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**RELATIONSHIP BETWEEN TOTAL IRON BINDING CAPACITY AND TRANSFERRIN
CONCENTRATION IN NEONATAL PIGLETS TREATED WITH IRON-DEXTRAN**

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Serum iron concentration and iron saturation of transferrin (Trf) are measures of body iron stores after administration of iron supplements. In clinical and experimental research, the complex determination of Trf was replaced by the simple determination of total iron binding capacity (TIBC). The objective of this work was to define if TIBC could be an adequate measure for Trf in neonatal piglets after i.m. iron administration.

Treated piglets received 150 mg of iron-dextran i.m. the first day of life, and were compared to the untreated control group. Prior to iron administration, as well as on days 2, 8 and 12 after iron administration, serum iron and TIBC concentration were analyzed by an automatized chemical analyzer and Trf was determined by densitometry of electrophoretic strips.

Our results show that regardless of iron treatment, TIBC is not a measure of Trf concentration in neonatal piglets two days after birth. At day 8 of their life a high correlation coefficient of these two parameters was established in non-treated animals, while in iron-treated piglets the same correlation was established 12 days after iron treatment. Thus, we suggest that in neonatal piglets, TIBC could be used as a measure of Trf concentration only 12 days after i.m. iron treatment.

Key words: TIBC, transferrin, neonatal, piglets, iron-dextran

INTRODUCTION

In modern farming neonatal piglets have a particular sensitivity to iron deficiency anemia (IDA). In order to prevent IDA, they are supplemented with high doses of iron between the first and third day of life. The effects of iron treatment are commonly followed by determination of hematological parameters. However, changes in iron metabolism precede these changes and give a more precise information about body iron stores.

To clinically assess body iron stores, different tests have been developed (Cook, 1999). Serum iron and TIBC, although not considered to be the most sensitive tests, are widely used as a measure of iron deficiency or iron excess. Serum iron is considered as a direct measure of iron bound to Trf, and TIBC is an indirect measure of Trf concentration. Studies over a wide range of clinical conditions indicate that it is not possible to develop a universal algorithm for the conversion of Trf values into TIBC (Gottschalk *et al.*, 2000). Chemically based formulas for transformation predict that TIBC ($\mu\text{mol/L}$)/ Trf (g/L) ratio should be 25, but the reported TIBC/Trf ratio has ranged from 11 to 26 in humans (Gambino *et al.*, 1997). Moreover, when iron overload exists (iron poisoning or haemochromatosis), a significant amount of serum iron is bound to other molecules, and the predicted correlation is not valuable (Guyader et Gandon, 2000).

For neonatal piglets there are no data concerning TIBC - Trf relationship. The purpose of this work was to investigate TIBC, as well as Trf changes and their relationship in neonatal piglets treated with iron to prevent IDA.

MATERIAL AND METHODS

Animals and experimental design

The trial was carried out on 14 Swedish Landrace piglets. Piglets were allowed to suckle freely and they received no other food during the experiment. The animals were kept on a concrete floor. All piglets received a vitamin and mineral supplement but no iron (2 ml of Vitoligam[®]), and received daily a bolus of *Streptococcus faecium* (Biopremix[®]) to prevent diarrhea. One group of piglets ($n=7$) received 150 mg of iron-dextran (Miofer[®]) *i.m.* the first day of birth and the other group ($n=7$) was the control with no iron fortification. Clinical symptoms of iron toxicity (incoordination, shivering and convulsions) were not observed after iron-dextran administration. Blood samples were collected from the intraorbital sinus. The first blood sampling was done between the 6th and 9th hour after birth (prior to iron application), and then on days 2, 8 and 12 thereafter. Serum was separated from the blood after 2 hours of coagulation at room temperature, and immediately stored at -20 C° for subsequent analysis.

Biochemical analysis of serum iron status

Serum iron and TIBC were analyzed by automated clinico-chemical analyzer with defined end-points assays (Model «Express 550» Corning – Gilford, Oberlin, USA). Serum iron concentration was determined by Ferrozine[®] method without deproteinisation and TIBC concentration by the same method, but after saturation and adsorption of iron excess.

To determine serum Trf concentration Rivanol[®] precipitation method (Heide *et al.*, 1978) was used to separate Trf and IgG from other serum proteins. The concentration of Rivanol precipitated proteins (Trf and IgG) was determined by tannin-turbidimetric method (Mejbaum-Katzenellenbogen, 1955). Electrophoretic separation of Rivanol precipitated proteins was performed in 1% agarose

(Johanson, 1972) on Gel-fix plastic sheets. The concentration of Trf was assessed by densitometry of electrophoretic strips.

