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Research article

Effect of Estrogen and Sodium Chloride on Fasting Blood Sugar and Weight-Gain in Female Diabetic Rats

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ABSTRACT: The effect of estrogen and sodium chloride (NaCl) on fasting blood sugar and weight gain was investigated in female diabetic rats. Changes in serum sodium/potassium ratio on fasting blood sugar (FBS) was also investigated. Female wister rats with an average weight of 150gms were used for this study. 32 healthy rats were used for the control experiments. They were divided into 4 subgroups of 8 rats each which served as control, estrogen treated, NaCl treated, and combination of estrogen and NaCl treated. The remaining 32 rats were made diabetic by intraperitoneal injection of alloxan (100mg/kg body weight) and divided into 4 sub groups as in the healthy rats. FBS, body weights and serum sodium/potassium ratio were determined in all the animals. The study indicates that both estrogen and sodium chloride significantly ($P<0.05$) lowered FBS in the female diabetic rats. However, the reduction of FBS level in the healthy (non-diabetic) rats - was significant ($P<0.001$) - though not sustained throughout the study. Treatments of the rats by using a combination of estrogen with sodium chloride showed a significant ($P<0.001$) reduction of FBS level. However, this reduction was not more than observed by treatment with only estrogen. This means that the substances do not have a cumulative effect in both diabetic and healthy rats. There was however, no significant difference ($P>0.05$) in serum sodium/potassium ratio in all the subgroups. The result of the investigation also demonstrates that there is significant ($P<0.05$) retardation of the weight-gain due to estrogen while sodium chloride significantly enhanced weight-gain ($P<0.001$) in both healthy and diabetic female rats. It was therefore concluded that both estrogen and sodium chloride enhance glucose utilization.

Key Word: Estrogen, NaCl, FBS, Diabetes, Weight-gain, Female Rats

INTRODUCTION

Estrogen has been reported to play a major role in lowering of blood sugar [Alonso *et al* 2006].This explains why female Wistar rats fed with fructose did not develop insulin resistance and hyper-insulinaemia compared with age-matched male [Galipeau *et al* 2002]. Administration of sodium chloride (NaCl) to rats was reported to increase glycogen deposit in the liver, thereby causing a lower blood level of glucose [Crabtree and Longwell, 1936]. The tolerance of the diabetic rat to intraperitoneally administered glucose was improved when NaCl was co-administered with the glucose [Orten and Delvin, 1940].

Although is generally known that women using estrogen containing oral contraceptive pills complain of weight gain, the role of estrogen on body weight are still doubtful [Oelkers, 1995]. However, estrogen has been shown to increase fat metabolism by a mechanism that is still not fully understood [Ashley *et al*, 2000]. The daily ingestion of between 1-2g of sodium chloride per kilogram of body weight resulted in gain of about 4%-5% of body weight within 2-4 days [McQuarrie *et al*, 1936].The mechanism of this weight gain that is

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apparently caused by sodium chloride has not been clearly explained.

An increase in serum sodium/potassium ratio was suggested as a possible mechanism by which NaCl enhances glucose utilization [Orten and Delvin, 1940].This postulation was investigated in this study. Estrogen which is known to cause sodium retention [British National Formulary, 2001] and NaCl were used to verify whether the lowering of FBS was induced by an increase in the serum sodium/potassium ratio.

The effects of estrogen and sodium chloride on FBS and weight-gain in healthy and diabetic female rats were compared. This study also investigated the possible synergistic action of estrogen and sodium chloride.

MATERIALS AND METHODS

Animals

Sixty four (64) female wistar rats with average weight of 150g were used for this study. Rats were procured from the animal house of the College of Medicine, University of Ibadan. They were fed with normal rat pellets and watered *ad libitum*. They were allowed to acclimatize for two weeks before commencement of the experiment.

Drug Solutions/Reagents

Estrogen: A Premarin® tablet which is a brand of conjugated estrogen produced by Weyth Pharmaceuticals Inc. was used in this study. 12.5 mg (i.e. 10 tablets) of Premarin tablets was dissolved in 52 ml of distilled water at a temperature of 37^0C using Paddle method of invitro dissolution of estrogen tablets. The solution was stirred until all particles of the tablet were totally dissolved to form a stock solution of 0.24 mg of conjugated estrogen per milliliters (0.24 mg/ml). The solution was prepared fresh and kept refrigerated until use.

Sodium Chloride: 25.6g of Sodium chloride was dissolved in 160 ml of distilled water to obtain a stock solution of 0.16g NaCl/ml. The solution was prepared fresh and kept refrigerated until use.

