Comparison of thermoregulatory responses between men and women immersed in cold water

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Comparison of thermoregulatory responses between men and women immersed in cold water. J Appl Physiol 89: 1403–1411, 2000.—Eleven women (age = 24.4 ± 6.3 yr, mass = 65.0 ± 7.8 kg, height = 167 ± 8 cm, body fatness = 22.4 ± 5.9%, mean ± SD) were immersed to neck level in 18°C water for up to 90 min for comparison of their thermal responses with those of men (n = 14) in a previous similarly conducted protocol. Metabolic rate increased about three times resting levels in men and women, whereas the rate of rectal temperature cooling (ΔTr/Δt) in women (0.47°C/h) was about one-half that in men. With use of all data, ΔTr/Δt correlates with the ratio of body surface area to size and the metabolic rate of shivering correlates inversely to the square root of body fatness. No significant gender differences in total metabolic heat production normalized for body mass or surface area were found among subjects who completed 90 min of immersion (9 women and 7 men). Nor was there a gender difference in the overall percent contribution (~60%) of fat oxidation to total heat production. Blood concentrations of free fatty acids, glycerol, β-hydroxybutyrate, and lactate increased significantly during the 90-min immersion, whereas muscle glycogen sampled from the right quadriceps femoris vastus lateralis decreased (free fatty acids, glycerol, and β-hydroxybutyrate were higher in women). When the subjects were subgrouped according to similar body fatness and 60 min of immersion (6 women and 5 men), no significant gender differences emerged in ΔTr/Δt, energy metabolism, and percent fat oxidation. These findings suggest that no gender adjustments are necessary for prediction models of cold response if body fatness and the body surface area (SA)-to-size ratio (19, 26, 37). If this is the case, then models that factor these variables into account might be valid as gender invariant.

Another important factor for prediction modeling of survival time for cold exposure is substrate utilization. A greater dependence on the contribution of fat oxidation (fatox) to fuel shivering thermogenesis could be advantageous by preserving carbohydrate (CHO) stores and thereby potentially extending shivering endurance. This benefit of CHO sparing has been demonstrated during exercise when the endurance time during cycling at 80% of maximal aerobic power (V02 max) was extended in active women compared with men because of a greater reliance on lipid and, consequently, a lower reliance on muscle glycogen (8). Although shivering thermogenesis occurs at low levels relative to V02 max, it has been proposed that shivering endurance depends not on the absolute intensity of shivering but its value relative to maximal shivering and is glycogen dependent (38). Use of intramuscular glycogen reserves to fuel intensive thermogenic activity has been reported by Martineau and Jacobs (17).

The proportion of specific energy substrates utilized during exercise depends on a number of factors, including exercise intensity and duration, cardiorespiratory function, hormonal components, and diet. Several studies (11, 12, 24, 28, 29) have reported that women utilize more fat than men during exercise at the same relative intensity. More recently, Pettit et al. (23) examined the contributions of fatox and CHO oxidation (CHOox) in eight men and nine women during a 2-h resting exposure to 5°C air. They found that these respective substrates contributed 53 and 47% to total heat production in men compared with 64 and 36% in women (P < 0.05), consistent with the exercise studies. Although expected, it is uncertain whether this relationship also holds for higher metabolic levels observed during shivering in cold water, rather than in cold air.

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Validation of a model prediction of survival time for men and women requires comparative data of thermoregulatory responses and substrate utilization to cold exposure. The prediction of survival time is not necessarily limited to extreme cold strain, since it also involves calculations that encompass conditions from thermoneutrality through lethal hypothermia. Consequently, if differences in thermoregulatory responses between genders are found under conditions of mild cold strain, such as at the beginning of a cold exposure, then these differences might propagate and ultimately impact survival time predictions. Hence, this investigation is focused on experimental conditions that involve a level of cold stress that might be considered moderate during the initial exposure but is potentially lethal in the long term. Furthermore, the aforementioned survival time prediction model (30, 31) assumes that casualties are in a sedentary posture; therefore, this study will also be limited to resting individuals exposed to cold.

The aims of the present study involving cold water immersion are to confirm that the rates of body cooling and metabolic heat production (M) are gender invariant when corrected for anthropometric differences and to determine gender differences in substrate utilization. As a result, this study will explore any adjustments that might be necessary to improve the accuracy of prediction models of cold exposure survival time. More specifically, we followed the experimental protocol developed by Martineau and Jacobs (17, 18) for men to test women. The hypotheses of the present study are as follows: 1) women and men exhibit similar changes in body cooling and metabolism during cold exposure when subject responses are corrected for body fatness (BF) and size, and 2) in women, a higher percentage of M during cold water immersion would be due to fat metabolism than was previously observed for men.

The experimental procedure has been described in detail by Martineau (16) and Martineau and Jacobs (17, 18). The primary differences are that women were used in the present study, and they were immersed in a different water tank for ease of operation, yet the level of water agitation was moderate in both cases. All data cited for men were obtained from the work of Martineau (16). Data on all 14 subjects (17), as well as the rectal temperature ($T_r$) response for a subgroup of 8 subjects (18), have been reported previously.

