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Effect of road transport stress on Erthrocyte Osmotic Fragility (EOF) of healthy young adult Nigerians during the harmattan season

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Summary: Road transportation and harmattan season have been reported to be stressful to live stock species. This study was conducted with the aim of investigating the effect of two and half hours of road transportation on the erythrocyte osmotic fragility of 23 healthy young adults Nigerians (15 males and 8 females) during the harmattan season. After an overnight fast, venous blood was collected from each subject for the determination of serum cortisol, glucose concentration and erythrocyte osmotic fragility. The subjects were then transported at a speed of 65 – 75Km/h covering a distance of 180km. Thereafter venous blood was again collected (within 10 minutes) for the determination of serum cortisol concentration, glucose concentration and erythrocyte osmotic fragility using standard methods. There was a statistically significant decrease ($P < 0.05$) in percent haemolysis recorded at Nacl concentration of 0.50% after transportation. There was also significant decrease ($P < 0.001$) in percent haemolysis at Nacl concentration of 0.60 and 0.70% in the male subject after road transportation as compared to values obtained before transportation. There was a statically significant difference ($P < 0.05$) between the serum concentrations of cortisols in the subjects before and after road transportation. The results of this study indicated that road transportation was stressful to the subjects and measurement of erythrocyte osmotic fragility (EOF) could be used as a biomarker of stress in humans.

Keywords*:* Road transport, Stress, Harmattan, Season, Erythrocyte Osmotic Fragility, Humans, Zaria, Northern Nigeria

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INTRODUCTION

Road transportation is the commonest means of transporting live-stocks and humans and have been shown to be stressful to animals (Buckhan- Sporer *et al* 2008, Ayo and Oladele 1996, Giovagnoh *et al* 2002, von Borell 2001, Pineiro *et al* 2007). For example, road transport stress has been reported to cause increase serum levels of cortisol, prolactin and glucose, decrease haematocrit, increase total Neurophil and Neutrophil to lymphocyte (N/L) ratio (Dixit et al 2001., Buckhan-Sporer *et al*, 2008, Parker *et al,* 2007, Krawczel *et al,* 2007.). Olorunshola *et al* (2011) have demonstrated the stressful effect of road transportation on the cardiopulmonary parameters of young adult Nigerians.

Stress induced changes in lower animal are affected by factors such as nature of road, quantity of gas emission, loading, distance and duration of journey. Other factors are vibration of vehicle, environmental temperature, season and space availability between the animals (Piccione *et al*., 2005; Peterson *et al.*, 2006; Voslarova *et al*., 2007; Minka and Ayo, 2007, 2008).

There are three (3) distinct seasons in the guinea savannah region of Northern Nigeria. They are the Harmattan (November-February) which is also known as the cold and dry season, hot dry (March-May) and hot-humid or rainy season (June - October), (Igono and Aliu, 1982). Seasonal variations in ambient temperatures and temperature-humidity index during these seasons are stressful with the harmattan season being thermally more stressful to lower animals (Igono *et al*., 1982, 1983., Fisher *et al* 2005., Piccione *et al* 2005., Minka and Ayo, 2007, 2008).

Stress factors have been shown to cause oxidative stress and impair activity of the antioxidant ascorbic acid (Halliwell, 1996, Sahin e*t al* 2001) and depletion of some antioxidant systems could increase the vulnerability of tissues and cellular components to reactive oxidation species (Piccione *et al* 2005 and

Nayanatara *et al* 2009). Free radicals are known to play a vital role in tissue damage and they have been demonstrated to have adverse effects on erythrocyte (Sumikawa. *et al*, 1993 and Avellini *et al* 1995).

Erythrocyte osmotic fragility is a measure of erythrocyte strength and its ability to withstand varying osmotic gradients. It is increased in haemolytic anaemia associated with haemolytic blood transfusion reactions, haemolytic disease of the newborn, genetic disorders such as hereditary spherocytosis and sickle cell disease and auto-immune haemolytic disease in which the membrane of the red blood cell is defective (Aldrich *et al*, 2006 and Pocock and Richards, 2006). Adenkola and Ayo (2009a) demonstrated increased erythrocyte osmotic fragility which was prevented with administration of Ascorbic acid in pigs subjected to road transport stress.

