RETROSPECTIVE REVIEW OF PHYSICAL ACTIVITY AND NUTRITIONAL QUESTIONNAIRE DATA COLLECTED FROM AN EXERCISE INTERVENTION PROGRAM

By

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A Thesis Submitted to The W.A. Franke Honors College

In Partial Fulfillment of the Bachelors degree
With Honors in

Physiology

THE UNIVERSITY OF ARIZONA

MAY 2023

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Abstract

In 2022, Garcia et al. published a study entitled "Can Exercise Training Alter Human Skeletal Muscle DNA Methylation?" [6]. That study found significant evidence that 8-weeks of exercise training altered DNA methylation patterns in skeletal muscle. This paper is a retrospective review of the data collected by the physical activity and nutritional questionnaires used in that study, which had yet to be analyzed. Here, we performed an analysis of the questionnaire data before and after the 8-weeks of exercise training. The retrospective analysis provides complementary findings to the previously published methylation study of Garcia et al. [6]. Additionally, this study examines and discusses participant compliance, limitations, and future recommendations for monitoring physical activity and nutritional diet as part of interventional studies.

Introduction

The Garcia et al. study aimed to determine the plasticity of skeletal muscle DNA methylation in response to exercise in participants with varying degrees of insulin sensitivity [6]. The study was an 8-week exercise intervention that included supervised visits at the research clinic. Each week of the intervention, blood pressure and weight were measured. In addition, insulin action on glucose metabolism and peak VO2 were measured before and after the 8-weeks of exercise. During the first 4-weeks, the participants exercised 3 times a week, and during the second half, they exercised 4 times a week. The participants began exercising at 60% of their VO2 peak for 20 min on a stationary cycle ergometer 3 times per week and, over the course of the study, increased exercise intensity, duration, and frequency to 70% of VO2 peak for 45 min, 4 times per week.

The participants were asked not to exercise outside of the study and to maintain their regular diet during the study. In addition, a study staff member (interviewer) administered a monthly recall diet questionnaire and a 7-day physical activity recall questionnaire at the baseline, 4-weeks, and 8-weeks of the training study to check for compliance. The Garcia et al. study describes the findings of the global methylation analysis along with the effects of the training on insulin action and VO2 peak [6]. This study analyzes the physical activity and nutritional (diet) compliance questionnaire data collected as part of the Garcia et al. study [6]. Since the study staff advised no changes to either physical activity or diet (beyond the supervised training in the research clinic), we hypothesized that the participants' amount of exercise and diet would not alter during the 8-weeks of study.

Physiologically, physical activity and diet have many effects on the body. In addition to the epigenetic effects described by Garcia et al., regular exercise has been shown to have positive effects on the body, such as increasing VO_2 max, reducing insulin resistance, and preventing chronic disease, such as obesity, hypertension, diabetes, and depression [6, 18]. Diet, both the types and amounts of food, also has an effect in preventing chronic disease [2].

Over-consumption of calories can increase weight, reduce insulin sensitivity, and reduce VO_2 max, while healthy weight loss can do the opposite [2]. Because physical activity and diet can have such a huge effect on the function of the human body, they must be taken into consideration when research is being conducted.

Survey Methods Used

The physical activity survey used in the present study was the Stanford Seven-Day Physical Activity Recall questionnaire (PAR) [11]. The PAR questionnaire has been used in several studies with various participants, including chronic obstructive pulmonary disease (COPD) patients [7], obese participants, and individuals undergoing a 24-month exercise intervention [5]. Additionally, the PAR questionnaire provides an estimate of total energy expenditure. For example, a previous study found the PAR questionnaire to be an accurate measure of energy expenditure compared with other methods, such as doubly labeled water, for measuring the mean level of total daily energy expenditure (TDEE) or physical activity energy expenditure (PAEE) for a group [17]. For the Garcia et al. study, an interviewer (i.e., study staff) asked the questionnaire questions to the participant and completed the answers on the sheet [6].

The nutritional survey that we used was a Dietary Screener Questionnaire (DSQ) developed in the National Health and Nutrition Examination Survey (NHANES) 2009-2010 series [10]. A study staff/interviewer asked the questions of the participant and recorded the answers on the Self-Administered Questionnaire sheet [6, 10]. This questionnaire has been used in multiple studies to assess the nutrition of specific populations and the overall US population [4]. However, it has mainly been used with other survey methods, such as the 24-hours recall by phone, and larger sample sizes [12, 3].

