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<b>Grant Number</b> 5R01TW6974-3		<b>Total Project Period</b> From: 08/01/2006 To: 05/31/2009	
<b>EIN:</b> 1900216383A1	<b>Review Group:</b> ZRG1 BDA-G (52)	<b>Requested Budget Period:</b> From: 06/01/2008 To: 05/31/2009	
<b>Title of Project:</b> Circadian Role of Vitamin A in Regulation of Redox State			<b>Due Date:</b> 04/16/2008 <b>Submitted Date:</b> 04/01/2008
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<b>Human Subjects:</b> <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes <b>Research Exempt:</b> <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes <b>Exemption No:</b> <b>FWA Number:</b> <b>Full IRB:</b> <input type="checkbox"/> No <input type="checkbox"/> Yes <b>Phase III Clinical Trial:</b> <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes		<b>Vertebrate Animals:</b> <input type="checkbox"/> No <input checked="" type="checkbox"/> Yes <b>Animal Assurance Number:</b> A5713-01  <b>Inventions and Patents:</b> <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> <b>Previously Reported</b> <input type="checkbox"/> <b>Not Previously Reported</b>	
<b>Program Income:</b> <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes			
<b>Budget Period</b>		<b>Anticipated Amount</b>	
<b>Budget Period</b>		<b>Source</b>	
<b>F&amp;A Changes:</b>			
<b>Performance Sites:</b> NATIONAL UNIVERSITY OF SAN LUIS NATIONAL UNIVERSITY OF SAN LUIS CHACABUCO Y PEDERNERA EDIF. EL BARCO, 2DO.PISO CP 5700 SAN LUIS SAN LUIS NATIONAL UNIVERSITY OF SAN LUIS EJERCITO DE LOS ANDES 950 CP 5700 SAN LUIS SAN LUIS			

<b>Principal Investigator:</b> ANA C ANZULOVICH	<b>Grant Number</b> 5R01TW6974-3
<b>Applicant Organization:</b> NATIONAL UNIVERSITY OF SAN LUIS	<b>Period Covered by this Report:</b> 06/01/2007 - 05/31/2008
<b>Title of Project:</b> Circadian Role of Vitamin A in Regulation of Redox State	
<b>SNAP Questions:</b>	
<p><b>Has there been a change in the other support of key personnel since the last reporting period?</b></p> <p><input checked="" type="checkbox"/> No <input type="checkbox"/> Yes</p> <p><b>Justification:</b></p>	
<p><b>Will there be, in the next budget period, a significant change in the level of effort for the PI or other personnel designated on the Notice of Grant Award from what was approved for this project?</b></p> <p><input checked="" type="checkbox"/> No <input type="checkbox"/> Yes</p> <p><b>Justification:</b></p>	
<p><b>Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total budget?</b></p> <p><input checked="" type="checkbox"/> No <input type="checkbox"/> Yes</p> <p><b>Justification:</b></p>	
<p><b>Changes in Select Agent Research?</b> <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes</p> <p><b>Changes in Multiple PI Leadership plan?</b> <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes</p>	
<b>Human Subject Education Requirement:</b>	
<p><b>Has the Involvement of Human Subjects changed since previous submission?</b> <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes</p> <p><b>Has the Involvement of Animal Subjects changed since previous submission?</b> <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes</p>	
<b>Publications:</b>	
<b>Citation ID:</b>	<b>Citation Text:</b>
	<p>Navigatoro Fonzo LS, Delgado SM, Bonomi MR, Rezza IG, Gimenez MS, Anzulovich AC. Circadian Variation Of Antioxidant Enzymes And TBARs In Hippocampus Of Vitamin A deficient rats. (Abstract). Biocell.2007. 31(Suppl): 153.ISSN 0327-9545.</p> <p>Ponce IT, Rezza IG, Bonomi MR, Delgado SM, Gimenez MS, Anzulovich AC. Circadian Variation Of Glutathione Metabolism Is Modified In The Vitamin A Deficiency.(Abstract). Biocell. 2007. 31 (Suppl.): 94.ISSN 0327-9545.</p>

Personnel Report						
Principal Investigator:				Grant Number		
ANA C ANZULOVICH				5R01TW6974-3		
Name:	Degree(s) Name:	SSN:	Role on Project:	Months Devoted to Project		
				Cal	Acad	Sum
ANA C ANZULOVICH	PHD	9751	PI	12.0		
Ruben Baler	PhD		Consultant	12.0		
Silvia M Delgado	PhD		Investigator/Statistician	12.0		
Maria Sofia Gimenez	PhD		Consultant	12.0		
Rebeca Golini			PhD student	12.0		
Lorena Silvina Navigatore			PhD student	12.0		
Ivana Tamara Ponce			PhD student	12.0		
Irma Rezza	PhD		Investigator	12.0		

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Manuscript Draft

Manuscript Number: NSC-08-397

Title: Temporal patterns of lipoperoxidation and antioxidant enzymes are modified in the hippocampus of vitamin A-deficient rats. Possible impact on memory and learning.

