

**Short Communication:****The effect of glucose and insulin on in vitro proliferation of Plasmodium falciparum**H. Humeida<sup>1</sup>, G. Pradel<sup>2</sup>, A. Stich<sup>3</sup>, \*M.B. Krawinkel<sup>1</sup>**Abstract:**

With increasing prevalence rates of diabetes mellitus in tropical countries, malaria and diabetes often coincide. The study was designed to investigate the effects of glucose and insulin upon in vitro proliferation of Plasmodium falciparum, the causative agent of malaria tropica. Plasmodium falciparum proliferation was determined via the Malstat™ assay, following incubation of the parasites at varying concentrations of glucose (0 - 27.7mM) or insulin (10 pM - 100µM) for 24 and 48 hrs. While Plasmodium falciparum proliferation was unaffected at concentrations of 5.5 – 27.7mM glucose, growth was impaired below a threshold of 5.5mM. No effect was seen following incubation of the parasites in the presence of 10pM – 100µM insulin. Insulin levels did not affect parasite proliferation whilst glucose levels below 5.5mM reduced parasite growth. The Malstat™ assay was identified as a suitable screening assay to assess the effects of glycemic control upon Plasmodium falciparum growth.

**Key words:** Diabetes, Malaria, Malstat™-Assay, glucose, insulin

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**Findings:**

The non-communicable disease diabetes is a major health problem affecting more than 200 million people worldwide. It has been projected that the number of afflicted patients will more than double by 2030, with the largest increase expected for Africa and India [1]. The tropical disease, malaria, affects an estimated 250 million people every year, mostly in tropical and subtropical countries, of which 1 million cases are fatal [2,3]. In spite of huge medical and scientific

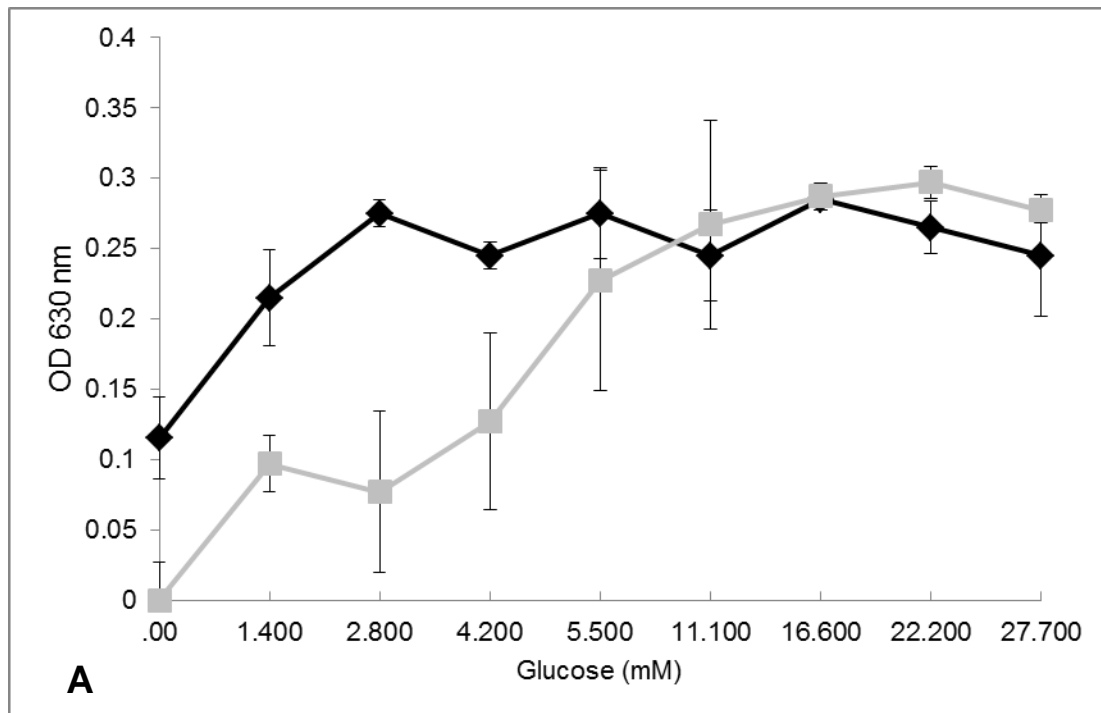
attention as well as economic efforts directed at combating these two diseases, virtually nothing is known as to how a malaria infection proceeds in a diabetes patient [4]. The Plasmodium falciparum parasites fully depend on glucose as an energy source [5]. As abnormally high blood glucose levels are the hallmark of all types of diabetes mellitus, it is reasonable to assume that the disease profiles should converge in an afflicted patient. It can furthermore be anticipated that the medications might have reciprocal effects on the diseases. For example, it is well known that the anti-malarial drug quinine stimulates diabetes relevant parameters, such as increased plasma insulin concentrations and hypoglycemia [6]. Much less is known about the possible effects of diabetes medications upon malaria infection. In the present study, we thus seek to investigate the effects of glucose and insulin on Plasmodium falciparum proliferation in vitro.

