*	127.6 ± 8.7	13.9 ± 13.9*	-
13	pm	3700	120.8 ± 14.7	3.6 ± 15.9*	129.2 ± 8.0	15.2 ± 12.3*	AG>CG**

Values are mean ± SD for Systolic Blood Pressure (SBP), millimetres of mercury (mmHg); Control Group (CG); Acetazolamide Group (AG); Morning (AM); Afternoon (PM); Sea Level (SL); Meters (m).

* Indicates significant differences above sea level ($P<0.05$).

** Indicates significant differences between groups ($P<0.05$).

On day 4, CG's SBP was significantly greater than AG's SBP; this was reversed on day 13 (*P*<0.05). DBP was significantly greater than at SL (CG: days 6, 7, 9, 11, 12, and 13; AG: days 3 to 9 and 11 to 13) (*P*<0.05; Table 6). The groups did not differ significantly from each other on any occasion.

The pre- and post-blood analysis were analysed whereby Hb and Hct groups were significantly different to SL values (*P*<0.01; Table 7). There was no significant difference between the groups' pre- and post-altitude for Hb and Hct. PV decreased (CG: 18.4 ± 11.3 %; AG: 14.0 ± 10.0 %) with no significant difference found between groups (Table 8).

Table 6. Diastolic blood pressure at high altitude

Day	am/pm	Altitude	DBP Control (mmHg)	DBP Control (mmHg > SL)	DBP Act. (mmHg)	DBP Act (mmHg > SL)	Con v Act
1	pm	3500	74.7 ± 14.7	0.7 ± 12.8	78.3 ± 9.9	6.0 ± 12.6	-
2	pm	3500	70.1 ± 10.3	-3.9 ± 13.8	74.0 ± 11.8	1.7 ± 13.0	-
3	pm	3500	78.4 ± 10.2	4.4 ± 9.7	76.5 ± 6.7	3.5 ± 7.7*	-
4	pm	3500	77.9 ± 9.9	3.9 ± 11.5	80.0 ± 8.8	7.1 ± 10.7*	-
5	pm	3940	79.1 ± 11.4	5.2 ± 14.4	77.6 ± 8.4	5.3 ± 10.6*	-
6	pm	4600	82.1 ± 12.6	8.1 ± 11.5*	86.1 ± 7.4	13.2 ± 9.2*	-
7	pm	4600	85.1 ± 9.5	11.1 ± 10.8*	84.4 ± 4.7	11.5 ± 9.5*	-
8	pm	3500	78.2 ± 13.2	4.2 ± 13.2	78.0 ± 7.2	4.7 ± 9.7*	-
9	pm	3800	84.8 ± 9.6	10.8 ± 6.6*	86.6 ± 9.7	13.8 ± 8.7*	-
10	pm	4000	77.8 ± 8.7	3.8 ± 9.0	76.4 ± 8.5	3.6 ± 8.2	-
11	pm	4800	86.5 ± 9.0	12.5 ± 8.2*	86.6 ± 9.0	13.8 ± 8.7*	-
12	pm	4800	82.1 ± 7.4	8.1 ± 7.7*	85.2 ± 8.5	12.4 ± 10.8*	-
13	pm	3700	80.6 ± 7.4	6.6 ± 7.1*	84.0 ± 7.7	11.4 ± 8.2	-

Values are mean ± SD for Diastolic Blood Pressure (DBP), millimetres of mercury (mmHg); Acetazolamide Group (AG); Control Group (CG); Sea Level (SL).
* Indicates significant differences above sea level (*P*<0.05).

Table 7. Hematocrit and haemoglobin data of subjects

Variables	Control (n=12)		Acetazolamide (n=12)	
	Pre	Post	Pre	Post
Hematocrit (Hct) (%)	40.3 ± 2.7	47.2 ± 3.9	42.0 ± 2.2	46.9 ± 3.0
Haemoglobin (Hb) (g/dl)	14.4 ± 1.3*	15.4 ± 1.6*	14.7 ± 0.8*	15.6 ± 1.2*

Values are mean ± SD for Hematocrit (Hct), percent (%); Haemoglobin (Hb), grams per decilitre (g/dl).
* indicates significance differences pre- and post- altitude in both groups (*P*<0.01).

Table 8. Plasma volume change of subjects

% change in PV (CG)	% change in PV (AG)
-18.4 ± 8.5	-14.0 ± 10.0

Values are mean ± SD for percent change in Plasma Volume (PV), percent (%) between Control Group (CG) and Acetazolamide Group (AG).
* indicates significant difference between groups (*P*<0.05).

AMS scores were recorded using the Lake Louise questionnaire throughout the stay at HA. There was no significant difference between groups except on day 11(am) when the AMS scores of AG were significantly greater than CG (Table 9). A trend of low scores was seen for several days in both groups after the first HA point (4,900 m) had been crossed. The correlational data for AMS v HA, RHR v HA and RBP v HA are indicated in Table 10.

Table 9. AMS scores at high altitude

Day	am/pm	Altitude (m)	AMS CG	AMS AG
1	pm	3500	2.1 ± 0.67	2.3 ± 1.5
2	am	3500	1.8 ± 1.7	1.0 ± 1.7
2	pm	3500	1.5 ± 1.6	2.0 ± 1.3
3	am	3500	2.8 ± 2.6	1.5 ± 1.6
3	pm	3500	1.2 ± 1.3	0.9 ± 1.2
4	am	3500	2.2 ± 1.8	2.1 ± 2.5
4	pm	3500	1.9 ± 2.1	1.1 ± 1.6
5	am	3500	1.4 ± 2.1	1.9 ± 2.1
5	pm	3940	1.4 ± 1.7	1.5 ± 1.6
6	am	3940	1.3 ± 2.1	1.9 ± 1.8
6	pm	4600	2.0 ± 2.5	1.8 ± 2.3
7	am	4600	1.6 ± 1.9	2.5 ± 1.8
7	pm	4600	1.6 ± 1.4	0.9 ± 1.2
8	am	4600	1.4 ± 1.9	1.9 ± 1.5
8	pm	3500	0.5 ± 0.9	0.6 ± 0.8
9	am	3500	0.5 ± 0.9	0.6 ± 0.8
9	pm	3800	0.8 ± 0.9	0.4 ± 0.7
10	am	3800	0.8 ± 1.1	0.6 ± 1.0
10	pm	4000	0.2 ± 0.4	0.2 ± 0.4
11	am	4000	0.9 ± 1.2	0.7 ± 1.2
11	pm	4800	0.7 ± 1.5	1.6 ± 1.7
12	am	4800	1.0 ± 1.0	1.5 ± 1.5
12	pm	4800	0.7 ± 1.2	1.3 ± 1.5
13	am	4800	1.4 ± 1.6	1.5 ± 2.0
13	pm	3700	0.9 ± 1.7	1.1 ± 1.4

Values are means ± SD for Acute Mountain Sickness (AMS), Afternoon (PM), Morning (AM), Meters (m), Acetazolamide Group (AG), Control Group (CG).
No significant difference was found between AG and CG ($P<0.05$).

Table 10. Correlation of physiological variables and high altitude

	Act r	Control r
RHR v HA	0.69	0.83
DBP v HA	0.7	0.71
SBP v HA	0.59	0.45
AMS (am & pm) v HA	0.03	-0.26
AMS (am) v HA	-0.29	0.21
AMS (pm) v HA	-0.04	-0.14

Values are coefficient of correlation for Resting Heart Rate (RHR), High Altitude (HA), Diastolic Blood Pressure (DBP), Systolic Blood Pressure (SBP), Acute Mountain Sickness (AMS), Morning (am), Afternoon (pm), Coefficient of Correlation (r).

4. Discussion

4.1. Resting Heart Rate (RHR)

RHR has been shown to increase at HA [7, 26, 27] and in this study, AG showed significantly lower RHR on three occasions during drug administration during the first 5 days at HA (day 2: morning, day 3: morning, and day 5: afternoon) ($P<0.05$; Table 4). This increase was due to stimulation of the systemic circulation by the sympathetic nervous system whereby RHR (69.5 beats•min^{-1} to 91.5 beats•min^{-1}) and blood pressure can increase after 48 hours at 3,800 mean [7]. In addition, resting and exercise HR were found to have rose with altitude, specifically a significant increase in RHR ranging from altitudes

5,400-6,300m and greatest increase at 6,300m [27, 28]. However, one study reported no significant increase in RHR and RBP above SL at altitudes of 2,700m and 3,700m (*P*<0.01) [29]. Interestingly, increases in RHR were less pronounced with slow ascent [26]. Therefore, we can conclude that RHR increases with HA.

