Impact of Microgravity Environment on Body Mass: Case Study of Lizards

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Abstract-Studies of animal behavior on spaceflight had shown that animals could survive trips away from the earth. This study examined the impact of microgravity environment on the body mass of lizards. Data was collected from the experiment conducted at the Microgravity Simulations Laboratory of the Engineering and Space Systems (ESS) Department, National Space Research and Development Agency (NASRDA), Abuja, Nigeria. A 2D clinostat was used as the microgravity simulations device. Three lizards were used for this experiment; sample A and sample B with body mass 7.5g and 8.0g respectively were impacted with simulated microgravity, while sample C (control) of body mass 7.2g was under normal earth gravitational influence. The data collected of their body masses after a period of observation was analyzed using regression analysis with other mathematical analyses. Resorptions were discovered in the body mass of samples A and B. Resorption increased as the period of microgravity simulations increased and lizard B being heavier than lizard A in body mass (equivalent to bone mass) had slower rate of resorption. The rate at which bone and muscles (body) mass declined under simulated microgravity was inversely proportional to the body mass. The non-linear curve therefore provides the most accurate and realistic comparative analysis for sample A and B as it gave realistic evidence that the sample A lizard had more body mass loss than lizard of sample B. The angle (θ) of rotation of the femur at midstance increased as the period of flight increased due to decrease in body mass. The decline in body mass of lizard samples A and B was more than lizard sample C, because sample A and sample B were under the influence of microgravity. Following non-linear body mass loss, it was agreed that the control lizard had little or no evidence of body mass loss since the non-linear curve is approximately parallel to the horizontal axis. Homeostatic stage was attained with sample A and sample B, with sample A 4.3g at t (2) and sample B with 4.5g at t (3) i.e. the lizard of greater body mass attained homeostatic stage later than the smaller body mass and that the linear regression analysis gave no indication of homeostatic, but the non-linear regression indicated homeostatic.

Keywords: Microgravity, Simulated Microgravity, Body mass, Lizards.

I. INTRODUCTION

The space environment is a microgravity environment; and the characteristics of a microgravity environment are weightlessness, low pressure and minute gravity. Astronauts are those people that travel to space. They could be scientists, engineers, medical personnel and pilot astronauts. There are two effects of microgravity on the astronauts; these are physiological and psychological effects. The effect of space environment on bones is physiological. Life in the microgravity environment of space brings many changes to the human body (Shelley and Brian, 2009). In the microgravity environment, astronauts lose calcium, nitrogen, and phosphorus (Berry and Catterson, 1967). The loss of bone and muscle mass (musculoskeletal), change in cardiac performance, variation in behavior, and body-wide alterations initiated by a change in nervous system are some of the most apparent and potentially detrimental effects of microgravity.

In 1948, Albert (a rhesus macaque monkey) was flown inside a V2 rocket into space, Laika (a dog) was also flown into orbit by Russians to test the survival of human beings in a trip away from the earth on spaceflights as weightlessness and/or microgravity environment was a subject of serious debate for people in the past. Animals were thought to be the appropriate objects to be used to find out the possibility of life surviving in space especially in regions of low gravity, but now most experiments could be conducted in space without involving animals simply because these previous experiments had shown sufficiently that humans could survive trips away from the earth; at least human beings have recorded 437 days spent in space as evidenced by Valeri Polyakov in his second flight while his first flight was for 240 days; while Sergi Krikalev spent a record 803 days altogether in his six flights. (Robert Frost, 2014), according to NASA (2011). NASA Astronaut Mike Copez-Alegrita has flown the longest U.S Space Station Mission to date at 215 days.

Reptiles are rare models for space and microgravity research and some reptiles' species demonstrated effective adaptation to spaceflight conditions. Generally, space research on animal models may provide good information for the manned space mission organization; and data are normally generated on various species (i.e. data based on adaptive scopes and perspectives of various species). According to Gulimova *et al.*, (2019) tortoises have been used for a 7-day spaceflight aboard the Zond 5 and Zond 7 satellites (USSR) as well as flown for 19, 22, 60 and 90 days. Also, turtles and other species of amphibians and reptiles, including geckos were studied in parabolic flights with short-term (7 and 20-25 s) weightlessness.

Researchers have studied behaviors, blood, internal organs, central nervous system, setae, skeletal bones, and excrement within a period of 7s – 45.5 day main orbital and parabolic flight experiments with reptiles while this study only considered the effect of microgravity environment on the body mass of lizards using a clinostat – as the microgravity simulation device.

Lizard (e.g. gecko) bones grow throughout life, it is a unique natural model object (especially its gripper system), that retain practically normal locomotion and behavior in weightlessness; but there is the tendency towards demineralization during (60-90day) flight (Gulimova *et al*., 2019). The demineralization can cause body mass loss in the lizard. Since there is body mass resorption (declination) as the period of flight increases, this may lead to speed declination under microgravity. It is reported that geckos flight experiment showed that the detailed μCT (Computed Microtomography) analysis of bone mass and architecture revealed significant loss of cancellous bone in the distal femur (Gulimova *et al*., 2019).

The pre-flight body mass and the post-flight body mass are usually compared together to know the extent of the damage done in the body mass during in-flight (microgravity environment). The assessment of post-flight body mass gives insight to the in-flight effect. The consequence of body mass loss during flight, can be better assessed by a decrease in post-flight body mass (Zwart *et al*., 2014). Also, if the crew members did not meet their caloric requirements before space flight it can lead to severe damage in the bone and muscle mass, quick demineralization of bone, reduction in bone mineral density (BMD) and muscles strength. The loss in the body mass (body flesh and fluid, bone mass and muscle mass) of animals during flight caused their basic activities pattern to be altered. The tibia, hip and femur, ankles and foot bones together with their muscle become weakened during flight. So perfect normal tibia, hip, ankles and foot bones and muscles that aid normal perfect locomotive system in pre-flight will be denied at the post-flight. On return to the 1g environment, those bones and muscles bearing loads are weak to withstand 1g gravitational pull. The body mass is pulled more to the earth surface during post-flight than during the pre-flight and this makes center of gravity in most four-footed animals (e.g. rats and lizards) much lower than normal. Their body weight will no longer be supported by those bones and muscle bearing loads (Riley, 1996). There are many things that can happen to a lizard in microgravity environment in contrasts to their features under the Earth's gravity. The speed of a lizard under gravity is scaled at $M^{0.17}$ (Christofer *et al.*, 2011) where M is the body mass of the lizard, but on returning to the Earth's gravity after flight, the lizard may not quickly attain to its normal speed due to microgravity resorption. For astronauts, gradual effective countermeasures for mitigating the effects of microgravity can be achieved through regular performing weight loading physical exercises and good nutrition are usually practiced for the purpose of stimulating the Earth's gravity.

According to Amin (2015), reloading of the skeleton in the 1g environment can stimulate bone formation with improvement in bone density lost during spaceflight and the time to recover bone density back in a 1g environment is considerably longer than the time it takes to lose it in microgravity. These will enable lizards regain formation of bone mineral density and muscles mass and strength; build flesh; and increase body fluid that will eventually make the lizard to come up with the normal body mass under Earth's

gravity, making it to continue to increase speed as the body mass increases.

