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Indices of Insulin Sensitivity and Oral Disposition Index in Adult Nigerians with Sickle Cell Anaemia: A Pilot Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author KSA designed the study. Author SKR performed the statistical analysis. Authors KSA, COA, SKR and FAF wrote the protocol. Authors SKR, KSA and FAF wrote the first draft of the manuscript. Authors VOD and COA supervised the tolerance test. Author COA managed the analyses of the study. Authors SKR and KSA managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To determine indices of insulin sensitivity and oral disposition index (D_{lo}) derived from 30 min of glucose challenge in adults with sickle cell anaemia; a group in whom there is chronic inflammation.

Study Design: Case-controlled study.

Place of Study: Department of Chemical Pathology and Department of Haematology, University of Ibadan/University College Hospital, Ibadan, Nigeria.

Methodology: Twenty five (25) adults with sickle cell anaemia (SCA) in steady state and 25 age, sex and body mass index (BMI) matched healthy individuals with HbAA genotype were recruited into this study. After an overnight fast of about 10 hr, 5 ml of venous blood was obtained from each participant for the determination of plasma glucose and serum insulin. Thereafter, each subject underwent a 75-g oral glucose

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tolerance test and at 30min, 5ml of venous blood was obtained for the determination of plasma glucose and serum insulin. Serum insulin was determined using ELISA while the plasma glucose was estimated using glucose oxidase method and indices of insulin sensitivity and β -cell function were calculated appropriately. Differences between variables with Gaussian distribution were determined using independent Student's t-test while Mann-Whitney U was used for the non-Gaussian variables. P -values less than 0.05 were considered significant.

Results: The mean fasting plasma glucose (FPG) was within the normal limit but was significantly lower in subjects with SCA compared with controls. All other indices of insulin sensitivity (insulinogenic index, fasting insulin resistance index, modified Matsuda index of insulin sensitivity and insulin secretion/insulin resistance index) and oral disposition index (DI_o) were similar in both groups.

Conclusion: It could be concluded from this study that SCA subjects have a similar insulin sensitivity status with HbAA subjects. This suggests that SCA subjects might not be more predisposed to the development of type 2 diabetes mellitus than those with HbAA despite the chronic inflammation associated with the former.

Keywords: Insulin sensitivity; oral disposition index; sickle cell anaemia; type II diabetes mellitus.

1. INTRODUCTION

Clinical experience in tropical countries with high prevalence of sickle cell anaemia (SCA) indicates that the concurrence of SCA with diabetes mellitus (DM) is not common. A Multi-Centre Study of Iron Overload reported that diabetes mellitus affects 2% of patients with SCA [1] while a number of studies failed to detect a single case of diabetes mellitus among patients with sickle cell anaemia [2]. A couple of studies reported concurrence of type 1 DM with SCA [3-5] however; there is little information on SCA concurrence with type 2 DM.

Reports have shown that there is a clear pathophysiologic role for inflammatory mediators in the initiation and progression of SCA complications [6]. This inflammation is triggered by the abnormal erythrocyte membrane and the presence of chronic haemolysis. Furthermore, many lines of evidence have shown that chronic activation of intracellular pro-inflammatory pathways within insulin target cells can lead to insulin resistance [7]. This occurs through phosphorylation of certain serine residues on insulin receptor, a major mechanism through which insulin signaling is negatively regulated by inflammatory mechanisms [8,9]. Despite the reported persistent chronic inflammation in patients with sickle cell anaemia [10,11] and the role of inflammatory cytokines in slow damage of the β -cell and insulin resistance [8,12], prevalence of diabetes mellitus is still reported to be very low among patients with sickle cell anaemia.

Over the years, various indices of insulin sensitivity and resistance obtained from both steady state (fasting) and dynamic testing have been used to predict the future development of type 2 diabetes mellitus (T2DM) and in the management of individuals with T2DM. These include the euglycaemic clamp [13], homeostasis model assessment-estimated insulin resistance (HOMA-IR) [14], quantitative insulin sensitivity check index (QUICKI) [15], Matsuda index of insulin sensitivity [16], oral disposition index (DI_o) [17], insulinogenic index of β -cell function [18] and insulin secretion/insulin resistance index [19] among others.

