Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1735 (2005) 185 – 191
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Culture age and carbamoylcholine increase the incorporation of endogenously synthesized linoleic acid in lipids of *Trypanosoma cruzi* epimastigotes

A.L. Villasuso[a], M. Aveldaño[b], A. Vicario[a], E.E. Machado-Domenech[a], M. Garcia de Lema[a,*]

[a]*Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, CP X5804BYA, Río Cuarto, Córdoba, Argentina*
[b]*Instituto de Investigaciones Bioquímicas de Bahía Blanca, CONICET-UNS, 8000, Bahía Blanca, Argentina*

Received 3 December 2004; received in revised form 1 June 2005; accepted 6 June 2005
Available online 28 June 2005

Abstract

Physiological and cellular adaptations to environmental changes are known to be related to modifications in membrane lipids. This work provides metabolic and compositional evidence that *Trypanosoma cruzi* epimastigotes are able to synthesize and desaturate fatty acids, to incorporate them into their lipids, and to modify this incorporation when carbamoylcholine is present in the medium. The fatty acids formed from [2-^{14}C]acetate in the period from 2 to 9 days were mostly (70%) incorporated in phospholipids, the remainder 30% being recovered in neutral lipids, such as triacylglycerols (TAG) and diacylglycerols (DAG). The main fatty acids formed from [2-^{14}C]acetate were saturates (16:0, 18:0), monoenes (16:1, 18:1) and dienes (mostly 18:2). The ratios between labelled unsaturated and saturated fatty acids increased continuously with growth, consistent with a precursor–product relationship between the main fatty acids, and with the occurrence in *T. cruzi* of Δ^9- and Δ^{12}-desaturases. From days 2 to 5, [^{14}C]18:2 was the main fatty acid produced. Accordingly, the fatty acid profiles showed a significant increase in the percentage of 18:2 in all lipids in the period under study, especially in the first 2 to 5 days. In the presence of carbamoylcholine, the labelling of DAG and TAG with [^{14}C]18:2 augmented. The results indicate that *T cruzi* is able to synthesize the main types of fatty acids required to form its membrane lipids, and to exchange them actively in response to environmental stimuli.

1. Introduction

Since unicellular organisms as protozoan parasites are frequently exposed to unpredictable changes in their environment, they must display a multiplicity of biochemical mechanisms in order to survive [1,2]. Among these, many physiological and cellular responses to environmental changes occur concurrently with modifications in membrane lipids. For example, the capacity of *Histoplasma capsulatum* to undergo a stress response is determined by the saturated/unsaturated fatty acid ratio in the lipid of their membranes [3]. In both, *Trypanosoma brucei* [4] and *Dictyostelium discoideum* [5], calcium entry is regulated by unsaturated fatty acids and phospholipase A_2 activity.

Trypanosoma cruzi, a flagellated protozoan that is the agent of Chagas' disease, has a life cycle that includes a stage in the gut of a blood-sucking reduviid host insect, where epimastigote forms are able to differentiate into metacyclic trypomastigotes, the forms that are eventually able to infect mammalian hosts. During their transit through the host gut, *T. cruzi* epimastigotes face an environment with variable temperature, osmolality, pH, and availability of nutrients [6]. Similarly, during growth in culture medium *T. cruzi* must cope with an osmolality increase between days 2 and 9, a pH decrease between days 2 and 5 and a pH increase between days 5 and 9 [7]. Previous work from our laboratory suggests that some of these changes, particularly the increase in the osmolality of the medium, may be a factor promoting the differentiation of epimas-

* Corresponding author. Tel./fax: +54 358 4676232.
E-mail address: mgarcia@exa.unrc.edu.ar (M. Garcia de Lema).

1388-1981/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2005.06.004

tigotes to trypomastigotes (Santander V. et al, unpublished observations).

Furthermore, we demonstrated that epimastigotes of *T. cruzi* are able to incorporate [1-^{14}C]acetate into fatty acids of total lipids and different lipid fractions [8,9]. This organism is also able to cope with some environmental changes by altering the fatty acids of some of their lipids. For example, when the concentration of fetal bovine serum (FBS) in the culture medium was reduced from 10% to 5%, the percentage of linoleic acid (18:2) in phosphoinositides decreased while that of palmitic and palmitoleic acid increased [10]. Changes in *T. cruzi* lipid composition occur during aging and in response to temperature variations [11,12]. Metabolic changes occurring in the parasite after cholinergic stimulation have been reported. *T. cruzi* is able to respond to cholinergic stimulation inducing significant changes in the inositol cycle and calcium signaling [13–15] and is also able to bind nicotinic ligands and carbamoylcholine [16] from the medium. A question raised by all these findings was how charbamoylcholine could affect the fatty acids of *T. cruzi* lipids.

