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Body Heat Storage and Entropy in Sickle Cell Anaemia

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ABSTRACT

Background: Sickle cell anaemia patients (SCAP) have been reported to live with higher basal metabolic rate (BMR) than their non-sickle cell counterparts (NSCP). This higher BMR has been attributed to higher cardiac work load and erythropoietic activity among other causes.

Objectives: The study estimated and compared oxygen consumption rate (VO_2), metabolic heat production rate (M), basal metabolic rate (BMR), resting energy expenditure –estimated (REEp), heat losses by radiation (Lr), convection (C), evaporation (E) and respiration (Res), in order to estimate and compare the change in heat storage (ΔH) and entropy change (ΔS) in SCAP and NSCP.

Methods: Forty-two (42) adult male SCAP and NSCP were studied. Their physical and blood pressure parameters, arterial pulse oxygen saturation (SPO_2), mean skin temperature (Tsk) and VO_2 were measured. M, BMR, REEp, E, C, Lr, Res, ΔH and ΔS were calculated. Full blood count, serum malondialdehyde ([MDA]) and triiodothyronine ($[T_3]$) concentrations were also measured.

Results: haemoglobin concentration ([Hb]), $[T_3]$, mean arterial pressure (MAP) and SPO2 were lower in SCAP than in NSCP ($p < 0.01$ or $p < 0.001$). Conversely, WBC count, [MDA], HR, PP, rate-pressure product (RPP), Tsk, VO2, M, BMR, REEp, ΔH and ΔS were higher in SCAP than in NSCP ($p < 0.05$, $p < 0.01$ and $p < 0.001$). There were greater heat losses via Lr, C and Res in SCAP than in NSCP ($p < 0.05$). ΔH correlated positively with RPP ($p < 0.01$), WBC ($p < 0.05$), MDA ($p < 0.001$), but correlated negatively with [Hb] ($p < 0.001$), SPO2 ($p < 0.05$), and [T3] ($p < 0.05$) in SCAP.

Conclusion: SCAP had higher ΔH, ΔS, VO_2, M, BMR, REEp, Tsk, Lr, C and Res than the NSCP. The positive correlation of ΔH with HR, RPP, WBC, [MDA] and the negative correlation of ΔH with [Hb], SPO_2 and $[T_3]$ obtained in SCAP show cardiovascular, haematological and metabolic bases for the higher ΔH with ΔS, VO_2, M, BMR and REEp obtained in them.

Key words: sickle cell anaemia, metabolic heat production, heat loss, heat storage, entropy change

INTRODUCTION

Sickle cell anaemia (SCA) is an inherited genetic and molecular blood disorder in which beta (β) globin genes on chromosome 11 (a mutant gene) produces a mutant of adult haemoglobin (HbA) called sickle haemoglobin (HbS)[1]. This mutation is precipitated by a single base change of thymine to adenine at codon 6 of the β-globin gene[2]. It has already been established that in the HbA to HbS mutation, there is a replacement of hydrophilic glutamic acid with a covalent-active hydrophobic valine amino acid at the sixth position of the β-chain of the haemoglobin, giving rise to HbS[3]. In spite of the simple genetic analysis, the various phenotypic manifestations of the disease have been published[2]. While SCA is regarded as a qualitative genetic and molecular disease, the causative genotype is a key determinant of its severity[2]. The frequency and severity of its phenotypic complications vary considerably within each genotypic group[2].

Polymerisation of deoxygenated HbS, production of irreversibly sickled cells and vaso-occlusion are the basic precipitants of sickle cell pathology which lead to acute pain crisis and chronic organ damage[2]. Exposure to cold, heat and physical exercise has been recognised for many years as precipitants of acute pain and vaso-occlusive crises[2]. The rate of HbS polymerisation depends on factors like hypoxia, pH, dehydration and cold. These factors are potentially alterable by extreme cold in the environment[2]. Sickle cell anaemia (SCA) has been linked with an increase in the resting energy expenditure (REE)[4] and increase in heat energy production has been severally reported in SCA patients[5]. Heat balance in human deals with dynamics by which heat gain and heat production are matched and balanced with heat loss in the body[6].

The addition of the heat gain and heat loss parameters has been used to evaluate the body net heat storage[7]. In order to maintain the deep body core temperature within acceptable range of about 37 ± 1°C equilibrium, a constant exchange of heat between the body and the environment is constantly maintained[7].

The quantity of heat exchange is a function of total heat production and heat gained from the environment. The components of human heat balance have been listed to include metabolic heat gain; direct, diffuse and reflected solar and radiative heat gain; terrestrial (or long wave) radiative heat exchange; convective (sensible) heat exchange with the atmosphere; evaporative (latent) heat loss and respiratory heat loss[7;8]. The body energy expenditure has been taught to result from five mechanisms of heat production: basal metabolic rate (BMR); diet-induced thermogenesis; exercise-induced thermogenesis; non-exercise activity thermogenesis and weather-induced thermogenesis[9]. The BMR refers to the heat energy rate of biochemical reactions in the body at rest[9;10]. These reactions are necessary to provide energy for maintenance of normal body temperature, cardiovascular and respiratory functions, erythropoiesis and protein synthesis, muscle tone, activities of the central nervous system, kidneys, liver and other essential activities of tissues and cells in the resting body[9]. The BMR constitutes about 60% of body energy needs, which has been reported to be elevated beyond the 60% in SCA4;[10]. In SCA patients, BMR has been estimated to be about 20% higher than those of non-sickle cell controls[11]. In addition, several studies have been documented to show an increase in REE in children and adults living with SCA[4;12;13].

Nevertheless, these earlier studies had focused on REE and/or BMR which represented only a part of the energy balance equation, which was not investigated in the SCA participants under studies4;14. There are little or no studies on heat balance, heat exchange, heat storage and entropy in SCA participants by these earlier scholars. Their studies have only reported and focused on energy expenditure; dietary intake; body growth and development mainly in preschool and prepubertal children and teenagers but not on heat balance: changes in heat storage and entropy (4;10;12;13). This study hypothesised that SCA individuals may live with higher body heat content and entropy with anomalous basal metabolic energy balance. The study estimated and compared change in heat storage (ΔH), entropy change (ΔS) in sickle cell anaemia participants (SCAP, as the test group) and non-sickle cell participants (NSCP, as the control group).

METHODOLOGY

Participants

Twenty-one (21) apparently healthy male adult non-sickle cell participants (NSCP) and 21 sickle cell anaemia participants (SCAP) were studied. The participants were age-matched. Their genotypes (HbAA for NSCP and HbSS for SCAP) were determined by electrophoresis. Ethical approval was granted by Lagos University Teaching Hospital Health Research Ethics Committee (ADM/DCST/HREC/APP/1359). Each participant gave informed consent and was given Subject Information, Consent, Data and Record Forms.

The SCAP were patients attending Sickle Cell Out-Patient's Clinic of the Lagos University Teaching Hospital, Surulere, Lagos, Nigeria, at the time of study. They were in steady state. They were neither hospitalised nor been on blood transfusion in the six (6) months prior to this study. The SCAP were not carrying any other forms of genotypes apart from HbSS. The sickling test was done using solubility test (hard red band on top and colourless solution, using a freshly prepared buffer mixture and packed red cell from EDTA anticoagulation blood). The haemoglobin electrophoretic pattern was determined for confirmation. The NSCP were students of the College of Medicine of the University of Lagos, Lagos, Nigeria. and they were non-smokers, non-alcoholics, not on any medications, coffee consumption, any supplements, not having thyroid or any metabolic diseases, and not on blood transfusion in the 6 months before the study.

