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REVIEW ARTICLE

Changes in lipid composition of different tissues of common octopus (*Octopus vulgaris*) during short-term starvation

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Abstract

In this work, we study the variations in concentration (mg 100 g^{-1} dry weight) and total content (mg $individual^{-1}$) of different lipid classes in muscle and the digestive gland of *Octopus vulgaris* during an 8-day starvation period. In all the samples analysed, polar lipids (PL) predominated in muscle (267.3–337.2 mg 100 g^{-1}) compared with neutral lipids (66.9–104.7 mg 100 g^{-1}). A significant positive tendency was observed in muscle for the content and concentration of monoglycerides as a consequence of starvation ($P < 0.05$). Furthermore, a higher cholesterol (CHO) content was detected in this tissue after the fourth day of starvation compared with the fed animals ($P < 0.05$). Neutral lipids predominated in the digestive gland (12 958–14 151 mg 100 g^{-1}) compared with PL (3157–6517 mg 100 g^{-1}), with triglycerides, free fatty acids and monoglycerides being the major lipid classes. The concentration of PL and monoglycerides showed a positive trend with starvation, while the triacylglycerol showed a negative tendency ($P < 0.05$). The results suggest that triglycerides and CHO are transported simultaneously from the digestive gland to the muscular tissues during starvation and the preferential use of PL and CHO during growth phases. It was estimated that lipids contributed 26% of the energy costs of the animals during starvation, mainly in the form of triglycerides from the digestive gland.

Keywords: *Octopus vulgaris*, lipid classes, starvation, muscle, digestive gland, bioenergetics

Introduction

Among different cephalopod species, common octopus (*Octopus vulgaris*) has awakened much interest in recent years for marine intensive culture (Vaz-Pires, Seixas & Barbosa 2004; Iglesias, Sánchez, Bersano, Carrasco, Dhont, Fuentes, Linares, Muñoz, Okumura, Roo, Van der Meeren, Vidal & Villanueva 2007; García García, Cerezo Valverde, Aguado-Giménez, García García & Hernández 2009). However, unlike in the aquaculture section dedicated to fish rearing, our knowledge of the nutritional requirements of cephalopods is not very advanced and is further limited by the poor availability of formulated diets that are both acceptable and nutritional (Lee, Forsythe, Dimarco, DeRusha & Hanlon 1991; Castro & Lee 1994; Domingues, López, Muñoz, Maldonado, Gaxiola & Rosas 2007). For this reason, several research groups are working towards this end with, so far, promising results (Cerezo Valverde, Hernández, Aguado Giménez & García García 2008; Quintana, Domingues & García García 2008; Rosas, Tut, Baeza, Sánchez, Sosa, Pascual, Arena, Domingues & Cuzon 2008).

It has been deduced that cephalopods are exclusively carnivorous and, while they commonly use proteins, they rarely use carbohydrates

or lipids as energy source (Lee 1994). However, there is still controversy – generated by previous studies – concerning the capacity of cephalopods to use lipids for energy and the way in which they do so. The body composition of cephalopods is less than 2% lipids on a wet weight basis, but show high levels of phospholipids, cholesterol (CHO) and polyunsaturated fatty acids, especially series n-3 HUFA (Sinanoglou & Miniadis-Meimaroglou 1998; Navarro & Villanueva 2000, 2003), suggesting a predominantly structural role for lipids rather than for energetic functions (O'Dor, Mangold, Boucher-Rodoni, Wells & Wells 1984; Moltschaniwskyj & Johnston 2006). Supporting this idea, low levels of enzymatic activity and a limited capacity for the oxidation of lipid substrates have been detected in muscle (Ballantyne, Hochachka & Mommsen 1981; Mommsen & Hochachka 1981). In contrast, evidence exists to support their use as energy source, as deduced from the O/N ratio observed in several species of cephalopod (Segawa & Hanlon 1988; Katsanevakis, Stephanopoulou & Miliou 2005), especially in starvation situations (Boucher-Rodoni & Mangold 1985, 1988), or the gradual release of marked carbon from previously supplied fatty acids (O'Dor *et al.* 1984). Furthermore, lipase enzymes are found throughout the digestive tract of cephalopods (Boucher-Rodoni 1982; Caruso, Giordano, Mancuso & Genovese 2004; Moltschaniwskyj & Johnston 2006), and although the digestibility of lipids is low compared with proteins (Petza, Katsanevakis & Verriopoulos 2006; Mazón, Piedecausa, Hernández & García García 2007), cephalopods store them as triglycerides in substantial quantities in the digestive gland (Sieiro, Aubourg & Rocha 2006; Cerezo Valverde, Hernández, García-Garrido, Rodríguez, Estefanell, Gairín, Rodríguez, Tomás & García García 2012) and mobilize them during periods of starvation (O'Dor & Wells 1987; Castro, Garrido & Sotelo 1992; García-Garrido, Hachero-Cruzado, Garrido, Rosas & Domingues 2010). According to Rosa, Pereira and Nunes (2005), the wide variations observed in this organ both as regards the lipid content and the distribution of different lipid classes suggest different roles that depend on the lifestyle and feeding strategy of the species. In the mantle, triglycerides are scarce although the concentration depends on the species in question (Sinanoglou, Meimaroglou & Miniadis-Meimaroglou 2008).

Since the aim of aquaculture is to optimize feed efficiency, captive animals are not commonly exposed to long period of starvation since it is more useful to study the biochemical changes that take place during the first few days of inanition. For all this, the objective of this study was to determine the variation in the lipid content and the classes of lipids, and their energetic contribution in different tissues of *O. vulgaris* during short-term starvation.