In many developing countries like Nigeria, the poor conditions of roads and of many commercial vehicles with attendant over- crowding, over loading and the emergence of night buses call for assessment of effect of road transportation stress and harmattan season on physiological systems. To the best of our knowledge, the effect of road transport stress on physiological systems has not been previously studied in humans, hence the present study aim to investigate the effect of road transportation stress during the harmattan season on erythrocyte osmotic fragility (EOF) in young adult Nigerians.

MATERIALS AND METHODS

Study site

The study was conducted in the laboratory of Human Physiology Department, Faculty of Medicine, Ahmadu Bello University, Samaru, Zaria, Kaduna state, Nigeria. Zaria lies in the Guinea Savannah belt, latitude 11°3′N, longitude 7°42′ E with a mean annual temperature of 27°C. The monthly temperature is highly variable, varying between 15.6°C and 32.1°C in the different seasons (Ati, 2004; Mortimore, 1970).

Meteorological data of the study area

The dry-bulb temperature (DBT) and relative humidity (R.H) were measured using a wet and dry bulb thermometer (Ellab Inc. USA) at the experimental site at 6.00, 14.00 and 18.00 hours daily for three consecutive days before and after transportation. The sunshine duration and wind direction were also recorded.

Study Population

Twenty-three healthy adult volunteers (15 males, 8 females) between the ages of 20 -35years (mean age 24.8 ± 0.6yrs) were recruited into the study. Exclusion criteria included smoking, alcohol consumption, hypertension, diabetes mellitus, obesity, musculo-skeletal disorders, sickle cell disease and goiter. Written informed consent was obtained from each volunteer before the commencement of study and approval from the Ethical Committee on Human Research of Ahmadu Bello University, Zaria was obtained.

Vehicle design and transportation

A standard Toyota Coaster 32 seater bus (Toyota, Japan) was used for transporting the subjects. The body of the vehicle was made of steel and the floor of steel covered with a rug carpet. The inner compartment of the vehicle measured 15.5 X 4.8m. The side walls of the body of the vehicle to a height of 2.8m was made of steel sheets above which were 6 windows of 1.2 X 0.8m dimension on each side for ventilation. The steel roof was lined by foam and upholstery leather. Boarding the bus was by a swinging door which had a windscreen on the right hand side of the bus. Each subject was on a seat and the whole group accompanied by three research assistants. No artificial air cooling was used and the subjects sat facing the direction of movement of the vehicle.

Transportation of the subjects was conducted from the Faculty of Medicine complex on the main campus of Ahmadu Bello University, Samaru, Zaria (11°3′N and 7°42′ E) to Mando, Kaduna (11°10′N and 7°38′ E) and back to Zaria nonstop. They covered a distance of 180km at a speed of 65-75km/hr over a period of two (2) hours.

Collection of Data

Subjects were fasted for 10 hours before the commencement of study. Basal blood pressure, pulse rate, core body temperature (oral), body weight, height and the body mass index were noted and recorded before commencement of the trip. All the parameters were re-assessed after the trip except for the weight and height of the subjects.

The weights of the subjects were measured while wearing light clothing to the nearest 0.2 kg with a calibrated scale. Height (without shoes) was measured to the nearest 0.5 cm with a standiometer. The body mass index was calculated as weight (kg)/ height (m^2), (Guyton and Hall, 2006). Core body temperature was determined using a digital thermometer (Hangzhou Sejoy Electronics & Instruments Co., China) placed sublingually for 2 -3 minutes and the temperature measured in degrees centigrade orally before the reading was noted and recorded for each subject.

Blood Collection

After the journey (within 10 minutes) 8mls, blood was again collected aseptically from the cubital vein using 10mls syringe and 21Gauje x 1½ inch sterile needle. Four (4) mls of blood collected from each

subject was poured into a sample bottle, containing anticoagulant disodium salt of ethylene diaminetetra-acetic acid at the rate of 2mg/ml of blood (Oyewale 1992) for the determination of erythrocyte osmotic fragility (EOF) test performed as described by Faulkner and King 1970 and used by Adenkola *et al* (2010). The remaining 4mls of the blood was poured into a plain bottle and allowed to clot and centrifuged using the bench centrifuge (*BTL La Lock*, England) The serum was harvested and stored at a temperature of +4oC and used for the analysis of serum cortisol levels in the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Shika, Zaria, Nigeria.

