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# Alterations in kidney enzyme pattern in acute hypervitaminosis A

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**SUMMARY.** The relation of excessive doses of vitamin A with various kidney pathologies is well known however, information concerning the relation of kidney enzyme activity with acute hypervitaminosis A is rather scarce. In this study we describe the kidney enzymatic alterations observed in rats that received daily intramuscular injections of 10.000, 30.000, 50.000 and 100.000 IU of vitamin A palmitate (VA) during seven days (TREATED GROUPS). A comparison is made with the enzyme activity in healthy rats pair-fed and treated with sodium palmitate by intramuscular injection (CONTROL GROUP). The treated rats showed a proportional increase ($p<0.05$) in activity of acid maltase, transaminases or aminotransferases (GOT and GPT), alkaline phosphatase (ALP) and acid protease with all doses of VA administered. Amylase, lipase and arginase tend to decrease ($p<0.05$) in activity only with doses of 50.000 and 100.000 I.U. of VA. Several factors are responsible for these findings, such as kidney necrosis due to release of lysosomal acid hydrolases produced by hypervitaminosis A.
**Key words:** Hypervitaminosis A, kidney enzyme pattern, renal enzyme.

**RESUMEN. Alteraciones enzimáticas renales en la hipervitaminosis A aguda.** Es un hecho conocido la relación existente entre las dosis excesivas de vitamina A y diversos trastornos renales, y viceversa. Sin embargo, es muy poco lo que se conoce en relación a la actividad enzimática renal en la hipervitaminosis A aguda. En el presente trabajo se describen las alteraciones enzimáticas renales producidas en ratas mediante la inyección intramuscular diaria de 10.000, 30.000, 50.000 y 100.000 U.I. de vitamina A durante 7 días (Hipervitaminosis A aguda). Los resultados muestran en estos animales un incremento ($p<0.05$) en la actividad de las siguientes enzimas: maltasa ácida, transaminasas o aminotransferasas (TGO y TGP), fosfatasa alcalina y proteasas ácidas con las diferentes dosis administradas de vitamina A mientras que la amilasa, la lipasa y la arginasa disminuyen ($p<0.05$) con las dosis de 50.000 y 100.000 U.I. de vitamina A. Diversos factores son los responsables de los hallazgos obtenidos, entre los cuales cabe citar la necrosis tisular renal debida a la liberación de la hidrolasas ácidas lisosomales determinada por la hipervitaminosis A.
**Palabras clave:** Hipervitaminosis A aguda, ratas, mapa enzimático renal, riñón, enzimas renales.

## INTRODUCTION

Several publications suggest there is a close relation of vitamin A metabolism with kidney function both in normal and pathological conditions (1). The kidney contains an enzyme that converts retinol to retinoic acid, which is excreted in the bile with glucuronides (2). Kleiner-Bossaler and De Luca (3) also found that in vitamin A-deficient rats, the kidney forms retinoic acid after the administration of a physiological dose of $^{3}$[H]-retinyl acetate. On the other hand, several authors have reported increased plasma levels of vitamin A in patients affected by kidney problems (4,5). Elevated vitamin A levels (6,8) and symptoms of vitamin A intoxication such as hypercalcemia (9) and skin dryness (7) also have been reported in patients with renal insufficiency undergoing chronic haemodialysis. Previous studies have showed a positive correlation between the increased plasma vitamin A level, and the degree of magnitude of kidney damage, clinically evaluated by the severeness of uremia present (10).

It has been also established that administration of excessive amounts of vitamin A during a long period of time determines histopathological kidney damage (11), which depends on the animal species studied, on the dosis given, and on the route and time of administration of the compound. Biochemical and histochemical studies of chronic hypervitaminosis A in relation to kidney enzymes are definitely scarce, and refer only to acid and alkaline phosphatase and succinic dehydrogenase (12).

Arvy (11) has found that high doses of vitamin A have a relevant and multiple effect on renal tissue. In this study we describe the change in activity of the following enzymes: glutamic-pyruvic transaminase (GPT or ALT), glutamic-oxaloacetic transaminase (GOT or AST), amylase, acid maltase, arginase, alkaline phosphatase (ALP), acid protease and lipase produced in kidney, due to intramuscular administration of increasing doses of vitamin A palmitate to rats, during a short period of time (7-days acute hypervitaminosis A). These enzymes were chosen either because they normally ocurr in kidney tissue, they are well known and their evaluation is practical and precise, or because their known properties make it likely that they are involved in cell damage (acid protease and acid maltase). Moreover, transaminases and acid maltase increase its activity in various kidney diseases.

