Serum lipids may not be potential markers in staging of Human African Trypanosomiasis

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Abstract. Successful management and prognosis of Human African Trypanosomosis (HAT) is dependent on proper diagnosis and staging of the disease. Lipid profiles appear to have a profound relationship to the stage and severity of disease and hence possible effect on the outcome of chemotherapy. This study was designed to determine the profiles of cholesterol and triglycerides in Trypanosoma brucei brucei infected vervet monkeys as potential markers for staging of the disease. Three vervet monkeys were infected intravenously with $10^4$ T. b. brucei (isolate GUTat 1) while two monkeys served as non-infected controls. Late stage disease was induced by sub-curative treatment using diminazene aceturate (DA) 28 days post infection (dpi). On relapse, the animals were treated curatively with melarsoprol. Blood for serum preparation was collected at weekly intervals for 300 days post infection. Biochemistry analysis was performed using the Humalyzer 2000 (Lab-chem, Germany). The results showed a decrease in both total cholesterol and low density lipoprotein cholesterol (LDL) and significant increase in high density lipoprotein cholesterol (HDL) and triglycerides levels in early stage disease. In the late stage disease (35-77) there was a significant increase in total cholesterol. Thereafter there was a significant decline corresponding to the trypanosome relapse. After curative treatment the levels declined to pre-infection levels. The disease was associated with marked alterations in the cholesterol and triglycerides levels. However the changes in the lipids tested were not sustained either during early or the late stage disease. This implies that none of these lipids can be used for diagnosis or staging of the HAT. These changes however could affect the pharmacokinetics of trypanocidal drugs and complicate management of HAT.

Keywords: Vervet monkey; Lipids; Markers; Sleeping sickness; T. b. brucei.

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Introduction

Human African trypanosomiasis (HAT, sleeping sickness) is still endemic in several parts of sub-Saharan Africa and it constitutes a major health hazard in endemic countries (Janning, 2004). It is one of the most neglected diseases (Morel, 2003) and its management is constrained by poor diagnostics and treatment regimens. In Kenya, the disease has been endemic in Western part of the country and has lately been reported in a non-endemic location within Maasai Mara Game Reserve (Clerinx et al., 2012; Wolf et al., 2012).

Staging of the disease is currently done in cerebrospinal fluid (CSF) by determination of presences of trypanosomes and number of white blood cells (WBC). This is compounded by low parasitosis and the fact that WBC cut-off is still questionable. In search for markers of staging, studies have focused on the immunological changes (Maina et al., 2004; Ngotho et al., 2006; 2009). Trypanosome infection leads to multiple organ damage whose severity depends on the stage of the disease and analysis of biomolecules in serum is known give an indication of not only the degree of damage to the host tissue as well as severity of infection (Cano et al., 2004).

Cholesterol is transported in the body in lipoprotein. Elevated serum LDL carries a risk for metabolic and cardiovascular disorders, whereas HDL in blood is thought to be beneficial (Perez-Tilve et al., 2010). Dramatic increase in triglycerides and cholesterol has been reported in the Rhodesian monkey model of HAT (Ngure et al., 2008) and also in sleeping sickness patients (Huet et al., 1990). In an earlier study, elevated serum cholesterol levels were reported and was suggested it would be adjunct for advanced late stage disease (Gaithuma et al., 2011) These changes were however noted in an experimental period of 56 days. This study was therefore aimed at determining lipoproteins and triglycerides levels in a more chronic disease and during follow-up after curative treatment.

Materials and methods

Trypanosomes

Trypanosoma brucei brucei GUTat 1 isolate was used. The isolate is a clone of TREU (Van Deursen et al., 2001) which was isolated from tsetse in Kiboko, Kenya (Goedbloed et al., 1973). The isolate was obtained from International Livestock Research Institute (ILRI) Biobank, passaged thrice in mice and preserved at Institute of Primate Research (IPR) trypanosome cryo-bank. The cryopreserved isolate was thawed and injected into donor Swiss White mice for expansion. At peak parasitaemia, the mice were euthanized and blood harvested by cardiac puncture.