Experimental Protocol

The 64 rats were divided into two (2) main groups; A (Healthy group, i.e. non-diabetic) and B (Diabetic group). Each group contained 32 rats that were further divided into four subgroups of 8 animals each.

Group A (Healthy Rats): Group A contained 4 subgroups (A1, A2, A3 and A4) of 8 animals each. These represented; control, estrogen treated, NaCl treated and combination of estrogen and NaCl treated: A1 - was given no treatment (control), A2 - received oral estrogen at a daily dose of 0.6 mg/kg body weight [Noris *et al*, 2000]. A3 - received oral NaCl at a daily dose of 0.8 g/kg bodyweight [Furihata *et al*, 1996].A4 - Received both oral estrogen (0.6 mg/kg body weight) and NaCl (0.8 g/kg body weight) daily.

Group B (Diabetic Rats): Group B also contained 32 rats. All animals in this group were made diabetic by intraperitoneal injection of alloxan (100mg/kg body weight) after an overnight fast (minimum of 14 hours) [Carvalho *et al*, 2003]. Thereafter, the animals were allowed free access to food and water. The rats were divided into four subgroups (B1, B2, B3 and B4) as in the healthy group. Blood obtained from the tip of the tail was used to determine FBS in each rats daily until diabetes was established. Rats with fasting blood sugar levels above 150 mg/dl were considered as having diabetes mellitus [Carvalho *et al* 2003; Harkness and Wagner, 1993]. Following establishment of diabetes mellitus, B1 - were given no treatment, this served as diabetic control. B2 - were given oral estrogen (0.6 mg/kg body weight) daily. B3 - were given oral NaCl (0.8 g/kg body weight) daily. B4 - were given both oral estrogen (0.6 mg/kg body weight) and NaCl (0.8 g/kg body weight) combined daily. FBS was determined in all the animals on days; 0, 4, 8, 12, 16, 20, 24, 28 and 32 of the study. The animals were weighed weekly and serum sodium and potassium was analyzed in all the subgroups on days; 0, 8, 16, 24 and 32 of the study.

The oral administration of drugs was done using oro-gastric cannula.

Determination of Fasting Blood Sugar (FBS)

FBS of the rats was measured at intervals using a glucometer with strips (Prestige IQ ® blood monitoring system, AR-Med LTD, Runny Mede Malthouse, Egham TW209BD, UK). A drop of blood is placed on the strip and the appropriate blood sugar concentration is displayed on the glucometer screen after 10 – 50 seconds. The glucometer employs glucose oxidase principle for blood glucose measurement (Trinder, 1969)

Determination of Serum Sodium /Potassium

2.5mls of blood was obtained through the retro-orbital sinus into lithium heparin bottle. The blood was centrifuged to obtain serum and the serum sodium/potassium ratio was analyzed using flame photometer.

$$\text{Serum Sodium/Potassium Ratio} = \frac{\text{Average Serum Sodium Level}}{\text{Average Serum Potassium Level}}$$

Animal Weight

Animals in all the groups were weighed on a weekly basis. Weighing was done with the aid of a digital electronic kitchen weighing scale (SF-400).

Statistical Analysis

Data on rats which died in the course of the study were excluded from the analysis. Mean and standard deviation were used to report variables. Trend charts for change in means of fasting blood sugar, weight and sodium/potassium ratio were presented. The General Linear Model repeated measures procedure was used to analyze repeated measurements. The F statistic was used as the test statistic and level of statistical significance was 5%.

RESULTS

Effect of Estrogen and Sodium Chloride on Fasting Blood Sugar: Figure 1 shows the pattern of blood sugar changes across the various treatment groups for healthy rats. There were reductions in blood sugar for those on estrogen, sodium chloride (NaCl) and combined estrogen and NaCl after 4days of treatment while the control group showed relatively constant blood sugar levels over the period. There was no significant difference ($P>0.05$) between the lowering effect caused by estrogen and that caused by NaCl after 4 days of treatment. Both substances significantly ($P<0.05$) lower FBS in isolation than when given combined. However, the reduction in blood sugar was not sustained with continuing treatment.