Each of the participants had a measured arterial blood pressure below 140 mm Hg systolic and 90 mm Hg diastolic before been recruited for the study.

Methods

After 12 hours of fasting, (food abstinence from 8.00 pm of the night before to 8.00 am the following morning), the NSCP and SCAP were received into the Human Physiology Laboratory. On entering the laboratory, the age (years), height (cm), weight (Kg), of each participants were measured (Avery Stadiometer, England). The participants laid supine on a couch (80 cm high) for thirty (30) minutes in order to return to physiological baseline. After which arterial blood pressure (BP), electrocardiograph R-R interval, and oxygen consumption rate were measured in the supine position.

Measurement of Arterial Pulse Oxygen Saturation, Arterial Blood Pressure and Heart Rate of the Participants

Arterial pulse oxygen saturation (SPO_2) was measured on the index finger using Deluxe Fingertip Pulse Oximeter (Drive Medical, China). The SPO_2 was then read on the digital screen of pulse oximeter. The systolic and diastolic blood pressures were measured on a brachial artery of each participant using Omron Automated Sphygmomanometer (Healthcare Company Ltd., Kyoto, Japan). Electrocardiogramme (ECG) R-R interval was measured for the calculation of heart rate, using lead V4 of a 12-lead MAC 1200 ST V1.2 ECG machine (GE Medical Systems, Freiburg, Germany). R-R interval was standardised at 25 mm/s throughout the recording (17).

Determination of Oxygen Consumption Rate

The participants were (in supine position) relaxed for 30 minutes. Each was connected to spirometer machine in the Physiology Teaching Laboratory via a mouth piece. A nose clip was worn to prevent breathing through the nostrils. A 3-way valve placed in the circuit close to the mouth piece allowed the participant to inhale from the closed circuit and exhale into it. The test was run for about five minutes. The best straight line was drawn through the tips of the wave tracings on the recording sheet which corresponded to the expiratory points. The slope of the line was converted into oxygen consumption rate[15;16].

Determination of the Mean Skin Temperature and the Temperature of the Surrounding Walls and Items

The mean weighted skin temperature was determined from a calculated average of the following skin temperature measured using non-contact infrared thermometer (DT-8806C, Body Infrared Thermometer, China) at a room temperature of 25°C. The temperature of the forehead, arms, hands, feet, legs, thighs, trunk, were then measured bilaterally (7). The temperature of the surrounding walls, tables, chairs, couch, and light equipment were also measured.

Measurement of Full Blood Count

Full blood count: red blood cell count, haematocrit, haemoglobin concentration, white blood cell count, and platelet count were measured from heparinised blood samples using Mindray Haematology Automated System (China) (17).

Measurement of Serum Malondialdehyde Concentration

Serum malondialdehyde concentration ([MDA]), a secondary product of lipid peroxidation (LPx), was determined for serum LPx using Thiobarbituric acid method as follows: 1.0 mL of serum was combined with 2.0 mL Thiobarbituric acid-trichloro Acetic acid-Hydrochloric acid (TBA-TCA-HCl) reagent to form a reacting mixture. The TBA-TCA-HCl reagent was prepared using 0.3% TBA-TCA stock solution and 0.25% HCl in ratio 1:1:1 by volume. The reacting mixture was then heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuging at 1000 rpm for 10 minutes. The absorbance of the sample was then determined at 535 nm against a blank that contained all reagent except serum sample. The [MDA] of the serum was then calculated (17).

Determination of Serum Triiodothyronine Concentration

Total serum triiodothyronine concentration ([T_3]) was measured in the subjects using EIA-Thyroid T_3 total kit by microplate enzyme immunoassay. The microplate wells for each calibrator was formatted, and the participants' serum samples were assayed in duplicate. 25 µL of appropriate calibrator and serum samples poured into the assigned well. 150 µL of enzyme T_3-conjugate working solution into all the wells and the microplates swirled gently for 30 seconds to mix the resulting enzyme T_3-conjugate–serum mixtures. A protective lid was then used to cover the wells and incubated for 90 minutes at room temperature. After drying the reacting mixture with 300 µL of washing solution per well in order to get 10 µL residual volume, 100 µL of TMB-substrate was pipette into each well and mixed for about 10 seconds. Absorbance was then read on the microplate reader at 450 nm within 20 minutes after stopping the reaction. The mean absorbance value for each calibrator duplicate was calculated and calibrated graph plotted (absorbance on Y while serum sample on X). The mean absorbance value for total T_3 for each serum sample was calculated in ng/mL (18).

Data Analysis

Body mass index (BMI) was calculated as BMI (Kgm^{-2}) = $W \div H^2$

Body surface area (BSA) was calculated as BSA (m^2) = $0.001315 \times H^{1.214}$ (cm) × $W^{0.262}$ (Kg)

Where W = body weight of the subject; H = standing height of the subject (15;16).

Pulse pressure (PP) was calculated as PP (mm Hg) = SBP – DBP

Mean arterial pressure (MAP) was calculated as MAP (mm Hg) = ⅓PP + DBP

Heart rate (HR) was calculated as HR (b/min) = 25 × 60 ÷ RR interval15;17.

Rate-Pressure product (RPP) was calculated as RPP (AU) = SBP × HR (17).

Where AU = arbitrary unit; DBP = diastolic blood pressure; SBP = systolic blood pressure

Oxygen consumption rate (VO_2 in L/h) was calculated from the slope of the spirometry tracing using Pythagoras equation, wherein 1 mm = 30 mL of oxygen on the graph sheet (15;16).

Metabolic heat production rate (M) was calculated as M (J/h) = VO_2 × 4.83 Kcal/L converted to J/L (15;16).

Where VO_2 = oxygen consumption rate (L/h); 4.83 Kcal/L = average calorific equivalent of oxygen at an average respiratory quotient (RQ) of 0.82 for carbohydrate (1.0), fat (0.7) and protein (0.8)

Basal metabolic rate (BMR) was calculated as BMR ($Kcal/h^{-1}m^{-2}$) = metabolic heat production rate ÷ BSA = VO2 × 4.83 ÷ BSA

Resting energy expenditure - estimated (REEP) was calculated as REE_P (Kcal/day) = 1305 + (18.6 × weight (Kg)) – (55.7 × Hb (g/dL)) (20).

Mean weighted skin temperature (tsk) was calculated as tsk (0C) = (0.071 tfh + 0.14ta + 0.05 tha + 0.07 tf + 0.13 tl + 0.19 tth + 0.35 tt) where tfh = temperature of the forehead skin; ta = arm skin temperature; tha = hand skin temperature; tf = feet skin temperature; tl = leg skin temperature; tth = thigh skin temperature; tt = trunk skin temperature (7;8).

Heat exchange by long radiation (Lr) was calculated as Lr = 6.6 (tw – tsk).

Where tw = mean temperature of the room walls and solid surrounding (tw = 25°C) (7;8).

