Article:
Tan, PY orcid.org/0000-0003-1281-4928, Amini, F and Mitra, SR (2022) Dietary protein interacts with polygenic risk scores and modulates the serum concentrations of C- reactive protein in overweight and obese Malaysian adults. Nutrition Research, 107. pp. 75-85. ISSN 0271-5317

https://doi.org/10.1016/j.nutres.2022.09.002

Dietary protein interacts with polygenic risk scores and modulates the serum concentrations of C- reactive protein in overweight and obese Malaysian adults

Pui Yee Tan[1] (Correspondence), Farahnaz. Amini[2] & Soma Roy Mitra[3]

[1]School of Food Science and Nutrition, Faculty of Environment, University of Leeds, Leeds LS2 9JT, United Kingdom

[2]School of Healthy Aging, Medical Aesthetics & Regenerative Medicine, UCSI University, KL Campus, Malaysia

[3]School of Biosciences, Faculty of Science and Engineering, University of Nottingham Malaysia, Semenyih, Selangor Darul Ehsan, Malaysia

Pui Yee Tan[1] (First author and corresponding author)

Tel: +44 7311442255

Postal address: School of Food Science and Nutrition, Faculty of Environment, University of Leeds, Woodhouse, Leeds LS2 9JT, United Kingdom.

Email: p.y.tan@leeds.ac.uk

ORCID: 0000-0003-1281-4928

Farahnaz. Amini[2]

School of Healthy Aging, Medical Aesthetics & Regenerative Medicine, UCSI University, KL Campus, Malaysia

Postal address: Faculty of Medicine and Health Sciences, Kuala Lumpur Campus, No.1, Jalan Menara Gading, UCSI Heights 56000 Cheras, Kuala Lumpur, Malaysia

Tel: +603 - 9101 8880; Ext: 2269

Email: farahnaz@ucsiuniversity.edu.my

ORCID: 0000-0002-9491-0851

Soma Roy Mitra[3]

School of Biosciences, Faculty of Science and Engineering, University of Nottingham Malaysia, Semenyih, Selangor Darul Ehsan, Malaysia

Postal address: The University of Nottingham Malaysia, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia

Tel: +919147005851

Email: mitra.somaroy@gmail.com

ORCID: 0000-0001-7045-1550

Abstract words count: 250 words

Number of figures: 1; number of tables: 5

List of abbreviation: *ADRB2*, beta- 2 adrenergic receptor; ANCOVA, analysis of covariance; BMI, body mass index; CIs, confidence intervals; CVD, cardiovascular diseases; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; FFQ, food frequency questionnaire; *FTO*, fat mass and obesity-associated; HDL- C, high- density lipoprotein cholesterol; HOMA- IR, homeostatic model assessment- insulin resistance; CRP, C- reactive protein; IL, interleukin; LDL- C, low- density lipoprotein cholesterol; MET, metabolic equivalent; ORs, odd ratios; PRS, polygenic risk score; RAF, risk allele frequency; RNI, Recommended Nutrient Intakes; SNPs, single nucleotide polymorphisms; TC, total cholesterol; TE, total energy; UNM, University of Nottingham Malaysia; WC, waist circumference; WHR, waist- hip ratio.

48 **Abstract**

49 Dietary intake may interact with gene variants and modulate inflammatory status. This study
50 aimed to investigate the combined effect of *FTO* (fat mass and obesity- associated) rs9930501,
51 rs9930506 and rs9932754 and *ADRB2* (beta- 2 adrenergic receptor) rs1042713 on C- reactive
52 protein (CRP) concentrations using polygenic risk scores (PRS), and modulatory effect of
53 dietary nutrients on these associations. We hypothesized that higher protein intake is
54 associated with lower inflammatory status in individuals genetically predisposed to obesity.
55 PRS was computed as weighted sum of the risk alleles possessed, and was stratified into first
56 (0 to 0.64), second (0.65 to 3.59) and third (3.60 to 8.18) tertile. 128 overweight and obese
57 Malaysian adults were dichotomized into groups of low and elevated inflammatory status (CRP
58 concentrations ≤3 and >3 mg/L, respectively). Half of the study participants (51%) were found
59 to have elevated inflammatory status. Second tertile and third tertile PRS were significantly
60 associated with increased odds of elevated inflammatory status, 7.56 (CI= 1.98-28.80;
61 adjusted p= 0.003) and 3.87 (CI= 1.10-13.60; adjusted p= 0.035) respectively. Individuals in
62 the third tertile PRS had significantly lower CRP concentrations (4.61 ± 1.3 mg/L vs 9.60 ± 2.6
63 mg/L, p= 0.019) when consuming ≥14% energy from protein (with an average of 18.0 ± 2.4%,
64 43.0 ± 7.7% and 39.0 ± 8.0% energy from protein, carbohydrate and fat per day). In conclusion,
65 third tertile PRS was significantly associated with increased odds of elevated CRP and higher
66 protein intake may alleviate inflammatory status and reduce CRP concentrations systemically
67 in those individuals.

