Plasma acid-base regulation above and below ventilatory threshold in late gestation

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Heenan, Aaron P., and Larry A. Wolfe. Plasma acid-base regulation above and below ventilatory threshold in late gestation. J. Appl. Physiol. 88: 149–157, 2000.—Stewart's physicochemical approach was used to study the effects of pregnancy on acid-base regulation in arterialized blood. Responses of 15 healthy pregnant women (PG; gestational age, 37.1 ± 0.2 wk) were compared with those of 15 nonpregnant controls (CG) at rest and during cycling at 70 and 110% of the ventilatory threshold (Tvent). Hydrogen ion concentration ([H+]1) was lower in the PG vs. CG at rest and during exercise (P < 0.05 at rest and 70% Tvent). Exercise-induced changes in [H+]1 were similar between groups. Lower resting [H+]1 values in the PG vs. CG resulted from lower values for arterialized PCO2 (Paco2) and total weak acid ([A]tot), which were partly offset by a lower strong-ion difference ([SID]). Reductions in [A]tot and [SID] at rest were primarily the result of reductions in albumin [Alb] and sodium [Na+], respectively. In the transition from rest to 70% Tvent, small increases in PaCO2 and [A]tot contributed to moderate increases in [H+]1 in both groups, however [SID] increased in the PG and decreased in the CG (P < 0.05 between groups). In the transition from rest to 110% Tvent, decreases in [SID] made a significantly greater contribution to changes in [H+]1 in the CG vs. PG. Exercise-induced increases in [H+]1 are similar in the pregnant vs. nonpregnant state, but there is a reduced contribution of [SID] both above and below Tvent during pregnancy.

hydrogen ion; carbon dioxide tension; strong-ion difference; total weak acid

PREGNANCY-INDUCED CHANGES in respiratory control and acid-base regulation have been studied extensively in the resting state. These include increases in minute ventilation (Ve), tidal volume, and alveolar ventilation and a reduction in arterial PCO2 (PaCO2) (1, 17, 25, 30). In accordance with conventional acid-base theory, these changes are accompanied by renal excretion of bicarbonate ([HCO3–]), resulting in a state of partly compensated respiratory alkalosis (arterial pH ≅ 7.43–7.47) (13, 17). These effects appear in the first trimester and may promote placental gas exchange before development of an effective fetal circulatory system (13).

Existing studies of acid-base balance during and after maternal exercise have reported conflicting results. Lehmann and Regnat (12) studied healthy pregnant women at rest and during 6 min of stationary cycling at both 50 and 80 W. They reported greater decreases in blood pH during exercise at both 50 and 80 W in pregnancy compared with measurements at 12 wk postpartum. Significantly lower absolute pH values were also encountered during the exercise at 80 W in pregnancy compared with the nonpregnant state. Conversely, Pivarnik et al. (25) reported higher arterial pH values in pregnant women at 37-wk gestation compared with postpartum during exercise bouts lasting 6 min on a cycle ergometer (50 and 75 W) and on a treadmill (67 m/min, 2.5% grade, and 67 m/min, 12% grade). Similar changes in pH in the transition from rest to exercise were reported under all exercise conditions in both groups, but [HCO3–] values decreased to a lesser extent in the pregnant group.

Lehman and Regnat (12) and Pivarnik et al. (25) employed the conventional Henderson-Hasselbalch approach to the study of acid-base balance in pregnancy and exercise. Unfortunately, this provides limited insight into the mechanisms controlling acid-base balance (6). In contrast, Stewart's physicochemical approach to acid-base analysis (26, 27) allows examination of the specific physicochemical determinants of changes in hydrogen ion concentration ([H+]1) in individual fluid compartments. All variables are defined as independent or dependent, and all systems are assumed to behave in accordance with the principles of electroneutrality, conservation of mass, and dissociation equilibria. If the values for the independent variables (i.e., PCO2, the strong ion difference ([SID]), and total weak acid ([A]tot)) and dissociation constants are known, values for dependent variables ([H+]1, [HCO3–], dissociated weak acid, weak acid, carbonate, and hydroxide) can be determined mathematically. This approach to acid-base analysis has been validated in an animal model (23) and at rest and during recovery from exercise in young men (11, 14).

Only one study has used Stewart's approach to study acid-base regulation in healthy women. Kemp et al. (9) compared the responses of physically active pregnant women (mean gestational age, 33 ± 1 wk) to those of a nonpregnant control group in both the resting state and during recovery from a maximal cycle ergometer test. During pregnancy significantly lower values for [H+]1 in venous plasma in the resting state were the result of lower values for PaCO2 and [A]tot (which would reduce [HCO3–]). This effect was only partly offset by a lower [SID]...
findings suggested that pregnancy-induced changes in postexercise recovery (1–7 min postexercise), and during the transition from rest to peak exercise, during early pregnancy are not affected by human pregnancy. Unfortunately, the value of the study by Kemp et al. (9) to describe the effects of human pregnancy on [H\(^+\)] and its independent determinants was limited by the use of venous plasma in the analyses. The composition of venous blood reflects the metabolic activity of the tissues that it drains, whereas that of arterial blood is consistent throughout the body. In this regard, Kemp et al. (9) studied venous blood drawn from the antecubital vein in the arm, whereas exercise was performed on a leg cycle ergometer. Clearly, the use of arterial (or arterialized) blood would have been more accurate and sensitive for analyzing changes in acid-base regulation induced by pregnancy and exercise.