Subjects. Eleven women completed the study conducted in accordance with a protocol approved by the Human Ethics Committee. Subjects signed an informed consent and underwent a preexperimental assessment, during which height and weight were measured, and $BF$ was estimated (27) after determination of body density by hydrostatic weighing, and $V_{O_2}$ was determined through a standard exercise test to exhaustion on a treadmill. The body SA-to-size ratio will be represented by the SA-to-volume (mass/density) ratio (SA/vol) instead of the conventional measure of SA/mass, since the ratio is intended to reflect the exposure area relative to overall body size.

Protocol. At least 1 wk before the experimental trial, subjects were immersed in 18°C stirred water for 15 min to familiarize them with the laboratory setting and test procedures. On the day of the experimental trial, subjects reported to the laboratory at the same time of day to avoid possible diurnal effects. They were asked to abstain from alcohol for 48 h and to fast for 12 h before the trial and not to exercise within 24 h of the trial; otherwise there were no dietary or mobility restrictions. No attempt was made to standardize the menstrual cycle phase for the cold water immersions. After the subject dressed down into a two-piece bathing suit and self-inserted a rectal probe, she was instrumented with bipolar electrocardiogram skin electrodes and an intravenous catheter. The subject then lay quietly on an open mesh cot in a supine position for 30 min at 23°C air temperature so that her resting metabolic rate could be measured. Then the subject, while remaining in the same position on the cot, was lowered into the water bath at a water temperature that averaged $-$18°C (within 0.2°C) during the immersion. Thereafter, the subject remained in a supine position immersed to the neck level until one of the following criteria was reached: 90 min elapsed, $T_r$ decreased to 35.5°C, or the subject asked to be removed.

Measurements. $T_r$ (measured at 15 cm past the anal sphincter) was measured continuously (Pharmaseal 400 Series, Baxter Healthcare) during the immersion and averaged each minute. Respiratory gases were monitored using a semi-automated metabolic cart system (model OCM-2, Ametek, Pittsburg, PA) during the last 10 min of the thermoneutral rest period before immersion and continuously throughout the immersion, with the exception of a 5-min break for recalibration purposes after 25 min of immersion. During monitoring, the subject was connected to a mouthpiece, breathing valve, and hose assembly, which directed the expired gases to a 5-liter mixing box connected in series to a ventilation module that measured the expired ventilation rate (VMM Ventilation Measurement Module, Interface Associates, Irvine, CA). A sample line directed gases from the mixing box to O$_2$ and CO$_2$ analyzers (models S-3A11 and CD-3A, respectively, Ametek, Applied Electrochemistry, Poli, PA). Commercially available microcomputer-based software (Vista/Turbofit Software, version 3.10, Vacumetrics, Ventura, CA) was used to register the data each minute and to convert the values of $O_2$ consumption ($V_{O_2}$) and $CO_2$ production into g/min units.

Muscle samples were taken from the right quadriceps femoris vastus lateralis by use of the percutaneous needle biopsy technique (1). Skin and the underlying fascia were anesthetized with 3 ml of xylocaine (2% epinephrine) after the area was cleansed with an antiseptic solution. Pre- and postimmersion samples were taken from the same incision (which was closed using a Steri-Strip) at $15$ min before and within 5 min after the immersion, respectively. An elasticized bandage was wrapped around the thigh during the immersion and removed for the postexposure biopsy, and then dry Steri-Strips and a dry elasticized bandage were placed on the leg on completion of the experiment. Muscle tissue samples were freeze-dried for $8$ h. Glycogen was assayed as glucose units after hydrochloric acid hydrolysis with use of a fluorometric enzymatic method (14).

Venous blood samples (10 ml) were scheduled to be drawn before immersion and after 5, 30, 60, and 90 min of immersion from an antecubital vein with use of a 20-gauge 1-in. catheter and heparin lock (10 U/ml). A waterproof dressing (Tegaderm) was placed over the site where the catheter pierced the skin to help stabilize the catheter. Two milliliters of the blood sample were dispensed into heparin-treated tubes for subsequent determinations of glucose, lactate, $\beta$-hydroxybutyrate (beta-OH), hematocrit, and Hb; 4 ml were dispensed into a tube treated with EGTA (90 mg/ml) and glu-
tathione (60 mg/ml) for subsequent determinations of catecholamines; 2 ml were dispensed into an EDTA-treated tube for subsequent determinations of free fatty acids (FFA), glycerol, and insulin; and 2 ml were dispensed into an EDTA-treated tube containing Trasylol for subsequent determination of glucagon. All samples were centrifuged, and the plasma was stored at −70°C until assayed.

Hematocrit was determined by centrifugation (Autocrit Ultra3 Centrifuge, Clay Adams, Parsippany, NJ). Commercially available kits were used to measure concentrations of plasma glucagon (Glucagon RIA kit, Diagnostic Products, Los Angeles, CA), plasma insulin (Pharmacia Insulin RIA 100, Pharmacia, Uppsala, Sweden), and FFA (WAKO NEFA kit, Osaka, Japan). Glucose and Hb were assayed using automated spectrophotometric techniques (Hemocue, Mission Viejo, CA). Plasma samples were analyzed for glycerol concentration after deproteinization (3), lactate, and β-OH (21). Plasma epinephrine and norepinephrine levels were measured using negative ion chemical ionization gas chromatography-mass spectrometry (40). Changes in plasma volume were calculated from the changes in hematocrit and Hb concentration (6).

