J Med Primatol doi:10.1111/j.1600-0684.2011.00523.x

ORIGINAL ARTICLE

Lipid metabolism and other metabolic changes in vervet monkeys experimentally infected with *Trypanosoma brucei rhodesiense*

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Keywords
creatinine kinase – lipids – sleeping sickness – staging – vervet monkeys

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Accepted September 28, 2011.

Abstract

Background Human African trypanosomiasis is associated with metabolic changes which have not been well characterized.
Methods Chlorocebus aethiops were experimentally infected with *Trypanosoma brucei rhodesiense* and late-stage disease induced at 28 days post-infection. Ear prick blood for glucose determination and blood samples were obtained at weekly intervals for 56 days. Analysis was carried out using dry chemistry analysis.
Results In early infection, there was a significant increase in creatine kinase, while during early and transitional stage of infection there was a significant decrease in glucose and high-density lipoprotein and an increase in triglyceride levels. In the late stage, there was a significant increase in both total cholesterol and LDL levels.
Conclusions Further investigations should focus on levels of total cholesterol during the follow-up period in curatively treated vervet monkeys. Apart from their importance in disease staging, the changes in lipids levels may also affect the pharmacokinetics of some trypanocides.

Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is the third most important vector-borne parasitic disease in Africa today and is considered a neglected disease. It is estimated that about 60 million people in 36 countries in sub-Saharan Africa are at a risk of HAT [4]. The control of HAT depends mainly on chemotherapy, and current drugs have drawbacks including severe toxicity, mode of administration which requires lengthy hospital administration and increasing incidence of treatment failure [2, 14, 24].

Trypanosome infection leads to multiple organ damage with histopathological changes whose severity depends on the stage of the disease. These clinical and pathological alterations are thought to be caused by various factors including toxins produced by the trypanosome, immunological factors (auto-antibodies, immune complexes, cytokines, kinins and nitric oxide) and metabolism factors [6]. In an effort to understand the pathogenesis of the disease, many studies have focused on the immunological changes [10, 11, 15, 17, 18, 23]. However, less emphasis has been put on metabolic changes.

The most conspicuous alternations in visceral organs involve the adipose tissue that is markedly lost [6]. The few reports available, however, have contradictory findings and only on a few lipids. Tumour necrosis factor (TNF) which is produced during acute *Trypanosoma brucei* infections inhibits triglyceride removal [3, 21] and this may lead to rise in triglycerides. In the vervet monkey model of HAT, dramatic increase in triglycerides and cholesterol has been reported [19]. However, in sleeping sickness patients, lowered triglyceride and cholesterol levels have been reported [1]. Muscle wasting and weight loss have been reported in sleeping sickness

patients and animal models of HAT [6, 7]. However, the kinetics of creatine kinase (CK) which rises during muscle wasting has not been reported. Profiling various lipids, glucose and CK may help in understanding how they interrelate during the course of disease progression. Using the vervet monkey model of HAT [7], we investigated the changes in various lipids (triglycerides, total cholesterol and lipoproteins), glucose and creatine kinase, during early (1–7 dpi), transitional stage (14–28 dpi) and advanced late-stage disease (35–61 dpi).

Materials and methods

Trypanosomes

Trypanosoma brucei rhodesiense isolate IPR 001 was used in this study. It was isolated from the cerebrospinal fluid of a late-stage HAT patient in Bugiri, Uganda in 2008 [16]. The isolate was passaged in irradiated (500 Rads) Swiss White mice before cryopreservation in liquid nitrogen.

Experimental animals

Seven vervet monkeys of both sexes, weighing 2.0–6.0 kg were recruited for the study. They were trapped from the wild in an area known to be non-endemic for human trypanosomiasis. The animals underwent to a 90-day quarantine, during which they were screened for zoonotic diseases and treated for ecto- and endoparasites before being subjected to the experiment. They were trained for ease of adaptation and maintained on commercial chew (Unga Feeds® Ltd., Nairobi, Kenya) supplemented with fresh fruits and vegetables. Drinking water was provided *ad libitum*. The monkeys were housed in stainless steel cages at ambient room temperatures of 18–25°C, under biosafety level II animal holding conditions.

