Blood oxygen binding in double-muscled calves and dairy calves with conventional muscle conformation

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Objective—To assess in vivo blood oxygen binding in double-muscled calves and dairy calves with conventional muscle conformation.

Animals—58 dairy and 48 double-muscled calves.

Procedure—Calves were classified as neonatal (24 hours old) or older calves (2 to 26 days old). Venous and arterial blood samples were collected, and hemoglobin concentration, pH, P_{CO_2}, and P_{O_2} were determined. Blood oxygen equilibrium curves (OEC) under standard conditions were constructed, and the oxygen exchange fraction (OEF) and the amount of oxygen released at the tissue level by 100 ml of blood (OEF Vol%) were calculated.

Results—In each breed, partial pressure of oxygen at 50% saturation of hemoglobin (P_{50}) under standard conditions was significantly higher in older than in neonatal calves, indicating a right shift in OEC with age. Venous P_{50} was significantly lower in neonatal double-muscled calves than in neonatal dairy calves, but arterial and venous P_{50} were significantly higher in older double-muscled calves than in older dairy calves. In double-muscled, but not in dairy, calves, OEF was significantly higher in older than in neonatal calves. In neonatal calves, OEF Vol% was not significantly different between breeds, but OEF Vol% was significantly higher in older double-muscled calves than in older dairy calves.

Conclusions and Clinical Relevance—The lower OEF in neonatal double-muscled calves, compared with dairy calves, could contribute to the higher sensitivity of double-muscled calves to hypoxia. Blood oxygen affinity decreased with age, but OEF and OEF Vol% were unchanged with age in dairy calves, whereas they increased with age in double-muscled calves. (*Am J Vet Res* 2000;61:299–304)

Double-muscled calves of the Belgian White and Blue breed are more sensitive to hypoxia than dairy calves with conventional muscle conformation.[1-3,a] In a previous study,[4] the influence of growth on the oxygen equilibrium curve (OEC) in double-muscled and Friesian calves was measured under standard conditions (pH, 7.4; P_{CO_2}, 40 mm Hg; temperature, 37 C), and it was found that blood function changes markedly in calves during the first month of life and blood oxygen affinity, assessed under these standard conditions, is altered in double-muscled calves. It was suggested that an impairment in blood oxygen binding in double-muscled calves could contribute to this pathologic status. However, other factors, such as hemoglobin concentration, blood gas partial pressures, and pH, influence blood oxygen transport in vivo. Therefore, the purpose of the study reported here was to assess in vivo blood oxygen binding in double-muscled calves and dairy calves with conventional muscle conformation, taking into account temperature, acid-base balance, and partial pressures of oxygen and carbon dioxide in arterial and venous blood. The influence of potential regulating factors such as 2,3 diphosphoglycerate (DPG), adenosine triphosphate (ATP), inorganic phosphate (Pi), and chloride ions was also investigated.

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Materials and Methods

Animals and sample collection—Fifty-eight healthy male and female dairy calves with conventional muscle conformation and 48 double-muscled Belgian White and Blue calves between 24 hours and 26 days old were used in the study. Samples were collected once from each calf. Heparinized plastic syringes were used to collect, under anaerobic conditions, 1 ml of venous and 1 ml of arterial blood from each calf. Venous and arterial blood samples were collected from the jugular vein and brachial artery, respectively. Syringes were placed on ice, and the pH, P_{O_2}, and P_{CO_2} were measured immediately.[b] Rectal temperature was measured at the same time blood samples were collected to correct blood gas values and pH.

Jugular vein blood samples were also collected into 20-ml syringes containing heparin for determination of the OEC and for biochemical and hematologic tests. A 2 ml aliquot was centrifuged at 2,600 X g for 15 minutes, and plasma was harvested for chloride and inorganic phosphate concentration determination. Whole blood and plasma samples were stored at 4 C until tested. The OEC was measured within 1 day after samples were collected.

