



## **Plasma Level of Macromolecules and Mathematical Calculation of Potential Energy in Type 2 Diabetic Individuals at NAUTH, Nnewi, Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Diabetes mellitus is associated with neutered metabolism and higher Energy Expenditure. This study aimed on the use of Adenosine diphosphate (ADP), Flavin adenine dinucleotide (FAD), AcetylCo-enzyme A (ACA) and Nicotinamide Adenine Dinucleotide (NADH) as an index of energy utilization, storage and energy balance in Diabetic individuals. This is a longitudinal, prospective, case-controlled study involving seventy seven (77) diabetic individuals newly diagnosed attending diabetic clinic of Nnamdi Azikiwe University University Teaching Hospital (NAUTH) aged 18-60 years both male and female not on anti-diabetic drug, were enrolled in the study as test subjects and thirty six (36) apparently healthy non-diabetic individuals both male and female as control subjects. ADP, FAD, ACA and NADH were estimated by enzyme linked immunosorbent assay (ELISA), while, energy balance from macromolecules was determined by calculation. The data obtained were subjected to statistical analysis using SPSS software application (version 21.0) and

the results expressed as mean  $\pm$  standard deviation. The Plasma Adenosine diphosphate (ADP), Flavin adenine dinucleotide (FAD), AcetylCo-enzymeA (ACA) and Nicotinamide Adenine Dinucleotide (NADH), were significantly lower ( $P < 0.05$ ) in both Diabetic pre-treatment and diabetic post-treatment group compared with control groups. Furthermore, the plasma level of ACA and NADH were significantly lower ( $P < 0.05$ ) in DM pre-treatment group compared with DM post-treatment group. While, the plasma concentration of ADP was significantly lower in DM post-treatment groups compared with DM pre-treatment groups. However, the Calculated energy from Macromolecules was lower ( $P < 0.05$ ) in DM groups compared with control group. Meanwhile, the calculated energy from Macromolecules in DM pre-treatment was significantly lower ( $P < 0.05$ ) compared with DM post-treatment. In conclusion, the significant changes in the biochemical parameters measured suggest altered metabolism, increased energy expenditure and energy deficit/energy imbalance in diabetic subjects resulting from increased energy expenditure. Hence, energy from macromolecules such as ADP, FAD, ACA and NADH can be used to predict early energy deficit and manage energy imbalance in diabetic individuals.

**Keywords:** DM; energy balance; adenosine diphosphate; flavin adenine dinucleotide; acetylco-enzymeA and nicotinamide adenine dinucleotide.

## 1. INTRODUCTION

Diabetes mellitus (DM), is a group of metabolic disorder characterized by chronic hyperglycemia caused by defects in insulin secretion, insulin action, or both [1]. Diabetes mellitus is also associated with altered metabolism and higher Energy Expenditure. Diabetes mellitus is a growing public health problem affecting people worldwide both in developing and developed countries, and poses a major socio-economic challenge [2,3]. It is assuming epidemic proportions worldwide [4]. According to the WHO standard, Nigeria has a comparative prevalence of 4.83% with over 88,681 Diabetes-related deaths. In South Eastern Nigeria the prevalence of diabetes mellitus is about 6.7%. [5]. Diabetes mellitus results in aberrations in carbohydrate, fat and protein metabolism which arise due to defects in insulin secretion, and/or action and is the major cause of energy imbalance in such individuals. The combined effect of the components of energy balance (energy intake/storage and energy expenditure) in individual with type 2 diabetes (T2D) has not been adequately investigated. Study has shown decreased Energy intake in diabetes mellitus individuals when compared to Non diabetic individuals [6]. Subjects with diabetes mellitus presented a higher Basal Energy Expenditure (BEE) than healthy people which may be due to an increase in Fasting Blood Glucose resulting in a higher glycosuria or gluconeogenesis [7]. Further study has shown 3–8% increase in BEE in subjects with DM with high FBG ( $> 10$  mmol/l), which returns to normal after insulin therapy [8,9].

