Intravascular infusions of plasma into fetal sheep cause arterial and venous hypertension

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Submitted 30 December 2004; accepted in final form 19 April 2005

Intravascular infusions of plasma into fetal sheep cause arterial and venous hypertension. J Appl Physiol 99: 884–889, 2005. First published May 5, 2005; doi:10.1152/japplphysiol.01429.2004.—Fetal volume control is driven by an equilibrium between fetal and maternal hydrostatic and oncotic pressures in the placenta. Renal contributions to blood volume regulation are minor because the fetal kidneys cannot excrete fluid from the fetal compartment. We hypothesized that an increase in fetal plasma protein would lead to an increase in plasma oncotic pressure, resulting in an increase in fetal arterial and venous pressures and decreased angiotensin levels. Plasma or lactated Ringer solution was infused into each of five twin fetuses. After 7 days, fetal protein concentration was 71.2 ± 4.2 g/l in the plasma-infused fetuses compared with 35.7 ± 6.3 g/l in the lactated Ringer-solution-infused fetuses. Arterial pressure was 68.0 ± 3.6 compared with 43.4 ± 1.9 mmHg in the lactated Ringer solution-infused fetuses (P < 0.0003), whereas venous pressure was 4.8 ± 0.3 mmHg in the plasma-infused fetuses compared with 3.3 ± 0.4 mmHg in the lactated Ringer solution-infused fetuses (P < 0.036). Six fetuses were studied on days 0, 7, and 14 of plasma protein infusion. Fetal protein concentration increased from 31.1 ± 1.5 to 84.8 ± 3.8 g/l after 14 days (P < 0.01), and arterial pressure increased from 43.1 ± 1.8 to 69.1 ± 4.1 mmHg (P < 0.01). Venous pressure increased from 3.0 ± 0.4 to 6.2 ± 1.3 mmHg (P < 0.05). Fetal heart rate did not change. Angiotensin II concentration decreased, from 24.6 ± 5.6 to 2.9 ± 1.3 pg/l, after 14 days (P < 0.01). Fetal plasma infusions resulted in fetal arterial and venous hypertension that could not be corrected by reductions in angiotensin II levels.

According to a widely accepted model of adult long-term cardiovascular regulation (10, 17, 18), the adult body regulates arterial pressure by modulating renal excretion of sodium and fluid. Fetal cardiovascular regulation, on the other hand, is likely somewhat different because fetal urine is not lost to the external environment. Rather, fetal urine becomes part of the amniotic fluid, a fetal compartment. As a result, fetal urination does not constitute elimination because exchange of electrolytes and fluid between amniotic fluid and fetal extracellular fluid takes place by at least four different pathways (8). Because fetal urination cannot regulate fetal arterial blood pressure by altering fetal renal excretion of sodium and fluid, control of fetal vascular volume and arterial blood pressure must lie elsewhere. Exchange between amniotic fluid and maternal extracellular fluid (without first passing through the fetus and the placenta to the maternal circulation) is only barely detectable, even under the influence of extreme osmotic gradients (5). There is a further difference between adult and fetal physiology. During the infusion of a volume of isotonic saline as large as 4 liters into a 3-kg fetal sheep over a period of 4 h, arterial pressure increases only 7 mmHg and just for a short period of time (9). The modest elevation in arterial pressure returns to normal within 1 h after the majority of the infusate is lost to the maternal circulation. Infusion of a similar volume per kg body weight into an adult would result in a prolonged increase in extracellular volume, if it could be tolerated at all. Clearly, the fetus is able to make rapid adjustments in response to excess levels of sodium and fluid.

The renin-angiotensin system is an important regulator of blood pressure and fluid volume in the fetus and in the adult. Infusions of angiotensin I or angiotensin II into fetal sheep produce substantial increases in fetal blood pressure (4, 12, 26). Similarly, infusions of angiotensin I or angiotensin II increase arterial pressure in the adult (11, 16, 20, 28). Blockade of type 1 angiotensin receptors results in a decrease in blood pressure (23, 24, 27) in the fetus and in the adult (19, 28, 29). Graded decreases in renal perfusion pressure result in a substantial activation of the renin-angiotensin system in the fetus (6). Bilateral nephrectomy results in a decrease in plasma renin activity, which not only stops the normal gestational increase in arterial pressure but also leads to a progressive decline in fetal arterial pressures (3). It is clear from these observations that the renin-angiotensin system plays a central role in the regulation of fetal arterial pressure, as it does in the adult. It is important to know whether fetal arterial pressure can be persistently elevated under conditions of low circulating angiotensin.

