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EFFECTS OF CORTISOL AND GROWTH HORMONE ON THE METABOLISM OF LIVER AND BONE IN CHILDREN WITH MALNUTRITION*

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A protein-energy deficit produces stress in the organism affecting all systems. Proportional to the degree of disease, cortisol and GH are mostly responsible for some of these effects. To investigate the effects on liver and bone, cortisol, GH, AST, ALT, ALP activities and Ca_T and P_I in serum were measured in 21 marasmus, nine kwashiorkor and 34 control children. In the marasmus group, we found a positive correlation between cortisol and AST, ALT and Ca_T and a negative correlation between cortisol and ALP. In the kwashiorkor group there were positive correlations between the same parameters, although, they were of a lesser degree. Furthermore, in the kwashiorkor group we established a positive correlation between GH and ALP. Cortisol stimulates transaminases directly and suppresses ALP activity, thus indirectly increasing Ca_T, whereas GH has no direct effect on these enzymes. As the disease progresses and as liver functions deteriorate, AST, ALT and ALP increase in serum. *Key words: malnutrition, cortisol, growth hormone, stress.*

Malnutrition is a condition that affects almost all the systems of an organism. A group of clinical findings are observed in the malnourished as a result of the disturbances of some systems; the most important being growth retardation[1]. Whatever the etiology, an inadequate diet and stress are the predominant causes for these changes[2]. In response to stress, some hormone levels of the endocrine system decrease, while others increase. Although some differences are seen during the course of the disease, the most marked difference is an increase in cortisol and growth hormone (GH) levels[2,3]. Even though GH levels are found to be elevated in malnourished children, growth retardation is present. The reason for this paradox is the breaking down of the metabolic integrity and the protein of the organism. The disturbances exhibit themselves through various clinical and laboratory findings. For example, as a result of liver failure, carbohydrate, lipid and protein metabolisms are affected[4,5]. In addition, secondary to an inadequate diet, some changes are also seen in bone metabolism.

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The purpose of this study was to evaluate the fore-mentioned changes, and investigate the effects of cortisol and GH on liver and bone metabolism. It was therefore necessary to measure the cortisol, GH, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities, and total calcium (Ca_T) and inorganic phosphorus (P_i) levels in the serum of children suffering from malnutrition and to investigate correlations of these hormones with the biochemical parameters mentioned in patient groups.

Material and Methods

The study population consisted of 35 subjects chosen at random from children with protein-energy malnutrition (PEM) who had been seen at the Pediatric Polyclinic of Uludağ University Faculty of Medicine. Using the Wellcome Classification[6], marasmus was diagnosed in 21 children, kwashiorkor in nine children, marasmic kwashiorkor in two children and three children were underweight. The latter five children were excluded from the study because their number was insufficient to comprise a group for statistical evaluation. The control group consisted of 34 healthy children whose ages approximately matched those of the subjects.

The patients fasted nearly ten hours before venous blood was drawn from the forearm. On the same day, serum concentrations of AST and ALT using the Biotrol A 03011 AST/TGO monoreactive kit and the Biotrol A 03021 ALT/TGP monoreactive kit, respectively, were determined. These kits were based upon the continuous monitoring principle[7].

The optimized kinetic method devised by the Coulter firm (Reagent Ref. No. 9866357) was used to determine ALP activity. P-nitrophenyl phosphate was the substrate used. The Biotrol / A 02478 phosphorus U.V. kit was used to measure P_1 levels and the classical "Complexometry method"[8] was used to measure Ca_T levels.

Cortisol levels were determined by using the Quanticoat Cortisol kit (Cat. No. 825, Kallestad Laboratories Inc., USA) and GH levels were arrived at by using the Quantitope^R HGH kit (Cat. No. 850, Kallestad Diagnostics, USA). These kits were based on the RIA principle.

Statistically significant differences between patient groups and correlations of cortisol and GH levels with enzyme activities and Ca_T and P_i levels were calculated using the Student's *t* test.

Results

The mean values of AST, ALT and ALP were within normal ranges in the patient groups, but when compared with the control group, highly significant differences ($p<0.001$) were found (Table I).