68 **Keywords:** C- reactive protein, polygenic risk score, dietary protein, nutrient- gene interaction,
69 overweight and obese Malaysian adults

70 1. Introduction

71 Prevalence of obesity is increasing worldwide and has emerged as an important public health
72 issue in many countries. High prevalence of overweight and obesity has been reported in the
73 Malaysian population amongst other Asian populations, with 50.1% of the population being

either overweight or obese [1]. Higher prevalence was found in females (54.7%) and in the Indian ethnicity (63.9%). Obesity has been well known to be a major risk factor of non-communicable diseases including cardiovascular diseases (CVD), atherosclerosis, diabetes, and some types of cancer, where the fundamental pathology underlying the diseases, is inflammation [2,3].

C- reactive protein (CRP) is an acute- phase protein secreted by hepatocytes following Interleukin- 6 (IL- 6) secretion by macrophages and T- cells. It appears to be the most commonly examined systemic inflammatory marker for both communicable and non-communicable diseases [4,5], and has been widely accepted to be an independent risk factor for predicting future cardiovascular events [6]. Researchers have reported significant positive associations between CRP concentrations and obesity as also with biochemical parameters of metabolic syndrome including hyperinsulinemia, insulin resistance, hypertriglyceridemia, and low high-density lipoprotein cholesterol (HDL- C) [7]. CRP has been incriminated as a cardiovascular risk factor in severe obesity [8] and in patients with diabetes mellitus II [9].

Although it has been demonstrated adequately that elevated inflammation is driven by excess fat [10], however the inter-individual variability of the inflammatory profile assessed by CRP concentrations was observed in obese populations [11], suggesting that environmental and genetic factors may play an important role in modulating CRP concentrations [12]. In our previous investigation, we reported significant elevated CRP concentrations in Malaysian adults carrying the risk allele (G allele) of fat mass and obesity- associated protein (*FTO*) rs9939506 [13]. A similar finding by Sun et al. reported that the risk allele of *FTO* rs9939609 (A allele) was strongly associated with increased odds of obesity and elevated CRP concentrations in the Chinese Han population [14].

Multiple dietary nutrients have been reported to have significant effects on serum inflammatory markers [15]. Framingham Heart Offspring cohort study by Hruby and Jacques, proposed that dietary protein, particularly from plant- based sources may have a protective effect on inflammatory markers in the elderly population [16]. A decreasing trend in CRP concentrations

was observed in participants with renal disease (but not on dialysis) when consuming plant proteins compared to those consuming animal proteins (e.g., egg and red meat) [17]. Supplementations of essential amino acids were also found to be significantly associated with decreased CRP concentrations and improved circulating lymphocytes in participants with both high and low inflammatory status [18].

However, to date, there is limited research investigating the interactions between gene variants and dietary nutrients in modulating inflammatory markers. Therefore, in this study, a Mendelian randomization was conducted to investigate the combined effect of single nucleotide polymorphisms (SNPs) including *FTO* rs9930501, rs9930506 and rs9932754, and beta- 2 adrenergic receptor (*ADRB2*) rs1042713 on CRP concentrations and further, their interaction with dietary nutrients. The SNPs reported were found to be significantly associated with increased risk of obesity and obesity-related phenotypes in our previous investigation [13,19]. The authors computed polygenic risk scores (PRS), and using interaction analysis, assessed the modulatory effect of dietary nutrients (total energy and 26 macro and micro-nutrient intakes were analyzed) on the association between PRS and CRP concentrations, in the study participants. Based on our initial research, we hypothesized that dietary nutrients (e.g., protein intake) may positively modulate and decrease the CRP concentrations in individuals who are genetically predisposed to obesity.

