

**A STUDY OF HYPERCROTONAEMIA
IN SRI LANKAN CHILDREN AND ITS
POSSIBLE AETIOLOGY**

By

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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.

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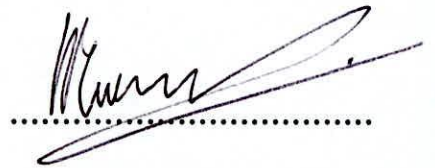


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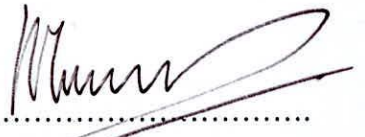
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TABLE OF CONTENTS

	Page no.
LIST OF TABLES	vii
LIST OF FIGURES	ix
LIST OF PLATES	xii
ABBREVIATIONS	xiii
ACKNOWLEDGEMENTS	xv
ABSTRACT	xviii
1. INTRODUCTION	01
1.1 General Introduction	01
1.2 Justification	02
1.3 Scope of thesis	03
2. LITERATURE REVIEW	05
2.1 Carotenoids	05
2.1.1 Occurrence carotenoids	05
2.1.2 General structure of carotenoids	06
2.1.3 Classification of carotenoids	06
2.1.4 Absorption of carotenoids	08
2.1.5 Formation of Vitamin A	13
2.1.6 Other benefits of carotenoids	13

2.2 Metabolism of carotenoids after uptake	16
2.2.1 Excretion	19
2.2.2 Distribution of carotenoids in blood and tissues	19
2.3 Hypercarotenaemia	21
2.3.1 Definition	21
2.3.2 Diagnosis	21
2.3.3 Causes of hypercarotenaemia	23
2.3.4 Past studies in Sri Lanka	26
2.4 Vitamin A	27
2.4.1 15,15'- dioxygenase activity	27
2.4.2 Vitamers	30
2.4.3 Vitamin A deficiency	30
2.4.4 Hypervitaminosis	32
2.5 High Performance Liquid Chromatography	34
2.5.1 Reverse Phase HPLC (RP-HPLC)	36
2.5.2 Advantages and draw backs of HPLC in carotenoid analysis	37
3. MATERIALS AND METHODS	39
3.1 Materials	39
3.1.1 Water	39
3.1.2 Chemicals	39

3.1.2.1 Solvents	39
3.1.2.2 Special Chemicals	39
3.1.3 Instruments	39
3.1.4 Subjects	40
3.1.4.1 Ethical Clearance	40
3.1.4.2 Inclusion and Exclusion Criteria	40
3.1.4.3 Hypercarotenaemics	40
3.1.4.4 Control/ non hypercarotenaemics	41
3.1.5 Wistar rats	41
3.1.6 ICR mice	41
3.2 Methods	43
3.2.1 Human study	43
3.2.1.1 Isolation of carotenoids from blood for carotenoid and vitamin A analysis	43
3.2.1.2 Blood for longitudinal study	43
3.2.1.3 Extraction of carotenoids their metabolites and vitamin A for HPLC detection	43
3.2.1.4 Spectral fine structures of carotenoids	44
3.2.1.5 Quantification	46
3.2.1.6 Construction of standard curves	46
3.2.2 Proportion of children who developed hypercarotenaemia	48

3.2.3 Sibling study of hypercarotenaemics	50
3.2.4 Carotenoids in faeces of hypercarotenaemics and non-hypercarotenaemics	50
3.2.5 Animal studies	51
3.2.5.1 Wistar rats	51
3.2.5.2 ICR mice	51
3.2.5.3 Inducing hypercarotenaemia in rats and mice	53
3.2.5.4 Isolation of carotenoids from blood, faeces, liver, Adipose tissue and digesta – Wistar rats	53
3.2.5.5 Isolation of carotenoids from blood, faeces, liver, adipose tissue and bile – ICR mice	56
3.2.6 Validation of the RP-HPLC technique	56
3.2.6.1 Precision (within day variation) and precision of day to day variation	57
3.2.6.2 Linearity	57
3.2.6.3 Limit of detection (LOD)	58
3.2.7 Statistical analysis	58
4. RESULTS	59
4.1 Verification of accuracy of HPLC procedures	59
4.1.1 Precision	59

