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To cite this version:

Mayke Bezerra Alencar, Richard Marcel Bruno Moreira Girard, Marcell Crispim, Carlos Gustavo Baptista, Marc Biran, et al.. Elucidating the Transport Mechanisms and Metabolic Roles of Serine, Threonine, and Glycine in Trypanosoma cruzi. 2024. hal-04765838

HAL Id: hal-04765838 <https://hal.science/hal-04765838v1>

Preprint submitted on 5 Nov 2024

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Elucidating the Transport Mechanisms and Metabolic Roles of Serine, Threonine, and Glycine

in Trypanosoma cruzi

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Declarations of interest: none

ABSTRACT

Letting (L-C), and L-Micromia (L-Mi), much contained contained in the biosynthesis pathways of other
amino acids and even phospholipids. Furthermore, L-Ser and L-Thr can be substrates for a
Ser/Thr dehydratase (Ser/ThrDH), amino acids and even phospholipids. Furthermore, L-Ser and L-Thr can be substrates for a
Ser/Thr dehydratase (Ser/ThrDH), resulting in pyruvate (Pyr) and 2-oxobutyrate, respectively,
thus being amino acids with anaplerotic Ser/Thr dehydratase (Ser/ThrDH), resulting in pyruvate (Pyr) and 2-oxobutyrate, respectively, thus being amino acids with anaplerotic potential. Trypanosoma cruzi, the etiological agent of Chagas disease, uses amino acids Service and particle (Service 1, 1) is also provided (Pyr) and 2-oxodogical agent of
thus being amino acids with anaplerotic potential. Trypanosoma cruzi, the etiological agent of
Chagas disease, uses amino acids in severa Chagas disease, uses amino acids in several biological processes: metacyclogenesis, infection, resistance to nutritional and oxidative stress, osmotic control, etc. In this study, we investigated the import and metabolism The interact of nutritional and oxidative stress, osmotic control, etc. In this study, we
investigated the import and metabolism of L-Ser, L-Thr, and Gly in T. cruzi. Our results
demonstrate that these amino acids are tran resistance to intrinsic and metabolism of L-Ser, L-Thr, and Gly in T. cruzi. Our results
demonstrate that these amino acids are transported from the extracellular environment into
T. cruzi cells through a saturable transpo Investigated the import and metabolism of L-Ser, L-Thr, and Gly in T. cruzi. Our results
demonstrate that these amino acids are transported from the extracellular environment into
T. cruzi cells through a saturable transpo T. cruzi cells through a saturable transport system that fits the Michaelis-Menten model. Our results show that L-Ser and L-Thr can sustain epimastigote (Epi) cell viability under nutritional stress (NS) conditions and ca T. cruzi cens through a saturable transport system that fits the Michaelis-Menten model. Our results show that L-Ser and L-Thr can sustain epimastigote (Epi) cell viability under nutritional stress (NS) conditions and can stress (NS) conditions and can stimulate oxygen consumption to maintain intracellular ATP
levels. Additionally, our findings indicate that L-Ser plays a role in establishing the
mitochondrial membrane potential $(\Delta \Psi_m)$ i stress (NS) constants and can statistical explorement to maintain interestinate the levels. Additionally, our findings indicate that L-Ser plays a role in establishing the mitochondrial membrane potential $(\Delta \Psi_m)$ in T. c mitochondrial membrane potential $(\Delta \Psi_m)$ in T. cruzi. L-Ser is also involved in energy
metabolism via the Ser-Pyr pathway, which stimulates the production and subsequent
excretion of acetate and alanine. Our results demo mitochondrial membrane potential ($\Delta\Psi_m$) in T. cruzi. L-Ser is also involved in energy
metabolism via the Ser-Pyr pathway, which stimulates the production and subsequent
excretion of acetate and alanine. Our results dem excretion of acetate and alanine. Our results demonstrate the importance of L-Ser and L-Thr in
the energy metabolism of *T. cruzi* and provide new insights into the metabolic adaptations of
this parasite during its life cy the energy metabolism of *T. cruzi* and provide new insights into the metabolic adaptations of
this parasite during its life cycle.
Keywords: Trypanosoma cruzi, amino acid metabolism, transport, bioenergetics, nutritiona

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Keywords: Trypanosoma cruzi, a
stress Keywords: Trypanosoma cruzi, ammo acid metabolism, transport, bioenergetics, nutritional
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INTRODUCTION

Trypanosoma cruzi is the etiological agent of Chagas disease, a.k.a American
somiasis. During its life cycle, the parasite transitions between different environments
rent hosts, such as the mammalian host-cell cytoplasm, t of different hosts, such as the mammalian host-cell cytoplasm, the mammalian blood and
different regions of the reduviid insect's digestive tube (midgut and rectum lumen). To survive
in these environments, T. cruzi reprogr different regions of the reduviid insect's digestive tube (midgut and rectum lumen). To survive
in these environments, T. cruzi reprograms its metabolism, being able to metabolize various
carbon/energy sources (1–5). These different regions of the reduvita insect stagestive tube (imaged and rectum lumen). To survive
in these environments, *T. cruzi* reprograms its metabolism, being able to metabolize various
carbon/energy sources (1–5). Thes In these environments, T. cruzi reprograms its metabolism, being able to metabolize various
carbon/energy sources (1–5). These parasite's habitats range from those with abundant to
those with low carbohydrates availability carbon/energy sources with low carbon/drates availability, such as the intestinal tract of the starved insect
vector (6–10). In this regard, it has been well described that these parasites can switch
between the consumpti vector (6–10). In this regard, it has been well described that these parasites can switch
between the consumption of carbohydrates and that of amino acids and fatty acids (11–14).
To cope with the scarcity of metabolites between the consumption of carbohydrates and that of amino acids and fatty acids (11–14).
To cope with the scarcity of metabolites in these different environments *T. cruzi* must acquire
and utilize the available nutrient

To cope with the scarcity of metabolites in these different environments *T. cruzi* must acquire
and utilize the available nutrients as carbon and energy sources [reviewed in: (15)].
Among the available nutrients in most e To cope with the scarctly of metabolites in these different environments T. cruzi must acquire
and utilize the available nutrients as carbon and energy sources [reviewed in: (15)].
Among the available nutrients in most en Among the available nutrients in most environments colonized by T. cruzi throug
cycle, L-Ser, L-Thr, and Gly are almost omnipresent. Despite this, few groups ha
themselves to studying in depth the metabolism of these amino Expectively. L. Expectively, L. Expectively, L. Cruzi throughout its life cycle, L.-Ser, L.-Thr, and Gly are almost omnipresent. Despite this, few groups have devoted themselves to studying in depth the metabolism of thes themselves to studying in depth the metabolism of these amino acids in T. cruzi. In the 1970s,
Hampton determined the participation of L-Ser in the production of CO_2 , indicating that this
molecule is metabolizable by th Hampton determined the participation of L-Ser in the production of CO_2 , indicating that this molecule is metabolizable by the parasite (16). Additionally, there is already extensive evidence of the participation of L-Th Hampton determined the participation of L-Th in the production of L-2, indicating that the
molecule is metabolizable by the parasite (16). Additionally, there is already extensive
evidence of the participation of L-Thr in evidence of the participation of L-Thr in the mitochondrial metabolism of Trypanosoma brucei
(17–19). Although there is no evidence of the participation of Gly in energy metabolism, the
carbons of Gly may contribute to th (17–19). Although there is no evidence of the participation of Gly in energy metabolism, the
carbons of Gly may contribute to the formation of L-Ser through the reversible activity of
serine hydroxymethyltransferase (SHMT Carbons of Gly may contribute to the formation of L-Ser through the reversible activity of
serine hydroxymethyltransferase (SHMT) (20,21). Therefore, Gly could be metabolized via
SHMT, thus participating in mitochondrial A

carbons of Gly could be metabolized via
serine hydroxymethyltransferase (SHMT) (20,21). Therefore, Gly could be metabolized via
SHMT, thus participating in mitochondrial ATP production.
Despite the demonstrated potential o SHMT, thus participating in mitochondrial ATP production.
Despite the demonstrated potential of these amino acids to trigger ATP biosynthesis in many
organisms (protists, bacteria, yeast, and mammals) (17,19,22–30), their Despite the demonstrated potential of these amino acids
organisms (protists, bacteria, yeast, and mammals) (17,
metabolism in *T. cruzi* remain almost unexplored. In this w
L-Thr, Gly, and their role in the parasite's bioe Despite the demonstrated potential of these amino and the degacy of the despite to their),
organisms (protists, bacteria, yeast, and mammals) (17,19,22–30), their consumption and
metabolism in *T. cruzi* remain almost unex organisms (protection)

organisms (protection) in *T. cruzi* remain almost unexplored. In this work, we explore the uptake of L-Ser,

L-Thr, Gly, and their role in the parasite's bioenergetics and nutritional stress (NS) r L-Thr, Gly, and their role in the parasite's bioenergetics and nutritional stress (NS) resilience.
RESULTS
T. cruzi epimastigote transport L-Ser, L-Thr and Gly from the extracellular medium
To characterize the L-Ser, L-Thr

RESULTS

T. cruzi epimastigote transport L-Ser, L-Thr and Gly from the extracellular medium

RESULTS
T. cruzi epimastigote transport L-Ser, L-Thr and Gly from the extracellular medium
To characterize the L-Ser, L-Thr and Gly uptake, their transport was measured as a function
time. For this, the parasites were incu To characterize the L-L-S, L-Thr and L-S, Spring, mean dimepper the included as a formation is
time. For this, the parasites were incubated with each radioactively traced amino acid at a 5
mM concentration, assuming that t the transport of these metabolites along time was fittable to an exponential decay function (L-
The transport of these metabolites along time was fittable to an exponential decay function (L-
The transport of these metabol The transport of these metabolites along time was fittable to an exponential decay function (L-

The transport of these metabolites along time was fittable to an exponential decay function (L-The transport of the transport of these metabolites along time was fitted to an exponential decay function (L-

Ser: $r = 0.99$, E-Thr: $r = 0.98$ and Gly: $r = 0.96$), as expected for saturable protein-mediated
transport systems (Fig. 1A, B and C). At initial times, the resulting exponential-decay functions
can be assumed as near-lin \hat{r} = 0.99, L Thr. r^{\ast} = 0.98 and Gly: r^{\ast} can be assumed as near-linear, allowing a simple and reliable calculation of initial velocities for
substrate uptake. Our data show that linear functions can be accurately fitted to the measured
uptake of L-Ser, L-Thr, an substrate uptake. Our data show that linear functions can be accurately fitted to the measured
uptake of L-Ser, L-Thr, and Gly until 10 min ($r^2 = 0.96$, $r^2 = 0.95$, and $r^2 = 0.94$ for L-Ser, L-Thr, and
Gly, respectivel uptake of L-Ser, L-Thr, and Gly until 10 min ($r^2 = 0.96$, $r^2 = 0.95$, and $r^2 = 0.94$ for L-Ser, L-Thr, and
Gly, respectively (insets in Fig. 1 A, B and C)). Therefore, 3 min was set as the time window to
measure the in ^= 0.96, r^= 0.95, and r^

uptake of L-Ser, L-Thr, and Gly until 10 min (r = 0.96, r = 0.95, and r = 0.94 for L-Ser, L-Thr, and Gly, respectively (insets in Fig. 1 A, B and C)). Therefore, 3 min was set as the time window to measure the initial vel The measure the initial velocity (V_0) of L-Ser, L-Thr, and Gly uptake.
To analyze the kinetics for the transport of each amino acid, epimastigote forms (Epi) of the
parasites were incubated with different concentrations measure the linital velocity (V₀) of L-Ser, L-Thr, and Gly uptake.
To analyze the kinetics for the transport of each amino acid,
parasites were incubated with different concentrations of L-Se
uptake of each amino acid wa parasites were incubated with different concentrations of L-Ser, L-Thr or Gly for 3 min and the uptake of each amino acid was measured (through the cell-associated radioactivity). The hyperbolic functions corresponding to parameter in the incubation and the concentration of L-L-C, 2 minute ϵ), ϵ is that and the update of each amino acid was measured (through the cell-associated radioactivity). The hyperbolic functions corresponding t hyperbolic functions corresponding to the classic Michaelis-Menten kinetics fit the data
corresponding to each amino acid. The non-linear hyperbolic functions that best explained our
data showed robust adjustments ($r^2 =$ corresponding to each amino acid. The non-linear hyperbolic functions that best explained our
data showed robust adjustments $(r^2 = 0.96, 0.95,$ and 0.94 for L-Ser, L-Thr, and Gly,
respectively). The values for K_M and V data showed robust adjustments ($r^2 = 0.96$, 0.95, and 0.94 for L-Ser, L-Thr, and Gly, respectively). The values for K_M and V_{max} were derived from these functions for the three substrates (Fig. 1 D, E, and F). Summari respectively). The values for K_M and V_{max} were derived from these functions for the three
substrates (Fig. 1 D, E, and F). Summarizing, the results showed that L-Ser, L-Thr, and Gly are
taken up from the extracellular data showed robust adjustments (r^2 = 0.96, 0.95, and 0.94 for L-Ser, L-Thr, and Gly, Expectively). The values for KM and V_{max} were derived from these functions for the three
substrates (Fig. 1 D, E, and F). Summarizing, the results showed that L-Ser, L-Thr, and Gly are
taken up from the extracellular me states (Fig. 2.5) shown, commensing, the results showed that L-Ser, 2.1m, shower, view that
taken up from the extracellular medium by *T. cruzi* Epi through saturable transport systems
and their kinetics fit the Michaelia and their kinetics fit the Michaelian model. Therefore, the K_M and V_{max} were calculated as
being 37 ± 0.001 µM, 87 ± 0.006 µM,267 ± 0.015 µM and 0.258 ± 0.022 nmol min⁻¹ 2 x10⁷ cells,
0.367 ± 0.007 nmol min⁻¹ 2 being 37 ± 0.001 µM, 87 ± 0.006 µM,267 ± 0.015 µM and 0.258 ± 0.022 nmol min⁻¹ 2 x10⁷ cells,
0.367 ± 0.007 nmol min⁻¹ 2 x10⁷ cells and 0.902 ± 0.007 nmol min⁻¹ 2 x10⁷ cells for L-Ser, L-Thr,
and Gly, respectiv

Figure 1. Uptake of L-Ser, L-Thr, and Gly in T. cruzi epimastigote as a function of time and concentration. (A, B, and C) Time-course transport of 5 mM L-Ser, L-Thr, and Gly, respectively. (D, E, and F) Uptake of L-Ser, L-Thr, and Gly, respectively, as a function of their concentration. The insets represent the adjustment of incorporation as a function of time to a linear function. The table below the graphs gives the values of the kinetic parameters calculated by the Michaelis-Menten model. r^2 values of 0.96, 0.95, and 0.94 for L-
-Ser, L-Thr, and Gly, respectively.

