Clinical Biochemistry 52 (2018) 80–84

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Lutein levels in arterial cord blood correlate with neuroprotein activin A in healthy preterm and term newborns: A trophic role for lutein?

Simonetta Picone[a], Alberto Ritieni[b], Adele Fabiano[a], Giulia Graziani[b], Piermichele Paolillo[a], Giovanni Livolti[c], Fabio Galvano[c], Diego Gazzolo[d,e,*]

[a] Neonatal Intensive Care Unit, Policlinico Casilino, Rome, Italy
[b] Dept. of Pharmacy, Federico II Naples University, Italy
[c] Dept. of Drug Science, University of Catania, Italy
[d] Dept. of Maternal, Fetal and Neonatal Medicine, C. Arrigo Children's Hospital, Alessandria, Italy
[e] Neonatal Intensive Care Unit G. d'Annunzio University, Chieti, Italy

ARTICLE INFO

Keywords:
Newborn
Cord blood
Lutein
Activin A
Biomarkers
Brain development

ABSTRACT

Background: Lutein (LT) is a naturally occurring xanthophyll carotenoid most predominant in the central nervous system (CNS), but its neurotrophic role is still debated. We therefore investigated whether cord blood concentrations correlated with a well-established neurobiomarker, namely activin A.
Methods: We conducted a prospective study on the distribution of LT and activin A in arterial cord blood of healthy preterm (n = 50) and term (n = 82) newborns according to weeks of gestational age (wGA) and gender.
Results: LT and activin A showed a pattern of concentration characterized by higher levels ($P < 0.01$, for all) at 33–36 wGA followed by a progressive decrease ($P < 0.01$, for all) from 37 onwards with a dip at term. Both LT and activin A were gender-dependent with significantly ($P < 0.01$, for all) higher levels in all recruited females and after sub-grouping for preterm and term births. LT ($R = 0.33$; $P < 0.001$) correlated with wGA at sampling. There were significant positive correlations between lutein and activin A in male ($R = 0.93$; $P < 0.001$) and female ($R = 0.89$; $P < 0.001$) groups.
Conclusions: The present data showing a correlation between LT and activin A support the notion of a neurotrophic role gender-dependent for LT and open the way to further investigations correlating LT with well-established biochemical markers of CNS development/damage.

1. Introduction

Lutein (LT) is a naturally occurring xanthophyll carotenoid, not synthesized by humans, found in fruits and vegetables such as spinach and kale, which are the most abundant sources in nature [1]. Both in humans and in animals, LT concentration in the body is dependent to its dietary intake [2]. Although the several functions of LT are still a matter of debate, it has been shown in adults to protect skin from UV exposure, to moderate atherosclerosis progression and to decrease the risk of some cancer types [3–5]. In the perinatal period, LT is one of the prevalent carotenoids in mature breast milk [6,7]. LT and its isomer zeaxanthin are the only carotenoids that make up the yellow pigment of the macula, thus supporting the latter's crucial role in the protection from oxidative damage and the occurrence of retinopathies in premature infants [8,9]. In this regard, LT supplementation associated with lycopene and beta-carotene, in preterm newborns, has been shown to improve neuro-retinal health [8]. LT is also the predominant carotenoid in the central nervous system (CNS) [9,10], where it is highly concentrated in the frontal and occipital cortex and hippocampus areas, which are important for learning and memory [10]. These brain LT-specific relationships with metabolic, energy, neurotransmission and antioxidant pathways have shown that LT may be involved in CNS development [9,10]. A trophic role of LT in the third trimester of pregnancy has recently been suggested by its correlation with gestational age and gender in a cohort of healthy preterm and term newborns [11]. Notably, the higher LT levels were observed at gestational ages in which CNS development was at its highest level in terms of brain weight and volume, synaptogenesis, dendritic arborization and axonal elongation [12–17]. However, the mechanisms through which LT may participate in CNS development are not understood and LT correlations

Abbreviations: LT, Lutein; CNS, central nervous system; wGA, weeks of gestational age; BW, birth weight; HPLC, high performance liquid chromatography; MRI, magnetic resonance imaging; NIRS, near infrared spectroscopy

* Corresponding author at: Dept. of Maternal, Fetal and Neonatal Medicine, C. Arrigo Children's Hospital, Alessandria, Italy, Spalto Marengo 46, 15100 Alessandria, Italy.
E-mail address: dgazzolo@hotmail.com (D. Gazzolo).

https://doi.org/10.1016/j.clinbiochem.2017.11.017
Received 26 July 2017; Received in revised form 26 November 2017; Accepted 27 November 2017
Available online 28 November 2017

with consolidated neurobiomarkers of CNS development and damage are also still lacking. This is true also for activin A, which is a growth factor composed of two beta subunits belonging to the transforming growth factor beta superfamily of dimeric proteins. Activin A, its receptors, and binding proteins are widely distributed throughout the brain [18,19]. Among several activin A functions, in human and in animal models, it has been shown that the protein plays a relevant role in the common response to acute neuronal damage of various origins (hypoxic/ischemic injury, mechanical irritation, and chemical damage) and in the neuroprotection [20–22]. Elevated activin A levels in different biological fluids (cerebrospinal, cord, peripheral blood, urine) were observed at an earlier stage in fetuses/newborns complicated by acute and chronic hypoxia, perinatal asphyxia and cerebral hemorrhage [23–26].