Future development of T2DM has been predicted in different cohorts (such as first degree relatives of T2DM patients) using these indices [20]; no such information is available in subjects with SCA. Due to recent advances in the management of SCA subjects culminating in improved quality of life and longer life expectancy, it is desirable to assess their risk of developing T2DM. This study therefore, determined oral disposition index (DI_o) and selected indices of insulin sensitivity obtained from 30 min of oral glucose challenge in adult Nigerians with sickle cell anaemia.

2. MATERIALS AND METHODS

2.1 Subjects

Fifty participants comprising 25 adults (12males, 13females) with SCA in steady state and 25 age, sex and body mass index (BMI) matched healthy individuals (12 males, 13 females) with HbAA genotype were recruited into this study. The SCA subjects were recruited from the Haematology Day Care Unit, Department of Haematology, University College hospital, Ibadan.

2.2 Inclusion Criteria

Steady state was defined as absence of acute complicating factors or acute clinical symptoms or crisis requiring blood transfusion for at least three months. This was established by a careful history and complete physical examination.

2.3 Exclusion Criteria

Excluded from the study were subjects with other forms of genotype apart from HbSS and HbAA (this was confirmed through haemoglobin electrophoresis), diabetes mellitus, hypertension, human immunodeficiency virus (HIV), hepatitis, cancer and established endocrine dysfunctions. Pregnant and lactating mothers, SCA subjects in any form of crisis and obese individuals were also excluded from this study.

2.4 Data collection

Drug history, family history of DM and/or hypertension, and frequency of blood transfusion were obtained using a short structured questionnaire.

2.5 Blood Pressure and Anthropometric Measurement

Height (m) was taken using a Stadiometer while body weight (kg) was taken using a body weight weighing scale with the subject wearing light clothing and without shoes. Body mass Index (BMI) was calculated as the ratio of weight (kg) to the square of height (m²). Percentage body fat was determined using bioelectric impedance method (Omron BF300, UK). Blood pressure (BP) was obtained using a Mercury Sphygmomanometer after at least 10minutes of rest.

2.6 Sample Collection

After an overnight fast of about 10 hrs, 5 ml of venous blood was obtained from each participant. About 2 ml of each sample was dispensed into fluoride oxalate bottles while 3 ml was dispensed into plain bottles. Thereafter, a standard 75-g oral glucose tolerance test according to World Health Organization (WHO) criteria was performed on all the subjects and at 30mins, 5ml of venous blood was obtained and dispensed into fluoride oxalate and plain sample bottles as earlier stated. Plasma and serum were appropriately obtained and stored at -20°C and -70°C respectively until analyses were done.

2.7 Laboratory Analysis

Serum level of insulin was determined using ELISA (Genway Biotechnology, USA) following the manufacturers' instructions while the plasma glucose was estimated using glucose oxidase method. Standard method was used for the determination of packed cell volume (PCV) while total white blood cell count (WBC) was determined using Turk solution.

2.8 Ethical Approval

The study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee (UI/EC/12/0194). Also, written informed consent was obtained from each participant.

2.9 Calculation of Indices of Insulin Resistance/Sensitivity

- a. Computer-based homeostatic model assessment (HOMA) index of insulin sensitivity (HOMA-S%), computer-based homeostatic model assessment (HOMA) index of beta-cell function (HOMA-B%), and computer-based homeostatic model assessment (HOMA) index of insulin resistance (IR) were calculated using homeostasis model assessment-2 (HOMA-2) calculator (www.dtu.ox.ac.uk/homa).
- b. Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated as the product of fasting insulin and fasting glucose divided by 22.5
- c. Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated as $1/[\log I_0 (\mu\text{U/ml}) + \log G_0 (\text{mg/dl})]$
- d. Insulinogenic index (IGI) [18,21] was estimated as the change in insulin divided by change in glucose from 0 to 30 min ($\Delta \text{insulin}_{0-30 \text{ min}}/\Delta \text{glucose}_{0-30 \text{ min}}$).
- e. $1/\text{FI}$ was calculated as the reciprocal of fasting insulin
- f. Fasting insulin resistance index (FIRI) was calculated as (fasting glucose \times fasting insulin)/25.
- g. Modified Matsuda index [16] was calculated as $10,000/\sqrt{[(G_{\text{fasting}} \times I_{\text{fasting}}) \times (G_{\text{OGTT}} \times I_{\text{OGTT}})]}$, where fasting glucose and insulin data are taken from time 0 of the OGTT and 30 min after a 75g glucose load. The square root is used to correct for nonlinear distribution of insulin, and 10,000 is a scaling factor in the equation.
- h. Insulin secretion/insulin resistance index was calculated as the product of IGI and modified Matsuda index [19].
- i. Oral disposition index (DI_0) was calculated as the product of $1/\text{fasting insulin} \times \text{IGI} (\Delta I_{0-30}/\Delta G_{0-30})$ [17].

