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ORIGINAL ARTICLE

Postnatal Changes in Ghrelin, Adiponectin, Insulin and Leptin Concentrations in Term Newborns

Hytinantti T[a], Kajantie E[b], Karonen SL[c], Andersson S[d]

[a,b,d]Hospital for Children and Adolescents, [d] Department of Clinical Chemistry [a] Helsinki City Maternity Hospital, Helsinki University Central Hospital, [b] National Public Health Institute, Helsinki, Finland

Birth denotes a sharp transition from constant placental nutrient delivery to independent regulation of energy intake and expenditure. Adiponectin, ghrelin, insulin and leptin are key hormones regulating appetite and energy homeostasis and could therefore be crucial components of postnatal adaptation.

We studied the changes and relationships in these hormones and their correlation to clinical variables during adaptation to extrauterine life.

Materials and Methods: Plasma venous adiponectin, ghrelin, insulin and leptin concentrations were measured at birth and controlled during early postnatal days (59.4+15.4 [SD] h, range 36-93 h) for 38 healthy newborn infants [(20 male, 18 female), gestational age 40.2+0.9 wk (mean+SD), range 37.9 to 42.1 wk] born from uncomplicated pregnancies.

Results: Median adiponectin concentration at birth was at 25. 1 μg/mL (interquartile range 20. 0–29. 1 μg/mL) and during early postnatal days 17. 8 μg/mL (10.7-23.3; 95% CI of difference 26 to 46%). Median ghrelin concentration in cord plasma was 651 pg/mL (539-830) which during early postnatal days had decreased to 537 pg/mL (476-723; 95% CI 6 to 23%). At birth, median insulin concentration was 7.35 mU/L (6.53-9.43) and, during early postnatal days age, this was 4.35 mU/L (3.43-6.03; 95% CI 33 to 53%). At birth, median leptin concentration was 4.60 μg/L (3.51-5.84) and during early postnatal days was 0. 83 μg/L, (0. 65-1. 06; 95% CI 78 to 85%). At birth no correlation between hormone concentrations and gestational age or birth weight existed. During early postnatal days, adiponectin was positively (r=0. 49) correlated with ghrelin concentrations.

Conclusion: In healthy term infants, the change from fetal to extrauterine life is accompanied by a decrease in circulating adiponectin, ghrelin, insulin and leptin suggesting distinct roles of these hormones in postnatal adaptation.

Correspondence: Timo Hytinantti, Helsinki City Maternity Hospital, 00029 HUS Helsinki, Finland
E-mail: timo. hytinantti@hus.fi

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Introduction

Birth denotes a sharp transition from constant placental nutrient delivery to independent regulation of energy intake and expenditure. Therefore adaptation to extrauterine life may result in rapid changes in the concentrations of adiponectin, ghrelin, insulin and leptin hormones that are involved in energy metabolism and the control of appetite.[1] However, the roles of these hormones during the postnatal transition period remain poorly known.

The regulation and function of adipocytokines, such as adiponectin and leptin, may be different during fetal development, postnatal adaptation, childhood and adulthood; obser-

vation of postnatal changes of these hormones may provide valuable information about their roles during different phases of human growth and development. For example, based on studies in adults, leptin and adiponectin were initially thought to originate exclusively from adipocytes. However, high fetal leptin concentrations decline sharply after birth, a phenomenon which was subsequently thought to be due to removal of leptin synthesized by the placenta.[2] Later studies have shown that placenta contributes minimally of its leptin release to the fetal circulation suggesting that other mechanisms could be responsible for the sudden decline of leptin concentrations after birth.[3] Correspondingly, a distinct origin of adiponectin in the fetus was suggested by observations of adiponectin concentrations increasing towards term, to values 2 to 3-fold higher than in adults. [4] Recently, adiponectin was found to be synthesized by several fetal non-adipose tissues, and also by the placenta.[5,6]

Another key regulator of food intake and energy homeostasis is ghrelin. [1] It is a peptide hormone produced mainly by the stomach, but also by the placenta. [1] Most studies have shown increased cord plasma ghrelin concentrations during late gestation,[7] suggesting a specific role of ghrelin during this period of fetal development and possibly during postnatal adaptation. However, little is known about the course and regulation of circulating ghrelin during the early postnatal period in term infants.

