EXOGENOUS TESTOSTERONE STIMULATES GLUCONEOGENESIS IN HYPOPROTEINEMIC ALBINO RAT

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ABSTRACT

Changes in plasma glucose and protein concentrations in two experimental groups of albino rats, weighing 250 - 300g, were evaluated after 7 days of acclimatization to laboratory conditions and another 14 days of feeding the rats with low protein diets. Frank hypoproteinmia was evident by the low plasma protein levels and some clear physical manifestations, such as hair loss, change in skin colour and edema. Edema was caused by lowered plasma protein concentrations. Daily intraperitoneal (i.p.) injections of 0.2 ml of testosterone for a period of 7 days produced a statistically significant increase in plasma glucose concentrations (P < 0.01) when compared with the saline-treated controls. There was a statistically significant decrease (P <0.05) in total protein concentrations in testosterone injected hypoproteinemic rats when compared with the control rats. These findings suggest that testosterone, in addition to its anabolic function of protein build up in muscles, may also be involved in gluconeogenesis, the formation of plasma glucose from non-carbohydrate substrates. Apparently, the hypoproteinemic rats require enough glucose to survive since glucose is the only source of energy for the mammalian brain. The mechanism of action of steroid hormones on target organ cells, and the role of testosterone as a performance enhancing drug are discussed.

Keywords: Exogenous testosterone, Protein, Glucose, Gluconeogenesis, Hypoproteinemic rat

INTRODUCTION

Protein-energy malnutrition, also referred to as kwashiorkor, is a disease condition that results from hypoproteinemia (Heaton, 1986). Hypoproteinemia means a drop in blood protein. This condition often leads to edema since a drop in blood protein, chiefly serum albumin, means a decrease in colloid osmotic pressure (COP) which, in turn, means an increase in filtration pressure (FP). This explains the edema of malnutrition (Pihs, 1963; Zaloga and Roberts, 1994).

The major source of blood glucose during prolonged periods of fasting comes from protein (Mayer and Thomas, 1967). For survival of the brain, plasma glucose concentration must be maintained. Glycogen stores, particularly in the liver, act as the first line of defense. They are quickly broken down to glucose by a process known as hepatic glycogenolysis (Meyer and Thomas, 1967) and released into the bloodstream. They can supply the body's needs for several hours, but under prolonged conditions of starvation protein and fat are mobilized. Utilization of substrates, such as amino acids and glycerol, provides a glucose-sparing action, and the molecules may also be employed directly in glucose formation, a process known as gluconeogenesis (Meyer and Thomas, 1967)

In human beings, kwashiorkor kills more people than all other malnutrition diseases put together (Fakunle, 1986). Five million children in Africa, Latin America, and the Far East, die every year from kwashiorkor. It is a very common disease in tropical countries where the population lives on staple food such as plantains, cassava, or maize, which provides enough energy as carbohydrates but not enough protein for a growing child (Fakunle, 1986). Added factors are poverty, ignorance and taboos against giving milk, eggs, fish or meat to children (Heaton, 1986). In mammals, treatment for kwashiorkor or hypoproteinemia has often centered mainly on protein and amino acids supplementations (Heaton, 1986).

Androgens are known to inhibit the pituitary gland, stimulate protein anabolism or buildup in muscles, and cause the retention of potassium and phosphate (Olsen, 1979; Maclusky and Naftolin, 1981; Sairam *et al.,* 1981). Testosterone is the chief androgen, and the present investigation was designed to assign a possible role for exogenous testosterone in the treatment and maintenance of hypoproteinemic albino rats.

MATERIALS AND METHODS

Experimental Animals: 15 adult male albino rats, weighing 250 – 300 g, were collected from the department's animal house and acclimatized in the laboratory for 7 days. During acclimatization, all the animals were fed and watered *ad libitum* with diet formulated from mixture of maize 50g, fishmeal 20g, and groundnut cake 40g. The proximate composition of the balanced diet is presented in Table 1.

