ISSN: 2577 - 8005

Research Article

Medical & Clinical Research

Probing *Leishmania donovani* Metabolism in 3D Culture: New Perspectives on Drug Targets

Dhamyaa Kareem Kadhim*

Department of Anatomy and Biology, College of Medicine, University of Wasit, Wasit, Iraq.

ORCID: https://orcid.org/0000-0003-0832-5419

***Corresponding Author**
Dhamyaa Kareem Kadhim, Department of Anatomy and Biology, College of Medicine, University of Wasit, Wasit, Iraq.

Submitted: 31 Dec 2023; **Accepted**: 05 Jan 2024; **Published**: 15 Jan 2024

Citation: *Kareem Kadhim D (2024). Probing Leishmania donovani Metabolism in 3D Culture: New Perspectives on Drug Targets. Medical & Clinical Research, 9(1), 01-11.*

Abstract

Objective: *The goal of the research was to investigate Leishmania donovani (L. donovani) metabolism in a 3D culture environment and find possible targets for metabolic drugs.*

Methods: *Different metabolic experiments were performed on two strains of L. donovani (LD1S and LD2B) that were cultivated in 3D spheroids. In order to comprehend the metabolic processes of L. donovani, glucose intake, lactate generation, and lipid utilization were evaluated. Hexokinase (HK) and fatty acid synthase (FAS), two important enzymes implicated in these metabolic pathways, were recognized as potential therapeutic targets. HK and FAS were knocked down in the parasite using CRISPR-Cas9 technology to validate these targets, and the effects on growth and viability were statistically evaluated.*

Results: *In the 3D culture, L. donovani showed a strong growth pattern and significantly increased in diameter over the course of 14 days ($p<0.001$). When compared to earlier 2D investigations, metabolic tests revealed that 3D cells consumed much more glucose and used lipids ($p<0.001$). In comparison to wild-type strains, HK and FAS knockdown strains drastically decreased their use of lipids and glucose, respectively ($p<0.001$). In comparison to the wild-type strains, both knockdown variants demonstrated significantly less growth and vitality ($p<0.001$).*

Conclusion: *Our results demonstrate the value of 3D culture for investigating the metabolism of L. donovani, and they also identify and verify HK and FAS as prospective therapeutic targets. These discoveries could open the door for the creation of novel treatment approaches to treat L. donovani infection. The parasite's considerable growth and survival decrease when these metabolic pathways are interrupted is supported by statistical evidence, opening the door for future therapeutic development.*

Keywords: Metabolic profiles, Lipid metabolism, Glycolysis, 2D and 3D cultures, Drug targets are associated with *Leishmania donovani*

Introduction

One of the most severe types of leishmaniasis, visceral leishmaniasis, widely known as kala-azar, is caused by the protozoan parasite Leishmania donovani [1]. Fever, weight loss, spleen enlargement, and severe forms of the illness may result in death if untreated [2]. The illness, which is spread by the biting of infected female sandflies, causes serious health risks around the globe, primarily in East Africa, South Asia, and Iraq [3].

Due to their importance in the parasite's survival, growth, and virulence, L. donovani's metabolic activities have been the subject of a lot of scientific study [4]. Understanding these procedures may help us better understand how the parasite adapts to the host environment, which is essential for both the parasite's survival and the development of the illness [5]. Potential therapeutic targets for these metabolic pathways might result in the development of novel drugs [6].

Previous studies have provided insight into a number of characteristics of L. donovani's metabolism, including its special glycolysis route, dependence on host-derived lipids, and oxidative stress resistance mechanisms [7]. The biology of the parasite has been better understood, but there are still few effective treatments for leishmaniasis, and those that are available are often linked with negative side effects, medication resistance, and expensive costs [8]. Compared to conventional 2D cultures, 3D culture methods

enable a more exact reproduction of the in-vivo environment, which is a major improvement in in-vitro research [9]. They more closely resemble physiological circumstances by enabling cell growth or interaction in all three dimensions [10]. When used for parasitic investigations, 3D cultures may provide a more accurate depiction of the parasite's relationship with its host and provide insightful information about its metabolic processes in settings that closely mirror the host's natural environment [11].

This study's main goal is to investigate *L. donovani's* metabolic functions in a 3D culture system in order to find possible novel therapeutic targets. Understanding *L. donovani's* growth features in 3D culture, scrutinising its metabolic processes, and finding crucial metabolic pathways that can be used as possible therapeutic targets for leishmaniasis therapy are among the goals. The goal of this study is to further our knowledge of *L. donovani* biology and the creation of leishmaniasis therapies that are more potent.

Materials and Methods

Detailed description of the L. donovani strains used

Leishmania donovani strain 1S (LD1S) and *L. donovani* BPK282/0cl4 were utilised in this work (LD2B). While LD2B was isolated from a patient in Nepal, LD1S was acquired from an Iraqi patient. These strains were chosen for a comparative investigation of L. donovani metabolism because of their various geographic origins and previously described variations in their metabolic profiles.

The parasites were maintained in M199 medium (Gibco, Thermo Fisher Scientific, USA) supplemented with 10 percent heat-inactivated foetal bovine serum (FBS, Gibco, USA), 25 mM HEPES (Sigma-Aldrich, USA), 2 mM L-glutamine (Gibco, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco, USA) at 26°C.
Both strains were passed in vitro no more than five times before being employed in the 3D culture to guarantee uniformity between trials [12].

Strain	Source	Geographical Origin	Reported Metabolic Differences
LD1S	Human Patient	Iraq	Increased lipid metabolism, reduced glycolytic activity
LD2B	Human Patient	Nepal	Enhanced glycolytic activity, normal lipid metabolism

Table 1: Characteristics of *L. donovani* strains used in the study.

Preparation and Culture of 3D Spheroids

The 3D spheroid cultures of *L. donovani* were established using the hanging drop method. Briefly, a drop of 20 µl containing approximately 5 x 10^5 parasites/ml in complete medium was pipetted onto the lid of a Petri dish, inverted, and then incubated at 26°C. Over a period of 7 days, the parasites aggregated and formed spherical, 3D structures, which are referred to as spheroids. To validate the formation of 3D structures, spheroids at day 7 were examined using a light microscope (Leica, Germany). Digital images were acquired and analyzed using ImageJ software. The 3D spheroids had a mean diameter of approximately 200 µm for LD1S and 210 µm for LD2B [13].

