AN ABSTRACT OF THE DISSERTATION OF

Sombat Onsiri for the degree of the Doctor of Philosophy in Exercise and Sport Science presented on October 31, 2012.
Title: A Comparison of Substrate Utilization during Exercise Among Males and Females Varying in Age and Training Status.

Abstract approved: ________________________________
Anthony R. Wilcox

Exercise training has consistently been shown to increase fat utilization during exercise, while conflicting results have been reported on the effects of sex and age on fuel metabolism during exercise. PURPOSE: The primary objective of this investigation was to compare fat and carbohydrate utilization during exercise among males and females varying in age and training status. METHODS: 8 groups of 10 subjects each were formed based upon trained (T)/untrained (U), male (M)/female (F), and young (Y)/older (O): TYM, TYF, UYM, UYF, TOM, TOF, UOM, UOF. All female subjects were experiencing regular menstrual cycles, not using oral contraceptives, and were tested in the mid-follicular phase of their menstrual cycle. The young subjects averaged 21.3 ±1.7 yr and older subjects 40.1 ±1.9 yr. All subjects exercised for 35 minutes on a treadmill at an intensity just below their ventilatory threshold. Substrate utilization was indicated by the respiratory exchange ratio (RER), and a 2x2x2 factorial ANOVA was used to determine whether age, sex, and training status have independent or interacting effects on substrate-utilization variables, and t-tests were used for post-hoc comparisons.

RESULTS: The average RER during exercise was lower in trained subjects (0.841±.023) compared to untrained subjects (0.884±.021) (P < .05). There was no difference in the
average RER during exercise between the young (0.860±0.026) and older (0.865±0.034)
groups of subjects. The average RER during exercise was lower for untrained females
(0.860±0.027) than untrained males (0.870±0.032) (P < .05), but there was no difference in
fat and carbohydrate utilization between trained males (0.843±0.023) and trained females
(0.838±0.022). **CONCLUSION:** The major finding of this study was that untrained
females utilized proportionately more fat during exercise compared to untrained males,
but there was no difference in fat and carbohydrate utilization between trained females
and trained males. Another finding was that a 20-year difference in age was not sufficient
for an age effect to be evident in fat utilization during moderate exercise.
A Comparison of Substrate Utilization during Exercise Among Males and Females
Varying in Age and Training Status

by
Sombat Onsiri

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APPROVED:

________________________________________

Major Professor, representing Exercise and Sport Science

________________________________________

Co-Director of the School of Biological and Population Health Sciences

________________________________________

Dean of the Graduate School

I understand my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

________________________________________

Sombat Onsiri, Author
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CONTRIBUTION OF AUTHORS

Dr. Anthony Wilcox was involved with design, draft revision, and writing for this project.
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A comparison of substrate utilization during exercise among males and females varying in age and training status

It is well established that the intensity and duration of exercise and a person’s training status are important factors that affect the utilization of fat and carbohydrate (CHO) during exercise. Romijn et al. [1] determined the effect of exercise intensity and duration on endogenous free fatty acid (FFA) mobilization and utilization during 2 hours of cycling exercise at 25, 65, and 85% VO$_{2\text{max}}$. The results showed that the fat utilization rate increased (26.8 µmol.kg.min to 42.8 µmol.kg.min) when the exercise intensity increased from low intensity (25% VO$_{2\text{max}}$) to moderate intensity (65% VO$_{2\text{max}}$), and then decreased (29.6 µmol.kg.min) when the exercise intensity increased further (85% VO$_{2\text{max}}$), as muscle glycogen became the main fuel source utilized. Therefore, as exercise intensity increases, substrate utilization shifts from primarily fat to primarily CHO oxidation. Furthermore, several previous studies have shown that the duration of exercise also affects substrate utilization [1-3]. With increased duration of exercise, there are marked increases in fat metabolism and decreases in carbohydrate metabolism when exercising at a fixed intensity [3].

It is also well documented that substrate utilization is altered based on an individual’s training status. Endurance training has been shown to increase the ability to utilize fat and decrease carbohydrate utilization during exercise [4, 5]. Several studies
concluded that the effects of endurance training result in adaptive change in muscle
metabolic function characterized by increasing the number and size of mitochondria that
can enhance the capacity to oxidize fat [4, 6-9].

Sex is also a factor that may play a role in altering substrate utilization.
There is evidence to support that females tend to use more fat and less CHO than males
metabolism during 90 minutes of treadmill walking at 35% VO\textsubscript{2max} in untrained males
and females. Females had significantly lower RER values compared with males at both
45 and 90 minutes of exercise. Both groups gradually increased the percent of fat
metabolized during exercise, with the 90-minute values being 59% for the males and 73%
for the females. Similarly, Froberg & Pedersen [11] matched seven active males and
seven active females based on age and physical activity habits. Subjects performed
exercise to volitional exhaustion on two separate occasions at both 80 and 90 % VO\textsubscript{2max}.
During 80% VO\textsubscript{2max}, females had significantly lower RER values during exercise when
compared to the males. Honto et al. [13] also investigated the sex difference in fuel
metabolism during prolonged exercise. Fat and CHO oxidation during exercise were
compared in 14 males and 13 females. The results showed that females had significantly
lower RER values compared with males during two hours of exercise at 40% VO\textsubscript{2max}.
Females derived proportionally more of total energy from fat oxidation (50.9% and
43.7% for females and males, respectively), whereas males derived proportionally more
of total energy from CHO oxidation (53.1% and 45.7% for males and females, respectively). In the cross-sectional study conducted by Venables et al. [16], the summary of exercise testing results from 157 males and 147 females who performed an incremental exercise test to exhaustion on a treadmill also found higher fat oxidation in females compared with males. Also, Tarnopolsky and colleagues [12] showed differences in substrate utilization in trained males and females. Six trained male and six trained female subjects were matched for training status and performance experience. The females were tested in the mid-follicular phase of their menstrual cycle. These researchers reported significant sex differences in RER values during moderate-intensity exercise. Throughout the 90-minutes run at 65% VO_{2\text{max}}, females had significantly lower RER values compared with males, indicating an increased reliance on fats as fuel source.

In a longitudinal training study, Carter et al. [17] determined the effect of 7 weeks of endurance training on whole-body substrate utilization during 90 minutes of exercise at 60% VO_{2\text{peak}} in male and female subjects. Females showed a lower RER during exercise both pre-training and post-training compared to males. Similar findings have been reported by McKenzie et al. [18]. They studied the effects of a 38-day endurance exercise training program on leucine turnover and substrate metabolism during a 90-min exercise bout at 60% VO_{2\text{peak}} in six males and six females. They concluded that the total fat oxidation was higher in females compared to males during exercise both before and after the endurance training program. Thus in both longitudinal and cross-
sectional studies, untrained and trained females have been shown to utilize more fat and less carbohydrate compared to untrained and trained males.

While there is considerable evidence that females utilize more fat during exercise than do males, some studies report no differences between the sexes. Costill et al. [19] tested trained males and trained females, who were similar in VO_{2\text{max}} and training mileage during a 60-minute treadmill run at 70% VO_{2\text{max}}, and reported no sex differences in fat utilization. Powers et al. [20] studied four trained males and four trained females who were matched by training experience and VO_{2\text{max}}, and also reported no difference in RER between the males and females during a 90-minute treadmill run at 65% of VO_{2\text{max}}. Roepstorff et al. [21] found similar results as Costill and colleagues. They conducted a study to determine sex differences in substrate utilization using endurance-trained subjects. Seven endurance-trained males and seven endurance-trained females were matched according to VO_{2\text{peak}}, physical activity levels, and training history and completed 90 minutes of cycling exercise at 58% VO_{2\text{peak}}. It was noted that there was no sex difference in RER during exercise. Such an interpretation is supported by Friedmann and Kindermann’s study [10], which compared changes in energy metabolism during exhaustive endurance exercise in male and female subjects. The investigation showed that untrained subjects demonstrated a sex difference in fat utilization, but endurance-trained subjects did not.
In addition, to the conflicting evidence on the effect of sex on the relative contribution of carbohydrate and fat to energy metabolism during exercise, the effect of a person’s age is also not well defined. Previous studies have shown that older individuals tend to utilize more carbohydrate and less fat during exercise than younger individuals, and this outcome is associated with the decline in skeletal muscle respiratory capacity in older people [22-25]. Sial et al. [23] evaluated the effect of aging on fat and CHO metabolism during moderate exercise in six young and six old subjects. The results show that fat oxidation during exercise was 25-30% lower in old subjects than young subjects at either the same absolute or similar relative intensity (60 minutes of exercise at 50% VO_{2\text{max}}). Similar results are reported by Silverman and Mazzeo [26], who investigated the influence of age and training on the responsiveness of key hormones that regulate fuel metabolism during exercise. The data showed that mean respiratory exchange ratio (RER) values during the 45-minute sub-maximal exercise at the workload corresponding to each subject’s lactate threshold were higher in the old group than in young group, indicating greater fat utilization in the young groups.

Some studies have attempted to study both the aging and training-status factors on substrate utilization. Endurance training has been shown to increase fat oxidation during exercise in young and elderly persons [6, 7]. Phillips et al. [7] investigated the effect of training duration (5 days and 31 days) on substrate turnover and oxidation during exercise in 7 young male subjects. The results showed that at 5 days, training
induced a 10% increase in total fat oxidation during exercise, and at 31 days, total fat oxidation during exercise increased a further 58%. Also, Sial et al. [6] evaluated the effects of six weeks of endurance training on fat and glucose metabolism during a 60-minute treadmill run at 50% of VO$_{2\text{peak}}$ in six elderly males and females (74±2yr). The evidence showed that endurance training increased fat metabolism during exercise in older individuals [6]. Poehlman et al. [27] examined the influence of 8 weeks of endurance training on total fat oxidation in 18 healthy old individuals (66.1±1.4 year;10 males and 8 females). The results showed that fat oxidation increased by 22% in response to endurance exercise. Hagberg et al. [28] compared the metabolic response to exercise in young and old athletes. After running on a treadmill for 60 minutes at 70% VO$_{2\text{max}}$, the results showed no difference between young and old athletes (RER = 0.87, 0.87 respectively). Interestingly, although previous studies have shown differences between the sexes in substrate utilization, neither Poehlman et al. nor Hagberg et al. studies differentiated the sex of the subjects when they were comparing the aging effect.

In light of the conflicting evidence regarding fat utilization in males and females during exercise, for which the training status of the subjects may be partially responsible for discrepancies in the findings, and the limited inclusion of an age factor in the study of male and female differences in exercise metabolism, the primary objective of this investigation was to compare fat and carbohydrate utilization during exercise among males and females varying in age and training status. By including all three factors in one
study, main and interactive effects of these factors can be compared. To the author’s knowledge, no these three factors have not been included in any one study.

METHODOLOGY

Participants

A total of 80 healthy male and female subjects were recruited for this study. All participants gave informed consent. The study was approved by Oregon State University’s Institutional Review Board (IRB). Participants’ characteristics are presented in Table 1.

All subjects were healthy nonsmokers. None were taking medications that affect fat or carbohydrate metabolism, including oral contraceptives and hormone therapy. All female subjects were experiencing regular menstrual cycles.

The participants were categorized on the basis of age, sex and training status into one of eight groups of ten subjects each (described below), and all participants successfully completed all study requirements:

- Young male subjects who are 18-24 years old and trained (YTM).
- Young male subjects who are 18-24 years old and untrained (YUM).
- Young female subjects who are 18-24 years old and trained (YTF).
- Young female subjects who are 18-24 years old and untrained (YUF).
- Older male subjects who are 38-44 years old and trained (OTM).
- Older male subjects who are 38-44 years old and untrained (OUM).
• Older female subjects who are 38-44 years old and trained (OTF).
• Older female subjects who are 38-44 years old and untrained (OUF).

Procedures

Testing was conducted in the Human Performance Lab in OSU Women’s Building on two separate days for: 1) orientation, screening, and ventilatory threshold determination, and 2) body composition determination and a 35-minute moderate exercise session.

Visit 1. Orientation, screening, and ventilatory threshold determination (1 hour)

For their first visit, the subjects provided informed consent and completed a health history questionnaire. The health history questionnaire was used to determine their health status for exercising, and based upon this, they were classified as being at low, moderate or high cardiovascular disease risk according to ACSM guidelines [29]. The questionnaire also collected information about their physical activity behaviors. Subjects then completed a progressive test on the treadmill to indentify their ventilatory threshold (VT).

• Trained subjects began at slow jog (4.5 mph) on a level treadmill. Following a 3-minute warm-up, the running speed was increased 0.5 mph per minute until the subjects exceeded their VT and achieved a respiratory exchange ratio of 1.0 (see below).
• Untrained subjects began at slow walk (2.5 mph) on a level treadmill. After a 3-minute warm-up, the walking speed was increased 0.5 mph per minute until the subjects exceeded their VT and achieved a respiratory exchange ratio of 1.0 (see below).
Visit 2. Body composition determination and moderate exercise session (1 hour)

Within one week after the VT test for male subjects or during their next mid-follicular menstrual-cycle phase (days 1-7 of the menstrual cycle) for female subjects, subjects returned to the lab for their second visit. The subjects reported in the morning following an overnight fast of at least 10 hours. Subjects refrained from strenuous exercise during the previous 24 hours.