Material and methods

Experimental animals and maintenance

Octopuses (*O. vulgaris*) were caught at sea (Murcia, SE Spain) and kept in 2000 L tanks in the laboratory with the water being completely renewed every 60–90 min. The experiments began when the animals (700–1100 g) had acclimatized during 2 weeks in the same necessary conditions for the experimental development and were seen to be feeding on the amount of feed calculated according to García García and Cerezo Valverde (2006). Bogue (*Boops boops*) and crab (*Carcinus mediterranus*) were supplied on alternate days before the experiment started. Subsequently, the animals were transferred to individual 216 L circular tanks – both to avoid cannibalistic behaviour and for an exact determination of food intake – containing PVC tubes as shelters and connected to a recirculation seawater system with controlled temperature, UV lamps and mechanical and biological filtration systems. The water temperature varied between 17 and 20°C (18.19 ± 1.13), which is within the optimal range of temperatures for this species (Aguado Giménez & García García 2002). The photoperiod was 12L:12D, salinity 37‰, pH 7.7–8.0, while dissolved oxygen was maintained at above 90% saturation, so that this factor was not limiting (Cerezo Valverde & García García 2005). Total ammonia nitrogen was below 0.2 mg L^{-1}.

Experimental design

After acclimation in individual tanks (2 weeks), the animals were weighed and sexed, before being divided into five experimental groups of males ($n = 4$), each with a similar initial weight (1034 ± 148 g; see Table 1). Subsequently, four groups were sacrificed 1, 2, 4 and 8 days after the last meal. A final control group was fed through-

Table 1 Initial weight (W_i), final weight (W_f), weight increase (WI), eviscerated weight (EW), digestive gland weight (DGW), eviscerated weight index (EWI) and digestive gland index (DGI) and water and lipid contents in muscle and digestive gland of *Octopus vulgaris* exposed to different periods of starvation

						$y = a + b^*$ days starved	
Group	Fed $N = 4$	1 day starved $N = 4$	2 days starved $N = 4$	4 days starved $N = 4$	8 days starved $N = 4$	*a*	*b*
W_i (g)	998 ± 157	974 ± 116	1034 ± 166	1050 ± 163	1099 ± 182	981.56	15.44
W_f (g)	1066 ± 154	918 ± 121	968 ± 164†	959 ± 169†	979 ± 177†	932.54	6.36
WI (g)	68 ± 62	−56 ± 11‡	−66 ± 28‡	−91 ± 14‡	−120 ± 31‡	−49.03	−9.08*
EW (g)	921 ± 117	819 ± 111	879 ± 150	882 ± 148	912 ± 150	832.93	9.32
DGW (g)	44.0 ± 6.5	36.5 ± 8.1	31.9 ± 7.9	24.1 ± 6.4‡	19.6 ± 3.0‡	36.72	−2.32*
EWI (%)	86.6 ± 3.4	89.0 ± 0.5	89.8 ± 0.3	92.0 ± 1.1‡	92.0 ± 1.4‡	89.16	0.42*
DGI (%)	4.2 ± 0.9	3.9 ± 0.4	3.3 ± 0.3	2.5 ± 0.3‡	2.0 ± 0.1‡	3.87	−0.25*
Muscle							
Moisture (g 100 g^{-1} wet weight)	80.2 ± 0.4	79.4 ± 0.5	79.2 ± 0.4‡	79.3 ± 0.6‡	79.6 ± 0.2‡	79.25	0.03
Lipids (g 100 g^{-1}dry weight)	0.41 ± 0.22	0.51 ± 0.09	0.43 ± 0.17	0.48 ± 0.12	0.39 ± 0.10	0.50	−0.01
Lipids (g $individual^{-1}$)	0.76 ± 0.43	0.87 ± 0.28	0.81 ± 0.43	0.89 ± 0.30	0.73 ± 0.30	0.89	−0.02
Digestive gland							
Moisture (g 100 g^{-1} wet weight)	62.3 ± 1.9	60.6 ± 2.3	65.3 ± 4.7	66.3 ± 5.5	70.2 ± 3.3‡	61.13	1.19*
Lipids (g 100 g^{-1} dry weight)	21.6 ± 5.4	21.0 ± 3.2	22.4 ± 9.2	20.9 ± 12.4	20.7 ± 12.6	21.75	−0.13
Lipids (g $individual^{-1}$)	3.6 ± 0.9	3.1 ± 1.0	2.6 ± 1.3	2.0 ± 1.7	1.4 ± 1.1‡	3.12	−0.23*

*$P < 0.05$ for the slope of the regression line.
†$P < 0.05$ with respect to initial weight of the same experimental group.
‡$P < 0.05$ with respect to fed animals.

out the 8-day experimental period. All the animals were anaesthetized by immersion in cold seawater before sacrifice. After sacrifice, the muscle tissue (whole animal without viscera) and the digestive gland were weighed, ground and mixed separately three times consecutively until a homogenous sample was obtained. All the samples were vacuum-packed (Popova, Marinova, Vasileva & Krasimira Lidji 2009; Summo, Caponio, Paradiso, Pasqualone & Gomes 2010) and frozen at −20°C until use (a maximum of 3 months until lipids were extracted).

Analytical method and determination of lipid classes

Moisture was obtained by drying (105 ± 1°C, 24 h) to constant weight in a drying chamber KOWELL D2 NOVA (AOAC 1997; Method no. 930.15). The total lipid content (TL) was obtained from a 2-g sample, using ethyl ether in a SOXTEC AVANTI 2058 extractor (AOAC 1997; Method no. 920.39) and analyses were made in triplicate. Based on TL, the quantity of sample necessary to extract 10 mg of lipids was calculated according to Folch, Lees and Sloane-Stanley (1957). The lipids were kept dissolved in chloroform:methanol (2:1 v/v) containing butylated hydroxytoluene (0.01%) as antioxidant at −80°C (Christie 1982), adjusting the concentration to 10 μg lipids μL^{-1} before analysis. The lipid classes were separated by high performance thin layer chromatography (HPTLC) according to Olsen and Henderson (1989). Lipids (15 μg) were applied to 20 × 10 cm silica gel plates (Merck, Darmstadt, Germany) using an automatic injector (Linomat 5, CAMAG) according to Cerezo Valverde *et al.* (2012).