Acetazolamide acts primarily as a ventilatory stimulant (CAI) [8, 9, 10]. Acetazolamide may reduce the physiological impact of hypoxia on the body; whereby HA causes a rise in blood pressure, however, it can be reduced when an individual consumes acetazolamide [30]. Some studies have suggested that acetazolamide at a larger dose (750mg/day) is more effective [31]. However, administration of acetazolamide to adolescents should also be carried out with caution as there are known side effects (e.g. depression, nausea, anorexia, paraesthesia, weight loss and fatigue) [3, 4].

In two out of the three mornings, AG had a significantly lower RHR (*P*<0.05) than the CG and on the afternoon of day 5 after the first major gain in altitude from 3,500 to 3,940m had taken place (Table 4). This could be due to effects of acetazolamide that causes the vasodilation in the human forearm [32] and increases the oxygen supply to the heart in hypoxia [33]. Beyond day 5, no significant difference was found for RHR between the two groups.

CG's RHR were significantly correlated to altitude *r*= 0.83 (CG) versus *r*= 0.69 (AG) (Table 10) which indicated that acetazolamide has the effect of "lowering" altitude by increasing alveolar and arterial oxygen tension. Morning RHR were only used in this analysis. Despite an hour's rest prior to data collection, afternoon RHR may have been elevated by other factors such as heat, exertion and dehydration that may not lead to accurate results. Therefore, administration of acetazolamide may also prevent elevation of RHR and its relationship with altitude would be expected to be less than in CG.

4.2. Resting Blood Pressure (RBP)

There is no immediate change in the rise in BP upon arrival at HA [34], progressive rises occurred during the first few days. In Wolfel et al.'s (1994) study on systemic hypertension at 4,300m, the authors found that participants with the greatest increases in BP also had highest levels of norepinephrine, which had a vasoconstrictive action [34]. They concluded that this may have cause a change in sympathetic neural activity in some participants and brought about an increase in BP.

The results of this study found that SBP had mostly significantly greater values than SL values whereas increases in DBP values were less consistent. Although not conclusive, there is some suggestion that systolic pressure is more affected than diastolic pressure [7]. On ascent to 4,300m, SBP in another group of participants, rose from pre-ascent values of 103 mmHg to 121 mmHg and remained raised with no change in DBP [9]. Another factor in elevation of blood pressure is a decrease in atrial natriuretic peptide (ANP) secretion. ANP causes vasodilation, however, secretion of this hormone is reduced under hypoxic conditions and this may be partly responsible for increases in BP [35].

4.3. Acute Mountain Sickness (AMS)

It was noted that from day 8 (pm) to day 11 (am), AMS showed lower rated scores than at any other time for both groups. At this stage, both groups had completed the first high pass (4,900 m) and were at relatively lower altitudes (3,500-4,000 m) (Table 9)

feeling much better acclimatised. Anecdotal observations of the participants being in good spirits at this stage also supported this. From days 11 (pm) to 13 (pm), increases in altitude were accompanied by an increase in AMS scores although these increases were much less pronounced than at the start of the trip. AG rated higher scores on AMS during the latter stage of the trek (Table 9).

Many previous studies have found that AMS symptoms such as nausea, headache, insomnia, lassitude and dizziness were reduced in adults taking acetazolamide [15, 17, 22, 36]. Ten in the latter study had previously climbed up to 5,400m.

The adolescents in this study were fifteen-year olds who seemed to experience similar degrees of AMS regardless whether or not they consumed acetazolamide. It may be the case that participants of this age combined with little HA experience made it difficult for them to discriminate between the effects of fatigue and the effects of altitude. This study found that on the morning of day 12 after a night at 4,800 m (highest altitude overnight camp), AG had significantly higher AMS scores than CG. This may be a statistical outlier that has no merit for further discussion; or, given that the study involved three major ascents in altitude, rather than a constant ascent. The first descent being within the drug administration period, the second and third after drug administration. Perhaps further studies would indicate how long before complete acclimatisation takes place under these conditions.

These findings may also suggest that adolescents or children who are not acclimatized to HA tend to have lower oxygen saturation of Hb and higher HR, as compared to those who grow up on HA tend to have a better degree of acclimatization to altitude [37].

4.4. Blood Analyses

Hct increased by 5-7%, which was similar to a study by Stokke et al. (1986) where 10 male participants at a mean altitude of 4,100m for 20 days showed an increase of 5.4% in Hct. Results were also similar in this study where pre- and post-altitude blood analyses in both groups showed a significant increase ($P<0.05$) after 17 days at HA (3, 500-5, 100 m). A decrease in PV leads to hemoconcentration at HA, which could be misinterpreted as increased erythropoietic activity. Their data showed wide individual variation with one participant recording very high Hct levels (69%), which required hemodilution [38].

Hb concentration also increased in this study after exposure to HA by about 1g/dl or 6-7% for both groups (Table 7). Other studies have shown greater increases in Hb; Böning (1997) reported increases of 14% 7-8 days after return to SL, however their study lasted 26-29 days at 4,700-7,600m which lasted longer and altitude was higher than this study, consisting of 12 experienced male mountaineers as participants [35].

Regardless of methodology, haemoconcentration occurred at HA in response to decreases in plasma volume; the longer the stay at altitude and higher the elevation, the greater the response [39]. During the first 3-4 weeks at HA erythrocyte volume, it was stable but PV reductions were apparent whilst longer stays of several months showed an increase in erythrocytes [39].

This study involved a 17-day stay at HA which would fall into the first stage described by Sawka et al. (2000) [39]. Data obtained by Robach et al. (2000) supported the theory that an increase in red cell volume takes more than 2 weeks despite immediate increases in levels of

erythropoietin (EPO) [40].

4.5. Plasma volume

Decreases in PV after ascent to HA have been attributed to dehydration, diuresis, plasma protein loss and increased capillary hydrostatic pressures. Sawka et al. (1995) found 11% and 9% decreases after day 1 and 9 at HA respectively. The authors concluded that PV decreases were mediated by protein loss that reduces vascular oncotic pressure (pressure from circulating proteins) [41]. Their study carefully monitored fluid intake, so it is unlikely that dehydration was the sole cause of PV losses, they calculated that even if dehydration had occurred it would only account for 2% of PV losses.

The participants in this study had blood samples taken 40 hours after departure. PV losses were due to dehydration that may only account for a small percentage. Other possible reasons for PV losses were likely to be related to HA exposure, namely plasma protein loss, increased capillary permeability and diuresis[40]. Longer sojourns at higher altitude have found different results regarding PV; whereby prolonged stays at extreme altitudes could cause salt and water retention due to an increase in circulating stress hormones, which reduces renal blood flow [38]. The results of this study corroborate with other studies that a short duration (2-3 weeks) at HA causes a significant decrease in PV.

In addition, these adolescents were still undergoing puberty and have yet to reach their full maturation growth, whereby their physiological attributes (i.e. aerobic, anaerobic and strength power) have distinct potential to grow with training [42]. Therefore, their physiological adaptations and perceptions differ from adults during the climb. Although the greatest increase of cardiovascular capacity occurs between 11-

15 years of age, these participants were lacking in experience in hiking HA terrains. Simultaneously, maximal oxygen consumption (VO_{2max}) of an individual also reduces at HA but increases at a lower altitude [42, 43, 44]. Therefore, these reasons may suggest the insignificant difference between both AG and CG. This may also explain the significant difference between adults' and adolescents' consumption of acetazolamide, where the former experienced significant improvements in AMS scores.

5. Limitations

For many of the participants, the experience of trekking at HA was a new and challenging one. Initially, the participants were unable to distinguish between symptoms caused by fatigue or exertion and symptoms caused by HA. Some participants may have overestimated their symptoms. However, participants seemed to have acclimatised and were able to distinguish between symptoms. Additionally, during this study, PV changes during the drug administration period could not be analysed as it was not possible to take blood samples during the HA stay.

6. Conclusions

Taking moderate doses (250 mg/day) of acetazolamide may cause less pronounced increases in adolescents' RHR. SBP showed significant increases on exposure to HA, however, consuming acetazolamide did not significantly affect this increase ($P<0.05$). DBP often showed significant increases at HA although not as consistently as SBP ($P<0.05$). There was no evidence to suggest that taking acetazolamide affected DBP. Results also indicated that there was no significant difference between AG and CG's AMS scores and Hct during the drug administration period. Adolescents may not

react physiologically to hypoxic environments in the same way as adults, especially during travel and trekking at HA. An adolescent's perception of AMS may be different to an adult and may be affected by factors such as the ability to cope with fatigue from walking in a hot dry environment for up to 10 hours a day and dealing with extreme temperatures. Given that the predominance of literature is on acetazolamide on adult males' physiology responses at HA, the findings from this investigation could add to the limited literature on HA physiology responses among adolescents.