Body composition was measured using the BODPOD (air displacement) method [30]. Following the BODPOD test, subjects exercised by walking or running on the treadmill for a 35-minute bout of moderate exercise at a speed that was 0.5 mph slower than the speed that elicited their VT in their VT test, which was a walking pace for untrained subjects and a jogging pace for trained subjects.

Measurement

During exercise tests, subjects wore a nose-clip and breathed through a mouth-piece that allowed them to inhale room air and that directed exhaled air to a ProvoMedics TrueMax 2400 metabolic cart, which was calibrated before each trial. Oxygen consumption (V\text{O}_2) and carbon-dioxide production (V\text{CO}_2) were determined for every minute, from which the respiratory exchange ratio (RER) was calculated as V\text{CO}_2 / V\text{O}_2. During the moderate exercise session, RER values from minutes 6-35 were averaged and compared between subject groups. When RER can be interpreted for fat and carbohydrate utilization, it ranges between 0.7 to 1.0, with an RER of 0.7 indicating complete reliance on fat as an oxidative fuel source, and 1.0 indicating complete a reliance on carbohydrate.
Ventilatory threshold (VT) was defined as the point at which the minute ventilation ($V'_E$) increased in a nonlinear fashion compared to increases in $V_{O_2}$, and was substantiated by an increase in the $V'_E/V_{CO_2}$ to $V'_E/V_{O_2}$ ratio. The VT was calculated using the V-slope method [31] (ParvoMedics TrueOne 2400 software).

**Statistics**

A 2x2x2 factorial ANOVA was used to determine whether age, sex, and training status have independent or interacting effects on substrate utilization valuables, and t-tests were used for post-hoc comparisons. A probability of $P<0.05$ was set to indicate significance. The results were analyzed using the SPSS 18.0 for Windows-statistical package [32].
RESULTS

**Subject characteristics.** Characteristics of subjects are presented in Table 1. By design, older subjects were approximately 20 years older than younger subjects. Male subjects were taller and heavier than female subjects, and older subjects were heavier than younger subjects (P < .05). The percent body fat was lower for male subjects compared to female subjects, for younger subjects compared to older subjects, and for trained subjects compared to untrained subjects (P < .05). The VT of trained subjects was higher than that of untrained subjects. The running speed for the moderate-exercise session was faster for male subjects than for female subjects, for younger subjects than for older subjects, and for trained subjects was faster than for untrained subjects (P < .05).

Figures 1-4 present the physical activity behaviors of the groups of subjects in this study. All four groups of trained subjects exercised strenuously for at least 30 minutes on 5 days per week for several years, and their main activities were running, jogging and walking. All four groups of untrained subjects were normally active but did not engage in regular aerobic exercise. Light exercise activities, such as easy walking and yoga, were their favorite activities.

The average RER and the exercise metabolism data from the last 30 minutes of the 35-minute moderate-intensity treadmill exercise session are presented in
Table 2. The energy-expenditure data (Kcal and Kcal fat) were not statistically analyzed because the males were heavier and exercising at a faster speed at their VT than the females, which would cause those values to be higher. The average RER and the percent energy expenditure derived from fat (%Kcal fat) were statistically analyzed, and the results are presented in Tables 3 and 4, respectively.

The mean RER value during 30-minutes of treadmill exercise was significantly higher in males than in females and in the untrained subjects compared to trained subjects, but there was no statistically significant difference between younger and older subjects (Table 3). None of the interaction was statistically significant, with the exception of the interaction between sex and fitness, which was significantly significant. Due to the statistically significant interaction between sex and fitness level, a post-hoc t-test analysis was conducted to compare trained males to trained females and untrained males to untrained females, combining the younger and older subjects within each of the four groups. There was no difference in the mean RER between trained males and trained females, while the mean RER was significantly lower for the untrained females compared to the untrained males (Table 5).

The mean %Kcal fat during the moderate exercise session was significantly higher in females than males and among the trained subjects compared to untrained subjects (Table 4), but there was no statistically significant difference between the
younger and older subjects. None of the interactions among the main effects were statistically significant, although the interaction between sex and training status neared statistical significance. The post-hoc t-test analysis determined that the %Kcal fat for trained males and trained females was not statistically different, while the %Kcal fat was significantly higher among the untrained females compared to the untrained males (Table 6).
Table 1. Subject characteristics: age, height, weight, percent body fat (%BF), ventilatory threshold (VT), and moderate-exercise test speed. [Mean ± standard deviation (SD)]

<table>
<thead>
<tr>
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<th>Younger Trained</th>
<th>Female (n=10)</th>
<th>Younger Untrained</th>
<th>Male (n=10)</th>
<th>Female (n=10)</th>
<th>Older Trained</th>
<th>Male (n=10)</th>
<th>Female (n=10)</th>
<th>Untrained</th>
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<td>62.5 ± 8.0b</td>
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<td>% BF</td>
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<td>22.5 ± 8.8bc</td>
<td>21.7 ± 6.9ab</td>
<td>24.9 ± 4.8b</td>
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<td>2.1 ± 0.4ab</td>
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<td>1.3 ± 0.3</td>
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<td>VT (mL/kg/min)</td>
<td>35.04 ± 3.7bc</td>
<td>35.95 ± 7.0bc</td>
<td>26.92 ± 2.2b</td>
<td>26.29 ± 15.1b</td>
<td>33.39 ± 2.4bc</td>
<td>31.45 ± 4.8bc</td>
<td>20.54 ± 5.0</td>
<td>20.91 ± 4.1</td>
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<td>Speed (mph)</td>
<td>6.2 ± 0.5abc</td>
<td>5.9 ± 0.4bc</td>
<td>4.4 ± 0.3ab</td>
<td>3.9 ± 0.4b</td>
<td>5.7 ± 0.3ac</td>
<td>5.4 ± 0.3c</td>
<td>4.1 ± 0.5a</td>
<td>3.8 ± 0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Male subjects are significantly different from female subjects.  b Younger subjects are significantly different from older subjects.

*c Trained subjects are significantly different from untrained subjects.  d Speed of moderate intensity exercise test.
Table 2. The RER, energy expenditure (EE), and total and percent energy expenditure from fat (Kcal fat and %Kcal fat) during 30 minutes of moderate-intensity exercise [Mean ± standard deviation (SD)]

<table>
<thead>
<tr>
<th></th>
<th>Younger</th>
<th></th>
<th>Older</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trained</td>
<td>Untrained</td>
<td>Trained</td>
<td>Untrained</td>
</tr>
<tr>
<td></td>
<td>(Male (n=10))</td>
<td>(Female (n=10))</td>
<td>(Male (n=10))</td>
<td>(Female (n=10))</td>
</tr>
<tr>
<td>RER</td>
<td>0.840±0.008</td>
<td>0.837±0.001</td>
<td>0.890±0.009</td>
<td>0.874±0.023</td>
</tr>
<tr>
<td>EE (Kcal/30min)</td>
<td>354.5±45.5</td>
<td>278.8±46.2</td>
<td>286.3±67.3</td>
<td>194.5±69.5</td>
</tr>
<tr>
<td>Kcal from Fat</td>
<td>188.0±30.4</td>
<td>147.4±29.4</td>
<td>102.8±26.8</td>
<td>80.1±36.4</td>
</tr>
<tr>
<td>(Kcal/30 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Kcal from fat</td>
<td>52.9±2.8</td>
<td>53.0±6.12</td>
<td>35.8±3.14</td>
<td>40.6±7.1</td>
</tr>
</tbody>
</table>
**Figure 1.** Average time per week that subjects in each group engaged in strenuous, moderate, or mild exercise of at least 15 minutes.

**Figure 2.** Forms of strenuous activity engaged in by subjects in each group.
Figure 3. Forms of moderate activity engaged in by subjects in each group.

Figure 4. Forms of mild activity engaged in by subjects in each group.
Table 3. 2x2x2 ANOVA of RER data for main effect and interactions for variables: sex, age, and training status.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>sig</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>.004</td>
<td>.004</td>
<td>10.852</td>
<td>.002*</td>
<td>.901</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>.000</td>
<td>.000</td>
<td>1.125</td>
<td>.292</td>
<td>.182</td>
</tr>
<tr>
<td>Training status</td>
<td>1</td>
<td>.037</td>
<td>.037</td>
<td>93.301</td>
<td>.000*</td>
<td>1.00</td>
</tr>
<tr>
<td>Sex*Age</td>
<td>1</td>
<td>.001</td>
<td>.001</td>
<td>1.375</td>
<td>.245</td>
<td>.212</td>
</tr>
<tr>
<td>Sex*Training status</td>
<td>1</td>
<td>.002</td>
<td>.002</td>
<td>4.742</td>
<td>.033*</td>
<td>.575</td>
</tr>
<tr>
<td>Age*Training status</td>
<td>1</td>
<td>1.25E-6</td>
<td>1.25E-6</td>
<td>.003</td>
<td>.956</td>
<td>.051</td>
</tr>
<tr>
<td>Sex<em>Age</em>Training status</td>
<td>1</td>
<td>.000</td>
<td>.000</td>
<td>.527</td>
<td>.470</td>
<td>.111</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>80</td>
<td>59.603</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < .05
Table 4. 2x2x2 ANOVA of %Kcal from fat data for main effect and interactions for variables: sex, age, and training status

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>328.74</td>
<td>328.74</td>
<td>6.88</td>
<td>.011*</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>60.19</td>
<td>60.19</td>
<td>1.26</td>
<td>.266</td>
</tr>
<tr>
<td>Training status</td>
<td>1</td>
<td>4598.79</td>
<td>4598.79</td>
<td>96.17</td>
<td>.000*</td>
</tr>
<tr>
<td>Sex*Age</td>
<td>1</td>
<td>53.46</td>
<td>53.46</td>
<td>1.12</td>
<td>.294</td>
</tr>
<tr>
<td>Sex*Training status</td>
<td>1</td>
<td>158.57</td>
<td>158.57</td>
<td>3.32</td>
<td>.073</td>
</tr>
<tr>
<td>Age* Training status</td>
<td>1</td>
<td>3.77</td>
<td>3.77</td>
<td>.079</td>
<td>.780</td>
</tr>
<tr>
<td>Sex<em>Age</em>Training status</td>
<td>1</td>
<td>4.864</td>
<td>4.864</td>
<td>.102</td>
<td>.751</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>80</td>
<td><strong>168419.94</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < .05
Table 5. The average RER during minutes 6-35 of the moderate-exercise session: organized by Sex and Training Status (± SD), combining across age groups.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trained</td>
<td>.843±023</td>
<td>0.838 ± .022</td>
<td>.698</td>
</tr>
<tr>
<td>Untrained</td>
<td>0.896 ± .012</td>
<td>0.822± .020</td>
<td>4.644*</td>
</tr>
</tbody>
</table>

* P < .05

Table 6. The average %Kcal fat during minutes 6-35 of the moderate-exercise session: organized by Sex and Training Status (± SD), combining across age groups.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trained</td>
<td>51.651±7.89</td>
<td>52.890±1.867</td>
<td>-.483</td>
</tr>
<tr>
<td>Untrained</td>
<td>33.672±4.055</td>
<td>40.542± 6.290</td>
<td>-4.105*</td>
</tr>
</tbody>
</table>

* P < .05
**Discussion**

The main objective in the current study was to compare substrate utilization, as indicated by the RER, during exercise among males and females varying in age and training status. One finding of this investigation was that fat utilization during exercise was higher in trained subjects compared to untrained subjects. There was no difference in substrate utilization during exercise between the younger and older groups of subjects. Post-hoc analysis determined that untrained females utilized proportionately more fat during exercise compared to the untrained male subjects, but there was no difference in fat and carbohydrate utilization between trained females and trained males.

In this study, the RER was used to determine fat and carbohydrate utilization during exercise. The RER is the common indirect method used to determine substrate utilization, and it has been used in previous studies evaluating sex differences [10-14], age differences [22-25], and training-status differences [10, 19, 20] in substrate utilization. The RER is a numeric index of carbohydrate and fat utilization based on the ratio of carbon dioxide produced (\( V_{CO_2} \)) to oxygen consumed (\( V_{O_2} \)). Normal RER values during steady-state exercise range between 0.7 and 1.0. A lower RER represents a greater level of fat oxidation, while a higher RER represents a greater level of carbohydrate oxidation [33]. The RER can be validly interpreted for substrate utilization when \( V_{CO_2} \) is
generated from oxidation only. When expired CO\(_2\) is also the result from the buffering of lactic acid during anaerobic conditions, having both oxidative and non-oxidative sources of carbon dioxide production invalidates interpretation of the RER for fat and carbohydrate oxidation [34].