Identification and calibration for quantifying lipid classes

To identify the lipid classes, their order of appearance and reference position was determined by applying each one separately and using solutions made up with pure standards (Larodan Fine Chemicals, Malmo, Sweden). Polar and neutral lipids were included (PL and NL respectively): lysophosphatidylcholine (LPC), sphingomyelin (SM),

phosphatidylcholine (PC), lysophosphatidylethanolamine (LPE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylethanolamine (PE), monoacylglycerols (MG; monoolein), diacylglycerols (DG; diolein), CHO, free fatty acids (FFA; oleic acid), triacylglycerols (TG; triolein) and steryl esters (SE; Cholesteryl oleate). For quantification purposes, the area, expressed in arbitrary units (AU) corresponding to the application of up to 12 different quantities (0.1–15 μg) of each lipid class, was obtained in triplicate. The values obtained fit the equation for a straight line ($y = a + bx$) or a non-lineal saturation-type function ($y = y0 + ax/(b+x)$) depending on the lipid class (see Figs 1 and 2). In the present study, all the equations fit the values obtained to a statistically significant degree ($P < 0.001$) and explain more than 99% of the variability observed. Since no pure standard was available for sulpholipids (SL), their content was estimated as the product of the area detected and the total quantity of lipid applied (15 μg).

Parameters obtained and data analysis

The mean was obtained for each experimental group and the standard deviation corresponding to initial weight (W_i), final weight (W_f), weight increase (WI), eviscerated weight (EW) and weight of the digestive gland (DGW), eviscerated weight index (EWI% = EW/W_f × 100) and digestive gland index (DGI% = DGW/W_f × 100). The total concentration of lipids was expressed in g and each lipid class in mg 100 g^{-1} of dry weight. The total content of PL and NL was obtained as the sum of the lipid classes corresponding to each of these groups. The content of lipids accumulated in muscle tissue and in the digestive gland of each individual was also calculated separately to prevent misinterpretation of the concentrations due to change in the size of the fractions, according to the formula:

Lipid content (g ind^{-1}) = (dry weight of muscle tissue or of the digestive gland (g) × lipid concentration (g 100 g^{-1} dry weight)/100).

To compare the means of the initial and final weights corresponding to each starvation period, Student's *t*-test for paired samples was used. A simple regression analysis was applied to determine the variation in concentration or individual content of lipids as a function of the number of starvation days. A Student *t*-test for independent samples was used to compare the concentration or content of lipids between groups of starved animals and control (fed animals), establishing a significance level of $P < 0.05$ for all the analyses tested.

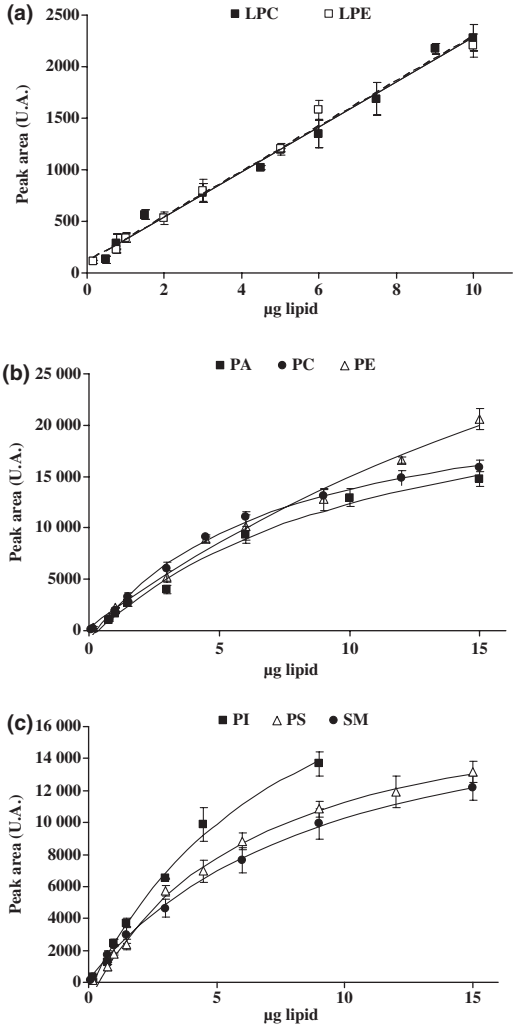

Figure 1 (a–c) Calibration curves for polar lipids. (a) Lysophosphatidylcholine (LPC): $y = 106.5 + 217.9x$; lysophosphatidylethanolamine (LPE): $y = 110.2 + 218.9x$. (b) Phosphatidic acid (PA): $y = -844.1 + (27\,968.5x)/(11.2 + x)$; phosphatidylcholine (PC): $y = -982.8 + (25\,156.0x)/(7.1 + x)$; phosphatidylethanolamine (PE): $y = 237.8 + (62\,514.2x)/(32.5 + x)$. (c) Phosphatidylinositol (PI): $y = -560.4 + (27\,426.0x)/(8.1 + x)$; phosphatidylserine (PS): $y = -1230.9 + (20\,161.3x)/(6.2 + x)$; sphingomyelin (SM): $y = 137.5 + (19\,488.1x)/(9.3 + x)$, where '$y$' is the peak area in arbitrary units and 'x' the quantity of lipids in μg.