The human malaria pathogen, Plasmodium falciparum strain 3D7, was cultivated in a 5% suspension of A+ erythrocytes (Bavarian Red Cross, Wuerzburg) in RPMI-1640 medium (Gibco, Invitrogen, Karlsruhe) with 25mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES, pH 7.4), 5 g/l Albumax (Invitrogen) and 0.37 mM hypoxanthine. The cultures were maintained as described previously [7] and medium was changed on a daily basis. Synchronized ring stages of Plasmodium falciparum strain 3D7 were plated in 96 - well

plates at a parasitaemia of 1%. The effects of glucose and insulin upon parasite proliferation were tested each at final concentrations of 0 - 27.7mM for glucose and 10pM - 100µM for insulin.

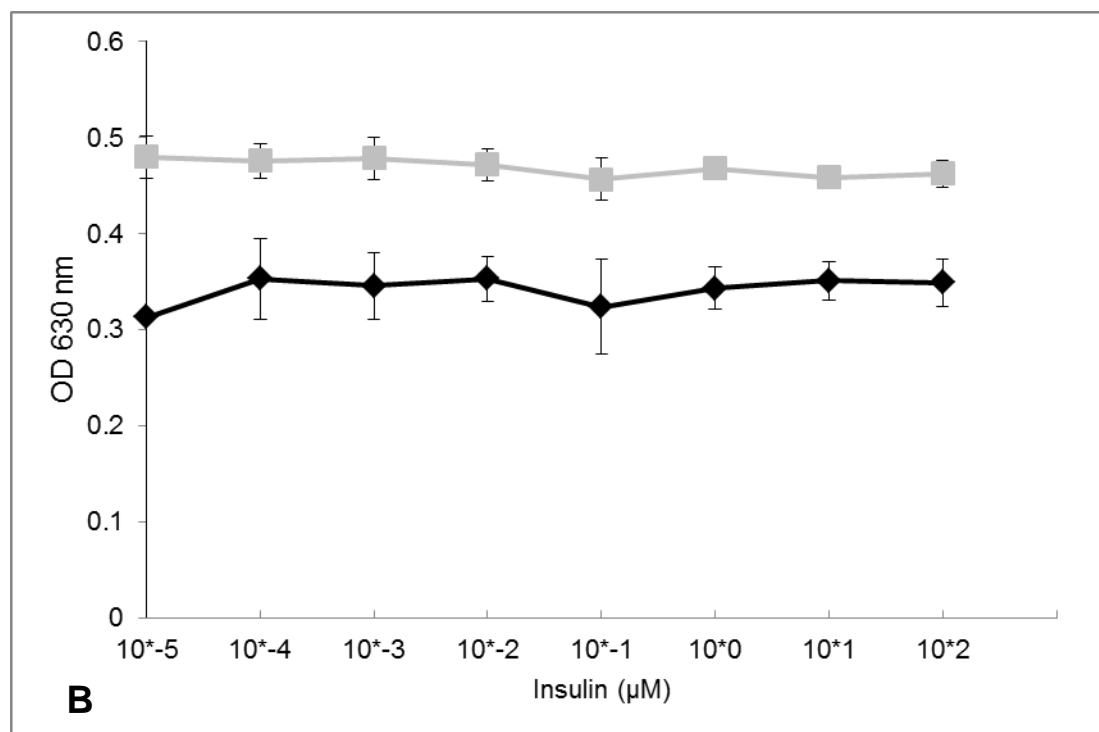
**Table 1. Effects of glucose concentrations on Plasmodium falciparum proliferation in vitro.**

Glucose concentration (mM)	Optical Density OD600 (24h) (n = 4 ± S.D.)	Parasite Growth (%)	Optical Density OD600 (48h) (n = 4 ± S.D.)	Parasite Growth (%)
27.7	0.25 ± 0.04	86	0.28 ± 0.01	93
22.2	0.27 ± 0.02	93	0.30 ± 0.01	100
16.6	0.29 ± 0.01	100	0.29 ± 0.01	96
11.1	0.25 ± 0.03	86	0.27 ± 0.07	90
5.5	0.28 ± 0.03	97	0.23 ± 0.08	77
4.2	0.25 ± 0.01	86	0.13 ± 0.06	43
2.8	0.28 ± 0.01	97	0.08 ± 0.06	26
1.4	0.22 ± 0.03	76	0.10 ± 0.02	33
0	0.12 ± 0.03	41	0.02 ± 0.03	6



**Figure 1 (A): The effect of glucose on Plasmodium falciparum proliferation (n = 4 ± S.D.).**

Rhombuses = 24h, squares = 48h time point.