Based on serum Fe and TIBC concentration transferrin saturation with iron can be calculated (transferrin saturation (%) = serum Fe / TIBC x 100). Because at some points of the experiment the concentration of serum Fe exceeded the concentration of serum TIBC we decided to change the term transferrin saturation into the term saturation of iron binding sites (transferrin saturation = saturation of iron binding sites).

Statistical analysis was performed by descriptive statistic tools, unpaired student t-test, and correlation analysis using Excel PC Program.

RESULTS

At birth, serum iron concentration and TIBC values were low and similar in all tested animals. In the iron-treated group of piglets the increase in both serum iron concentration and TIBC values was observed on day two after iron-dextran administration (Figure 1). As the values obtained for serum iron were higher than TIBC values the saturation of serum iron binding sites was over 100% (Table 1). Both values decreased on the following days, but remained relatively constant sustaining saturation of binding sites at 50% (Table 1). In non-treated, control piglets, throughout the experiment decreasing serum iron concentrations were observed, while in the same samples increasing TIBC values were detected (Figure 2). In these piglets saturation of existing iron binding sites in serum rapidly decreased from nearly 40% at birth to 4% on day 12 (Table 2).

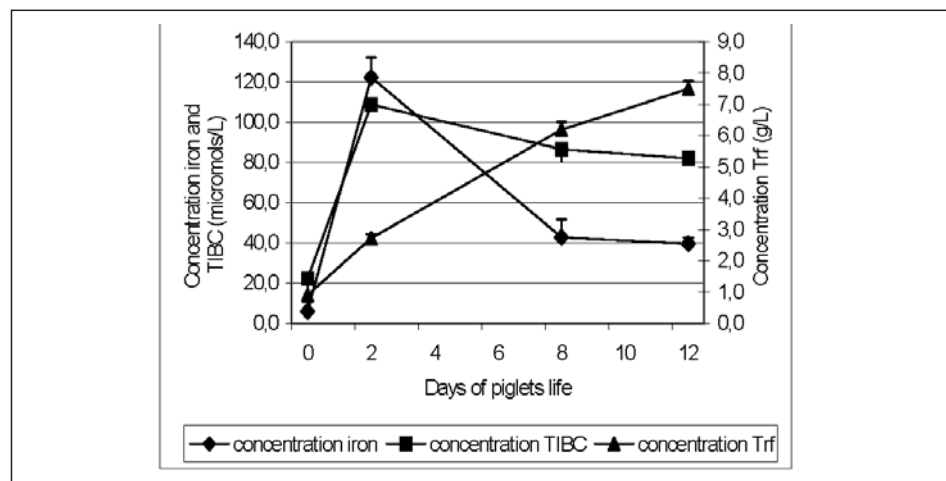


Figure 1. Serum iron concentrations, TIBC and Trf concentrations determined in neonatal piglets after iron-dextran administration (150mg *i.m.* between 6th and 9th hour after birth). The data are shown as mean \pm SE of values obtained from seven piglets

Table 1. Saturation of serum iron binding sites and TIBC/Trf relationship in iron-treated piglets (150 mg *i.m.* between 6th and 9th hour after birth)

	Saturation of serum iron binding sites (%) (mean±s.e.)	Ratio TIBC/Trf (X (min-max))	TIBC/Trf correlation coefficient (r ²)
Time zero (n=7)	29 ± 5.5	/	/
day 2 (n=7)	114 ± 4.5	44 (23-77)	r ² = -0.43
day 8 (n=7)	49 ± 7.7	14 (11-18)	r ² = 0.65
day 12 (n=7)	50 ± 5.6	11 (9-12)	r ² = 0.92

