THE BIO-AVAILABILITY OF VITAMIN A IN ADULTS AND CHILDREN WITH PERSISTENT DIARRHOEA IN ZAMBIA

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A Dissertation submitted to the University of Zambia in Partial Fulfilment of the Requirement of the award of the Degree of Master of Medicine in Paediatrics.

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE

YEAR 2001

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DECLARATION

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ACKNOWLEDGEMENTS

I would like to express my gratitude to the following people for their support during the study period and dissertation write up.

Prof. Bhat and Dr. Chewe Luo for their guidance and encouragement during the dissertation write up.

- **Dr.Paul Kelly for supervising me from proposal to dissertation write up and material support.**
- **Dr. Mubiana Mubita for corrections made in my dissertation write up.**
- **Dr. Emmanuel Kafwembe for analysing blood and urine samples for retinol levels.**

  Miss Albertina C. I. Katungu and Mrs Emma Mutale for typing my proposal and dissertation.
- **Mrs. Chizya Musuku being so supportive and encouraging during this period.**

ABSTRACT

HIV infection has had a major impact on the health of the Zambian population. Micronutrient deficiencies are common in people with AIDS, and include Vitamin A, Vitamin E, Folic Acid, Zinc and Selenium deficiencies.

There is a strong relationship between low serum retinol concentration and mortality in Zambian AIDS patients with diarrhoea. In order to understand the above relationship, serum retinol concentration changes over 6 hours following oral mega-dose therapy (60,120 or 180 mg retinol) were analysed.

The study was done in two phases. In phase 1, it was demonstrated that absorption of retinol was not delayed at 6 hours after administration of 60 mg of retinol in both diarrhoeal patients and controls (patients without diarrhoea).

In phase 2, we studied a total of fifty patients that included 24 adults without diarrhoea, 15 adults with persistent diarrhoea and 11children, 6 girls and 5 boys with persistent diarrhoea.

We did not include women in the study because of the teratogenicity of Vitamin A. Lower baseline serum retinol levels (medium 0.29 mmo/lL, interquartile range 0.21 – 0.56) were found in 24 adults with persistent diarrhoea than controls (1.6, 0.84 – 1.47; p = 0.003). After administering 60 mg retinol, it was noted that the rise in serum retinol in HIV seropositive controls (0.63, 0.35 – 0.77) did not differ significantly from that observed in HIV seronegative controls (0.35, 0.04 – 0.56; p = 0.20).

After administering a mega dose of 120 mg or 180 mg, no significant rise in serum retinol concentration was noted in adults without diarrhoea. However, there was significant rise of retinol in adults without diarrhoea (0.44, 0.25 –0.74; p = 0.03) when compared to adults with persistent diarrhoea (0.25, 0.04 – 0.35 mg\ and in children with persistent diarrhoea (0.11, 0.04 – 0.46).

Patients with persistent diarrhoea lost large amounts of retinol in urine over a 24 hour period than controls though less than 1% of the ingested dose.

Further work is required to determine physiological and metabolic fate of therapeutic doses of retinol since this study confirms that persistent diarrhoea is associated with reduced bio-availability of retinol.

CHAPTER ONE

INTRODUCTION

Diarrhoea is a frequent problem in patients with HIV infection and AIDS. Primary infection with rotavirus, adenovirus, mycobacterium intracellulare, giardia lamblia cryptosporidia and microsporidia are among the most common causes of diarrhoea in patients with HIV infection. In addition, bacterial overgrowth secondary to the frequent administration of antibiotic can cause diarrhoea.

In a minority of HIV-infected patients with diarrhoea, no intestinal pathogen can be identified. Intestinal biopsies from the patients reveal villus atrophy and crypt hyperplasia. Additional pathologic features include disruption of brush-border hydrolases, reduction of mucosal surface area, and decreased crypt mitotic figures [1].

Vitamin A is an important micronutrient comprising several retinoids: retinol, retinoic acid, and retinol esters. Its biological activities encompasses effects on epithelial integrity and gene transcription and there is considerable evidence that vitamin A supplementation has important benefits for children in the tropics [2, 3].

The Zambian population has been found to have a high prevalence of Vitamin A deficiency as demonstrated by low serum retinol concentration and a high prevalence of night blindness [4]. The HIV epidemic has had a severe impact on the Zambian population, with HIV sero-prevalence reaching 25 – 35% in many cities [5] and a concomitant rise in adult mortality levels [6].

Micronutrient deficiencies are common in people with AIDS, and include Vitamin A, Vitamin E, Folic Acid, Zinc and selenium deficiencies [7, 8, 9, 10]. These deficiencies are clearly related to increased mortality in North American population. In a Zambian study, low serum concentration of Vitamin A and E were strongly associated with death [6]. It is less clear, however, how this increased mortality is mediated. Possible explanations include specific effects on lymphocytes or other components of the immune system, or it may be that these deficiencies are merely makers of the severity of inflammation or immune dysfunction.