We, therefore, set out to study how plasma ghrelin concentrations change between birth and the first postnatal days in healthy term infants and whether they are related to concurrent changes in adiponectin, insulin and leptin concentrations, and also to evaluate their relevance to clinical variables.

Materials and Methods

Patients

We studied 38 healthy newborn infants (20 males, 18 females; gestational age 40.2+0.9 wk [mean+SD], range 37.9 to 42.1 wk) born to healthy, non-smoking mothers after uncomplicated pregnancies at Helsinki City Maternity Hospital. Table 1 shows the clinical data. Only term infants of normal relative birth weight (between -2.0 to +2.0 SD) were recruited to minimize the confounding effects of preterm birth and deviant fetal growth.[4,7-11] Gestational age was confirmed by ultrasonography before 20 wk of gestation. The infants and their placentas were measured immediately after birth, and the infants were weighed again, in conjunction with the early postnatal blood sample. All infants were exclusively breast fed.

Birth weight relative to gestational age, expressed in SD units, was determined separately for both sexes with reference to current Finnish standards. [12] Ponderal index at birth was calculated as weight Kg/m^3.

Umbilical cord venous blood samples were drawn at birth, and peripheral venous control blood sample was obtained during early postnatal days (59.4+15.4 [SD] h, range 36-93 h). Blood was drawn into aprotinin-EDTA-containing tubes, with plasma separated and frozen without delay, and stored at -70°C. Prior to the puncture of the peripheral vein, local anaesthetic cream (EMLA ®, Astrazeneca, Sweden) was applied to the skin.

The study was conducted in accordance with the Declaration of Helsinki. Written informed parental consent was obtained before participation in the study. The study was approved by the Ethics Committee of the Department of Obstetrics and Gynaecology of Helsinki University Central Hospital.

Table 1. Patients' data

Variables	Mean (SD)	Range
Gestational age (weeks)*	40. 2 (0. 9)	37. 9-42. 1
Birth weight (g)	3555 (384)	3015-4380
Relative birth weight (SDS)	-0. 14 (0. 93)	-1. 8-+2. 0
Length at birth (cm)	50. 5 (1. 5)	47. 5-54. 0
Head circumference at birth (cm)	35. 4 (1. 4)	33. 0-39. 0
Ponderal index at birth (kg/m3)	27. 6 (2. 1)	23. 2-33. 5
Placental weight (g)	614 (113)	400-920
Weight at 2days (g)	3360 (382)	2760-4085
Weight reduction; birth–2 days (g)	200 (86)	20-375
Upper arm circumference (at 2 days) (cm)	12. 3 (0. 8)	10. 5-14. 0
Maternal height (cm)	166. 5 (6. 3)	155-181
Maternal pre-pregnancy BMI (kg/m2)	23. 3 (3. 2)	19. 0-31. 6

* Male/female and vaginal cesarean section ratios were 20/18 and 33/5 respectively.

Biochemical assays

Total ghrelin concentrations were measured by means of a commercial RIA (Phoenix Pharmaceuticals, Belmont, CA, USA). Fifty percent binding was at 190 pg/mL. The sensitivity in undiluted samples was 15 pg/mL. Inter-and intra-assay confidence intervals, as given by the manufacturer, were 7.5% and 4.0%, respectively.

Total adiponectin concentrations were determined by ELISA (R&D Systems, Inc., Minneapolis, MN, USA). Intra-and inter-assay coefficients of variation of the assay were between 2.5% and 4.7% and between 5.8% and 6.9%, respectively.

Leptin concentrations were measured by RIA (Linco Research, St. Charles, MO, USA). Intra-and inter-assay coefficients of variation at low concentration (2.8+0.2 μg/L) were 4.7% and 2.6%, and at medium concentration (15.6 μg/L) 6.7% and 2.2%, respectively.

Insulin concentrations were determined by immunoradiometric assay (Biosource, Nivelles, Belgium). Intra-assay coefficients of variation at low concentration (6.6+0.2 mU/L) and at high concentration (53.0 +1.1 mU/L) were 4.5% and 21% respectively.