2. Methods and materials

2.1. Ethics approvals

Ethical approval was obtained from the Science and Engineering Research Ethics Committee, University of Nottingham Malaysia (UNM) (ID- SM190614), and this study was also registered under the Medical Research and Ethics Committee (MREC) of the National Medical Research Registry (Research ID- 25110), Ministry of Health Malaysia (MOH). Written informed consent was requested and obtained from each participant. The study protocol was prepared in accordance with the ethical standards laid down by the Declaration of Helsinki, 1961 and

followed the Good Clinical Practice Guidelines of the government of Malaysia (Third Edition Oct 2011© Ministry of Health Malaysia).

2.2. Study design and study population

This study is nested in a broader study investigating the effect of dietary nutrients on obesity-related phenotypes conducted from 2015 to 2018, the detailed information on the methodology undertaken has been described in our previous publications [13,19,20]. Moreover, a Mendelian randomization was conducted to search for gene variants genetically predisposing individuals to the risk of overweight and obesity, captured from published GWAS studies. *FTO* (rs9930501, rs9930506 and rs9932754) and *ADRB2* (rs1042713) were found to be significantly associated with increased odds of obesity and obesity-related phenotypes [13,19], and these genes and their variants have been investigated in this study. A total of 128 overweight and obese Malaysian adults (Malaysian Chinese, Malays and Indians) aged 18 years and above were recruited randomly through advertisements and flyers, distributed at the University of Nottingham in Malaysia (UNM) and at schools and community centers in the vicinity of UNM. Structured questionnaire was used to record information on race and ethnicity, and the questions were directed to trace back three generations to confirm ancestry. Participants completed a health and lifestyle questionnaire which included questions on past diseases, family history of past diseases, physical activity status and substance abuse. Smoking status and alcohol consumption were reported as i) never, ii) former and iii) current. However, none of the participants were found to be smoking nor drinking, therefore the said data will not be reported.

Interested individuals attended an initial screening to determine whether the participant met the specified inclusion and exclusion criteria. Individuals diagnosed with cardiovascular diseases, stroke, diabetes, renal and endocrine disorders such as hypothyroidism were excluded from the study. Exclusion criteria also included pregnant woman, those on drugs such as cholesterol lowering, hypoglycemic and psychiatric medication.

153 ### 2.3. Measurement of anthropometric parameters

154 Height of the individual was measured with light clothes and barefoot measured using
155 standard height rod. Obesity-related anthropometric parameters including weight (kg), fat
156 mass (kg), skeletal muscle mass (kg), fat free mass (kg) and percent body fat (%) were
157 measured using body composition analyzer direct segmental multi-frequency bioelectrical
158 impedance analysis (DSM-BIA) (InBody 230, Seoul, Korea). Body mass index (BMI) was
159 defined as weight in kilograms divided by the square of height in meters (kg/m^2) [21]. Waist
160 circumference (WC) was measured at the midpoint between the top of iliac crest and the lower
161 margin of the last palpable rib [22].

162 ### 2.4. Assessment on dietary intake

163 Energy, macro and micronutrient intakes were assessed by an interviewer-administered
164 validated food frequency questionnaire (FFQ) developed by Loy et al. [23]. Food items listed
165 in the validated FFQ were then modified to include items popular to different ethnic groups
166 (Malaysian Malays, Chinese and Indians), based on three-day 24-hour recalls collected at the
167 beginning of the study period. The modified FFQ consisting of 156 food items were listed
168 under 12 categories (grain, meat and poultry, fish and seafood, egg and eggs products,
169 legumes, milk and milk products, vegetables, fruits, drinks, confectionary, bread spread and
170 flavorings) [23]. For each food item, participants indicated the frequency of consumption for
171 the past week. The number of standard portions consumed per sitting was recorded with the
172 aid of photographs of standard portion sizes [24]. Detailed information related to the brands,
173 methods of cooking, supplementation of vitamins and minerals and oil consumption were
174 collected and documented to avoid under- reporting and to capture macro and micronutrient
175 intakes as accurately as possible. Total daily intake of a particular food item was calculated
176 by multiplying the unit portion of each food item, by the frequency of consumption of each food
177 per week, times the number of portions consumed per sitting, and finally divided by 7 days to
178 give an estimate of the food intake per day. The per day food consumption data was entered
179 into an energy and nutrient assessment software, Dietplan7 (Forestfield Software Ltd., UK) to

