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SUSTAINED OPPOSITE RELATIONSHIPS BETWEEN ANABOLIC HORMONES IN PREWEANING TRIPLET LAMBS BORN TO OBESE MOTHERS

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Abstract

The aim of the study was to analyse the effects of maternal obesity and the litter size on the growth rate and plasma concentrations of GH, IGF-1, insulin, and glucose in ewe lambs during the first 6 weeks of their postnatal life. Seventy-six SCP sheep: 35 ewes and 41 female offspring were used. Before gestation, the ewes were divided into two groups: N – normally weighing and O - obese sheep. After the parturition, the lambing rate and the birth type were estimated. The born female lambs were separated into five groups: IN - singleton and IIN - twin offspring of normally weighing mothers; IO - singleton, IIO – twin and IIIO – triplet offspring of obese mothers. They were weighed at birth and weekly thereafter, until the completion of the 6th week of their postnatal life. Afterward, daily weight gains and concentrations of biochemical parameters were analysed. No significant differences in GH, IGF-1, insulin, and glucose levels between the groups of lambs born to non-obese and obese sheep, both carrying singletons and twins, were found. In contrast, significantly increased concentrations of GH ($P \leq 0.001$), insulin ($P \leq 0.001$), and glucose ($P \leq 0.05$) and markedly dropped level of IGF-1 ($P \leq 0.001$), as well as reduced daily body mass gains in triplets in comparison to other groups of lambs were observed. Maternal obesity caused significant lambing rate's accretion with the rise in triplets' frequency. However, in ewe lambs of this birth type, the disrupted relationships between plasma levels of GH, IGF-1 and growth rate, and between plasma levels of insulin and glucose were found.

Key words: ewe, lambs, maternal obesity, somatotropin, IGF-1, insulin, glucose.

Maternal obesity in sheep increases the tendency of their offspring to develop hypertension, adiposity, and insulin resistance in adult life (7, 15, 20-22, 26, 27). It affects lambs weight, body composition, and lambing rate. Low birth weight, resulting from high litter size - induced intrauterine growth retardation, and placenta insufficiency, (10, 17) predisposes to growth rate and energy storage disorders, central and visceral adiposity, as well as metabolic diseases and reproductive disturbances in mature sheep. There are a number of studies concerning the mechanisms of growth and adaptations to postnatal life and consequences of prenatal growth retardation in low birth weight lambs and/or multiple offspring of obese sheep (8, 9, 22). However, most of them applies to lambs, whose growth was experimentally modified by different postnatal nutrition (5, 8, 9) or to the offspring of sheep, which were over- or undernourished before or during gestation (19, 22, 24-26). Additionally, their results, especially regarding the beginning of the catch-up growth of lambs and maturation of somatotropic/IGF axis, which together with insulin, has a pivotal role in regulating postnatal growth (6, 18), are still equivocal**.** Therefore, the aim of the study was to analyse the effects of maternal obesity and litter size on the growth rate and plasma concentrations of GH, IGF-1, insulin, and glucose in ewe lambs of high prolific SCP line during the first 6 weeks of their postnatal life.

Material and Methods

Animals and experimental design. Seventy-eight female, multiparous, highly-prolific SCP sheep (Suffolk + Charolaise + Polish Lowland Sheep + Romanov + Olkuska): 37 ewes (4-5 years of age, mean body weight 78.26 ±11.50 kg) and 41 ewe lambs (the offspring of the experimental adult sheep) were used. The experiment was carried out from September to March. All adult ewes were given two injections of 200 µg of $PGF_{2\alpha}$ analogue (Cloprostenol, Oestrophan, Leciva, Czech Republic) at 11-d intervals to synchronise the oestrous cycle. 48 h after the second $PGF_{2\alpha}$ injection, at the expected oestrus time, blood samples from the jugular vein were collected to check the efficiency of synchronisation by high performance liquid chromatography (HPLC) analysis of 17β-oestradiol (E-2) and progesterone (P-4). The obtained data (E-2 concentration: 26.67 ±2.05 pg/mL; P-4 concentration: 0.68