Calculations. The subject’s body SA (in m²) was estimated using the following formula regressed specifically for women from the data of Jones et al. (13)

\[ SA = 0.205 \cdot Wt^{0.479} \cdot Ht^{0.324} \]  

(1)

where Wt and Ht are the subject’s mass and height in kilograms and meters, respectively. The subject’s volume was determined from the ratio of body mass to density, the latter determined by hydrostatic weighing.

\[ M = (281.65 + 80.65 \cdot RER) \cdot \dot{V}O_2 \]  

(2)

Data collected during the first 5 min of immersion were not used in any related calculations because of the reflex hyperventilation caused by cold water immersion. The energy rates of CHOox and fatox were calculated using the nonprotein RER via the CO₂ production according to Peronnet (21), lactate, and β-OH (21). Plasma epinephrine and norepinephrine levels were measured using negative ion chemical ionization gas chromatography-mass spectrometry (40). Changes in plasma volume were calculated from the changes in hematocrit and Hb concentration (6).

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(2)

Data collected during the first 5 min of immersion were not used in any related calculations because of the reflex hyperventilation caused by cold water immersion. The energy rates of CHOox and fatox were calculated using the nonprotein values of VO₂ and RER. Details on these calculations and justification for use of the nonprotein RER are found in the report of Vallerand et al. (36).

Data analysis. The thermoregulatory variables measured were Tₑₑ and the metabolic responses to cold water immersion. Because subjects were immersed in water up to the neck level, it was assumed that skin temperature and its effect on thermoregulatory responses were not different between the men and women.

The data involving all subjects (All) were analyzed for the duration of immersion (Δt), the rate of change of Tₑₑ over the immersion period (ΔTₑₑ/Δt), the M at the lowest common Tₑₑ attained by all subjects, percent contribution of fatox to total heat production, and the proportionality constant (A) for the prediction of the metabolic rate due to shivering (Mshiv). According to Tikuisis and Giesbrecht (32), Mshiv is inversely proportional to √%BF for given core and mean skin temperatures

\[ M_{shiv} = A \cdot √%BF \]  

(3)

Therefore, A can be estimated from Mshiv/√%BF. The value of Mshiv was determined from the difference between the subject’s metabolic rates measured at the lowest common Tₑₑ and at rest.

Additionally, the data of all subjects who completed 90 min of immersion (subgroup S90) were separately analyzed for ΔTₑₑ/Δt, the total heat production, percent contribution of fatox to total heat production, and muscle glycogen depletion (ΔGly). This discrimination allows a direct comparison of substrate utilization between men and women over the maximum period of immersion.

For comparative purposes, a second subgroup involved subjects of similar body fatness (subgroup SBF) who completed ≥60 min of immersion. Data from this subgroup were specifically selected to allow a direct comparison of results with those of McArdle et al. (19), whose male and female subjects were immersed in 20°C water for 60 min and subgroup according to %BF of 15–18 and 15–21%, respectively (n = 4 in each). Hence, our data analyses involved ΔTₑₑ/Δt and mean VO₂ over 60 min but also included %fatox and muscle ΔGly. Male and female subjects were selected on the basis of whether their %BF was higher or lower than the respective mean values for the All group.

A t-test was applied to test for gender differences within each group at P < 0.05. Statistical analyses of substrate utilization, muscle glycogen content, and blood metabolites were conducted using a mixed ANOVA design with a between-subject variable (gender) and repeated measures for within-subject comparisons. A Huynh-Feldt score of <0.05 was applied for significance testing. Multiple linear regressions were performed on the All data to identify significant parameters, with acceptance at P < 0.05.

RESULTS

Table 1 summarizes the subject characteristics in each group. When data were used from all subjects (group All), gender differences were observed for height and BF. Gender differences were found in all subject characteristics except age in those subjects who were immersed for 90 min (subgroup S90). Body mass, SA, and SA/vol were significantly different between men and women of comparable BF that were immersed for 60 min (subgroup SBF). Whereas SA/vol was significantly different between genders in subgroups S90 and SBF, SA/mass (not shown) was significantly different in all groups.

Figure 1 shows the mean Tₑₑ profile of all the female subjects immersed in the cold water. All but three subjects responded with an initial transient rise in Tₑₑ, and none reached the cutoff criterion of 35.5°C. Two subjects requested early withdrawal after 60 min of immersion. Net decreases in Tₑₑ ranged from 0.27 to 1.47°C. Figure 2 shows the mean M of all female subjects, which increased to ~3.2 times the resting metabolic rate after 30 min of immersion. Also shown in Figs. 1 and 2 are the mean responses of all the male subjects, of whom six reached 35.5°C before 90 min of immersion and one requested early withdrawal (16, 17).

