Effects of Blood pH and Blood Lactate on Growth Hormone, Prolactin, and Gonadotropin Release after Acute Exercise in Male Volunteers (44082)

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Abstract. **It has recently been found that prevention of the acidosis of anaerobic exercise blocks β-endorphin release. Because heavy exercise affects secretion of other anterior pituitary hormones, we studied the results of alkali infusion and ingestion upon blood levels of four hormones: luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone (GH), and prolactin (PRL). Eight male subjects were studied after either 2 mEq/kg placebo (NaCl) or alkali ($NaHCO_3$) administered before and during exercise to exhaustion. Blood samples were obtained before exercise and then 15, 30, 60, 90, 120, and 180 min postexercise. GH and PRL but not FSH or LH increased significantly postexercise, with a peak at 60 min, and subsequently declined back to baseline by 180 min. Base treatment reduced GH at baseline and postexercise (except at 60 min) and increased PRL significantly, particularly at 60 min. While the precise mechanisms on how acid/base changes affect hormone release remain to be defined, there are possible consequences on gonadal function and substrate availability during exercise.** [P.S.E.B.M. 1997, Vol 214]

Exercise, both acute and sustained, is associated with changes in the secretion of several pituitary hormones (1–9). Pituitary hormones that are consistently released with exercise include growth hormone (GH), prolactin (PRL), and pro-opiomelanocortin-derived peptides such as ACTH and β-endorphin (1, 10–13). Gonadotropins, particularly luteinizing hormone (LH), often show a fall after exercise (3–7). The potential mechanisms responsible for the release or suppression of release of pituitary hormones after exercise has been extensively investigated (14). It has recently been shown that avoidance of acidosis by infusion and ingestion of alkali prevents β-endorphin release (15). Hence, we utilized this approach to evaluate acidosis as a potential cause of control of hormone release.

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Materials and Methods

Subjects. Eight male volunteers aged 20 to 25 years (mean ± SEM: 22.10 ± 0.58) participated in the study which was approved by the Human Subjects Research Committee of the University of California, Irvine. The subjects' mass ranged from 54.5 to 90.9 kg (mean ± SEM: 76.90 ± 3.45), and their height ranged from 1.62 to 1.83 m (mean ± SEM: 1.76 ± 0.03). None of the volunteers suffered from any medical illness, and none was on any medication. All subjects had a normal sleep-wake pattern. Studies were initiated between 0900 and 1000 hr. Prior to exercise testing, all subjects were screened for medical illness. Screening tests included spirometry and electrocardiogram (ECG).

Experimental Protocol. Each subject performed an incremental cycle test on three occasions. The first was for screening and familiarization. The second and third tests were incremental cycle tests with randomized double-blind administration of either base (B) or placebo (C). The subjects pedaled a cycle ergometer (Monark # 668 or Collins Pedalmate, Braintree, MA) at 60 rpm; individual subjects used only one of these cycles. Following 2 min of pre-exercise period of sitting on the cycle, subjects pedaled for 2 min without any load. Power was increased by 15 W each minute until exhaustion. Time to exhaustion ranged from 13

to 21.25 min for the B-treated volunteers (mean ± SEM: 17.43 ± 0.80 min). Time to exhaustion after placebo treatment also ranged from 13 to 21.25 min (mean ± SEM: 16.93 ± 0.80 min, P = NS).

Alkali and Placebo Administration. The antecubital vein was used for access using an 18-gauge catheter. Two 3-way stopcocks were connected to the intravenous tubing to switch between iv infusion of either B or C or for blood removal for hormone and other measurements.

Subjects were asked to ingest, within 30 min, an appropriate number of capsules (No. 0330) with water. The capsules were filled with either 1 mg/kg $NaHCO_3$ or NaCl. The same compound as ingested was infused through the iv line in order to provide most of another 1 mg/kg of isotonic B or C before exercise was started about 30 min after capsule ingestion. During exercise, the remainder of the infusion was administered until the end of the test.

