Scientific Research Publishing

Open Journal of Preventive Medicine, 2019, 9, 69-79
http://www.scirp.org/journal/ojpm
ISSN Online: 2162-2485
ISSN Print: 2162-2477

Chronic Physical Activity Does Not Impact Metabolic Responses to Low or High Doses of Resistant Starch: A Crossover Trial

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How to cite this paper: Kim, J.S., Emerson, S.R., Haub, M.D. and Rosenkranz, S.K. (2019) Chronic Physical Activity Does Not Impact Metabolic Responses to Low or High Doses of Resistant Starch: A Crossover Trial. *Open Journal of Preventive Medicine*, **9**, 69-79.
https://doi.org/10.4236/ojpm.2019.97007

Received: June 26, 2019
Accepted: July 22, 2019
Published: July 25, 2019

Abstract

Physical activity and resistant starch are known independently, to beneficially affect metabolic health especially in the gut. However, little is known about the combined effects of physical activity and resistant starch. Thus, the purpose of this study was to investigate the effects of physical activity at different dosages of resistant starch on gut and metabolic health, represented by breath hydrogen and blood glucose responses. Twenty young, healthy participants were stratified into two physical activity groups based on seven-day accelerometry data. Participants visited the lab twice in random order and consumed a meal with either 5 g or 25 g of resistant starch. Breath hydrogen and blood glucose were measured at baseline and serially for six and two hours after meal consumption respectively. Total area under the curve (AUC) for breath hydrogen and incremental AUC for blood glucose were not different between physical activity groups or resistant starch conditions. Thus, chronic physical activity status did not impact breath hydrogen or blood glucose responses to either low or high resistant starch meals.

Keywords

Dietary Fiber, Physical Activity, Resistant Starch, Fermentation, Diabetes

1. Introduction

The association between gut microbiota and chronic diseases is becoming well established [1]. Microbiota primarily live in the large intestine, and dysbiotic microbiota has been found to be associated with diabetes, heart disease, and ob-

esity [2]. A gut microbial population with higher diversity and a lower ratio of *Firmicutes* to *Bacteridetes* has been found to be an indicator of a healthy state of the gut [3] [4]. For analyzing gut status, a direct measure of microbiota analysis via cecum/feces has been widely applied to examine the association between gut microbiota and lifestyle factors such as diet and physical activity (PA) [3] [4] [5].

From a dietary perspective, dietary fiber and resistant starch (RS) are associated with decreased risk of developing several chronic diseases and have frequently been shown to significantly change the bacteria present within an individual's gastrointestinal system [6] [7] [8]. The assessment of breath hydrogen concentration has been used as an indirect evaluation of gut bacteria metabolism of dietary fiber [9] [10]. Specifically, one study that employed a cross-over design among healthy subjects, reported that consuming RS increased gut fermentation as evidenced by increased breath hydrogen concentration in as little as seven days [11]. Additionally, a double-blinded cross-over study in healthy human subjects demonstrated that RS type 4 elicited significant microbiome changes in as little as two to three weeks [12]. Results from a single-blinded cross-over study revealed that high RS intake (60 grams in 24 hrs) significantly increased breath hydrogen among healthy adult individuals [13]. However, there are limited data available to indicate whether breath hydrogen increases more acutely, such as in within the postprandial period, immediately following RS consumption.

Unlike RS, there is little evidence including the effects of PA on gut bacteria within humans. However, a cross-sectional study testing rugby players found that PA altered the composition of gut microbiota compared to a control group at one-time point [3]. This study analyzed fecal microbiota composition and showed 22 distinct phyla with a higher diversity of gut microbiota in the rugby players as compared to the control group. Having a higher diversity of gut microbiota is commonly found among lean individuals compared to obese individuals, potentially indicating better health outcomes [14] [15]. However, due to the cross-sectional nature of the study, causality for PA on gut microbiota could not be determined.

