Identifying potential biomarkers of iron-induced inflammation in the association between body mass index (BMI) and selected micronutrients with haemoglobin concentration: A bioinformatics-assisted review (BaR) nutrition study

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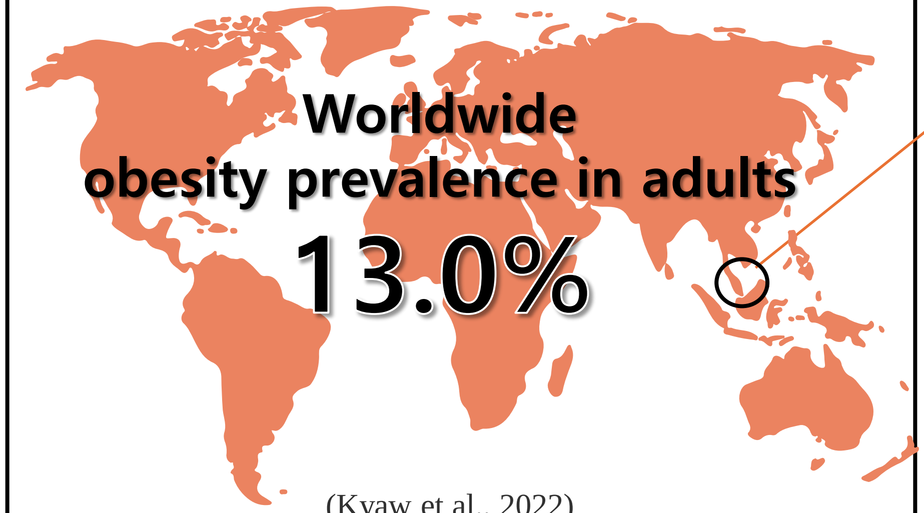
Introduction



19.9%

Obesity prevalence in Malaysian adult

(IPH, 2019)

NEW UPDATE!!

54.4%

HALF OF MALAYSIAN are overweight and obese

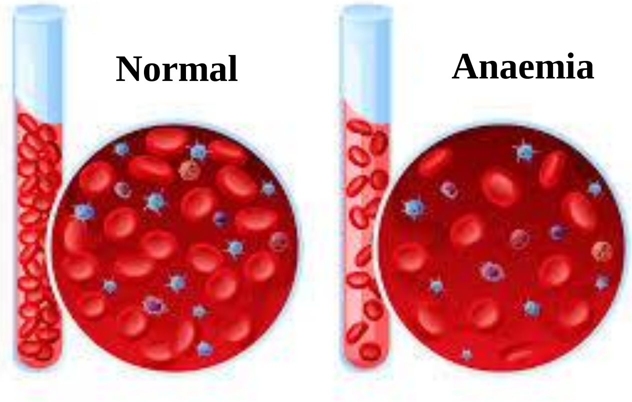
(IPH, 2023)

INTRODUCTIONS

Abnormalities at the endothelium, hormonal, and inflammatory levels
+
excessive visceral fat distribution