Therefore, in the present study we aimed at investigating whether in a cohort of healthy preterm and term infants LT assessment in arterial cord blood correlated with activin A levels measured in the same samples, thereby supporting its involvement in CNS development.

2. Materials and methods

The local Ethics Committees approved the study protocol and the parents of the examined subjects gave informed consent.

We recruited 132 women with consecutive singleton physiological pregnancies (82 at term and 50 preterm), whose deliveries were between 33 and 42 weeks of gestational age (wGA). Arterial cord blood samples were taken from newborns at birth for standard laboratory monitoring parameters, LT and activin A assessment.

GA was determined by clinical data and by first trimester ultrasound scan. Appropriate growth was defined by the presence of ultrasonography signs (when biparietal diameter and abdominal circumference were between the 10th and the 90th centiles), according to the normograms of Campbell and Thoms [27], and by postnatal confirmation of a birth weight (BW) between the 10th and 90th centiles according to our population standards, corrected for the mother's height, weight, parity and the sex of the newborn. Newborns were classified as preterm when born < 37 wGA and as term when born > 37 wGA, with a BW ranging from the 10th to 90th centile according to our population standards. In all cases, GA was determined by the last menstrual period and confirmed by a first trimester ultrasound scan. At birth, both term and preterm newborns showed a normal postnatal neurologic outcome at the 7th day of age and at discharge from hospital fulfilled all of the following criteria: no maternal illness; no signs of fetal distress; pH > 7.2 in cord or venous blood; and Apgar scores > 7 at 1 and 5 min. Exclusion criteria were: multiple pregnancies, intrauterine growth retardation, gestational hypertension, diabetes and infections, fetal malformations, chromosomal abnormalities, perinatal asphyxia and dystocia. At birth arterial cord blood samples were collected, centrifuged at 2500*g* and stored at − 80 °C for LT and activin A measurement.

2.1. Neurological examination

Neurological examination was performed daily during hospitalization. Neonatal neurological conditions were classified using a qualitative approach, as described by Prechtl [28], assigning each infant to one of three diagnostic groups: normal, suspect or abnormal. An infant was considered to be abnormal when one or more of the following neurological syndromes were present: hyper- or hypokinesia, hyper- or hypotonia, hemisyndrome, apathy syndrome, hyperexcitability syndrome. An infant was classified as suspect if, in absence of a defined syndrome, only isolated signs were present. On admission to the Neonatal Intensive Care Unit, all newborns routinely underwent an assessment of clinical parameters (red blood cell count, venous blood pH, ion concentrations, plasma glucose level, arterial blood pressure), cerebral ultrasound and neurological examination.

2.2. Lutein extraction and high performance liquid chromatography (HPLC) analysis

We decided to investigate LT pattern of concentration in newborns since it represents the most predominant carotenoid found in the CNS. Thus, LT extraction and HPLC analysis were performed using analytical conditions reported in our previous work [11]. Briefly, an aliquot of 500 μL of serum was treated with 500 μL of ethanol (1% BHT) in order to precipitate proteic pellet. Hydroalcoholic fraction and pellet were separately extracted twice with 500 μL of hexane. Hexanic fractions, containing serum carotenoids, were combined, evaporated under nitrogen and solubilized in 50 μL of chloroform before HPLC analysis. Chromatographic separation of LT was performed on a LC-10 AD Shimadzu HPLC system equipped with binary pump and a column compartment, coupled to a UV-diode array detector. Separation was performed on a Develosil 5 μm RP-AQUEUOUS C30, 250 × 4,6 mm column (Phenomenex Torrance, CA, USA) using chromatographic conditions previously reported [11].

Quantification of LT was done by the external standard method using a calibration curve built with LT as reference standard. The lowest point of the calibration curve (LOD, limit of detection) was 0.017 nmol/mL.

2.3. Mass spectrometry equipment and condition

The identification of LT was confirmed by liquid chromatography coupled to a tandem mass spectrometer (LC/MS/MS). The chromatographic separation was performed by HPLC connected with two micropumps 200 (Perkin Elmer, Carlsbad, CA, USA), using the same column and the same chromatographic conditions described for HPLC analysis. The API 3000 tandem mass spectrometer (API 3000, Applied Biosystem, Waltham, MA, USA) equipped with an atmospheric pressure chemical ionization (APCI) source was used for mass spectrometry analysis. The optimum settings of the mass spectrometer were: probe temperature 500 °C, the nebulizer current 4 μA, declustering potential 45 V and focusing potential 300 V.