Article Type: Research Paper

Keywords: circadian rhythm; catalase; glutathione peroxidase; lipid peroxidation; retinoid; brain

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**Abstract:** Daily rhythms of superoxide dismutase, glutathione peroxidase (GPx), and glutathione reductase activities, as well as day-night oscillations of glutathione and malondialdehyde levels, have been described in different tissues, including brain. In the present study, we investigate the temporal fluctuations of lipoperoxidation and antioxidant enzymes in the rat hippocampus and evaluate to which extent vitamin A deficiency may affect their amplitude or phase. Male rats from control, vitamin A-deficient and vitamin A-recovered groups were sacrificed by decapitation throughout a 24-h period. Levels of lipoperoxidation, catalase (CAT) and GPx mRNA, protein and activity were determined in the rat hippocampus obtained every 4 or 5 h. mRNA expression of RAR $\alpha$  and RXR $\beta$  was also quantified in the hippocampus of the three groups of rats. Our results show significant daily variations of lipid peroxidation levels, CAT and GPx expression and activity, with maximal levels of lipoperoxidation and enzymatic activities occurring during the dark period. Vitamin A deficiency reduced significantly RXR $\beta$  mRNA level, phase shifted the daily pattern of lipoperoxidation and had a differential effect on CAT and GPx mRNA, protein, and activity oscillating levels. Learning how vitamin A deficiency affects the circadian expression of genes involved in the antioxidant defense system in the hippocampus may have an impact on the nutritional, neurobiology and chronobiology fields, emphasizing for the first time the importance of nutritional factors, such as dietary micronutrients, in the regulation of circadian parameters in brain.

## Dr Anzulovich updated biosketch

NAME Anzulovich, Ana Cecilia		POSITION TITLE Associate Investigator (CONICET) Teaching Assistant (UNSL)	
eRA COMMONS USER NAME ANZULOVA			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
National University of San Luis	Chem. Profes.	1992	Chemistry
National University of San Luis	Lic. Biochem	1993	Biochemistry
National University of San Luis	PhD	1998	Biochemistry and Nutrition
Milan University	Post-doc	1999	Molecular Biology
NIMH/NIH	Post-doc	2001-2004	Molecular Chronobiology

### A. Positions and Honors.

#### Positions and Employment

**1994-present.** Teaching assistant in the Chair of Biological Chemistry, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis (Argentina).

**1994-2001.** Investigator in CONICET and 8I04/C-T UNSL, Project entitled: Nutrition, Environment and Cellular Metabolism, PI: Dr. Sofia Gimenez, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis (Argentina).

**2001-2004.** Pos-doctoral fellow in biomedical research, studying the mechanisms of transcriptional regulation in the rodent liver as a model of circadian peripheral oscillations, under supervision of Dr. Ruben Baler, in the Unit of Temporal Gen Expression of Laboratory of Cellular and Molecular Regulation at the National Institute of Mental Health, NIH, Bethesda, MD, (USA).

**2005-present.** Investigator in Project #6223 (CONICET) and #8I04/C-T (UNSL). Project entitled: Nutrition, Environment and Cellular Metabolism, PI: Dr. Sofia Gimenez, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis (Argentina).

**2006-present.** Principal Investigator (Grant # R01-TW006974-01A2, FIC/NIH). Project entitled: Circadian role of vitamin A in the regulation of redox state. Studies performed at the National University of San Luis.

**2007-present.** Associate Investigator at the Argentine National Council of Science and Technology (CONICET, Res n° 2727/07).

#### **Awards and fellowships:**

**1995** - Award from of the VI Ibero-American Society of Cellular Biology Congress Committee, Oaxtepec, Mexico.

**1997.-** Award from Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, Res. n° 033-97

for post-graduate studies at University of Rosario.

**1999.-** Research fellow of the Italian Government in the Laboratory of Biochemistry and Molecular Biology of Lipids, at the Institute of Pharmacology, University of Milan (Italy). Director: Prof. Giovanni Galli. Supervisor: Dr. Maurizio Crestani.

**2000.-** Second Prize, XVIII Scientific Meeting of Biology Society of Cuyo, Mendoza, Argentina.

**2005-2007-** Pos-doctoral fellowship from CONICET (Argentina) to study the Role of vitamin A in the circadian regulation of lipoperoxidation and antioxidant enzyme systems. Studies performed at the National University of San Luis.

## B. Selected peer-reviewed publications (in chronological order).

2000.- **Anzulovich A.C.**, Oliveros L., Muñoz E., Martínez L.D. and Giménez M.S.; "Nutritional Vitamin A Deficiency Alters Antioxidant Defenses and Modifies the Liver Histoarchitecture in Rat", *J. Trace Elem. Exp. Med.* 13 (4):343-357.

2000.- Oliveros L., Vega V., **Anzulovich A.C.**, Ramírez, D. and Giménez M.S.; "Vitamin A Deficiency Modifies Antioxidant Defenses and Essential Element Contents in Rat Heart", *Nutrition Research* 20(8): 1139-1150.

2000.- Oliveros L., Vega V., **Anzulovich A.C.**, Varas S. y Giménez M.S.; "Estudio de la Deficiencia de Vitamina A sobre Variables Ponderales y Séricas en la Rata", *Anuario Latinoamericano de Educación en Química*.