There are few studies that have been designed to better understand the impact of PA at different levels of RS consumption on gut microbiota. A better understanding of the effects of PA on gut fermentation following consumption of different levels of RS is important given the recommendations to include both in a healthy lifestyle [16] [17]. Therefore, the purpose of the current study was to investigate the possible effects of chronic PA on breath hydrogen production and blood glucose, at different dosages of RS, indicating potential metabolic health effects. We hypothesized that more physically active individuals would exhibit greater breath hydrogen production due to a potentially greater diversity of gut microbiota, and decreased two-hour blood glucose response as compared to the less physically active group. We utilized a simple, fast and non-invasive measure of gut fermentation (breath hydrogen production), and also assessed the acute

postprandial period, to further explore the interaction between gut fermentation and metabolic health in a true-to-life scenario.

2. Methods

Twenty healthy young participants (9 M/11 F; age: 24.5 ± 3.4 yrs) were recruited from the local area. All participants were non-smokers, free from ongoing dysmetabolic conditions, and had not used antibiotics for at least the past three months. Participants were recruited from posted flyers and contacted a research assistant via phone or email. Participants were screened for whether they met the American College of Sport Medicine (ACSM) physical activity guidelines of 30 minutes or more of moderate-to-vigorous physical activity (MVPA) five times a week, via a short form International physical activity questionnaire (IPAQ-SF) for seven days [17] [18]. We further assessed PA using Actical accelerometers (Respironics Inc., Bend, OR, USA) as an objective PA assessment. Participants wore accelerometers on their wrist, and based on seven consecutive days of accelerometer data, were divided into two different groups by median split: a "more active group" (MA, more than 160 minutes per day MVPA) or "less active group" (LA, less than 116 minutes per day MVPA). The study was approved by the Institutional Review Board of Human Subjects at Kansas State University, Manhattan, Kansas (protocol #7901).

All participants were required to attend three appointments at the Physical Activity and Nutrition Clinical Research Consortium facility (PAN-CRC). On the first visit, participants provided both oral and written informed consent for the study. Then, participants listened to a brief introduction of the study, provided required information through documents (medical history form, IPAQ, 3-day food record), and accelerometers were attached on their non-dominant wrist. Each participant's height and weight, used to calculate Body Mass Index (BMI), were measured. Three-day food record data were analysed using Nutritionist Pro software by the same technician (Axxya Systems LLC, WA, USA). For the food records, participants were instructed to write down the food items consumed, the brand/type of food, and portion sizes based on the instructions.

Participants each completed two separate meal sessions, following a 10 - 12 hour fast and exercise avoidance for 48 hours. At each meal session, participants consumed a commercially available breakfast bowl (Great Value, Walmart, AR, USA), with the main ingredients being potatoes, eggs, cheese, and sausage. The amount of food consumed was calculated based on participant's body weight (10 kcal/kg). This dose was chosen based on the previous literature [19] to represent a high-fat meal (~58% fat), with kcals ranging from 466 to 1002 kcals. Either 5 g or 25 g of RS type 4 powder (MGP Ingredients, KS, USA) was added to a lemonade beverage that was consumed along with the breakfast bowl (Minute Maid Premium Lemonade, 250 mL, The Coca-Cola Company, GA, USA). Participants were blinded to the RS dosage, and the meal was consumed within 20 minutes

on each visit.

For each meal session, breath hydrogen (H_2) was measured at baseline and every hour up to the fourth hour using Quintron Breath Tracker Analyzer (Breath Tracker SC, Quintron Instrument Company, Inc., WI, USA). After the fourth hour, measurements were taken every 30 minutes. Exhaled breath collection was performed according to manufacturer standards (Alveosampler, cat.#: QT00827-P, Quintron Instrument Company, Inc., WI, USA) and processed within 2 hours of collection. The present study assessed the fourth-hour breath hydrogen AUC as well, due to the consideration of individual variability in oro-cecal transit time (OCTT) between 192 and 232 minutes following consumption of a solid meal [20]. Additionally, a previous study measured the breath hydrogen AUC from 180 minutes until the end of the study [5]. To assess glucose, there were seven serial finger pricks at baseline, 15, 30, 45, 60, 90, and 120 minutes after the consumption of the test meal, performed according to standard protocol using a Bayer Countour blood glucose meter (Bayer HealthCare LLC, Mishawaka IN, USA) [21].