Table 2 summarizes all the subjects’ thermoregulatory responses grouped by gender. The only significant gender difference was in the rate of change of Tₑₑ. No significant differences were found in M when further normalized against lean body mass (LBM). Nor was there any gender difference in the resting metabolic rates (48.5 ± 14.1 and 43.7 ± 6.8 W/m² for men and women, respectively) used to determine Mshiv.
Although the female trials were not controlled for menstrual cycle, the above analysis was repeated with women subgrouped according to their menstrual cycle phase (follicular ($n=4$) and luteal ($n=7$)). There were no differences between these subgroups in any of their physical characteristics or thermoregulatory responses listed in Tables 1 and 2. The only difference was in their immediate preimmersion $T_{re}$ [36.85 ± 0.07°C and 37.12 ± 0.15°C ($P=0.01$)] for women in follicular and luteal phases, respectively, yet this had no impact on their subsequent thermoregulatory responses.

The regression of the pooled data of $\Delta T_{re}/\Delta t$ against the physiological variables listed in Table 1 yielded $\text{SA/vol}$ as the only significant independent variable [$\Delta T_{re}/\Delta t$ ($^\circ\text{C/h}$) = 3.36 – 0.144·$\text{SA/vol}$; $P=0.04$, $r=0.42$]. The regression of $M_{\text{shiv}}$ (by use of data at the lowest measured common value of $T_{re} = 36.8^\circ\text{C}$) yielded $\text{BF}$ as the only significant independent variable [$M_{\text{shiv}}$ (W/m$^2$) = 160.8 – 3.49·%BF; $P=0.00$, $r=0.56$]. The fit of $A$ (see Eq. 3) against the pooled data yielded a closer fit: $M_{\text{shiv}}$ (W/m$^2$) = 371.5/$\%\text{BF}$ (root mean square error = 1,542 vs. 1,757 with use of the above linear regression).

Table 3 summarizes the responses by gender of the subjects who completed 90 min of cold water immersion (subgroup S90). The only significant gender difference was in the absolute total heat production. However, when normalized against total body mass, LBM, or body SA, no significant differences in heat production emerged. If these data were further confined to subjects of similar $\text{BF}$ (by use of the selection criterion in subgroup SBF resulting in 5 subjects/gender group),

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Group-Gender</th>
<th>$n$</th>
<th>Age, yr</th>
<th>Mass, kg</th>
<th>Height, cm</th>
<th>SA, m$^2$</th>
<th>SA/vol, m$^{-1}$</th>
<th>BF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>25.5 ± 4.3</td>
<td>72.3 ± 11.1</td>
<td>176 ± 11*</td>
<td>1.89 ± 0.19</td>
<td>28.3 ± 1.8</td>
<td>10.6 ± 4.4*</td>
</tr>
<tr>
<td></td>
<td>(19–33)</td>
<td>(53.9–90.0)</td>
<td>(165–200)</td>
<td>(1.58–2.19)</td>
<td>(25.1–31.4)</td>
<td>(3.5–19.7)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>24.4 ± 6.3</td>
<td>65.0 ± 7.8</td>
<td>167 ± 8</td>
<td>1.80 ± 0.13</td>
<td>29.1 ± 1.6</td>
<td>22.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>(18–37)</td>
<td>(52.3–80.8)</td>
<td>(151–179)</td>
<td>(1.56–2.03)</td>
<td>(25.6–31.3)</td>
<td>(14.3–35.0)</td>
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<tr>
<td><strong>S90</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>25.9 ± 4.6</td>
<td>78.9 ± 8.4a</td>
<td>179 ± 7*</td>
<td>1.99 ± 0.13a</td>
<td>27.1 ± 1.5*</td>
<td>12.7 ± 5.2a</td>
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<tr>
<td></td>
<td>(19–33)</td>
<td>(66.6–90.0)</td>
<td>(171–191)</td>
<td>(1.80–2.19)</td>
<td>(25.1–28.9)</td>
<td>(3.5–19.7)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>24.0 ± 6.0</td>
<td>66.5 ± 7.3</td>
<td>170 ± 6</td>
<td>1.82 ± 0.11</td>
<td>28.8 ± 1.5</td>
<td>23.0 ± 6.3</td>
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<td></td>
<td>(18–37)</td>
<td>(58.2–80.8)</td>
<td>(160–179)</td>
<td>(1.70–2.03)</td>
<td>(25.6–30.5)</td>
<td>(14.3–35.0)</td>
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<td><strong>SBF</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
<td>25.0 ± 3.9</td>
<td>81.4 ± 8.9a</td>
<td>179 ± 8</td>
<td>2.02 ± 0.14a</td>
<td>26.5 ± 1.4*</td>
<td>15.3 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>(19–29)</td>
<td>(66.6–90.0)</td>
<td>(171–191)</td>
<td>(1.80–2.19)</td>
<td>(25.1–28.9)</td>
<td>(13.3–19.7)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>26.5 ± 6.8</td>
<td>67.1 ± 4.1</td>
<td>171 ± 5</td>
<td>1.84 ± 0.06</td>
<td>29.0 ± 1.0</td>
<td>18.3 ± 3.0</td>
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<tr>
<td></td>
<td>(20–37)</td>
<td>(61.7–70.9)</td>
<td>(165–179)</td>
<td>(1.76–1.91)</td>
<td>(27.7–30.5)</td>
<td>(14.3–21.6)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD, with ranges in parentheses; $n$, no. of subjects. SA, surface area; BF, body fat. All, S90, and SBF refer to groups containing all subjects, subjects that were immersed for 90 min, and subjects of similar BF, respectively. aSignificant gender difference within a group ($P<0.05$).
there were also no differences in any of the heat production variables, including the absolute value.

