



UNIVERSITI PUTRA MALAYSIA

**IRON BIOAVAILABILITY FROM SPIRULINA (*ARTHROSPIRA
PLATENSIS*) AND ITS INTERACTIONS WITH OTHER DIETARY
FACTORS IN VITRO AND IN VIVO**

LOH SU PENG

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Philosophy**

June 2004



**I lift up my eyes to the hills
Where does my help come from?
My help comes from the LORD
the Maker of heaven and earth**

Psalm 121:1-2



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Deficiency of iron is common worldwide. Various approaches have been used to improve iron intake and absorption. These include the use of spirulina, a microalga that is already popular in many Asian countries as a functional food supplement. The main objective of this study was to determine the iron bioavailability from spirulina and its interactions with other dietary factors both *in vitro* and *in vivo*.

In vitro digestion/Caco-2 cell culture system accompanied by either centrifugation or dialysis step was used to assess the availability of iron from spirulina. Using the centrifugation method, the cultured and commercial spirulina yielded significantly higher results ($P < 0.05$) than the dialysis method, both in the form of iron available for uptake and the actual amount of iron being transported across the Caco-2 cells. The amount of available iron and iron being transported from ferrous sulphate (FeSO_4) did not differ significantly for both the dialysis and centrifugation method. The effects of

different molar ratios of nutrients (calcium, ascorbic acid, zinc, tannic acid and caffeine) to iron on the availability of iron from cultured spirulina differs in comparison with FeSO_4 . In the presence of lower concentrations of calcium (1:5, 1:10, 1:15 and 1:20 Fe:Ca molar ratios), iron from spirulina was not significantly inhibited compared to FeSO_4 , but at higher concentrations (1:37.34, 1:74.67 and 1:149.34 Fe:Ca molar ratios) iron from both spirulina and FeSO_4 was significantly inhibited. The availability of iron from spirulina in the presence of ascorbic acid were not significantly enhanced at all the molar ratios tested (1:0.5, 1:1, 1:1.5 and 1:2 Fe:AA molar ratios) whereas iron availability from FeSO_4 were significantly higher for all the molar ratios. Both zinc and tannic acid were more inhibiting on iron availability from spirulina in comparison to FeSO_4 . As for caffeine, it did not show any significant inhibitory effects on both iron availability from spirulina and FeSO_4 . Two iron pools could coexist in the spirulina, one containing organic iron and another comprising inorganic iron. Organic iron is known to be more bioavailable and less affected by the presence of other nutrients. This could be one of the explanations why the iron from this algae is highly available and its bioavailability is not significantly affected by other nutrients as in FeSO_4 .

Haemoglobin repletion assay was used to further investigate the effect of calcium on absorption of iron in spirulina and its comparison with FeSO_4 . In this study, haemoglobin and haematocrit levels of male Sprague-Dawley rats fed both spirulina and FeSO_4 were found similar although the dose of FeSO_4 used had twice the amount of iron compared to that in spirulina. The

presence of calcium did not significantly reduced the haematological value in rats fed spirulina and FeSO₄. The percentage of haemoglobin regeneration efficiency (HRE) obtained was significantly higher in rats fed spirulina compared with rats fed FeSO₄ indicated that the absorption efficiency were better from iron in spirulina compared to iron in FeSO₄.

The distribution study of iron from spirulina and FeSO₄ in the presence of calcium was done using iron deficient and iron normal male ICR mice fed either spirulina or FeSO₄ tagged extrinsically with ⁵⁹Fe. The amount of ⁵⁹Fe being absorbed by the iron deficient mice fed spirulina was comparable with those fed FeSO₄ at 6 h and 24 h. However at 7 d, the FeSO₄ group showed better absorption than the spirulina group. In the iron normal mice, a significantly lower percentage of ⁵⁹Fe was observed in mice fed spirulina compared to mice fed FeSO₄ at 6 h and 24 h indicating that iron from spirulina were not readily absorbed in iron normal states, which could prevent iron overload and toxicity. The presence of calcium did not significantly inhibit iron absorption in spirulina as shown in the *in vitro* study.