4.1.2	Repeatability	59
4.1.3	Linearity	61
4.1.4	Limit of detection	61
4.2.	Serum carotenoids and vitamin A in hypercarotenaemics	61
4.2.1	Serum carotenoids and vitamin A levels after withdrawal of carotenoid rich foods	61
4.2.2	Serum carotenoids and vitamin A levels of identical twins	70
4.2.3	Serum carotenoids, their metabolites and vitamin A levels of controls (non hypercarotenaemics)	70
4.3	Surveys on hypercarotenaemia	76
4.3.1	Hypercarotenaemia in siblings of hypercarotenaemics	76
4.3.2	Preliminary survey on hypercarotenaemia in children in the Western province	76
4.4	Carotenoids in faeces of hypercarotenaemic and non hypercarotenaemic children	81
4.5	Studies of Wistar rats	81
4.5.1	Serum carotenoid and vitamin A profiles	81
4.5.2	Digesta, liver and adipose profiles	86
4.5.3	Faeces study	86
4.6	Studies on ICR mice	86
4.6.1	Mice vitamin A profile	91
4.6.2	Mice faeces profile	91

5. DISCUSSION		96
6. CONCLUSION		109
7. REFERENCES		110
8. APPENDICES		127
APPENDIX	- List of Publications, Communications and Awards from work on this thesis	127
APPENDIX II	- Letter of ethical clearance	130
APPENDIX III (a)	- A copy of the questionnaire given to parents of hypercarotenaemics	131
APPENDIX III (b)	- A copy of the sinhala translated questionnaire given to parents of hypercarotenaemics	133
APPENDIX IV (a)	- A copy of the questionnaire given to parents who are having children below 5 years of age	135
APPENDIX IV (b)	- A copy of the sinhala translated questionnaire given to parents who are having children bellow 5 years of age	137

LIST OF TABLES

		Page No.
Table 3.1	Ingredients for preparing 1 Kg of standard rat/mice pellet	52
Table 4.1	Observed retention times of β -carotene and all <i>trans</i> - retinol in the determination of the precision of the RP- HPLC technique	60
Table 4.2	Observed retention times β - carotene and all <i>trans</i> - retinol in the determination of repeatability of the used RP-HPLC technique	60
Table 4.3	Observed chromatographic areas for different concentrations of standard β - carotene in determination of LOD	62
Table 4.4	Observed chromatographic areas for different concentrations of standard all- <i>trans</i> - retinol in determination of LOD	62
Table 4.5 (a)	Serum levels of carotenoids, their metabolites and vitamin A on day of reporting to Consultant	63
Table 4.5(b)	Continuation of serum levels of carotenoids, their metabolites and vitamin A on day of reporting to Consultant	64
Table 4.5(c)	Summary of serum carotenoids and vitamin A on day of reporting to Consultant	65
Table 4.6 (a)	Summary of parents' data on feed intake of hypercarotenaemics	66
Table 4.6 (b)	Continuation of table 4.6 (a); summary of parents' data on feed intake of hypercarotenaemics	67
Table 4.7	Serum carotenoids, their metabolites and vitamin A levels after a month from cessation of intake of carotenoid rich foods	68

Table 4.8	Serum carotenoids, their metabolites and vitamin A levels after two month from cessation of intake of carotenoid rich foods	69
Table 4.9	Levels of serum carotenoids, their metabolites and vitamin A levels of identical twins on reporting	74
Table 4.10	Vitamin A levels of controls/non hypercarotenaemics	74
Table 4.11	Major carotenoids levels present in faeces of hypercarotenaemics and non-hypercarotenaemics	82
Table 4.12	Final body weights of test and control group rats	83
Table 4.13	Serum vitamin A levels in test and control Wistar rats	85
Table 4.14	Final body weights of the test and control ICR mice	90
Table 4.15	Vitamin A levels of the two groups of the ICR mice after 2 ½ months time	92