With these factors in mind, the present study is an attempt to explore one of the potential differences in the regulation of arterial pressure in the fetus compared with the adult. In the adult, vascular volume is modulated by renal excretion of sodium and water. In the fetus, vascular volume control is effected in the placenta and is driven by the requirement that there is equilibrium between fetal and maternal hydrostatic and oncotic pressures in the presence of zero net fluid transfer (1, 13, 15). Because the renin-angiotensin system plays an important role in the determination of fetal arterial pressure, angiotensin II could be involved in the mechanism underlying the plasma protein-induced fetal hypertension. We hypothesized that an increase in fetal plasma protein would lead to an increase in plasma oncotic pressure. The increase in fetal oncotic pressure would lead to net fluid flow from the mother.
to the fetus, resulting in an increase in fetal arterial and venous pressures, but a decrease in the circulating angiotensin II levels. To test this hypothesis, we divided the study into two parts: a short-term, 1-wk-long protocol, to establish that plasma-infused fetuses develop hypertension compared with control twins that are given equal volumes of lactated Ringer solution; and a long-term protocol, of 2-wk duration, to establish the nature of the hypertensive response to continued plasma protein infusion and to identify the role played by angiotensin.

**METHODS**

**Animal preparations.** Methods of anesthesia and surgery on pregnant ewes and their fetuses and all experimental procedures were approved by the Oregon Health and Sciences University Animal Care and Use Committee. Details have been published (4, 12). Time-bred ewes of mixed Western breeds were obtained from a commercial source. In short, the ewes were premedicated with 7.5 mg atropine im, 10 mg diazepam iv, and 400 mg ketamine iv; intubated; and continued on a 75% oxygen and 25% nitrous oxide mixture with 1% halothane. The halothane concentration was adjusted as necessary, and additional doses of diazepam or ketamine were administered to ensure a surgical level of anesthesia in both the ewe and the fetus.

The fetal sheep were instrumented in a sterile fashion at a gestational age of 118–122 days (mean 120 days). Indwelling pedal artery and saphenous vein catheters were placed in each fetus and amniotic fluid catheters were sewn to the fetal skin. All incisions were closed in layers, and the catheters were tunneled underneath the skin of the ewe. Catheters emerged at the ewe’s flank and were stored in a pouch sewn to the skin. The ewes received routine postoperative pain medication (0.6 mg buprenorphine, twice a day) for 2 days. In five ewes with twin fetuses, each fetus was instrumented for daily study of the short-term effects of plasma infusion over 7 days, one fetus receiving an infusion of plasma and the other fetus an infusion of lactated Ringer solution. Two of these twins underwent plasma infusion for 14 days. An additional four single fetuses were instrumented for study at 0, 7, and 14 days of plasma infusion to understand the long-term effects of plasma infusion.

**Experimental protocol.** During the experiments, the ewes were kept in stanchions in the laboratory (4–6). The baseline experiments were performed at an average gestational age of 128 days (range 125–131 days). Fetal arterial and venous blood pressures and amniotic fluid pressures were measured continuously. The results taken over a 60-min period were averaged and recorded. Heart rates were obtained from arterial pressure records. Fetal arterial blood samples were taken for determination of angiotensin II concentration, for fetal blood gases and pH, and for plasma protein concentration.

After baseline measurements were made, intravenous infusion of adult sheep plasma or lactated Ringer solution was started using a Gilson Minipuls-3 roller pump. In two fetuses, no venous catheter was available (due to a kink of the catheter in the subcutaneous tunnel), and the infusions were administered intra-arterially. The plasma infusion rate was started at 229 ± 5 (SE) ml/day and increased by 3.5%/day to correspond to the percent increase in fetal size due to growth. Preliminary experiments for the present study demonstrated that infusion of this volume of (protein-free) fluid into the fetus results in no changes in blood pressures or heart rate. Other researchers have infused normal saline at 150 ml/h and observed no change in arterial blood pressure in the ovine fetus (22). Initially, the plasma protein infusion rate was 14.1 ± 0.5 g/day. In the lactated Ringer solution-infused group, the infusion was started at 229 ± 5 (SE) ml/day and also increased by 3.5%/day. These infusions were continued for 7 days. After 7 days, the plasma infusion group received a total of 108 ± 3.6 g protein and 1,821 ± 31 ml of fluid. The short-term lactated Ringer solution group received 1,821 ± 31 ml of fluid. In the fetuses receiving a 14-day infusion, the plasma protein infusion rate was started at 219 ± 6 ml/day (13.4 ± 0.4 g/day) and increased by 3.5%/day. After 14 days, the long-term plasma infusion group received a total of 238 ± 15 g protein and 3,885 ± 251 ml of fluid.

