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A STUDY OF HYPERCROTENAEMIA IN SRI LANKAN CHILDREN AND ITS POSSIBLE AETIOLOGY

By

Nekadage Don Amal Wageesha

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DECLARATION BY THE CANDIDATE

The work described in this thesis was carried out by me under the supervision of Prof. S. Ekanayake (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura), Prof. E.R. Jansz (Emeritus Professor, University of Sri Jayewardenepura) and Prof. S.P.Lamabadusuriya (Emeritus Professor, University of Colombo) and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma.

16th March 2010 ..... Achala Cageesha .....

Date Signature of candidate

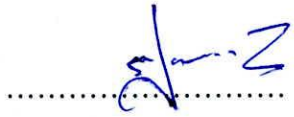
We certify that the candidate has incorporated all corrections amendments and additions recommended by the examiners.

.....	.....	.....
Prof. S. Ekanayake	Prof. E.R. Jansz	Prof. S.P. Lamabadusuriya
	26/11/2010	

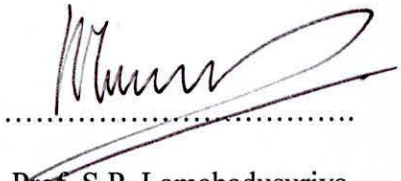
CERTIFICATION BY SUPERVISORS

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

Prof. S. Ekanayake



Prof. E.R. Jansz



Prof. S.P. Lamabadusuriya

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ABBREVIATIONS

AR	Analytical Reagent
BCO	β –carotene 15,15′ monooxygenase
BMR	Basel metabolic rate
CMO	Carotenoid mono oxygenase
CRBP	Cellular retinoid binding protein
DTUL	Daily tolerance upper limit
DW	Dry weight
FW	Fresh weight
HDL	High density lipoprotein
HC	Hypercarotenaemia
HPLC	High performance liquid chromatography
ICR	Institute of cancer research
IU	International units
LDL	Low density lipoprotein
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
MRI	Medical research institute
MS	Mass spectrometry
NHC	Non hypercarotenaemic
NMR	Nuclear magnetic resonance
PDA	Photodiode array
PE	Petroleum ether

R_f	Retardation factor
RP	Reversed phase
TLC	Thin layer chromatography
TSH	Thyroid stimulating hormone
UV	Ultra violet
VAD	Vitamin A deficiency
VLDL	Very low density lipoprotein
WHO	World Health Organization

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I dedicate this thesis to all my teachers and to my family.

N.D.A.Wageesha

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A STUDY OF HYPERCAROTENAEMIA IN SRI LANKAN CHILDREN AND ITS POSSIBLE AETIOLOGY

N.D.A. Wageesha

ABSTRACT

Hypercarotenaemia is clinically diagnosed by yellowing of the dermal tissue particularly of the palms and soles but not the sclera of the eye. It is reported in Sri Lanka particularly, but not exclusively in infants and young children. This study excludes hypercarotenaemia caused by liver and thyroid pathology and is confined to the feeding of carotenoid rich foods. In this case there are relatively few subjects who develop hypercarotenaemia. In Sri Lanka the carotenoid rich foods fed to infants and children are boiled, carrot, pumpkin and ripe papaw. In the present study an attempt was made to study hypercarotenaemic serum profiles, time course on serum carotenoid profiles on cessation of feeding carotenoids, proportion of hypercarotenaemics in the Western province and the aetiology using animal models.

Hypercarotenaemic subjects (n=36) were tested for carotenes in their serum. The carotenoids were classified as α and β carotenes, monohydroxy metabolites and polyhydroxy metabolites which varied from 119 μg/dL to trace amounts, 148.7 μg/dL to trace amounts, 214 μg/dL to non detectable or less than 0.5 μg/dL, 822.7 μg/dL to 7.0 μg/dL respectively. It was possible to predict the contribution of carotenoid rich foods to the condition by the presence of α-carotene (carrot), β-cryptoxanthin (papaw). There was no hypervitaminosis due to vitamin A.

Longitudinal studies following withdrawal of carotenoid rich food indicated serum carotenoid levels declining at slightly varying rates in each individual, while vitamin A

levels were more or less maintained, probably due to the liver 15-15′-dioxygenase activity.

Classically hypercarotenaemia is termed to be genetic or metabolic. However, in types of hypercarotenaemia studied it can be predicted to be in some way or another due to genetics. In the present study, identical hypercarotenaemic twins had similar serum carotenoid profiles which may be due to genetics. However, this needs further studies with statistically significant number of such twins before it can be confirmed.

Preliminary tests on faeces to study the effect of absorption of carotenoids in the development of hypercarotenaemia with 08 hypercarotenaemic and 10 non-hypercarotenaemic subjects showed that no α and β carotenes were present (LOD = 0.5 μg/dL) in hypercarotenaemic patient's faeces while the faeces of the 10 non hypercarotenaemics showed 1.53 μg/dL and 0.7μg/dL, 1.74 μg/dL and 1.0 μg/dL, 1.0 μg/dL and trace amounts, 1.8 μg/dL and 1.4 μg/dL, 1.2 μg/dL and 1.0 μg/dL, 1.6 μg/dL and 0.9 μg/dL, 1.0 μg/dL and 1.3 μg/dL and trace amount and 0.7 μg/dL of β and α carotenes respectively per one gram of freeze dried faeces. This indicated that the mechanism responsible for controlling absorption of carotenoids in hypercarotenaemics to be deranged which again could be due to a genetic factor.

A study in determining the proportion of hypercarotenaemics among pre-school children aged below 5 years in the Western Province showed that the proportion was 1.5%. However, the proportion of hypercarotenaemia among subjects fed carotenoid rich food was approximately 2%. This observation was made irrespective of vitamin A mega dosing.

Animal studies were designed originally to determine the bilary excretion product(s) of carotenoids in hypercarotenaemia induced Wistar rats and ICR mice with the intention

of studying the carotenoid metabolism. This was unsuccessful as neither breed, despite being fed on high carotenoid rich diet, developed hypercarotenaemia. The serum, liver, adipose tissue around the kidneys, bile (in mice), digesta (in rats) did not show carotenoids. However both types of rodents had high levels of α and β carotenes in the faeces similar to the human fecal study. This indicates that one natural way of preventing hypercarotenaemia is by controlling the absorption of carotenoids.

This study proves that the genes responsible for synthesis of proteins which are responsible for metabolism and/or absorption of carotenoids are not common in the group studied.