Statistical analysis

Hormone concentrations were logtransformed to normality and are presented as median (interquartile range). Other raw data are presented as mean, SD, and range. Paired samples t-test was used to compare hormone concentrations in cord vein plasma with those during early postnatal days, and linear correlation coefficients were calculated to assess associations between variables; $p<0.05$ were considered statistically significant.

Results

Table 2 shows the hormonal data at birth and at the time of early postnatal blood sampling. Median plasma concentrations of ghrelin, adiponectin, leptin and insulin were lower during early postnatal days than in cord plasma (Fig 1). The reductions in mean concentrations were: ghrelin, 15% (95% CI 6 to 23%; p=0. 002), adiponectin, 36% (95% CI 26 to 46%; p<0. 0001), leptin, 82% (95% CI 78 to 85%; p<0. 0001), and insulin, 44% (95% CI 33 to 53%; p<0. 0001).

Table 2. Hormone concentrations at birth

Hormone concentration	Median (25-75 percentile)	
	At birth	At two days of age
Adiponectin (μg/mL)	25. 1 (20. 0-29. 1)	17. 8 (10. 7 -23. 3)
Ghrelin (μg/L)	651 (539-830)	537 (476-723)
Insulin (mU/L)	7. 35 (6. 53-9. 43)	4. 35 (3. 43-6. 03)
Leptin (μg/L)	4. 60 (3. 51-5. 84)	0. 83 (0. 65-1. 06)

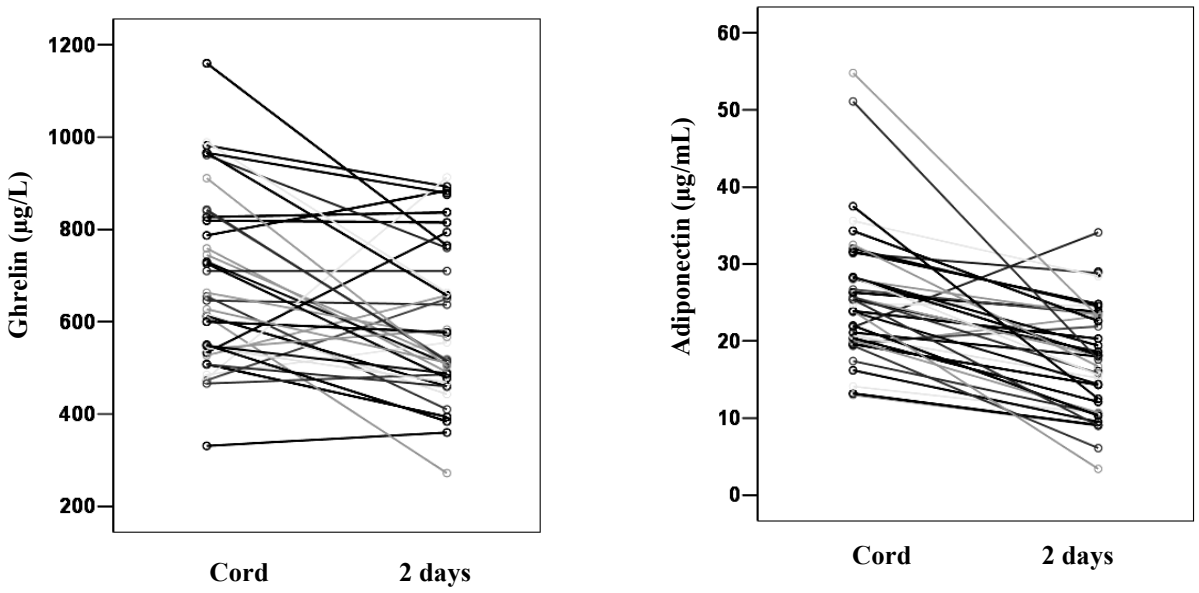

Fig. 1. Ghrelin and adiponectin concentrations at birth and at 2 days of age. Each line connects the concentrations of an individual infant

Ghrelin concentrations in cord plasma correlated with those during early postnatal days, (r=0.45; p=0.005), the same being true for adiponectin (r=0.47; p=0.003), but not for leptin (r=0.22; p=0.2) or insulin (r=0.02; p=0.9).