Results

There were no significant differences between the mean weights of the different groups at the beginning

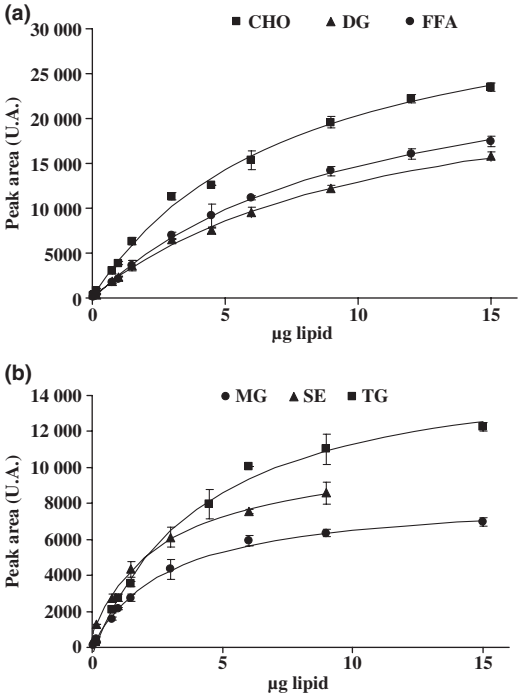

Figure 2 (a–b) Calibration curves for neutral lipids. (a) Cholesterol (CHO): $y = 46.9 + (35\ 297.6x)/(7.4 + x)$; diacylglycerols (DG): $y = 134.6 + (26\ 607.9x)/(10.8 + x)$; free fatty acids (FFA): $y = -179.9 + (29\ 138.6x)/(9.6 + x)$. (b) Monoacylglycerols (MG): $y = 1.6 + (8401.0x)/(2.9 + x)$; steryl esters (SE): $y = 684.1 + (10\ 246.5x)/(2.8 + x)$; triacylglycerols (TG): $y = -481.5 + (16\ 700.7x)/(4.2 + x)$, where '$y$' is the peak area in arbitrary units and 'x' the quantity of lipids in µg.

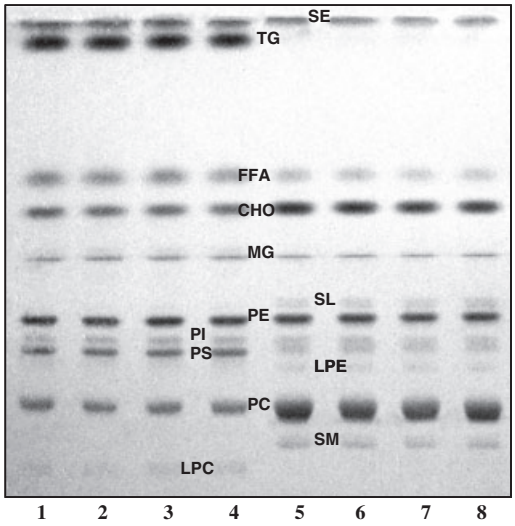

Figure 3 HPTLC chromatogram, where the different lipid classes detected can be visualized in their order of appearance (tracks 1–4 for digestive gland and tracks 5–8 for muscle in one sample of *Octopus vulgaris*). Polar lipids: LPC, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid (include cardiolipin and phosphatidylglycerol); PE, phosphatidylethanolamine; SL, sulpholipids. Neutral lipids: MG, monoacylglycerols; DG, diacylglycerols; CHO, cholesterol; FFA, free fatty acids; TG, triacylglycerols; SE, steryl esters (include waxes).

of the experiment. From the second day of starvation onwards, there began to appear differences between W_i and W_f with a significant weight decrease of 9 g day^{-1} of starvation ($P < 0.05$; Table 1). Similarly, DGW and DGI decreased at a rate of 2.32 g day^{-1} and 0.25% day^{-1}, respectively, although the EWI increased with starvation at 0.42% day^{-1}. In contrast, fed animals gained an average of 68 g, showing greater DGW and DGI and a lower EWI than the animals starved for 4 or 8 days ($P < 0.05$). Both the lipid concentration and content were lower in muscle (0.39–0.51 g 100 g^{-1}; 0.73–0.89 g ind^{-1}) than in the digestive gland (20.7–22.4 g 100 g^{-1}; 1.4–3.6 g ind^{-1}). No significant trend was observed in muscle tissue for the water or lipid content as a consequence of starvation, whereas in the digestive gland the percentage of water increased significantly with starvation days ($P < 0.05$). However, the TL showed a significantly negative trend with starvation (−0.23 g ind^{-1}day^{-1} of starvation; $P < 0.05$; Table 1).

As regards lipid classes, as many as 15 different bands were detected in the chromatograms, nine corresponding to PL and six to NL (see Fig. 3). In muscle the PL predominated (267.3–337.2 mg 100 g^{-1}) over NL (66.9–104.7 mg 100 g^{-1}), with PC (100.2–120.6 mg 100 g^{-1}), PE (68.7–94.5 mg 100 g^{-1}) and CHO (31.6–62.2 mg 100 g^{-1}) being the major lipid classes, together representing more than 60% of the total lipids detected. The lipid composition of muscle remained constant throughout the starvation period, except for the significant positive tendency observed for the concentrations of SM and MG ($P < 0.05$; Table 2). Furthermore, the concentrations of MG on day 8 and of CHO on day 4 were higher than in the fed animals ($P < 0.05$). In the case of lipids per individual, a positive and significant trend was only observed for MG (2.96 mg ind.$^{-1}$ day^{-1}), while all the others remained constant (Table 3).