2001.- Emma De Fabiani, Nico Mitro, **Ana Cecilia Anzulovich**, Alessandra Pinelli, Giovanni Galli, and Maurizio Crestani; "The Negative Effects of Bile Acids and Tumor Necrosis Factor- $\alpha$  on the Transcription of Cholesterol 7 $\alpha$ -Hydroxylase Gene (*CYP7A1*) Converge to Hepatic Nuclear Factor-4. A Novel Mechanism of Feedback Regulation of Bile Acid Synthesis Mediated by Nuclear Receptors", *JBC* 276(33): 30708-30716.

2003.- Zirulnik F, **Anzulovich AC**, Larregle E, Jahn GA, Gimenez MS. "Role of prolactin in the regulation of cytosolic NADP isocitrate dehydrogenase in the liver of the male rat. *Endocr Res.* 29(2):201-10.

2005.- Appelbaum L., **Anzulovich A.**, Klein DC., Baler R., and Gothilf Y., "Homeobox-Clock proteins interaction, a mechanism for pineal-specific and rhythmic gene expresión", *J. Biol. Chem.* 280 (12): 11544-11551.

2005.- Brewer M., Lange D., Baler R. and **Anzulovich A**; "SREBP-1 as a transcriptional integrator of circadian and nutritional cues in the liver", *Journal of Biological Rhythms*,20 (3):195-205-.

2006- Lior Appelbaum, Daniela Vallone, Ana Anzulovich, Limor Ziv, Moshe Tom, Nicholas SFoulkes and Yoav Gothilf; "Zebrafish arylalkylamine-N-acetyltransferase genes-targets for regulation of circadian-clock". *J. Molec. Endocrin.* 36:337-347.

2006- **Ana Anzulovich**, Alain Mir, Michelle Brewer, Gabriela Ferreyra, Charles Vinson, and Ruben Baler. Elov13: A model gene to dissect homeostatic links between the circadian clock and nutrition status. *J. Lipid Res.* 47(12):2690-2700.

2007- Bina Zilberman-Peled, Lior Appelbaum, Daniela Vallone, Nicholas S. Foulkes, Sarit Anava, **Ana Anzulovich**, Steven L. Coon, David C. Klein, Jack Falcon, Benny Ron and Yoav Gothilf. Transcriptional regulation of arylalkylamine-N-acetyltransferase-2 gene in the pineal gland of the gilthead seabream, *J. Neuroendocrinol.* 19: 46-53.

2008- **Ana Cecilia Anzulovich**. Circadian regulation of lipid metabolism. In *Advances in Lipid Metabolism*, ISBN: 978-81-308-0246-6. Research Signpost 37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India.

2008- L.S. Navigatore Fonzo, R. Golini, S.M. Delgado, M.R. Bonomi, I.G. Rezza, M.S. Giménez And A.C. Anzulovich. Temporal patterns of lipoperoxidation and antioxidant enzymes are modified in the hippocampus of vitamin a-deficient rats. Possible impact on memory and learning. Sent to Neuroscience, Elsevier Ed. Ms. No.: NSC-08-397.

## **Published abstracts:**

2000.- E. De Fabiani, M. Crestani, D. Caruso, M. N. Orsini, A. C. Anzulovich, A. Pinelli and G. Galli; Regulation of cholesterol 7 $\alpha$ -hydrolase gene (*CYP7A1*) by bile acids, *Chem. Phys. Lipids*, 107 (1).

2000.- Anzulovich, A.C., Oliveros, L., Sterin-Speziale N.B., Speziale E.H. and Giménez M.S.; Phospholipid content and fatty acid composition of liver from vitamin A deficient rats, *Chem. Phys. Lipids*, 107 (1).

2003.- Baler R. and Anzulovich A. C., The Cold Inducible Glycoprotein (CIG) 30 gene promoter: rhythmic control by clock and sterol signaling pathways. *MBC, suppl.14:493a-494a*.

2007.- Ferrari Vivas MC, Reinchisi GE, Varas SM, Anzulovich AC y Gimenez MS. Diagnóstico de Fibrosis Quística . Uso de PCR en Tiempo Real. *J.Basic and Applied Genetics XVII (Suppl. II): 86*.

2007- Ponce IT, Rezza IG, Bonomi MR, Delgado SM, Gimenez MS, Anzulovich AC. Circadian Variation Of Glutathione Metabolism Is Modified In The Vitamin A Deficiency. *Biocell* 31 (Suppl.), 94. ISSN 0327-9545.

2007- Ferramola MI, Coria M, Biaggio V, Anzulovich AC, Giménez MS. Do You Want A Drink? Cardiovascular Oxidative Stress Produced By Drinking Cd Contaminated Water. *Biocell* 31 (Suppl.), 96. ISSN 0327-9545.

2007- Vega V, Anzulovich AC, Giménez MS, Oliveros L. Expression Of Retinoic Acid Receptors (RAR and RXR) and PPARs In Heart: Effect Of vitamin A deficiency. *Biocell* 31 (Suppl.), 125. ISSN 0327-9545.