±0.14 ng/mL) enabled us to recognise oestrus and confirmed the efficacy of the synchronisation. Only the synchronised ewes (n=35) were used in the next step of the experiment. They were divided into two groups, similar with regard to the mean age, but differing in the body mass and fat ratio: N - normally weighing, non-obese ewes (70.14 ±4.10 kg, n=18) and O – heavy, obese ewes (86.37 ±4.80 kg, n=17). Afterwards, all sheep have been mated. The pregnant ewes were fed the same standard feed suitable for particular stages of gestation and foetal numbers anticipated on the ground of ovulation rate. After the parturition, lambing rates were estimated. The ewes with one lamb (group N: n=5; group O: n=4) were fed meadow hay, mangolds, grass silage, and oats grain (1.75, 1, 1, and 0.2 kg/d/ewe, respectively) (2.03 kg of dry matter, total gross energy: 9.57 MJ, 8.90% digestible protein on a dry matter basis). Each sheep nursing two (group N: n=13; group O: n=6) or three lambs (group N: n=0; group O: n=7) received additionally 0.5 kg or 1 kg of meadow hay/d (0.43 or 0.86 kg of dry matter, total gross energy: 1.89 or 3.78 MJ, respectively, 9.3% digestible protein on a dry matter basis). Next steps of the experiment were carried out only with the female lambs (group N: total offspring number - 31; females - 18; group O: total offspring number - 37; females - 23). Neonatal ewes were divided into five experimental groups: IN - singleton offspring of non-obese mothers (n=4); IIN – twin offspring of non-obese mothers (n=14); IO - singleton offspring of obese mothers (n=4); IIO – twin offspring of obese mothers (n=6), and IIIO - triplet offspring of obese mothers (n=13). The lambs were suckled by their dams and kept in sheep pens. All lambs had free access to water and feeding stuff from the beginning of the 3rd week. During the 3rd and 4th week, they were given meadow hay (50 and 75 g/d/lamb, respectively) and crushed oats grain (50 and 75 g/d/lamb, respectively). In the 5th and 6th week of life, in turn, each suckling lamb received meadow hay (100 and 125 g/d, respectively), crushed oats grain (100 g/d), concentrated feeding stuff (10 and 30 g/d, respectively), mineral fodder (2 g/d) and dried beet pulp (15 and 30 g/d, respectively). Lambs were weighed at birth and weekly thereafter, until the completion of the 6th week of their postnatal life. Afterward, daily body mass gains from the 1st to 42nd d were calculated. On the 14th, 28th, and 42nd d (the 2nd, 4th, and 6th week, respectively) of postnatal life, 5-ml blood samples were collected from the jugular vein every 15 min for 3 h starting at 8.00 h, in order to assess somatotropin (GH), insulin, insulin-like growth factor-1 (IGF-1), and glucose concentrations. The blood was centrifuged (20 min at 4˚C, 1,000 *g*) and the obtained plasma was stored at -20˚C. All procedures used in this experiment were approved by the 2nd Animal Care and Use Committee of the University of Life Sciences in Lublin.

Analytical procedures. Steroid hormones (E-2 and P-4) were extracted from the plasma with dichloromethane and analysed by HPLC (Beckman, Gold System, USA) with UV detection (14). Growth hormone concentrations were measured by immunoradiometric assay (GH-IRMA [I^{125}]; Biosource, Belgium). Intra- and inter-assay coefficients of variation were 7.4% and 8.6%, respectively. IGF-1 concentrations were determined using radioimmunoassay (IGF-1-D-RIA-CT; Biosource, Belgium). Intra- and inter-assay coefficients of variation were 8.4% and 9.4%, respectively. The plasma insulin was estimated by immunoradiometric assay (Insulin-IRMA; Biosource, Belgium). Intra- and inter-assay coefficients of variation were 6.7% and 7.6%, respectively. Glucose level was measured with the glucometer One Touch ™ II (Lifescan Inc., USA).

Statistical analysis. Statistical analysis of the obtained results was performed using Statistica 5.0 PL (Statsoft Inc., Tulsa, USA). The results were expressed as means and standard deviations. Comparisons between the groups were performed using ANOVA and the paired *t*-tests. Differences were considered as significant at $P \leq 0.05$ or $P \leq 0.001$.

Results

Birth type. The number of born lambs and that of multiple lambing was higher in the group of obese ewes (lambing rate – 2.18, lambs of multiple lambing – 89.2% (36.4% - twin and 52.8% - triplet lambing) than in the non-obese mothers (lambing rate – 1.72, lambs of multiple lambing – 83% (only twin lambing). There was a very high positive correlation between adult sheep body mass before pregnancy and the lambs birth type (r=0.92).