The purpose of this study was to examine, by using Stewart's physicochemical approach, the effects of human pregnancy on arterialized plasma [H\(^+\)] and its determinants in the resting state, as well as changes in [H\(^+\)] in the transition from rest to both steady-state and non-steady-state exercise. It was hypothesized that the results would confirm the earlier findings of Kemp et al. (9) from venous blood that lower plasma [H\(^+\)] in the resting state is the combined result of lower values for P\(_{\text{CO}_2}\) and [A\(_{\text{tot}}\)] and that this effect is partly offset by a lower [SID]. It was further hypothesized that changes in [H\(^+\)] in the transition from rest to both steady-state and non-steady-state exercise would be similar in the pregnant vs. nonpregnant state and that the contributions of arterialized P\(_{\text{CO}_2}\) (P\(_{\text{CO}_2}\)), [A\(_{\text{tot}}\)], and [SID] to these changes would not differ significantly among groups.

METHODS

Subjects. Subjects were 15 healthy, nonsmoking, physically active pregnant women (pregnant group; PG). Results from the PG were compared with those of 15 healthy nonpregnant women with similar physical and demographic characteristics (control group; CG). Prospective subjects were recruited from local prenatal fitness classes and from the general population via media advertisements, posters, flyers, and contact with local obstetricians. Medical clearance for pregnant subjects was obtained from the physician or midwife monitoring their pregnancy by using a standard form (Pregnancy Activity Readiness Medical Examination for Pregnancy). Nonpregnant subjects completed the revised Physical Activity Readiness Questionnaire.

Written informed consent was obtained from all subjects before entry into the study. The study protocol, described in Exercise testing protocols, and consent form were approved by the Research Ethics Board, Faculty of Medicine, Queen's University, and the US Army Medical Research and Material Command, Human Subjects Protection Branch.

Basic physical measurements included body height, body mass, and resting blood pressure. Body mass index (BMI) was calculated as body mass (kg)/body height\(^2\) (m\(^2\)). The PG and CG were matched for mean age, body height, prepregnant body mass, parity, and aerobic fitness. Members of the PG were tested between 34 and 38 wk of their gestation. Subjects in the CG were not using oral contraceptives, and menstrual cycle status at the time of the second exercise test was determined by using the first day of their last menstrual cycle and the average length of their cycle and was verified by using serum progesterone samples taken at rest on the day of the test (30).

Exercise testing protocols. Subjects performed two exercise tests at least 1 day apart on a SensorMedics (model 8800) constant-work-rate cycle ergometer. Subjects consumed a standard meal (350 kcal, 40% carbohydrate, 40% fat, 20% protein) 1–2 h before both tests and avoided strenuous physical activity and caffeine on the day of testing. The first test was used to determine the ventilatory threshold (T\(_{\text{vent}}\)) and to assess aerobic working capacity. The protocol involved 5 min of resting data collection and a 4-min warm-up at 20 W, followed by an increase in work rate of 20 W/min until a heart rate (HR) of 170 beats/min was reached (9, 16). Respiratory responses were measured on a breath-by-breath basis by using a computerized system (First Breath) that incorporates a respiratory mass spectrometer (Perkin-Elmer, MGA 1100) with a volume turbine (VMM-1100) as described byHughson et al. (7). Breath-by-breath alveolar gas exchange was calculated by using the algorithm of Beaver et al. (3), and T\(_{\text{vent}}\) was identified by using the V-slope method (4). HR was monitored with both a Polar Vantage monitor and a Marquette Max-1 electrocardiograph. Oxygen pulse (\(\text{VO}_2/\text{HR}\)) at a heart rate of 170 beats/min was calculated as an index of aerobic working capacity (30).

The second exercise test involved a 10-min period of resting data collection, followed by a 3-min warm-up at 0 W and a ramp increase in work rate, over a 30-s time period, to one corresponding to 70 or 110% of T\(_{\text{vent}}\). Both work rates were continued for 7 min after achievement of the prescribed work rate. Subjects rested for 20 min between levels. Before this test, an indwelling catheter was inserted into a dorsal hand vein, as far from the thumb as possible. The hand and lower arm were soaked in a warm-water bath before insertion of the catheter and then placed in a Plexiglas heating box (45°C) to promote vasodilation. Arterialized blood samples were then collected at rest and during minute 6 of exercise at 70 or 110% of T\(_{\text{vent}}\). Measured arterial PO\(_2\) values averaged were 79 ± 4, 74 ± 3, and 71 ± 2 Torr at rest, 70% T\(_{\text{vent}}\), and 110% T\(_{\text{vent}}\), respectively, and confirmed adequate arterIALIZATION.

Blood samples for the determination of PO\(_2\), P\(_{\text{CO}_2}\), [HCO\(_3\)-], and [H\(^+\)] were collected in a syringe containing lyophilized heparin and analyzed immediately by using a Radiometer ABL 30 acid-base analyzer at a standard temperature of 37°C. Correction of blood-gas values for changes in temperature were not necessary, because tympanic temperature measurements confirmed no significant deviation from 37°C with pregnancy or exercise by using the present protocol. Quality control checks using four control liquids were done on all testing days. The remaining blood was then centrifuged for 10 min at 2,500 rpm and frozen at −80°C for later analysis, as described below.

