Research Article

Synergistic insulinotropic effect of metformin-Mg²⁺ adjunct supplement: A case study of streptozotocin-induced type 2 diabetes in sprague dawley rats

Abstract

Aim: To determine the effects of metformin alone versus metformin and magnesium supplemented diet on insulin sensitivity and insulin receptor in type 2 diabetic Sprague Dawley rats.

Material and Methods: Total of 24 Sprague dawley rats (Four groups of six rats each were used for this study, namely:

a. **Group 1:** Normal control rats (CONTROL) given distilled water for 4 weeks;

b. **Group 2:** Diabetic untreated control rats (DU) given distilled water for 4 weeks;

c. **Group 3:** Metformin treated diabetic rats (DM), orally given 100mg/kg body weight for 4 weeks;

d. **Group 4:** Metformin treated diabetic rats (DMM), orally given 100mg/kg body weight with 2000mg/kg body weight magnesium supplemented feed for 4 weeks). Measured data was analyzed statistically.

Observations and Result: DMM (45.14±0.34) was significantly ($p<0.05$) higher when compared with DM (33.57±1.25) in body weight at week 4. There was significant ($p<0.05$) increase in the feed and water intake of the treated rats but the metformin-magnesium supplement treated group showed more increase when compared with only metformin treated group. The insulin receptor of DU, DM and DMM were significantly ($p<0.05$) lower when compared with CONTROL (9.68±0.42). However when compared with DU (3.83±0.23), DM (6.33±0.31) and DMM (7.10±0.17) were significantly ($p<0.05$) higher. DU (93.33±2.40) was significantly ($p<0.05$) higher than CONTROL (72±1.16) while DM (82±3.06) and DMM (81.67±2.85) were significantly ($p<0.05$) lower than DU in plasma insulin level.

Conclusion: This difference between DM and DMM was considered highly significant in body weight, feed and water intake. This suggested magnesium supplementation as an adjunct therapy with metformin helps in improving insulin sensitivity and insulin receptor.

Keywords: Metformin, magnesium, insulin receptor, insulin sensitivity, adjunct therapy

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Introduction

Diabetes mellitus (DM) is currently a major public health concern, because its incidence and prevalence are elevated and increasing, reaching epidemic proportions.[1] Diabetes mellitus (DM) has been distinguished with persistently elevated blood glucose leading to acute or long term complications. Globally, DM presents increased public health issue. The prevalence of DM in all age groups rate is expected to rise by 8% to 170 million in 2000 and by 4.4% to 366 million in 2030.[2] Normal non-diabetic patients maintain plasma glucose <100 mg/dl in the fasting and <135 mg/dl in the post prandial period. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both.[1] Diabetes mellitus is classified as type 1, type 2, other specific types and gestational diabetes.[1] Type1diabetes is known as insulin dependent diabetes or Juvenile-onset diabetes and type 2 diabetes is known as non-insulin dependent or adult onset diabetes.[1] Metformin is currently the drug of choice in patients with type 2 diabetes mellitus, as indicated in the guidelines published by the European Association for the Study of Diabetes and American Diabetes Association.[3] Magnesium is an essential mineral with several dietary sources including whole grains, green leafy vegetables, legumes, and nuts. It is the fourth most abundant ions present in living cells and its plasma concentration is remarkably constant in healthy subjects. Homeostasis of magnesium is tightly regulated and depends on the balance between intestinal absorption and renal excretion. Insufficient cellular magnesium levels set the stage for deterioration of proper metabolic function that typically snowballs into more significant health problems. Researchers have detected 3751 magnesium binding sites on human protein, reflecting how important this mineral is to biological processes.[4] Emerging evidence has indicated a genetic basis for magnesium metabolism in human. In prospective observational studies, dietary magnesium intake has been inversely associated with the incidence of the metabolic syndrome and associated chronic diseases includes type 2 D.[5]

The link between diabetes mellitus and magnesium is well established. A grouping body of evidence shows that magnesium plays a crucial role in reducing cardiovascular risk and may be involved in the pathogenesis of diabetes itself.[6] Studies have shown

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that both mean plasma and intracellular free magnesium levels are lower in patients with diabetes than in general population. This magnesium deficiency which may take the form of a chronic latent magnesium deficit rather than clinical hypomagnesaemia may have clinical importance because the magnesium ion is a crucial cofactor for many enzymatic reactions involved in metabolic processes.[6] This study was aimed at determining the effects of metformin alone versus metformin and magnesium supplemented diet on insulin sensitivity and insulin receptor in type 2 diabetic *Sprague dawley* rats.

Materials

Experimental animals

Twenty-four (24) adult female Sprague Dawley rats (average weight 130g) were obtained from the Animal Unit of the University of the Lagos Teaching Hospital (LUTH), Lagos, Nigeria. The rats were kept in a well-ventilated room, with 12h light and 12h dark cycles at 24 ± 2^0C. They were given free access to food (standard pelleted feed) and water and allowed to acclimatize for three weeks before the commencement of the study. Treatment of the animals conformed to the National Academy of Science's Guide for Care and Use of Laboratory Animals (1996).

