

# Changes in rectal temperature and hematologic, biochemical, blood gas, and acid-base values in healthy Labrador Retrievers before and after strenuous exercise

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**Objective**—To measure changes in rectal temperature and hematologic, biochemical, blood gas, and acid-base values before and after exercise.

**Animals**—14 healthy adult Labrador Retrievers.

**Procedure**—Dogs exercised continuously for 10 minutes by repeatedly retrieving a dummy thrown approximately 40 to 50 yards on land. The ambient temperature during each exercise period was recorded. Rectal temperature, pulse, and respiratory rate were measured; CBC and serum biochemical profile were determined; and arterial blood gas tensions, acid-base status, and plasma lactate and pyruvate concentrations were measured at rest and immediately after exercise. Rectal temperature, pulse, respiratory rate, and lactate and pyruvate concentrations were evaluated at intervals up to 120 minutes after exercise.

**Results**—Immediately after exercise, rectal temperature increased markedly; ambient temperature did not affect rectal temperature. Arterial blood pH and  $P_{aO_2}$  were significantly increased after exercise, and  $P_{aCO_2}$  and bicarbonate concentration were significantly decreased after exercise. Also, statistically, but not clinically, significant increases were observed in RBC, WBC, and segmented neutrophil counts; hemoglobin, total protein, and serum sodium and potassium concentrations; PCV; anion gap; and creatine kinase activity. Plasma lactate and pyruvate concentrations increased significantly after exercise, but there was no change in the lactate-to-pyruvate ratio.

**Conclusion and Clinical Relevance**—Reference values for healthy Labrador Retrievers during a standardized exercise protocol were established to compare data obtained from Labrador Retrievers with exercise intolerance and collapse. Important characteristics of lactate and pyruvate metabolism were documented that will enable more precise evaluation of exercise intolerance in this breed. (*Am J Vet Res* 1999;60:88–92)

recognized during retrieving drills. A syndrome that develops in young, adult, working Labrador Retrievers, whereby affected dogs became ataxic and collapsed after 5 to 15 minutes of strenuous exercise, with rectal temperatures  $> 41.5$  C and plasma lactate concentration between 8 and 10 mmol/L, has been reported.<sup>1</sup> The dogs were clinically normal at rest and after mild-to-moderate activity. Extensive workups, including cardiovascular and orthopedic evaluations, yielded normal results.<sup>1</sup> Rectal temperature and plasma lactate concentration in affected Labrador Retrievers were higher than those observed experimentally in mixed-breed dogs exercised on a treadmill for similar duration, prompting speculation that muscle oxidative metabolism may be abnormal in affected dogs.<sup>2</sup> Exercise causes significant alterations in rectal temperature, pulse, respiratory rate, blood-gas tensions, acid-base status, and indicators of oxygen debt, including lactate and pyruvate concentrations and the lactate-to-pyruvate ratio (L/P). These changes vary with the type and intensity of exercise performed. To assess the significance of the findings in Labrador Retrievers with exercise intolerance and collapse, it is necessary to compare their data with reference values for dogs of the same breed and age performing a standardized exercise. The study reported here was done to measure changes in rectal temperature and hematologic, biochemical, blood-gas, and acid-base values in healthy Labrador Retrievers before and after strenuous exercise.

## Materials and Methods

**Dogs**—Fourteen working Labrador Retrievers (6 males, 1 neutered male, 6 females, 1 spayed female), ranging in age from 1 to 8 (mean, 3.4) years, were healthy on the basis of history, results of physical examination (including orthopedic and neurologic examinations), CBC, serum biochemical profile, and urine specific gravity. All dogs were competitive field trial dogs with normal capacity for exercise.

**Study design**—Dogs exercised continuously for 10 min-

Exercise intolerance is frequently encountered in working Labrador Retrievers, and is often initially

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Table 1—Mean  $\pm$  SD rectal temperature, pulse, and respiratory rate before and immediately after exercise in 14 Labrador Retrievers

