POSITIVE CORRELATION BETWEEN PROTEASOME ACTIVITY AND POLYPHENOLS IN THE TELENCEPHALON OF ADULT FEMALE MICE.

CORRELACIÓN POSITIVA ENTRE LA ACTIVIDAD PROTEASOMAL Y LOS POLIFENOLES EN EL TELENCÉFALO DE RATONES HEMBRAS ADULTAS.

Agustín Ramiro Miranda[1,2], Mariela Valentina Cortez[1,3], Silvana Valeria Serra[2], Elio Andrés Soria[1,4,5]

Abstract:

Introduction: Proteasome regulates proteostasis, and can be compromised in neurodegenerative diseases. Thus, our aim was to correlate the activity of proteasome to the level of polyphenols in telencephalon during murine adulthood. Methods: Proteasome activity, polyphenols and other variables (glucose and hydroperoxides) were analysed in Balb/c female telencephala (n = 20, age = 4-12 months), using multivariate methods. Results: The following values were found: proteasome activity = 3.1 ± 0.6 FI/µg of tissue proteins, glucose = 0.1 ± 0.0 µg/µg, hydroperoxides = 363.4 ± 96.6 OD/µg, and polyphenols = 0.1 ± 0.0 ng/µg. Polyphenols reduced during aging showed a direct correlation with proteasome (Pearson's coefficient = 0.43, p = 0.0590, and a multivariate linear regressive coefficient = 17.85, p = 0.0216), with glucose and hydroperoxides being not involved (p>0.1). This correlation was confirmed by partial least square regression (beta = 0.67). Conclusion: Proteasome activity can be affected during ageing, and promoted by telencephalic polyphenol levels. Thus, these diet compounds might exert benefits in adult brain.

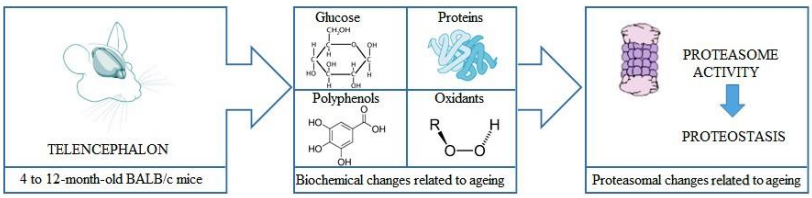
Palabras clave: central nervous system; aging; proteostasis; phytochemicals; multivariate statistics.

Resumen:

Introducción: El proteasoma regula la proteostasis y puede verse comprometido en enfermedades neurodegenerativas. Por lo tanto, nuestro objetivo fue correlacionar la actividad del proteasoma con el nivel de polifenoles en el telencéfalo durante la edad adulta de ratones. Métodos: Se analizaron la actividad del proteasoma, polifenoles y otras variables (glucosa e hidroperóxidos) en telencéfalos de ratones hembras Balb/c (n = 20, edad = 4-12 meses), utilizando métodos multivariados. Resultados: Se encontraron los siguientes valores: actividad proteasomal = 3,1 ± 0,6 FI/µg de proteínas tisulares, glucosa = 0,1 ± 0,0 µg/µg, hidroperóxidos = 363,4 ± 96,6 OD/µg y polifenoles = 0,1±0,0 ng/µg. Los polifenoles reducidos durante el envejecimiento mostraron una correlación directa con el proteasoma (coeficiente de Pearson = 0,43, p=0,0590 y un coeficiente de regresión lineal multivariante = 17,85, p=0,0216). Glucosa e hidroperóxidos no estuvieron implicados (p>0,1). Esta correlación fue confirmada por regresión parcial de mínimos cuadrados (beta = 0,67). Conclusión: La actividad proteasomal puede afectarse durante el envejecimiento y ser promovida por el nivel telencefálico de polifenoles. Así, estos compuestos dietéticos podrían ser beneficiosos para el cerebro adulto.

Keywords: sistema nervioso central; envejecimiento; proteostasis; fitoquímicos; estadística multivariada.