A positive association between Vitamin A deficiency and diarrhoeal morbidity has been reported [11, 12]. In Brazil, routine administration of Vitamin A (60 mg) at four monthly intervals to children age 1 – 5 years reduced the incidence of severe diarrhoea by 20% and the diarrhoea prevalence by 23% [13].

Micronutrients such as zinc and Vitamin A reduce the severity and duration of diarrhoea either by promoting rapid and effective repair of the intestinal epithelium after an acute enteric infection due to their role in the regulation of cell division or enhancing the immune response.

CHAPTER TWO

JUSTIFICATION

Persistent diarrhoea and weight loss are common problems for AIDS patients, occurring in as many as 80% of cases in some series [13].

HIV/AIDS is now probably the most important health issue in Sub-Saharan Africa including Zambia. It has become the most deadly epidemic that has affected human kind throughout its history. It is estimated that the number of fatalities by the Year 2000 will be more than 30 million adults, and 10 million children.

Studies on Vitamin A supplementation have proven the effectiveness of Vitamin A in reducing the severity and duration of diarrhoeal disease. There has been no studies to show whether Vitamin A supplementation at the recommended dosages ameliorates diarrhoea and wasting in AIDS patients.

Mortality in AIDS patients is higher in those with lower retinol levels. Supplementing Vitamin A in infected infants significantly reduced morbidity [14].

Given the many potential biological benefits of multiple micronutrient supplementation, its use as a therapy may be attractive. However, in a randomised controlled trial, no clinical benefit was detected from a two week course of modest doses of Vitamin A, C, E, seleniam and zinc given to AIDS patients with diarrhoea-wasting syndrome [12].

In order to begin to understand this finding, we set out to test the hypothesis that adults and children with persistent diarrhoea and HIV have reduced bio-availability of Vitamin A following oral mega dose therapy as this is the form in which Vitamin A supplements would usually be administered to malnourished patients with persistent diarrhoea in Zambia and other parts of Africa.

CHAPTER THREE

3.0 LITERATURE REVIEW

3.1 CONSEQUENCES OF VITAMIN A DEFICIENCY

A number of potential biological mechanism which normally limit infections could be altered in Vitamin A deficiency. These include increased penetration of bacteria, viruses and parasites through altered epithelial barriers, changes in lymphoid cell maturation, abnormal production of cytokines and lymphokines that regulate the immune response and altered membrane structures that could affect the cells receptors for antigens and regulatory molecules. The clearance of pathogens by cytotoxic phagocytic cells might also be impaired [15].

Cross sectional studies of impaired Vitamin A status and various infections have been carried out in many developing countries [15], the strongest association in most cases was with diarrhoea especially when it was persistent, chronic or severe. Vitamin A reduces diarrhoea related deaths by reducing the duration and severity. It was recently reported from Peru that diarrhoeal disease especially that due to rotavirus infection and with accompanying high fever led to a tenfold increase in the urinary excretion of Vitamin A, this also applied to respiratory diseases [16].

3.2 IMPACT ON VISION

The role of vitamin A in the eye is two-fold. The retinoldehyde molecule, formed from retinol functions as the chromophore for visual pigment, rhodopsin. Vitamin A, probably in the form of retinoic acid is also essential for the development of the neural tissue of the eye and for maintaining ocular epithelial cells. Thus, night blindness is caused by inadequate vitamin A to regenerate rhodopsin, involved in vision in dim light, in the photoreceptor cells after bleaching due to bright light. In contrast, xerophthalmia involving dryness of the cornea and progressive corneal deterioration is almost certainly due to lack of retinol for conventions to retinoic acid necessary for normal differentiation of cornea [17].

3.3 IMPACT ON GROWTH

Vitamin A is among the nutrients whose deficiency the growing organism appears to be most sensitive.

Work with experimental animals has demonstrated that chronic Vitamin A deficiency results in loss of taste, smell and appetite leading to inanition [18].

A decreased rate of growth is reliable maker of vitamin A deficiency in experimental animals where other variables can be controlled. Rats reach a weight plateau after all liver reserves are exhausted and plasma retinol concentration has fallen to 5 – 10 mg/dl.

Within a few days of providing either retinol or retinoic acid to retinol deficient animals, weight gain and growth are restored. Impaired growth may be related to both inanition and to metabolic changes such as disturbance in water balance and protein utilization. The weight plateau is probably not related to infection per see because germ free or antibiotic animals survived longer than conventionally housed rats while still exhibiting reduced growth [19, 20]. Thus vitamin A is required for sustained growth even in the absence of infection. It appears that periodic large doses of vitamin A supplementation has a significant impact on growth in xerophthalmic children [21]. In addition, three studies in which pre-formed vitamin A was consumed in adequate amounts regularly showed slight improvement in linear growth [21].