Blood Sampling. The infusion was briefly interrupted for blood sampling. The initial 10 ml of blood drawn through the distal stopcock was discarded. Sample blood was then drawn from the proximal stopcock to avoid contamination of the sample with iv fluid. Serum sodium (Na) and hemoglobin (Hgb) were measured to check for the diluting effect of the iv fluids on the sample. Hemoglobin and serum electrolytes (Na, K, Cl, HCO_3, Ca, and ionized Ca), and blood gases (Corning 178; Novostat Profile, Waltham, MA) were determined from each sample.

Blood was drawn at 1, 3, 6, 9, 12, and 15 min for measurement of arterial blood gases, lactate and electrolytes. Blood samples for lactate analysis were collected in pre-chilled heparinized 3-ml blood gas syringes and transferred to pre-chilled 2-ml centrifuge tubes. Analysis was performed within 3 min after collection (YSI 23L; Yellowspring Instruments, Inc., Yellowspring, Ohio). The analysis employs a membrane-bound lactate oxidase reaction coupled to an electromechanical detector that measures oxidation of the hydrogen peroxide reaction product. The machine was calibrated with stock 5.0 and 15 m*M* lactate standards as well as 5, 7, 10, and 12.5 m*M* dilutions. A 25-μl syringe pipette was rinsed three times with distilled water and at least one additional time with sample before injection. Each sample was analyzed in duplicate.

Physiological Measurements. Heart rate (f_c) was continuously monitored on a 3-channel electrocardiograph (ECG 3A; Brentwood Instruments, Torrance, CA) and recorded the last 10 sec of each minute. Expired minute ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$), and respiratory exchange ratio (R) were determined as previously described (8).

Respiratory measurements were made on a breath basis, averaged, and reported for each 15-sec interval. Subjects breathed through a two-way non-rebreathing valve (Model 2700; Hans-Rudolph, Kansas City, MO). Expired air was connected to a pneumotachograph (Model 50 MC; Miriam, Cleveland, OH) *via* 3 m of flexible tubing (3.5 cm i.d.) to attain thermal stability. The pneumotachograph was connected to a differential pressure transducer and carrier demodulator (CD15; Validyne, Northridge, CA) to generate an analog flow signal. This digitized signal was sampled every 15 ms (Keithly System 570; Data Acquisition Control, Cleveland, OH) and recorded with a computer (IBM-AT clone; Microexpress 286, Santa Anna, CA). The flow signal was integrated to determine minute ventilation ($\dot{V}_E$). Expired air was sampled at the mouth and routed *via* capillary tubing to a mass spectrometer (Perkin-Elmer 1100, Pomona, CA) at a rate of 60 ml/min; the mass spectrometer signal was digitized and computer recorded every 15 ms. Oxygen uptake ($\dot{V}_{O_2}$) was calculated from $\dot{V}_E$ in real time using the Haldane transformation (16). Carbon dioxide production ($\dot{V}_{CO_2}$), $\dot{V}_{O_2}$, $\dot{V}_E$, f_c, rate of perceived exertion (R_{PE}) were monitored for each test.

Hormone Measurements. Pre-exercise blood was removed 2 min before free pedaling. Blood was subsequently removed at 15, 30, 60, 90, 120, 150, and 180 min after start of exercise for measurement of GH, PRL, LH, and FSH. Blood was collected in pre-chilled polystyrene tubes containing no anticoagulant. Serum was immediately separated in a cold centrifuge and stored at −70°C until assayed.

Follicle-stimulating hormone. FSH was assayed using an immunochemiluminometric (ICMA) assay technique. The assay uses two monoclonal antibodies, a capture antibody and an enzyme-labeled monoclonal antibody. Light generated by the action of the enzyme (alkaline phosphatase) on dioxatane is measured in a luminometer. The standard was calibrated against WHO second reference preparation (2nd IRP 78/549). The assay has 100 times greater sensitivity than radioimmunoassays. Sensitivity of the assay is 0.02 IU/L. Coefficient of variation is 10% (17).

Luteinizing hormone. LH was assayed using a technique similar to that for FSH. The standard was calibrated against WHO first reference preparation (1st IRP 68/40). The assay has 100 times greater sensitivity than radioimmunoassays. Sensitivity of the assay is 0.02 IU/l. Coefficient of variation is 12% (17).

