Influence of age and breed on the binding of oxygen to red blood cells of bovine calves

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Influence of age and breed on the binding of oxygen to red blood cells of bovine calves. J. Appl. Physiol. 82(3): 784–790, 1997.—The influence of somatic growth and genetic selection on the whole blood oxygen equilibrium curve (OEC) was measured under standard conditions in double-muscled and dairy calves during their first 3 mo of life. Crossbreed animals were also investigated. Hemoglobin, 2,3-diphosphoglycerate (DPG), Cl, and Pi concentrations were also measured. The percentage of fetal hemoglobin (HbF) was determined. The influence of exogenous Cl, P, and pH on the OEC was also assessed. The PO2 at 50% hemoglobin saturation (P50) increased during somatic growth, probably because of the increase in DPG recorded in double-muscled neonates and to the progressive disappearance of HbF in both breeds. The oxygen exchange fraction (OEF%) was used to assess the combined influence of the OEC shift and OEC shape changes on blood oxygen desaturation under standard conditions, when the PO2 decreases within a physiological range. The OEF% showed an increase during the first month, then a stabilization. The effects of CI, P, and pH in Friesian calves were similar as in adult cattle. Double-muscled neonates had a lower P50, OEF% values, and DPG concentrations and higher hemoglobin and CI concentrations than Friesian neonates. The P concentration and the percentage of HbF were similar in both breeds. The pH and the CI concentration had significantly less effect on the OEC in double-muscled than in Friesian calves. Crossbreed animals exhibited intermediate parameter values, between those recorded for double-muscled and Friesian calves. All differences between breeds progressively disappeared during the first month. These data show that blood function changes markedly in calves during the first month of life and that genetic selection can alter blood function.


MATERIALS AND METHODS

Blood samples. Venous blood samples from healthy male and female Friesian (n = 47) and double-muscled Belgian White and Blue breed (n = 50) between 0 and 100 days of age were collected from the jugular vein into syringes containing heparin (10,000 IU/l; blood; Heparin Novo, Novo Nordisk). Blood was also sampled in six crossbreed neonates (Friesian × Belgian White and Blue). The blood was immediately stored at 4°C and the OEC recorded within 24 h, a period during which no modification in our control group can be observed.

Curve plotting. The methodology used in this study has been extensively described previously (7, 10). Briefly, the entire OEC (PO2 ranging from 0 to 320 Torr) was recorded dynamically with a homemade analyzer in which oxygen saturation was measured by photometry as a function of PO2, the latter being measured polarographically (PO2 electrode). Changes in plasma pH were corrected automatically in the
analyzer by addition of NaOH (1N) or HCl (1N). The pH was continuously measured at 0.001 units by a glass combined electrode (Radiometer), and a digital comparator computed the pH difference between the blood pH and the pH required for the experiment. Depending on this difference, a titration liquid was injected automatically by a micropump in amounts sufficient to maintain the pH at a constant value, with no hemolytic and dilution effects. A temperature of 37°C and a PCO₂ of 40 Torr were maintained throughout the experiment. For each curve, 100 points were measured automatically: their PO₂ and oxygen saturation coordinates were digitized and stored on a floppy disk on an IBM-compatible personal computer (HP Vectra, QS/16S, Hewlett-Packard). The computer reproduced the curves on a laser printer (Hewlett-Packard) and processed the data as follows.

The oxygen exchange fraction (OEF %) (22) was calculated as the difference in saturation between a PO₂ of 110 and 40 Torr, with the mean PO₂ values classically measured in arterial and mixed venous blood, respectively, in cattle (12, 16). These values were fixed to study the functional behavior of blood under standard conditions. This in vitro functional parameter is known, by contrast to the P50, to better describe the OEC function by quantifying the combined influence of the OEC shift and OEC shape changes on the ability of blood to release oxygen in standard conditions when the PO₂ decreases within a physiological range. This parameter can also be expressed as volume percent, i.e., as the amount of oxygen released by 100 ml of blood by taking into account the Hb concentration, the Hb oxygen capacity, and the release from dissolved oxygen.

The precision of our method for measuring the OEC, expressed by the SD of the P50, was 0.1 Torr for six curves recorded from the same blood sample (analytical error of the analyzer) and 0.3 Torr for 11 samples taken from the same control subject over a 30-day period (analytical error associated with intra-individual variations).

Influence of Cl and P_i values on the OEC. In 41 animals, 20 ml of blood were divided into two equal parts for a control and a test sample. After sedimentation of the red blood cells, the plasma was removed, and phosphate and chloride ions were added separately to the test samples (70 mmol/l plasma). K⁺, HCO₃⁻, and KCl were used. The red blood cells were then resuspended in plasma enriched with ions. Thereafter, blood was tonometered with a deoxygenating gas mixture (94.4% N₂-5.6% CO₂) for 60 min, a time sufficient to reach a steady state (9). The OEC was then recorded as previously described.