3.4 VITAMIN A AND IMMUNITY

Vitamin A is involved in immune response by maintaining epithelial cell lining of organs and tissues, thus providing a first line defence in resistance to infection.

In vitamin A deficiency, cell mediated immunity is markedly impaired. The production and maturation of lymphocytes are reduced. In a study in Indonesia, it was found that the ratio of T-cells bearing CD4+ and CD8+ antigen was lower in the peripheral blood lymphocytes of xerophthalmic children compared with non xerophthalmic controls [22].

After vitamin A supplementation, the proportion of CD4+ to CD8+ T-cells and percentage of naive CD4+ T-lymphocytes increased.

Cell mediated immunity has been assessed in humans and animal studies by the delayed type hypersensitivity reactions. In vitamin A deficient mice, the delayed type hypersensitivity was significantly reduced [23]. The function of cytotoxic T-lymphocytes may also be reduced during vitamin A deficiency. When vitamin A deficient chicks were challenged with Newcastle disease virus, the cytotoxic activity of spleen cells was low implying that the recovery from viral infection would be poor.

Vitamin A deficiency may also affect the recovery of natural killer cells which mediate natural cytotoxicity killing virus infected cells.

These cells also secrete a number of cytokines such as interferon (IFN) gamma which have regulatory roles in hematopoiesis and antibody formation. The released interferon can further increase the cytotoxic activity of natural killer cells and regulate the production of certain classes of immunogloblin.

The ability of supplemented vitamin A to hasten bacterial clearance even in normal animals and its adjuvant properties in some immune responses are consistent with the hypothesis that reactions to infection could be improved following vitamin A supplementation.

The therapeutic effect of high doses of vitamin A such as has been reported in children with measles might also result from stimulation of normal immune response mechanism by supplemental vitamin A.

3.5 HIV AND VITAMIN A

Taking into consideration the fact that vitamin A and its metabolites act as immune enhancers, it therefore has a beneficial role in immune deficiency disorders including HIV. In the United States, serum levels of vitamin A were found to be low in HIV seropositive type-1 adults as compared to seronegative adults [24]. HIV positive mothers who transmitted the infection to their infants were most likely to have lower serum vitamin A levels than infected mothers who did not transmit the virus [24].

However, it is not clear yet whether low serum vitamin A levels in HIV infected individuals are due to recurrent diarrhoea or to an acute phase response to infection [25]. The acute phase response involves an increase in levels of C-reactive proteins and other proteins. This results in suppression of synthesis of transport proteins like albumin and retinol binding proteins by hepatocytes [26].

Infant mortality in off-springs of HIV infected mothers with low serum retinol was as high as 90% [27].

3.6 VITAMIN A ABSORPTION CURVES

Mendeloff in 1954 demonstrated that using single dose of 60 mg (200.000 iu) of vitamin A followed by a test two hours later, highly reproducible vitamin A absorption curves could be obtained [28].

These protocols have been useful in the past in defining regimes for vitamin A treatment of xerophthalmia in Indonesian children and the gastrointestinal tract reaction to radiation therapy in cervical cancer [29 30].

Serum vitamin A was determined before, and 30 – 45 days after the administration of 60 mg vitamin A to 544 Brazilian children residing in slum areas of Recife.

The frequency distribution curves were compared in a subgroup of children whose vitamin A status was assessed initially by the relative dose response (RDR) test. The curves of children with negative (adequate status) and positive (inadequate status) RDR tests were different. However, the difference disappeared after supplementation. The shape of the distribution curve after supplementation was close to normal with a mean, median and 95% confidence intervalof $1.78^{\pm}$ 0.49, 1.68 and 1.02 – 2.90 mmol per litre respectively.

The post supplementation curve derived from this study may serve as a reference for diagnostic, surveillance and program evaluation purposes [31].

CHAPTER FOUR

4.0 AIMS AND OBJECTIVES

4.1 MAIN OBJECTIVE

To define the bio-availability of Vitamin A in Zambian adults and children with persistent diarrhoea in Zambia with a view of designing more effective treatment regimes.

4.2 SPECIFIC OBJECTIVES

1. To determine whether Vitamin A deficiency can be overcome by using oral mega doses.
2. To determine the absorptive pattern of Vitamin A when given orally.
3. To assess the urinary excretion of retinol.
4. To determine whether HIV infection has an effect on Vitamin A absorption and plasma levels.
5. To determine baseline serum retinol level.

CHAPTER FIVE

5.0. PATIENTS AND METHODS

5.1 STUDY DESIGN

Studies were performed in two groups of adult in-patients in the University Teaching Hospital, Lusaka, Zambia. The first group included patients with persistent diarrhoea and weight loss, and the second group included convalescent patients on traction for femoral fracture in surgical wards with no history of diarrhoea in the previous month.

