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Trypanosoma brucei brucei induced hypoglycaemia resulted in severe reduction in hexokinase activity in liver, kidney, brain and heart of untreated *Trypanosoma brucei brucei* infected mice

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Abstract

Trypanosoma brucei brucei depends on mammalian host glucose for survival and establishment of infection. The interference with host glucose results in disturbance of pathways for glucose metabolism and their enzymes. Therefore, this study tried to examine the relationship between untreated *Trypanosoma brucei brucei* infection and the host hexokinase activity in selected organs that depend on glucose for their normal biological and biochemical functions. The mice for this study were grouped into two: control (uninfected) and infected group. Baseline values for PCV, serum glucose and protein; hexokinase activity and protein concentration in the liver, kidney, heart and brain were obtained for each group. The infected group was intraperitoneally inoculation by 1×10^4 parasites/mice and monitor for the presence of *Trypanosoma brucei brucei* from the second day post infection. The same parameters were collected again on days 4 and 11 before the death of the infected group. Protein was determined by colorimetric method using Bradford reagent. PCV was analysed using a sysmex haematology analyser while hexokinase activity was measured spectrophotometrically by a coupled reaction with glucose-6-phosphate dehydrogenase at 340 nm. The result of this study showed that untreated *Trypanosoma brucei brucei* infection resulted in decrease in PCV, serum glucose, hexokinase activity in the liver, kidney, heart and brain but increase in serum protein. In conclusion, untreated *Trypanosoma brucei brucei* infection resulted in reduction in host liver, kidney, brain and heart hexokinase activity which probably deprived them of the needed energy and may be the cause of early death in untreated trypanosomiasis.

Keywords: African trypanosomiasis; Energy production; Glycolysis; Hexokinase activity *Trypanosoma brucei brucei*

1. Introduction

African trypanosomiasis (HAT) is a threat to food security in sub-Saharan Africa due to large arable land that is rendered uncultivable and incapacitation of the infected individuals [1, 2, 3, 4]. To establish infection and survive in mammalian host, the bloodstream form (BSF) *Trypanosoma brucei brucei*, relies solely on glycolysis for energy (ATP) production because of its lack of active component of citric acid cycle and electron transport chain for oxidative metabolism of pyruvate and other reducing equivalent generated during glycolysis [5].

The rate of glucose utilisation in bloodstream *T. brucei brucei* is 50-fold higher than that of mammalian tissues which has been linked to hypoglycaemia associated with trypanosomiasis [6, 7,8, 9,10]. The interference with host glucose results in reduction of available glucose to the host for energy production and other biological processes that require glucose. In addition, pathways and enzymes for glucose metabolism will be affected. One of such enzyme that may be affected is hexokinase, an enzyme in the glycolytic pathway that catalyses the conversion of glucose to glucose 6-phosphate [6]. This is evident in most of the symptoms associated with the disease such as weakness, confusion, sleeping and neurological disorder which can be clearly linked to interference in the process of energy generation of

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which hexokinase plays a key role. Therefore, it is necessary to study the relationship between untreated *Trypanosoma brucei brucei* infection and the host hexokinase activities in selected organs that depend on glucose for their normal biological and biochemical functions during the course of the infection.

2. Material and methods

2.1. Parasite Strain

Trypanosoma brucei brucei (Federe strain) was obtained from the Institute of Trypanosomiasis Research Centre, Kaduna State, Nigeria and maintained in the laboratory by serial blood passage in rats until when required.

2.2. Experimental Animal and Housing

Twenty adult male mice weighing between 25g and 35g were obtained from Nigerian Institute of Trypanosomiasis Research Centre, Vom, Plateau State, Nigeria. They were housed in fly proof well-ventilated experimental animal house in Bingham University Karu, Nasarawa state, Nigeria. Animals were humanely cared for in compliance with the principle of laboratory animal care. They were fed with pelletized grower feed and water was available *ad libitum* throughout the period of the study. Animal experiments were carried out in accordance with the guidelines for the Care and Use of Laboratory animals.

2.3. Experimental Design

The mice were divided into two groups:

GROUP A (CONTROL): This group comprises of ten (10) mice that were not infected with *Trypanosoma brucei brucei*

GROUP B (INFECTED): This group comprises of ten (10) mice that were infected with *Trypanosoma brucei brucei*

2.4. Animal inoculation

Parasite free healthy mice were infected by intraperitoneal inoculation of $1x10^4$ parasites/mice in 0.3ml of phosphate saline glucose (PSG) (pH 7.8). Parasitemia was monitored daily from the second day after the infection by bleeding the tail of each animal and the mobile parasites counted using haemocytometer.