compute energy, macro and micronutrient intakes per day. Malaysian food items not found in the Dietplan7 database were keyed in from the "Recommended Nutrient Intakes (RNI), Malaysia 2017" [25] and "Nutrient Composition of Malaysian Foods" [26]. The intakes of macronutrients were expressed as a percentage of total energy (TE) consumed (% of TE). TE per basal metabolic rate (BMR) ratio <1.2 was used to exclude under- reporters [27]. In the current study, none of the participants were found to be under- reporting. Therefore, all participant data were included for further analysis.

2.5. Assessment on physical activity

Physical activity data were collected through a structured questionnaire including a list of physical activities with corresponding metabolic equivalent (MET) values [28]. Details of activities asked included (1) the type and intensity of the activity; (2) the time and duration (mins) of each activity performed per day; and (3) the number of days each activity was performed in a week. The reported physical activity was then categorized into light (<3 METs), moderate (3 to 6 METs) and vigorous (>6 METs) intensity physical activity, according to Ainsworth et al. [28]. The total time (mins) spent on sedentary, moderate and vigorous intensity physical activity in a week was computed for each participant. Physically active was defined as accumulation of at least 150 minutes per week of moderate intensity physical activity or 60 minutes per week of vigorous intensity physical activity, according to the guidelines published by the Ministry of Health Malaysia, else they were considered as physically inactive [29]. Participants of the current study were then categorized into two groups: 'physically active' and 'physically inactive'.

2.6. Blood biochemical analysis

Fasting blood samples were collected from the antecubital vein into vacutainer tubes containing fluoride oxalate for plasma glucose analysis, and vacutainer tube with clot activator and gel for serum insulin, lipid profile including total cholesterol (TC), triglyceride, HDL- C, and low- density lipoprotein cholesterol (LDL- C) and CRP analysis (Becton Dickinson, Oxford,

United Kingdom). The blood biochemical analyses were assessed using Abbott Architect CI8200 Automatic System according to manufacturer's instructions. Homeostatic model assessment-insulin resistance (HOMA- IR) was calculated as the product of fasting plasma glucose (mmol/L) and fasting serum insulin (uU/ml) divided by 22.5 according to homeostatic model assessment (HOMA) [30]. Insulin resistance (IR) was defined as HOMA- IR ≥1.7 [31]. LDL- C was determined using the Friedewald formula: LDL- C = TC – [(Triglyceride / 5) + HDL-C] [32]. Elevated inflammatory status was defined as CRP concentrations >3 mg/L, indicating higher risk of cardiovascular events [33].

2.7. DNA extraction and genotyping assay

5 mL of whole blood was drawn from an antecubital vein into Lavender top Vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ) containing Ethylenediaminetetraacetic acid (EDTA). Genomic Deoxyribonucleic acid (DNA) was extracted from blood leukocytes using MasterPure DNA Purification kit according to manufacturer's instructions. DNA samples were then stored at -20 °C until use. A DNA fragment of 237 bp containing *FTO* rs9930501, rs9930506 and rs9932754 was amplified by using polymerase chain reaction (PCR) with specific primers (Forward primer: 5'-TGATGAGAATGTAAGAAGGGAGA-3' and reverse primer: 5'-TCATTTGACAGATGGACTTTTCA-3'). A DNA fragment of 310 bp containing *ADRB2* rs1042713 was amplified by using PCR with specific primers (Forward primer: 5'-CCGCCGTGGGTCCGCC-3' and reverse primer: 5'-CCATGACCAGATCAGCAC-3'). Detailed information of the PCR protocol has been previously described in our earlier publications [13,19]. The PCR amplicons were then verified by using electrophoresis on 2% agarose gel and visualized under ultraviolet illumination after staining by ethidium bromide. The verified amplicons were then sequenced by using the BigDye® Terminator v3.1 cycle sequencing kit chemistry.