By using [2-^{14}C]acetate, in the present work we followed the labeling of the fatty acids acyl moieties of lipids in *T. cruzi* epimastigotes cultured under control and stimulated conditions. The results show that the parasite is able to perform an active synthesis of saturated, monounsaturated, and diunsaturated fatty acids, which are efficiently incorporated into phospholipids (PL) and neutral lipids (NL). 18:2, the major polyunsaturated component formed, varies during parasite growth in culture in a way that is consistent with the modifications observed with the fatty acids formed with ^{14}C-acetate as precursor. The muscarinic agonist evokes changes in the labelling of 18:2 of DAG and TAG.

2. Materials and methods

2.1. Organism and growth conditions

The Tulahuen strain of *Trypanosoma cruzi* was used in this study. The epimastigote forms were grown at 28 °C in a modified Warren medium as described by [17]. Ten percent of FBS and 1,000,000 U of penicillin per 4×10^7 parasites or cells/ml were used. After growth, the weight of the harvested cells and the number of the mobile cells per ml of culture medium were measured.

2.2. Incorporation of labelled acetate

Previously sterilized [2-^{14}C]acetate, sodium salt (43 mCi/mmol), New England Nuclear, was added to the media (0.4 μCi/ml). The growth was allowed to proceed for 2, 5, and 9 days at 28 °C. Cells were harvested at the indicated times by centrifugation at $6000\times g$ for 10 min and washed three times with KRT buffer, as previously described [17]. The same procedures and conditions were used for unlabelled samples.

2.3. Stimulation conditions

In order to determine the effects of carbamoylcholine, three bottles incubated with [2-^{14}C]acetate at the time of inoculation were used. One of them, with no additions, was used as control, the second one was incubated in the presence of carbamoylcholine (2×10^{-5} M) and the third one contained carbamoylcholine (2×10^{-5} M) plus atropine (1×0^{-4} M). Solutions of the above mentioned drugs were sterilized in autoclave for 10 min at 1 atm before being added to the media.

2.4. Lipid extraction

Total lipids were extracted from the washed parasites according to the acidified extraction procedure of Bligh and Dyer [18], 0.1 M KCl in 50% methanol was added to obtain a lower chloroform phase and an upper phase. The lower phase, containing the lipids, was washed once with the KCl solution, dried under N_2 and dissolved in a suitable volume of chloroform/methanol 2:1 (v/v).

2.5. Processing of radioactive samples

2.5.1. Separation and analysis of phospholipids and neutral lipids

Aliquots of the total lipid extracts were subjected to thin-layer chromatography to separate the total phospholipid fraction from the neutral lipids, using hexane/ethyl ether/acetic acid 80:20:1 (v/v/v) as solvent. After TLC, the lipids were located by exposing the plates to iodine vapours when destined to radioactivity analysis or by exposing the plates to UV light after spraying them with 2,7′dichlorofluoresce in methanol when destined to further analysis of fatty acids.

2.5.2. Separation and analysis of labelled fatty acids from lipids

Fatty acid methyl esters (FAME) were prepared from the lipid fractions separated by TLC with 10% BF_3 in methanol [19]. The labelled FAME were resolved according to their number of double bonds on TLC plates of silica gel G impregnated with $AgNO_3$ (4%, w/w) using toluene as solvent. The FAME bands were located under UV light after spraying the plates with dichlorofluorescein, eluted [20] and taken to dryness in counting vials. Radioactivity was measured in a liquid scintillation counter (Beckman LS 60001 C).

2.6. Processing of non radioactive samples

2.6.1. Separation of neutral lipids and phospholipids

Aliquots of the extracts were taken for the determination of total lipid phosphorus [21] and for the separation of lipid

classes for fatty acid analysis. Neutral lipids were resolved from PL using the TLC procedure just described for labelled samples. Phospholipids were subjected to TLC [22] to separate the choline and ethanolamine glycerophospholipids. All lipids were eluted from the silica support with chloroform/methanol/acetic acid/water 50:39:1:10 (v/v/v/v) [23].

2.6.2. Analysis of lipid fatty acids

Aliquots of lipid extracts (total lipid), as well as some of the lipid classes separated by TLC, were taken to dryness and subjected to methanolysis as described for labelled samples in order to prepare FAME by means of BF_3 in methanol. After adding N_2, the tubes were closed and placed overnight at 45 °C. The resulting FAME were purified by TLC using hexane/diethyl ether 95:5 (v/v), on plates of silica gel G that had been previously washed with methanol/diethyl ether 75:25 (v/v). FAME were recovered from the silica support by agitation with water/methanol/hexane 1:1:1 (v/v/v), followed by centrifugation, repeating the hexane extraction 3 times. Fatty acid analysis was performed using a Varian 3700 gas chromatograph equipped with two (2 m×2 mm) glass columns packed with 15% SP 2330 on Chromosorb WAW 100/120 (Supelco Inc., Bellefonte, PA) and two flame ionization detectors. The column oven temperature was programmed from 155 °C to 230 °C at a rate of 5 °C/min. Injector and detector temperatures were 220 °C and 230 °C, respectively; the carrier gas was N_2 (30 ml/min).