Evaporative heat loss (E) was calculated as E = 14 × Va × 0.6 (P_{sk} – Pa)

Va = air speed (Va = 2.0 ms^{-1}); Pa = water vapour pressure of ambient air (Pa = 42.1 mm Hg); P_{sk} = vapour pressure of water on the skin (42 mm Hg). P_{sk} for each subject was calculated as 42 (tsk + 273.15) ÷ (35 + 273.15) at 35^{0}C (7;8).

Convective heat exchange (C) was calculated as C (J/h) = 7.0 × Va × 0.6 (ta – tsk). Where ta = air temperature (25°C) (7;8).

Respiratory heat loss (Res) was calculated Res (J/h) = 0.0014 M (ta – tea) + 0.00173 M (avp – vpea); avp = air vapour pressure (23.8 mm Hg); tea = temperature of exhale air 35.2 ± 0.7 in NSCAP and 36.8 ± 0.5 in SCAP); vpea = vapour pressur of exhale air (47 mm Hg) (7;8)

Change in body heat storage (ΔH) was calculated as ΔH (J/h) = (M-W) + E + C + Lr + Res; where W = external work done by the participants ≈ zero (for fasting participants in quiescence) (7;8;20).

Therefore, if M – W = H (heat production), M = H (when W ≈ 0) (7;20).

Entropy change, ΔS, was calculated as ΔS = M ÷ Tsk for both groups of participants (20).

Serum lipid peroxidation (LPx) was calculated as [MDA] = (A ÷ Σ) × (V÷ v) × 10^6 (nM/mL)

Where [MDA] = serum malondialdehyde concentration A = mean of 3 absorbance readings of serum samples; Σ = molar extinction coefficient for MDA; = 1.56 × 10^5; V = total reacting volume = 2.1 mL; v = serum sample volume = 0.1 mL (17).

Percent difference of parameters is calculated as δ (%) = (SCAP parameter – NSCP parameter) (100) ÷ (SCA parameter).

Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Percent differences of parameters were calculated. Data are also presented as bar charts. Statistical analyses were made using Graph Pad Prism 5 and Microsoft Excel 2010 softwares. Statistical comparisons were made using unpaired student t-test for the two (2) groups. Spearman's rank correlation coefficient, r, was calculated between the 2 groups among the measured parameters. Significance level was accepted at $p < 0.05$ for each comparison.

RESULTS

Physical Parameters of the Participants

Table 1 shows summary of the physical parameters of the participants. The non-sickle cell and sickle cell anaemia participants (NSCP and SCAP respectively) were of similar age (22.4 ± 0.6 years vs 24.0 ± 2.0 years), height (168.2 ± 2.8 cm vs 163.3 ± 1.6 cm), weight (59.8 ± 2.6 Kg vs 59.4 ± 2.1 Kg), body surface area (BSA: 1.94 ± 0.2 m^2 vs 1.86 ± 0.4 m^2), body mass index (BMI: 20.8 ± 0.5 Kgm^{-2} vs 22.2 ± 0.6 Kgm^{-2}), but the mean skin temperature was higher in SCAP than in NSCP (T_{sk}: 35.1 ± 0.1 vs 36.5 ± 0.4 0C; $p < 0.01$). There is little or no difference between the physical parameters measured in NSCP and SCAP except for the mean skin temperature.

Table 1: Physical Parameters of Non-Sickle Cell and Sickle Cell Anaemia Participants

Parameters	NSCP	SCAP
Age (years)	22.4 ± 0.6	24.0 ± 2.0 ^{NS}
Height (cm)	168.2 ± 2.8	163.3 ± 1.6 ^{NS}
Weight (Kg)	59.8 ± 2.6	59.4 ± 2.1 ^{NS}
BSA (m^2)	1.94 ± 0.2	1.86 ± 0.4 ^{NS}
BMI (Kgm^{-2})	20.8 ± 0.5	22.2 ± 0.6 ^{NS}
T_{sk} (^{0}C)	35.1 ± 0.1	36.5 ± 0.2*

BSA = body surface area; BMI = body mass index; T_{sk} = mean skin temperature; NSCP = non- sickle cell participants; SCAP = sickle cell participants; NS = not significant; * = $p < 0.05$

Haematological Parameters, Arterial Pulse Oxygen Saturation, Serum Malondialdehyde and Triiodothyronine Concentrations in the Participants

Table 2 shows the summary of haematological parameters of the participants. The haematocrit (Hct: 26.1 ± 1.1% vs 42.3 ± 1.2%), red blood cell (RBC) count (3.1 ± 0.1 × 106/μL vs 5.2 ± 0.2 × 106/μL), haemoglobin concentration ([Hb]) (9.2 ± 0.3 g/dL vs 14.4 ± 0.4 g/dL), arterial pulse oxygen saturation (SPO_2) (93.5 ± 0.2% vs 98.6 ± 0.3%) and serum triiodothyronine concentration ([T_3]) (1.04 ± 0.1 vs 1.6 ± 0.2 μg/dL) obtained in the SCAP were lower than those obtained in the NSCP ($p < 0.001$ in each case). On the other hand, white blood cell (WBC) count (8.6 ± 0.7 × 10^3/μL vs 5.9 ± 0.4 × 10^3/μL), platelet (Plt) count (282.5 ± 8.1 × 10^3/μL vs 202.3 ± 7.4 × 10^3/μL) and serum malondialdehyde concentration ([MDA]) (44.1 ± 2.5 mM/mg Prot vs 27.3 ± 2.1 mM/mg Prot) obtained in the SCAP were higher than those obtained in the NSCP ($p < 0.05$ or $p < 0.001$ as the case may be). The Hct, RBC count, [Hb], SPO_2, and [T_3] obtained in the SCAP were 62.1 ± 2.1%, 67.7 ± 3.2%, 56.5 ± 2.3%, 5.5 ± 0.5% and 49.0 ± 3.5% respectively lower than those obtained in the NSCAP ($p < 0.001$ in each case). On the other hand, the WBC count, Plt count and [MDA] obtained in the SCAP were (31.4 ± 3.5%, 28.4 ± 1.3%, 38.1 ± 1.1% respectively) higher than those obtained in NSCP ($p < 0.05$ or $p < 0.001$ as the case may be).

Arterial Blood Pressure and Heart Rate Parameters of the Participants

The mean arterial pressure (MAP) measured in SCAP was lower than that measured in NSCP (76.2 ± 1.9 mm Hg vs 88.7 ± 1.5 mm Hg, $p < 0.001$). However, pulse pressure (PP: 55.6 ± 1.2 mm Hg vs 42.3 ± 1.6 mm Hg, $p < 0.001$), heart rate (HR: 77.4 ± 1.6 b/min vs 67.8 ± 1.3 b/min, $p < 0.01$), and rate-pressure product (RPP: 91.7 ± 1.5 × 102 AU vs 82.9 ± 1.3 × 10^2 AU, $p < 0.05$) measured in the SCAP were higher than those measured in NSCAP (Figure 1). The MAP measured in the SCAP was 16.4 ± 1.3% lower than that measured in NSCP (p < 0.01). On the other hand, the PP, HR, and RPP measured in the SCAP were 23.9 ± 0.9%, 12.4 ± 1.1% and 9.6 ± 0.5% respectively higher than those measured in NSCP (p < 0.001 in each case).