Total protein concentration ([TP]) was measured by using the direct Biuret method. [A\(_{\text{tot}}\)] was calculated from [TP] (g/l) by using the conversion factor 0.243 (11). Albumin concentration ([Alb]) was determined by using a conventional dye-binding method. Plasma concentrations of sodium ([Na\(^+\)], potassium ([K\(^+\)]), calcium ([Ca\(^{2+}\)]), and chloride ([Cl\(^-\)]) were
analyzed by using ion-selective electrodes. Plasma osmolality was determined by using the freezing-point depression technique. Globulin concentration ([Glob]) was calculated by subtracting the [Alb] from [TP] so that the Alb-to-Glob (A/G) ratio could be determined. The interassay coefficient of variability was <3% for all of the procedures listed above.

Plasma lactate concentration ([La\(^{-}\)]) was determined by using an automated analyzer (model 2300, Yellow Springs Instruments). The analyzer was calibrated before analysis by using 5 and 15 mmol/l standards and at regular intervals during the analysis. The test-retest reliability of [La\(^{-}\)] measurements was described in an earlier publication from this laboratory (30). [SID] was then calculated as ([Na\(^{+}\) + [K\(^{+}\)] + 2[Ca\(^{2+}\)]) – ([Cl\(^{-}\)] + [La\(^{-}\)]).

Stewart's physicochemical analysis. Stewart's physicochemical equation (26, 27) was used to calculate [H\(^{+}\)] by using values for the three independent variables (9, 11) and to calculate the contributions of the three independent variables to changes in [H\(^{+}\)] in each group in response to exercise (9, 14)

\[
[H^{+}^3 + (K_{A} + [SID]) [H^{+}] + \frac{[K_{A}][SID] - ([A_{tot}] - (K_{C} \times PCO_{2} + K_{w}) [H^{+}]^2 - [K_{A}[^{3}P_{CO_{2} + K_{w}} + (K_{3} \times K_{C} \times PCO_{2})][H^{+}] - (K_{A} \times K_{3} \times K_{C} \times PCO_{2}) = 0]
\]

where the dissociation constant for water (Kw) = 4.4 × 10\(^{-14}\)(eq/l)\(^2\), the dissociation constant for carbonic acid (Kc) = 2.46 × 10\(^{-11}\)(eq/l)/Torr, the dissociation constant for bicarbonate (K3) = 6.0 × 10\(^{-11}\)(eq/l), and the dissociation constant for weak acid (Kw) = 3.0 × 10\(^{-7}\)(eq/l).

As previously described by Lindinger et al. (14), the contribution of each independent variable to a change in [H\(^{+}\)] can be determined by solving the above equation while using resting values for the variables and changing variables singly or in combination with their measured values.

Statistical analyses. Physical characteristics, T\(_{vent}\), oxygen pulse at 170 beats/min, and measured changes in [H\(^{+}\)] at the two exercise levels were compared between groups by using Student's t-statistics for independent samples. Data at rest and during exercise at 70 and 110% T\(_{vent}\) were compared within and between subjects by using a two-way ANOVA (groups vs. rest/exercise level) with repeated measures on the second factor. When a significant between-group main effect was observed, separate independent Student's t-statistics were used to identify significant differences among group means at rest, 70% T\(_{vent}\), and 110% T\(_{vent}\). When a significant within-subject main effect was observed, paired Student t-statistics were also used to detect significant differences among rest, 70% T\(_{vent}\), and 110% T\(_{vent}\) within each group.

[H\(^{+}\)] was calculated by using Stewart's physicochemical equation and compared with measured [H\(^{+}\)] within both groups under each experimental condition by using paired Student's t-statistics. The corresponding associations between calculated and measured [H\(^{+}\)] were examined by using Pearson's product-moment correlation coefficients (Pearson's r). Contributions of the three independent variables to a change in [H\(^{+}\)] at each work rate were compared within and between groups by using a two-way ANOVA (group vs. independent variables contribution) with repeated measures on the second factor. When a significant between-group main effect or a group × contribution interaction was observed, separate independent t-statistics were used to identify significant differences among group means at rest, 70% T\(_{vent}\), and 110% T\(_{vent}\). When a significant within-subject effect or a group × time interaction was observed, paired Student's t-statistics were used to detect significant differences among variables within each group.

All statistical tests were considered significant if P < 0.05. Because comparisons between groups and across variables were planned and the number of comparisons was small in each case, the critical alpha level for significance was maintained at P < 0.05 (10). Results were identified as trends when 0.05 < P < 0.08.

RESULTS

Subjects. Subjects in both groups were between 25 and 40 yr of age, and the mean values were 29.5 ± 0.9 and 26.9 ± 1.6 yr for the PG and CG, respectively (Table 1). Mean gestational age of the PG was 37.1 ± 0.2 wk. Within the CG, nine women were in the follicular phase of their menstrual cycle and six women were in the luteal phase. As expected, body mass and BMI were significantly higher in the PG compared with the CG at the time of testing. However, the PG's preganancy body mass and BMI were not different from those of the CG. There were no significant differences in mean age, body height, parity, V\(_{O_{2}}\) at T\(_{vent}\), and oxygen pulse (ml/beat) at 170 beats/min.