2.4. Activin A measurement

Activin A measurement was performed in duplicate using a specific two-site enzyme immunoassay purchased from Serotec (Oxford, Oxford, UK) as previously described [18]. The limit of detection for activin A was 10 pg/mL, and intra- and inter-assay coefficients of variation were 2.5% and 3.0% respectively. The activin A assay has no detectable cross-reaction with inhibin A, inhibin B, follistatin or activin B. Activin A plates were read at 490 nm on an automated ELISA plate reader.

2.5. Statistical analysis

Clinical data are reported as the mean and standard deviation (SD). LT and activin A data are reported as median and interquartile centiles. Statistical analysis was performed using XLStat-Pro v.7.2.5 (Addinsoft, New York, USA). The results of neonatal monitoring parameters were compared between groups by the two-sided Mann–Whitney *U* test and by Kruskal–Wallis one-way ANOVA followed by the Dunn post-hoc test when the data did not follow a Gaussian distribution. Comparisons between proportions were performed with the Fisher exact test. Linear regression analysis was performed for correlations between LT, activin A and GA, respectively. A value of $P < 0.05$ was considered significant.

3. Results

Table 1 shows maternal and perinatal characteristics in the term and preterm newborns.

Table 1
Perinatal characteristics in preterm and term newborns. Data are given as mean (SD).

Parameters	Preterm (n = 50)	Term (n = 82)
Maternal Age (y)	29 (4)	28 (5)
Mode of delivery, n (%)		
Caesarean	27 (54)	20 (24.4)
Vaginal	23 (46)	62 (75.6)
GA (weeks)	34 (2)	39 (2)*
BW (g)	2675 (489)	3311 (501)*
Gender (male/female)	27/23	43/39
Apgar score > 7 n (%)		
At 1 min	50 (100)	82 (100)
At 5 min	50 (100)	82 (100)
SaO_2 (%)	96 ± 2	97 ± 2
Red blood cell count (10^6/mm^3)	3.91 ± 0.1	3.9 ± 0.07
Hemoglobin (g/dL)	13.4 ± 0.03	13.6 ± 0.04
Hematocrit rate %	40.3 ± 0.4	41.1 ± 0.2
Venous blood pH	7.31 ± 0.01	7.36 ± 0.06
Partial venous CO_2 pressure (mmHg)	42.6 ± 1.5	41.3 ± 1.5
Partial venous O_2 pressure (mmHg)	39.1 ± 0.9	40.3 ± 0.9
Base excess	−0.5 ± 0.2	−0.2 ± 1.1
Na^+ (mmol/L)	139 ± 0.4	140 ± 0.5
K^+ (mmol/L)	4.1 ± 0.1	4.1 ± 0.2
Ca^{++} (mmol/L)	1.13 ± 0.03	1.14 ± 0.03
Prechtl score at admission to NICU (normal/total)		
Normal	50 (50)	82 (82)
Suspect	0 (50)	0 (82)
Abnormal	0 (50)	0 (82)
Cerebral ultrasound (normal/total)	50 (50)	82 (82)

Abbreviations: Gestational age, GA; Birth-weight, BW; arterial oxygen saturation, SaO_2; carbon dioxide partial pressure, CO_2; oxygen partial pressure, O_2.
* $P < 0.05$.

In particular, maternal age, delivery mode, gender, Apgar scores at 1st and 5th minutes, arterial cord blood pH, SaO_2, neurological examination, cerebral ultrasound patterns and the incidence of cases with a BW between the 10th and 90th centiles did not differ ($P > 0.05$, for all) in preterm and term groups. Moreover, at discharge from hospital, no overt neurological syndrome was detectable in all infants admitted to the study. As expected, wGA age and BW were significantly lower ($P < 0.001$, for both) in PN.

Laboratory and monitoring parameters (red blood cell count, venous blood pH, ion concentrations, plasma glucose level, arterial blood pressure) were superimposable ($P > 0.05$, for all) in the two groups.

Activin A concentrations were measurable in all the samples collected. The protein showed a pattern of concentration characterized by higher levels in the early wGA ($P < 0.01$, for all) with a peak at 33–36 wGA (activin A median: 475.73 pg/mL; 25th–75th centile: 328.37–1076.07 pg/mL) and by a progressive decrease, with a dip at 42 wGA (activin A median: 130 pg/mL; 25th–75th centile: 120–240 pg/mL). After sub-dividing for gender, activin A levels were found to be higher ($P < 0.01$, for all) in the female study population and after sub-grouping for preterm and term birth (Fig. 1, panel A).

LT concentrations were measurable in all the samples collected. LT showed a pattern of concentration characterized by higher levels in the early wGA ($P < 0.01$, for all) with a peak at 33-36 wGA (LT median: 76.50 pmol/mL; 25th–75th centile: 65.86–135.95 pmol/mL), followed by a progressive decrease in LT levels from 37 wGA onwards, with a dip at 42 wGA (LT median: 52.27 pmol/mL; 25th–75th centile: 36.97–65.83 pmol/mL). LT correlated significantly ($P < 0.01$) with wGA at sampling ($R = 0.31$; $P < 0.001$).

