

Evaluation of Sub-acute Effects of Exogenous Insulin on White Blood Cells and Erythrocyte Sedimentation Rate in Mice

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ABSTRACT

The effect of experimentally induced hyper-insulinaemia on total white blood count (WBC) and differential counts, platelets count, ESR and total plasma proteins have been studied following sub-acute insulin administration. Sixteen mice, weighing 25 – 30 g were divided into 2 groups and treated with insulin (10 I.U/kg) or distilled water (control) for seven days. Haematological parameters were determined using standard laboratory methods. Total WBC count of the insulin-treated group ($7.74 \pm 0.6 \times 10^9/L$) was higher than that of the control ($6.14 \pm 0.3 \times 10^9$ cells/L). Similarly, lymphocytes and platelet counts of the insulin-treated group ($27.88 \pm 1.1\%$ and $190.13 \pm 2.2 \times 10^9$ cells/L) were higher than those of the control ($20.50 \pm 1.0\%$ and $175.63 \pm 3.6 \times 10^9$ cells/L). ESR of the insulin-treated mice was lower than that of the control. It was concluded that sub-acute insulin treatment significantly increased total WBC, neutrophil and platelet counts, decreased ESR, and did not have any significant effect on other differential WBC counts and plasma proteins.

Key words: Insulin, hyper-insulinaemia, WBC, Lymphocytes, Platelets, ESR, Serum Proteins

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INTRODUCTION

The burden of diabetes mellitus (DM) is increasing worldwide. International Diabetes Federation (IDF, 2014) (1) reported the world prevalence of type 2 diabetes mellitus in 2014 to have increased to 8.33% with estimated number of 387 million people, and by 2035 the number might reach 592 million. In Africa, the estimated prevalence of type 2 diabetes mellitus (T2DM) is 1% in rural areas, up to 7% in urban sub-Saharan Africa (2). Hyperglycaemia and its consequences have been implicated in the pathophysiology of DM complications (3). However, in DM and pre-diabetic states hyperglycaemia coexists concurrently with hyper-insulinaemia (4,5). There is the possibility that some of the pathophysiological effects ascribed to hyperglycaemia are actually due to hyper-insulinaemia. To resolve this, there is the

need to study the effects of hyper-insulinaemia in non-diabetic normoglycaemic subjects without the interference of hyperglycaemia. Several effects of hyper-insulinaemia have been studied so far, but there is dearth of information on the effects of hyper-insulinaemia on haematological parameters. Previously, hyper-insulinaemia was induced experimentally by administration of exogenous insulin (6,7,8,9). Thus, this study was aimed at evaluating the sub-acute effects of insulin on total white blood count (WBC) and platelets counts, erythrocyte sedimentation rate and total serum proteins in mice administered with exogenous insulin.

MATERIALS AND METHODS

Animal Care, Grouping and Treatments

Young mice of both sexes, weighing between 25 – 30 g, were used for the study. They were kept in large aerated cages and allowed free access to feed and drinking water. They were maintained under the prevailing natural light-dark cycle (photophase: 6:22 – 18:11). Experimental protocols were approved by local Institutional Research Committee and were in accordance with the guidelines for animal research, as stated in the NIH Guidelines for the Care and Use of Laboratory Animals (National Academy of Sciences and National Institutes of Health Publications, 2011).

Insulin (Novo Nordisk A/S, Denmark) was reconstituted 1:3 with deionized water for ease of dosing; and administered subcutaneously using insulin syringe daily between the hours of 8:00 – 9:00 am for seven days. Mice in the control group received deionized water, while those in the insulin group received insulin at 10 I.U./kg/day, subcutaneously (10).

Laboratory Procedures

Laboratory procedures were carried out as described by Sirois (1995) (11) and briefly stated below. Total and differential leukocyte counts: 0.5cm³ of blood was drawn into the WBC pipette and diluted with WBC diluting fluid (1:20), mixed and allowed to stay for 5 – 10 minutes. The diluted blood was introduced into the improved Neubauer Counting Chamber and the cells counted using HM – Lux Microscope (Germany).

Platelets counts: 0.5 cm³ of blood was drawn into the WBC pipette and diluted with Rees-Ecker solution to achieve a dilution ratio of 1:20. The diluted blood was introduced into the improved Neubauer Counting Chamber and the cells counted using HM – Lux Microscope (Germany).