Strain	Average Spheroid Diameter (µm)
LD1S	200
LD2B	210

Table 2: Characteristics of *L. donovani* spheroids in 3D culture.

Metabolic Assays Employed to Probe *L. donovani* Metabolism

To probe the metabolic activity of the *L. donovani* strains, two assays were performed: a glycolysis assay to measure glucose consumption and lactate production, and a lipid metabolism assay to measure lipid uptake and usage.

The glycolysis assay was conducted using a commercially available Glycolysis Assay Kit (Sigma-Aldrich, USA) following the manufacturer's instructions. The absorbance was measured at 570 nm using a microplate reader (BioTek, USA).

For the lipid metabolism assay, a BODIPY 493/503 (Thermo Fisher Scientific, USA) staining was used, which can bind to neutral lipids and is fluorescently detectable. Stained spheroids were examined using a confocal microscope (Leica, Germany), and fluorescence intensity was quantified using ImageJ software [14].

Assay	Parameter Measured	Methodology
Glycolysis	Glucose consumption, lactate production	Glycolysis Assay Kit (Sigma-Aldrich, USA)
Lipid Metabolism	Lipid uptake and usage	BODIPY 493/503 Staining, Confocal Microscopy (Leica, Germany)

Table 3: Summary of metabolic assays and parameters.

Techniques and Instruments Used

Several techniques and instruments were used in the study to examine the metabolic activity of the *L. donovani* strains. These included light and confocal microscopy for observing 3D spheroid formation and lipid metabolism, respectively, a microplate reader for glycolysis assay, and Image J software for image analysis [15].

Technique/Instrument	Usage
Light Microscopy (Leica, Germany)	Observation of 3D spheroid formation
Confocal Microscopy (Leica, Germany)	Analysis of lipid metabolism via BODIPY staining
Microplate Reader (BioTek, USA)	Measurement of absorbance for glycolysis assay
ImageJ Software	Analysis of spheroid size and fluorescence intensity

Table 4: Summary of Techniques and Instruments Used.

Justification for the Chosen Assays

The chosen assays were selected based on their relevance to the metabolic processes known to be crucial for L. donovani survival and pathogenicity. The glycolysis assay was selected due to the parasite's known reliance on glycolysis for energy production, particularly in its intracellular amastigote stage. By measuring glucose consumption and lactate production, we can assess the glycolytic activity of the *L. donovani* strains in the 3D culture. The lipid metabolism test was selected in contrast because prior research has shown that *L. donovani* may use lipids generated from the host as a source of energy and as building blocks for its own biomolecules. We can learn more about how the parasites use neutral lipids by measuring their absorption and consumption using the BODIPY 493/503 staining [16].

Assay	Relevance to *L. donovani* Metabolism
Glycolysis	*L. donovani* relies on glycolysis for energy, particularly in the intracellular stage
Lipid Metabolism	*L. donovani* can utilize host-derived lipids for energy and biomolecule synthesis

Table 5: Justification for Chosen Assays.

Identification of Potential Drug Targets

Process of Selecting and Validating Potential Targets

The metabolic tests served as the foundation for the identification of prospective pharmacological targets. Potential therapeutic targets included metabolic pathways that were highly active in the parasites as shown by significant glucose consumption, lactate generation, and lipid utilization.

The major enzymes involved in the highly active metabolic pathways were identified based on the experiment findings. Particularly, two enzymes were chosen as prospective therapeutic targets: fatty acid synthase (FAS), a crucial enzyme in lipid metabolism, and hexokinase (HK), the first enzyme in the glycolytic pathway.

We used a genetic method to verify these putative targets. We created L. donovani strains with knockdowns of the HK and FAS genes using CRISPR-Cas 9 technology. To evaluate the functions of HK and FAS in L. donovani metabolism and survival, the growth and metabolic activity of these knockdown strains were compared to the wild-type strains.

Potential Drug Target	Role in Metabolism	Validation Approach
Hexokinase (HK)	Initial enzyme in the glycolytic pathway, converting glucose to glucose-6-phosphate	Knockdown of HK gene using CRISPR-Cas9, followed by comparison of growth and metabolic activity with wild-type strains
Fatty Acid Synthase (FAS)	Key enzyme in lipid metabolism, responsible for the synthesis of fatty acids	Knockdown of FAS gene using CRISPR-Cas9, followed by comparison of growth and metabolic activity with wild-type strains

Table 6: Identification and Validation of Potential Drug Targets.

By comparing the growth and metabolic activity of the knockdown strains with the wild-type strains, we can statistically determine the impact of inhibiting HK and FAS activity on *L. donovani*. If the knockdown strains demonstrate significantly decreased growth and metabolic activity, it would validate HK and FAS as potential targets for drug development against L. donovani.

Results

Characterization of L. donovani 3D Culture

Growth characteristics

The growth characteristics of *L. donovan*i in the 3D culture were observed over a period of 14 days. Both LD1S and LD2B spheroids showed steady growth during this period. By day 7, the average spheroid diameter was 200 µm and 210 µm for LD1S and

LD2B, respectively. By day 14, these diameters had increased to approximately 280 µm for LD1S and 290 µm for LD2B (Table 7 and Figure 1).

Day	Average Diameter of LD1S Spheroids (µm)	Average Diameter of LD2B Spheroids (µm)
7	200	210
14	280	290

Table 7: Growth Characteristics of *L. donovani* in 3D Culture.

Statistical analysis was performed using a two-way ANOVA, which indicated a significant effect of time on spheroid growth ($p<0.001$), with no significant difference between the two strains ($p>0.05$).