Variable	Time in relation to exercise							
	Before	Immediately	5 min	10 min	15 min	30 min	60 min	120 min
Rectal temperature (C)	39.4 $\pm$ 0.5	41.8 $\pm$ 0.3*	41.8 $\pm$ 0.3*	41.6 $\pm$ 0.3*	41.1 $\pm$ 0.4*	39.8 $\pm$ 0.5†	39.0 $\pm$ 0.3*	38.8 $\pm$ 0.4*
Pulse (beats/min)	99 $\pm$ 16	150 $\pm$ 20*	135 $\pm$ 16*	123 $\pm$ 20†	112 $\pm$ 11†	98 $\pm$ 21	93 $\pm$ 15	95 $\pm$ 15
Respiratory rate (breaths/min)	82 $\pm$ 12	183 $\pm$ 34*	231 $\pm$ 38*	220 $\pm$ 35*	218 $\pm$ 60*	160 $\pm$ 77*	79 $\pm$ 53	53 $\pm$ 40†

\*Significantly ( $P < 0.01$ ) different from pre-exercise value. †Significantly ( $P < 0.05$ ) different from pre-exercise value.

utes by repeatedly retrieving a soft plastic tube (retrieving dummy) thrown approximately 40 to 50 yards on land. This consisted of short bursts of strenuous exercise interspersed with brief (< 5 seconds) rest periods each time the dummy was thrown. Dogs typically sprinted at full speed to retrieve the dummy and returned to the handler, running at a slightly slower pace. Ambient temperature during each exercise period was recorded. Rectal temperature, pulse, respiratory rate, results of CBC, serum biochemical profile, arterial blood-gas and acid-base status, and plasma lactate and pyruvate concentrations were measured at rest and immediately (within 1 to 2 minutes) after exercise. Blood for measurement of arterial gas tensions was taken 15 minutes after exercise. Rectal temperature, pulse, and respiratory rate were measured 5, 10, 15, 30, 60, and 120 minutes after exercise, and venous blood was obtained for measurement of lactate and pyruvate concentrations at 15, 30, 60 and 120 minutes after exercise.

**Measurement of variables**—Venous blood was collected into plain and EDTA-containing tubes. Complete blood counting was done within 30 minutes of sample collection. Red and white blood cells and platelets were counted, and hemoglobin concentration was measured, using an electronic counter.<sup>a</sup> A blood smear was stained with Wright-Giemsa, and a differential WBC count was obtained. Serum was separated and frozen at  $-20$  C for biochemical analysis, which was done by use of an automated analyzer.<sup>b</sup> Blood from either the femoral or dorsal metatarsal artery was collected into a heparinized syringe and was analyzed immediately after collection by use of an analyzer.<sup>c</sup> Oxygen tension ( $P_{aO_2}$ ), carbon dioxide tension ( $P_{aCO_2}$ ), and pH were measured and corrected for rectal temperature, and bicarbonate concentration and base deficit were calculated. Venous blood for lactate analysis was collected into sodium fluoride-containing tubes. Plasma was separated within 30 minutes of collection, and was frozen and stored at  $-20$  C until analyzed. Lactate concentration was determined by use of an immobilized enzyme membrane system.<sup>d</sup> For pyruvate analysis, 1 ml of venous blood was mixed with an equal volume of 10% perchloric acid, then was centrifuged for 10 minutes. The supernatant was removed and frozen at  $-20$  C until analyzed. Pyruvate concentration was quantified by enzymatic determination, using lactate dehydrogenase, in a spectrophotometric assay.<sup>e</sup>

**Analysis of data**—Paired  $t$ -tests were used to identify significant differences between hematologic and serum biochemical variables before and immediately after exercise. Repeated measures ANOVA was used to compare values before exercise with values at each interval after exercise for temperature, pulse, respiratory rate, blood gas tensions, lactate and pyruvate concentrations, and L/P.<sup>f</sup> For dogs with rec-

Table 2—Mean  $\pm$  SD and range of arterial blood gas and acid-base variables before and after exercise in 14 Labrador Retrievers

Variable	Time in relation to exercise		
	Before	Immediately	15 min
pH	7.391 $\pm$ 0.023 (7.35–7.42)	7.600 $\pm$ 0.081* (7.42–7.66)	7.464 $\pm$ 0.062* (7.39–7.59)
$P_{aCO_2}$ (mm Hg)	33.7 $\pm$ 3.6 (27.5–40.1)	14.7 $\pm$ 4.9* (5.2–24.2)	24.5 $\pm$ 6.8* (13.9–33.0)
$P_{aO_2}$ (mm Hg)	102.0 $\pm$ 6.5 (91.6–113.1)	140.3 $\pm$ 17.8* (124.0–182.9)	101.6 $\pm$ 17.0 (72.7–122.3)
Bicarbonate (mmol/L)	19.6 $\pm$ 1.8 (15.7–21.4)	13.0 $\pm$ 3.2* (6.0–18.2)	16.3 $\pm$ 2.9* (10.5–20.8)