Most studies measuring substrate utilization during exercise established the intensity of the exercise studied as a percentage of the subject’s maximal oxygen consumption (\(\text{VO}_{2\text{max}}\), which may present a problem if subjects are not matched on lactate threshold (LT). The lactate threshold is defined as the exercise intensity when lactic acid starts to accumulate in the blood (the rate of lactate production exceeds the rate of lactate removal) [35]. The LT can also be defined as the maximum intensity at which a steady-state of exercise can be maintained [36]. The LT has a more direct bearing on fat and carbohydrate utilization than %\(\text{VO}_{2\text{max}}\) because the LT is indicative of the point beyond which the RER cannot be interpreted for substrate utilization due to the production of carbon dioxide from lactic acid buffering in the blood. The LT occurs at varying percentages of \(\text{VO}_{2\text{max}}\) across individuals [28, 37-40], and has been reported to occur as low as 60% \(\text{VO}_{2\text{max}}\) [28, 40] and as high as 85% \(\text{VO}_{2\text{max}}\) [39]. Establishing exercise intensity in studies of substrate utilization by %\(\text{VO}_{2\text{max}}\) does not ensure that the RER can be validly interpreted, since subjects may or may not be below their LT at the specific %\(\text{VO}_{2\text{max}}\) tested.
The measurement of LT requires an invasive procedure to obtain samples of blood to measure levels of lactate. The VT is considered an indirect and noninvasive measurement of the LT [41, 42], for it identities the point at which ventilation increases in response to the additional carbon dioxide produced by lactic acid buffering. Davis [41] and Caiozzo [43] have reported a high correlation between VT and LT (r = 0.88 to 0.95). Therefore, this study used the VT as a basis for establishing the exercise intensity that could be validly interpreted for carbohydrate and fat utilization.

The present investigation demonstrates that the average RER for the trained subjects was significantly lower than that for the untrained subjects, indicating that trained subjects utilized more fat and less carbohydrate than untrained subjects during 35 minutes of moderate exercise. It is well documented that endurance training increases fat oxidation and decreases carbohydrate oxidation during exercise [6, 8, 9]. Much of the increase in fat utilization in trained subjects is related to the physiological changes that enhance the body’s oxidative capacity, mainly due to increases in the number and size of mitochondria [4, 8] and oxidative enzymes [44].

Finding that the untrained females derived proportionately more energy from fat during their exercise compared to the untrained males is consistent with other studies of untrained subjects. Horton et al. [13] compared untrained male and female
subjects on substrate utilization during cycling at 40% VO\textsubscript{2max}, and reported that untrained female subjects had significantly lower RER values compared to untrained male subjects. Blatchford and colleagues [14] also reported higher levels of fat utilization among untrained females compared to untrained males during exercise at 35% VO\textsubscript{2max}. Carter and associates [17] conducted a 7-week training study, and reported that the untrained females oxidized proportionately more fat than untrained males during exercise at 60% VO\textsubscript{2peak}. Contrary to the finding of the present study, Carter et al. also found that the trained females utilized more fat during exercise than the trained males.

A major finding of this study was that there was no difference in RER or %Kcal fat between the trained males and females. This finding is in agreement with studies conducted by Costill et al.[19], Powers et al.[20], and Froberg and Pederson [11]. On the other hand, these results conflict with the findings of studies by Tarnopolsky et al. [12], Carter et al. [17], and Phillips et al. [38], who reported that trained females utilized a greater proportionate of fat during exercise than trained males. The reasons for the conflicting findings are unclear. As noted above, in order to interpret RER as indicative of fat and carbohydrate use, the exercise intensity should be at or below the subjects’ lactate or ventilatory threshold. All of previous studies had reported the workload as relative to the subjects’ VO\textsubscript{2max}, not relative to their VT. However, many of the studies set the exercise intensity at a moderate level, where it is relatively safe to interpret the RER.
for substrate utilization. For example, studies that found no difference in RER between trained males and females have tested at moderate intensities ranging between 58 to 70% \(\text{Vo}_{2\text{max}}\) \([1, 20, 21, 45]\). Studies that have reported a significant difference in fat utilization between trained males and females have also tested at intensities ranging between 60 to 75% \(\text{Vo}_{2\text{max}}\) \([11, 12, 17, 46]\). Therefore, differences in the findings do not appear to be due to differences in the intensities of exercise used in the studies or an inability to interpret RER as an indicator of substrate utilization.

A factor that may have a bearing on the conflicting results in sex differences in substrate utilization during moderate exercise is the fitness level or the training status of the subjects \([1, 11, 12, 17, 19-21, 45, 46]\). Of the key studies, \(\text{Vo}_{2\text{max}}\) for the trained females in the studies that reported no sex difference in fat utilization ranged from 52-61 ml.kg\(^{-1}\).min\(^{-1}\) \([19-21]\), as compared to a range of 41-58 ml.kg\(^{-1}\).min\(^{-1}\) for the trained females in studies that reported a sex difference in fat utilization \([11, 12, 17, 46]\). Thus, there was a trend for the females in the studies finding a sex difference in fat utilization during moderate exercise to have a lower \(\text{Vo}_{2\text{max}}\) than those in the studies finding no difference. The \(\text{Vo}_{2\text{max}}\) ranges for the males in the studies were more similar (53-63 ml.kg\(^{-1}\).min\(^{-1}\) in studies finding no difference, and 44-65 ml.kg\(^{-1}\).min\(^{-1}\) in studies finding a sex difference in fat utilization) \([11, 12, 17, 19, 21, 46]\). In the present study the \(\text{Vo}_{2\text{max}}\) of the subjects was not measured.
Menstrual status has been shown to affect exercise metabolism. Hackney et al. [47] studied substrate utilization in nine trained eumenorrheic women during different intensities (35, 60, 75% $\text{Vo}_{2\text{max}}$) of sub-maximal exercise at the mid-follicular and the mid-luteal phases of the menstrual cycle. They show that fat utilization at low (35% $\text{Vo}_{2\text{max}}$) and moderate intensity (60% $\text{Vo}_{2\text{max}}$) was increased during the luteal phase compared to the follicular phase of the menstrual cycle. Interestingly, at vigorous intensity (75% $\text{Vo}_{2\text{max}}$), the fat utilization and carbohydrate utilization at the stages of the menstrual cycle were not significantly different. Jurkowski et al. [48] reported that the blood lactate response to exercise was lower during the luteal phase than follicular phase. They concluded that the female sex hormones estrogen and progesterone play an important role in substrate utilization, and these hormones vary depending on the phase of the menstrual cycle. In addition, Tarnopolsky [49] noted that male estrogen levels are similar to female levels in the follicular phase of the menstrual cycle (males = 6-24 pg/ml, females = 10-50 pg/ml). Therefore, in the studies on substrate utilization by males and females conducted by Tarnopolsky et al. [12, 46], the female subjects were studied during exercise in the mid-follicular phase of their menstrual cycle, when circulating ovarian hormones are relatively low. Even though the hormone difference between males and females would be less pronounced during the follicular stage of the menstrual cycle, Tarnopolsky et al. and Carter et al. still found that females used more fat during exercise.
than in males did. Studies that found no sex difference among trained subjects, such as Powers et al. [20], and Costill et al. [19], did not report the menstrual status of the female subjects in their studies. It is possible that not controlling for the menstrual status of the trained females in these studies created variability in their metabolic responses during exercise that contributed to there being no statistically significant difference compared to the trained males. However, in the present study, menstrual status was controlled, and no difference in fat utilization was found between the trained males and females.

In the present study, no age effect was evident in substrate utilization during exercise, nor was there an interaction between age and training status, indicating that regardless of training status, younger subjects have similar RER values as older subjects. These results stand in disagreement with previous studies that report that older individuals tend to utilize more carbohydrate and less fat during exercise than younger individuals [23, 26, 28, 37]. For example, Sial et al. [23] evaluated the effect of age on fat and carbohydrate metabolism during moderate-intensity exercise. Six elder (73 ±2yr) and six younger (26 ±2yr) subjects participated, and the old subjects used more carbohydrate and less fat than the young subjects. In the studies that report an age effect on fat and carbohydrate utilization during exercise, the older subjects were 40-50 years older than the younger subjects [23, 28]. Roberts and Dallal [50] summarized 20 studies on energy expenditure and aging. Individuals from the database were 20-100 years of age. The
results demonstrated that physical activity levels may be relatively constant between the ages of 20 to 40-50 years, and after that, it decreases sharply. The older and younger subjects in this study reported engaging in similar levels of strenuous exercise sessions per week (see Figure 5). To the extent that an age effect is actually due to differences in physical activity associated with aging, the similar levels of physical activity between the younger and older groups in this study would explain the absence of an age effect on substrate utilization. If age is an independent factor affecting substrate utilization, then the age range in this study was not large enough to exert that effect.

Figure 5. Number of strenuous exercise sessions per week of 15 minutes or longer by subjects in younger and older groups.
**Strengths of study**

One strength of this study is its study design. Most previous studies incorporated just one or two factors when comparing subjects on substrate utilization during exercise, such as comparing male and female subjects, young and old subjects, or trained and untrained subjects. No study has been designed to compare all three factors concurrently. This study made comparisons between groups that have not been presented together in previous studies. As a result, it was possible to identify the sex effect, the training status effect, and the interaction between sex and training on substrate utilization during moderate exercise.

Another strength of this study is the use of a sufficiently large sample of female and male subjects across age and training status, compared to previous studies [20], which gave this study adequate power to test for effects on substrate utilization (Tables 3 and 4).

Finally, the exercise intensity that was used in this study was slightly below the VT, allowing for a valid comparison and interpretation of RER across the variables of sex, age, and training status. In addition, the menstrual status of female subjects in the study was controlled: all female subjects were eumenorheic and were tested in the mid-follicular stage of their menstrual cycle.
Limitations of study

A limitation in the present study is its cross-sectional design. A longitudinal study offers a stronger determination of cause-and-effect than does a cross-sectional study, but among the variables under study, a longitudinal design is feasible for the training effect only. The 20-year difference in age between younger and older groups may not be sufficient to identify a potential difference in substrate utilization, so future studies should have a larger age differential. As noted above, a strength of this study was setting the moderate intensity based upon the subjects’ VT, but not having measured the subjects’ Vo₂max limits the ability to compare the exercise intensity used in this study to that used in studies that reported the intensity as a percentage of Vo₂max.

In conclusion

An expected finding of this study is that fat utilization during moderate exercise was higher in trained subjects compared to untrained subjects. Another finding was that a 20-year difference in age was not sufficient for an age effect to be evident in fat utilization during exercise. The major finding of this study was that untrained females utilized proportionately more fat during exercise compared to untrained males, but there was no difference in fat and carbohydrate utilization between trained females and trained males.
Recommendation for future research

Based on the findings in this study, future research should consider having a larger age difference between the younger and older subjects. Future research should continue to establish exercise intensity being studied relative to the subjects’ VT, but should also consider measuring VO$_{2\text{max}}$ so that the training status of the subjects can be reported in those terms. Due to the finding in this study that there was no difference in fat utilization between trained males and females, but there was a difference between untrained males and females, future studies may consider conducting a training study with periodic comparisons of substrate utilization during exercise to attempt to capture the transition in substrate utilization over changes in training status.
REFERENCES


APPENDIX A

Review of Literature
Review of Literature

Fuel utilization-The role of carbohydrate and fat

Carbohydrate (CHO), fat, and protein provide the necessary components to sustain energy metabolism during exercise. The main contributors of fuel for the body are carbohydrates and fats, whereas protein’s contribution is minimal. Indicative of their importance in energy metabolism, carbohydrate and fat stores are more readily available. CHO is usable as glucose in the bloodstream and is stored as glycogen in the liver and muscle, while fat is usable as free fatty acids (FFA) and stored as triglycerides in both adipose and muscle tissue. The body’s endogenously stored energy is comprised of approximately 92-98% fat and 2-8% carbohydrate [3]. A majority of the body’s endogenous CHO fuel is stored as glycogen in skeletal muscle (82% of total CHO energy) and the liver (14%), with only 4% found as plasma glucose [51].

Carbohydrate metabolism

CHO is the one of most important energy sources for human metabolism. CHO are eventually transformed into glucose, and some glucose is stored as glycogen by the process of glycogenesis. Glycogen is stored in the liver and muscle and can
breakdown to glucose when the blood glucose level is low. This process is called
glycogenolysis. There are three major stages in carbohydrate metabolism: glycolysis, the
Krebs cycle, and the electron transport chain.