Data are presented as Mean ± SD in tables and Mean ± SE in figures. The sample size was determined based on the previous literature, indicating that 10 to 20 subjects were required for adequate power to detect differences in breath hydrogen response or blood glucose, respectively [5] [13]. Shapiro-Wilk tests were performed for determining the normality distribution of each variable [22]. A Mann-Whitney U test and student t-test were performed for determining differences between the groups. Spearman correlations were conducted for MVPA and self-report MVPA as well as with hydrogen data with PA level. The incremental area under the curve (iAUC) for postprandial glucose (mmol/l × 2 hr), and total area under the curve (tAUC) for breath hydrogen (ppm × 6 hr), fourth hour AUC breath hydrogen were calculated using the trapezoid model with v6.0 Prism (GraphPad Software, Inc., La Joya, CA, USA). Two-way mixed analysis of variance (ANOVA) was performed for postprandial glucose iAUC and log-transformed breath hydrogen AUC and fourth-hour hydrogen AUC, with RS and PA as two independent factors. Three-way ANOVA with repeated measures was performed for the postprandial glucose response. Freidman's tests and Wilcoxon signed rank tests were conducted for breath hydrogen data analysis within the groups and between the groups at the different level of RS. All the above statistics were completed using IBM SPSS Statistics 23 (SPSS Inc., Chicago, IL, USA). The level of significance was set at $p < 0.05$.

3. Results

Participant characteristics and PA status are presented in **Table 1**. A total of eleven females and nine males completed the study. The more active (MA) group was older than the less active (LA) group ($p < 0.05$). The other descriptive variables were not significantly different between groups. The MA group achieved more daily steps and MVPA (19,728 steps/day and 204 mins/day) compared

with the LA group (11,486 steps/day and 96 mins/day) ($p < 0.05$). Data from the 3-day food records are displayed by the group in Table 2. There were no differences in dietary intake between the two groups.

Postprandial breath hydrogen responses are displayed in Figure 1(a). Postprandial breath hydrogen total AUC (tAUC) data are displayed in Table 3. Based on the two-way ANOVA results, there was no interaction effect for postprandial

Table 1. Participant characteristics and physical activity (PA) summary.

	More Active group (n = 10)		Less Active group (n = 10)		*p*-value
Sex	Male (n = 3)	Female (n = 7)	Male (n = 6)	Female (n = 4)	0.178
	Mean ± SD		Mean ± SD		
Age (yrs)	26.0 ± 3.5		22.8 ± 2.4*		0.300
BMI (kg/m²)	23.75 ± 3.84		25.33 ± 4.12		0.384
Height (m)	1.70 ± 0.09		1.67 ± 0.08		0.565
Weight (kg)	69.0 ± 16.5		71.8 ± 17.1		0.717
Baseline Glucose (mmol/L)	4.76 ± 0.38		4.82 ± 0.41		0.677
Baseline Hydrogen (ppm)	10 ± 13		11 ± 11		0.436
Steps (day)	19728 ± 5256		11486 ± 3997*		0.001
MVPA (mins/day)	204.2 ± 41.2		95.8 ± 24.1*		<0.001
(mins/week)	1429.4 ± 288.5		670.6 ± 168.9		
MVPA (self-report) (mins/week)	141.5 ± 173.6		109.5 ± 125		0.853

*Less active group is significantly different from more active group ($p < 0.05$) MVPA: moderate-to-vigorous physical activity.