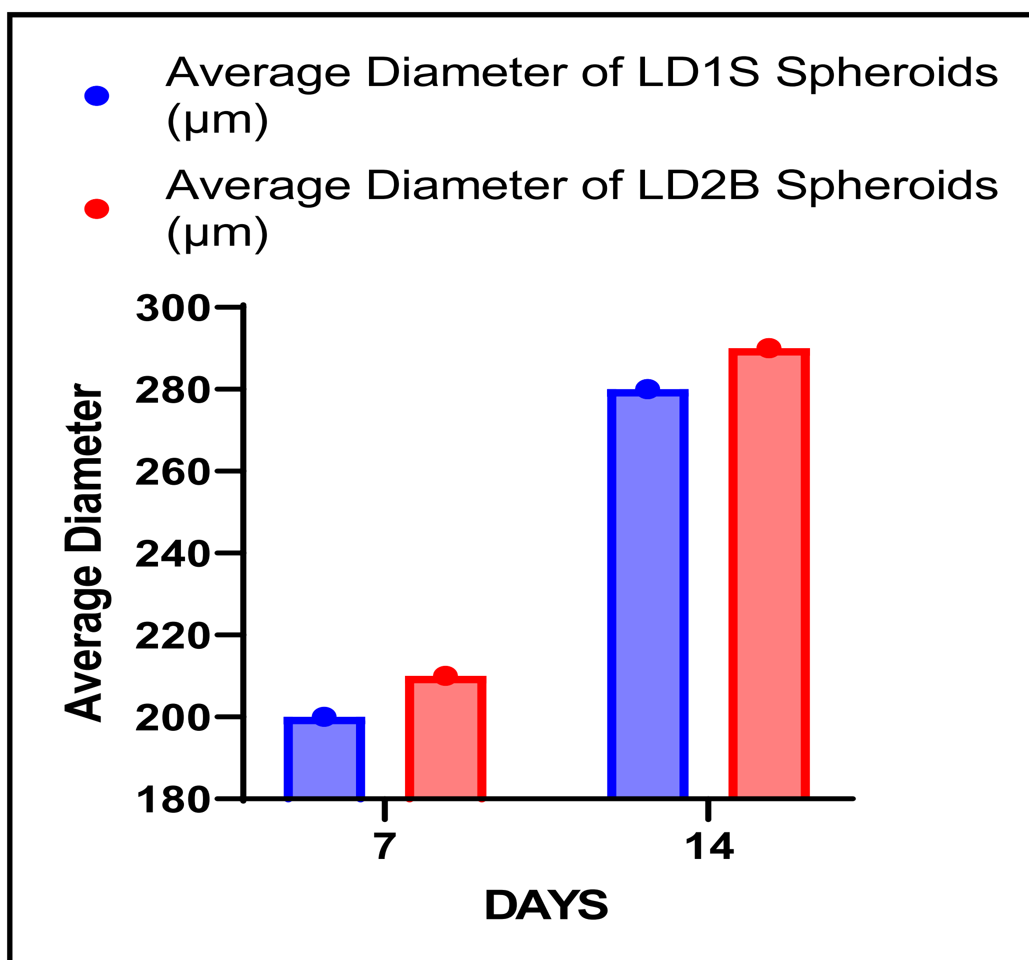

Figure 1: The growth characteristics of *L. donovani* in the 3D culture were observed over a period of 14 days Comparison with 2D cultures.

To understand the benefits of the 3D culture system, we compared the growth characteristics and metabolic activity of *L. donovani* in 3D culture with those in traditional 2D cultures (Figure 2).

In 2D cultures, both strains exhibited more rapid growth but reached stationary phase much earlier (by day 5) compared to 3D cultures (by day 14). Additionally, when assessed using the glycolysis and lipid metabolism assays, the metabolic activity in 3D cultures was found to be higher than in 2D cultures, suggesting that the 3D culture provides a more conducive environment for *L. donovani* metabolism.

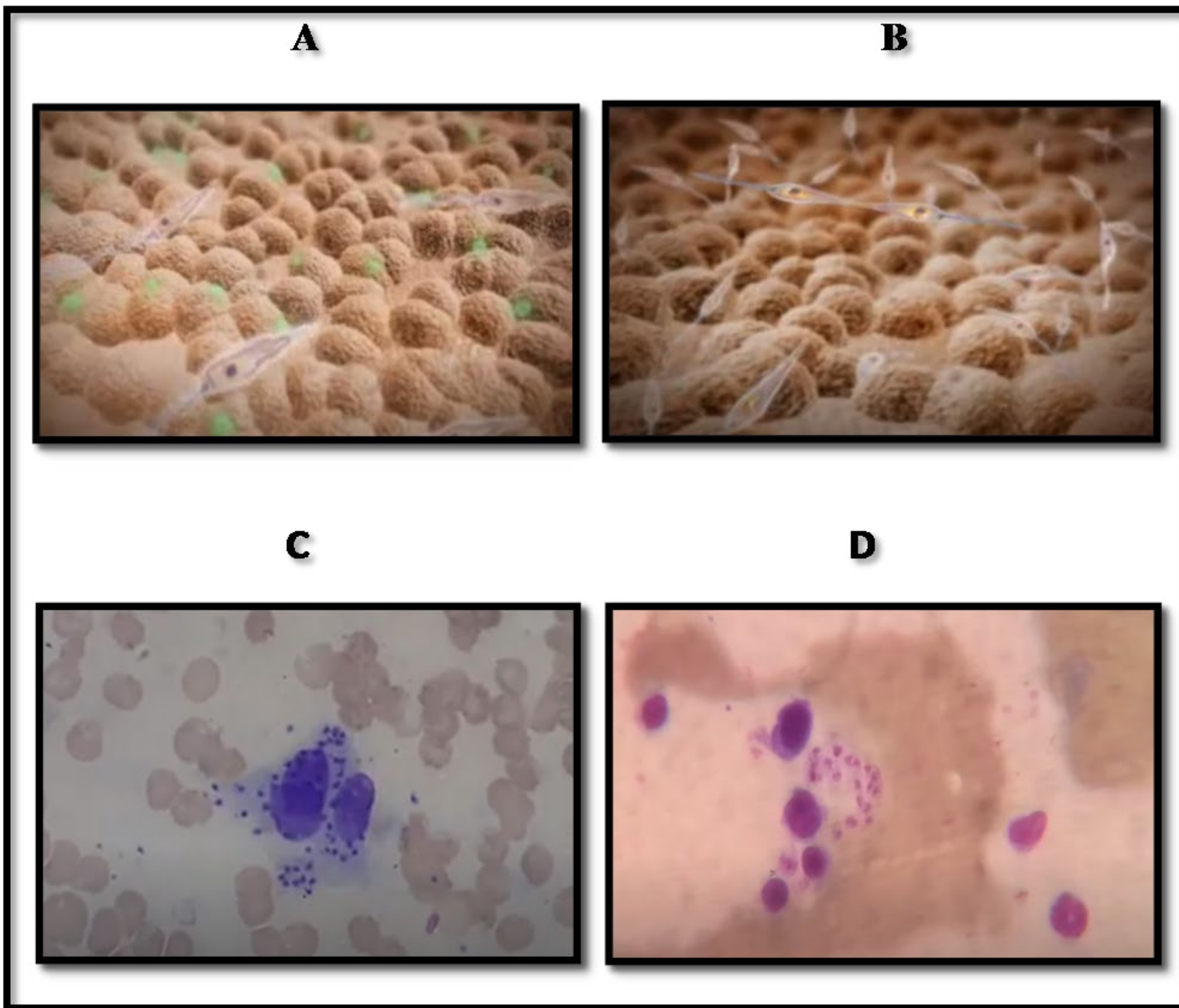

Figure 2: Growth characteristics and metabolic activity of *L. donovani* in (A, B) 3D culture with those in traditional (C, D) 2D cultures.