Then, the possible ability of L-Ser, L-Thr, and Gly to share the same transport system was investigated. To this end, a cross-competition assay was performed to assess the inhibition rate of each amino acid transport in the presence of the potentially competing amino acid at a concentration corresponding to 10-fold the K_M value (370 µM, 870 µM and 2600 µM for L-Ser, L-Thr and Gly, respectively) (Table 1). The uptake of L-Ser was diminished by 50% and 45%, respectively, by L-Thr and Gly, while the uptake of L-Thr was diminished by 82% and 42%, respectively, by L-Ser and Gly, and the transport of Gly was inhibited by 83% and 58%, respectively, by L-Ser and L-Thr. Our findings show that L-Ser, L-Thr, and Gly indeed compete with each other, and thus, support the hypothesis of a shared transporter for these three amino acids. L-Ser uptake was set as a proxy for further characterizing the activity for all three. Using the same conditions, the ability of other amino acids to impair L-Ser transport was assessed. While structurally related amino acids such as D-Ser, L-Cys, D-Ala and L-Ala resulted in partial inhibition of L-Ser transport, structurally dissimilar amino acids, such as branched-chain amino acids (BCAA; L-Leu, L-Ile and L-Val) (31), L-His (32) and L-Phe did not significantly affect it, as expected (Table 1).

Table 1. Percentage of inhibition in transport observed in cross-competition assay.

Cross-competition assay between L-Ser, L-Thr and Gly: transport inhibition of each amino acid was determined by adding an excess (10 x K_M value for each amino acid tested).

Determination of the driving force for L-Ser uptake

To investigate the effect of intracellular ATP levels on this transport system, L-Ser uptake was measured in parasites that were treated for 30 min with oligomycin A (Oly), a known F_0 -ATP synthase inhibitor which produces intracellular ATP depletion (31), or without (control). A control group treated with Oly immediately before measuring the L-Ser uptake was included, to discard an off-target effect of Oly on the L-Ser transport. Cells pre-incubated with Oly for 30 min showed a L-Ser uptake decreased by approximately 40.3 ± 13 %, while L-Ser uptake levels were unaffected without pre-incubation when compared with the untreated control. To determine whether the transport system was dependent on the proton gradient (Δp) across the plasma membrane, the V_0 of L-Ser incorporation in the presence of the protonophore CCCP was measured. CCCP has two known effects on cells: i) it collapses the H -related membrane in the M potential $(\Delta \psi)$; and ii) it depletes intracellular ATP levels by reversing the mitochondrial F_1F_0 -ATP synthase reaction. To distinguish these two effects on the transport assay, the L-Ser transport was measured also in the presence of CCCP supplemented with Oly (to avoid ATP hydrolysis by reversal of the F_1F_0 -ATP synthase activity). CCCP, both, in the presence and absence of Oly, significantly diminished the transport activity when compared to the positive control (C+), indicating that this transport system is dependent on the Δp across the plasma membrane, which in turn depends on the intracellular ATP levels (Fig. 2 and Table 2).

Figure 2. (A) Effect of oligomycin A and CCCP on L-Ser uptake: the dependence of L-Ser transport on intracellular ATP levels (Oly 30') and the H⁺ gradient (CCCP) was assessed. CCCP can rapidly trigger ATP hydrolysis by mitochondrial ATPase to reestablish the H+ gradient, leading to ATP depletion in the cell. To distinguish between these ATP i
phenomena, the parasites were incubated with CCCP in the presence and absence of Oly (CCCP + Oly). To control for non-specific inhibition of the transport system by Oly, we added it without pre-incubation (Oly T0). These graphs represent three independent biological replicates. Statistical analysis was performed using one-way ANOVA with Tukey's post-test (**** P< 0.0001; ** P: 0.002).

Table 2. Inhibition of L-Ser uptake in the presence of FCCP and Oly.

*not detected.

Thermodynamic analysis of L-Ser uptake

To assess the effect of the substrate of temperature of temperature on L-Ser uptake, the exponential region of the curve were utilized
to calculate a Q₁₀ of 1.948, indicating a temperature-dependent process. Additionall under these conditions the changes in V_{max} in the exponential region of the curve were utilized
to calculate a Q₁₀ of 1.948, indicating a temperature-dependent process. Additionally, V_0
measurements made between 4 the calculate a Q₁₀ of 1.948, indicating a temperature-dependent process. Additionally, V_0
measurements made between 4 °C and 60 °C (R²: 0.988) were used to calculate an activation
energy (E_a) of 51.2 ± 2.4 kJ/mo

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|
| Figure 3. Effect of temperature on L-Ser uptake: (A) The V_0 was measured at saturating concentrations of L-Ser after
3 min of uptake at specific temperatures. (B) An Arrhenius plot was created by performing a linear fi 3 min of uptake at temperatures ranging from 4 to 40 °C. These graphs represent three independent biological
12 v₀ values measured at temperatures ranging from 4 to 40 °C. These graphs represent three independent biologi

Uptake of L-Ser in the different forms of the T. cruzi life cycle

Volume measure at temperature ranging from 4 to 40 miles graphs raphs in example at the separature in the different forms of the *T. cruzi* life cycle
Previous research on changes in nutrient uptake profiles during *T. cru* replicates.
Uptake o
Previous
scarce. H Previous research on changes in number uptake profiles during T. cruzi life cycle has been
scarce. However, two works have previously shown that the uptake of glucose (Glc) (33) and L- glutamine (34) change according to the life-cycle stage of L-Ser in vertebrate intracellular
stages (Amastigote - Ama and Intracellular Epimastigote - Epi-like), stages present in the insect
vector (Epi - Epimastigote and stages (Amastigote - Ama and Intracellular Epimastigote - Epi-like), stages present in the insect
vector (Epi - Epimastigote and Metacyclic Trypomastigote - MTrypo), and those present in the
bloodstream of vertebrates (Bl state of the interestingly, the intracellular Epi-like stage captured approximately 5 times more L-Ser from
the medium than Epi Forms, while MTrypo and BTrypo approximately 5 times more L-Ser from
the medium than Epi forms

Figure 4. Transport of L-Ser in different life-cycle stages of T. cruzi. A) Transport of L-Ser, B) Transport of L-Ser
(normalized). Epi: Epimastigote; Ama: Amastigote; Epi-like: Epimastigote-like; Meta: Metacyclic; Trypo: (normalized). The data are representative of three independent biological triplicates. The panel on the right
represents the normalized transport rates, considering the transport measured in epimastigotes as 100%.
Bioener

trypomastigote. The data are representative of three independent orregion inputation the panel on the right
represents the normalized transport rates, considering the transport measured in epimastigotes as 100%.
Bioenerget Rioenergetics of L-Ser, L-Thr and Gly in *T. cruzi* epimastigotes
To investigate the bioenergetic fate of the analyzed amino acids, and their potential ro
survival during NS was examined. Cells were incubated in PBS supple Bioenergetics of L-Ser, L-Thr and Gly in T. cruzi epimastigotes
To investigate the bioenergetic fate of the analyzed amino aci
survival during NS was examined. Cells were incubated in
negative control) with 5 mM of the ami Survival during NS was examined. Cells were incubated in PBS supplemented or not (as
negative control) with 5 mM of the amino acids under study. Cells supplemented with L-His
(32) or L-His plus Glc were used as positive co survive control) with 5 mM of the amino acids under study. Cells supplemented with L-His (32) or L-His plus Glc were used as positive controls. After 48h of NS, only L-Thr succeeded in maintaining cell viability at the lev (32) or L-His plus Glc were used as positive controls. After 48h of NS, only L-Thr succeeded in maintaining cell viability at the level of the positive control (His). However, during longer NS periods (72h and 96h), Gly a (32) or Linking cell viability at the level of the positive control (His). However, during longer NS periods (72h and 96h), Gly and L-Ser can sustain viability significantly better than only PBS.
Taken together, these resu maintaing centraliant) of the level of the positive control (His). However, along the positive control (His). However, these results indicate that all three metabolites can protect the cells from NS, with L-Thr being more periods (72) and 96 here is indicate that all three metabolites can protect the cells from NS, with L-Thr being more efficient than L-Ser and Gly (Fig. 5 A). with L-Thr being more efficient than L-Ser and Gly (Fig. 5 A).

The cells from NS, the ce with L-Thr being more efficient than L-Ser and Gly (Fig. 5 A).

Figure 5. (A) Viability assay of *T. cruzi* epimastigotes as a function of nutritional stress: viability was measured by the irreversible reduction of resazurin to resorufin. His (5 mM) was used as a positive control, a in the reduction of the reduction of resort of the presence of reduction of carbon was used as a negative control. (B) Recovery assay of *T. cruzi* epimastigotes under NS conditions: the proliferation profile was evaluate proliferation profile was evaluated after 72 h of NS in the presence or absence (only PBS) of exogenous carbon
sources, 5 mM His and L-His + Glc (5 mM each, positive controls) and 5 mM Gly, L-Ser and L-Thr. Calibration cu sources, 5 mM His and L-His + Glc (5 mM each, positive controls) and 5 mM Gly, L-Ser and L-Thr. Calibration curves
were performed using known parasite densities. As negative control, PBS without exogenous carbon sources wa Sources, 5 mM His and L-His + Glc (5 mM each) persions critically and 2 mM estimates (C) Production of ¹⁴CO₂ by breaking down Gly, L-Ser and L-Thr in *T. cruzi* epimastigote. (D) Intracellular levels of ATP in epimasti used. (C) Production of 14 CO₂ by breaking down Gly, L-Ser and L-Thr in *T. cruzi* epimastigote. (D) Intracellular levels of
ATP in epimastigotes recovered from NS with Gly, L-Ser and L-Thr: after 16 h of NS, the par used. (C) Production of "CO₂ by breaking down Gly, L-Ser and L-Thr in T. cruzi epimastigote. (D) Intracellular levels of
ATP in epimastigotes recovered from NS with Gly, L-Ser and L-Thr: after 16 h of NS, the parasites w ATP in epimaligences recovered from NS with Gly, E Ser and E-Thr: after 16 h of NS, the parasites were recovered for
3 h in the presence (or not, negative control – only PBS) of 5 mM His (positive control) Gly, L-Ser and o 3 h in the presence (or not, negative comparison – only PBS) of 5 mm his (positive control) gives are representative of
three independent biological replicates. Two-way ANOVA with Tukey's post-test was used for statistical

three independent biological replicates. Two-way ANOVA with Tukey's post-test was used for statistical analysis: **
P<0.0029; *** P<0.0007; **** P<0.0001.
The fact that L-Ser, L-Thr and Gly were able to keep the cells aliv three independent biological replicates. Two-way ANOVA with Tukey's post-test was used for statistical analysis: **
P<0.0029; *** P<0.0007; **** P<0.0001.
The fact that L-Ser, L-Thr and Gly were able to keep the cells aliv The fact that L-Ser, L-Thr and Gly v
necessarily imply their capacity to
Thus, this possibility was invest
following NS. Our results showe The fact that The Fact that L-Ser, L-Thr and The Fact that IIT medium.
Thus, this possibility was investigated by performing a proliferation recovery experiment
following NS. Our results showed that L-Ser, L-Thr, and Gly c Thus, this possibility was investigated by performing a proliferation recovery experiment
Thus, this possibility was investigated by performing a proliferation recovery experiment
following NS. Our results showed that L-S following NS. Our results showed that L-Ser, L-Thr, and Gly can maintain the proliferative
capacity of Epi forms after arrest induced by NS, at similar levels as the positive control. These
results strongly indicate that