Birth weight. Lower mean birth weight was observed in twin lambs (IIN – 2.76 ±0.39 kg; IIO – 2.89 ±0.32 kg) compared to singletons (IN – 2.97 ±0.35 kg; IO – 3.18 ±0.40 kg), in case of the offspring of both: non-obese and obese ewes. However, singleton and twin lambs born to obese mothers (IO and IIO) had slightly higher body weight at birth compared with singleton and twin offspring of normally weighing ewes (IN and IIN). The lowest birth weight was recorded in triplet lambs born to obese sheep (IIIO - 2.36 ±0.24 kg).

Daily body mass gains between the 1st and the 42nd d of postnatal life. In both groups of lambs born to non-obese and obese sheep, the average daily gains of body mass were higher in the animals derived from single pregnancies (IN – 0.198 ±0.049 kg; IO – 0.174 ±0.045 kg) in comparison to the lambs from multiple pregnancies (IIN – 0.181 ±0.036 kg; IIO – 0.159 ±0.039 kg; IIIO – 0.121 ±0.033 kg). The negative correlation between birth type (singleton, twins, triplets) and body mass gain (r= -0.94) was observed. Moreover, the offspring of obese ewes (IO, IIO, and IIIO) had decreased body mass gains in comparison to lambs born to lighter mothers (IN and IIN) (mothers body weight *vs.* lambs daily body mass gains – r= -0.96). However, when daily gains were expressed as a percentage of the birth weight, only the difference between the offspring born to normally weighing (IN – 6.67%; IIN – 6.55%) and obese sheep (IO – 5.47%; IIO – 5.50%) were noted, but not between singletons and twins in the group I and II, respectively. The lowest body mass gain, in comparison to other groups of lambs ($P \leq 0.05$), was recorded in triplets (IIIO - 5.12% of the birth weight).

Somatotropin. Mean plasma concentrations of GH were insignificantly higher in singleton and twin lambs born to non-obese mothers (IN – 3.05 ±0.19, 3.15 ±0.22, and 3.30 ±0.26 ng/mL; IIN – 2.70 ±0.19, 3.00 ±0.17, and 2.85 ±0.11 ng/mL, respectively in the 2nd, 4th, and 6th week

of the postnatal life) in comparison to the singleton and twin offspring of obese ewes (IO – 2.40 ±0.18, 2.45 ±0.19, and 2.70 ±0.29 ng/mL; IIO – 2.40 ±0.16, 2.55 ±0.19, and 2.70 ±0.14 ng/mL) (Fig. 1). In the lambs born to normally weighing sheep, singletons had slightly increased somatotropin levels in comparison to twins. In the 2nd, 4th, and 6th week of the life, the significantly augmented (P≤0.001) GH levels in the triplet offspring of obese ewes (IIIO – 4.80 ±0.16, 4.55 ±0.16, and 4.65 ±0.15 ng/mL) were revealed, when compared to the other groups (IN, IIN, IO, IIO) (Fig. 1).

Insulin-like growth factor-1. Although the mean IGF-1 plasma concentrations were increased in singleton and twin offspring of normally weighing ewes (IN - 216.30 ±7.31, 196.20 ±6.54, and 209.19 ±11.79 ng/mL; IIN – 176.72 ±5.89, 191.70 ±6.39, and 194.40 ±6.48 ng/mL, respectively in the 2nd, 4th, and 6th week of the postnatal life) in comparison to the same birth type lambs derived from obese ewes (IO – 149.40 ±4.98, 145.84 ±4.86, and 166.81 ±5.56 ng/mL; IIO – 149.74 ±4.99, 150.63 ±5.02, and 170.16 ±5.67 ng/mL, respectively in the 2nd, 4th, and 6th week of the life), they did not differ significantly between the groups. Among the offspring of non-obese sheep, the increased IGF-1 levels in singleton lambs compared to twins were observed. Significantly lower (P≤0.001) IGF-1 concentrations in triplets (IIIO – 57.35 ±1.91, 56.17 ±1.87, and 60.26 ±2.02 ng/mL, respectively in the 2nd, 4th, and 6th week) were found, when compared to the other groups (IN, IIN, IO, IIO) (Fig. 1).

