Composition of neutral lipids and phospholipids in tegu lizard *Tupinambis merianae* fat bodies

Composición de lípidos neutros y fosfolípidos en cuerpos grasos de lagartos overos Tupinambis merianae

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Summary
A detailed analysis of neutral and polar lipids in fat bodies of *Tupinambis merianae* lizards raised in captivity and fed on a standard farm diet was performed. Lipids were separated by thin-layer chromatography. For determining fatty acid composition, methyl esters were analyzed by gas-liquid chromatography. Cholesterol was measured by commercially available enzymatic assay. Triacylglycerols represented about 97% of the total, followed by free fatty acids (~1.8%), and diacylglycerols (~1.3%). Oleic (18:1), palmitic (16:0), linoleic (18:2), and palmitoleic (16:1) acids were the most abundant fatty acids in triacylglycerols. In diacylglycerols, palmitic, stearic (18:0), oleic and linoleic acids were the major fatty acids. In free fatty acids, palmitic acid was followed by palmitoleic and stearic acids, and this lipidic fraction evidenced the lowest unsaturation index. Phospholipids were present in low quantities in fat bodies, with phosphatidylcholine being the major constituent (it amounted to approximately 65% of the total content). The fatty acid composition of the total phospholipids showed that they were enriched with fatty acids of 18 carbon chain lengths, with the principal component being oleic acid. The prevalence of unsaturated fatty acids makes fat bodies of *Tupinambis merianae* a potential source of fat for producing cosmetics, medicines and food products.
Key words: tegu lizard, *Tupinambis merianae*, fat bodies, lipids, fatty acids.

Resumen
Se realizó un análisis detallado de los lípidos neutros y polares en los cuerpos grasos de lagartos *Tupinambis merianae*, criados en cautiverio y alimentados con una dieta estándar de criadero. Los lípidos se separaron por cromatografía de capa fina. Para determinar la composición en ácidos grasos, los metilésteres se analizaron mediante cromatografía de reparto gas-líquida. El colesterol se cuantificó mediante un ensayo enzimático disponible comercialmente. Los triacilglicéridos representaban aproximadamente el 97% del total de lípidos, seguido por los ácidos grasos libres (~1,8%) y diacilglicéridos (~1,3%). Los ácidos grasos oleico (18:1), palmítico (16:0), linoleico (18:2) y palmitoleico (16:1) fueron los más abundantes en los triacilglicéridos. En diacilglicéridos, los ácidos grasos palmítico, esteárico (18:0), oleico y linoleico fueron los principales. En los ácidos grasos libres, el ácido palmítico

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fue seguido por los ácidos palmitoleico y esteárico, presentando esta fracción lipídica el índice de insaturación más bajo. Los fosfolípidos están presentes en bajas cantidades en los cuerpos grasos, siendo la fosfatidilcolina el constituyente principal (aproximadamente el 65% del contenido total). La composición de ácidos grasos de los fosfolípidos totales mostró que estaban enriquecidos con ácidos grasos con cadenas de 18 átomos de carbono, siendo el componente principal el ácido oleico. La prevalencia de ácidos grasos insaturados hace de los cuerpos grasos de *Tupinambis merianae* una potencial fuente de grasa para propósitos cosméticos, farmacéuticos o alimentarios.
Palabras clave: lagarto tejú, *Tupinambis merianae*, cuerpos grasos, lípidos, ácidos grasos.

Introduction

The *Tupinambis* genus (Squamata: Teiidae) comprises a group of large South American lizards, which are widely spread in regions with tropical to temperate climates. *T. merianae* and *T. rufescens*, the southernmost representatives (Cei and Scolaro, 1982), can be as long as 1.2 m and reach a 7 kg body weight as adults. They were traditionally hunted by indigenous communities for their meat, fat and leather (Donadío and Gallardo, 1984; Norman, 1987).

Currently, the *Tupinambis* genus is included in the Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), so the trade of these species and their parts remains under control.