Conflict of interest
The authors declared no conflicts of interest.

Authors' contributions
Conceptualization [Balasekaran G., Thompson S.]; Methodology [Balasekaran G., Thompson S.]; Investigation [Balasekaran G., Thompson S.]; Writing – Original Draft [Balasekaran G., Thompson S., Govindaswamy V.V.]; Writing – Reviewing & Editing [Balasekaran G., Govindaswamy V.V., Ng Y.C.]; Resources [Balasekaran G., Thompson S.]; Supervision [Balasekaran G.. Govindaswamy V.V.]..

Ethical considerations
The author has completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc.

Data availability
The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

References
[1] Hannon JP, Shields JL, Harris CW. "A comparative review of certain responses of men and women to high altitude". *Symp. Arctic Med. BioZ. Physiology of Work in Cold and Altitude*. Arctic Aeromed. 1966; 6: 113-245.

[2] Lipman GS, Pomeranz D, Burns P, Phillips C, Cheffers M, Evans K, Jurkiewicz C, Juul N, Hackett P. "Budesonide versus acetazolamide for prevention of acute mountain sickness". *Am J Med*. 2018; 131(2): 200.e9-200.e16. doi: 10.1016/j.amjmed.2017.05.034. PMID: 28668540.

[3] Kakunje A, Prabhu A, Priya Es S, Karkal R, Pookoth RK, Pd R. "Acetazolamide for antipsychotic-associated weight gain in schizophrenia". *J Clin Psychopharmacol*. 2018; 38(6): 652-653. doi: 10.1097/JCP.0000000000000940. PMID: 30192255; PMCID: PMC6221403.

[4] Teppema LJ. "Multifaceted clinical effects of acetazolamide: will the underlying mechanisms please stand up?". *J Appl Physiol*. 2014; 116(7): 713-4. doi: 10.1152/japplphysiol.00141.2014. Epub 2014 Feb 20. PMID: 24557795.

[5] Todd TJ. "Pediatric Injectable Drugs". *The Teddy Bear Book*. Stephanie J, Phelps Tracy M, Hagemann Kelly, Lee R. Jill Thompson A. American Society of Health-System Pharmacists Publication. Bethesda, MD. 2018.

[6] Krasney JA. "A neurogenic basis for acute altitude illness". *Med Sci Sports Exerc*. 1994; 26(2): 195-208. doi: 10.1249/00005768-199402000-00010. PMID: 8164537.

[7] Zafren K, Honigman B. "High-altitude medicine". *Emerg Med Clin North Am*. 1997; 15(1): 191-222. doi: 10.1016/s0733-8627(05)70291-1. PMID: 9056576.

[8] Stager JM, Tucker A, Cordain L, Engebretsen BJ, Brechue WF, Matulich CC. "Normoxic and acute hypoxic exercise tolerance in man following acetazolamide". *Med Sci Sports Exerc*.

1990; 22(2): 178-84. PMID: 2355814.

[9] Ward M, West JB, Milledge JS. "High altitude medicine and physiology". Michael P, Ward JS, Milledge JB. West Chapman & Hall Medical London. 1995.

[10] Pollard AJ, Murdoch DR. *The High Altitude Medicine Handbook*. Radcliffe Medical Press, Inc. 1997.

[11] Logan RW, Crooks SM, Hutchison JH, Kerr MM. "Blood carbonic anhydrase activity in the newborn". *Arch Dis Child*. 1973; 48(4): 256-8. doi: 10.1136/adc.48.4.256. PMID: 4196214; PMCID: PMC1648337.

[12] Berfenstam R. "Studies on carbonic anhydrase activity in children". *Acta Paediatr (Stockh)*. 1952; 41(1): 32-52. doi: 10.1111/j.1651-2227.1952.tb17839.x. PMID: 14933012.

[13] Ucrós S, Granados CM, Castro-Rodríguez JA, Hill CM. "Oxygen saturation in childhood at high altitude: A systematic review". *High Alt Med Biol*. 2020; 21(2): 114-125. doi: 10.1089/ham.2019.0077. Epub 2020 Apr 2. PMID: 32239983.

[14] Eskandari D, Zou D, Grote L, Hoff E, Hedner J. "Acetazolamide reduces blood pressure and sleep-disordered breathing in patients with hypertension and obstructive sleep apnea: A randomized controlled trial". *J Clin Sleep Med*. 2018; 14(3): 309-317. doi: 10.5664/jcsm.6968. PMID: 29510792; PMCID: PMC5837832.

[15] Grissom CK, Roach RC, Sarnquist FH, Hackett PH. "Acetazolamide in the treatment of acute mountain sickness: Clinical efficacy and effect on gas exchange". *Ann Intern Med*. 1992; 116(6): 461-5. doi: 10.7326/0003-4819-116-6-461. PMID: 1739236.

[16] Van Berkel MA, Elefritz JL. "Evaluating off-label uses of acetazolamide". *Am J Health Syst Pharm*. 2018; 75(8): 524-531. doi: 10.2146/ajhp170279. PMID: 29626002.

[17] Greene MK, Kerr AM, McIntosh IB, Prescott RJ. "Acetazolamide in prevention of acute mountain sickness: a double-blind controlled cross-over study". *Br Med J (Clin Res Ed)*. 1981; 283(6295): 811-3. doi: 10.1136/bmj.283.6295.811. PMID: 6794709; PMCID: PMC1507046.

[18] Larson EB, Roach RC, Schoene RB, Hornbein TF. "Acute mountain sickness and acetazolamide" Clinical efficacy and effect on ventilation". *JAMA*. 1982; 248(3): 328-32. PMID: 7045433.

[19] Gertsch JH, Basnyat B, Johnson EW, Onopa J,

Holck PS. "Randomised, double blind, placebo controlled comparison of ginkgo biloba and acetazolamide for prevention of acute mountain sickness among Himalayan trekkers: the prevention of high altitude illness trial (PHAIT)". *BMJ*. 2004; 328(7443): 797. doi: 10.1136/bmj.38043.501690.7C. Epub 2004 Mar 11. PMID: 15070635; PMCID: PMC383373.

[20] Leaf DE, Goldfarb DS. "Mechanisms of action of acetazolamide in the prophylaxis and treatment of acute mountain sickness". *J Appl Physiol*. 2007; 102(4): 1313-22. doi: 10.1152/japplphysiol.01572.2005. Epub 2006 Oct 5. PMID: 17023566.

[21] Chao CC, Chen LH, Lin YC, Wang SH, Wu SH, Li WC, Huang KF, Chiu TF, Kuo IC. "Impact of a 3-day high-altitude trek on Xue Mountain (3886 m), Taiwan, on the emotional states of children: A prospective observational study". *High Alt Med Biol*. 2019; 20(1): 28-34. doi: 10.1089/ham.2018.0086. PMID: 30543474.

[22] Shlim DR. "The use of acetazolamide for the prevention of high-altitude illness". *J Travel Med*. 2020; 27(6): taz106. doi: 10.1093/jtm/taz106. PMID: 31897486.

[23] Harris MD, Terrio J, Miser WF, Yetter JF 3rd. "High-altitude medicine". *Am Fam Physician*. 1998; 57(8): 1907-14, 1924-6. PMID: 9575328.

[24] Sutton CG, Houston CS. "Hypoxia and mountain medicine". *7th International Hypoxia Symposium*. Lake Louise, Canada, February 1991.

[25] Dill DB, Costill DL. "Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration". *J Appl Physiol*. 1974; 37(2): 247-8. doi: 10.1152/jappl.1974.37.2.247. PMID: 4850854.

[26] Malconian M, Rock P, Hultgren H, Donner H, Cymerman A, Groves B, Reeves J, Alexander J, Sutton J, Nitta M, et al. "The electrocardiogram at rest and exercise during a simulated ascent of Mt. Everest (Operation Everest II). *Am J Cardiol*. 1990; 65(22): 1475-80. doi: 10.1016/0002-9149(90)91358-d. PMID: 2353654.

[27] Reeves JT, Groves BM, Sutton JR, Wagner PD, Cymerman A, Malconian MK, Rock PB, Young PM, Houston CS. "Operation Everest II: Preservation of cardiac function at extreme altitude". *J Appl Physiol*. 1987; 63(2): 531-9. doi: 10.1152/jappl.1987.63.2.531. PMID: 3654411.

[28] Karliner JS, Sarnquist FF, Graber DJ, Peters RM Jr, West JB. "The electrocardiogram at extreme altitude: Experience on Mt. Everest".