## 2. MATERIALS AND METHODS

The study was carried out at diabetic clinic of Nnamidi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State of Nigeria.

### 2.1 Study Design

This is a longitudinal, prospective, case-controlled study. Subjects were recruited by random sampling, in this case every subject has the same probability of being chosen. Subjects newly diagnosed and confirmed with type 2 diabetes were followed up from the time of diagnosis, till the subjects start treatment over a period of 12 months.

### 2.2 Study Population

The study population consist of Group one: A total number of seventy seven (77) Diabetic individuals newly diagnosed attending diabetic clinic of NAUTH aged 18-60 years both male and female not on anti-diabetic drug, were enrolled in the study as test subjects and were followed up till they start treatment and during treatment for a period of 12 months.

**Group two:** A total number of thirty six (36) apparently healthy non-diabetic individuals both male and female were recruited as control subjects.

### 2.3 Inclusion Criteria

Subjects for the study include male and female subjects between the ages 18-60 years. The

subjects that were confirmed to be diabetic not on drug yet, were enrolled in the study and were followed up after commencement of drug administration for at least 12 months.

## 2.4 Exclusion Criteria

Control subjects with underlying history of chronic illness, such as diabetic were excluded from the study.

Test subjects without diabetes mellitus was excluded, subjects with hypertension, tuberculosis, pregnant women were also excluded.

## 2.5 Blood Sample Collection

Six milliliters (6ml) of fasting blood Sample was drawn aseptically by venepuncture from all subjects into a heparin specimen containers then allowed to clot, centrifuged for 10 minutes at 3500rpm. Plasma separated, aliquoted into two parts for estimation of all the analytes. All samples were kept frozen at  $-20^{\circ}$  or  $-80^{\circ}$ c until the time of analysis.

### 2.5.1 Laboratory for analysis

Nnamidi Azikiwe University Teaching Hospital (NAUTH), Anambra State.

## 2.6 Assay Methodology

Determination of Plasma Nicotinamide Adenine Dinucleotide (NADH), was done using Enzyme linked Immunoassay (ELISA) as described by Castro-Marrero et al., [10], Flavin Adenine Dinucleotide (FAD), by Enzyme linked Immunoassay based method as described by [11].

AcetylCoA (ACA), was also done using Enzyme linked Immunoassay based method as described by [12].

Adenosine diphosphate (ADP) was estimated by ELISA based method as described by Perez-Ruiz et al., [13].

Energy Balance was Determined Mathematically; Energy balance equation is equal to rate of energy intake (EI)/energy storage minus rate of energy expenditure (EE). [14].

1. Calculation of energy balance from macromolecules (NADH, FAD and ACA) this depicts energy storage.

Complete utilization of NADH through kreb's cycle and electron transport chain give equivalent of three (3) ATP. FAD gives equivalent of two (2) ATP. While, ACA gives twelve (12) ATP.

2. Two ADP and Two GDP are used up in the kreb's cycle, totally Four (4) ADP equivalent, this depict energy expenditure.

Total ATP from the macromolecule =  $(ACA \times 12) + (FAD \times 2) + (NADH \times 3)$  which is the energy stored. Total ADP equals  $(ADP \times 4)$  which is the energy used. Therefore total energy balance from macromolecules = summation of  $(ACA \times 12) + (FAD \times 2) + (NADH \times 3)$  minus  $(ADP \times 4)$ .

## 2.7 Statistical Analysis

The data obtained were statistically analyzed using SPSS Version 23.0 statistical package. Independent sample t-test was used to assess the mean difference between two dependent variable and Analysis of variance (ANOVA) were used to compare the differences in the parameters measured among groups, post hoc multiple comparison was used to assess inter group variability and all variables were expressed as mean  $\pm$  standard deviation ( $M \pm SD$ ). Significant level was considered at  $p < 0.05$ .