As an alternative to wild capture, captive breeding programs have been proposed for a sustainable use of these lizards (Mercolli and Yanosky, 1990; Noriega et al., 1996). The success of these programs will depend largely on an integral use of these lizards, which includes the utilization of not only their skin, but also their meat (Caldironi and Manes, 2006; Cossu et al., 2004; Panella et al., 2003) and fat (Ferreira et al., 2009; 2010).

Tupinambis fat bodies constitute a pair of large abdominal organs, representing between 7 and 9% of the body mass, and they store most of the body lipids. As in other lizards, these reservoirs would participate as energy sources during hibernation, arousal, gonadal recrudescence, and starvation stress (Afroz et al., 1971; Avery et al., 1974; Derickson, 1976; Fitzgerald et al., 1993). In addition to being of physiological interest in research, *Tupinambis merianae* fat bodies are known to have been traditionally used in folk medicine to treat several illnesses (Donadio and Gallardo, 1984), and recent laboratory studies have shown that they have anti-inflammatory properties (Ferreira et al., 2010).

Lipid composition of fat bodies was analyzed in a few reptile species, with results showing that triglycerides are the most abundant lipid class, and that their composition is dominated by unsaturated fatty acids (Afroz et al., 1971; Avery et al., 1974; Brian et al., 1972; Greenberg et al., 1984). Fatty acid composition of fat bodies from wild-caught *Tupinambis merianae* specimens was also studied (Ferreira et al., 2009), although there have been no reports on the main lipid classes in these organs.

Because of their theoretical and economic interest, and because their composition can be considerably influenced by diet (Gist, 1972; Salgado et al., 1992; Simandle et al., 2001), this paper studied the lipidic composition of fat bodies in *Tupinambis merianae* specimens raised in captivity and fed on a standard farm diet.

Materials and Methods

Our study was conducted with *Tupinambis merianae* specimens raised in captivity in the province of Tucumán, Northern Argentina, which is a region characterized by a temperate climate and a dry winter season. The animals were fed on a standard farm diet consisting of 85% of ground chicken heads and feet (1:2), 15% soybean meal, 0.25% vitamin-mineral

supplement for broilers (Micromix, Biofarma), 0.25% sodium chloride and 0.1 % butyl hydroxy toluene (Vega Parry and Manes, 2004). From an adult population of 20 individuals of 21 to 33 months of age, 4 specimens were randomly selected, weighing between 2.7 and 3.7 kg. They were euthanized at arousal (in the pre-reproductive stage) by cranial concussion, followed by immediate spinal cord transection (AAZV, 2006; AVMA, 2001). The fat bodies were removed and maintained at -20ºC until lipid extraction.

For analysis, 4 replicate samples of about 1 g per animal were taken. Lipids were extracted from fat bodies as described by Folch et al. (1957). Total lipids were separated by monodimensional thin-layer chromatography (TLC) on silica gel G gradient plates (250-1000 µm thickness), using hexane/diethylether/acetic acid (80:20:1, by vol.) to avoid chromatographic interference of the abundant triacylglycerides. Triacylglycerides (TAG), diacylglycerides (DAG), free fatty acids (FFA) and phospholipids (placed in the plate origin) were eluted following Arvidson (1968). Then, eluted phospholipids were separated by two-dimensional TLC on silica gel H, according to Rouser et al. (1970). They were identified by iodine vapors, the silica was scraped off, and phosphorus was quantified after digestion with perchloric acid (Rouser et al., 1970).