Am Heart J. 1985; 109(3 Pt 1): 505-13. doi: 10.1016/0002-8703(85)90555-1. PMID: 3976477.

[29] Nishihara F, Shimada H, Saito S. "Rate pressure product and oxygen saturation in tourists at approximately 3000 m above sea level". *Int Arch Occup Environ Health*. 1998; 71(8): 520-4. doi: 10.1007/s004200050317. PMID: 9860159.

[30] Parati G, Revera M, Giuliano A, Faini A, Bilo G, Gregorini F, Lisi E, Salerno S, Lombardi C, Ramos Becerra CG, Mancia G, Salvi P. "Effects of acetazolamide on central blood pressure, peripheral blood pressure, and arterial distensibility at acute high altitude exposure". *Eur Heart J*. 2013; 34(10): 759-66. doi: 10.1093/eurheartj/ehs140. Epub 2012 Jun 17. PMID: 22711752.

[31] Dumont L, Mardirosoff C, Tramèr MR. "Efficacy and harm of pharmacological prevention of acute mountain sickness: quantitative systematic review". *BMJ*. 2000; 321(7256): 267-72. doi: 10.1136/bmj.321.7256.267. PMID: 10915127; PMCID: PMC27441.

[32] Pickerodt PA, Francis RC, Höhne C, Neubert F, Telalbasic S, Boemke W, Swenson ER. "Pulmonary vasodilation by acetazolamide during hypoxia: impact of methyl-group substitutions and administration route in conscious, spontaneously breathing dogs". *J Appl Physiol*. 2014; 116(7): 715-23. doi: 10.1152/japplphysiol.01235.2013. Epub 2014 Jan 30. PMID: 24481964; PMCID: PMC3972746.

[33] Salvi P, Revera M, Faini A, Giuliano A, Gregorini F, Agostoni P, Becerra CG, Bilo G, Lombardi C, O'Rourke MF, Mancia G, Parati G. "Changes in subendocardial viability ratio with acute high-altitude exposure and protective role of acetazolamide". *Hypertension*. 2013; 61(4): 793-9. doi: 10.1161/HYPERTENSIONAHA.111.00707. PMID: 23438935.

[34] Wolfel EE, Selland MA, Mazzeo RS, Reeves JT. "Systemic hypertension at 4,300 m is related to sympathoadrenal activity". *J Appl Physiol*. 1994; 76(4): 1643-50. doi: 10.1152/jappl.1994.76.4.1643. PMID: 8045844.

[35] Böning D. "Altitude and hypoxia training--a short review". *Int J Sports Med*. 1997; 18(8): 565-70. doi: 10.1055/s-2007-972682. PMID: 9443586.

[36] "Acetazolamide in control of acute mountain sickness". *Lancet*. 1981; 1(8213): 180-3. PMID: 6109857.

[37] Huicho L, Pawson IG, León-Velarde F, Rivera-Chira M, Pacheco A, Muro M, Silva J. "Oxygen saturation and heart rate in healthy school children and adolescents living at high altitude". *Am J Hum Biol*. 2001; 13(6): 761-70. doi: 10.1002/ajhb.1122. PMID: 11748815.

[38] Stokke KT, Rootwelt K, Wergeland R, Vale JR. "Changes in plasma and red cell volumes during exposure to high altitude". *Scand J Clin Lab Invest Suppl*. 1986; 184: 113-7. PMID: 3473608.

[39] Sawka MN, Convertino VA, Eichner ER, Schnieder SM, Young AJ. "Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness". *Med Sci Sports Exerc*. 2000; 32(2): 332-48. doi: 10.1097/00005768-200002000-00012. PMID: 10694114.

[40] Robach P, Déchaux M, Jarrot S, Vaysse J, Schneider JC, Mason NP, Herry JP, Gardette B, Richalet JP. "Operation Everest III: role of plasma volume expansion on VO₂max during prolonged high-altitude exposure". *Journal of Applied Physiology*. 2000; 89(1): 29-37. https://doi.org/10.1152/jappl.2000.89.1.29.

[41] Sawka MN, Young AJ, Rock PB, Lyons TP, Boushel R, Freund BJ, Muza SR, Cymerman A, Dennis RC, Pandolf KB, Valeri CR. "Altitude acclimatization and blood volume: effects of exogenous erythrocyte volume expansion". *J Appl Physiol*. 1996; 81(2): 636-42. doi: 10.1152/jappl.1996.81.2.636. PMID: 8872628.

[42] Pearson DT, Naughton GA, Torode M. "Predictability of physiological testing and the role of maturation in talent identification for adolescent team sports". *J Sci Med Sport*. 2006; 9(4): 277-87. doi: 10.1016/j.jsams.2006.05.020. Epub 2006 Jul 17. PMID: 16844415.

[43] West JB. "Oxygen conditioning: A new technique for improving living and working at high altitude". *Physiology (Bethesda)*. 2016; 31(3): 216-22. doi: 10.1152/physiol.00057.2015. PMID: 27053735.

[44] *Dempsey* JA, McKenzie DC, Haverkamp HC, Eldridge MW. "Update in the understanding of respiratory limitations to exercise performance in fit, active adults". *Chest*. 2008; 134(3): 613-622. doi: 10.1378/chest.07-2730. PMID: 18779196.

The effect of a selected exercise protocol on trunk and lower limb muscle activity of older adults with both low back pain and pronated feet during walking

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Article Info	Abstract
Original Article **Article history:** Received: 18 August 2021 Revised: 26 August 2021 Accepted: 21 October 2021 Published online: 08 December 2021 **Keywords**: electromyography, foot pronation, intervention, low back pain, training.	**Background:** Low back pain is a common musculoskeletal condition that can impact a person's ability to walk and move comfortably. Pronated foot posture has been suggested as a potential contributor to low back pain, and this study examines its impact on muscle activity during gait in individuals with low back pain. **Aim:** This study aimed to investigate whether pronated foot alters the activity timing of trunk and lower limb muscles during gait in low back pain patients. **Materials and Methods:** The sample of this study included 32 men with low back pain and pronated foot. Participants were divided into control (n=15, with foot pronated only) and experimental (n=17, with both low back pain and foot pronated) groups. The experimental group did resistance training with Thera-band for 12 weeks, 3 sessions per week. A wireless electromyography system with 9 pairs of bipolar surface electrodes was used to record the electromyography activity timing of back and lower limb muscles (sample rate: 2000 Hz). Two-way ANOVA was used for statistical analysis. **Results:** Significant between-group differences were found at baseline onset of EMG activity timing for gastrocnemious medialis ($P<0.001$), gluteus medius ($P<0.001$) and erector spinae at 3rd lumbar vertebral level ($P=0.001$) muscles. Results indicated significant main effects of "Time" for erector spinae at 3rd lumbar vertebral level offset ($P=0.023$), significant main effects of "group" for tibialis anterior offset ($P=0.039$) and for erector spinae at 3rd lumbar vertebral level offset ($P=0.010$). **Conclusion:** The selected training program changed the timing of erector spinae at 3rd lumbar vertebral level in older adults with both low back pain and pronated feet during walking.

1. Introduction

Low back pain (LBP) is among the most common health problems seen in primary care [1]. Because of its frequency and its mostly benign character, LBP is often seen as a trivial problem compared to other afflictions that generate a higher mortality risk, like cancer or infectious diseases [2]. The first episode of LBP occurs between 20 and 40 years of age [3], and the prevalence is high at ages from 35 to 55 years old [4]. LBP is among the four most commonly reported symptoms in the elderly [5]. It has been reported that up to 84% of older adults suffer from LBP [6]. Despite its high incidence, there is no clear consensus on its etiology.

One possible cause leading to LBP is abnormal foot function [7, 8]. Abnormal foot pronation, which is pronation occurring in an excessive way, causes increased tibia and femur internal rotation [8, 9], anterior inclination of the pelvis [8, 10], and therefore increases mechanical forces on the lumbar spine [8, 11]. This increases tension on the muscles of this region and rotation of the lumbar vertebrae during gait [12]. Therefore, pronated foot (PF), in association with other factors, should be taken into account when evaluating imbalances in the LBP patients with PF [13].