The analysis methods we used for this review were ANOVA (analysis of variance: single factor) and t-Test (t-Test: Paired Two Sample for Means). The ANOVA was used to compare the means of the three time points: baseline, 4-weeks, and 8-weeks. A significant p value of < 0.05 for the ANOVA test would indicate that there was significant variation between the three time points. The t-Test was used to compare the answers for two time points. We did a t-Test to compare baseline to 4-weeks and another to compare baseline to 8-weeks. A significant p value of < 0.05 for the t-Test would indicate that there was a significant difference between the means of the two time points.

The surveys used in this study were validated measures of physical activity and diet recall [3, 4, 7, 10, 12, 17]. For our analysis, we relied on previous studies that used these questionnaires to guide how we interpreted our findings [7, 12, 17]. For this retrospective analysis, it was important to measure environmental (i.e.m exercise/diet) contributions that may be important to the methylation findings described by Garcia et al. [6]. We hypothesized that any observed methylation changes in the original Garcia et al. study [6] were due to the 8-weeks of supervised training and not due to environmental changes, such as changes to diet or additional exercise outside the program.

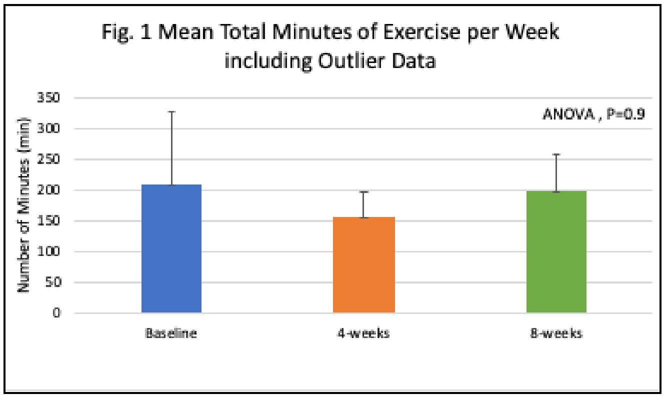
Results

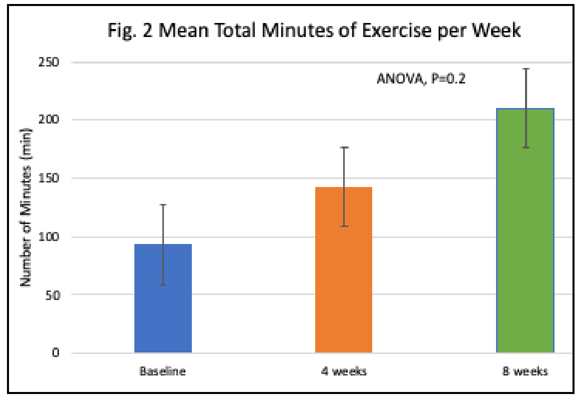
Participant Data

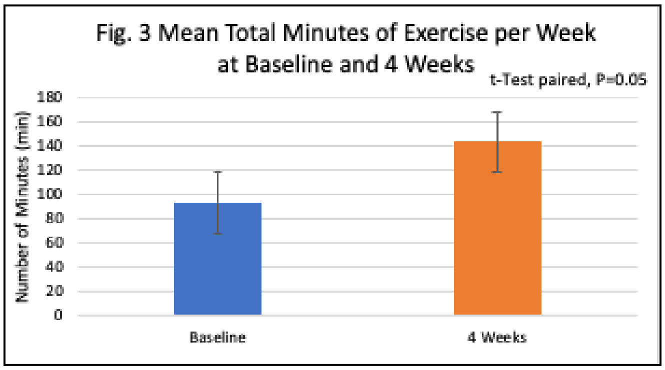
We included 15 participants in the retrospective analysis: 4 were lean (body mass index < 25 kg/m^2), and 11 were obese (body mass index ≥ 30 kg/m^2). Originally, we had 16 participants, but one volunteer was removed as an outlier as the questionnaire data was skewed (see below). Two of the obese participants had type 2 diabetes mellitus (T2DM). The average age of the participants was 36.6 years old. The average age for participants in the lean category was 33.2 years old and the average age for the obese group was 38.2 years old. Six participants were male and 9 were female.