For determining fatty acid composition, lipids were separated by TLC as described above, and spots were visualized under ultraviolet light after spraying with 2'-7'-dichlorofluorescein in methanol. Methanolysis was directly performed on the silica spots scraped off from the plates according to Morrison and Smith´s method (1964), using boron trifluoride (14% w/v in methanol, Sigma, St. Louis, MO). Fatty acid methyl esters (FAME) were purified by TLC using hexane/diethylether (95:5, by vol.) on silica gel 60 plates, prewashed with methanol/diethylether (75:25, by vol.). FAME were analyzed by gas-liquid chromatography (GLC) using 21:0, added before methanolysis, as internal standard. Two glass columns (2 m x 0.2 cm i.d.), packed with 10% SP2330 on 80-120 Chromosorb WAW (Supelco, Bellefonte, PA), were connected to two flame ionization detectors operated in the dual-differential mode. Initial and final oven temperatures were 160 and 220°C, respectively, and increase rate was 5°C/min. Injector and detector temperatures were 220º and 230°C, respectively. The carrier gas was N2 (30 ml/min). Chromatograms were quantified with a CDS-111 Varian integrator (Palo Alto, CA). Peaks were identified by comparing retention times with those of standards. This procedure led to a tentative identification of polyunsaturated fatty acids (PUFA). The unsaturation index (UI) was determined as the sum of percentages of individual unsaturated fatty acids times the number of double bonds.

Cholesterol was eluted from the chromatographic plates with chloroform and measured by commercially available enzymatic assay (Wiener Laboratories, Rosario, Argentina).

During all the procedures (lipid extraction, solvent evaporation, TLC spotting, drying and spraying of the TLC plates, and derivatization), the lipids were kept in a N2 atmosphere. All organic solvents were of analytical grade.

Proteins were determined by the method proposed by Lowry et al. (1951) after extraction with 1N NaOH, using crystalline bovine serum albumin as standard.

Statistical analysis was carried out using Student´s t-test, with the values representing the mean ± standard deviation of the total number of samples indicated in each legend.

Results

At arousal, in the pre-reproductive stage *Tupinambis merianae* fat bodies had an average weight of 276.8 ± 91.5 g, and an organ to body weight relationship of 8.2%. They were almost exclusively compounded by neutral lipids of which triacylglycerols (TAG) represented a 96.92%, followed by free fatty acids (FFA) and diacylglycerol (DAG), at 1.77 and 1.31%, respectively (Figure 1).

Compositional studies indicated that oleic acid was the most abundant fatty acid in TAG, followed by similar proportions of palmitic and linoleic acids (Table 1).

With respect to DAG and FFA, palmitic, stearic and oleic acids comprised about 82% and 60% of the total fatty acids. Palmitic acid

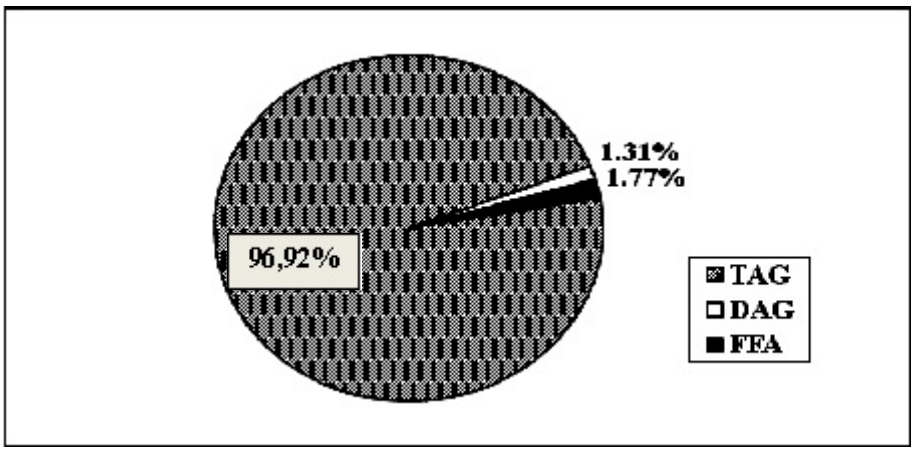

Figure 1: Percentual distribution of neutral lipids in *Tupinambis merianae* fat bodies. TAG: triacylglycerols; DAG: diacylglycerols; FFA: free fatty acids. Data are mean ± SD from four independent samples.

Figura 1: *Distribución porcentual de lípidos neutros en cuerpos grasos de Tupinambis merianae. TAG: triacilglicéridos, DAG: diacilglicéridos; FFA: ácidos grasos libres. Los datos representan la media ± DE de cuatro muestras independientes.*

Table 1: Fatty acid composition of triacylgycerols (TAG), diacylglycerols (DAG) and free fatty acids (FFA) in *Tupinambis merianae* fat bodies (mean ± s.d.; n=4).