Oxygen Consumption Rate, Metabolic Heat Energy Parameters of the Participants

Figure 2 shows that oxygen consumption rate (VO2: 25.2 ± 2.1 L/hr vs 19.3 ± 1.7 L/hr; p < 0.001), metabolic heat production rate (M: 419.2 ± 34.9 KJ/hr vs 321.1 ± 28.3 KJ/hr; p < 0.001), basal metabolic rate (BMR: 225.4 ± 18.8 KJ/hr/m^2 vs 165.5 ± 14.6 KJ/hr/m^2 p < 0.001), resting energy expenditure –estimated (REEp: 134.1 ± 7.5 KJ/Kg/day vs 113.1 ± 5.9 KJ/Kg/day; p < 0.01), change in heat storage (ΔH: 225.1 ± 10.2 KJ/hr vs 150.4 ± 7.6 KJ/hr; p < 0.001) and entropy change (ΔS: 1.4 ± 0.1 KJ/hr/K vs 1.1 ± 0.1 KJ/hr/K; p < 0.001) were higher in SCAP than those obtained in NSCP. In other words, VO$_2$, M, BMR, REEp, ΔH and ΔS were 23.4 ± 1.6%, 23.4 ± 1.6%, 26.6 ± 1.9%, 18.6 ± 2.2%, 33.2 ± 1.2% and 28.6 ± 1.0% higher in SCAP than in NSCP (p < 0.01 or p < 0.001 as the case may be). Similarly Figure 3 shows that the long wave radiative heat loss (Lr: -75.9 ± 3.6 KJ/hr vs -66.7 ± 3.2 KJ/hr; p < 0.05), convective heat loss (C: -96.6 ± 5.7 KJ/hr vs -84.84 ± 4.6 KJ/hr; p < 0.05) and respiratory heat loss (Res: -23.3 ± 3.3 KJ/hr vs -17.5 ± 2.7 KJ/hr; p < 0.05) were higher in SCAP than in NSCP. Nevertheless, there was little or no change in the evaporative heat loss in SCAP and NSCP (-1.7 ± 0.3 KJ/hr vs -1.7 ± 0.2 KJ/hr).

Change in heat storage (ΔH) correlated positively with HR (r = 0.7; p < 0.001), RPP (r = 0.6, p < 0.01), WBC (r = 0.5; p < 0.05), MDA (r = 0.7; p <0.01). ΔH correlated negatively with [Hb] (r = -0.7; p < 0.001), SPO2 (r = -0.6; p < 0.05), T$_3$(r = -0.6; p < 0.05) in SCAP (Table 3). On the other hand, ΔH correlated positively with plasma T3 (r = 0.6; p < 0.01) in NSCP (Table 3).

Table 2: Haematological Parameters, Arterial Pulse Oxygen Saturation, Serum Malondialdehyde and Triiodothyronine Concentrations in Non-Sickle Cell and Sickle Cell Anaemia Participants

Parameters	NSCP	SCAP	Δ (%)
Hct (%)	42.3 ± 1.2	26.1 ± 1.1	-62.1 ± 2.1***
RBC count (10^6/μL)	5.2 ± 0.2	3.1 ± 0.1	-67.7 ± 3.2***
[Hb] (g/dL)	14.4 ± 0.4	9.2 ± 0.3	-56.5 ± 2.3***
WBC count (10^3/μL)	5.9 ± 0.4	8.6 ± 0.7	31.4 ± 3.5***
Plt count (10^3/μL)	202.3 ± 7.4	282.5 ± 8.1	28.4 ± 1.3***
Arterial SPO$_2$ (%)	98.6 ± 0.3	93.5 ± 0.2	5.5 ± 0.5*
[MDA] (mM/mg Prot)	27.3 ± 2.1	44.1 ± 2.5	38.1 ± 1.1***
[T$_3$] (μg/dL)	1.6 ± 0.2	1.04 ± 0.1	-49.0 ± 3.5***

Hct = haematocrit; RBC = red blood corpuscles; white blood cell; Plt = platelet; [MDA] = malondialdehyde concentration; [Hb] = haemoglobin concentration; SPO$_2$ = arterial pulse oxygen saturation; [T$_3$] = triiodothyronine concentration; NSCP = non-sickle cell participants; SCAP = sickle cell participants; * = p < 0.05; *** = p < 0.001 = significant

Table 3: Showing the Coefficient (r) between Change in Heat Storage and other Measured Parameters in the Non-Sickle Cell and Sickle Cell Anaemia Participants

Parameter	Correlation Coefficient (r) in NSCP vs ΔH	Correlation Coefficient (r) in SCAP vs ΔH
HR	0.3	0.7***
RPP	0.3	0.6**
[Hb]	0.1	-0.7***
WBC	-0.1	0.5*
[MDA]	0.1	0.7**
SPO$_2$	0.1	-0.6*
[T$_3$]	0.7**	-0.6*

NSCP = non-sickle cell participant; SCAP = sickle cell anaemia participant; vs = versus; ΔH = change in heat storage; HR = heart rate; RPP = rate-pressure product; [Hb] = haemoglobin concentration; WBC = white blood cell; [MDA] = malondialdehyde concentration; SPO$_2$ = pulse oxygen saturation; [T$_3$] = triiodothyronine concentration; * = p < 0.05; ** = p < 0.01 and *** = p < 0.001 = significant

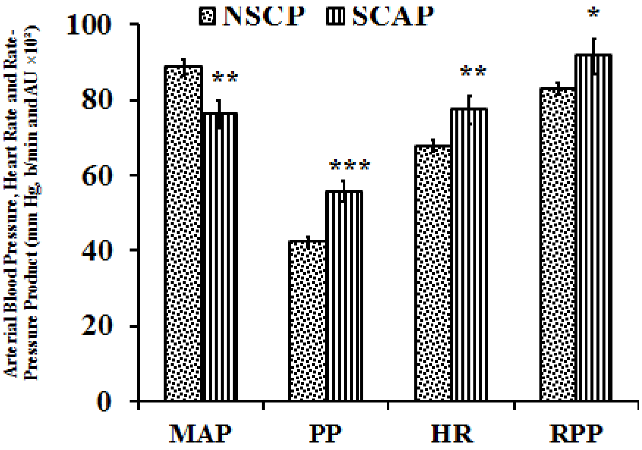

Figure 1: Showing comparison of the mean arterial pressure, pulse pressure, heart rate and rate-pressure product in non-sickle cell and sickle cell anaemia participants

NSCP = non-sickle cell participants; SCAP = sickle cell anaemia participants; MAP = mean arterial pressure; pulse pressure; HR = heart rate; RPP = rate-pressure product; AU = arbitrary unit; * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$ = significant

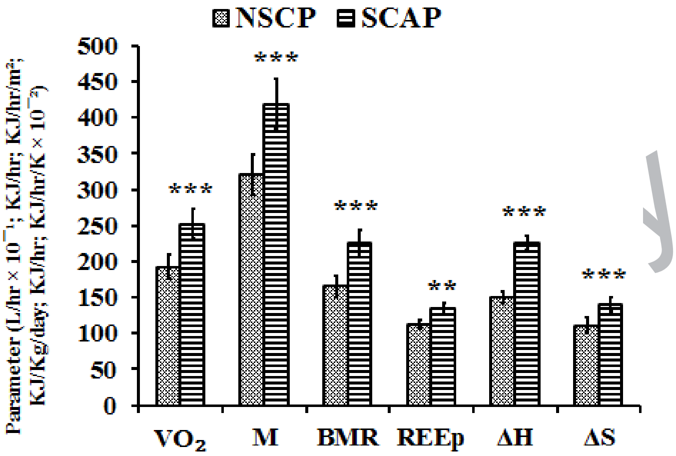

Figure 2: Showing comparison of oxygen consumption, metabolic energy production, basal metabolic rate, resting energy expenditure-estimated, change in heat energy storage and entropy change