Obesity

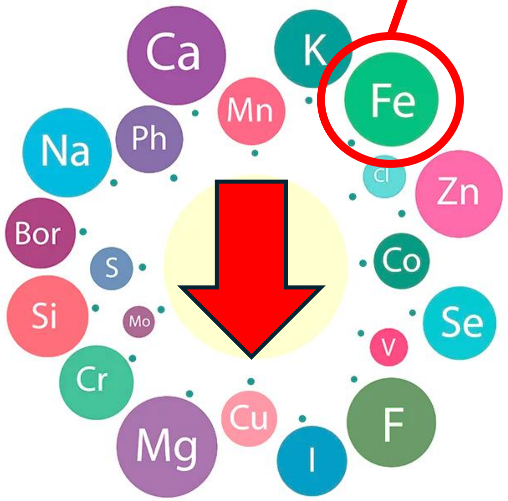
Increase hepcidin level



Anaemia

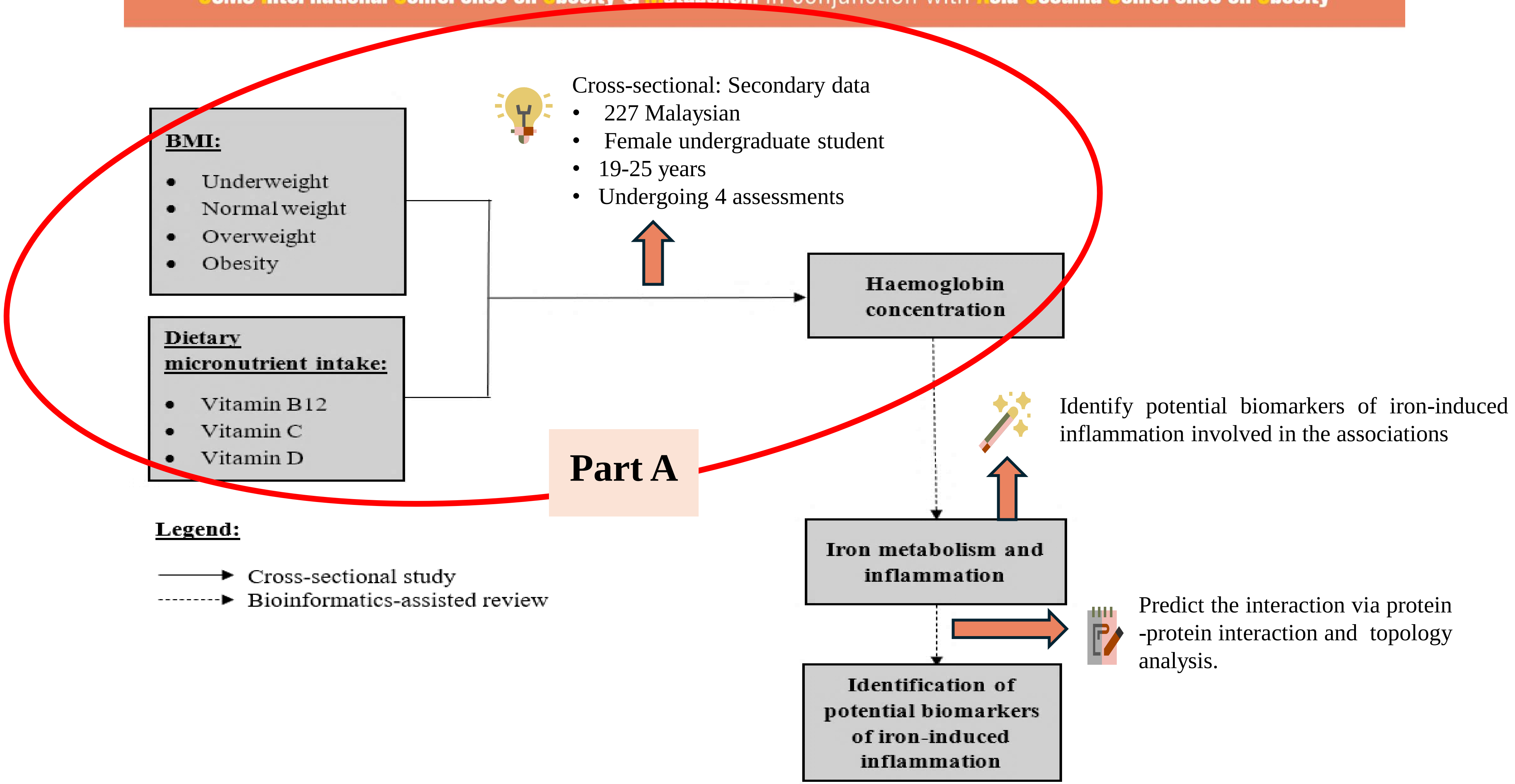
MAIN OBJECTIVE:

To identify the potential biomarkers of iron-induced inflammation in the association between BMI and dietary vitamin B12, C, and D intake with hemoglobin concentration by using bioinformatics-assisted review (BaR)

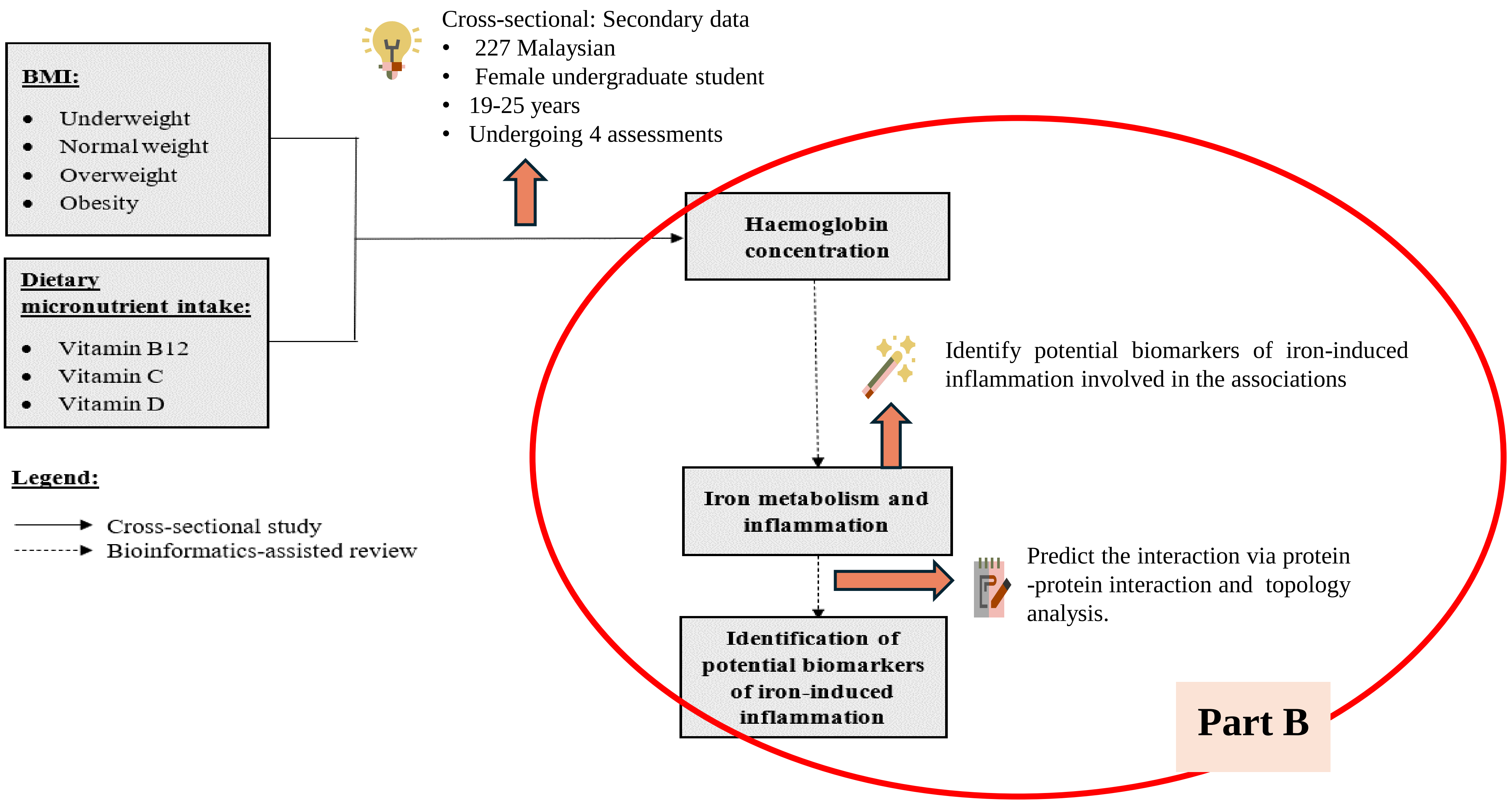


Micronutrients deficiencies

GENERAL METHODS



GENERAL METHODS



METHODS PART A

Part A: BMI and selected micronutrients with haemoglobin concentration

BMI Classification	Range (kg/m^2)
Normal	≥18.5 to 24.9
Underweight	<18.5
Overweight	≥25 to 29.9
Obesity	≥30