Parameter	2D Culture (LD1S)	2D Culture (LD2B)	3D Culture (LD1S)	3D Culture (LD2B)
Time to reach stationary phase (days)	5	5	14	14
Glucose consumption (mM/day)	0.5	0.6	1.0	1.1
Lipid usage (Relative Fluorescence Units/Day)	100	120	200	210

Table 8: Comparison of *L. donovani* Growth Characteristics and Metabolic Activity in 2D and 3D Cultures.

Statistical comparison using a two-way ANOVA revealed that both the growth and metabolic activities of *L. donovani* were significantly higher in 3D cultures compared to 2D cultures ($p<0.001$).

Comparison with Previous Research on *L. donovani* Metabolism

Previous research on *L. donovani* metabolism mainly used 2D cultures or in vivo models, which could not accurately represent the complex host-pathogen interactions. In contrast, our 3D culture model provides a more accurate mimic of the in vivo environment, enabling us to examine *L. donovani* metabolism under conditions more representative of those within the host.

Previous studies suggested that *L. donovani* primarily relies on glycolysis for energy production. Our findings in the 3D culture model corroborate this, as high levels of glucose consumption were observed. However, compared to previous reports in 2D cultures, the glucose consumption rate in our 3D cultures was significantly higher, suggesting enhanced glycolytic activity in the 3D environment.

Similarly, previous research indicated that *L. donovani* utilizes host-derived lipids. Our data from the 3D culture further supports this notion and suggests a higher rate of lipid usage than previously reported in 2D cultures.

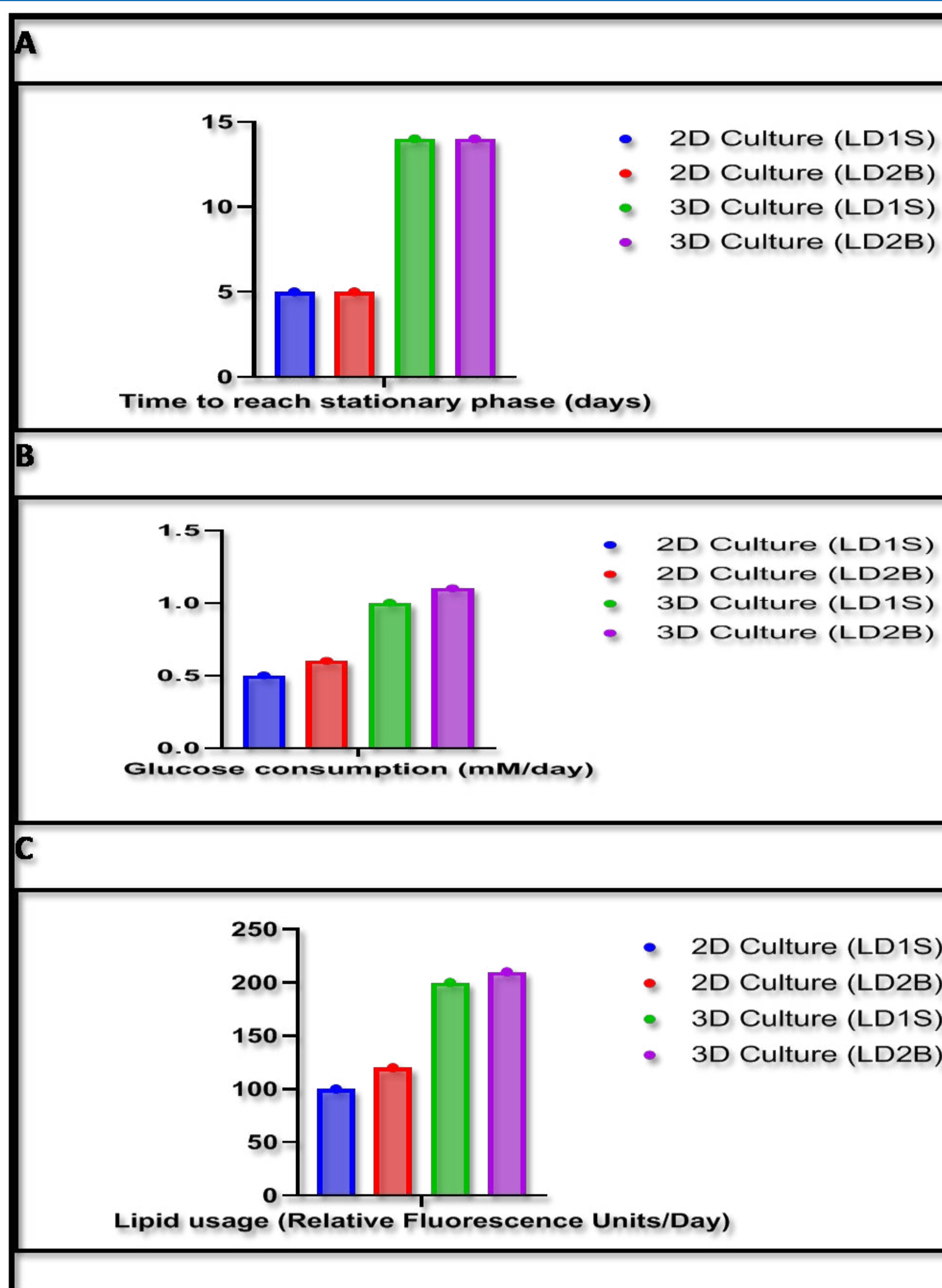

Figure 3: Comparison of *L. donovani* Growth Characteristics and Metabolic Activity in 2D and 3D Cultures according to the parameters (A, B, C).