Graphical abstract:



1. Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, INICSA. Bv. de La Reforma, Ciudad Universitaria, Córdoba 5014, Argentina.
2. Universidad Nacional de Córdoba. Facultad de Ciencias Médicas. Escuela de Fonoaudiología. Bv. de La Reforma, Ciudad Universitaria, Córdoba 5014, Argentina.
3. Universidad Nacional de Córdoba. Facultad de Ciencias Médicas. Escuela de Nutrición, CENINH. Bv. de La Reforma, Ciudad Universitaria, Córdoba 5014, Argentina.
4. Universidad Nacional de Córdoba. Facultad de Ciencias Médicas. Cátedra de Biología Celular, Histología y Embriología, Instituto de Biología Celular. Bv. de La Reforma, Ciudad Universitaria, Córdoba 5014, Argentina.
5. Email de contacto: easoria@fcm.unc.edu.ar

Recibido: 2018-04-24 **Aceptado:** 2018-05-19
DOI: 10.31053/1853.0605.v75.n3.19930

Introduction

Proteasome is crucial for cellular proteostasis, by removing abnormal and unnecessary proteins[1], such as those involved in cell cycle, apoptosis, transcription, DNA repair, protein quality control, antigenic presentation, and differentiation[2]. Proteasome is a 26S multicatalytic protease with a 20S proteolytic core and two 19S regulatory caps[3]. Different agents compromise this structure and functions, leading to pathological development[4]. Concerning this, deficient proteostasis is associated with mitochondrial dysfunction, oxidative stress, and apoptosis[5], which is implicated in neurodegenerative diseases and other age-related pathologies[6]. Senescence impairs therefore proteasome activity by different mechanisms with the consequent brain damage[7].

Dietary plant compounds, such as glucose and polyphenols, arrive at brain and modify its age-dependent metabolism and redox state, which might affect the activity of proteasome[8]. Given the relation of oxidative metabolism and proteostasis[7,9], their markers should be analysed together. Furthermore, polyphenols are antioxidant and chemopreventive phytochemicals[10], with multitarget neuroactivity in the central nervous system[11]. In consequence, the aim of the present study was to correlate proteasome activity and polyphenols in murine female telencephalon using multivariate methods to integrate different concerns.

Methods

Conditions

Healthy adult female Balb/c mice (n = 20, age range = 4-12 months, body weight range = 18-26 g) were maintained under physiological conditions supported by *ad libitum* access to standard diet (200±13 g/Kg/d; Cargill SACI, Argentina) and potable water (150±10 mL/Kg/d; Aguas Cordobesas SA, Argentina), a light-dark cycle, avoiding overcrowding in cages. They were sacrificed by isoflurane inhalation, with all procedures being in accordance with ethical concerns and good laboratory practices (86/609/CEE).

Proteasome activity (response), glucose, polyphenols (dietary plant compounds), and hydroperoxides (oxidative markers) were in telencephala (weight = 150.9 ± 5.6 mg, protein content determined by the Biuret's reaction = 13.2 ± 1.2 µg/mg of tissue), using a GloMax® Multi Microplate Multimode Reader (Promega Corp., Madison, WI, USA), and chemicals from Sigma-Aldrich Co. (St. Louis, MO, USA).

Telencephalic samples were homogenised at 4 °C in a solution of 50 mM Tris and 5 mM $MgCl_2$ (pH 7.5 by HCl addition), to determine proteasome and glucose. Another aliquot (400 µL) was incubated for 30 minutes at 37°C in darkness with methanol (600 µL) and 50% trichloroacetic acid (50 µL), to obtain the supernatant by centrifugation (5 minutes at 10000 rpm) and determine polyphenols and hydroperoxides.

Proteasome activity

A mixture of 8 µL of homogenate and 2 µL of glycerol was incubated for 2 hours at 37 °C in darkness with 100 µL of substrate (0.3 mM Z-Leu-Leu-7-amido-4-methylcoumarin, 50 mM Tris, 10 mM $MgCl_2$, 1 mM 1,4-dithiothreitol). Reaction was stopped with 200 µL of 4°C ethanol, to measure the methylcoumarin-amide product as fluorescence intensity (FI, spectrofluorometry: Ex 380 nm/Em 440 nm)[12].