Pronated foot along with LBP may develop from a lack of muscle strength and stability or from overuse. To quantitatively evaluate the effects of LBP along with PF on lower extremity biomechanics, existing studies have principally focused on three measurement techniques: electromyography (EMG), kinematics, and kinetics. With regard to kinetics, LBP along with PF is associated with increased vertical reaction forces and loading rate, impulse in posterior-anterior reaction force, and

positive peak free moments during walking [14]. Also, LBP along with PF is associated with decreased ankle plantar flexion moment, hip flexion moment, and peak positive ankle power during walking [15]. Concerning kinematics, LBP with PF is associated with a decreased peak ankle inversion, peak knee flexion, internal rotation, and peak hip internal rotation [15]. Concerning EMG, LBP along with PF is associated with higher activity of gastrocnemius medialis, gluteus medius, erector spinae and internal oblique muscles during stance phase of walking [15]. Different treatments are often recommended to control excessive PF and LBP [15]. However, their biomechanical mechanism of action on LBP patients with PF is still not fully clear.

Various treatment programs, such as rest, medications, exercise therapy, traction, osteopathic treatment, manipulation, massage, and electrotherapy (e.g., diathermy, laser, musculocutaneous and nerve electrical stimulation, and interferential currents) have been suggested for patients with LBP [16]. Among them, exercise protocols have received more attention within the past 2 decades [16, 17]. This program is commonly used for its short- and long-term advantages, including pain reduction and facilitation of the neuromuscular control at lumbar spine [16, 18].

van Tulder et al. (2000) reported a significant effect of exercise therapy on chronic LBP [19]. Recently, exercises that are related to the engagement of stabilizing muscles such as transverse abdominis (TrA), lumbar multifidus (MF), and internal oblique (IO) have received more attention [16]. These muscles provide segmental stabilization by maintaining a neutral intervertebral position during exercises and

functional activities unlike erector spinae and rectus abdominis (which seem to be involved in movement production) [16]. Co-contraction of deep muscles such as TrA, lumbar MF, and IO as well as pelvic floor muscles produce a force that may contribute to the stability of the spine through thoracolumbar fascia and interabdominal pressure mechanisms [16].

Accordingly, these muscles play a supportive role for the spine. For example, lumbar MF muscle resists the force coming from outside by engagement during the full range of spine movement and also during movements of upper and lower extremities. Because these muscles are always active during daily activities, they do not provide much power. However, good endurance and coordination are required to keep the back in normal position through their constant activities (they don't furnish a whole lot power, in spite of the fact that desirable perseverance and coordination are essential to preserve the back in expected function via their constant activities) [16, 20]. Specific exercises that activate abdominal and/or back extensor muscles are advocated to reduce pain and disability [21]. It is claimed that there is a link between local muscle dysfunction and low back pain, with the development of clinical instability in which there is an excessive range of abnormal segmental movement without muscular control [22]. A trunk stabilization training program reduced low back pain in pain developers, but only corrected gluteus medius co-contraction in males [23, 24].

Although, several studies have investigated the effect of exercises on reducing pain and/or improving muscle function in patients with LBP, the effect of exercise on electromyography of the lower limb and trunk muscles in the LBP patients with PF is very scarce in the literature.

Therefore, the aim of this study is to investigate the effect of a special training program (reinforcing ankle anti-pronators, knee and hip external rotators, and abdominal muscles) on timing of muscle activity of the lower limb and trunk muscles in the LBP patients with PF during walking.

2. Methods

2.1. Participants

In this experiment study, 15 male older adults with PF only (age: 26.0 ± 2.9 years; height: 174.5 ± 5.5 cm; mass: 78.7 ± 9.9 kg; body mass index: 25.9 ± 3.2 kg/m^2) formed the control group and 17 other male older adults with both LBP and PF formed the experimental group (age: 25.3 ± 2.7 years; height: 173.8 ± 4.9 cm; mass: 79.4 ± 10.0 kg; body mass index: 26.3 ± 3.0 kg/m^2). Participants were recruited from a local clinic. All subjects were right foot dominant as determined by kicking ball test.

An orthopedic surgeon in the local clinic assessed all subjects prior to selection. For any of both the control and experimental groups, subjects required a navicular drop of more than 10mm, and a foot posture index of greater than 10 [25]. Navicular drop was measured as the difference in navicular height between non-weight bearing and full weight bearing conditions of the foot in standing position [26]. Additional inclusion criteria were a LBP index of>30 based on visual analog pain scale, and a disability index of>10 based on Roland-Morris disability questionnaire [27].

For all groups, the exclusion criteria were a history of major musculoskeletal surgery at trunk and/or lower limbs, neuromuscular disorders, orthopedic related diseases or postural disorders (except feet pronation for experimental groups, and LBP for PF+LBP), limb length discrepancies of greater than 5 mm, and if

heavy physical tasks or exercises leading to fatigue were performed in the previous two days prior to the experimentation. The research protocol was approved by the ethical committee of Medical Sciences University of Ardabil (IR-ARUMS-REC-1397-031). All subjects gave their informed consent to participate in the study.

2.2. Apparatus

A portable EMG system (BIO SYSTEM, UK) with nine pairs of bipolar pre-gelled Ag/AgCl surface electrodes (circular in shape with 11 mm in diameter; 25 mm center-to-center distance; input impedance of 100 MΩ; and common mode rejection ratio of >110 dB at 50–60 Hz) was used to record the activity of the tibialis anterior (TA), gastrocnemious medialis (Gas-M), long head of biceps femoris (BF), vastus lateralis (VL), gluteus medius (Glut-M), erector spinae at 3rd lumbar vertebral level (ES_{L3}), rectus abdominus (RA), external oblique (EO), and internal oblique (IO) muscles of the right side at a sampling frequency of 2000 Hz.

Skin surface over the selected muscles was shaved, cleaned with alcohol (70% Ethanol–C_2H_5OH) and abraded gently, according to the SENIAM recommendations prior to the electrodes placement [28].

The electrodes' location for TA, Gas-M, BF, VL, and Glut-M muscles was determined based on the recommendation by SENIAM [8, 28]. For ES_{L3} muscle electrodes were placed vertically on the skin, 3 cm lateral to the related spinous process [8, 28, 29, 30]. For the RA muscle, electrodes were placed 3 cm from the midline of the abdomen and 2 cm above the umbilicus [8, 31]. The electrodes for IO muscle were placed 2 cm inward and distal to the anterior superior iliac spine oriented toward umbilicus at an angle of 45° [15].

2.3. Task, procedure, and data processing

Subjects had five minutes for warm up exercises including walking. During both pre- and post-test, the starting point was set appropriately so that the subject had at least eight steps before entering the calibrated space and stepped on the force plate with his right foot. Three successful barefoot walking trials were analyzed during both pre- and post-test. A trial was considered successful if the foot was landed in the middle of the force plate, all markers were visible, and the EMG signals of all muscles were recorded correctly, EMG signals were processed as described in Farahpour et al. (2018) [15].

2.4. Corrective Exercise Intervention

Elastic resistance bands (Thera-Band®, Akron, Ohio, US) ranging from very low to very high resistance (yellow, red, green, blue, black and silver) were used in this intervention. The 1 m long elastic bands were stretched to twice their resting length prior to each exercise [32, 33]. Following the pre-test, the experimental group performed resistance band exercises for both legs three times per week for eight weeks (i.e., 24 strength training sessions). The participants were familiarized with the exercises prior to training. Each exercise session consisted of a general warm-up (10 min), followed by a resistance training session (35-40 min) and was completed with a cool-down routine. Following an adaptation phase of two weeks using low external resistance (yellow TheraBand®, unless the participant was obviously unchallenged, 1 set of 14 repetitions per exercise [34, 35]), exercise intensity was progressively increased by adapting the resistance of the elastic band (based on the Thera-Band® force elongation table [35] from yellow to red, and further to black). In

addition, the exercise volume was extended by increasing the number of sets from one to two. The rate of progression was based on individual improvements (band colour was changed if participants were able to perform 16 repetitions or more in the second set) and reported that they were below seven on the OMNI resistance for active muscle scale (0 extremely easy to 10 extremely hard [36]). The movement velocity during exercises was very low.

The intervention in the experimental group was conducted individually in a physiotherapy clinic, with every session being supervised by a physiotherapist to ensure the correct technique or to modify the exercise or the progression to suit the participants' needs. After the intervention, participants of the experimental group were re-evaluated following the same procedure as the first evaluation. The re-evaluation was scheduled six days after the final intervention session to guarantee that acute physiological responses to training did not interfere with the measurements [8, 37]. The control group did not perform any exercise and was re-evaluated after 8 weeks. All participants were asked not to participate in any other forms of sports or exercise during the intervention period.

2.5. Statistical analysis
The normality of the distribution for outcome measures was confirmed by the Shapiro-Wilk test. Independent t tests were used to analyze any significant differences on the timing of selected muscles between two groups. Statistical within-and between-group differences were assessed by a mixed ANOVA (Time×Group). The effect sizes (Cohen's d) were computed as a ratio of the mean difference divided by the pooled standard deviation. Statistical significance was set at $p<0.05$. Data were analyzed using the SPSS version 28 software (SPSS Inc., Chicago, IL). All values are reported as mean (standard deviation).