(Zierle-Ghosh & Jan, 2018)

Dietary vitamins	RNI
Vitamin B12	4 µg/day
Vitamin C	70 mg/day
Vitamin D	400 IU/day

(RNI, 2017)

Hb Classification	Hb concentration
Normal	≥12.0
Mild Anaemia	11.0 to 11.9
Moderate Anaemia	8.0 to 10.9
Severe Anaemia	<8.0

(WHO, 2000)

Sampling design:
Sampling Location (faculty): Multi-stratified
Sampling participants: Convenience

Application for an **approval** from JKEUPM ethical

Participants recruitments
(n = 225, 20% drop out)
formula from(Hulley et al., 2013).

Assessment 1: Anthropometric measurements determination
SECA 803 flat (weight)
SECA 213 stadiometer (height)
(Boone et al., 2016).

Assessment 2:
Vitamin C intake (24-hours dietary recall)
Vitamin B12 Food Frequency Questionnaires (B12FFQ) (Mearns & Rush, 2017)
VIDSUN questionnaire (Nabak et al., 2014)

Assessment 3: Haemoglobin concentration determination
HemoCue Hb 201+ Analyzer
(Parker et al., 2018)

The association between variables determination using software **IBM SPSS Statistic 26**

BMI and haemoglobin concentration (Khan et al., 2018)

$(r = 0.199)$ $\quad c = 0.5 * In\left[\frac{(1+0.199)}{(1-0.199)}\right] = 0.21$

$$N = \left[\frac{(1.96+0.84)}{0.21}\right]^2 + 3 = \mathbf{180}$$

Adjustment in computing the sample size with 80% response rate:

$$n = \frac{180}{80} \times 100$$

$= 225$ participants

- ❖ Normality: Cronbach's alpha, and Shapiro-Wilk test (Ghasemi & Zahediasl, 2012)
- ❖ Descriptive statistics – Baseline characteristics
- ❖ The Pearson correlation for continuous data (Williams et al., 2020)
- ❖ Binary Logistic regression - weightage determination of each variable (OR > 1 indicates increased risk) – factors contribute to association

Results for Part A: Baseline characteristics of the participants (n=227)

RESULTS FOR PART A (1)

Variables	n	%	Mean ± SD
Haemoglobin concentration (g/dL)			11.57 ± 1.45
Anaemia	138	60.8	
Mild	75	33.0	
Moderate	58	25.6	
Severe	5	2.2	
Non anaemia	89	39.2	
Body mass index (BMI) (kg/m²)			23.20 ± 5.54
Normal	117	51.5	
Underweight	43	18.9	
Overweight	39	17.2	
Obese	28	12.3	

Based on WHO classification (WHO, 2020), if the percentage of anaemia in target population is higher than 40.0%, considered as severe health problem that maybe cause to:

Insufficient sleep

Meal skipping

Irregular eating pattern

Hectic and stressful lifestyle

Poor dietary habit

(Monika et al., 2019)

Variables	n	%	Mean ± SD
Vitamin B12 intake per day (μg)			5.37 ± 7.91
Adequate (≥ 4)	99	43.6	
Inadequate (<4)	128	56.4	
Vitamin C intake per day (mg)			29.31 ± 45.12
Adequate (≥70)	24	10.6	
Inadequate (<70)	203	89.4	
Vitamin D intake per day			
Milk servings (cup/day)			0.54 ± 0.72
0 - 2	223	98.2	
3 - 4	4	1.8	
Fish oil/fish (servings/week)			0.29 ± 0.77
0 - 2	221	97.4	
3 - 4	5	2.2	
≥5	1	0.4	
Vitamin D supplementation per day (IU)			0.01 ± 0.94
Adequate ≥ 400	2	0.9	
Inadequate < 400	225	99.1	

This study revealed that most of the participant have inadequate in al l micronutrients studied which due to poor dietary choices that beco ming a factor contribute to the prevalence of anaemia.