Ventilatory variables and HR. HR was significantly higher in the PG compared with the CG at rest and 70% T\(_{vent}\), but the difference narrowed with increasing exercise intensity (Table 2). V\(_{O_{2}}\) and the respiratory exchange ratio (RER) did not differ significantly between groups at rest or at either exercise level. VE and the end-tidal P\(_{O_{2}}\) (PET\(_{O_{2}}\)) were significantly higher in the PG at all measurement times. Ventilatory equivalents for oxygen (VE/V\(_{O_{2}}\)) and carbon dioxide (VE/P\(_{CO_{2}}\)) were significantly higher in the PG at all measurement times except for VE/P\(_{CO_{2}}\) at rest. The end-tidal P\(_{CO_{2}}\) (PET\(_{CO_{2}}\)) was significantly lower in the PG at all measurement times and increased from rest to both work rates.

Dependent acid-base variables. During all three experimental conditions [H\(^{+}\)] and [HCO\(_{3}^{-}\)] were lower in the PG vs. CG (Fig. 1, A and B). Results were statisti-

### Table 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>PG (n = 15)</th>
<th>CG (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>29.5 ± 0.9</td>
<td>26.9 ± 1.6</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>37.1 ± 0.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163.6 ± 2.0</td>
<td>163.5 ± 1.5</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>77.9 ± 2.7*</td>
<td>61.9 ± 1.9</td>
</tr>
<tr>
<td>Body mass index</td>
<td>29.0 ± 0.7*</td>
<td>23.1 ± 0.6</td>
</tr>
<tr>
<td>Prepregnancy body mass, kg</td>
<td>64.4 ± 2.7</td>
<td>N/A</td>
</tr>
<tr>
<td>Prepregnancy body mass index</td>
<td>24.0 ± 0.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Parity</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>V(<em>{O</em>{2}}) at T(_{vent}), l/min</td>
<td>1.76 ± 0.06</td>
<td>1.81 ± 0.04</td>
</tr>
<tr>
<td>V(<em>{O</em>{2}}) pulse at 170 beats/min, ml/beat</td>
<td>12.7 ± 0.5</td>
<td>13.4 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of subjects; PG and CG, pregnant and control group, respectively; V\(_{O_{2}}\), VO\(_{2}\) uptake; T\(_{vent}\), ventilatory threshold; N/A, not applicable. *Significant difference (P < 0.05) between groups.
calculated variables: $P_aCO_2$, was significantly lower in the PG vs. CG at rest and both exercise levels (Fig. 3). $P_aCO_2$ did not change significantly with exercise, except for an increase from rest to 70% $T_{vent}$ in the PG. However, a trend for $P_aCO_2$ values to decrease from 70 to 110% $T_{vent}$ was present in the CG. Calculated means changed in measured $[H^+]$ from rest to 70 and 110% $T_{vent}$ were similar between groups (Fig. 2).

Independent acid-base variables: $P_aCO_2$, was significantly lower in the PG vs. CG at rest and both exercise levels (Fig. 3). $P_aCO_2$ did not change significantly with exercise, except for an increase from rest to 70% $T_{vent}$ in the PG. However, a trend for $P_aCO_2$ values to decrease from 70 to 110% $T_{vent}$ was present in the CG. Calculated means changed in measured $[H^+]$ from rest to 70 and 110% $T_{vent}$ were similar between groups (Fig. 2).

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Both $Na^+$ and $K^+$ increased in the transition from rest to 70% $T_{vent}$ and from 70 to 110% $T_{vent}$ in both groups (Table 3). $Na^+$ was significantly lower at rest and both exercise levels in the PG compared with the CG. $K^+$ was significantly lower at rest and 70% $T_{vent}$ in the PG compared with the CG. As expected, osmolality exhibited the same significant trends between groups and measurements as those for $Na^+$. $Ca^{2+}$ was greater at 110% $T_{vent}$ in the PG than at rest and 70% $T_{vent}$. Values for $Ca^{2+}$ in the PG were significantly higher than those of the CG at 70 and 110% $T_{vent}$. There were no differences in $[Cl^-]$ between groups at any level. $[Cl^-]$ increased significantly in the transition from rest to both 70 and 110% $T_{vent}$ except for in the PG at 70% $T_{vent}$. $[La^-]$ increased significantly from rest to 70% $T_{vent}$ and from 70 to 110% $T_{vent}$ as expected. There were no significant between-group differences in absolute values for $[La^-]$, and increases from rest to both exercise intensities were not significantly different.

In both groups, $[TP]$ and $[Glob]$ increased significantly in the transition from rest to both 70 and 110% $T_{vent}$ (Table 4). $[Glob]$ significantly increased from rest to 110% $T_{vent}$ in the PG. $[TP]$, $[Alb]$, and $[Glob]$ also increased significantly from 70 to 110% $T_{vent}$, except for $[TP]$ and $[Glob]$ in the CG. There were no significant changes across measurement conditions for the $A/G$ ratio. At rest, 70% $T_{vent}$, and 110% $T_{vent}$, the values for $[TP]$, $[Alb]$, and the $A/G$ ratio were significantly lower in the PG compared with the CG. The values for $[Glob]$ were significantly higher in the PG compared with the CG at rest and both exercise levels. Differences between groups and across exercise levels for the calculated values for $[La^-]$ (Fig. 5) followed those of $[TP]$. The relationship between measured and calculated $[H^+]$ values. Calculated $[H^+]$ values from Stewart’s equation are compared with measured in Table 5. Calculated $[H^+]$ was not significantly different from measured $[H^+]$ at rest or during exercise in the CG. However, a small but statistically significant underestimation was present in the PG. Strong statistically significant correlations were also observed between measured and calculated $[H^+]$ in both groups. The only exception to this was the relationship at rest in the CG. This effect was attributable to the low variability of data in the resting state.