2007- Quiroga C, Anzulovich AC, Bonomi M, Oliveros L, Giménez MS. Srebp Expression On Fatty Acid Metabolism In Vitamin A deficient liver. *Biocell* 31 (Suppl.), 125. ISSN 0327-9545.

2007- Navigatore Fonzo LS, Delgado SM, Bonomi MR, Rezza IG, Gimenez MS, Anzulovich AC. Circadian Variation Of Antioxidant Enzymes And TBARs In Hippocampus Of Vitamin A deficient rats. *Biocell* 31 (Suppl.), 153. ISSN 0327-9545.

## **Progress Report (5R01-TW006974-03)**

### **A. Specific Aims.**

Even though the two specific aims originally proposed in the application (R01-TW006974-01A2) have not been modified, we altered the chronological order of the studies directed toward them. That was because of the easier accesibility to the methods described below, in the meantime we were setting up all cell culturing conditions and getting the reagents to perform transfection experiments (some times getting some reagents can take up to 3-4 months for us).

## B. Studies and Results.

**Animal Model.** Last year we had some problems in getting the adequate number of Wistar rats as proposed in the original proposal and, even keeping exactly the same animal protocol and maintaining conditions, we had to switch to Holtzman strain in order to get the right number of animals. To compare with our previous results in Wistar rats, we repeated the key experiments using Holtzman strain and found results matched perfectly with those previously obtained with Wistars, then we continued and will continue working with Holtzman rats.

**Statistic analysis.** Daily variations were statistically analyzed using One way ANOVA followed by Tukey-Kramer post test with significance for  $P < 0.05$ .

**B.1. Studies in hippocampus:** In the previous progress report we showed that vitamin A deficiency modifies temporal gene expression and activity of CAT and GPx in the rat hippocampus.

Here, we found lipoperoxidation follows a daily rhythm in the rat hippocampus with a peak at the end-of-the-night-beginning-of-the-day, ZT22-ZT2 ( $P < 0.01$ ). Interestingly, we observed a significant phase-shift in the pattern of lipoperoxidation in vitamin A-deficient rats ( $P < 0.0001$ ). Refeeding vitamin A-deficient rats with the control diet shortened in 4 hours, but didn't completely reversed, the phase-shift observed in vitamin A-deficient animals in comparison to controls (Figure 1).

Daily rhythms of CAT expression at protein level ( $P < 0.005$ ) were abolished in the hippocampus of vitamin A-deficient rats ( $P > 0.05$ ) (Figure 2 left and central panels) and 15 days of vitamin A refeeding reverted the effects of vitamin A deficiency and started recovering protein rhythms (Figure 2 right panel). In the case of GPx, vitamin A deficiency attenuated ( $P < 0.05$ ) the daily rhythm of protein level observed in controls ( $P < 0.0001$ ) (Figure 3 left and central panels). Daily oscillation of GPx protein level started been restored in vitamin A-recovered rats ( $P < 0.01$ ).

We propose vitamin A deficiency might affect transcriptional and/or post-transcriptional processes, either affecting the transcriptional regulation by RARs or RXRs through specific response elements on CAT and GPx non-coding regulatory regions either affecting the interaction of BMAL:CLOCK heterodimer with the antioxidant enzymes promoters, both of those possibilities will be tested in the next coming project period.

Althought, we have done only one experiment with pooled hippocampus samples ( $n=2$  per time point), up to this moment, we observed a daily variation in Bmal1 and Per mRNA expression ( $\beta$ -actin corrected) in the hippocampus of rat. Interestingly, vitamin A deficiency would attenuate rhythmic expression of those clock genes (Figure 4) and, in that way, might interfere with the circadian expression of clock-controlled genes, such as BDNF, CAT and GPx. A daily variation in the expression of BDNF, a learning-and-memory-related gene, was also observed. Such rhythmicity was abolished in the vitamin A-deficient rats as well, probably, as a consequence of the loss of Bmal1 and Per1 rhythmic expression (Figure 5), and was recovered after 15 days of vitamin A refeeding.

**B.2. Studies in liver:** Glutathione (GSH) is essential for the maintenance of an optimal cellular redox state and the production of  $\text{NADPH} + \text{H}^+$ , which are critical for the transcriptional activity of the biological clock (Rutter et al., *Science*, 2001). Retinoid receptor binding sites (RAREs and RXREs) and E-box sites were found in the regulatory regions of Glutathione Peroxidase (GPx) and Glutathione reductase (GR) genes and shown in the original proposal of this work. Here, we report a daily variation of RARs, RXRs, GPx and GR mRNA levels ( $P < 0.025$  and  $P < 0.05$ ) as well as of GPx and GR activity and GSH/GSSG levels in the liver of control rats (Figs. 6, 7, 8 and 9). Vitamin A deficiency abolished (RARalpha,  $P > 0.05$  ns) or attenuated (RXRalpha,  $P < 0.05$ ) daily rhythmicity which starts recovering after 15 days of vitamin A refeeding.