Influence of pH on OEC. Effects of pH were measured in blood samples from 69 randomly selected animals that were 0–100 days old. OEC values were recorded at three pH values (7.2, 7.4, and 7.6) at 37°C. The Bohr effect was derived from a linear regression of d log PO₂ vs. d pH, taking pH 7.4 as the reference at 20 different levels of saturation. The d log PO₂/d pH coefficient was calculated.

Biochemical and hematological determinations. Hb (in g/100 ml), carboxyhemoglobin (in %), and methemoglobin (in %) were determined with an OSM 3 radiometer (Radiometer, Denmark). Hematocrit was measured by microcentrifugation (Martin Christ), and the mean corpuscular Hb content was calculated by dividing the total Hb content by hematocrit. There was no hemolysis, as shown by determination of the plasma Hb content.

The DPG level was determined by an enzymatic method (Sigma Chemical technical bulletin no. 35 UV, 2.3-DPG, St Louis, MO). Cl concentrations were determined by a titrimetric method using Hg(NO₃)₂, as described in Merckotest no. 3311, and P_i was determined by the Sigma Diagnostics procedure (kit no. 670).

Determination of the percentage of HbF. Red blood cells were washed four times with 0.9% saline and centrifuged at 2,000 g for 15 min. After the last centrifugation, packed red blood cells were hemolyzed by addition of an equal volume of deionized water. The hemolsate was then centrifuged at 2,000 g for 15 min and the supernatant stored at –70°C.

To separate the hemoglobins, we electrophoresed them for 60 min in Rosa buffer on cellulose-acetate membranes (pH 8.6; 25°C; 250 V). The membranes were then stained with a Ponceau stain as described by Lee et al. (18) and dried at 100°C for 15 min. HbF and adult hemoglobin A (HbA) were clearly separated.

The percentage of HbF was determined by quantitative analysis of the electrophoresis membranes. This was done with the Biocom image-analysis system (version 2a; Lecphor program). This procedure is a two-dimensional analysis of the digitized pictures of the electrophoresis membranes in 256 gray levels. Peaks were detected automatically by routine of the program and quantified by fitting with Gaussian functions. Results were expressed as percentages of the sum of all peaks identified as Hb in a given lane. In most samples, two peaks were found. The peak corresponding to HbA was identified by its rate of migration, which was identical to that of the single band observed in blood samples from two adult cows of each breed. The second peak was attributed to HbF.

In some cases, a third peak was found, migrating faster than HbF. This was attributed to hemoglobin B (HbB) on the basis of the results of Lee et al. (18).

Statistical analysis. Statistical analysis was performed with BMDP New System Professional Edition 1.0 statistical software (Statistical Solutions, Univ. of California Press, Bekerley, CA). All data are expressed as means ± SD. Results were considered significant when the P value did not exceed 0.05. Because the OEF varies with age, the calves were grouped by age: <10 days (group 1), between 10 and 30 days (group 2), and >30 days (group 3). To compare double-muscled and Friesian calves, variables were submitted to two-way analysis of variance with F-tests or Welch tests in cases of unequal variances. When the interaction between breed and age was significant, the two breeds were compared within each age subgroup by using Student’s t-test for unequal or equal variances and Bonferroni’s adjustment for P values. Equality of variances was tested by Levene’s F statistic. In newborn calves, a three-class breed factor was defined to separate double-muscled, Friesian, and crossbred calves. Variables were compared among these classes by using a one-way analysis of variance with the F-test. Only when the result was significant were two breeds contrasted by using Scheffé’s criteria. The Bohr effect was estimated from the slope of the linear regression of d log P50 vs. d pH within each breed, and the slopes were compared by using a Z-test. Because of its comparable distribution in the two breeds, age was not a confounding variable in these two regressions.

RESULTS

The influence of age on the OEC is illustrated in Fig. 1. In both breeds, the OEC was found to shift to the right during the first month of life, which indicated a decrease in the blood oxygen affinity. Neonatal Friesian and double-muscled calves displayed markedly different curves, whereas no breed-related difference was recorded in animals between 30 and 100 days of age. Figure 2 shows the relationship between animal age and the individual P50 values measured in Friesian and double-muscled calves. In Friesian calves, the P50 in-
creased for 30 days from a mean value of 22.2 Torr at birth, stabilizing thereafter at a plateau value of ~26.0 Torr. A similar pattern was observed in double-muscled calves, but a wider dispersion of data was recorded.

Table 1 provides a detailed statistical analysis of these data. In the table, individual values for each breed are grouped by age as described above, taking into account the general evolution of $P_{50}$. In groups 1 and 2, the recorded $P_{50}$ values were significantly lower for double-muscled than for Friesian calves. No interbreed difference was observed in group 3. The OEF% evolved similarly over the investigated period (Fig. 2).