2.7. Materials

All solvents were of analytical or HPLC grade. Lipid standards were obtained from Sigma Chemical Co. The culture medium used was from Merck or from Difco. Fetal bovine serum was from Natocor (Argentina).

3. Results

3.1. Incorporation of [2-^{14}C]acetate in T. cruzi lipids

Fig. 1 shows that the incorporation of [2-^{14}C]acetate into the total lipid of *T. cruzi* increased continuously throughout the incubation interval studied. The precursor was incorporated mostly (60–70%) into phospholipids (PL), the rest being recovered in the neutral lipid (NL) fraction. Most of the radioactivity incorporated was in fatty acids esterified to lipids. Among the components of the NL fraction, those most actively labelled were the free sterols (FS), while an important proportion of label was also present in steryl esters (SE). The incorporation of [2-^{14}C]acetate into PL and FS markedly augmented with growth between days 2 and 5. The labelling of DAG, TAG and free fatty acids (FFA) tended to increase in the last stage studied (day 9). The total amount of label in lipids increased nearly twice (an average of 85%) in the 5–9 day period, even though the distribution of label among lipids did not change significantly (data not shown).

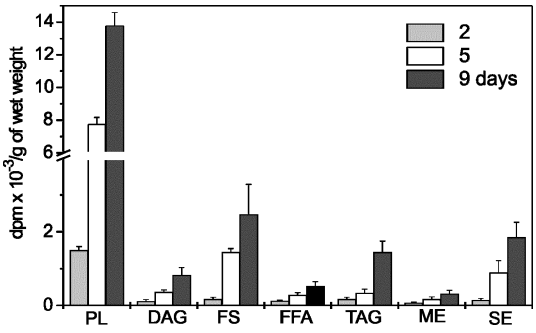

Fig. 1. Incorporation of [2-^{14}C]acetate into lipids during growth of *T. cruzi* epimastigote forms. The parasites were incubated with [2-^{14}C]acetate (0.4 Ci ml^{-1}, added at inoculation time) and harvested after the indicated days. Lipids were extracted and separated by TLC. Radioactivity was measured by Liquid Scintillation. Results are expressed as dpm×10^{-3}/g of wet weight. Values represent means±S.E.M. of three independent experiments. PL; phospholipids, DAG: diacylglycerols, TAG: triacylglycerols, FS: free sterols, FFA: free fatty acids, ME: methyl esters, SE: sterol esters.

3.2. Labelling of lipid fatty acids with [2-^{14}C] acetate

Fig. 2 depicts the incorporation of [2-^{14}C]acetate into the fatty acids of DAG, TAG and PL of *T. cruzi* epimastigotes during the 2–9 days growth period studied. In the three lipids examined, there were significant changes with growth time in the distribution of the label among saturated, monounsaturated and dienoic fatty acids, especially between days 2 and 5, but in all of them the labelled dienes predominated at day 9.

In DAG (Fig. 2A and B), monoenes predominated at day 2 while at day 5 diunsaturated fatty acids became more important and they were the major fatty acids at day 9. In TAG (Fig. 2C and D), monoenes predominated at day 2, dienes at day 5 and saturated fatty acids at day 9. In the major PL fraction (Fig. 2E and F), the saturates were the main fatty acids present at day 2, their percentage decreasing with time due to a several fold increase in the proportion of mono and diunsaturated fatty acids.

Although to different extents, the ratio between diunsaturaded and monounsaturated fatty acids in the tested period increased in all three lipids: DAG (0.3–2.1), TAG (0.7 to 1.1), and PL (0.5–3.1).

3.3. Effect of carbamoylcholine on the incorporation of [2-C^{14}]acetate in fatty acids of specific lipids

The effects of carbamoylcholine on the labelling of the fatty acids of DAG and TAG are presented in Fig. 3. While the total radioactivity incorporated into total NL and PL was not significantly altered (data not shown), the presence of the agonist resulted in a significant increase in the percentage of diunsaturated fatty acids incorporated into