Animal grouping

Four groups of six rats each were used for this study, namely:

a. **Group 1:** Normal control rats (CONTROL) given distilled water for 4 weeks;

b. **Group 2:** Diabetic untreated control rats (DU) given distilled water for 4 weeks;

c. **Group 3:** Metformin treated diabetic rats (DM), orally given 100mg/kg body weight for 4 weeks;

d. **Group 4:** Metformin treated diabetic rats (DMM), orally given 100mg/kg body weight with 2000mg/kg body weight magnesium supplemented feed for 4 weeks; This dose was chosen from a pilot dose-response study; it reflects a balance between the toxic and therapeutic dose of the supplement. The rats were weighed weekly.

Reagents

Streptozotocin was purchased from Sigma-Aldrich (St Louis, MO, USA). Standard drug, Metformin was obtained from Bernados Pharmacy at Idi-Araba, Lagos, Nigeria and it was branded as Glucophage by a local manufacturer (swiss pharma) in Nigeria. For the insulin assay, (ELISA) kit (Ultrasensitive Rat Insulin ELISA from Mercodia) and insulin receptor assay, ELISA kit (Elabscience Biotechnology limited, Hubei, Province, Sourthern, China), diagnostic kit was purchased from Reckon Diagnostic Pvt. Ltd., Lagos, Nigeria. All the chemicals used in the experiment were of analytical grade.

Feeding

Pelletized feeds were given to the rats. The animals were given distilled water.

Methodology

Administration of streptozotocin/ induction of diabetes

Streptozotocin, dissolved in (pH 4.5) cold citrate buffer, was administered to the rats, by intra-peritoneal injection, at a dose of 60mg/kg body weight after a 12-hour fast. This was followed by administration of nicotinamide dissolved in normal saline at a dose of 110mg/kg body weight after 5 minutes. Diabetes was confirmed after seventy-two (72) hours of STZ administration by measuring fasting blood sugar (FBS). Only rats with glucosuria and FBS higher than 250mg/dl were used.[7]

Administration of magnesium and anti-diabetic drugs

Magnesium supplemented feed was prepared by adding 2000mg/ kg diet in normal rat chow and thereafter pelletized. The magnesium supplement was added to the normal rat chow and the anti-diabetic drugs were given orally to the animals in quantity based on their body weights as well as the distilled water.

Assessment of body weight

The weight of animals were measured weekly during the experimental period with the aid of a digital weighing scale.

Assessment of feed intake

The feed intake of animal was determined by deducting the amount of feed left the next day from the amount given the day before, measurement was done using a digital weighing scale.

Assessment of water intake

The amount of water taken by the animals was gotten by deducting the amount of water left the next day from the amount of water given the previous day. The amount of water given each day and the amount of water left were measured using measuring cylinder.

Oral glucose tolerance test

At the second and fourth week, the oral glucose tolerance test was carried out. Blood was collected from the tip of the tail of the animals with the aid of a surgical blade and applied on the blood glucose strip which was inserted into the glucometer. The animals were fasted for 16 hours prior to the oral glucose tolerance test. The body weight of animals were measured and recorded just before taken the fasting blood glucose level on the prepared record sheet. Snipping the tail by placing the rats on the table and making small incision over the lateral vein (1-2cm from tail tip). Fasting blood glucose levels of the animals were determined prior to glucose administration with the aid of glucose strips inserted in a glucometer. 2g/kg body weight of glucose was freshly prepared in distilled water and administered orally with the aid of an oral cannula and syringe. Prior to the administration of glucose, fasting blood glucose level was measured (baseline). The blood glucose level was also repeatedly measured at 30, 60,120 and 180 minutes after glucose administration.

Insulin tolerance test

At the second and fourth week, the animals' blood glucose level was carried out. The tails of the animals were cut off to get blood; they were applied on the blood glucose strip which has been inserted into the glucometer. Then Accu check glucometer was used afterwards their blood glucose levels were recorded. The weight of animal was taken and recorded on the prepared record sheet. Snipping the tail by placing the rats on the table and making small incision over the lateral vein (1-2cm from tail tip). Baseline of the animals fasting blood glucose concentration level was recorded using glucose strips and glucometer. 0.5IU/ml per body weight of insulin was given intraperitoneally. Glucose level was measured at 15 minutes, 30

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minutes, 90 minutes and 120 minutes. During this experiment it was ensured that there was no excessive bleeding before returning animals to their cages.

Sample collection

Blood was drawn from the tail vein of each rat before the administration of streptozotocin (STZ) to obtain the basal levels of all parameters. After the administration of STZ and the commencement of treatment, FBS was assessed at 7 days interval. Other parameters were assessed once every week. Blood samples were used to check the blood glucose level using glucometer. At the end of the monitoring phase, the rats were sacrificed; blood was obtained through retro orbital puncture. Blood for insulin assays was collected in heparin zed bottle and centrifuged at 7000rpm for 10mins to separate blood plasma. The plasma was transferred into eppendrof bottles with the aid of a micro pipette. 0.5g of liver and 0.5g gastronomies muscle were removed for assay they were kept in universal sample bottles and homogenized using 5.0ml of phosphate buffer solution with concentration 1:10 in a sterilized homogenizer. They were centrifuged at 7,000 rev/minute for 15 minutes. 1000ul of each supernatant was collected, placed in eppendorf tube and stored in the freezer.