NSCP = non-sickle cell participant; SCAP = sickle cell anaemia participant; VO_2 = oxygen consumption; M = metabolic energy production; BMR = basal metabolic rate; REEp = resting energy expenditure-estimated; ΔH = change in heat energy storage; ΔS = entropy change; ** = $p < 0.01$ and *** = $p < 0.001$ = significant

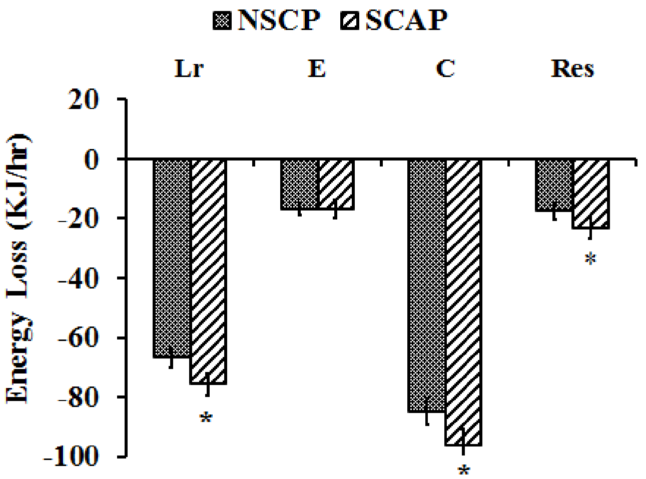

Figure 3: Showing comparison of long wave radiative heat loss, evaporative heat loss, convective heat loss, and respiratory heat loss in non-sickle cell and sickle cell anaemia participants

DISCUSSION

Physical, Arterial Blood Pressure and Haematological Parameters of the Participants

The findings show that the non-sickle cell participants (NSCP) and the sickle cell anaemia participants (SCAP) who volunteered for this study were of similar age and physique. The similarity in physique is not in concordance with earlier findings and reports from other researchers[17;21;22]. Be that as it may, the similarity in physique between NSCP and SCAP is of a coincidental importance in this study, because body weight and body surface area are key factors in the study of heat energy production, dynamics and homeostasis[15;20]. The similarity in the physical parameters validates the evaluation and comparison of metabolic heat energy production rate, heat energy loss, change in heat energy storage and entropy change of the SCAP with those of NSCP controls. The higher mean skin temperature (T_{sk}) obtained in the SCAP than that obtained in NSCP suggests a higher energy metabolism and higher heat content in the SCAP than the NSCP counterparts. This higher body temperature has been suggested as a compensation to prevent red blood cell coagulation and vaso-occlusive crisis in individuals living with SCA[2;23;24]. The T_{sk} of the SCAP (36.5 ± 0.4^{o}C) was within physiologic range (36.5-38^{o}C). Higher body temperatures (within physiologic range) in SCA have been considered to have physiological compensation for wellbeing and prevention of vaso-occlusive crisis[2].

The low mean arterial pressure (MAP) and the high pulse pressure (PP), heart rate (HR) and rate-pressure product (RPP) obtained in the SCAP compared to those obtained in the NSCP are in agreement with those obtained in SCA patients who were not in crisis[17;22]. Similar values of low blood pressure (BP) and HR parameters in SCA subjects in the steady state have been published[17]. Our findings show a co-existence of low MAP with low haematocrit (Hct), low RBC count, low haemoglobin concentration ([Hb]) as obtained in SCAP compared to those obtained in NSCP. Ogungbemi (2014) has linked low MAP in SCA subjects with low RBC count, low Hct, and high total bilirubin concentration as an adaptation to prevent peripheral resistance and improve peripheral tissue perfusion in SCA patients[17]. Similarly, from our results, high PP, HR and RPP obtained in SCAP compared to those obtained in NSCP suggest cardiovascular compensation to produce high ventricular beat-to-beat-volume (or cardiac stroke volume) and ventricular minute-volume (or cardiac output) for SCAP living with a compromised arterial BP. The high RPP obtained in SCAP compared to that obtained in NSCP is an index of high basal myocardial oxygen consumption and energy expenditure in the SCAP compared to NSCP or controls[12;17]. The normal BP and HR obtained in the NSCP show that they were apparently healthy controls during the study.

The low Hct level, RBC count, and [Hb] obtained in the SCAP compared to those obtained in NSCP confirm earlier findings that SCAP live with chronic anaemia[17;22]. Anaemia in SCAP has been linked to impaired erythropoiesis, intravascular haemolysis owing to RBC sickling and vaso-occlusive crisis[17;22]. The high white blood cell (WBC) and platelet (Plt) counts obtained in SCAP compared to those obtained in NSCP were typical of SCAP17. These findings suggest that SCAP live with chronic inflammation with the evidence of high WBC and Plt counts, and high oxidative stress[17, 22].

Arterial Pulse Oxygen Saturation, Serum Malondialdehyde and Triiodothyronine Concentrations in the Participants

The low arterial pulse oxygen saturation (SPO$_2$) obtained in the SCAP compared to that obtained in the NSCP is an index of hypoxia. Published data on oxygen-haemoglobin-dissociation curve suggests that SPO$_2$ (of 93.5 ± 0.2%) obtained in SCAP is an indication of hypoxia in them, because SPO$_2$ of 93.5 ± 0.2% is equivalent to arterial partial pressure of oxygen (PO$_2$) below 80 mm Hg on published oxygen-haemoglobin-dissociation curve[15]. On the other hand, SPO$_2$ of 98.6 ± 0.3% in NSCP indicates that the NSCP were having normal arterial oxygen tension and not living with hypoxia, having had normal PO$_2$ above 100 mm Hg at SPO$_2$ of 98.6 ± 0.3%15. The co-existence of low Hct, RBC count, low [Hb] and the low arterial SPO$_2$ in the SCAP is a strong indication of anaemic hypoxia in them. High PP, HR, and RPP co-existed with the low Hct, RBC count, [Hb] and SPO$_2$ in the SCAP. The high PP, HR and RPP and low MAP bed indicates low peripheral resistance and high peripheral vasodilation responses to high physiological oxygen demand in the tissues of the SCAP as the compensatory cardiovascular biomechanics in response to chronic anaemic hypoxia for increasing oxygen perfusion into tissues in the SCAP[17;22].

The high serum concentration of malondialdehyde ([MDA]) obtained in SCAP compared to that obtained in NSCP is a biomarker of high lipid peroxiadation (LPx) in the SCAP[17;22]. Ogungbemi (2014) and Jaja (2017) have shown that there is higher LPx in Nigerians living with SCA[17;22]. In addition, HbS has been known to be a lipophilic pro-oxidant that could damage the endothelial cells to produce RONS[17;22]. Higher rate of [MDA] production or LPx have been observed in the RBC of SCA volunteers[17;22]. It is known that WBC produces superoxide ions via (nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and acting as a major source of RONS in SCA[17;22]. This study has shown a coexistence of high LPx with high WBC and Plt. counts in SCAP.