## MATERIAL AND METHODS

One hundred (100) albino male Wistar rats were studied,

each weighing 180 to 200 g. They were kept in individual metabolic cages in the Laboratory for ten days, previous to experimentation. The animals had free access to water and food during the experimental period. The formula for the basal diet was similar to the recommended by the American Institute of Nutrition (13). After this period of adaptation, the animals were distributed randomly in 5 groups of 20 rats each. No significant difference among mean weight was detected. Groups one to four received equivolumetric intramuscular injections of 10.000, 30.000, 50.000 and 100.000 IU/kg/day, respectively, of vitamin A palmitate (Merck, hydrosoluble vitamin A palmitate; 1 mL=100.000 IU) for seven days (TREATED GROUPS). Group five was pair-fed and received equivolumetric injections of 10 mg/kg/day of sodium palmitate intramuscularly for seven days (CONTROL GROUP). Sodium palmitate was used in this group because vitamin A palmitate is dissociated into vitamin A and palmitate anion in rat liver. In view of vitamin A metabolism it seems unlikely that the palmitate factor could have any particular inluence of its own.

During experimentation all animals had access to the same type of food and water. They were weighed daily and examined to detect any pathological sign. At the end of this period, the animals were decapitated with a Harvard guillotine, at the same time, to avoid any possible circadian influence on enzyme activity, and allowed to bleed 2-3 min. A median laparatomy was performed, leaving the kidneys exposed, which were immediately removed for preparating tissue homogenates in a proportion of 1 g of tissue per 100 mL of bidestilled adn deionized water.

**Estimation of enzymes activity**

Glutamic-pyruvic transaminase (GPT or alanine aminotransferase; E.C. 2.6.1.2) and glutamic-oxaloacetic transaminase (GOT or aspartate aminotransferase; E.C. 2.6.1.1) activities were determined according to the procedure described by Reitman and Frankel (14), using alanine and aspartate as substrates, respectively. Results were expressed in UK per g of fresh tissue. Amylase (E.C. 3.2.1.1) activity was assayed as described by Mordoch et al. (15), using hydrosoluble starch as the substrate and estimating the glucose liberated by the method of glucose-oxidase (16). Results were expressed in mg of liberated glucose per g of tissue, per hour of incubation. Acid maltase acid α-1,4-glucosidase; E.C.3.3.1.20) activity was determined according to the method of Gamklou and Scherstén (17) for liver tissue homogenates, using maltose (Sigma) as substrate and estimating the glucose liberated by the method of glucose-oxidase (16). Results were expressed in mg of liberated glucose per g of tissue, per hour of incubation. Alkaline phosphatse (A1P; E.C.3.1.3.1) activity was assayed as described by Berger and Rudolpyh (18), using phenolthalein monophosphate as substrate. Results are expressed in IU per mg of tissue per mg of protein. Proteolytic activity (acid protease; E.C.3.4.1.14) was determined using 4% hemoglobin in 0.1 M acetate buffer (pH: 4.5) as the substrate and determining the amount of tyrosine liberated by the method of Folin and Ciocalteu (19). Results were expressed in μg of lieberated tyrosine per mg of fresh tissue, per hour of incubation. Lipase (E.C, 3.1.1.3) activity was determined by the method of Tietz et al. (20), using olive oil as substrate. Results were expressed as IU per kg of fresh tissue. Arginase (E.C., 3.5.3.1) activity was assayed according to the procedure described by Bhide et al. (21), using arginine as substrate. Results were expressed in μg of lieberated urea per g of tissue, per hour of incubation. The vitamin A in kidney was evaluated according to the technique of Neelds and Pearson (22) and results were expressed as μg of vitamin A per g of wet tissue.

**Statistical analysis**

Mean±standard deviation values were calculated and used in the basical statistical analysis. Differences among the enzyme activity values in treated and control groups were estimated by the ANOVA test. Significant differences between each subgroup and the corresponding controls were calculated by t Student. Linear regression analysis was used to assess correlations and differences in the slopes and/or intercepts between the groups. Statistical significance was considered present when $p<0.05$.