Experimental design

Four monkeys were infected intravenously with approximately 10^4 trypanosomes, delivered in 1 ml of phosphate saline glucose, while three monkeys comprised a non-infected control group. The infected animals were given sub-curative treatment with Diminazene aceturate (DA) (Veriben®; Sanofi, Paris, France) at 5 mg/kg body weight (bwt) intramuscularly for three consecutive days, from 28 days post-infection (dpi). The vervets were sampled a week before the experimental infections to provide baseline data. A daily clinical evaluation of the appetite, clinical appearance and disease symptoms was conducted before and during the course of infection. The parasitaemia was scored daily using the method previously described [9] while weekly ear prick blood was obtained for glucose determination.

The animals were anaesthetised at weekly intervals with ketamine hydrochloride (Agrar®; Agrar Holland BV, Soest, the Netherlands) at 10 mg/kg bwt, weighed and clinically examined. The animals were euthanized upon presentation of late-stage clinical signs.

Blood samples were obtained from the femoral vein weekly starting 1 week before infection to 56 dpi. Blood for serum separation was collected in plain vacutainers, incubated at room temperature for 1 hour then at 10°C overnight, while blood for plasma was collected in Ethylene di-amine tetra-acetic acid tubes and kept in ice. Plasma and serum samples were prepared by spinning blood at 1,500 *g* for 10 minutes and aliquoted before storage at −20°C.

Blood glucose determination and biochemical assays

Ear prick blood was collected weekly on test strips which were read immediately in a glucometer (Accu-Chek® Go; Roche Diagnostics GmbH, Mannheim, Germany) for measurement of glucose levels. Biochemical assays were performed using the dry chemistry Reflotron® plus biochemical analyzer (Roche Diagnostics GmbH). Briefly, the samples were thawed and a 32 μl aliquot placed on biomolecule assay-specific reaction strip which were read by the biochemical analyser.

The CK assay was carried out on serum while lipids assays (triglyceride, total cholesterol and HDL) were carried out on plasma as recommended by the manufacturer. The LDL cholesterol levels were calculated using the Friedwald formula [8].

Data analysis

Data were managed using an Ms Excel (Microsoft, SAS Institute Inc., Cary, NC, USA) spreadsheet and analysis was undertaken using Minitab version 15 (Minitab Inc, State College, PA, USA). Descriptive statistics and summary tables were used to initially describe the data using Ms Excel (XlStat Version 2010.2.03, Addinsoft, NY, USA). Paired clustered means were compared using the Student's *t*-test. Means were deemed significant when the indicated probability for test of equality was $< 5\%$ ($P < 0.05$).

Ethical review

All protocols and procedures used in the current study were reviewed and approved by the Institutional

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Review Committee (IRC) of the Institute of Primate Research (IPR), Kenya.

Results

Clinical signs

The infected animals developed symptoms characteristic of trypanosomiasis including: fever, dullness, increased respiratory and pulse rates, pallor of mucous membranes, enlarged lymph nodes and spleen, raised hair coat and erythema on the skin around the eyes. The infected animals showed a steady decrease in body weight and a significant drop ($P < 0.05$) was noted at 28 dpi. Other notable clinical signs included poor appetite and difficulty in perching. The animals presented with late-stage clinical signs between days 56 and 63 post-infection and were euthanized.

Two infected monkeys lapsed into coma 9 dpi. Efforts to resuscitate the monkeys using intravenous (i.v.) glucose were successful only for one monkey which immediately regained consciousness. The other monkey did not respond and was promptly euthanized.