Polyphenols

Supernatants (100 µL) were mixed with 0.1% Fast Blue BB (10 µL) and 20% sodium bicarbonate (10 µL) for 30 min in darkness at 37ºC. Polyphenols were measured at 450 nm and calculated according to a standard gallic acid curve[13].

Hydroperoxides

These oxidative markers were analyzed in 10 µL of supernatant mixed with 100 µL of chromogen (100 mM sorbitol and 0.125 mM xylenol orange) and 1 µL of catalyser (25 mM ferrous ammonium sulphate in 2.5 M sulphuric acid). After 30 minutes at room temperature, optical density (OD) was measured at 540 nm[14].

Glucose

The Trinder's method was used using a commercial kit according to a previous work[14]. Results at 540 nm were calculated according to a standard glucose curve.

Statistical analysis

Proteasome data were analyzed with different statistical methods using the Infostat v.2012 software as previously done[15]. Variables were correlated by the Pearson´s coefficient followed by the path analysis to discriminate interactions of these correlations. Also, age was categorised according to the mean < or ≥ 7.6 ± 0.5 months as younger and older mice, respectively, for *T* test. Multivariate linear regression was used to confirm results, and partial least squares regression to obtained betas in case of covariate predictors[16].

Results

The following values were found per µg of tissue proteins: proteasome activity = 3.1 ± 0.6 FI (40.6 ± 5.4 FI/mg of tissue), glucose = 0.1 ± 0.0 µg (0.8 ± 0.1 µg/mg), polyphenols = 0.1 ± 0.0 ng (1.4 ± 0.2 ng/mg), and hydroperoxides = 363.4 ± 96.6 OD (3361.7 ± 436.6 OD/mg). No significant differences in proteasome activity were found according to age (p=0.2473, Table 1).

Table 1. Neurochemical variables found in telencephala of female Balb/c mice (n = 20)

	Younger mice*	Older mice**
Proteasome activity	3.5 ± 0.9 FI/µg of proteins (33.9 ± 7.4 FI/mg of tissue)	2.9 ± 0.7 FI/µg of proteins (44.9 ± 7.4 FI/mg of tissue)
Glucose	0.1 ± 0.0 µg/µg of proteins (0.7 ± 0.1 µg/mg of tissue)	0.1 ± 0.0 µg/µg of proteins (0.9 ± 0.1 µg/mg of tissue)
Polyphenols	0.2 ± 0.0 ng/µg of proteins (1.6 ± 0.2 ng/mg of tissue)	0.1 ± 0.0 ng/µg of proteins (1.2 ± 0.2 ng/mg of tissue)
Hydroperoxides	327.0 ± 87.7 OD/µg of proteins (3235.0 ± 659.3 OD/mg of tissue)	383.0 ± 143.4 OD/µg of proteins (3430.0 ± 588.4 OD/mg of tissue)

* age = 5.4 ± 0.6 months, weight = 21.7 ± 0.9 g. ** age = 8.8 ± 0.4 months, weight = 22.0 ± 0.6 g (mean ± error).

Pearson's coefficient of proteasome activity and age was -0.14 (p=0.5464). This weak inverse correlation was indirect and mainly depended on aging-related reduction of polyphenols (-0.10 of -0.14). Moreover, the proteasome-polyphenol coefficient was direct and equal to 0.43 (p=0.0590). Although hydroperoxides and glucose were reciprocally correlated (coefficient=0.92, p<0.0001), they were not associated with proteasome (p>0.7) and age (p>0.8) (Table 2).