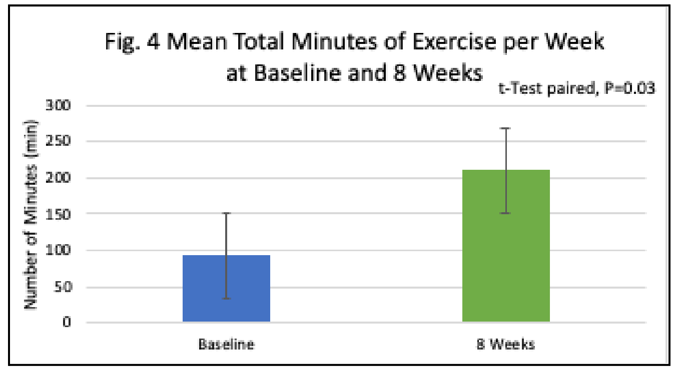
Physical Activity Questionnaire

The physical activity data was analyzed by the number of minutes per 7-day recall. Our analysis showed no change in activity throughout the program (Fig. 1). However, we noticed that one participant's baseline data was at least 10-fold higher than the majority of the group. We removed this outlier and reanalyzed the data. For ease of interpretation, this outlier was removed from downstream analyses. In our ANOVA: Single Factor reanalysis (Fig. 2), there was clearly an upward trend in the number of minutes per 7-day recall. Thus we analyzed the data by t-Test for Baseline *versus* 4-Weeks and Baseline *versus* 8-Weeks. Both of the t-Tests had significant p values, as seen in Fig. 3 and Fig. 4.









We set out to see if age contributed to the number of minutes of exercise performed over the 7-day recall period. Age cut-offs were determined to allow for equal numbers per each age category. The age categories were < 30 years old, between 30 and 50 years old, and 50 years and older. Fig. 5, Fig. 6, and Fig. 9 showed no significant difference across the baseline, 4-weeks, and 8-weeks for each of the age groups. However, there was an increased trend in the amount of exercise performed in a week. We suspect that one of the reasons for the lack of significance across the three groups is the low number of people in each age category. The t-Tests for the middle-aged group (30-50 years old) were significant with p values of 0.03 for both the comparison of Baseline *versus* 4-Week data (Fig. 7) and the comparison of Baseline *versus* 8-Week data (Fig 8).

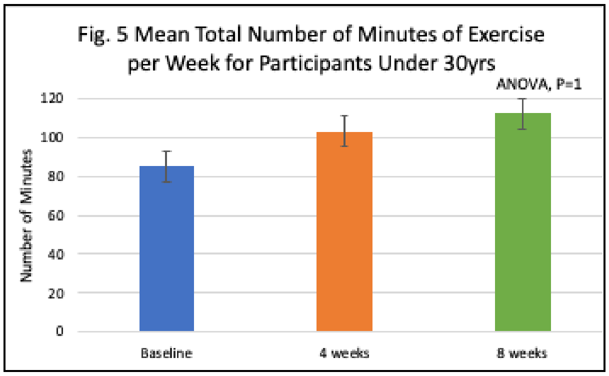


Fig. 5 Mean Total Number of Minutes of Exercise per Week for Participants Under 30yrs

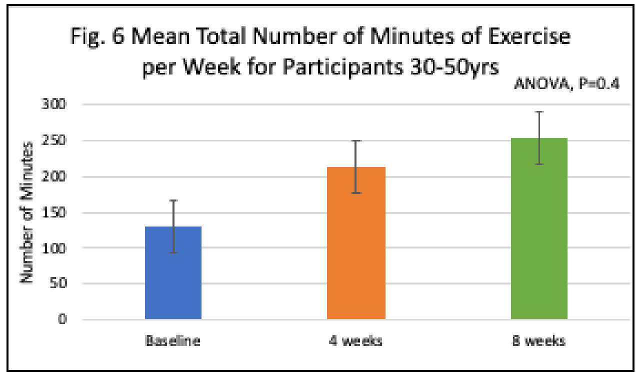


Fig. 6 Mean Total Number of Minutes of Exercise per Week for Participants 30-50yrs