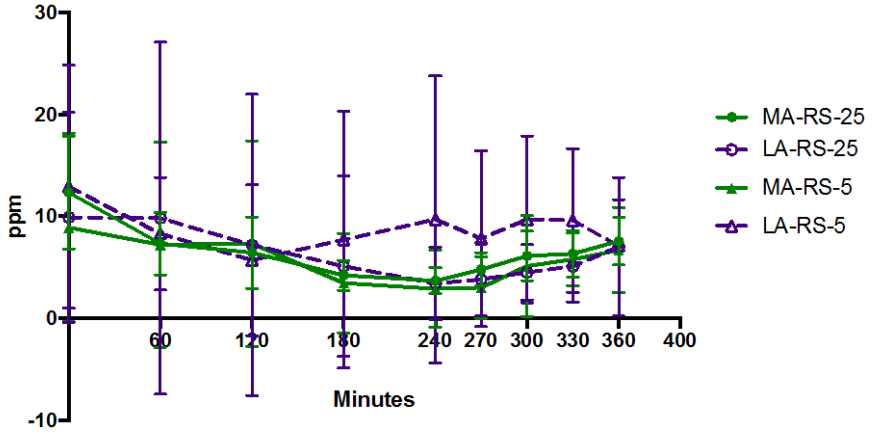
Table 2. Three-day food record dietary summary.

	More Active Group (N = 10)	Less Active Group (N = 10)	*p*-value
	Mean ± SD	Mean ± SD	
Total KJ **- Kilocalorie**	8002 ± 2680 1912 ± 641	8182 ± 3483 2034 ± 832	0.718
Carbohydrate (g)	202 ± 70	252 ± 77	0.146
g/kg bw	3.1 ± 1.4	3.5 ± 0.8	0.105
% energy	44.0 ± 11.9	51.9 ± 7.8	0.095
Protein (g)	89 ± 36	81 ± 44	0.481
g/kg bw	1.34 ± 0.53	1.10 ± 0.50	0.329
% energy	18.96 ± 4.30	15.29 ± 4.22	0.071
Fat (g)	86 ± 53	78 ± 46	0.739
g/kg bw	1.26 ± 0.71	1.05 ± 0.48	0.447
% energy	38.06 ± 10.28	32.30 ± 6.05	0.144
Fiber (g)	17 ± 10	14 ± 10	0.481
g/1000 kcal	8.80 ± 4.15	6.92 ± 2.97	0.436

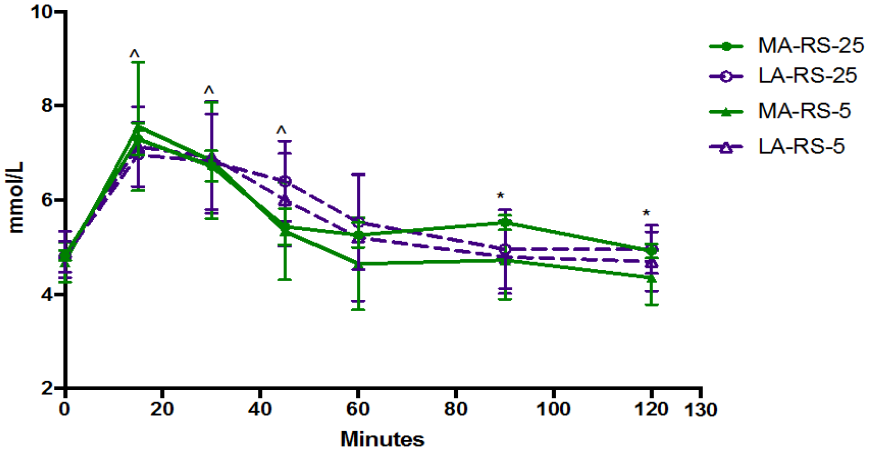
bw: body weight.

breath hydrogen AUC between PA groups at different levels of RS dosage, and no interaction effect in fourth-hour breath hydrogen AUC between PA groups at the different levels of RS dosage ($p = 0.281$). Additionally, in the LA group, 5 g of RS showed higher fourth hour AUC compared to 25 g, however this result was not significant according to the a priori p-value cutpoint ($X^2(1) = 3.600$, $p = 0.058$). There was an inverse correlation for AUC hydrogen at the 25 g RS dose and MVPA ($r = -0.869$, N = 10, $p = 0.001$).