At birth no correlations were found between ghrelin, adiponectin, leptin or insulin concentrations. In the early postnatal days sample there was a positive correlation between ghrelin and adiponectin concentrations (r=0. 49; p=0. 002) and an inverse correlation between leptin and adiponectin concentrations (r= -0.39; p=0.01, Fig. 2). However, the two lowest adiponectin concentrations, 3.4 and 6.1 μg/mL, were seen with two exceptionally high leptin concentrations, 2.48 and 4.31 μg/L. If these two outliers were ex-

cluded from the analysis the correlation between postnatal leptin and adiponectin was no longer statistically significant (r=0. 03; p=0. 8, fig 2). No association of insulin with ghrelin, adiponectin or leptin concentration reached statistical significance.

Neither ghrelin nor adiponectin concentrations in cord plasma or in the early postnatal days sample were correlated to infant sex, mode of delivery or any of the measurements shown in tables 1 and 2.

The time interval between the previous feed and venous sample collection (55+45 min, range 10-180 min), and the relative (%) or absolute (g) change in weight from birth to time of control blood sample collection did not correlate with the concentrations of ghrelin or other hormones at that age.

As for leptin, gestational age at birth was related to its concentrations in cord plasma (r=0. 33; p=0. 04) but not to that at 2-4 d of age (r=0. 16; p=0. 3) whereas the correlation of birth weight with cord leptin was weak (r=0. 29; p=0. 09). Furthermore, there was a correlation between cord plasma insulin and relative birth weight (p=0. 04).

Discussion

We found a significant decline in ghrelin, adiponectin, leptin and insulin concentrations during early postnatal days. Characteristic phenomena of postnatal adaptation include weight loss mainly due to loss of edema, and low energy intake until the breast milk supply becomes established, and the infant is dependent on hepatic glycogen stores in order to maintain euglycemia. [13]

The cord plasma ghrelin concentrations we found are in keeping with other reports,[11] being about 2-fold higher than adult preprandial values.[14] Ghrelin expressions in human placenta is probably confined to the first trimester.[15] Thus, other sources of fetal ghrelin may contribute to the elevated umbilical cord ghrelin concentrations. For example, from week 10 of gestation, human fetal pancreas and lung have been demonstrated to produce ghrelin, in contrast to respective tissues in adults.[16] Our assay measured total ghrelin concentration which has been shown to have an excellent correlation (r=0. 81 in cord plasma) with active ghrelin concentration.[17]

In spite of the decline in ghrelin concentrations during the early postnatal days the hormone concentrations still remain at adult pre-prandial levels. [14] Relatively high ghrelin levels may help to sustain the infant's efforts to promote breast milk production by sucking. Interestingly, in newborns and children, in contrast to adults, food intake does not seem to produce a decline in ghrelin concentrations.[18,19] In addition, ghrelin induces hyperglycemia and decreases plasma insulin concentrations. [20]

In contrast to ours, a previous study of healthy, appropriately grown term infants showed significantly higher postnatal plasma ghrelin concentrations compared with those in cord plasma. [11] However, in that study, cord vein ghrelin concentrations were lower than in the present study, and the infants were 3-7 days older, at which age most infants are already gaining weight after the usual nadir at about two to three days after birth. An increased ghrelin concentration after this period could contribute to weight gain because of the positive effects of the hormone on energy balance. [1] It is interesting to note that different studies have reported varying concentrations of ghrelin,[2] including a study which obtained samples within 2 hours after birth and reported ghrelin concentrations 6-fold higher than ours.[21,22] Whether or not ghrelin concentrations fluctuate similarly to thyroid hormones for example during the first days of life, requires further study.