Contributions of independent variables to changes in $[H^+]$. $[SID]$ was the only variable to make a significantly different contribution to a change in $[H^+]$ between the groups (Fig. 6). At 70% $T_{vent}$ an increase in $[SID]$ in the PG attenuated the rise in $[H^+]$, whereas a decrease in $[SID]$ in the CG contributed to the rise. At 110% $T_{vent}$, the contribution of $[SID]$ to the rise in $[H^+]$ was significantly greater in the PG vs. CG. This appeared to be offset by a modest reduction in $P_aCO_2$ ($P < 0.05$) in the CG. This was reflected in a trend for a different contribution of $P_aCO_2$ between groups from rest to 110% $T_{vent}$, in which $P_aCO_2$ contributed to the rise in $[H^+]$ in the PG but attenuated the rise in $[H^+]$ in the CG.

**DISCUSSION**

The central objective of this study was to examine, using the physicochemical approach of Stewart (26, 27), the effects of human pregnancy on mechanisms of acid-base regulation at rest and during exercise above and below $T_{vent}$. The two exercise intensities were chosen to examine the effects of pregnancy on acid-base regulation during moderate steady-state exercise and
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During strenuous non-steady-state exertion that represented a more severe challenge to maternal acid-base homeostasis. This study adds significantly to information available from the earlier study of Kemp et al. (9) because arterialized rather than venous plasma was employed for the analysis and the effects of exercise both below and above $T_{vent}$ (i.e., steady-state and non-steady-state exercise conditions) were examined. It was hypothesized that acid-base balance would be significantly altered at rest in pregnancy and that absolute changes in [H+] in the transition from rest to exercise and the mechanisms responsible would be similar to the nonpregnant state.

Generalized metabolic and cardiorespiratory responses at rest and during both exercise tests and differences between groups were consistent with previous studies of exercising pregnant women. It is well documented that respiratory sensitivity to CO₂ is increased, resulting in higher values for $V_{E} / V_{O2}$, and $V_{E} / V_{CO2}$, as well as increased $PET_{O2}$ and reduced $PET_{CO2}$.

![Fig. 1](image1.png)

**Fig. 1.** A: measured hydrogen ion concentration ([H⁺]) at rest and at 2 work rates. B: calculated bicarbonate ion concentration ([HCO₃⁻]) at rest and at 2 work rates. *Significant difference between groups. †Significant change within group from rest. ‡Significant change within group from cycling at 70% ventilatory threshold ($T_{vent}$) to cycling at 110% $T_{vent}$, $P < 0.05$.

![Fig. 2](image2.png)

**Fig. 2.** Changes in measured [H⁺]. Changes in measured [H⁺] from rest to both 70 and 110% $T_{vent}$ were not significantly different between groups, $P < 0.05$.

![Fig. 3](image3.png)

**Fig. 3.** Arterialized plasma $PCO_{2}$ ($PaCO_{2}$) at rest and at 2 work rates. *Significant difference between groups, $P < 0.05$. †Significant change within group from rest, $P < 0.05$.
both at rest and during submaximal exercise relative to the nonpregnant state (21, 22). RER does not appear to be altered at work rates below the onset of blood lactic acid accumulation (16, 29). It is well documented that HR is augmented both at rest and during submaximal exercise and that these differences in HR compared with the nonpregnant state increase with increasing exercise intensity (21). Finally, although the effects of pregnancy on maximal aerobic power are controversial (29), existing studies indicate that $T_{\text{vent}}$ is not changed with the nonpregnant state (21, 22). RER does not appear to be altered at work rates below the onset of blood lactic acid accumulation (16, 29). It is well documented that HR is augmented both at rest and during submaximal exercise and that these differences in HR compared with the nonpregnant state increase with increasing exercise intensity (21). Finally, although the effects of pregnancy on maximal aerobic power are controversial (29), existing studies indicate that $T_{\text{vent}}$ is not changed with the nonpregnant state (21, 22).

Fig. 4. Calculated plasma strong-ion difference ([SID]) at rest and at 2 work rates. *Significant difference between groups, P < 0.05. †Significant change within group from rest, P < 0.05. §Significant change within group from cycling at 70% $T_{\text{vent}}$ to cycling at 110% $T_{\text{vent}}$. P < 0.05.

Table 3. Plasma strong ions at rest and at 2 work rates

<table>
<thead>
<tr>
<th>Variable</th>
<th>[Na$^+$], mmol/l</th>
<th>[K$^+$], mmol/l</th>
<th>[Ca$^{2+}$], mmol/l</th>
<th>[Cl$^-$], mmol/l</th>
<th>[La$^-$], mmol/l</th>
<th>Osmolality, mosmol/kg H$_2$O</th>
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</thead>
<tbody>
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<td>Rest</td>
<td></td>
<td></td>
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<tr>
<td>PG</td>
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<td>3.9 ± 0.1*</td>
<td>1.18 ± 0.02</td>
<td>104 ± 0.6</td>
<td>1.5 ± 0.1</td>
<td>277 ± 1*</td>
</tr>
<tr>
<td>CG</td>
<td>139 ± 0.4</td>
<td>4.3 ± 0.1</td>
<td>1.15 ± 0.01</td>
<td>105 ± 0.5</td>
<td>1.2 ± 0.2</td>
<td>284 ± 1</td>
</tr>
<tr>
<td>70% $T_{\text{vent}}$</td>
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</tr>
<tr>
<td>PG</td>
<td>137 ± 0.4†</td>
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<td>141 ± 0.4†</td>
<td>4.8 ± 0.1†</td>
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<td>106 ± 0.6†</td>
<td>2.5 ± 0.4†</td>
<td>288 ± 1†</td>
</tr>
<tr>
<td>110% $T_{\text{vent}}$</td>
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<td></td>
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<tr>
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<td>5.9 ± 0.6‡</td>
<td>293 ± 1†‡</td>
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</tbody>
</table>

Values are means ± SE; n = 15 in PG and CG, respectively. Brackets denote concentration. *Significant difference (P < 0.05) between groups. †Significant change (P < 0.05) within groups from rest. ‡Significant change (P < 0.05) within groups from cycling at 70% vs. 110% $T_{\text{vent}}$. P < 0.05.