Table 2: Path analysis of proteasome activity (FI/µg of proteins, n=20)

Effect	Path	Coefficient	p-value
Polyphenols (ng/µg)	Direct	0.65	
Polyphenols (ng/µg)	Body weight (g)	-0.15	
Polyphenols (ng/µg)	Age (months)	0.01	
Polyphenols (ng/µg)	Glucose (µg/µg)	-0.19	
Polyphenols (ng/µg)	Hydroperoxides (OD/µg)	0.11	
Total correlation		**0.43**	**0.059**
Body weight (g)	Direct	0.43	
Body weight (g)	Polyphenols (ng/µg)	-0.24	
Body weight (g)	Age (months)	-0,02	
Body weight (g)	Glucose (µg/µg)	0.12	
Body weight (g)	Hydroperoxides (OD/µg)	-0.13	
Total correlation		**0.15**	**0.523**
Age (months)	Direct	-0.06	
Age (months)	Polyphenols (ng/µg)	-0.13	
Age (months)	Body weight (g)	0.13	
Age (months)	Glucose (µg/µg)	-0.06	
Age (months)	Hydroperoxides (OD/µg)	-0.02	
Total correlation		**-0.14**	**0.546**
Glucose (µg/µg)	Direct	-0.62	
Glucose (µg/µg)	Polyphenols (ng/µg)	0.21	
Glucose (µg/µg)	Body weight (g)	-0,08	
Glucose (µg/µg)	Age (months)	-0.00	
Glucose (µg/µg)	Hydroperoxides (OD/µg)	0.41	
Total correlation		**-0.08**	**0.724**
Hydroperoxides (OD/µg)	Direct	0.45	

Hydroperoxides (OD/µg)	Polyphenols (ng/µg)	0.17	
Hydroperoxides (OD/µg)	Body weight (g)	-0.13	
Hydroperoxides (OD/µg)	Age (months)	0.00	
Hydroperoxides (OD/µg)	Glucose (µg/µg)	-0.57	
Total correlation		**-0.07**	**0.758**

Multivariate linear regression of proteasome activity modelled age (months), body weight (g), polyphenols (ng/µg) and glucose (µg/µg) as independent variables, which respectively showed these regressive coefficients: -0.13 (p=0.6216), 0.42 (p=0.1319), 17.85 (p=0.0216) and -10.98 (p=0.4064). Regression was repeated using hydroperoxides instead of glucose, given their covariation, with similar results and a nule regressive coefficient for these oxidative markers (p=0.6189) (Figure 1).

