EXERCISE: A POSSIBLE MEDIATOR IN THE ENHANCED ANTIBODY RESPONSE TO INFLUENZA VACCINATION IN HEALTHY WEIGHT AND OVERWEIGHT INDIVIDUALS

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Honors Thesis

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ABSTRACT

In recent years, the obesity epidemic has continued to grow and has emerged as a huge public health issue over the last decade. Obesity has been found to be associated with a multitude of health problems including cardiovascular disease, metabolic syndrome, arthritis and overall morbidity. Additionally, obesity has been linked to a decreased antibody immune response to influenza vaccination. Exercise is commonly understood to have health benefit, both in mental and physical health, but the association between exercise and immune response is an area of limited research. The present study aimed to investigate whether exercise in both healthy weight and overweight individuals would enhance the antibody immune response following influenza vaccination. Pre- and post- vaccination serum samples from the 2013-2014 study year were used to determine vaccine antibody titer response using haemagglutination inhibition assays. Exercise data for the subjects was collected from a self-reported questionnaire administered upon pre-vaccination blood draw. There were no significant differences between pre- and post-vaccination antibody titers between non-exercisers and exercisers nor was there one between the four body weight-exercise subgroups. As such there was also no significant difference between antibody titer fold change between subgroups. Although the data was restricted by limited sample size and possible subgroup misclassifications and no significant differences were found, there were trends that may suggest that exercise plays a role in mediating the antibody immune response in healthy weight and overweight individuals.
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I. Introduction

a. Obesity

Obesity has become an epidemic among both adults and children in the United States in recent years. In fact, the most recent data shows over one-third of adults and 16% of children are classified as being obese. Obesity has numerous implications on human health, including both the psychological and physiological states. It is well established that obesity significantly increases the risk for a multitude of chronic illnesses such as diabetes, cardiovascular disease, arthritis, cancer, and overall morbidity. A link between excess body weight in obese individuals and impaired immune response has also been found. This decrease in immune response has been investigated in bacterial and viral infections, and proven to be impaired in both.

b. Influenza

Influenza virus is an annual epidemic that causes serious respiratory infection in 3-5 million people each year. Of these cases, over an estimated 250,000 result in death. As studies have shown that obese individuals have a decreased immune response to infection, this finding is also consistent with decreased influenza immunity in obese individuals. Specifically, an association between obesity and decreased antibody response to the influenza vaccination has been found. Influenza vaccination has proven to serve as an effective tool in influenza infection prevention. The vaccine introduces an inactive form of the virus that allows the body to develop antibodies against the particular virus strain. The production of these antibodies produces a memory response specific to the viral strain. In the case that an individual is later exposed to the live virus, the body elicits this antibody memory response and the body is more effective in fighting the virus. The most common assessment of influenza antibody response is
measurement of the haemagglutination inhibition (HAI) titer\textsuperscript{3}. HAI’s determine the circulating level of influenza-specific antibodies that can inhibit the ability of the virus to bind to lung epithelial cells.

c. Exercise

It is widely-accepted that exercise has a variety of systemic affects on the body, including reduced risk of cardiovascular disease, increased muscle and bone strength, and increased mental health\textsuperscript{6}. Prior studies also show that exercise has positive effects on the immune response. Furthermore, increased antibody response following influenza vaccination has been found as well. Although most current published studies on this topic focus on older adults rather than the general population as a whole, the results support exercise as a mediator of immune response. Kohut et. al found that in older adults with an age-declined influenza vaccine response, exercisers experienced a greater fold increase in antibody titer in response to vaccination as compared to non-exercising older adults\textsuperscript{5}. A similar study of an elderly population found significant results of increased immune vaccination response following a 10-week cardiovascular exercise intervention\textsuperscript{12}.