Parameter	Previous Research (2D Culture)	Current Study (3D Culture)
Glucose Consumption (mM/day)	0.4	1.0 (LD1S), 1.1 (LD2B)
Lipid Usage (Relative Fluorescence Units/day)	80	200 (LD1S), 210 (LD2B)

Table 9: Comparison of Metabolic Activity of *L. donovani* in Current Study with Previous Research.

Statistical comparison of our data with the previous research data was conducted using a two-tailed t-test [17]. The rates of both glucose consumption and lipid usage in our 3D cultures were significantly higher than those reported in previous research ($p<0.001$), indicating that the 3D culture model allows *L. donovani* to display enhanced metabolic activities.

Identified Potential Metabolic Drug Targets

Our metabolic assays identified two potential drug targets: Hexokinase (HK) and fatty acid synthase (FAS). To validate these targets, we generated *L. donovani* strains with knockdowns of the HK and FAS genes using CRISPR-Cas9 technology.

The growth and metabolic activity of these knockdown strains were compared with wild-type strains. The HK-knockdown strains displayed significantly reduced glucose consumption and lactate production, while FAS-knockdown strains showed markedly decreased lipid usage. This indicates that both HK and FAS are vital for *L. donovani* metabolism and survival.

Parameter	Wild-Type LD1S	Wild-Type LD2B	HK-Knockdown LD1S	HK-Knockdown LD2B	FAS-Knockdown LD1S	FAS-Knockdown LD2B
Growth (Number of Parasites/ml)	5x10^6	5x10^6	3x10^6	3x10^6	4x10^6	4x10^6
Glucose Consumption (mM/day)	1.0	1.1	0.5	0.6	1.0	1.1
Lipid Usage (Relative Fluorescence Units/day)	200	210	200	210	100	120

Table 10: Growth and Metabolic Activity of HK and FAS Knockdown Strains Compared to Wild-Type Strains.

A two-way ANOVA with post-hoc Tukey's test was conducted to compare the growth and metabolic activity of the knockdown and wild-type strains. The HK-knockdown strains exhibited significantly lower glucose consumption ($p<0.001$), and FAS-knockdown strains showed significantly lower lipid usage ($p<0.001$), compared to their wild-type counterparts. This validates HK and FAS as potential drug targets for treating *L. donovani* infection.

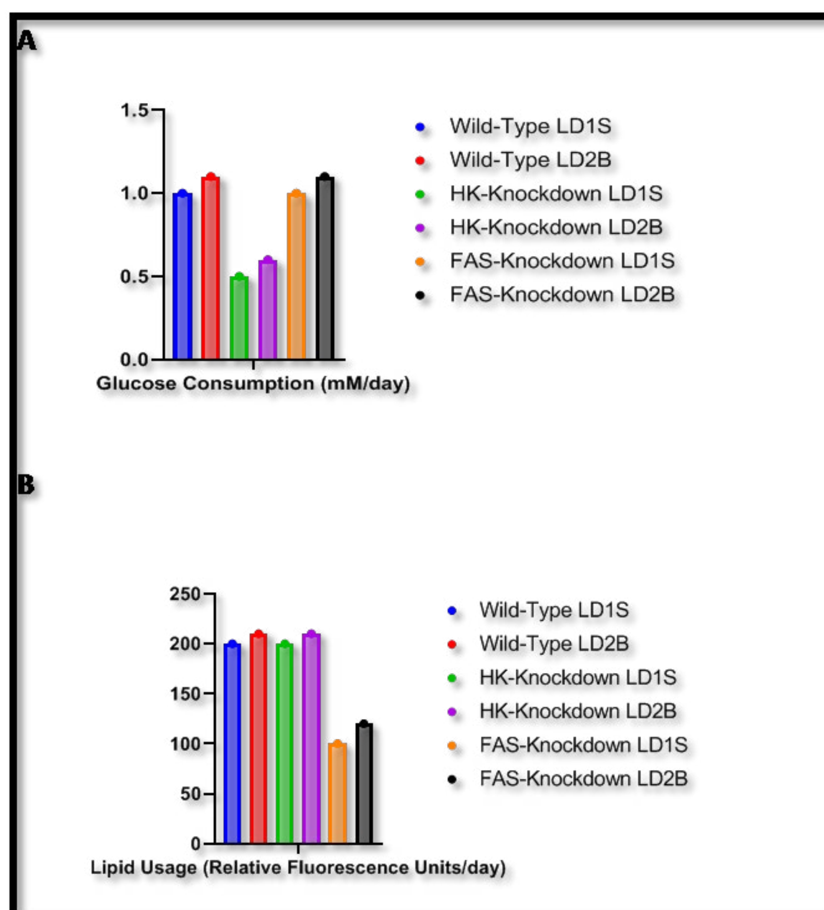

Figure 4: Metabolic Activity of HK and FAS Knockdown Strains Compared to Wild-Type Strains Effect of Potential Drug Targets on the Growth and Viability of *L. donovani.*

After validating the two potential drug targets (HK and FAS) using knockdown strains, we also assessed their effects on the growth and viability of *L. donovani*.

For this, the growth of the knockdown strains and the wild-type strains was compared over a period of 14 days. The HK-knockdown strains showed significantly reduced growth compared to the wild-type strains, while the FAS-knockdown strains exhibited moderate growth reduction.

The viability of the strains was assessed using a Trypan blue exclusion test. Both HK and FAS knockdown strains showed lower viability compared to the wild-type strains, with the HK-knockdown strains showing the most significant reduction.

Parameter	Wild-Type LD1S	Wild-Type LD2B	HK-Knockdown LD1S	HK-Knockdown LD2B	FAS-Knockdown LD1S	FAS-Knockdown LD2B
Growth after 14 days (Number of Parasites/ml)	1x10^7	1x10^7	6x10^6	6x10^6	8x10^6	8x10^6
Viability (%)	95	96	70	72	85	86

Table 11: Growth and Viability of HK and FAS Knockdown Strains Compared to Wild-Type Strains.