Determination of blood glucose level

Blood glucose concentration for each subject was determined using a rapid glucometer (ACCU-CHEK Meter Systems, U.S.A) based on the glucose oxidase method as described by Yuen and Mc- Neil (2000). A small drop of blood from each specimen collected before and after transportation was placed on a disposable test strip and inserted into the glucometer which displayed the blood glucose concentration in mmol/L.

Determination of Serum Cortisol level

The assay for serum cortisol was carried out using BLK Cortisol Enzyme Immunoassay Kit (BLK diagnostics, France) according to the manufacturer's instruction as described by Dhar *et al* 1986 and Candito *et al.* (1992) Absorbance at 450nm was determined by a microplate reader and cortisol concentration was determined using the standard curve.

Determination of Erythrocyte Osmotic Fragility (EOF).

Erythrocyte Osmotic fragility was carried out by a modification of the method described by Faulkner and King (1970) and used by Adenkola *et al* (2010). A Sodium Chloride (Nacl) solution was prepared as described by Faulkner and King (1970) in volume of 500mls for each of the samples in concentration ranging from 0.00 to 0.90% at PH 7.4. A set of 11 test tubes with 10 containing 10 mls of Nacl solution of concentration ranging from 0.0 to 0.90% and 1 test tube containing 10mls of distilled water were arranged serially in a test tube rack. One set was used to analyze each sample. The test tubes were labeled with corresponding Nacl concentration. A one milliliter pipette was used to transfer exactly 0.02ml of blood sample into 10 of the 11 test tubes (Adenkola *et al* 2010). Mixing was performed by gently inverting the covered test tubes for about 5 times. The test tubes were allowed tostand at room temperature (26-27^{0c}) for 30 minutes. The contents of the test tubes were remixed and centrifuged at 1500 *rpm* for 15 minutes. The supernatant of each test tube was transferred into a glass cuvette and the concentration of haemoglobin in the supernatant solution was measured using a Spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, UK) at 540 nanometer by reading the absorbance.

The percent haemolysis was calculated using the formula of Faulkner and King (1970) as:

$$\frac{\text{Optical Density (OD) of test}}{\text{O.D of distilled water}} \text{ X } 100$$

EOF curve was obtained by plotting percent haemolysis against the saline concentration.The same procedure was repeated for every blood sample of each subject for the study.

Statistical analysis

All data obtained were recorded as mean ± SEM and subjected to statistical analysis using student's ´t´ test with Graph Pad Prism version 4.00 for windows. P values of ≤ 0.05 were considered significant.

RESULTS

The mean ambient temperatures, relative humidity, sunshine and rainfall during the harmattan season of year 2011 are presented in Table 1. The mean ambient temperature in the study site ranged from 10.9±0.12 to 28.3±0.21ºC with a mean of 19.6±0.20ºC corresponding to a diurnal cycle of cold nights alternating with warm days. The relative humidity ranged from 16 to 22% with a mean of 18.0±0.43% during the the study period. Duration of sunshine was shorter (mean of 8.2±0.25hours/day). The wind during harmattan was cold, dry and dusty and there was no rainfall

Table 1: Meteorological data during the study.

Ambient temperature (°C)	
Minimum ambient temperature (°C)	10.9 ± 0.12
Maximum ambient temperature (°C)	28.3 ± 0.21
Mean ambient temperature (°C)	19.6 ± 0.20
Relative Humidity (%)	18.0 ± 0.43
Sunshine (hr/day)	8.2 ± 0.25
Rainfall (mm)	
Amount	0.00
Days	0.00

Mean±SEM, Data collected from Meteorological Unit, Institute of Agricultural Research, Ahmadu Bello University, Zaria; during the 2010/2011 harmattan season.

Blood Pressure, Pulse Rate and Body Temperature

The diastolic blood pressure, mean arterial blood pressure (MABP) and body temperature were elevated after transportation, but the values were not significantly different from values obtained before transportation ($p>0.05$), see table 2

Table 2: Arterial Blood Pressure, Pulse Rate and Temperature of the subjects before and after transportation.

Variables	Before Transportation	After Transportation
MABP (mmHg)	92.2±1.91	93.2±2.31
PR Beat/Minutes)	83.27±2.94	78.69±2.86
Body Temp. (^{o}C)	36.72±0.17	36.74±13.35
Glucose (mmol/L)	3.25±0.65	3.08±0.70
Cortisol (umol/L)	0.57±0.45	1.05±0.49*

MABP =Mean Arterial Blood Pressure, PR=Pulse Rate, *p<0.001

Serum Cortisol and Glucose

Mean serum cortisol level significantly increased from 0.57±0.45 before transportation to 1.05±0.49 mol/L after transportation (p<0.001), but there was no significant change in the serum glucose concentration before and after transportation, (Table 2).