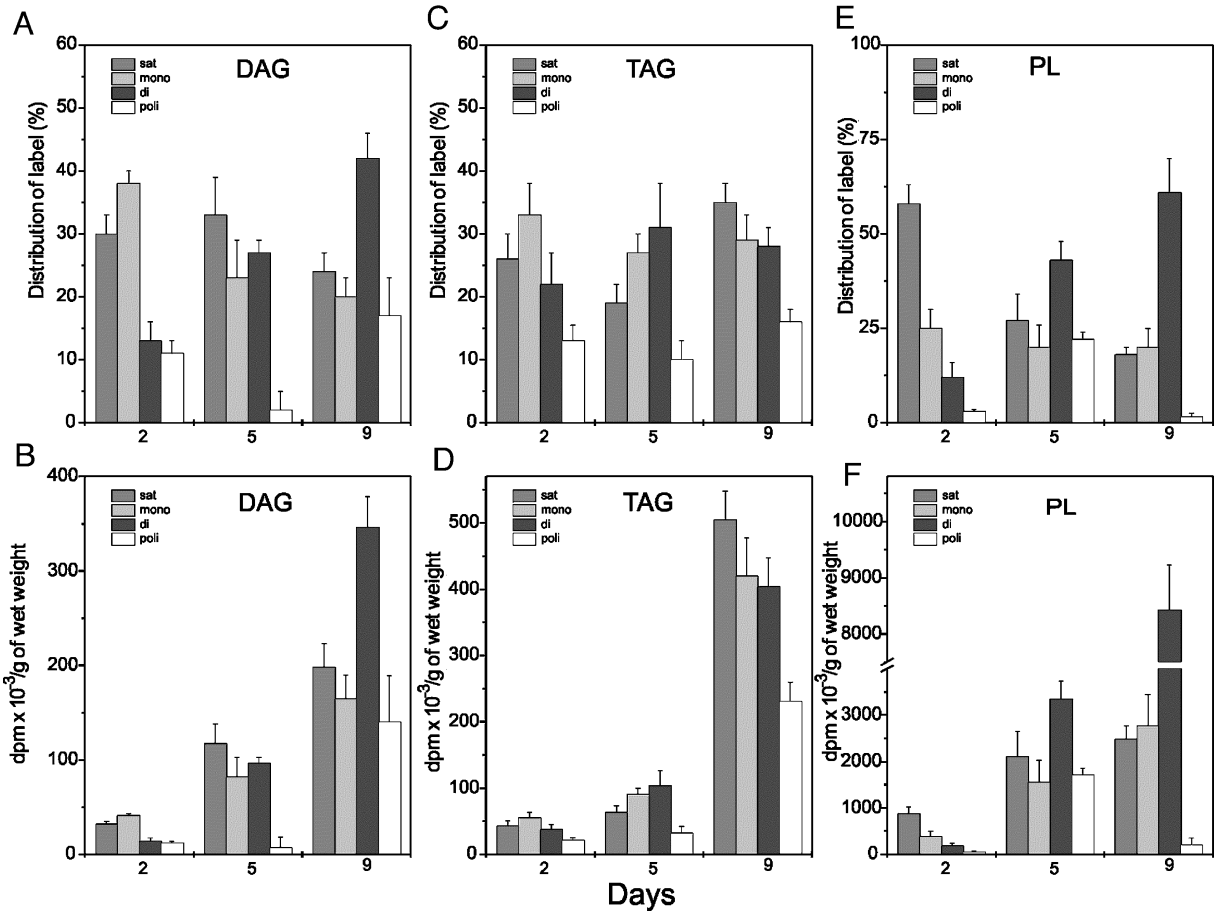

Fig. 2. Incorporation of [2-^{14}C]acetate in fatty acids of *T. cruzi* lipids. After the indicated days in culture, lipids were extracted from epimastigotes and separated by TLC. Fatty acid methyl esters were prepared from DAG (A and B), TAG (C and D) and PL (E and F) and separated according to unsaturation degree by using TLC plates impregnated with 4% $AgNO_3$. Results are expressed as percentage of the total radioactivity incorporated in each of the fatty acid fractions of each lipid (upper panels) and as dpm$\times 10^{-3}$/g of wet weight (lower panels). Values represent the means ± S.E.M. of three independent experiments.

DAG (Fig. 3A) (62% higher than that of controls) and surprisingly, also in TAG (Fig. 3B) (42% higher).

3.4. Changes in lipid and fatty acid composition associated with growth

Consistent with the results from [^{14}C]acetate labelling, the most significant change associated with epimastigote growth was a sustained increase in the total amount of lipids, particularly of PL. There was a significant increase with time in the content of PL, FS, SE, TG and ME per gram of wet weight between days 2 and 5. In the period between days 5 and 9, the rate of PL and FS accumulation decreased, whereas that of TAG and SE did not. This produced a relative increase in the percentage of the latter lipids at day 9 (Table 1).

In all lipids examined, but especially in the major PL classes, there was a sustained increase in the content of the main unsaturated fatty acids, 18:1 and 18:2 with growth (Table 2). The total unsaturated to total saturated fatty acid ratio tended to increase in all lipids examined throughout the incubation period. It is remarkable that PC showed higher percentages of 22:6n−3, 20:4n−6 and 22:5n−6 at day 2 than at days 5 and 9. These fatty acids, probably derived from FBS included in the incubation medium, became minor components as the parasite was able to synthesize its own lipids. Taking into account their amount (45–50% of the PL), the choline glycerophospholipids (CGP) were the components showing the largest and most rapid increase in 18:2.