Insulin. There were no significant differences in plasma insulin concentration between singleton (IN – 2.29 ±0.46, 2.03 ±0.14, 2.29±0.23 µIU/mL; IO – 2.76 ±1.08, 2.71 ±0.18, 2.87 ±0.16 µIU/mL in the 2nd, 4th, and 6th week, respectively) and twin lambs (IIN – 2.27 ±0.56, 2.29 ±0.21, 2.60 ±0.18 µIU/mL, IIO – 2.74 ±0.68, 2.69 ±0.62, 2.74 ±0.45 µIU/mL in the 2nd, 4th, and 6th week, respectively). However, the offspring of obese sheep (IO, IIO) had slightly higher insulin concentrations in comparison to ewe lambs originating from normally weighing mothers (IN and IIN). Conversely, in triplets insulin levels were significantly increased (P≤0.001), when compared to the singleton and twin lambs (IIIO – 12.81 ±3.18, 12.41 ±2.97, 9.11 ±2.14 µIU/mL) (Fig. 2).

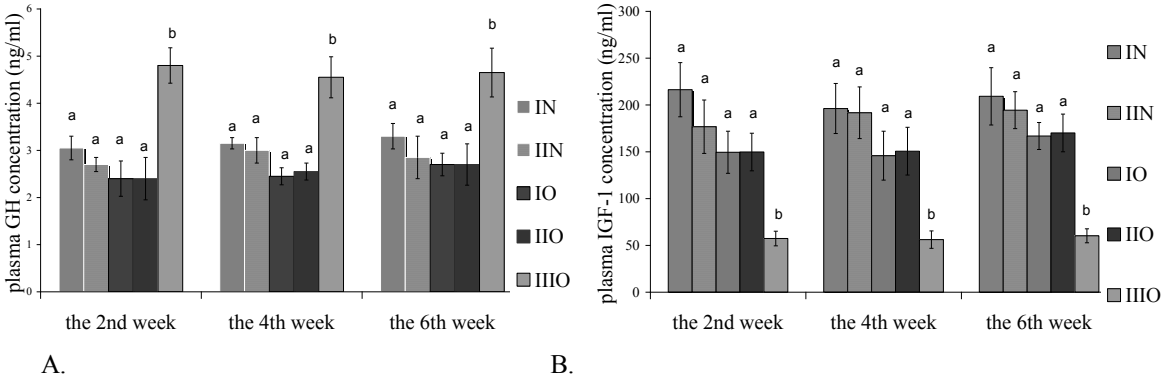


Fig. 1. Plasma concentrations of GH (ng/mL) (A) and IGF-1 (ng/mL) (B) (mean ±SD) in the experimental SCP ewe lambs during the 2nd , 4th, and 6th week of their postnatal life: IN - singleton offspring of non-obese mothers (n=4); IIN - twin offspring of non-obese mothers (n=14); IO - singleton offspring of obese mothers (n=4); IIO – twin offspring of obese mothers (n=6); IIIO – triplet offspring of obese mothers (n=13).
a,b – the values signed with various letters differ significantly (P≤0.001).