This study indicated that spirulina is a concentrated source of iron for both supplementation and fortification. Iron from spirulina is highly bioavailable and easily absorbed by the body especially in the iron deficient state. Beside providing the necessary iron, it could also prevent iron overload and toxicity in normal iron status and thus making spirulina suitable for both the iron deficient and normal iron status.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIOAVAILABILITI FERUM DARIPADA SPIRULINA (*ARTHROSPIRA PLATENSIS*) DAN INTERAKSINYA DENGAN FAKTOR DIETARI LAIN SECARA IN VITRO DAN IN VIVO

Oleh

Loh Su Peng

Jun 2004

Pengerusi : Profesor Madya Maznah Ismail, Ph.D.

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Masalah kekurangan ferum merupakan perkara biasa di seluruh dunia. Pelbagai cara telah digunakan untuk meningkatkan pengambilan dan penyerapan ferum. Ini termasuk penggunaan spirulina, sejenis mikroalga yang telah popular di banyak negara di Asia sebagai makanan suplemen berfungsi. Objektif utama kajian ini adalah untuk menentukan bioavailabiliti ferum daripada spirulina dan interaksinya dengan faktor dietari lain secara *in vitro* dan *in vivo*.

Gabungan proses penghadaman *in vitro* dan sel kultur Caco-2 yang diikuti samada dengan langkah emparan atau dialisis telah digunakan untuk menilai keberolehan (*availability*) ferum daripada spirulina. Dengan menggunakan kaedah emparan, spirulina yang dikultur dan spirulina komersial telah memberikan hasil yang lebih signifikan ($P < 0.05$) berbanding dengan kaedah dialisis dari segi bentuk ferum yang tersedia untuk penyerapan dan juga jumlah sebenar ferum yang diangkut melalui sel Caco-

2. Jumlah ferum yang tersedia dan ferum yang diangkut dari ferus sulfat (FeSO_4) tidak berbeza secara signifikan untuk kedua-dua kaedah dialisis dan emparan. Kesan pelbagai nisbah molar nutrien (kalsium, asid askorbic, zink, asid tanik dan kafein) terhadap kepadatan ferum daripada spirulina yang dikultur bebeza berbanding dengan FeSO_4 . Kehadiran kalsium pada kepekatan rendah (nisbah molar Fe:Ca 1:5, 1:10, 1:15 and 1:20), tidak merencat penyerapan ferum daripada spirulina secara signifikan berbanding FeSO_4 tetapi pada kepekatan tinggi (nisbah molar Fe:Ca 1:37.34, 1:74.67 dan 1:149.34) penyerapan ferum daripada kedua-dua spirulina dan FeSO_4 didapati terencat secara signifikan. Keperolehan ferum daripada spirulina dengan kehadiran asid askorbik pada nisbah molar yang diuji (nisbah molar Fe:AA 1:0.5, 1:1, 1:1.5 and 1:2) tidak ditingkatkan secara signifikan manakala keperolehan ferum FeSO_4 dapat ditingkatkan secara signifikan untuk semua nisbah molar. Kedua-dua zink dan asid tanik menunjukkan kesan rencatan terhadap keperolehan ferum daripada spirulina berbanding dengan FeSO_4 . Kafein pula tidak menunjukkan sebarang kesan signifikan terhadap ferum dari kedua-dua spirulina dan FeSO_4 . Dua tadahan ferum wujud bersama di dalam spirulina, satu mengandungi ferum organik dan satu lagi ferum tak organik. Ferum organik telah dikenalpasti menunjukkan bioavailabiliti ferum yang lebih baik dan tidak dipengaruhi oleh kehadiran nutrien-nutrien lain. Ini mungkin merupakan salah satu penjelasan mengapa keperolehan ferum dari alga ini adalah sangat tinggi dan bioavailabilitinya tidak dipengaruhi secara signifikan oleh kehadiran nutrien-nutrien lain seperti dalam FeSO_4 .