Total plasma proteins: After reading the PCV, the content of each capillary tube was

used to determine the total plasma proteins. The capillary tube was broken above the sedimented cells to collect the plasma. The plasma was drained under a glass slide of the refractometer (Catalog 10400 by American Optical Corp Keene, N. H., U. S. A), and the total plasma protein was read (12).

Erythrocyte sedimentation rate: The mixture of 2 cm³ of blood and 0.5 cm³ of sodium citrate was drawn into a Westergren-Katz tube to the 200 mm mark. The tube was kept in a strictly vertical position at room temperature for 1 hour, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment was measured.

Statistical analyses

Results were presented as mean ± SD and analysed using SPSS® software, version 14.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Differences in continuous haematological values between control and insulin treated groups were assessed by independent Student's *t*-test. A *P*-value <0.05 was considered statistically significant.

RESULTS

Total and Differential White Blood Cells Count

The values of total WBC and differential counts are presented in table 1. Total WBC count of the insulin-treated group was significantly higher than that of the control (*P* = 0.025). Neutrophil count of the insulin-treated group was also significantly higher than that of the vehicle-treated control (*P* = 0.001). However, the other differential leucocyte counts of the insulin-treated animals - bands, lymphocytes, monocytes and eosinophils - were not significantly different from that of the control (*P* = 1.000, *P* = 0.254, *P* = 0.292, *P* = 0.373, *P* = 0.671, respectively). There were no basophils found

in the blood of animals in both groups. Platelets count of the animals treated with insulin ($190.13 \pm 2.2 \times 10^9$ cells/L) was significantly different compared with that of the control ($175.63 \pm 3.6 \times 10^9$ cells/L) ($P = 0.005$). The results suggest that insulin treatment significantly increased total WBC, neutrophil and platelet counts, and did not have any effect on the other differential leucocytes counts of the treated animals.

Erythrocyte Sedimentation Rate and Total Plasma Proteins

Erythrocyte sedimentation rate of the animals in the insulin-treated group (2.27 mm/hr) was significantly lower compared to control (5.17 mm/hr) ($P = 0.001$) (Figure 1). This result suggests that insulin treatment has reduced ESR in the treated animals. Similarly, the value of total plasma proteins of the insulin-treated group ($6.80 \pm$ mg/dL) was statistically similar to that of control. (6.85 ± 0.2 mg/dL). The results indicate that insulin treatment did not have a significant effect on these parameters in the treated mice.

Table 1: Total and differential leucocyte count of control and insulin-treated mice.

Parameters	Control	Insulin-treated	<i>P</i> value
WBC ($\times 10^9$ /L)	6.14 ± 0.3	$7.74 \pm 0.6^*$	0.025
Bands (%)	0.25 ± 0.25	0.25 ± 0.25	1.000
Neutrophils (%)	20.50 ± 1.0	$27.88 \pm 1.1^*$	0.001
Lymphocytes (%)	74.38 ± 4.5	67.63 ± 4.2	0.292
Monocytes (%)	1.25 ± 0.8	2.5 ± 1.1	0.373
Eosinophils (%)	1.75 ± 1.2	1.13 ± 0.9	0.671
Basophils (%)	0.00 ± 0.0	0.00 ± 0.0	NC
Platelets ($\times 10^9$ /L)	175.63 ± 3.6	$190.13 \pm 2.2^*$	0.005

WBC- white blood cells, NC- not calculable; (Mean \pm S.E.M, n = 8)

* = significant compared to control.

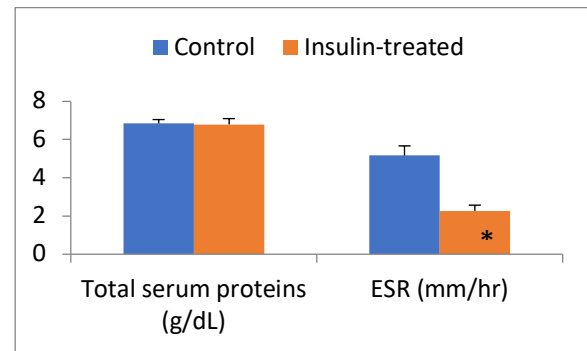


Figure 1: Total plasma protein and ESR of insulin-treated and control mice. (Mean \pm S.E.M) * = significant compared to control.

