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ORIGINAL ARTICLE

Serum and hepatic lipid levels in rats infected with *Trypanosoma brucei*

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Abstract *Trypanosoma brucei*, Federe strain, caused an acute infection in rats after intraperitoneal inoculation of 10^6 trypanosomes. Parasitemia occurred from day 3 post-infection (pi) with peak parasitemia from day 7 pi. Anemia was observed between days 7 and 11 pi. The serum triglyceride concentration was comparable with the control value on day 7 pi, but increased ($P<0.05$) above the control value on day 11 pi. The serum total cholesterol, high density lipoprotein (HDL), and low density lipoproteins (LDL) cholesterol concentrations decreased ($P<0.0.05$) when compared with the control values on days 7 and 11 pi. The LDL cholesterol decreased more on day 11 than day 7 pi. The liver content of triglycerides and total cholesterol decreased ($P<0.05$) during the infection from control values on days 7 and 11 pi. The decrease in hepatic triglyceride concentration was more on day 11 than day 7 pi, while the hepatic total cholesterol content decreased to comparable extents on days 7 and 11 pi. Hepatic LDL cholesterol content was unaffected on day 7 pi, but decreased ($P<0.05$) on day 11 pi. The content of HDL cholesterol in the liver did not vary ($P>0.05$) significantly during the infection. It was concluded that the decreased hepatic contents of these lipids were consistent with the serum lipid concentration, which did not seem to favor lipid uptake by hepatocytes.

Keywords Lipids · Cholesterol · Triglyceride · Serum · Liver · Rat · Trypanosome

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Introduction

Trypanosomes are unicellular hemoprotozoan parasites which cause infection in humans and animals. Humans are susceptible to *Trypanosoma rhodesiense* and *Trypanosoma gambiense*, but a related subspecies, *Trypanosoma brucei*, does not cause infection in humans because they are lysed by lipoprotein-related lytic factor in the human blood (Gillet and Owen 1992). Trypanosomes require lipoprotein to multiply in axenic culture conditions and bloodstream forms incorporate lipids into their membranes from the circulation since they lack the ability to synthesize their own lipids (Mellor and Samad 1989; Green et al. 2003; Bansal et al. 2005).

Trypanosome-infected animals lose body fat (Stephen 1970; Ikede and Losos 1975) due to lipolysis. The infection of rabbits with *T. gambiense* (Diehl and Risby 1974) and *T. brucei* (Goodwin and Guy 1973) caused hypercholesteroleremia. Also, there was elevated plasma cholesterol concentration in *T. brucei*-infected dogs (Egbe-Nwiyi et al. 1993). In rodent infections, hypercholesterolemia occurs due to increased hepatic cholesterol synthesis and decrease in low density lipoprotein clearance, although hypocholesterolemia occurs in infections of humans and non-human primates (Khovidhunkit et al. 2008). Hypocholesterolemia has also been reported in trypanosome-infected ruminants

(Katunguka-Rwakishaya et al. 1992, 1997; Biryomumaisho et al. 2003; Taiwo et al. 2003). Humans infected with *T. gambiense* had decreased triglyceride concentration (Awobode 2006). Circulating non-esterified fatty acids increased in trypanosome-infected goats (Akinbamijo et al. 1992).

In *T. congolense*-infected rats, cholesterol accumulated in the brain (Nok et al. 1992). An electron microscopic study of the liver in deer mice infected with *T. brucei* indicated necrosis of hepatocytes without lipid droplets (Anosa and Kaneko 1984). It is unclear whether the changes in the lipid composition of the blood during trypanosome infections relate to the lipid content of the liver. This study was an attempt to assess the lipid levels of the serum and liver of *T. brucei*-infected rats.

Materials and methods

Healthy albino rats of both sexes, weighing 98–205 g, obtained from the Nigerian Institute for Trypanosomiasis Research (NTIR), Vom, Nigeria, were housed in cages and freely offered commercial diet (Noma Feeds, Kaduna) and water. Twenty (group B) were infected with *T. brucei* (Federe strain) and ten (group A) served as uninfected controls.

The infective trypanosome was originally isolated from cattle in Federe, Kaduna State, Nigeria and maintained in liquid nitrogen in NITR, Vom, from where it was passaged into donor rats. Parasitemic blood from the donor rat was diluted with physiological saline and each rat in group B was inoculated intraperitoneally with an inoculum containing 10^6 trypanosomes.

The infected rats were monitored daily for parasitemia by wet mount examination of the tail blood and after the onset of parasitemia, the level of parasitemia and severity of anemia were determined at 2-day intervals. Wet mount scores of parasitemia per microscopic field were adapted from Woo (1969) as 0 (no trypanosome), 1 (1–2 trypanosomes), 2 (3–6 trypanosomes), 3 (7–20 trypanosomes) and 4 (>20, swarming trypanosomes). The determination of packed cell volume (PCV) was by microhematocrit method.

Five rats were killed in each group (control, A and infected, B) on days 7 and 11 post-infection (pi) by decapitation under ether anesthesia. The blood exanguinated from the cervical blood vessels was collected without anticoagulant. Serum was harvested from the blood after clot separation.