The fatty acids of DAG and TAG also showed proportions and modifications in their fatty acids with time that were consistent with their labelling with [^{14}C]acetate. In both, the proportion of monoenoic and saturated fatty acids was much larger than the dienoic fatty acids at day 2. Dienoic fatty acid increased at day 5 in DAG and at day 9 in TAG.

4. Discussion

The present results show that the generation of linoleic acid-containing lipids is concomitant with *T. cruzi* growth. Metabolic and compositional data coincide in showing that

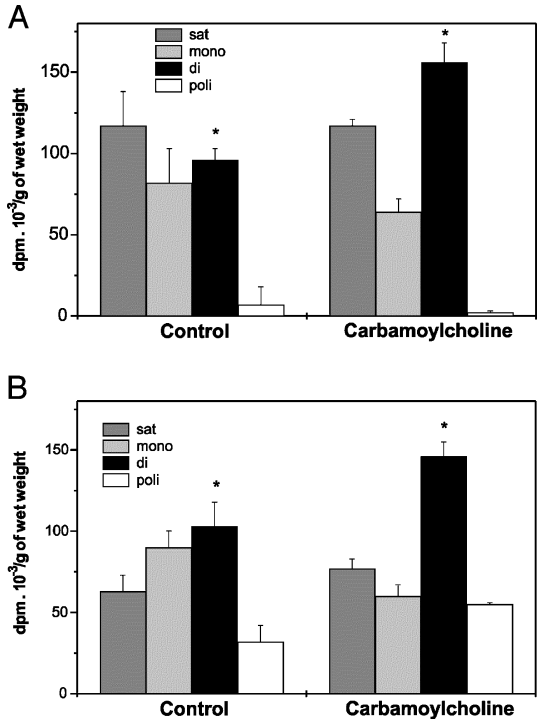

Fig. 3. Effects of carbamoylcholine on the incorporation of [2-^{14}C]acetate into fatty acids of *T. cruzi* DAG and TAG. Parasites were incubated for 5 days with [2-^{14}C]acetate (0.4 Ci ml^{-1}) in the presence or absence of carbamoylcholine (2×10^{-5} M, added from inoculation time). Lipids were extracted and separated by TLC. Fatty acids methyl esters of DAG (A) and TAG (B) were prepared and separated on TLC plates containing $AgNO_3$. Results are expressed as dpm$\times10^{-3}$/g of wet weight and represent the means ± S.E.M. of three independent experiments $^*P<0.05$.

fatty acids are not only synthesized de novo, but also actively desaturated in epimastigotes in culture. In agreement with previous results [8,9], we show that epimastigote forms of *T. cruzi* are able to incorporate [^{14}C]acetate into fatty acids that are mostly incorporated as acyl groups of PL. Both the synthesis of fatty acids and their esterification into lipids were most active in the logarithmic phase of growth (2–5 days), the rate of both processes slowing down during the stationary phase (5–9 days).

The time course of the production of saturated, monoenoic and dienoic fatty acid production, and the changes in their ratios, are consistent with the interpretation that palmitic and stearic acids are synthesized from acetate by fatty acid synthase (FAS) and then they serve as substrates for at least two desaturases [9,24,25]. Monounsaturated fatty acids are indeed produced from [1-^{14}C]palmitic and [1-^{14}C]stearic acids by a microsomal fraction isolated from the parasite after 5 days in culture [25]. Thus, the products of FAS could be substrates for a Δ^9 desaturase, and at least one of their products, 18:1, could in turn be the substrate for a second desaturase, identified as Δ^{12} desaturase [26] to produce 18:2. The synthesis of this major fatty acid requires 18:1 to be available, the present results indicating that both desaturases were more actively expressed at day 5 than at day 2 in culture. Therefore, variations in the ratio between linoleic and oleic acids are likely to play a major role in the response of the parasite to environmental conditions.

The time-course of the labelling of saturated, monoenoic, and dienoic fatty acids of TAG and PL suggests that part of the fatty acids synthesized early and stored in TAG could in part have been transferred to PL. Besides providing fatty acids as energetic fuels, one of the functions of TAG could be to serve as a reservoir of polyunsaturated fatty acids, providing them to PL when required to sustain the active biosynthesis of membrane PL that is associated with *T. cruzi* development in culture. The possible relationship between PL and TAG through fatty acids is strengthened by the observation [11] that in the stationary phase of growth when the rate of PL biosynthesis decreased, TAG started to accumulate. Moreover, the present observation that the proportion of TAG carrying [^{14}C]18:2 is increased by carbamoylcholine supports the idea that this lipid class could have a more dynamic involvement in cellular processes than it is usually thought. In relation to this, Lerique et al. [27] proposed that a small proportion of the total TAG of cells could be involved in physiological and pharmacological modifications, and even in cell transformation and metastatic processes.