Table 2 Concentration of the different lipid classes (mg 100 g^{-1} dry weight) in muscle of *Octopus vulgaris* submitted to different starvation periods

Group	Fed *N* = 4	1 day starved *N* = 4	2 days starved *N* = 4	4 days starved *N* = 4	8 days starved *N* = 4	$y = a + b^{*}$ days starved	
						a	*b*
Polar lipids (PL)							
LPC	ND	14.4 ± 20.4	7.2 ± 6.1	10.3 ± 20.6	ND	14.46	−1.73
SM	4.4 ± 3.6	4.9 ± 1.9	6.5 ± 2.0	6.7 ± 2.9	9.2 ± 3.0	4.74	0.55*
PC	106.9 ± 58.8	120.6 ± 21.0	102.6 ± 33.6	104.0 ± 27.3	100.2 ± 29.3	114.58	−2.06
LPE	10.8 ± 10.9	16.5 ± 8.1	17.2 ± 17.5	23.9 ± 12.8	17.8 ± 0.4	18.21	0.19
PS	22.3 ± 12.1	29.3 ± 3.7	28.4 ± 1.7	33.1 ± 8.9	31.7 ± 6.4	28.96	0.44
PI	18.4 ± 10.0	29.4 ± 4.3	41.5 ± 19.9	40.4 ± 17.4	32.7 ± 6.9	36.61	−0.15
PA	18.6 ± 6.2	23.3 ± 6.4	21.1 ± 6.0	24.2 ± 8.4	16.8 ± 4.1	24.44	−0.82
PE	82.0 ± 44.3	88.9 ± 7.9	94.2 ± 32.9	94.5 ± 26.1	68.7 ± 14.5	98.83	−3.27
SL	3.8 ± 3.1	6.9 ± 4.9	1.9 ± 3.8	ND	2.1 ± 2.3	4.48	−0.46
Neutral lipids (NL)							
MG	10.1 ± 7.9	11.7 ± 0.8	12.0 ± 3.2	17.2 ± 2.4	21.4 ± 4.1†	10.14	1.45*
DG	ND	ND	ND	ND	ND		
CHO	31.6 ± 11.8	58.6 ± 20.2	49.9 ± 12.9	62.2 ± 13.3†	45.1 ± 19.8	59.34	−1.43
FFA	16.4 ± 21.4	13.5 ± 1.5	14.4 ± 4.9	17.4 ± 10.6	15.6 ± 9.5	14.13	0.29
TG	4.5 ± 2.6	3.8 ± 2.7	1.8 ± 2.0	6.1 ± 8.9	5.2 ± 4.1	2.89	0.35
SE	3.4 ± 6.8	2.3 ± 1.6	3.8 ± 3.0	1.8 ± 3.7	7.9 ± 5.6	1.21	0.73
PL (total)	267.3 ± 138.6	334.2 ± 29.5	316.4 ± 95.0	337.2 ± 100.0	280.8 ± 61.4	342.62	−6.79
NL (total)	66.9 ± 48.8	89.9 ± 22.1	81.9 ± 15.1	104.7 ± 22.8	95.1 ± 41.6	87.76	1.38

LPC, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid (include cardiolipin and phosphatidylglycerol); PE, phosphatidylethanolamine; SL, sulpholipids; MG, monoacylglycerols; DG, diacylglycerols; CHO, cholesterol; FFA, free fatty acids; TG, triacylglycerols; SE, steryl esters (include waxes); ND, no detect.

*$P < 0.05$ for the slope of the regression line.

†$P < 0.05$ with respect to fed animals.

The CHO content was significantly higher in the starved animals at day 4 (115.1 mg $ind.^{-1}$) and day 8 (101.1 mg $ind.^{-1}$) than in the fed animals (58.0 mg $ind.^{-1}$).

The NL predominated in the digestive gland (12 958–14 151 mg 100 g^{-1}) compared with the PL (3157–6517 mg 100 g^{-1}), with TG (1799–7952 mg 100 g^{-1}), FFA (2862–5376 mg 100 g^{-1}) and MG (595–2469 mg 100 g^{-1}) being the major lipid classes (Table 4). A positive trend was detected for the PL concentration (428 mg 100 g^{-1} day^{-1}), being significant in the case of SM, PC, PS and PE ($P < 0.05$). No trend was observed for the total NL concentration, although MG showed a positive tendency (190 mg $ind.^{-1}$ day^{-1}) and TG negative (−595 mg $ind.^{-1}$ day^{-1}), being significant in both cases ($P < 0.05$). The TG concentration in animals starved for 8 days was lower and the MG concentration higher than in fed animals ($P < 0.05$; Table 4).

As regards the individual lipid content of the digestive gland, a negative trend was observed for both the PL and NL, although the trend was only significant in the latter (−133.31 mg $ind.^{-1}$ day^{-1}; $P < 0.05$; Table 5). This decrease can be mainly attributed to the significant decrease in TG (−103.67 mg $ind.^{-1}$ day^{-1}). Generally, individuals starved for 8 days showed lower levels of all the lipid classes than the normally fed animals, the difference being significant in the cases of the NL, LPE, PA and TG contents ($P < 0.05$; Table 5).

Discussion

In the present work, the method of HPTLC described previously by Olsen and Henderson (1989) has showed effective for the quantification of the distinct lipid classes when it accompanies of calibration curves elaborated with pure standards. As it observes in the Figs 1 and 2, this method has the advantage of taking into account the different response of the lipid classes in spite of applying similar quantities of lipids. Therefore, this method could comport results more precise for quantitative lipid classes estimations compared

Table 3 Mean content of the different lipid classes (mg ind.$^{-1}$) in muscle tissue of an *Octopus vulgaris* specimen submitted to different starvation periods