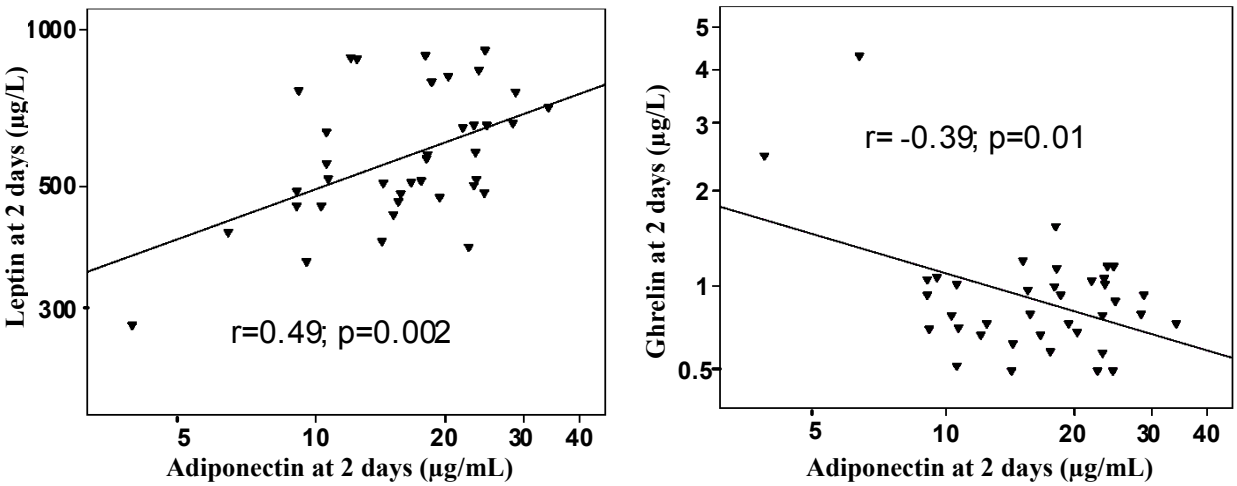

Fig. 2. Relationship of adiponectin concentration with leptin and ghrelin concentrations at 2 days of age. After exclusion of the two outliers, the correlation between leptin and adiponectin concentrations becomes non-significant (r=0. 03; p=0. 8)

We tried to eliminate the effect of possible circadian pattern of circulating ghrelin concentrations, which is observed at least in adults,[14] by timing our sampling to noon, although it is not yet known at what age the circadian pattern emerges. We also evaluated the possible connection between fasting and ghrelin concentrations by recording the time elapsed between breastfeeding and the collection of the postnatal plasma sample. Although no such correlation was observed, the result may have been masked by large inter-individual variation in circulating ghrelin concentrations, as demonstrated in adults. [14] Interestingly, in older infants, fasting increases ghrelin concentrations,[23] a recent study in preterm infants showed that ghrelin concentrations were not influenced by eating. [18] We also found, as previously demonstrated,[2] a marked postnatal decline in leptin concentrations, the mechanisms of which are not known.[2,3] The orexigenic properties of ghrelin,[1] and the opposing ones of leptin[1] would make our observation of concomitantly high ghrelin and a more marked decline in leptin concentrations physiologically feasible.

Adiponectin concentrations at birth were 2- to 3-fold higher than those reported in adults. [4] We also observed a decrease in adiponectin concentrations during early postnatal days. In a recent study, adiponectin concentrations at four days of age were similar to those observed at birth in healthy, term infants.[10] The main difference between these studies is the postnatal age at the time of the plasma sample. It is possible that chronologically distinct changes in these hormones occur rapidly during adaptation to extrauterine life.

High adiponectin concentrations at the time of birth might be beneficial for the infant during the transition to a colder extrauterine environment, as adiponectin has been shown to increase thermogenesis in mice,[24] and in rats, brown adipose tissue actively produced adiponectin during perinatal period. [25] Whether this is the case in humans is not known, and the high adiponectin concentrations at term may result from adiponectin synthesis in a number of different fetal tissues. [5]

A high concentration of adiponectin, which has marked insulin sensitising properties,[1] might be inappropriate during the initial pe-

riod of breast feeding when a constant milk supply is not yet established. Adiponectin has been shown to inhibit hepatic glucose production. [1] This could be harmful after birth, since in newborn infants, gluconeogenesis provides about 10% of the glucose metabolized during the early adaptation period. [26] In rats, fasting and refeeding have been shown to diminish and reverse both adiponectin and leptin gene expression respectively. [27] Adiponectin has also been shown to decrease body weight by stimulating energy expenditure in mice. [28] The high adiponectin concentration at birth and the observed decrease thereafter may suggest a role for adiponectin as a modifier of insulin and glucose metabolism and thermoregulation during this period.