Tabla 1: *Composición en ácidos grasos de los triacilglicéridos (TAG), diacilglicéridos (DAG) y ácidos grasos libres (FFA) en los cuerpos grasos de Tupinambis merianae (media ± DE, n= 4).*

Fatty acids	TAG	DAG	FFA
		mol %	
14:0	0.64 ± 0.08	0.70 ± 0.09	1.99 ± 0.03
16:0	19.82 ± 4.54	29.32 ± 3.71	44.29 ± 2.75
16:1	11.98 ± 0.90	5.86 ± 1.21	28.13 ± 1.92
18:0	4.49 ± 0.30	27.11 ± 3.89	10.80 ± 0.70
18:1	44.12 ± 5.51	25.30 ± 3.19	8.47 ± 0.54
18:2	18.73 ± 1.20	11.67 ± 1.78	5.32 ± 0.08
20:4n6	0.19 ± 0.04	0.14 ± 0.02	1.01 ± 0.03
		µmol/mg protein	
SFA	13.82 ± 2.85	0.43 ± 0.09	0.58 ± 0.04
MUFA	31.07 ± 1.90	0.23 ± 0.07	0.37 ± 0.02
PUFA	10.48 ± 2.11	0.10 ± 0.01	0.06 ± 0.01
UI	94.32 ± 2.73	55.06 ± 9.13	51.28 ± 8.23

Neutral lipids were isolated by TLC as described in Materials and Methods, and fatty acid composition was analyzed by GLC. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UI: unsaturated index.

was the main component, representing 29% and 44% of DAG and FFA total fatty acid content, respectively (Table 1). In all lipid fractions, n3 fatty acids were null or scarce.

TAG fraction was enriched with monounsaturated fatty acids (MUFA), which amounted to 56% of the total fatty acids and had the highest unsaturation index (UI) (Table 1). In contrast to TAG, DAG and FFA evidenced higher percentages of saturated fatty acids (SFA), low quantities of polyunsaturated fatty acids (PUFA), and comparable UI values (Table 1).

Phospholipids were also present but in much smaller quantities: less than 80 ng per mg of protein. Phosphatidylcholine (PC) was the most abundant (66% of the total phospholipids), followed by sphingomyelin (SM) and phosphatidylethanolamine in similar amounts (Figure 2). Fatty acid composition of total phospholipids is shown in Figure 3. Oleic and palmitic acids were the major fatty acids, representing about 66% of the total, followed by similar amounts of linoleic, stearic and palmitoleic acids.

Enzymatic estimates of cholesterol gave 0.94 mg per gram of fat bodies.