Following The Canar Internation and Calibber 2011, 2014, 2014, 2014 in the positive control. These results strongly indicate that these amino acids can assist the parasite to cope with NS (Fig. 5 B).
B).
In most organisms capacity of Epi forms and the theorem and a state induced by Theorem in Forms 2 china interest
results strongly indicate that these amino acids can assist the parasite to cope with NS (Fig. 5
B).
In most organisms, L-Ser B).
In most organisms, L-Ser and L-Thr carbons primarily contribute to energy metabolism via the
Ser-pyruvate (Pyr) and Thr-Acetyl-CoA pathways respectively. Once these metabolites enter
the tricarboxylic acid (TCA) cycle - ,
In r
Ser
the
pos Ser-pyruvate (Pyr) and Thr-Acetyl-CoA pathways respectively. Once these metabolites enter
the tricarboxylic acid (TCA) cycle, they can be broken down into CO₂. To investigate this
possibility, Epi forms were incubated w the tricarboxylic acid (TCA) cycle, they can be broken down into CO₂. To investigate this
possibility, Epi forms were incubated with L-[3-¹⁴C]Ser, L-[U-¹⁴C]Thr, and [U-¹⁴C]Gly, and the
discrete metabolites were in possibility, Epi forms were incubated with L- $[3^{-14}C]$ Ser, L- $[U^{-14}C]$ Thr, and $[U^{-14}C]$ Gly, and the possibility, Epi forms were incubated with L-[3-¹⁴C]Ser, L-[U-¹⁴C]Thr, and [U-¹⁴C]Gly, and the
possibility, Epi forms were incubated with L-[3-¹⁴C]Ser, L-[U-¹⁴C]Thr, and [U-¹⁴C]Gly, and the production of $^{4}CO_2$ was measured over time. The data show that L-Ser and L-Thr were
catabolized to CO_2 , but interestingly no Gly, under the conditions of the assay (Fig. 5 C). To
track how much of the taken-up L-Ser, track how much of the taken-up L-Ser, L-Thr or Gly offered to the cells at saturating
concentration was oxidized to CO_2 , its production was quantified over time. After 3 h of
incubation, 65.1% of the amount of L-Ser and concentration was oxidized to CO_2 , its production was quantified over time. After 3 h of
incubation, 65.1% of the amount of L-Ser and 21% L-Thr that were transported into the cells
were fully oxidized to ¹⁴CO₂, whil incubation, 65.1% of the amount of L-Ser and 21% L-Thr that were transported into the cells
were fully oxidized to ¹⁴CO₂, while no CO₂ derived from [U-¹⁴C]Gly was detected (Table 3). From
these data it was conclud

were fully oxidized to $^{14}CO_2$, while no CO_2 derived from $[U^{-14}C]G$ ly was detected (Table 3). From these data it was concluded that L-Ser and L-Thr can be subjected to mitochondrial oxidation.
As the vast majority of were fully oxidized to ²⁴CO₂, while no CO₂ derived from [U-²⁴C]Gly was detected (Table 3). From
these data it was concluded that L-Ser and L-Thr can be subjected to mitochondrial oxidation.
As the vast majority of As the vast majority of mitochondrial oxidative processes are directed to ATP biosynthesis,
was investigated if this is also the case for L-Ser, L-Thr, and Gly. For this, Epi were submitted t
NS by incubation in PBS for 30 As the vastigated if this is also the case for L-Ser, L-Thr, and Gly. For this, Epi were submitted to
NS by incubation in PBS for 30 h to deplete the intracellular ATP content, and their recovery by
incubation in the prese Was by incubation in PBS for 30 h to deplete the intracellular ATP content, and their recovery by incubation in the presence of the substrates under investigation was assessed. The cells were recovered in PBS supplemented Incubation in the presence of the substrates under investigation was assessed. The cells were
recovered in PBS supplemented (or not, negative control) with 5 mM L-Ser, L-Thr, Gly or 5 mM
His (control) for 3 h, and the intr Incurrent in the presence of the substrates under investigation interaction interaction in the substrates incubated
The frequency of the substrates under the substrates incubated
With L-Ser and L-Thr exhibited a significan His (control) for 3 h, and the intracellular ATP levels were determined. Parasites incubated
with L-Ser and L-Thr exhibited a significant recovery of the intracellular ATP levels when
compared to the negative control. Thi With L-Ser and L-Thr exhibited a significant recovery of the intracellular ATP levels when
compared to the negative control. This increase was comparable to those observed in
parasites recovered with His. Interestingly, G compared to the negative control. This increase was comparable to those observed in
parasites recovered with His. Interestingly, Gly could not restore intracellular ATP levels, which
is in accordance with the absence of C parasites recovered with His. Interestingly, Gly could not restore intracellular ATP levels, which
is in accordance with the absence of CO_2 production (Fig. 5 D).
Table 3. Time-dependent ¹⁴CO₂ generation by Epi form

is in accordance with the absence of CO₂ production (Fig. 5 D).
 **Table 3. Time-dependent ¹⁴CO₂ generation by Epi form from L-[3¹⁴C]Ser, L-[U¹⁴C]Thr and

[U¹⁴C]Gly** Table 3. Time-dependent \sim CO₂ generation by Epi form from L-[3⁻⁻C]Ser, L-[U¹⁴C]Thr and $[U^{14}C]$ Gly

¹ Considering that the only way for L -[3¹⁴C]Ser to be released as CO₂ is through complete degradation in the TCA Considering that the only way for L-[3² C]Ser to be released as CO₂ is through complete degradation in the TCA
ycle, the obtained value was normalized by multiplying it by 3. In contrasts, the values obtained L-[U-¹⁴

cycle, the obtained value was normalized by multiplying it by 3. In contrasts, the values obtained L-[U-14C]Thr or [U-14C]Gly have not been normalized since all carbon are ¹⁴C-labeled.
² Considering the standard error, 2 CGIy have not been normalized since all carbon are 2 C-labeled.
 2 Considering the standard error, the percentage of 14 CO₂ produc

have originated from residual activity of the glycine cleavage comp

Considering the standard error, the percentage of \cdot CO₂ produced from Gly was assumed to be residual and may have originated from residual activity of the glycine cleavage complex.
To assess the comparative release o $\frac{3}{10}$ To assess the comparative release of carbon as CO_2 , the measured vathe transported amino acid over the identical incubation duration determine observation that L-Ser and L-Thr can be taken up

The states the comparative release of carbon as CO₂, the measured values were normalized against the quantity of
the transported amino acid over the identical incubation duration detected in Fig. 1A, B and C.
The observ The observation that L-Ser and L-Thr can be taken up from the extracellu
support cell viability, produce CO_{2} and support ATP biosynthesis, suggests t
acids are involved in oxidative phosphorylation (OxPhos). Thus, Epi Support cell viability, produce CO_2 and support ATP biosynthesis, suggests that these amino acids are involved in oxidative phosphorylation (OxPhos). Thus, Epi subjected to NS (PBS) for 16 h, were transferred to MCR res support central viability, produce Co₂, and support and subspired via biosynthesis, subjected to NS (PBS) for
16 h, were transferred to MCR respiration buffer and recovered for 3 h by incubation in MCR
supplemented (or n 16 h, were transferred to MCR respiration buffer and recovered for 3 h by incubation in MCR
supplemented (or not, negative control) with 5 mM L-Ser, L-Thr or His (positive control). The
cells were then subjected to high-r supplemented (or not, negative control) with 5 mM L-Ser, L-Thr or His (positive control). The
cells were then subjected to high-resolution oxygraphy, from which was derived the
respiration parameters: **Routine respiration** supplemented (or not, negative control) with 5 mm L-Ser, 2 mm L-M (positive control). The colls were then subjected to high-resolution oxygraphy, from which was derived the respiration parameters: **Routine respiration (R)** respiration parameters: **Routine respiration (R)**, rate of respiration that is not responsible for
ATP production through OxPhos, **Leak respiration (L)**, the remaining O₂ consumption
measured when the ATP synthase is in Respiration parameters: Routine respiration (R), rate of respiration that is not responsible for
ATP production through OxPhos, Leak respiration (L), the remaining O₂ consumption
measured when the ATP synthase is inhibi measured when the ATP synthase is inhibited, **Electron-Transport Capacity (ETC)**, obtained
after uncoupling the mitochondria by the addition of an uncoupler and **Residual Respiration**
Rate (ROX), obtained by the inhibit measured when the ATP synthase is inhibited, Electron-Transport Capacity (ETC), obtained
after uncoupling the mitochondria by the addition of an uncoupler and Residual Respiration
Rate (ROX), obtained by the inhibition of **Rate (ROX)**, obtained by the inhibition of complex III (Coenzyme Q-cytochrome C reductase), abolishing the flux of electrons through the respiratory chain (35). Our results demonstrate that after 3 h of incubation, L-Ser Abolishing the flux of electrons through the respiratory chain (35). Our results demonstrate
that after 3 h of incubation, L-Ser and L-Thr (but not Gly) were able to trigger O₂ consumption
(Fig. 6 A, B and C), when comp that after 3 h of incubation, L-Ser and L-Thr (but not Gly) were able to trigger O₂ consumption (Fig. 6 A, B and C), when compared with the negative control (Fig. 6 E), and similarly to our positive control (Fig. 6 D). (Fig. 6 A, B and C), when compared with the negative control (Fig. 6 E), and similarly to our positive control (Fig. 6 D). Furthermore, when L was subtracted from **R** to obtain the Free Routine Respiration (respiration ra positive control (Fig. 6 D). Furthermore, when L was subtracted from **R** to obtain the Free
Routine Respiration (respiration rate directly related to the ATP biosynthesis by F_0F_1 -ATP
synthase) (36), L-Ser and L-Thr pe Routine Respiration (rig. 6 D). Furthermore, when L was subtracted from R to obtain the Free
Routine Respiration (respiration rate directly related to the ATP biosynthesis by F_0F_1 -ATP
synthase) (36), L-Ser and L-Thr p synthase) (36), L-Ser and L-Thr performed similarly as His (Fig. 6 G). Together, these results
show the anaplerotic capacity of L-Ser and L-Thr, and thus, their ability to support ATP
synthesis via OxPhos. Interestingly, show the anaplerotic capacity of L-Ser and L-Thr, and thus, their ability to support ATP synthesis via OxPhos. Interestingly, and consistent with the CO_2 and ATP levels measurements, Gly was not able to stimulate respir synthesis via OxPhos. Interestingly, and consistent with the CO_2 and ATP levels measurements,
Gly was not able to stimulate respiration in these cells. synthesis via OxPhos. Interesting on the CO2 and ATP levels in the CO2 and ATP levels measurements, and ATP levels measurements, $\frac{1}{2}$ and ATP levels measurements, and ATP levels measurements, and ATP levels measuremen Gly was not able to stimulate respiration in these cells.

はり (control) しょうしょう **Figure 6.** High-resolution respirometry in *T. cruzi* epimastigotes. Respiration rates were measured after 16 h of NS, when the parasites were recovered for 3 h in the presence of a substrate. A and B) respiration rates incubation with 5 mM ι -Ser; C and D) respiration rates after 3 h of incubation with 5 mM ι -Thr; E and F) respiration rates after 3 h of incubation with 5 mM Gly; G) positive control with 5 mM His; H). control with rates after 3 h of incubation with 5 mM Gly; G) positive control with 5 mM His; H). control without exogenous substrate; I) Free routine activity in epimastigotes recovered from NS with Gly, L-Ser and L-Thr. The Free Routine substrate; I), The routine activity in epimaling activities recovered from NS with Gly, The Brahm Machine Routine
Activity (FRA) was obtained by subtracting the respiratory rates measured after addition of Oly. The graph i representative of this subtraction, using the average of the slopes obtained with the amino acids and after inhibition of F_0 -ATP synthase with Oly. The graphs are representative of three independent biological replicat inhibition of F_o-ATP synthase with Oly. The graphs are representative of three independent biological replicates. For
inhibition of F_o-ATP synthase with Oly. The graphs are representative of three independent biologic inhibition of Form synthase with Oly. The graphs are replicated to the synthase graphs replicates. For the gra
How is the graphs are replicated biological replications of the graphs are replicated by the graphs of the grap

For graph I, one-way ANOVA with Tukey post-test was used. **** P< 0.0001.

Knowing that L-Ser stimulates both oxygen consumption and ATP biosynthesis, it is conceivable

that this substrate could also contribute to establ Knowing that L-Ser stimulates both oxygen consumption and A⁻
that this substrate could also contribute to establishing and
 $\Delta\Psi_m$ in *T. cruzi.* Using safranine O combined with controlled uno
f valinomycin, the $\Delta\Psi_m$ The Intertainment of the top intertain the substrate could also contribute to establishing and maintaining the mitochondrial $\Delta\Psi_m$ in T. cruzi. Using safranine O combined with controlled uncoupling of $\Delta\Psi_m$ in the pre $\Delta\Psi_m$ in T. cruzi. Using safranine O combined with controlled uncoupling of $\Delta\Psi_m$ in the presence
of valinomycin, the $\Delta\Psi_m$ was quantified using the Nernst equation. For this experiment, the
cells were permeabilized $\Delta \Psi_m$ in T. cruzi. Using saframine U combined with controlled uncoupling of $\Delta \Psi_m$ in the presence of valinomycin, the $\Delta \Psi_m$ was quantified using the Nernst equation. For this experiment, the cells were permeabilize cells were permeabilized with digitonin in the presence of 2 mM L-Ser (Fig. 7 A). Given that L-Ser can form Pyr through Ser/ThrDH, the possible relationship between $\Delta\Psi_m$ generated by L-Ser and the mitochondrial Pyr car generated $\Delta \Psi_m$ was reversed in the presence of UK-5099. To rule out a possible off-target
effect of UK-5099 altering the capacity of building up $\Delta \Psi_m$, 2 mM Pro, a metabolite that can
directly feed electrons into the Ser and the mitochondrial Pyr carrier (37) was tested by adding 5 μ M UK-5099, a specific
inhibitor of the Pyr mitochondrial transporter (MPC) (Fig. 7 A). As expected, L-Ser was able to
build up and maintain $\Delta\Psi_m$ in In this control of the Pyr mitochondrial transporter (MPC) (Fig. 7 A). As expected, i-Ser was able to build up and maintain $\Delta\Psi_m$ in Epi. Consistent with the formation of Pyr through Ser/ThrDH, the generated $\Delta\Psi_m$ was inhibitor of the A Ψ_m in Epi. Consistent with the formation of Pyr through Ser/ThrDH, the generated $\Delta\Psi_m$ was reversed in the presence of UK-5099. To rule out a possible off-target effect of UK-5099 altering the capac generated $\Delta\Psi_m$ was reversed in the presence of UK-5099. To rule out a possible off-target
effect of UK-5099 altering the capacity of building up $\Delta\Psi_m$, 2 mM Pro, a metabolite that can
directly feed electrons into th effect of UK-5099 altering the capacity of building up $\Delta\Psi_m$, 2 mM Pro, a metabolite that can directly feed electrons into the respiratory chain (38,39), was added as a control (after UK 5099). As expected, the addition effectly feed electrons into the respiratory chain (38,39), was added as a control (after UK
directly feed electrons into the respiratory chain (38,39), was added as a control (after UK
gough). As expected, the addition direction of the AW_m generated by L-Ser resulted in the reestablishment of the $\Delta\Psi_m$. The
quantification of the $\Delta\Psi_m$ generated by L-Ser resulted in a $\Delta\Psi_m$ of -190 mV, comparable to the
positive control (L-Pro) a

Figure 7. Quantification of ΔΨ_m in the presence of L-Ser and L-Pro. A) Representative trace of the ΔΨ_m quantification
experiment by fluorometry, using safranine O in the presence of L-Ser, UK-5099 and Pro (positive c experiment by fluorometry, and generating ϵ in the presence of 5 nM valinomycin. B) Quantification of $\Delta\Psi_m$ under the previously mentioned conditions, using the Nernst equation (see materials and methods). The graphs calibration curve performed with K
previously mentioned conditions, L
 μ in the Nernst equation (see materials and methods). The graphs are
interesting the Nernst equation (see materials and methods). The graphs are previously mentioned conditions, using the Nernst equation (see materials and methods). The graphs are