Group	Fed $N = 4$	1 day starved $N = 4$	2 days starved $N = 4$	4 days starved $N = 4$	8 days starved $N = 4$	$y = a + b^*$ days starved	
						a	*b*
Polar lipids (PL)							
LPC	ND	21.0 ± 29.6	14.1 ± 13.8	19.8 ± 39.6	ND	23.81	−2.69
SM	8.3 ± 6.7	8.7 ± 4.6	11.9 ± 5.2	12.2 ± 5.6	17.6 ± 8.6	8.26	1.16
PC	196.4 ± 112.2	208.5 ± 65.7	192.6 ± 96.5	192.3 ± 65.0	191.7 ± 90.8	202.52	−1.65
LPE	20.0 ± 20.2	27.2 ± 11.8	30.8 ± 28.9	41.7 ± 19.1	32.3 ± 5.2	30.66	0.64
PS	41.9 ± 23.4	50.4 ± 14.0	51.3 ± 9.2	60.6 ± 20.1	59.7 ± 23.0	50.22	1.41
PI	34.1 ± 19.7	50.1 ± 11.6	79.3 ± 54.0	73.7 ± 33.6	61.5 ± 23.9	65.75	0.11
PA	34.6 ± 13.2	39.4 ± 11.5	39.4 ± 17.3	45.1 ± 19.5	31.8 ± 13.2	42.95	−1.07
PE	151.2 ± 84.8	151.7 ± 33.8	177.3 ± 93.7	175. ± 65.5	129.5 ± 49.3	175.91	−4.62
SL	7.0 ± 6.0	12.4 ± 8.9	4.4 ± 8.8	ND	3.8 ± 4.1	8.49	−0.88
Neutral lipids (NL)							
MG	18.7 ± 14.7	20.0 ± 3.9	22.0 ± 7.5	31.0 ± 3.0	40.2 ± 15.1	17.19	2.96*
DG	ND	ND	ND	ND	ND		
CHO	58.0 ± 23.3	96.7 ± 28.2	92.9 ± 38.8	115.1 ± 35.4†	101.1 ± 30.2†	97.54	1.04
FFA	30.4 ± 39.3	23.1 ± 5.9	26.4 ± 10.4	30.1 ± 14.4	31.4 ± 22.9	23.65	1.09
TG	8.3 ± 4.9	5.8 ± 4.2	2.9 ± 3.3	12.1 ± 17.0	10.8 ± 9.1	4.24	0.97
SE	6.3 ± 12.5	5.2 ± 1.0	9.1 ± 2.7	2.7 ± 5.4	17.9 ± 12.7	1.95	1.71
PL (total)	493.6 ± 266.7	569.4 ± 122.4	593.4 ± 288.8	621.5 ± 227.3	530.8 ± 211.2	603.93	−6.71
NL (total)	123.5 ± 90.9	149.0 ± 29.0	151.0 ± 51.0	191.0 ± 51.2	185.5 ± 104.7	147.93	5.65

LPC, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid (include cardiolipin and phosphatidylglycerol); PE, phosphatidylethanolamine; SL, sulpholipids; MG, monoacylglycerols; DG, diacylglycerols; CHO, cholesterol; FFA, free fatty acids; TG, triacylglycerols; SE, steryl esters (include waxes); ND, no detect.

*$P < 0.05$ for the slope of the regression line.

†$P < 0.05$ with respect to fed animals.

with other studies where percentages from the areas detected in densitometry are showed (i.e. Almansa, Domingues, Sykes, Tejera, Lorenzo & Andrade 2006; García-Garrido *et al.* 2010). Equally, in other studies the preparation of calibration curves for quantitative analysis by means of HPTLC has applied with good results for lipids of the nervous tissue including SM, PC, PE, PI, PS, cerebrosides and SL (Macala, Yu & Ando 1983) or TG of long and medium chain and their products derived of the digestion (Sek, Porter, Kaukonen & Charman 2010).

Weight loss in *O. vulgaris* as a consequence of starvation involves a greater loss of weight in the digestive gland than in the eviscerated fraction, an observation that suggests that there is a greater energetic contribution on the part of the digestive gland during the first few days of starvation. Similarly, O'Dor *et al.* (1984) in *O. vulgaris*, Tait (1986) in *Todarodes japonicus* and Castro *et al.* (1992) in *Sepia officinalis* described how the digestive gland showed faster weight loss rates than the weight animal during starvation.

Variations in the lipid composition of muscle

Our results suggest that lipids present in muscle tissue in *O. vulgaris* make no significant contribution to the maintenance of the energy metabolism during short-term starvation. First, both the lipid concentration and total content were extremely low (<0.51 g 100 g^{-1} and < 0.89 g ind.$^{-1}$), and remained constant during the 8 days of the starvation period applied. These findings coincide with those described for other octopods (Rosa *et al.* 2005). Second, the lipid composition of muscle hardly varied during the starvation period, the PL always predominating over the neutral. As in the studies of Navarro and Villanueva (2000), Almansa *et al.* (2006) and Cerezo Valverde *et al.* (2012), PC, PE, PS and PI were the best represented PL. Besides their merely structural role, these lipids intervene in the regulation of membrane

Table 4 Concentration of the different lipid classes (mg 100 g^{-1} dry weight) in the digestive gland of *Octopus vulgaris* submitted to different starvation periods