There was no significant gender difference in substrate utilization in those subjects who completed 90 min of immersion (subgroup S90). To examine whether the pattern of substrate utilization was different over this period of immersion, the relative contributions of %fatox and %CHOox were determined during 30-min intervals. Men showed a significant increase in %fatox (from 54 to 66%) and a corresponding significant decrease in %CHOox (from 46 to 34%) from 30 to 60 min, which leveled off during the last 0.5 h of immersion. Women, on the other hand, showed no significant changes over time (%fatox and %CHOox were 64 and 36%, respectively).

For additional comparisons to other published studies, subgroup S90 was further segregated (subgroup S90Rel) to involve only subjects with similar relative intensities of shivering heat production compared with their V\textsubscript{O2 max}. No significant gender difference in contributions of substrate utilization was found among the subjects in this subgroup [5 women (V\textsubscript{O2 max} = 41.1 ± 6.6 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) and 5 men (47.0 ± 3.9 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}), P > 0.05] whose relative shivering intensities were 31 ± 2 and 29 ± 6% of V\textsubscript{O2 max}, respectively. Yet, the same shift in substrate utilization with time from CHO to fat occurred in the men in subgroups S90Rel (Fig. 3) and S90.

Muscle glycogen samples were obtained from all but two women in subgroup S90. Concentrations decreased significantly between the pre- and postimmersion samples (from 493 ± 132 to 382 ± 76 mmol glucose/kg). Compared with the corresponding decrease in the male sample concentrations (from 405 ± 64 to 312 ± 42 mmol glucose/kg), no main effect of gender was found.

Difficulty was encountered in obtaining blood samples from several subjects during the immersion and was attributed to cold-induced vasoconstriction in the subject's forearm. Consequently, the statistical analysis was limited to comparison of the pre- and postimmersion values. Blood analyses for the men were further limited to FFA, β-OH, glycerol, glucose, and lactate (Table 4). FFA, β-OH, glycerol, and lactate concentrations increased significantly during the immersion in subgroup S90. Gender comparisons in subgroup S90 revealed a greater increase in FFA (119.7 vs. 36.2%), glycerol (255 vs. 54.8%), and glucose (15.9 vs. −3.7%) in women than in men. There was no significant gender difference in the magnitude of plasma volume change (Table 4).

The following results pertain to the four to five women in subgroup S90 from whom pre- and postimmersion blood samples were obtained (Table 4). Although cold water immersion did not affect the levels of β-OH, epinephrine, and glucagon, significant increases were observed in all other metabolite and hormone concentrations (i.e., FFA, glycerol, glucose, insulin, lactate, and norepinephrine). The decrease in plasma volume (19.3 ± 4.7%) may be partly responsible for the increased blood metabolite levels of glucose and norepinephrine. However, the changes in the concentrations of FFA (120%), glycerol (255%), insulin (41%), and lactate (163%) were too large to be attributed only to the change in hemocconcentration.

Table 5 summarizes the responses by gender of the subjects selected to have similar BF and to have completed ≥60 min of immersion (subgroup SBF). The variables were the same as those reported by McArdle et al. (19) for comparative purposes and also include %fatox and muscle ΔGly. No significant gender differences were found in any of these variables. However, the analysis for ΔGly (on the basis of values before and 90 min after immersion) excluded one woman who was immersed for only 60 min. In addition, an analysis of the blood samples taken for all subjects in subgroup SBF before and after 60 min of immersion indicated metabolite concentrations that mirrored those shown in Table 4. Specifically, similar gender differences were found in FFA, glycerol, glucose, and lactate, and a main effect of time was found in the same components except for glucose. These results, however, must be

Table 2. Responses of all subjects during immersion in 18°C water

<table>
<thead>
<tr>
<th>Gender</th>
<th>ΔT, min</th>
<th>ΔT\textsubscript{ax}, °C</th>
<th>ΔT\textsubscript{ax}/Δt, °C/h</th>
<th>M, W/m\textsuperscript{2}</th>
<th>M\textsubscript{shivering}, W/m\textsuperscript{2}</th>
<th>Fat\textsubscript{ox}, %</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>66.1 ± 27.8</td>
<td>0.86 ± 0.34</td>
<td>1.01 ± 0.67*</td>
<td>164 ± 57</td>
<td>115 ± 60</td>
<td>48.7 ± 17.1</td>
<td>342 ± 132</td>
</tr>
<tr>
<td>Female</td>
<td>83.6 ± 11.8</td>
<td>0.65 ± 0.45</td>
<td>0.47 ± 0.32</td>
<td>137 ± 33</td>
<td>94 ± 34</td>
<td>58.6 ± 11.4</td>
<td>425 ± 124</td>
</tr>
</tbody>
</table>

Values are means ± SD. Δt and ΔT\textsubscript{ax} duration of and decrease in rectal temperature during immersion; M and M\textsubscript{shivering} total and shivering component of the metabolic heat production at T\textsubscript{re} = 36.8°C; fat\textsubscript{ox}, contribution of fat oxidation toward total heat production; A, proportionality constant of M\textsubscript{shivering}·√BF (Eq. 3). *Significant gender difference (P < 0.05).