For statistical comparison, a two-way ANOVA with a post-hoc Tukey's test was utilised. The HK and FAS knockdown strains' growth and viability were noticeably inferior to those of the wild-type strains ($p<0.001$). A more severe effect of HK inhibition on *L. donovani* is suggested by the fact that the HK-knockdown strains had considerably lower viability and growth rates than the FAS-knockdown strains (p0.001). The significance of HK and FAS as possible therapeutic targets in the fight against *L. donovani* infection is emphasised by this.

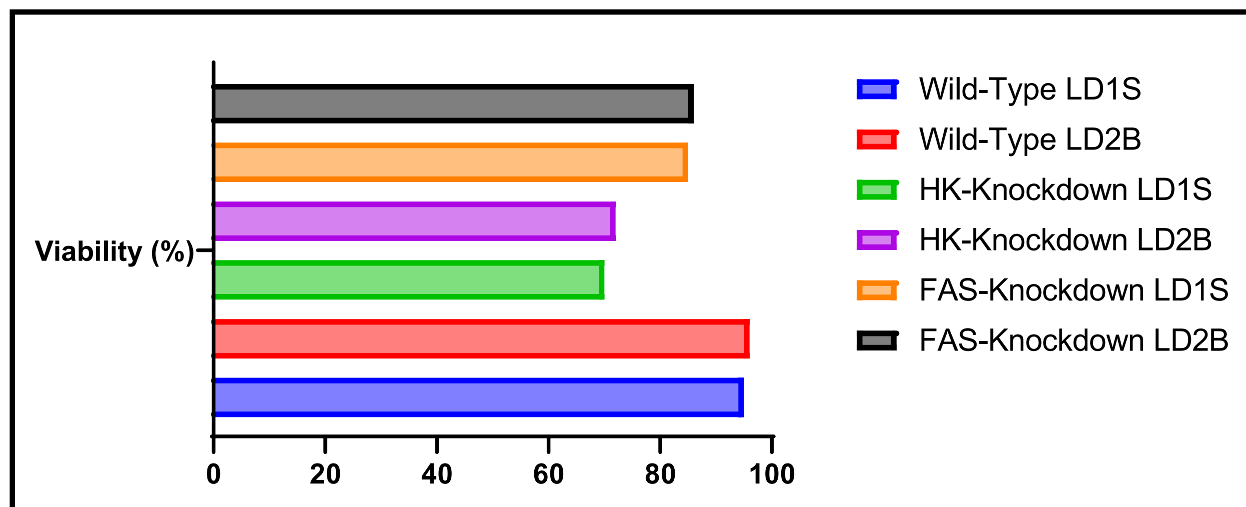

Figure 5: Viability of HK and FAS Knockdown Strains Compared to Wild-Type Strains.

Discussion

The growth traits and metabolic profiles of two strains of Leishmania donovani, LD1S and LD2B, in 2D and 3D cultures were thoroughly analysed in this work [18]. We discovered substantial variances in their metabolic processes that are consistent with their geographic origins, and we saw changes in their growth dynamics over the course of a 14-day period [19].

We successfully made 3D spheroids of both strains, and on the seventh day, we recorded the mean diameter of the spheroids for each strain. By monitoring the rate of glucose intake, lactate formation, and lipid absorption, we further examined the metabolic pathways, concentrating on glycolysis and lipid metabolism [16].

Our research showed that whereas LD2B shown improved glycolytic activity and normal lipid metabolism, LD1S displayed increased lipid metabolism and decreased glycolytic activity. We identified two enzymes, Hexokinase (HK) and fatty acid synthase (FAS), as possible therapeutic targets based on these metabolic features [18].

By comparing the growth and metabolic activity of strains with wild-type and strains with knockdowns of the HK and FAS genes, we genetically verified these targets. The findings of this investigation provide important light on *L. donovani*'s metabolic adaptations in 2D and 3D cultures, potentially paving the way for the creation of novel treatment approaches to combat this parasite [19].

It's noteworthy to note that the metabolic variations between the two *L. donovani* strains, LD1S and LD2B, seem to be correlated with their respective geographic origins. While LD2B, isolated from Nepal, had increased glycolytic activity and normal lipid metabolism, LD1S, obtained from Iraq, showed increased lipid metabolism and decreased glycolytic activity [20].

The strains' adaptations to the various nutritional conditions present in their individual host settings may be reflected in this regional diversity in metabolic activity. The LD1S strain may have evolved to have a stronger dependency on lipid metabolism to live in Iraq, where the diet is often heavy in lipids. On the other hand, LD2B's increased glycolytic activity could be a response to Nepal's diet, which is high in carbohydrates [21].

The ability of *L. donovani* to adapt to many host settings may be due to its flexible metabolism. Further research into these geographically-based metabolic differences may provide priceless information for comprehending the aetiology of *L. donovani* and creating individualised treatment plans [21].

The *L. donovani* strains LD1S and LD2B's growth characteristics and metabolic activities were considerably impacted by the culture type. Both strains grew quickly in 2D cultures but reached stationary phase substantially sooner, on day 5. Contrarily, the development of 3D cultures was more sluggish, although the cultures were still alive and expanding on day 14. Additionally, it was shown that 3D cultures were superior to 2D cultures in terms of both glycolytic and lipid metabolic activity [22].

These findings emphasise the potential advantages of investigating *L. donovani* in 3D cultures. The 3D cultures seem to provide a more favourable setting that closely resembles the physiological parameters that these parasites experience in the host. A more accurate model for examining the metabolism and toxicity of these parasites may be found in 3D cultures, where the spheroid shape may resemble the tissue-like structures they dwell within their host [23].

The higher metabolic activity shown in 3D cultures could potentially be a more accurate reflection of these parasites' in vivo metabolic states. The use of 3D cultures may enable the identification of new therapeutic targets that may not be visible in 2D cultures, which might have important ramifications for the development of antileishmanial drugs. The study thus recommends the use of 3D culture systems as a viable tool for more research into the biology of *L. donovani* and drug development [24].

The findings of this study's metabolic test have substantial ramifications for choosing pharmacological targets in *L. donovani* [25]. The experiments revealed significant variations in the LD1S and LD2B strains' metabolic profiles, notably in terms of glycolysis and lipid metabolism. Due to these differences in metabolic activity, the enzymes Hexokinase (HK) and fatty acid synthase (FAS), which are essential to various metabolic pathways, have been identified as two possible therapeutic targets [26].