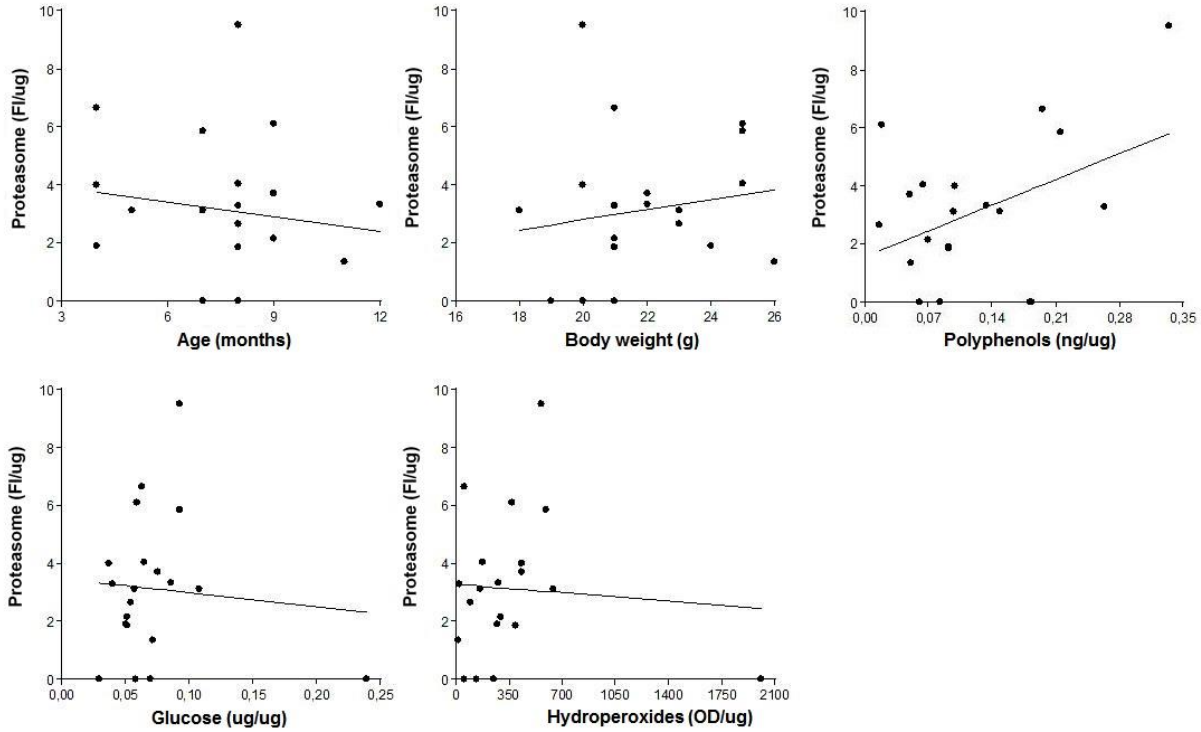


Figure 1. : Linear regression of proteasome activity (FI/?g of proteins, n=20) according to different variables.

PLS allowed modelling age categories, glucose, hydroperoxides, body weight, polyphenols (predictors), telencephalic weight and proteasome activity, to confirm that this activity correlated to polyphenols (beta=0.67), which decreased in telencephalon during aging (Figure 2).

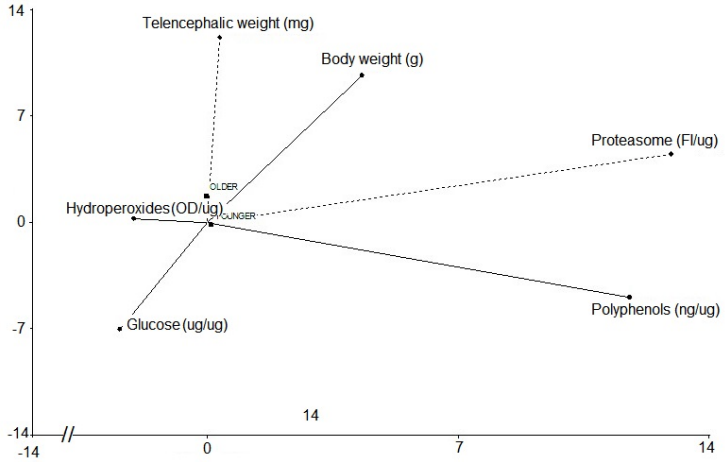


Figure 2. Partial least square regression of proteasome activity (FI/?g of proteins, n=20) and telencephalic weight (mg) according to different variables.

Discussion

Although the synthesis of proteasome can be reduced during aging, this is compensated in brain by proteasome plasticity[17]. Activity is therefore conserved, as demonstrated here. Proteasome deficiency has been also reported, but it is described by univariate methodology under pathological conditions, which disturb proteostasis by different mechanisms[18]. Moreover, healthy conditions were supported by the no involvement of glucose and hydroperoxide, which are dysmetabolic markers[19]. Contradictory data about age-related proteasome compromise are revised by Dantuma and Bott in accordance with different experimental conditions[20].

The neuroprotective activity of polyphenols depends on their capacity to stabilise degradation machinery and promote quality control of proteins with subsequent increase of lifespan[21]. Accordingly, polyphenols are neurotropic compounds[11], which showed a positive relation with proteasome in the current work. Telencephalic decrease of these compounds during aging can respond to changes on their organic absorption and distribution. A diet supply might be therefore suggested to prevent brain-affecting non-communicable diseases[11]. Summing up, healthy female mice do not exhibit proteasome modifications during the first year of life, which supports the concept of normal ageing at molecular level, when appropriate diet compound supply is given.

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