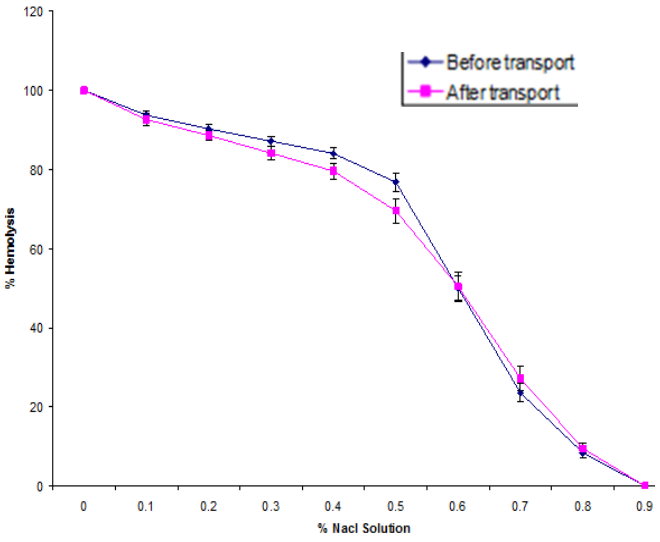

Figure 1: Percentage heamolyssis before and after transportation. N=23

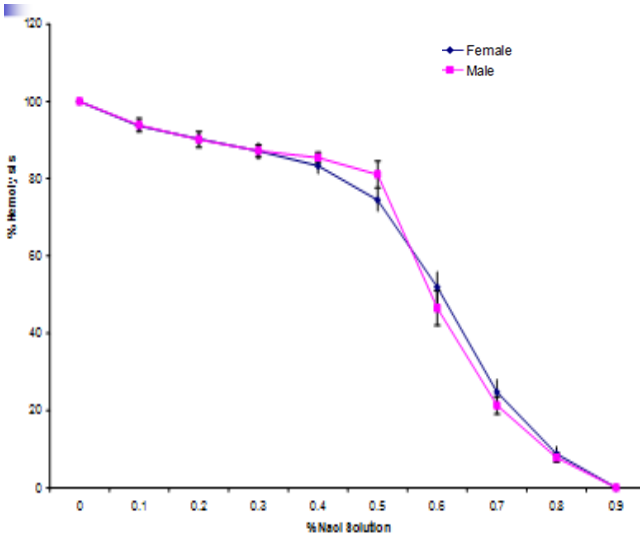

Figure 2: Percentage heamolysis of male and female subjects before transportation

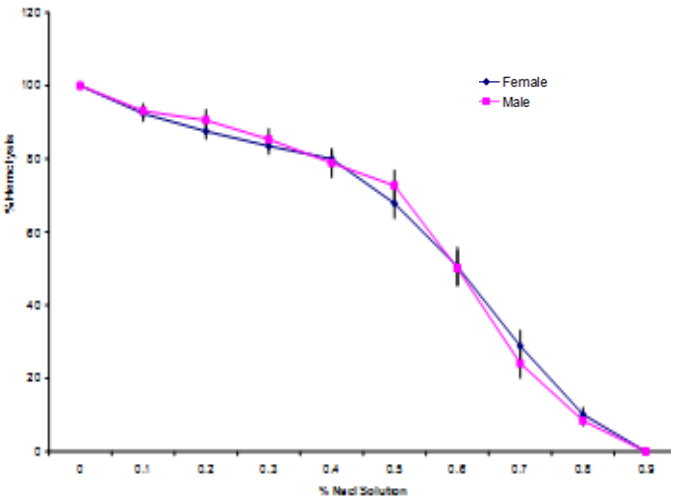

Figure 3: Percentage heamolysis of male and female subjects after transportation.