d. Study Aim

In order to better understand the effectiveness of exercise as a mediator of antibody response to influenza vaccination, we used HAI titers as a means to measure antibody concentration. We were interested in investigating whether exercise can enhance influenza vaccine immune response and potentially outweigh the impaired immunity that has been found as result of obesity. To best determine this, serum samples from Dr. Melinda Beck’s 2013-2014 study year were used to collect antibody responses and these antibody titers were then
compared between 4 sample subgroups: healthy weight exercisers, obese exercisers, healthy weight non-exercisers, and obese non-exercisers. We hypothesized that exercise in both healthy weight and obese individuals would produce a greater influenza antibody response to vaccination compared to non-exercisers. The circulating virus strain during the study year from which serum samples were used included the 2009 influenza A (H1N1) and corresponding vaccine produced by the A/California/7/2009 (H1N1)pdm09-like virus.
II. Methods

a. Study Population

This study is part of a larger, ongoing clinical study within Dr. Melinda Beck’s clinical biochemistry research lab at UNC Gilling’s School of Global Public Health. Participants are annually recruited through the University of North Carolina’s Family Medicine Center located in Chapel Hill, North Carolina. The subjects for this particular study included 587 subjects 18.6-83.5 years old from the 2013-2014 study year (Table 1). In mid-September of 2013, background demographics such as height, weight, sex, race, and calculated body mass index (BMI) as well as a self-reported exercise questionnaire was collected upon subject enrollment.

Table 1. Demographic characteristics of 2013-2014 study subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sample Population</th>
<th>Total Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex—no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9 (60.0)</td>
<td>209 (35.6)</td>
</tr>
<tr>
<td>Female</td>
<td>6 (40.0)</td>
<td>378 (64.4)</td>
</tr>
<tr>
<td>Age—year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>49.0 ± 18.1</td>
<td>54.3 ± 15.2</td>
</tr>
<tr>
<td>BMI—kg/m$^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Overall</td>
<td>24.3 ± 3.2</td>
<td>30.6 ± 8.0</td>
</tr>
<tr>
<td>Mean in Healthy Weight</td>
<td>21.5 ± 2.5</td>
<td>22.3 ± 2.10</td>
</tr>
<tr>
<td>Mean in Overweight</td>
<td>26.7 ± 1.0</td>
<td>33.4 ± 7.18</td>
</tr>
<tr>
<td>Race—No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>12 (80.0)</td>
<td>386 (62.7)</td>
</tr>
<tr>
<td>African American</td>
<td>1 (6.7)</td>
<td>181 (30.8)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (6.7)</td>
<td>20 (3.4)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1 (6.7)</td>
<td>13 (2.1)</td>
</tr>
<tr>
<td>Mixed Race</td>
<td>0 (0.0)</td>
<td>4 (0.68)</td>
</tr>
<tr>
<td>Southeast Asian</td>
<td>0 (0.0)</td>
<td>1 (0.17)</td>
</tr>
<tr>
<td>Exercise Level—No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Exerciser</td>
<td>7 (46.7)</td>
<td>--</td>
</tr>
<tr>
<td>Exerciser</td>
<td>8 (53.3)</td>
<td>--</td>
</tr>
<tr>
<td>Weight Status—No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy Weight</td>
<td>7 (46.7)</td>
<td>151 (25.7)</td>
</tr>
<tr>
<td>Overweight</td>
<td>8 (53.3)</td>
<td>436 (74.3)</td>
</tr>
</tbody>
</table>
Out of the total 587 subject study population, 15 subjects were chosen to make up the sample population for this specific study (Table 1) (Table 2). The subjects were placed into the following subsets based on their classifications for weight status and exercise level: healthy weight non-exercisers, overweight non-exercisers, healthy weight exercisers, and overweight exercisers. BMI was used to define body weight status. A BMI less than 25 kg/m$^3$ classified individuals as healthy weight, while a BMI in the 25-29.99 kg/m$^3$ range was overweight.
b. Determination of Exercise Activity Level

Exercise level was determined through a self-reported questionnaire that ranked exercise frequency and type from on a scale from 1-7. The Rapid Assessment of Physical Activity (RAPA) questionnaire and was used (Fig. 2). The University of Washington Promotion Research Center provided examples of activity intensity levels defined as light, moderate, and vigorous (Fig. 1). This questionnaire is approved as a valid measure for physical activity status. According to the guidelines set forth by the University of Washington Health Promotion Research Center, those who answered 1 and/or 2 were classified as non-exercisers while 6 and/or 7 were classified as an exerciser.