2.5. Determination of Packed cell volume

Packed cell volume was determined using the automated haematologic analyzer (Sysmex, KX-21, Japan).

2.6. Duration of the experiment and Preparation of Samples

Trypanosoma brucei brucei infection was allowed to run its course naturally without treatment until first mortality was recorded and all the mice exhibiting the chronic symptoms of the second phase of the disease before the research was terminated. The mice were euthanized by intramuscular injection of ketamine (24 mg/kg/body) and sacrificed by cervical decapitation. The liver, kidney, heart and brain were dissected and washed with ice-cold saline immediately to remove blood. Each of them were weighed and the whole organ was homogenized in ice-cold sucrose (0.25M), centrifuged at 10,000 rpm for 30 minutes, and the supernatant was collected and used for biochemical analysis.

2.7. Determination of Hexokinase Activity in the Total Protein Extract

The hexokinase activity was measured spectrophotometrically in triplicate by a coupled reaction with glucose-6-phosphate dehydrogenase. Hexokinase activity was measured by following the reduction of $NADP^+$ at 340 nm. That is, the phosphorylation of glucose (hexokinase activity) was measured by monitoring the production of NADPH.The reaction mix included 50 mM Tris-HCl (pH 7.6), 5.0 mM magnesium chloride, 10 mM glucose, 5.25 mM ATP, 1.0 mM $NADP^+$ and 1 IU glucose- 6-phosphate dehydrogenase. The reaction mix was run at 25°C for 6 min on a UV-VIS spectrophotometer before cell extract was added and changes in absorbance (A_{340}) was measured over time. The change of absorption over time was converted to change of $NADP^+$ concentration using a molar extinction coefficient of 6,220 $M\ cm^{-1}$. The stoichiometry of the coupled enzyme assay dictates that changes in concentration of $NADP^+$ correspond to equal changes in glucose concentration, that is, the change in $NADP^+$ concentration is a measure of the hexokinase activity.

2.8. Determination of the Total Protein in the Extract

The protein concentration was determined according to the method described by Bradford [11].

2.9. Statistical Analysis

Statistical analysis of the data was done using Graph Pad prism version 5. The differences in various parameters within the groups and across the groups were investigated using one-way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test. The $p < 0.05$ were considered significant.

3. Results

From this study, we observed that the infection resulted in gradual decrease in packed cell volume (PCV) of the infected mice (Table 1) until the decrease became significant ($p<0.05$) compare to the packed cell volume of the control mice on day 11 when the mice were sacrifice when first death was recorded and the other mice became immobile as a result of the infection. On the other hand there was no significant difference ($p<0.05$) between the baseline PCV of control and the value recorded on day 11(Table 1). Packed cell volume (PCV) gives a reliable indication of the disease status and anaemia of trypanosome infected organisms. This clearly showed that the disease became severe as the day progresses.

Table 1 Effect of Trypanosomiasis on Pack Cell Volume (%) of *T. brucei brucei* infected mice

Group	DAY 0	DAY 4	DAY 11
Control	37.77 ± 0.87^{a}	38.57 ± 1.63^{a}	38.77 ± 0.29^{a}
Infected	37.28 ± 0.86^{a}	33.18 ± 1.43^{b}	$20.63\pm0.37^{c,*}$

The values are expressed as mean ± standard deviation of the number of the surviving animals
Values with different alphabets are significantly different within group across the days ($p<0.05$)
* Significant different from the last value of the control ($p<0.05$)

All the infected mice exhibited parasitaemia on the fourth day post infection (pi) (Figure 1). There was a gradual increase in the parasitaemia in the first 3 days post infection, followed by a sharp rise to a peak value on day 8 post infection and followed by a drop. However, the parasitaemia began to rise until the death of one of the animals before the experiment was terminated on day11.