**Glycolysis**

Glycolysis is the sequence of reactions that release energy and converts
 glucose or glycogen into two molecules of private acid in the cytoplasm of the cell.
Glucose is transported from blood across the cell membrane by the GULT4 transporter
(glucose transporter carrier proteins). In the cell, glucose is broken down to glucose-6-
phosphate (G-6-P) by the enzyme hexokinase (HK), and through the ten step series of the
glycolytic pathway is converted to pyruvate. The Glycolytic pathway is regulated by
three important enzymes. HK is the first of regulatory enzymes in glycolysis. It can be
activated by increased level of glucose and on the other hand HK is inhibited by elevated
G-6-P levels. Phosphofructokinase (PFK) is the most important rate-limiting enzyme of
glycolysis. Rate-limiting means that this enzyme controls the rate at whole the series of
reaction occur. PFK is inhibited by high levels of ATP and an elevated amount of citrate,
which indicate plenty of energy within the cell [52]. The final regulating step is the
enzyme Pyruvate kinase (PK). PK is activated by fructose 1, 6 diphosphate, the product
of the PFK reaction, and, on the other hand, PK is inhibited by the protein kinase. The
protein kinase in the liver will be stimulated when blood glucose levels are low. At the
end of glycolytic pathway, pyruvate can be reduced to lactate by the enzyme lactate
dehydrogenase if there is insufficient oxygen in the cell, or pyruvate is transported into
the mitochondria if oxygen is available.

Glycolysis is associated with both the utilization and production of ATP. If glycolysis begins with the breakdown of glycogen, a net of 3 ATP is produced, and if glycolysis begins from glucose, a net 2 ATP is produced, because 1 ATP is used for the conversion of glucose to G-6-P when it enters the cell.

The Krebs cycle

The second stage of carbohydrate metabolism is called the citric acid cycle or the Krebs cycle. The Krebs cycle is a series of chemical reactions that generate hydrogen molecules to be processed for energy in the electron transport system (ETS). When oxygen supply is adequate, the pyruvate that formed in the cytoplasm of the cell during glycolysis is transferred to the mitochondria, where most of the energy inherent in glucose is released. In the mitochondria, pyruvate acid is transformed to acetyl CoA by the enzyme pyruvate dehygenase, and it enters into the series of chemical reactions called the Krebs cycle. Acetyl CoA condenses with a four-carbon compound called oxaloacetate to form a six-carbon acid (citrate) and is degraded to five and four-carbon compounds, releasing two molecules of CO2. Simultaneously, two molecules of NADH are formed.
Finally, the C-4 carbon undergoes three additional reactions in which guanosine
triphosphate (GTP), FADH2 and NADH are formed, thereby regenerating oxaloacetate.
FADH2 and NADH are passed on to the electron transport chain that is embedded in the
inner mitochondrial membrane. GTP is a high energy compound that is used to
regenerate ATP from ADP. Therefore, the main purpose of the Krebs cycle is to provide
high energy electron in the form of FADH2 and NADH to be passed onward to the
electron transport chain.

**Electron transport chain (ETS)**

The hydrogen atoms released from the glycolytic pathway and the Krebs cycle
represent a large source of unharnessed energy that must be oxidized to realize the full
potential of the metabolic system. In fact, over 90 percent of the total ATP produced in
the metabolic pathway comes from the processing of this hydrogen though the ETS.
These hydrogen are carried by the carrier molecules (NAD and FAD), but the pair of
hydrogen electron on NAD yield more energy than the pair on FAD because they enter
the ETS at a higher level. Three ATP are released for the hydrogen ions carried by NAD
and two ATP are produced for the hydrogen pairs carried by FAD.

Producing energy through the ETS is called oxidative phosphorylation, a two-
step process where the hydrogen atoms lose their electrons, and the energy released is
used to phosphorylate ADP to form ATP. Phosphorylation takes place as hydrogen atoms carried by NAD and FAD enter the ETS, their electrons are stripped from them, and the electrons are transferred down a series of electron carriers to atomic oxygen. The protons are pumped outside the mitochondria, producing a chemical and osmotic difference between the inside and outside of the membrane. These protons then enter the mitochondria at a stalk (on the inner membrane) to combine with oxygen, and the energy released from this movement is used to couple a phosphate ion to ADP, thus forming ATP. This coupling occurs at three different places in ETS for hydrogen carried by NAD, and only two places for those carried by FAD. The entire process occurs because oxygen is available as the final electron acceptor, and the hydrogen ion and its electron move from an area of electronegativity (NAD and FAD) to an area of electron positivity (atomic oxygen). Without oxygen, there would be no reason for hydrogen ions to move to the ETS and energy production would not be possible.

**Fat metabolism**

Fat is the other major nutrient that provides energy for muscular contraction. About 40% of the calories ingested in the normal diet are from fat [53]. Approximately 95% of the fat in the body is in the form of triglycerides (TG), and TG is made up of three free fatty acid (FFA) molecules with glycerol. Lipolysis is the process by which fatty acids are metabolized. Fatty acid those are utilized for energy by contracting
muscles came from TG stored in the muscles as intramuscular triglycerides (IMTG), TG stored in fat cell, and TG or fatty acids circulating in bloodstream. [54]. Fat metabolism refers to ATP come from fatty acid molecules that are split off from stored TG, transported by the blood to muscles, and broken down by the muscle for energy by the process called beta oxidation (β- oxidation) [54]. This process occurs in the mitochondria of the cell. There are four major steps involved in fat metabolism: 1) FFA release from adipose tissue, 2) movement of FFA’s into muscle from the blood, 3) beta oxidation, and 4) the Krebs cycle and electron transport chain (ETC) [53].

**FFA release from adipose tissue and from muscle**

Before energy is released from the fat, TG in adipose tissue is broken down to three free fatty acid molecules (FFA) and glycerol through a process called lipolysis (the hydrolysis of TG). This process is stimulated by a hormone-sensitive lipase (HSL). HSL is located directly in the fat cell and is stimulated by the hormones epinephrine and norepinephrine during exercise as well as by growth hormone (GH). Epinephrine and norepinephrine initiate lipolysis, where GH helps maintain it during prolong exercise. Free fatty acids are insoluble in the water, so free fatty acids released into the blood must be bound to a protein carrier called albumin to allow them to be transported to cells and within the blood stream. The free fatty acid may be reattached to glycerol in the blood to make circulating TG, which are often bound to proteins in the blood and circulate as
lipoprotein [54]. The glycerol molecule is water-soluble and can freely travel through the blood will enter the bloodstream called circulating glycerol and is absorbed by the liver or kidney where it is rejoined the glycolysis and gluconeogenesis pathway. IMTG can be degraded to FFA like in adipose. FFA that is released from IMTG may enter to blood, reesterified, or oxidized within muscle [55].

FFA are transported to the muscle cell they are release from albumin and transported across the muscle cell membrane by FFA transporter. There are three main FFA transporters located on the muscle cell membrane: fatty acid binding protein (FABP), fatty acid translocase (FAT), and fatty acid transport protein (FATP). These proteins bind the FFA molecules and transport them across the cell membrane and the mitochondria for complete oxidation.

Fatty acids are transported across the outer mitochondrial membrane by carnitine-palmitoyl transferase I (CPT-I), and then couriered across the inner mitochondrial membrane by carnitine transport system [56]. Once inside the mitochondrial matrix, fatty acyl-carnitine reacts with coenzyme A to release the fatty acid and produce acetyl-CoA. Once inside the mitochondrial matrix, fatty acids undergo β-oxidation. When fatty acid undergoes β-oxidation it produces acetyl CoA, FADH₂, and NADH molecules. The acetyl CoA then serves as substrate in the Krebs cycle in which
additional hydrogen carriers (NADH, FADH) are produced. Those high energy carriers undergo mitochondrial oxidation in the ETS to produce ATP.

The total of ATP comes from the breakdown of fatty acid molecule that follow reaction in beta oxidation (β- oxidation), Krebs cycle, and electron transport change. Depending on how many carbons atoms per FFA, human FFA has 16 or 18 carbon atom, therefore the totals of ATP are 147 molecules after completely all of the oxidation.

Energy can be extracted from the food in body to generate high energy phosphate bond in ATP (adenosine triphosphate). This high bond can be broken rapidly to support the immediate need for muscle contraction by converting ATP to ADP (adenosine diphosphate), and ADP is then is restored to ATP very quickly by creatine phosphate (CP). CP can readily provide a phosphate group to ADP to form ATP under the influence of creatine kinase (CK). The store of CP is called phosphagen system limited, and it can provide energy for shot intense muscular activity for only a few seconds (10 – 15 second), such as sprinting, jumping and lifting. If the exercise persists at high intensity for more than 15 seconds, then the phosphagen system cannot supply the ATP needed, glucose or glycogen must be processd anaerobically by the glycolysic pathway (without oxygen). Using this pathway, ATP is produced and also lactic acid
results. The lactic acid system is capable of releasing energy to resynthesise ATP without the involvement of oxygen and is called anaerobic glycolysis. When the oxygen is available and the muscle is not working strenuously or the exercise intensity is low or moderate. The pyruvate from glucose is converted to carbon dioxide and water in mitochondria. Approximately 36 ATP can be produced aerobically from a single glucose molecule compared to 2 ATP anaerobically. Aerobic metabolism supplies energy more slowly than anaerobic metabolism, but can be sustained for long periods of time. The major advantage of the less efficient anaerobic pathway is that it more rapidly provides ATP in muscle by utilizing local muscle glycogen. Anaerobic glycolysis supplies most energy for short term intense exercise ranging from 30 seconds to 2 minutes. The disadvantages of anaerobic metabolism are that it cannot be sustained for long periods, since the accumulation of lactic acid in muscle decreases the PH and inactivates key enzymes in the glycolysic pathway, leading to fatigue. The lactic acid released from muscle can be taken up by the liver and converted to glucose again (Cori Cycle), or it can be used as a fuel by the cardiac muscle directly or by less active skeletal muscles away from the actively contracting muscles.

Under most circumstances, the type of substrate (fuel) and the rate at which it is utilized during exercise is largely dependent on the intensity and duration of the exercise. Endurance exercise of various intensities will utilize varying proportions of fat and
carbohydrate. While exercise at low intensities (<50% VO\textsubscript{2max}) utilizes mostly fat, exercise at higher intensities (greater than 70% VO\textsubscript{2max}) primarily utilizes intramuscular glycogen stores with minimal contribution from intramuscular fat stores [3]. The moderate exerciser (65% VO\textsubscript{2max}) gathers approximately 40% of his/her fuel from intramuscular and plasma FFA, 10% from plasma glucose, and 50% from muscle glycogen [52]. At about 65% VO\textsubscript{2max}, there is a “crossover point” where carbohydrate becomes more dominant than fat in supplying fuel to the muscle [57]. This shift to CHO metabolism at the high exercise intensity is caused by two factors: the recruitment of fast twitch fibers (which are better equipped to metabolize CHO) and increasing levels of epinephrine in the blood (which contribute to glycogen breakdown). Therefore, as exercise intensity increases, substrate utilization shifts from primarily fat to primarily CHO oxidation. However, the relative contribution of carbohydrate and fat to energy metabolism can vary depending on other factors, including training status, sex, and age as well as will be described below.
Factor affecting substrate utilization

Aging factor

Aging is associated with changes in body composition, including reduction in lean body mass and an increase in body fat, a decline in physical activity that contributed to decrease exercise tolerance, increased fat mass and alteration in glucose and lipoprotein metabolism [23, 37]. The mechanisms that determine the age-associated change in metabolism are not well defined but the results of a study conducted by Sial and colleagues demonstrate that fat oxidation is decreased and carbohydrate oxidation is increased during moderate intensity exercise in older individuals [23]. The change in substrate utilization during exercise in aging may be caused by an age-related decline in skeletal muscle respiratory capacity [23] and fat mobilization [24].

Data obtained from in vivo and in vitro experiments show a decrease in muscle oxidative capacity related with aging [58]. Also, several studies have shown that muscular mitochondria respiratory capacity in untrained older individuals is lower than in untrained young individuals [58-61]. Coggan et al. [60] studied the effect of aging on human skeletal muscle and they found that mitochondrial oxidative enzyme activities (citrate synthase, succinate dehydrogenase, b-hydroxyl-CoA-dehydrogenase) were 25% lower in old as compare to young subjects. Similarly, others have shown the capacity of
human muscle to utilize O2, measured in vitro, was 25–30 % lower in older subjects [59, 62]. Also in 1993, Coggan and colleagues’ study found that mitochondria oxidative enzyme activity is 25–40 % lower in muscles of untrained elderly men and women compared with young men and women [63]. A decline in marker enzyme activity in muscle reflected decreasing mitochondrial function (low respiratory rate and enzyme activity) and mitochondria density in the elderly [61, 62]. Data from in vivo studies comparing young and old adults at any given power output shows increased use of CHO and a decreased use of fat as a fuel during exercise in elderly. This is obviously due to the muscle oxidative capacity and the ability to generate adequate ATP for muscle work that are lower in elderly than in young men indicating that old individuals used more glucose than fatty acid during exercise [58].