Figure 1. Examples of physical activity intensity levels defined by the University of Washington Promotion Research Center for the Rapid Assessment of Physical Activity (RAPA) questionnaire.
c. Serum Collection

Prior to influenza vaccination, baseline blood samples were also collected from subjects. Subjects then received the 2013-2014 seasonal trivalent influenza vaccine which included the A/California/7/2009 (H1N1)pdm09-like virus, the A(H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011, and B/Massachusetts/2/2012-like virus. At follow up period 30 days after vaccination, a second blood sample was collected from subjects.
The blood was collected into a non-heparinized tube and allowed to clot. The serum was then removed and stored at -80 °C until use.

d. Haemagglutination inhibition assay (HAI)

The prevalent virus that circulated the United States for the 2013-2014 influenza season was the 2009 influenza A (H1N1), influenza A (H3N2), and influenza B. Haemagglutination inhibition assays were run to determine the antibody titer present in the serum samples specifically for the 2009 influenza A (H1N1) virus. The serum samples were treated with prepared Receptor Destroying Enzyme (RDE) to a final dilution of 1:10. This was performed by first adding 90 µL of RDE to 30 µL serum, the samples were incubated at 37 °C for 18 hours then heated at 56 °C for 1 hour and allowed to cool to room temperature. A total of 180 µL of 0.85% physiological saline was added to each sample. Samples were then placed in the freezer for a minimum of 4 hours until use. Prior to each HAI, a sample of 2009 influenza A H1N1 virus was diluted to 8HAU/50µL using sterile PBS. To ensure the virus was properly diluted to this ratio, the diluted virus was tested to determine the haemagglutination unit titer using fresh turkey red blood cells at 0.5%. Following the HAU titer test, an HAI was performed using the 0.5% turkey RBC, diluted 1:10 serum samples, and 8HAU/50µL prepared virus in a 96 well plate. Serial dilutions were performed followed by a 20-30-minute incubation period. The HAI titer was determined as the last serum dilution which prevented red blood cell agglutination.
e. Data Analysis

The HAI titer was recorded for each individual. In addition to pre and post vaccination HAI titer, the fold change between the pre and post time point was determined for each subject by dividing the post-vaccination titer by the pre-vaccination titer. Data was analyzed using GraphPad Prism 6.0. An unpaired t-test was used to analyze the statistically significant difference of pre and post-vaccination titers between the non-exercisers and exercisers. The four subgroups’ pre- and post-vaccination titer values were then compared using multiple t tests and statistical significance was determined using the Holm-Sidak method. Titer fold change comparisons between the four subgroups were performed and statistical significance was determined using a one-way ANOVA.
III. Results

a. Demographics of the Study Population

For this study, 15 subjects were chosen from the larger 2013-2014 study. In order to investigate a possible association of exercise influencing the influenza vaccine antibody response, subjects were split into 4 subgroups: healthy weight non-exercisers, overweight non-exercisers, healthy weight exercisers, and overweight exercisers. Subjects were chosen to best represent the total population while also controlling for potential confounding factors. In terms of demographic characteristics, the sample population was a fair representation of the total 2013-2014 study population (Table 1). Age was the closest match, with the samples average age falling within 5.3 years of the total population’s. The mean overall BMI in the sample was 6.3 kg/m$^3$ lower than that of the total population, which was expected as the sample population was selected purposefully to have equal healthy weight and overweight subjects while the total population was based on volunteer subject enrollment and not controlled. Caucasians represented the majority, comprising 80% in the sample population versus 62.7% in the total population. African American (6.7%), Asian (6.7%), and Hispanic (6.7%) were additional races included in the sample population. On the other hand, sex was the least representative of the total population as females comprised the majority of the total population (64.4%) but only made up 40% of the sample subject population.