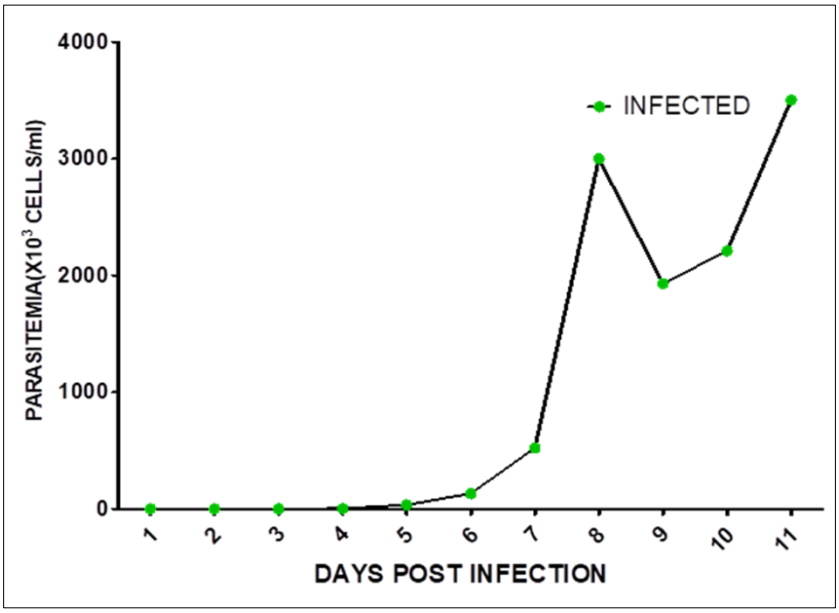

Figure 1 Parasitaemia Level in *Trypanasoma brucei brucei* infected mice

Decrease in serum glucose concentration was observed in all the infected mice but gradual increase was observed in the control group (Table 2). It is important to know that there was a significant difference ($p<0.05$) between the base line serum glucose and the final serum glucose concentration in both control and the infected groups. Significant

decrease was observed in the infected group while increase was observed in the control. It is interesting to note that about 43.43% of the baseline glucose was lost to the infection in the infected group which is deleterious to the host.

Table 2 Effect of Trypanosomiasis on Serum Glucose Level (mg/dl) of *Trypanasoma brucei brucei* Infected Mice

Group	DAY 0	DAY 4	DAY 11
Control	143.2±2.62[a]	148.6 ± 3.31[a,b]	154.8±4.11[b]
Infected	146.± 3.80[a]	133.9 ± 5.90[b]	78.25 ± 5.91[c*]

The values are expressed as mean ± standard deviation of the number of the surviving animals
Values with different alphabets are significantly different within group across the days ($p<0.05$)
* Significant different from the last value of the control ($p<0.05$)

Table 3 Effect of trypanosomiasis on hexokinase activities (U/ml) of the liver, kidney, and heart and brain of T*rypanasoma brucei brucei* infected mice

Group		DAY 0	DAY 4	DAY 11
LIVER	CONTROL	3.89±0.74[a*]	4.47±0.41[a*]	7.10±0.20[b*]
	INFECTED	3.72±0.74[a*]	1.52±0.10[b**]	0.27±0.06[c**]
KIDNEY	CONTROL	2.32±0.18[a*]	2.48±0.08[a*]	2.58±0.43[a*]
	INFECTED	2.45±0.31[a*]	1.15±0.17[b**]	0.41±0.08[c**]
HEART	CONTROL	2.53±0.02[a*]	2.80±0.58[a*]	3.23±0.54[a*]
	INFECTED	2.46±0.10[a*]	1.56±0.10[b**]	1.12±0.02[c**]
BRAIN	CONTROL	7.91±0.02[a*]	8.09±0.68[a*]	9.97±0.81[b*]
	INFECTED	7.81±0.28[a^]	4.83±0.27[b**]	2.23±0.17[c**]

The values are expressed as mean ± standard deviation of three observations
Values with different alphabets are significantly different within group across the days ($p<0.05$)
* Significantly different from the control ($p<0.05$)

Table 4 Protein concentration (g/dL) in the serum. liver, kidney, heart and brain of *Trypanasoma brucei brucei* infected mice

GROUP		DAY 0	DAY 4	DAY 11
LIVER	CONTROL	2.77± 0.04[a*]	4.17±0.06[b*]	4.15±0.93[b*]
	INFECTED	2.54±0.21[a*]	2.04 ±0.05[b**]	1.77 ±0.04[a**]
KIDNEY	CONTROL	1.71±0.29[a]	1.51±0.01[a*]	1.21±0.02[b*]
	INFECTED	1.77±0.03[a]	0.58±0.08[b**]	0.34±0.05[c**]
HEART	CONTROL	2.14±0.29[a*]	2.79±0.61[a*]	2.05±0.04[a*]
	INFECTED	2.13±0.29[a*]	2.14±0.01[a*]	1.83±0.11[b**]
BRAIN	CONTROL	2.14±0.48[a*]	2.01±0.1[a*]	1.79±0.03[a*]
	INFECTED	2.20±0.13[a*]	2.05±0.45[a*]	2.04±0.04[b**]
BLOOD	CONTROL	0.77 ± 0.06[a*]	0.81±0.08[a*]	0.87± 0.13[a*]
	INFECTED	0.70 ± 0.02[a*]	0.82 ±0.06[a*]	2.10 ±0.27[b**]