Postprandial glucose (mmol/L) responses following the two meal sessions are displayed in Figure 1(b). Based on the three-way RM ANOVA, there was no significant interaction effect between RS dose and PA group. However, there was a significant Time × RS dose interaction effect in the glucose response ($p < 0.05$), indicating the higher dose of RS showed less fluctuation throughout the period.



(a)



(b)

Figure 1. Postprandial breath hydrogen and glucose responses. (a) Postprandial breath hydrogen for 360 minutes, (b) Postprandial glucose for 120 minutes for More active group with 25 g of resistant starch received (MA-RS-25) and 5 g of resistant starch received (MA-RS-5), and Less active group with 25 g of resistant starch received (LA-RS-25) and 5 g of resistant starch received (LA-RS-5), Mean ± SE, *MA with 25 g intake significantly different from MA with 5 g intake ($p < 0.05$) ^significant time effect compared to baseline ($p < 0.05$).

Table 3. Incremental area under the curve (iAUC) for postprandial glucose and postprandial breath hydrogen area under the curve (tAUC) by group and RS dosage.

	More Active group (n = 10)		Less Active group (n = 10)		RS × PA[&]	RS[&]	PA[&]
	25 g RS	**5 g RS**	**25g RS**	**5 g RS**			
iAUC glucose[1]	118.2 ± 52.7	125.6 ± 40.7	126.6 ± 45.3	122.6 ± 62.7	0.659	0.894	0.891
tAUC Hydrogen[2]	2424 ± 2100	2129 ± 2065	2283 ± 3110	3045 ± 2962			
AUC Hydrogen[3]	3.2 ± 0.5	3.2 ± 0.4	3.2 ± 0.4	3.3 ± 0.4	0.369	0.415	0.622
AUC Hydrogen[4] (%[5])	963 ± 1096 (40.1)	524 ± 405 (40.3)	659 ± 462 (30.6)	1053 ± 879 (39.0)			

Mean ± SD [1]units for iAUC: mmol/minutes/L; [2]units for total AUC: ppm/minutes; [3]Log10 transformed data were used; [4]fourth hour AUC hydrogen calculation: 240 minutes (baselines) - 360 minutes; [5]denotes the proportion of fourth hour AUC in total AUC hydrogen in percentage; [&]indicates two way mixed analysis of variance was conducted: interaction effect of RS and PA, main effect from RS and PA, *p*-values all >0.05. All of the AUC hydrogen variables were calculated with ppm * minutes.

Among LA, there was no difference in glucose response at 90 and 120 minutes. Postprandial glucose iAUC is represented in Table 3. Additionally, there was a significant time effect for glucose values at 15, 30 and 45 minutes compared to the baseline ($p < 0.05$). At 90 and 120 minutes following meal consumption, MA showed a higher glucose response for the 25 g dosage (5.53 ± 0.50 vs. 4.73 ± 0.83 and 4.92 ± 0.46 vs. 4.36 ± 0.58) compared to the 5 g dosage ($p < 0.05$). However, LA did not show any difference between the 5 g or 25 g RS dosage.

4. Discussion

We hypothesized that chronic level of PA would have an impact on the breath hydrogen and blood glucose responses to different doses of RS. Our results support the null hypothesis, indicating that there was no effect for chronic level of PA on breath hydrogen or glucose responses to high or low doses of RS during the acute postprandial period in apparently healthy adults. To our knowledge, there are no previous studies which have examined the impact of chronic PA level on breath hydrogen production, either in the fasted or postprandial state. With regard to the glucose response, there was a significant difference at 90 and 120 minutes when comparing the 25 g RS dosage to the 5 g RS dosage in the MA group, supporting a potentially beneficial effect of the high level of RS among the more active participants.