**Figure 1 (B): The effect of insulin on Plasmodium falciparum proliferation (n = 4 ± S.D.).**  
**Rhombuses = 24h, squares = 48h time point.**

For the glucose experiments, glucose free RPMI-1640 medium supplemented with Albumax-II (5g/l) as serum replacement, was used to which glucose was added at defined concentrations. For the insulin experiments, Albumax-II medium containing 11 mM glucose was used. The assays were performed in triplicate in 96-well plates, that were maintained in an air-tight exsiccator, in a humid atmosphere of 5% O<sub>2</sub> and 5% CO<sub>2</sub> + 90% O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>. Negative controls were performed by the omission of either glucose or insulin. Dead controls were performed by addition of 20µl chloroquine (100µM final concentration) prior to the assay. The viability of the parasites was screened after 24 and 48 hrs, using the Malstat™ assay. This assay measures the activity of the malaria parasite-specific enzyme, lactate dehydrogenase spectrophotometrically, at an OD of 630nm [8-10]. All experiments were repeated four times each. The standard deviations are reported (Table 1).

In the present study, the lower glucose concentration threshold for normal parasite proliferation in vitro, was defined at 5.5mM. At concentrations

between 5.5 and 27.7mM, no effect of varying glucose concentrations on parasite proliferation was observed, indicating that the medium was glucose saturated. At concentrations below 5.5mM, the growth of the parasites decreased notably, suggesting that glucose concentration was a limiting factor (Fig. 1a, Table 1). As expected, no proliferation was observed when glucose was lacking or when the parasites were killed by addition of chloroquine at final concentration of 100µM prior to the assay. We further investigated, whether varying insulin concentrations have an effect on Plasmodium falciparum proliferation. The insulin concentrations were chosen to resemble and exceed the physiological concentrations in healthy people and diabetic patients. The concentrations range in healthy people from 17 pM at fasting to 0.59 nM without fasting and can reach up to 1.2 nM following stimulation with glucose or glucagon [11]. Insulin had no effect on Plasmodium falciparum growth when tested at eight different concentrations between 10pM to 100µM. This suggests that insulin exerts neither

inhibitory nor stimulating effect on parasite proliferation. These findings are in agreement with these of a recent study, where human insulin at concentrations from 170pM to 17µM did not affect growth of asexual stage Plasmodium falciparum NF54, in vitro [12].

Malaria parasites were discovered to be heterotrophic and dependent on glucose as a nutrient source, 100 years ago [9]. As the parasites have no capacity to store energy in the form of glycogen or other polysaccharides, they rely entirely on an exogenous supply of glucose [13]. For the antimalarial drug quinine, an effect on insulin release has been shown [14]. Blood glucose levels of healthy (normoglycemic) individual range between 3.3 and 6.7mM. Severe malaria, particularly in children, is often associated with hypoglycemia [15] and blood glucose concentrations can drop below 3mM. The threshold below which parasite proliferation decreases in vitro falls well in the range of normal blood glucose levels in vivo. This can be interpreted as a possible evolutionary adaptation of the parasite to the human blood as its natural habitat. A recent review on heterotrophic intracellular bacteria highlights the relevance of intracellular nutrient availability for intracellular pathogens and parasites [16]. Its relevance to malaria is to be further studied, employing methods like differential gene expression profiling methods (DGEP). Moreover, it has recently been shown that type 2 diabetes mellitus patients in urban Ghana are more likely to be infected with Plasmodium falciparum [17]. The relevant clinical implications are therefore that people with abnormally high blood glucose levels may suffer more likely from malaria.

Insulin is the intrinsic blood glucose regulating hormone as well as an extrinsic therapeutic agent used to lower the blood glucose. Even though different insulin concentrations showed no effect on parasite proliferation it is conceivable that antidiabetic drugs, such as sulfonylureas, glitazones, glinides, metformin or miglitole might be able to affect Plasmodium falciparum growth through an impact on the glucose availability and uptake. This effect could be either indirectly via the reduction of blood glucose levels or possibly also directly as these structurally small molecules (in comparison to insulin) might get access to the parasites inside the erythrocytes. Indeed, sulfonylureas have shown antimalarial activities, including inhibition of in vitro development of a chloroquine-resistant

strain of Plasmodium falciparum as well as that of in vivo development of Plasmodium berghei in murine malaria [18]. This aspect deserves further studies because findings would be particularly relevant in case a proliferation effect of one of the drugs is found. The Malstat™ assay is typically used to screen compounds for antiplasmodial activities [19]. We have found it to be suitable for studying antidiabetic drugs also. It is easy to handle, has low costs and is highly reproducible.