We observed different effects of vitamin A deficiency on the circadian expression of GR and GPx. On one hand, Vitamin A deficiency phase shifted the peak of GR mRNA expression from ZT12 to ZT2 ( $P < 0.05$ , Fig. 7) and, on the other hand, it increased GPx expression at different time points throughout the day, without affecting its temporal pattern (Fig. 8). GPx mRNA levels returned to controls in vitamin A-recovered rats (Fig. 8) while phasing of GR mRNA expression was just partially recovered in those animals (Fig. 7). Following temporal patterns in mRNA expression, enzymatic

activity of GR and GPx vary in a 24 h cycle ( $P < 0.0001$  and  $P < 0.001$ , respectively). We observed a phase shift in the daily activity of GR, from ZT2 to ZT7 ( $P < 0.0001$ ), in the liver of vitamin A-deficient rats and a significant increase in the GPx activity at the beginning of the day (ZT2-ZT7,  $P < 0.025$ ), in comparison to controls (Fig 8), being the last consistent with previous results from our lab (Anzulovich et al., 2000).

GSH levels decreased and its rhythmic variation was abolished in the liver of vitamin A-deficient rats which start recovering after 15 days of vitamin A refeeding (Figure 9).

These results would suggest retinoids would participate in the circadian regulation of GSH/GSSG levels and thus, in the circadian regulation of the cellular redox state in liver, a peripheral clock with a relevant function in the control of circadian metabolism, and coordination with other peripheral oscillators. The intrinsic mechanisms of such regulation will be probably elucidated during the next project period.

**C. Significance.** The results reported here would contribute to define for the first time a putative role for vitamin A as a rhythm regulator in the establishment of circadian antioxidant enzyme systems in peripheral clocks such as hippocampus and liver, as well as in the circadian expression of memory- and learning-related genes. Learning how vitamin A deficiency affects the circadian expression of clock and clock-controlled genes may have an impact on the nutritional, neurobiology and chronobiology fields, emphasizing for the first time the importance of nutritional factors such as dietary micronutrients in the regulation of circadian parameters in peripheral clocks. VAD is a serious concern and has a clinical and socio-economical significance worldwide, and particularly, in Argentina where the alarming incidence of VAD reaches a fifth of the school-aged children. We would expect emerging data from these and future studies will also highlight retinoid signalling pathways as potential novel therapeutic targets for cognitive deficits as well as for circadian rhythms disorders.

**C. Plans.** Above results encourage us to continue with the studies proposed originally. Keeping same original specific aims (#1 and 2), our timetable is as follows:

Years	Studies
2008-2009	<ul style="list-style-type: none"> <li>-Obtention of the Animal Model (twice per year).</li> <li>- Behavioral studies (Specific Aim 1).</li> <li>- Study of the circadian expression of clock genes in liver and hippocampus.(Specific Aim 1, continuation)</li> <li>- Study of the circadian expression of BDNF at the protein level in the hippocampus (Specific Aim 1).</li> <li>- Studies in vitro of the regulatory mechanisms through which vitamin A might modulate the circadian expression of clock genes. (Specific Aim 1)</li> <li>- Studies in vitro of the regulatory mechanisms through which vitamin A might modulate the circadian expression of antioxidant enzymes genes. (Specific Aim 2)</li> </ul>

## E. Publications

Some of the results reported here have been presented in two posters to the Argentine Society of Biochemical and Molecular Biological Research and their abstracts published as follow:

2007- Navigatore Fonzo LS, Delgado SM, Bonomi MR, Rezza IG, Gimenez MS, Anzulovich AC. Circadian Variation Of Antioxidant Enzymes And TBARs In Hippocampus Of Vitamin A deficient rats. Biocell 31 (Suppl.), 153. ISSN 0327-9545.

2007- Ponce IT, Rezza IG, Bonomi MR, Delgado SM, Gimenez MS, Anzulovich AC. Circadian Variation Of Glutathione Metabolism Is Modified In The Vitamin A Deficiency. Biocell 31 (Suppl.), 94. ISSN 0327-9545.

The following manuscript was recently sent to Neuroscience:

- L.S. Navigatore Fonzo, R. Golini, S.M. Delgado, M.R. Bonomi, I.G. Rezza, M.S. Giménez And A.C. Anzulovich. (2008) Temporal patterns of lipoperoxidation and antioxidant enzymes are modified in the hippocampus of vitamin A-deficient rats. Possible impact on memory and learning. Sent to Neuroscience, Elsevier Ed. Ms. No.: NSC-08-397.

Manuscript in preparation:

- Ponce IT, Rezza IG, Delgado SM, Bonomi MR, Gimenez MS and Anzulovich AC. Daily variation of glutathione metabolism in the rat liver. Effect of nutritional vitamin A deficiency.