Bone remodels in response to stress in order to maintain constant strain energy per bone mass (Marzban, 2008). To do this, it grows more in density in areas experiencing high stress (e.g. gravity environment or Earth's gravity), while resorbing density in areas experiencing low stress (e.g. microgravity environment or Mars gravity). The Planet Mars, where gravity is about one-third that of Earth's, the gravitational forces acting on astronauts' bodies would be much lower, causing bones to decrease in mass and density (ACSM, 2006). Bearing weight or gravitational stress on the feet is an important event that helps to strengthen the feet's bones as work or exercise is done against the weight or gravitational stress. So, not having to bear weight on the feet sounds relaxing (as it's no longer exerting against the pull of gravity), but in the long-term there are many health problems associated with it. Thus bones and muscles weaken, and other additional changes also take place within the body. One of the functions of the International Space Station (ISS) is to study how astronaut health is affected by long periods in weightlessness. Microgravity slightly deteriorates cognitive ability (thinking speed and accuracy) and bone formation. Reductions in body mass is due to reduction in bones mass-density, demineralization of bones and loss of muscles (mass, strength and endurance), and calcium in the bones been lost through urine secretions (Kanas and Dietrich, 2008). Average bone loss of 1-2% was recorded in astronauts on MIR each month (NASA, 2001), meaning that the rate at which bones are been broken down to minerals is more than the rate at which bones are formed (Rodan, 1998). This causes bones to diminish constantly with no recovery.

Exposure to microgravity alters the ability of bones to heal after fractures because there is decrease in bone density which can increase the risk of kidney stones and bone fractures (NASA, 2001, Blaber *et al.*, 2013). In animals (e.g. mammals and birds) the peak bone stresses scale with $M^{0.28}$ (Biewener, 1982). This is applicable under Earth gravitation influence. The peak bone stress increases with increase in the body mass, while it decreases as the body mass decrease. Since the microgravity influence affect body mass through resorption of bone and muscles mass, it is expected that the peak bone stress will decline under the microgravity influence due to decline in the body mass as the period of stay in the microgravity increases. This decline in the peak bone stress can lead to bone fracture during post-flight. According to Axpe *et al*., (2020) it is important to note that during missions, the risk of fracture is minimal as sudden mechanical overloads are very unlikely to happen in microgravity environment. But there is a decline in the peak bone stress as the body mass decreases due to increase in the time spent in microgravity environment compared with pre-launch stage. When they return to the influence of Earth's gravity, the risk of fracture increases, because the reduced peak bone stress may not be able to withstand the reloading effect on the Earth's surface as the astronauts are working against gravitational pull of the Earth for effective dynamic motion or locomotion processes.

Terrestrial mammals maintain constant stress by changing the way bones and muscles are loaded. The changing in the molecular mechanism of species of animals (fish, amphibians and birds) under microgravity experiments is determined mainly by biological and phylogenetic history of each species (Khvatov *et al*., 2014).

II. METHODOLOGY

Data was collected from the experiment conducted at the Microgravity Simulations Laboratory of Engineering and Space Systems (ESS) Department, National Space Research and Development Agency (NASRDA), Abuja, Nigeria. 2D Clinostat was used as the microgravity stimulations device on which the lizard samples were subjected. Three lizards were used for this experiment, two of the lizards (sample A and sample B) were subjected to simulated microgravity influence with body masses 7.5g and 8.0g respectively; and lizard sample C (control: under normal Earth gravitational influence) had body mass 7.2g. The body mass of all the samples were been measured hourly for 4 hours (Table 1). The weight of the lizards were

measured using laboratory weighing balance, the lizards were put into petri-dishes and mounted differently and individually on the Clinostat and rotated at a fast rotation per minute (rpm) of 85 for 4 hours, with the weight checked every 1 hour.

The following methods were used for the analysis and discussion.

- Linear and Non-linear graph from regression analysis
- Energy Strain and Body Mass Relation
- Energy Density and Body Mass Relation
- Assumption of Hysteresis *(H)* Principle

$$H = 8.89M - 0.03$$

- Torsional Stress (TS) and Gravitational Force Effect (GFE); while the Torsional stress (τ) is scaled at:

$$\tau \; \alpha \; M^{-0.049}$$

- The angle (θ) of rotation of femur at midstance was scaled at

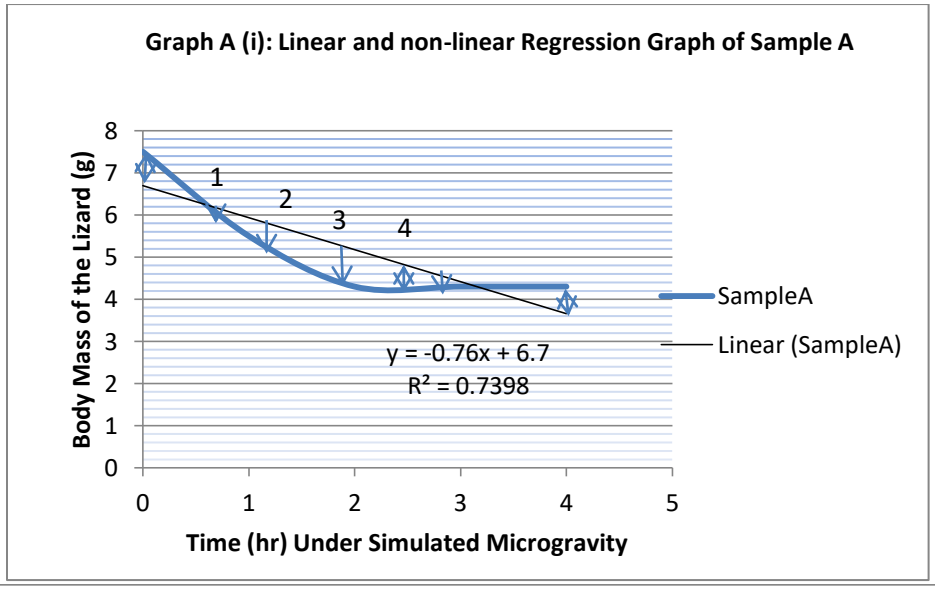
$$\theta \; \alpha \; M^{-0.064}$$

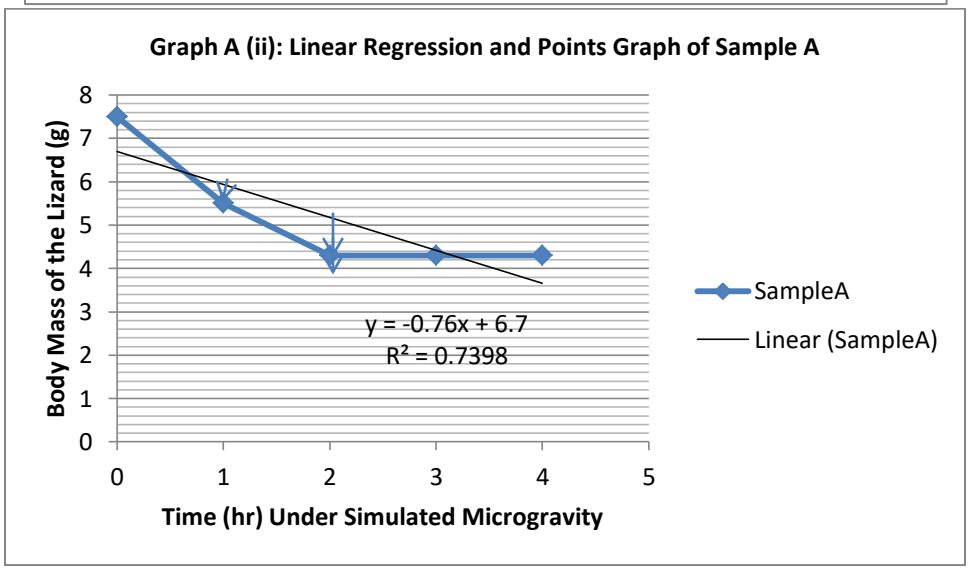
III. DATA PRESENTATION AND ANALYSIS

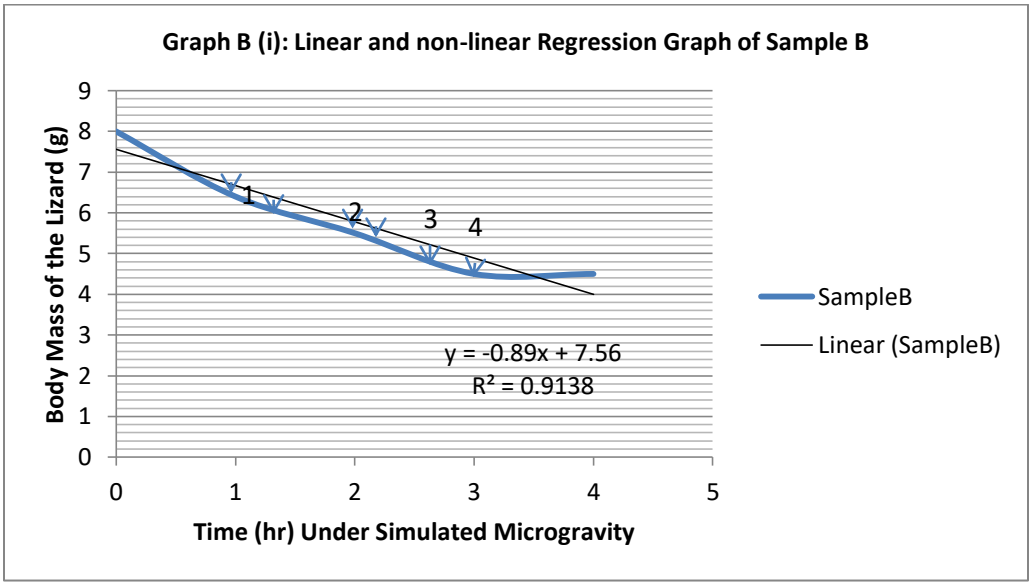
3.1 Data Presentation

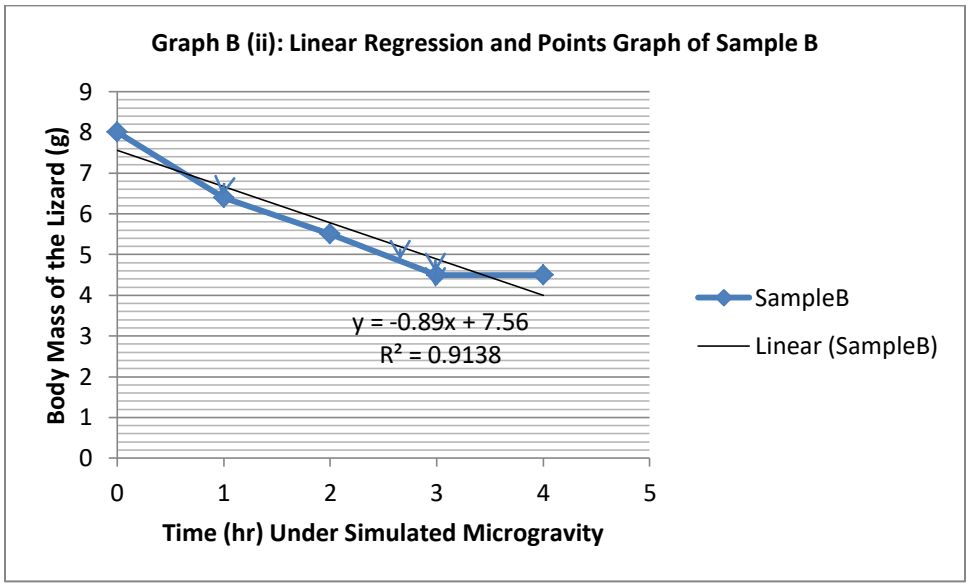
Table 1: Hourly Body Mass of Lizards of Samples A, B and C

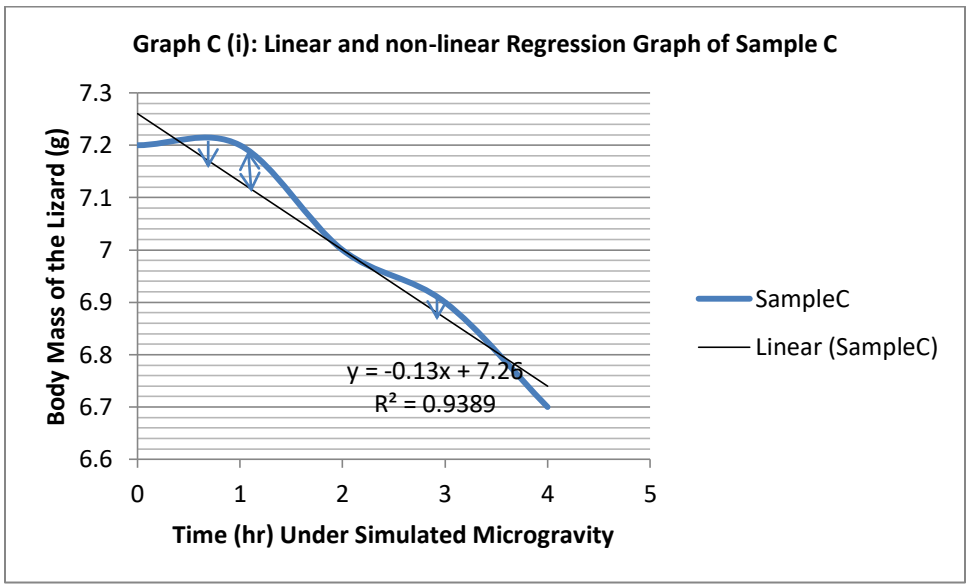
Hour	Sample A	Sample B	Sample C
0	7.5	8.0	7.2
1	5.5	6.4	7.2
2	4.3	5.5	7
3	4.3	4.5	6.9
4	4.3	4.5	6.7