Asai *haemoglobin repletion* telah digunakan untuk mengkaji secara *in vivo* kesan kalsium terhadap penyerapan ferum daripada spirulina dan perbandingannya dengan FeSO_4 . Dalam kajian ini, didapati hemoglobin, hematokrit dan paras *mean corpuscular volume* (MCV) tikus jantan Sprague-Dawley yang diberi diet spirulina dan FeSO_4 adalah serupa walaupun dos ferum daripada FeSO_4 adalah dua kali ganda lebih tinggi dari jumlah ferum spirulina. Kehadiran kalsium tidak merencat secara signifikan nilai hematologi tikus yang diberi spirulina tetapi tidak dalam FeSO_4 . Peratusan "kecekapan pembaharuan hemoglobin" (*haemoglobin repletion efficiency*) yang didapati adalah lebih tinggi secara signifikan dalam tikus yang diberi spirulina berbanding tikus yang diberi FeSO_4 . Ini menunjukkan kecekapan penyerapan ferum daripada spirulina adalah lebih baik jumlah ferum daripada FeSO_4 .

Kajian penyebaran ferum daripada spirulina dan FeSO_4 dengan kehadiran kalsium telah dijalankan dengan menggunakan mencit jantan ICR yang kekurangan ferum dan normal ferum. Mereka diberi sama ada diet spirulina atau FeSO_4 yang telah dilabel ^{59}Fe secara ekstrinsik. Jumlah ^{59}Fe yang diserap oleh mencit kekurangan ferum adalah serupa antara kumpulan yang diberi spirulina dengan kumpulan yang diberi FeSO_4 pada jam ke-6 dan ke-24. Walau bagaimanapun, pada hari ke-7, kumpulan FeSO_4 menunjukkan penyerapan yang lebih baik berbanding dengan kumpulan spirulina. Dalam mencit normal ferum, peratus ^{59}Fe yang lebih rendah dalam spirulina berbanding dengan FeSO_4 pada jam ke-6 dan ke-24 menunjukkan ferum dari spirulina tidak dapat tersedia untuk diserap yang mana dapat

menghalang ketoksikan ferum dari berlaku. Kehadiran kalsium tidak menghalang secara signifikan penyerapan ferum daripada spirulina seperti yang ditunjukkan oleh kajian *in vitro*.

Keseluruhan kajian ini menunjukkan spirulina merupakan sumber ferum yang berkepekatan tinggi untuk dijadikan suplemen dan fortifikasi makanan. Bioavailabiliti ferum dari spirulina adalah tinggi dan mudah diserap oleh tubuh terutamanya dalam keadaan kekurangan ferum. Selain dapat memberi ferum yang diperlukan, ia juga dapat menghalang berlakunya kesaratan ferum dan ketoksikan dalam keadaan normal ferum dan oleh itu spirulina adalah sesuai untuk kedua-dua keadaan kekurangan ferum dan normal ferum.

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I acknowledge, first and foremost, my dependence on God. I want to thank Him, who has sustained me through these, the best and toughest years of my life, providing for all my needs, giving me the strength to study and finally granting me the ability to finish this thesis. He ordered my steps in every aspect of this study, and, as with all of my life. I recognize my utter reliance on Him. I thank God for the marvelous ways He has brought people into my life that have helped me achieve my goals.

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TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	x
APPROVAL	xii
DECLARATION	xiv
LIST OF TABLES	xviii
LIST OF FIGURES	xxi
CHAPTER	
1 INTRODUCTION	1
1.1 Objective	6
1.1.1 General objective	6
1.1.2 Specific objectives	6
2 LITERATURE REVIEW	7
2.1 Iron functions and chemistry	7
2.2 Iron absorption and metabolism	10
2.3 Factors influencing iron absorption	10
2.3.1 Luminal factors	11
2.3.2 Dietary factors	13
2.3.3 Physiological factors	24
2.4 Mechanism of iron absorption	24
2.4.1 Mucosal uptake of iron from the lumen	26
2.4.2 Movement of iron within the intestinal cell	29
2.4.3 Transfer of iron from the cell to the circulation	31
2.5 Measurement of iron absorption	33
2.5.1 <i>In vitro</i> methods	34
2.5.2 <i>In vivo</i> methods	41
2.6 <i>Arthrospira platensis</i> (Spirulina)	49
2.6.1 Introduction	49
2.6.2 Morphology and Taxonomy	50
2.6.3 Nutritional Value of Spirulina	52
2.6.4 Health benefits of Spirulina	58
3 THE EFFECTS OF VARIOUS DIETARY FACTORS ON THE BIOAVAILABILITY OF IRON FROM <i>ARTHROSPIRA PLATENSIS</i> (SPIRULINA) <i>IN VITRO</i>	
3.1 Introduction	61
3.2 Materials and methods	63
3.2.1 Materials	63