Sample storage

The plasma and homogenates were stored at 2°C in the freezer and placed on ice when use.

Biochemical assays

Insulin assay

All reagents and samples were brought to room temperature before use. The required amount of enzyme conjugate 1X solution was prepared. Wash buffer 1X solution was prepared. The samples, insulin control solutions, and calibrators were prepared. Sufficient microplate wells were prepared to accommodate Calibrators and samples in duplicate. A plate plan was made. 25μl each of Calibrators was pipetted into appropriate wells. 25μl each of samples was pipetted into appropriate wells. 100μl of enzyme conjugate 1X solution was added into each well. The mixture was incubated on a plate shaker (700-900 rpm) for 2 hours at room temperature (18-25°C). Each well was washed 6 times with wash buffer 1X solution. The reaction volume was discarded by inverting the microplate over a sink. 350μl wash solution was added to each well. The wash solution was discarded, tapped firmly several times against adsorbent paper to remove excess liquid. This was repeated 5 times. Prolonged soaking was avoided during washing procedure. 200μl Substrate TMB was added into each well. Incubated for 15 minutes at room temperature (18 - 25°C). 50μl Stop Solution was added to each well. The plate was placed on the shaker for approximately 5 seconds to ensure mixing. Optical density was read at 450 nm. Read within 30 minutes. The results were calculated.

Insulin receptor assay

100uL of standard, Blank or sample was added per well. The lank well was added with Reference standard and sample diluents. Solutions were added to the bottom of micro ELISA plate well and mixed gently. The plate was covered with the provided sealer and incubated for 90 minutes at 37°C. Liquid was removed from each well without washing. Immediately, 100Ul of Biotynylated Detection Ab working solution was added to each well. The wells were again covered with the plate sealer and incubated for one hour 37°C. Each well was aspirated and was washed for about three times. Washing was done by filling each well with wash buffer. After the last wash, the remaining wash buffer was removed b decanting. Then the plate was inverted and patted against thick clean absorbent paper. 100ul of HRP conjugate working solution was added to each well and covered with the plate sealer. The well was then incubated for 30 minutes at 37°C. The wash process was repeated for five times as conducted in step 3. 90ul of substrate solution was added to each well and covered with a new plate sealer. This is then incubated for 15 minute at 37°C. 50ul of stop solution was added to each well. Then, the water turned to yellow immediately. The other used to add stop the solution was the same as the substrate solution. The optical density of each well was determined using a micro-plate reader set to 450nm.

Statistical analysis

All data were analyzed using student t-test and one way analysis of variance (ANOVA) and displayed as mean± standard error of mean (SEM). Graph pad version 5.0 (Graph pad software, San Diego California, USA) was used for the analyses p<0.05 was considered as statistically significant.

Results

Effect of magnesium supplementation on body weight

(Table 1) shows the effect of magnesium supplementation on the body weight of the animals. The body weight of the DU (117.67±0.88) animals showed a significant (p<0.05) decrease when compared with the control at the week 3 and week 4. In addition a significant (p<0.05) reduction in the body weight was also observed at the 0 week, week 1 and week 2 of experiment in the DM (115.17±2.47) animals when compared with CONTROL (129.4±1.21) (Figure 1).
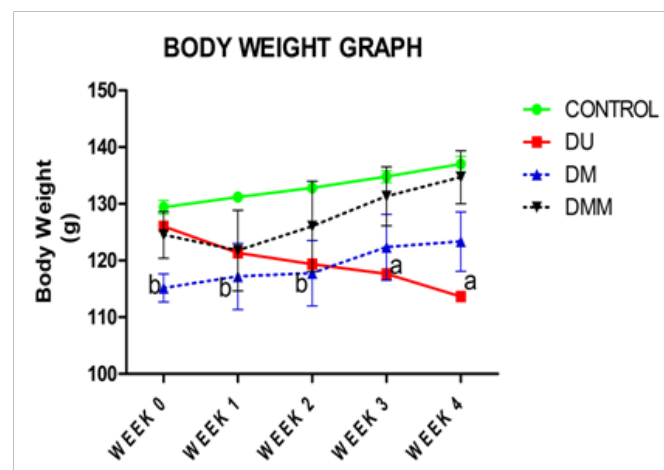

Figure 1 Effect of magnesium supplementation on body weight.
Abbreviations: DU, Diabetic untreated**;** DM, Diabetic Metformin**;** DMM, Diabetic Metformin Magnesium.

Effect of magnesium supplementation on feed intake

(Table 2) shows that a significant (p<0.05) decrease in food intake was observed in DU (28.04±0.62), DM (28.86±0.77) and DMM (28.63±0.93) at week 1 when compared with CONTROL (40.57±0.53). Week 2 showed that there was a significant (p<0.05) increase in DM (25.71±0.81) and DMM (32.29±1.43) but DU

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(24.14±0.51) showed significant decrease when compared with CONTROL. DMM (32.29±1.43) showed a significant increase while DM (25.71±0.81) showed no significant difference when compared with DU (24.14±0.51). DMM also significantly (p<0.05) increased when compared with DM. Week 3 table also showed that there was a significant (p<0.05) increase in DM (33.57±1.25) while DU (21.96±0.66) showed significant (p<0.05) decrease when compared with CONTROL. DMM (42.57±1.13) and DM (33.57±1.25) when compared with DU (21.96±0.66) showed a significant (p<0.05) increase. DMM also showed no significant (p<0.05) increase when compared with DM. Week 4 showed that DU (23.43±0.48), DM (42.86±1.06) and DMM (45.14±0.34) showed a significant (p<0.05) increase when compared with CONTROL (45.29±0.68), DM (42.86±1.06) and DMM (45.14±0.34) showed a significant (p<0.05) increase when compared with DU (23.43±0.48). DMM also showed significant (p<0.05) difference when compared with DM (Figure 2).