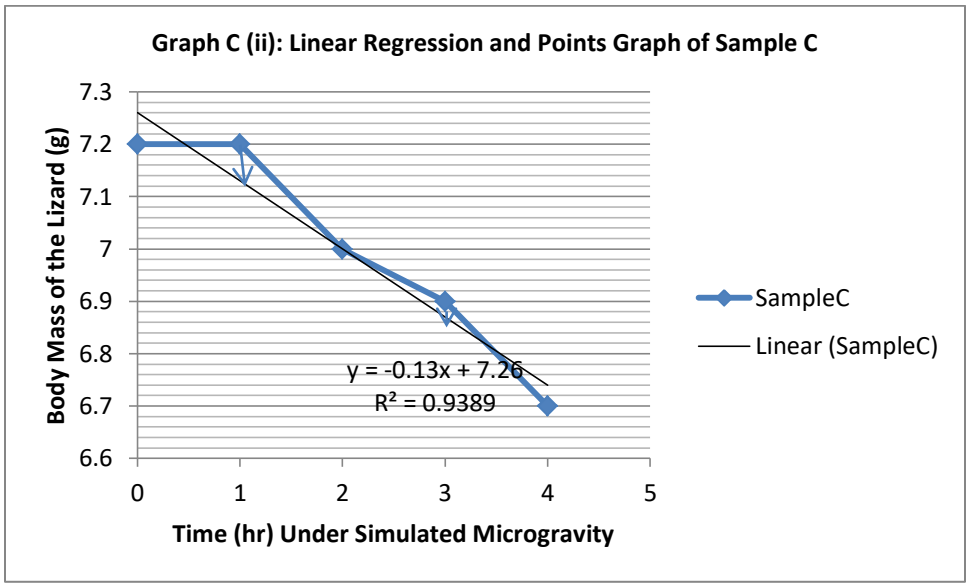


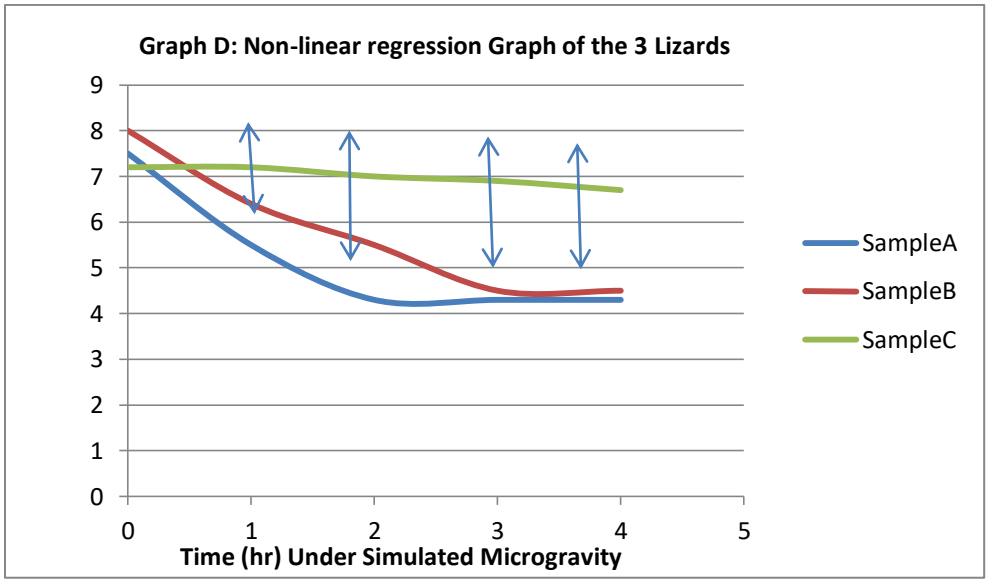



Graph B (i): Linear and non-linear Regression Graph of Sample B
Body Mass of the Lizard (g)
Time (hr) Under Simulated Microgravity
y = -0.89x + 7.56
R² = 0.9138
SampleB
Linear (SampleB)


Graph B (ii): Linear Regression and Points Graph of Sample B
Body Mass of the Lizard (g)
Time (hr) Under Simulated Microgravity
y = -0.89x + 7.56
R² = 0.9138
SampleB
Linear (SampleB)


Graph C (i): Linear and non-linear Regression Graph of Sample C
Body Mass of the Lizard (g)
Time (hr) Under Simulated Microgravity
y = -0.13x + 7.26
R² = 0.9389
SampleC
Linear (SampleC)





3.2 Data Analysis and Findings

- The linear regression shows that there are decline in the mass of the lizards of the sample A and sample B as the period of flight increases because the graph slopes down, indicating negative association between the two masses and the period of flight. Also, the non-linear curve equally indicates that there is decline in the body mass of the lizards (sample A and sample B) as the period of simulated microgravity increased.
- In comparing the linear with non-linear regression curve of the samples, the deviation from the linear regression is much in sample A than in sample B at each point considered (Graph A (i-ii) and B (i-ii)).

Table 2: Magnitude of Deviation of Non-linear from Linear Graph: Samples A, B and C

	Sample A			Sample B		
	Linear Curve Point	Non-linear Point	**Magnitude of Deviation**	Linear Curve Point	Non-linear Point	**Magnitude of Deviation**
I	7.50	6.70	**0.80**	8.00	7.40	**0.60**
II	6.20	5.50	**0.70**	6.60	6.40	**0.20**

III	5.80	4.30	**1.50**	5.80	5.50	**0.30**
IV	5.20	4.30	**0.90**	4.85	4.30	**0.55**
	4.80	4.30	**0.50**	4.50	4.0	**0.50**
Average	5.90	5.02	**0.88**	5.95	5.52	**0.43**

From Table 2, it is seen that at each point I, II, III, IV and at the average value, the larger mass has lower resorption than the smaller mass.

- In comparing the non-linear curve of the three samples, the deviation of experimental or test samples (A and B) from the control sample C, shows that sample A deviated from the control sample C more than sample B (Graph A, B and C).

Table 3: Magnitude of Deviation of Non-linear of Sample A and Sample B from Sample C: (Graph D)

	Sample A	Sample B	Sample C	**Magnitude of Deviation of A from C**	**Magnitude of Deviation of B from C**
	X	Y	Z	**Z – X**	**Z – Y**
	Non-linear Point	Non-linear Point	Non-linear Point	Linear Curve Point	Non-linear Point
	5.50	6.40	**7.20**	1.70	0.80
	4.30	5.50	**7.00**	2.70	1.50
	4.30	4.50	**6.70**	2.40	2.20
	4.30	4.50	**6.70**	2.40	2.20
Average	4.60	5.23	**6.90**	2.30	1.68

From Table 3:

- The linear regression curve seem to be below that of the non-linear curve for sample C (Graph C (i-ii), while the linear regression curve seem to be above the non-linear regression curve for sample A and sample B (Graph A (i-ii) and B (i-ii)).
- Sample A and sample B shows a constant mass at 2 hours and 3 hours respectively (Table 1).
- The non-linear curve of both samples (A and B) show that the control sample C curve did not deviate so much from the original mass at time $(t = 0)$ because it nearly maintained approximately straight line parallel to the x-axis (Graph A, B and C).
- From graph D: The deviation of sample A curve from sample C curve is more than the deviation of sample B curve from sample C curve.

IV. DISCUSSION OF FINDINGS

Although, those lizards' mass were measured and recorded, but the mass of the lizards (sample A and sample B) microgravity simulation and at a faster rate compared to then lizard of sample C, while their outer body seemed not to be affected. There is the possibility that some components of the body (mass bone, bone mineral density, muscles mass, frequent urination) that can be easily affected by microgravity environment, caused a decline in the mineral calcium in the body; such as the lizard defecation during the experiment. All these may have caused decline in the mass of their bodies, as bone and muscles mass are the major mass in the body.