\*Significantly ( $P < 0.01$ ) different from pre-exercise value.

tal temperature above the upper limit of the thermometer (42.0 C), a value of 42.0 C was used for statistical analysis. For dogs with plasma pyruvate concentration less than the lowest detectable value of 0.05 mmol/L, this concentration was used for statistical analysis. Linear regression and correlation analysis were used to evaluate the effect of ambient temperature on plasma lactate concentration and rectal temperature immediately after exercise. For all hypothesis tests, values of  $P < 0.05$  were considered significant.

## Results

Over the 10-minute exercise period, each dog retrieved the dummy  $43.6 \pm 4.9$  (mean  $\pm$  SD) times, with a range of 36 to 51 times. Dogs were noticeably tired at the end of the exercise period and panted heavily, but all indicated eagerness to continue retrieving.

The effects of exercise on rectal temperature, pulse, and respiratory rate were determined (Table 1). Pulse and respiratory rate increased immediately after exercise, as would be expected. Immediately after exercise, all dogs had rectal temperature  $> 41.1$  C; 7 of the 14 dogs had temperature  $> 42.0$  C. Rectal temperature had returned to nearly baseline within 30 minutes, but was still significantly ( $P < 0.01$ ) increased. Rectal temperature 60 and 120 minutes after exercise was significantly lower than baseline values ( $P < 0.01$ ). Ambient temperature during the exercise periods ranged from 11 to 28 C, with a mean value of 16.7 C. In these dogs, there was no significant effect of ambient temperature during exercise on rectal temperature measurements immediately after exercise.

Arterial blood pH and Pao<sub>2</sub> were significantly ( $P < 0.01$ ) increased after exercise, and PaCO<sub>2</sub> and bicarbonate concentration were significantly ( $P < 0.01$ ) decreased after exercise (Table 2). Fifteen minutes after exercise, Pao<sub>2</sub> had returned to baseline. Arterial pH, PaCO<sub>2</sub>, and bicarbonate concentration were moving toward baseline at 15 minutes after exercise, but were still significantly ( $P < 0.01$ ) different from pre-exercise values. There were significant increases in RBC, WBC, and segmented neutrophil counts, PCV, hemoglobin and serum sodium and potassium concentrations, anion gap ( $P < 0.01$ ), total protein concentration, and creatine kinase activity ( $P < 0.05$ ) after exercise (Table 3).

The effect of exercise on plasma lactate and pyruvate concentrations was determined (Table 4). Plasma lactate concentration ranged from 0.53 to 3.07 mmol/L before exercise and from 0.80 to 9.86 mmol/L immediately after exercise. Plasma lactate concentration returned to baseline by 60 minutes after exercise. At 120 minutes after exercise, plasma lactate concentration was significantly ( $P < 0.01$ ) lower than baseline

Table 3—Mean  $\pm$  SD hematologic and biochemical values before and immediately after exercise in 14 Labrador Retrievers

Variable	Time in relation to exercise	
	Before	Immediately after
RBC ( $10^{12}/L$ )	7.14 $\pm$ 0.54	7.61 $\pm$ 0.57*
Hemoglobin (g/L)	172 $\pm$ 11	183 $\pm$ 11*
PCV (L/L)	0.47 $\pm$ 0.03	0.51 $\pm$ 0.03*
WBC ( $10^9/L$ )	9.51 $\pm$ 1.85	10.29 $\pm$ 1.92*
Neutrophils ( $10^9/L$ )	5.90 $\pm$ 1.32	6.77 $\pm$ 1.74*
Eosinophils ( $10^9/L$ )	0.77 $\pm$ 0.44	0.59 $\pm$ 0.36
Lymphocytes ( $10^9/L$ )	2.23 $\pm$ 0.78	2.47 $\pm$ 1.09
Monocytes ( $10^9/L$ )	0.59 $\pm$ 0.23	0.45 $\pm$ 0.23
Sodium (mmol/L)	152 $\pm$ 2	154 $\pm$ 2*
Potassium (mmol/L)	4.6 $\pm$ 0.3	5.0 $\pm$ 0.3*
Chloride (mmol/L)	122 $\pm$ 2.2	123 $\pm$ 1†
Calcium (mmol/L)	2.65 $\pm$ 0.13	2.63 $\pm$ 0.13
Urea (mmol/L)	7.3 $\pm$ 2.2	6.9 $\pm$ 2.1
Glucose (mmol/L)	5.4 $\pm$ 0.5	6.0 $\pm$ 1.7
ALP (U/L)	43 $\pm$ 21	46 $\pm$ 22
ALT (U/L)	64 $\pm$ 43	66 $\pm$ 41
Anion gap (mmol/L)	18 $\pm$ 3	23 $\pm$ 3*
CK (U/L)	105 $\pm$ 29	143 $\pm$ 62†
Total protein (g/L)	61 $\pm$ 3	61 $\pm$ 4†