Table 1 Effect of magnesium supplementation on body weight

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
CONTROL	129.4±1.21	131.2±0.86	132.8±0.66	134.8±1.07	137±1.34
DU	126±0.58	121.33±0.67	119.33±0.67	117.67±0.88[a]	113.67±0.88[a]
DM	115.17±2.47[b]	117.2±5.83[b]	117.75±5.76[b]	122.33±5.81	123.33±5.24
DMM	124.5±4.09	121.75±7.09	126±7.99	131.33±5.21	134±4.67

Data expressed as Mean ± SEM (n=6).
[a]p<0.05 DU vs. CONTROL,
[b]p<0.05 DM vs. CONTROL

Table 2 Effect of magnesium supplementation on feed intake

Groups	Week 1	Week 2	Week 3	Week 4
CONTROL	40.57±0.53	40.71±0.68	42.71±1.15	45.29±0.68
DU	28.04±0.62[a]	24.14±0.51[a]	21.96±0.66[a]	23.43±0.48[a]
DM	28.86±0.77[b]	25.71±0.81[b]	33.57±1.25[b,d]	42.86±1.06[d]
DMM	28.63±0.93[c,e]	32.29±1.43[c,e,f]	42.57±1.13[e,f]	45.14±0.34[e]

Data expressed as Mean ± SEM (n=6).
[a]p<0.05 DU vs. CONTROL, [d]p <0.05 DM vs. DU
[b]p<0.05 DM vs. CONTROL, [e]p <0.05 DMM vs. DU
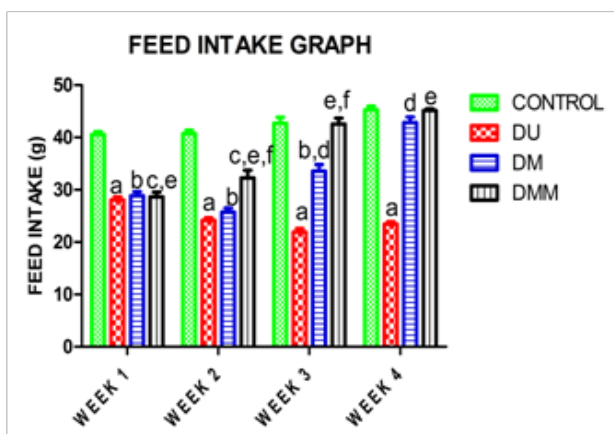[c]p<0.05 DMM vs. CONTROL, [f]p <0.05 DM vs. DMM

Figure 2 Effect of magnesium supplementation on feed intake.
Abbreviations: DU, Diabetic untreated; DM, Diabetic Metformin; DMM, Diabetic Metformin Magnesium

Effect of magnesium supplementation on water intake

(Table 3) Shows that at week 1 there was a significant (p<0.05) decrease in water intake in DU (38±0.82) and DM (36.43±0.78) when compared with CONTROL (48.29±0.84) but DMM (48.54±0.84) showed no significant (p<0.05) difference. Compared with DU (38±0.82), DM (36.43±0.78) showed no significant (p<0.05) difference but DMM (48.54±0.84) showed a significant (p<0.05) increase. Also when compared with DM (36.43±0.78), DMM (48.54±0.84) showed a significant (p<0.05) increase. At week 2, a significant (p<0.05) increase in water intake in DM (42.86±0.99) was observed when compared with CONTROL (47.54±1.27) but DMM (46.46±0.92) showed no significant (p<0.05) difference while DU (35.57±0.78) showed significant (p<0.05) decrease. When compared with DU (35.57±0.78), DM (42.86±0.99) and DMM (46.46±0.92) showed a significant (p<0.05) increase. Also when compared within, no significant (p<0.05) difference between DM (42.86±0.99) and DMM (46.46±0.92) was observed. At week 3, DM (41.33±1.02) and DMM (41.29±1.02) showed a significant (p<0.05) increase when compared to CONTROL (46.66±1.72) while DU (32.57±0.48) showed significant (p<0.05) decrease. When compared with DU (32.57±0.48) DM (41.33±1.02) and DMM (41.29±1.02) showed significant (p<0.05) increase. Finally when compared within groups DM (41.33±1.02) and DMM (41.29±1.02) showed no significant (p<0.05) difference. At week 4, DM (42.07±1.32) and DMM (42.07±1.32) showed a significant (p<0.05) increase when compared to CONTROL

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(54.89±2.06) while DU (30.57±0.65) showed significant (p<0.05) decrease. When compared with DU (30.57±0.65) DM (42.07±1.32) and DMM (48±1.54) showed significant (p<0.05) increase. Finally when compared within groups DMM (48±1.54) was significantly (p<0.05) higher than DM (42.07±1.32) (Figure 3).