2.8. Computation of PRS

Allele frequency was estimated by gene counting and Chi- square test was used to assess deviation from Hardy- Weinberg Equilibrium (HWE) [34]. Genetic predisposition to obesity was assessed using PRS. PRS was computed as the sum of individual's risk alleles across the 4 SNPs (*FTO* rs9930501, rs9930506 and rs9932754 and *ADRB2* rs1042713), weighted by the effect size of each risk allele using their respective natural log of OR for obesity. The 4 SNPs were selected as they were found to be significantly associated with increased risk of obesity and obesity- related phenotypes in our previous investigation [13,19]. The formula to evaluate PRS is as follows [35–37]:

$$PRS = \sum_{i=1}^{n} W_i \, X_i$$

where X_i represents the number of the risk alleles; W_i represents the natural log of the odds ratio (ORs) of obesity associated with the respective risk allele of the SNP; n represents the number of the SNPs included in PRS. The risk alleles of each SNP were captured from published GWAS studies, *FTO* rs9930501 (G allele), rs9930506 (G allele) and rs9932754 (C allele) and *ADRB2* rs1042713 (G allele) [38–41]. The odds of obesity for each SNP have been reported in our earlier investigation (**Table 1**), using logistic regression to determine the risk of obesity associated with gene variants. ORs with 95% confidence intervals (95% CIs) were estimated for each genotype. Participants were then stratified into three equal groups as the first tertile (PRS 0 to 0.64), second tertile (PRS 0.65 to 3.59) and third tertile (PRS 3.60 to 8.18) PRS, with higher PRS indicating greater predisposition to obesity.

2.9. Power and sample size calculation

This is a secondary analysis of the original data set. Therefore, post hoc power analysis was performed to compute the minimum detectable effect using the sample size of the current study. Power and sample size calculation was performed using software- QUANTO, Version 1.2.4. For computation of power and effect size the following parameters were used: type 1 error of 0.05, population prevalence of elevated inflammatory status (CRP >3 mg/L), 51%, (as

256 in our study); minor allele frequency (G) of the selected gene variant, 0.37 (*FTO* rs9930506);
257 65 participants with elevated CRP and 63 with low CRP concentrations, we calculated 93%
258 power to detect an effect of 3.87 (ORs in **Table 4**). Regarding the PRS-diet interaction, given
259 that the mean of CRP concentrations in the study participants was 5.72 mg/L, environmental
260 effect (differences in CRP concentrations between low and high percent energy from protein)
261 was 7.57 (2.03 mg/L to 9.60 mg/L), genetic effect was 0.76 {differences in CRP concentrations
262 between the first tertile and third tertile PRS (2.03 mg/L to 2.79 mg/L)} and interaction effect
263 was 4.99 (9.60 mg/L to 4.61 mg/L), a power of 44% for the PRS-diet interaction was computed.

264 **2.10. Statistical Analyses**

265 Statistical analysis was performed using the Statistical package for social sciences (IBM SPSS
266 statistic, Chicago, IL, USA, version 22). Participants were categorized into two groups, low
267 and elevated CRP concentrations. Data were expressed as mean ± standard error (SE) or
268 number (percentage). Kolmogorov–Smirnov test was applied to assess the normality of the
269 continuous variables. Independent t- test and Chi- square tests were performed to assess the
270 differences in general characteristics with continuous and categorical variables respectively
271 and between the groups with low and elevated inflammatory status. Analysis of covariance
272 (ANCOVA) was performed to assess the differences in dietary intake, anthropometric and
273 blood biochemical parameters between the groups with low and elevated inflammatory status.
274 Adjustments on covariates age, gender, physical activity, BMI fat mass, percent body fat, and
275 total energy intake were applied where appropriate. Variance inflation factor (VIF) was taken
276 as a measure for testing multicollinearity among related parameters [42], with VIF value ≥10
277 indicating high collinearity. Due to high collinearity among the anthropometric parameters, only
278 BMI, fat mass and percent body fat were adjusted as covariates in all analysis.