Parasitaemia

The pre-patent period ranged from 2 to 4 days. The parasites multiplied rapidly, giving a first parasitaemia peak of approximately 1×10^9 trypanosomes/ml at 9 dpi. Thereafter, the parasitaemia remained high, characterized by minor fluctuations (Fig. 1). Treatment with DA, 28 dpi resulted in clearance of the trypanosomes in the blood. The parasites reappeared in blood from 51 to 56 dpi.

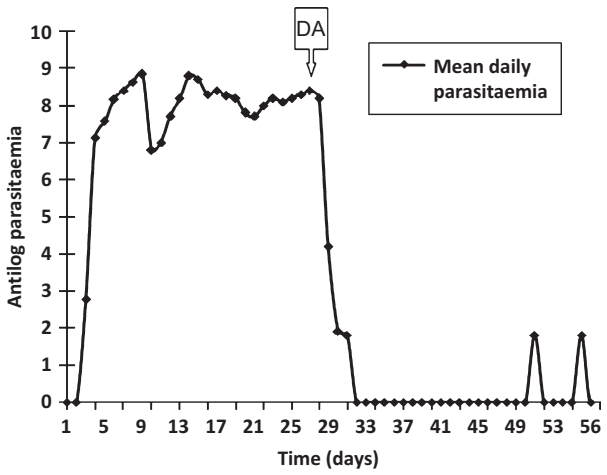

Fig. 1 Mean daily parasitaemia of monkeys infected with *Trypanosoma brucei rhodesiense* IPR 001. Sub-curative treatment with Diminazene aceturate to induce late stage was undertaken at 28 dpi.

Blood glucose levels

The glucose levels in the uninfected animals ranged from 6.5 to 2.9 mM during the experimental period. Prior to infection, the mean baseline glucose levels in the test animals were 4.9 mM (ranging from 3.6 to 6.7). There were no changes in glucose levels during the first 7 days after infection. However, during the transitional stage, glucose levels decreased and significantly dropped ($P < 0.05$) to a mean of 2.133 ± 0.669 mM being recorded 21 dpi (Fig. 2). One vervet monkey that had been in coma exhibited the lowest glucose levels (0.8 mM) and was resuscitated by glucose intravenous infusion. After sub-curative treatment, the glucose levels returned to pre-infection values.

Creatine kinase

Pre-infection, CK levels were 369 ± 50 U/l. In early infection (7 dpi), CK levels increased significantly ($P < 0.05$) to 1668 ± 242 U/l. Thereafter, there was sustained decrease reaching pre-infection levels by 28 dpi (Fig. 3). In the uninfected (control) animals, CK varied between 115 and 705 U/l during the experimental period.

Lipids

Triglycerides

Before infection, the baseline pre-infection triglyceride levels were 0.8211 ± 0.020 mM. In the control animals, triglyceride varied only slightly ranging from 0.8 to 0.97 mM. However, in early infection (7 dpi), the levels of triglycerides significantly increased ($P < 0.05$)

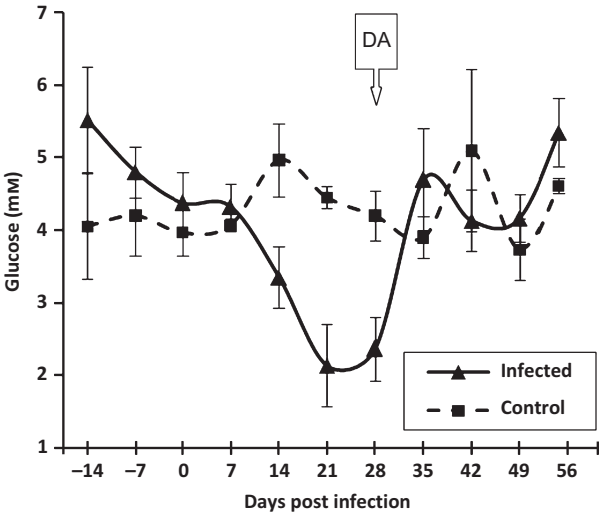

Fig. 2 Mean changes in weekly glucose levels of control and *Trypanosoma brucei rhodesiense* IPR 001 infected vervet monkeys.