In the sample population, an age difference of 11.6 years was found between non-exercisers and exercisers. Equal amounts of females were found in both groups, with one more male found in exercisers. Caucasian subjects made up 5 of the 7 non-exercisers with two
subjects identified as African American and Asian. In the exercisers, 7 of the 8 subjects identified as Caucasian and 1 as Hispanic.

b. HAI Titer

In fall 2015, all pre- and post-vaccination serum HAI s for the 15 subjects selected for the sample population were completed successfully and HAI titers were determined (Table 3).

Table 3. Demographic and HAI titer data from the 2013-2014 sample study population.

<table>
<thead>
<tr>
<th>BMI</th>
<th>Age</th>
<th>Race</th>
<th>Sex</th>
<th>Exercise</th>
<th>Pre-</th>
<th>Post-</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Weight Non-exercisers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1505</td>
<td>24</td>
<td>83.5</td>
<td>Caucasian</td>
<td>Female</td>
<td>1&amp;2</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>1644</td>
<td>19.4</td>
<td>44.4</td>
<td>African American</td>
<td>Male</td>
<td>1</td>
<td>20</td>
<td>160</td>
</tr>
<tr>
<td>1945</td>
<td>23.3</td>
<td>61.5</td>
<td>Caucasian</td>
<td>Male</td>
<td>1&amp;2</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Overweight Non-exercisers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1850</td>
<td>26.2</td>
<td>61.2</td>
<td>Caucasian</td>
<td>Female</td>
<td>1&amp;2</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>1893</td>
<td>27</td>
<td>32.1</td>
<td>Caucasian</td>
<td>Male</td>
<td>1&amp;2</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>1748</td>
<td>27.3</td>
<td>64.9</td>
<td>Caucasian</td>
<td>Female</td>
<td>1&amp;2</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>1979</td>
<td>28.2</td>
<td>39</td>
<td>Asian</td>
<td>Male</td>
<td>1&amp;2</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Healthy Weight exercisers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1667</td>
<td>22.7</td>
<td>34.1</td>
<td>Caucasian</td>
<td>Female</td>
<td>6&amp;7</td>
<td>40</td>
<td>160</td>
</tr>
<tr>
<td>1963</td>
<td>23.6</td>
<td>23</td>
<td>Hispanic</td>
<td>Female</td>
<td>6&amp;7</td>
<td>80</td>
<td>320</td>
</tr>
<tr>
<td>1955</td>
<td>17.6</td>
<td>18.6</td>
<td>Caucasian</td>
<td>Male</td>
<td>7</td>
<td>320</td>
<td>640</td>
</tr>
<tr>
<td>1936</td>
<td>19.8</td>
<td>67.4</td>
<td>Caucasian</td>
<td>Male</td>
<td>7</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Overweight Exercisers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1679</td>
<td>25.4</td>
<td>55.4</td>
<td>Caucasian</td>
<td>Male</td>
<td>6&amp;7</td>
<td>80</td>
<td>160</td>
</tr>
<tr>
<td>1886</td>
<td>25.7</td>
<td>38.8</td>
<td>Caucasian</td>
<td>Male</td>
<td>6&amp;7</td>
<td>40</td>
<td>320</td>
</tr>
<tr>
<td>1698</td>
<td>27.9</td>
<td>53.4</td>
<td>Caucasian</td>
<td>Female</td>
<td>6&amp;7</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>1938</td>
<td>26</td>
<td>58.3</td>
<td>Caucasian</td>
<td>Male</td>
<td>7</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>

Overall, there was a trend for both pre- and post-vaccinated HAI titers of exercisers to be higher than non-exercisers, although this was determined to not be statistically significant (Fig. 3). Observing the 4 sub-groups, healthy weight exercisers, overweight exercisers, healthy weight non-exercisers, and overweight non-exercisers, all experienced an increase in antibody response from pre- to post-vaccination (Fig. 4). Differences in pre- and post-vaccinated HAI
titers between healthy weight exercisers and non-exercisers were found to be statistically insignificant (pre: $P=0.416$, post $P=0.253$). Pre/post vaccination comparisons of overweight exercisers and non-exercisers were also found to be statistically insignificant (pre: $P=0.301$, post: $P=0.174$). The HAI titer fold change was used as the main determinant for the role of exercise in antibody response to influenza vaccination (Fig. 5). One-way ANOVA analysis determined no statistically significant difference of titer fold change between the four sub-groups ($P=0.541$).