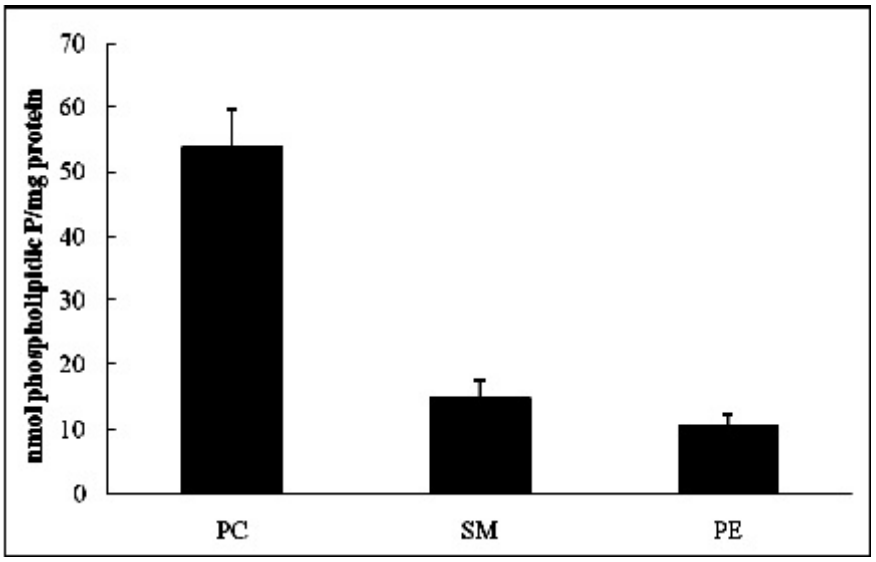

Figure 2: Phospholipid content in *Tupinambis merianae* fat bodies. Phospholipidic phosphorus was measured according to Rouser et al. (1970). Results are presented as nanomoles of phosphorus per milligram protein and are mean ± SD from four independent samples. PC: phosphatidylcholine; SM: sphingomyelin; PE: phosphatidylethanolamine.

Figura 2: *Contenido en fosfolípidos de los cuerpos grasos de Tupinambis merianae. El fósforo fosfolipídico se midió según Rouser et al. (1970). Los resultados se presentan en nanomoles de fósforo por miligramo de proteína y representan la media ± DE de cuatro muestras independientes. PC: fosfatidilcolina; SM: esfingomielina; PE: fosfatidiletanolamina.*

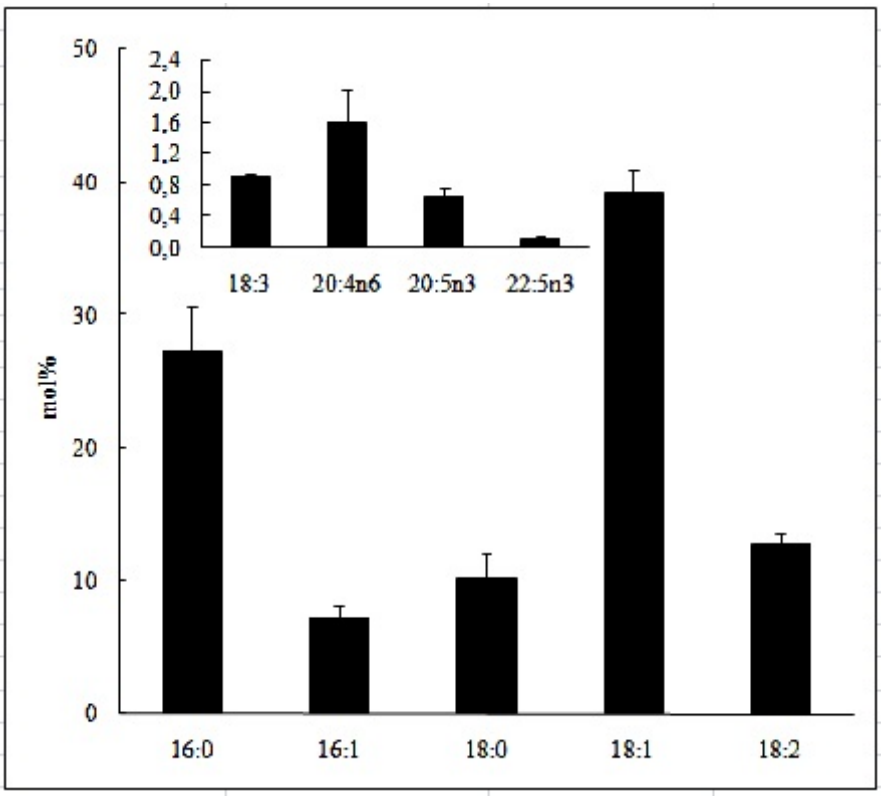

Figure 3: Fatty acid composition of total phospholipids in *Tupinambis merianae* fat bodies. Results are mean values ± SD from four independent samples.

