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*Research Article*

# Effect of high plant (cowpeas) and animal (casein) proteins on urinary –N-acetyl-beta-D- glucosaminidase (NAG) and micoalbuminuria in rats

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*High-protein diet, Urinary NAG, Microalbumin*

## ABSTRACT

**Background:** Urinary NAG activity is one of the most frequently evaluated urinary enzymes used in the diagnosis of renal tubular toxicity in recent time. Urinary NAG activity has been reported to precede changes in serum creatinine and endogenous creatinine clearances while microalbumin is the excretion of albumin in urine; this is highly variable, ranging from non detectectable quantities to milligrams of albumin. The aim of this study is to determine the effect of the intake of high plant (Cowpeas) and animal (Casein) proteins on urinary NAG and microalbuminuria in rats. **Methods:** One hundred and eighty wistar rats were used in this study. The rats were randomly distributed into 8 experimental groups (20 per group) and control (20). Blood and 24 hour urine samples were collected at baseline, 1 month, 3 months and 6 months intervals. Urinary NAG and microalbumin were determined spectrophotometrically. **Results:** Urinary NAG concentration was observed to be significantly higher ($P<0.01$) in urine of rats on casein diets when compared to baseline values, increases of over tenfold were observed. When NAG values of cowpeas fed rats was compared to that of casein fed rats, there was a significant increase ($P<0.01$) in urinary NAG values of rats fed with casein diet and the increase seen was proportional to dosage and duration. However for urinary microalbumin concentration, there was a significant increase in urinary microalbumin concentration of 30% casein fed rats when compared to baseline at 1month ($P<0.05$), which dropped at 3 months and 6 months and for the 40% casein diet, there was a significant increase ($P<0.01$) at 1 and 3 months but dropped at 6 months. Values of 30% cowpeas fed rat were significantly higher ($P<0.01$) than baseline values at 1 month and at 3 months for 35% cowpeas fed rats. Others were not significant. **Conclusion:** This study has clearly shown that the intake of a high plant protein, cowpeas leads to mild increase in NAG and microalbumin in urine. On the other hand, an intake of high casein diet, an animal protein, resulted in a marked increase in urinary NAG and mild increase in microalbumin. This study has also shown that the increase of urinary NAG precedes that of microalbumin



## INTRODUCTION

N-acetyl-β-D- glucosaminidase (NAG) is a lysosomal enzyme abundantly present in the renal proximal tubular cell, (Welwood *et al.*, 1978). It has a large molecular weight of 130,000 to 140,000 daltons which therefore its urinary excretion is relatively constant with minimal diurnal changes. Urinary NAG activity is one of the most frequently evaluated urinary enzymes used in the diagnosis of renal tubular toxicity in recent time. It is a very sensitive marker of renal tubular impairment (Price 1992). Furthermore, the increased urinary activity of NAG precedes changes in the serum creatinine or endogenous creatinine clearance; and the urinary NAG activity has been reported to correlate with the activity of the disease (Chiu, 1992). Caliskan *et al.*, (1996), in a study in Turkey on children having Nephrotic Syndrome, with and without steroid–resistance, was compared with healthy children. They found a significantly higher level of NAG in the

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nephrotic groups compared to the controls as well as in steroid – sensitive patients the level of NAG was significantly higher than those in remission. Finally, they also found positive correlation between proteinuria and urinary NAG excretion in nephrotic patients when compared with controls. The researchers argued that massive glomerulo-proteinuria might cause urinary NAG excretion in primary renal disease. Dillion *et al.*, (1998), Fede *et al.*, 1999 and Tomlinson *et al.,* (1997) also reported a positive correlation between proteinuria and urinary NAG. In another study by Tomlinson *et al.*, (1997), there was correlation between urinary NAG excretion and albumin excretion when pediatric patients with steroid sensitive nephrotic syndrome were compared with those with steroid resistant nephrotic syndrome. Brenner (1982) and many other researchers, have proposed that habitual consumption of excessive dietary protein negatively impacted on kidney function by a sustained increase in glomerular pressure and renal hyperfilteration. It has also been established that NAG excretion precedes that of GFR, due to the large functional reserve of the kidney; (Chiu, 1972).

Protein is a nutrient that is required for growth and maintenance and the normal requirement for rat is 5% for maintenance and 15% for growth and lactation while dietary reference guideline for human is 0.8g/kg body weight. Carbohydrate make up the bulk of rats diet, however requirements for dietary energy are often expressed as a function of basal metabolic rate (BMR). In general, energy requirements for rats can be met by diets with a wide range of energy densities, and animals will adjust their intakes to meet their requirement, (National Research Council, 1995).