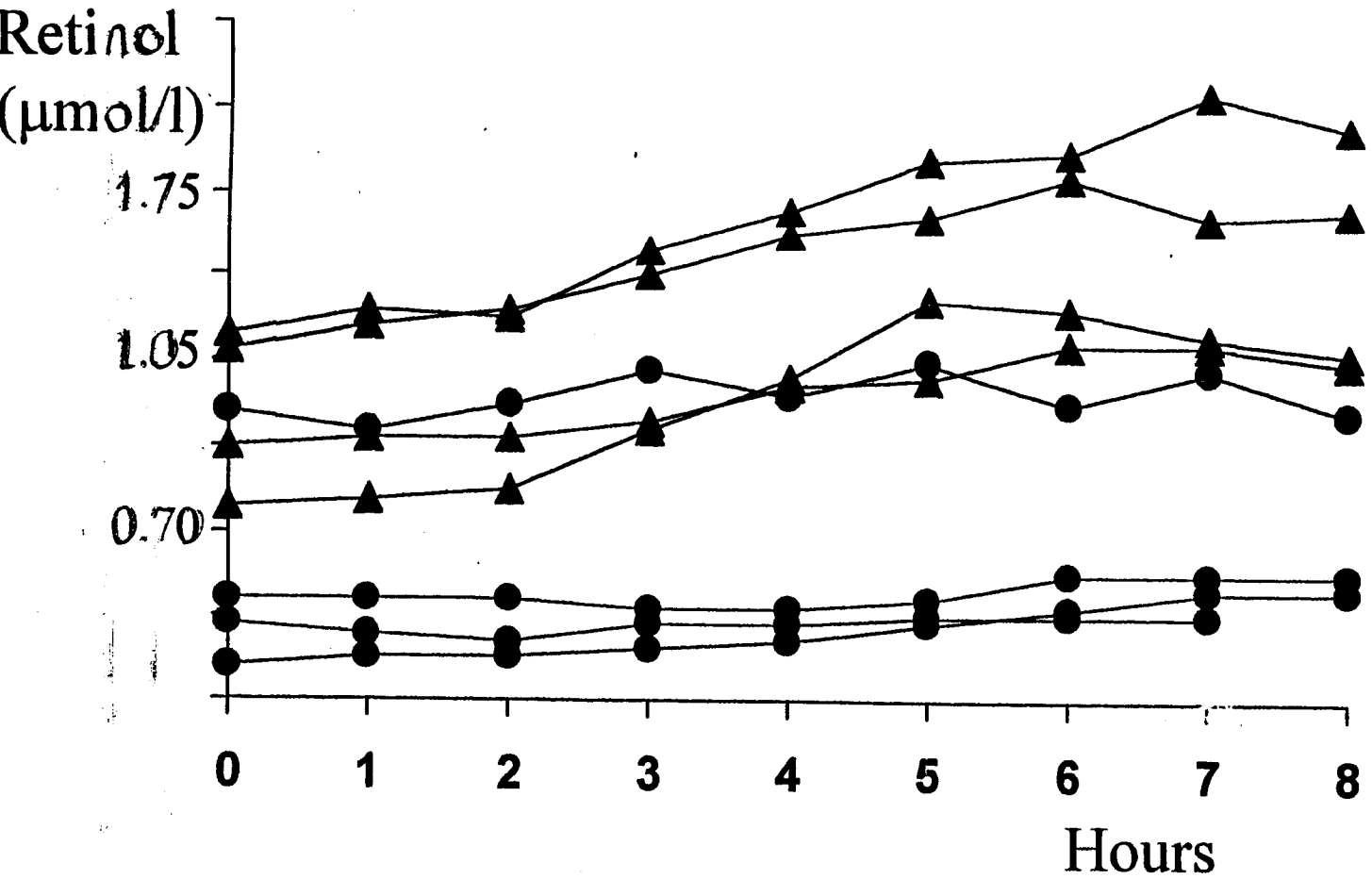
We also studied Vitamin A handling in children of both sexes with persistent diarrhoea and malnutrition aged 12 – 24 months while under treatment in the Paediatric Nutrition Ward, including children with marasmus, marasmic kwashiorkor and kwashiorkor during the recovery phase of the illness. We did not study any children without diarrhoea.

Written consent to participate in the studies was obtained in all cases and the studies were approved by the Research Ethics Committee of the University of Zambia.

5.2 PROTOCOL FOR STUDIES

In Phase 1, studies were carried out in four adult patients with diarrhoea and four controls to determine the time course and peak of serum concentrations of retinol. Blood samples were collected at baseline and hourly for 8 hours. These profiles demonstrated that although there was no rise in serum retinol in diarrhoea patients compared to controls (Figure I), there was no evidence of delayed absorption so that blood sampling for subsequent (PHASES) studies could be performed at 0 and 6 hours.