Table 4. Plasma protein at rest and during 2 work rates

<table>
<thead>
<tr>
<th>Variable</th>
<th>[TP], g/l</th>
<th>[Alb], g/l</th>
<th>[Glob], g/l</th>
<th>A/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG</td>
<td>62 ± 1.1*</td>
<td>30 ± 0.6*</td>
<td>32 ± 0.9*</td>
<td>0.93 ± 0.03*</td>
</tr>
<tr>
<td>CG</td>
<td>71 ± 1.6</td>
<td>42 ± 0.6</td>
<td>28 ± 1.4</td>
<td>1.55 ± 0.06</td>
</tr>
<tr>
<td>70% $T_{\text{vent}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG</td>
<td>66 ± 1.2†</td>
<td>32 ± 0.6†</td>
<td>34 ± 0.8*</td>
<td>0.94 ± 0.02*</td>
</tr>
<tr>
<td>CG</td>
<td>74 ± 1.7†</td>
<td>44 ± 0.5†</td>
<td>31 ± 1.3</td>
<td>1.46 ± 0.05</td>
</tr>
<tr>
<td>110% $T_{\text{vent}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG</td>
<td>69 ± 1.6‡‡</td>
<td>33 ± 0.8‡‡</td>
<td>36 ± 1.1†‡‡</td>
<td>0.93 ± 0.03*</td>
</tr>
<tr>
<td>CG</td>
<td>76 ± 1.4‡</td>
<td>46 ± 0.4‡</td>
<td>31 ± 1.4</td>
<td>1.51 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15 in PG and CG, respectively. TP, total protein; Alb, albumin; Glob, globulin; A/G, albumin-to-globulin ratio. *Significant difference (P < 0.05) between groups. †Significant change (P < 0.05) within groups from rest. ‡Significant change (P < 0.05) within groups from cycling at 70% vs. 110% $T_{\text{vent}}$. P < 0.05.
and Regnat (12), who found that exercise-induced decreases in base-excess were greater in pregnancy and those of Pivarnik et al. (25), who reported smaller exercise-induced decreases in $[\text{HCO}_3^-]$.

In accordance with either Stewart’s physicochemical approach or traditional acid-base analysis, significantly lower PaCO$_2$ values in the PG vs. the CG would contribute to the maintenance of a lower [H$^+$] in the PG at all measurement times. This confirms the importance of pregnancy-induced increases in respiratory sensitivity (2) to reduce PaCO$_2$ and to maintain a lower [H$^+$] than in the nonpregnant state. Augmented respiratory sensitivity in pregnancy has been attributed to increased circulating levels of progesterone, a known respiratory stimulant, and estrogen (5, 18). The increased estrogen level elevates the number of progesterone receptors in the hypothalamus (2, 5). In accordance with recent findings of Jennings and associates (8), the increase in Ve in pregnancy may also be due to changes in osmolality, [SID], and angiotensin II levels, which have been implicated in the control of ventilation. During pregnancy, osmolality decreases, [SID] decreases, and angiotensin II levels increase. In theory, all of these effects could contribute to the increase in Ve in pregnancy in concert with the effects of progesterone (28).

The lower [SID] in the PG compared with the CG at rest and both work rates resulted primarily from lower values for [Na$^+$] and, to a lesser extent, [K$^+$], in the PG, because there were no significant differences in [Cl$^-$] or [La$^-_2$] between groups. The reductions in [Na$^+$] and [K$^+$] may have been the result of pregnancy-induced hemodilution (or perhaps other mechanisms), because blood volume in pregnancy increases by 40–50% over nonpregnant levels (15, 24). Expansion of blood volume during pregnancy is attributable to an estrogen-mediated stimulation of the renin-angiotensin system, which, in turn, augments aldosterone secretion, as well as Na$^+$ and water retention (15). Reduced [Na$^+$] and [K$^+$] values at rest in pregnancy have been observed previously (9, 17). As reported previously, pregnancy did not cause reductions in [Cl$^-$] (9, 18). This could be due to changes in renal absorption of Cl$^-$, ionic shifts between fluid compartments, or altered binding of Cl$^-$ by plasma proteins.

The decrease in [SID] observed in both groups during the transition from rest to exercise at 110% T$_{vent}$ was primarily the result of an increase in [La$^-_2$]. [La$^-_2$] did not differ significantly between groups at either exercise intensity, and increases were not significantly different between groups, although there have been reports of lower [La$^-_2$] for exercising pregnant women at work rates above T$_{vent}$ (19, 30). Blunted [La$^-_2$] responses in pregnancy would, in theory, contribute to the maintenance of a lower [H$^+$].