Figures are shown on the following pages

**FIGURE 1**

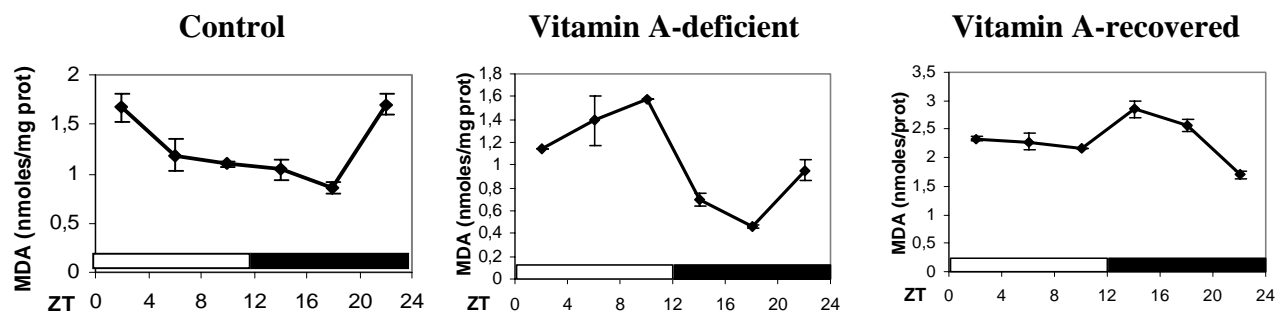


Figure 1. Day-night cycles of lipoperoxidation in the hippocampus of control, vitamin A-deficient and vitamin A-recovered rats. MDA was measured by the thiobarbituric acid method. Each value represents the mean $\pm$ SE of 4 animals. Horizontal bars represent the distribution of light (open) and dark (closed) phases of the 24 h photoperiod. ZT is zeitgeber time, with ZT=0 when light is on.

**FIGURE 2**

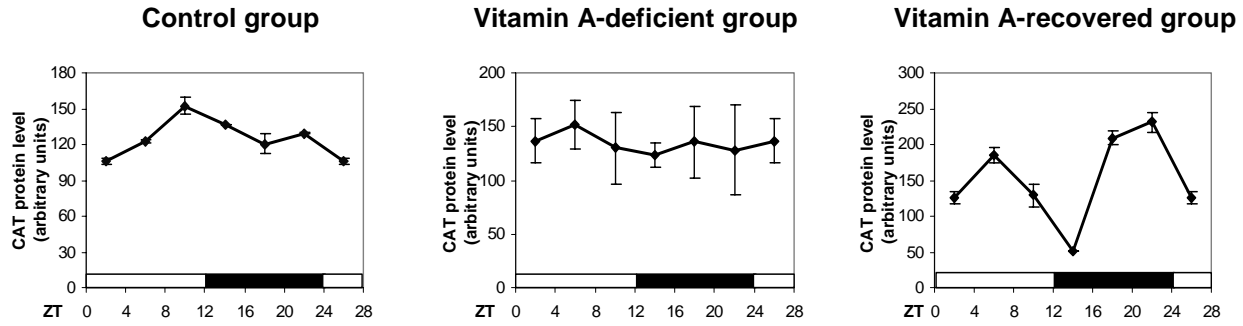


Figure 2. Daily rhythms of CAT protein levels in the hippocampus of control, vitamin A-deficient and vitamin A-recovered rats. Protein levels were determined by immunoblotting against a goat anti-CAT IgG. Each value represents the mean $\pm$ SE of 2 pools of n=3 hippocampi. Horizontal bars represent the distribution of light (open) and dark (closed) phases of the 24 h photoperiod. ZT is zeitgeber time, with ZT=0 when light is on.

**FIGURE 3**

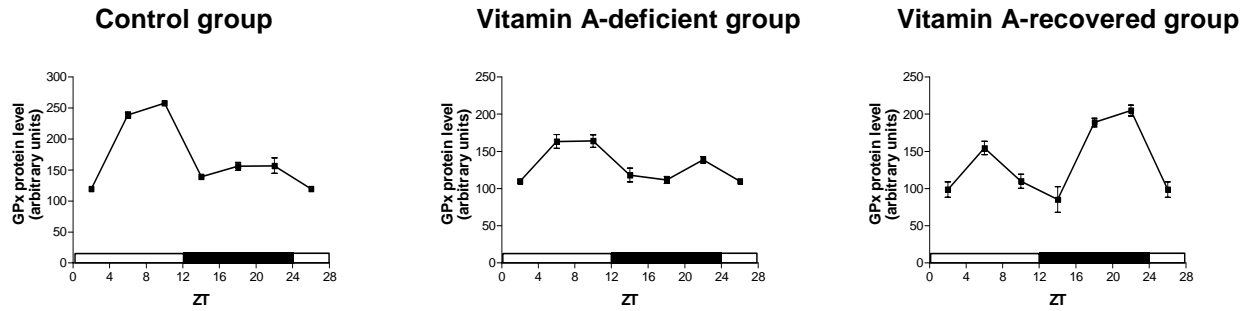


Figure 3. Daily rhythms of GPx protein levels in the hippocampus of control, vitamin A-deficient and vitamin A-recovered rats. Protein levels were determined by immunoblotting against a goat anti-GPx IgG. Each value represents the mean $\pm$ SE of 2 pools of n=3 hippocampi. Horizontal bars represent the distribution of light (open) and dark (closed) phases of the 24-h photoperiod. ZT is zeitgeber time, with ZT=0 when light is on.