Figure 2 shows the evolution of individual DPG levels in double-muscled and Friesian calves. The corresponding mean values for the three pooled groups are given in Table 1. Table 2 shows the mean values for purebreed and crossbreed animals <2.5 days of age (neonates). DPG concentrations were significantly lower in double-muscled than in Friesian neonates. Cross-breeds displayed intermediate values, differing significantly from those recorded for Friesian calves but not significantly from those measured in double-muscled animals. In double-muscled calves, the DPG concentration rose abruptly, peaked at around 10 days, then decreased rapidly. In Friesian animals, the DPG concentration was high from the first days of life but then decreased rapidly. One-hundred-day-old calves displayed similar low DPG levels, whatever the breed.
BLOOD OXYGEN AFFINITY IN GROWING CALVES

In double-muscled calves, the Cl and P_i concentrations did not change significantly during the first 100 days of life, but among the youngest animals the levels varied widely. In Friesian animals, the Cl concentration was slightly but significantly lower in calves <10 days of age than in older animals or in double-muscled calves of the same age. Double-muscled calves, on the other hand, displayed slightly higher P_i levels throughout the investigated period (Fig. 2; Table 1).

Figures 2 and 3 show the evolution of individual Hb concentrations and HBF percentages. The corresponding mean data are given in Table 1. Whereas no significant change in the total Hb concentration was recorded in Friesian calves during the period of interest, this parameter decreased significantly in double-muscled calves during the first 10 days of life. After this period, no significant change was observed. The Hb concentration was significantly higher in double-muscled calves <10 days old than in the corresponding Friesian animals. Intermediate values were observed in crossbred animals (Table 2).

The mean HBF percentages decreased linearly from birth (80%) to day 30 in both breeds, reaching ~20% by the end of this period. Whereas the means calculated for Friesian and double-muscled calves were not significantly different, intrabreed variances were significantly greater in the latter than in the former. Four of eight double-muscled calves presented traces of HbB in red blood cell of group 1 (i.e., <10 days old). No traces of HbB were recorded in groups 2 and 3. Three of five Friesian calves presented traces of HbB in groups 1 and 2, but HbB disappeared thereafter.

Both Cl and P_i caused the OEC to shift significantly to the right (Fig. 4). This effect depended on Hb saturation, being most marked at the highest saturation levels. In Friesian calves, the recorded effects were similar to those previously measured in adult cows (15). Whereas the P_i effect was not significantly different in double-muscled calves, Friesian calves, and adult cattle, the Cl effect was significantly (P < 0.05) weaker in double-muscled calves than in Friesian calves and in adult animals.

The Bohr effect was significantly weaker (P < 0.05) in double-muscled calves. Table 2 shows that the P_50 values for Hb and blood increase from 18.6 and 19.3 Torr in neonates.

DISCUSSION

These data show that marked changes in Hb oxygen binding occur in calves during the first 30 days of life and that this blood function can be altered by genetic selection.

It is well known that fetal bovine Hb and blood oxygen affinity are greater than in adults (5, 6). The changes in oxygen affinity occurring during the fetal and postnatal periods are illustrated by the data of Smith et al. (20), who showed that the P_50 values for Hb and blood increase from 18.6 and 19.3 Torr in neonates to 22.2 and 21.9 Torr in adult cattle.
to 29.3 and 32.1 Torr in cows. The breed was not specified in these experiments. The high DPG concentrations recorded in red blood cells from young fetuses and calves (23) contrast with the absence of this metabolite in adult cattle. In calves, DPG might indirectly modulate the oxygen affinity by increasing the internal acidity of the red blood cells (2, 6). Direct interaction between DPG and Hb is weaker in bovines than in humans (6). In adult cattle, other factors, such as Cl, are suggested to play an important role in regulating the blood oxygen affinity, along with the pH and temperature (11, 15). The kinetics of the disappearance of HbF in calves has been documented, but the wide variations reported within and between studies remain largely unexplained (17–19, 21). The data of Breepoel et al. (5, 6) suggest that the P50 increase observed in calves is due to two combined factors: the DPG level increase occurring in newborn calves and the drop in HbF observed during the first 3 mo of life. Other evolving factors such as the Cl concentration or the pH, previously identified as modulators of oxygen affinity in adult cattle (11, 15) and other species (1), might also contribute. These, however, have never been investigated.