\*Significantly ( $P < 0.01$ ) different from pre-exercise value. †Significantly ( $P < 0.05$ ) different from pre-exercise value.

Seg = segmented; ALP = alkaline phosphatase; ALT = alanine transaminase; CK = creatine kinase.

values. There was no significant effect of ambient temperature during exercise on plasma lactate concentration immediately after exercise. Plasma pyruvate concentration ranged from 0.05 to 0.12 mmol/L before exercise and from 0.05 to 0.32 mmol/L immediately after exercise. Serum pyruvate concentration returned to baseline within 60 minutes. There was no significant difference in the L/P value at any interval after exercise, compared with the pre-exercise value.

## Discussion

We identified changes in rectal temperature, pulse, respiratory rate, acid-base and blood gas values, and plasma lactate and pyruvate concentrations after exercise in healthy Labrador Retrievers. We established reference values for Labrador Retrievers during a standardized exercise protocol to compare data with those obtained from Labrador Retrievers with exercise intolerance and collapse. To assess the significance of the findings in affected Labrador Retrievers, reference ranges for Labrador Retrievers after a similar period of strenuous exercise were needed. To evaluate sporting dogs for exercise intolerance, control values for each type of exercise are necessary because response varies with the type, duration, and intensity of exercise performed.<sup>2,5</sup> Differences may also exist among dogs of various breeds or body types, but this has not been critically evaluated.

Working Labrador Retrievers will voluntarily exercise strenuously to the point of exhaustion because of their drive to retrieve. Exercise intolerance in these dogs is most often recognized during retrieving drills; therefore, a standardized exercise regimen similar to that used in intensive field training was developed for this study. Retrieving handthrown dummies on land provided a clinically relevant way to strenuously exercise Labrador Retrievers that could be readily reproduced for evaluation of clinical patients. This type of exercise was preferable to use of a treadmill because it would be difficult, using a treadmill, to mimic the repeated short bursts of intense exercise characteristic of retrieving.

After 10 minutes of strenuous exercise, all of our dogs had a marked increase in rectal temperature, indicating that an increase of up to 3 C is normal for healthy, strenuously exercising Labrador Retrievers. Rectal temperature increases after exercise because a portion of nutrient energy is converted to heat during

Table 4—Mean  $\pm$  SD lactate and pyruvate concentrations and the lactate-to-pyruvate ratio before and after exercise in 14 Labrador Retrievers

Variable	Time in relation to exercise					
	Before	Immediately	15 min	30 min	60 min	120 min
Lactate (mmol/L)	1.31 $\pm$ 0.61	3.57 $\pm$ 2.22*	3.31 $\pm$ 1.50*	2.04 $\pm$ 0.67*	1.08 $\pm$ 0.35	0.80 $\pm$ 0.37*
Pyruvate (mmol/L)	0.082 $\pm$ 0.022	0.192 $\pm$ 0.081*	0.172 $\pm$ 0.049*†	0.113 $\pm$ 0.047‡	0.074 $\pm$ 0.0285	0.073 $\pm$ 0.031
Lactate/Pyruvate	17.0 $\pm$ 8.2	20.5 $\pm$ 5.9	21.2 $\pm$ 5.7†	20.6 $\pm$ 5.2	15.7 $\pm$ 3.25	12.1 $\pm$ 4.3