TABLE I: Evaluation of AST, ALT and ALP Mean Values in Children With Marasmus and Kwashiorkor Compared to the Control Group

Parameters	Control n = 34 $\bar{X} \pm SE$	Groups: Marasmus n = 21 $\bar{X} \pm SE$	p	Groups: Kwashiorkor n = 9 $\bar{X} \pm SE$	p	p*
AST (U/L)	24 ± 2.3	31.7 ± 1.9	<0.001	38.2 ± 2.9	<0.001	<0.02
ALT (U/L)	12.4 ± 1.8	28.4 ± 3.1	<0.001	37.1 ± 4.0	<0.001	<0.02
ALP (U/L)	173.5 ± 7.0	328.5 ± 22.3	<0.001	375.2 ± 31.7	<0.001	N.S.

N.S.: Non-significant.

p* : Statistically significant differences between patient groups.

In the marasmus and kwashiorkor groups, serum Ca_T levels were found to be significantly lower ($p<0.01$ and $p<0.001$, respectively) than in the control group. However, only in the kwashiorkor group were serum P_i levels significantly higher ($p<0.01$) than in the control group (Table II).

When AST and ALT were compared between the two patient groups, the mean values of the kwashiorkor group were found to be higher ($p<0.02$) than the marasmus group (Table I). When the mean values of the two groups were compared with each other, Ca_T levels were observed to be higher in the marasmus group ($p<0.01$) while P_i levels were found to be significantly higher in the kwashiorkor group ($p<0.01$) (Table II).

TABLE II: Investigation of Serum Ca_T and P_i Levels

Parameters	Control n = 34 $\bar{X} \pm SE$	Groups: Marasmus n = 21 $\bar{X} \pm SE$	p	Groups: Kwashiorkor n = 9 $\bar{X} \pm SE$	p	p*
Ca_T (%mg)	10.2 ± 0.1	9.9 ± 0.1	<0.01	9.1 ± 0.3	<0.001	<0.01
P_i (%mg)	4.1 ± 0.2	4.3 ± 0.3	N.S.	5.3 ± 0.3	<0.01	<0.01

N.S.: Non-significant.

p* : Statistically significant differences between patient groups.

Serum ALP, GH and cortisol levels were not significantly different in the two patient groups (Tables I, III). However, in comparison with the control group, the GH and cortisol values in the marasmus group were seen to be significantly higher ($p<0.01$ and $p<0.001$, respectively). In the kwashiorkor group, the serum GH

level was noted to be significantly elevated ($p<0.001$) while the cortisol value was slightly elevated ($p<0.05$) (Table III).

In the marasmus group, we found a positive correlation between cortisol values and AST, ALT and Ca_T and a negative correlation between cortisol and ALP (Table IV). However, in the kwashiorkor group, the most marked positive correlation was between GH and ALP activity ($r = 0.92$) (Table V).

TABLE III: Serum Cortisol and GH Compared to Control Group

		Groups				
	Controls n = 34	Marasmus n = 21		Kwashiorkor n = 9		
Parameters	$\bar{X} \pm SE$	$\bar{X} \pm SE$	p	$\bar{X} \pm SE$	p	p*
GH (ng/ml)	6.7 ± 1.7	11.8 ± 1.7	<0.01	14.7 ± 1.5	<0.001	N.S.
Cortisol (ng/ml)	184.0 ± 8.6	285.6 ± 23.5	<0.001	244.7 ± 24.9	<0.05	N.S.

N.S.: Non-significant.
p* : Statistically significant differences between patient groups.

TABLE IV: Correlations of Cortisol and GH With Various Parameters in the Marasmus Group

	Cortisol		GH	
AST	$y = 1.8x + 210$	$r = 0.94$	$y = 0.24x + 6.9$	$r = 0.17$
ALT	$y = 1.5x + 265$	$r = 0.91$	$y = 0.30x + 14.7$	$r = -0.15$
ALP	$y = -0.5x + 390$	$r = -0.85$	$y = 0.09x + 17$	$r = -0.04$
Ca_T	$y = 59x + 478$	$r = 0.79$	$y = 6.84x + 79.5$	$r = 0.30$
P_i	$y = -45x + 414$	$r = -0.28$	$y = -4.28x + 29$	$r = 0.21$

TABLE V: Correlations of Cortisol and GH With Various Parameters in the Kwashiorkor Group

	Cortisol		GH	
AST	$y = 1.2x + 260$	$r = 0.75$	$y = 0.07x + 11$	$r = 0.06$
ALT	$y = 0.4x + 285$	$r = 0.79$	$y = -0.04x + 17$	$r = -0.3$
ALP	$y = -0.54x + 410$	$r = -0.50$	$y = 0.08x + 3.7$	$r = 0.92$
Ca_T	$y = 93x + (-659)$	$r = 0.40$	$y = 1.3x + 1.9$	$r = 0.04$
P_i	$y = 44x + 109$	$r = 0.27$	$y = 0.13x + 16$	$r = -0.03$