Table 1. Corrective exercise program used in this research

Movement	Description
Hip abductor strength training	Four exercises: in the side-lying position with limb to be strengthened on top; in the standing position; side-stepping with elastic resistance in the distal region of the thigh [38], and the exercise was performed seated on massage table adjusted to position the hip at 60° (from this position, the participant was instructed to activate the hip abductors while keeping the knee at about 90°)
Invertor strength training	The invertors were strengthened in side-lying position [38].
Abdominal strength training	After a light warm-up, subjects performed 2 sets of 8 repetitions of the abdominal crunch, right/ left oblique crunch, abdominal drawing-in, and abdominal bracing. Subjects were instructed to hold each contraction for 5 sec, and a 2-min rest followed each set [39]
Hamstring strength training	In standing position, their legs up from the back upwards and doing the extension of the hip against resistance [40]
Hip external rotation training	This exercise was performed on the hip external rotation muscles while the subject was sitting on a table with a 90° flexion angle [40]

3. Results
The between-group differences on age, height, mass, and body mass index were not significant (*P*>0.05). The difference between the navicular drop of PF (12.15 ± 1.51 mm) and PF+LBP (12.24 ± 1.54 mm) groups was also not significant (*P*>0.05).

Significant between-group differences

were found at the baseline onset of EMG activity for Gas-M ($P<0.001$), Glut-M ($P<0.001$) and ES_{L3} ($P<0.001$) muscles (Table 2).

Table 3 describes pre-and post-data for the onset and offset of EMG activity in the trunk and lower limb muscles. The statistical analyses indicated significant main effects of "Time" for ES_{L3} offset ($P=0.023$; d=0.873) muscle. The statistical analysis indicated significant main effects of "group" for TA offset ($P=0.039$; d=0.773) and for ES_{L3} offset ($P=0.010$; d=1.000) muscles.

Table 2. Group-specific baseline values of reported timing of selected muscles electromyography amplitude

Muscles	Timing	CG	EG	*P*-value
TA	Onset	-271.5±177.4	-300.9±192.9	0.659
	Offset	225.1±69.0	256.3±73.3	0.226
Gas-M	Onset	364.2±83.9	213.1±101.2	<0.001*
	Offset	659.5±74.1	657.1±67.9	0.926
BF	Onset	-146.2±28.6	-129.9±31.8	0.139
	Offset	282.2±76.45	245.1±81.2	0.196
VL	Onset	-105.6±86.2	-65.1±46.8	0.104
	Offset	360.1±84.5	424.0±178.9	0.201
Glut-M	Onset	-87.2±58.8	18.0±66.7	<0.001*
	Offset	510.4±117.6	515.9±121.6	0.897
ES_{L3}	Onset	-237.0±238.4	21.6±98.2	0.001*
	Offset	104.1±250.5	202.9±50.8	0.154

Note: CG = control group; EG = experimental group; TA = tibialis anterior; Gas-M = gastrocnemious medialis; BF = biceps femoris; VL = vastus lateralis; Glut-M = gluteus medius; ES_{L3} = erector spinae at 3rd lumbar vertebral level.
*Significant level $P<0.05$
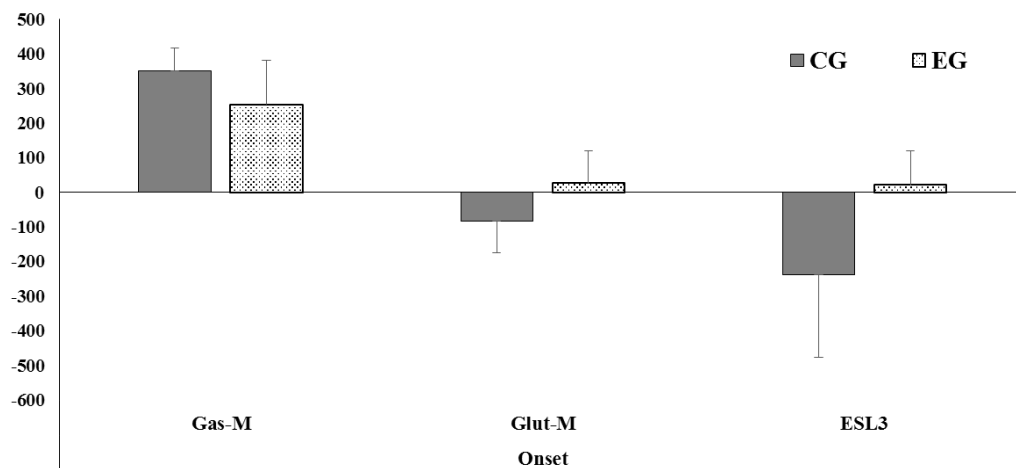
Figure 1. Effects of selected exercises on timing amplitude for trunk and lower limb muscles activity during walking

Table 3. Selected exercises effects on timing amplitude of trunk and lower limb muscles activity during walking

Muscles	Timing	CG (n=15)					EG (n=17)					p-value (Partial Eta Square)		
		Pre-test		Post-test		Δ (%)	Pre-test		Post-test		Δ (%)	Main effect: Time	Main effect: Group	Interaction: Time×Group
		M	SD	M	SD		M	SD	M	SD				
TA	Onset	-271.5	177.4	-251.4	197.4	-7.4	-300.9	192.9	-280.5	201.9	-6.7	0.367 (0.02)	0.654 (0.00)	0.995 (0.00)
	Offset	225.1	69.0	188.4	55.8	16.3-	256.3	73.3	245.9	91.3	-4	0.161 (0.06)	*0.039 (0.13)	0.429 (0.02)
Gas-M	Offset	659.5	74.1	646.7	62.3	-1.94	657.1	67.9	652.1	128.4	-0.76	0.559 (0.01)	0.956 (0.00)	0.798 (0.00)
BF	Onset	-146.2	28.6	-146.7	37.4	0.3	-129.9	31.83	-130.0	75.39	769.8	0.978 (0.00)	0.239 (0.04)	0.985 (0.00)
	Offset	282.2	76.2	260.5	89.6	-7.6	245.1	81.2	264.5	54.7	7.9	0.932 (0.00)	0.481 (0.01)	0.147 (0.06)
VL	Onset	-105.6	86.2	-79.3	41.9	-24.9	-65.1	46.8	-43.8	91.6	-32.7	0.060 (0.11)	0.091 (0.09)	0.841 (0.00)
	Offset	360.1	84.5	338.8	97.9	-5.9	424.0	178.9	408.2	144.7	-3.7	0.388 (0.02)	0.128 (0.07)	0.897 (0.00)
Glut-M	Offset	510.4	117.6	522.1	109.6	2.29	515.9	121.6	521.3	134.6	1.04	0.689 (0.00)	0.950 (0.00)	0.882 (0.00)
ES_{L3}	Offset	104.1	250.5	8.4	185.9	-91.9	202.9	50.8	183.8	57.5	-9.41	0.023* (0.16)	*0.010 (0.20)	0.121 (0.07)

Note: CG = control group; EG = experimental group; TA = tibialis anterior; Gas-M = gastrocnemious medialis; BF = biceps femoris; VL = vastus lateralis; Glut-M = gluteus medius; ES_{L3} = erector spinae at 3rd lumbar vertebral level; M = mean; SD = standard deviation.

*Significant level $P<0.05$, 2-w ANOVA

4. Discussion

The aim of this study was to investigate the effect of a special training program (reinforcing ankle anti-pronators, knee and hip external rotators, and abdominal muscles) on timing of muscle activity of the lower limb and trunk muscles in the LBP patients with PF during walking.

In this study, significant between-group differences were found at baseline onset of EMG activity for Gas-M, Glut-M and ES_{L3} muscles. In describing pre and post data for onset and offset of EMG activity in trunk and lower limb muscles, the statistical analyses showed significant main effects of "Time" for ES_{L3} offset muscle. Also, the statistical analysis indicated significant main effects of "group" for TA offset ($P=$ 0.039; d= 0.773) and for ES_{L3} offset ($P=$ 0.010; d= 1.000) muscles.

An increased activity of the ES_{L3} [15, 41, 42] and hamstring [15, 43] muscles was reported in LBP patients. Besides, in LBP patients with PF, higher activity of Gas-M, Glut-M, ES_{L3}, and IO muscles compared with healthy controls was reported [15]. In the early stance phase, TA acts to decelerate the ankle plantar flexion allowing a smooth flat foot phase [15, 44].