It is the aim of this study to look at the effect of plant (cowpeas) and animal (casein) proteins as high protein diets on urinary excretion of NAG and microalbumin as an early marker in the diagnosis of renal impairment.

## MATERIALS AND METHODS

*Number of rats /group:*

One hundred and sixty wistar rats of both sexes were used for this study; the rats were divided into two groups of A and B based on the amount of protein in their diet with 80 rats per group. Groups A and B were further divided into subgroups of 20 per subgroup. A1 (25% cowpeas diet), A2 (30% cowpeas diet), A3 (35% cowpeas diet) and A4 (40% cowpeas diet) and group B were divided into subgroups of B1 (25% casein diet), B2 (30% casein diet), B3 (35% casein diet) and A4 (40% casein diet). Group C (20) was the control group and the rats were fed the normal rat chows (15% protein). The rats were allowed to acclimatize for two weeks before administration of experimental diet. Rats were housed in the Department of Pharmacology animal house in wooden cages according to their groups with males separated from the females. The constituted feeds were made into pellets; rats were grouped based on the diet they were fed with, the rats were allowed free access to food and water throughout the duration of the study. Rats were transferred to individual metabolic cages 48 hour prior to sample collection; weights of the rats were taken at the beginning of the study; at 1 month; 3 months and 6 months prior to collection of samples.

*Preparation of diet:*

Cowpea: This was bought in our local market, cleaned and oven dried at $100^{o\ c}$ for 45 minutes to remove the anti-nutritional factor (Osman, 2007). The oven heated cowpeas was then blended and added to the other constituents of the diet. For the casein diet, casein powder was bought and used to constitute diet B. The proximate composition of the diet is as shown below.

**Table 1**: Proximate composition of experimental diet a (100%) - cowpeas

| Constituents | Control | A1 (25%) | A2 (30%) | A3 (35%) | A4 (40%) |
|---|---|---|---|---|---|
| Protein (%) | 15 | 25 | 30 | 35 | 40 |
| Carbohydrate | 47.19 | 39.95 | 32.26 | 26.45 | 20.55 |
| Moisture | 10.38 | 6.70 | 6.92 | 7.25 | 7.17 |
| Fiber | 16.35 | 15.35 | 15.32 | 15.25 | 15.38 |
| Fat/oil | 5.0 | 9.9 | 10 | 9.8 | 10.3 |
| Ash | 6.08 | 3.10 | 5.5 | 6.25 | 6.60 |

At the start of the study, all rats were weighed and rats were housed in metabolic cages for urine collection. Urine samples were collected into universal containers and refrigerated at -20$^{o}$C. Samples were analyzed within one week of collection. NAG value was determined by method of Price and Whiting (1992). Urinary microalbumin was determined by method of Feldt-Rasmussen *et al*., (1994).

*Statistical analysis*

Results were analyzed using SPSS version 20.0, and one-way analysis of variance (ANOVA), LSD post-hoc test was used to determine pair wise differences among groups. Students't -test was used to determine the difference between cowpeas fed rats (A) and casein fed rats (B).

**Table 2:** Proximate composition of experimental diet (b)- (casein)

| Constituents | C (15%) | B1 (25%) | B2 (30%) | B3 (35%) | B4 (40%) |
|---|---|---|---|---|---|
| **Protein (%)** | **15** | **25** | **30** | **35** | **40** |
| Carbohydrate | 47.19 | 31.66 | 26.47 | 22.07 | 16.89 |
| Moisture | 10.38 | 9.54 | 9.56 | 9.55 | 9.63 |
| Fat/oil | 5.0 | 10.01 | 10 | 9.8 | 10.4 |
| Fiber | 16.35 | 17.0 | 17.22 | 17.23 | 17.25 |
| Ash | 6.08 | 6.79 | 6.75 | 6.35 | 5.83 |

**RESULTS:**

**Table 3:** Mean ± SEM) of urine n-acetyl-beta-D-glucosaminidase (NAG) concentrations (IU/l) of rats fed cowpea (A1), casein (B1) and control diets.