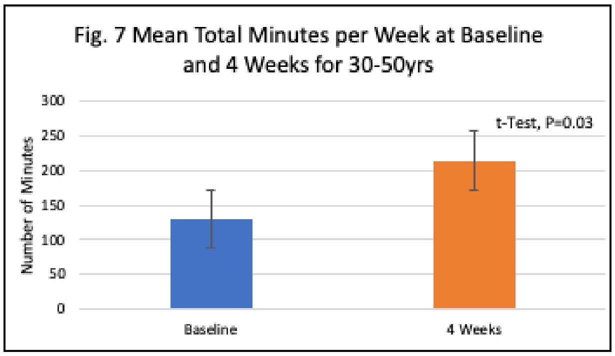


Fig. 7 Mean Total Minutes per Week at Baseline and 4 Weeks for 30-50yrs

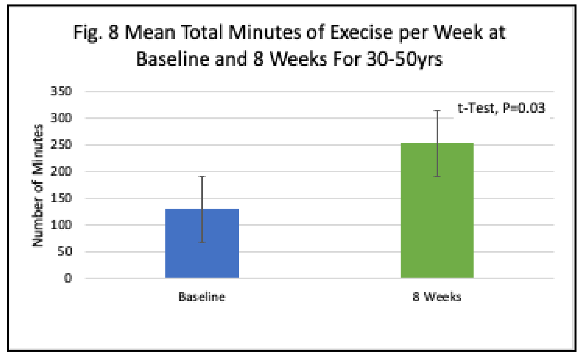


Fig. 8 Mean Total Minutes of Execise per Week at Baseline and 8 Weeks For 30-50yrs

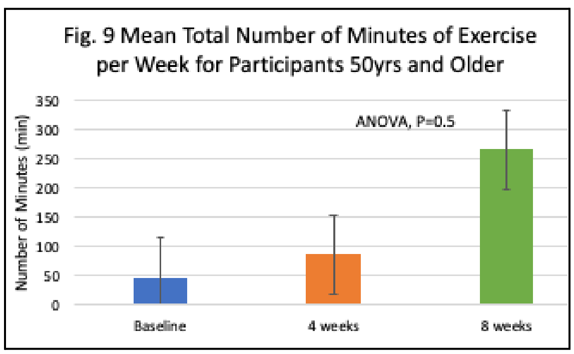


Fig. 9 Mean Total Number of Minutes of Exercise per Week for Participants 50yrs and Older

Similarly, we set out to see if gender was a factor in the number of minutes of exercise per week measured. Again, the increasing trend appears in the ANOVA analysis for both males and females (Fig. 10 and Fig. 12), but is not significant, again, likely due to the low number of participants. However, the t-Test comparing the Baseline *versus* 4-Weeks Data for the males was significant, with a p value of 0.046 (Fig. 11).

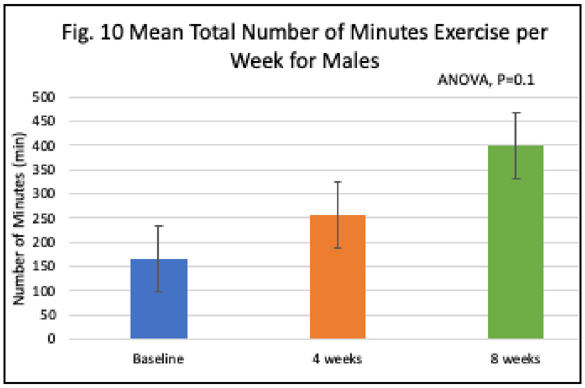


Fig. 10 Mean Total Number of Minutes Exercise per Week for Males

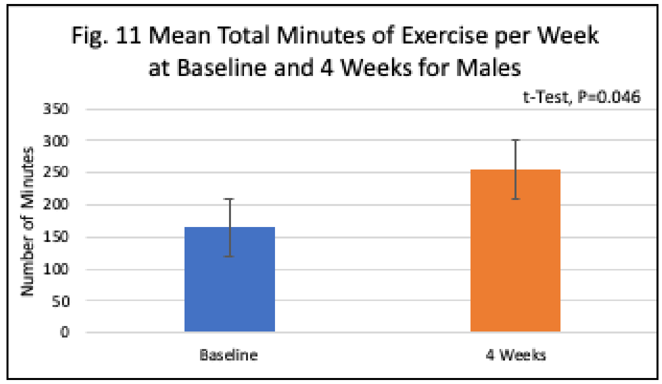


Fig. 11 Mean Total Minutes of Exercise per Week at Baseline and 4 Weeks for Males