Results for Part A: Correlation between anthropometric measurement and BMI, selected vitamin intakes with haemoglobin concentration by using Pearson correlation test

Variables	r	p-value
Height	0.103	0.120
Weight	**0.137***	**0.039**
BMI	0.118	0.077
Vitamin B12 intake	-0.103	0.121
Vitamin C intake	-0.086	0.197
Milk servings	0.013	0.845
Fish oil/fish	-0.007	0.913
Vitamin D supplement	0.011	0.865

*Correlation is significant at the 0.05 level (2-tailed). r, correlation coefficient.

1. There is **no significant association** between BMI and the intake selected micronutrient with haemoglobin concentration.
2. A **significant linear association** was observed, between weight and haemoglobin concentration (r=0.137, p=0.039).

Haemoglobin is blood biomarker to represent anaemia, which generally affected only at final stage of iron deficiency.

Early and indirect changes in iron homeostasis from inadequate nutrient intake from diet, may not be readily observable.

(Chaparro & Suchdev, 2019).

Also influenced by other cofounder such as inflammation that is not assessed.

(Bagni et al., 2013)

RESULTS FOR PART A (3)

Results for Part A: Prediction of selected micronutrients and BMI in contributing to anaemia by logistic regression

Indicators		p-value	Odds ratio (OR)	95% CI for OR	
				Lower	Upper
BMI	Underweight				
	Normal	0.382	1.533	0.588	4.000
	Overweight	0.018*	2.772	1.194	6.437
	Obese	0.133	2.133	0.794	5.730
Vitamin B12 intakes per day (µg)	Adequate (≥ 4)				
	Inadequate (<4)	0.619	0.872	0.509	1.495
Vitamin C intakes per day (mg)	Adequate (≥70)				
	Inadequate (<70)	0.856	1.084	0.453	2.595
Vitamin D intakes	Milk servings (cup/s)				
	(0 -2)	0.564	0.511	0.052	4.995
	(3-4)				
	Fish oil/fish (servings/week)				
	0 - 2	1.000	<0.001	<0.001	
	3 - 4	1.000	<0.001	<0.001	
	≥5				
	Vitamin D supplementation per day (IU)				
	Adequate ≥ 400				
	Inadequate < 400	0.755	1.557	0.096	25.213

* p-value <0.05. Dependent variable: Haemoglobin classification (Normal and anaemia). CI, Confidence interval; OR, Odd ratio.

Overweight participants significantly contributed to anaemia 2.772 times more compared to others BMI category (OR=2.772, 95% CI 1.194-6.437, p=0.018)

- Haemoglobin concentration influenced by muscle mass, body fat percentage, overall body size, and can vary among individuals with the same weight but different heights
- Mechanism of action:
  - **Increase BMI→ increased body composition, adiposity, inflammation, organ size → disrupt physiological body function**
  - Example:
    - Increased adiposity → increase Vitamin D stored → lower vitamin D bioavailability and distribution
    - Obesity associated with poor dietary habit → lower water-soluble vitamin (Vitamin C and B12)
    - Increased cholesterol, TGA and FFA → Impact protein-bound micronutrients distribution

(Guardiola-Márquez et al., 2022; Lapik et al., 2020).