n = number of animals

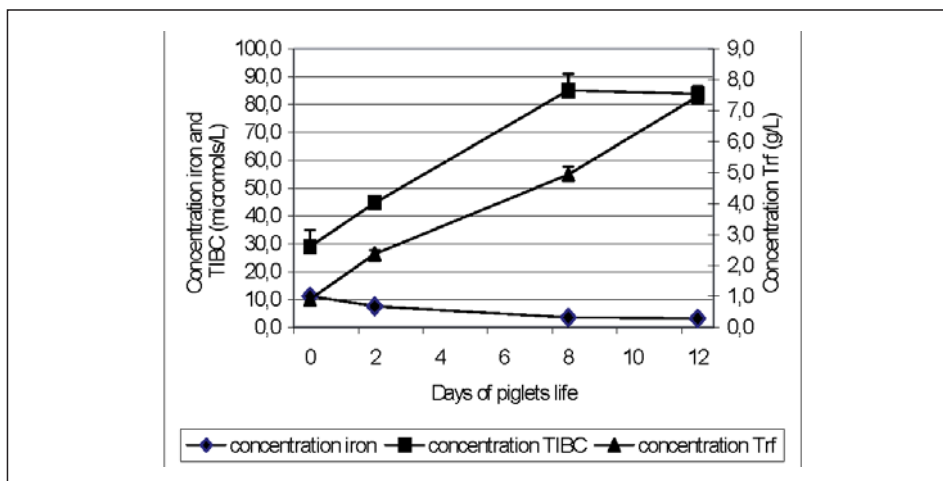


Figure 2. Serum iron concentrations, TIBC and Trf concentrations determined in control neonatal piglets without iron-dextran treatment. The data are shown as mean ± SE of values obtained from seven piglets

Table 2. Saturation of serum iron binding sites and TIBC/Trf relationship in control, non-treated piglets

	Saturation of serum iron binding sites (%) (mean±s.e.)	Ratio TIBC/Trf (mean (min-max))	TIBC/Trf correlation coefficient (r ²)
Time zero (n=7)	39 ± 6.4	/	/
day 2 (n=7)	17 ± 1.5	18 (15-21)	r ² = 0.67
day 8 (n=7)	4 ± 0.6	17 (15-20)	r ² = 0.83
day 12(n=7)	4 ± 0.2	11 (11-12)	r ² = 0.95

n = number of animals

With the method we used Trf concentrations were below detectable level (<1 g/L) in the samples taken from piglets prior to iron administration (between the sixth and ninth hour of life). From these nearly undetectable levels at birth, the concentration of Trf in both groups increased continuously afterwards (Fig 1 and 2). Comparing the values for TIBC and Trf for each day and every piglet, in the iron-treated group no correlation between these two values ($r^2=-0.43$) was observed on day two after iron administration. On day 8 after the treatment a correlation ($r^2=0,65$) was established, with a very high correlation coefficient on day 12 ($r^2=0.92$) (Table 1). The non-treated, control piglets had positive TIBC/Trf correlation throughout the experiment, with permanent increase of the correlation coefficient from day two onward (Table 2).

When TIBC/Trf ratio was calculated, the obtained values showed that high TIBC/Trf ratio corresponded to higher saturation of serum iron binding sites, but lower correlation coefficient between these two parameters (Table 1 and 2).

However, on day 12 after birth the TIBC/Trf ratio equals 11 both in iron-treated and non-treated piglets, thus corresponding to a wide range of values of saturation of serum iron binding sites 4%-50%, while the correlation coefficient between TIBC and Trf at same time point was very high.

DISCUSSION

Treatment of IDA in neonatal piglets is a problem that has been frequently studied in the past 50 years, but some phenomenons are still interesting to be investigated from at least two points of view, i.e. in modern farming, as well as due to the extensive use of pigs as experimental models for different pathology phenomena. In this study we investigated the changes in TIBC and Trf in neonatal pigs with and without iron treatment after birth in order to determine if TIBC could be an adequate measure for Trf in neonatal piglets. Namely, in clinical laboratories the expensive evaluation of Trf is replaced with the determination of TIBC, since the quantitative transformation between these values is established in humans.

The results revealed decreased serum iron concentrations and elevated TIBC values in neonatal piglets without iron treatment, which is in accordance with the findings obtained for sideropenic anemia in both humans (Rajamaki *et al.*, 1979, Brugnara, 2003) and piglets (Furuguri 1975, Egeli *et al.*, 1998). In addition, in this sideropenic group of piglets, better correlation between TIBC and Trf was obtained compared with the treated animals. The increase in the correlation coefficient obtained in the first days after delivery was accompanied by a decrease in TIBC/Trf ratio.