2.10 Statistical Analysis

Statistical analysis was done using SPSS version 17.0. The distribution of the data was assessed using histogram with normal curve. Results are presented as mean \pm standard deviation or as median (interquartile range) for Gaussian and non-Gaussian distributed data respectively. Differences between variables with Gaussian distribution were determined using independent Student's t-test while Man-Whitney *U* was used for the non-Gaussian variables. *P*-values less than 0.05 were considered significant.

3. RESULTS

In Table 1, the mean pulse and WBC were significantly higher while PCV was significantly lower in subjects with sickle cell anaemia (SCA) compared with the control group. Body fat, BP and BMI were similar in both groups.

In Table 2, the mean fasting plasma glucose (FPG) was significantly lower in SCA subjects compared with controls. D₁₀ and all other indices of insulin sensitivity obtained from the steady state (fasting) and dynamic testing were similar in both groups.

Figs. 1 and 2 showed the hyperbolic relationship between HOMA-B and HOMA-S in SCA and controls. The SCA subjects and the controls had similar hyperbolic curves.

Table 1. Characteristics of the study participants

	SCA (n = 25)	Controls (n = 25)	P-value
Age (years)	31.60 \pm 8.42	28.68 \pm 6.71	0.181
Body Weight (Kg)	53.22 \pm 7.30	52.74 \pm 3.39	0.896
Height (m)	1.62 \pm 0.07	1.66 \pm 0.09	0.074
BMI (Kg/m ²)	20.42 \pm 2.78	20.96 \pm 2.83	0.500
Body fat (%)	20.84 \pm 7.04	21.31 \pm 8.65	0.836
Systolic BP (mmHg)	111.20 \pm 11.66	109.20 \pm 9.09	0.502
Diastolic BP (mmHg)	72.00 \pm 8.66	71.40 \pm 6.54	0.783
Pulse (beats/min)	77.96 \pm 9.58	69.72 \pm 5.43	0.000*
PCV (%)	21.28 \pm 3.84	36.24 \pm 3.71	0.000*
WBC (10 ⁶ / μ L)	9476.00 \pm 3136.52	3688.00 \pm 876.70	0.000*

*Significant at *P*<0.05, BMI=body mass index, BP=blood pressure, PCV=packed cell volume, WBC=total white blood count