Figure 1 Serum profiles following oral administration of 60 mg retinol in four patients with persistent diarrhoea and four controls on surgical wards without diarrhoea



5.3 STUDY PROCEDURE

After an overnight fast, a baseline blood sample was collected and fatty breakfast (either peanut butter, sandwich or fried egg roll depending on availability; approximate fat content 20g) was given. Vitamin A was administered orally as a capsule of retinol palmitate in oil (60 mg => 200,000 iμ), 120 mg = 400,000 iμ or 180 mg = 600,000 iμ), at a time 0. Only 60 mg or 120 mg doses were given to children. 5 ml blood samples were collected and protected from light in a light-proof box. Serum was separated and frozen at 80°C until assays were performed. Urine was collected for 24 hours following administration of the Vitamin A dose from 10 men with diarrhoea and 10 men without diarrhoea.

Retinol for oral administration was given as capsules of retinal palmitate in oil, obtained from LAMBO laboratories n.v, 7-9 Pieter de Smethstraat, Antwerp, Belgium.

Vitamin A was assayed in blood and urine by high performance liquid chromatography (HPLC). 200 μl of serum was added to an equal volume of ethanol and an internal retinyl acetate standard and extracted into 500 μln-hexane. The hexane was evaporated under a nitrogen stream and the residue dissolved in 200 μl dicloromethane:propanol (4:1 v/v). 25 μl was applied to a supelcasil column (LC 18, 25cm x 4.6 mm, 5μm) and a methanol: water solvent (98:2) used at 2ml/min . Elution was detected at 325nm.

HIV tests were performed using rapid antibody tests (Capillus 1+2, Trinity Biotech, Ireland) on adult controls only in order to assess whether bio-availability was affected by HIV infection in men without diarrhoea. It was previously demonstrated that the aetiology of persistent diarrhoea in adult patients in this hospital is HIV-related in over 75% of cases (32). We did not perform HIV tests in the diarrhoea patients as the comparison we undertook was between patients with or without persistent diarrhoea. Since HIV testing was performed after the individual studies were completed, it was not possible to ensure that HIV seropositive and HIV seronegative subjects were evenly distributed among groups receiving different doses.

5.4 STATISTICAL ANALYSIS

Serum retinol concentration at baseline and changes over 6 hours are presented as median with interquartile range (IQR). Significance testing was performed using the Kruskal-Wallis non parametric test. The proportion of patients with detectable and undetectable urine retinol excretion was compared using the x^2 test.

CHAPTER SIX

6.0. RESULTS

6.1 PHASE 1

Median (IQR) baseline concentration was 1.24 (0.92-1.47) μmol/L in the four men without diarrhoea. Men with persistent diarrhoea had a median (IOR) base line level of 0.36 (0.23-0.81) μmol/L which was lower than men without diarrhoea. Three out of four of these men would be classified as Vitamin A deficient using the World Health Organisation (15) classification (figure 1) by which serum concentration of under 1.05 μmol/L would be regarded as indicative of deficiency.

Following an oral dose of 60 mg retinol, the serum retinol increased in all men without diarrhoea giving a peak concentration of 1.90 (1.54-2.20) μmol/L at 6 hours. In contrast the retinol concentration did not change and remained at baseline levels in patients with diarrhoea. At all time points after 5 hours the difference between men with and without diarrhoea was significant ($P<0.05$). Based on these observations in the men without diarrhoea and the time at which retinol concentration reached a peak, it was decided to take blood samples for PHASE 2 studies at baseline and at 6 hours after the dose.

6.2 PHASE 2

24 adult controls, 15 adults with persistent diarrhoea and 11 children with persistent diarrhoea – malnutrition syndrome were studied. Among the controls, 10 were HIV seropositive and 14 were HIV seronegative.

6.3 RETINOL CONCENTRATION AT BASELINE

In the 24 adults without diarrhoea, baseline values were 1.16 (0.84-1.47) μmol/L. Within the group, there was no significant difference between the 14 men who were HIV seronegative (1.23; 0.88-144 μmol/L) and 10 men who were HIV seropositive (1.09; 0.77 – 1.51 mmol/L). Baseline values were significantly lower in adults with persistent diarrhoea (0.39; 0.21 – 0.56 mmol/L) and children with persistent diarrhoea (0.28; 0.18 – 0.45 mmol/L) than in adults with no diarrhoea ($p < 0.001$; Table 1).

6.4. EFFECT OF VITAMIN A SUPPLEMENTATION ON RETINOL IN ADULTS WITHOUT DIARRHOEA

6.4.1 60 mg RETINOL

Sixteen (16) of the 24 adults without diarrhoea were given 60 mg retinol (Table 2). The median increase in retinol concentration was 0.39 (0.14 – 0.70) mmol/L. In this group 11 were HIV seronegative and 5 HIV seropositive. There was no significant difference in the rise in serum retinol between seronegative adults (0.35; 0.04 – 0.56 mmol/L) and in the HIV seropositive adults (0.65; 0.35 – 0.77 mmol/L).