## 3. RESULTS

Table 1 showed comparison of the mean level of Adenosine diphosphate (ADP), Flavin adenine dinucleotide (FAD), AcetylCo-enzymeA (ACA) and Nicotinamide Adenine Dinucleotide (NADH) in control and Diabetic subjects before and during treatment.

The result showed that Adenosine diphosphate (ADP), Flavin adenine dinucleotide (FAD), AcetylCo-enzymeA (ACA) and Nicotinamide Adenine Dinucleotide (NADH), were significantly lower ( $P < 0.05$ ) in both Diabetic pre-treatment and diabetic post-treatment group compared with control groups. Furthermore, the plasma level of ACA and NADH were significantly lower ( $P < 0.05$ ) in DM pre-treatment group compared with DM

**Table 1. Comparison of mean levels of ADP, FAD, ACA and NADH in control and diabetic subjects before and during treatment**

Parameters	Control N = 36	Pre - DM-Treatment N = 77	12 Months Post- DM - Treatment N = 45	F – Value	P – Value
ADP (nmol/l)	1036.7±380.1	871.6±279.2 <sup>a,c</sup>	584.4±242.9 <sup>a</sup>	18.85	0.001
FAD (ng/ml)	1.49±0.57	0.98±0.37 <sup>a</sup>	0.94±0.22 <sup>a</sup>	24.40	0.001
ACA (ng/ml)	23.5±7.5	10.5±2.8 <sup>a,c</sup>	18.0±4.9 <sup>a,c</sup>	78.62	0.001
NADH (ng/ml)	2.1±0.8	1.23±0.4 <sup>a,c</sup>	1.53±0.4 <sup>a,c</sup>	22.11	0.001

\*P is significant at <0.05; a- Significant When Compared with control groups, c- Significant When Compared With both DM Groups (pre- and post- treatment),ADP-Adenosine Diphosphate, FAD-Flavin Adenine Dinucleotide, ACA-Acetyl Co-enzyme A, NADH-Nicotinamide Adenine Dinucleotide.

**Table 2. Comparison of calculated energy from macromolecules in control and dm subjects before and during treatment**

Parameter	Control N = 36	Pre -dm Treatment N = 77	12 Months Post -DM Treatment N = 45	F-Value	P-Value
ENERGY BAL. (kilocal)	2133.33±673.28	969.28±246.09 <sup>a,c</sup>	1630.51±431.45 <sup>a,c</sup>	80.49	0.001

\*P is significant at <0.05; Keys: Kilocal- kilocalories; a-Significant When Compared with control group; c- Significant When Compared with DM post- treatment group

Post-treatment group. While, the plasma concentration of ADP was significantly lower in DM post-treatment groups compared with DM pre-treatment groups.

The results of Table 2 comparison of Calculated Energy from Macromolecules in Control and DM Subjects before and during Treatment.

The result showed that the Calculated energy from Macromolecules was lower ( $P<0.05$ ) in DM groups compared with control group. However, the calculated energy from Macromolecules in DM pre-treatment was significantly lower ( $P<0.05$ ) compared with DM post-treatment.

#### 4. DISCUSSION

The present study focused on the use Adenosine Diphosphate, Flavin Adenine Dinucleotide, Acetyl Co-enzyme A, Nicotinamide Adenine Dinucleotide and mathematical calculation of potential energy as an index of energy utilization and storage to predict and manage energy balance in Diabetic individuals.

In this present study, ADP, ACA, NADH and FAD were observed to be lower in DM subjects as a result of increased activity of glycogenolysis, lipolysis and gluconeogenesis which are energy consuming metabolic activity and increased energy expenditure in diabetes. Subjects with diabetes have higher energy expenditure, likely a consequence of higher gluconeogenic activity. There are many mechanism implicated in increased energy expenditure in diabetes such as increased oxidation of carbohydrates, augmentation of gluconeogenesis and hepatic glucose output. A higher fasting plasma glucose concentration is associated with a high energy expenditure [15,16,7].

ACA is the most abundant high energy molecule stored, ACA is equivalent to twelve ATP, as a result of high energy demand its level is depleted in diabetes.