Bone remodels respond to stress in order to maintain constant strain energy per bone mass throughout (Marzban, 2008). In the process of remodeling, the bone becomes denser in areas where it experiences higher stress (e.g. under Earth gravity) while resorbing density (i.e. decline in density) in areas experiencing low stress (e.g. under microgravity). The lizard of sample A and sample B at t (0) under Earth gravitational force have more body mass than when they were placed under microgravity simulated environment and they were found to be declined with increase in the simulated microgravity period.

$$\beta = \frac{Strain\ Energy}{Bone\ Mass} \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Let the bone of mass (m) be equivalent to mass of the lizard (M) been strain by Δl with force (F). The energy (E) which is work done on the bone can be written as:

$$E = F.\Delta l$$

$$\beta = \frac{Strain\ Energy}{Bone\ Mass} = \frac{\Delta l}{l} F.\Delta l \frac{1}{M} = \frac{\Delta l}{l}\frac{F}{A} A.\Delta l \frac{1}{M} = Srain\ stress \frac{\Delta V}{M}$$

This can be manipulated to be:

$$\frac{E}{\Delta V} = stress = Energy\ Density$$

So,

$Stress = Energy\ Density$…… (2)

And from

$$Young\ Modulus\ (\gamma) = \frac{Stress}{Strain}$$

$Stress = \gamma \times Strain$…… (3)

Expression like this can also be given:

$$\beta = \frac{Energy\ Density}{Bone\ Mass} \ldots\ldots\ldots\ldots (4)$$

Checking equation 1, 2, 3 and 4, as the stress increases, the Strain, Strain Energy, Energy Density and β increases.

The body mass of the lizards was made to be equivalent to the bones and muscles mass in the equation (i.e. the heavier the bone of man, the more the measurement of the overall weight of the man), since their bones were not measured. It is agreed that gravity induce high bone mass density, and evidence has shown that heavy-weight people exhibit higher bone density (Iwase *et a.l*, 2013). The value β, is the extent of bone formation (osteoblast) especially under stress (or Earth gravitational loading) or extent of bone resorption (osteoclast) especially unloading (reduced stress under microgravity). When the stress increases, the β increases, an indication of bone formation, but the larger the mass the smaller the β; larger mass use little counter force to overcome the applied stress, and then the risk of bone fracture is lower with the larger mass, indicating bone development. The bone peak stress will not be quickly reached with larger bone mass while with the smaller mass β increases; the smaller mass allows the application of greater counter force to overcome the stress; then the risk of bone fractures is higher with smaller mass because it will quickly attain bone peak stress. While under microgravity there is reduced stress or nearly zero loading (i.e. little or no gravitational pull), β-value decreases with decrease in the applied stress, an indication of resorption. But the larger mass has low resorption compared with the smaller mass. This is to say that the rate at which bone and muscle mass decline in microgravity is inversely proportion to the mass.

The risk of fractures is higher with smaller mass than the larger mass i.e. Bone Mineral Density (BMD) decreases faster with smaller bones than with larger bones. The risk of fracture is higher with the low BMD than the larger BMD. According to Lauder *et al*. (2000) multivariate analysis revealed a strong negative association between femoral neck BMD and the probability of stress fractures (i.e. the lower the BMD, the higher the risk). The big bone mass (or higher BMD) has low rate of resorption than the small bone mass (or low BMD) under microgravity environment as it is seen in the lizard of sample B that has low rate of resorption than the lizard of sample A. Having it in mind that the low body weight is a well-known risk factor associated with low BMD (Lauder *et al*., 2000). It is also reported that strong negative association between BMD of the femoral neck and probability of stress fractures indicate that lower levels of BMD are associated with an increase in likelihood of stress fractures.

Another indication that resorption of bone and muscle mass is inversely proportional to the body mass (bone mass or BMD) has been proved according to Amin (2015), that bone loss seems to occur predominantly in the trabecular more than cortical bone and that there was greater cancellous than cortical bone loss that occurred as early as

after 1 month of exposure to microgravity (Vico *et al.*, 2000). In summary cortical bone compositions make it denser than the cancellous or trabecular bone (Osterhott *et al.*, 2017).

- In comparing the linear regression line with non-linear curve of the sample, the deviation from the linear regression is much in sample A than in Sample B (Table 2).
- In comparing the non-linear curve of the three samples, the deviation of experimental or test samples from the control sample shows that sample A deviated from the control sample C more than sample B (Table 3).

The deviation of sample A and sample B non-linear curve from the linear regression curve, and from sample C are indications that changes in body mass occur and that sample A with greater deviation have higher resorption or declined rate.

The decline in the mass of lizard B is at a lower rate than lizard A, due to fact that the body mass of lizard of sample B is greater than the mass of lizard of sample A. From the Table 1, sample A lizard reached minimum decline stage after 2hours with 4.3 g, while that of sample B after 3 hours at 4.5g. Under microgravity Sample B been of higher mass will reached is bone peak stress later than sample A. On returning to the gravity environment the bone risk factures with be less in sample B than in sample A.

The study at this instance agree with Narici *et al* (2011) and Smith *et al* (2015) that in addition to muscle loss, microgravity leads to increased bone resorption, decreased bone mineral density, and increased fracture risks. Bone resorption leads to increased urinary levels of calcium, which can subsequently lead to an increased risk of nephrolithiasis (or kidney stones) and the loss of muscle mass occurs because of imbalances in protein synthesis and breakdown, the loss of muscle mass is also accompanied by a loss of muscle strength and also decreases in the generation of contractile forces and whole muscle power have also been found in response to microgravity (Narici *et al.*, 2011; Smith *et al.*, 2015).

In the petri dish where the lizards were put individually after which it was mounted on the Clinostat, it was discovered that the lizards defecated and urinated during the microgravity simulation in the absence of food and water, which might have led to dehydration and reduction in calcium level resulting into decline in the mass or the BMD of the lizards.

4.1 Assumption of Hysteresis Principle

Hysteresis in the field of economy refers to an event in the economy that persists into future, even after the factors that led to that event have been removed. Also, it can be narrated as the property by which some neurons do not return to their basal/initial condition from a stimulated condition immediately after removal of the stimulus.

This principle can be applied to verify the fact that the resorption at microgravity environment decrease with increase in the body mass or bone, and muscle mass and bone mineral density; and increases with decrease in the body mass or bone mineral density, or bone and muscle mass.

The development of body mass bone and muscle mass, or mass mineral density through loading before flight can be an advantage to withstand the microgravity environment and resorption. The loading can persist for some time in the microgravity and reduce the bone degradation because of unloading effect.

The hysteresis (H) as a Percentage (%) of total strain energy is related to the body mass (M) as (Pollock and Shadwick, (1994):

$$H = 8.89M - 0.03 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

In comparison equation (5) with equation (1) therefore, the strain energy increases as the body (bone) mass increases. Hysteresis increases along with increase in the strain energy, also indicating increase in stress. Hysteresis depends on the mass of the body or loading or stress before the flight. The hysteresis increases with increase in loading. When the effect of Earth's gravitational force is removed, the loading effect due to Earth's gravitational activities on the body can persist to the microgravity environment. The training of the astronauts described as core building prepares them for space missions, before the flight pre-flight), during the flight (in-flight), and after the flight

(post-flight) are complex physical processes. (Kale *et al.*, 2013), and medical tests (Lewis, 2017), extra-vehicular activity (EVA) training, procedure training, rehabilitation process (Oddsson, 2007) as well as training on experiments they will accomplish during their stay in space. The higher body mass or bone and muscle mass or BMD can be built-up during Astronaut training before flight. The physical training is into three phases: pre-flight, in-flight, and post-flight (Loehr *et al.*, 2015).