Measurement of OEC—Oxygen equilibrium curves were measured by use of a dynamic method under standard conditions (pH, 7.4; P_{CO_2}, 40 mm Hg; temperature, 37 C).[5] A 15-ml blood sample was deoxygenated in a rotary tonometer with a gas mixture composed of 5.6% CO_2 and nitrogen. The sample was placed in an analyzer and equilibrated with this first gas mixture. For 15 minutes, the oxygen tension was slowly increased from 0 to 320 mm Hg by introducing a second gas mixture composed of 5.6% CO_2 and oxygen. Oxygen saturation as a function of P_{O_2} was measured by means of photometry[c]; P_{O_2} was measured polarographically.[d] Changes in plasma pH were corrected automatically to a pH of 7.4 by addition of 1N NaOH or 1N HCl. A temperature of 37 C and P_{CO_2} of 40 mm Hg were maintained throughout the experiment. For each curve, 100 data points were automatically measured. Values for P_{O_2} and oxygen saturation were stored on a computer, which processed the data and printed out the

curves. Accuracy of this method of measuring OEC, expressed as the SD of the P_{O_2} at 50% saturation of hemoglobin (P_{50}), was 0.1 mm Hg for 6 curves recorded from the same blood sample and 0.3 mm Hg for 11 samples obtained from the same control subject over a 30-day period. Changes in oxygen affinity were evaluated by measuring P_{50} under standard conditions (standard P_{50}).

Hemoglobin content was determined with a OSM3 hemoximeter.[e] The DPG and ATP concentrations were determined by use of enzymatic methods.[f,g] Concentration of Pi was determined by use of a commercially available kit.[h] Plasma chloride concentration was determined by use of a titrimetric method.[i]

Calculation of oxygen exchange fractions—The **oxygen exchange fraction (OEF)** represents the difference in saturation calculated for P_{O_2} values measured in arterial and venous blood samples collected at the same time, taking into account the position and shape of the OEC for the compartments. In practice, arterial and venous OEC were calculated from the standard OEC corrected for the effects of pH, temperature, and P_{CO_2}.[6]

The **amount of oxygen released at the tissue level in vivo by 100 ml of blood (OEF Vol%)** was calculated by use of the following equation:

$$\text{OEF Vol\%} = \text{Hb} \times \text{Pox} \times (\text{OEF}/100) + \alpha(\text{Pa}_{O_2} - \text{Pv}_{O_2})$$

where Hb represents hemoglobin concentration (g/100 ml), Pox is the hemoglobin oxygen capacity (1.39 ml of O_2/g of Hb),[6,7] α represents the oxygen solubility coefficient for blood at the temperature of the experiment (0.003 ml $\times$ 100 ml^{-1} $\times$ mm Hg^{-1}), and Pa_{O_2} and Pv_{O_2} represent the partial pressures of oxygen in arterial and venous blood (mm Hg).

Statistical analyses—Data were expressed as mean ± SD. Values were tested for normal distribution by use of the Omnibus test. Data that were normally distributed were compared between groups by use of Student *t*-tests or Aspin-Welch tests, depending on whether variances were or were not equal. Data that were not normally distributed were compared between groups by use of Mann-Whitney tests. For all analyses, values of $P < 0.05$ were considered significant.

For the parameters that had the most marked changes with age, a semilogarithmic regression line was calculated with age as the independent variable. The effects of DPG, ATP, chloride ion, and Pi concentrations on the standard P_{50} were assessed by use of simple linear regression.

Results

In both breeds, standard P_{50} was significantly higher in older calves (ie, calves ≥ 2 days but ≤ 26 days old) than in neonatal calves (ie, calves that were 24 hours old), indicating that the OEC was shifted to the right and that blood oxygen affinity was decreased (**Table 1**). Standard P_{50} was significantly correlated with age in both groups (**Fig 1**). In both groups, the standard P_{50} first increased rapidly, then more slowly, tending toward a plateau at the end of study period (26 days).