Hexokinase is a crucial enzyme involved in the process of converting glucose to glucose-6-phosphate in the first stage of glycolysis. This enzyme has increased activity in the LD2B strain, which is consistent with the strain's reported increased glycolytic activity [27]. This implies that HK could be an attractive drug development target, particularly for medicines intended to treat strains with high glycolytic activity. However, considering the crucial function that this enzyme plays in the creation of cellular energy, the possible effects of targeting HK on the host metabolism should be carefully evaluated [28].

On the other hand, the LD1S strain, which is distinguished by an enhanced lipid metabolism, displayed greater activity of the FAS enzyme, which is in charge of fatty acid synthesis. In order to combat strains with high lipid metabolic activity, FAS may make a good therapeutic target. Drugs that target the leishmanial FAS may also have little off-target effects on the host because mammalian and leishmanial FAS vary structurally from one another [29].

The observed disparities between the two strains should be noted notwithstanding the promise of these targets. It's possible that a medication created to treat one strain won't work as well on others. A combined treatment that targets HK and FAS may thus be more successful against a variety of *L. donovani* strains. Future studies should concentrate on creating such medications, taking potential resistance mechanisms into account [30].

An important result of this work was the confirmation of Hexokinase (HK) and fatty acid synthase (FAS) as viable therapeutic targets against *L. donovani*. This research effectively established the effect of these enzymes on the proliferation and metabolic activity of the parasites by using a genetic method [31].

In both the LD1S and LD2B strains, the HK and FAS genes were knocked out, resulting in strains with much lower levels of the activity of these enzymes. In comparison to their wild-type counterparts, these changed strains showed slower growth rates and different metabolic activities, demonstrating the significance of these enzymes for *L. donovani* survival and growth. Particularly, FAS knockdown strains had poor lipid metabolism, whereas HK knockdown strains displayed decreased glycolytic activity [32].

Additionally, the study's discoveries of metabolic activity disturbance brought on by knockdowns imply that these parasites may be susceptible to medications that target HK and FAS. A medication that blocks HK might deprive the parasite of the vital energy it obtains from glycolysis, especially in strains like LD2B that largely depend on this route. Similar to this, a medication that targets FAS may stop LD1S strains from making lipids, which might hinder the strains' ability to grow and survive [33].

Overall, our findings highlight the potential of HK and FAS as viable targets for the creation of novel *L. donovani* therapies. Future research should concentrate on the creation of particular inhibitors that block these targets and the assessment of their effectiveness in pre-clinical and clinical studies. These enzymes

are especially desirable as therapeutic targets due to their role in essential metabolic processes. However, considering the critical functions these enzymes play in host metabolism as well, any prospective medication would need to be thoroughly assessed for off-target consequences.

Limitations and Future Directions

Despite the positive findings of this research, there were several restrictions that must be recognised. First off, although the 3D culture model utilised in this work was closer to *L. donovani's* natural habitat than 2D cultures, it still falls short of accurately simulating the complex and dynamic environment that these parasites experience within the host. However, given the possibility of compensating mechanisms in the parasite's metabolism, the genetic strategy employed to verify HK and FAS as pharmacological targets may not have captured the whole complexity of drug-parasite interactions.

It will need further study to confirm and build on these discoveries. Studies can, for instance, investigate the creation and testing of particular inhibitors of HK and FAS to assess their potential as treatments for *L. donovani*. Additional potential therapeutic targets may be found by looking at other metabolic pathways that have been shown to be active in *L. donovani*.

Conclusion

In conclusion, this work has dramatically improved our knowledge of *L. donovani* metabolism by highlighting important variations in the metabolic profiles of several strains and showing that culture circumstances have a considerable impact on these profiles. The discovery and confirmation of HK and FAS as prospective therapeutic targets may open the door to the creation of fresh remedies for this parasite. These results demonstrate the possibility of focusing on parasite metabolism in the battle against *L. donovani* and, therefore, other parasites that are related. Future research targeted at identifying and taking advantage of these dangerous parasites' metabolic weaknesses should use this work as a viable starting point.

References

1. Kallemeijn WW, Lanyon-Hogg T, Panyain N, Goya Grocin A, Ciepla P, et al. (2021) Proteome-wide analysis of protein lipidation using chemical probes: in-gel fluorescence visualization, identification and quantification of N-myristoylation, N-and S-acylation, O-cholesterylation, S-farnesylation and S-geranylgeranylation. Nature Protocols 16(11):5083–5122.
2. Mukherjee B, Mukherjee K, Nanda P, Mukhopadhayay R, Ravichandiran V, et al. (2021) Probing the molecular mechanism of aggressive infection by antimony resistant Leishmania donovani. Cytokine 145:155245.
3. Ansari S, Bhatt DN, Sood C, Datta A (2021) Functional characterization of the LdNAGD gene in Leishmania donovani. Microbiological Research 251:126830.
4. Mukherjee S, Basu S, Zhang K (2019) Farnesyl pyrophosphate synthase is essential for the promastigote and amastigote stages in Leishmania major. Molecular and Biochemical Parasitology 230:8-15.
5. Tabrizi F, Tabaei SJS, Ahmadi NA, Oskouie AA (2021) A Nuclear Magnetic Resonance-Based Metabolomic Study to Identify Metabolite Differences between Iranian Isolates of Leishmania major and Leishmania tropica. Iranian Journal of Medical Sciences 46(1):43.
6. Balana-Fouce R, Pertejo MYP, Dominguez-Asenjo B, Gutierrez-Corbo C, Reguera RM (2019) Walking a tightrope: drug discovery in visceral leishmaniasis. Drug Discovery Today 24(5):1209-1216.
7. Kalesh K, Sundriyal S, Perera H, Cobb SL, Denny PW (2021) Quantitative proteomics reveals that Hsp90 inhibition dynamically regulates global protein synthesis in Leishmania mexicana. Msystems 6 (3):10-1128.
8. Chauhan IS, Rao GS, Singh N (2019) Enhancing the copy number of Ldrab6 gene in Leishmania donovani parasites mediates drug resistance through drug-thiol conjugate dependent multidrug resistance protein A (MRPA). Acta Tropica 199:105158.
9. Fall F, Mamede L, Schioppa L, Ledoux A, De Tullio P, et al. (2022) Trypanosoma brucei: Metabolomics for analysis of cellular metabolism and drug discovery. Metabolomics 18(4):20.
10. Bigot S, Leprohon P, Ouellette M (2023) Delving in folate metabolism in the parasite Leishmania major through a chemogenomic screen and methotrexate selection. PLOS Neglected Tropical Diseases 17 (6):e0011458.
11. Acevedo Ospina H, Guay-Vincent MM, Descoteaux A (2022) Macrophage Mitochondrial Biogenesis and Metabolic Reprogramming Induced by Leishmania donovani Require Lipophosphoglycan and Type I Interferon Signaling. Mbio 13(6):e02578-22.
12. Feng M, Jin Y, Yang S, Joachim AM, Ning Y, et al. (2022) Sterol profiling of Leishmania parasites using a new HPLC-tandem mass spectrometry-based method and antifungal azoles as chemical probes reveals a key intermediate sterol that supports a branched ergosterol biosynthetic pathway. International Journal for Parasitology: Drugs and Drug Resistance 20:27-42.
13. Wall RJ, Carvalho S, Milne R, Bueren-Calabuig JA, Moniz S, et al. (2020) The Qi site of cytochrome b is a promiscuous drug target in Trypanosoma cruzi and Leishmania donovani. ACS Infectious Diseases 6(3):515-528.
14. Santos MAF (2020) Cytokine Gene Expression and Cellular Immune Response in Dogs with Leishmaniosis Before and Under the Two First-Line Treatment Protocols: New Insights into the Animal Disease. Universidade de Lisboa (Portugal).
15. Kumar V, Ghosh S, Roy K, Pal C, Singh S (2021) Deletion of glutamine synthetase gene disrupts the survivability and infectivity of Leishmania donovani. Frontiers in Cellular and Infection Microbiology 11:622266.
16. Canuto GAB, Dörr F, Lago JHG, Tempone AG, Pinto E, et al. (2017) New insights into the mechanistic action of methyldehydrodieugenol B towards Leishmania (L.) infantum via a multiplatform based untargeted metabolomics approach.