279 Multivariate binary logistic regression was performed to determine the effect of PRS on the
280 odds of elevated inflammatory status. ORs with 95% CIs were estimated for each tertile of
281 PRS, and the first tertile PRS was used as the reference group. Adjustments for covariates
282 age, gender, ethnicity, height, BMI, fat mass, percent body fat, HDL- C, physical activity status,

smoking status, alcohol consumption and total energy intake were applied. Nutrient- gene interaction was evaluated by using general linear model after adjusting for age, gender, ethnicity, physical activity status, BMI, fat mass, percent body fat, and total energy intake. In brief, total energy and 26 macro and micronutrient intakes were analyzed to investigate the nutrient- gene interactions on CRP concentrations, however significant association was only found with dietary protein. Therefore, data from other dietary parameters are not reported. Percent energy from dietary protein was dichotomized into 2 groups by using the median value of 14% per day (for all participants), for further analysis. A statistical probability of $p< 0.05$ (two-sided) was considered significant.

3. Results

3.1. Differences in general characteristics between the overweight and obese Malaysian adults with low and elevated inflammatory status

In total, 51% of our study participants were found to have elevated inflammatory status (CRP concentrations >3 mg/L), indicating high risk of cardiovascular events, in our study population (**Table 2**). None of the physically active participants had elevated inflammatory status, whereas 54% of the physically inactive had elevated inflammatory status. No significant difference was found between the two groups in age, gender, and in the distribution of ethnicity.

3.2. Differences in the mean values of anthropometric, blood biochemical and dietary parameters between overweight and obese Malaysian adults with low and elevated inflammatory status

Differences in the mean values of anthropometric, blood biochemical and dietary parameters between the groups with low (CRP concentrations ≤3 mg/L) and elevated inflammatory status (CRP concentrations >3 mg/L) are reported in **Table 3**. Our results revealed that individuals with elevated inflammatory status had significantly higher body weight ($p= 0.025$), BMI ($p= 0.003$), WC ($p< 0.001$), waist- hip ratio (WHR) ($p= 0.033$), fat mass ($p= 0.001$) and percent body fat ($p< 0.001$), compared to those with low inflammatory status.

3.3. Association between PRS and the odds of elevated inflammatory status in the overweight and obese Malaysian adults

Multivariate binary logistic regression was performed to examine the effect of PRS on the odds of elevated inflammatory status. Our results revealed that the second tertile (PRS 0.65 to 3.58) and the third tertile PRS (PRS 3.59 to 8.18) were significantly associated with increased odds of elevated inflammatory status, 5.18 (CI= 1.67-16.09; p= 0.004) and 3.49 (CI= 1.15-10.62; p= 0.028), respectively, compared to the first tertile PRS (PRS 0 to 0.64) (**Table 3**). After adjusting for covariates age, gender, ethnicity, height, BMI, fat mass, percent body fat, physical activity status, and total energy intake, the odds of elevated inflammatory status in both second tertile and third tertile PRS increased to 7.56 (CI= 1.98-28.80; adjusted p= 0.003) and 3.87 (CI= 1.10-13.60; adjusted p= 0.035) respectively. Due to high collinearity among the anthropometric parameters, only BMI, fat mass and percent body fat were adjusted as covariates in all the analysis.

3.4. Difference in the distribution of PRS between the overweight and obese Malaysian adults of three different major ethnic groups

There were significant differences in the distribution of PRS between the different ethnic groups (p= 0.016) (**Table 4**). Higher number of Malaysian Indians were categorized under the third tertile PRS (3.59 to 8.18), compared to the Malays and the Chinese (42% vs 32% and 21%), whereas higher number of Malaysian Chinese were categorized under first tertile PRS (0 to 0.64) compared to the Malays and the Indians (50% vs 35% and 13%).

3.5. Effect of the interaction between PRS and percent energy from protein on CRP concentrations in the overweight and obese Malaysian adults

To study the effect of the interaction between PRS and dietary intake on CRP concentrations, dietary nutrients were dichotomized into 2 groups by using the median value of per day intake, for analysis. A significant association was only found in dietary protein, therefore 26 other dietary parameters are not reported (p> 0.05). Percent energy from dietary protein was

dichotomized into 2 groups by using the median value of 14% protein energy intake per day (for all participants). Results from general linear model analysis revealed that overweight and obese individuals in the third tertile PRS had significantly lower CRP concentrations (4.61 ± 1.3 mg/L vs 9.60 ± 2.6 mg/L, p= 0.019 and p interaction= 0.045) (**Figure 1**) when consuming ≥14% energy from dietary protein per day (with an average of 18.0 ± 2.4%, 43.0 ± 7.7% and 39.0 ± 8.0% energy from protein, carbohydrate and fat, respectively), compared to those consuming <14% energy from dietary protein per day (with an average of 11.5 ± 1.7%, 51.0 ± 5.7% and 36.5 ± 5.1% energy from protein, carbohydrate and fat, respectively), even after adjusting for covariates age, gender, physical activity status, smoking status, alcohol consumption, BMI, fat mass, percent body fat, HDL- C, and total energy intake.