**FIGURA 4**

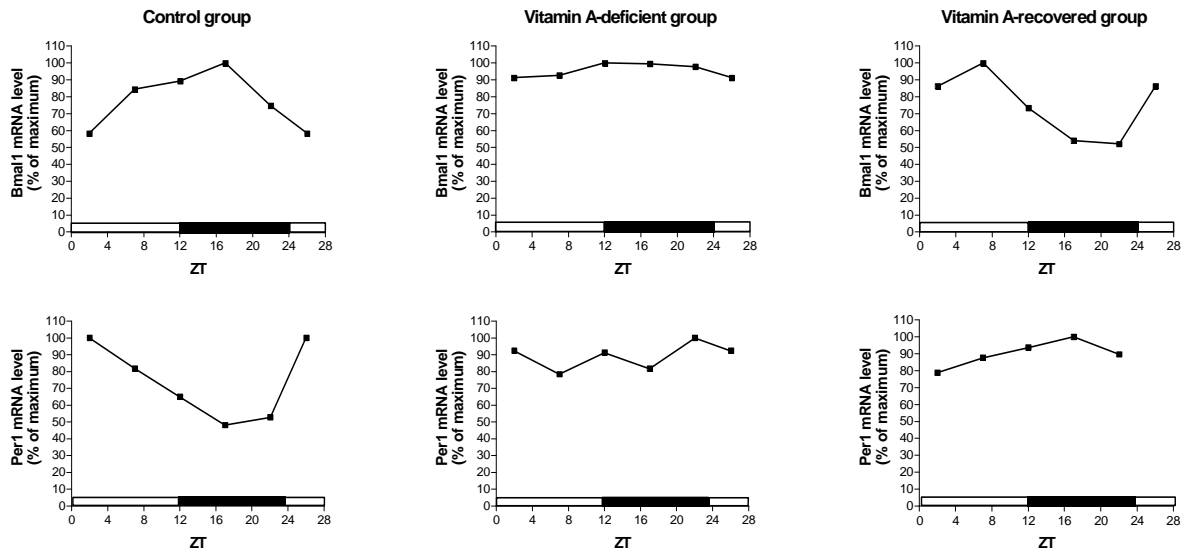


Figure 4. Daily rhythms of clock, *Bmal1* and *Per1*, gene expression in the hippocampus of control, vitamin A-deficient and vitamin A-recovered rats. mRNA levels were determined by RT-PCR and normalized to  $\beta$ -actin. Each value represents the mean $\pm$ SE of n=2 pooled hippocampi. Horizontal bars represent the distribution of light (open) and dark (closed) phases of the 24-h photoperiod. ZT is *zeitgeber time*, with ZT=0 when light is on.

## FIGURA 5

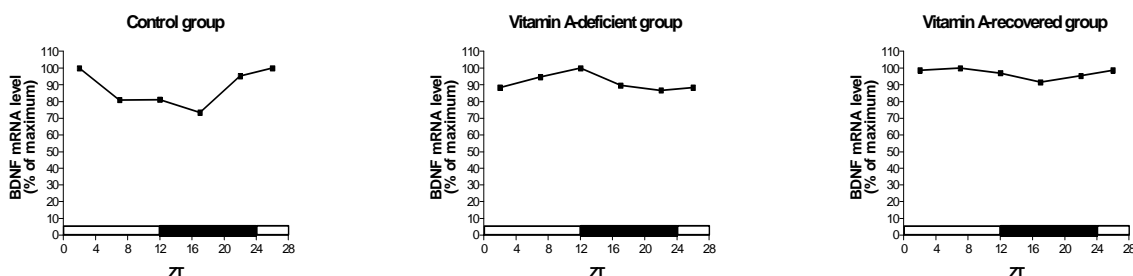


Figure 5. Daily variation of *BDNF* gene expression in the hippocampus of control, vitamin A-deficient and vitamin A-recovered rats. mRNA levels were determined by RT-PCR and normalized to  $\beta$ -actin. Each value represents the mean $\pm$ SE of n=2 pooled hippocampi. Horizontal bars represent the distribution of light (open) and dark (closed) phases of the 24-h photoperiod. ZT is *zeitgeber time*, with ZT=0 when light is on.

## FIGURE 6

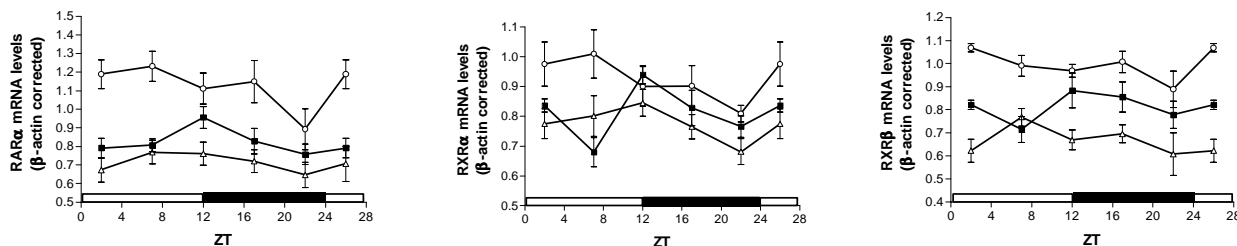
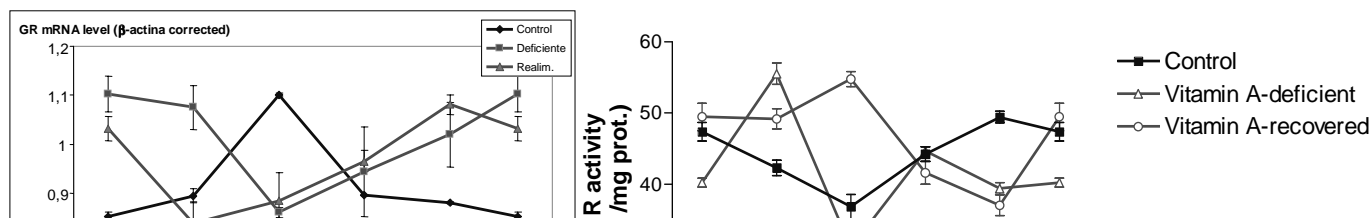


