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SERUM ATP AND ADP VALUES IN MODERATELY EXERCISED APPARENTLY HEALTHY MALE AND FEMALE SUBJECTS WITH DIFFERENT BODY MASS INDEX

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ABSTRACT

Cells are involved in generation and utilization of energy for the purpose of well-being during basal metabolism and any activity. The balance of energy is better if surplus. This study was designed to observe energy expenditure in undergraduate male and female students that engaged on trekking for a period of 20minutes. The aim was to know if there was gender bias in energy balance. Forty five students randomly recruited participated in the study and consist 21 males and 24 females. They trekked from their hostel to the school where the blood sample was collected. This was a cross sectional study. After arriving at the school, 5mls of blood sample was collected from all the participants and dispensed into plain sample container and serum extracted after retraction of the clot and stored frozen at -20oC until analysed same day. ELISA was used to determine the ATP and ADP content of the serum samples while their Body mass index was determined using weight (kg) /height (m^2). Significant value was recorded at P<0.05. The finding showed that serum ATP (nmol/l) value was significantly higher in female undergraduates (500±100nmol/l) compared with 423±89nmol/l observed in male undergraduates (P=0.016). However, BMI and ADP were not significantly different between the male and female undergraduate (P>0.05). The minimum and maximum value of ATP (nmol/l) for the female undergraduates (286 to 659) was wider than for male undergraduates 325 to 607). However, the minimum and maximum value of ADP (nmol/l) for female undergraduates (156 to 525) was shorter compared with that of male undergraduate (163 to 732). There was no noticeable difference in minimum and maximum range of BMI in the male undergraduates and female undergraduates. There was a very strong positive association between ATP and ADP values in the study (r = 0.663; p=0.000). This was not the case for ATP and BMI nor ADP vs BMI. The study concludes that female undergraduate subjects conserved energy better than the male counterparts during the 20minutes trekking. They also had wider range for ATP but not for ADP. Therefore, the rate of energy utilization and generation seem better in female undergraduates than their male counterparts. It is our opinion that energy utilization may be gender biased.

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Keywords: Energy; ATP; ADP; BMI; undergraduate; male; female

Introduction

A cell is a living unit of an organism. For cells to remain viable in-vivo and in-vitro there must be available source of energy (D'Alessandro et al 2015, Coss-bu and Mehta 2016). The whole essence of metabolic process is to sustain the cells of the body through generation and supply of energy in form of ATP. Therefore for effective generation and supply of energy to the cells of the body the energy balance must be in surplus (Dunn and Grider, 2020). Therefore, these high energy molecules could be used to implicate the state of health or diseases (Chida et al 2013, Ezugwu et al 2021). Any activity or performance that initiates a metabolic process consumes energy. The quantum of energy consumed differs between anabolic and catabolic processes. Anabolic process eventually produces surplus energy while catabolic process produces negative energy balance.

Basal metabolic rate consumes energy. That is energy consumed at resting state while activity is almost non-existent except sleeping. Ambulation, reading, thinking, stressor, drugs and sexual activities all consume energy. Eating, digestion, assimilation and transportation of nutrients also make demand on energy though some of these processes also generate energy. In our previous observation we did show that HIV and Diabetes Mellitus which are chronic diseases were associated with high level of energy degradation that predisposes the subjects to negative energy balance (Ezugwu et al 2021). We observed that HIV which is a chronic viral infection degrades much more energy than Diabetic Mellitus which was a chronic metabolic disease. It has also been reported that blood donations stored in blood bags enriched with ATP will after some days use up the enriched energy in the blood bags (Xu et al; 2019).

This source of this energy is from foods consumed. The breakdown of such macromolecules into micro molecules as usable fuel for the cellular activities also produces the stored energy informs of ATP within the mitochondria molecules. Understanding the energy need of routine activities or diseases states may help to adjust diet for appropriate energy balance. Without this understanding excessive heating may result in overweight or obesity which are forms of metabolic syndrome associated with morbidity and mortality. Therefore this study was designed to observe the close-to basal metabolic rate energy status of undergraduate student after trekking from their hostel accommodation to the classroom. This was considered a moderate exercise since none of the participants trekked for more than 20minutes to the point of sample collection.

Research Method

Subject recruitment

Undergraduate students between the ages of 18 and 25 years were which were apparently healthy were randomly recruited for the study. Forty-five participants consisting of 21male and 24 female subjects participated in the study. They were told to trek to the spot for sample collection from their hostels which was not more than 20minutes trekking.

The study design was a cross sectional study. 5ml of blood sample was collected from each of the participants and dispensed into plain tubes and allowed to cloth and retract and the serum samples collected and used for the analysis. The cells were separated within 1hr of sample collection and stored frozen. The samples were analysed later same day. The serum sample was used for the determination of ATP and ADP concentration while the height and weight of the participants were taken to determine their BMI.