6.4.2 120 mg AND 180 mg RETINOL

The median increase in retinol following 120 mg and 180 mg retinol was 0.81 (0.60 – 0.98) mmol/L and 0.39 (0.26 – 0.56) mmol/L respectively. These values werenot significantly different to the rise in serum retinol observed for all the adults given 60 mg retinol.

6.5 EFFECT OF VITAMIN A SUPPLEMENTATION ON RETINOL IN ADULTS WITH PERSISTENT DIARRHIEA

Four men with persistent diarrhoea were given 60 mg, 6 were given 120 mg and 5 were given 180 mg retinol (Table 2). There was an increase in serum retinol of 0.07 (0.02 – 0.18) mmol/L in those receiving 60 mg retinol. The rise was slightly higher in those receiving 120 mg (0.28: 0.07 – 0.35 mmol/L) or 180 mg (0.28: 0.25 – 0.35 mmol/L) but this was not statistically significant ($p = 0.23$). Taking all the men with persistent diarrhoea together irrespective of dose, the median increase in retinol (0.25; 0.04 – 0.35 mmol/L) was significantly lower than that observed for all men without diarrhoea taken together irrespective of dose (0.44; 0.26 – 0.74 mmol/L; $p < 0.01$).

6.6 EFFECT OF VITAMIN A SUPPLEMENTATION IN RETINOL IN CHILDREN WITH PERSISTENT DIARRHOEA

In the six children who were given 60 mg retinol, the increase in retinol was 0.11 (0.07 – 0.25) mmol/L and was similar to the 5 children receiving 120 mg retinol 0.11; 0.04 – 0.46 μmol/L). Taking these children together irrespective of dose, there was no significant difference in the rise in retinol between children with persistent diarrhoea and adults with persistent diarrhoea.

6.7 URINARY EXCRETION OF RETINOL

Urine collection over 24 hours were completed in 10 men without diarrhoea and 10 men with diarrhoea. Retinol was undetectable in urine of 9 out f 10 controls, but detectable in 9 out of 10 patients with diarrhoea ($p = 0.000\ 3$ by X^2 test). However, the diarrhoea patients whose urine collections were complete had received higher doses (120 or 180 mg) than the controls, who all received 60 mg. In those whose collections over 24 hours were complete, the range of excretion (when detectable) was 33 – 683 μg over 24 hours.However ,the amount excreted was less than 1% of the ingested doses.

Urine collection proved difficult to achieve in children due to spillage from collecting bags and contamination from diarrhoeal stools

TABLE 1: CHARACTERISTICS OF PARTICIPANTS IN STUDIES OF BIO AVAILABILITY OF RETINOL FOLLOWING MEGA DOSE ORAL THERAPY

	ADULT CONTROLS HIV NEGATIVE	ADULT CONTROLS HIV POSITIVE	ADULT PATIENTS WITH DIARRHOEA	CHILDREN WITH DIARRHOEA
N	14	10	15	11
Sex	All Male	All Male	All male	5 Male, 6 Female
Age mean year	30	28	30	2
Baseline Serum Retinol	1.23 (0.88 - 1.44)	1.09 (0.77 – 1.51)	0.39(0.21 – 0.35)	0.28 (0.18 – 4.6)
Change in Serum Retinol	0.35 (0.4 – 0.56)	0.65 (0.35 – 0.77)	0.25 (0.04 – 0.35)	0.11(0.04 – 0.46)