Growth hormone. GH was measured using a rabbit polyclonal anti-human GH antiserum and ^{125}I-GH. Bound/free separation was achieved using goat anti-rabbit γ-globulin. The radioimmunoassay measures both 20K and 22K GH species. Assay sensitivity was 0.6 μg/l at 90% B/Bo. Coefficient of variation is 8%. WHO standard (1st international standard, 80/505) for HGH was used as the reference preparation (18).

Prolactin. Prolactin was measured using an immunoradiometric assay (IRMA). The assay employs antiprolactin and [^{125}I]prolactin. Highly purified prolactin is calibrated against WHO first reference preparation. Bound/free separation is achieved using a second antibody. Assay sensitivity is 2 μg/l. Coefficient of variation is 6%. WHO standard (1st IRP 75/504) was used as the reference preparation. The assay was performed using Nichols Institute Diagnostic kit (Corning Nichols Institute, San Juan, CA) and the procedure was followed per the manufacturer's in-

structions (Nichols Institute Prolactin Assay Kit Insert, kit 60-4130, effective 10/1992).

Statistical Analysis. Statistical analysis was performed using non-parametric tests (Wilcoxon and Mann Whitney *U* tests), ANOVA, and repeated measures ANOVA design with orthogonal decomposition. Student's *t* tests for selected paired and grouped observations were performed when ANOVA was significant. Level of significance was 0.05 or less.

Results

Effect of Exercise on Heart Rate, Respiratory Variables, and Venous Electrolytes. The effects of exercise on heart rate, respiratory variables, and venous electrolytes are outlined in Table I. The maximum f_c reached ranged from 82% to 102% of the maximum f_c predicted for individual subjects. Maximal heart rates and maximal values for respiratory variables (V_{O_2max}, V_{Emax}, and R_{PEmax}) were not significantly different between placebo and base-loading. Blood pH over time is shown in Figure 1. Time to exhaustion was similar in both base- and placebo-treated subjects (mean ± SEM: base treatment, 17.43 ± 0.80 min; placebo treatment, 16.93 ± 0.80 min; P = not significant). Electrolytes, with the exception of ionized calcium, were all significantly different between conditions; significance was tested by paired comparison. The differences between HCO_3 and base excess were 11–12 mEq/l (base higher), while the difference for Na was 2.3 and that for Ca 0.42 mEq/l (placebo higher). The nonsignificant difference for ionized Ca was 0.13 ± 0.17 mEq/l.

Baseline Serum FSH, LH, GH, and PRL Concentrations. Baseline FSH, LH, GH, and PRL concentrations in placebo-treated volunteers were 2.49 ± 0.95 IU/l, 2.98 ± 0.31 IU/l^{-1}, 3.26 ± 1.72 μg/l, and 5.88 ± 0.96 μg/l, respectively. Baseline FSH, LH, GH, and PRL concentrations in base-treated volunteers were 2.60 ± 0.51 IU/l, 3.22 ± 0.35 IU/l^{-1}, 1.78 ± 0.67 μg/l, and 4.84 ± 1.16 μg/l, respectively. There was no significant difference between baseline placebo-treated and base-treated hormone concentrations, except for GH, which was lower with base treatment ($P < 0.02$).

Table I. Effect of Exercise on Maximum Values of Heart Rate, Respiratory Variables, and Venous Electrolytes

	Placebo	Base
Heart rate (f_c) (bpm)	182 ± 4	185 ± 4
Blood pH	7.22 ± 0.01	7.43 ± 0.02[a]
Maximum O_2 uptake—$\dot{V}_{O_2max}$ (l/min)	3.30 ± 0.20	3.31 ± 0.26
Expired Minute Ventilation—$\dot{V}_E$ (l/min)	124.2 ± 6.5	115.0 ± 7.1
Rate of perceived exertion (R_{PE})	19.9 ± 0.1	19.4 ± 0.3
HCO_3 (mEq/l)	22.2 ± 0.6	32.5 ± 1.3[a]
Base excess (BE) (mEq/l)	6.2 ± 0.7	5.1 ± 1.1[a]
Blood lactate (mEq/l)	5.86 ± 0.42	6.45 ± 0.90
Na (mEq/l)	150 ± 0.4	147 ± 0.8[a]
Ca (mg/dl)	4.57 ± 0.08	4.17 ± 0.14[a]
Ionized Ca (mg/dl)	4.16 ± 0.05	4.04 ± 0.14

Note. Values are expressed as mean ± SEM.
[a] Significant difference.