Numerous studies have shown that a high dose of RS elicits a large breath hydrogen response 24 hours or later [11] [13]. The current study investigated breath hydrogen for 6 hours post-meal, potentially explaining the null findings, since evidence suggests that additional time is required to detect breath hydrogen [20] [23]. It is possible that our assessment was insufficient in duration for accurately observing the production of hydrogen from gut to oral assessment. Thus, there is a discrepancy between our results and results from a previous study that examined the acute post-exercise breath hydrogen response. Participants exercised for 5 minutes on a treadmill at 10 km/h with an incline of 20%,

and results indicated a consistently increased breath hydrogen response when comparing the breath hydrogen tAUC and third hour AUC until the end of the study [5]. Despite the lack of differences in our study, the proportion of hydrogen produced between hours 4 and 6 reached almost 40% of the total hydrogen production in MA and LA, indicating a greater time requirement for accurately assessing gut fermentation. This result suggests that breath hydrogen response is likely initiated at least 4 hours after a high RS intake.

The current study results indicated no difference in glucose iAUC between the MA and LA participants; however, there was a higher glucose response in the MA for the 25 g RS dosage as compared with the 5 g RS dosage at 90 and 120 minutes. This result may be due to a protective effect of more RS type 4, in the gut, indicating a prolonged time for digestion and inflation of stomach, and thus possibly providing longer satiety. Additionally, the MA group had a higher peak glucose at 15 minutes for the 5 g RS dosage compared to 25 g RS dosage, pointing to a potentially beneficial effect of RS [21] [24]. However, in the LA, we saw the opposite trend with high glucose levels at peak. Overall, RS type 4 seemed to be advantageous for maintaining glucose homeostasis within the MA group, but not in the LA group.

We believe our study is novel and contributes to the larger body of research knowledge regarding the interactions between PA and diet. Particularly, examining the effect of chronic PA status on acute breath hydrogen production and blood glucose following a high and low dosage of RS was innovative. Our study utilized a true-to-life meal which was relative to the size of the individual. Also, use of the objective PA assessment substantially strengthened the group assignment by PA status. However, there are limitations to be considered when interpreting the results. Despite the utilization of participants' self-reported levels of PA for screening for the purpose of recruiting sufficiently and insufficiently active participants, according to objective accelerometry, both groups exceeded the ACSM physical activity guideline recommendation of 150 minutes per week of MVPA. We observed that four out of ten of the LA group subjects preferred to commute by walking and biking, with the rest commuting by automobiles. It is possible that college students who frequently engage in walking between classes and home, may not perceive these activities as MVPA according to IPAQ definitions. Inferring from a very low correlation between objective MVPA and self-report MVPA, it is possible that wrist-based accelerometers may have overestimated MVPA in this population, and wrist-based accelerometer cut-points may need to be revised. However, there was a consistency with regard to some additional unexpected findings for the LA group. Especially, within the LA group at the 5 g RS dosage. An inverse correlation was found between breath hydrogen AUC and MVPA which is the opposite of what we expected. These results may be due to a small sample size combined with a high variability in the LA group's postprandial breath hydrogen, reflecting the complexity of analyzing breath hydrogen in human participants.

5. Conclusion

In conclusion, chronic PA levels did not modify the acute breath hydrogen or blood glucose responses to high or low RS doses during a six-hour assessment period. However, given that the duration of the assessment was likely insufficient to adequately understand breath hydrogen responses following the consumption of different RS doses, further studies are warranted. In addition, future studies may need to include more participants in order to generalize the effects of chronic PA with different dosage of RS on metabolic health outcomes.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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