Neonatal double-muscled calves had significantly lower DPG concentration and significantly higher chloride and ATP concentrations than did calves with conventional muscle conformation; however, Pi concentrations were not significantly different between groups (Table 1). Among double-muscled calves, DPG concentration was significantly higher in older than in neonatal calves, but the same was not true in calves with conventional muscle conformation, and older double-muscled calves had significantly higher DPG

Table 1—Assessment of in vivo blood oxygen binding and related factors in double-muscled (DM) calves and dairy calves with conventional muscle conformation

Variable	Breed	Neonatal calves*	Older calves†
Age (d)			
	DM calves	1 ± 0	10.1 ± 7.1
	Dairy calves	1 ± 0	12.9 ± 7.1
Standard P_{50} (mm Hg)			
	DM calves	18.7 ± 1.1	22.5 ± 2.3‡
	Dairy calves	19.3 ± 1.8	22.0 ± 2.2‡
DPG (µmol/g Hb)			
	DM calves	6.6 ± 1.6	14.2 ± 4.7‡
	Dairy calves	8.3 ± 2.6§	10.5 ± 4.7‖
ATP (µmol/dl)			
	DM calves	28.9 ± 7.2	26.9 ± 6.1
	Dairy calves	21.8 ± 6.2§	20.4 ± 6.8‖
Chloride (mmol/L)			
	DM calves	106.4 ± 5.0	105.4 ± 5.5
	Dairy calves	99.4 ± 4.7¶	102.2 ± 5.3¶ #
Inorganic phosphates (mmol/L)	DM calves	2.1 ± 0.2	2.5 ± 0.4**
	Dairy calves	2.3 ± 0.3	2.3 ± 0.3§
Rectal temperature (C)			
	DM calves	38.6 ± 0.5	39.1 ± 0.4**
	Dairy calves	38.7 ± 0.2	39.0 ± 0.4**
Arterial pH			
	DM calves	7.41 ± 0.03	7.34 ±0.04‡
	Dairy calves	7.40 ± 0.04	7.38 ± 0.03‖
Venous pH			
	DM calves	7.37 ± 0.03	7.30 ± 0.04‡
	Dairy calves	7.35 ± 0.03	7.34 ± 0.03‖
$Paco_2$ (mm Hg)			
	DM calves	44.6 ± 3.2	45.9 ± 3.9
	Dairy calves	45.1 ± 3.7	47.2 ± 4.0
$Pvco_2$ (mm Hg)			
	DM calves	50.6 ± 3.3	56.8 ± 5.7**
	Dairy calves	55.1 ± 4.5§	55.2 ± 5.0
Arterial P_{50} (mm Hg)			
	DM calves	21.0 ± 1.6	26.8 ± 2.7‡
	Dairy calves	21.7 ± 2.6	25.9 ± 2.5‡ ¶
Venous P_{50} (mm Hg)			
	DM calves	22.6 ± 1.7	29.4 ± 2.9‡
	Dairy calves	24.7 ± 2.2§	27.9 ± 2.6‡ ¶
Pao_2 (mm Hg)			
	DM calves	77.2 ± 11.8	86.8 ± 11.5#
	Dairy calves	88.2 ± 10.8¶	85.9 ± 11.3
Pvo_2 (mm Hg)			
	DM calves	35.7 ± 5.4	37.4 ± 7.8
	Dairy calves	34.3 ± 7.3	37.8 ± 7.0
OEF (%)			
	DM calves	18.7 ± 7.5	30.8 ± 13.9**
	Dairy calves	27.8 ± 12.7§	27.2 ± 10.9
Hb (g/dl)			
	DM calves	10.7 ± 1.2	10.6 ± 1.5
	Dairy calves	9.2 ± 2.6	9.5 ± 2.3¶
OEF Vol%			
	DM calves	2.8 ± 1.1	4.5 ± 2.0**
	Dairy calves	3.7 ± 2.3	3.5 ± 1.4§

Values are given as mean ± SD.