LT levels after sub-dividing for gender were found to be higher ($P < 0.01$, for all) in the female study population and after sub-grouping for preterm and term birth (Fig. 1, panel B).

3.1. Lutein and activin A correlations

LT and activin A inversely correlated ($R = -0.22$; $P = 0.017$) when the whole study population was considered (data not shown). However, when divided for gender, we found that LT positively correlated with activin A both in whole male ($R = 0.93$; $P < 0.001$) and

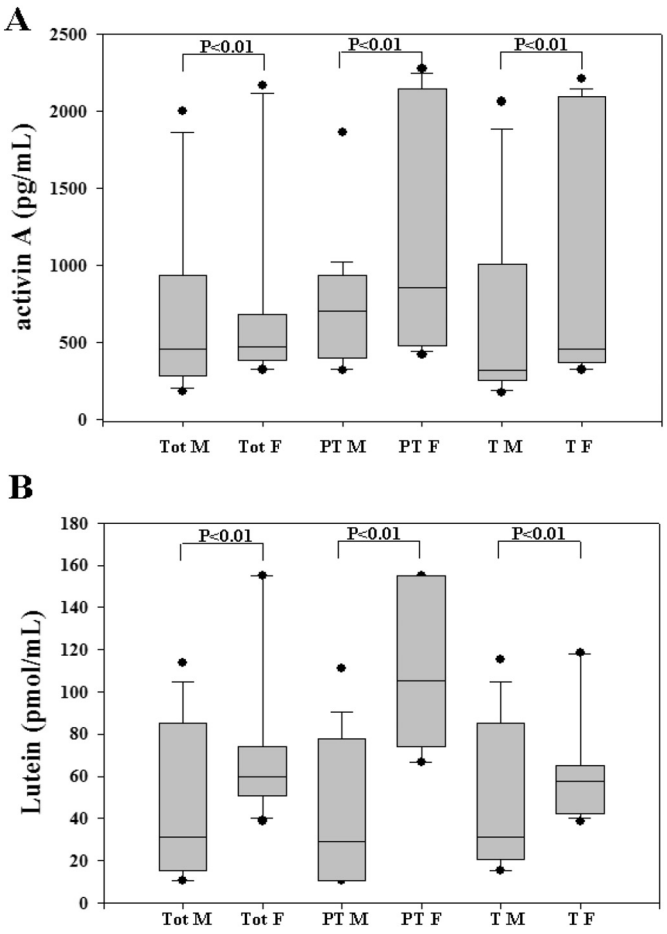

Fig. 1. Panel A. Activin A (pg/mL) arterial cord blood concentrations in whole studied population (Tot) and when divided for gender (male: M; female: F) and sub-grouped for preterm (PT) and term (T) births. Data are given as medians and 5th–95th centiles. Panel B. Lutein (pmol/mL) arterial cord blood concentrations in studied population when divided for gender and sub-grouped for preterm and term births. Data are given as medians and 5th–95th centiles.

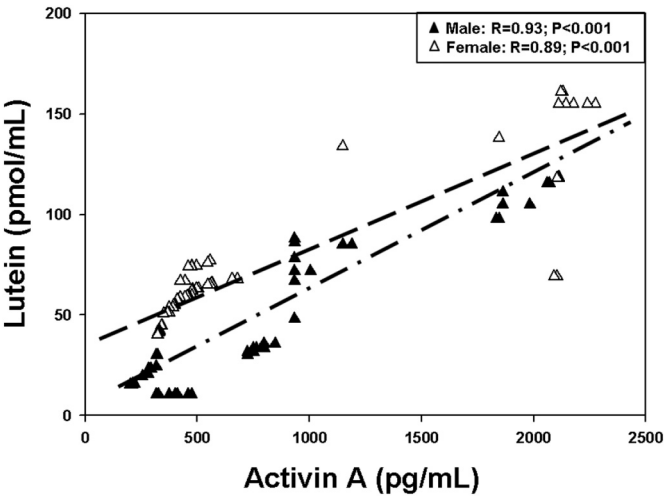

Fig. 2. Lutein (pmol/mL) correlations with activin A (pg/mL) in samples collected from arterial cord blood of preterm, term male (▲) and female (Δ) newborns. There were significant positive correlations between lutein and activin A in male ($R = 0.93$; $P < 0.001$) and female ($R = 0.89$; $P < 0.001$) groups.

female (R = 0.89; P < 0.001) populations (Fig. 2). Of note, identical correlations were found when sub-grouping for preterm (male: R = 0.92; female: R = 0.97; P < 0.001, for both) and term (male: R = 0.96; female: R = 0.75; P < 0.001, for both) (data not shown).