Table 3 Effect of magnesium supplements on water intake

Groups	Week 1	Week 2	Week 3	Week 4
CONTROL	48.29±0.84	47.54±1.27	46.66±1.72	54.89±2.06
DU	38±0.82[a]	35.57±0.78[a]	32.57±0.48[a]	30.57±0.65[a]
DM	36.43±0.78[b]	42.86±0.99[b,d]	41.33±1.02[b,d]	42.07±1.32[b,d]
DMM	48.54±0.84[e,f]	46.46±0.92[e]	41.29±1.02[c,e]	48±1.54[c,e,f]

Data expressed as Mean ± SEM (n=6).

[a]p<0.05 DU vs. CONTROL, [d]p <0.05 DM vs. DU

[b]p<0.05 DM vs. CONTROL, [e]p <0.05 DMM vs. DU

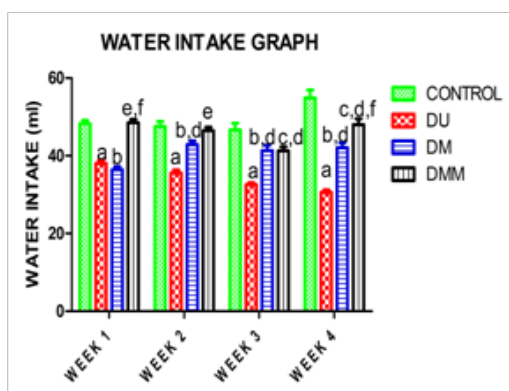[c]p<0.05 DMM vs. CONTROL, [f]p <0.05 DM vs. DMM

Figure 3 Effect of magnesium supplementation in water intake.
Abbreviations: DU, Diabetic untreated**;** DM, Diabetic Metformin**;** DMM, Diabetic Metformin Magnesium

Effect of magnesium supplementation on glucose tolerance test at week 2

(Table 4) Below shows that at 0 minute which was the baseline there was no significant (p<0.05) difference in DM (84.33±15.86) and DMM (55.25±14.68) when compared with CONTROL (71.8±2.63) but there was a significant (p<0.05) increase at DU (282.5±31.73). When compared with DU (282.5±31.73), DM (84.33±15.86) and DMM (55.25±14.68) showed a significant (p<0.05) decrease. At 30 minutes a significant (p<0.05) increase at DU (422 ±13.84), DM (284±38.50) and DMM (273.25±45.08) was observed when compared with CONTROL (104.67±2.40), but DM (284±38.50) and DMM (273.25±45.08) were significantly (p<0.05) lower when compared with DU (422 ±13.84) and there was no significant (p<0.05) difference between DM (284±38.50) and DMM (273.25±45.08). At 60 minutes table shows a significant (p<0.05) decrease at DU (352±13.75), DMM (249±59.41) but significant (p<0.05) increase at DM (294±68.68) when compared with CONTROL (88±0.41). At 120 minutes table showed a significant (p<0.05) decrease at DU (323.5±10.5), DM (219±69.20) when compared with CONTROL (81.25±0.95) but when compared with DU (323.5±10.5), DMM (94.50±27.35) showed a significant (p<0.05) decrease. DMM (94.50±27.35) also significantly (p<0.05) decreased compared with DM (219±69.20). At 180 minutes table showed a significant (p<0.05) decrease at DU (286±19.25) when compared with CONTROL (53.50±4.50) while DM (147±39) and DMM (48.50±16.19) showed significant (p<0.05) decrease when compared with DU (286±19.25) but no significant (p<0.05) difference was observed between DM (147±39) and DMM (48.50±16.19) (Figure 4).
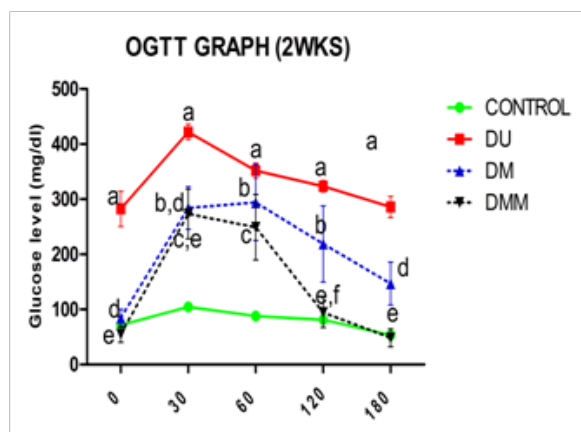
Table 4 Effect of magnesium supplementation on glucose tolerance at 2 weeks

Groups	0 Min	30 Min	60 Min	120 Min	180 Min
CONTROL	71.8±2.63	104.67±2.40	88±0.41	81.25±0.95	53.50±4.50
DU	282.5±31.73[a]	422 ±13.84[a]	352±13.75[a]	323.5±10.5[a]	286±19.25[a]
DM	84.33±15.86[d]	284±38.50[b,d]	294±68.68[b]	219±69.20[b]	147±39[d]
DMM	55.25±14.68[e]	273.25±45.08[c,e]	249±59.41[c]	94.50±27.35[e,f]	48.50±16.19[e]

Data expressed as Mean ± SEM (n=6).