Figura 3: *Composición en ácidos grasos de los fosfolípidos totales en cuerpos grasos de Tupinambis merianae. Los resultados representan valores medios ± DE de cuatro muestras independientes.*

Discusion

Broadly speaking, the lipidic composition of *Tupinambis merianae* fat bodies coincided with that reported for other lizards (Afroz et al., 1971; Avery et al., 1974; Brian et al., 1972; Greenberg et al., 1984). These are mainly made up by neutral lipids, of which 97% correspond to TAG. Predominant fatty acids in this fraction (TAG) were oleic (18:1), palmitic (16:0), linoleic (18:2), palmitoleic (16:1) and stearic (18:0).

As expected, there were clear differences between our captive raised animals and wild specimens (Ferreira et al., 2009) in relation to fat bodies composition. Fat bodies of the former had higher oleic and linoleic acid levels, together with minor amounts of palmitic and stearic fatty acids, which are factors that partially account for the higher unsaturation rate found in our studies (75% vs 57%). These different values are probably related to the dissimilar diets of these two groups of animals (Gist, 1972; Salgado et al.; 1992; Simandle et al., 2001).

In addition, the high content of oleic, linoleic and palmitoleic fatty acids in *Tupinambis merianae* fat bodies also results in significant differences in UFA values with respect to the adipose (subcutaneous) tissues of farm animals (46, 65 and 61% in cattle, pigs

and poultry, respectively) (Crespo and Esteve-García, 2001; Estévez et al., 2006; French et al., 2000; López-Ferrer et al., 2001; Orellana et al., 2009; Renaudeau and Mourot, 2007). Similar differences are detected when comparing our value (75%) with these animals intramuscular (interstitial) fat (49, 60 and 58%, respectively) (Crespo and Esteve-García, 2001; Estévez et al., 2006; French et al., 2000; López-Ferrer et al., 2001; Orellana et al., 2009; Renaudeau and Mourot, 2007).

Phospholipids were minor components of *Tupinambis merianae* fat bodies. These polar lipids correspond to those found in *Lacerta vivipara* fat bodies (Avery et al., 1974) and in *Sphenodon punctatus* adipose tissue (Body and Newman, 1989). In many systems, the sum of the two choline lipids constitutes about half of the total phospholipids, even though the ratio of their amounts varies greatly (White, 1973). In *Tupinambis merianae* fat bodies, these two lipids represented about 84%.

The total amount of cholesterol existing in *Tupinambis merianae* fat bodies (0.94 mg/g tissue) is equivalent to the values found in cattle (Eichhorn et al., 1986) and rat (Angel and Farkas, 1975) adipose tissues.

The presence of some light grease, constituted mainly by short-chain fatty acids with a high unsaturation degree, as that found in *Tupinambis merianae* fat bodies, seems appropriate to the physiology of a poikilotherm animal (Scapin et al., 1990).

Because of its abundance of triglycerides and prevalence of oleic, palmitic and linoleic fatty acids, *Tupinambis merianae* fat bodies have a remarkable similarity to ratites fat (Shimizu and Nakano 2003; Grompone et al., 2005; Márquez et al., 2007), which has interesting cosmetic properties (Zemstov et al., 1996; Wang et al., 2000; Márquez et al., 2007), as well as pharmaceutical ones (Ashe and Zimmerman, 1977; Ferreira et al., 2010; Politis and Dmytrowich, 1998; Zemstov et al., 1996.

On the other hand, the favorable PUFA / SFA relationship would make fat bodies of *Tupinambis merianae* a potential source of fat for food industry purposes (Department of Health, United Kingdom, 1994; Moreno and Mitjavila, 2003; Wood et al., 2003). However, some consideration should be given to the seemingly poor n6/n3 ratio (Holman, 1998).

Conclusions

Tegu lizard *Tupinambis merianae* fat bodies were almost exclusively compounded by neutral lipids, which mainly consisted of triacylglycerols. This fraction was enriched with a high proportion of unsaturated fatty acids, particularly oleic acid, in contrast to what is found in the adipose tissues of farm animals. Cholesterol content was similar to that found in the adipose tissue of other mammals. The predominance of unsaturated fatty acids makes *Tupinambis merianae* fat bodies convenient raw materials for producing cosmetic and pharmaceutical products, and incidentally food as well.

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