Table 1
Lipids of *Trypanosoma cruzi* epimastigotes during growth in culture

Time (days)	mg lipid/g wet wt			%			μmol/g wet wt			mw[a]
	2	5	9	2	5	9	2	5	9	
Steryl esters	0.4	2.0	2.1	11.2	11.7	13.4	0.6	3.1	3.2	643
Methyl esters	0.1	0.5	0.8	2.1	3.1	5.3	0.3	1.8	2.7	297
Triacylglycerols	0.1	0.2	0.7	2.2	1.4	4.9	0.1	0.3	0.8	890
Free fatty acids	0.3	0.4	0.3	7.8	2.4	2.0	1.0	1.5	1.1	283
Diacylglycerols	0.1	0.4	0.3	1.5	2.1	1.9	0.1	0.6	0.5	601
Free sterols	0.3	1.6	0.7	7.0	9.5	4.4	0.7	4.6	1.9	360
Phospholipids (PL)	2.5	12.1	10.4	68.3	69.8	68.1	3.3	15.6	13.4	775
Totals	3.6	17.3	15.3	100	100	100	5.8	27.1	23.4	

T. cruzi epimastigotes were harvested after the specified periods in culture and lipid extracts were prepared. Days 2, 5 and 9 correspond to lag, logarithmic and stationary stages, respectively. Lipid classes were resolved by TLC and quantified as described in Materials and methods. Results are from one experiment, representative of three.

[a] Average molecular weights used for estimating the μmol/g of each lipid.

Table 2
Fatty acid composition of lipid classes from epimastigotes of *Trypanosoma cruzi* at different growth periods

Time (days)	Steryl esters			Triacylglycerols			Diacylglycerols			Ethanolamine glycerophospholipids			Choline glycerophospholipids			Total lipids		
	2	5	9	2	5	9	2	5	9	2	5	9	2	5	9	2	5	9
14:0	3.4	0.8	1.0	1.6	1.7	0.4	1.0	0.3	0.1	0.5	1.6	0.4	0.7	1.8	0.7	1.3	1.7	0.8
15:0	2.1	1.0	1.0	0.4	0.6	0.2	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16:0	29.9	28.5	26.0	27.1	24.1	14.5	21.3	10.8	5.8	18.3	19.8	14.3	9.4	10.6	7.2	15.4	14.7	14.7
16:1	15.9	10.0	11.0	7.4	6.9	4.4	7.7	3.1	2.0	2.3	2.0	1.6	4.9	7.7	3.6	4.7	4.5	3.3
17:0	1.3	0.4	0.4	1.7	1.0	0.5	1.3	0.4	0.9	1.0	0.3	0.5	0.7	0.2	0.2	0.8	0.2	0.3
17:1	0.8	0.3	0.4	1.4	0.8	0.6	1.2	0.7	1.1	1.0	0.4	0.3	0.1	0.1	0.1	0.1	0.1	0.1
18:0	9.6	2.0	2.5	16.5	12.8	9.8	15.0	12.9	13.3	12.6	11.1	16.1	6.1	4.1	5.1	10.5	8.1	9.6
18:1	21.3	30.7	27.8	21.5	24.6	23.9	25.0	19.0	26.0	28.4	21.9	33.1	12.3	11.8	18.4	18.1	16.2	24.3
18:2	8.6	12.0	17.4	14.6	21.6	41.0	21.4	39.1	40.1	31.3	40.5	31.3	38.9	56.9	58.2	31.0	46.6	40.7
18:3n−3	0.2	0.2	0.3	0.8	0.7	0.6	0.7	0.6	0.6	0.4	0.5	0.4	0.5	0.5	0.5	0.4	0.5	0.5
20:3n−6	0.3	1.2	1.1	0.8	0.3	0.3	0.3	0.5	0.4	0.6	0.3	0.2	2.4	0.8	0.8	1.1	0.6	0.4
20:4n−6	1.9	9.3	7.9	2.7	0.8	0.5	1.5	0.3	0.2	0.6	0.1	0.1	6.3	0.7	0.4	3.8	1.1	0.9
22:4n−6	1.5	0.2	0.2	0.7	0.6	0.3	0.8	1.1	1.2	0.2	0.1	0.1	0.3	0.1	0.1	0.4	0.3	0.5
22:5n−6	1.7	0.1	0.2	0.8	0.9	0.5	1.0	4.3	3.1	0.3	0.1	0.2	3.4	1.2	1.0	1.9	1.1	0.9
22:5n−3	–[a]	0.3	0.2	0.4	0.1	0.1	0.2	0.2	0.1	0.1	–	–	0.2	0.1	0.2	0.1	0.1	0.1
22:6n−3	0.1	1.7	1.4	1.1	1.4	0.8	0.1	5.3	4.3	0.7	0.2	0.3	10.3	2.5	2.1	6.4	2.1	1.5
Others[b]	1.3	1.2	1.3	0.8	0.9	1.6	1.3	1.3	0.7	1.5	1.1	0.9	3.5	1.0	1.2	4.0	1.9	1.4
NS/S[c]	1.1	2.1	2.2	1.1	1.5	2.9	1.5	3.0	3.9	2.0	2.0	2.2	4.8	5.0	6.4	2.5	3.0	2.9

T. cruzi epimastigotes grown for the specified times were harvested and lipids were extracted. The fatty acids of lipid classes resolved by TLC were converted to methyl esters and analyzed by GC. The figures represent the results of one representative of three experiments, expressed as wt.%.