3.2.2	Methods	65
3.2.3	Statistical analysis	78
3.3	Results	79
3.3.1	Mineral content	79
3.3.2	Confluency assay	80
3.3.3	Morphological aspects of Caco-2 cell differentiation	83
3.3.4	Protein concentration	85
3.3.5	Iron displacement from <i>Spirulina</i>	87
3.3.6	Iron bioavailability from <i>Spirulina</i> using two different <i>in vitro</i> methods	90
3.3.7	Effect of dietary factors on iron bioavailability from <i>Spirulina</i>	93
3.4	Discussion	111
4	THE EFFECTS OF CALCIUM SUPPLEMENTATION ON THE BIOAVAILABILITY OF IRON FROM <i>ARTHROSPIRA PLATENSIS</i> (SPIRULINA) <i>IN VIVO</i>	
4.1	Introduction	122
4.2	Materials and methods	124
4.2.1	Animals and diets	124
4.2.2	Hemoglobin repletion assay	126
4.2.3	Iron content in liver and spleen	129
4.2.4	Statistical analysis	129
4.3	Result	130
4.3.1	Food intake evaluation and growth	130
4.3.2	Hematological variables	132
4.3.3	Iron content of liver and spleen	138
4.4	Discussion	140
5	THE EFFECTS OF CALCIUM SUPPLEMENTATION ON IRON DISTRIBUTION FROM FeSO_4 AND <i>ARTHROSPIRA PLATENSIS</i> (SPIRULINA) <i>IN VIVO</i>	
5.1	Introduction	146
5.2	Materials and methods	147
5.2.1	Animals and diets	147
5.2.2	Establishment of iron status	147
5.2.3	Experimental design	148
5.2.4	Determination of haematological parameter	151
5.2.5	^{59}Fe analyses	152
5.2.6	Statistical analysis	153
5.3	Result	153
5.3.1	Growth and Iron status	153
5.3.2	Standardization of μCi and count per minute (cpm)	159
5.3.3	^{59}Fe uptake and distribution	160
5.4	Discussion	169

6	GENERAL DISCUSSION AND CONCLUSION	176
6.1	Recommendations for further studies	182
6.2	Recommendations for public health nutrition and consumer health promotion	182
	BIBLIOGRAPHY	183
	BIODATA OF THE AUTHOR	206

LIST OF TABLES

Table		Page
2.1	Host related factors that affect iron absorption	25
2.2	Techniques used to study iron bioavailability	34
2.3	Definitions of absorption	42
2.4	Advantages and disadvantages of using stable isotopes	44
2.5	Proximate composition (% dry weight) of <i>A. platensis</i> , <i>A. maxima</i> and soy bean meal	53
2.6	Distribution of fatty acids in two strains of spirulina namely <i>A. maxima</i> and <i>A. platensis</i>	55
2.7	Vitamins content of spirulina	57
2.8	Mineral content (range mg/kg dry weight) in spirulina	58
3.1	Tissue dehydration for transmission electron microscopy	69
3.2	Tissue infiltration with resin and acetone mixture	69
3.3	Various amount of protein used for a standard curve	71
3.4	Ratios of Fe to nutrients used for experiment	78
3.5	Mineral composition of cultured spirulina and commercial spirulina	79
3.6	The absorbance and protein concentration values for Caco-2 cells grown for 14 day on polycarbonate membrane	87
3.7	Percentage of ⁵⁹ Fe found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation	92
3.8	Effect of calcium on percentage of ⁵⁹ Fe found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation	95
3.9	Effect of ascorbic acid on percentage of ⁵⁹ Fe found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation	99