The sliced liver of each rat was washed with physiological saline and homogenized. Total lipid extraction from the homogenized liver sample was performed using organic solvents in sequence (Bligh Dyer 1959; St John and Bell 1989). Briefly, 5 ml methanol was added to 0.5 g homogenized liver sample and after heating (50–60°C, 2 min) and cooling, 5 ml diethyl ether was added, thoroughly mixed, allowed to stand for 5 min and was filtered through glass wool; the residue was rinsed twice with 10 ml methanol-ether (1–1) and twice with 5 ml diethyl ether to complete the extraction.

The concentrations of triglycerides, total cholesterol, high density lipoprotein (HDL), and low density lipoproteins (LDL) cholesterol were estimated in serum samples and aliquots of the lipid extract from the liver. Triglycerides (Rifai et al. 1999) and total cholesterol (Allain et al. 1974) were determined by enzymatic methods using commercial reagent kits (DIALAB, Austria; Fortress Diagnostics Limited, Antrim, http://www.fortressdiagnostics.com). After precipitation of chylomicrons, very low density lipoproteins and low density lipoproteins with phosphotungstic acid and magnesium chloride, high density lipoprotein cholesterol was estimated as total cholesterol in the supernatant fluid (Allain et al. 1974). The LDL cholesterol was calculated from total cholesterol in the whole sample, HDL cholesterol and triglyceride concentrations according to the formula of Fiedewald et al. (1972). The liver contents of the various lipids were, thereafter, calculated in weights using their concentrations in the aliquots of the extracts.

The data were summarized as means±standard deviations and variations in means were assessed by analysis of variance and Student's *t*-test using computer software (GraphPad Instat 1993 version, http://www.graphpadinstat.com).

Results

The PCV and parasitemia scores of the control and infected rats are presented in Table 1.The infected rats became parasitemic from day 3 pi after which a peak was reached with swarming parasitemia from day 7 pi. Mortality (30%)

Table 1 Packed cell volume and parasitemia scores of *Trypanosoma brucei*-infected and control rats

Days post-infection	Packed cell volume (%)		Parasitemia score
	Control	Infected	
0	49.2 ± 5.0^{a} (10)	48.6 ± 4.9^{a} (20)	–
3	48.9 ± 5.2^{a} (10)	47.8 ± 4.7^{a} (20)	0.5 ± 0.5^{d}
5	46.9 ± 5.7^{a} (10)	43.6 ± 4.1^{a} (20)	3.5 ± 0.5^{e}
7	46.9 ± 5.7^{a} (10)	40.8 ± 4.0^{b} (20)	4.0 ± 0.0^{f}
9	50.0 ± 4.8^{a} (5)	41.5 ± 2.9^{b} (10)	4.0 ± 0.0^{f}
11	49.6 ± 5.0^{a} (5)	40.2 ± 1.3^{b} (7)	4.0 ± 0.0^{f}

Number of rats in parenthesis; parasitemia score: 0, no trypanosome; 1, 1–2 trypanosomes; 2, 3–6 trypanosomes; 3, 7–20 trypanosomes; 4, >20, swarming trypanosomes from wet mount microscopic examination (per field) adapted from Woo (1969). Means±SD with different superscript letters are significantly ($P<0.05$) different

occurred between days 9 and 11 pi within the first wave of parasitemia. The PCV significantly ($P<0.05$) decreased between days 7 and 11 pi.

The levels of lipids in the serum and livers of control and infected rats are presented in Tables 2 and 3, respectively. The serum triglyceride concentration was comparable with the control value on day 7 pi, but increased ($P<0.05$) above the control value on day 11 pi. The serum total cholesterol, HDL, and LDL cholesterol concentrations decreased ($P<$ 0.0.05) when compared with the control values on days 7 and 11 pi. The LDL cholesterol decreased more on day 11 than day 7 pi. The liver content of triglycerides and total cholesterol decreased ($P<0.05$) during the infection from control values on days 7 and 11 pi. The decrease in hepatic triglyceride concentration was more on day 11 than day 7 pi, while the hepatic total cholesterol content decreased to comparable extents on days 7 and 11 pi. Hepatic LDL cholesterol content was unaffected on day 7 pi, but decreased ($P<0.05$) on day 11 pi. The content of HDL cholesterol in the liver did not vary ($P>0.05$) significantly during the infection.