[a]p<0.05 DU vs. CONTROL,

[b]p<0.05 DM vs. CONTROL,

[c]p<0.05 DMM vs. CONTROL,

[d]p <0.05 DM vs. DU

[e]p <0.05 DMM vs. DU

[f]p <0.05 DM vs. DMM

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Figure 4 Effect of magnesium supplementation on glucose tolerance at week 2.
Abbreviations: DU, Diabetic untreated; DM, Diabetic Metformin; DMM, Diabetic Metformin Magnesium

Effect of magnesium supplementation on glucose tolerance test at week 4

(Table 5) below shows that there were no significant (p<0.05) differences in all the comparison made (Figure 5).

Effect of magnesium supplementation on insulin tolerance test at week 2

(Table 6) shows the glucose level of control, DU, DM and DMM group at 0, 15, 30, 90,120 and 180 minutes during the insulin tolerance test at week 2. Before insulin administration (0 minute), there was a significant increase (p<0.05) in the glucose level of DM and DMM when compared with CONTROL. After 15 and 30 minutes, there was a significant (p<0.05) decrease in DM but no significant (p<0.05) change in DMM and DU compared with the CONTROL. There was also significant (p<0.05) decrease in DM when compared with DU. At 90 and 120 minutes, there were no significant (p<0.05) changes among the different groups (Figure 6).

Table 5 Effect of magnesium on glucose tolerance test at week4

Groups	0 Min	30 Min	60 Min	120 Min	180 Min
CONTROL	68.67±2.35	46±3.50	56.83±4.90	81.83±2.65	91.67±2.58
DU	164.67±12.35	144.67±11.68	136.33±4.18	139.33±10.40	156±12.22
DM	171.33±48.25	202.33±64.13	165±68.15	103±44.14	69±13.58
DMM	145±24.43	158±70.49	89±29.10	30±4.04	52±6.81

Data expressed as Mean ± SEM (n=6).

Table 6 Effect of the magnesium supplementation on insulin tolerance at week 2

Groups	0 Min	15 Min	30 Min	90 Min	120 Min
CONTROL	97.4±1.44	83±3.58	54.4±8.02	45.4±7.10	70.20±6.91
DU	136.50±12.34	92±10.92	44 ±5.43	23.75±2.18	36.25±2.36
DM	240±74.85b	208.67±74.37[b,d]	186.33±75.29[b,d]	76.67±22.70	111.67±31.97
DMM	205.33±52.19[c]	167±44.64	93±26.10	61±19.52	93.33±27.75

Data expressed as Mean± SEM (n=6).
[a]p<0.05 DU vs. CONTROL,[d]p <0.05 DM vs. DU
[b]p<0.05 DM vs. CONTROL, [e]p <0.05 DMM vs. DU
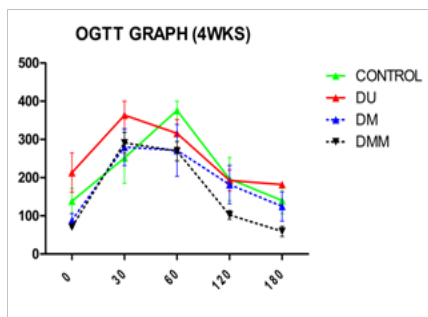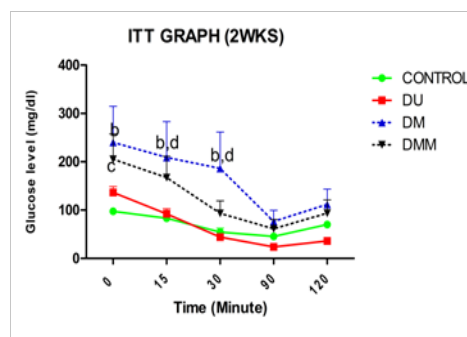[c]p<0.05 DMM vs. CONTROL, [f]p <0.05 DM vs. DMM

Figure 5 Effect of magnesium on glucose tolerance test at week 4.
Abbreviations: DU, Diabetic untreated; DM, Diabetic Metformin; DMM, Diabetic Metformin Magnesium

Figure 6 Effect of magnesium supplementation on insulin tolerance test at week 2.
Abbreviations: DU, Diabetic untreated; DM, Diabetic Metformin; DMM, Diabetic Metformin Magnesium

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Effect of magnesium supplementation on insulin tolerance test at week 4

(Table 7) below shows the glucose level of control, DU, DM and DMM groups at 0, 15, 30, 90, and 120 minutes during the insulin tolerance test week 4. At 0 minutes, the glucose level of DU and DM was significantly (p<0.05) higher when compared with CONTROL. At 15 minutes, there was a significant (p<0.05) increase in DM and DMM but significant (p<0.05) decrease in DU when compared with CONTROL. After 30 minutes, DM decreased significantly (p<0.05) when compared with CONTROL. After 90 and 120 minutes, there was a significant (p<0.05) decrease in DMM when compared with DU (Figure 7).