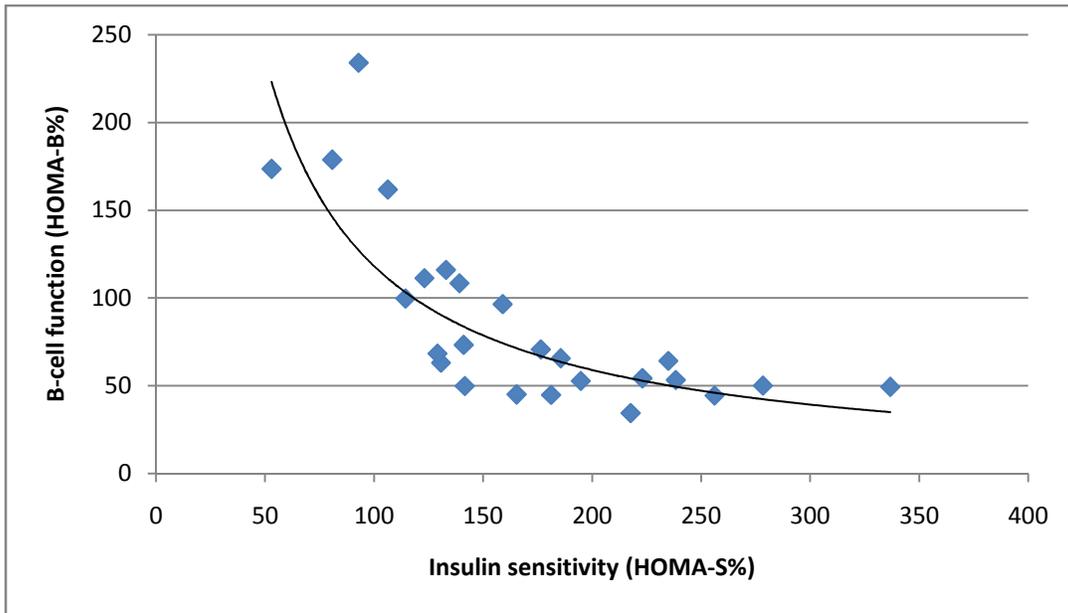


Fig. 1. Hyperbolic curve for SCA subjects plotted for HOMA-B% vs HOMA-S%

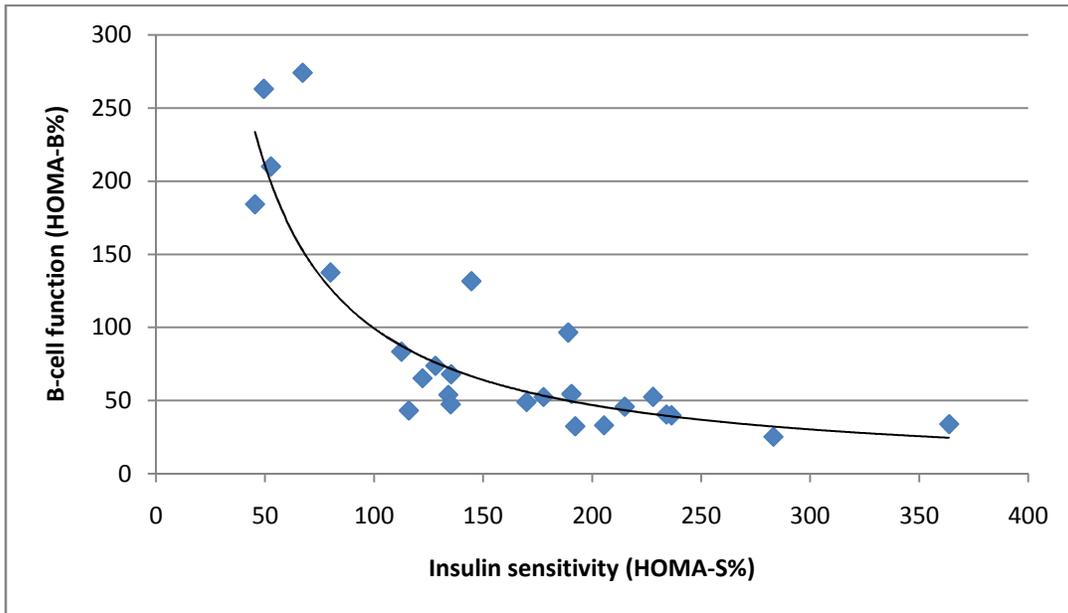


Fig. 2. Hyperbolic curve for the control subjects plotted for HOMA-B% vs HOMA-S%

Table 2. Indices of insulin sensitivity and β -cell function in subjects with sickle cell anaemia (SCA) and controls

	SCA (n=25)	Controls (n=25)	P-value
FPG (mg/dL)	78.84 \pm 8.89	85.64 \pm 10.42	0.019*
30min PG (mg/dL)	115.27 \pm 21.38	110.30 \pm 19.84	0.399
Fasting insulin (pmol/L)	85.42(51.05-112.86)	102.79 (52.09-131.26)	0.426
30min insulin (pmol/L)	188.21(130.57-259.40)	212.52 (159.04-255.23)	0.265
Surrogates derived from steady state (fasting) condition			
1/FI (pmol/L) ⁻¹	0.01 (0.01–0.02)	0.01 (0.01–0.02)	0.367
HOMA-B (%)	159.00 (126.00-220.40)	144.60 (114.20-210.15)	0.621
HOMA-S (%)	65.70 (50.00-109.85)	54.00 (41.95-114.15)	0.327
IR	1.50 (0.90-2.00)	1.90 (0.90-2.40)	0.336
QUICKI	0.34 (0.33–0.36)	0.33 (0.31–0.37)	0.184
HOMA-IR	2.31 (1.37–2.92)	2.89 (1.33–3.96)	0.184
Log HOMA-IR	0.32 \pm 0.23	0.37 \pm 0.33	0.485
FIRI	37.50 (22.22-47.35)	46.89 (21.47-64.15)	0.184
Surrogates derived from dynamic testing (OGTT)			
IGI (pmol/mmol)	55.00 (27.09–246.32)	54.57 (26.01–115.34)	0.426
Modified Matsuda	6.62 \pm 3.10	5.92 \pm 2.35	0.372
Insulin secretion/insulin resistance index	3.11 (1.05-4.52)	2.63 (1.36-11.32)	0.607
Dlo (mM ⁻¹)	0.77 (0.25–4.17)	0.73 (0.28–1.82)	0.677