![Graphs showing antibody titer response](image)

Figure 3. Pre- and post-vaccination antibody titer response in exercisers and non-exercisers: (a) pre-vaccination $P=0.251$, (b) post-vaccination $P=0.073$. 
Figure 4. Pre- and post-vaccination antibody titer levels of body weight and exercise status subgroups. No significant difference in titer level between healthy weight non-exercisers and exercisers in both pre- and post-vaccination (pre: P=0.416, post: P=0.253). No significant difference in titer level between overweight non-exercisers and exercisers in both pre- and post-vaccination (pre: P=0.301, post: P=0.174).
Figure 5. H1N1 Titer Fold Change for body weight and exercise subgroups. No significant difference (P=0.541).
IV. Discussion

a. Findings

Influenza is considered to be one of the most serious communicable diseases due to its contagious nature and potential for severe health complications. The 2009 influenza season was classified as the first pandemic in over four decades. In the United States alone, there were an estimated 60.8 million cases reported for influenza A (H1N1) infection, 12,469 of which resulted in death\(^8\). Global mortality estimates from the 2009 H1N1 influenza season are between 151,700 and 575,400 people\(^2\). Seasonal vaccination has been found to be the most effective protective measure against acquiring influenza. Prior studies within the Beck lab have shown that obesity can impair the antibody response to influenza vaccination\(^9\). Given this study’s finding and the fact that obesity is now considered an epidemic, overall decreased influenza protection by vaccination becomes a serious public health concern.

Nutrition, among other factors, plays a role in both obesity and the immune response. A multitude of other factors such as genetic susceptibility, age, certain exposures, and various lifestyle habits can also play a role in the functionality of an individual’s immune system. Exercise is often suggested as one of the most important health behaviors for improving health. Although it is known that exercise has health benefits that extend beyond body weight control, few studies have looked into the effects on antibody response to vaccination, specifically influenza vaccination. The purpose of this study was to investigate whether or not exercise can serve a mediating role in antibody immune response to influenza vaccination in healthy weight and overweight individuals.
Based on evidence from limited prior studies investigating the association between exercise and antibody response, it was hypothesized that exercise in both healthy weight and overweight individuals would result in an increased influenza-specific antibody response following flu vaccination. This study predicted that healthy weight and overweight exercisers would have a larger increase in the titer from pre to post vaccine than non-exercising healthy weight and overweight individuals. However, data from this study did not support the hypothesis. Although no significant associations were found, the data suggests possible trends that warrant further investigation.

Strictly comparing exercise status only, Fig. 3 suggested a trend toward higher antibody titers in exercisers both pre- and post-vaccination as compared to non-exercisers. Similarly, as shown in Fig. 4, pre- and post-vaccination antibody titers are higher in healthy weight exercisers and overweight exercisers than their non-exercising counterpart subgroups. Titer fold changes from Fig. 5 seem to be comparable between healthy weight non-exercisers, healthy weight exercisers, and overweight exercisers. Overweight non-exercisers differ by almost an entire 2-fold difference.

It is important that these trends be viewed with caution due to the small sample size and therefore strong effect of outliers on individual subgroup data. Outliers seemed to play a noteworthy role in this study. The healthy weight exerciser subject with a pre-vaccination titer of 320 and post-vaccination of 640 classified as an outlier for the subgroup and the sample population as a whole. This needs to be taken into consideration when considering the trends.