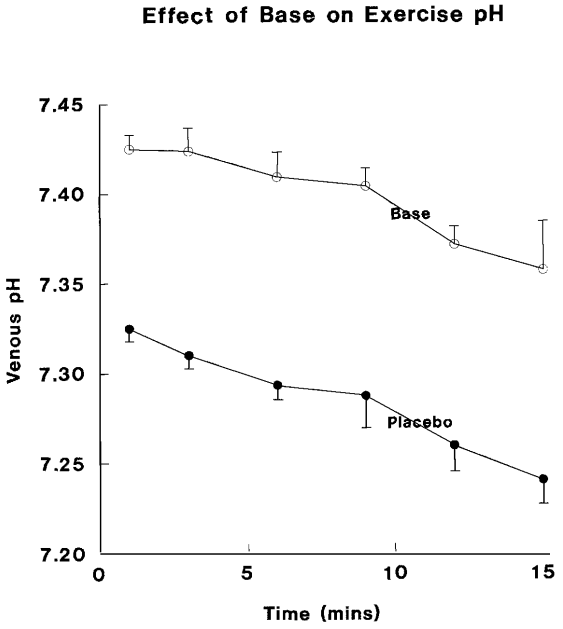

Figure 1. Venous pH over time.

Effects of Exercise on Serum FSH, LH, GH, and PRL Concentrations. The effects of exercise on serum FSH, LH, GH, and PRL concentrations are shown in Figures 2 and 3. As is evident from the figures, GH and PRL ($P < 0.001$), but not FSH or LH, increased at 60 min postexercise and declined steadily thereafter, reaching baseline values at 180 min. Repeated measure ANOVA confirmed that this pattern (quadratic) was the best fit. Additionally, base administration enhanced PRL ($P < 0.035$) but, except for peak value at 60 min, tended to suppress GH ($P < 0.042$). LH decreased following exercise, as previously reported (4–7). However, nadir serum LH concentrations occurred at different times in different subjects, so LH decrease in the group was not significant.

Discussion

Acute exercise is associated with the release of a number of pituitary hormones (14). This is particularly true of GH, PRL, and pro-opiomelanocortin-derived peptides such as ACTH, β-endorphin, and β-lipotropin (1–7, 9, 11, 19, 20). Other hormones such as the gonadotropins, particularly LH, show a transient rise followed by depression (3–5). The mechanism(s) responsible for altered pituitary hormone release with exercise are not clearly defined. Earlier studies showed a relationship between exercise intensity and β-endorphin release (21–23), a finding not supported by subsequent investigations (24, 25); the latest findings suggest that