| | Baseline | 1 month | 3 months | 6 months | |
|---|---|---|---|---|---|
| A1 | $0.48^{c}$±0.05 | $0.96^{c}$±0.16 | $2.7^{b}$±0.20 | $4.4^{a}$±0.6 | **P<0.01 |
| B1 | $0.48^{b}$±0.05 | $25.9^{a}$±1.8 | $22.72^{a}$±2.73 | $4.72^{b}$±2.16 | **P<0.01 |
| T-test | P>0.05 | P<0.05 | **P<0.01 | P>0.05 | |
| A2 | $0.48^{b}$±0.05 | $5.84^{a}$±1.32 | $0.98^{b}$±0.3 | $2^{b}$±0.32 | **P<0.01 |
| B2 | $0.48^{c}$±0.05 | $55.96^{b}$±12.23 | $61.58^{b}$±13.16 | $87.92^{a}$±7.46 | **P<0.01 |
| T-test | P>0.05 | **P<0.01 | **P<0.01 | **P<0.01 | |
| A3 | $0.48^{b}$±0.05 | $10.74^{a}$±1.55 | $0.72^{b}$±0.2 | $3.3^{b}$±1.75 | **P<0.01 |
| B3 | $0.48^{b}$±0.05 | $93.65^{a}$±8.45 | $59.56^{a}$±4.31 | $88.4^{a}$±4.62 | **P<0.01 |
| T-test | P>0.05 | P>0.05 | **P<0.01 | **P<0.01 | |
| A4 | $0.48^{c}$±0.05 | $11.16^{a}$±1.61 | $5.78^{b}$±1.29 | $8.64^{b}$±0.96 | **P<0.01 |
| B4 | $0.48^{c}$±0.05 | $107.48^{a}$±15.09 | $60.82^{b}$±6.92 | $91.46^{b}$±4.45 | **P<0.01 |
| T-test | P>0.05 | **P<0.01 | **P<0.01 | **P<0.01 | |

**Table 4:** Mean ± SEM of urinary microalbumin of rats fed cowpeas (A), casein (B) and control diets

| | Baseline | 1 month | 3 months | 6 months | |
|---|---|---|---|---|---|
| A1 | 0.79±0.2 | 1.64±0.5 | 0.68±0.21 | 1.74±0.65 | P>0.05 |
| B1 | 0.79±0.2 | 2.42±0.67 | 2.3±0.36 | 1.76±0.28 | P>0.05 |
| T-test | P>0.05 | P>0.05 | P<0.05 | P>0.05 | |
| A2 | $0.79^{a}$±0.2 | $1.06^{a}$±0.29 | $0.05^{b}$±0 | $0.1^{b}$±0.03 | **P<0.01 |
| B2 | $0.79^{b}$±0.2 | $3.46^{a}$±1.15 | $2.7^{b}$±0.25 | $2^{b}$±0.3 | *P<0.05 |
| T-test | P>0.05 | P>0.05 | **P<0.01 | **P<0.01 | |
| A3 | $0.79^{b}$±0.2 | $1.36^{b}$±0.39 | $3.28^{a}$±0.71 | $0.06^{b}$±0.01 | **P<0.01 |
| B3 | $0.79^{b}$±0.2 | $6.06^{a}$±1.76 | $1.66^{b}$±0.33 | $1.64^{b}$±0.24 | **P<0.01 |
| T-test | P>0.05 | P>0.05 | P>0.05 | **P<0.01 | |
| A4 | 0.79±0.2 | 1.19±0.36 | 0.79±0.21 | 2.5±1.28 | P>0.05 |
| B4 | $0.79^{b}$±0.2 | $3.44^{a}$±0.51 | $2.7^{a}$±0.64 | $1.3^{b}$±0.22 | **P<0.01 |
| T-test | P>0.05 | P<0.05 | P<0.05 | P>0.05 | |

*P<0.05- Significant, **P<0.01 = Highly Significant. Different superscript letters across the rows show that the means are significantly different from each other.