Figure 2 shows the pattern of blood sugar changes across the various treatment groups for diabetic rats. After sixteen days of treatment, estrogen significantly ($P<0.05$) lowered blood sugar, likewise combined estrogen and NaCl. The lowering caused by NaCl only after 16days of treatment was not significant ($P>0.05$). At the end of 32 days of treatment, there was significant ($P<0.05$) reduction in blood sugar in diabetic rat taking estrogen only, NaCl only and combined estrogen and NaCl. However, while treatment with estrogen only caused about 50.58% reduction in blood sugar, combined estrogen and NaCl caused 44.29% reduction and treatment with NaCl only resulted in 20.35% reduction in blood sugar.

Effect of Estrogen and Sodium Chloride on Weight-Gain: There were increases in weight for all healthy treatment groups. The most rapid weight gain was recorded in rats given combined estrogen and NaCl, followed by NaCl only, then controls and least for estrogen only group (Table 1). Those on combined estrogen and NaCl and those on estrogen alone however recorded a decline in weight gain by the third week.

The pattern of change for those on estrogen after the second week appeared to be different from other groups and they had a significant decline by week 4. After 32 days of treatment, the average total weight gain among the control rats was 28.7%, while those on estrogen only recorded just 13.6% weight gain. Those on NaCl treatment had 26.2% total weight gain while rats treated with both estrogen and NaCl combined recorded 23.3% average total weight gain. The differences were statistically significant ($P<0.001$)

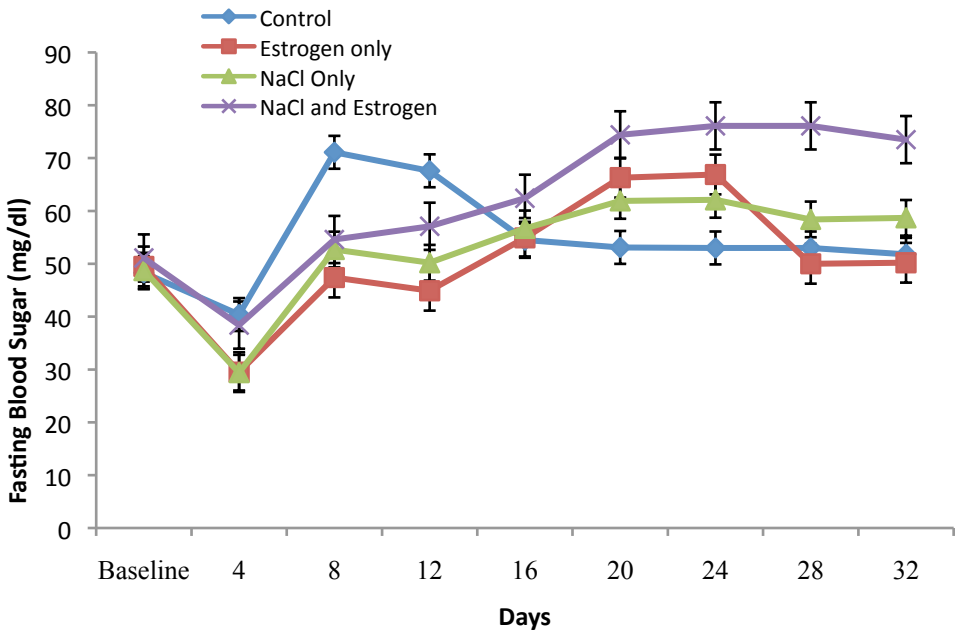

Figure 1:
Pattern of Change in Mean Fasting Blood Sugar Among Healthy Rats Treated With Estrogen and Sodium Chloride