Exclusion criteria

Those who showed sign of exhaustion after the moderate trekking exercise were excluded from the study. Those who were scared of needle prick were also excluded from the study.

Ethical approval and informed consent

Ethical clearance was obtained from the Ethics Committee of Federal medical centre Asaba (NHREC/TR/FMCASABA-HREC/31/7/24/14). The participants also gave informed consent.

Determination of ATP and ADP

Determination of ATP and ADP were carried out in serum. ELISA technique was used to determine the serum value of ATP and ADP. The procedure was as described by the manufacturer of the Kit. In summary, into the micro well already coated with purified human ATP antibody was added 10ul of test sample and 40ul of sample diluent. This gave 5 fold dilution. This was incubated for 30 minutes at 37oC. Thereafter the micro-wells were washed with washing fluid enclosed and rinsed. This was carried out thrice. Then 50ul of HRP-conjugate reagent was added to each well except for the blank. This process was incubated for 30 minutes at 37oC after which micro wells were washed and rinsed thrice. Then 50ul of the chromogen was added to the micro wells, the reaction was incubated in the dark for 10 minutes at 37oC. The 50ul of the stopping solution is added to stop the reaction. The colour changes from blue to yellow upon adding the stopping solution. The absorbance was read at 450nm against reagent blank within 15minutes of adding the stopping solution. The standards were treated as for the test. The concentration of the ATP and ADP were calculated.

Determination of body Mass index

Determination of BMI was done by calculation using the formula height (m^2)/ weight (kg)/ height (m^2).

Statistical analysis

The variables were expressed in mean ±Standard deviation (SD). The descriptive statistic was used to determine the range of the parameters while the independent Student T- test was used to consider for gender bias. Levels of association between parameters were assessed using Pearson correlation coefficient. Significant level was put at $P<0.05$.

Presentation of Results

The mean ± SD BMI (kg/m^2) was 22.8 ±3.0 in male subjects and 23.0 ±3.8 in female subjects (P=0.807). Similarly, the mean ±SD ATP (nmol/l) was 422.53 ±88.89 in male subjects and 499.85±100.18 in female subjects (P=0.016). While the mean ±ADP (nmol/l) was 323.40±140.77 in male subjects and 366.70±86.31 in female subjects (P=0,273). Table 1.

The minimum limit for BMI (kg/m^2) was 17.40 while the maximum limit was 30.40

and the mean value was 22.89±3.41. Similarly, the minimum limit for ATP (nmol/l) was 286 while the maximum limit was 659 and the mean ±SD was 467.0±102. Furthermore, the minimum limit for ADP (nmol/l) was 156 and maximum was 732 and the mean ±SD was 348±113. Table 2.

There was a strong positive correlation between ATP and ADP (r= 0.663; P=0.000). However, there was a weak negative correlation between BMI and ADP (r=-0.149; P=0.359). The positive correlation between ATP and BMI was very weak (r=0.045; P=0.781).

Discussion of Findings

This study was designed to observe the serum levels of ATP, ADP and BMI value of undergraduate male and female students after trekking for about 20minutes from hostel to school. The BMI of the undergraduate Students revealed no significant difference in body mass index of the students between the male and female subjects. Study amongst healthy adolescents in Kano, Nigeria showed that female subjects have higher BMI compared with their male counterpart. They also observed that BMI value increased with age (Atiku and Yunusa 2009). Other reports showed that the percentage of women that are obese were more than men (Remero-Corral et al 2008) while in another study a significant association was observed between gender and BMI amongst Students (Singh and Desbey 2018). However among Irish Students, the male students had higher incidence of overweight compared with women (McCarthy and Warnes (2022). The discrepancies in gender related BMI may be indication of body builds associated with different ethnic groups.

In this study there was no significant difference in serum ADP (nmol/l) level between the male and female undergraduate students. However, the serum ADP range observed in female undergraduate students had wider range for (286 – 659 nmol/l for ATP and 156 – 525 nmol/l for ADP) compared with that observed in male undergraduate students (325 – 607 nmol/l for ATP and 163 – 732 nmol/l for ADP). The female undergraduate range was wider with ATP while the male undergraduate range was wider with ADP. Not much disparity was observed with the ranges of BMI between the female and male subjects. Studies have shown that ATP and ADP values dropped post –exercise (Mortiz et al; 2017).

In this study, ATP (nmol/l) was significantly higher in the female undergraduate students compared with the male undergraduate students (P=0.016). This implied that male undergraduate students might have expanded much energy during the trekking than their female counterparts. This might be a likely explanation. Another possible explanation might be basal ATP. Do females have better basal energy level than men are a likely probe? Do men exert more muscular activities while trekking than women? These are the likely variables that could be implicated for a given physical activity that men consume much more energy than women. Repeated sprints have been shown to induce smaller reduction of ATP and smaller accumulation of IMP and Inosine in women than in men in type-II muscle fibers. This was an indication that women possess faster ATP recovery mechanism through reamination of IMP during recovery periods (Mona et al 2002).