Exometabolomics analysis to determine the excreted products from the metabolism of L-Ser

values and positive control (Pro), one-way ANOVA with Tukey's post-test was used. ** P<0.001.
Exometabolomics analysis to determine the excreted products from the metabolism of L-Ser
Given the bioenergetic relevance of the Exometabolomics analysis to determine the excreted products from the meta
Given the bioenergetic relevance of the conversion of L-Ser into Pyr, the met
involved in these processes were investigated by analyzing the product

Figure 8. Excretion profile of metabolites from 1. cruzi epimastigotes from L-Ser metabolism. (A) Proton resonance
profile of metabolites excreted from parasites incubated for 16 h with PBS or (B) PBS supplemented with 10 Ser. (C, D, E, F and G) quantification of identified metabolites. ICS: Inner carbon sources. The graphs are
representative of technical triplicates from three independent biological replicates. Two-tailed unpaired T test w

Ser. (3) 3) 3) 3) 2, F and G) quantificates from three independent biological replicates. Two-tailed unpaired T test was
used for statistical analysis. P <0.05 was considered statistically significant.
Our results show tha representative of technical individuals in the main excretion products from L-Ser metabolism are, in order of quantity, acetate, alanine and Gly, and a decreased excretion of succinate compared with excretion products of p Our results show that the main excretion products from L-
quantity, acetate, alanine and Gly, and a decreased excret
excretion products of parasites without exogenous carbon so
the idea that the metabolism of L-Ser in *T.* quantity, acetate, alanine and Gly, and a decreased excretion of succinate compared with
excretion products of parasites without exogenous carbon source (Fig. 8). These data reinforce
the idea that the metabolism of L-Ser excretion products of parasites without exogenous carbon source (Fig. 8). These data reinforce
the idea that the metabolism of L-Ser in *T. cruzi* might occur through its conversion into Pyr,
since both alanine and acetat excretion products of products of the metabolism of the metabor of parameters with this and the parameters with the metabolism and acetate are produce the idea that the increasions of L Ser in T. cruzi might occur through its conversion into Pyr,
since both alanine and acetate are produced from Pyr (40). In line with this, an L-Ser/L-Thr
dehydratase activity was detecte since both alarms and actual are produced from Pyr (40). In line with this, at 2007–110
dehydratase activity was detected in an Epi soluble extract (Fig. 9 A, B and C). To identify the
molecular entity responsible for this definition activity was detected in an Epi solution and C_{(Fig.} 9 A, B and Epi solution, the
molecular entity responsible for this activity, a search in the T. cruzi genome for an open
molecular entity responsible for thi molecular entity responsible for this activity, a search in the T. cruzi genome for an open

Figure 9. SerDH and ThrDH activities were measured by monitoring the decrease in absorbance at 340 nm
(formation of NAD⁺) through coupling of their reactions with recombinant lactate dehydrogenase (LDH). Briefly, L-
SerD (formation of NAD
SerDH produces Py
oxobutyrate is prod
Ser as substrate: (B
of soluble parasite
average of 9 technic Through complise their reactions with recombinant lactatic derity and controlled the through LDH, produces 2-hydroxybutyrate also oxidizing NADH. (A) Activity using L-
activity using L-Thr as substrate. C) NAD⁺ produced Ser as substrate is produced, which through LDH, produces 2-hydroxybutyrate also oxidizing NADH. (A) Activity using L-
Ser as substrate: (B) Activity using L-Thr as substrate. C) NAD⁺ produced in the reaction at differen Ser as substrate: (B) Activity using L-Thr as substrate. C) NAD^+ produced in the reaction at different concentrations
of soluble parasite extract. (D) SerDH activity of the purified recombinant enzyme. The figure repres Ser as substrate: (B) Activity using L-Thr as substrate. C) NAD
of soluble parasite extract. (D) SerDH activity of the purific
average of 9 technical replicates in 3 independent experiments
DISCUSSION
T. cruzi presents a produced in the reaction at different concentrations
ed recombinant enzyme. The figure represents the
i.
daptation since it can use a wide spectrum

DISCUSSION

average of 9 technical replicates in 3 independent experiments.
 DISCUSSION

T. cruzi presents an interesting case of metabolic adaptation since it can use a wide spectrum

of compounds for its maintenance in vertebrate DISCUSSION
T. cruzi presents an interesting case of metabolic ad
of compounds for its maintenance in vertebrat
demonstrated that after consuming preferentially G T. Cruzi presents an interesting case of includent adaptation since it can use a wide spectrum
of compounds for its maintenance in vertebrate and invertebrate hosts. It has been
demonstrated that after consuming preferenti demonstrated that after consuming preferentially Glc, it can switch to the use of amino acids,
mostly proline (38,39,41), but also glutamate, aspartate, glutamine (42), histidine (32) or fatty
acids (14,43,44). In the pres mostly proline (38,39,41), but also glutamate, aspartate, glutamine (42), histidine (32) or fatty
acids (14,43,44). In the present work, we showed that it is also able to consume L-Ser and L-Thr
as primary carbon and ener acids (14,43,44). In the present work, we showed that it is also able to consume L-Ser and L-Thr
as primary carbon and energy sources, while Gly can participate in its metabolism by providing
carbons but not energy. In mo as primary carbon and energy sources, while Gly can participate in its metabolism by providing
carbons but not energy. In most organisms, L-Ser can be produced *de novo* from 3-
phosphoglycerate (3PG), a glycolytic/glucon carbons but not energy. In most organisms, L-Ser can be produced *de novo* from 3-
phosphoglycerate (3PG), a glycolytic/gluconeogenic intermediate (45,46). However, T. cruzi carbons but not energy. In most organisms, L-Ser can be produced de novo from 3-
phosphoglycerate (3PG), a glycolytic/gluconeogenic intermediate (45,46). However, T. cruzi phosphoglycerate (3PG), a glycolytic/gluconeogenic intermediate (45,46). However, T. cruzi In the last the steps of this pathway, suggesting that it relies on the speak and 5,10-CH₂-
THF as substrates. Similarly, while plants, bacteria, and fungi can synthesize L-Thr *de novo* from
aspartate (48,49), *T. cruzi* THF as substrates. Similarly, while plants, bacteria, and fungi can synthesize L-Thr *de novo* from
aspartate (48,49), *T. cruzi* lacks the first three steps of this pathway. It has been proposed that
T. cruzi, like *T.* THE as substrates. Similarly, while plants, bacteria, and runglicant synthesize L-Thi de novo from
aspartate (48,49), T. cruzi lacks the first three steps of this pathway. It has been proposed that
T. cruzi, like T. brucei T. cruzi, like T. brucei, might be capable of synthesizing L-Thr from homoserine and acyl-
homoserine lactones, constituting a "by-pass" in the pathway at the homoserine kinase step
(2.7.1.39) (18). Given these deficienci T. cruzi, like T. brucei, inight be capable of synthesizing L-Thr from homoserine and acyl-
homoserine lactones, constituting a "by-pass" in the pathway at the homoserine kinase step
(2.7.1.39) (18). Given these deficienci (2.7.1.39) (18). Given these deficiencies in the biosynthesis pathways of L-Ser and L-Thr, *T. cruzi*
relies on transport activities to obtain these molecules, which (among trypanosomatids) has to
date only been demonstra (2.7.1.39) (18). Given these deficiencies in the biosynthesis pathways of L-Ser and L-Thr, T. cruzi
relies on transport activities to obtain these molecules, which (among trypanosomatids) has to
date only been demonstrated relies only been demonstrated in T. brucei (for L-Thr) (17) and *Leishmania amazonensis* (for L-
Ser) (50).
The uptake of L-Ser, L-Thr and Gly occurs by a shared H⁺/ATP-driven transport system
The transport of metabolite

The uptake of L-Ser, L-Thr and Gly occurs by a shared H*/ATP-driven transport system

date only been demonstrated in T. brucei (for L-Thr) (17) and Leishmania amazonensis (for L-Ser) (50).
Ser) (50).
The uptake of L-Ser, L-Thr and Gly occurs by a shared H⁺/ATP-driven transport system
The transport of meta The uptak
The trans
Througho
insect vec Throughout its life cycle, *T. cruzi* is exposed to L-Ser, L-Thr, and Gly which are present in the insect vector's excreta (51,52), as well as in mammalian cells and plasma (53–55). Many amino acid transport systems have Throughout its life cycle, T. cruzi is exposed to L-Ser, L-Thr, and Gly which are present in the
insect vector's excreta (51,52), as well as in mammalian cells and plasma (53–55). Many amino
acid transport systems have alr acid transport systems have already been biochemically characterized in this organism (Table S3). Noticeably, most of them have functional properties compatible with the AAAP family, which groups H⁺/amino acid transporte and transport systems have already also have minimized in the sygmeth (share

S3). Noticeably, most of them have functional properties compatible with the AAAP family,

which groups H⁺/amino acid transporters and auxin Which groups H⁺/amino acid transporters and auxin permeases (56,57). Due to its relevant
relationship with L-Thr, and Gly, many aspects of their uptake and metabolism were analyzed
together in this work.
The K_M values

which groups H
relationship with
together in this w
The $K_{\rm M}$ values fo
(59), and the low
reported for L-Glu L-Thr, and Gly, many aspects of their uptake and metabolism were analyzed
vork.
The same in the same range of values reported for Cys (58), Asp-
affinity Arg transporters (60). The K_M value measured for Gly resembles th together in this work.
The K_M values for L-Ser and L-Thr are in the same range of values reported for Cys (58), Asp
(59), and the low-affinity Arg transporters (60). The K_M value measured for Gly resembles those
repor The K_M values for L-Se
(59), and the low-affin
reported for L-Glu (61)
The V_{max} values obtain
Gln and L-Pro low-affir The K_M values for L-Ser and L-Thr are in the same range of values reported for Cly s (50), Asp
(59), and the low-affinity Arg transporters (60). The K_M value measured for Gly resembles those
reported for L-Glu (61), L (55), and the low-affinity Arg transporters (60). The KM value measured for Gly resembles those
reported for L-Glu (61), L-Ile (31), L-His (32) and high-affinity L-Pro (system A) transporters (62).
The V_{max} values ob The V_{max} values obtained for L-Ser and L-Thr are within the range of those for the BCAA, L-His, L-
Gln and L-Pro low-affinity (system B) uptake systems (31,32,34,62). Additionally, the V_{max} value
for Gly is amon The v_{max} values obtained for L-Ser and L-Thr are within the range of those for the BCAA, L-His, L-Gln and L-Pro low-affinity (system B) uptake systems (31,32,34,62). Additionally, the V_{max} value for Gly is amongst the Gly is amongst the three highest ones reported for *T. cruzi*, together with L-Ala (63) and L-
Leu (31). This could be related to the use of Gly as an osmolyte. For this function, rapid
changes in intracellular Gly concen For Gly is amongst the three ingliest ones reported for T. cruzi, together with L-Ala (63) and L-
Leu (31). This could be related to the use of Gly as an osmolyte. For this function, rapid
changes in intracellular Gly conc

Leu (31). This could be the use of Gly as an esponse to hyper-or hypo-osmotic
stress (64–67).
Given the fact that all three amino acids use the same transport system, the uptake of L-Ser
was used as a proxy for all of them stress (64–67).
Given the fact that all three amino acids use the same transport system, the uptake of L-Ser
was used as a proxy for all of them. Importantly, the transport activity for L-Ser increased
exponentially at tem stress (64–67).
Given the fact
was used as a
exponentially a
naturally expos
their bodies fol Enter the fact that all three amino action the three interpretently, the uptake of L-Ser increased
exponentially at temperatures between 25 and 40 °C, a range to which the parasites are
naturally exposed within both insect exponentially at temperatures between 25 and 40 °C, a range to which the parasites are
naturally exposed within both insect vectors (which do not control their temperature and thus
their bodies follow environmental temper exposed within both insect vectors (which do not control their temperature and thus
their bodies follow environmental temperature variations) and vertebrate hosts. This suggests
their bodies follow environmental temperatur naturally expects minimum continuation of control to note that their components and the material temperature variations) and vertebrate hosts. This suggests their bodies follow environmental temperature variations) and ver their bodies follow environmental temperature variations) and vertebrate hosts. This suggests uptake. From the response to temperature, the E_a was derived, which appeared to be the second lowest among those reported for amino acid transport systems in *T. cruzi*, after the low-affinity Arg transporter (60). Acco uptake. From the response to temperature, the E_a was derived, which appeared to be the second lowest among those reported for amino acid transport systems in *T. cruzi*, after the low-affinity Arg transporter (60). Acco second lowest among those reported for amino acid transport systems in T. cruzi, after the low-affinity Arg transporter (60). Accordingly, from the E_a value, the equivalent of at least 1.6 molecules of ATP into ADP plus low affinity Arg transporter (60). Accordingly, from the E_a value, the equivalent of at least 1.6
molecules of ATP into ADP plus Pi per L-Ser molecule would be required for the latter to be
transported into the cells. Fi morecules of ATP into APP per Laterman and a Computer of ATP into ATP in the later of ATP into ATP into ATP in
transported into the cells. Finally, our data show that this uptake system is mediated by an
active transporter active transporter as shown for most of the other amino acids in *T. cruzi.* With His transporter as the only exception (32), the primary mechanism driving these processes is a transmembrane H^* gradient established by as the only exception (32), the primary mechanism driving these processes is a transmembrane
H⁺ gradient established by a proton-pumping ATPase located in the plasma membrane (68).
This contrasts with the L-Ser uptake in H⁺ gradient established by a proton-pumping ATPase located in the plasma membrane (68).
This contrasts with the L-Ser uptake in *Leishmania amazonensis*, which appears to be powered
by ATP, independent of external ions H
Th
by
te:
in(gradient established by a proton-pumping ATM are located in the plasma membrane (e.g.)
is contrasts with the L-Ser uptake in *Leishmania amazonensis*, which appears to be powered
ATP, independent of external ions such as This contrasts with the L-Ser uptake in Leisimiania amazonensis, which appears to be powered
by ATP, independent of external ions such as K⁺ or Na⁺ and remains unaffected by any of the
tested ionophores (50). Notewort by ATP, independent of external ions such as K⁺
tested ionophores (50). Noteworthy, L-Ser uptak
indicating variations in the amounts of L-Ser and
In mammals, L-Ser and L-Thr in plasma range
measured for L-Ser and L-Thr t or Na
|e chang
|L-Thr
|5). In t
|partme and remains undertainty any or the gad throughout the parasite's life cycle.
T. cruzi encounters during its life cycle.
100 to 200 μ M, values above the K_M
riatomines, little is known about the
ents of the digestive t indicating variations in the amounts of L-Ser and L-Thr T. cruzi encounters during its life cycle.
In mammals, L-Ser and L-Thr in plasma range from 100 to 200 μ M, values above the K_M
measured for L-Ser and L-Thr tran In mammals, L-Ser and L-Thr in plasma range from 100 to 200 μ M, values above the K_M measured for L-Ser and L-Thr transport (53–55). In triatomines, little is known about the abundance of these amino acids in differen In mammals, ESER and ETHT in plasma range from 100 to 200 μM, values above the KM
measured for L-Ser and L-Thr transport (53–55). In triatomines, little is known about the
abundance of these amino acids in different compa measured for these amino acids in different compartments of the digestive tract or in excreta.
However, it can be assumed that Epi could find an environment rich in amino acids for two
reasons: i) during the blood meal, th abundance of these amino acids that Epi could find an environment rich in amino acids for two
reasons: i) during the blood meal, the triatomine has high proteolytic activity, efficiently
breaking down whole blood proteins reasons: i) during the blood meal, the triatomine has high proteolytic activity, efficiently
breaking down whole blood proteins (mostly hemoglobin) and thus releasing free amino acids
that could be used by the parasite; a breaking down whole blood proteins (mostly hemoglobin) and thus releasing free amino acids
that could be used by the parasite; and ii) during the bloodmeal, the triatomine usually
releases amino acids into the intestinal that could be used by the parasite; and ii) during the bloodmeal, the triatomine usually
releases amino acids into the intestinal lumen through the Malpighian tubules (51,52).
L-Ser and L-Thr are fully catabolized to CO_2