Effects of magnesium supplementation on insulin receptor

(Table 8) Below shows that the insulin receptors of DU, DM and DMM were significantly (p<0.05) lower when compared with CONTROL. However when compared with DU, DM and DMM were significantly (p<0.05) higher (Figure 8).

Effects of magnesium supplementation on plasma insulin

(Table 9) Shows that the plasma insulin level of DU was significantly (p<0.05) higher than CONTROL while that of DM and DMM were significantly (p<0.05) lower than DU (Figure 9).

Table 7 Effect of magnesium supplementation on insulin tolerance at week 4

Groups	0 Min	15 Min	30 Min	90 Min	120 Min
CONTROL	68.68±2.35	46±3.50	56.83±4.90	81.83±2.65	91.67±2.58
DU	164.67±12.35[a]	144.67±11.68[a]	136.33±4.18	139.33±10.40	156±12.22
DM	171.33±48.25[b]	202.33±64.13[b]	165±68.15[b]	103±44.14	69±13.58
DMM	145±24.43	158±70.49[c]	89±29.10	30±4.04[e]	52±6.81[e]

Data expressed as Mean± SEM (n=6).
[a]p<0.05 DU vs. CONTROL,[d]p <0.05 DM vs. DU
[b]p<0.05 DM vs. CONTROL, [e]p <0.05 DMM vs. DU
[c]p<0.05 DMM vs. CONTROL, [f]p <0.05 DM vs. DMM

Table 8 effect of magnesium supplementation on insulin receptor

Groups	INSR
CONTROL	9.68±0.42
DU	3.83±0.23[a]
DM	6.33±0.31[b,d]
DMM	7.10±0.17[c,e]

Data expressed as Mean ± SEM (n=6).
UNIT- pg/ml
[a]p<0.05 DU vs. CONTROL,[d]p <0.05 DM vs. DU
[b]p<0.05 DM vs. CONTROL, [e]p <0.05 DMM vs. DU
[c]p<0.05 DMM vs. CONTROL, [f]p <0.05 DM vs. DMM

Figure 8 Effect of magnesium supplementation on insulin receptor.
Abbreviations: DU, Diabetic untreated; DM, Diabetic Metformin; DMM, Diabetic Metformin Magnesium

Figure 9 Effect of magnesium supplementation on plasma insulin.
Abbreviations: DU, Diabetic untreated; DM, Diabetic Metformin; DMM, Diabetic Metformin Magnesium
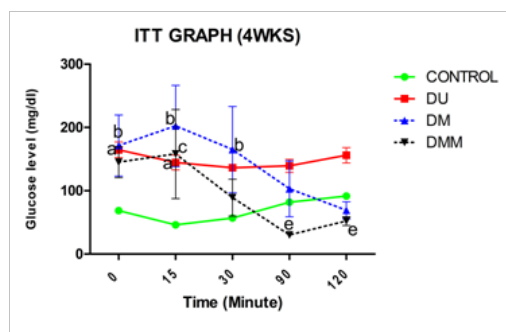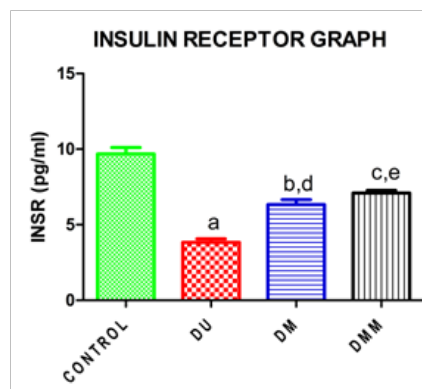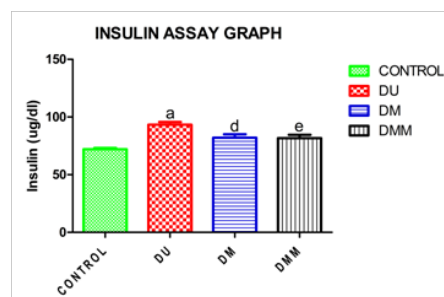

Figure 7 Showing the glucose level of the control, diabetic untreated, metformin and metformin with magnesium on insulin tolerance test at week 4.
Abbreviations: DU, Diabetic untreated; DM, Diabetic Metformin; DMM, Diabetic Metformin Magnesium

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Discussion

Diabetes mellitus (DM) is currently a major public health concern, because its incidence and prevalence are elevated and increasing, reaching epidemic proportions.[1] Metformin is currently the drug of choice in patients with type 2 diabetes mellitus, as indicated in the guidelines published by the European Association for the Study of Diabetes and American Diabetes Association.[3] Magnesium is an essential mineral with several dietary sources including whole grains, green leafy vegetables, legumes, and nuts. It is the fourth most abundant ions present in living cells and its plasma concentration is remarkably constant in healthy subjects. Homeostasis of magnesium is tightly regulated and depends on the balance between intestinal absorption and renal excretion.[8] Insufficient cellular magnesium levels set the stage for deterioration of proper metabolic function that typically snowballs into more significant health problems. Researchers have detected 3751 magnesium binding sites on human protein, reflecting how important this mineral is to biological processes.[4] Studies on the effect of magnesium supplementation on type 2 diabetes have produced several contradictory results, some studies indicate that magnesium supplementation plays a key role in glucose control and insulin sensitivity in type 2 diabetes and vice versa.[9–11] Magnesium is one of the most promising nutritional factors for prevention of type 2 diabetes.[12] Despite a century of research on potential health effects of magnesium, there has been a longstanding debate over the inconsistent results of dietary or supplemental magnesium against diabetes from observational studies.[8] It is obvious that future large well-conducted secondary prevention trials are warranted to unravel the efficacy and safety of magnesium supplements. In addition, emerging evidence has suggested that several genetic factors play central roles in magnesium metabolism in the human body.[13] This study was designed to investigate the effect of magnesium supplementation as adjunct therapy on physical parameters such as body weight, food and water intake, glucose tolerance test, insulin and insulin receptor assay in comparison with metformin. The data gotten from the result clearly demonstrated that the supplementation of magnesium compared with control and untreated diabetes illustrated certain changes.