\*Significantly ( $P < 0.01$ ) different from pre-exercise value. †Measurements available from 12 dogs. ‡Significantly ( $P < 0.05$ ) different from pre-exercise value. §Measurements available from 13 dogs.

cellular metabolism.<sup>6</sup> Rectal temperature in our Labrador Retrievers after this exercise regimen was higher than that observed in mixed-breed dogs running on a treadmill for approximately 45 minutes or in Greyhounds racing for 48 seconds ( $40.1 \pm 0.5$  C and  $40.6 \pm 0.3$  C, respectively).<sup>3,5</sup> Temperature of our dogs was similar to that documented in mixed-breed dogs run to exhaustion on a treadmill, defined as inability to keep pace with the treadmill, evidenced by stumbling and collapse (mean duration of run to exhaustion was  $57 \pm 8$  minutes; mean rectal temperature after exercise was  $41.8 \pm 0.2$  C).<sup>7</sup> In contrast, the Labrador Retrievers of this study tired and panted heavily after strenuous exercise, but did not have difficulty completing the exercise period; they had only a slight decrease in speed, and all dogs were eager and willing to continue the exercise.

Concurrent respiratory alkalosis and metabolic acidosis reflected hyperventilation and strenuous anaerobic activity in these dogs. A similar metabolic pattern in Greyhounds immediately after racing was reported,<sup>5,8</sup> although the respiratory alkalosis was more marked and the metabolic acidosis was less pronounced in our Labrador Retrievers, compared with the racing Greyhounds. Because hydrogen ions generated in association with the production of lactate are buffered principally by bicarbonate, lactate accumulation results in decreased bicarbonate concentration and metabolic acidosis. Hyperventilation caused increased  $\text{PaO}_2$ , decreased  $\text{PaCO}_2$ , and respiratory alkalosis. The magnitude of this change was striking; 1 of our dogs had  $\text{PaCO}_2$  of 5.2 mm Hg and pH of 7.68. In dogs performing mild to moderate exercise on a treadmill, hyperventilation, hypocapnia, and increased pH occurred almost immediately, suggesting that hyperventilation was not simply a consequence of metabolic acidosis.<sup>2</sup> The degree of hypocapnia and hyperventilation did not change even as the dogs worked toward maximal exercise with increasing lactic acid and decreasing pH values.<sup>2</sup> Possible explanations for the hyperventilation include response to increased  $\text{O}_2$  demand, response to increased body temperature, concurrent transmission of neurologic impulses to contracting muscles and the respiratory center, and stimulation of the respiratory center by neurologic impulses from joint proprioceptors.<sup>2,6</sup>

Significant increases in many hematologic and serum biochemical variables were observed in these Labrador Retrievers, similar to those reported in racing Greyhounds.<sup>5,9</sup> Although the changes were significant, none of the values in the dogs of this study were increased outside of the reference ranges and, thus, are of limited clinical relevance.

After 10 minutes of strenuous exercise, plasma lactate concentration increased markedly in the clinically normal Labrador Retrievers of this study. Increased plasma lactate concentration after exercise has traditionally been attributed to anaerobic muscle metabolism as portions of muscle become hypoxic during intense exercise.<sup>10</sup> To generate ATP for energy during exercise, glucose is converted to pyruvate, which enters the Krebs cycle and undergoes oxidative metabolism, liberating ATP. Oxygen is required for this path-

way of pyruvate metabolism. If oxygen demand is greater than the oxygen supply to muscle, anaerobic conversion of pyruvate to lactate occurs (anaerobic glycolysis).<sup>6,11</sup> The dogs of our study had increased plasma lactate concentration, but were not hypoxemic and actually had increased  $\text{PaO}_2$ . It is likely that arterial oxygen tension does not accurately reflect intracellular oxygen content. Intracellular oxygen concentration was not measured because the procedure is technically difficult and has limited availability.<sup>12</sup> The role of oxygen in lactate metabolism is controversial, and factors other than inadequate mitochondrial oxygen may be responsible for increased lactate concentration during exercise. Muscle fiber type,  $\beta$ -adrenergic stimulation, muscle contraction pattern, duration of muscle contraction, and substrate availability have been implicated.<sup>13,14</sup>