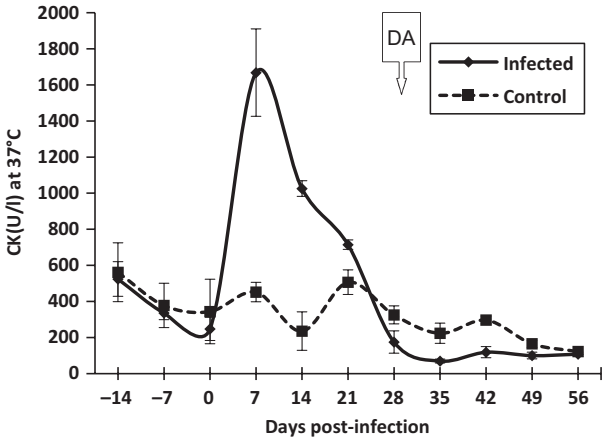

Fig. 3 Mean changes in weekly creatine kinase levels of control and *Trypanosoma brucei rhodesiense* IPR 001 infected vervet monkeys.

to 4.78 ± 0.98 mM. In the transition stage (14–28 dpi), there was a gradual decrease in triglyceride but nevertheless, the levels were significantly higher than pre-infection. During the advanced late stage, the pre-infection levels of triglyceride were attained (Fig. 4).

Total cholesterol

The total cholesterol levels of control animals ranged between 2.599 and 3.57 mM during the experimental period. The total cholesterol levels of infected vervet monkeys were within the normal range (2.342 ± 0.16 mM) during the acute and transitional stages of infection (7–28 dpi). However, during the late advanced stage, the levels of cholesterol increased significantly ($P < 0.05$) and the highest level recorded was 4.367 ± 0.13 mM at 42 dpi (Fig. 5).

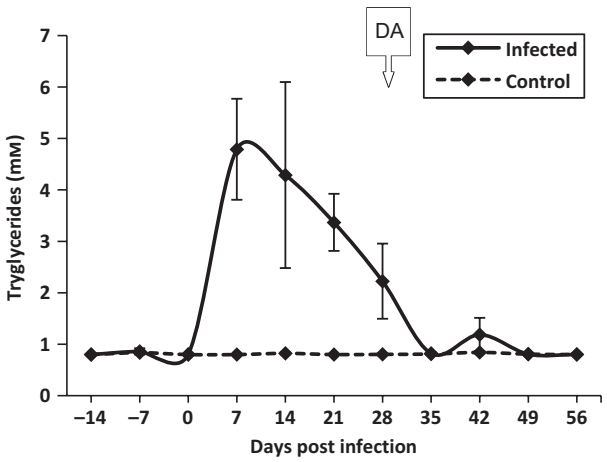

Fig. 4 Mean changes in weekly triglycerides levels of control and *Trypanosoma brucei rhodesiense* IPR 001 infected vervet monkeys.

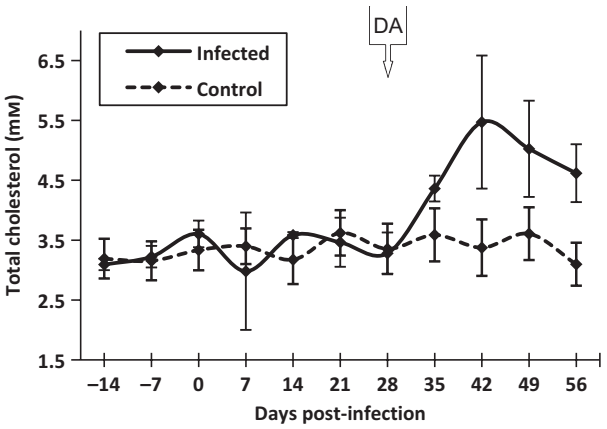

Fig. 5 Mean changes in weekly total cholesterol of control and *Trypanosoma brucei rhodesiense* IPR 001 infected vervet monkeys.