The present data confirm the previously described age-related changes in the P50, DPG level, and HbF percentage. Similar evolution has also been reported in lambs (2, 3). Moreover, our results concerning the changes of the whole OEC and of the factors that regulate oxygen binding to red blood cells in growing animals (pH, Cl, Pi) provide a basis for a functional assessment of Hb oxygen binding in calves. The data of Table 1 and Fig. 2 show that under standard conditions the OEF% increases significantly from birth until day 30, because of the changes in the OEC. These changes include the right shift of the OEC illustrated by the increase in the P50 values and also by the evolution of the slope of the OEC illustrated in Fig. 1. The assessment of the relative influence of both factors on the degree of saturation of Hb was not considered in this paper because of the difficulty in dissociating their respective influence when the changes occur simultaneously and also because their effects depend on the level of saturation. There may be a link between the low OEF values measured in neonates of both breeds and the high sensitivity of young animals to tissue hypoxia. Finally, since in Friesian calves the effects of pH, Cl, and Pi, on the standard OEC (Fig. 4) are similar to those observed in adult cattle (12), one can calculate the amount of oxygen released in vivo at the tissue level by 100 ml of blood, taking into account the levels of these regulatory factors in arterial and venous blood (8, 14, 15).

The data of Table 2 clearly show that blood oxygen transport depends on the genetically selected breed.
The OEC recorded for double-muscled neonates lies to the left of the curve obtained with Friesian animals (Fig. 1). The consequence is a significantly lower OEF% value in double-muscled neonates. The lower OEF% is compensated by a higher Hb concentration. The volume of oxygen released per 100 ml of blood under standard conditions is thus 2.9 ml in double-muscled neonates, 2.3 ml in crossbreed neonates, and 2.6 ml in Friesian neonates. These values were calculated under the assumption that, as in adult cattle (14), the oxygen capacity of Hb is the same for double-muscled and Friesian neonates (1.39 ml O2/g) and that HbF and HbA have a similar oxygen capacity (4, 14).

During the first 10 days of life, however, the Hb concentration evolves toward similar values in the two breeds, so that the OEF value calculated for 10- to 30-day-old animals is 2.6 ml O2/100 ml for double-muscled calves and 3.1 ml O2/100 ml for Friesian animals. This parameter thus decreases in the former during the first month of life while it increases in the latter.

Whereas the lower OEF% of double-muscled calves is partly balanced by a higher Hb concentration, the amount of oxygen released by 100 ml of blood could be very low in some double-muscled animals. Most of the parameters measured here display a wider dispersion of values among double-muscled than among Friesian calves (Table 1, Fig. 2) as well as other physiological parameters such as arterial blood gases and the mechanical properties of the respiratory system (12). The OEF (vol%) values calculated for calves between 10 and 30 days of age ranged from 2.1 to 3.2 ml O2/100 ml in double-muscled calves and from 2.5 to 3.6 ml O2/100 ml in Friesian animals. The low OEF% values recorded in some double-muscled calves may be related to the extremely high sensitivity to hypoxia observed clinically in some animals of this breed. However, it must be emphasized that, in vivo, other factors can also compensate for the relative functional deficiency of double-muscled calves blood, compared with Friesian animals. For example, the double-muscled animals with their large muscle mass could have a lower mixed venous oxygen pressure. However, no systematic study has been performed so far to compare this parameter in neonates from both breeds. In older animals, no difference has been recorded (16). All the differences between breeds recorded in calves progressively disappear after the first month of life.

The cause of the low mean standard P50 value recorded in double-muscled neonates cannot be conclusively deduced from this study, but the low DPG level and the low sensitivity to Cl (Fig. 4) may contribute to lowering the blood P50. In vivo, the rather limited effect of pH might also play a role. There may be a link between the low sensitivity to Cl and the higher plasma CI and P1 concentrations recorded in double-muscled animals, a difference that might be interpreted as a compensatory mechanism tending to increase the P50. The mean HbF percentages were similar in both breeds and in agreement with previously published data (18, 21). The electrophoretic patterns for Hb were also similar for double-muscled and Friesian calves. The fact that DPG is higher in double-muscled than in Friesian calves at 10–30 days, whereas the P50 is lower, illustrates the fact that the regulation of the OEC is not monofactorial.

We conclude that 1) Hb oxygen binding is more limited in neonates than in adult cattle due to the position and shape of the OEC. Modulating factors such as P1, CI, and pH exert the same effect in Friesian calves as in adult animals; 2) the present data can be used to calculate the amount of oxygen released by the blood, taking into account the influence of the main modulating factors. Such measurements can thus be used for experimental and clinical applications in bovines; and 3) in double-muscled neonates, the lesser delivery of oxygen from Hb (as compared with Friesian neonates) is balanced by a higher Hb concentration. The rapid disappearance of this compensatory mechanism in the first 10 days of life and the wide dispersion of individual values explain the marked deficiency in blood oxygen transport observed in some individuals of this breed. Moreover, CI and pH exert a lesser influence in double-muscled than in Friesian animals.

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