Experimental animals

Five wild caught vervet monkeys weighing 2-4 Kg were used. Initially, these wild trapped monkeys underwent quarantine for 90 days during which they were screened for zoonotic diseases, and treated for ecto- and endoparasites before being recruited in the study. The monkeys were housed in stainless steel cages at room temperatures of 18-25°C. They were fed twice daily with monkey cubes while water was provided ad libitum. Twice a week, the animals were given fruits and vegetables.

Experimental design

Three monkeys were infected intravenously (IV) with 1 ml of the suspension containing $10^4$ T. b. brucei (GUTat 1) while two monkeys comprised a non-infected control group. The infected monkeys were monitored daily for parasitaemia as earlier described (Herbert and Lumsden, 1976). At 28 dpi the monkeys were treated sub-curatively using diminazene aceturate (DA) at a dose rate of 5 mg/kg bwt intramuscularly for three consecutive days to induce late stage disease. The monkeys were then monitored for parasitaemia and on relapse they were curatively treated with melarsoprol at dose rate of 3.6 mg/kg body weight, intravenously (IV) for four consecutive days. The animals were monitored for 180 days post-treatment (dpi) when the experiment was terminated. The animals were
euthanised by injection of Euthatal (20%, sodium pentobarbionate, Rotexmedica, Trittau, Germany) via the femoral vein.

**Ethical review and permit**

The monkeys used in this study were caught from wild in an area known to be non-endemic for trypanosomiasis. A permit allowing the capture of the monkeys from the wild was approved by Kenya Wildlife Services. Prior to commencement of the study, all protocols and procedures used in this study were reviewed and approved by the Institute of Primate Research (IPR) Institutional Animal Care and Use Committee (IRC/19/10).

**Blood sampling**

Blood was collected in plain vacutainers, incubated at room temperature for one hour then at 10°C overnight. Serum was prepared by spinning blood at 2500 rpm for ten minutes and aliquoted before storage at -20°C.

**Biochemistry analysis**

Serum assays for LDL, HDL, total cholesterol and triglycerides (TG) were performed using the Humalyzer 2000 (Lab-chem, Germany) according to manufacturer instructions.

**Disease staging**

The disease stage was determined in accordance with the WHO criteria and as previously described (Gould and Sayer, 1983; Ngotho et al., 2010). Late stage infection was diagnosed when trypanosomes were detected in CSF or presence of CSF white blood cell (WBC) count of >5/mm (Clerinx et al., 2012).

**Data analysis**

Data was managed using Microsoft Excel (Microsoft USA, version 2007) which acted as the database and analysis was undertaken using GraphPad Prism version 5.0. Means and standard errors of the means were computed. Differences between means were compared using the Student’s t-test. Differences between parameters of estimate were deemed statistically significant at p < 0.05.

**Ethical consideration**

All the protocols and experimental procedures used in this study were reviewed and approved by the Animal Care and Use committee of Institute of Primate Research (IPR).

**Results**

**Parasitaemia and CSF parasitosis**

All experimentally infected animals had a prepatent period ranged from two to four days and the first parasitaemia peak about 10^7 trypanosomes/ml of blood occurred 7-9 dpi. Treatment with diminazene aceturate (DA) resulted in clearance of the trypanosomes in the blood by the last day of treatment. The parasites relapsed in blood starting 114 dpi in all the monkeys. The trypanosomes were detected in the CSF of two monkeys on day 28 and 105 PI. Treatment with Melarsoprol at 119 dpi led to clearance both the parasitaemia and CSF parasitosis by the last day of treatment.

**Serum cholesterol levels**

During the entire experimental period the mean total cholesterol level of control animals was 154.31 ± 12.2 mg/dL while the total cholesterol levels of infected vervet monkeys decreased significantly from a pre-infection value of 139.8 ± 0.8 mg/dL by 7 dpi. After induction of late stage disease, the level of total cholesterol increased significantly (p<0.05) in the following four weeks to reach peak levels of 257.2 ± 10.25 mg/dL by 77 dpi (figure 1). Thereafter, the levels declined significantly until commencement of curative treatment with MelB 119 dpi. After curative treatment, there was recovery of total cholesterol to pre-infection values.