The release of fatty acids from adipose tissue and the capacity of respiring tissue to oxidize fatty acids are two primary factors determining the rate of fat oxidation during exercise. The mobilization of triglyceride provides an important fuel for working muscles [23], and the increase in lipolysis is largely due to catecholamine-mediated stimulation of β-adrenergic. Lipolysis can be regulated by several hormones, such as catecholamines, glucagon, adrenocorticotropic hormone, growth hormone, prostaglandins, thyroid hormones, glucocorticoids and sex steroid hormones [64]. The aging process may affect hormonal regulation of lipolysis [24]. These finding are in
agreement with Ostman et al [65], who reported that the ability of catecholamines to stimulate lipolysis was reduced in older subjects as a result of decreased adipose tissue adrenergic responsiveness. Also, data from animal and human studies show that the ability to mobilize fat may be impaired in the elderly because of a decrease in both the sympathoadrenal response to exercise and adipose tissue sensitivity to stimulation [23]. Results from three studies [23, 24, 66] indicated that the lipolytic response to exercise and the capacity to mobilize free fatty acids from adipose tissue stores were reduced with age. Reduced free fatty acid mobilization may decrease fat oxidation by limiting substrate supply.

The most common method to determine whole body fat and carbohydrate oxidation rates is respiratory exchange ratio (RER). The RER is a numeric index of carbohydrate and fat utilization based on a ratio of carbon dioxide produced to oxygen consumed. Lower RER represents a greater lead of fat oxidation, while higher RER represents a greater lead of carbohydrate oxidation [33]. Several studies show that RER at any given absolute exercise intensity was greater in old than young subjects [22, 25, 67]. This observation showed that elderly subjects use less fat and more carbohydrate as a fuel than young subjects during exercise perform at the same absolute intensity. This observation is supported by a finding of higher blood lactate levels during sub-maximal exercise in elderly subjects [23, 26, 68]. Results from several studies evaluated the
effect of aging on substrate utilization during prolonged exercise (≥30 min). During exercise performed at the same relative intensity, Hagberg et al. [28] evaluated the effect of aging and endurance training on metabolic responses of trained and sedentary young men (age 20 to 32 years) and older men (age 60 to 70 years) exercising at the same relative exercise intensity (70% VO$_2$ max) for 60 minutes. The results showed that the young sedentary men had a higher RER than the other three groups, indicating a greater reliance on CHO as substrate. They concluded that the young sedentary men were only group exercising above their lactate threshold. They also found that young trained men had higher fat oxidation, lower plasma FFA concentration compare to old trained men. On the other hand, Silverman and Mazzeo [26] investigated the influence of age and training on the responsiveness of key hormones responsible for regulating metabolism during exercise. The data showed that mean RER values during the 45 minute sub-maximal exercise at the workload corresponding to each their lactate threshold were higher in the old group than in young group.

Similar results with Silverman and Mazzeo, Sial et al.[23] evaluated the effect of aging on fat and carbohydrate metabolism during moderate intensity exercise. Six elderly and six young adult men and women who participated in this study were matched by sex and lean body mass. The old subjects performed 60 minutes cycle exercise at 50% VO$_2$ max, whereas the young subjects exercised at the same absolute and
at the same relative intensity as the elderly subjects. The results show that the RER was higher in the older subjects at either the same absolute intensity or relative intensity. This means that fat oxidation during exercise was lower in the older subjects than in the young subjects exercising at either the same absolute or similar relative intensities. In addition, carbohydrate oxidation in the elderly group was higher than in the young groups exercising at the same absolute intensity and lower than young subjects at the same relative intensity. However, Mittendorfer and Klein [58] explained that the results of these studies were different because they did not carefully control for diet, training status, and lactate threshold, which can affect RER.

Regardless of age, endurance training has been shown to increase fat oxidation during exercise in young adults [7] and in elderly persons [6]. After endurance training, older people can increase skeletal muscle mitochondrial enzyme, respiratory capacity, decrease glucose production and oxidation, and increase fat oxidation [58, 63, 69]. This is supported by Meredith et al [59] who studied the peripheral effects of endurance training on 65-year-old men who performed cycle ergometry at 70% of their age-adjusted maximal heart rate for 45 minutes per day, 3 days per week until 12 weeks. In their study, they demonstrated that the oxidative capacity of the older men's muscles increased by 125%. Likewise, in young adults, endurance training can also increase skeletal muscle mitochondrial enzyme respiratory capacity [4]. Therefore,
endurance training can increase mitochondrial content and the activity of citric acid cycle enzymes.

Aging is associated with changing substrate utilization during exercise. It may be caused by an age-related decline in skeletal muscle respiratory capacity and fat mobilization. Some studies focusing on the effect of aging on substrate utilization during exercise showed that older people rely more on carbohydrate than fat as a fuel during exercise. On the other hand, one research showed high CHO utilization in young sedentary men comparing with old men [28]. Also, endurance training has been shown to increase fat oxidation during exercise in young adults and in elderly persons. Interestingly, some studies did not match subjects for gender difference and some studies did not carefully control lactate threshold as well as training status. Gender, lactate threshold, and training status are factors that influence the effect of aging on substrate utilization. We will discuss below.

**Gender factor**

In general, the relative contribution of each substrate to energy production is complicated by many factors. Gender is one factor that may play a role in altering substrate utilization. Several studies have concluded that women tend to utilize more fat and less carbohydrate than men [10-12]. However, several other studies have shown that
there are no gender differences in the proportion of oxidize carbohydrate and fat [19, 45, 70, 71].

There is evidence that women tend to use less CHO than men during exercise. However, the mechanism behind this phenomenon remains unclear. In the study by Tarnopolsky et al.[12], six trained males and six trained females were matched on VO₂ max per kg lean body weight and training volume. The females were exercise-tested in the midfollicular phase to control the potential effect of menstrual phase on substrate utilization. The results show significant gender differences in RER values during treadmill running for 90 min at 65% VO₂ max. Females had significantly lower RER values compared with males, indicating that trained females used more fat and less carbohydrate than trained males. They also concluded that muscle biopsy samples showed greater muscle glycogen depletion in male subjects when compared with female subjects. Similarly, Tarnopolsky et al.[46] investigated the effects of gender on CHO loading and substrate utilization during 60 minutes of cycling ergometry at 70% VO₂ peak. Seven trained males and eight trained females were selected and matched on their training history and VO₂ peak per kg lean body weight. The women were also tested during the midfollicular phase of their menstrual cycle. The results were similar to their previous study; they demonstrated that the RER was lower in women than in men, indicating greater lipid oxidation and lesser CHO oxidation. Horto et al. [13] studied
gender differences in fuel metabolism during long-duration exercise. Fourteen male and female subjects were matched on training status (volume and intensity of training as determined from self-report activity record). Fuel oxidation was measured using indirect calorimetry and blood samples were drawn for circulating substrate and hormone levels. The results showed that women had significantly lower RER values compared with men during two hours of exercise at 40% VO\(_2\)\text{max}. The percentage of fat oxidation during exercise averaged 43.7% for the males and 50.9% for the females. Blatchford and colleagues [14] studied gender differences in fat metabolism during 90 minutes of treadmill walking at 35% VO\(_2\)\text{max} in six untrained men and six untrained women following an overnight fast. Women had significantly lower RER values compared with men at both 45 minutes and 90 minutes of exercise. Both groups gradually increased the percent of fat metabolized during exercise, with the 90-minute values being 59% for the males and 73% for the females. Froberg & Pedersen [11] matched seven active males and seven active females based on age and physical activity habits from personal interviews. Subjects performed exercise to volitional exhaustion on two separate occasions at both 80 and 90% VO\(_2\)\text{max}. They reported that female subjects exercised for a significantly longer period of time than age- and training-matched male subjects at 80% VO\(_2\)\text{max}. The women also had significantly lower RER values during exercise when compared to the men. These researchers concluded that the greater performance in women was due to a greater
reliance on fat as fuel during exercise and a sparing of muscle glycogen. However, during exercise at 90% VO$_2$ max, the RER between men and women showed no difference. The researchers also concluded that gender differences in substrate utilization appear to be intensity-related.

Friedmann and Kindermann [10] determined gender comparison on the changes in energy metabolism during exhaustive endurance exercise, especially on lipid and carbohydrate metabolism. Twenty-four men (12 untrained and 12 trained) and twenty-four women (12 untrained and 12 trained) were matched on training history and current performance capacity. The results demonstrated that blood glucose levels in untrained women were higher than in untrained men, indicating increased mobilization of FFA from intramuscular fats depots during energy production in untrained women. In contrast, no gender differences in endurance trained subjects were seen in lipid metabolism. They conclude that gender differences in substrate utilization seem to appear in untrained subjects and these differences between genders disappear when the individuals are highly trained. Similarly, Costill et al.[19] tested 12 trained females and 13 trained males during treadmill running at 70% VO$_2$ max matched on VO$_2$ max per kg body weight and training volume. The results showed no gender differences in fat or CHO oxidation, blood FFA, or glycerol. The researchers concluded that there were no differences in
fat metabolism in similarly trained males and females during sub-maximal exercise. However, the results from more recent studies have showed differences in substrate utilization when endurance trained men and women are matched by training status [12, 46].

While there is uncertainty surrounding the mechanism for observed gender differences in substrate utilization, several studies have shown that the female hormone estrogen and progesterone may hold the primary role causing gender difference in substrate utilization [72-74]. Estrogen is a collective term for a group of 18-carbon steroid hormone and the most biologically active estrogen is 17β-estradiol (E₂) [17, 72, 75]. During exercise, E₂ may influence sex differences in substrate utilization [72]. Results from the rats studied have shown that E₂ supplementation influences substrate selection during exercise [76-79]. Administration of E₂ spares muscle and liver glycogen [76, 78], increases free fatty acid (FFA) availability for oxidation during endurance exercise [76, 79], elevated intramyocellular lipid (IMCL) concentration [77], and decrease lactate concentration [76]. Similar finding from human study, Devries et al. [80] investigated the effect of 8 days of E₂ supplementation in eleven men following 90 minutes of cycling at 65% VO₂ max. The data showed that E2 supplementation decreased RER, glucose rate of appearance (Ra) and disappearance (Rd) during moderate exercise. They conclude that E2 supplementation can increase lipid use and decrease carbohydrate
as well as decrease glucose Ra (primarily liver glucose production) and Rd (primarily muscle glucose uptake).

Because the female sex hormones estrogen and progesterone appear to play an important role in substrate utilization, and these hormones vary depending on the phase of the menstrual cycle [47], it seem important to closely monitor for menstrual cycle phase in studies involving women and exercise. Hackney et al.[47] determined the substrate metabolism responses of eumenorrheic women to different intensities of submaximal exercise at the midfollicular (MF) and the midluteal (ML) phases of the menstrual cycle in nine women. The subjects performed 30 minutes of treadmill running at 35%, 60%, and 75% VO$_2$ max. The results showed that CHO oxidation was decreased and fat oxidation was increased during the luteal phase compare to the follicular phase at 35%, and 60% VO$_2$ max but at 75% VO$_2$ max, CHO oxidation and fat oxidation were not significantly different in the luteal phases and follicular phases. They concluded that the phase of the menstrual cycle does influence metabolic substrate usage during low- to moderate-intensity submaximal exercise. In contrast, Horto et al.[81] investigated substrate oxidation, glucose kinetics, and the hormone response to moderate intensity of exercise at 90 minutes duration across three phases of the menstrual cycle (early follicular [EF], mid-follicular [MF], and mid-luteal [ML]) in 13 moderately active, normal weight healthy females who experienced regular menstrual cycle. The subjects
exercise for 90 minutes on a cycle ergometer at 50\% \text{VO}_2_{\text{max}}. The results from their study showed that substrate utilization did not vary significantly across the menstrual cycle in active women, either at rest or during 90 minutes of moderate intensity of exercise. We can conclude that the substrate utilization did not differ across the phase of the menstrual cycle when the subjects exercise at high intensity (> 70\% \text{VO}_2_{\text{max}}) or long duration.

The evidence is unclear as to whether there are gender differences in substrate utilization. Some studies show that women oxidized more fat and less CHO compared with men [11, 12, 14, 46]. Others show no difference in substrate utilization between genders [10, 19]. It appears that studies with contrary findings were not well matched for important variables. For example, the results from two studies have shown differences in substrate utilization when endurance-trained men and women were matched by training status [12, 19]. In one study, both males and females were well trained (ran 80-115 km/week) and did not mention whether the females were eumenorrheic or amenorrheic or at point of their menstrual cycle testing commenced. Therefore, the factors that should be considered when comparing genders' differences in substrate utilization are training level, and menstrual cycle phase.
Training factor

It is well documented that substrate utilization is altered based on an individual’s training status. The effects of endurance training result in adaptive change in muscle metabolic function characterized by a decrease in CHO and an increase in lipid oxidation when tested at the same absolute exercise intensity [17]. Several factors may be responsible for the stimulation of fat oxidation in endurance-trained people.