4. Discussion

There is growing evidence that the pathophysiological steps involved in CNS development and damage are still not fully understood and they are therefore of interest as a subject of investigation. Technological improvements have made it possible to identify and assess new neuro-biomarkers. This is especially true for new laboratory procedures such as metabolomics, which can identify the roles and activities of metabolites [10]. In this respect, several biomarkers have recently been proposed and investigations are in progress for their validation in accordance with Food and Drugs Administration and European Medicine Association statements [29].

In the present study we showed that LT concentrations in the arterial cord blood of healthy preterm and term newborns were gestational age- and gender-dependent and correlated with a well-established marker of CNS development and damage, namely activin A [21–26,29]. Moreover, activin A and LT patterns of concentration showed higher levels in the early weeks of the third trimester of gestation (i.e. 33–36 weeks), starting to decrease from 37 weeks onwards and reaching their dip at term.

The finding that LT is gestational age-dependent is not surprising; it fits previous observations and offers additional support in the debate concerning a putative trophic role for LT [8–11]. It is noteworthy, in this respect, that LT levels were higher at 33–36 weeks, i.e. in the so-called late preterm period, in parallel with higher activin A concentrations. This finding warrants further consideration bearing in mind that at this stage CNS development is at its highest level in terms of brain volume, weight and structure [12–15]. In particular, brain volume and weight increase respectively to 35% and 37% of the total amount during pregnancy, when myelinization and arborization processes are at their maximum peaks [12–15]. These findings were further corroborated by recent observations of CNS development and function monitoring, in humans, by magnetic resonance imaging (MRI), neuro-biomarker assessment in different biological fluids and non-invasive techniques such as near infrared spectroscopy (NIRS) [15–17,29–31]. MRI provided evidence on the timing and duration of CNS development at the stage of investigation [15]. Moreover, the assessment in biological fluids of consolidated neuro-biomarkers of CNS development and function such as S100B protein and activin A were in agreement with MRI patterns and with the biochemical, morphological and electrophysiological maturation of the CNS [29–31]. In this regard, the mechanisms through which LT and activin A can both act as markers of CNS development/damage are not fully understood and merit further consideration. On the one hand trophic and protective roles for LT in humans have been suggested by: i) its predominant localization in CNS areas crucial for learning and memory [10], ii) its correlation with fatty acids and lysophospholipids involved in cortical development and folding [32–34], in oligodendrocyte maturation [35] and in intracellular and cell-cell signalling; iii) the correlation with 1-octadecanol, phosphate and NADH, metabolites associated with energy pathways, supporting the notion that LT participates in myelination during development as oligodendrocytes require extremely high metabolic rates during peak myelination [34,35], and iv) the correlation with the aminoacid neurotransmitters GABA and aspartate, involved in the modulation of neuronal proliferation and maturation, neurite outgrowth and synapse formation and neurotransmission [36–39]. On the other hand, activin A: i) shows an instructive neuronal effect in cortical and hippocampal neuronal progenitor cells, playing a relevant role in their specification towards a neuronal phenotype [18], ii) increases survival of differentiated neurons [40]; iii) promotes neuron-regeneration after hypoxic insults [18,30], iii) exaggerated releasing after hypoxic insult as expression of increased neuro-protection and neuro-regeneration processes [18,30,31], and iv) following hypoxia, correlates with biochemical markers of oxidative stress, nucleated red blood cell counts, hypoxanthine, xanthine, base deficit levels, and pH [25]. Altogether, on the basis of the above findings it is possible to argue that LT and activin A participate in a cascade of events modulating CNS development and protecting it from damage. The possibility that LT and activin A may be triggered by a hypoxic insult is thus consistent and, together with their effects on CNS development and damage, is a promising avenue of investigation.

Finally, NIRS studies in healthy infants have provided evidence that in the late preterm period CNS pattern of development is characterized by an improved oxygenation status and increased tissue function [16,17]. Thus, it is reasonable to conclude that the pattern of concentration of LT in the third trimester of pregnancy, which is not significantly different on that of other monitoring and well-established biochemical markers, offers additional support to its CNS trophic role.

In the present study, we also found that LT and activin A correlated in a gender-dependent manner: these differences could be explained by different patterns of brain maturation in the two sexes and are in agreement with previous observations comparing several fetal/neonatal parameters such as growth, metabolic, biochemical and hormonal patterns [16,18,29].