This study also shows a low serum triiodothyronine concentration ([T$_3$]) and activity in the SCAP compared to their NSCP counterparts. The low serum [T$_3$] suggests that SCAP live with compromised thyroid function or thyroxine insufficiency. Similar findings about low T$_3$ level in SCA patients have been published[18;26;27]. Despite the low serum T$_3$ (which is known as a metabolic hormone) commonly and severally reported in the SCA patients, there have been always reports about increase in oxygen consumption and production of high metabolic rate or energy expenditure in SCA individuals contrary to expected low metabolic rate or energy expenditure. Similarly, unexpected increase in erythropoiesis has been reported despite the low level of thyroxine hormones that are needed for stimulation of more erythropoietin hormone needed for the increased erythropoiesis (26;27). The compromised function of the thyroid gland as suggested by this study is supported by Prasad et al. (1989), Philips et al. (1992) and Olatunji-Bello and Igbinova (2002)[18,26;27], because they reported that thyroid gland was unresponsive to high level thyroid stimulating hormone (TSH) obtained in the SCA patients studied, indicating abnormal pituitary thyroid axis and thyroid failure[18]. Phillips et al. (1992) have linked low levels of thyroid hormones in SCA patients to iron overload (from haemolysis and blood transfusion in three SCA patients)[26] that might have been deposited in the thyroid follicles, causing extensive thyroid fibrosis and failure, iron poisoning and low thyroid hormones[26].

Anomalies in Oxygen Consumption, Metabolic Heat Production, Heat Energy Balance, Heat Storage and Entropy Dynamics in the Sickle Cell Anaemia Participants

Sickle cell anaemia (SCA) is energy costly and the higher Tsk obtained in the SCAP is a sign of energy wastage[28;31]. Basically, RBC sickling and the subsequent haemolysis push SCA bio-system to compensate for cardiovascular and haematological homeostatsis[28;29;31]. The compensations shift the energy equilibrium in SCA bio-system to yield extra energy for its functioning[28;31]. SCA has been reported to have displacement from energy homeostasis-cum-homeokinesis equilibrium to altered system functions[28;31]. Anaemia from frequent haemolysis has been reported to put SCA bio-systems into a state of low oxygen tension and anergy, which overwork the heart and increase protein turnover and erythropoiesis to compensate for anaemia and low oxygen[2;28;29;31]. These compensations might be the bases for increase oxygen consumption rate, metabolic energy production rate and resting energy expenditure –estimated in the participants[28;29;31].

Our results suggest that SCAP live with higher body heat storage and entropy than the NSCP, because they consume more oxygen and produce more metabolic energy in spite of higher body heat loss (in them) via radiation, convection, and expiration. Change in heat storage (ΔH) is the heat energy freely available (in excess of heat energy storage, content or enthalpy in which a bio-system structures subsist and hold together) for dynamics of bio-system functions at nuclear, cellular, molecular, electrochemical, tissue-organ-system and whole organism levels of the participants[20]. Whereas, entropy change (ΔS) is the degree of functional kinetics of cellular particles of an intact bio-system of the participants or the heat energy available for the dynamics of bio-system structures and functions in the participants[20]. These findings are intriguing owing to little or no reports of body heat balance, body heat storage and entropy on SCA in previous studies by other researchers. Previous studies have been much concerned about oxygen consumption, basal metabolic rate, resting energy expenditure (which were reported to be elevated or higher in SCA volunteers) and pain reactions to cold, warm and windy environments[2;18;30]. Our results show that oxygen consumption rate (VO_2), metabolic energy production rate (M), basal metabolic rate (BMR) and resting energy expenditure-estimated (REEp) obtained in SCAP were more than those obtained in the NSCP. The higher VO_2 obtained in the SCAP translated to the higher M and higher BMR in them. The high REEp estimated in the SCAP (compared to that estimated in the NSCP) is a function of the high haemoglobin deficiency (i.e. low haemoglobin concentration) and their body weight. Several methods have been employed in studying VO_2[30]; BMR[4;10] and REEp[19]. However, our results are comparable to the findings reported by these earlier authors. The higher VO_2, M, BMR and REEp obtained in the SCAP than those obtained in the NSCP are in agreement with findings of earlier researchers. Previous studies have shown that microvascular oxygen consumption, VO_2, BMR, REE and energy intake are elevated in SCA patients[4;19;30]. Several explanations have been reported to provide physiological bases for these elevated energy parameters in SCA subjects. These physiological bases for the high VO_2, M, BMR, REE include: increase cardiac compensation (29); increase rate of protein synthesis and erythropoiesis[12]; increase phagocyte mitochondrial activity and lipid peroxidation[17;22]. While earlier studies by various authors reported mainly the VO_2, BMR and REE[4;19;30], our study reports findings about heat loss by radiation, evaporation, convection and respiration (in addition to VO_2, M, BMR and REEp) in order to bring out the ΔH and ΔS in the SCAP compared to NSCP. Our findings also show that the SCAP lost more body heat to the environment than the NSCP via radiation, convection and expiration. Currently, there are little or no reports on heat loss dynamics in SCA studies. However, consequences of exposure to cold and windy environments (on the basis of body heat loss) have been studied[2]. The greater heat loss from the SCAP is a function of the higher Tsk of the SCAP. The higher temperature gradient 11.5°C of the SCAP (i.e. T_{sk} of 36.5 ± 0.2°C in the SCAP vs 25°C of room temperature) compared with the temperature gradient 10.1 °C of the NSCP (i.e. 35.1 ± 0.1 °C vs 25°C) by 1.4^0C allowed these higher heat losses in SCAP. The higher temperature gradient is a secondary factor for heat loss over the BSA which was only slightly higher (by 0.08 m^2) in the NSCP than in the SCAP. It has been reported that 1°C in temperature above or below 35°C produces greater heat exchange20. Nevertheless, the BSA (1.94 ± 0.2 m^2 for NSCP vs 1.86 ± 0.04 m^2 for SCAP) of both groups of participants are comparable values and factors of the various heat losses produced. So, our findings show that there was more heat flown away from the hypermetabolic and hyperthermic SCAP bio-system to the room environment at 25°C than from normometabolic and normothermic NSCP bio-system to the same room environment °C. The greater ΔH and ΔS (in spite of greater heat loss) obtained in the SCAP than those obtained in the NSCP is a function of the greater M in them. And these higher ΔH and ΔS suggest that the SCAP were hypermetabolic and hyperthermic – as it is evident from the higher Tsk. These hypermetabolic and hyperthermic nature suggest an anomalous metabolic heat energy homeostasis. From our results, the higher ΔH and ΔS obtained in the SCAP than those obtained in the NSCP are supported by the explanation of Fakhri et al. (1991) and Kiessling et al. (2000) that SCA individuals may suffer a greater or extra disorderliness in their bio-systems (in compensation for low [Hb] and low arterial oxygen saturation)[28;31], because of their increase in body temperature and entropy[29]. Kiessling et al. (2000) and Reid et al. (2006) also explained that SCA patients may go into the dissipative chaos of entropy, instead of being organised as parts of stable system structures such as fat, healthy nerves, muscles, blood components and blood flow which are lacking in SCA bio-system[31;32]. Our findings are supported with these explanations. The higher ΔH and ΔS in the SCAP from our study supports the suggestions that SCA is an energy and body tissue wasting disease (as body mass can be converted to excessive heat or Gibbs free energy via oxidation), and as a consequence, energy deficiency, delay in growth and development, macronutrient and micronutrient wastage have been reported[18;28;31]. Our results also show that the higher ΔH and ΔS obtained from the SCAP than those obtained from NSCP suggest metabolic energy wastage, because SCAP were on fasting without external energy source in form glucose or any food. The metabolic energy produced was rather from the body storage (i.e. glycogen and lipid Gibbs free energy) of these fasted participants[20]. These findings support the fact that there are macronutrient insufficiency and body tissue wasting in SCA patient as commonly reported in them[28;29;31]. The source of the higher M, BMR, ΔH and ΔS in the SCAP is from glycogen and fat store in the tissues as the Gibbs free energy without any external work done during the study, because they were in resting state[20]. From our findings, the higher ΔH with the subsequent higher ΔS obtained in the SCAP is associated with the higher HR, RPP, WBC count, serum [MDA] and lower [Hb], arterial SPO_2 and $[T_3]$. From our results, the positive correlations of ΔH in the SCAP with HR, RPP, WBC count, serum [MDA] as well as the negative correlations of ΔH in the SCAP with [Hb], arterial SPO_2 and serum $[T_3]$ show possible bases for the higher ΔH and ΔS in the SCAP. On the other hand, the positive correlation of ΔH with serum $[T_3]$ in NSCP indicates that T_3 is responsible for heat production and metabolism in the NSCP controls15;20.