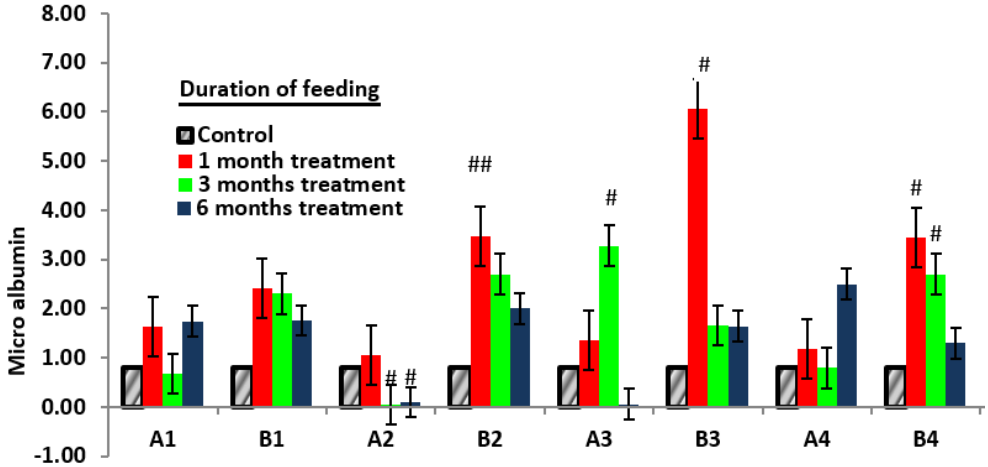


**Fig.1**: Mean ± SEM) of urinary microalbumin of rats fed cowpea (A), casein (B) and control diets. Key: A1= 25% Cowpeas, B1= 25% Casein, A2= 30% Cowpeas, B2= 30% Casein, A3= 35% Cowpeas, B3= 35% Casein, A4= 40% Cowpeas, B4= 40% Casein. Note: ## (P<0.01) and # (P<0.05) for control vs various months.

There was a significant increase in urinary NAG values in rats fed casein diet when compared to control and rats fed cowpeas diets in the various doses of the diet and at varying durations of feeding.

When urinary microalbumin of control was compared with cowpeas and casein fed rats, there was no significant difference in the 25%. But there was a significant increase in urinary microalbumin at 30% cowpeas and casein fed rats for one month which dropped at 3 and 6 months. When rats were fed 35% cowpeas diet, there was a significant increase in urinary microalbumin concentrations at 3 months which dropped at 6 months, while for the 35% casein fed rats there was a significant increase in 1 month but later dropped at 3 and 6 months. Rats fed 40% casein diets had significantly high microalbumin values in urine while cowpeas fed rats did not show any significant difference with control.

## DISCUSSION

Urinary NAG concentrations in cowpeas fed rats and casein fed rats were significantly higher than control values, with values of rats fed casein diet higher than cowpeas fed rats and control. A significant positive correlation has been found between chronic renal insufficiency patients and 24 hr urinary NAG levels (Tassi *et al*., 2003). The researchers reported that total effective daily excretion of urinary NAG could have a practical importance not only for early renal recognition or renal co-involvement but also that it could be repeated in serial measurement to monitor the progress of the disease. Szechinski and Wiland (1997); carried out a study on 63 renal failure patients and they observed that 92.2% of the patients with chronic renal failures had abnormal NAG values. In our study, we observed a significant increase in NAG values of rats fed with casein diet. Urinary NAG has also been predictive marker for the development of micro albuminuria in adolescents with diabetes, as urinary NAG excretion preceded the increase of albumin excretion (Kordonouri *et al*., 1994). Goragen and Simoni, (2012) and Goray *et al.*, (2013) have reported reduction in urinary NAG and albuminuria in hypertensive nephropathy placed on increased fruits and vegetables. These agree with our findings in this study, rats fed on cowpeas (plant protein) diet had a significantly lower NAG values when compared to casein fed rats. Goraya *et al.*, (2013), in their study on the effect of increased fruits and vegetables to reduce dietary acid by half, they observed that there was a decline in urinary NAG and albuminuria when patients with stage 4 chronic kidney disease were placed on increased fruits and vegetables for one year. We also observed a significant difference in urinary micro albumin concentrations between control and casein fed rats as well as cowpea fed rats. In our study, rats that were fed cowpeas diet, had lower excretion of albumin in urine while rats fed with casein diet had significantly higher excretion of albumin in urine. Values of control were lower than those of casein fed rats. In a study carried out by Caliskan *et al.*, (1996) on children having Nephrotic Syndrome, they reported a correlation between proteinuria and urinary NAG excretion in nephritis patients when compared to controls. They argued that massive glomerulo-proteinuria might cause urinary NAG excretion in primary renal disease. In our study, there was no significant correlation between urinary NAG excretion and urine microalbumin. Dillion (1998), Fede (1999) and Tomlinson (1997) also reported a positive correlation between proteinuria and urinary NAG. We also observed that rats fed with casein diet, had significantly higher levels of NAG and microalbumin and urinary NAG excretion in casein fed rats were very consistent and also proportional to

dosage and duration. We conclude that in apparently healthy rats, high casein diet resulted in urinary excretion of NAG.

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