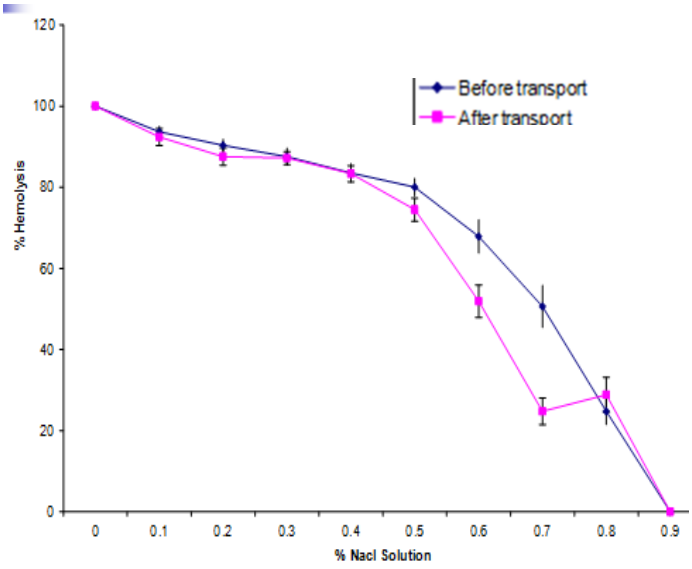

Figure 4: Percentage haemolysis of males before and after transportation

Table 3: Percentage haemolysis before and after transportation, n=23

% Concentration of Nacl	Before	After	T	P
0.0% Nacl (Control)	100 ± 0.000	100 ± 0.000		
0.1% Nacl	93.643 ± 1.367	92.361 ± 2.107	0.434	0.671
0.2% Nacl	90.301 ± 1.492	87.527 ± 2.107	0.886	0.391
0.3% Nacl	87.527 ± 2.109	87.168 ± 1.625	0.115	0.91
0.4% Nacl	83.515 ± 2.267	83.327 ± 2.005	0.0567	0.956
0.5% Nacl	80.002 ± 2.172	74.471 ± 2.816	1.718	0.108
0.6% Nacl	*67.844 ± 4.113	*51.902 ± 3.993	4.293	<0.001
0.7% Nacl	*50.598 ± 5.190	* 24.755 ± 3.268	4.467	<0.001
0.8% Nacl	24.755 ± 3.268	28.868 ± 4.230	-0.756	0.462
0.9% Nacl	0.000 ± 0.000	0.000 ± 0.000		

*p<0.001

Erythrocyte Osmotic Fragility

The percentage haemolysis significantly decreased from 76.77±2.24% to 69.52±3.04 before and after

transportation respectively at Nacl solution of 0.5% ($P<0.05$) in the whole subjects, see figure 1.There was significant sex variation in the erythrocyte osmotic fragility in the subjects. For example, there was significant decrease ($P<0.001$) in percent haemolysis from 67.84±4.11% before transportation to 51.90±40% after transportation and from 50.60 ±5.20% before transportation to 24.76±3.27% after transportation at Nacl concentration of 0.60% and 0.70% respectively in the male subjects. See table 3 and figure 4. There was no significant difference ($P > 0.05$) between percent haemolysis before and after transportation in the female subjects and there were no significant differences in the percentage haemolysis between male and female subjects before and after transportation, see figure 2 and 3. The maximum haemolysis in all the subjects was recorded at Nacl solution of 0.1% while the minimum haemolysis was recorded at 0.8% Nacl solution.

DISCUSSION

The climatic condition in the study area during the road transportation of the subject was characterized by fluctuations in environmental temperatures with diurnal cycle of cold nights alternating with warm days, shorter duration of sunshine and cold, dry and dusty wind without rainfall (see table 1). The harmattan season is reported to be the most thermally stressful to lower animals than the other two seasons (hot dry and rainy) in the guinea savannah region of Northern Nigeria (Igono and Aliu, 1982 and Ayo and Oladele, 1996).

Road transportation is a major transport mode due to its flexibility. In many developing countries including Nigeria, expenditure on road construction and maintenance is low when compared to other countries and road networks are overused as alternative modes of transport are poorly developed (Oni, 2004 and Oyesiku and Odufuwa, 2002). This results to poor state of roads, high travel time and a high rate of mobility stress. In addition, a large percentage of the population cannot afford private vehicles and public/commercial transport facilities are often utilized. Transport stress is also worsened by the poor state of these vehicles with overcrowding and overloading due to non standardization of seat size and space with passengers sitting and standing on the aisle of vehicle (Helaakoski and Merilainen, 2001., Haider and Badam, 2004., World Bank, 2002 and Odufuwa, 2003). The emergence of *Night Buses* in the West African sub region where traders travel through the whole night, transact their businesses in the day and travel back during the second night to continue trading the morning after calls for special interest in the physiological systems of these traders and the drivers of the vehicles.