*Calves that were 24 hours old (n = 10 for each group). †Calves that were between 2 and 26 days old (n = 38 for DM calves and 48 for dairy calves). ‡Significantly ($P < 0.001$) different from value for neonatal calves of the same breed. §Significantly ($P < 0.05$) different from value for double-muscled calves. ‖Significantly ($P < 0.001$) different from value for double-muscled calves. ¶Significantly ($P < 0.01$) different from value for double-muscled calves. #Significantly ($P < 0.05$) different from value for neonatal calves of the same breed. **Significantly ($P < 0.01$) different from value for neonatal calves of the same breed.

Standard P_{50} = P_{O_2} at which hemoglobin is 50% saturated, measured under standard conditions (pH, 7.4; P_{CO_2}, 40 mm Hg; temperature, 37 C). DPG = 2,3 Diphosphoglycerate. ATP = Adenosine triphosphate. OEF = Oxygen exchange fraction. Hb = Hemoglobin. OEF Vol% = Amount of oxygen released at the tissue level by 100 ml of blood.

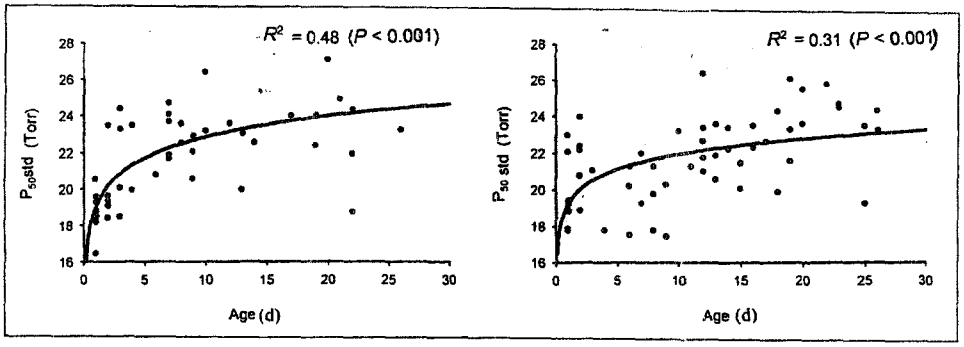

Figure 1—Scatter plot of partial pressure of oxygen at 50% saturation of hemoglobin, measured under standard conditions (P_{50} std) in 48 double-muscled calves (left) and 58 dairy calves with conventional muscle conformation (right).

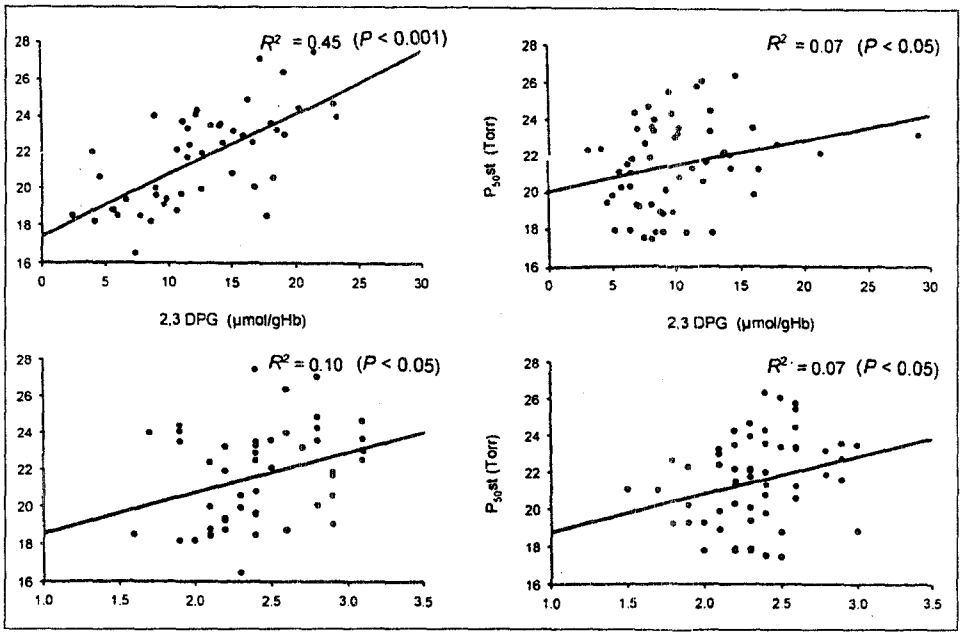

Figure 2—Scatter plot of P_{50} std versus 2,3 diphosphoglycerate concentration (DPG) and versus inorganic phosphate concentration (Pi) in double-muscled (left) and dairy (right) calves.