Low-density lipoprotein (LDL)

Prior to infection, the levels of LDL in test animals were 1.293 ± 0.060 mM and were within the range of the control animals. In early stage of infection, the LDL levels decreased but this drop was not significant ($P > 0.05$) (Fig. 6). In the transition stage of infection, LDL levels were similar to those in pre-infection. However, during the late advanced stage of infection, the LDL cholesterol levels significantly increased ($P < 0.05$) and highest levels of 2.977 ± 0.35 mM occurred 42 dpi.

High-density lipoprotein cholesterol (HDL)

The HDL levels in the uninfected animals ranged from 0.69 to 2.2 mM during the experimental period. Pre-infection HDL was 1.55 ± 0.061 mM. In early stage of infection, HDL levels decreased significantly

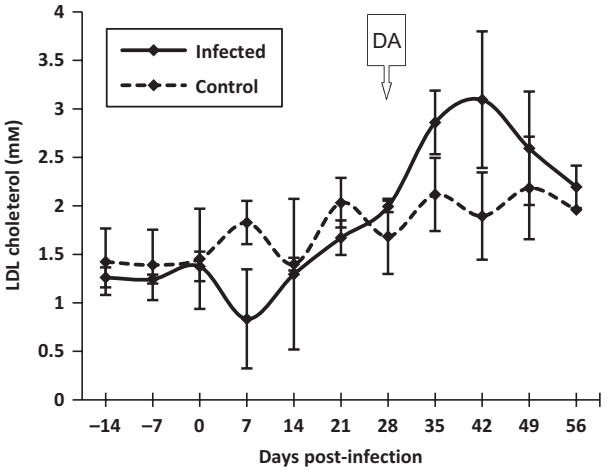

Fig. 6 Mean changes in low density lipoprotein cholesterol of control and *Trypanosoma brucei rhodesiense* IPR 001 infected vervet monkeys.

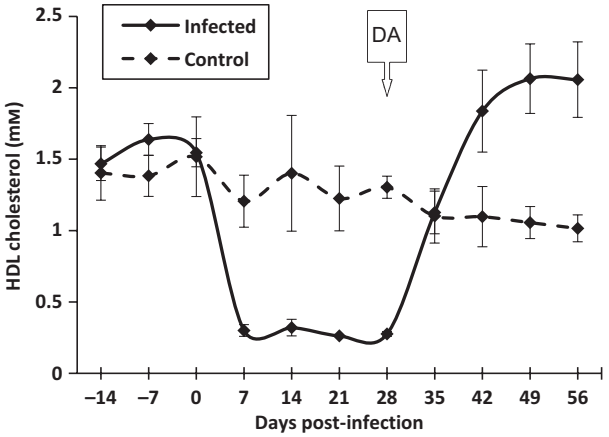

Fig. 7 Mean changes in high density lipoprotein cholesterol of control and *Trypanosoma brucei rhodesiense* IPR 001 infected vervet monkeys.

($P < 0.05$) to 0.3 ± 0.041 mM and remained low in the transitional stage of infection (Fig. 7). However, in the late advanced stage of infection, there was a sharp increase in the HDL returning to pre-infection levels.

There was a significant correlation ($P < 0.05$; $R^2 = 79.3\%$) between LDL and total cholesterol but there was no correlation between HDL and total cholesterol ($R^2 = 29.9\%$).

Discussion

In this study, a recently isolated trypanosome was used. The infected vervet monkeys displayed classical clinical symptoms associated with HAT. The clinical features observed were also similar to those in monkeys infected with *T. b. rhodesiense* KETRI 2537 [16]. This experimental HAT model may therefore be used to study the changes that occur during infection. In this study, we report the changes in various lipids, glucose and CK during early (1–7 dpi), transitional (14–28 dpi) and advanced late-stage disease (35–61 dpi).