The values are expressed as mean ± standard deviation of three observations
Values with different alphabets are significantly different within group across the days ($p<0.05$)
* Significant different from the control ($p<0.05$)

From the results, there was a steady decrease in the hexokinase activity of the host liver, kidney, heart and brain until they become significant on day 11 when the mice started showing the chronic symptoms of the second stage of the

infection (Table 3).The decrease in the hexokinase activities also occur at different rate in the different organs examined. For example, on day 4 post infection when the parasite were first sighted, the hexokinase activity in the liver, kidney, heart and brain has reduced by 59.14%,53.06%, 36.59,38.16 respectively, making the liver the most affected and the brain the least affected. At the chronic last stage when the animal were immobilised by the infection, the hexokinase activity has reduced by 92.74%,83.27%,54.47% and 71.45% in the liver, kidney, heart and brain respectively. This still made the liver the most affected organ followed by kidney then brain and the least affected being the heart.

Generalised decrease in protein concentration was observed in the liver, kidney, brain and heart of the infected mice compare to the control but increase was observed in the serum (Table 4).The decrease was gradual until it become significant in all the organs examined. The significant increase in the serum as oppose to the decrease in the organs may be due to the release of protein from the host destroyed cells and dead parasites.

4. Discussion

There was a significant decrease in Pack Cell Volume (PCV) of the infected group compared to the control group. The significant decrease in Pack Cell Volume (PCV) of the infected group signified blood loss or anaemia in the infected groups which is one of the major signs of trypanosomiasis [12]. This agrees with previous studies on *Trypanosoma* infection [13, 14, 15]. Many factors might have contributed to the significant decrease in PCV observed in infected groups. These include haemolysis [16], mechanical damage of red blood cells by the parasite movement [16]. Other possible cause of the anaemia is malnutrition which result from inadequate food intake by the animals as the disease progresses which affects the availability of essential nutrients necessary for haematopoesis [16]. Increase in the production of hydrogen peroxide and superoxide radicals by macrophages of the activated mononuclear phagocytic system and by *T. brucei* which deplete the blood of the endogenous antioxidants which are necessary for the protection of the red blood cells from the free radicals have been shown to increase the susceptibility of red blood cell membrane to oxidative damage resulting in low PCV observed in the infected group [17, 18,19,20,21,22]

All the infected animals became parasitaemic by day 4 post infection. The infection was acute in nature reaching peak parasitaemia on day 8 post infection. The short pre-patent period observed in this study was comparable with the observation of Egbe-Nwiyi et al.[23] and Ezeokonkwo *et al*.[14].The rapid multiplication is also confirm by accelerated fall in packed cell volume (PCV) of the infected group[21,22,24].

Significant decrease in serum glucose of the infected group compare to the control is an indication of *T. brucei brucei* metabolic activity since blood stream form *T. brucei brucei* is known to solely depend on the host blood glucose for their growth and survival in mammalian hosts [25,26]. Many authors have also reported the same observations in trypanosomal infected animals [27, 28,29]. The current finding was also supported by the works of Mazet et al.[7] and Nwagwu & Opperdoes [6] who discovered that the rate of glucose consumption in bloodstream *T. brucei brucei* to be 50 times higher than that of mammalian tissues leading to a sharp decrease in host blood glucose. Hypoglycaemia observed in this study is likely due to excessive utilization of glucose by the parasites [7, 8, 9, 10].

Many reasons have been given for high rate of glucose utilisation by the bloodstream form *T. brucei brucei*. Most important of this, is that, bloodstream (BSF) *T. brucei brucei* replicate faster to replace parasites destroyed by the immune system [10]. This implies that bloodstream *T. brucei bruce*i must produce ATP at faster rate to meet its energy demand for replication. Secondly, the ATP molecules produced per glucose is lower in bloodstream *T. brucei brucei* than in the procyclic form of *T. brucei brucei* found in insects [10,30,31]. Finally, some biological processes consume more ATP in blood stream form of the parasite compare to the procyclic form (PFs), for example, endocytosis is 10-fold upregulated in bloodstream form compared to the procyclic form [32,33],this is essential for rapid recycling of cell-surface glycosylphosphatidylinositol (GPI)-anchored variant surface glycoprotein (VSG) for internalisation and removal of bound antibodies which is crucial for their escape from the host immune defenses and also help in obtaining nutrient from the host[10,32,33]

The ability of the blood stream form *T. brucei brucei* to generate ATP from glycerol as the alternative source to glucose have been reported [7, 34, 35], but there is relatively low level of glycerol in mammalian bloodstream [36].In addition to this, another major obstacle to the use of glycerol by the parasite for ATP generation is the discovery that more oxygen are consumed by the parasite when glycerol is used. That is, two times more oxygen is consumed per carbon atom when glycerol is the carbon source for ATP generation yet with a reduced ATP production rate that does not allow cells to grow. This implies that glycerol catabolism is strictly oxygen dependent while glycolysis can occur under both aerobic anaerobic conditions [25, 26].