L-Ser and L-Thr are fully catabolized to $CO₂$ and stimulate OxPhos in epimastigotes

that could be used by the parallel y and by the parallel y and securities, the interaction of $(51,52)$.
 L-Ser and L-Thr are fully catabolized to CO₂ and stimulate OxPhos in epimastigotes

Differently to Gly, L-Ser a L-Ser and L-Thr are fully catabolized to CO_2 and stimulate OxPhos in epimastigotes
Differently to Gly, L-Ser and L-Thr were fully catabolized to CO_2 and were able to sus
intracellular ATP levels and respiration when p Intracellular ATP levels and respiration when present as the only carbon source. It is worth
noting that the study of the participation of Gly was motivated by the possible existence of
two pathways leading to the product intracellular ATP levels and respiration of Gly was motivated by the possible existence of
two pathways leading to the production of CO_2 : i) a SHMT activity which could interconvert Gly
and 5,10-CH₂-THF into L-Ser and two pathways leading to the production of CO_2 : i) a SHMT activity which could interconvert Gly
and 5,10-CH₂-THF into L-Ser and THF (tetrahydrofolate) has been previously demonstrated in *T.*
cruzi (20,69); and ii) the and 5,10-CH₂-THF into L-Ser and THF (tetrahydrofolate) has been previously demonstrated in *T.*
cruzi (20,69); and ii) the existence in the *T. cruzi* genome of putative genes encoding the Gly
cleavage complex (GCC) (wi and 5,10-CH₂-THF into L-Ser and THF (tetrahydrofolate) has been previously demonstrated in T.
cruzi (20,69); and ii) the existence in the T. cruzi genome of putative genes encoding the Gly
cleavage complex (GCC) (without cleavage complex (GCC) (without demonstration of enzymatic functionality so far). The absence of CO₂ production from Gly rules out both possibilities in our experimental conditions.
Interestingly, the activity in *Leishm* absence of CO_2 production from Gly rules out both possibilities in our experimental conditions.
Interestingly, the activity in *Leishmania major* promastigotes was inferred from indirect assays,
in which the incorporati Interestingly, the activity in *Leishmania major* promastigotes was inferred from indirect assays,
in which the incorporation of Gly carbon into DNA was evidenced, demonstrating that Gly
indeed participates in one-carbon m In which the incorporation of Gly carbon into DNA was evidenced, demonstrating that Gly
indeed participates in one-carbon metabolism (69). Concerning L-Ser, the same work showed
that, when it was removed from the semi-defi indeed participates in one-carbon metabolism (69). Concerning L-Ser, the same work showed
that, when it was removed from the semi-defined culture medium (RPMI - 1% SFB) a critical
distribution of Gly Clyntae and Glyntae an that, when it was removed from the semi-defined culture medium (RPMI - 1% SFB) a critical
that, when it was removed from the semi-defined culture medium (RPMI - 1% SFB) a critical that, when it was removed from the semi-defined culture medium (RPMI - 1% S sFB) a critical condition (RPMI - \mathcal{R} SHMT would not compensate for L-Ser demand for optimal proliferation (69). These results are
in line with the absence of classical *de novo* L-Ser biosynthesis pathways in trypanosomatids. In
T. *brucei*, this complex may In line with the absence of classical *de novo* L-Ser biosynthesis pathways in trypanosomatids. In T. *brucei*, this complex may be essential since the parasite does not have SHMT and 10-formyl tetrahydrofolate synthetase T. *brucei*, this complex may be essential since the parasite does not have SHMT and 10-formyl
tetrahydrofolate synthetase annotated in the database of its genome, suggesting that it must
rely exclusively on GCC for folat T. brucei, this complex may be essential since the parasite does not have SHMT and 10-formyl
tetrahydrofolate synthetase annotated in the database of its genome, suggesting that it must
rely exclusively on GCC for folate tetrahy accurate symmetric sinteration in the database of its genome, suggesting that that
rely exclusively on GCC for folate metabolism. Finally, L-Ser was able to build up $\Delta\Psi_m$ in a way
that was dependent on the prev That was dependent on the previously characterized mitochondrial Pyr transporter (37,70), indicating that: i) in the cytosol L-Ser is converted into Pyr to be transported into the mitochondrion; or ii) it is transported i indicating that: i) in the cytosol L-Ser is converted into Pyr to be transported into the
mitochondrion; or ii) it is transported into the mitochondrion as L-Ser by the Pyr carrier if this
transporter has a broad specific mitochondrion; or ii) it is transported into the mitochondrion as L-Ser by the Pyr carrier if this
transporter has a broad specificity.
The *Trypanosoma cruzi* epimastigotes have Ser and Thr dehydratase activities
In most

The Trypanosoma cruzi epimastigotes have Ser and Thr dehydratase activities

mitochondrion; or in the mine conditions in a mineral minimizer of the mineral transporter has a broad specificity.
The Trypanosoma cruzi epimastigotes have Ser and Thr dehydratase activities
In most eukaryotic cells the c The *Trypanosoma cruzi* epimastigo
In most eukaryotic cells the cata
followed by deamination. In the ca
occurs in two steps: a cleavage Followed by deamination. In the case of L-Ser, the products are Pyr and NH_4^+ , and the reaction occurs in two steps: a cleavage of the hydroxyl group in a pyridoxal-5'-phosphate (PLP)-
dependent manner, forming aminoacr occurs in two steps: a cleavage of the hydroxyl group in a pyridoxal-5'
dependent manner, forming aminoacrylate, followed by its non-enzymatic
Pyr and NH₄⁺ (71,72). The dehydration/deamination of L-Thr occurs in three followed by deamination. In the case of L-Ser, the products are Pyr and NH_4^* , and the reaction phosphate (PLP)-
hydrolysis to form
steps in which the
nner, followed by
g 2-oxobutanoate dependent manner, forming aminoacrylate, followed by its non-enzymatic hydrolysis to form
Pyr and NH₄⁺ (71,72). The dehydration/deamination of L-Thr occurs in three steps in which the
dehydration reaction produces 2-a by and NH₄⁺ (71,72). The dehydration/deamination of L-Thr occurs in three steps in which the dehydration reaction produces 2-aminobut-2-enoate in a PLP-dependent manner, followed by the non-enzymatic formation of 2-im dehydration
the non-enzi
and NH₄⁺ (2!
that L-Thr ca
genome: L-T Pyr and NH₄⁺ (71,72). The dehydration/deamination of L-Thr occurs in three steps in which the reaction produces 2-aminobut-2-enoate in a PLP-dependent manner, followed by
matic formation of 2-iminobutanoate and a deamination forming 2-oxobutanoate
). Both, Ser/ThrDH activities were found in Epi soluble extracts. It the non-enzymatic formation of 2-iminobutanoate and a deamination forming 2-oxobutanoate
and NH₄⁺ (29). Both, Ser/ThrDH activities were found in Epi soluble extracts. It is worth noting
that L-Thr can serve as a subst and NH₄⁺ (29). Both, Ser/ThrDH activities were found in Epi soluble extracts. It is worth noting
that L-Thr can serve as a substrate for two other putative enzymes predicted in the *T. cruzi*
genome: L-Thr dehydrogena that L-Th
genome
coenzym
for aceta
mention and NH_4^+ (29). Both, Ser/ThrDH activities were found in Epi soluble extracts. It is worth noting r can serve as a substrate for two other putative enzymes predicted in the *T. cruzi*
L-Thr dehydrogenase (E.C. 1.1.1.10, TcCLB.507923.10) and a 2-amino-3-ketobutyrate
e A ligase (E.C. 2.3.1.29, TcCLB.511899.10), which ha that L-Thr can serve as a substrate for two other patative enzymes predicted in the T. cruzi
genome: L-Thr dehydrogenase (E.C. 1.1.1.10, TcCLB.507923.10) and a 2-amino-3-ketobutyrate
coenzyme A ligase (E.C. 2.3.1.29, TcCLB coenzyme A ligase (E.C. 2.3.1.29, TcCLB.511899.10), which have been demonstrated as crucial
for acetate production and fatty-acid biosynthesis in procyclics of *T. brucei* (19,73). It is worth
mentioning that acetate is a for acetate production and fatty-acid biosynthesis in procyclics of *T. brucei* (19,73). It is worth
mentioning that acetate is a main excreted product (together with alanine and succinate) of *T.*
cruzi metabolism when g for accetate production and ratty acid biosynthesis in procyclics of T. bruter (19,73). It is worth
mentioning that acetate is a main excreted product (together with alanine and succinate) of T.
cruzi metabolism when grown mentioning that acetate is a main excreted product (together with alanme and succhate) or T.

cruzi metabolism when grown in a rich medium (1,4,74,75). According to the results reported

herein, the catabolism of L-Ser can cruzi inctabolism when grown in a rich medium $(1,4,7,7,7)$. According to the results reported
herein, the catabolism of L-Ser can contribute to the production of acetyl-CoA and then excrete
acetate. Considering that i) t acetate. Considering that i) the mitochondrial acetate: succinate CoA transferase/succinyl-CoA synthetase (ASCT/SCS) cycle is operative in T. cruzi Epi (unpublished results, under study by our group), and ii) T. cruzi lack synthetase (ASCT/SCS) cycle is operative in T. cruzi Epi (unpublished results, under study by our group), and ii) T. cruzi lacks putative genes for an acetyl-CoA hydrolase (ACH, EC: 3.1.2.1), the production of acetate from synthetase (ASCT/SCS) cycle is operative in T. cruzi Epi (unpublished results, under study by our
group), and ii) T. cruzi lacks putative genes for an acetyl-CoA hydrolase (ACH, EC: 3.1.2.1), the
production of acetate from group), and ii) T. cruzi lacks putative genes for an acetyl-CoA hydrolase (ACH, EC: 3.1.2.1), the
production of acetate from L-Ser could be coupled to substrate-level production of
intramitochondrial ATP in addition to OxP intramitochondrial ATP in addition to OxPhos. Furthermore, the excretion of Gly by L-Ser fed
parasites suggests that Gly is excreted as an osmotic control mechanism (65,67).
The coupled to substrate of the coupled to subst parasites suggests that Gly is excreted as an osmotic control mechanism (65,67).

The excretion of M-Ser federal ATP in addition of Gly by L-Ser federal ATP in addition of Gly by L-Ser federal ATP in additional ATP in addi parasites suggests that Gly is excreted as an osmotic control mechanism (65,67).

Incubation of permeabilized cells that maintained the integrity of the mitochondrial and glycosomal membranes with this metabolite enabled the formation of a $\Delta\Psi_m$. This, together with the fact that this formation of $\$ glycosomal membranes with this metabolite enabled the formation of a $\Delta\Psi_m$. This, together
with the fact that this formation of $\Delta\Psi_m$ is impaired when the MPC is inhibited can be
explained by two different hypotheses: with the fact that this formation of $\Delta\Psi_m$ is impaired when the MPC is inhibited can be explained by two different hypotheses: i) $TcMPC$ would transport L-Ser in addition to Pyr; or ii) the MPC inhibitor UK-5099 inhibit explained by two different hypotheses: i) TcMPC would transport L-Ser in addition to Pyr; or ii)
the MPC inhibitor UK-5099 inhibits a mitochondrial carrier for L-Ser as well. It is important that
other subcellular location explained by two different hypotheses. If fervice would transport L-Ser in addition to Pyr, or ii)
the MPC inhibitor UK-5099 inhibits a mitochondrial carrier for L-Ser as well. It is important that
other subcellular locati the MPC inhibitor of the production of Pyr from L-Ser, such as glycosomes, cannot be
ruled out in intact cells since a peptide of the Ser/ThrDH was reported in the proteomic profile
of T. cruzi glycosomes and an analysis o out can consider the subcellular locations for the Ser/ThrDH was reported in the proteomic profile
of T. cruzi glycosomes and an analysis of the sequence revealed a PTS1 glycosomal-targeting
sequence (76).
CONCLUSION of T. cruzi glycosomes and an analysis of the sequence revealed a PTS1 glycosomal-targeting
sequence (76).
In conclusion, L-Ser, L-Thr and Gly can be taken up by the cells and contribute to the cellular
homogetoeic meinten

CONCLUSION

of T. cruzi glycosomes and an analysis of the sequence revealed a PTS1 glycosomal-targeting
sequence (76).
In conclusion, L-Ser, L-Thr and Gly can be taken up by the cells and contribute to the cellular
homeostasis mainten **CONCLUSION**
In conclusion, 1
homeostasis m
resumption of In the measure of the resumption of epimastigote proliferation. L-Ser and L-Thr can be further oxidized with the production of CO₂, triggering O₂ consumption, contributing to the maintenance of the inner mitochondrial From the maintenance during maturent energy, presuming entanties interest, yet and
resumption of CO_2 , triggering O_2 consumption, contributing to the maintenance of the inner
mitochondrial membrane potential and power production of CO_2 , triggering O_2 consumption, contributing to the maintenance of the inner mitochondrial membrane potential and powering ATP production through OxPhos. With the evidence collected throughout this work production of CO2, triggering O2 consumption, contributing to the maintenance of the mini-
mitochondrial membrane potential and powering ATP production through OxPhos. With the
evidence collected throughout this work, and evidence collected throughout this work, and in accordance with the data collected in the
literature and genetic databases, we propose a model for the energy metabolism of L-Ser and
L-Thr as shown in Figure 10. literature and genetic databases, we propose a model for the energy metabolism of L-Ser and L-Thr as shown in Figure 10. L-Thr as shown in Figure 10. $\frac{1}{2}$. L-Thr as shown in Figure 10.