In the loading response phase, the onset time of BF and ES_{L3} muscles in experimental group during post-test was significantly sooner than during pre-test. Also, in the loading response phase, the onset time of TA, Gas-M, VL and Glut-M muscles in experimental group during post-test was significantly later than during pre-test. In the loading response phase, the offset time of TA, Gas-M, VL and ES_{L3} muscles in experimental group during post-test was significantly sooner than during pre-test. Also, in the loading response phase, the offset time of BF and Glut-M muscles in experimental group during post-

test was significantly later than during pre-test. Some studies have postulated that the higher muscle activity in LBP patients is a neuromuscular response to reduce pain by better lumbopelvic stabilization [15, 41, 45, 46, 47]. However, this higher muscle activity in the lumbopelvic region could be associated with trunk inclination control that normally occurs in walking [48].

Ntousis et al. (2013) reported that pronating or supinating bilaterally or unilaterally a normal foot, did not alter the EMG activity of the RA muscle in a quiet standing position [49]. The statistical analyses yielded significant "Time×Group" Interaction during mid-stance for EO muscle and during push off phase for RA muscle.

Murley et al. (2009) [50], and Hunt, Smith and Torode (2004) [51] also reported higher TA activity in PF individuals compared to healthy control group during walking. In the early stance phase, TA acts to decelerate the ankle plantar flexion allowing a smooth flat foot phase [15, 44, 50, 51]. The activity of TA muscle in this phase was not associated with the strong coupling co-activation of tibialis posterior muscle [15, 51]. It appears that the higher TA muscle activity in PF individuals is a neuromuscular adaptation to compensate the PF. Since the higher activity of TA muscle was observed in both PF with and without LBP, it should be excluded as an indication of LBP.

5. Conclusion

The selected training program changed the timing of erector spinae at 3rd lumbar vertebral level in older adults with both low back pain and pronated feet during walking. Since erector spinae muscles play an important role in low back pain and lumbar stability in daily activities, this study's

training program may improve recruiting motor units of erector spinae muscles, resulting in improvement of low back pain and lumbar stability.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to the original idea, study design.

Ethical considerations

The author has completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc.

The research was conducted based on the Helsinki Declaration.

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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References

[1] Vader K, Donnelly C, French SD, Grady C, Hill JC, Tripp DA, et al. "Implementing a new physiotherapist-led primary care model for low back pain: a qualitative study of patient and primary care team perspectives". *BMC Primary Care*. 2022; 23(1): 1-12. https://doi.org/10.1186/s12875-022-01817-5.

[2] Al Sad S, Start AR. "Primary care providers' experiences treating low back pain". *Journal of Osteopathic Medicine*. 2022; 122(5): 263-9. https://doi.org/10.1515/jom-2021-0229.

[3] Rahman MA. "To evaluate the different causes and preventive measures of low back pain: a retrospective study". *SAR J Med*. 2021; 2(3): 22-31.

[4] Grabovac I, Dorner TE. "Association between low back pain and various everyday performances". *Wiener Klinische Wochenschrift*. 2019; 131(21): 541-9. https://doi.org/10.1007/s00508-019-01542-7.

[5] Bazargan M, Loeza M, Ekwegh T, Adinkrah EK, Kibe LW, Cobb S, et al. "multi-dimensional impact of chronic low back pain among underserved African American and Latino older adults". *International Journal of Environmental Research and Public Health*. 2021; 18(14): 7246. https://doi.org/10.3390/ijerph18147246.

[6] Majumder MSM, Hakim F, Bandhan IH, Razzaque MA, Zahid-Al-Quadir A, Ahmed S, et al. "Low back pain in the Bangladeshi adult population: a cross-sectional national survey". *BMJ Open*. 2022; 12(9): e059192. https://doi.org/10.1136/bmjopen-2021-059192.

[7] Yang J, Ou Z, Mao Z, Wang Y, Zhong Y, Chen Z, et al. "Reliability and validity of Foot Posture Index (FPI-6) for evaluating foot posture in participants with low back pain". *Research Sququre*. 2022. https://doi.org/10.21203/rs.3.rs-1466101/v1.

[8] Madadi-Shad M, Jafarnezhadgero AA, Sheikhalizade H, Dionisio VC. "Effect of a corrective exercise program on gait kinetics and muscle activities in older adults with both low back pain and pronated feet: A double-blind, randomized controlled trial". *Gait & Posture*. 2020; 76: 339-45. https://doi.org/10.1016/j.gaitpost.2019.12.026.

[9] Jafarnezhadgero A, Musavi SH, Mehr SMA, Madadi-Shad M. "the long-term wearing of foot orthoses can change the frequency domain of ground reaction forces in children with flexible flat feet". *JPO: Journal of Prosthetics and Orthotics*. 2022; 34(1): 22-32. https://doi.org/10.1097/jpo.0000000000000386.

[10] Yar UB. "A review: Biomechanical substitutions during pregnancy and application of orthoses". *Journal of University Medical & Dental College*. 2022; 13(3): 446-52. https://doi.org/10.37723/jumdc.v13i3.715.

[11] Gevers-Montoro C, Murray KJ, Santamaría B, Dominguez-Vera G, Álvarez-Galovich L, Vindigni D, et al. "Combined chiropractic and podiatric treatment for chronic low back pain concomitant with a unilateral pronated foot: protocol for a multicenter pilot randomized controlled trial". *Journal of Chiropractic Medicine*. 2021; 20(4): 177-82.

https://doi.org/10.1016/j.jcm.2021.12.012.

[12] Tirtashi FH, Eslami M, Taghipour M. "Effect of shoe insole on the dynamics of lower extremities in individuals with leg length discrepancy during walking". *Journal of Bodywork and Movement Therapies*. 2022; 31: 51-6. https://doi.org/10.1016/j.jbmt.2022.03.006.

[13] Castro-Méndez A, Munuera PV, Albornoz-Cabello M. "The short-term effect of custom-made foot orthoses in subjects with excessive foot pronation and lower back pain: a randomized, double-blinded, clinical trial". *Prosthetics and Orthotics International*. 2013; 37(5): 384-90. https://doi.org/10.1177/0309364612471370.

[14] Farahpour N, Jafarnezhad A, Damavandi M, Bakhtiari A, Allard P. "Gait ground reaction force characteristics of low back pain patients with pronated foot and able-bodied individuals with and without foot pronation". *Journal of Biomechanics*. 2016; 49(9): 1705-10. https://doi.org/10.1016/j.jbiomech.2016.03.056.

[15] Farahpour N, Jafarnezhadgero A, Allard P, Majlesi M. "Muscle activity and kinetics of lower limbs during walking in pronated feet individuals with and without low back pain". *Journal of Electromyography and Kinesiology*. 2018; 39: 35-41. https://doi.org/10.1016/j.jelekin.2018.01.006.

[16] Nabavi N, Bandpei MAM, Mosallanezhad Z, Rahgozar M, Jaberzadeh S. "The effect of 2 different exercise programs on pain intensity and muscle dimensions in patients with chronic low back pain: a randomized controlled trial". *Journal of Manipulative and Physiological Therapeutics*. 2018; 41(2): 102-10. https://doi.org/10.1016/j.jmpt.2017.03.011.

[17] Chasouraki A, Kourek C, Sianis A, Loritis K, Kostakou P, Tsougos E, et al. "Practical approaches to build and sustain a cardio-oncology clinic". *Journal of Cardiovascular Development and Disease*. 2022; 9(5): 158. https://doi.org/10.3390/jcdd9050158.

[18] Motallebi L, Mohseni Bandpei MA, Rahmani N. "Effects of stabilization exercises on pain intensity, functional disability and cross sectional area of multifidus muscle in women with non-specific chronic low back pain". *Journal of Mazandaran University of Medical Sciences*. 2013; 23(100): 11-19. https://doi.org/10.1016/j.ultrasmedbio.2013.02.270.

[19] van Tulder M, Malmivaara A, Esmail R, Koes B. "Exercise therapy for low back pain: a systematic review within the framework of the cochrane collaboration back review group". *Spine*. 2000; 25(21): 2784-96. https://doi.org/10.1097/00007632-200011010-00011.

[20] Bogduk N. *Clinical Anatomy of the Lumbar Spine and Sacrum*: Elsevier Health Sciences; 2005.

[21] Singh SK, Khan N, Agarwal R. "Effect of rhythmic stabilization exercise v/s conventional physiotherapy on pain and disability with patients of chronic mechanical low back pain". *The Indian Journal of Physiotherapy & Occupational Therapy*. 2019; 13(4): 4122. https://doi.org/10.5958/0973-5674.2019.00146.1.