## RESULTS

The renal content of vitamin A in the experimental and control groups is presented in Table 1. This shows that vitamin A content increases proportionally to doses of vitamin A applied ($r=0.997$; $p<0.05$). The effect of excessive doses of vitamin A on enzyme activity is shown in Table 2. Linear regression analysis proves that liver GPT ($r=0.954$), GOT ($r=0.972$), acid maltase ($r=0.961$), alkaline phosphatase (0.978) and acid protease ($r=0.972$) increase its activity significantly ($p<0.01$) with vitamin A administration, reaching a maximum with 100.000 I.U. Since the P-value in the ANOVA table is less than 0.01, there is a statistically significant relation between GPT, GOT, acid maltase, alkaline phosphatase, acid protease and vitamin A at the 99% confidence level. The R-Squared (91.97, 94.56, 92.35, 95.68 and 96.64% for GPT, GOT, acid maltase, alkaline phosphatase and acid protease, respectively) indicate that the model as fitted explains 92, 95, 92, 96 and 97% of the variability in the mentioned enzymes. The correlations coefficient > 0.95, in all cases, indicate a relatively strong relationship between the variables enzyme activity and vitamin A. Linear regression analysis and slope differences in the respective regression lines showed that the observed variation of enzyme activity values is positive and highly significant ($p<0.01$) and the increase in the activity of these liver enzymes is proportional to vitamin A administered. On the other hand, in rats treated with excessive doses of vitamin A linear regression analysis shown that amylase ($r=-0.964$), arginase ($r=-0.917$) and lipase (-0.92) diminished significantly ($p<0.05$). Since the P-value in the ANOVA table

is less than 0.01, there is a statistically significant relation between the activity of these enzymes and vitamin A at the 99% confidence level. The R-Squared obtained indicate that the model as fitted explains 93, 84 and 96% of the variability in amylase, arginase and lipase, respectively. The correlations coefficient -0.91, in all cases, indicate a relatively strong relationship between the enzyme activity and vitamin A. Linear regression analysis and slope differences in the respective regression lines shown that the observed variation of enzyme activity values is negative and highly significant ($p<0.01$). Thus, the diminution in the activity of these enzymes is proportional to vitamin A administration.

TABLE 1
Effects of increasing doses of retinol on the renal concentration of vitamin A

| Doses | Kidney vitamin A concentration | "p" |
|---|---|---|
| 10.000 U.I. | 108±2.365 | <0.05 |
| 30.000 U.I. | 363±11 | <0.05 |
| 50.000 U.I. | 638±23 | <0.05 |
| 100.000 U.I. | 1450±104 | <0.05 |
| Control Group | 14±1.08 | |

Kidney concentration of vitamin A is expressed in µg/g of fresh tissue (average±standard deviation). $p<0.05$ is statistically significant on comparing with the respective control group.

TABLE 2
Effects of increasing doses of vitamin A on several renal enzymes

| Enzymes | Controls | 10.000 | 30.000 | 50.000 | 100.000 | F[1] | P[2] |
|---|---|---|---|---|---|---|---|
| Amylase | 0.29±0.03 | 0.25±0.08 | 0.20±0.11[a] | 0.13±0.25[a] | 0.08±0.02[a] | 15.03 | <0.05 |
| Acid maltase | 0.59±0.08 | 1.02±0.16 | 1.35±0.14[a] | 1.60±0.16[a] | 2.06±0.18[a] | 39.4 | <0.05 |
| Arginase | 0.31±0.03 | 0.28±0.04 | 0.18±0.08[a] | 0.12±0.02[a] | 0.08±0.01[a] | 19.44 | <0.05 |
| GPT | 89±13 | 113±12[a] | 135±17[a] | 220±11[a] | 259±14[a] | 32.2 | <0.05 |
| GOT | 130±18 | 166±14 | 260±18[a] | 312±49[a] | 398±43[a] | 17.02 | <0.05 |
| ALP | 1346±74 | 2101±117[a] | 2442±134[a] | 2986±186[a] | 3968±228[a] | 18.7 | <0.05 |
| Acid Protease | 3.82±0.38 | 7.45±0.48[a] | 10±0.55[a] | 16±0.67[a] | 21±1.5[a] | 57.23 | <0.05 |
| Lipase | 149±25 | 138±16 | 124±12[a] | 119±18[a] | 97±17[a] | 13.56 | <0.05 |

Enzyme activity is expressed as average±standar deviation. DOSES: Administered doses of IU vitamin A/kg/day, intramuscular.
[1]F= Analysis of variance of averages in the respective groups, statistically significant on comparing the averages of the groups.
[a]$p<0.05$ is statistically significant on comparing with the respective control group.

## DISCUSSION

The presence of acute hypervitaminosis A was confirmed by the increased kidney vitamin A levels. Administration of vitamin A at doses much higher than daily permited or recommended requirement for males rats, during short period of time, significantly modified the enzymes studied. We have found that the activity of acid maltase, transaminases (aminotransferases), alkaline phosphatase and acid protease is increased. In contrast, the other enzymes showed a significant ($p<0.05$) decrease in activity with high doses of vitamin A.