Inconsistent result

↓

Needs of comprehensive interpretation of the results

↓

Indicate possible pathways at molecular level

↓

Investigate direction of causality

Part B: Bioinformatics-assisted review (BaR)

(Bonilla et al., 2022)

Literature review

Search scientific literature by using: :

Google Scholar, PubMed.gov, Scopus

Enrichment and protein-protein interaction mapped into STRING

Search Tool for the Retrival of Interacting Genes (STRING) for potential interactor evaluation

Significance: PPIN p-value = <1.0e-16)

Evidence based verification

NIH National Library of Medicine – National Center for Biotechnology Information

Immunome KnowledgeBase, MARKER database, InnateDB

1 2 3 4 5

Gene/Protein priortization

Manual curation for 5 months and cross-referring:

GENEONTOLOGY Unifying Biology, EMBL-EBI, UniProt, BioGPS.ORG, Worldwide PDB Protein Data Bank

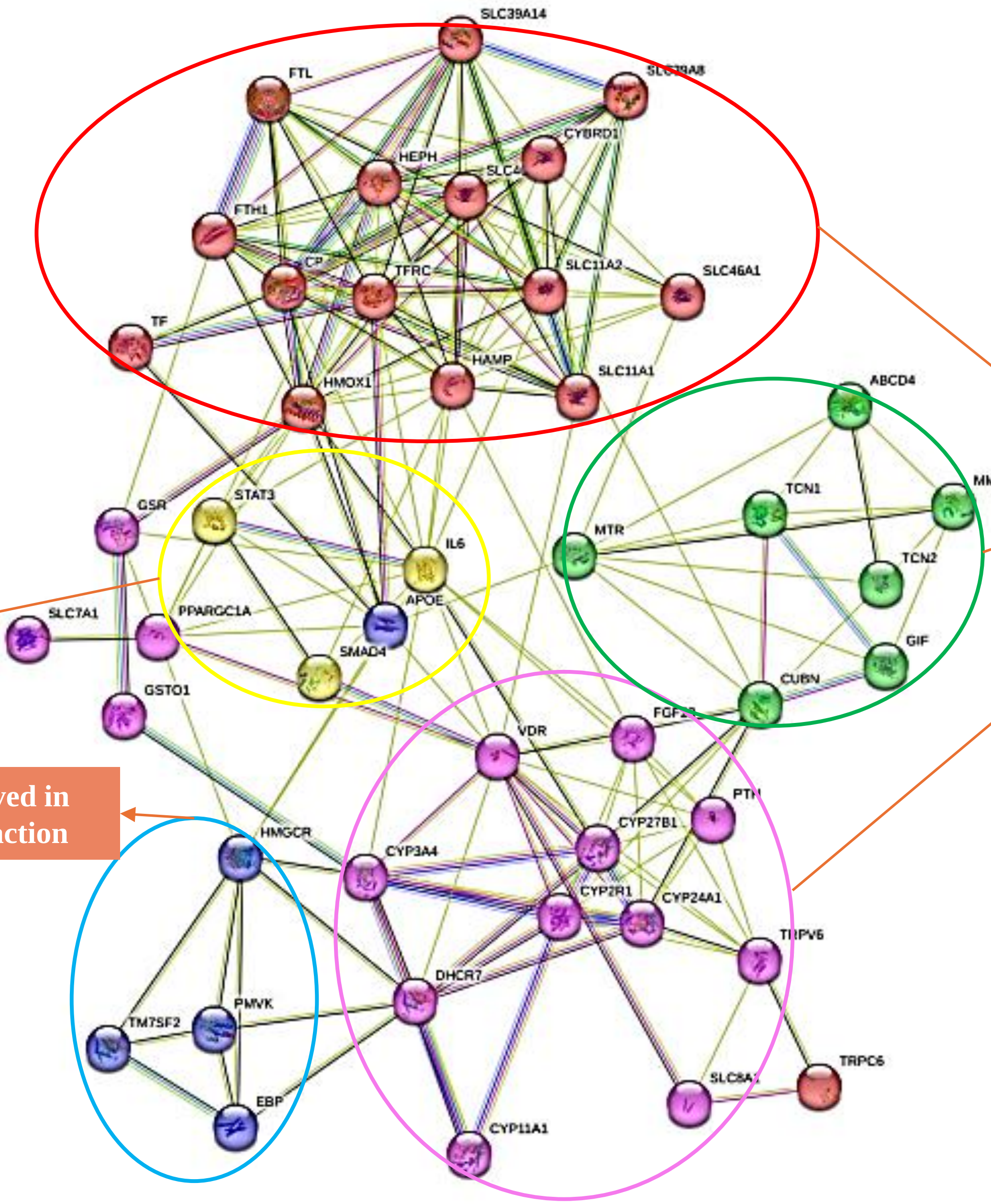
Netwrok topology and hub

- To identify hub nodes
- Ranked from 0 – 1 based on centrality measures (Koutrouli et al., 2020):
  - 1 representing the highest level of confidence
  - 0.5 mean that one out of every two interaction might be false positive (Szklarczyk et al. 2019)

Results for Part B: Analysis of protein-protein interaction (PPIN) for potential biomarkers of iron-induced inflammation identification

Total nodes: 46 nodes
Total edges: 194 edges
Clustering coefficient: 0.67
Average node degree: 8.43
P-value: (10e-16) = (<0.001)

The very low PPIN enrichments p-value indicated that the nodes were significant and not random.



Iron metabolism and regulation protein/gene

Vitamin B12 metabolism and regulation protein/gene

Vitamin D metabolism and regulation gene/protein

Inflammation indicators (Gene/protein) involved in iron transportation and regulations

Gene/ protein involved in lipid and iron interaction

(Bonilla et al., 2022)

RESULTS FOR PART B (2)

Table 4.6 Functional enrichment analysis of PPIN