In five of the twins, hemodynamic measurements were measured continuously. Reported pressures were averaged over a 60-min period. Blood samples were taken on day 0 and day 7. In fetuses that received 14 days of plasma protein infusion, hemodynamic measurements and blood samples occurred on day 0, day 7, and day 14.

**Instrumentation.** Pressures were measured with Abbott Transpac pressure transducers and a computerized recording system. The system was calibrated daily against a mercury manometer to a scale value of better than 1% and rezeroed for drift before each measurement. Repeat calibrations established an accuracy of 0.5 mmHg. All fetal intravascular pressures were referred to amniotic fluid pressure as zero.

**Laboratory measurements.** Two-milliliter arterial blood samples for determining plasma angiotensin II concentrations were collected in EDTA (Becton Dickinson), they were immediately separated in a refrigerated centrifuge, and the plasma samples were stored in a freezer. Angiotensin II concentrations were determined with Peninsula Laboratories radioimmunoassay kit RIK 7002. Arterial samples for other analyses were anticoagulated with heparin. A refractometer was used to obtain rapid estimates of plasma protein concentrations during the experiments. These estimates were not used in the final data analysis. Plasma protein concentrations were measured with a commercially produced version of the Lowry method (Sigma Diagnostics). Blood-gas partial pressures and pH were determined in an Instrumentation Laboratories model IL-1610 blood-gas analyzer at 39°C. Hemoglobin concentrations and oxygen content were determined in an IL-842 CO-oximeter.

**Plasma source.** Sheep plasma was obtained from ewes that were to be euthanized. The ewes were medicated as described above for induction of anesthesia and given additional doses of ketamine to maintain a deep level of anesthesia. About 10,000 units of heparin were administered intravenously. A large-bore sterile catheter was inserted into a carotid artery and the animal was exsanguinated into 1-liter sterile bottles. Each bottle contained 10,000 units of heparin. The collected blood was immediately centrifuged in sterile containers, and the plasma was separated. Our main concern with the methodology was to not raise the peripheral resistance of the plasma-infused fetuses by microembolization. For this reason, as well as to ensure final sterility, all collected adult plasma was subjected to ultrafiltration first through a sterile Corning cellulose acetate 0.45-µm filter and next through a similar 0.22-µm filter. Because capillary diameters are typically 20 times larger, this eliminated physical blockage as a cause of concern. We also observed that the two fetuses that were given intra-arterial infusions (for lack of venous access) responded like the other fetuses. Cultures confirmed the sterility of the prepared plasmas. One million units of penicillin-G per liter were then added, and the plasmas were stored at room temperature until use.

**Statistical methods.** All statistical analyses were performed with GraphPad Prism (GraphPad Software, San Diego, CA). In the twins that were studied daily for 7 days, repeated-measures analysis of variance with Dunnett’s multiple comparison test was used to determine whether and when the daily measurement value differed from the baseline, day 0 value. A level of 0.05 was used to determine whether the day 0 value for the lactated Ringer solution infusion group differed from the plasma infusion group. Similarly, the nonpaired t-test was used to determine whether the day 7 values for the lactated Ringer solution infusion group differed from the plasma infusion group.

In the studies done on day 0, day 7, and day 14, repeated-measures analysis of variance with Dunnett’s multiple comparison test was used to determine whether the day 7 and/or day 14 measurement values differed from the baseline, day 0 value. A level of $P < 0.05$ was
required for any result to be considered statistically significant. Results are presented as means ± SE.