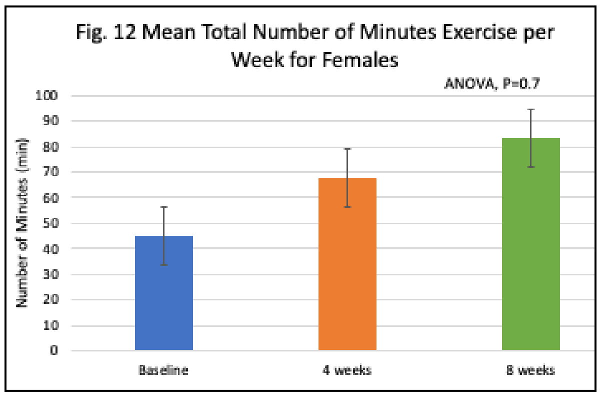
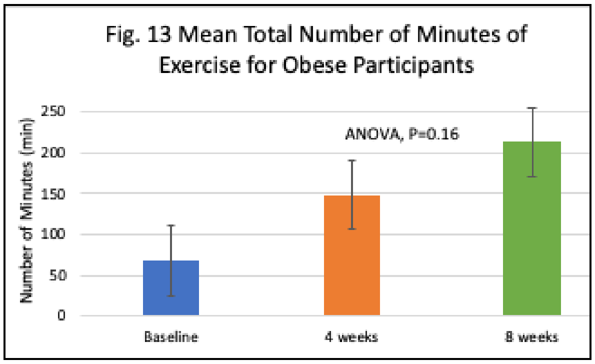
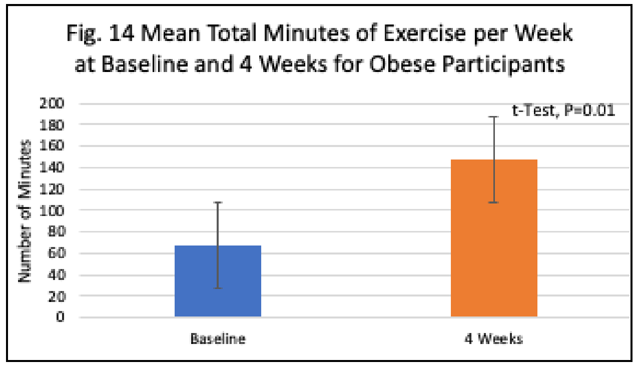
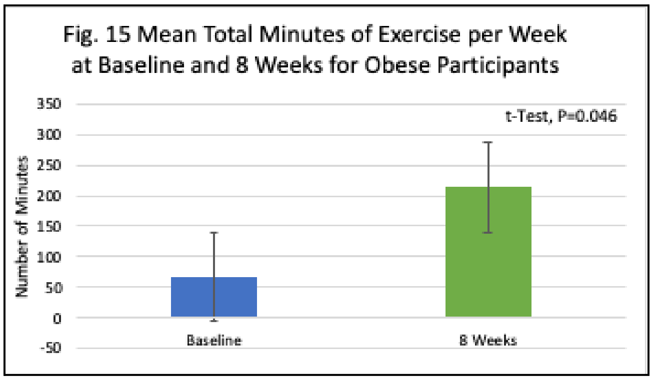


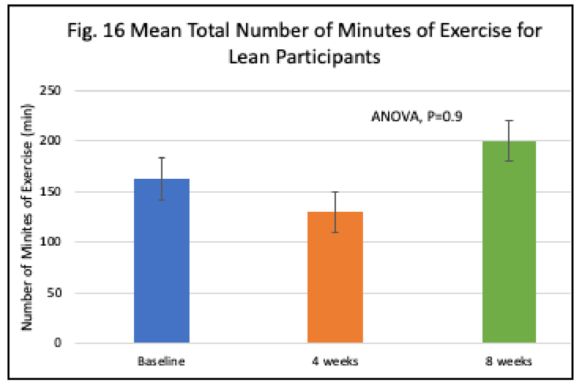
Fig. 12 Mean Total Number of Minutes Exercise per Week for Females