Comparing this data to the Kohut and Woods prior studies previously discussed, although our data is statistically insignificant, the trends follow the similar findings. Both of
these studies found a greater titer response in exercising individuals versus non-exercisers. The most significant differences between our study and the published literature was age of subjects, sample population size, and previous exercise status. Both these case-control studies focused more on the older population, as this population is found to have reduced immune responses and the greatest influenza illness and death rates. The Kohut study included 27 adults greater than 64 years old with no meaningful exercise history. Similarly, the Woods study included 144 adults aged 60 to 83 who had all been considered sedentary prior to the study intervention. Our study included 15 participants ages 18.6-83.5 whose exercise status was at the discretion of the subject due to the nature of the study design and therefore exercise duration could not be accurately accounted for.

The earlier studies included several risk factors: older age and prior sedentary lifestyle. As such, it would be expected for a significant, more dramatic changes in overall health and immune response to vaccine after exposing these individuals to exercise and vaccination compared to our subjects who were at younger ages and possible prior exercisers. In addition, both the Kohut and Woods studies had a larger sample population size, allowing the studies more statistical power and significance.

b. Study Limitations

There were several limitations within this study. As there were only 15 subjects within the sample population, sample size was the most significant limiting factor for the study. It was difficult to find any statistically significant differences between groups when each group only contained 3 or 4 subjects. As previously discussed, the small sample size increases distortion of statistic estimates due to the strong influence of the outliers. The age range for this sample
population was quite large (64.9 years, 18.6-83.5 y.o.) and as such age could have played a strong confounding role.

The small sample size was due to the limited number of subjects in the entire study population reporting substantial exercise. Although the original study did not primarily target exercise, exercise data was collected from a self-reported questionnaire completed at the time of pre-vaccination blood draw. Due to the self-reported nature of the questionnaire, it was found that a fair percentage of the questionnaires were not filled out accurately or completely. These subjects were therefore not able to be considered as part of the sample population and limited subject selection for the sample population. Inaccurate reporting and possible recall bias from the self-reported questionnaire could have resulted in an underestimation or overestimation of the true exercise estimations and as such distorted exercise subgroup classification.

Body Mass Index (BMI) was used as the measure to classify subjects as healthy weight or overweight. BMI is simply the ratio of weight to height and therefore only an indirect measure of body fat as it does not take into account body mass type, specifically fat versus muscle. Individuals with high muscle mass can often be classified as overweight or obese when their body fat is much less than that for defined obesity. Because we used BMI as our measure, subjects with high muscle mass could have been wrongly classified into the overweight category when actually their body fat percentage is below the overweight cut point. This could have resulted in misclassification, especially among the exercisers, and therefore skewed the data.
c. Future Studies

As important trends associated exercise with enhanced influenza vaccination antibody response were seen in this study, future studies are recommended. Unfortunately, due to small sample size, the power of this study was low and as such statistical significance was not found. In order to increase the power of this study, the subject sample size must be larger. Expanding the sample size will better allow control for potential confounders such as age and gender as well as limit the effect of outliers.

As exercise data was scarce, increasing exercise data collection and accuracy would contribute to a much larger subject pool. Exercise data accuracy can be achieved through stricter exercise control such as a monitored implemented regimen. Regimens could be implemented using group fitness classes, personal trainers, or even step monitors. In addition, exercise training types should be investigated to determine the benefits of cardio versus strength training.

To best determine body weight categories in future studies, BMI should not be used. There are several other body fat measurement techniques that can better differentiate between muscle mass and fat mass and therefore provide a truer classification of weight status. If cost is an issue, a relatively cheap device such as calipers can be used to measure skin fold thickness to determine relative fatness. More advanced methods that measure actual body composition include bioelectrical impedance, air displacement plethysmography, hydrostatic weighing, and dual-energy X-ray absorptiometry. It is also important to take into account the locations of extra body fat, as abdominal fat in particular has been linked to higher cases of metabolic and cardiovascular diseases and morbidity\(^1\).
References


*PMC.*