Table 3. Responses of subjects who completed 90 min of immersion in 18°C water

<table>
<thead>
<tr>
<th>Gender</th>
<th>ΔT\textsubscript{ax}/Δt, °C/h</th>
<th>HP</th>
<th></th>
<th>Fat\textsubscript{ox}, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.50 ± 0.29</td>
<td>1,732 ± 407*</td>
<td>22.5 ± 7.1</td>
<td>25.5 ± 6.7</td>
</tr>
<tr>
<td>Female</td>
<td>0.45 ± 0.33</td>
<td>1,257 ± 247</td>
<td>19.0 ± 4.0</td>
<td>24.8 ± 3.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. HP, total heat production; fat\textsubscript{ox}, contribution of fat oxidation toward HP; LBM, lean body mass. *Significant gender difference (P < 0.05).
weighed against the small number (i.e., 2–5) of blood samples involved.

**DISCUSSION**

Although no difference in the rate of shivering heat production between genders emerged in our study, the pooled data indicated a significant correlation between $\dot{M}_{\text{shiv}}$ and BF. That an improvement in fit was further obtained by the attenuation of $\sqrt{\%BF}$ (Eq. 3) confirms the earlier finding of such a dependency (32). Because no difference was found in the proportionality constant $A$ between genders (Eq. 3), it is concluded that the shivering metabolic drive was not different, at least when $T_{\text{ev}} = 36.8^\circ\text{C}$ during immersion to neck level in 18°C water.

Cold exposure has been shown to be a significant stimulus for lipolysis and glycogenolysis (17, 18, 35). These earlier findings are substantiated in the present study by the changes observed in FFA, glycerol, $\beta$-OH, lactate, and muscle glycogen content during cold water immersion. Furthermore, the assessment of $\text{fat}_{\text{ox}}$ and $\text{CHO}_{\text{ox}}$ supports the contention that the increased metabolic demand during cold exposure is fueled by $\text{fat}_{\text{ox}}$ and $\text{CHO}_{\text{ox}}$.

Despite gender differences in body size, BF, total heat production, and the relative intensity of shivering, there were no gender differences in the relative contributions of fat and CHO to fueling metabolism over the 90-min immersion period (Table 3). Similar changes in muscle glycogen concentration during immersion also suggest that men and women relied on CHO to a similar extent during shivering.

The gender difference in plasma FFA, glycerol, and glucose might appear to contradict the absence of a significant gender difference in $\text{fat}_{\text{ox}}$. However, gender differences in these metabolite responses may reflect the greater relative intensity of shivering in women (34 ± 5 and 26 ± 7% $\dot{V}_{\text{O}_2\text{max}}$ for women and men, respectively) rather than an inherent difference in the type of energy substrate that is preferentially oxidized. To support this contention, statistical analysis of the blood metabolites was performed on subjects in subgroup $S90_{\text{Rel}}$ (subjects with similar relative shivering intensities), and only glucose showed a significant gender difference, although blood data were available only for three women and five men in subgroup $S90_{\text{Rel}}$. Furthermore, there was no gender difference in muscle glycogen utilization in subgroup $S90_{\text{Rel}}$.

Yet these results are in contrast to those reported by Pettit et al. (23) involving 5°C air exposure. In that study involving nine women and eight men (mass = 55.6 and 76.6 kg, height = 165 and 178 cm, SA/mass = 0.0290 and 0.0254 m²/kg, and BF = 23.2 and 19.5%, respectively; mass, height, and SA/mass are significantly different), the ratio of $\%\text{fat}_{\text{ox}}$ to $\%\text{CHO}_{\text{ox}}$ was 1.84 for women and only 1.19 for men. In the present study, the ratio was similar for both genders (1.76 and 1.58, respectively, in subgroup $S90$ and 1.41 and 1.30, respectively, in subgroup $S90_{\text{Rel}}$).

**Table 4. Gender response comparisons in blood metabolites of subjects who completed 90 min of immersion**

<table>
<thead>
<tr>
<th>Gender</th>
<th>$n$</th>
<th>FFA,† μM</th>
<th>β-OH, μM</th>
<th>Glycerol,† mM</th>
<th>Glucose,† mM</th>
<th>Lactate, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post*</td>
<td>Pre</td>
<td>Post*</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>553 ± 200</td>
<td>753 ± 285</td>
<td>156 ± 83</td>
<td>237 ± 172</td>
<td>0.093 ± 0.062</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>393 ± 182</td>
<td>683 ± 202</td>
<td>213 ± 132</td>
<td>347 ± 242</td>
<td>0.051 ± 0.012</td>
</tr>
</tbody>
</table>

Values are means ± SD of 7 men and 5 women unless otherwise noted. FFA, free fatty acid; β-OH, β-hydroxybutyrate; PV, plasma volume; Pre and Post, before and after cold water immersion. *Significant gender difference ($P < 0.05$); †main effect of time between Pre and Post. §$n = 6$; ¶$n = 4$. 