Discussion

Former opinions to the effect that serum enzyme levels in malnourished children, were reduced because of protein deficiency are no longer accepted[9]. Pancreatic enzymes are found to be reduced only in PEM and the decrease is parallel to the severity of the disease. The reason for this is probably decreased external stimulation of pancreatic enzyme secondary to a low diet intake and probable decrease of the "growth hormone-releasing factor" which stimulates exocrine pancreatic enzyme secretion[10]. As a result, malabsorption occurs which worsens the child's nutritional status[11].

In the liver, inadequate protein intake in particular causes liver functions to become abnormal. Although, the histological changes in the liver cannot explain and do not show a correlation with the biochemical changes in serum, both show an increase proportional to the severity of PEM[12].

As a result of inadequate lipoprotein synthesis in the liver, lipids cannot form lipoproteins and their release into the circulation becomes limited. Therefore, fat, primarily as triglyceride, accumulates in the liver parenchyma[13]. The individual liver parenchymal cells bloat and appear to block the narrow sinusoids giving rise to the theory of an intrahepatic obstructive cause for the presenting jaundice[14]. The liver cells, though distended, rarely undergo necrosis. In epidemiological studies, no direct relation has been found between PEM and cirrhosis or other chronic liver diseases[15]. Kwashiorkor is found to play a role in the etiology of cirrhosis because malnourished children are more sensitive to toxic or infectious agents but viral hepatitis is believed to be the responsible factor in cirrhosis occurring in these cases[12]. The increased immunosuppressive effect of cortisol in addition to protein inadequacy, reduces the resistance of an organism, causing a greater risk of infection.

It is observed in PEM that alterations in the concentration of serum amino acids are correlated with changes in hormonal balance[16]. In normal children, serum insulin concentrations tend to be high, while cortisol and GH values are normal or subnormal. It is postulated that relatively high insulin levels could reduce the plasma concentration of essential amino acids by stimulating their movement out of the plasma into the muscle. When total food intake is curtailed, the metabolic response becomes similar to that of a wasting situation, that is, serum cortisol concentrations become high, and insulin correspondingly low. The main metabolic difference between this phase in the development of kwashiorkor and that found in uncomplicated marasmus is a concomitant rise in serum GH concentration. The high level of GH, even in the presence of elevated cortisol concentrations, might be the major reason for the even more dramatic fall in amino acid concentrations. The effects of high concentrations of serum cortisol, coupled with low insulin levels, would normally have replenished plasma aminoacid concentrations by

reducing amino acid utilization for protein synthesis in muscle in addition to increased proteolysis. Although the actions of cortisol and GH are complementary in maintaining blood glucose concentrations, they are antagonistic in their effects on amino acid metabolism. In contrast to cortisol, GH stimulates the passage of amino acids out of the serum into the tissues as part of its anabolic action.

In malnutrition another factor which might further decrease the concentration of serum amino acids is the increased activity of the transaminases in muscle[17]. Transaminases are activated by cortisol. This action of cortisol actually increases amino acid catabolism. To meet energy requirements, first transamination then the oxidation of alpha-ketoacids increase. As the severity of the disease increases, especially in severe kwashiorkor cases, serum amino acid levels drop. Alanine is also one of these amino acids, however its marked elevation can be found in pre-kwashiorkor cases. It is suggested that where protein is deficient and energy is adequate in the diet (as in the pre-kwashiorkor states), the alanine level rises, since it is neither utilized for gluconeogenesis nor metabolized to urea[18-19]. In marasmus there is an inadequate caloric intake due to insufficiency in the diet[1] and, therefore, in these children, alanine and aspartate are used for gluconeogenesis[18].

Increased cortisol in marasmus affects protein metabolism in three ways: increases protein catabolism, increases the mobilization of amino acids from muscle and stimulates the activity of transaminases. In kwashiorkor, although there is adequate energy, the utilization of these amino acids in gluconeogenesis is decreased by the effect of GH. As a result of protein inadequacy, fatty liver occurs. Structural and functional disturbances of the liver cause these enzymes to seep into plasma. Although the increasing mechanisms of transaminases in serum are different in PEM, they can be used as an index for establishing the stage of the disease. For example, in addition to the increase in hormone levels, the presence of hepatomegaly and elevated bilirubin and transaminase (AST, ALT) levels indicate that there is liver insufficiency, and a diagnosis of kwashiorkor can be made. A rise only in serum transaminase levels can be interpreted solely as a compensation or adaptation effect of cortisol and this increase is seen predominantly in marasmus patients.