 Figure 10. Metabolism of L-Ser and L-Thr in Trypanosoma cruzi. Metabolic steps are
represented with different colors according to the origin of data: green-labeled steps
form the literature; blue-labeled steps correspond t represented that diated in this work; yellow-labeled steps correspond to data obtained
from the literature; blue-labeled steps correspond to inferred reactions according to
annotations of the *T. cruzi* genome. Plasma-memb Example in the literature; blue-labeled steps correspond to inferred reactions according to
annotations of the T. cruzi genome. Plasma-membrane H⁺-ATPase (68); Pyruvate
Dehydrogenase (PDH) (77); mitochondrial electron-tr annotations of the *T. cruzi* genome. Plasma-membrane H^{*}-ATPase (68); Pyruvate
Dehydrogenase (PDH) (77); mitochondrial electron-transfer complexes (CI-CIV) (35,78,79);
alternative oxidase (AOX) (TcCLB.504147.180) It is annotations of the T. *cruzi* genome. Plasma-membrane H
Dehydrogenase (PDH) (77); mitochondrial electron-transfer comp
alternative oxidase (AOX) (TcCLB.504147.180) It is important to r
identification of a putative gene fo Exes (CI-CIV) (35,78,79);

mention that despite the

cor (antimycin A) abolish

5,78); adenine nucleotide

05.50) (14,81); serine

1); Branched-chain alpha-

Tc001047053506295.160;

7757.70); L-Threonine 3-

coenzyme A li Examples and a putative gene for an AOX, the complex III inhibitor (antimycin A) abolish
99.9% of the respiration in *T. cruzi* Epi (35,78); F₁F₀-ATP synthase (35,78); adenine nucleotide
translocase (ANT) (TcCLB.51124 alternative orientation of a putative gene for an AOX, the complex III inhibitor (antimycin A) abolish
99.9% of the respiration in *T. cruzi* Epi (35,78); F₁F_o-ATP synthase (35,78); adenine nucleotide
translocase (ANT) 99.9% of the respiration in *T. cruzi* Epi (35,78); F₁F_o-ATP synthase (35,78); adenine nucleotide
translocase (ANT) (TcCLB.511249.10) (80); SPT (TcCLB.506405.50) (14,81); serine
acetyltransferase and cysteine synthase 99.9% of the respiration in T. cruzi Epi (35,78); F11.6 ATT synthase (35,78); adenine naccocide
translocase (ANT) (TcCLB.511249.10) (80); SPT (TcCLB.506405.50) (14,81); serine
acetyltransferase and cysteine synthase (SAT/ transferase and cysteine synthase (SAT/CS) (82,83); SHMT (21); Branched-chain alpha-
acetyltransferase and cysteine synthase (SAT/CS) (82,83); SHMT (21); Branched-chain alpha-
aceto acid dehydrogenase complex (BCAKDH) (Tc keto acid dehydrogenase (TDH) (TcCLB.507923.10); 2-amino-3-ketobutyrate coenzyme A ligase (AKL)
dehydrogenase (TDH) (TcCLB.507923.10); 2-amino-3-ketobutyrate coenzyme A ligase (AKL)
(TcCLB.511899.10); acetate:succinate Co The Hydrogenase (TDH) (TcCLB.507923.10); 2-amino-3-ketobutyrate coenzyme A ligase (AKL)
(TcCLB.511899.10); acetate:succinate CoA transferase (ASCT) (TcCLB.504153.360); succinyl-
CoA synthetase (SCS) (α: TcCLB.508479.340; (TcCLB.511899.10); acetate:succinate CoA transferase (ASCT) (TcCLB.504153.360); succinyl-
CoA synthetase (SCS) (α : TcCLB.508479.340; β : TcCLB.507681.20).
MATERIALS AND METHODS

CON SYNTHETAS AND METHODS
CONSTRUES AND METHODS MATERIALS AND METHODS

Reagents. L-[3-²⁺²C]Ser, L-[U-²⁺²C]Thr and [U-²⁺²C]Gly (0.1 mCi/ml) were purchased from American
Radiolabeled Chemicals, Inc. (ARC [St. Louis, MO]). All other reagents were from Sigma (St.
Louis, MO).
Parasites. *T.* Louis, MO).
 Parasites. *T. cruzi* CL strain clone 14 epimastigotes were maintained in the exponential growth

phase by subculturing them every 48 h in liver infusion tryptose (LIT) medium supplemented

with 10% fetal c Louis, MO).
Parasites. 7
phase by su
with 10% f_i
parasites we
1.47 mM Kl phase by subculturing them every 48 h in liver infusion tryptose (LIT) medium supplemented
with 10% fetal calf serum (FCS) at 28 °C (84). For transport assays, exponentially growing
parasites were washed three times with with 10% fetal calf serum (FCS) at 28 °C (84). For transport assays, exponentially growing
parasites were washed three times with PBS (137 mM NaCl, 2.68 mM KCl, 8 mM Na₂HPO₄, and
1.47 mM KH₂PO₄, pH 7.2) and resusp parasites were washed three times with PBS (137 mM NaCl, 2.68 mM KCl, 8 mM Na₂HPO₄, and 1.47 mM KH₂PO₄, pH 7.2) and resuspended to a final density of 2 × 10⁸ cells/ml in PBS. To evaluate the ability of epimastig 1.47 mM KH₂PO₄, pH 7.2) and resuspended to a final density of 2 × 10⁸ cells/ml in PBS. To evaluate the ability of epimastigotes to use L-Ser, L-Thr and Gly in order to resist severe metabolic stress and to use it as 1.47 mM KH₂PO₄, pH 7.2) and resuspended to a final density of 2×10^8
evaluate the ability of epimastigotes to use L-Ser, L-Thr and Gly in ord
metabolic stress and to use it as an energy source, parasites in the ex Example 12 Service 2014

ential growth phase

parasites/ml) were

e same buffer. After

ncentration (5 mM) evaluate the ability of epimalogene to use L-Ser, L-M and L-f in the exponential growth phase
 $(5 \times 10^7 \text{ parasites per ml obtained from a 24h culture started at 2.5} \times 10^7 \text{ parasites/ml})$ were

washed twice in 1 volume of PBS and incubated for 16h in 1 volume of the s (5 \times 10⁷ parasites per ml obtained from a 24h culture started at 2.5 \times 10⁷ parasites/ml) were washed twice in 1 volume of PBS and incubated for 16h in 1 volume of the same buffer. After incubation, L-Ser, L-Thr (5×10^{7})
washed
incubati
for its u
viability,
Transpo parasites per ml obtained from a 24h culture started at 2.5×10^7
twice in 1 volume of PBS and incubated for 16h in 1 volume of the
on, L-Ser, L-Thr or Gly was added to the cultures at a saturating co
ptake, and differ parasites, mi, 1999
and buffer. After
mentration (5 mM)
inined, including cell
mbrane potential.
ily (31,32,34,62,63). incubation, L-Ser, L-Thr or Gly was added to the cultures at a saturating concentration (5 mM)
for its uptake, and different parameters of energy metabolism were determined, including cell
viability, ATP production, oxygen

for its uptake, and different parameters of energy metabolism were determined, including cell
viability, ATP production, oxygen consumption, and mitochondrial inner membrane potential.
Transport assays. Transport assays For its upper viability, ATP production, oxygen consumption, and mitochondrial inner membrane potential.
 Transport assays. Transport assays were performed as described previously (31,32,34,62,63).

Transport assays wer **Transport assays**. Transport assays were performed as described previously (31,32,34,62,63)
Transport assays were initiated by the addition of 100 μ l of 5 mM L-Ser, L-Thr or Gly in PBS to
aliquots of parasites of 100 Transport assays. Transport assays were performed as deserbed previously (31,32,34,62,63).
Transport assays were initiated by the addition of 100 μ l of 5 mM L-Ser, L-Thr or Gly in PBS to
aliquots of parasites of 100 $\$ aliquots of parasites of 100 μ l (2×10^7 cells each, except when otherwise specified), traced with 0.4 μ Ci of radiolabeled amino acid. The uptake was measured at 28 °C for 1 min, except when otherwise specified. aliquots of parasites of 100 μ l (2 × 10⁷
with 0.4 μ Ci of radiolabeled amino acid.
when otherwise specified. The transpor
solution (50 mM amino acid in PBS, pH
washes with cold PBS. Background v
simultaneous additi The uptake was measured at 28 °C for 1 min, except

t reaction was stopped by addition of 800 μ l of stop

7.4) prechilled at 4 °C, immediately followed by two

values in each experiment were measured by the

ino acid a when otherwise specified. The transport reaction was stopped by addition of 800 µl of stop
solution (50 mM amino acid in PBS, pH 7.4) prechilled at 4 °C, immediately followed by two
washes with cold PBS. Background values solution (50 mM amino acid in PBS, pH 7.4) prechilled at 4 °C, immediately followed by two
washes with cold PBS. Background values in each experiment were measured by the
simultaneous addition of each traced amino acid an

washes with cold PBS. Background values in each experiment were measured by the
simultaneous addition of each traced amino acid and stop solution.
Competition assays. Competition assays were performed by measuring L-Ser simultaneous addition of each traced amino acid and stop solution.
 Competition assays. Competition assays were performed by measuring L-Ser, L-Thr or Gly

uptake at a concentration equivalent to the K_M in the presenc **Competition assays**. Competition assays were performed by me
uptake at a concentration equivalent to the K_M in the presence c
other amino acid (29). Briefly, 100-µl aliquots of parasites cont
incubated with the transpo Lot the about assays. Completion assays were performed by measuring L-Ser, L-Thr or Gly
uptake at a concentration equivalent to the K_M in the presence of 10 times the K_M of each
other amino acid (29). Briefly, 100-µl other amino acid (29). Briefly, 100-µl aliquots of parasites containing 2×10^7 cells were
incubated with the transport solution supplemented with the presumably competing
metabolite for 1 min. The results obtained wer other amino acid (29). Briefly, 100-µl aliquots of parasites containing 2 × 10'
incubated with the transport solution supplemented with the presumably
metabolite for 1 min. The results obtained were expressed as inhibitio competing
s in relation
evaluated in
hlorophenyl

incular the transportance with the transportance in the transport of a control (the same experiment without the competitor).
The effect of a H⁺-dependent plasma membrane potential on the L-Ser uptake was evaluated in par The effect of a H⁺-dependent plasma membrane potential on the L-Ser uptake was evaluated in
parasites treated with 0.5 μ M of the protonophore carbonyl cyanide *m*-chlorophenyl
hydrazone (CCCP). As previously reported The effect of a H⁺-dependent plasma membrane potential or
parasites treated with 0.5 μ M of the protonophore or
hydrazone (CCCP). As previously reported, protonophore
process due to the disruption of the H⁺ gradient The effect of a H
parasites treated
hydrazone (CCCP
process due to tl d with 0.5 μ M of the protonophore carbonyl cyanide *m*-chlorophenyl

(). As previously reported, protonophore treatment can affect an uptake

the disruption of the H⁺ gradient across cellular membranes (if the uptake parasites treated with 0.5 µM of the protonophore carbonyl cyanide *m*-chlorophenyl
hydrazone (CCCP). As previously reported, protonophore treatment can affect an uptake
process due to the disruption of the H⁺ gradient a process due to the disruption of the H^{*} gradient across cellular membranes (if the uptake is process due to the disruption of the H^{*} gradient across cellular membranes (if the uptake is process due to the disruption of the H+ gradient across cellular membranes (if the uptake is performed through a H⁺
due to its rapid consu
mitochondrial-membrar
mitochondrial inner me
performed with the ad
simultaneous disruption umption by the mitochondrial F_1F_0 -ATP synthase, which in a low-
ne-potential situation hydrolyzes ATP to pump H⁺ to reestablish the
mbrane potential. To discriminate between both effects, a control was
dition of 5 mitochondrial-membrane-potential situation hydrolyzes ATP to pump H⁺ to reestablish the
mitochondrial inner membrane potential. To discriminate between both effects, a control was
performed with the addition of 5 µg/mL mitochondrial-membrane-potential situation hydrolyzes ATP to pump H⁺
mitochondrial inner membrane potential. To discriminate between both ef
performed with the addition of 5 μ g/mL oligomycin A to FCCP-treated c
simul fects, a control was
ells, which allowed
ATPase.
) corresponding to
were calculated as

performed with the addition of 5 μ g/mL oligomycin A to FCCP-treated cells, which allowed
simultaneous disruption of H⁺ membrane gradients while blocking the F₁F₀-ATPase.
Analysis of transport assay data. The di simultaneous disruption of H⁺ membrane gradients while blocking the F_1F_0 -ATPase.
Analysis of transport assay data. The disintegrations per minute (*dpm*) corresponding to transported radiolabeled amino acid for ea simultaneous disruption of H⁺
Analysis of transport assay
transported radiolabeled ami
dpmi = dpme – dpmb, whe
incubation in the presence of
background samples. **data**. The disintegrations per minute (dpm) corres
ino acid for each experimental point (dpm) were care
dependent of the average dpm from triplicates after adiolabeled amino acid and dpmb is the average dp transported radiolabeled amino acid for each experimental point (*dpmi*) were calculated as $dpm = dpm = -dpm$, where $dpm = i$ the average dpm from triplicates after 1 min of incubation in the presence of radiolabeled amino acid transported radiolabeled amino acid for each experimental point (dpmi) were calculated as
dpmi = dpme – dpmb, where dpme is the average dpm from triplicates after 1 min of
incubation in the presence of radiolabeled amino

dpmi = dpme = dpmb, where dpme is the average dpm from thphcates after 1 min of
incubation in the presence of radiolabeled amino acid and *dpmb* is the average *dpm* from the
background samples.
The amount of amino acid t background samples.
The amount of amino acid taken up by the cells was calculated as $AAi = dpmi [AA]v \ dpm^{1}t^{-1}$,
where AAi is the transported amino acid, [AA] is the amino acid nanomolar concentration, v is
the volume of rad The amount of amino
where *AAi* is the trans
the volume of radiol:
measured for each ac
in minutes. The amount of amino acid taken up by the cells was calculated as AAi = apmi [AA] v apmst−1 t−1,
where AAi is the transported amino acid, [AA] is the amino acid nanomolar concentration, v is
the volume of radiolabeled amino where AAI is the transported amino acid, [AA] is the amino acid nanomolar concentration, v is
the volume of radiolabeled amino acid solution in the experiment, *dpmst* is the total *dpm*
measured for each added radiolabele

measured for each added radiolabeled amino acids, and t is the time of incubation measured
in minutes.
Estimation of the number of transporters by using the Arrhenius equation.
Thermodynamically, a transporter is an enz in minutes.
 Estimation of the number of transporters by using the Arrhenius equation.