In the early postnatal samples, the inverse relation between adiponectin and leptin may be a result of the regulatory action of adiponectin on adipose tissue. [1] A similar relationship has been found in adolescents and adults. [29,30] Our results may thus be a sign of a rapid shift of the actions of adiponectin on the endocrine function of the adipose tissue after birth.

The conflicting results observed at birth between adiponectin and leptin concentrations may be due to different study populations, and the effect of the placenta on these hormones. [31-33]

The positive correlation between the concentrations of ghrelin and adiponectin during early postnatal days may reflect the complex interactions of energy balance and glucose utilization during adaptation.

In conclusion, in healthy term infants, we have demonstrated a decline in ghrelin, adiponectin, leptin and insulin hormone concentrations in response to the change from fetal to extrauterine life. These changes may act in concert to stimulate food intake and to support metabolic adaptation during the first days of life.

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References

1. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004; 50: 1511-25.
2. Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat Med 1997; 9: 1029-33.
3. Linnemann K, Malek A, Sager R, Blum WF, Schneider H, Fusch C. Leptin production and release in the dually in vitro perfused human placenta. J Clin Endocrinol Metab 2000; 85: 4298-301
4. Kajantie E, Hytinantti T, Hovi P, Andersson S. Cord plasma adiponectin: a 20-fold rise between 24 weeks gestation and term. J Clin Endocrinol Metab 2004; 89: 4031-6.
5. Corbetta S, Bulfamante G, Cortelazzi D, Barresi V, Cetin VI, Mantovani G, et al. Adiponectin expression in human fetal tissues during mid-and late gestation. J Clin Endorcinol Metab 2004; 90: 2397-402.
6. Chen J, Tan B, Karteris E, Zervou S, Digby J, Hillhouse EW, et al. Secretion of adiponectin and its receptors by cytokines. Diabetologia 2006; 49: 1292-302.
7. Bellone S, Rapa A, Vivenza D, Vercellotti A, Petri A, Radetti G, et al. Circulating ghrelin levels in the newborn are positively associated with gestational age. Clin Endocrinol 2004; 60: 613-7.
8. Harigaya A, Nagashima K, Nako Y, Morikawa A. Relationship between concentration of serum leptin and fetal growth. J Clin Endocrinol Metab1997; 82: 3281-4.
9. Farquhar J, Heiman M, Wong A, Wach R, Chessex P, Chanoine J-P. Elevated umbilical cord ghrelin concentrations in small for gestational age neonates. J Clin Endocrinol Metab 2003; 88: 8324-7.
10. Sivan E, Mazaki-Tovi S, Pariente C, Efraty Y, Schiff E, Hemi R, et al. Adiponectin in human cord blood: relation to fetal birth weight and gender. J Clin Endocrinol Metab 2003; 88: 5656-60.
11. Kitamura S, Yokota I, Hosoda H, Kotani Y, Matsuda J, Naito E, et al. Ghrelin concentration in cord and neonatal blood: relation to fetal growth and energy balance. J Clin Endocrinol Metab 2003; 88: 5473-7.