DISCUSSION

In this study, insulin treatment has significantly increased WBC count, which could be a sign of inflammation in the treated mice. Type 2 diabetes is a state of chronic inflammation (13,14,15) with increased WBC count associated with some of its risk factors and/or pre-diabetic states (16,17,18). In fact, Twig et al. (2016) (19) demonstrated in young normoglycaemic men that an increase in WBC count (even within the normal range) was associated with increased risk of diabetes. Most of the pathophysiologic changes seen in type 2 diabetes and pre-diabetic states were mainly associated with the effects of hyperglycaemia (3). However, in type 2 diabetes mellitus (T2DM) and pre-diabetic conditions, insulin resistance and hyperglycaemia exists concurrently with compensatory hyperinsulinaemia (4,5). It is proposed here, that the increased WBC count seen in T2DM and pre-diabetes could be caused by the excess insulin (hyper-insulinaemia) observed in this condition. The finding of this study could mimic a pre-diabetic condition when compensatory (due to insulin resistance) hyper-insulinaemia induces inflammation and subsequent changes in diabetes and its complications. Evidence has been provided of hyper-insulinaemia resulting from preceding insulin administration (6,7,8,9) consistent with the design of this study. The findings of this study thus provided evidence to support this proposition. Anti-inflammatory effect of insulin has also been reported (20,21), which is contrary to the findings of this study.

Neutrophil count was also higher in the insulin-treated group in line with the increase in total WBC observed in this study. Corroborating this finding, Walrand *et al.* (8) in a similarly designed study, reported increased neutrophil count, chemotaxis, phagocytosis and bactericidal capacity of polymorphonuclear neutrophils due to hyper-insulinaemia in healthy normoglycaemic

human subjects. Rassias et al. (2002) (22) also reported increased neutrophil count and phagocytic activity in non-diabetic cardiac surgical patients. These results corroborate the increase WBC count seen in this study. Insulin administration did not significantly affect other differential WBC counts, thereby making the leucocyte change seem more specific and mimic that of response to bacterial infection.

Platelets are small anucleated cell fragments that circulate in blood playing crucial role in managing vascular integrity and regulating hemostasis. Platelets are also involved in the fundamental biological process of chronic inflammation associated with disease pathology. Platelets activation and dysfunction have been implicated in diabetes, renal diseases, tumorigenesis, Alzheimer's, and CVD (23). In the present study insulin/hyper-insulinaemia increased platelets count in the treated animals. It was previously reported that platelets from patients with type 2 diabetes had increased reactivity and baseline activation (24), which could be due to hyper-insulinaemia, in agreement with other findings of this study. Platelet function is influenced by its redox state, determined by the formation of reactive oxygen and nitrogen species (25). Insulin possesses anti-aggregation effect on human platelets, mediated through increase NO synthesis and subsequent enhancement of cAMP and cGMP in these cells (26). Decreased platelet aggregation in vivo may be observed as an increase in platelets count supporting the finding of this study. However, contrary to this study, Xiao et al. (27) reported that short-term intensive insulin treatment reduces the enhanced platelet function in newly diagnosed type 2 diabetics. In another study, insulin resistance was reported to have no effect on mean platelet volume in non-obese women (28); and a more intensive insulin treatment failed to affect platelet reactivity (29).

Insulin administration in this study did not significantly affect total plasma proteins in the treated mice. In previous studies, insulin administration was found to improve body protein balance by suppressing protein degradation in non-diabetic (30), type 2 diabetic (31) and type 1 diabetic subjects (32). Insulin oedema with increased total plasma proteins was reported following excessive insulin treatment in type 1 diabetes (33). The absence of increased serum protein in the current study could be because the mice were fed *ad libitum* with normal diet compared to other studies in which the diet was improved in protein and energy. This anti-proteolytic, anabolic effect of exogenous insulin could be of advantage to diabetic subjects treated with insulin instead of oral hypoglycaemic agents.

Insulin significantly decreased ESR, a marker of systemic inflammation (34), in the current study. ESR is expected to increase in line with increased WBC and neutrophils counts reported in this study and by others (17,18). Diabetes, an insulin-resistant state accompanied by hyper-insulinaemia (4,5), was reportedly associated with anaemia, due to increased haemolysis (35,36). Increased haemolysis could be an explanation for the low ESR reported in this study, since a decrease in erythrocyte count may result in faster rate of sedimentation.

CONCLUSION

Sub-acute insulin treatment has significantly increased total WBC, neutrophil and platelet counts, decreased ESR, and did not have any significant effect on other differential WBC counts and serum proteins.

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