GO-term	Biological Process (GO) Descriptions	FDR *p*-value
GO:0042369	Vitamin D catabolic process	4.35×10^{-5}
GO:0010980	Positive regulation of vitamin D 24-hydroxylase	4.35×10^{-05}
GO:0006876	activity	0.00034
GO:0036378	Cellular cadmium ion homeostasis	7.11×10^{-5}
GO:0006824	Calcitriol biosynthetic process from calciol	2.03×10^{-8}
	Cobalt ion transport	

GO-term	Molecular Function (GO) Descriptions	FDR *p*-value
GO:0015086	Cadmium ion transmembrane transporter activity	$1.29 \text{ X } 10^{-6}$
GO:0070643	Vitamin D 25-hydroxylase activity	0.00014
GO:0015093	Ferrous iron transmembrane transporter activity	0.00014
GO:1902271	D3 vitamins binding	0.0060
GO:0070576	Vitamin D 24-hydroxylase activity	0.0060

GO-term	Cellular Components Description	FDR *p*-value
GO:0008043	Intracellular ferritin complex	0.0051
GO:1990712	HFE-transferrin receptor complex	0.0171
GO:0031232	Extrinsic component of the external side of the plasma	0.0171
GO:0044754	membrane	0.0225
GO:0031526	Autolysosome	0.0033
	Brush border membrane	

Pathway ID	KEGG Pathways Descriptions	FDR *p*-value
hsa00100	Steroid biosynthesis	4.39×10^{-9}
hsa04216	Ferroptosis	7.07×10^{-15}
hsa04978	Mineral absorption	4.42×10^{-17}
hsa04977	Vitamin digestion and absorption	5.65×10^{-5}
hsa00900	Terpenoid backbone biosynthesis	0.0498

Domain	Protein Domain (PFAM) Descriptions	FDR *p*-value
PF00067	Cytochrome P450	0.0010
PF14478	The domain of the unknown function (DUF4430)	0.0010
PF01222	Ergosterol biosynthesis ERG4/ERG24 family	0.0419
PF01122	Eukaryotic cobalamin-binding protein	0.0010
PF01566	Natural resistance-associated macrophage protein	0.0315

The p-values, which indicate the significance of the enrichment, were corrected for multiple testing within each category using the Benjamini-Hochberg method generated by STRING. FDR, false discovery rate; GO, gene ontology; HFE, homeostatic iron regulator; KEGG, Kyoto Encyclopedia of Genes and Genome; PFAM, Protein Families database.