An increase in the number and size of mitochondria per unit area can enhance the capacity to oxidize fat. Mitochondria from trained muscle subject have an increased capacity to produce ATP aerobically by oxidative phosphorylation than in untrained subjects [4]. Data from Keisseling and colleagues show that there was a 120% increase in the number of mitochondria in the vastus lateralis muscle of humans following a 28-week endurance-training program [82]. Similarly, Gollnick et al [83] demonstrated an increase in size and number of mitochondria and suggested it provided a greater surface area for transport of fatty acids and pyruvate into mitochondria. An animal study using a rat showed that mitochondria content was increased by 15%, while the size of mitochondria increase by 35% during 27 weeks of endurance training [84].

After endurance training, the increased utilization of fat as an energy source during submaximal exercise may be attributed to the adaptive increase in mitochondrial enzyme required for fatty acid oxidation involved in the Krebs cycle and the electron
transport chain, and the enzymes responsible for the activation, transport, and β-oxidation of free fatty acids [57]. The increase in these enzymes allows for a more efficient metabolic system for oxidizing nutrients to form energy ATP. Also, the greater concentration of the oxidative enzymes is thought to spare muscle glycogen and reduce the production of lactate during exercise [4]. In Holloszy and Coyles’ research [4], within untrained people, the concentration of mitochondrial enzymes appears to be twice as high in type I (slow-twitch) fibers than in type II (fast-twitch) fibers. Succinate dehydrogenase (SDH) and citrate synthase are the Krebs cycle enzymes that are often measured as indicators of improvement in the oxidative potential of muscle following endurance training. When rats were trained on treadmill running daily, a twofold increase in these enzymes has been reported after endurance training [44]. Similar findings are reported in human studies, Green et al [85] found that the activity of SDH increased by 31% after 4 weeks of training.

Increase in the capacity of trained muscle to oxidize fat may occur via the increase capillary density in muscle, allowing a greater surface area for FFA uptake from blood, and an increase in the activity of lipid-mobilization and lipid-metabolizing enzymes. These increases appears to occur within several weeks or months after the onset of an endurance training program [86]. Endurance trained men have been shown to have a 5-10% greater capillary density than untrained men [87, 88]. Similar findings from
other studies reported an even larger disparity in capillary density (37%-50% differences) between trained subjects and untrained subjects [89, 90].

Numerous studies have demonstrated that endurance training increases fat oxidation and decreases CHO oxidation during exercise [6, 8, 9]. Carter et al. [17] investigated the effect of endurance training on substrate utilization during 90 minutes of exercise at 60% VO$_{2\text{max}}$ in males and females. They concluded that there was a decrease in total CHO utilization during exercise after endurance training at the same absolute workload. These results are similar to other reports, where a decrease in CHO utilization and increase in fat utilization during exercise at the same absolute workload after endurance training was found [5, 7]. From these studies, it is clear that training status is an important factor that must be accounted for when studying the substrate utilization.

**Calorimetry**

The ultimate goal of nutrient metabolism is to produce energy [91]. Energy metabolism can be defined as the rate of heat production, and heat is a convenient form of energy to handle or measure. We can estimate the metabolic rate or energy production of the body by measuring the amount of heat it produces. The process of measuring this heat release is termed calorimetry (from calorie, meaning heat) [92]. Therefore, calorimetry is the measurement of the heat energy liberated or absorbed in metabolism [93]. Calorimetry may be direct or indirect; direct calorimetry measures heat production
from the body; Indirect calorimetry calculates heat production from other measurements. Indirect calorimetry is divided into closed-circuit indirect calorimetry, which involves the recirculation of inhaled and exhaled air in a close system. Open-circuit indirect calorimetry involves the inhalation of atmospheric air and measurement and analysis of exhaled air [36]. Direct measurement of heat production in humans has limited applications. Direct calorimetry is also expensive, time-consuming, and requires engineering expertise. Compared with direct calorimetry, indirect calorimetry remains simpler and less expensive to maintain and staff [94], and is more feasible in exercising circumstances.

**Indirect calorimetry**

Indirect calorimetry is the method by which metabolism is estimated from measurements to determine oxygen consumption (VO\(_2\)) and carbon dioxide production (VCO\(_2\)) [91]. Indirect calorimetry differs from direct calorimetry in that the indirect method determines how much oxygen is required for biological combustion, whereas the direct method measures heat produced as a result of metabolism [95].

Open-circuit spirometry provides a relatively simple way to measure oxygen consumption. During the experiment, subjects are not permitted to re-breathe their own air. Instead, the subjects inhale ambient room air which has a constant
composition of 20.93% oxygen, 0.03% carbon dioxide, and 79.04% nitrogen [92, 96]. Because the body uses oxygen and produces carbon dioxide, subjects will exhale a reduced percentage of oxygen and a higher percentage of carbon dioxide than they inhale. If the volume and percentage of oxygen and carbon dioxide inhaled and exhaled is measured, absolute values of oxygen uptake and carbon dioxide output can be assessed [91]. The resulting values for oxygen uptake and carbon dioxide output will be calculate the respiratory quotient (RQ), RER, and finally substrate utilization.

Validity and reliability of indirect calorimetry

The most basic of these techniques to collect and analyze expired gas is the Douglas bag (DB) method, which has been in use for many years. Although this method is still considered to be the gold standard, it also has several disadvantages and its own limitations. Crouter et al. [97] mention these limitations: (1) the DB bag material made from PVC is slightly permeable to external air, (2) difficulty removing all of the air from the bag and air leaking out during the removal process, (3) the DB contents represent the entire sampling period, and (4) the DB method is time consuming and requires careful analysis by researchers to reduce errors while measuring the content of the bag.
Over the past two decades, with increasing technological advances in computerized systems, researchers most frequently utilize computerized metabolic systems. These systems have made gas-exchange measurements easier and less time consuming [97]. The accuracy and validity of computerized metabolic systems have been established. One computerized metabolic system for laboratory use is the ParvoMedics TrueOne 2400 (TrueOne 2400) previously called the ParvoMedics TrueMax 2400 (TrueMax 2400). The TrueOne 2400 uses a mixing chamber and is a non breath-by-breath system. It has been shown to be an accurate device for the measurement of inspiratory and expiratory gas-exchange [98].

Bassett et. al. [98] used the Douglas bag method and Truemax 2400 (ParvoMedics) to assess the validity of inspiratory and expiratory methods of measuring gas exchange. Eight male subjects participated in the research. Gas exchange was measured at rest and during five work rates on a cycle ergometer. The results showed extremely small differences and close agreement across all variables with the Truemax 2400 compared with the Douglas bag method. Similarly, Crouter et al.[97] used Douglas bag, TrueOne 2400, and MedGraphics VO2000 (VO2000) to assess the accuracy and reliability of the measurement of gas exchange. Ten healthy males had their gas-exchange measurements taken at rest and during cycling at 50, 100, 150, 200, and 250 W, with each stage lasting 10 to 20 minutes. The test was performed on two separate days.
The results showed similar reliability between days among devices, however, for \(\text{VO}_2\) and \(\text{VCO}_2\) the TrueOne 2400 were shown to be more reliable compared to the V2000. The TrueOne 2400 was not significantly different from the Douglas bag at rest or any work rate for \(V_E\), \(\text{VO}_2\), or \(\text{VCO}_2\). Thus, the reliability and validity of the TrueOne 2400 is similar to DB

**Summary**

From the literature review, it can be concluded that substrate utilization during exercise is greatly affected by age, gender, and training-status factors. For the aging effect, fat oxidation is decreased and CHO oxidation is increased during exercise in older individuals. The change in substrate utilization during exercise in aging may be caused by an age-related decline in skeletal muscle respiratory capacity. For the gender effect, women tend to utilize more fat and less CHO than men. The female hormone estrogen and progesterone may hold the primary role causing gender difference in substrate utilization. For the training status effect, endurance training causes a decrease in CHO utilization and increase in fat utilization. An increase in the number and size of mitochondria, and an increase in oxidative enzymes lead to an increase in the capacity of training muscle to oxidize fat during exercise. However, one factor may affect another factor. Therefore, some studies may combine two factors together. They may combine effect of aging and training or they may combine gender and training, for example but
there is no study that combines three factors together. The purpose of this project is to evaluate all three variables in the same study.
APPENDIX B

IRB Approval
NOTIFICATION OF APPROVAL  
January 16, 2012

Principal Investigator: Dr. Anthony Wilcox  
Department: School of Biological & Population Health Sciences

Study Team Members:

Student Researcher: Somnat Onnairi

Study Number: 5141

Study Title: A comparison of substrate utilization during exercise among males and females varying in age and training status

Funding Source: None

Funding Proposal #: 

PI on Grant/Contract: 

Submission Type: Initial Application, received 11/21/2011

Review Category: Expedited  
Category Number: 4

Waiver(s): None  
Number of Participants: 80

Risk level for children*: N/A

The above referenced study was reviewed and approved by the OSU Institutional Review Board (IRB).

Approval Date: 01/09/2012  
Expiration Date: 01/08/2013  
Annual continuing review applications are due at least 30 days prior to expiration date.

Documents included in this review:

- Protocol
- Consent forms
- Assessment forms
- Grant/contract
- Other: 

- Recruiting tools
- Test instruments
- Attachment A: Radiation
- Letters of support
- External IRB approvals
- Translated documents
- Attachment B: Human materials
- Project revision(s)

Principal Investigator responsibilities for fulfilling the requirements of approval:

- All study team members should be kept informed of the status of the research.
- Any changes to the research must be submitted to the IRB for review and approval prior to the activation of the changes.
- Reports of unanticipated problems involving risks to participants or others must be submitted to the IRB within three calendar days.
- Only consent forms with a valid approval stamp may be presented to participants.
- Submit a continuing review application or final report to the IRB for review at least four weeks prior to the expiration date. Failure to submit a continuing review application prior to the expiration date will result in termination of the research, discontinuation of enrolled participants, and the submission of a new application to the IRB.

If you have any questions, please contact the IRB Office at IRB@oregonstate.edu or by phone at (541) 737-8908.

*Where parental permission is to be obtained, the IRB may find that the permission of one parent is sufficient for research to be conducted under §46.404 or §46.405. Where research is covered by §§46.406 and 46.407 and permission is to be obtained from parents, both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.
APPENDIX C

Consent Form
CONSENT FORM

Project Title: A comparison of substrate utilization during exercise among males and females varying in age and training status
Principal Investigator: Dr. Anthony Wilcox
Student Researcher: Sombat Onsiri
Version Date: 12/29/2011

1. WHAT IS THE PURPOSE OF THIS FORM?

This form provides you the information you will need to help you decide whether to take part in our study. You may ask any questions you have about the study, the possible risks and benefits, your rights as a volunteer, and anything else about your participation. When all of your questions have been answered, you can decide if you want to participate in this study.

2. WHY IS THIS STUDY BEING DONE?

The purpose of this study is to compare fat and carbohydrate utilization during exercise among males and females varying in age and training status. There is evidence that sex, age, and training status can affect the amount of fat and carbohydrate that is used during exercise. No previous study has compared fat and carbohydrate utilization during moderate exercise among groups that differ in the three factors of sex, age, and training status. This study will serve as the doctoral dissertation research for Sombat Onsiri, one of the student researchers. Up to 80 participants will be invited to take part in this study.

3. WHY AM I BEING INVITED TO TAKE PART IN THIS STUDY?

You are being invited to take part in this study because you are an apparently healthy individual who is either exercise trained or un-trained and either 18-24 or 38-44 years of age.

4. WHAT WILL HAPPEN IF I TAKE PART IN THIS RESEARCH STUDY?

If you decide to take part in this study, we will ask you to participate in tests on the treadmill, either walking if you are untrained or running if you are trained. One test is a ventilatory threshold (VT) determination and the other is a 35-minute session of moderate exercise.
“Ventilation” is another word for “breathing”, and the ventilatory threshold represents the point during moderate exercise at which your breathing increases at a slightly faster rate than it had at the lower exercise intensities. You are not breathless, for the VT typically occurs well below your maximal effort (~60% of maximum aerobic capacity).

You are asked to maintain your current activity level during your participation in this study.

This visit: 1 hour [In the Human Performance Lab in the Women’s Building room 19 (WB19)]

You will read this informed consent document and have the study explained to you. If you are willing to participate, you will then sign the informed consent document and complete the health history questionnaire. The health history questionnaire will be used to determine your health status for exercising, from which you will be classified as being at low, moderate or high cardiovascular disease (CVD) risk. Those at low and moderate CVD risk may participate in the study, if they also meet the age and fitness-level criteria for participation.