The higher bone mineral density built-up during pre-flight can be an advantage to withstand microgravity effect because 90% of Earth gravity reaches the space station (NASA, 2012) Even when the effect of gravity is removed the bone mineral density built-up can still persist for some time to withstand microgravity effect before the in-flight effects sets in. This can be called "*Earth Gravity induced Bone mineral density hysteresis (BMDH)"* supporting bone strength against microgravity resorption. Bone mineral density hysteresis is the stored energy or strength acquired during the pre-flight that can persist for some time in the microgravity in the absence of Earth's induced gravitational force. The higher the body mass, the greater the strength of the hysteresis. The hysteresis strength helps to resist the resorption bone rate. It can also be the reason why the body of a higher mass have slow rate of resorption. Therefore, it can be said that the hysteresis strength in the lizard of sample B is higher than that of sample A; and that sample B lizard had lower rate of bone resorption than sample A.

Table 4: Hysteresis of Samples A, B and C

Hour	Sample A	Sample B	Sample C			**Hysteresis (H)**		
	Mass (Kg)	Mass (Kg)	Mass (Kg)	Constant	Constant	Sample A	Sample B	Sample C
0	0.0075	0.008	0.0072	8.89	0.03	0.0367	0.0411	0.034008
1	0.0055	0.0064	0.0072	8.89	0.03	0.0189	0.0269	0.034008
2	0.0043	0.0055	0.007	8.89	0.03	0.0082	0.0189	0.03223
3	0.0043	0.0045	0.0069	8.89	0.03	0.0082	0.0100	0.031341
4	0.0043	0.0045	0.0067	8.89	0.03	0.0082	0.0100	0.029563

At t (0), the hysteresis induced by the gravitational force was of higher value in lizard of sample B than lizard of sample A. The Earth's gravity induced Hysteresis decreases under microgravity environment, indicating bone and muscle or bone mineral density resorption i.e. decline in body mass (bone or muscle mass or BMD). At t (2), samples A, B and C hysteresis are 0.0082, 0.0189 and 0.03223 respectively. At time t (2) they have lost 0.0284, 0.0222 and 0.0018 hysteresis respectively. The hysteresis loss at t (2) is more with sample A than sample B. If these values are approximated to only two decimal places, they will be 0.03, 0.02 and 0.00 respectively. It is therefore seen clearly that sample C which was the control lizard have lost approximately none of its hysteresis because it was placed under Earth's gravitational influence at t (2). It can be said here that microgravity environment is an anti-hysteresis induced by Earth's gravitational force i.e. it serves to reduce the influence of Earth's gravitational force. At t (4) which is final stage of the experiment, the hysteresis for lizard of the samples A, B and C are 0.0082, 0.0100 and 0.029563 respectively, it is also seen that at the final stage, the hysteresis declined more in A than B, also B than C. Since hysteresis directly depend on the mass of the body, the hysteresis increases as the body mass of the lizard increases, while the effect of staying in the anti-hysteresis environment (i.e. microgravity) increases with decrease in the body mass i.e. microgravity decreases the body mass with time then it decreases the hysteresis. This is an

indication that the strain energy as well as stress on bone decreases under microgravity. The resorption rate decrease with increase in hysteresis; resorption increases with length of flight under microgravity environment; and then hysteresis induced in the lizards under the influence of the Earth's gravitational force decreases with the time of flight under microgravity environment. Therefore, resorption increases with decrease in the strain energy as well as decrease in the bone stress.

Microgravity influence can be regarded as anti-hysteresis induced under the influenced of Earth gravitational force.

The important events are the homeostatic hysteresis induced by microgravity, which has more damaging effect on body (bone and muscles) mass than Earth's gravity-induced hysteresis. The homeostatic hysteresis occurred under microgravity influence at a time when the body mass started to remain constant. Lizard of sample A lost 77.57 % of hysteresis to microgravity while lizard sample of B lost 75.67%, but the hysteresis remains 22.43% and 24.33% for sample A and sample B respectively. 22.43% and 24.43% hysteresis remained constant at t (2) and t (3) for sample A and sample B respectively. These are referred to as homeostatic hysteresis induced by microgravity, but when they were returned to Earth's gravity, microgravity-induced homeostatic hysteresis may have negative effect on the lizard by accommodating Earth's gravity stress. The Earth's gravity induced hysteresis has positive impact during spaceflight (in-flight) while microgravity induced hysteresis will have negative impact during post-flight. The percentage hysteresis loss is much in lizard of sample A than sample B lizard, indicating higher resorption rate of lizard A body mass than lizard B. Notwithstanding, lizard of sample B still has ability to withstand the effect of Earth's gravity better than lizard of sample A.

4.2 Torsional Stress (TS), Ground Reaction Force (GRF) and Gravitational Force Effect (GFE)

According to Richards *et al*., (2013) a ground reaction force (GRF) is the force that acts on a body as a result of the body resting on the ground or hitting the ground. Studying forces is referred to as kinetics. The GRF is opposite the weight of the body and this might cause additional stress to the bone and muscles. The GRF seems to work opposite to the direction of the Earth's gravitational pull on the body.

If someone stands on a floor without moving, the person is exerting a force (the person's weight) on the floor, but the floor exerts an equal and opposite reaction force on the person. That is an example of the simplest GRF, but it never happens as easily as that with human balancing because of sway.

The torsional Stress (τ) for four-footed reptile is scaled approximately at:

$$\tau \; \alpha \; M^{-0.049}$$

Table 5: Torsional Stress for Samples A, B and C

Hour	**Sample A**	**Sample B**	**Sample C**		**Torsional Stress** (τ)		
	Mass (Kg)	Mass (Kg)	Mass (Kg)		A	B	C
0	0.0075	0.008	0.0072	-0.049	1.2709	1.2669	1.273476
1	0.0055	0.0064	0.0072	-0.049	1.2904	1.2808	1.273476
2	0.0043	0.0055	0.007	-0.049	1.3061	1.2904	1.275235
3	0.0043	0.0045	0.0069	-0.049	1.3061	1.3031	1.276134
4	0.0043	0.0045	0.0067	-0.049	1.3061	1.3031	1.277975

From Table 5:

The torsional stress (TS) at midstance resulting from the GRF should actually decrease as body size increases (Christofer *et al.*, 2011), because torsional stress needed to overcome the force of gravity or to adjust to the gravitational activities on the body will lessen with higher mass body than the low mass. The torsional stress needed to overcome the Earth's gravitational force activities that make the lizard adjust to Earth's gravitational effect is decreasing with increase in the mass. The lizard of sample A returned to Earth's gravitational environment with mass 0.0043kg while B with 0.0045 kg. Lizard of sample B quickly adjusted to the Earth's gravitational effect before sample A lizard as they were placed on the same gravitational force condition and motion. Sample A might experience fractures in a greater dimension than sample B lizard on returning to the Earth's gravitational environment because the microgravity-induced *homeostatic torsional stress* is higher in sample A than in sample B after 4 hours of the experiment at the point of returning to the Earth's gravitational effect. Microgravity induced homeostatic torsional stress for sample A is 1.31 while for sample B is 1.30. Higher torsional stress is needed to overcome the gravitational pull of the Earth for sample A than B when taken back to Earth's gravity.