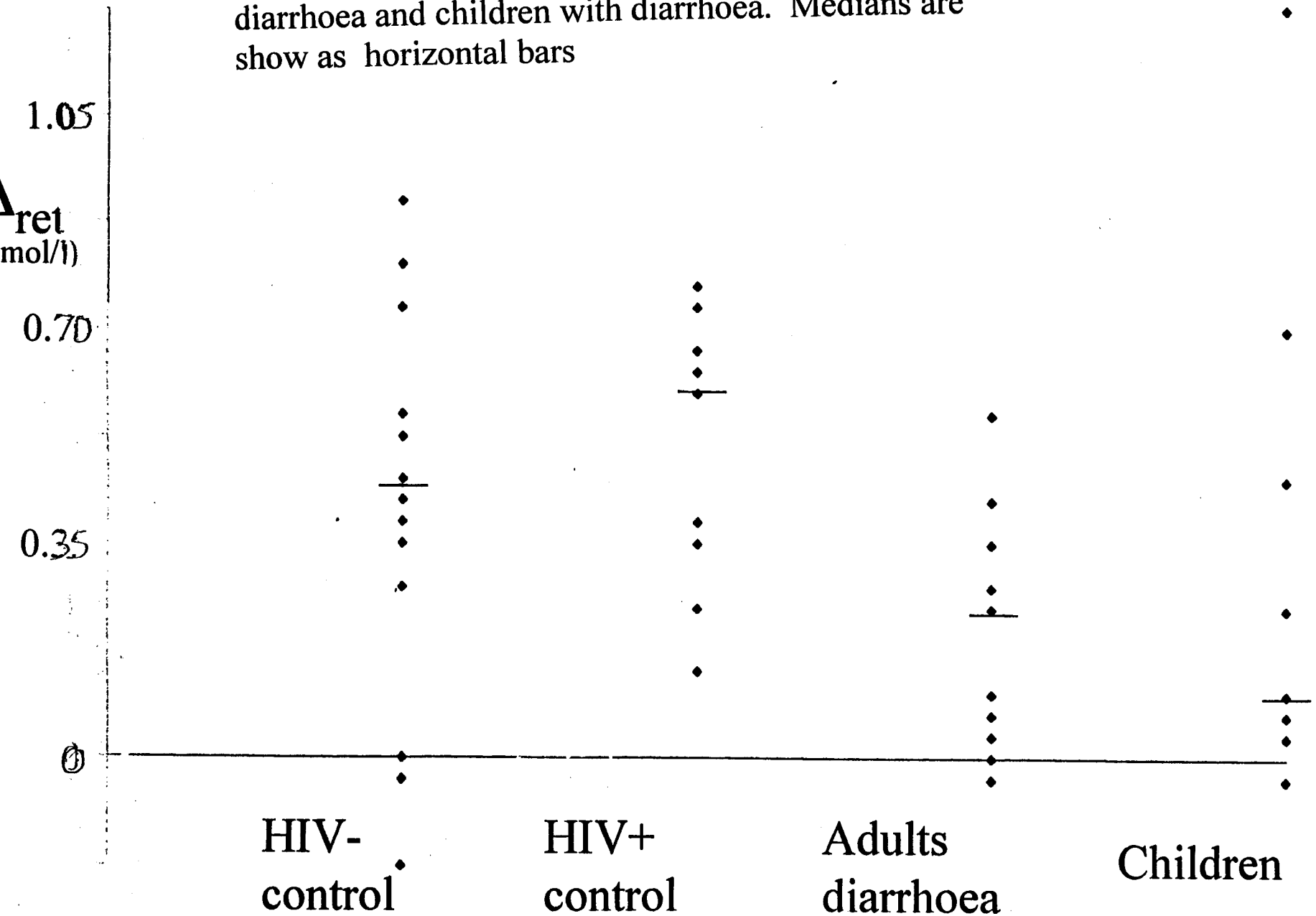
Ages given are median (range). Values are expressedin μmol/L for purpose comparison, 200000 iu palmitate is the equivalent of 60 mg retinol. A retinol concentration of 1 μmol/L is equivalent to 28.5 μg/dl. The dose administered is shown in Table 2

TABLE 2: ALLOCATION OF MEN AND CHILDREN WITHIN EACH GROUP TO DIFFERENT DOSE REGIMES

SUBJECTS	DOSE OF RETINOLS		
	60 mg	120 mg	180mg
24 men with no diarrhoea	16	4	4
15 men with diarrhoea	4	6	5
11 children with diarrhoea	6	5	-

Figure 2

diarrhoea (with or without HIV infection), men with diarrhoea and children with diarrhoea. Medians are show as horizontal bars



CHAPTER SEVEN

7.0 DISCUSSION

World Health Organization recommendation for vitamin A supplementation suggest that 60 mg retinol given as retinyl palmitate is an appropriate dose for adults and children over one year of age [33]. However, relatively few data is available on the fate of such doses following administration. The data in this study indicate that bioavailability of vitamin A is reduced in patients with persistent diarrhoea in this population as shown by the clear difference in serum retinol concentration profile in patients with diarrhoea compared to controls.

Giving higher doses did not overcome the impaired bioavailability. Patients with diarrhoea had lower baseline serum concentrations of retinol than control suggesting that the reduced bioavailability probably leads to reduced body vitamin A stores in this group of patients as a whole.

We found no evidence that HIV status per se influences baseline retinol concentration, or the rise in concentration following oral administration. This suggests that in apparently healthy men who are HIV infected, absorption of vitamin A is unlikely to be severely impaired.

The most important factor is likely to be impaired uptake across the alimentary tract, but other processes could contribute to the impaired bioavailability we observed. It could be that the failure to respond to the dose administered in some individuals might have been a consequence of infections, induced changes in the transport of retinol from the liver. It is also possible that retinol sensitive tissues also alter the concentration of retinol by increase uptake, but this possibility has not been explored to date. It would not appear to be explained by increased urine losses as the amount received from urine over 24 hours was small (33 –683 μg) compared to the quantities administered (60 – 180 mg).