After only a brief period of strenuous voluntary exercise, the healthy Labrador Retrievers of this study generated plasma lactate concentration similar to that of dogs forced to undergo maximal or exhaustive exercise on a treadmill. Plasma lactate concentration in foxhounds after maximal exercise (defined as no further increase in heart rate and cardiac output with increasing work intensities) was  $2.71 \pm 0.4$  mmol/L.<sup>15</sup> Maximal lactate concentration of 9.86 mmol/L observed in 1 of our clinically normal Labrador Retrievers was approaching  $11.7 \pm 0.7$  mmol/L, the reported concentration in dogs run to exhaustion (maximal heart rate and animal beginning to falter) on a treadmill.<sup>3</sup> These concentrations are higher than mean arterial lactate concentration  $< 2$  mmol/L in mixed-breed dogs exercising moderately on a treadmill for 4 to 6 minutes,<sup>2</sup> but are lower than the plasma lactate concentration of  $28.93 \pm 2.99$  mmol/L in Greyhounds immediately after racing for 48 seconds in another report.<sup>5</sup> It is clear that lactate concentration varies in dogs depending on the duration and intensity of exercise performed. Lactate concentration that exceeds the concentration in clinically normal dogs performing exercise of the same duration and intensity may be evidence of an underlying disorder of metabolism.<sup>16-18</sup> Defects of oxidative metabolism, including deficiency of pyruvate dehydrogenase or pyruvate carboxylase, and defects of the Krebs cycle or the mitochondrial respiratory chain will result in increased blood lactate concentration.<sup>16,19</sup>

Alteration in the L/P is determined to help localize the enzymatic defect in human beings with pathologic lactic acidemia.<sup>19</sup> Increase in lactate and pyruvate concentrations with normal L/P suggests a defect in pyruvate dehydrogenase or gluconeogenic enzymes, whereas increased L/P is consistent with a defect in the electron transport chain, pyruvate carboxylase deficiency, or mitochondrial myopathy.<sup>19</sup> In healthy human beings, the L/P increases during exercise because of a marked and more rapid increase in lactate than pyruvate concentration.<sup>20</sup> Within 5 minutes after exercise, the L/P begins to decrease, as lactate concentration rapidly decreases, but pyruvate concentration still increases slowly.<sup>20</sup> Because there was no significant difference between L/P before exercise, compared with any point evaluated after exercise, in our clinically nor-

mal Labrador Retrievers, direct correlations with alterations in the L/P in human beings with pathologic lactic acidemia are not possible.

Although the precise explanation for the lack of increase in L/P after exercise in the clinically normal Labrador Retrievers is not known, it may be explained by the difference in muscle fiber types between dogs and people. Human skeletal muscle is composed of 4 fiber types: type I (slow-twitch, oxidative, fatigue-resistant), type IIA (fast-twitch, oxidative/glycolytic, fatigue-resistant), and type IIB (fast-twitch, glycolytic fatiguing); type-IIC fibers are rare.<sup>21</sup> There is greater lactate concentration in type-IIB fibers than in type-I fibers, owing to their predominantly glycolytic activity.<sup>14</sup> There are no classic type-IIB fibers in canine skeletal muscle; thus, it is highly oxidative, compared with human muscle.<sup>22</sup> Because of the high oxidative capacity and the absence of the highly glycolytic type-IIB fibers, lower lactate concentration and less difference between lactate and pyruvate values (resulting in unaltered L/P) may be expected in exercising dogs, compared with people.

In conclusion, significant changes in temperature, blood gas tensions, and acid-base status occur in healthy Labrador Retrievers after they perform strenuous exercise for 10 minutes. The magnitude of change that developed in these healthy Labrador Retrievers, in conjunction with the wide variation in values reported from other studies, underscores the necessity for using a standardized exercise protocol and appropriate control values when evaluating dogs with exercise intolerance. In addition, important characteristics of lactate and pyruvate metabolism were documented that will enable more precise evaluation of exercise intolerance in this breed.

\*Coulter Counter S + IV, Coulter Electronics, Hialeah, Fla.

<sup>b</sup>Abbott Spectrum II, Abbott Park, Chicago, Ill.

<sup>c</sup>ABL 330, Radiometer, Copenhagen, Denmark.

<sup>d</sup>YSI Inc, Yellow Springs, Ohio.

<sup>e</sup>Test run by Dr. Mitch Halperin, St. Michael's Hospital, Toronto, Ontario, Canada.

<sup>f</sup>BMDP Statistical Software (1990), Los Angeles, Calif.

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