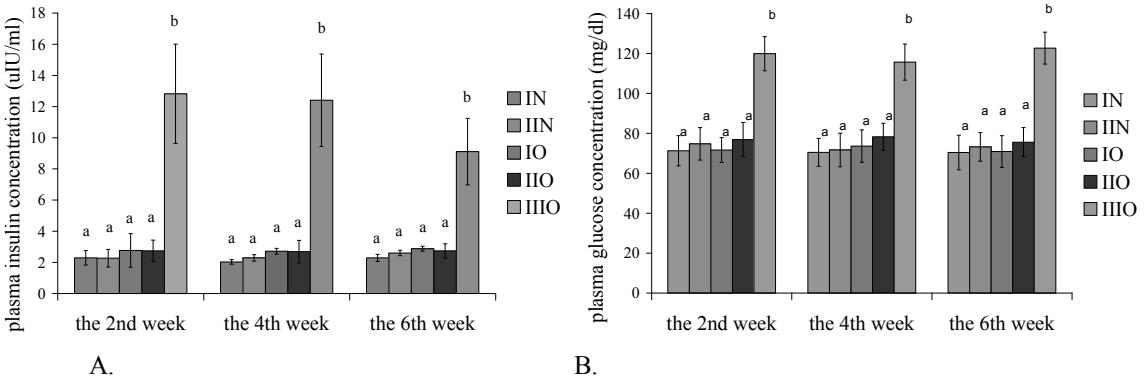


Fig. 2. The averaged plasma concentrations of insulin (µIU/mL) (A) and blood glucose levels (mg/dl) (B) (mean ±SD) in SCP ewe lambs during the 2nd , 4th, and 6th week of their postnatal life: IN - singleton offspring of non-obese mothers (n=4); IIN - twin offspring of non-obese mothers (n=14); IO - singleton offspring of obese mothers (n=4); IIO – twin offspring of obese mothers (n=6); IIIO – triplet offspring of obese mothers (n=13).
a,b – the values signed with various letters differ significantly (A: P≤0.001; B: P≤0.05)

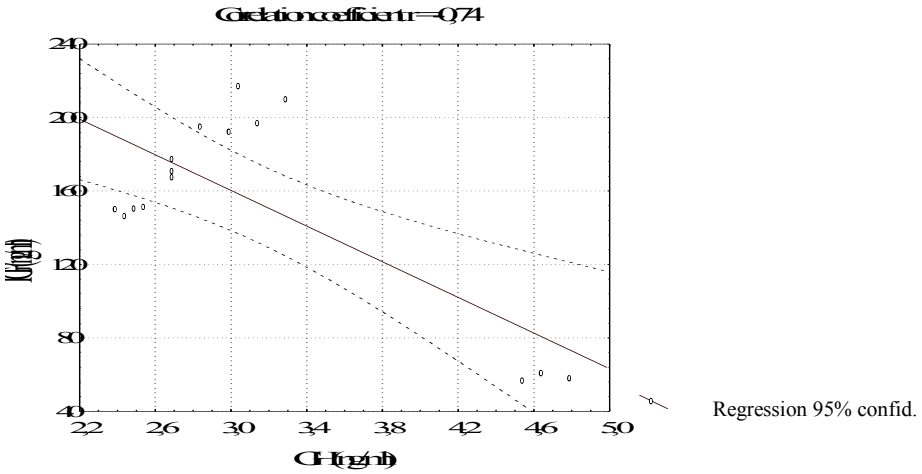


Fig. 3. The inverse relationship between GH (ng/mL) and IGF-1 (ng/mL) plasma concentrations in triplet lambs born to obese ewes (IIIO).

Glucose. Blood glucose concentrations did not differ markedly between the experimental groups of lambs born to non-obese and obese ewes, both carrying singletons and twins (IN – 71.25 ±7.56, 70.45 ±6.98, and 70.40 ±8.65 mg/dl; IIN – 74.76 ±8.14, 71.68 ±8.40, and 73.20±7.17 mg/dl; IO – 71.65 ±6.24, 73.60 ±8.12, and 70.87 ±7.95 mg/dl; IIO – 76.89 ±8.56, 78.24 ±6.79, and 75.55±7.34 mg/dl in the 2nd, 4th, and 6th week, respectively). However, the glucose levels were significantly augmented ($P \leq 0.05$) in triplets (IIIO – 119.89 ±8.55, 115.67 ±9.05, and 122.67±7.99 mg/dl, in the 2nd, 4th, and 6th week, respectively) (Fig. 2).

Relationship between GH, IGF-1, insulin, and body mass gains. In the groups of singletons and twins, both born to normally weighing and obese mothers, high positive correlations between GH and IGF-1 (IN – r = 0.94; IIN – r = 0.91; IO – r = 0.92; IIO - r = 0.89), as well as between GH and daily weight gains (IN – r = 0.92; IIN – r = 0.90; IO – r = 0.88; IIO – r = 0.85) were found. However, in triplets, inverse GH/IGF-1 and GH/weight gains relationships were observed (r = -0.74 and r = -0.73, respectively) (Fig. 3). The analysis of relation between GH and insulin showed a positive correlation in all groups of experimental ewe lambs (IN – r = 0.91; IIN – r = 0.89; IO – r = 0.88; IIO – r = 0.86; IIIO – r = 0.78). Moreover, a high positive relationship between insulin and glucose plasma level in triplets was revealed (r = 0.96).