concentration than did calves with conventional muscle conformation. Concentration of ATP was not significantly different between age groups, and older double-muscled calves had significantly higher ATP concentration than did older calves with conventional muscle conformation. Among calves with conventional muscle conformation, but not among double-muscled calves, plasma chloride concentration was significantly higher in older than in neonatal calves; however, plasma chloride concentration was still significantly higher in older double-muscled calves than in older calves with conventional muscle conformation. Among double-muscled calves, but not among calves with conventional muscle conformation, the Pi concentration was significantly higher among older than among neonatal calves. Thus, older double-muscled calves had a significantly higher Pi concentration than did calves with conventional muscle conformation. In both groups, standard P_{50} was significantly correlated with 2,3 DPG and Pi concentrations (Fig 2); however, standard P_{50} was not significantly correlated with ATP or chloride concentrations.

Rectal temperature was not significantly different between neonatal calves of the 2 breeds; however, in both breeds, it was significantly higher in older than in neonatal calves (Table 1). Arterial and venous blood pH was not significantly different between neonatal calves of the 2 breeds. However, pHa and pHv were significantly lower in older than in neonatal double-muscled calves. Neonatal calves with conventional muscle conformation had a significantly higher $PvCO_2$ than did double-muscled calves, but $PvCO_2$ for older calves was not significantly different between groups.

In both breeds, arterial and venous OEC for older calves were shifted to the right, compared with OEC for neonatal calves (Table 1), as was the case for the standard OEC. However, because of the combined influence of various regulating factors, some breed-related differences in arterial and venous P_{50} were recorded. Mean venous P_{50} was significantly lower in neonatal double-muscled calves than in neonatal calves with conventional muscle conformation, but arterial and venous P_{50} were both significantly higher in older double-muscled calves than in older calves with conventional muscle conformation.

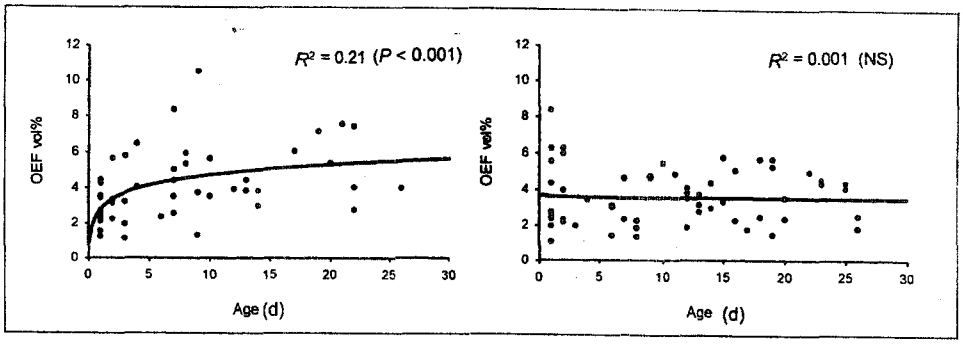

Figure 3—Scatter plot of amount of oxygen released at the tissue level by 100 ml of blood (OEF Vol%) versus age in double-muscled (left) and dairy (right) calves.

Neonatal double-muscled calves had a significantly lower Pa_{O_2} than did calves with conventional muscle conformation (Table 1); however, a difference between breeds was not detected for older calves. The Pv_{O_2} was not significantly different between breeds among neonatal or older calves. Neonatal double-muscled calves had a significantly lower OEF than did neonatal calves of conventional muscle conformation, but OEF Vol% was not significantly different between groups because of differences in hemoglobin concentrations. Among double-muscled calves, but not among calves with conventional muscle conformation, the OEF and, consequently, the OEF Vol% was significantly higher in older than in neonatal calves (Fig 3), so that OEF Vol% for older double-muscled calves was significantly higher than OEF Vol% for older calves with conventional muscle conformation.