In our study, we also found AST and ALT mean values to be significantly higher in both patient groups than in the control group (Table I). Although the mean values of both groups were within the normal ranges, when compared with each other, the mean levels of these enzymes in serum were found to be higher in kwashiorkor patients. As a result of our correlations, we found a more marked, positive correlation of cortisol with AST and ALT in the marasmus group ($r = 0.94$, $r = 0.91$) when (Table IV) compared with the kwashiorkor group ($r = 0.75$, $r = 0.79$) (Table V), respectively.

Even though ALP levels were found to be within normal ranges, they were significantly higher than the mean of the control group. However, when the mean values of the two patient groups were compared with each other, the mean value of the kwashiorkor group was found to be higher (Table I). ALP, a membrane-bound enzyme found in the liver, forms the cell and biliary canalicula, and is located on the sinusoidal border of the parenchymal membrane[21]. In any of the obstructive liver states, as a result of cholestasis, ALP regurgitates into the plasma and its level is found to be high. In bone, ALP catalyzes the breakdown of inorganic pyrophosphates, and thus, plays an important role in the deposition of calcium salts, i.e., calcification. An increase of ALP in serum is generally proportional to an increase of osteoblastic activity. These processes are complex in malnutrition. In order to maintain blood Ca_T at normal levels, as a result of nutritional inadequacy, decalcification is generally seen in PEM. Ca_T levels in malnourished children are generally found to be around 8 mg/dl[9]. These values are lower than those normally found in children, but, if a correction is made for the reduced serum protein, the ionic calcium values are found to be within normal limits. In our study Ca_T and P_i values were established to be at normal levels. However, when compared with the mean values of the controls, we found that there were very significant differences between them (Table II).

Since we did not perform ALP isoenzyme electrophoresis, we could not ascertain whether or not the increase in ALP was from the liver or bone. However, when transaminase levels are considered, it may be argued that in kwashiorkor, in addition to the effect of GH, an obstruction secondary to a mild degenerative process in the liver explains the relative increase of this enzyme observed in the plasma. GH increases the intestinal absorption of calcium and the renal retention of phosphorus, which in a way increases the metabolic activity in bone, so that ALP in bone is also found to be activated. However, in marasmus, as a result of dietary inadequacy, and to compensate and maintain blood calcium levels within normal ranges, mineral depletion occurs in bone, leading to suppression of osteoblastic activity. Only after initiation of treatment, are signs of rickets and an increase in ALP observed.

In conclusion, it is suggested that this enzyme level is normally expected to decrease in marasmus and increase in kwashiorkor. We believe that the reason for the relatively high enzyme level observed in the marasmic patients we studied was either because the pathology of these cases was more severe than it had appeared in reference to the clinical picture or that treatment had been initiated in some patients. However, we tend to give more weight to the former explanation.

We found a negative correlation between cortisol and ALP ($r = -0.85$) in the marasmus group, suggesting ALP is relatively suppressed. There was somewhat of a negative correlation between the same parameters in the kwashiorkor group

(Tables IV, V). The positive correlation established between GH and ALP in the kwashiorkor group supports the afore-mentioned conjectures. In the marasmus group, the positive correlation found between cortisol and Ca_T indicates that cortisol attempts at maintaining metabolic integrity and at keeping serum calcium at normal levels (Table IV).

As a result of this study, it may be said that in marasmus, an increase in cortisol stimulates transaminase activity and gluconeogenesis. In bone, it increases calcium mobilization and prevents blood Ca_T from falling below normal levels, but this compensation effect causes osteoporosis. Whereas in kwashiorkor, the accumulation of lipid in the liver probably causes enzyme levels to increase in the plasma. Although GH is found to be elevated in these patients, and attempts at displaying its anabolic effect, GH cannot be useful in both calcium deposition in bone and in maintenance of normal protein metabolism in the liver as long as dietary inadequacy continues. It is known that when GH is found to be predominantly increased in PEM patients, it signifies the terminal stage of the disease, and that dysadaptation has begun.

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