In humans and animal models it has been shown that there is a developmental period for each species in which the CNS is more sensitive to gonadal hormones effects than at any other time. In mammals, the heterogametic sex differentiation occurs as a result of gonadal hormones releasing in the preterm period: the modulation and the timing of estrogen and androgen releasing play a relevant role [41]. It has been shown that fetus is continuously exposed to endogenous estrogen from placenta-maternal bloodstream trough a mechanism, mediated by specific receptors, that protect fetus from estrogen side-effects and promote CNS growth. Conversely, androgen can act directly on the CNS without mediated mechanisms [41]. In the CNS newborn, the receptorial system is not static but change rapidly during and after the perinatal period and the regional distribution of estrogen receptors changes during development. These findings are supported by data in animal model where estrogen receptors are the first to increase, in parallel with the onset of sensitivity of the CNS to the hormonal action, leading to brain sexual differentiation [42]. Thus, CNS has a different gender answer to gonadal steroids in a time-dependent manner [42] in whom interaction among gonads, adenohypophysis and inhibin-activin family play a crucial role [43]. Altogether, it is reasonable to infer that CNS development in the preterm period is significantly precocious in female [41]. The finding is also corroborated by results in clinical and biochemical studies suggestive of an earlier CNS female maturation [44–49]. However, further studies aimed at elucidating the mechanism trough which LT and activin A participate in the cascade of events leading to CNS development are so justified. The issue is of utmost relevance bearing in mind that a later CNS development in male is related with a higher incidence of prematurity and cerebral hemorrhage as well as of multiorgan failure [47–49].

Finally, this study had a number of limitations. In particular, we conducted a cross-sectional design and we did not record maternal diet diary in order to know LT intake. Further investigations aimed at correlating these two measures and potential implications to prenatal dietary recommendations are therefore requested.

5. Conclusions

In conclusion, the present data showing a correlation between LT and activin A support the notion of a neurotrophic role of LT and open the way to further investigations correlating LT with well-established biochemical markers of CNS development/damage.

Conflicts of interest

None.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] A. Perry, H. Rasmussen, E.J. Johnson, Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products, J. Food Compos. Anal. 22 (2009) 9–15.

[2] M.E. O'Neill, Y. Carroll, B. Corridan, B. Olmedilla, F. Granado, I. Blanco, H. Van den Berg, I. Hininger, A.M. Rousell, M. Chopra, S. Southon, D.I. Thurnham, A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study, Br. J. Nutr. 85 (2001) 499–507.

[3] J.H. Dwyer, M. Navab, K.M. Dwyer, K. Hassan, P. Sun, A. Shircore, S. Hama-Levy, G. Hough, X. Wang, T. Drake, C.N. Merz, A.M. Fogelman, Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study, Circulation 103 (2001) 2922–2927.

[4] S. Gonzalez, S. Astner, W. An, D. Goukassian, M.A. Pathak, Dietary lutein/zeaxanthin decreases ultraviolet B-induced epidermal hyperproliferation and acute inflammation in hairless mice, J. Invest. Dermatol. 121 (2003) 399–405.

[5] P. Palombo, G. Fabrizi, V. Ruocco, E. Ruocco, J. Fluhr, R. Roberts, P. Morganti, Beneficial long-term effects of combined oral/topical antioxidant treatment with the carotenoids lutein and zeaxanthin on human skin: a double-blind, placebo-controlled study, Skin Pharmacol. Physiol. 20 (2007) 199–210.

[6] F.J. Schweigert, K. Bathe, F. Chen, U. Büscher, J.W. Dudenhausen, Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma and plasma lipoprotein fractions, Eur. J. Nutr. 43 (2004) 39–44.

[7] C.P. Gossage, M. Deyhim, S. Yamini, L.W. Douglass, P.B. Moser-Veillon, Carotenoid composition of human milk during the first month postpartum and the response to beta-carotene supplementation, Am. J. Clin. Nutr. 76 (2002) 193–197.

[8] L.P. Rubin, G.M. Chan, B.M. Barrett-Reis, A.B. Fulton, R.M. Hansen, T.L. Ashmeade, J.S. Oliver, A.D. Mackey, R.A. Dimmit, E.E. Hartmann, D.H. Adamkin, Effect of carotenoid supplementation on plasma carotenoids, inflammation and visual development in preterm infants, J. Perinatol. 32 (2012) 418–424.

[9] R. Vishwanathan, M.J. Kuchan, S. Sen, E.J. Johnson, Lutein and preterm infants with decreased concentrations of brain carotenoids, J. Pediatr. Gastroenterol. Nutr. 59 (2014) 659–665.

[10] J.C. Lieblein-Boff, E.J. Johnson, A.D. Kennedy, C.S. Lai, M.J. Kuchan, Exploratory metabolomic analyses reveal compounds correlated with lutein concentration in frontal cortex, hippocampus, and occipital cortex of human infant brain, PlosOne 10 (2015) 8 e0136904.

[11] S. Picone, A. Ritieni, A. Fabiano, A.D. Troise, G. Graziani, P. Paolillo, G. Li Volti, N. D'Orazio, F. Galvano, D. Gazzolo, Arterial cord blood lutein levels in preterm and term healthy newborns are sex and gestational age dependent, Clin. Biochem. 45 (2012) 1558–1563.