The implication of the finding was that men may be prone to negative energy balance faster than females. Although the ADP was not significantly difference due to wider standard deviation observed in the study but the absolute value of ADP was insignificantly raised in women than was observed in men. This finding is a pointer to the fact that more energy seemed to be expanded by the male undergraduate students than the female undergraduates for the same activity. This finding may align with the report that men spend much energy in exercise due to hypermuscular activity (Mona et al 2002).

A possible challenge encountered in this study was the use of serum for the analysis of ATP. We decided to use serum because most of our clinical analysis uses serum. However, it is important to note that ATP rapidly degrade within few minutes in serum (Kobayashi et al; 2010) but in the presence of pyruvate and lactate in serum the recovery of ATP may be faster. Similarly, if the clotted blood was not allowed to retract properly it may result in some degree of heamolysis and leakage of ATP from the damage red blood cells into the serum (Manson et al; 2012; Ellsworth et al; 2009).

Energy is essential for human metabolic processes and physical activities (Coss-bu and Mehta, 2016). Daily intake of nutrients is required for sustained energy generation because the stored high energy molecules are generated from metabolism of the nutrients (Dunn and Grider, 2020). Since energy is essential for metabolic processes, it meant that any disruptor of the metabolic system can cause energy deficit or surplus. In our observation, we have reported that disease states such as HIV/AIDS and Diabetics Mellitus can deplete energy (Ezugwu et.al; 2021). We have also reported that during exercise there was expression of some enzymes involved in glycolytic and cellular metabolic pathways (Ehiaghe et.al; 2017), this implies that exercise may disrupt energy expenditure.

During fed state the excess energy generated are stored in high molecules such as ATP through phosphorylation of lower energy molecules such as ADP and AMP (Morgan et.al; 2015) while during fasted states the high energy molecules such as ATP are consumed and drop energy to become ADP or AMP (Berg et.al., 2017). Therefore, it is our view that all life processes require energy for their sustenance whether in basal state of metabolism or active state of metabolism. Similarly academic activities has been associated with different levels of stress and is regarded as an active stressors (Onyenekwe et.al; 2014, Osakue et.al; 2015). Stressors are widely known to disrupt energy expenditure because that can induce enhanced glucose metabolism. For instance glycolytic pathway of metabolism of glucose consumes energy in form of ATP but the cellular metabolism of a molecule of glucose yields ATP molecules

A weak negative association was observed between BMI and ADP in the study population. This could suggest that overweight and/ or obesity may be associated with low ADP turnout while healthy BMI range may be associated with conservation of ADP and by extension conservation of energy. However, the correlation was positive between ATP and BMI though very weak, indicating no

possible drag between ATP and BMI unlike between ADP and BMI.

It is our opinion that energy generation and expenditure may be gender biased. The strong positive correlation between ATP and ADP, though expected was an indication that both could serve as source for either. What could explain the likely energy conservation in women? Finding this factor may be of significant impact on cell metabolism.

Conclusion

The study seem to reveal that energy balance may be gender biased and that most likely BMI did not influence energy balance.

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Table 1: mean ± (SD) serum levels of ATP (nmol/l), ADP (nmol/l) and BMI (kg/m^2) of apparently healthy male and female subjects.

Parameters	n	BMI (Kg/M^2)	ATP (nmol/l)	ADP (nmol/l)
Male	21	22.8±3.0	423±89	323±141
Female	24	23.0±3.8	500±100	367±86
p-value		0.807	0.016	0.273

Table 2: The minimum and maximum limits of ATP and ADP of apparently healthy undergraduate males and females

Parameters	**n**	**Minimum limit**	**Maximum limit**	**mean**
BMI female subjects	24	17.40	30.00	23.01
BMI Male subjects	21	18.90	30.40	22.76
ATP Female subjects	24	286	659	500
ATP Male subjects	21	325	607	423
ADP Female subjects	24	156	525	367
ADP Male subjects	21	163	732	323

Table 3: The minimum and maximum range of BMI, ATP and ADP of the apparently healthy subjects

Parameters	Minimum limit	Maximum limit	Mean
BMI	17.40	30.40	22.89
ATP	286	659	467.0
ADP	156	732	348.0

Table 4 levels of associations between BMI, ATP and ADP among apparently healthy subjects

Parameters	**n**	**r**	**p-value**
BMI vs ATP	**45**	**0.045**	**0.781**
BMI vs ADP	**45**	**-0.149**	**0.359**
ATP vs ADP	**45**	**0.663**	**0.000**