(Table 4) shows the effect of magnesium supplementation on the body weight of the animals. The body weight of the DU (117.67±0.88) animals showed a significant ($p < 0.05$) decrease when compared with the control at the week 3 and week 4 this may be due to Diabetes type 2 with attending symptoms such excessive urination, reduction in feed and water intake.[14,15] In addition a significant ($p < 0.05$) reduction in the body weight was also observed at the 0 week, week 1 and week 2 of experiment in the DM (115.17±2.47) animals when compared with CONTROL (129.4±1.21). There was reduction in the body weight of the DMM group though not significant ($p < 0.05$) but the body weight increased after week 1 suggesting that magnesium supplementation in combination with the standard drug metformin offers a better weight management in treating diabetes type 2 compared to when metformin was used alone which does not improve weight loss. Omotayo et al.[16] also reported on the inability of metformin to prevent weight loss resulting from induction of diabetes by STZ. There was significant ($p < 0.05$) increase in the feed and water intake of the treated rats but the metformin-magnesium supplement treated group showed more increase when compared with only metformin treated group. The increase in water and feed intake in the DM and DMM could be due to increased excretion of magnesium which is in line with the report of Ammerman et al.[17] but reduced urinary magnesium wasting was noticed along the line of treatment of the metformin-

magnesium supplement treated group suggesting good metabolic control.[18] The glucose tolerance test showed that the blood glucose for the treated groups and the untreated groups were different. There was no significant ($p < 0.05$) difference in DM and DMM when compared with CONTROL but there was a significant ($p < 0.05$) increase in the blood glucose level of DU when compared with CONTROL.[19] When compared with DU, DM and DMM groups showed a significant ($p < 0.05$) decrease. This depicts that magnesium supplementation helps in regulating blood sugar level.[20] This is in line with previous theories that magnesium causes an increase in the rate of glucose utilization by cells in the body thereby reducing blood glucose level in type 2 diabetes as reported by Singh et al.[21]

At week 2, the fasting blood glucose of the animals treated and the diabetic untreated during insulin tolerance test decreased after 15, 30 and 60 minutes compared to CONTROL. After 90 and 120 minutes, there was a slight increase in the animals treated and diabetic untreated compared to CONTROL. At week 4, the glucose level of the diabetic untreated increased rapidly after 15 minutes while the group treated with metformin and magnesium supplement started increasing at 90 minutes being more tolerant to insulin. This establishes the fact that magnesium supplementation in combination with metformin improves insulin sensitivity and insulin in turn stimulates magnesium uptake in insulin sensitive tissues.[21] These findings are in line with the view of Rodriguez-Moran,[11] who reported that oral magnesium supplementation improves insulin sensitivity[22] and metabolic control in type 2 Diabetes. A 2013 study in the journal of nutrition found that higher magnesium intake was associated with lower levels of fasting glucose and insulin. Also Cong et al.[23] reported that magnesium intake decreases fasting blood glucose in type 2 diabetic rat. Diminished level of magnesium was observed to decrease tyrosine kinase activity at insulin receptors.[24] In this study, the treated rats showed an important increase in insulin receptors when compared with diabetic untreated, with the metformin-magnesium supplemented group slightly higher than only metformin treated group, but the control group showed a higher insulin receptor than all the diabetic groups. This study showed that magnesium supplement when combined with metformin as an adjunct therapy increases insulin receptor. These present result is in line with the view of Cong et al.[23] who reported the effect of magnesium on expression of insulin receptor in type 2 diabetes. This is also in association with Paolisso et al.[25] who reported the view, insulin-mediated glucose uptake. This study showed increase plasma insulin in the untreated diabetic group suggesting insulin resistance.[26] The plasma insulin level was lower in the metformin treated and the metformin-magnesium supplement treated group suggesting improvement in insulin resistance.[27] This finding is similar to the report by Song et al.[28] that dietary magnesium intake has inverse association with plasma insulin level in type 2 diabetes.

Conclusion

Based on the research carried out and documented in this report, magnesium supplement offered potential anti-diabetic property when used as adjunct therapy with the standard anti-diabetic drug metformin. More research work should be carried out testing for more parameters on the anti-diabetic property of magnesium supplement using varying doses as a mono-dietary therapy and as a combination therapy with other standard drugs.

Acknowledgement

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Conflict of interest

None.

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