### Table 5. Values of measured [H$^+$] and calculated [H$^+$] at rest and at 2 work rates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Measured [H$^+$], neq/l</th>
<th>Calculated [H$^+$], neq/l</th>
<th>Mean Difference, neq/l</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG</td>
<td>37.6 ± 0.4</td>
<td>35.4 ± 0.7*</td>
<td>2.2</td>
<td>0.78</td>
</tr>
<tr>
<td>CG</td>
<td>39.9 ± 0.4</td>
<td>39.6 ± 0.7</td>
<td>0.3</td>
<td>0.10</td>
</tr>
<tr>
<td>70% T$_{vent}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG</td>
<td>39.5 ± 0.5</td>
<td>36.4 ± 0.8*</td>
<td>3.1</td>
<td>0.86</td>
</tr>
<tr>
<td>CG</td>
<td>41.8 ± 0.6</td>
<td>42.2 ± 1.3</td>
<td>−0.4</td>
<td>0.80</td>
</tr>
<tr>
<td>110% T$_{vent}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG</td>
<td>43.5 ± 0.7</td>
<td>40.8 ± 1.1*</td>
<td>2.7</td>
<td>0.74</td>
</tr>
<tr>
<td>CG</td>
<td>45.1 ± 0.8</td>
<td>45.9 ± 1.1</td>
<td>−0.8</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15 in PG and CG, respectively.

*Significant difference (P < 0.05) between measured and calculated [H$^+$].

With recent findings of Jennings and associates (8), the increase in Ve in pregnancy may also be due to changes in osmolality, [SID], and angiotensin II levels, which have been implicated in the control of ventilation. During pregnancy, osmolality decreases, [SID] decreases, and angiotensin II levels increase. In theory, all of these effects could contribute to the increase in Ve in pregnancy in concert with the effects of progesterone (28).

The lower [SID] in the PG compared with the CG at rest and both work rates resulted primarily from lower values for [Na$^+$] and, to a lesser extent, [K$^+$], in the PG, because there were no significant differences in [Cl$^-$] or [La$^-_2$] between groups. The reductions in [Na$^+$] and [K$^+$] may have been the result of pregnancy-induced hemodilution (or perhaps other mechanisms), because blood volume in pregnancy increases by 40–50% over nonpregnant levels (15, 24). Expansion of blood volume during pregnancy is attributable to an estrogen-mediated stimulation of the renin-angiotensin system, which, in turn, augments aldosterone secretion, as well as Na$^+$ and water retention (15). Reduced [Na$^+$] and [K$^+$] values at rest in pregnancy have been observed previously (9, 17). As reported previously, pregnancy did not cause reductions in [Cl$^-$] (9, 18). This could be due to changes in renal absorption of Cl$^-$, ionic shifts between fluid compartments, or altered binding of Cl$^-$ by plasma proteins.

The decrease in [SID] observed in both groups during the transition from rest to exercise at 110% T$_{vent}$ was primarily the result of an increase in [La$^-_2$]. [La$^-_2$] did not differ significantly between groups at either exercise intensity, and increases were not significantly different between groups, although there have been reports of lower [La$^-_2$] for exercising pregnant women at work rates above T$_{vent}$ (19, 30). Blunted [La$^-_2$] responses in pregnancy would, in theory, contribute to the maintenance of a lower [H$^+$].
[A\textsubscript{tot}] was lower, as a result of reductions in [Alb], in the PG vs. the CG at rest and both work rates, thus contributing to the lower [H\textsuperscript{+}] in the PG. The lower [A\textsubscript{tot}] in the PG was attributable to pregnancy-induced blood volume expansion and altered synthesis of proteins by the liver, as suggested by the decreased A/G ratio. The present results for [TP], [Alb], [Glob], and A/G ratio also agree with previous findings. [TP] and [Alb] decrease in pregnancy whereas [Glob] increases moderately (9). The overall result is a decrease in the A/G ratio (9, 24), [TP], and thus [A\textsubscript{tot}]. Increase during exercise, likely the result of exercise-induced hemococoncentration.

Similar absolute changes in [H\textsuperscript{+}] in the transition from rest to 70 and 110% T\textsubscript{vent} in the PG and CG suggested that the contributions of independent variables to the increase in [H\textsuperscript{+}] in response to strenuous exercise were similar in the pregnant vs. nonpregnant state, as reported previously by Kemp et al. (9) in venous plasma. To examine this hypothesis further, Stewart’s equation was utilized to calculate the contributions of the independent variables to changes in [H\textsuperscript{+}] from rest to each work rate. The contribution of [A\textsubscript{tot}] was not significantly different between groups at either exercise intensity. As discussed below, a trend (P = 0.07) was present for a different contribution of \textit{PaCO\textsubscript{2}} between groups at 110% T\textsubscript{vent}. The contributions of [SID] to changes in [H\textsuperscript{+}] were significantly different between groups at both exercise intensities, and a significant group \times time interaction was observed for the absolute [SID] values. The altered behavior of [SID] in the pregnant state resulted in a negative contribution to the rise in [H\textsuperscript{+}] at the 70% T\textsubscript{vent} work rate and a smaller positive contribution to the [H\textsuperscript{+}] rise at the 110% T\textsubscript{vent} work rate. These findings are different from those of Kemp et al. (9), who found no pregnancy-induced effect on the contributions of any of the independent variables to changes in [H\textsuperscript{+}] in recovery from a maximal exercise test. This discrepancy was attributable to the use of venous as opposed to arterial or arterialized plasma in this earlier study.