Figure 6. Temporal expression of *RAR $\alpha$* , *RXR $\alpha$*  and *RXR $\beta$*  in the liver of control (closed squares), vitamin A-deficient (open triangles) and vitamin A-recovered (open circles) rats. mRNA levels were determined by RT-PCR and normalized to  $\beta$ -actin. Each value represents the mean $\pm$ SE of 4-5 liver samples. Horizontal bars represent the distribution of light (open) and dark (closed) phases of the 24-h photoperiod. ZT is *zeitgeber time*, with ZT=0 when light is on.

## FIGURE 7



**FIGURE 8**

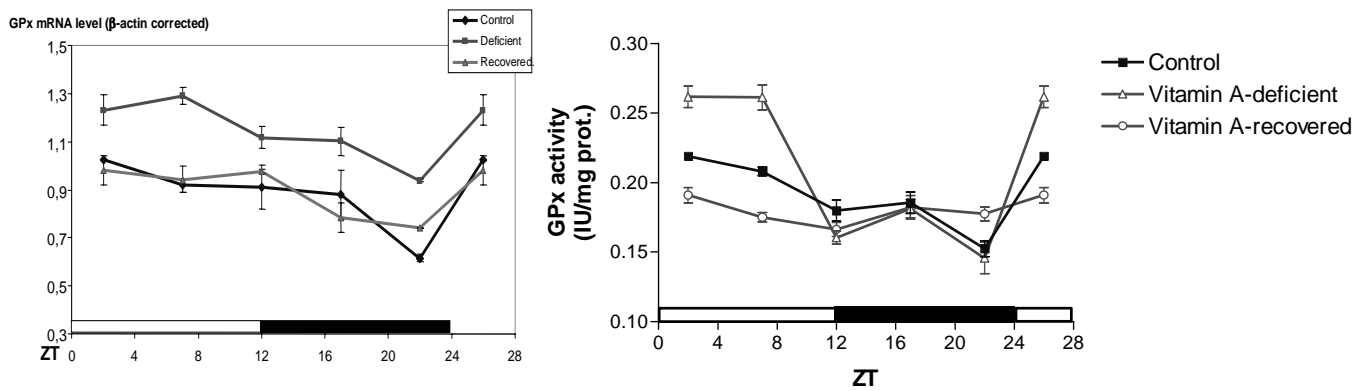


Figure 8. Temporal expression and activity of GPx in the liver of control (blue line), vitamin A-deficient (red line) and vitamin A-recovered (green line) rats. mRNA levels were determined by RT-PCR and normalized to  $\beta$ -actin. Each value represents the mean $\pm$ SD of 4-5 liver samples. Horizontal bars represent the distribution of light (open) and dark (closed) phases of the 24-h photoperiod. ZT is zeitgeber time, with ZT=0 when light is on.

**FIGURE 9**

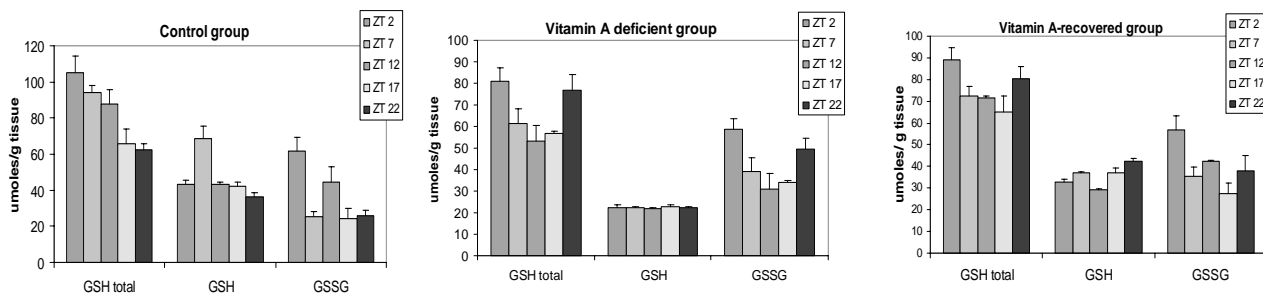


Figure 9. Daily variation of total GSH, GSH and GSSG levels in the liver of control, vitamin A-deficient and vitamin A-recovered rats. Bars represent the mean $\pm$ SD of 4 liver samples. ZT is zeitgeber time, with ZT=0 when light is on and ZT=12 when light is off.