3.10	Effect of zinc on percentage of ^{59}Fe found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation	103
3.11	Effect of tannic acid on percentage of ^{59}Fe found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation	106
3.12	Effect of caffeine on percentage of ^{59}Fe found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation	109
4.1	Iron and calcium contents of experimental diets	125
4.2	Body weight of rats by experimental groups and days of experiment	130
4.3	Feed intake of rats by experimental groups and weeks of experiment	131
4.4	Haematological variables in rats with iron-deficient-induced anaemia and subsequently fed different type of diets	133
4.5	Haemoglobin regeneration efficiency (HRE) values in rats with iron-deficient-induced anaemia and subsequently fed different type of diets	135
4.6	Serum iron and TIBC values in rats with iron-deficient-induced anaemia and subsequently fed different type of diets	137
4.7	Transferrin and transferrin saturation values in rats with iron-deficient-induced anaemia and subsequently fed different type of diets	138
4.8	Weight and iron content of liver in rats with iron-deficient-induced anaemia and subsequently fed different type of diets	139
4.9	Weight and iron content of spleen in rats with iron-deficient-induced anaemia and subsequently fed different type of diets	139
5.1	Iron and calcium contents of experimental diets	149

5.2	Distribution of unabsorbed ^{59}Fe (% of dose) in the lumen of the gastrointestinal tract of iron deficient mice fed FeSO_4 or spirulina diet with or without addition of Calcium carbonate	163
5.3	Distribution of unabsorbed ^{59}Fe (% of dose) in the lumen of the gastrointestinal tract of iron normal mice fed FeSO_4 or spirulina diet with or without addition of Calcium carbonate	164
5.4	Distribution of absorbed ^{59}Fe (% of dose) in various organ of iron deficient mice fed FeSO_4 or spirulina diet with or without addition of Calcium carbonate	167
5.5	Distribution of absorbed ^{59}Fe (% of dose) in various organ of iron deficient mice fed FeSO_4 or spirulina diet with or without addition of Calcium carbonate	168

LIST OF FIGURES

Figure		Page
1.1	Estimated population affected by anaemia and iron deficiency, by WHO region	1
2.1	Iron distribution (mg) and metabolism within the body	8
2.2	Mechanism of iron absorption	26
2.3	The influence of iron stores in cells on iron uptake	27
2.4	Diagram of <i>in vitro</i> digestion/Caco-2 cell culture model utilising radiolabeled iron as developed by Glahn <i>et al.</i> , (1996)	40
2.5	Life cycle of Spirulina	52
3.1	Experimental design for iron displacement from spirulina	73
3.2	TEER value of Caco-2 cells grown on polycarbonate membrane	81
3.3	Percentage of phenol red diffusion of Caco-2 cell lines grown on polycarbonate membrane	82
3.4	Ultrastructural features of Caco-2 cell monolayers grown on polycarbonate membranes	84
3.5	High magnification of brush border showing microvilli and tight junction for cell at 3 days of culture and 13 days of culture	85
3.6	Protein dye binding response pattern for standard using bovine serum albumin	86
3.7	Percentage of ⁵⁹ Fe displaced from spirulina and found in the supernatant after centrifugation (4 000 rpm X 10 min) at different period of time	88
3.8	Percentage of ⁵⁹ Fe retained in the cells after centrifugation (4 000 rpm X 10 min) at different period of time	89
3.9	Total soluble iron present at the end of dialysis and centrifugation	91
3.10	Total iron uptake by Caco-2 cell	93