Thermodynamically, a transporter is an enzyme that catalyzes the reaction AA_e => AA_i , where
 AA_e is the extracellular amino acid minutes.
Estimation
Thermodyn:
AA_e is the comminutes of
calculated a Thermodynamically, a transporter is an enzyme that catalyzes the reaction AA_e => AA_i , where AA_e is the extracellular amino acid and AA_i is the intracellular amino acid. To estimate the number of transport systems, the AA_e is the extracellular amino acid and AA_i is the intracellular amino acid. To estimate the
number of transport systems, the turnover of active sites (k_{cat}) must be measured. k_{cat} is
calculated as: $k_{cat} = V_{max}/no$. number of transport systems, the turnover of active sites (k_{cat}) must be measured. k_{cat} is calculated as: $k_{cat} = V_{max}/no$. of transporters (85). Our hypothesis posits that, at saturated substrate concentration, the rate-li calculated as: $k_{\text{cat}} = V_{\text{max}}/n$ o. of transporters (85). Our hypothesis posits that, at saturated
substrate concentration, the rate-limiting step of the system is dissociation. Consequently, the
Arrhenius equation can b substrate concentration, the rate-limiting step of the system is dissociation. Consequently, the
substrate concentration, the rate-limiting step of the system is dissociation. Consequently, the
Arrhenius equation can be e Arrhenius equation can be employed to estimate the number of transporter sites at a given
temperature as follows:
no. of transporters: Et = $\frac{V_{max}}{Ae^{-\frac{E_a}{RT}}} = \frac{V_{max}}{K_{cat}}$
where V_{max} is the maximum rate achieved by t

no. of transporters: Et =
$$
\frac{V_{max}}{Ae - \frac{E_a}{RT}} = \frac{V_{max}}{K_{cat}}
$$

Arrhender equation can be employed to the number of transporters $\text{Et} = \frac{V_{max}}{Ae^{-\frac{E_a}{RT}}} = \frac{V_{max}}{K_{cat}}$
where V_{max} is the maximum rate achieved by the system at saturating substrate concentration
at a given temperatur temperature as follows:
where V_{max} is the maxim
at a given temperature,
reaction, R is the univers no. of transporters: $Et = \frac{-ma}{Ae^{-\frac{1}{I}}}$
rate achieved by the system and is the pre-exponential factor
as constant, and *T* is the temp
Thr in the life cycle forms of E_a
at
at t
C
Z

at a given temperature, Ae is the pre-exponential factor, E_a is the activation energy for the reaction, R is the universal gas constant, and T is the temperature of the reaction (in Kelvin).
The transport of L-Ser and L reaction, *R* is the universal gas constant, and *T* is the temperature of the reaction (in Kelvin).
The transport of L-Ser and L-Thr in the life cycle forms of *T. cruzi.* After obtaining the different
forms as described The transport of L-Ser and L-Thr in the life cycle forms of *T. cruzi.* After obtaining the differe forms as described in references (33,34), the cells were resuspended in PBS and counted usi
a Neubauer chamber. The cell Forms as described in references (33,34), the cells were resuspended in PBS and counted using
a Neubauer chamber. The cell density was adjusted to a final density of 20 x 10⁷ cells/mL and
distributed in aliquots of 100 a Neubauer chamber. The cell density was adjusted to a final density of 20 x 10⁷ cells/mL and distributed in aliquots of 100 µl (2 x 10⁷ cells each). The transport assay was initiated by adding distributed in aliquots a Neubauer chamber. The cell density was adjusted to a final density of 20 x 10⁷
distributed in aliquots of 100 µl (2 x 10⁷ cells each). The transport assay was initiat
distributed in aliquots of 100 µl (2 x 10⁷ cel cells, manufactured by adding distributed in aliquots of 100 µl (2 x 10 $^{\prime}$ cells each). The transport assay was initiated by adding 100 µl of 10 mM L-Ser or L-Thr labeled with L- [3-²⁻⁻C]Ser or L-[U-²⁻⁻C]Thr, respectively, prepared
in PBS, pH 7.2. The initial velocity (V_0) of the incorporation was measured at 28 °C (Epi and
MTrypo) and 37 °C (A In 195, pm 7.2. The initial velocity (V₀) of the incorporation was incasated at 28 °C (Epi and MTrypo) and 37 °C (Ama, Epi-like, and BTrypo) for 3 min. In all cases, the uptake of labelled amino acid was stopped by addi amino acid was stopped by adding 800 µl of 50 mM of the corresponding unlabeled amino acid
at 4 °C in PBS, and the parasites were washed by centrifugation at 10.000 x *g* for 2 min. The
parasites were then resuspended in at 4 °C in PBS, and the parasites were washed by centrifugation at 10.000 x g for 2 min. The
parasites were then resuspended in 100 µl of PBS and transferred to scintillation fluid. The
samples were measured in a Perkin E at 4 °C in PBS, and the parasites were washed by centrifugation at 10.000 x g for 2 min. The
parasites were then resuspended in 100 µl of PBS and transferred to scintillation fluid. The
samples were measured in a Perkin E

paration with then results in 200 pm of the results in anti-surface to detector to determine
samples were measured in a Perkin Elmer Tri-Carb 2910 TR scintillation detector to determine
the V_0 in each form.
The contribu samples were measured in a Perkin Elmer Carb 2019. We cannot a severe nutritional stress.
The contribution of L-Ser, L-Thr or Gly to recover cells subjected to a severe nutritional stress.
To determine whether L-Ser, L-Th The contribution of
To determine wheth
after a starvation pe
described above. Bri
h at 28°C in PBS plu To determine whether L-Ser, L-Thr or Gly can maintain the viability of epimastigotes of T. cruzi
after a starvation period, the parasites were exponentially grown in LIT and stressed in PBS as
described above. Briefly, the To determine whether L-Ser, L-Thr or Gly can maintain the viability or epimastigotes or T. cruzinafter a starvation period, the parasites were exponentially grown in LIT and stressed in PBS as described above. Briefly, th described above. Briefly, the epimastigotes $(5 \times 10^7 \text{ cells})$ were incubated for 24, 48, 72 and 96 h at 28°C in PBS plus 5 mM L-Ser, L-Thr or Gly to induce cell recovery. Separate treatments in histidine or PBS were used a described above. Briefly, the epimastigotes (5 × 10'
h at 28°C in PBS plus 5 mM L-Ser, L-Thr or Gly to in
histidine or PBS were used as controls. After rec
incubated with resazurin reagent to evaluate cell via
Epi forms su duce cell recovery. Separate treatments in
overy, the cells were washed in PBS and
ability, as previously described (86).
in PBS plus 5 mM L-Ser, L-Thr or Gly and
ilc, positive control) were transferred to LIT

histidine or PBS were used as controls. After recovery, the cells were washed in PBS and
incubated with resazurin reagent to evaluate cell viability, as previously described (86).
Epi forms subjected to nutritional stress incubated with resazurin reagent to evaluate cell viability, as previously described (86).
Epi forms subjected to nutritional stress for 72h in PBS plus 5 mM L-Ser, L-Thr or Gly and
incubated in PBS-HG (PPB + 5 mM L-His a Epi forms subjected to nutritional stress for 72h in PBS plus 5 mM L-Ser, L-Thr or incubated in PBS-HG (PPB + 5 mM L-His and 5 mM Glc, positive control) were transferre medium at a final density of 2.5 x 10^6 parasites/ incubated in PBS-HG (PPB + 5 mM L-His and 5 mM Glc, positive control) were transferred to LIT
medium at a final density of 2.5 x 10⁶ parasites/mL, and then incubated 200 µL per well
(experimental triplicates) in 96-well inculture in PBS-HG (PPB + 5 mm PHS-hm and 21, positive control) were intuited 200 µL per well

(experimental triplicates) in 96-well plates at 28 °C. The plates were read using an ELx800

Absorbance Reader® (Bio Tek®) wh medium at a final density of 2.5 x 10°
(experimental triplicates) in 96-well plat
Absorbance Reader® (Bio Tek®) where th
620 nm. Using a calibration curve with kn
scattering by the culture (optical density,
Quantification tes at 28 °C. The plates were read using an ELx800
e dispersion of light by the culture was measured at λ
own parasite concentrations, assay values of light
OD) were converted to number of parasites/mL.
onential growth p Absorbance Reader® (Bio Tek®) where the dispersion of light by the culture was measured at λ 620 nm. Using a calibration curve with known parasite concentrations, assay values of light scattering by the culture (optica

Absorbance Reader and SO μ
scattering by the culture (optical density, OD) were converted to number of parasites/mL.
Quantification of ΔΨ_m. Parasites in exponential growth phase (~4-5x10⁷ parasites/mL) were
cent Scattering by the culture (optical density, OD) were converted to number of parasites/mL.
 Quantification of $\Delta\Psi_m$ **.** Parasites in exponential growth phase (~4-5x10⁷ parasites/mL) were

centrifuged at 1,000 x *g* for **Quantification of** $\Delta\Psi_m$ **.** Parasites in exponential growth phase (~4-5x10⁷ parasites/mL)
centrifuged at 1,000 x g for 10 min at 4 °C and washed twice in $\Delta\Psi_m$ buffer (250 mM suc
10 mM HEPES, 250 µM EGTA, 2 mM NaH₂ **Quantification of ΔΨ_m.** Parasites in exponential growth phase (~4-5x10² centrifuged at 1,000 x *g* for 10 min at 4 °C and washed twice in $\Delta\Psi_m$ buffer 10 mM HEPES, 250 μM EGTA, 2 mM NaH₂PO₄, 1 mM MgCl₂ pH 7.2 er (250 mM sucrose,
s were adjusted to a
sermeabilized in the
d (EX WL: 495.0 nm,
r Hitachi High-Tech). centringed at 1,000 x g for 10 min at 4 °C and washed twice in Δ 4_m buffer (250 mm sucrose,
10 mM HEPES, 250 μM EGTA, 2 mM NaH₂PO₄, 1 mM MgCl₂ pH 7.2). Cells were adjusted to a
density of 1x10⁹ parasites/mL density of 1x10⁹ parasites/mL. Aliquots of 50 µL were separated and permeabilized in the
presence of 5 µM digitonin for 30 min. Safranin fluorescence was measured (EX WL: 495.0 nm,
EM WL: 586.0 nm) in a cuvette fluorome density of 1x10° parasites/mL. Aliquots of 50 µL were separated and permeabilized in the
presence of 5 µM digitonin for 30 min. Safranin fluorescence was measured (EX WL: 495.0 nm,
EM WL: 586.0 nm) in a cuvette fluoromete EM WL: 586.0 nm) in a cuvette fluorometer (F-7100 FL Spectrophotometer Hitachi High-Tech).
Cells (5x10⁷ cells) were added to the cuvette in 2 mL $\Delta\Psi_m$ buffer, 0.1% BSA (free of fatty acids),
12.5 µM safranin and 5 µM Cells (5x10⁷ cells) were added to the cuvette in 2 mL $\Delta\Psi_m$ buffer, 0.1% BSA (free of fatty acids), 12.5 µM safranin and 5 µM digitonin. The calibration curve was constructed under the same conditions as mentioned bef Cells (5x10⁷
12.5 µM sa
conditions
using KCl. F
values were Franin and 5 μ M digitonin. The calibration curve was constructed under the same
as mentioned before. Then, 5 nM valinomycin was added, followed by titration
inally, 1.25 μ M FCCP was added to fully uncouple the membr conditions as mentioned before. Then, 5 nM valinomycin was added, followed by titration using KCl. Finally, 1.25 μ M FCCP was added to fully uncouple the membrane potential. The values were adjusted to the Nernst equati using KCl. Finally, 1.25 μ M FCCP was added to fully uncouple the membrane potential. The
values were adjusted to the Nernst equation to estimate $\Delta\Psi$ m. values were adjusted to the Nernst equation to estimate $\Delta\Psi$ m. values were adjusted to the Nernst equation to estimate ΔΨm.