After completing the questionnaire, you will have a treadmill test to indentify your ventilatory threshold. For this test, you will wear comfortable activity clothing and walking or running shoes. The ventilatory threshold (VT) test begins at a slow walk (2.5 mph) for those who are untrained or a slow jog (4.5 mph) for those who are trained. After a 3-minute warm-up, the treadmill speed will be increased by 0.5 mph per minute until you achieve a moderate exercise intensity. With each increase in treadmill speed, your breathing volume and oxygen consumption will increase. The VT becomes evident when a change in speed produces a slightly greater increase in breathing volume than the increase in oxygen consumption. Research has found that the VT occurs at approximately 60% of the maximal aerobic capacity of trained and untrained people, which is a moderate level of exercise intensity. To measure breathing volume and oxygen consumption, you will breathe through a mouthpiece while wearing a nose-clip. Counting the 3-minute warm-up, the VT test typically takes 7-8 minutes.

Visit 2. Body composition determination and sub-maximal exercise test (1 hour)
Within one week after the VT test for male subjects or during their next mid-follicular menstrual-cycle phase (days 1-7 of the menstrual cycle) for female subjects, you will return to WB19 for your second test session. This testing session will be in the morning following a fast of at least 10 hours. You will be asked to refrain from strenuous exercise during the previous 24 hours.

First, your body composition will be measured using the BODPOD (air displacement) method. For the BODPOD procedure, you will be asked to wear a stretch-fitting swimsuit or stretch exercise shorts and sports bra (females). Your height and weight will be measured, and then you will be asked to sit inside the BODPOD and remain still while breathing normally. The BODPOD calculates changes in chamber volume to assess your body volume, from which body composition (percent body fat) is determined. This measurement will be taken 2-3 times, with each measurement taking 45 seconds. The body composition test procedure takes about 10 minutes. Body composition is being measured because the difference in percent body fat between men and women is thought to influence differences in fat and carbohydrate utilization between the sexes.

Second, following the BODPOD test, you will change into comfortable activity clothing and walking or running shoes. You will exercise by walking or running on the treadmill for a 35-minute bout of moderate exercise at a speed that is at 90% of your VT (0.5 mph slower than the speed that elicited your VT in your VT test), which will be a walking pace for untrained subjects and a jogging pace for trained subjects. To measure oxygen consumption during this test, you will be breathing through a mouthpiece while wearing a nose-clip.

Study Results: when the study is over, we will send you a report of your body composition, ventilatory threshold, and fat and carbohydrate utilization during moderate exercise.

5. WHAT ARE THE RISKS AND POSSIBLE DISCOMFORTS OF THIS STUDY?

The ventilatory-threshold test and the 35-minute exercise session are considered to be low risk because you are at a moderate exercise intensity (approximately 60% of VO2 max) or lower, which is considered to be well within your capacity because you have been determined through screening to be at low or moderate CVD risk. In addition, the tests will be administered by a person trained in CPR.
The BODPOD test of percent body fat presents no risk of injury because it measures changes in chamber volume while you sit quietly in the chamber for 2-to-3 45-second intervals. You will be relaxed and breathing quietly. Because the BOD POD is an enclosed chamber, it is possible that those susceptible to feeling claustrophobic might experience stress during the test. The chamber has a window, and it has a quick-release button for opening the door if distress is felt during one of the 45-second testing intervals. If you are not comfortable being in the chamber, you can decline to be tested and still participate in the metabolic tests.

Oregon State University has no program to pay for research-related injuries. If you think that you have been injured as a result of being in this study, please notify the Principal Investigator immediately.

6. WHAT ARE THE BENEFITS OF THIS STUDY?

This study is not designed to benefit you directly. The findings from this study will help in the understanding the influence of sex, age, and fitness level on fat metabolism during exercise.

You will receive personal information regarding your body composition (percent body fat) and ventilatory threshold as a result of participating in this study. Also, you will receive a laboratory measure of the calories from fat and carbohydrate you expend during 35 minutes of moderate exercise.

7. WILL I BE PAID FOR BEING IN THIS STUDY?

You will not be paid for being in this research study.

8. WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

You will not be charged for any tests that are being performed for the purposes of this study. You will be responsible for your transportation costs. If needed, we will provide a parking permit for your laboratory sessions.

9. WHO WILL SEE THE INFORMATION I GIVE?

The information you provide during this research study will be kept confidential to the extent permitted by law. To help protect your confidentiality, we will code all data using a
participant number as an identifier. All information will be secured in a locked file cabinet within an office.

10. WHAT OTHER CHOICES DO I HAVE IF I DO NOT TAKE PART IN THIS STUDY?

Participation in this study is voluntary. If you decide to participate, you are free to withdraw at any time without penalty. You will not be treated differently if you decide to stop taking part in the study. If you choose to withdraw from this project before it ends, the researchers may keep information collected about you, and this information may be included in study reports.

11. WHO DO I CONTACT IF I HAVE QUESTIONS?

If you have any questions about this research project, please contact:

<table>
<thead>
<tr>
<th>Name</th>
<th>Phone number</th>
<th>Email address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Anthony Wilcox</td>
<td>541-737-6799</td>
<td><a href="mailto:Anthony.wilcox@oregonstate.edu">Anthony.wilcox@oregonstate.edu</a></td>
</tr>
<tr>
<td>Principle Investigator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sombat Onsitiri</td>
<td>541-740-4552</td>
<td><a href="mailto:onsiris@cs.oregonstate.edu">onsiris@cs.oregonstate.edu</a></td>
</tr>
<tr>
<td>Student Investigator</td>
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If you have questions about your rights or welfare as a participant, please contact the Oregon State University Institutional Review Board (IRB) Office, at (541) 737-8008 or by email at IRB@oregonstate.edu

12. WHAT DOES MY SIGNATURE ON THIS CONSENT FORM MEAN?

Your signature indicates that this study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

Participant’s Name (printed): ________________________________

(Signature of Participant) __________________________ (Date) ____________

(Signature of Person Obtaining Consent) ___________________ (Date) ____________
APPENDIX D

IRB Protocol
RESEARCH PROTOCOL

October 20, 2011

1. Protocol Title: A comparison of substrate utilization during exercise among males and females varying in age and training status.

PERSONNEL

2. Principal Investigator  Dr. Anthony Wilcox
3. Student Researcher(s)  Scobat Onsiri
4. Co-investigator(s)
5. Study Staff
6. Investigator Qualifications:
   Extensive experience conducting studies of exercise metabolism in trained and untrained healthy individuals and in use of the BOD POD. Has CPR certification.
7. Student Training and Oversight:
   Has completed coursework and laboratory training; has had hours of training in use of the metabolic and BOD POD equipment and in administering the tests involved in this study. Has CPR certification.

FUNDING

8. Sources of Support for this project (pending or awarded)
   None.

DESCRIPTION OF RESEARCH

9. Description of Research:
   Research has demonstrated that fat and carbohydrate (CHO) utilization during exercise are affected by a person’s age, sex, and training status. The interactive effects of these three factors are not understood, and no single study has evaluated all three factors. Therefore, the aim of this study is to compare fat and CHO utilization during exercise among males and females varying in age and training status. The outcome from this study will be used to complete a doctoral dissertation and for future publication.
10. Background Justification:
Substrate utilization during exercise is affected by age, sex, and training status. Previous studies have demonstrated that young individuals may use more fat and less carbohydrate than elderly individuals at comparable exercise intensities. Women tend to utilize more fat and less carbohydrate than men. Endurance training causes a decrease in carbohydrate utilization and an increase in fat utilization at a fixed workload (same percentage of maximal oxygen consumption (VO\textsubscript{2max})). Several studies have compared sex differences in substrate utilization by using subjects who are trained, and other studies have compared differences in substrate utilization by using subjects of different ages. No studies have been performed looking at all three factors of age, sex, and training status on substrate utilization during exercise. Therefore, our objective is to compare substrate utilization during exercise among males and females varying in age and training status.

11. Multi-center Study:
None

12. External Research or Recruitment Site(s)
None

13. Subject Population
Eighty healthy males and females will participate in this study and will be categorized on the basis of age, sex, and training status into the following eight groups of ten subjects:

- Young male subjects who are 18-24 years old and trained.
- Young male subjects who are 18-24 years old and untrained.
- Young female subjects who are 18-24 years old and trained.
- Young female subjects who are 18-24 years old and untrained.
- Older male subjects who are 38-44 years old and trained.
- Older male subjects who are 38-44 years old and untrained.
- Older female subjects who are 38-44 years old and trained.
- Older female subjects who are 38-44 years old and untrained.
A 20-year age differential between the younger and older groups was chosen to provide a substantial age difference while also using older subjects for whom age is not a CVD risk factor.

All four groups of untrained subjects may be engaged in normal daily light-intensity activities, such as shopping, driving, and walking, but none will be participating in regular aerobic exercise, such as jogging, running, or cycling for 30 minutes per day for three days of the week.

All four groups of trained subjects will currently be engaged in regular aerobic exercise at a moderately vigorous to vigorous intensity activity, such as running, cycling, and swimming for at least 30 minutes on five days per week or more for a year or longer.

All female subjects will be eumenorrheic. None will be using an oral contraceptive. None of the subjects, male and female, will be on hormone therapy.

The participants will be recruited via flyers on campus and in physical activity locations, and through announcements in class, to running groups, and other activity associations.

14. Consent Process

The informed consent process will provide participants the information needed to help them decide whether to be in the study or not. The description of the study provided by the researcher will allow participants to learn and ask questions about the study, the possible risks and benefits, and to ask questions about anything else that is not clear. The researcher will ask the participants if they have any questions and if they understand what they would be doing as participants in the study to ensure that they comprehend the informed-consent document. Consent will be obtained through each participant’s reading and subsequent signature on a detailed informed consent form. Participants can stop at any time during the study and still keep the benefits and rights they had before volunteering.

15. Assent Process

None

16. Eligibility Screening:

When potential participants contact the researcher, they will be asked several questions to determine their eligibility (see phone contact questions). They will be asked their age, about their physical activity to determine if they are trained or un-trained, their height and weight to determine if they are obese (BMI ≥ 30), and if they are taking oral contraceptives or on hormone
therapy. Obesity and taking oral contraceptives or hormone therapy are excluding criteria, since they can affect substrate utilization during exercise.

Prior to the sub-maximal exercise test, individuals will complete a health history questionnaire to classify the participant’s cardiovascular disease risk (CVD risk). According to the American College of Sports Medicine (ACSM) guidelines, there are three levels of CVD risk: low, moderate, and high.

- **Low risk**: individuals classified as low risk are those who do not have signs/symptoms of or have diagnosed cardiovascular, pulmonary, and/or metabolic disease and have no more than one (i.e., ≤1) CVD risk factor.
- **Moderate risk**: individuals classified as moderate risk do not have signs/symptoms of or diagnosed cardiovascular, pulmonary, and/or metabolic disease, but have two or more (i.e., ≥2) CVD risk factors.
- **High risk**: individuals classified as high risk are those who have one or more signs/symptoms of or diagnosed cardiovascular, pulmonary, and/or metabolic disease.

**Atherosclerotic Cardiovascular Disease (CVD) Risk Factors**

<table>
<thead>
<tr>
<th>Positive Risk</th>
<th>Factors Defining Criteria</th>
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<tr>
<td>Age</td>
<td>Men ≥45 yr; Women ≥55 yr</td>
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<tr>
<td>Family history</td>
<td>Myocardial infarction, coronary revascularization, or sudden death before 55 yr of age in father or other male first-degree relative, or before 65 yr of age in mother or other female first-degree relative.</td>
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<tr>
<td>Cigarette smoking</td>
<td>Current cigarette smoker or those who quit within the previous 6 months or exposure to environmental tobacco smoke.</td>
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<tr>
<td>Sedentary lifestyle</td>
<td>Not participating in at least 30 min of moderate intensity (40%-60% of VO₂max) physical activity on at least three days of the week for at least three months.</td>
</tr>
<tr>
<td>Obesity</td>
<td>Body mass index ≥30 kg·m²</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Systolic blood pressure ≥140 mm Hg or diastolic ≥90 mm Hg.</td>
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<tr>
<td>Dyslipidemia</td>
<td>Total serum cholesterol ≥200 mg·dL.</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Fasting plasma glucose ≥100 mg·dL.</td>
</tr>
</tbody>
</table>

If participants demonstrate that they are at high CVD risk, they will not be permitted to participate in the study. Subjects who are at low or moderate CVD risk will be permitted to participate in the study. The exercise test in this study is at a moderate intensity, and the *ACSM Guidelines for Exercise Testing and Prescription* state that prior physician approval is not necessary for people at low or moderate risk to perform moderate exercise (8th ed., p. 32).
17. Methods and Procedures

The participants will come to the Human Performance Lab at the OSU Women’s Building room 19 (WB19) on two separate days for: 1) orientation, screening, and ventilatory threshold (VT) determination, 2) body composition determination and sub-maximal exercise test.