Hypoproteinization of Rats: 10 of the acclimatized rats were placed on a diet of carbohydrates consisting of a mixture of maize, plantain, cassava, and rice for 14 days. This feeding regime (Table 1) rendered the rats hypoproteinemic as compared with the rats maintained on the balanced diet. The hypoproteinemic rats were divided into 2 groups, B and C, and housed in separate rat cages. The

proximate composition of the high carbohydrate diet is presented in Table 1.

Group A rats served as controls and were given daily intraperitoneal (i.p.) injections of physiological saline (0.6 % saline) of 2.5 ml/kg body weight. Group B rats were given daily i.p. injections of saline, while group C rats were injected with 0.2 mg/kg body weight of testosterone for a period of 7 days.

At the end of the experiment, animals were properly anaesthetized with chloroform prior to being sacrificed. Blood was collected by cardiac puncture into heparinized test tubes. The test tubes were centrifuged for 15 minutes at 2000 revolutions per minute (rpm). The plasma rich supernatant was decanted into clean test tubes for protein and glucose determination and the packed blood cells residue were discarded. This was repeated three times and the mean values recorded.

Table 1: Gross and Proximate compositions of balanced and high carbohydrate diets

Food items	A	B
Maize	50g	20g
Plantain	-	20g
Rice	-	20g
Fishmeal	20g	-
Groundnut cake	40g	-
Composition		
Moisture	19.9%	24.4%
Ash	1.6%	0.7%
Lipid	25.8%	11.6%
Protein	12.1%	4.6%
Carbohydrates	40.7%	44.8%

A - Balanced diet, B - High carbohydrate diet

Analytical Procedure: The proximate compositions of both the balanced diet and the hypoproteinemic diet were analyzed by methods described by Windham (1996)

Physical Changes in Experimental Albino Rats: The five rats, which served as controls were fed *ad libitum* with balanced diet. The 10 rats, which served as experimentals were fed with high carbohydrate diet throughout the period of experimentation. Physical changes between the control and hypoproteinemic rats were observed and recorded daily.

Weighing of Liver and Kidneys: After drawing the blood from the control and experimental rats, livers and kidneys were removed, cleaned with blotting paper and weighed on a Mettler balance. The weights of the livers and kidneys of each group were added together and the mean calculated by dividing the total weight of each group by the number of rats in that group, which is 5 rats.

Quantitative Determination of Total Plasma Protein and Glucose Levels: The glucose concentrations of plasma were determined by the glucose oxidase method (Folin and Malmrose, 1929; modified by Free, 1963). The experiment was replicated three times.

Quantitative Determination of Total Plasma Protein Concentration: The total protein concentrations of plasma were determined by the biuret method (Tietz, 1995, 1999). The experiment was repeated three times and the mean value taken.

Statistical Analysis: All results were expressed as means ± standard deviation (SD). Statistical comparisons between the two groups of hypoproteinemic albino rats were performed using the Student's two - tailed test for unpaired data. The analysis of variance (ANOVA) for statistical difference among the different groups of rats was performed by means of Steel and Torrie (1980).

RESULTS

Physical Manifestations of Hypoproteinemia: Physical manifestations of hypoproteinemia were evident from this investigation. Normal albino rats had weight gains, no hair losses, and no changes in colour. In contrast, the hypoproteinemic rats developed edematous appearances, hair losses and changes in skin colour. They remained miserable or listless as compared with the normal rats.

Mean Weights of Livers and kidneys: There was no significant change in the mean weights of the livers of Group A rats (7.2 ± 0.33 mg) and the Groups B and C rats (7.1 ± 0.30 mg). Similarly, there was no significant change in the mean weights of the kidneys of Group A rats (1.2 ± 0.12 mg) and Groups B and C rats (1.1 ± 0.12 mg)

Mean Plasma Glucose Concentration of the Groups of Rats: The mean plasma glucose concentrations of the three groups of rats (A, B, and C) are presented in Table 2. There was a statistically significant increase ($P < 0.01$) between the plasma glucose concentrations in Group A rats (84.75 ± 1.35 mg%), Group B rats (104.69 ± 3.46 mg%) and the Group C rats (128.50 ± 1.89 mg%).