*Significant at $P < 0.05$, FPG=fasting plasma glucose, PG=plasma glucose, FI=fasting insulin, %B=homeostasis model assessment-B (HOMA-B), %S=homeostasis model assessment-S (HOMA-S), IR=insulin resistance, FIRI=fasting insulin resistance index, IGI=insulinogenic index, Dlo = Oral disposition index

4. DISCUSSION

Metabolic and endocrine disorders as well as evidence of insulin resistance have been reported in subjects with SCA [1,22]. Adekile et al. [23] showed that children with sickle cell anaemia have marginally higher glucose intolerance. Similarly, Alsultan et al. [24] reported that fasting blood glucose, insulin and some surrogates derived from steady state (fasting) condition were significantly elevated in SCA subjects compared to the controls.

In this study, the FPG of SCA subjects was within the normal range (≥ 60 mg/dl but < 100 mg/dl) but was significantly lower when compared with the controls. The reason for our observed lower FPG in SCA subjects is not immediately clear as our observation contradicts the report of Alsultan et al. [24] which showed elevated FPG (although still within the normal range) in SCA subjects. Although their subjects and ours were in steady state and did not receive blood transfusion in the previous three months, subject selection as well as duration of overnight fast might be responsible for the discrepancies observed.

Oral disposition index (Dlo) is a composite measure of β -cell function which has been used to predict future development of T2DM independent of other risk factors including fasting and 2-h glucose concentrations [25]. In a 10-year follow up study, Utzchneider et al. [17] showed that subjects who progressed to T2DM had a lower Dlo compared with the non-progressors. This suggests that higher Dlo is a good indicator of decreased risk of T2DM. After confirming the hyperbolic relationship between HOMA-B and HOMA-S in both SCA subjects and controls (Figs. 1 and 2), it was observed that Dlo was not significantly different in SCA

subjects compared with the controls. However, SCA subjects had a slightly higher level of D₁₀ compared with the controls. This observation could indicate that SCA subjects might enjoy some form of protection from T2DM.

Despite a better understanding of insulin resistance and β -cell dysfunction, as well as improved methods of treating insulin resistance, prevention of T2DM on a global scale is still a public health problem [26]. Therefore, prevention of T2DM requires an insight into the underlying differences between subgroups at risk such as SCA subjects who are exposed to chronic inflammation even, in the steady state [11,26]. In this study, SCA subjects have comparable insulin sensitivity status with the control subjects. This probably, indicates that the SCA-associated chronic inflammation does not make individuals with SCA more prone to T2DM or perhaps, the SCA-associated inflammation does not directly cause insulin resistance as seen in obesity-induced insulin resistance where chronic inflammation has been implicated. Therefore, studies that will reveal the interplay between chronic inflammation and the insulin homeostasis in SCA subjects are required.

It must be noted that small sample size was a major limitation of this study. Therefore, there is need for a prospective larger population study to further elucidate the interplay between inflammation, insulin sensitivity and risk of T2DM in SCA subjects. Lack of information on ferritin level in the SCA subjects is another limitation of this study.

5. CONCLUSION

It could be concluded from this study that SCA subjects have relatively lower fasting plasma glucose but a comparable status of insulin sensitivity and β -cell function compared with HbAA subjects. These observations suggest that subjects with sickle cell anaemia do not seem to be more predisposed to the development of type 2 diabetes mellitus despite the prevailing chronic inflammation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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