We also wanted to see if body mass index was a factor in the number of minutes of exercise per week measured. The increasing trend appears in the data for the obese participants but not for the lean participants. The graph for the lean participants has higher values at the study's beginning and end and lower values in the middle. Neither ANOVA tests (Fig. 13 and Fig. 16) for the groups had significant p values, again, likely due to the low number of participants in the study and in each category. However, the t-Tests comparing the Baseline *versus* 4-Weeks data (Fig. 14) and the Baseline *versus* 8-Weeks data (Fig. 15) were both significant, with p values of 0.01 and 0.046, respectively.





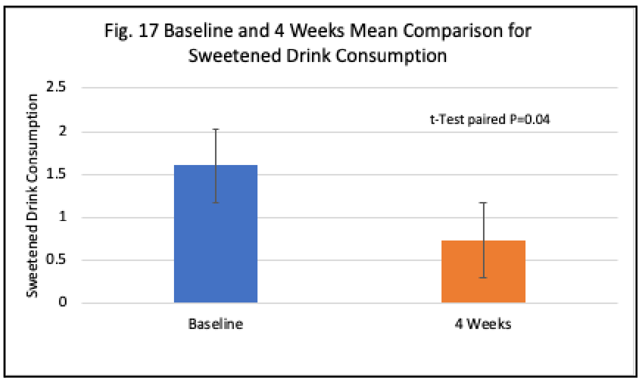


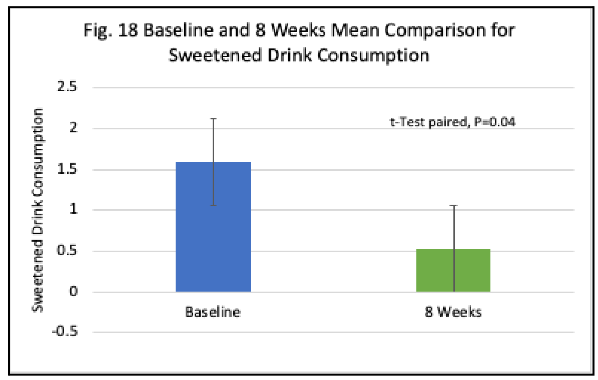


Diet Questionnaire

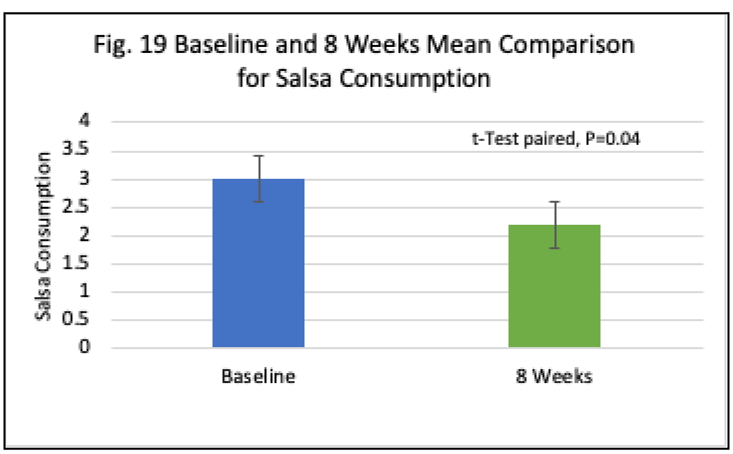
The diet questionnaire was composed of 30 questions, 27 of which assessed the frequency that the participants eat specific foods or food types within the past month. We tried to determine the extent to which participants fulfilled the nutritional requirements of the daily recommended 2,000 calorie diet. However, we could not accurately measure this because of how the questions were asked and the lack of data on the portion sizes eaten by the participants [16]. In addition, the diet data gathered was not connected to a more extensive diet database; thus extrapolation could not occur. Instead, all questions were then analyzed by assigning a coded value to each possible answer. For example, the answer "never" was coded as "0" and the answer "1 time per day" was coded as "7". Using the coded values, the participants' responses at baseline, 4-weeks, and 8-weeks were compared using ANOVA single factor and paired t-Tests. None of the ANOVA comparisons had significant p values (data not shown). However, the t-Tests with significant values are shown below (Fig. 17 - Fig. 23). All the t-Tests with significant p values showed a decrease in food consumption.