Group	Fed $N = 4$	1 day starved $N = 4$	2 days starved $N = 4$	4 days starved $N = 4$	8 days starved $N = 4$	$y = a + b^{*}$ days starved	
						a	*b*
Polar lipids (PL)							
LPC	282.5 ± 114.8	306.9 ± 37.9	218.4 ± 173.1	298.1 ± 178.1	367.1 ± 185.8	244.37	14.21
SM	14.5 ± 29.1	ND	ND	25.0 ± 29.1	40.2 ± 54.8	−6.80	6.16*
PC	1567.0 ± 794.8	819.7 ± 154.8	960.1 ± 268.1	1623.2 ± 668.2	1940.6 ± 580.8	720.37	164.14*
LPE	292.6 ± 71.1	186.3 ± 70.6	263.0 ± 99.5	241.8 ± 147.7	265.6 ± 128.6	210.96	7.53
PS	807.3 ± 286.6	673.4 ± 101.2	856.9 ± 278.9	969.9 ± 515.1	1207.2 ± 473.4	663.16	70.32*
PI	439.3 ± 160.5	399.0 ± 45.3	629.2 ± 362.9	558.8 ± 275.7	683.9 ± 174.1	457.14	29.49
PA	265.2 ± 37.4	161.9 ± 25.9†	194.1 ± 62.7	109.5 ± 43.3†	200.4 ± 149.3	154.24	3.27
PE	965.7 ± 465.5	383.5 ± 178.3	591.5 ± 177.1	700.9 ± 212.9	1357.4 ± 834.7	255.60	134.07*
SL	ND	ND	ND	ND	ND		
Neutral lipids (NL)							
MG	595.6 ± 204.8	1184.2 ± 281.8†	1250.5 ± 631.8	1684.1 ± 621.6†	2469.4 ± 1579.9†	933.43	190.29*
DG	405.9 ± 141.8	690.9 ± 258.4	562.0 ± 355.8	740.2 ± 216.3	1102.9 ± 1469.3	520.95	69.43
CHO	529.6 ± 227.4	666.1 ± 19.2	1186.4 ± 967.4	934.6 ± 426.8	1901.9 ± 1477.5	597.21	153.3
FFA	2862.9 ± 980.2	3635.0 ± 366.9	4813.5 ± 1920.0	3621.7 ± 2471.5	5376.3 ± 5149.5	3665.79	185.56
TG	7952.4 ± 1924.3	5949.8 ± 1252.0	5680.6 ± 927.3	6157.6 ± 5151.1	1799.1 ± 1130.4†	7129.48	−595.39*
SE	645.3 ± 104.9	1068.1 ± 338.8	686.2 ± 283.2	968.3 ± 959.9	919.2 ± 920.8	935.55	−3.42
PL (total)	5116.4 ± 2702.1	3157.7 ± 531.6	4534.7 ± 1270.8	4949.5 ± 2132.1	6516.7 ± 2032.7	3181.6	428.22*
NL (total)	12 958.6 ± 2316.6	13 492.5 ± 1249.7	14 151.4 ± 3896.8	13 239.2 ± 8962.4	13 864.1 ± 10 921.9	13 639.49	12.62

LPC, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid (include cardiolipin and phosphatidylglycerol); PE, phosphatidylethanolamine; SL, sulpholipids; MG, monoacylglycerols; DG, diacylglycerols; CHO, cholesterol; FFA, free fatty acids; TG, triacylglycerols; SE, steryl esters (include waxes); ND, no detect.

*$P < 0.05$ for the slope of the regression line.

†$P < 0.05$ with respect to fed animals.

Table 5 Mean content of the different lipid classes (mg ind^{-1}) in the digestive gland of an *Octopus vulgaris* specimen submitted to different starvation periods

Group	Fed $N = 4$	1 day starved $N = 4$	2 days starved $N = 4$	4 days starved $N = 4$	8 days starved $N = 4$	$y = a + b^*$ days starved	
						a	*b*
Polar lipids (PL)							
LPC	44.5 ± 9.6	43.6 ± 8.7	25.1 ± 20.4	28.8 ± 25.1	24.3 ± 18.1	37.41	−1.85
SM	2.8 ± 0.9	ND	ND	1.4 ± 1.7	3.1 ± 1.9	−0.64	0.48*
PC	259.8 ± 130.6	113.3 ± 8.1	108.5 ± 51.2	149.6 ± 113.1	115.9 ± 42.2	118.13	0.99
LPE	48.4 ± 12.6	27.2 ± 11.6†	29.6 ± 14.1	23.4 ± 20.6	15.6 ± 6.9†	31.04	−1.89
PS	131.8 ± 44.0	96.3 ± 22.7	98.5 ± 55.0	92.6 ± 78.8	76.9 ± 44.9	102.43	−3.03
PI	72.3 ± 27.9	58.6 ± 17.9	71.8 ± 46.3	53.3 ± 45.6	42.1 ± 18.5	68.86	−3.30
PA	120.5 ± 4.3	58.3 ± 5.9†	68.4 ± 40.5	26.4 ± 11.9†	45.5 ± 37.0†	60.10	−2.78
PE	169.3 ± 99.8	50.8 ± 12.6	66.8 ± 31.5	63.7 ± 46.5	75.1 ± 39.4	53.87	2.73
SL	ND	ND	ND	ND	ND		
Neutral lipids (NL)							
MG	95.3 ± 24.2	164.0 ± 23.4†	165.1 ± 116.2	118.1 ± 74.3	168.6 ± 145.2	153.13	0.22
DG	68.4 ± 31.2	108.8 ± 64.2	57.5 ± 29.5	84.2 ± 46.4	85.9 ± 119.8	85.85	−0.47
CHO	84.1 ± 27.8	96.4 ± 23.6	144.8 ± 130.8	83.4 ± 55.1	133.7 ± 131.2	105.41	2.45
FFA	456.5 ± 101.9	536.1 ± 180.7	558.6 ± 321.0	331.3 ± 259.9	391.3 ± 441.9	546.34	−24.53
TG	1341.6 ± 460.8	903.7 ± 402.3	627.8 ± 194.4†	642.2 ± 698.9	104.2 ± 56.7†	958.22	−103.67*
SE	109.4 ± 13.3	143.4 ± 8.4†	80.2 ± 56.01	116.8 ± 159.8	71.5 ± 99.5	132.97	−7.61
PL (total)	843.8 ± 353.2	439.8 ± 42.9	517.7 ± 246.9	460.6 ± 371.4	402.4 ± 187.5	492.94	−10.08
NL (total)	2146.9 ± 480.2	1994.6 ± 668.6	1613.9 ± 762.2	1304.8 ± 905.1	976.6 ± 765.3†	1972.42	−133.31*

LPC, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid (include cardiolipin and phosphatidylglycerol); PE, phosphatidylethanolamine; SL, sulpholipids; MG, monoacylglycerols; DG, diacylglycerols; CHO, cholesterol; FFA, free fatty acids; TG, triacylglycerols; SE, steryl esters (include waxes); ND, no detect.

*$P < 0.05$ for the slope of the regression line.

†$P < 0.05$ with respect to fed animals.

fluidity, transport processes, metabolism and energy production (Tocher, Bendiksen, Campbell & Bell 2008).