**Serum LDL levels**

Pre-infection levels of LDL cholesterol had a mean of 213.2 ± 7.4 mg/dL. After infection, there was a significant decrease in serum LDL cholesterol concentration with the lowest value of 74.4 ± 4.7 being recorded 14 dpi (figure 1). On induction of late stage disease, there was a significant increase and normal pre-infection levels were attained and
remained so until 140 dpi. Thereafter, the levels dropped and fluctuated without exhibiting significant undulations.

**Serum HDL levels**

The mean HDL level of the control animals was 98.3 ± 5.8 mg/dL. In early infection HDL levels increased significantly to a peak of 329.8 ± 18.2 mg/dL 14 dpi (figure 1). After induction of late stage disease, there was recovery of pre-infection values without significant fluctuations throughout experimental period.

**Serum triglycerides levels**

Mean serum concentration of triglycerides in the control was 108.3 ± 5.3 mg/dL. For the treatment group, the levels of triglycerides increased to reach the peak value of 303.5 ± 17.25 mg/dL by 35 dpi (figure 2). The mean PI values of triglycerides decreased rapidly following sub-curative treatment with DA. There was a slight increase in triglycerides levels on relapse and after curative treatment with MelB, the levels recovered and were maintained at normal pre-infection values for the rest of experimental period.

**Discussion**

In the current study, differences in levels of lipids within the early and late stages of infection were observed. During the early stage, there was a marked elevation in triglycerides and HDL and significant decreases in both total cholesterol and LDL cholesterol. The marked elevation in triglycerides observed in the first week of infection may be as a result of mobilization of lipids from adipose tissues. This elevation is attributable to the inhibitory effect of TNF on lipoprotein lipase activity with resultant disturbances in lipid metabolism that mediate acute phase reactions (Khovidhunkit et al., 2004). The elevated triglycerides could also result from defective plasma triglyceride degradation making free fatty acids unavailable for importation into hepatocytes despite serum triglyceride elevation (Adamu et al., 2009). Hypertriglyceridemia observed in this study is in agreement with that observed in vervet monkeys infected with *T. b. rhodesiense* (Ngure et al., 2008; Gaithuma et al., 2011), HAT due to *T. b. rhodesiense* infection (Huet et al., 1990) and in rabbits infected with *T. b. brucei* (Rouser and Cerami, 1980).

![Figure 1](image-url)  
*Figure 1.* Changes in mean total cholesterol, high density lipoprotein and low density lipoprotein cholesterol levels of control and *T. b. brucei* GUTat 1 experimentally infected and treated vervet monkeys.
Decreased cholesterol levels observed at 7 dpi in this study is in agreement with that observed in T. b. brucei infected pigs and T. b. rhodesiense infected vervet monkeys (Gould and Sayer, 1983; Ngure et al., 2008). This observation corresponded to the height of parasitaemia in the experimental animals. Trypanosomes are unable to synthesize cholesterol and other lipids required for multiplication and depend on the host source (Green et al., 2003; Nok and Balogun, 2003). The rapid growth leads to high cholesterol utilization and thus, the lowered serum levels of total cholesterol. In addition, TNF produced during the acute phase response is a potent inhibitor of lipoprotein lipase (Beutler and Cerami, 1988). As a result of this inhibition, conversion of VLDL to LDL is hampered; hence the low levels of LDL. The significant rise in HDL levels during early disease could be two-pronged; as a product of the acute phase response and due to conversion of excess VLDL to HDL. As a result, the high levels of HDL registered during the acute phase support the immune system in control of the infection and indeed in this model, parasitaemia levels decreased following a rise in HDL.

Late stage sleeping sickness is largely marked by trypanosome-CNS invasion and the disruption of the normal neural circuit system in the brain (Perez-Tilve et al., 2010) and could therefore directly affect the control of lipid metabolism. In this study, high cholesterol levels in the late stage disease (35-77 dpi) were consistent with the hyperlipidaemia observed in late stage disease (Ngure et al., 2008; Gaithuma et al., 2011). However, in late stage T. b. gambiense patients, hypercholesterolemia is reported (Huet et al., 1990).

Human African Trypanosomiasis is associated with marked alterations in the composition and levels of host lipids; this could be used to monitor the progression of the disease. However, this study has demonstrated that none of the lipid tested, total cholesterol, triglycerides, HDL and LDL, are sustained during infection. This indicates that their role as markers of disease stage in the vervet monkey model of HAT is doubtful.

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References