Question 11 on the questionnaire asked about the participants' consumption of sweetened fruit drinks, sports/energy drinks, and fruit juices made at home with added sugar. Fig.17 and Fig. 18 compared the participants' sweetened drink consumption at baseline to the consumption at 4-weeks and 8-weeks. The p values of 0.04 indicate that the participants' consumption decreased significantly.

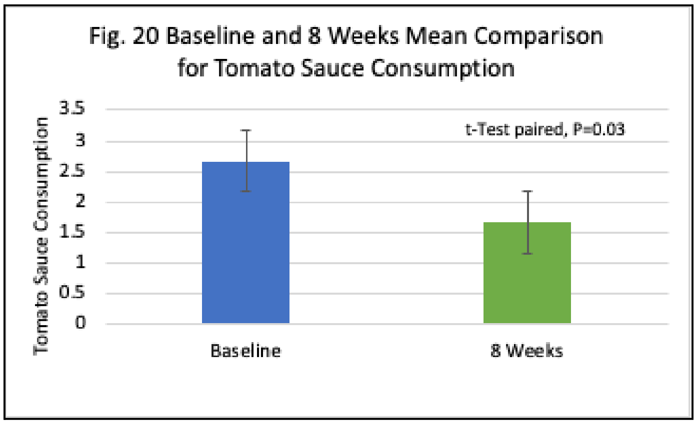




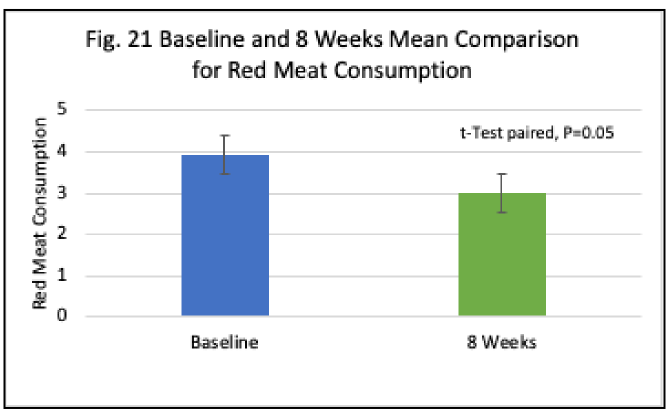
Question 19 asked how often the participants ate Mexican-style tomato salsa. Fig. 19 shows the t-test results for the comparison of salsa consumption between the beginning and end of the study. The amount of consumption decreased significantly during the study with a p value of 0.04.



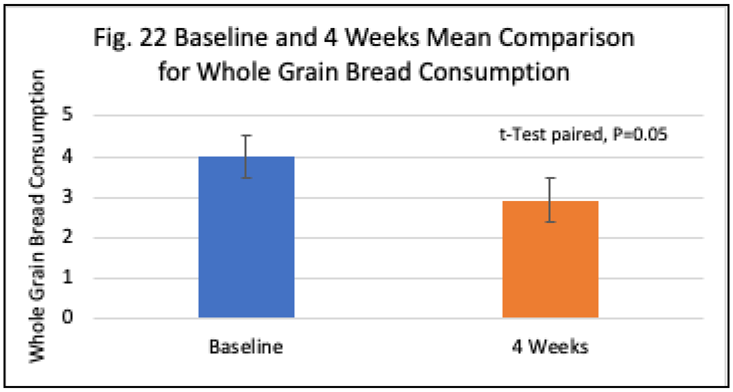
Question 21 asked how often the participants consumed tomato sauces. This question included sauces such as the ones used in pasta but specifically excluded pizza. Again, the amounts of consumption decreased significantly from the start of the study to the end, as shown in Fig. 20 with a t-Test p value of 0.03.