A trend for \textit{PaCO\textsubscript{2}} values to decrease from 70 to 110% T\textsubscript{vent} was present in the CG (P = 0.06), but not in the PG (P = 0.32), suggesting that respiratory compensation for the rising [H\textsuperscript{+}] was present in the CG at this work rate but not in the PG. The reduction in \textit{PaCO\textsubscript{2}} in the CG but not the PG could be easily explained if the rise in [H\textsuperscript{+}] from 70 to 110% T\textsubscript{vent} was greater in the CG vs. PG, but this was not observed. However, if [SID] rather than [H\textsuperscript{+}] is the stimulus to chemoreceptors and ventilation as hypothesized by Jennings (8), the decrease in [SID] in the CG in the transition from rest to 110% T\textsubscript{vent} (approximately twice that of the PG) would account for the differences in the behavior of \textit{PaCO\textsubscript{2}} at 110% T\textsubscript{vent}.

Excellent agreement was observed between measured and calculated values for [H\textsuperscript{+}] both at rest and during exercise in the CG and, except for values in the CG at rest (owing to low variability of measurements), strong statistically significant correlations were observed between measured and calculated [H\textsuperscript{+}] in both groups under all experimental conditions. In accordance with earlier studies of exercising men (11), these results provide strong support for the validity and utility of Stewart’s approach.

Within the PG, there was a small (2–3 meq/l) but statistically significant underestimation of [H\textsuperscript{+}] using Stewart’s equation and values for the independent variables. In accordance with modern acid-base theory (8), this could be an effect of changes in temperature, [SID], and/or osmolality on the dissociation constants used in Stewart’s analysis. Indeed, our data showed significant differences in plasma osmolality and [SID] (but not temperature) in the pregnant vs. nonpregnant state. Given the small differences involved, it is unlikely that this introduced important mathematical errors in our calculation of contributions of the independent variables to changes in [H\textsuperscript{+}]. It is also important to keep in mind that the purpose of using Stewart’s approach is not to predict [H\textsuperscript{+}] but to allow a more mechanistic approach to acid-base analysis than is possible by using the conventional Henderson-Hasselbalch approach by itself. The prediction of [H\textsuperscript{+}] is usually done to show the validity of Stewart’s approach and to provide assurance that no serious errors are involved in the measurement or calculation of the three independent variables. Taken by themselves, the results from the PG provide strong support for Stewart’s approach on the basis of significant correlations between measured and calculated [H\textsuperscript{+}]. Indeed, although the difference between the measured and calculated [H\textsuperscript{+}] in the PG was significantly greater than in the CG, it was still quite small and in the same range observed in earlier validation studies (9, 11). Nevertheless, this effect should be considered in future studies of acid-base regulation in human pregnancy.

The present study confirmed earlier findings (1, 13, 17, 25, 30) that human pregnancy is accompanied by a partly compensated respiratory alkalosis as reflected by significantly reduced values for [H\textsuperscript{+}], \textit{PaCO\textsubscript{2}}, and [HCO\textsubscript{3}⁻] in arterialized plasma in the resting state. Application of Stewart’s physicochemical approach was helpful in clarifying the involvement of metabolic, hepatic, and renal contributions to a reduction in [H\textsuperscript{+}] in the resting state. The reduction of [A\textsubscript{tot}] can be attributable, in part, to dilution of plasma proteins (and other weak acids) in an expanded maternal blood volume. However, a significant decrease in the A/G ratio also suggests altered synthesis of plasma proteins by the liver. Pregnancy-induced reduction in [SID] was the result of lower values for [Na\textsuperscript{+}] and [K\textsuperscript{+}], again presumably as a result of hemodilution. The reasons why [Cl\textsuperscript{−}] and [La\textsuperscript{−}] were not significantly reduced should be examined in future investigations of metabolic, renal, and ionic factors. The present study results also confirmed earlier findings that exercise-induced reductions in [H\textsuperscript{+}] are quantitatively similar in the pregnant vs. nonpregnant state (9, 24). We also observed no significant differences between groups in the change in [HCO\textsubscript{3}⁻] in the transition from rest to either moderate (70% T\textsubscript{vent}) or strenuous (110% T\textsubscript{vent}) exercise. Application of Stewart’s physicochemical approach dem-
shown that there is a blunted [SID] response to exercise above and below \( T_{\text{vent}} \) in late pregnancy.

In conclusion, our results support our original hypothesis that lower [H\(^+\)] of arterIALIZED plasma in the resting state is the combined result of lower values for \( \text{PaCO}_2 \) and [A\(_{\text{tot}}\)] and that this effect is partly offset by a lower [SID]. Whereas exercise-induced increases in [H\(^+\)] are quantitatively similar in the pregnant vs. nonpregnant state, the mechanisms of adaptation from rest to exercise are different. The altered behavior of [SID] above and below \( T_{\text{vent}} \) in pregnancy is beneficial to maintain a lower [H\(^+\)]. In addition, because there is a lesser contribution of [SID] to changes in [H\(^+\)] induced by strenuous exercise in pregnancy, acid-base regulation may require less respiratory compensation at work rates above \( T_{\text{vent}} \).

This study was supported by US Army Medical Research and Materiel Command Contract DAMD17-96-C-6112, the Ontario Thoracic Society, and the Natural Sciences and Engineering Research Council of Canada.

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Received 5 January 1999; accepted in final form 14 September 1999.

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