In the present study, there was a sustained drop of blood glucose at 14–28 dpi which appeared to correspond to high parasitemia. Two of the infected vervet monkeys went into comatose at 9 dpi and one monkey recovered after glucose infusion pointing to a possible hypoglycaemic coma. Utilization of glucose by trypanosomes has been reported as the cause of hypoglycaemia in infected hosts [25] but the levels of glucose in HAT patients are, however, rarely studied. Hypoglycaemia may signal host metabolism towards mobilization of other energy sources such as lipids and proteins that may account for weight loss noted in this study. Further analysis indicated increased plasma CK, an enzyme that is raised in muscle wasting as result of rhabdomyolysis [12]. Elevation of creatinine kinase is also one of the acute-phase reactions during trypanosomiasis [19].

In the current study, we noted differences in trends of lipids during the early and late stage of infection. During the early stage, there was also marked elevation in triglyceride and a significant decrease in HDL. Elevation in triglycerides was also noted in an earlier study in *T. b. rhodesiense*-infected vervet monkeys [19] and in sleeping sickness patients [10]. These alterations in triglycerides and HDL could be owing to acute-phase responses during early infection [13]. TNF produced during acute infection has been shown to inhibit adipose tissue enzyme lipoprotein lipase [3, 21] that is responsible for clearing lipids from plasma. Secondly, in response to gluconeogenesis, there would be increased lipolysis of triglycerides from fat cells in adipose tissue. There are reports that the adipose tissue is markedly lost in trypanosomiasis [6].

In the advanced late stage of infection (35–61 dpi), there was significant increase in LDL and total cholesterol. Recent studies have shown that there is cholesterol regulatory mechanism in the brain that directly control cholesterol metabolism in the liver through melanocortin signalling in the CNS [20]. As late-stage sleeping sickness is largely marked by trypanosome-CNS invasion, the disruption of the normal neural circuit system in the brain could therefore directly affect the control of cholesterol metabolism in the liver, leading to the hypercholesterolaemia observed. Indeed, in this study, a correlation between hypercholesterolaemia and the elevated LDL was noted suggesting that cholesterol was most probably owing to LDL. At 42 dpi, both LDL and cholesterol were at peak levels. In a previous study, peak levels of serum IL-10, a late-stage cytokine, were also noted at 42 dpi [18]. The parasite has been observed in the CSF from as early as day six of infection but invasion of the brain tissue may not occur before 42 dpi [22]. Previous histopathological studies revealed that by 42 dpi, the parasites are starting to cause lesions in the brain [17]. Indeed, when killed at 56–61 dpi, brain lesions were distinct and uniformly observed among the infected monkeys [16].

Our findings suggest serum cholesterol may also be used as an adjunct for advanced late-stage disease. However, further investigations on cholesterol should focus on its trends in curatively treated vervet monkeys during the follow-up period. Subsequently, validation of the parameter in sleeping sickness patients needs to be performed.

Another important consideration is related to HAT chemotherapy. Changes in lipids levels may affect the pharmacokinetics of certain trypanocides in the sleeping sickness patients and hence affect the therapeutic outcome. In addition, bloodstream forms of trypanosomes are unable to synthesize their own cholesterol and obtain it from the host [5, 6]. This uptake may be exploited in trypanocidal drug formulation. Coupling of trypanocidal drugs to lipoproteins such as HDL may increase the uptake of coupled drugs by the trypanosomes. This would increase drug selectivity and hence decrease toxicity.

Acknowledgments

This work received financial support from Jomo Kenyatta University of Agriculture and Technology (JKUAT) RPE department and Institute of Primate Research (IPR) HAT program and Institute of Primate Research (IPR) HAT Research Program through EU FP7 funding of Nanotryp consortium (project reference number: 223048). The technical assistance particularly from Mr Christopher Kinyanjui and Mr Victor Mwadime of IPR-HAT program and Ms Grace Kimani of Biochemistry department JKUAT is gratefully acknowledged.

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