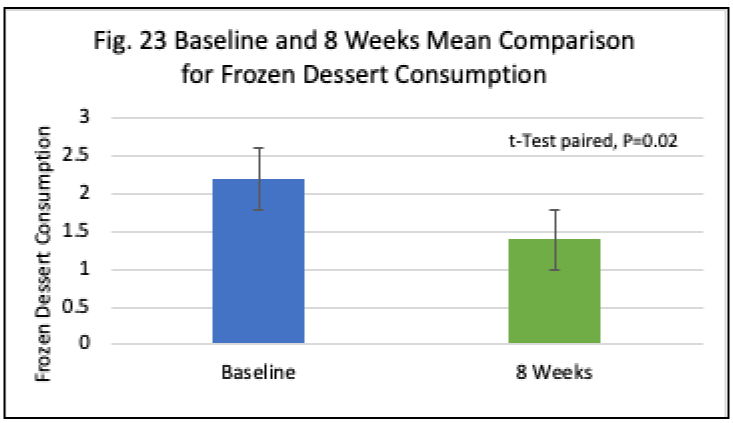
Question 23 asked how often the participants ate red meat, including beef, pork, ham, sausage, veal, and lamb, but excluding chicken, turkey, and seafood. As shown in Fig. 21, there was a significant decrease in the amount consumed at baseline compared to 8-weeks. The p value for the t-Test was 0.05.



Question 26 assessed the consumption of whole grain bread. There was a significant decrease in consumption between the beginning of the study and the 4-week mark, with a p value of 0.05 (Fig. 22).



Question 29 asked about the consumption of ice cream or other frozen desserts, excluding any sugar-free varieties. As shown in Fig. 23, there again was a decrease in the amount consumed at the beginning of the study and at the end, with a p value of 0.2.



Conclusion and Future Considerations

In the present study, we analyzed the physical and nutritional (diet) compliance questionnaires collected as part of the Garcia et al. study. As described above, the study staff advised no changes to either physical activity or diet (beyond the supervised training in the research clinic) to each participant that entered the study. Our hypothesis was that the participants' amount of exercise and diet would not alter during the 8-weeks of study. We showed that there were no changes to diet or exercise when analyzed using the ANOVA tests. However, we did demonstrate that there were some changes to the participants exercise and dietary habits during the study when analyzing across two groups using the t-Test analysis. Each significant t-Test p value indicates that the study participants changed their exercise or particular dietary habits during the study, which may have affected the DNA methylation findings described by Garcia et al. [6]. Moreover, the findings from this study showed that the participants did not comply across the 8-weeks of training since there were some changes to exercise and some of the diet questions.

Administering and collecting the questionnaire data has many benefits, but there are some limitations. Recalling exercise and diet information is often subject to bias and misremembering [1, 8, 9, 13, 14]. Also, we did not use technology, such as heart-rate monitors or exercise monitors, to verify the self-reported data [14]. The interviewers/study staff collecting the data were trained in the methods, however, there were some inconsistencies. For example, there were missing data points for sleep and exercise time/amounts questions. Specifically, for the diet data, the study attempted to account for 24-hour recall. Still, it did not use a daily phone call, food journal, or some other daily check-in tool to corroborate the data collected [8, 9, 13, 15]. The method of diet questioning also cannot be used to determine if the participants were eating the correct portion size of different food groups or meeting the recommended 2,000 daily calorie count [16].

The possible physiological effect of changes in diet or exercise must be taken into consideration when conducting research. Levels of physical activity as well as the types and amounts of food eaten have been shown to affect the body's response to insulin, VO_2 max, weight, and risk for chronic disease [2, 18]. If diet and physical activity levels are unaccounted for during research, there is always the possibility that a study's results will be influenced by one or both of these environmental factors.

In the future, there are several things that should be considered when doing an exercise study. Nutrition intake and outside physical activity must be measured during the study. This would prevent the study results from being skewed by environmental factors. Exercise studies should be separated by age and gender. This would ensure that the results are not skewed by a specific demographic. Technology, such as heart rate monitors, should also be used to confirm patient questionnaire answers and ensure daily exercise compliance [14]. Diet should be assessed daily, such as through a daily phone call by trained professionals that collect information about what a participant has consumed within the past 24hrs [15]. The daily assessment could then be combined with a weekly or monthly recall questionnaire, like the one used in the study, to assess participant compliance and whether participants are meeting the daily dietary recommendations [16].

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