![Fig. 3. Heat production due to carbohydrate (CHO) and fat metabolism of men and women who completed 90 min of immersion with similar intensities of shivering compared with their $O_2$ consumption (subgroup $S90_{\text{Rel}}$). Values are means ± SE. Differences between genders are significant. *Significant increase over time among the men ($P < 0.05$).](http://jap.physiology.org/.../ Downloaded from October 15, 2017)
Table 5. Responses of subjects of similar body fatness during immersion in 18°C water

<table>
<thead>
<tr>
<th>Gender</th>
<th>$T_{se}$, °C</th>
<th>$\Delta T_{se}/\Delta t$, °C/h</th>
<th>$V_{O2}$, l/min</th>
<th>$\text{fat}_{\text{ox}}$, %</th>
<th>$\Delta Gly$, mmol glucose/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>37.02 ± 0.30</td>
<td>0.42 ± 0.49</td>
<td>0.85 ± 0.13</td>
<td>65.0 ± 11.8</td>
<td>94.0 ± 32.9</td>
</tr>
<tr>
<td>Female</td>
<td>37.04 ± 0.19</td>
<td>0.31 ± 0.26</td>
<td>0.72 ± 0.15</td>
<td>61.8 ± 15.5</td>
<td>148.1 ± 73.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. $V_{O2}$, mean rate of O2 consumption; $\text{fat}_{\text{ox}}$, mean oxidation contribution of fat over 60 min; $\Delta Gly$, decrease in muscle glycogen concentration in all male and 4 female subjects over 90 min of immersion.

respectively, in subgroup S90Rel). The greater cold stress during the entire 90 min of water immersion at 18°C in contrast to the 2 h of cold air exposure at 5°C might account for the differences in substrate utilization between the two studies. Indeed, Pettit et al. reported a mean increase in $V_{O2}$ of only 80% during 2 h of the cold air exposure compared with a >200% increase in $V_{O2}$ during 90 min of immersion in our study. Total heat production for women and men in subgroup S90Rel was twice that reported by Pettit et al. (1,213 vs. 681 kJ for women and 1,897 vs. 904 kJ for men), although the ratio of total heat production in women to that in men was similar in both studies (0.75 reported by Pettit et al. and 0.73 in the present study). Furthermore, when substrate utilization was analyzed by 30-min intervals, our data indicate that substrate selection in men shifted toward more fat and less CHO with time in subgroups S90 and S90Rel (%fatox-to-%CHOox ratios at the end of the first 30 min of immersion were 1.17 and 0.89, respectively, closer to the value of 1.19 reported by Pettit et al.).

Other explanations for the differences between the two studies may be possible differences in the relative shivering intensities and/or $V_{O2}$max, although neither $V_{O2}$max nor subject training status was reported in Pettit et al. (23). As is the case with exercise, subjects shivering at a higher fraction of their $V_{O2}$max should demonstrate an increased CHOox. Also, there is evidence that gender differences in substrate utilization during exercise may be attenuated by physical training (5, 7, 25). Such findings are not universal, inasmuch as Tarnopolsky et al. (28) reported that well-trained female endurance athletes demonstrated a preference for lipid metabolism during submaximal exercise compared with male athletes.

Our results also contrast with those of Blatchford et al. (2), who studied substrate utilization in six women and six men during a 90-min walk at 35% $V_{O2}$max and reported a greater level of fat utilization in the women. Although cold exposure was not involved, their study was similar to the present investigation in terms of duration and energy expenditure of the subjects. The disparity in %fatox between the two studies raises the question of whether it is valid to expect substrate utilization of exercising muscle to be similar to that of involuntarily shivering muscle when $V_{O2}$ rates are the same as those addressed by Tipton et al. (33).

Aside from the shift in substrate utilization observed in men, the only other difference between responses of men and women related to the rates of deep body cooling. Interestingly, although BF was different between the two genders, it did not emerge as the dominant regressor with regard to the rate of body cooling. Instead, SA/vol (which was not different between the genders) did emerge as the dominant, although weak, regressor. This seemingly anomalous result can be explained through a closer inspection of the individual thermoregulatory responses. The subjects (both genders) with the lowest SA/vol also responded with the lowest $\Delta T_{se}/\Delta t$. In subjects with the highest SA/vol, $\Delta T_{se}/\Delta t$ values were near the highest observed, yet %BF values were near the average in their gender group. On the other hand, in subjects with the lowest %BF, $\Delta T_{se}/\Delta t$ values were the highest, which would mitigate heat debt and body cooling rate. A striking example of this was the male subject having the lowest %BF at 3.5%. This individual (72.0 kg, 187 cm) responded to the water immersion with an overall heat production of 2,466 kJ (or 34.2 kJ/kg, 35.5 kJ/kg LBM, 1,319 kJ/m$^2$), which was markedly higher than the average (Table 3).