Table 2 Changes in Plasma glucose and total protein concentrations of the groups of rats (mg/100 ml)

	Plasma glucose ± SD mg%	Total protein ± SD mg%
A	84.75 ± 1.35	7.45 ± 0.13
B	104.65 ± 3.46	6.41 ± 0.19
C	128.50 ± 1.89	5.90 ± 0.20

A = Normal rats treated with saline, B = Hypoproteinemic rats injected with saline, C = Hypoproteinemic rats injected with testosterone

Mean Plasma Total Protein Concentration of the Groups of Rats: The mean plasma total protein concentration of the three groups of rats (A, B and C) is presented in Table 2. There was a statistically significant decrease ($P < 0.05$) between the plasma total protein concentrations in Group A rats (7.45 ±

0.13 mg %), Group B rats (6.41 ± 0.19 mg %) and group C rats (5.90 ± 0.20 mg %).

DISCUSSION

Hypoproteinemia leads to a disease condition known as kwashiorkor. In mammals, treatment for kwashiorkor has often centered on amino acid supplementations. In humans, patients suffering from protein-energy deficiency, or kwashiorkor, manifest signs of edema (Heaton, 1986).

Androgens, like other steroid hormones, are known to act by stimulating protein synthesis within their target tissues (Chan and O'Malley, 1976; Vesely, 1980; Brooks, 1981). The major functions of testosterone include stimulation of the development of male secondary sexual characteristics, and protein anabolism (buildup) in muscles (Edelman and Marvar, 1980).

In the present investigation, some Albino rats were experimentally rendered hypoproteinemic by feeding them with high carbohydrate diets for a period of two weeks. Frank hypoproteinemia was established by lowered plasma protein concentrations in addition to physical manifestations. In normal rats there was increase in mean body weight, no hair losses, and no changes in skin colour. In contrast, the hypoproteinemic rats developed edematous appearances, hair losses, and changes in skin colour. They remained miserable or listless as compared with the normal rats. The results showed no significant changes in the mean weights of the livers and kidneys between the saline- treated rats and the testosterone-injected rats.

The mean plasma glucose concentration of the testosterone-treated rats was statistically higher ($p<0.01$) than the saline-treated hypoproteinemic controls. There was a statistically significant decrease ($P<0.05$) in plasma total protein concentration between the saline-treated control rats and the testosterone-injected experimental rats (Figs. 1, 2 and Table 2).

These findings suggest that testosterone significantly lowered the plasma total protein and elevated the plasma glucose levels. In fact, the rise in blood glucose concentration is concomitant and proportional to the fall in blood total protein concentration when compared with the control rats. It can be conclusively surmised that the androgen, testosterone, in addition to its anabolic function of protein buildup in muscles, may also be involved in the formation of plasma glucose from non-carbohydrate substrates, such as proteins, amino acids and lipids, a process referred to as gluconeogenesis (Perssin and Bell, 1992). Apparently, the hypoproteinemic rats require enough glucose to survive since glucose is the only source of energy for the brain of mammals, including human beings (Guillemin, 1978).

Recent report (Ndukuba, 2004) showed that exogenous thyroxine stimulated gluconeogenesis in normal albino rats. The present finding is interesting since, like thyroid hormones, steroid hormones employ the mobile receptor mechanism and act on the genome to induce messenger ribonucleic acid (mRNA) synthesis and subsequent protein synthesis (Jacobs and Cuatrecasas, 1977; Iynedjian, 1993). Testosterone has been classified as a performance enhancing drug by World Athletic Federations. Thus, athletes who test positive to testosterone during routine checks are banned for life from competitive sporting events.

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