[12] R.L. Haynes, N.S. Borenstein, T.M. Desilva, D. Folkerth, L.G. Liu, J.J. Volpe, H.C. Kinney, Axonal development in the cerebral white matter of the human fetus and infant, J. Comp. Neurol. 484 (2005) 156–167.

[13] A.M. Guihard-Costa, J.C. Larroche, Differential growth between the fetal brain and its infratentorial part, Early Hum. Dev. 23 (1990) 27–40.

[14] S.A. Back, N.L. Luo, N.S. Borenstein, J.M. Levine, J.J. Volpe, H.C. Kinney, Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury, J. Neurosci. 21 (2001) 1302–1312.

[15] P.S. Huppi, S. Warfield, R. Kikinis, P.D. Barnes, G.P. Zientara, F.A. Jolesz, M.K. Tsuji, J.J. Volpe, Quantitative magnetic resonance imaging of brain development in premature and mature newborns, Ann. Neurol. 43 (1998) 224–235.

[16] L.G. Tina, A. Frigiola, R. Abella, P. Tagliabue, L. Ventura, G. Paterlini, G. Li Volti, S. Pinzauti, P. Florio, V. Bellissima, C. Minetti, D. Gazzolo, S100B protein and near infrared spectroscopy in preterm and term newborns, Front. Biosci. 2 (2010) 159–164.

[17] L.G. Tina, A. Frigiola, R. Abella, B. Artale, G. Puleo, S. D'Angelo, C. Musmarra, P. Tagliabue, G. Li Volti, P. Florio, D. Gazzolo, Near infrared spectroscopy in healthy preterm and term newborns: correlation with gestational age and standard monitoring parameters, Curr. Neurovasc. Res. 6 (2009) 148–154.

[18] P. Florio, D. Gazzolo, S. Luisi, F. Petraglia, Activin A in brain injury, Adv. Clin. Chem. 43 (2007) 117–130.

[19] S. Luisi, P. Florio, F.M. Reis, F. Petraglia, Expression and secretion of activin a: possible physiological and clinical implications, Eur. J. Endocrinol. 145 (2001) 225–236.

[20] M. Lai, P. Gluckman, M. Dragunow, P.E. Hughes, Focal brain injury increases activin A mRNA expression in hippocampal neurons, Neuroreport 8 (1997) 2691–2694.

[21] M. Lai, E. Sirimanne, C.E. Williams, P.D. Gluckman, Sequential patterns of inhibin subunit gene expression following hypoxic-ischemic injury in the rat brain, Neuroscience 70 (1996) 1013–1024.

[22] D.D. Wu, M. Lai, P.E. Hughes, E. Sirimanne, P.D. Gluckman, C.E. Williams, Expression of the activin axis and neuronal rescue effects of recombinant Activin A following hypoxic-ischemic brain injury in the infant rat, Brain Res. 835 (1999) 369–378.

[23] P. Florio, S. Luisi, M. Bruschettini, D. Grutzfeld, A. Dobrzanska, P. Bruschettini, F. Petraglia, D. Gazzolo, Cerebrospinal fluid activin a measurement in asphyxiated full-term newborns predicts hypoxic ischemic encephalopathy, Clin. Chem. 50 (2004) 2386–2389.

[24] P. Florio, S. Perrone, S. Luisi, M. Longini, D. Tanganelli, F. Petraglia, G. Buonocore, Activin A plasma levels at birth: an index of fetal hypoxia in preterm newborn, Pediatr. Res. 54 (2003) 696.

[25] P. Florio, S. Perrone, S. Luisi, P. Vezzosi, M. Longini, B. Marzocchi, F. Petraglia, G. Buonocore, Increased plasma concentrations of activin A predict intraventricular hemorrhage in preterm newborns, Clin. Chem. 52 (2006) 1516–1521.

[26] P. Florio, S. Luisi, B. Moataza, M. Torricelli, I. Iman, M. Hala, A. Hanna, F. Petraglia, D. Gazzolo, High urinary concentrations of activin A in asphyxiated full-term newborns with moderate or severe hypoxic ischemic encephalopathy, Clin. Chem. 53 (2007) 520–522.

[27] S. Campbell, A. Thoms, Ultrasound measurement of the fetal head to abdomen circumference ratio in the assessment of growth retardation, Br. J. Obstet. Gynaecol. 84 (1977) 165–174.

[28] H.F.R. Prechtl, Assessment methods for the newborn infant: a critical evaluation, in: D. Stratton (Ed.), Psychobiology of the Human Newborn, John Wiley and Son, Chichester UK, 1982, pp. 21–52.

[29] L.D. Serpero, V. Bellissima, M. Colivicchi, M. Sabatini, A. Frigiola, A. Ricotti, V. Ghiglione, M.C. Strozzi, G. Li Volti, F. Galvano, D. Gazzolo, Next generation biomarkers for brain injury, J. Matern. Fetal Neonatal Med. 26 (2013) 44–49.