The controls we studied were convalescent male patients on surgical wards with fractures of femur, but we decided not to perform studies in healthy children because of anticipated difficuties in obtaining consent from caretakers. The adult controls had no evidence of pulmonary, intestinal or other infectious diseases and therefore we chose to use this group as they were receiving the same diet as the patients with intestinal diseases in the same hospital and were available for studies lasting for 6 hours. In contrast, it is likely that the patients with persistent diarrhoea, many of whom would have AIDS if we extrapolate from previous studies, would have had co-morbid pulmonary or systemic infections. These would contribute to the impaired bioavailability of vitamin A though the mechanism of hyporetinolaema related to systemic infection needs further clarification.

The interpretation of serum retinol concentration following oral administration is not complete because there are several steps in handling of retinol esters which would determine the bioavailability of the molecule once administered. Most of the early work using the "vitamin A absorption test" developed in the 1950s and 1960s used high doses of retinol esters, comparable with doses used in this study [34, 35]. But clinical and analytical protocols were not standardised and results are difficult to compare from one laboratory to another [36].

Subsequently, it was noted that hepatic retinol store influences the handling of retinol by the liver [37], so that in a state of repletion retinol is stored in the liver, but in deplete individuals retinol is released for transport by RBP for use by retinol – dependent tissues. This observations became the basis of the relative dose response (RDR) tests in which small doses of retinol or retinols esters are administered and serum concentration is used to make an assessment of body retinol stores.

Recently the RDR test have been criticised, and the reliability even of liver biopsy, previously regarded as the gold standard, has been questioned [38, 39, 40]. However, it is not clear whether the handling of mega doses of retinol is affected by hepatic stores in the same way as near physiological doses. .

CHAPTER EIGHT

8.0 CONCLUSION

The bioavailability of vitamin A is reduced in patients with persistent diarrhoea in this population as shown by the clear difference in serum retinol concentration profile in patients with diarrhoea compared to the controls.

Patients with persistent diarrhoea have lower baseline serum retinol levels.

HIV status per se does not influence baseline retinol concentration or the rise in concentration following oral administration. This suggests that in apparently healthy men who are HIV infected, absorption of vitamin A is unlikely to be severely impaired.

Vitamin A deficiency is difficult to overcome by using oral mega doses since excretion in urine increase with corresponding increase in the oral dose.

When Vitamin A is administered orally, it reaches a peak concentration at around 6 hours of administration. This is in line with Mendeloff's absorption curves.

CHAPTER NINE

9.0 RECOMMENDATION

- Current strategy should probably be to restore and maintain vitamin A repletion in adults and children with HIV infection before they develop intestinal disease. This may have prophylactic effects in delaying onset immune failure in HIV infected individuals. Though this strategy needs to be tested in a large population-based trial.

- However, there is an urgent need to define how best to achieve vitamin A repletion in adults and children with intestinal disease as our data suggest that orally administered retinal-palmitate is likely to be inadequately absorbed as in other small intestinal disorders [41] and they would be expected to have increased requirements for this important micronutrient.

Further work is needed to determine if the impaired bio-availability observed in this study might explain why vitamin A administration is less effective in treating severe illness than in preventing morbidity and mortality in children in developing countries [3].

STUDY LIMITATION

1. No women were involved in the study because Vitamin A has been known to be teratogenic in experimental animals and we could not exclude pregnancy since could not do pregnancy tests.

2. Urine collection proved difficult in children because of leakage and contamination with diarrhoeal stools.

3. No controls for children were recruited because of difficulties in obtaining content from caretakers.

4. The role of other micronutrient which have a role to play in the bio-availability of Vitamin A was not analysed.

5. Lack of resources prevented us from recruiting more patients with persistent diarrhoea .This resulted in a small sample size especially in children.

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APPENDIX I

STUDY FORM

STUDY ON BIOAVAILABILITY OF VITAMIN A IN ADULTS AND CHILDREN WITH PERSISTENT DIARRHOEA IN ZAMBIA

Name: _____

Age: _____

Sex: Male / Female

Address: _____

_____

_____

_____

Ward: _____

Duration of diarrhoea: _____

Type of fracture: _____

Weight: _____

URINE: Volume: _____ mls

Dose of Vitamin A: _____ iμ

APPENDIX 2

VITAMIN A ABSORPTION STUDY

UNIVERSITY TEACHING HOSPITAL

You have been asked to help us with a short study to work out why people in Zambia seem to have too little vitamin A in the blood. This seems to make people become very ill. so it is important for us to understand why.

We would like to give you one tablet of vitamin A and then we will measure the amount in your blood after 3 and 6 hours. followed by another sample at 24 hours and we will collect urine for this period.

Vitamin A is an important and widely used vitamin and we do not anticipate any problems or unwanted effects. The only discomfort is the needle. four times. If you do not agree or it you withdraw from the study. you will still be looked after as before.

If you have any questions. please ask one of the Doctors. If you are willing. please sign below to indicate your agreement.

I agree to be included in the Vitamin A Absorption Study.

Name : ----------

Signed : ----------

Date : ----------