From the results obtained, transportation of the young adults for 180 km over a period of 2hours during the harmattan season of year 2010 was stressful to the subjects as indicated by the significant rise in serum cortisol level from 0.57±0.45 µmol/L to 1.05±0.49 µmol/L ($p<0.001$) after the journey. Similarly, there was a non significant elevation of mean arterial blood pressure, diastolic pressure and body temperature of the subjects after road transportation. These effects have been reported to be features of physical stress and also manifest in adaptation to change in environmental temperature and road transport stress in humans and lives tocks (Weiss *et al* 1963., Alario *et al* 1987., Gamallo *et al* 1992,Masa-aki *et al*, 2004., Ramin *et al*, 2008.,Stall and Rodiek 2002 and Olorunshola *et al* 2011). WHO Regional office for Europe in 2003 reported heart rate variability and ectopic beats in human subjects after a patrol shift.

Erythrocyte osmotic fragility is a measure of erythrocyte strength and its ability to withstand varying osmotic gradients and it has being reported to be increased in situations of low oxygen tension, red blood cell membrane abnormality and during oxidative stress (Aldrich *et al*, 2006., Matsumura *et al*, 1996, Adenkola *et a*l 2010 and Ayo and Oladele, 1996). The result of this study showed a significant decrease in erythrocyte osmotic fragility after transportation in the study group at Nacl solution of 0.5%. This result is in contrast to reports in live stocks where road transport stress lead to significant increase in erythrocyte osmotic fragility during the harmattan season (Adenkola and Ayo, 2009b., Adenkola *et al*, 2010., Oladele *et al*, 2003 and Ayo and Oladele, 1996). The different results may be due to difference in species adaptation to stress. Oladele *et al* 2003 and Aldrich *et al* 2006 reported species variation in EOF related to adaptation to terrestrial, aquatic and semi-aquatic habitat, mean red blood cell volume and the presence or absence of nucleated red blood cells.

Age has been documented as a factor affecting EOF. For example, Bowdler *et al* (1981) and Oyewale (1991) found elevated EOF with increasing age in both human and poultry respectively. The subjects in the present study are young adult university students.

Antioxidants such as vitamins C and E, folic acid, zinc and carnitine are known to have protective effect on EOF (Matsumura *et al* 1996, Azeez et al 2001., Adenkola and Ayo 2009a., Minka and Ayo, 2007., Adenkola *et al* 2010a, b., Camdan *et al* 2002 and Celik and Oztunk, 2003). We did not measure the serum levels of antioxidant vitamins and enzymes in our subjects.

This study also showed significant sex variation in erythrocyte osmotic fragility of the young Nigerians after road transportation with statistically significant decrease ($p< 0.001$) in EOF of the male subjects after transportation at Nacl concentration of 0.60% and 0.70%. There was no significant change in EOF in the female subjects after transportation. Our result is in agreement with Olayemi *et al* 2009 and Oyewale *et al* 1998 that reported sexual dimorphism in EOF with females having higher osmotic fragility. Free radicals have been demonstrated to have deleterious effects on red blood cells membrane, causing increase EOF (Adenkola and Ayo, 2009a). The dual role of estrogen as a stimulator and protector of oxidative injury from free radicals may be a factor for the sex difference in EOF.

In human subjects, increased serum levels of parathyroid hormone and calcium has been reported to lead to increased calcium ion influx into red blood cells causing creanation, increased rigidity and increased haemolysis. Parathyroid hormone stimulate Ca-activated ATPase / adenylate cyclase and the resultant calcium ion influx affect the spectrin-actin cytoskeleton network of erythrocytes, altering the stability and integrity of the red blood cell membrane. This may be the basis of anemias of uremia which is reversed by verapamil (Bogin *et al* 1992). The serum level of calcium before, during and after road transportation of our subjects was not measured.

We conclude that road transportation during the harmattan season in the guinea savannah zone of Northern Nigeria was stressful to the young adult human subjects. The study also demonstrated a significant decrease in EOF in the male subjects after transportation. We recommend similar studies in humans subjected to longer distance and duration and during other seasons.

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