[a] Percentage values lower than 0.05% are not depicted.

[b] Others: Sum of all fatty acids not shown in this table.

[c] NS/S: Ratio between the sum of unsaturated and the sum of saturated fatty acids.

The present results also show that the proportion of DAG containing [^{14}C]18:2 increased due to carbamoylcholine stimulation. This increase may result from: (1) the release of labelled DAG from lipids formed more actively under the effect of the agonist, (2) the stimulation of PL biosynthesis by the agonist, accelerating the formation of DAG, or (3) the reduced utilization of labelled DAG in the formation of other lipids, like PC or TAG. However, the latter can be ruled out in view of the fact that TAG as DAG was more actively labelled in the presence than in the absence of carbamoylcholine. We favour the first possibility since carbamoylcholine has been shown to stimulate the formation of polyphosphoinositides and phosphatidic acid from [^{32}P] Pi in *T. cruzi* [14], as well as the release of inositol phosphates from these lipids [14,15], through the stimulation of a PIP_2-specific phospholipase C. This phospholipase could be responsible for the generation of the [^{14}C]18:2 containing DAG observed in this work. DAG might act as an effective activator of PKC in *T. cruzi* [28], as is the case in mammalian cells. The unsaturation of the fatty acids bound to DAG is an important factor in determining the potency of the latter as PKC activators [29]. In previous work, it was shown that the polyphosphoinositides of *T. cruzi* epimastigotes become richer in 18:2 when the culture medium is supplemented with 10% of fetal bovine serum [10]. Interestingly, when the FBS concentration was lower than 10%, the proportion of 18:2 in the parasite phosphoinositides was lower and the usual response to carbamoylcholine (increased labelling of PPI and PA) was not observed. Even when these phospholipids were not measured in the present work, it is likely that [^{14}C]18:2 was as actively incorporated into polyphosphoinositides as it was into the major PL.

Since arachidonic acid (20:4) is not a major component of *T. cruzi* lipids, and since 18:2 not only becomes the major fatty acid of lipids with development but is also a fatty acid increased in DAG by carbamoylcholine, our results support the possibility that in *T. cruzi* 18:2 could play an equivalent role to that played by 20:4 in the signalling processes of mammalian cells.

Experiments are in progress in our laboratory in order to characterize *T. cruzi* desaturases and their prospective modulators. The fact that successful development of *T. cruzi* requires the synthesis of unsaturated fatty acids like 18:2 turns the parasite's desaturases into promising targets for chemotherapeutic drugs.

Acknowledgements

This work was supported by CONICET, FONCyT, Argentina and SECyT, UNRC, Río Cuarto, Córdoba, Argentina. We thank the contribution of language consultant Professor Iliana A. Martínez.

References

[1] W.R. Fish, Lipid and membrane metabolism of the Malaria parasite and the African Trypanosome, in: J.J. Marr, M. Müller (Eds.),