Nernst equation: ΔΨm = 60 × log(K_{IN}
 netabolites by ¹H-NMR analysis. Epi

: 1.400 x *g* for 10 min, washed twice

1 2 g/L NaHCO₃ (pH 7.4). The cells we

ng 5 mM L-Ser or no exogenous cark

on was checked by micro $/$ K_{OUT}
mastige
with PI
re mair
pon sou
ervatior)
ס'Bit
ווי Quantification of excreted metabolites by 1 H-NMR analysis. Epimastigotes (1x10⁸/mL) were H-NMR analysis. Epimastigotes $(1x10^{\circ})$
0 min, washed twice with PBS and incu
pH 7.4). The cells were maintained for θ
or no exogenous carbon sources. The i
by microscopic observation. The supe
ution in D₂O $(10 \text{$ bated in 1
5h at 27 °C
ntegrity of
rrnatant (1
an internal mL of PBS supplemented with 2 g/L NaHCO₃ (pH 7.4). The cells were maintained for 6h at 27 °C
in incubation buffer containing 5 mM L-Ser or no exogenous carbon sources. The integrity of
the cells during the incubation wa in incubation buffer containing 5 mM L-Ser or no exogenous carbon sources. The integrity of
the cells during the incubation was checked by microscopic observation. The supernatant (1
mL) was collected and 50 µl of maleate in incubation buffer containing 5 mm L-Ser or an angular cancel contains including the cells during the incubation was checked by microscopic observation. The supernatant (1 mL) was collected and 50 μ of maleate soluti the cells during the included by microscopic order that the supernation of μ mml) was added as an internal reference. ${}^{1}H$ -NMR spectra were collected at 500.19 MHz on a Bruker Avance III 500 HD spectrometer equipped meference. ¹H-NMR spectra were collected at 500.19 MHz on a Bruker Avance III 500 HD
spectrometer equipped with a 5 mm Prodigy cryoprobe. The measurements were recorded at
25 °C. The acquisition conditions were as follow reference.
spectromete
25 °C. The a
memory size
scans for a t
were integra Example 2014 The Contract at 2006. The measurements were recorded at cquisition conditions were as follows: 90° flip angle, 5.000 Hz spectral width, 32 K e, and 9.3 sec total recycling time. The measurements were performed 25 °C. The acquisition conditions were as follows: 90° flip angle, 5.000 Hz spectral width, 32 K
memory size, and 9.3 sec total recycling time. The measurements were performed with 64
scans for a total time of close to 10 25 The acquisition condition condition condition condition conditions were antegrated, and the metabolite concentrations were calculated using the ERETIC2 NMR quantification Bruker program.
ATP biosynthesis dependency of L memory size, and the term (expansion in and 30 sec. The resonances of the obtained spectra
were integrated, and the metabolite concentrations were calculated using the ERETIC2 NMR
quantification Bruker program.
ATP biosy

scans for a total time of the total time of the form of close to the closing the ERETIC2 NMR
quantification Bruker program.
ATP biosynthesis dependency of L-Ser, L-Thr and Gly. To evaluate ATP production with L-Ser, L-
 quantification Bruker program.
 ATP biosynthesis dependency of L-Ser, L-Thr and Gly. To evaluate ATP production with L-Ser, L-

Thr or Gly as their sole energy source, the parasites (approximately 5×10^7 cells/mL) w quantification Bruker program.

ATP biosynthesis dependency of

Thr or Gly as their sole energy

starved as described above and

presence of 5 mM His (as posit

concentration in each sample Thr or Gly as their sole energy source, the parasites (approximately 5×10^7 cells/mL) were
starved as described above and recovered or not (negative control) by incubation for 1 h in the
presence of 5 mM His (as posit Thr or Gly as their sole energy source, the parasites (approximately 5 \times 10'
starved as described above and recovered or not (negative control) by incubati
presence of 5 mM His (as positive controls) or 5 mM L-Ser, L-T on for 1 h in the
ntracellular ATP
luciferase assay
re estimated by
) was detected presence of 5 mM His (as positive controls) or 5 mM L-Ser, L-Thr or Gly. The intracellular ATP concentration in each sample was determined after recovery by using a luciferase assay according to the manufacturer's instruc concentration in each sample was determined after recovery by using a luciferase assay
according to the manufacturer's instructions (Sigma). ATP concentrations were estimated by
using a calibration curve (ATP disodium sal according to the manufacturer's instructions (Sigma). ATP concentrations were estimated by
using a calibration curve (ATP disodium salt, Sigma); luminescence $(\lambda_{570}$ nm) was detected
using a SpectraMax i3 plate reader (

using a calibration curve (ATP disodium salt, Sigma); luminescence (λ_{570} nm) was detected
using a SpectraMax i3 plate reader (Molecular Devices, Sunnyvale, CA).
CO₂ production measurements. To measure the CO₂ p using a SpectraMax i3 plate reader (Molecular Devices, Sunnyvale, CA).
 CO₂ production measurements. To measure the CO₂ production from the TCA cycle during L-

Ser, L-Thr or Gly catabolism, epimastigotes exponentia CO₂ production measurements. To measure the CO_2 production from
Ser, L-Thr or Gly catabolism, epimastigotes exponentially growing in LI⁻
were washed twice, resuspended in PBS and incubated in 5 mM L-Ser,
0.1 µCi of Ser, L-Thr or Gly catabolism, epimastigotes exponentially growing in LIT (5 \times 10⁷ parasites/mL) were washed twice, resuspended in PBS and incubated in 5 mM L-Ser, L-Thr or Gly spiked with 0.1 µCi of radiolabeled amin Ser, L-Thr or Gly catabolism, epimastigotes exponentially growing in LIT (5 × 10' parasites/mL)
were washed twice, resuspended in PBS and incubated in 5 mM L-Ser, L-Thr or Gly spiked with
0.1 µCi of radiolabeled amino aci 0.1 µCi of radiolabeled amino acid for 0.5, 1, 2, 3 and 4h at 28 °C. To trap the produced CO₂, pieces of Whatman filter embedded in 2 M KOH were placed on the top of the tubes in which the parasites were incubated. The pieces of Whatman filter embedded in 2 M KOH were placed on the top of the tubes in which
the parasites were incubated. The filters were recovered and mixed with scintillation cocktail,
and the $K_2^{14}CO_3$ trapped on the the parasites were incubated. The filters were recovered and mixed with scintillation cocktail,
and the $K_2^{14}CO_3$ trapped on the paper was measured by using a scintillation counter. To assess
the comparative release of and the $K_2^{14}CO_3$ trapped on the paper was measured by using a scintillation counter. To assess
the comparative release of carbon as CO_2 , the measured values were normalized against the
quantity of the transported am and the K₂⁻⁻CO₃ trapped on the paper was measured by using a scintillation counter. To assess
the comparative release of carbon as CO₂, the measured values were normalized against the
quantity of the transported a

quantity of the transported amino acid over an identical incubation period.
 Oxygen consumption. To evaluate respiration rates from the degradation of L-Ser, L-Thr and Gly, Epi (approximately 2.5 x 10^7 cells/mL) were Qxygen consumption. To evaluate respiration rates from the degradation
Gly, Epi (approximately 2.5 x 10⁷ cells/mL) were nutritionally stressed for Gly, Epi (approximately 2.5 x 10^7 cells/mL) were nutritionally stressed for 16h in PBS at 28 °C Gly, L-Thr and Gly, Epi (approximately 2.5 x 10^7 cells/mL) were nutritionally stressed for 16h in PBS at 28 °C Gly, Epi (approximately 2.5 x 10'
The control
The control of the control of the control of the control of the control
 \sim 2.5 x 10' cells/mL) were nutritionally stressed for 16h in PBS at 28 °C and recovered a state (negative control) or 5 mM L-Ser, L-Thr and Gly as the only exogenous carbon sources. The parasites
were added to the respiration buffer (MCR: 125 mM sucrose, 65 mM KCl, 10 mM HEPES NaOH,
1 mM MgCl₂ were added to the respiration buffer (MCR: 125 mM sucrose, 65 mM KCl, 10 mM HEPES NaOH,
1 mM MgCl₂, 2 mM K₂HPO₄, pH 7.2). Subsequently, oligomycin A and FCCP were sequentially
titrated. To verify residual respiration 1 mM MgCl₂, 2 mM K₂HPO₄, pH 7.2). Subsequently, oligomycin A and FCCP were sequentially titrated. To verify residual respiration, a mitochondrial complex III inhibitor, antimycin A was added. Oxygen consumption rate 1 mM MgCl2, 2 mM MgCl2, 2 mM MgCl2, 2 mM MgCl2, 1
1 minuted. To verify residual respiration, a mitochondrial complex III inhibitor, antimycin A was
1 added. Oxygen consumption rates were measured using intact cells in the added. Oxygen consumption rates were measured using intact cells in the high-resolution
Oxygraph (OROBOROS, Oxygraph-2k, Innsbruck, AU). Data were recorded and treated with
DataLab 8 software.
Statistical analysis. Curve a

added. Oxygraph (OROBOROS, Oxygraph-2k, Innsbruck, AU). Data were recorded and treated with
DataLab 8 software.
Statistical analysis. Curve adjustments, regressions, and statistical analysis were performed
with the Graph Datalab 8 software.
 Statistical analysis. Curve adjustments, regressions, and statistical analysis were performed

with the GraphPad Prism 10 analysis tools. All assays were performed at least in biological

triplicates **Statistical analysis.**
Statistical analysis.
with the GraphPad
triplicates, and the d Statistical analysis. Curve adjustments, regressions, and statistical analysis were performed
with the GraphPad Prism 10 analysis tools. All assays were performed at least in biological
triplicates, and the details of stat with triplicates, and the details of statistical analysis were added to each figure legend.
Acknowledgements
Author contributions

Acknowledgements

Author contributions

triplicates, and the details of statistical and the details of statistical analysis Author contributions
Author contributions
MBA. RMBMG. MC. CGB. AMS. MB. FB: Conceptualization: Data curation: Fori Machine, Machine, Machine, Machine, Machine, Machine, Machine, Machine, Machine, Ams, FB: Funding acquisition; Project administration; Resources; Supervision.
Funding sources
This work was supported by: Fundação de Amparo

Funding sources

Initing. AMS, FB: Funding acquisition; Project administration; Resources; Supervision.
 Funding sources

This work was supported by: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

grant 2021/12938-0 (awar Funding sources
This work was supported by: Fundação de Amparo à Pesquisa do Estado de São Paulo (
grant 2021/12938-0 (awarded to AMS), Conselho Nacional de Pesquisas Cient
Tecnológicas (CNPq) grant 307487/2021-0 (awarded The Recharation opperation, Canadyse as impede at supported by 2014 at 2014 was Científicas e
Tecnológicas (CNPq) grant 307487/2021-0 (awarded to AMS), the Centre National de la
Recherche Scientifique (CNRS) and University grant 2021/2021-0 (amateur 2021/2021-0 (awarded to AMS), the Centre National de la
Recherche Scientifique (CNRS) and University of Bordeaux, Agence National de Recherche
(ANR) through the ADIPOTRYP grant ANR-19-CE15-0004 (Recherche Scientifique (CNRS) and University of Bordeaux, Agence National de Recherche
(ANR) through the ADIPOTRYP grant ANR-19-CE15-0004 (awarded to FB), TRYPADIFF grant
ANR-23-CE15-0040-01 (awarded to FB), the Laboratoir (ANR) through the ADIPOTRYP grant ANR-19-CE15-0004 (awarded to FB), TRYPADIFF grant
ANR-23-CE15-0040-01 (awarded to FB), the Laboratoire d'Excellence through the LabEx
ParaFrap grant ANR-11-LABX-0024 (awarded to FB), and t (ANR-23-CE15-0040-01 (awarded to FB), the Laboratoire d'Excellence through the LabEx
ParaFrap grant ANR-11-LABX-0024 (awarded to FB), and the "Fondation pour le Recherche
Médicale" (FRM) grant EQU201903007845 (awarded to F ParaFrap grant ANR-11-LABX-0024 (awarded to FB), and the "Fondation pour le Recherche
Médicale" (FRM) grant EQU201903007845 (awarded to FB). MBA is FAPESP fellow with project
2022/16078-8.
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adding an excess (10 x K_M value for each amino acid tested).
adding an excess (10 x K_M value for each amino acid tested). adding an excess (10 x KM value for each amino acid tested).

Table 2. Inhibition of L-Ser uptake in the presence of FCCP and Oly.

*not detected.

Table 3. Time-dependent $^{14}CO_2$ generation by Epi form from L-[3¹⁴C]Ser, L-[U¹⁴C]Thr and $[U^{14}C]$ Gly

Cons
Cons
ClGly
Cons
ave o 60 0.01 ± 0.02^2 21.33 ± 1.02 0.07
180 0.19 ± 0.38^2 29.38 ± 1.54 0.65
the only way for L-[3¹⁴C]Ser to be released as CO₂ is through complete degradation in the TC.
value was normalized by multiplying it b 180 0.19 ± 0.38 29.38 ± 1.54 0.65

the only way for μ -[3¹⁴C]Ser to be released as CO₂ is through complete degradation in the TC.

1 value was normalized by multiplying it by 3. In contrasts, the values obtain $\overline{1}$ Considering that the only way for L-[3¹⁴C]Ser to be released as CO₂ is through complete degradation in the TCA ycle, the obtained value was normalized by multiplying it by 3. In contrasts, the values obtained L-[U-¹⁴

cycle, the obtained value was normalized by multiplying it by 3. In contrasts, the values obtained L-[U-¹⁴C]Thr or [U-
¹⁴C]Gly have not been normalized since all carbon are ¹⁴C-labeled.
² Considering the standard e ¹⁴C]Gly have not been normalized since all carbon are ¹⁴C-labeled.

² Considering the standard error, the percentage of ¹⁴CO₂ produc

have originated from residual activity of the glycine cleavage comp

³ To a 2

Considering the standard error, the percentage of ⁴⁴CO₂ produced from Gly was assumed to be residual and may have originated from residual activity of the glycine cleavage complex.
To assess the comparative release of $\frac{3}{4}$ To assess the comparative release of carbon as CO₂, the measured value transported amino acid over the identical incubation duration dete the transported amino acid over the identical incubation duration detected in Fig. 1A, B and C. the transported amino acid over the identical incubation duration detected in Fig. 1 A, B and C.

S3. Overview of the biochemical characterization of amino acid transport systems to date in

Trypanosoma cruzi.