[22] Kliziene I, Sipaviciene S, Klizas S, Imbrasiene D. "Effects of core stability exercises on multifidus muscles in healthy women and women with chronic low-back pain". *Journal of Back and Musculoskeletal Rehabilitation*. 2015; 28(4): 841-7. https://doi.org/10.3233/bmr-150596.

[23] Nelson-Wong E, Callaghan JP. "Is muscle co-activation a predisposing factor for low back pain development during standing? A multifactorial approach for early identification of at-risk individuals". *Journal of Electromyography and Kinesiology*. 2010; 20(2): 256-63. https://doi.org/10.1016/j.jelekin.2009.04.009.

[24] Sipaviciene S, Kliziene I. "Effect of different exercise programs on non-specific chronic low back pain and disability in people who perform sedentary work". *Clinical Biomechanics*. 2020; 73: 17-27. https://doi.org/10.1016/j.clinbiomech.2019.12.028.

[25] Redmond AC, Crosbie J, Ouvrier RA. Development and validation of a novel rating system for scoring standing foot posture: The foot posture index". *Clinical Biomechanics*. 2006; 21(1): 89-98. https://doi.org/10.1016/j.clinbiomech.2005.08.002.

[26] Cote KP, Brunet ME, II BMG, Shultz SJ. "Effects of pronated and supinated foot postures on static and dynamic postural stability". *Journal of Athletic Training*. 2005; 40(1): 41.

[27] Mousavi SJ, Parnianpour M, Mehdian H, Montazeri A, Mobini B. The oswestry disability index, the Roland-Morris disability questionnaire, and the Quebec back pain disability scale: translation and validation studies of the Iranian versions". *Spine*. 2006; 31(14): E454-E9. https://doi.org/10.1097/01.brs.0000222141.61424.f7.

[28] Hermens HJ, Freriks B, Disselhorst-Klug C,

Rau G. "Development of recommendations for SEMG sensors and sensor placement procedures". *Journal of Electromyography and Kinesiology*. 2000; 10(5): 361-74. https://doi.org/10.1016/s1050-6411(00)00027-4.

[29] Cheung J, Halbertsma JP, Veldhuizen AG, Sluiter WJ, Maurits NM, Cool JC, et al. "A preliminary study on electromyographic analysis of the paraspinal musculature in idiopathic scoliosis". *European Spine Journal*. 2005; 14(2): 130-7. https://doi.org/10.1007/s00586-004-0780-7.

[30] de Sèze MP, Cazalets JR. "Anatomical optimization of skin electrode placement to record electromyographic activity of erector spinae muscles". *Surgical and Radiologic Anatomy*. 2008; 30(2): 137-43. https://doi.org/10.1007/s00276-007-0289-y.

[31] Mirka GA, Marras WS. "Coactivation during trunk bending". *Spine*. 1993; 18(11): 1396-409. https://doi.org/10.1097/00007632-199318110-00003.

[32] Andersen LL, Andersen CH, Mortensen OS, Poulsen OM, Bjørnlund IBT, Zebis MK. "Muscle activation and perceived loading during rehabilitation exercises: comparison of dumbbells and elastic resistance". *Physical Therapy*. 2010; 90(4): 538-49. https://doi.org/10.2522/ptj.20090167.

[33] Patterson RM, Jansen CWS, Hogan HA, Nassif MD. "Technical report-material properties of thera-band tubing". *Physical Therapy*. 2001; 81(8): 1437-45. https://doi.org/10.1093/ptj/81.8.1437.

[34] Franzke B, Halper B, Hofmann M, Oesen S, Pierson B, Cremer A, et al. "The effect of six months of elastic band resistance training, nutritional supplementation or cognitive training on chromosomal damage in institutionalized elderly". *Experimental Gerontology*. 2015; 65: 16-22. https://doi.org/10.1016/j.exger.2015.03.001.

[35] Page P, Ellenbecker TS, Page P. *Strength Band Training*. Human Kinetics USA; 2005.

[36] Lagally KM, Robertson RJ. "Construct validity of the OMNI resistance exercise scale". *Journal of Strength and Conditioning Research*. 2006; 20(2): 252. https://doi.org/10.1519/00124278-200605000-00003.

[37] Clarkson PM, Hubal MJ. "Exercise-induced muscle damage in humans". *American Journal of Physical Medicine & Rehabilitation*. 2002; 81(11): S52-S69. https://doi.org/10.1097/00002060-200211001-00007.

[38] Kamonseki DH, Gonçalves GA, Liu CY, Júnior IL. "Effect of stretching with and without muscle strengthening exercises for the foot and hip in patients with plantar fasciitis: a randomized controlled single-blind clinical trial". *Manual Therapy*. 2016; 23: 76-82. https://doi.org/10.1016/j.math.2015.10.006.

[39] Kordi R, Dehghani S, Noormohammadpour P, Rostami M, Mansournia MA. "Effect of abdominal resistance exercise on abdominal subcutaneous fat of obese women: A randomized controlled trial using ultrasound imaging assessments". *Journal of Manipulative and Physiological Therapeutics*. 2015; 38(3): 203-9. https://doi.org/10.1016/j.jmpt.2014.12.004.

[40] Haff GG, Triplett NT. *Essentials of Strength Training and Conditioning*. 4th edition: Human Kinetics; 2015.

[41] Hanada EY, Johnson M, Hubley-Kozey C. "A comparison of trunk muscle activation amplitudes during gait in older adults with and without chronic low back pain". *PM & R*. 2011; 3(10): 920-8. https://doi.org/10.1016/j.pmrj.2011.06.002.

[42] Lamoth CJ, Daffertshofer A, Meijer OG, Beek PJ. "How do persons with chronic low back pain speed up and slow down?: Trunk–pelvis coordination and lumbar erector spinae activity during gait". *Gait & Posture*. 2006; 23(2): 230-9. https://doi.org/10.1016/j.gaitpost.2005.02.006.

[43] Vogt L, Pfeifer K, Banzer W. "Neuromuscular control of walking with chronic low-back pain". *Manual Therapy*. 2003; 8(1): 21-8. https://doi.org/10.1054/math.2002.0476.

[44] Hunt AE, Smith RM. "Mechanics and control of the flat versus normal foot during the stance phase of walking". *Clinical Biomechanics*. 2004; 19(4): 391-7. https://doi.org/10.1016/j.clinbiomech.2003.12.010.

[45] Arab AM, Ghamkhar L, Emami M, Nourbakhsh MR. "Altered muscular activation during prone hip extension in women with and without low back pain". *Chiropractic & Manual Therapies*. 2011; 19(1): 18. https://doi.org/10.1186/2045-709x-19-18.

[46] Ferguson SA, Marras WS, Burr DL, Davis KG, Gupta P. "Differences in motor recruitment and resulting kinematics between low back pain patients and asymptomatic participants during lifting exertions". *Clinical Biomechanics*. 2004; 19(10): 992-9. https://doi.org/10.1016/j.clinbiomech.2004.08.007.

[47] van der Hulst M, Vollenbroek-Hutten MM, Rietman JS, Hermens HJ. "Lumbar and abdominal muscle activity during walking in subjects with chronic low back pain: support of

the "guarding" hypothesis?". *Journal of Electromyography and Kinesiology*. 2010; 20(1): 31-8. https://doi.org/10.1016/j.jelekin.2009.03.009.

[48] Begon M, Leardini A, Belvedere C, Farahpour N, Allard P. "Effects of frontal and sagittal thorax attitudes in gait on trunk and pelvis three-dimensional kinematics". *Medical Engineering & Physics*. 2015; 37(10): 1032-6. https://doi.org/10.1016/j.medengphy.2015.08.001.

[49] Ntousis T, Mandalidis D, Chronopoulos E, Athanasopoulos S. "EMG activation of trunk and upper limb muscles following experimentally-induced overpronation and oversupination of the feet in quiet standing". *Gait & Posture*. 2013; 37(2): 190-4. https://doi.org/10.1016/j.gaitpost.2012.06.028.

[50] Murley GS, Landorf KB, Menz HB, Bird AR. "Effect of foot posture, foot orthoses and footwear on lower limb muscle activity during walking and running: a systematic review". *Gait & Posture*. 2009; 29(2): 172-87. https://doi.org/10.1016/j.gaitpost.2008.08.015.

[51] Hunt AE, Smith RM, Torode M. "Extrinsic muscle activity, foot motion and ankle joint moments during the stance phase of walking". *Foot & Ankle International*. 2001; 22(1): 31-41. https://doi.org/10.1177/107110070102200105.