Table 6: Torsional Stress, Hysteresis and Angle of Rotation of Femur for Samples A, B and C

Hour	**Sample A**	**Sample B**	**Sample C**	**Torsional Stress** (τ)			**Hysteresis** (H)			**Rotation of the Femur at Midstance** (θ)		
	Mass (Kg)	**Mass (Kg)**	**Mass (Kg)**	**Sample A**	**Sample B**	**Sample C**	**Sample A**	**Sample B**	**Sample C**	**Sample A**	**Sample B**	**Sample C**
0	0.0075	0.008	0.0072	1.271	1.267	1.273	0.037	0.041	0.034	1.368	1.362	1.371
1	0.0055	0.0064	0.0072	1.290	1.281	1.273	0.019	0.027	0.034	1.395	1.382	1.371
2	0.0043	0.0055	0.007	1.306	1.290	1.275	0.008	0.019	0.032	1.417	1.395	1.374
3	0.0043	0.0045	0.0069	1.306	1.303	1.276	0.008	0.010	0.031	1.417	1.413	1.375
4	0.0043	0.0045	0.0067	1.306	1.303	1.278	0.008	0.010	0.030	1.417	1.413	1.378
t(4)-t(0)	0.003	0.004	0.001	-0.035	-0.036	-0.004	0.028	0.031	0.004	-0.050	-0.051	-0.006

From above Table 6:

The femur torsional stress on the lizard increases as the period under simulated microgravity increases. On return to Earth's gravitational influence, the femur torsional stress on the lizard will begin to reduce again as the lizard work against the Earth's gravitational force through locomotion activities to gain body mass or bone and muscle mass or BMD. Earth's gravitational force is an anti-torsional stress induced on femur under microgravity environment i.e. it counters the torsional stress induced in femur of the lizard during simulated microgravity. Under microgravity environment, the rotation of the femur at midstance

increases as the body mass decreases due to resorption as the period under microgravity environment increases. Microgravity could have equally aided the rotation of the femur, because any little force on the femur to rotate it could have been aggravated by the microgravity influence. The microgravity influence aiding the femur rotation increases as the body mass or bone and muscle mass or BMD decreases due to resorption. The gripping system in the lizard could have also increased the torsional stress of the muscle rotating femur due the GRF of the petri dish. The lizard gripping system and the increase in the torsional stress compensates for the microgravity damage and sustained the hysteresis induced by the Earth's gravity before the microgravity simulation. The compensation seems too slowly developed compared with fast rate of damage done by the microgravity. The resorption due microgravity overwhelmed the compensation. When returning from the simulated microgravity to Earth's gravity, the body mass (bones (BMD) and muscles mass, and also muscles strength) is gradually developing to gain loss body mass. According to Amin (2015), data following return from long-duration spaceflight suggest that reloading of the skeleton in the 1g environment can stimulate bone formation with improvement in bone density lost during spaceflight and that an increase in bone density seems to reflect an increase in size of bone. This also can reflect an increase in body mass. The gradual gaining of body mass decreases the torsional stress (due to muscles rotating the femur from the midstance and GRF), and increasing the Hysteresis loss during microgravity. The formula behind the computation is according to Christofer *et al.*, (2011).

The torsional stress (τ) of the muscle rotating femur is scaled at:

$$\tau \, \alpha \, M^{-0.049}$$

The angle (θ) of rotation of the femur at midstance, is scaled at:

$$\theta \, \alpha \, M^{-0.064}$$

The L is proportional to tibia length, and is scaled at:

$$L \, \alpha \, M^{0.349}$$

The muscle force (F_{mc}) scale is much lower than the body mass

$$F_{mc} \, \alpha M_{mc}^{-0.034}$$

The bone stress σ_b is predicted to be scaled at:

$$\sigma_b \, \alpha M_b^{0.166}$$

The torsional stress may result from the action of both the GRF and of muscles rotating the femur (Christofer *et al.*, 2011). This is only applicable in Earth's gravitational environment, but under the microgravity, torsional stress of the muscles rotating the femur is talked about whereby GRF is not applicable. For lizards (e.g. gecko) that have gripping system making it cling to walls with ease e.g. the ability of thick-toe geckos to remain attached to smooth surface during weightlessness allows the geckos and any such lizards to keep normal activities and behavior during weightlessness (Gulimova *et al*., 2019; Landau, 2014) and this might be due to the fact that geckos assume a skydiving posture when falling or in microgravity situations (Higham *et al.*, 2017).

It was also observed that the lizards struggled at every stage of the experiment for about 2-3mins immediately after weighing them at one-hour interval during the observation period and mounting the petri dish they are in on the Clinostat. Afterwards, they then stayed calm and remain cliff to the wall of the petri dish as the microgravity simulations continued. In case the GRF/WRF (Wall Reaction Force) of the petri dish was very small due to weightlessness, it might have added to femur torsional stress. The most essential factor of femur torsional stress is the muscle rotating the femur which is directly proportional to the angle of rotation of the femur at midstance. This angle of rotation seems to be less under Earth's gravity than in the microgravity environment, because the gravitational force may act to counter the rotation while the

microgravity environment eases the application of force to increase the rotation; heavy objects move around easily for example, astronauts can move equipment weighting hundreds of pound with their fingertips (NASA, 2012) of which it can be possibly assumed one can lift a load far more than one's weight with fingertips in the microgravity environment

4.3 Lizard of Sample C

The control lizard which was kept and made to remain under the influence of Earth's gravitational force served to observe the possible damage that microgravity environment could have done on the lizards – sample A and sample B. At end of the experiment, observations were made on the differences at t (0) and t (4) for the body mass, torsional stress and hysteresis. Observed differences was also made at the angle of rotation of the muscles rotating the femur at the midstance, which was calculated for sample C as:

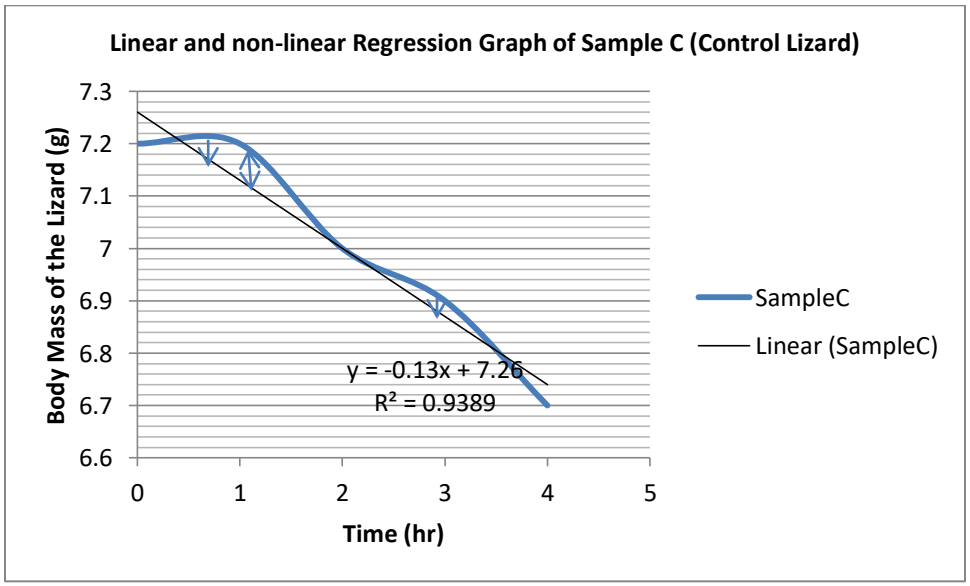
$$t(4) - t(0)$$

$$M = 0.001\ kg$$
$$\tau = 0.004\ Kg^{-1}$$
$$H = 0.004Kg$$
$$\theta = -0.006^0$$