The increase in activity of aminotransferase is due to the histopathological kidney damage caused by high doses of vitamin A, which depends on the animal species and the doses administered, a well as on the duration of vitamin A administration (11,23-26). These changes could explain the increase in transaminase or aminotransferase activity since these enzymes are considered markers for acute or chronic tissue damage (27).

Other enzymes that showed an increase in activity in kidney of vitamin A-treated rats were acid maltase and acid protease. The increment in activity of the latter enzyme is in agreement with previous studies by Dingle et al. (28). These vitamin A effects could be explained, as suggested by Fell et al. (29) by an alteration of the permeability of intracellular particles such as lysosomes and mitochondria. The concept that vitamin A has a direct action on membrane permeability gained further strength when Dingle and Lucy (30) demonstrated that rat liver lysosomes treated with vitamin A released a proteolytic enzyme with an optimum acid pH. Additional work on in vitro liver preparations was done by Dingle (31) who, by ultracentrifugation, isolated the mitochondrial fractionof rat liver and then subjected it to excess vitamin A. The latter author reported that a proteolytic enzyme was released by the mitochondria. Although the exact mechanism of altered mitochondrial membrane permeability is not known, the dependence of the vitamin A effect upon temperature and pH suggest an enzymatic mechanism. Swelling of mitochondria, due to vitamin A has been reported by Lucy et al. (32). The notion that vitamin A alters membrane

permeability was further substantiated when Dingle and Lucy (33) showed that vitamin A not only affects lysosomal membrane permeability but also influences the membrane stability of other entirely different structures such as the erythrocyte. It is tempting to speculate hat the site of action of vitamin A overdosage may be at the lipoprotein membrane of cells and their intracellular organelles (lysosomes). Should this assumption be correct, then vitamin A could be regarded as capable of releasing a variety of hydrolases (cathepsin, ribonuclease, deoxyribonuclease, phosphatase, glucuronidase, and sulfatase) which, according to de Duve (34) are present in lysosomes. Dingle (31) supports the view that some, or all, of the above mentioned bound hydrolases may be released by vitamin A overdosage, and that the above mentioned enzymes complex may be responsible for many of the changes observed in tissues treated with excessive vitamin A.

Our results clearly show a considerable increase in activity of the alkaline phosphatase in animals treated with vitamin A. This is in close agreement with previous biochemical studies by Rahalkar et al. (35) and Alarcón (36). This finding is also in accord with other histochemical studies conducted in rat liver and in rabbit kidney by Alarcón (36), which proved that the enzyme is more active in the brush border of the proximal tubule and in the cytoplasm of the vascular endothelial cells. Regarding this finding several studies suggest that the enzyme synthesis is a vitamin A-dependent phenomenon. Riley and Spearman (37) think that the vitamin A induces the enzyme synthesis by means of various mechanisms which could be by aggregation of inactive subunits or by diminishing its degradation. Studies of these authors suggest that, at least, at the epidermis the vitamin A induces specifically the alkaline phosphatase synthesis by its action on RNA. The functional significance of this incremented synthesis of alkaline phosphatase induced by vitamin A is not clear, although some of the effects of vitamin A, for example bone formation, transport and lipid accumulation (specially phospholipid), could be catalyzed by this enzyme; reactions in which the phosphate transference are mediated by this enzyme system (38).

The decrease in activity of α-amylase is in accord with studies of Rinaudo et al. (39) who worked with liver homogenates of vitamin A-treated rats. The relevance of this finding is not yet clear, but no doubt, it will be considered in future studies with experimental animals.

Reduction in activity of lipase could explain the previous work of Alarcón (40) in rabbit kidney, in which he detected the presence of fat droplets distributed around the surrounding proximal and distal tubules, using histochemical techniques. This result partially agrees with the hypothesis that vitamin A is related to lipid metabolism (41,42) and with the fat degenerationof the endothelial reticular system, described in connection with chronic intoxication, which mechanism of productionis not yet known (41).

An enzyme present in large amounts in liver, and in small amounts in kidney,is arginase (43), localized in the cellular cytoplasm and requiring the cofactor $Mn^{2+}$ for activation, also decreased its activity significaantly, exactly as it happens in liver (44). The decrease of enzyme activity may indicate a failure of aminoacid utilizationfor protein synthesis, although the function of this enzyme at kidney tissue is not yet known. This decrease could result from a diminution in food intake, among other factors, (43) determined by the excessive doses of vitamin A.

In conclusion, the present study shows that high doses of vitamin A changes the activity of several kidney enzymes. Therefore, the acute hypervitaminosis A produces serious renal cellular metabolic changes.

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