LIST OF FIGURES

		Page No.
Figure 2.1	Structure of pro-vitamin A carotenoids	07
Figure 2.2	Structures of non pro-vitamin A carotenoids with substituted β -ionone ring with oxygen functions	09
Figure 2.3	Structures of some non pro-vitamin A carotenoids	10
Figure 2.4	Structures of some carotenoids occurring in animals	11
Figure 2.5	Structures of some carotenoids used as food colorants	12
Figure 2.6	Central and stepwise cleavage pathways for the oxidative conversion of all- <i>trans</i> - β -Carotene to retinal	14
Figure 2.7	Metabolism and excretion pathway of carotenoids	18
Figure 2.8	Serum carotenoid metabolites	20
Figure 2.9	Central cleavage of β -carotene by enzyme -carotene 15,15'-monooxygenase	29
Figure 2.10	Structural formulae of major retinoids	31
Figure 2.11	General schematic representation of a HPLC apparatus	35
Figure 3.1	Schematic representations of serum carotenoid and vitamin A extraction and analysis	45
Figure 3.2	Standard curve for all- <i>trans</i> -retinol	47
Figure 3.3	Standard curve for β -carotene	49
Figure 4.1	Typical RP- HPLC chromatogram of a Hypercarotenaemic patient	71
Figure 4.2	RP-HPLC chromatogram of a hypercarotenaemic patient with β -cryptoxanthin	71

Figure 4.3	UV/ VIS spectrum of serum hexane extract of a typical hypercarotenaemic patient	72
Figure 4.4	Typical RP-HPLC vitamin A profile of a hypercarotenaemic patient	73
Figure 4.5	Typical Serum carotenoid RP- HPLC profile of non-hypercarotenaemic patient	75
Figure 4.6	Typical serum Vitamin A RP-HPLC profile of a non-hypercarotenaemic patient	75
Figure 4.7	Summary of the sibling study	77
Figure 4.8(a)	The relationship of hypercarotenaemia with high carotenoid diet and Vitamin A supplementation in the studied sample	78
Figure 4.8(b)	The relationship of hypercarotenaemia with high carotenoid diet and Vitamin A supplementation in the studied sample including siblings	80
Figure 4.9	RP-HPLC chromatogram of the test group serum carotenoid profile	84
Figure 4.10	RP-HPLC chromatogram of the control rat serum carotenoid profile	84
Figure 4.11	Test group rat faeces carotenoid extract profile	87
Figure 4.12	Control group rat faeces carotenoid extract profile	87
Figure 4.13	Diagrammatic representation of TLC of faeces of rats	88
Figure 4.14	RP-HPLC profile of scraped TLC UV active spots of test group rat faeces	89
Figure 4.15	Test group mouse faeces carotenoid RP-HPLC profile	93

Figure 4.16	Control group mouse faeces carotenoid RP- HPLC profile	93
Figure 4.17	Diagrammatic representation of TLC of faeces of mice	94
Figure 4.18	RP-HPLC profile of UV active spots of test mouse faeces	95
Figure 5.1	Schematic representation of alternatives for solubilisation of carotenoids	104

LIST OF PLATES

		Page No.
Plate 2.1	Yellow-orange discoloration of palm and a normal shown palm shown for comparison	22
Plate 2.2	Yellow orange peripheral corneal ring most prominent in the superior and inferior cornea	24
Plate 3.1	Male Wistar rats	42
Plate 3.2	Male ICR mouse	42

ABBREVIATIONS

AR	Analytical Reagent
BCO	β -carotene 15,15' monooxygenase
BMR	Basal 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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A STUDY OF HYPERCAROTENAEMIA IN SRI LANKAN CHILDREN AND ITS POSSIBLE AETIOLOGY

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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 $\mu\text{g/dL}$ to trace amounts, 148.7 $\mu\text{g/dL}$ to trace amounts, 214 $\mu\text{g/dL}$ to non detectable or less than 0.5 $\mu\text{g/dL}$, 822.7 $\mu\text{g/dL}$ to 7.0 $\mu\text{g/dL}$ 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 $\mu\text{g/dL}$) in hypercarotenaemic patient's faeces while the faeces of the 10 non hypercarotenaemics showed 1.53 $\mu\text{g/dL}$ and 0.7 $\mu\text{g/dL}$, 1.74 $\mu\text{g/dL}$ and 1.0 $\mu\text{g/dL}$, 1.0 $\mu\text{g/dL}$ and trace amounts, 1.8 $\mu\text{g/dL}$ and 1.4 $\mu\text{g/dL}$, 1.2 $\mu\text{g/dL}$ and 1.0 $\mu\text{g/dL}$, 1.6 $\mu\text{g/dL}$ and 0.9 $\mu\text{g/dL}$, 1.0 $\mu\text{g/dL}$ and 1.3 $\mu\text{g/dL}$ and trace amount and 0.7 $\mu\text{g/dL}$ 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 biliary 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.