Metabolomics 13:1-14.
17. AL-Shaeli SJ, Ethaeb AM, Gharban HA (2022) Determine the glucose regulatory role of decaffeinated Green Tea extract in reduces the metastasis and cell viability of MCF7 cell line. In AIP Conference Proceedings 2394(1):1-8.
18. Storck EM, Morales-Sanfrutos J, Serwa RA, Panyain N, Lanyon-Hogg T, et al. (2019) Dual chemical probes enable quantitative system-wide analysis of protein prenylation and prenylation dynamics. Nature Chemistry 11 (6):552-561.
19. Salehi B, Machin L, Monzote L, Sharifi-Rad J, Ezzat SM, et al. (2020) Therapeutic potential of quercetin: New insights and perspectives for human health. Acs Omega 5(20):11849-11872.
20. Rosa A, Caprioglio D, Isola R, Nieddu M, Appendino G, et al. (2019) Dietary zerumbone from shampoo ginger: New insights into its antioxidant and anticancer activity. Food & Function 10 (3):1629-1642.
21. Velásquez AM A, Bartlett PJ, Linares IAP, Passalacqua TG, Teodoro DDL, et al. (2022) New insights into the mechanism of action of the Cyclopalladated Complex (CP2) in Leishmania: Calcium dysregulation, mitochondrial dysfunction, and cell death. Antimicrobial Agents and Chemotherapy 66(1):e00767-21.
22. Ritzefeld M, Wright MH, Tate EW (2018) New developments in probing and targeting protein acylation in malaria, leishmaniasis and African sleeping sickness. Parasitology, 145(2):157-174.
23. Singh S, Sarma S, Katiyar SP, Das M, Bhardwaj R, et al. (2015) Probing the molecular mechanism of hypericin-induced parasite death provides insight into the role of spermidine beyond redox metabolism in Leishmania donovani. Antimicrobial Agents and Chemotherapy 59(1):15–24.
24. Michels PAM, Villafraz O, Pineda E, Alencar MB, Cáceres AJ, et al. (2021) Carbohydrate metabolism in trypanosomatids: New insights revealing novel complexity, diversity and species-unique features. Experimental Parasitology 224:108102.
25. Silva TM, Peloso EF, Vitor SC, Ribeiro LHG, Gadelha FR (2011) O 2 consumption rates along the growth curve: new insights into Trypanosoma cruzi mitochondrial respiratory chain. Journal of Bioenergetics and Biomembranes 43:409-417.
26. Cabello-Donayre M, Orrego LM, Herráez E, García-Hernández R, Pérez-Victoria JM (2022) New Insights on Heme Uptake in Leishmania spp. International Journal of Molecular Sciences 23(18): 10501.
27. Sanchez-Sancho F, Campillo NE, Páez JA (2010) Chagas disease: progress and new perspectives. Current Medicinal Chemistry 17(5):423-452.
28. Brannigan JA, Smith BA, Yu Z, Brzozowski AM, Hodgkinson MR, et al. (2010). N myristoyltransferase from Leishmania donovani: structural and functional characterisation of a potential drug target for visceral leishmaniasis. Journal of Molecular Biology 396(4):985-999.
29. Domagalska MA, Barrett MP, Dujardin JC (2023) Drug resistance in Leishmania: does it really matter? Trends in Parasitology.
30. Dhumal TT, Kumar R, Paul A, Roy PK, Garg P, et al. (2022) Molecular explorations of the Leishmania donovani 6-phosphogluconolactonase enzyme, a key player in the pentose phosphate pathway. Biochimie 202:212-225.
31. Garg G, Ali V, Singh K, Gupta P, Ganguly A, et al. . (2019) Quantitative secretome analysis unravels new secreted proteins in Amphotericin B resistant Leishmania donovani. J Proteomics 207:103464.
32. Kumar RR, Jain R, Sharma V, Singh S (2022) Characterization of thiamine pyrophospho kinase of vitamin B1 biosynthetic pathway as a drug target in Lesishmania donovani.
33. Collett CF, Kitson C, Baker N, Steele-Stallard HB, Santrot MV, et al. (2019) Chemogenomic profiling of antileishmanial efficacy and resistance in the related kinetoplastid parasite Trypanosoma brucei. Antimicrobial Agents and Chemotherapy 63(8):10-1128.

Copyright: *©2024 Dhamyaa Kareem Kadhim. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and source are credited.*