12. Pihkala J, Hakala T, Voutilainen P, Raivio K. Characteristic of recent fetal growth curves in Finland. Duodecim 1989; 105: 1540-6 (Finnish).
13. Polk DH. Disorders of carbohydrate metabolism. In: Taeusch HW, Ballard RA, editors. Avery's Disease of the Newborn. 7th ed. Pennsylvania: W.B. Saunders Company 1998. p.1235-41.
14. Cummings DE, Purnell JQ, Scott FR, Scmidova K, Wisse BE, Weigle DS. Preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001; 50: 1714-9.
15. Gualillo O, Caminos E, Blanco M, Garcia-Caballero T, Kojima M, Kangawa K, et al. Ghrelin, a novel placental-derived hormone. Endocrinology 2001; 142: 788-94.
16. Rindi G, Necchi V, Savio A, Torsello A, Zoli M, Locatelli V, et al. Characterisation of gastric ghrelin cells in man and other mammals: studies in adult and fetal tissues. Histochem Cell Biol 2002; 117: 511-9.
17. Yokota I, Kitamura S, Hosoda H, Kotani Y, Kangawa K. Concentration of the n-octanylated active form of ghrelin in fetal and neonatal circulation. Endocr J 2005; 52: 271-6.
18. Bellone S, Baldelli R, Radetti G, Rapa A, Vivienza D, Petri A, et al. Ghrelin secretion in preterm neonates progressively increases and is refractory to the inhibitory effect of food intake. J Clin Endocrinol Metab 2006; 91: 1929-33.
19. Bellone S, Castellino N, Broglio F, Rapa A, Vivienza D, Radetti G, et al. Ghrelin secretion in childhood is refractory to the inhibitory effect of feeding. J Clin Endocrinol Metab 2004; 89: 1662-5.
20. Broglio F, Arvat E, Benso A, Gottero C, Muccoli G, Papotti M, et al. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. J Clin Endocrinol Metab 2001; 86: 5083-6.
21. Ng PC, Lee CH, Lam CW, Wong E, Chan IH, Fok TF. Plasma ghrelin and resistin concentrations are suppressed in infants of insulin-dependent diabetic mothers. J Clin Endocrinol Metab 2004; 89: 5563-8.
22. Phillps AF. Ghrelin, energy balance, and weight in infants: The chicken or the egg? J Pediatr Gastroenterol Nutr 2005; 41: 575-7.
23. Savino F, Grassino EC, Fissore MF, Guidi C, Liguori SA, Silvestro L, et al. Ghrelin, motilin, insulin concentration in healthy infants in the first months of life: relation to fasting time and anthropometry. Clin Endocrinol 2006; 65: 158-62.
24. Yoda M, Nakano Y, Tobe T, Shioda S, Choi-Miura NH, Tomita M. Characterization of mouse GBP28 and its induction by exposure to cold. Int J Obes Relat Metab Disord 2001; 25: 75-83.
25. Fujimoto N, Matsuo N, Sumiyoshi H, Yamaguchi K, Saikawa T, Yoshimatsu H, et al. Adiponectin is expressed in the brown adipose tissue and surrounding immature tissues in mouse embryos. Biochim Biophys Acta 2005; 1731: 1-12.
26. Frazier TE, Karl IE, Hillman LS, Bier DM. Direct measurement of gluconeogenesis from [2,3] 13C2] alanine in the human neonate. Am J Physiol 1981; 240: E615-21.
27. Zhang Y, Matheny M, Zolotukhin S, Tumer N, Scarpace PJ. Regulation of adiponectin and leptin gene expression in white and brown adipose tissues: influence of beta3-adrenergic agonists, retinoic acid, leptin and fasting. Biochim Biophys Acta 2002; 1584: 115-22.
28. Qi Y, Takahashi H, Hileman SM, Patel HR, Berg AH, Pajvani UB, et al. Adiponectin acts in the brain to decrease body weight. Nat Med 2004; 10: 524-9.
29. Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. Eur J Endocrinol 2002; 147: 173-80.
30. Stefan N, Bunt JC, Salbe AD, Funahashi T, Matsuzawa Y, Tataranni PA. Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. J Clin Endocrinol Metab 2002; 87: 4652-6.
31. Tsai PJ, Yu CH, Hsu SP, Lee YH, Chiou CH, Hsu YW, et al. Cord plasma concentrations of adiponectin and leptin in healthy term neonates:positive correlation with birthweight and neonatal adiposity. Clin Endocrinol (Oxf) 2004; 61: 88-93.
32. Pardo I, Geloneze B, Tambascia MA, Barros-Filho AA. Hyperadiponectemia in newborns: Relationship with leptin levels and birth weight. Obes Res 2004; 12: 521-4.
33. Lindsay RS, Walker JD, Hovel PJ, Hamilton BA, Calder AA, Johnston FD; Scottish Multicentre Study of Diabetes Pregnancy. Adiponectin is present in cord blood but is unrelated to birth weight. Diabetes Care 2003; 26: 57-62.