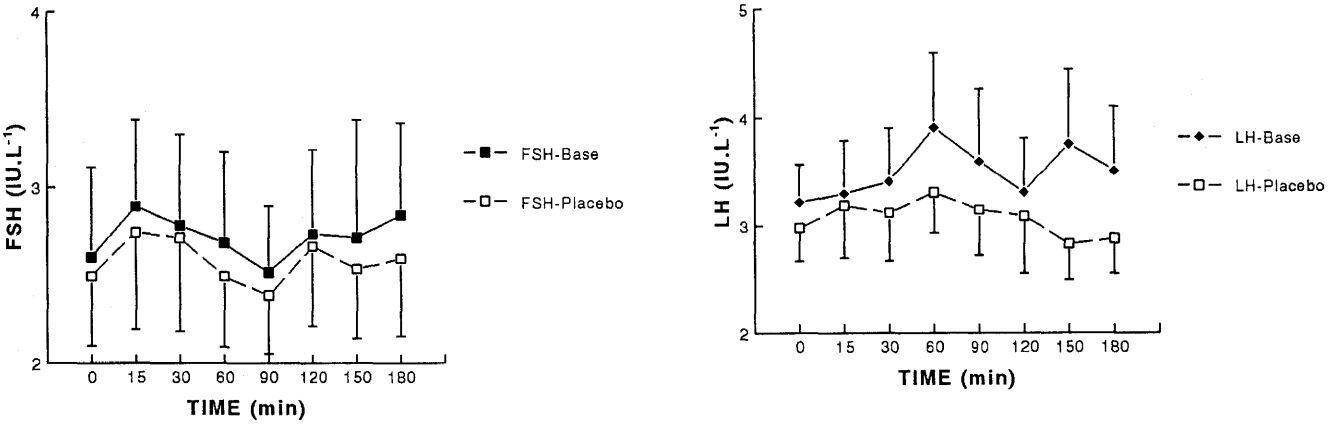

Figure 2. Serum FSH and LH concentrations during saline (placebo) and bicarbonate (base) treatment of male volunteers. Values are mean ± SEM.

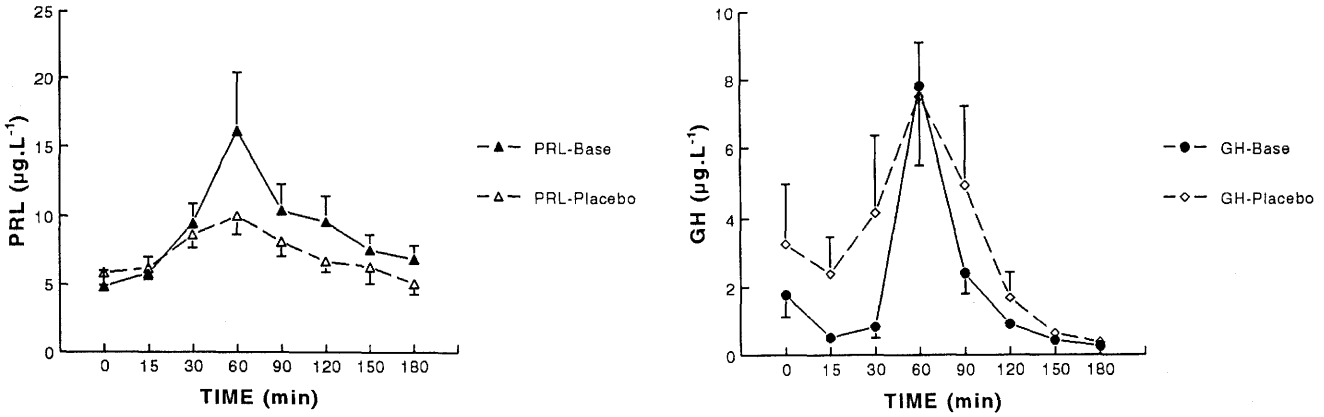

Figure 3. Serum GH and PRL concentrations during baseline (placebo) and bicarbonate (base) treatment of male volunteers. Values are mean ± SEM.

β-endorphin release during exercise is a simple function of the level of metabolic acidosis reached (15). Because of these reports, we investigated the role of metabolic acidosis in pituitary hormone levels following exercise.

In the present study, serum lactate concentrations rose significantly in both the saline- and base-treated volunteers. Serum LH and FSH release in the saline- and base-treated volunteers were not significantly different. However, GH was lower at baseline with base treatment but was not significantly different from placebo treatment at any other time of sampling following exercise. The lower baseline GH between base and placebo treatments may reflect day-to-day fluctuation in pulsatile GH secretion. Base treatment enhanced prolactin release except at the peak 60-min sample. This pattern of response suggests that pH drop with exercise might not be the mechanism responsible for previously reported changes in serum GH and gonadotropins. Similarly, pH-dependent events such as change in ionized calcium were not significantly different during placebo and base treatments. This does not, however, exclude the possibility of intracellular calcium changes being responsible for the hormonal profile with exercise.

The reasons for the enhanced PRL response to base treatment are unclear. Unlike the secretion of other anterior pituitary hormones which is stimulated by hypothalamic releasing hormones, prolactin release occurs primarily by modulation of inhibitory tone exerted by prolactin inhibitory factors the most important of which is dopamine. In animals pre-treated with sulpiride (a dopamine antagonist) prolactin response to exercise is significantly enhanced compared with non-sulpride-treated controls (26). In view of these observations one might speculate that exercise-induced drop in pH may have a limiting effect on PRL release which can be reversed by base treatment.

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