Of particular interest at muscular level were the changes observed in MG and CHO during starvation. The former can be considered intermediaries in the process of TG degradation to obtain energy and they gradually increased as starvation progressed. However, no significant quantities of TG or variations in the same were observed in muscle, suggesting that these MG came from a different tissue. At the same time, a higher CHO content was observed in the starved animals than in those fed regularly, suggesting that CHO may also be transported from other tissues and organs. These results led us to think that both TG and CHO may be transported simultaneously through the haemolymph during starvation towards the muscle cells. Earlier, Heras and Pollero (1990, 1992) described the presence of three lipoproteins of different densities (LP-I, LP-II and LP-III) and different lipid composition that might act as transports of lipids between organs in *Octopus tehuelchus*. Most of the CHO and phospholipids are transported by LP-I and LP-II while FFA, SE and TG are transported by LP-III. However, the fact that both MG and TG concentrations remained low in muscle suggests that they were metabolized immediately.

Variations in the lipid composition and content of the digestive gland

The above hypothesis would also explain the gradual decrease in TG levels observed in the digestive gland, and some of the results obtained by other authors as regards the lipid metabolism of cephalopods, among them: (a) improved nutritional efficiency of the diet and protein retention when mixed fish- and crustacean-based diets – moderate in lipids – are provided and compared with an exclusively crustacean-based diet – low in lipids (García García & Cerezo Valverde 2006; Prato, Portacci & Biandolino 2010); (b) the controversy surrounding some species in which digested lipids are not stored in the digestive gland (Semmens 1998; Moltschaniwskyj

& Johnston 2006); (c) the excellent on-growing results obtained for *O. vulgaris* fed *B. boops* from by-catch of fish farm cages despite the high NL and CHO contents (Estefanell, Socorro, Guirao, Fernández-Palacios, Izquierdo & Roo 2010); (d) the generalized presence of CHO in the muscle of cephalopods, and the improved growth observed when their prey contains higher CHO levels (Navarro & Villanueva 2000, 2003; Domingues, Sykes, Sommerfield, Almansa, Lorenzo & Andrade 2004; Almansa *et al.* 2006). Cholesterol is seen as important nutrient at structural level and is involved in the physiological properties of the cell membranes, although it also acts as the precursor of steroidal, hormones, biliary salts and vitamin D (Crockett & Hazel 1997; Kanazawa 2001). However, the diminution of CHO levels in muscle tissue for fed animals suggests its participation in the metabolic processes associated with growth.

In the digestive gland, there was a tendency for concentrate most of the PL, probably because the organ decreased in size. However, the total stored quantity of these lipids remained constant, so that this lipid class does not seem to be mobilized during short periods of starvation. In *S. officinalis*, too, the concentration of phospholipids was seen to increase as the starvation period lengthened (Castro *et al.* 1992), suggesting that their use may be limited to growth phases. The fed animals, on the other hand, showed a capacity to store these lipid classes to levels that doubled the values observed in the starved animals, although the great variability of the data did not permit us to detect differences for most of them and the observations may have been the result of previous feeding (Castro *et al.* 1992; Moltschaniwskyj & Johnston 2006).

Variations were also evident in the total NL, FFA, SE and TG, the content of which tended to decrease with starvation. The mobilization of lipids, TG and SE in this organ has also been demonstrated by other authors (e.g. O'Dor & Wells 1987; Castro *et al.* 1992; Moltschaniwskyj & Johnston 2006; García-Garrido *et al.* 2010). Moltschaniwskyj and Johnston (2006) did not detect increased lipid reserves in the digestive gland despite an increase in the amount of food supplied, which is why they suggested the existence of other lipid storage sites, their possible excretion or immediate mobilization, the last being the most plausible hypothesis accordant to the results obtained in our study. More recently, García-Garrido *et al.* (2010) observed a decrease of the SE and TG in the digestive gland of *O. vulgaris* after 10 and 21 days of starvation respectively. These authors only take into account the concentration values but not the clear changes detected in size. In this sense, if a constant concentration is detected, the total content of SE and TG will be lower in a smaller digestive gland, so an earlier response in this lipid classes should be expected.

Bioenergetic of lipids in *O. vulgaris*

From a bioenergetic point of view, the results obtained point to a significant contribution on the part of lipids to the energy metabolism in octopus. More specifically, the lipids stored in the digestive gland decreased at a rate of 0.23 g day^{-1} of starvation in a 1 kg specimen. From the coefficients proposed by Miglavs and Jobling (1989), 1 g of lipids provides 38.9 kJ, which would imply a caloric contribution of about 9 kJ day^{-1}, mostly from the TG in the digestive gland. In the case of a 1 kg specimen at 18°C (similar experimental conditions to those of the present study), Cerezo Valverde and García García (2004) estimated oxygen consumption to be 2.6 g day^{-1} and an energy cost of 34.7 kJ day^{-1} in routine conditions. In this case, it can be estimated that lipids provided 26% of the daily energy of the animals during short-term starvation, the rest supplied by other sources, e.g. proteins (Lee 1994) or carbohydrates (Morillo-Velarde, Cerezo Valverde, Serra Llinares & García García 2011). In *S. officinalis* lipids provided 39.8% of the energy from the digestive gland during prolonged starvation (Castro *et al.* 1992), reaching 57% during reproductive starvation in *Octopus minus* (Zamora & Olivares 2004).

Therefore, the results of the present study suggest that both TG and CHO may be transported simultaneously through the haemolymph during starvation towards the muscle cells and so, the TG levels were gradual decreasing in the digestive gland with this interesting hypothesis should be checked by lipid metabolism studies. Similarly, the use of formulated diets supplemented with different classes of lipids, especially phospholipids, triglycerides or CHO, could serve to throw light on their role and to confirm that they are used during periods of growth.

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