Discussion

In a previous study,[4] the influence of growth during the first 3 months on the OEC in double-muscled and Friesian calves was measured in vitro under standard conditions. In these breeds, the OEC shifted to the right during the first month of life, indicating a decrease in blood oxygen affinity. Double-muscled calves had lower standard P_{50} values during the first month of life than did Friesian calves, but breed-related differences were not observed for older animals. This difference in P_{50} values was detected even though plasma concentrations of chloride and Pi, which are known to decrease the oxygen affinity of hemoglobin, were higher in double-muscled than in Friesian calves. Likewise, the DPG concentration increased significantly during the first 10 days of life in double-muscled calves but not in Friesian calves. It was further demonstrated that factors that regulate the OEC exerted different effects according to breed. In particular, the chloride and Bohr effects were weaker in double-muscled than in Friesian calves.

On the basis of these observations, it was hypothesized that double-muscled calves may have a deficiency in blood oxygen release. Because other factors, such as hemoglobin concentration, body temperature, blood gas partial pressures, and pH, can influence blood oxygen transport in vivo, the purpose of the present study was to record in vivo OEC in the arterial and venous compartments so as to calculate the amount of oxygen released by 100 ml of blood, taking into account all these regulating factors.

The significantly higher standard P_{50} in older calves in the present study, compared with neonatal calves, can be related to a decrease in fetal hemoglobin concentration.[4,8-11] In double-muscled calves, the significantly higher DPG concentration among older, compared with neonatal, calves may also have contributed to a decrease in hemoglobin oxygen affinity. Standard P_{50} was significantly correlated with DPG concentration in double-muscled calves and calves with conventional muscle conformation. However, among calves with conventional muscle conformation, the coefficient of determination was lower, likely because DPG concentration was not significantly different between neonatal and older calves. Beyond the first month of life, DPG concentration decreases in both breeds, and DPG is not detectable in adult cattle.[7] In the present study, Pi concentration was also significantly correlated with standard P_{50}, but coefficients of determination were low, suggesting that the effect of Pi on the increase in P_{50} with age was slight. Even though chloride and organic phosphate can modulate in vitro OEC when added to plasma or hemoglobin solutions,[12,13] standard P_{50} was not significantly correlated with chloride or ATP concentrations in the present study, probably because chloride and ATP concentrations were not significantly different between neonatal and older calves.

In contrast with results of a previous study[4] in which standard P_{50} was significantly lower in double-muscled than in Friesian calves, we observed no significant difference in standard P_{50} between breeds. This reflects the fact that mean standard P_{50} for calves with conventional muscle conformation was lower in the present study than in the previous study,[4] and the SD was higher; whereas, results for double-muscled calves were similar for the 2 studies. In the previous study,[4] calves were selected from just a few farms. In the present study, double-muscled calves were also selected from these few farms, but dairy calves were obtained from the market. Thus, differences in environmental factors may help explain the high variability of the data for the dairy calves, along with differences in genetics, as indicated by breed-related differences in DPG, chloride, and Pi concentrations. None of the calves used in the present study were used in the previous study.[4]

In vivo, factors such as pH, Pc_{O_2}, and body temperature modulate the OEC and related parameters such as OEF and OEF Vol%. In the present study, Pvc_{O_2} was significantly higher among calves with conventional muscle conformation than among double-muscled

calves. Moreover, calves with conventional muscle conformation displayed relative, albeit non significant, acidosis. These results explain the significantly higher venous P_{50} among calves with conventional muscle conformation, compared with double-muscled calves.