Discussion

Maternal obesity caused significant increase in lambing rate with the rise in triplets' frequency. It was connected also with slightly increased birth weight of singleton and twin, but not triplet lambs. In triplets reduced birth weight and daily weight gains during the first 6 weeks of the postnatal life were observed.

It is well established that after birth the somatotropic axis regulates growth predominantly *via* growth hormone acting on GH receptors (GHR) to increase both tissue and circulating IGF-1 levels (1, 18). However, despite approximately twofold higher plasma concentration of GH in our SCP experimental triplet ewes, both IGF-1 levels and daily weight gains were dropped, compared to the other groups of female lambs. These results are in accordance with the data obtained by Greenwood *et al*. (8, 9) in the experiments with male crossbreed lambs. This disruption in GH *vs*. IGF-1 relationship, resulting in low growth rate, might be caused by immaturity of somatotropic axis. According to Klempt *et al*. (13), plasma GH levels in sheep are 10 to 20-fold higher during foetal life than after birth, whereas IGF-1 concentrations are relatively low. This discrepancy between GH and IGF-1 before birth results primarily from the decreased GHR expression in the foetal liver, which reaches only about 30% of its adult value (13). It may be caused also by altered signalling mediated by STAT5b (signal transducer and activator of transcription 5b), which is phosphorylated by the receptor-associated Janus kinase following binding of GH to its receptor (1). However, after birth GHR, as well as circulating IGF-1 levels increase quickly**,** whereas circulating GH levels diminish to adult concentrations in the properly developing sheep (2-4). Moreover, it is widely accepted that nutritional status plays a principal role in determining GH and IGF-1 concentrations (4). Plasma levels of GH are elevated as a result of undernutrition, which exerts an influence in hypoglycaemia and low serum free fatty acids on the pituitary. The increase in GH secretion at reduced nutritional intakes is also linked with diminished IGF-1 negative feedback within the somatotropic axis and a drop in somatostatin secretion. Additionally, a reduced metabolic clearance rate of GH at low nutrition is observed, which is facilitated by impaired GH-mediated feedback due to reduction of hepatic GHR. Concurrently, the ability of GH to maintain plasma IGF-1 is impaired (4). Although, our experimental SCP triplet ewe lambs were well fed, the above mentioned mechanism is possible because of observed discrepancy in insulin *vs* glucose levels. In triplets, 3.5 to 6-fold higher insulin concentrations, and simultaneously about 50% increase in

the glucose levels were observed, when compared to the other groups of lambs. Moreover, circulating GH levels in triplets were augmented despite high insulin concentrations, which under normal condition suppress GH secretion (11). This observation suggests the development of insulin resistance and inability to maintain adequate glucose uptake. Thereby, there could be a similar glucose availability as in a case of undernutrition causing disparity in somatotropic/IGF-1 axis. Additionally, insulin resistance itself can decrease the growth rate due to a drop in glucose uptake by target cells. Furthermore, it is known that GH elevates plasma glucose and afterwards insulin levels, which can create an insulin resistant state in the long term (4, 6). However, at the condition of granted low GHR expression levels (which was partially confirmed by decreased IGF-1 concentrations), the induction of hyperglycaemia and, after that hyperinsulinaemia, by GH appears to be doubtful. On the other hand, chronic hyperinsulinaemia itself may also induce hepatic GH resistance (12). Additionally, because IGF-1 under normal conditions contributes to cellulipetal glucose transport, its low levels could escalate the increment in plasma glucose and consequently also in insulin concentrations (5).

There were no significant differences in GH, IGF-1, insulin, and glucose levels between the singletons and twins, born to non-obese, as well as obese sheep. In these lambs, compared to triplets, higher values of birth weight and regular relationships between GH, IGF-1, insulin, and glucose were observed. They resulted in normal growth rate in these groups of ewes.

To sum up, transition from prenatal to postnatal life requires maturation of biological systems essential for growth, especially the GH/IGF-1 axis (8, 9, 16, 23). SCP triplet ewe lambs born to obese mothers, characterised by low birth weight, had the disrupted relationship between GH, IGF-1, and growth rate, as well as between insulin and glucose plasma levels during the first six weeks of their postnatal life. It suggests that their metabolic system requires much longer period to adapt to postnatal life and begin the rapid growth in comparison to singletons and twins bigger at birth.

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