These correlations of ΔH with other measured parameters in the SCAP compared to that of NSCP as shown in our findings further corroborate various mechanisms that have been published in explanation for higher M, BMR and REEp, in the SCA subjects by various authors. The positive correlation of ΔH with HR and RPP suggest higher cardiac work load in the SCAP which might have contributed to the higher ΔH and ΔS obtained in them. The higher HR, pulse pressure and RPP obtained in SCAP are indicators of higher cardiac output and myocardial oxygen consumption[12;17]. From our results, the higher pulse pressure (PP) obtained in the SCAP than that obtained in the NSCP indicates a compensation for stroke volume in the SCAP[17;21;22) Higher stroke volume in the SCA subjects of 75 mL in HbSS subjects has been reported against 49 mL in HbAA controls[13]. The higher PP and HR obtained in the SCAP than those obtained in the NSCP are indices of higher cardiac output in them. High cardiac output and myocardial oxygen consumption are both cardiovascular compensation in SCAP. These findings are supported by earlier works that higher cardiac workload in SCA patients contributes to higher BMR and REE in them[28;29;31]. So, the association of higher HR, PP, and RPP in the SCAP with higher ΔH implies a relationship between the ΔH with higher cardiac output and myocardial oxygen consumption (i.e. cardiac workload) as compensations in the SCAP. The higher cardiac workload has been reported to compensate for blood, nutrient and oxygen perfusions and blood flow turbulence[28;29;31]. The positive correlations of ΔH with WBC and serum [MDA] indicate that there were higher activities of the WBC and lipid peroxidation in the SCAP which might have contributed to the higher ΔH and ΔS in them. These findings are in consonance with the study of Serjeant (1974) that oxidative stress damage in SCA destroys the energy efficient pi-biconcave erythrocytes and then increase energy cost of blood stream transport, erythropoiesis, cardiac contraction, blood ejection and cardiac output[33]. On the other hand, the negative correlations of ΔH with lower [Hb] and SPO_2 obtained in SCAP indicates that lower [Hb] and arterial SPO_2 might have been responsible for the higher ΔH and ΔS obtained in them. Badaloo et al. (1996) have reported that the magnitude of the RBC protein turnover is six times greater than that estimated in the HbAA controls(34). This erythropoietic turnover compensates for the low [Hb] at a very high energy cost[28;31;34] and increase VO_2 (and the subsequent M, BMR, ΔH and ΔS) for cardiac load and RBC turnover (28;31;29). The negative correlation of higher ΔH with lower serum $[T_3]$ did not associate T_3 level with ΔH. Prasad et al. (1989) has shown that the mechanisms for increase in BMR and REE cannot be attributed to lower $[T_3]$ in SCA patients[18]. Our study suggests that higher red cell protein turnover and cardiac workload might have been responsible for the higher VO_2, ΔH, ΔS, BMR and M, in the SCAP. The positive correlation of ΔH with serum $[T_3]$ in NSCP is in concordance with basic physiology of metabolism that thyroxine is a positive factor of basal metabolic rate[15;20]. Although, higher ΔH and ΔS have been linked with energy and nutrient wastage and poor growth and development in SCA patients[28;31;34], the displacement from homeostasis-cum-homeokinesis equilibrium and altered system functions is a shift in energy equilibrium for survival. We agree with Fakhri et al (1991) and Kiessling et al (2000) that the higher ΔH and ΔS status in SCAP is their most energetically stable and efficient bio-systems for optimal function despite metabolic energy and body tissue wastage[28;31]. In addition, SCA patients have been reported to live with decrease threshold to cold, thermoreceptor hypersensitivity, in which a normal cool weather produces cold, vaso-occlusion, acute vaso-occlusive pain[2]. Therefore, SCA energy bio-system may prevent may prevent HbS polymerisation, irreversibly sickled RBC population, peripheral vasoconstriction, vaso-occlusion, ischaemic and acute vaso-occlusive pain to a degree by maintaining a body temperature that is about 1°C above normal body temperatures of controls[2]. Some studies have suggested hot bath and massage as adjunct therapy in the management of SCA patients (23;28;31). These earlier reports support our findings that SCAP might have lived with higher M, BMR, ΔH and ΔS most likely in order to maintain optimal condition for proper functions of their bodies' tissue and organ-system to a degree.

CONCLUSION

The study shows that SCAP lived with higher ΔH and ΔS, because they consumed more oxygen and generated more M than NSCP. These higher ΔH and ΔS were not only associated positively with higher HR, RPP, WBC, serum [MDA], but also negatively with lower [Hb], arterial SPO_2 and serum $[T_3]$ obtained in the SCAP, serving as indices of cardiovascular, haematological and metabolic compensations and explanations for the elevated ΔH and ΔS, VO_2, M, BMR and REEp obtained in them. These elevated enthalpy and entropy status in SCAP may be beneficial for the optimal function of their bio-systems in addition to being an energy and tissue-wasting status of the SCAP.

RECOMMENDATION

We recommend prevention of HbS polymerisation and repair of affected body tissues in SCA sufferers by developing HbS-target supplements, simply because the whole high ΔH and ΔS or energy wastage anomaly is a consequence and/or compensation for HbS pathology in SCA in the first place.