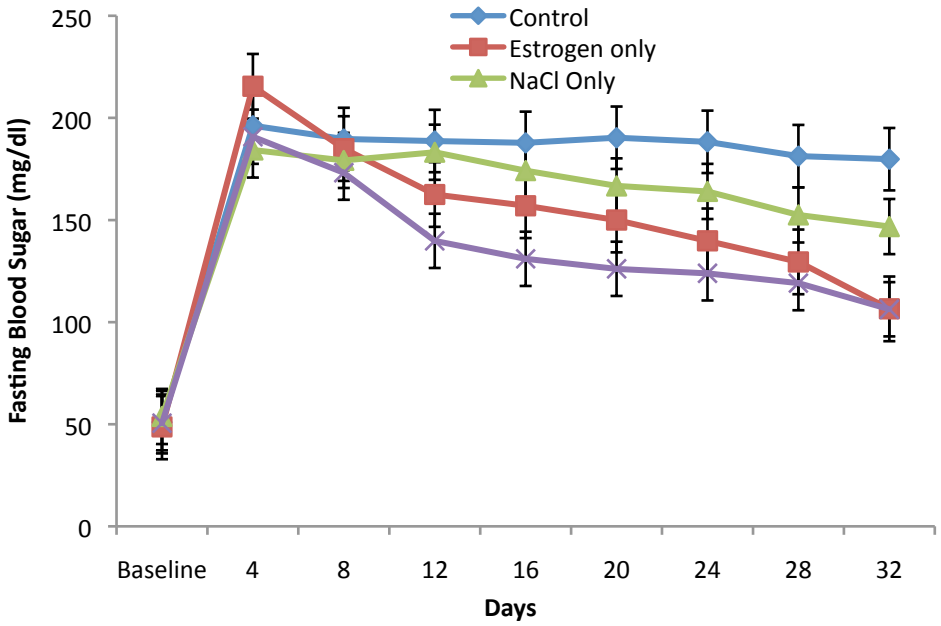

Figure 2:
Pattern of Change in Mean Fasting Blood Sugar among Diabetic Rats Treated With Estrogen and Sodium Chloride

Table 1:
Weight Changes in Healthy Rats Treated With Estrogen and Sodium Chloride

Treatment Group	Baseline	Week 1	Week 2	Week 3	Week 4
Controls	134.0±11.3	138.1±13.0	149.1±17.1	164.1±19.3	172.5±19.0***
Estrogen only	129.1±11.4	135.3±12.6	160.1±15.6	150.9±20.1	146.6±15.1**
NaCl only	140.6±11.4	149.7±10.8	172.7±8.2	174.7±5.2	177.4±4.5***
NaCl and estrogen	142.3±39.5	165.6±16.1	177.9±17.8	165.7±19.4	175.5±20.6*

*Values are expressed as Mean± SD. Asterisk indicates level of statistical significance between baseline values and Week 4 values. * p<0.05, **p<0.002; ***p<0.001*

Table 2:
Weight Change in Diabetic Rats Treated with Estrogen and Sodium Chloride

Treatment Group	**Baseline**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Controls	134.7±9.2	148.0±7.7	164.0±16.0	176.7±13.5	182.3±14.0***
Estrogen only	143.2±15.7	131.5±31.3	143.7±27.4	146.7±29.7	150.7±30.5
NaCl only	136.2±7.1	156.7±20.6	168.5±24.6	172.0±30.7	178.3±30.7
NaCl and Estrogen	142.6±14.4	151.1±13.7	161.4±15.0	162.2±19.1	165.6±20.5*

*Values are expressed as Mean± SD. Asterisk indicates level of statistical significance between baseline values and Week 4 values. * p<0.05, **p<0.002; ***p<0.001*

Table 3:
Sodium /Potassium Ratio in Healthy Rats Treated With Estrogen and Sodium Chloride

Treatment Group	Baseline	8 days	16 days	24 days	32 days
Controls	31.3±0.8	34.8±1.0	32.9±1.0	30.0±1.8	24.1±1.3
Estrogen treated	28.8±0.7	31.8±1.3	33.6±2.6	32.7±0.6	31.4±3.1
NaCl treated	30.5±3.2	28.8±4.0	28.1±1.2	27.9±2.0	29.7±2.4
Estrogen and NaCl treated	29.7±1.1	35.8±4.4	31.7±2.4	26.6±0.3	23.1±2.0

Values are expressed as Mean± SD

Table 2 shows the pattern of weight change for the treatment groups among diabetic rats. Similar to the pattern for healthy rats those on estrogen or estrogen combined with NaCl had a decline after the second week.

Effect of estrogen and sodium chloride on serum sodium / potassium ratio: The pattern of changes in sodium /potassium ratio for healthy and diabetic rats is shown in tables 3 and 4 respectively. In all the 8 subgroups, the values of sodium/potassium ratio remained within normal limits i.e. 27-40 [Feldman *et al*, 1992], throughout the study period. There were no significant differences ($P>0.05$) in the effects of all the treatment varieties for both healthy and diabetics rats.

DISCUSSION

A significant reduction in fasting blood sugar was observed in diabetic rats taking daily oral estrogen as compared to the diabetic control in which fasting blood sugar remained persistently high ($P<0.002$). This is in agreement with the report that a diabetic woman on estrogen replacement therapy consistently records a higher fasting blood sugar each time the oral estrogen therapy was suspended [Diabetic update, 2007].

Relatively low doses of 17β-estradiol is reported to have beneficial effects on glucose homeostasis [Alonso *et al*, 2006]. Additionally, insulin sensitivity and glucose tolerance are improved in postmenopausal women subsequent to treatment with estrogen, either as estrogen only tablets or in combination with progesterone [Howard *et al*, 2004].