RESULTS

To understand the short-term time course of the effects of plasma infusion on arterial pressure, plasma was infused for 7 days into one fetus of a twin pregnancy and lactated Ringer solution was infused for 7 days into the other fetus. Fetal plasma protein increased from 30.0 ± 1.3 to 71.2 ± 4.2 g/l after 7 days of plasma infusion. Fetal plasma protein was 30.0 ± 1.1 before and 35.7 ± 6.3 g/l after 7 days of lactated Ringer solution infusion. The daily measurements for arterial pressure, venous pressure and heart rate for the plasma-infused fetuses and for the lactated Ringer solution-infused control fetuses are shown in Fig. 1. There were no differences in arterial pressures, venous pressures, or heart rates at day 0 for the plasma-infused fetuses compared with the lactated Ringer solution-infused twin fetuses. Arterial pressure increased steadily over the 7-day infusion period in the plasma-infused fetus (\( P < 0.05 \) at day 2 of infusion and \( P < 0.01 \) at day 3 of infusion and beyond compared with baseline, day 0). At 7 days, arterial pressure was 68.0 ± 3.6 mmHg in the plasma-infused fetuses compared with 43.4 ± 1.9 mmHg in the lactated Ringer solution-infused fetuses (\( P < 0.0003 \)). There was a slight increase in arterial pressure in the lactated Ringer solution-infused twin fetuses that reached statistical significance (\( P < 0.05 \)) on day 7 of lactated Ringer solution infusion compared with baseline, day 0. This is the expected normal increase in arterial pressure observed during late gestation. There was a gradual increase in venous pressure in the plasma-infused fetuses. After 7 days, venous pressure was 4.8 ± 0.3 mmHg in the plasma-infused fetuses compared with 3.3 ± 0.4 mmHg in the lactated Ringer solution-infused fetuses (\( P < 0.036 \)). There was no significant change in heart rate over the 7-day period for the plasma-infused fetuses or the lactated Ringer solution-infused twin fetuses.

To understand the long-term effects of plasma infusion on fetal hemodynamics and angiotensin II arterial blood levels, six fetuses were studied on day 0 (before plasma protein infusion), after 7 days of plasma protein infusion, and after 14 days of plasma protein infusion. The results from the measurements for this series of experiments are illustrated in Figs. 2 and 3. Fetal plasma protein concentration at control, day 0, was 31.1 ± 1.5 g/l, and it rose to 79.1 ± 3.5 g/l after 7 days of plasma infusion (\( P < 0.01 \)) and to 84.8 ± 3.8 g/l after 14 days of infusion (\( P < 0.0003 \)).
blood pH, PCO2, PO2, hematocrit, hemoglobin, or oxygen concentration showed no significant change after 7 days of infusion. Fetal arterial blood pH was 7.370 ± 0.018 before lactated Ringer solution infusion and 7.353 ± 0.008 after 7 days. Fetal arterial PCO2 was 55 ± 1 Torr before lactated Ringer solution infusion and 55 ± 1 Torr after 7 days. Fetal arterial PO2 was 19 ± 1 Torr before lactated Ringer solution infusion and 18 ± 2 Torr after 7 days. Fetal hematocrit was 37 ± 2% before lactated Ringer solution infusion and 38 ± 6% after 7 days. Fetal hemoglobin concentration was 122.0 ± 7.1 g/l before lactated Ringer solution infusion and 134.8 ± 19.6 g/l after 7 days. Oxygen content was 79.5 ± 3.2 ml/l before lactated Ringer solution infusion and 70.3 ± 8.1 ml/l after 7 days.

Fetal arterial blood pH did not change significantly over the 14-day infusion period. Fetal arterial blood pH was 7.355 ± 0.007 before plasma protein infusion and 7.323 ± 0.007 after 7 days and 7.267 ± 0.039 after 14 days. Fetal arterial PCO2 showed a small increase from 53 ± 1 Torr before plasma protein infusion to 54 ± 2 Torr after 7 days and 56 ± 2 Torr after 14 days (P < 0.01). Fetal arterial PO2 declined from 19 ± 1 Torr before plasma protein infusion to 16 ± 2 Torr after 7 days and to 15 ± 1 Torr after 14 days (P < 0.01). Fetal hematocrit did not change significantly over the 14-day infusion period. Fetal blood hematocrit was 37 ± 1% before plasma protein infusion and 36 ± 2% after 7 days and 32 ± 3% after 14 days. Fetal hemoglobin concentration also did not change significantly over the 14-day infusion period. Fetal hemoglobin concentration was 121.5 ± 5.6 g/l before plasma protein infusion and 119.0 ± 6.2 g/l after 7 days and 100.6 ± 12.6 g/l after 14 days. Oxygen content decreased from 82.4 ± 3.4 ml/l before plasma protein infusion to 66.7 ± 11.6 ml/l after 7 days and to 43.4 ± 9.5 ml/l after 14 days (P < 0.05).