These biological mechanisms linked to poor dietary quality that may contribute to obesity-associated anaemia

(Guardiola-Márquez et al., 2022)

Results for Part B: Hub-protein identification based on network topology from the Network Analysis Profiler (NAP) v2.0

Protein Name	Centralisation Degree	Centralisation Betweenness	Eigenvector Centrality +	Subgraph Centrality
TFRC	34	32.54	1.00	3,491,864,896.57
HAMP	34	55.42	0.99	3,391,483,637.82
SLC11A2	30	50.63	0.94	3,076,936,707.97
CP	30	22.37	0.93	3,031,011,069.71
SLC40A1	28	11.40	0.93	3,004,681,454.04
HEPH	28	17.83	0.90	2,832,607,927.84
HMOX1	30	33.47	0.87	2,627,571,495.56
CYBRD1	24	2.33	0.83	2,416,019,436.33
FTH1	24	11.07	0.81	2,278,809,638.63
SLC39A14	22	0.70	0.77	2,070,462,694.19
SLC11A1	22	34.02	0.74	1,892,838,818.12
FTL	20	0.11	0.74	1,887,625,321.89
IL6	34	156.85	0.67	1,585,573,332.11
SLC39A8	16	0.00	0.59	1,215,307,696.66
SLC46A1	16	34.04	0.54	1,007,965,933.55

+ All scores rank from 0.5 -1, with 1 as the highest possible confidence meanwhile, 0.5 might indicate erroneous (for example: false positive) at every second interaction. TFRC, Transferrin receptor protein 1; HAMP, Hepcidin; SLC11A2, Natural resistance-associated macrophage protein 2; CP, Ceruloplasmin; SLC40A1, Solute carrier family 40 member 1; HEPH, Hephaestin; HMOX1, Heme oxygenase 1, CYBRD1, Cytochrome b reductase 1; FTH1, Ferritin heavy chain; SLC39A14, Zinc transporter ZIP14; SLC11A1, Natural resistance-associated macrophage protein 1; FTL, Ferritin light chain; IL6, Interleukin-6; SLC39A8, Zinc transporter ZIP8; SLC46A1, Mfs transporter, pcft/hcp family, solute carrier family 46.

TFRC
Transferrin receptor protein 1

HAMP
Hepcidin

TFRC- The most accurate indicator of iron metabolism in obese people
HAMP- Excess adiposity release IL-6 stimulate HAMP production

Sat et al. (2018)

These Top 10 identified biomarker had the highest influence of a node in the network with significance direct connections that impact the overall network

Lead to iron shortage in overweight and obese people

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In conclusions:

1. The prevalence of haemoglobin concentration was 60.8%, considered severe health problem as stated by the WHO 2020 and most of the participants reported inadequate intake of selected micronutrients.

2. The study indicate no association between BMI and selected micronutrients with Hb concentration. In further analysis, revealed that OW contributed 2.77 times higher of getting anaemia compared to other BMI.

3. The bioinformatics-assisted review (BaR) method enhances the detection of obesity-related anemia by combining data on iron, lipids, micronutrient deficiencies, and inflammation biomarkers.

4. This approach highlights the importance of bioinformatics in nutrition research, facilitating in understanding the causal pathways between diet, body functions, inflammation, and haemoglobin regulation

5. Insight of these analyses enabling more targeted interventions by elucidating the complex interplay between nutrition, genetics, and health.

Recommendations

Integrate Bioinformatics in Clinical Practice

Focus on Personalized Nutrition

Promote Interdisciplinary Research

Enhance Nutritional Education and Awareness

THANK YOU!!!!

Q&A Session