Generalised decrease in hexokinase activity was observed in all the organs examined in the infected mice. Similar alteration in hexokinase activity in different organs as a result of disease conditions have been observed by many authors with evidence of alteration in glucose metabolism[37,38,39,40,41,42,43,44,45]. The decrease in the hexokinase activity in this study could not be linked to the feedback inhibition by the glycolytic pathways products in the organs since severe reduction in the serum glucose was also observed in this study. It could only be due to excessive utilization of glucose by the *T. brucei brucei* which deprived the enzyme of its substrate (glucose) due decrease in glucose influx into the cells [7, 8, 9, 10].

Apart from the excessive consumption of glucose by the trypanosomes which might have affected the hexokinase activity, pancreatic beta cells are also sensitive to reactive oxygen species (ROS) generated by the parasite and the host macrophage due to their low endogenous antioxidants[46,47]. Increased reactive oxygen species (ROS) in pancreas can cause beta cell destruction or dysfunction which results in decreased insulin that is known to increase glucokinase and hexokinase activity [48]. Decrease in the hexokinase activity may therefore be as a result of the reduced serum insulin level [49, 50]. The oxidative stress that resulted from increased production of free radicals and hydrogen peroxide by *T. brucei* brucei, macrophages and the host phagocytic system[17] which deplete blood and organs their antioxidant reserves during *T. brucei brucei* infection,[18,19,51] can result in degenerative changes in organs and tissues which may cause a breakdown in hepatic or endocrine mechanisms controlling the mobilization of carbohydrate for metabolism[9,18,19,20,29,52]. The above facts made some authors to suggest that terminal hypoglycaemia in trypanosomiasis which affects the glycolysis is likely due to tissue/organ breakdown or derangement of endocrine mechanisms controlling the mobilization of carbohydrate reserves than a direct result of massive consumption of glucose by trypanosomes [53, 54].

High serum protein concentrations observed in the infected animals agree with previous reports [24, 55] but contradict the decreased serum total proteins reported by Abenga and Anosa [56] and Akpda [57]. Increase in serum total protein concentrations of infected animals could be as a result of cell derived proteins due to haemolysis. This suggests that infection by trypanosomes had led to tissue break down and inflammation in the host, especially in the liver, heart, muscle and kidney which resulted in the leakage of these enzymes from their intracellular stores into the plasma thus elevating their levels. This was also reflected in the lower protein concentration in all these organs [54].It may also be attributed to increase in parasite mass proteins derived from the lysis of parasites by host immune system and due to the released of intracellular enzymes due to increasing parasitaemia. Synthesis of immunoglobulin in response to infection and release of erythrocytes-derived enzymes may have contributed to the observed increases in serum protein [58]

5. Conclusion

From the result of this study, we therefore conclude that excessive consumption of host glucose and exhaustion of hosts' carbohydrate reserves which resulted in lethal hypoglycaemia and breakdown of organs' functions which drastically reduced host hexokinase activity and the ability of the host to generate energy may be the cause of early death in untreated trypanosomiasis. This is evident in most of the symptoms associated with trypanosomiasis which can be clearly linked to interference in the process of energy generation of which hexokinase plays a key role. Therefore inhibition of host glucose metabolism by the trypanosomes at the early stage of the disease which will make more glucose available to the host and prevent alteration in the energy generating pathway of the host may play a key role in the survival of the host at the early stage of the disease and also be a target for improving the condition of the patients during the infection.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declare that they have no competing interest

Authours' contributions

Rotimi Johnson Ojo conceived and designed the study. Lag-Ayan Chinyio and Grace Sabo Lot performed the experiments. Rotimi Johnson Ojo supervised the experiments. Rotimi Johnson Ojo, Lag-Ayan Chinyio and Grace Sabo Lot analysed the data. Rotimi Johnson Ojo, Chinyio Lag-Ayan and Grace Sabo Lot drafted the manuscript. Rotimi Johnson Ojo edited and finalized the manuscript. All the authours read and approved the final manuscript.

Statement of ethical approval

Animals were humanely cared for in compliance with the principles of laboratory Animal care as stated by Bingham University Karu Nasarawa State, Nigeria.

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