Arterial and venous P_{50} were 19 and 13% higher in older dairy calves than in neonatal dairy calves and 28 and 30% higher in older double-muscled calves than in neonatal double-muscled calves. The greater differences in double-muscled calves reflect decreases in pH and increases in $PvCO_2$ not observed in dairy calves. The relative acidosis in double-muscled calves has been reported.[3] Plasma pH is regulated by 3 independent factors: PCO_2, strong ion difference (ie, sodium, potassium, chloride, and lactate concentrations), and concentration of nonvolatile buffers (ie, proteins).[14-16] The higher chloride concentration in double-muscled calves in the present study would be expected to decrease the strong ion difference, resulting in a lower pH. On the other hand, a lower plasma protein concentration would be expected to increase pH. Differences in pH between double-muscled calves and calves with conventional muscle conformation in the present study could be attributable to differences in any or all of these independent factors. However, because the aim of this study was not to investigate causes of the differences in pH between breeds, data concerning the strong ion difference and total protein concentration were not collected.

In neonates, the higher oxygen affinity of venous blood and the relative arterial hypoxia help explain why the OEF was significantly lower in double-muscled calves in the present study, compared with calves with conventional muscle conformation. Yet the influence of PaO_2 on this breed-related difference was limited, because, at this point on the OEC, a difference in PaO_2 of 10 mm Hg causes only a 1% difference in hemoglobin saturation. We did not detect a significant difference in OEF Vol% between groups of neonatal calves, probably because of the greater variation in values for calves with conventional muscle conformation. This high interindividual variability was likely attributable to the relative nonhomogeneity of the calves in this group.

In the present study, the OEF was significantly higher among older double-muscled calves than among neonatal double-muscled calves, most likely because of the right shift of the arterial and venous OEC and, to a lesser extent, because of the increase in PaO_2. Among dairy calves, the OEF was not significantly different between age groups, most likely because of the slight, but nonsignificant, increase in PvO_2, which counterbalanced the right shift of the OEC. The higher hemoglobin concentration in double-muscled calves versus calves with conventional muscle conformation explains why the OEF Vol% was significantly higher among double-muscled calves (Fig 3). This peculiarity may explain why double-muscled calves 33 to 328 days old can maintain an oxygen consumption similar to that of dairy calves,[2j] despite a lower cardiac index.[17] Adaptive mechanisms enable double-muscled calves to maintain a level of ventilation similar to that of dairy calves.[18] Because of a smaller tidal volume, double-muscled calves must increase their respiratory frequency. Thus, one may assume that the high sensitivity of double-muscled calves to hypoxia, compared with dairy calves, reflects their need to use adaptive mechanisms to maintain a similar level of oxygen consumption.

In conclusion, we found that OEF was significantly lower in neonatal double-muscled calves than in calves with conventional muscle conformation. This deficiency may contribute to the higher sensitivity of double-muscled calves to hypoxia. In addition, blood oxygen affinity was significantly lower in older calves of both breeds, compared with neonatal calves. Finally, among calves with conventional muscle conformation, the OEF and, consequently, the OEF Vol% was not significantly different between older and neonatal calves; however, OEF and OEF Vol% were significantly higher in older double-muscled calves than in neonatal double-muscled calves.

[a]Gustin P. *Spécificités fonctionnelles du système respiratoire des bovins hyperviandeux.* PhD thesis. Departments of Physio-Pathology and Pharmacology-Toxicology. Université de Liège, Liège, Belgium, 1989.
[b]AVL, Biomedical Instruments, Graz, Austria.
[c]ED (660 nm), Monsanto, St Louis, Mo.
[d]Po_2 electrode, Eschweiler, Kiel, Germany.
[e]Radiometer, Copenhagen, Denmark.
[f]DPG kit No. 35A, Sigma Chemical Co, St Louis, Mo.
[g]ATP kit No. 366, Sigma Chemical Co, St Louis, Mo.
[h]Phosphorus inorganic kit n° 670, Sigma Chemical Co, St Louis, Mo.
[i]Merckotest, Merck, Dormstad, Germany.
[j]Desmecht D, Lekeux P. Oxygen uptake in relation to age and double-muscled conformation genetic selection in calves during complete rest in thermoneutral environment (abstr). *Arch Int Physiol Biochem* 1992;100:P34.

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