Toner et al. (34) provided an alternative explanation regarding enhanced heat loss with an increasing SA/vol. Essentially, they found that differences in heat loss diminished with exercise (vs. during rest) in men immersed in 26°C water. These investigators hypothesized that the increased blood perfusion to the muscles attenuated the insulative value of the larger relative muscle mass of the low SA/vol group. This hypothesis helps explain why differences in the rates of deep body cooling were not observed between the same subjects used by McArdle et al. (19) when they exercised in cold water (20). Regardless of the mechanism involved, SA/vol nevertheless provides a valid index of the susceptibility to heat loss in resting individuals, as previously concluded by McArdle et al. (19).

Graham et al. (10) found no significant differences in $\Delta T_{se}/\Delta t$ and an increase in $V_{O2}$ relative to $\Delta T_{se}$ between men and eumenorrheic women exposed to 5°C air for 60 min. Subject characteristics of mass, SA, and BF were significantly different between the genders. Although not provided, the mean SA/mass values were ~0.0256 and 0.0281 m$^2$/kg for the men and women, respectively. Despite these physical differences, the lack of differences in the subjects’ cold response is consistent with our own study if we consider subgroup S90, where the same physical characteristics cited above were also significantly different between genders.

Few studies have reported the effects of the menstrual cycle on thermoregulation in the cold. In a recent study, Gonzalez and Blanchard (9) exposed six resting women (60.9 kg, 165 cm, 23.9% BF) with cloth-
ing protection to a ramped decrease in air temperature during the follicular and midluteal phases. Their finding of a higher preexposure deep body temperature during the midluteal phase in the lightly clothed trial is consistent with our findings. However, the attenuation in shivering thermogenesis as a function of mean body temperature during the midluteal phase compared with the follicular phase was not evident in our study (through \(M_v\)). It is possible that the greater level of cold stress imposed on our female subjects, in whom shivering thermogenesis was at least twice as high, overwhelmed the effects observed by Gonzalez and Blanchard. In fact, the predictive equations of shivering thermogenesis that these investigators regressed are not applicable to conditions where mean skin temperature is <31°C, as in our study.

The results of the responses of subgroup SBF (Table 5) contrast with those of McArdle et al. (19), who reported considerably higher rates of decrease in \(T_{re}\) in both genders having similar BF in addition to a significantly higher rate of decrease in \(T_{re}\) in their female than in their male subjects (1.6 vs. 1.1°C/h). The higher rate in women was attributed to a larger SA/mass, and the overall rates for both genders were probably the result of the lack of transient rise in \(T_{re}\). The absence of a transient rise seems unusual given that cutaneous vasoconstriction, which normally causes a transient rise in \(T_{re}\) (4), should have occurred despite the slightly warmer water temperature (20°C) used. Indeed, the data reported by Martineau (16) and Young et al. (39) for men immersed to neck level in 18°C stirred water support this expectation. A transient rise in \(T_{re}\) (mean −0.07°C) was observed in the former study in all but one man \(n = 14;\) time to maximum \(T_{re}\) = 31.6 ± 23.2 (SD) min after the start of immersion. Young et al. showed a mean \(n = 7;\) transient increase of −0.4°C after 30 min of immersion. On the other hand, Kollias et al. (15) showed no transient increase in women of average BF \(n = 3,\) 22.2% BF) immersed to neck level in 20°C stirred water, whereas obese women \(n = 7,\) 34.0% BF) showed a transient rise. There is no obvious explanation for these disparities other than subject variability, possible differences in subject preparation before the immersion, and/or different degrees of cold stress due to differences in water temperature and turbulence level. Finally, no difference in the rate of deep body cooling was found between genders among all the subjects who completed 90 min of immersion in our study (subgroup S90), indicating a consistency in the cooling response during the latter stage of the immersion.

Furthermore, the metabolic responses of the SBF subjects were higher than those reported by McArdle et al. (19). \(V_o_2\) rates averaged 0.78 and 0.60 l/min for the respective studies (pooled data). The lower rate for the latter study helps explain the reported higher rate of deep body cooling (average respective values of \(\Delta T_{re}/\Delta t\) were −0.36 and −1.35°C/h). However, these large differences cannot be easily explained by differences in body characteristics (respective average values for our subjects \(n = 11;\) and those of McArdle et al. \(n = 4;\) were as follows: mass = 73.6 and 68.7 kg, height = 175 and 170 cm, SA/mass = 0.0263 and 0.0267 m²/kg, and BF = 16.9 and 17.7%). Gross methodological differences in subject preparation and immersion conditions are not readily apparent between the two studies, aside from the small difference in water temperatures used (18 vs. 20°C). Despite these disparities, including differences in the rate of body cooling, both studies are in agreement that thermoregulatory variations are explainable by anthropometric variations.

The aims of this study were to compare the rates of body cooling and energy metabolism of women and men immersed in cold water and to determine whether gender-related adjustments are necessary for prediction models of cold exposure survival time. On the basis of the above findings, we accept the first hypothesis that women and men exhibit similar changes in body cooling and M during cold water immersion at rest when subject responses are corrected for BF and size. On the other hand, we must reject the hypothesis that women metabolize a higher percentage of fat during cold water immersion lasting 90 min. The consequences of these findings with regard to modeling survival time for sedentary cold exposure is that no adjustments are necessary if BF and SA/VO2 are taken into account. That the %fat,ox was not different between women and men refutes a potential gender advantage with regard to CHO sparing during shivering thermogenesis.

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