[30] P. Florio, R. Abella, E. Marinoni, R. Di Iorio, G. Li Volti, F. Galvano, G. Pongiglione, A. Frigiola, S. Pinzauti, F. Petraglia, D. Gazzolo, Biochemical markers of perinatal brain damage, Front. Biosci. 2 (2010) 47–72.

[31] D. Gazzolo, R. Abella, E. Marinoni, R. Di Iorio, G. Li Volti, F. Galvano, G. Pongiglione, A. Frigiola, F. Bertino, P. Florio, Circulating biochemical markers of brain damage in infants complicated by ischemia reperfusion injury, Cardiovasc. Hematol. Agents Med. Chem. 7 (2009) 108–126.

[32] M.A. Kingsbury, S.K. Rehen, J.J. Contos, C.M. Higgins, J. Chun, Non-proliferative effects of lysophosphatidic acid enhance cortical growth and folding, Nat. Neurosci. 6 (2003) 1292–1299.

[33] L. Nogaroli, L.M. Yuelling, J. Dennis, K. Gorse, S.G. Payne, B. Fuss, Lysophosphatidic acid can support the formation of membranous structures and an increase in MBP mRNA levels in differentiating oligodendrocytes, Neurochem. Res. 34 (2009) 182–193.

[34] J.M. Bourre, O. Daudu, Stearyl-alcohol biosynthesis from stearyl-CoA in mouse brain microsomes in normal and dysmyelinating mutants (quaking and jimpy), Neurosci. Lett. 7 (1978) 225–230.

[35] M. Bradl, H. Lassmann, Oligodendrocytes: biology and pathology, Acta Neuropathol. 119 (2010) 37–53.

[36] Y. Ben-Ari, J.L. Gaiarsa, R. Tyzio, R. Khazipov, GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations, Physiol. Rev. 87 (2007) 1215–1284.

[37] G. Barbin, H. Pollard, J.L. Gaiarsa, Y. Ben-Ari, Involvement of GABAA receptors in the outgrowth of cultured hippocampal neurons, Neurosci. Lett. 152 (1993) 150–154.

[38] A. Represa, Y. Ben-Ari, Trophic actions of GABA on neuronal development, Trends Neurosci. 28 (2005) 278–283.

[39] R. Dingledine, C.J. Mc Bain, Glutamate and aspartate are the major excitatory transmitters in the brain, in: G.J. Siegel, B.W. Agranoff, R.W. Albers, S.K. Fisher, M.D. Uhler (Eds.), Basic Neurochemistry: Molecular, Cellular and Medical Aspects, 6th ed., Lippincott Williams & Wilkins, Philadelphia, 1999, pp. 267–289.

[40] S.Y. Li, Z.J. Fu, A.C. Lo, Hypoxia-induced oxidative stress in ischemic retinopathy, Oxidative Med. Cell. Longev. 2012 (2012) 426769, , http://dx.doi.org/10.1155/2012/426769.

[41] N.J. MacLusky, F. Naftolin, Sexual differentiation of the central nervous system, Science 211 (1981) 1294–1303.

[42] B.S. Mc Ewen, Neuronal gonadal steroids action, Science 211 (1981) 1303–1311.

[43] F. Naftolin, L.M. Garcia-Segura, T.L. Horvath, A. Zsarnovszky, N. Demir, A. Fadiel, C. Leranth, S. Vondracek-Klepper, C. Lewis, A. Chang, A. Parducz, Estrogen-induced hypothalamic synaptic plasticity and pituitary sensitization in the control of the estrogen-induced gonadotrophin surge, Reprod. Sci. 14 (2007) 101–116.

[44] B.S. McEwen, T.A. Milner, Understanding the broad influence of sex hormones and sex differences in the brain, J. Neurosci. Res. 95 (2017) 24–39.

[45] J. Marrocco, B.S. McEwen, Sex in the brain: hormones and sex differences, Dialogues Clin. Neurosci. 18 (2016) 373–383.

[46] D. Gazzolo, P. Vinesi, E. Marinoni, R. Di Iorio, M. Marras, M. Lituania, P. Bruschettini, F. Michetti, S100B protein concentrations in cord blood: correlations with gestational age in term and preterm deliveries, Clin. Chem. 46 (2000) 998–1000.

[47] J. Zeitlin, M.J. Saurel-Cubizolles, J. De Mouzon, L. Rivera, P.Y. Ancel, B. Blondel, M. Kaminski, Fetal sex and preterm birth: are males at greater risk? Hum. Reprod. 17 (2002) 2762–2768.

[48] E. Cuestas, J. Bas, J. Pautasso, Sex differences in intraventricular hemorrhage rates among very low birth weight newborns, Gend. Med. 6 (2009) 376–382.

[49] B. Skiöld, G. Alexandrou, N. Padilla, M. Blennow, B. Vollmer, U. Adén, Sex differences in outcome and associations with neonatal brain morphology in extremely preterm children, J. Pediatr. 164 (2014) 1012–1018.