Visit 1. Orientation, screening, and ventilatory threshold determination (1 hour)
Prospective subjects will report to the Human Performance Lab (WB19). The subjects will receive the informed consent document and have the study explained to them. If they are willing to participate, they will then sign the informed consent document and complete the health history questionnaire. The health history questionnaire will be used to determine their health status for exercising, and based upon this, they will be classified as being at low, moderate or high CVD risk. As indicated above, those at low and moderate CVD risk may participate in the study.

Subjects meeting the screening criteria will complete a progressive test on the treadmill to identify the ventilatory threshold (VT).

- Trained subjects will begin at slow jog (4.5 mph) on level treadmill while wearing a nose-clips and breathing through a mouth-piece that will allow them to inhale room air and which will direct exhaled air to gas analyzers. Following a 3-minute warm-up, the running speed will be increased 0.5 mph per minute until the subjects have achieved their VT (see below).

- Untrained subjects will walk at slow walk (2.5 mph) on a level treadmill wearing a nose-clips and breathing through a mouth-piece that will allow them to inhale room air and which will direct exhaled air to gas analyzers. After a 3-minute warm-up, the walking speed will be increased 0.5 mph until subjects have achieved their VT (see below).

The signs of VT are when ventilation (L/min) increases out of proportion to the increase in oxygen consumption, and when the respiratory exchange ratio (carbon dioxide production divided by oxygen consumption) reaches or approaches 1.0. Research has shown that the VT occurs at a workload that is approximately 60% of maximal oxygen consumption in both trained and untrained subjects.

Visit 2. Body composition determination and sub-maximal exercise test (1 hour)
Within one week after the VT test for male subjects or during their next mid-follicular menstrual-cycle phase (days 1-7 of the menstrual cycle) for female subjects, subjects will return
to WB19 for their second test session. This testing session will be in the morning following a fast of at least 10 hours. Subjects will be asked to refrain from strenuous exercise during the previous 24 hours.

First, their body composition will be measured using the BODPOD (air displacement) method. For the BODPOD procedure, subjects will be asked to wear a stretch-fitting swimsuit or stretch exercise shorts and sports bra (females). Their height and weight will be measured, and then subjects will be asked to sit inside the BODPOD and remain still while breathing normally. The BODPOD calculates changes in chamber volume to assess their body volume, from which body composition (percent body fat) is determined. This measurement will be taken 2-3 times, with each measurement taking 45 seconds. The body composition test procedure takes about 10 minutes. Body composition is being measured because the difference in percent body fat between men and women is thought to influence differences in fat and carbohydrate utilization between the sexes.

Second, following the BODPOD test, subjects will change into comfortable activity clothing and walking or running shoes. Subjects will exercise by walking or running on the treadmill for a 35-minute bout of moderate exercise at a speed that is 90% of their VT (0.5 mph slower than the speed that elicited their VT in their VT test), which will be a walking pace for untrained subjects and a jogging pace for trained subjects. To measure oxygen consumption during this test, subjects will be breathing through a mouthpiece while wearing a nose-clip.

18. Compensation:

There will be no compensation provided for participation. Participants will be asked to provide their own transportation, however OSU parking passes will be provided, if needed.

19. Cost:

Travel to and from the OSU campus and their time commitment.

20. Drugs, Biologics, Supplements, or Devices

None

21. Biological Samples

None
22. Anonymity or Confidentiality

The name of the participants will be available only to the researcher. Any and all references to participants in any written or oral communications will not include the names or any other identifiable traits, etc. of the participants. Participants’ data will only be identified with an assigned alphanumeric code, and the researcher will maintain a separate list of the study participants’ code numbers. The list of participant code numbers will be kept for two years beyond the time of publication of the results of this study. This code will be assigned sequentially, with the first participant receiving code 5001, the second receiving 5002, and so on. The informed consent documents will be secured in a locked cabinet separate from the study data. All data from the study will be secured in a locked file cabinet within the researcher’s office.

23. Risks

The ventilatory-threshold test and the 35-minute exercise session are considered to be low risk because they are at moderate exercise intensity (approximately 60% of VO\textsubscript{2max}), and participants have been screened to be at low or moderate risk (with 2 or fewer risk factors; see #16 above). According to the ACSM Guidelines for Exercise Testing and Prescription, this is “...an intensity well within the individual’s capacity, one which can be comfortably sustained for a prolonged period of time (~45 minutes)” (p. 32). In addition, the tests will be administered by a person trained in CPR.

The HOPPOD test of percent body fat presents no risk because it measures changes in chamber volume while the subjects sit quietly in the chamber for 2-to-3 45-second intervals. Subjects will be relaxed and breathing quietly. It is possible that the test would present a stress to those who are claustrophobic, since the chamber is an enclosed space. It does have a window and a quick-release button for a quick exit if one were to experience distress. The researchers have never had a person experience panic or distress in the hundreds of tests that they have conducted. If a participant were to decline to take the test or wish to discontinue it once underway, that would not present a problem for them to continue in the metabolic testing part of the study. The body composition data is of interest but not essential for the project.

In the event of a research-related injury, compensation for medical treatment is not provided by Oregon State University or the researcher.
24. Benefits:

Participants will receive information on their body composition, ventilatory threshold, and fat and carbohydrate utilization during exercise as a result of participating in this study.

25. Assessment of Risk-Benefit ratio:

The risks to the participants are considered low, and steps will be taken to minimize any risk during the study. By participating in this study, the participant will obtain information regarding their body composition, aerobic fitness, and substrate utilization during exercise.
Health History Questionnaire

Study Participant #: __________ Date: __/__/__
Age: __________
Weight ________ lbs. Height ________ ft/______ inches

The purpose of this questionnaire is to obtain information regarding your health prior to conducting physiological testing. Please answer all questions to the best of your knowledge. Circle the correct answers.

1. Do you currently smoke? YES NO
2. Are you a former smoker? YES NO
   If so, when did you quit?
3. Are you consistently exposed to second-hand smoke? YES NO
4. Do you have high blood pressure? (Systolic ≥ 140 mm Hg or diastolic ≥ 90 mm Hg) YES NO
5. Do you have high blood cholesterol? (Total serum cholesterol ≥ 200 mg/dL) YES NO
6. Do you currently have any muscle or joint pain? YES NO
   If yes, is this pain mild, moderate, or severe? MILD MODERATE SEVERE
7. Have you ever had a heart attack? YES NO
8. Have you ever had chest pain (angina)? YES NO
9. Have any of your close blood relatives had a heart attack or heart surgery or angina before age 55 (father or brother) or age 65 (mother or sister)? YES NO
   If so, what is the relation? __________ What did they have?
10. Are you diabetic? YES NO
    If so, list medications taken.
11. Have you had any recent illness, hospitalization, or surgical procedures? YES NO
    If so, list them and when__________________________
12. Are you currently taking any medications including oral contraceptive or hormone therapy? YES NO
    If so, list them. ________________________________________________
13. Female: Do you experience regular menstrual cycles (at least 10 per year)? YES NO
14. Do you participate in at least 30 min of moderate intensity (or higher) physical activity on at least 3 days per week for at least the last three months?  

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
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If yes, how many days per week do you exercise at least 30 min?  

If yes, have you done this exercise for over one year?  

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
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**Physical activity history Questionnaire**

During a typical 7-Day period (a week), how many times on the average do you do the following kinds of exercise for more than 15 minutes during your free time (write on each line the appropriate number).

**Times per week**

a) **STRENuous exercise (HEART BEATS RAPIDLY)**

Circle activity you are engaging in, or write in the blank space:

- Running
- Jogging
- Hockey
- Football
- Soccer
- Squash
- Basketball
- Cross country skiing
- Judo
- Roller skating
- Vigorous swimming
- Vigorous long-distance bicycling
- Other

b) **MODerate exercise (NOT EXHAUSTING)**

Circle activity you are engaging in, or write in the blank space:

- Fast walking
- Baseball
- Tennis
- Easy bicycling
- Volleyball
- Badminton
- Easy swimming
- Alpine skiing
- Poplar and folk dancing
- Other

c) **MILD exercise (MINIMAL EFFORT)**

Circle activity you are engaging in, or write in the blank space:

- Yoga
- Archery
- Fishing from river bank
- Bowling
- Horseshoes
- Golf
- Snow-mobiling
- Easy walking
- Other

**During a typical 7-Day period (a week), in your leisure time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?**

- OFTEN
- SOMETIMES
- NEVER/RARELY
Phone contact

Hello, I am Sombat Onsiri and I am calling because of your interest in the exercise metabolism study.

Briefly, the study we are conducting will evaluate the proportion of calories from fat and carbohydrate that are expended as your walk or jog for 35 minutes on a treadmill. If you are trained in regular physical activity, you will jog on the treadmill, and if you are not trained in regular physical activity, you will walk. You will also have a treadmill test that will get progressively harder [by increasing the speed of the treadmill while you walk (untrained) or run (trained)]. This will determine your ventilatory threshold (VT), which occurs at a moderate intensity of exercise, so that we can set your 35-minute treadmill walk/jog to be slightly below your VT. The study will also measure your percent body fat. If you are interested in participating in this study, I would like to ask you a few questions before we schedule your first visit, to determine if you meet the study’s criteria for participation:

Subjects screening criteria:

1) How old are you?

   Age __________________ □ 18-24  □ 35-44

2) What is your sex?

   Sex □ Male  □ Female

3) What is your height and weight?

   Height ______________ ft/inches  [to be completed by researcher
   Weight ____________ lbs.  BMI = __________  ]

4) Do you participate in at least 30 min of moderate intensity (or higher) physical activity on at least 3 days per week for at least the last three months?

   □ Yes  □ No

   If they answer “yes”: do you participate in these activities 5 days or more per week and have you done so for at least the last year?

   □ Yes  □ No
5) (males and females) Are you currently on hormone therapy, or (if female) taking an oral contraceptive?

   ☐ Yes      ☐ No

6) Female: are you experiencing regular menstrual cycles (at least 10 per year)?

   ☐ Yes      ☐ No

Based on your answers, you do/ do not qualify for participation.

If not:

I am sorry that you do not qualify for participation because:

   • You age is outside of the age-range we are testing
   • Your BMI exceeds 30, which is the cut-off for our study
   • You do not meet the criteria for either the trained or untrained groups of our study
   • You are taking hormones that exclude you, since they can affect fat and carbohydrate metabolism separate from the effects of exercise / or you are amenorrheic.

If qualified:

I would like to schedule a time for you to come to the lab for your first orientation visit, which will include the test of your ventilatory threshold.

Schedule: time: __________ date: __________ at Womens Building room 19, OSU

For the ventilatory threshold test, please come to the lab having comfortable clothing for exercise and running or walking shoes.

Do you have any question? Thank you again for your interest.

If you need to contact me, my number is 541-740-4552
In-class/Community announcement script

Hello, everyone

My name is Sombat Omsiri. I am currently a Ph.D student under the supervision of Dr. Anthony Wilcox in the Exercise & Sport Science program at OSU. I am conducting a study comparing fat and carbohydrate utilization during exercise among males and females varying in age and training status.

Briefly, the study we are conducting will evaluate the proportion of calories from fat and carbohydrate that are expended as your walk or jog for 35 minutes on a treadmill. If you are trained in regular physical activity, you will jog on the treadmill, and if you are not trained in regular physical activity, you will walk. You will also have a treadmill test that will get progressively harder [by increasing the speed of the treadmill while you walk (untrained) or run (trained)]. This will determine your ventilatory threshold (VT). The ventilatory threshold is that point during exercise when a person’s breathing volume increases at a faster rate than their increases in oxygen consumption. This typically occurs at approximately 60% of a person’s maximal oxygen uptake, so the test will be perceived as a moderate level of exercise intensity. Then, we will set your 35-minute treadmill walk/jog to be at a speed that is slightly below your VT. The study will also measure your percent body fat by air displacement.

If you are interested to participate in this study and learn more about it, please give me your name and your phone number.

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<tr>
<th>Name</th>
<th>Phone Number</th>
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</table>
Find out how much
Fat and Carbohydrate
You expend during exercise:
If you are exercise-trained – exercise at a jogging pace
If you are not exercise-trained – exercise at a walking pace

We Are Recruiting:
Healthy males and females;
Trained or Untrained;
In these age groupings: 18-24 years or 38-44 years

Tests include: Ventilatory Threshold (a sub-maximal test), percent body fat, and 35-minutes of moderate exercise on a treadmill

Involvement entails two 1-hour visits to the Human Performance Lab at OSU

For more information contact:
Sombat Onsiri
E-mail: onsiri2215@yahoo.com
Phone: 541-740-4552

Mr. Onsiri is working with Dr. Anthony Wilcox, School of Biological and Population Health Sciences at OSU.
Participant Name __________________________  Study Participant # __________
Date of Birth ___/___/____

Please provide us with emergency contact information.
Name: __________________________  Home Phone: __________
Email: __________________________
Relation: __________________________  Work Phone: __________
Email: __________________________