These corroborative evidences clearly justify the finding of a reduced fasting blood sugar with estrogen therapy in diabetic rats in this study.

In the healthy rats, estrogen therapy similarly reduced fasting blood sugar but this effect appears to be less apparent. This observed discrepancy in estrogen effect on fasting blood sugar between diabetic and healthy subjects cannot be well substantiated within the scope of this study.

On the contrary, the significant reduction in fasting blood sugar with sodium chloride treatment, observed in diabetic rats was not seen in the healthy counterpart. Rather, there was a slight rise in the mean FBS of healthy rats taking NaCl. Further work may be required along this line to fully elucidate this observation.

In previous works on human diabetics, high sodium chloride ingestion was found to result in lower levels of fasting blood sugar, less risk of ketonuria (complication of diabetes mellitus) and a high risk of insulin reaction. The high risk of insulin reaction occurred even with an insulin dosage that was otherwise optimal when sodium chloride ingestion was low (McQuarrie *et al*, 1936).This may imply that NaCl enhances insulin sensitivity or potentiates insulin action.

For the subgroups taking a combination of both estrogen and NaCl, since both substances separately lowers FBS, it is expected that there will be a further lowering of fasting blood sugar when compared to those taking either estrogen or NaCl alone. However, though there was a reduction in fasting blood sugar in the diabetic subgroup taking both treatments combined, the reduction is just similar to that observed in the subgroup taking estrogen only.

It may therefore not be impossible that both estrogen and sodium chloride lowers fasting blood sugar via a similar mechanism, and that the mechanism is through a system that is saturable. This means once the system is saturated, a maximum effect is seen and any further ingestion of the substances will not cause additional effect. This could be the reason why a combination of

both estrogen and sodium chloride did not cause any further reduction in fasting blood sugar than what was obtained with estrogen alone.

Also from the results of this study, it was clearly demonstrated that estrogen intake retards the rate of weight gain in both healthy and diabetic rats. This support the report that estrogen promotes fat metabolism and helps the body utilize fat and glucose as energy [Greenberg *et al*, 2005].

The observation from this study on the effect of estrogen on weight is in support of a previous report that estrogen stimulates the production of growth hormone (GH), which increases the mobilization of free fatty acid from adipose tissues [Robergs and Roberts, 1997]. An increased estrogen level in the body would be expected to augment the rate of adipose tissue breakdown, and since fat deposit constitutes a significant part of body weight, a retarded weight gain should be expected.

These findings also appear to be in line with the report of Lee and Howell, (2006), where estrogen was used to induce growth attenuation in tall girls and also to keep a developmentally disabled girl from growing to adult size [Gunther and Diekema, 2006].

On the contrary, sodium chloride was found to significantly enhance weight gain. This is in agreement with the work of McQuarrie *et al*. (1936), when the daily ingestion of between 1 – 2g of NaCl per Kg of body weight resulted in gain of about 4% - 5% of body weight within a period of 2 – 4 days.

The mechanism for this weight gain caused by NaCl has not been clearly reported; however it may not be unconnected with the enhanced glucose utilization and possibly water retention [British National Formulary, 2001].

In the group of rats given both estrogen and sodium chloride, the estrogen appears to neutralize some of the effects of NaCl on weight gain, and so the weight-gain in this group is not as pronounced as in those taking NaCl alone, though higher than what was observed with the estrogen only group. These effects on weight-gain follow the same trend in healthy as well as diabetic rats.

This study also attempted to find out if estrogen and sodium chloride increases the value of serum sodium to potassium ratio as a possible mechanism of lowering blood sugar as suggested by earlier researchers [Orten and Delvin, 1940]. There was no significant difference in this ratio with both treatments throughout the period of the study. It can therefore be concluded that even though both estrogen and NaCl enhance glucose utilization neither of the substances achieve this by increasing serum sodium to potassium ratio. Further research work will be required to ascertain the mechanism employed by these two substances.

Possible suggestions from this study is that both substances may enhance insulin production by the β-islet cells of the pancreas and/or increase target cell sensitivity to insulin.

In conclusion, estrogen and sodium chloride enhance glucose utilization significantly in diabetic female rats while this effect is minimal in healthy rats. Both substances do not act synergistically i.e. their blood sugar lowering effect is not cumulative and the effect was not achieved through increase in serum sodium/potassium ratio. Additionally, estrogen retards weight gain possibly by increasing fat metabolism, while sodium chloride on the other hand, causes a rapid weight-gain.

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