Discussion

The course of the infection in the rats was acute as was reported previously with various strains of *T. brucei* with the survival time ranging from 11.6±0.8 to 15.4±0.7 days pi (Egbe-Nwiyi 2002; Egbe-Nwiyi et al. 2005).The infection caused anemia by decreasing PCV as indication of the severity of the disease. The pathogenesis of the anemia had been earlier discussed by Igbokwe (1989). The invasion of the liver by trypanosomes and their antigens stimulate immune-mediated reactions with non-suppurative inflammatory response and hepatotoxic injuries arising from cytotoxic substances (Igbokwe 1994). Hepatic necrosis in *T. brucei* infections had been demonstrated ultrastructurally (Anosa and Kaneko 1984) and biochemically (Umar et al. 1999). This invariably suggests that hepatic functions relating to lipid metabolism may be deranged. The hepatocyte is highly susceptible to lipidosis when injured if there is excessive delivery of free fatty acids to them for triglyceride synthesis as body fats are mobilized during energy deficit (Slausson and Cooper 2002). The lipidotic condition is enhanced by impairment of lipoprotein synthesis in injured hepatocytes, which reduces triglyceride export from the cells. There is no available report clarifying the nature of the lipid metabolism in trypanosomosis. The present report shows that hepatic lipid depletion is observed in *T. brucei* infection of rats. No previous reports indicated similar findings or offered any hypothesis to suggest the event.

Current understanding is that energy deficit occurs in trypanosome-infected hosts (Igbokwe 1995) due to depletion of hepatic glycogen reserve (Lumsden et al. 1972; Ashman and Seed 1973), increasing glucose intolerance as the disease progresses, terminal hypoglycemia (Newton 1978; Igbokwe 1998; Igbokwe et al. 1998a, b, 1999) and loss of body fat with muscle wasting (Stephen 1970; Ikede and Losos 1975). Mobilization of body fat in the disease was indicated by increase in blood free or non-esterified fatty acids (Akinbamijo et al. 1992). Increased serum lipids, Hypertriglyceridemia and hypercholesterolemia were reported in infections of rabbits with *T. brucei* and *T. gambiense* (Diehl and Risby 1974; Rouzer and Cerami 1980). However, Awobode (2006) reported deceased plasma triglyceride concentrations in *T. gambiense* infection of humans.

Transportation of lipids into the liver ought to be accompanied by increased serum LDL cholesterol and free

Table 2 Serum concentrations of lipids in control (uninfected) and *Trypanosoma brucei*-infected rats

Parameters	Control (uninfected, $n=5$)	Infected rats at pi periods ($n=5$)	
		Day 7 pi	Day 11 pi
Triglycerides (mmol/L)	0.80 ± 0.10^{a}	0.78 ± 0.22^{a}	1.80 ± 0.24^{b}
Total cholesterol (mmol/L)	2.44 ± 0.09^{a}	2.06 ± 0.21^{b}	2.08 ± 0.18^{b}
HDL cholesterol (mmol/L)	0.52 ± 0.05^{a}	0.42 ± 0.01^{b}	0.38 ± 0.05^{b}
LDL cholesterol (mmol/L)	1.58 ± 0.13^{a}	1.34 ± 0.11^{b}	0.88 ± 0.08^{c}

Means±SD with different superscript letters are significantly ($P<0.05$) different
pi Post-infection

Table 3 Lipid contents of control (uninfected) and *Trypanosoma brucei*-infected rats

Parameters	Control rats (uninfected, $n=5$)	Infected rats at pi periods ($n=5$)	
		Day 7 pi	Day 11 pi
Triglycerides (mmol/g)	0.08 ± 0.01^{a}	0.06 ± 0.01^{b}	0.04 ± 0.01^{c}
Total cholesterol (mmol/g)	0.17 ± 0.03^{a}	0.14 ± 0.01^{b}	0.11 ± 0.01^{b}
HDL cholesterol (mmol/g)	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.02 ± 0.01^{a}
LDL cholesterol (mmol/g)	0.11 ± 0.02^{a}	0.09 ± 0.02^{ac}	0.07 ± 0.01^{bc}

Means±SD with different superscript letters are significantly ($P<0.05$) different
pi Post-infection

fatty acids concentrations, but this study showed decreased serum LDL cholesterol during the infection as was in earlier reports (Katunguka-Rwakishaya et al. 1992, 1997; Biryomumasho et al. 2003; Taiwo et al. 2003). HDL cholesterol did not vary during the infection making no contribution to the dynamics of lipid distribution between the plasma and the liver. Trypanosomes bind and take up LDL from the host, but their interaction with host's HDL is unclear (Gillet and Owen 1992; Bansal et al. 2005). Acute infections, generally, cause lipolysis and hypertriglyceridemia and decreased serum total cholesterol due to deceases in both LDL and HDL cholesterol (Sammalkorpi et al. 1988; Feingold et al. 1992). There was increased serum triglyceride concentration in our infected rats, especially towards the terminal phase of the infection. Rouzer and Cerami (1980) suggested that plasma triglyceride degradation was defective, probably making free fatty acid unavailable for importation into hepatocytes despite the increase in serum triglyceride concentration. Thus, the decreased hepatic contents of these lipids were consistent with the serum lipid concentration, which did not seem to favor lipid uptake by hepatocytes. The tendency towards lipid peroxidation which occurred in the circulating blood of rats with acute *T. brucei* infection (Igbokwe et al. 1994; Eze et al. 2008) did not extend to the liver of the infected animals (Eze et al. 2008), perhaps, because of the depletion of the hepatic lipid content.

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