3.11	Percentage of soluble iron present after centrifugation at different iron to calcium molar ratios	94
3.12	Total iron uptake by Caco-2 cell at different iron to calcium molar ratios	97
3.13	Percentage of soluble iron present after centrifugation at different iron to ascorbic acid molar ratios	98
3.14	Total iron uptake by Caco-2 cell at different iron to ascorbic acid molar ratios	101
3.15	Percentage of soluble iron present after centrifugation at different iron to zinc molar ratios	102
3.16	Total iron uptake by Caco-2 cell at different iron to zinc molar ratios	104
3.17	Percentage of soluble iron present after centrifugation at different iron to tannic acid molar ratios	105
3.18	Total iron uptake by Caco-2 cell at different iron to tannic acid molar ratios	107
3.19	Percentage of soluble iron present after centrifugation at different iron to caffeine molar ratios	108
3.20	Total iron uptake by Caco-2 cell at different iron to caffeine molar ratios	110
4.1	Experimental design	127
4.2	Percentage of haematological parameters changes in iron deficient rats fed different type of diets	134
5.1	Experimental design	150
5.2	Body weight of iron deficient mice for a period of 3 weeks	154
5.3	Body weight of iron normal mice for a period of 3 weeks	155
5.4	Haemoglobin level of iron deficient mice administered various iron supplement (ferrous sulphate or spirulina	157
5.5	Haemoglobin level of iron normal mice administered various iron supplement (ferrous sulphate or Spirulina)	158
5.6	Standard curve of of cpm and μ ci unit	159

5.7	Percentage of administered doses measured at different time period in mice provided different iron supplement	161
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CHAPTER 1

INTRODUCTION

Iron deficiency is the world's most widespread nutritional disorder, affecting both industrialised and developing countries. Iron deficiency and anaemia affect all age groups, particularly the young children and pregnant women and their impact present a major hurdle to national development. The World Health Organization estimated nearly 2 billion people worldwide are anaemic and over twice that number are iron deficient (WHO, 2000). Globally, 39% of preschool children and 52% of pregnant women are anaemic, of whom more than 90% live in developing countries (Figure 1.1).

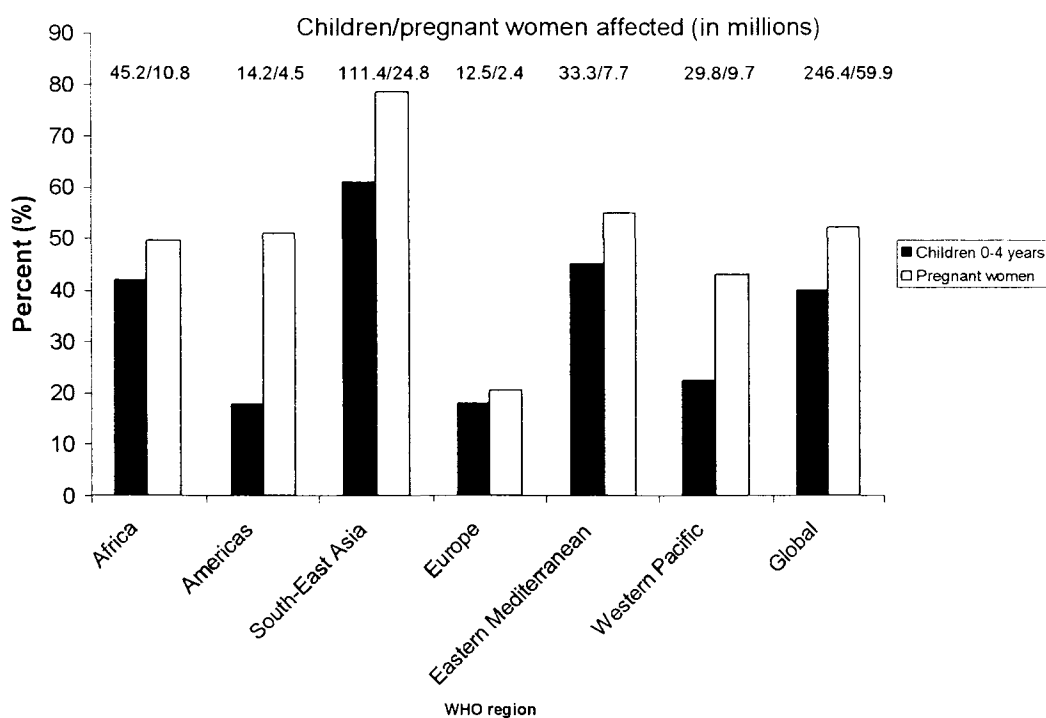


Figure 1.1: Estimated percentage of population affected by iron deficiency anaemia, by WHO region. Source: WHO (2000)