The difference in the values of the initial and final stage is very small and negligible for each quantity considered. The control lizard sample C was expected to maintain the same value as in t (0). The following reasons might have caused the changes, as the control lizard sample C was found to have been:

- Defecating
- Urinating
- Without food (hunger strike) and water (thirst)
- Resisted inside a petri dish under Earth's gravity

The above factors could be the reasons of it losing body mass. If the values gotten are approximated to two decimal places, these values will be $M = \tau = H =, \theta = 0^0$. Then, it may be said that the control lizard maintained its body mass feature throughout the course of the experiment under Earth's gravitational influence. But under normal condition of Earth's gravitational force, it is expected that the control lizard maintains normal body features such as body mass, that comprises of the flesh, body fluid, bones (with minerals component) and muscles without depreciation. It can be equally expected to have development in the body mass such as in the flesh, fluid, bone and muscles leading to increase in the body mass.



Looking critically at graph C (i-ii), it can be seen that there was a development from 7.2kg to 7.22kg before declining again to 7.00kg. Also, between t (2) and t (4) there was another x development. Actually, under Earth's gravitational force, there is an expectation of bone (BMD) and muscles to undergo development as there is: exercise against Earth's gravity as day to day activities go on; nourishing of the body with good food of nutritional mineral; living in a very good environmental condition; and doing bodily exercise every day. The evident that bodily development (bone, muscles and fluid) can occur under Earth's gravity can been seen in the control lizard subjected under gravity compared with lizards A and B subjected under microgravity influence.

4.4 New Homeostatic during Flight

Another feature noticed was the decline in body mass that was constant with 4.3g – for lizard of sample A from t (2) to t (4) and with 4.5g – for lizard of sample B from t (3) to t (4) under microgravity influence, which presently, there is no explanation for the constant values. The microgravity damage to bone or body mass is stocked and the damage seems not to go further. According to Ulbrich *et al.*, (2014), it is not yet clear whether loss in bone mass will continue as long as a person (or mammals) remains in the microgravity environment or level off in time, and according to Amin (2015) there are limited data available to date on whether bone (or body mass) loss will continue at an accelerated pace with longer consecutive exposure to microgravity, or whether the loss of bone will gradually reached a new homeostatic. According to Axpe *et al.*, (2020) the model in this study follows the terrestrial evidence that suggests that the BMD loss will eventually plateau.

Judging from the researchers and their colleagues mentioned above, the bone or body mass of the lizard of sample A and sample B continue the resorption until the resorption got stocked at 4.3g and 4.5g respectively, with no further decline in their body mass which can be called a stage of steady body mass. Lizard of sample A reached a new homeostatic at t (2) to t (4) with body mass 4.3g while lizard of sample B reached a new homeostatic at t (3) to t (4) with body mass 4.5g. The small body mass reached new homeostatic earlier than the higher body mass i.e. the loss of body mass of lizard of sample A is in higher accelerated rate than lizard of sample B. The linear regression analysis graph shows only negative relation between the period of microgravity simulations and the body mass without evidence of homeostatic, while non-linear curve indicates homeostatic stage. According to Axpe *et al.*, (2020) the mathematical model presented is the first to their knowledge that does not assume a linear decrease in the BMD of astronauts and that linear decrease is widely used in literatures; it will eventually predict a negative BMDs in long-duration spaceflights, which lack any physical meaning. However, the non-linear model provides the most accurate and realistic prediction of the BMD loss in long-duration spaceflights.

4.5 Test Lizards and Control Lizard

It has been said that three lizards were subjected into conditions of hunger and thirst (i.e. no food and water), also the lizards defecated and urinated during the experimental period. The lizards of sample A and sample B were subjected to simulated microgravity environment by putting them individually in a petri dish (constrained container with their movement equally restricted) and mounted on the Clinostat while sample C was under the influence of Earth's gravity. The three lizards both experienced resorption in body mass, but the rate of resorption of the body mass of lizards of sample A and sample B were more than the lizard of sample C. Apart from the hunger and thirst, defecation and urination experienced might have caused reduction in their body mass; it was therefore discovered that sample A and sample B under the influence of simulated microgravity made them to have high rate of resorption than control sample C. It may therefore be said that the resorption in the control sample C lizard might be due to hunger, thirst, defecation and urination. While the resorption of the lizards of sample

A and sample B might be due to hunger, thirst, defecation, urination and microgravity influence which might have further caused resorption in bone and muscles mass, and bone mineral density reduction due to demineralization effect. Therefore, simulated microgravity environment exacerbated resorption of body mass in sample A and sample B lizards.

V. CONCLUSION

The four hour experiment with the Clinostat – microgravity simulation device indicated decline in the body mass of the two lizards under simulated microgravity. The resorption increased as the period of the microgravity simulations increased, which is an evidence of microgravity effect. It is well known that long duration spaceflights induce BMD (body mass) loss in weight-bearing bones, although there is considerable individual variability (Axpe *et al.*, 2020). The variability in the sense that the resorption effect of microgravity on the two lizards (sample A and sample B) is more on lizard of sample A than B i.e. the lizard B with heavier body has slow resorption than lizard A. Since the heavier the bone of man, the more the measurement of the overall weight of the man, the resorption in bone is of higher rate in lizard A than in lizard B.

However, linear regression analysis alone may not be considered a good model to compare the decline rate of the body mass of the two lizards because the resorption bone rate is not linear. But the non-linear model provides the most accurate and realistic comparative analysis of the two isolated lizards as it gave realistic evidence that the lizard of sample A have more body mass loss than lizard of sample B. So, a linear decrease in the body mass (or BMD) is not realistic for very long duration, interplanetary missions, but non-linear exponential decline of BMD (or body mass) in the weight-bearing bones is a reasonable approach to model progressive bone loss in long-duration space missions (Axpe *et al.*, 2020).

The simulated microgravity environment made easy the rotational angle of femur at midstance, the angle (θ) of rotation of the femur at midstance increases as the period of the microgravity simulation impact increases due to decrease in the body mass.

The little decline rate of the body mass of the control lizard sample C can be attributed to lack of water, food, defecation and urination. But the decline rate in the body mass of lizard A and B is more compared to lizard C, because sample A and sample B are under the influence of microgravity. Following non-linear curve of the body mass loss, it can be agreed that the control lizard sample C had little or no evidence of body mass loss since the non-linear curve is approximately parallel to the horizontal axis.

The resorption of bone mineral density (body mass) of lizard samples A and B attained constant body mass under simulated microgravity as the period of isolation extended, this is an evidence that loss of bone or body mass will gradually reach a new homeostatic in microgravity environment. These two samples reached homeostatic at different periods under simulated microgravity influence as an evidence of individual variability. The lizard of greater body mass attained homeostatic stage later than the smaller body mass. Also, the linear regression analysis gave no indication of homeostatic, but the non-linear regression indicated homeostatic.

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