Biochemistry and Molecular Biology of parasites, Academic Press, New York, 1998, pp. 133–145.
[2] T. Kasai, T. Watanabe, H. Fucuchima, H. Lida, Y. Nozawa, Adaptative modification of membrane lipids in *Tetrahymena pyriformis* during starvation, Biochem. Biophys. Acta 666 (1981) 36–46.
[3] B. Maresca, G.S. Kobayashi, Changes in membrane fluidity modulates heat shock gene expression and produce attenuated strains in the dimorphic fungus *Histoplasma capsulatum*, Arch. Med. Res. 24 (1993) 247–249.
[4] J. Eintracht, R. Maathai, A. Mellors, L. Ruben, Calcium entry in *Trypanosoma brucei* is regulated by phospholipase A_2 and arachidonic acid, Biochem. J. 336 (1998) 659–666.
[5] R. Schaloske, J. Sonnemann, D. Malchow, C. Schlatterer, Fatty acids induce release of Ca^{2+} from acidosomal stores and activate capacitative Ca^{2+} entry in *Dictyostelium discoideum*, Biochem. J. 332 (1998) 541–548.
[6] A.H. Kollien, G.A. Schaub, *Trypanosoma cruzi* development in the vector, Parasitol. Today 16 (9) (2000) 381–387.
[7] V. Santander, M. Bollo, C. Pistelli, E.E. Machado, Changes in phospholipids induced by saline stress in *Trypanosoma cruzi*, Biocell 25 (Supp. II) (2001) C52.
[8] E.E. Aeberhard, E.R. Moretti, B. Basso, M.G. Lema, D.I.H. Bronia, Biosíntesis de lípidos en *Trypanosoma cruzi*, Medicina (Buenos Aires) 40 (Supl. 1) (1980) 165–170.
[9] E.E. Aeberhard, M.B.G. de Lema, D.H. Bronia, Biosynthesis of fatty acids by *Trypanosoma cruzi*, Lipids 16 (1981) 623–625.
[10] G. Racagni, M.G. de Lema, G. Hernández, E.E. Machado-Domenech, Fetal bovine serum induces changes in fatty acid composition of *Trypanosoma cruzi*: phosphoinositides, Can. J. Microbiol. 41 (1995) 951–954.
[11] D.H. Bronia, A.M. Aguerri, S.T. Bertetto, *Trypanosoma cruzi*: changes in lipid composition during aging in culture, Exp. Parasitol. 61 (1986) 151–159.
[12] M. Florin-Christensen, J. Florin-Christensen, E.D. de Isola, E. Lammel, E. Meinardi, R.R. Brenner, L. Rasmussen, Temperature acclimation of *Trypanosoma cruzi* epimastigote and metacyclic trypomastigote lipids, Mol. Biochem. Parasitol. 88 (1997) 25–33.
[13] E.E. Machado de Domenech, M.B. García, M.N. Garrido, G.E. Racagni, Phospholipid of *Trypanosoma cruzi:* increase of polyphosphoinositides and phosphatidic acid after cholinergic stimulation, FEMS Microbiol. Lett. 95 (1992) 267–270.
[14] M.N. Garrido, M.I. Bollo, E.E. Machado-Domenech, Carbamoylcholine stimulates inositol phosphate formation transiently in *Trypanosoma cruzi*, Cell. Mol. Biol. 42 (1996) 221–225.
[15] N. Marchesini, M. Bollo, G. Hernández, M.N. Garrido, E.E. Machado-Domenech, Cellular signalling in *Trypanosoma cruzi*: biphasic behaviour of inositol phosphate cycle components evoked by carbamoylcholine, Mol. Biochem. Parasitol. 120 (2002) 83–91.
[16] M. Bollo, G. Venera de Jimenez, M.B. Bonino, E. Machado-Domenech, Binding of nicotinic ligands to and nicotine-induced calcium signaling in *Trypanosoma cruzi*, Biochem. Biophys. Res. Commun. 281 (2) (2001) 300–304.
[17] G. Racagni, M. García de Lema, C.E. Domenech, E.E. Machado de Domenech, Phospholipids in *Trypanosoma cruzi*: phosphoinositide composition and turnover, Lipids 27 (1992) 275–278.
[18] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–918.
[19] W.R. Morrison, L.M. Smith, Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride, J. Lipid Res. 5 (1964) 600–608.
[20] R. Wilson, J.R. Sargent, High-resolution separation of polyunsaturated fatty acids by argentation thin-layer chromatography, J. Chromatogr. 623 (1992) 403–407.
[21] G. Rouser, S. Fleischer, A. Yamamoto, Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots, Lipids 5 (1970) 494–496.
[22] B.J. Holub, C.M. Skeaff, Nutritional regulation of cellular phosphatidylinositol, in: P.M. Conn, A.R. Means (Eds.), Meth. Enzymol., 141, Academic Press Inc., New York, 1987, pp. 234–422.
[23] G.A.E. Arvidson, Structural and metabolic heterogeneity of rat liver glycerophosphatides, Eur. J. Biochem. 4 (1968) 478–486.
[24] M. García de Lema, G. Lucchesi, G. Racagni, E. Machado-Domenech, Changes in enzymatic activities involved in glucose metabolism by Acyl-CoAs in *Trypanosoma cruzi*, Can. J. Microbiol. 47 (2001) 49–54.
[25] M. García, P. Moyano, M. Woelke, E. Machado-Domenech, Possible role of palmitic acid in *T. cruzi* environmental adaptation, Biocell 25 (Supplement II) (2001) 122.
[26] M.G. de Lema, E.E. Aeberhard, Desaturation of fatty acids in *Trypanosoma cruzi*, Lipids 21 (11) (1986) 718–720.
[27] B. Lerique, J. Lepetit-Thévenin, A. Vérine, C. Delpéro, J. Boyer, Triacylglycerol in biomembranes, Life Sci. 54 (13) (1994) 831–840.
[28] M. Gómez, L. Erijman, S. Arauzo, H. Torres, y M. Téllez-Iñón, Protein Kinase C in *Trypanosoma cruzi* epimastigote forms: partial purification and characterization, Mol. Biochem. Parasitol. 36 (1989) 101–108.
[29] Y. Nishizuka, Protein kinase C and lipids signaling for sustained cellular responses, FASEB J. 290 (1995) 327–333.