**DISCUSSION**

The main findings of this study are that fetal intravascular plasma infusion caused an increase in fetal plasma protein concentration, an increase in arterial blood pressure, and an increase in central venous pressure. Infusion of plasma into the fetus for 14 days led to a 60% increase in arterial pressure and a 107% increase in central venous pressure. The mechanism by which these vascular pressures increase is not clear. The only other intervention that produces similar severe and lasting arterial and venous hypertensions in fetal sheep is the infusion of angiotensin (4, 12, 21, 24). The hypertension previously produced with angiotensin infusion was not thought to be due to an immediate effect of angiotensin on somatic vascular smooth muscle (12, 14). Unlike the hypertension produced by infusion of epinephrine or norepinephrine (7), there was no immediate change in blood pressure when the angiotensin infusion was started. The fetuses also remained hypertensive after the angiotensin infusion was stopped (12).

Movement of fluid from the mother to the fetus across the placenta is affected by hydrostatic and oncotic pressure gradients. It is possible that angiotensin promotes transplacental fluid volume flow from mother to fetus by precapillary fetal placental vasoconstriction, as originally proposed by Adamson et al. (1, 2), and that the subsequent fetal vascular overload causes the increase in arterial pressure and central venous pressure, as would be predicted with adult theory (10, 17, 18). This explanation appeared to be contradicted by the observation that even large infusions of electrolyte solutions into fetal...
sheep produced only slight increases in arterial blood pressure and then only transiently (9). However, the authors of that study reasonably concluded that much of the infused volume was rapidly lost to the maternal circulation due to reversal of transplacental gradients (9). The present study supports the view that the relative ineffectiveness of saline infusions is indeed due to rapid fluid loss across the placenta and that this loss can be prevented by increasing the oncotic pressure of the fetal plasma. In the present study, infusion of plasma into the fetus led to a substantial increase in arterial pressure and in central venous pressure. These findings were associated with a decrease in angiotensin II levels consistent with a decreased activity of the fetal renin-angiotensin system. Fetal arterial angiotensin-II concentration decreased by 58% after 7 days of plasma infusion and by 88% after 14 days of plasma infusion. As a result, it is unlikely that the renin-angiotensin system induced the increase in fetal arterial and venous pressure seen with plasma infusion. In fact, the renin-angiotensin system intervened by decreasing its activity in an attempt to reverse the increase in blood pressure resulting from plasma protein infusion but was ineffective due to the large increase in fetal oncotic pressure. One likely mechanism for the increase in both arterial and venous pressure in the present study is that the increased oncotic pressure due to the elevated fetal plasma protein maintained a higher fetal fluid volume, resulting in increased fetal arterial and central venous pressure.

Compared with the adult, study of the regulation of blood pressure in the fetus is complicated by the growth of the fetus. The sheep fetus is growing at 3–4%/day, increasing body size, blood volume, arterial pressure, and cardiac output continuously over time. The relationship between the determinants of arterial pressure, i.e., peripheral vascular resistance and cardiac output, is more complicated in the fetus compared with the adult. In the fetus, total cardiac output is combined right ventricular and left ventricular output. As in the adult, the fetal left ventricular output goes to the aorta. However, in the fetus only a small proportion (10%) of right ventricular output goes to the lung, with the majority of right ventricular output going to the descending thoracic aorta via the ductus arteriosus. The biventricular output is divided between the somatic vascular bed of the fetus and the placental vascular bed and thus fetal arterial blood pressure is under the influence of fetal somatic vascular resistance and placental vascular resistance.