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REFERENCES

1. Morris CR. Mechanism of Vasculopathy in Sickle Cell Disease and Thalasaemia. Haematology American Society; Haematology Education Programme. 2008: 177-185.
2. Tewari S, Brousse V, Piel FB, Menzel S and Rees DC. Environmental Determinants of Severity in Sickle Cell Disease. Haematologica. 2015; 100 (9): 1108-1116.
3. Ingram VM. A Specific Chemical Difference between the Globins of Normal Human and Sickle-Cell Anaemia Haemoglobin. Nature. 1956; 178: 792-794.
4. Barden EM, Zemel BS, Kawchak DA, Goran MI, Obene-Frempong K and Stallings VA. Total and Resting Energy Expenditure in Children with Sickle Cell Disease. Journal of Paediatrics. 2000; 136: 73-79.
5. Odonkor PO, Addae SK, Apatu RS, Yamamoto S. Basal Metabolic Rate and Serum Thyroid Hormone Levels in Adolescent Sickle Cell Patients. IRCS Med Sci. 1982; 10: 891
6. Tikuisis P. Heat Balance Precedes Stabilization of Body Temperatures during Cold Water Immersion. Journal of Applied Physiology. 2003; 95: 89-96.
7. Blazejezyk K. Dry Heat Exchange and Skin Temperature in Subjects Outdoors at Fluctuated Cloud Cover. In: Thermal Physiology; Proceedings in International Symposium on Thermal Physiology. 1997: 115-118.
8. Havenith G. Heat Balance When Wearing Protective Clothing. Annals of Occupational Hygiene. 1999; 43 (5): 289-296
9. Grossman SC and Porth CM. Body Temperature Regulation In: Alterations in Temperature Regulation In: Porth's Pathophysiology: Concept of Altered Health States, 9th Edition; Lippincott Williams and Wilkins, Philadelphia. 2014: 216-221.
10. Kopp-Hoolihan LE, vanLoan MD, Mentzer WC, Heyman MD. Elevated Resting Energy Metabolism in Adolescents with Sickle Cell Anaemia; Journal of American Dietetic Association. 1999; 99 (2): 195-199
11. Singhal A, Davies P, Shotta A, Thomas PW and Serjeant GR. Resting Metabolic Rate in Homozygous Sickle Cell Disease. American Journal of Clinical Nutrition. 1993; 57: 32-34
12. Borel M.J., Buchowski M.S., Turner E.A., Peeler B.B., Goldstein R.E. and Flakoll P.J. Alteration in Basal Nutrient Metabolism Increases Resting Energy Expenditure in Sickle Cell. American Journal of Physiology Endocrinology and Metabolism. 1998; 274 (2): 257-264.
13. Salman E.K., Haymond M.W. and Bayne E., Sager BK, Wiisanen A, Pitel P. et al. Protein and Energy Metabolism in Prepubertal Children with Sickle cell Anaemia. Paediatric Research. 1996; 40: 34-40.
14. Singhal A, Parker S and Linsell L. Energy Intake and Resting Metabolic Rate in Preschool Jamaican Children with Homozygous Sickle Cell Disease. American Journal of Clinical Nutrition. 2002; 75: 1093-1097.
15. Barrett KE, Barman SM, Boitano S and Brooks HL. Nutritional Principles and Energy Metabolism In: Endocrine Functions of the Pancreas and Regulations of Carbohydrate Metabolism In: Ganong's Review of Medical Physiology. McGraw-Hill Companies Incorporation, United States. 2012; 24th Edition: 488-490.
16. Green JH. Nutritional Requirements In: An Introduction to Human Physiology, 2nd Edition; Oxford Medical Publication London. 1968: 95-98.
17. Ogungbemi SI. Contribution of L-Arginine to Autonomic and Haematologic Status, Antioxidant and Liver Enzymes Activities in Sickle Cell Anaemia Subjects. Ph.D Thesis. 2014; University of Lagos.
18. Prasad O, Stevens MCG, Hudson C, Rosenthal J, Melville GN, Dunn DT and Serjeant GR. Abnormal Thyroid Hormone and Thyrotropin Levels in homozygous Sickle cell Disease. Clinical laboratory Haematology. 1989; 11: 309-315.
19. Buchowski MS, Chen KW, Bryne D and Wang WC. Equation to Estimate Resting Energy Expenditure in adolescents with Sickle Cell Anaemia. American Journal of Clinical Nutrition. 2002; 76 (6): 1335-1344.
20. Boron WF and Boulpaep EL. Regulation of Body temperature In: Medical Physiology: A Cellular and Molecular Approach, Updated 2nd Edition; Elsevier Saunder, Philadephia. 2012: 2322-2346,
21. Jaja SI, Kehinde MO, Gbenebitse S, Mojiminiyi

FB and Ogungbemi AI. Effects of Vitamin C on Arterial Blood Pressure, Irreversibly Sickled Cells and Osmotic Fragility in Sickle cell Anaemia subjects. Nigerian Journal of Physiological sciences. 2000; 16:14-18.

22. Jaja SI. Low Dose Vitamin C, Vitamin E or L-Arginine supplementation and Sickle Cell Disease. Journal of African Association of Physiological Sciences. 2017; 5 (1): 7-14.
23. Shihabuddin BS and Scarfi CA. Fever in Children with Sickle Cell Diseases: Are All Fevers Equal? Journal of Emergency Medicine. 2014; 47 (4): 395-400.
24. Sokol E, Obringer E, Palama B, Hageman J and Peddinti R. (2016). Outpatients Management of Febrile Children with Sickle Disease. Clinical Paediatrics; 55 (3): 268-271.
25. Brewster B. Sickle Cell Anaemia: Causes, Signs, Symptoms and Treatment. Nursing Times. 2003; 99 (29): 30.
26. Philips GR, Becker B, Keller VA and Hartman J. 4th. Hypothyroidism in Adults with Sickle Cell Anaemia. American Journal of Medicine. 1992; 92 (5): 567-570.
27. Olatunji-Bello II and Igbinova E. Serum Levels of Thyroid Hormones and Thyrotropin in Some Sickle Cell Anaemia Patients in Lagos. Nigerian Quarterly Journal of Hospital Medicine. 2002; 12 (1-4): 26-28.
28. Fakhri ME, Aggarwal SK, Gayoum A and Sherrif DS. Reduced Plasma Ascorbic Acid and Red Cell Glutathione Contents in Sickle Cell Disease – A Preliminary Report. Indian Journal of biochemistry.1991; 6 (1): 47-50.
29. Hibbert JM, Creary MS, Gee BE. Erythropoiesis and Myocardial Energy Requirements Contribute to the Hypermetabolism of Childhood Sickle Cell Anaemia. Journal of Paediatrics, Gastroenterology and Nutrition. 2006; 43(5): 680-687.
30. Meyer D, McGraw M, George M, Mulier K and Beilman G. Tissue Haemoglobin Index: A Non-Invasive Optical Measure of Total Tissue Haemoglobin Oxygen saturation. Critical care. 2009; 13 (5): S2.
31. Kiessling K, Roberts N, Gibson JS and Ellroy JC. A comparison in normal Individuals and Sickle Cell Patients of Reduced Glutathione Precursor and Their transport between Plasma and Red Cells. Haematological Journal.2000; 1 (4): 243-249.
32. Reid M, Badaloo AV, Forrester T and Jahoor F. In Vivo Rates of Erythrocyte Synthesis in Adults with Sickle Cell Disease. American journal of Physiology, Endocrinology and Metabolism. 2006; 291: E73-E79.
33. Serjeant BE, Forbes M, Williams LL and Serjeant GR. Screening Cord Bloods for the Detection of Sickle Cell Disease in Jamaica. Clinical Chemistry.1974; 20: 666-669.
34. Badaloo AV, Singhal A, Forrester TE, Serjeant GR and Jackson AA. The Effect of Splenoctomy for Hypersplenism on Whole Body Protein Turnover, Resting Metabolic Rate and Growth in Sickle Cell Disease. European Journal of Clinical Nutrition.1996;50: 672-675.