On the other hand, in iron-treated piglets high serum iron concentrations and high TIBC values were detected two days after *i.m.* administration of 150 mg iron-dextran. Serum iron concentrations exceeded the TIBC values (saturation = 114%), similar results were obtained for marked iron overload in humans (Huebers *et al.*, 1987). These high serum iron concentrations pointed to the existence of unbound iron in the serum of iron-treated neonatal piglets. Additionally, two days after iron administration, TIBC values did not correlate with Trf concentrations, which is in accordance with the results obtained for

experimental iron poisoning in humans, (Burkhart *et al.*, 1991). Disproportion of TIBC vs. Trf values was also reported in patients with haemochromatosis (Grootveld *et al.*, 1988), renal dialysis patients with iron overload, as well as patients with transfusion dependent anemia (Levin *et al.*, 1995). This phenomenon was explained by iron binding to other serum proteins and low-molecular mass molecules (Hershko *et Peto*, 1987). In all these cases non-transferrin bound iron was a dominant form of serum iron and TIBC was not of diagnostic relevance for the determination of iron status. In iron-treated piglets, as the TIBC/Trf ratio decreased from day two after iron administration onwards the TIBC/Trf correlation coefficient increased and the high correlation between these two parameters was obtained just on day 12 after iron treatment. The ratio that corresponded to the highest correlation coefficient of these two parameters in neonatal piglets seems to be near 11, and the same value was reported for human newborns (Lentijes *et al.*, 1995).

As the concentration of Trf did not vary significantly between the two studied groups of piglets, we could suggest that this parameter is independent of iron metabolism during the first days of piglets' life. Data showing no relationship concerning serum iron and serum transferrin receptor concentration was reported in serum of newborn infants (Kuiper-Kramer *et al.*, 1998). On the contrary, Rusia *et al.*, (1996) found that serum transferrin receptor levels are a sensitive index of iron status in neonates.

Also, our experiments revealed that, in both groups, Trf concentration increased three times as compared to the values at birth on the second day of life (below 1g/L at birth). Our data for Trf levels at birth differ, but for the second day of piglets' life they are in accordance with other published data for the same period (Thoren-Tholling *et Martinsson*, 1974, Lampreave *et Pineiro*, 1992). Our results suggest that 1. the absorption of Trf from colostrum is abundant, or 2. that the intensive synthesis of Trf takes place the first days after birth. Also, at further days of the experiment, Trf concentration was constantly increasing in both groups, reaching higher levels than these reported by Thoren-Tholling and Martinsson (1974).

Taken together these results indicate that in iron overload states TIBC is not an adequate measure for Trf. This is also the case in neonatal piglets treated with iron for IDA prevention, in which TIBC could be used as a measure of Trf concentration only on day 12 after iron administration.

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**ODNOS IZMEĐU TOTALNOG KAPACITETA ZA VEZIVANJE GVOŽĐA I
KONCENTRACIJE TRANSFERINA KOD NOVOROĐENE PRASADI TRETIRANE
GVOŽĐE-DEKSTRANOM**

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SADRŽAJ

Određivanje statusa gvožđa u organizmu jedinke posle primene određenog preparata ovog mikroelementa moguće je utvrditi određivanjem njegove koncentracije u serumu i zasićenja transferina (Trf) gvožđem. U kliničkoj i eksperimentalnoj praksi složeno određivanje koncentracije Trf zamenjeno je jednostavnim određivanjem ukupnog serumskog kapaciteta za vezivanje gvožđa (TIBC). Cilj ovog rada je bio da se na modelu porasta serumskog Fe po *i.m.* aplikaciji Fe-dextrana novorođenoj prasadi, utvrdi odnos TIBC i Trf po aplikaciji ovog mikroelementa, kako bi se utvrdilo da li visoke doze gvožđa u serumu utiču na vrednost TIBC kao mere za određivanje koncentracije Trf.

Vrednosti za serumsko Fe, TIBC i Trf poređene su između grupe životinja koja je odmah po rođenju dobila 150 mg Fe-dextrana i kontrolne grupe u kojoj životinje nisu tretirane Fe-dextranom. Krv je uzorkovana pre aplikacije Fe-dextrana, drugog, osmog i dvanaestog dana po aplikaciji preparata gvožđa. Koncentracija gvožđa u serumu i TIBC su određivani standardnim kliničkim biohemijskim analizama, dok je koncentracija Trf određena denzitometrijom elektroforetskih traka.

Dobijeni rezultati ukazuju da bez obzira na primenu preparata Fe, TIBC nije adekvatna mera za Trf kod novorođene prasadi u prva tri dana po rođenju. Osmog dana života prasadi, utvrđen je visoki stepen korelacije ova dva parametra kod životinja koje nisu bile tretirane, dok je kod tretiranih jedinki taj stepen korelacije postignut dvanaestog dana. Na osnovu izloženih rezultata se može zaključiti da se kod novorođene prasadi TIBC može koristiti kao mera za Trf tek 12 dana nakon *i.m.* tretmana Fe-dextranom.