Any experimental changes in fetal blood volume are difficult to identify because they are superimposed on the natural increases in fetal blood volume of 3–4%/day associated with fetal growth. It is known that infusion of plasma protein increases oncotic pressure and expands blood volume. Therefore, fetal blood volume is increasing as a result of both an increase in oncotic pressure and growth over the time of the experiments. It is possible to predict changes in fetal blood volume using the computer model developed by Faber and Anderson (13). Taking into consideration the venous pressures and angiotensin II concentrations measured on days 0, 7, and 14 in the above experiments, the predicted fetal blood volumes would be 126.8, 134.0, and 139.5 ml/kg fetal weight. A fetus growing at 3.5%/day would show a 62% increase in size over the 14 days of the experiment. The level of error inherent in the determination of blood volume makes it unlikely that any change in fetal blood volume could be measured in these fetuses. These relatively small changes, combined with the limitations of blood volume measurement, make accurate determination of fetal blood volume during the conditions of these experiments unrewarding. As a result, fetal blood volume was not measured in the present study. Hematocrit or hemoglobin is sometimes used as a surrogate to estimate changes in blood volume over time. There was a downward trend in fetal hematocrit from 37% on day 0 to 32% on day 14. Hematocrit and hemoglobin are good markers of acute changes in plasma volume but are not good markers for chronic changes due to variations in erythropoiesis. However, in this study, the concurrent fetal hypoxemia may have driven fetal erythropoiesis, increasing fetal red cell mass, potentially masking an increase in blood volume as indexed by a decreasing hematocrit. The intermediate step after the increase in blood volume, that is the increase in venous pressure, was detectable in this study. All things taken together, a small increase in blood volume is a possible explanation.

Other mechanisms for the plasma protein-induced hypertension should be considered. It is possible that a vasoactive substance present in the infused plasma protein causes an increase in vascular resistance and thus fetal arterial pressure. This is an unlikely explanation because arterial blood pressure does not increase until ~24 h of plasma infusion. It is also possible that a vasoactive substance could be released by the fetus or by the placenta during plasma protein infusion that results in an increase in vascular resistance and thus fetal arterial pressures. Angiotensin is such a substance; however, angiotensin II levels sharply decreased during plasma protein infusion, as would be expected in view of normal fetal regulation of arterial blood pressure (6, 13). Other vasoactive substances present in maternal plasma could increase fetal arterial pressure. Such a vasoactive substance must have an exceptionally long half-life because arterial pressure remained elevated in fetuses studied up to 5 days after discontinuation of plasma infusion. In a fetus studied for 5 days after discontinuation of plasma infusion, mean arterial was 66.3 mmHg and fetal plasma protein was 86.2 g/l on the day plasma infusion was discontinued. Five days later mean arterial was 66.3 mmHg and fetal plasma protein was 75.6 g/l.

Perhaps determination of both somatic and placental resistance during plasma protein infusion could provide further insight. It is unlikely that the infused plasma protein solution obstructed the capillaries of the fetus or of the placenta and thus increasing resistance because the plasma protein solution was filtered through a 0.22-μm filter.

The goal of the present study was to explore one of the potential differences in the regulation of arterial pressure in the fetus compared with the adult. In the adult, vascular volume control is modulated by renal excretion of sodium and water. In the fetus, vascular volume is modulated in the placenta as a result of the requirement that there is equilibrium between fetal and maternal hydrostatic and oncotic pressures. The main findings of this study are that intravascular plasma infusions caused an increase in fetal plasma protein concentration, an increase in arterial blood pressure, an increase in central venous pressure and a decrease in angiotensin II levels. One likely explanation for these changes is that an increase in fetal plasma protein and oncotic pressure prevented fetal fluid volume from being cleared to the maternal circulation via the placenta and resulted in increased fetal arterial and venous pressure. We conclude from these observations that the main
difference between fetal long-term cardiovascular fluid regulation and adult regulation is the site of fluid exchange. Volume modulation in the fetus takes place in the placenta as opposed to in the kidney of the adult. It is to be noted, however, that if placental fluid exchange is affected by angiotensin control of the precapillary fetal placental resistance (1, 2), ultimate control of blood pressure in the fetus still resides in the kidney (13), as it does in the adult (17, 18).

ACKNOWLEDGMENTS

The authors thank Bob Webber, Lynn Allen, and Loni Socha for expert technical assistance.

GRANTS

This research was supported by National Institutes of Health Grants 5PO1-HD-34430, HD-37376, and HL-45043.

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J Appl Physiol • VOL 99 • SEPTEMBER 2005 • www.jap.org