FAD and NADH are also high energy molecule equivalent to two and three ATP respectively produced in all the metabolic pathway, this work has shown its depletion in diabetes as a result of increased energy expenditure and energy consuming process seen in diabetes. ADP depict energy used while FAD, NADH and ACA depict energy storage which are ATP equivalent. ADP is used up during oxidative phosphorylation to produce a new ATP in all metabolic pathway. All this could be attributed to reduction in energy

intake, energy storage, energy used and alteration in metabolism in DM subjects. A study observed loss of appetite and low energy intake and increased in energy expenditure in DM subjects [6].

Subjects with diabetes have higher energy expenditure, likely a consequence of higher gluconeogenic activity. There are many mechanism implicated in increased energy expenditure in diabetes such as increased oxidation of carbohydrates, augmentation of gluconeogenesis and hepatic glucose output. A higher fasting plasma glucose concentration is associated with a high energy expenditure [15,16,17].

The study also revealed that the plasma level of ACA and NADH in DM pre-treatment group were significantly lower ( $P<0.05$ ) compared to DM post-treatment group. ACA and NADH are high energy molecule produced during metabolism for energy release, this study has shown that DM pre-treatment group were more energy demanding compared with DM post treatment. This could be as a result of high energy expenditure in pre-treatment group basically because of high gluconeogenic activity seen in the early phase of diabetes which is an energy consuming process. Bock et al., [18], Basu et al., [17], Stephanie et al., [19], documented increased gluconeogenesis and glycogenolysis in drug naive newly diagnosed type 2 diabetes. Ferrannini, [20] also documented energy loss of about 120–320kcal/day in diabetes.

The present study has also Shown that calculated energy from macromolecules in DM group were significantly lower ( $P<0.05$ ) compared with control group. This implies that energy used in DM subjects (ADP) is greater than energy storage (ATP equivalents). However, this is a clear indication of energy deficit/ energy imbalance in DM subjects resulting from increased energy expenditure. Subjects with diabetes have higher energy expenditure, likely a consequence of higher gluconeogenic activity. This increased energy expenditure may be due to increased hepatic glucose production, a higher fasting plasma glucose concentration is associated with a higher basal metabolic rate (BMR). Another possible mechanism involved in raising the BMR is increased renal glucose reabsorption, which is an energy-dependent process. Hyperglycemia increases the amount of glucose filtered by the glomerulus and consequently, tubular glucose reabsorption is increased [21,22,23,24].

Several studies documented increased energy expenditure in DM subjects. Fontvieille et al., [25], weyer et al., [26], Gougeon *et al.*, [8], Bitz et al., [15], Miyake et al., [16]. Buscemi et al., [7], O'Neill et al., [27], Vallon and Thomson, [28]. All observed higher energy expenditure in DM subjects compared to health control subjects.

## 5. CONCLUSION

The present study thus concludes that the decrease in the level of high energy molecules (ACA, NADH, ADP and FAD) in Diabetic individuals were associated with altered metabolism and increased energy expenditure. Secondly, this present study also observed energy deficit, this implies that energy used in Diabetic (ADP) was greater than energy storage (ATP equivalents). However, this is a clear indication of energy deficit/ energy imbalance in diabetic subjects resulting from increased energy expenditure. An increase in energy expenditure can lead to nutritional imbalance, weight lost and wasting which is responsible for energy imbalance as seen in this present work. The energy imbalance was pronounced in the pre-treatment stage, but tends to drop after 12 months of treatment. High energy molecules such ACA, ADP, NADH and FAD can be used to predict early energy deficit and manage energy balance in Diabetic individuals.

## CONSENT

Informed consent was sought from all subjects before recruitment into the study.

## ETHICAL APPROVAL

Ethical approval was sought from ethics committee of Nnamidi Azikiwe University Teaching Hospital (NAUTH), with reference number NAUTH/CS/VOL.11/195/2018/129.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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