With respect to the increasing prevalence of diabetes mellitus in hyperendemic malaria areas as well as travelling of diabetic patients into these areas, the coincidence of malaria and diabetes mellitus is becoming common. This deserves more scientific attention with regard to the course of disease, pathophysiology and management of the afflicted patients.

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### References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27: 2568-2569.
2. Sachs J, Malaney P. The economic and social burden of malaria. *Nature* 2002, 415 :680 – 685.
3. World Health Organization (WHO). World Malaria Report. World Health Organization: Geneva, Switzerland, 2009
4. Mohapatra MK. Profile of severe falciparum malaria in diabetics. *Int J Diab Dev Ctries* 2001, 21:1561-1561.
5. Sherman IW: Biochemistry of Plasmodium (malarial parasites). *Microbiol Rev* 1979, 43:453-495.
6. White NJ, Warrell DA, Chanthavanich P, Looareesuwan S, Warrell MJ, Krishna S, et.al. Severe hypoglycemia and hyperinsulinemia in falciparum malaria. *N Engl J Med* 1983, 309:61-66.

7. Ifediba T, Vanderberg JP. Complete in vitro maturation of *Plasmodium falciparum* gametocytes Nature 1981, 294:364-366.
8. Gomez MS, Piper RC, Hunsaker LA, Royer RE, Deck LM, Makler MT, et.al. Substrate and cofactor specificity and selective inhibition of lactate dehydrogenase from the malarial parasite *Plasmodium falciparum*. Mol Biochem Parasitol 1997, 90: 235-246.
9. Goodyer ID, Taraschi TF. *Plasmodium falciparum*: a simple, rapid method for detecting parasite clones in microtiter plates. Exp Parasitol 1997, 86:158-160.
10. Makler MT, Ries JM, Williams JA, Bancroft JE, Piper RC, Gibbins BL, et.al. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. Am J Trop Med Hyg 1993, 48: 739-774.
11. Kerner W, Fuchs C, Redaelli M, Boehm BO, Köbberling J, Scherbaum WA, et.al. Definition, Klassifikation und Diagnostik des Diabetes mellitus. In: Evidenzbasierte Diabetes-Leitlinien. Scherbaum WA, Lauterbach KW, Joost HG (eds.), Deutsche Diabetes Gesellschaft, 1st edition.
12. Surachetpong W, Pakpour N, Cheung KW, Luckhart S. Reactive oxygen species-dependent cell signaling regulates the mosquito immune response to *Plasmodium falciparum*. Antioxid Redox Signal 2011, 14: 943-955.
13. Bass CC, Johns FM. The cultivation of malarial plasmodia (*Plasmodium vivax* and *Plasmodium falciparum*) in vitro. J Exp Med 1912, 16: 567-579.
14. White NJ, Warrell DA, Chanthavanich P, Looareesuwan S, Warrell MJ, Krishna S, et.al. Severe hypoglycemia and hyperinsulinemia in *falciparum* malaria. N Engl J Med. 1983; 309: 61-6
15. White NJ, Miller KD, Marsh K, Berry CD, Turner RC, Williamson DH, et.al. Hypoglycaemia in African children with severe malaria. Lancet 1987, 1: 708-711.
16. Eisenreich W, Dandekar T, Heesemann J, Goebel W. Carbon metabolism of intracellular bacterial pathogens and possible links to virulence. Nat Rev Microbiol 2010, 8: 401-412.
17. Danquah I, Bedu-Addo G, Mockenhaupt FP. Type 2 diabetes mellitus and increased risk for malaria infection. Emerg Infect Dis 2010, 16: 1601-1604.
18. León C, Rodrigues J, Gamboa-de-Domínguez N, Charris J, Gut J, Rosenthal PJ, et.al. Synthesis and evaluation of sulfonylurea derivatives as novel antimalarials. Eur J Med Chem 2007, 42: 735-742.
19. Tischer M, Sologub L, Pradel G, Holzgrabe U. The bisnaphthalimides as new active lead compounds against *Plasmodium falciparum*. Bioorg Med Chem 2010, 18: 2998-3003.