4. Discussion

Results from the current study support our hypothesis that an increased intake of dietary protein is associated with lower CRP concentrations in individuals with high- risk genetic predisposition to obesity. Besides, our results revealed that compared to those with lower CRP concentrations, individuals with elevated CRP concentrations were significantly associated with excess fat- related phenotypes including body weight, BMI, WC, WHR, fat mass and percent body fat. Positive correlations between CRP concentrations and anthropometric parameters including BMI, WC, WHR, and fat mass has been well established in different populations such as in the Taiwanese, Swiss and the Japanese populations [43–45]. These results corroborate the fact that adipose tissue, besides being an organ for storage of energy in the form of triglycerides, has an important function as an endocrine organ, producing a variety of pro-inflammatory adipokines such as interleukins (IL- 1, IL- 6, IL- 8), tumor necrosis factor-alpha, leptin and resistin [46]. The release of IL- 6 triggers hepatocytes to synthesize and secrete CRP, indicating a state of inflammation. A reduction in excess body fat can significantly reduce the inflammatory state of an individual [47].

The current study found that physically inactive individuals were associated with increased CRP concentrations, compared to physically active individuals. It has been well documented

that physical activity plays a role in modulating inflammatory status. A systematic review by Kasapis and Thompson, reported that in their investigation both cross-sectional and longitudinal, studies demonstrated inverse correlation between regular physical activity and serum inflammatory biomarkers. Physical activity directly reduced cytokine production by adipose tissue, skeletal muscles, endothelial and blood mononuclear cells, and indirectly improved insulin sensitivity and reduced body weight [48].

Results from logistic regression analysis of the current study, revealed that second tertile (PRS 0.65 to 3.58) and third tertile PRS (PRS 3.59 to 8.18) were significantly associated with increased odds of elevated inflammatory status, 5.18 (CI= 1.67-16.09) and 3.49 (CI= 1.15-10.62), respectively, independent of BMI, compared to the first tertile PRS (PRS 0 to 0.64). These findings suggest that CRP concentrations were significantly higher in those who were genetically predisposed (assessed by PRS) to overweight and obesity. These findings support and strengthen the conclusion of our earlier investigation which reported significantly higher CRP concentrations in individuals carrying the risk allele (G allele) of *FTO* rs9939506 in the Malaysian adults [13]. Although obesity is one of the main risk factors of low- grade inflammation, GWAS studies have confirmed that genetic regulation of the latter is largely independent of BMI and that their analysis revealed that a number of the risk variants of the *CRP* gene and different other genes (58 distinct genetic loci, 55 genes and their association with 29 signals) influence the serum concentrations of CRP even after adjusting for BMI [49]. Hence, we highlight the importance of the current study which has identified dietary protein out of 25 macro and micro-nutrients analyzed, as a moderator of low- grade inflammation in those who are genetically predisposed, irrespective of their BMI.

Sun et al. reported that the risk allele of *FTO* rs9939609 (A allele) was strongly associated with increased odds of obesity and elevated CRP concentrations in the Chinese Han population [14]. Similar findings were found in the middle- aged German population, the risk allele carriers (A allele) of *FTO* rs9939609 were associated with higher circulating CRP concentrations, independent of the degree of adiposity [50]. In the present study we found

389 significant differences in the distribution of PRS between the three major ethnic groups of the
390 Malaysian population. Higher proportion of Malaysian Indians were categorized under the third
391 tertile PRS compared to the Malays and the Chinese. Higher proportion of Malaysian Chinese
392 were categorized under the first tertile PRS. Our findings add to the existing evidence that
393 differences in genetic distribution do exist in various ethnic groups. Certain variants correlate
394 with CRP concentrations in Europeans such as *CRP, HNF1A* (hepatic nuclear factor 1- alpha),
395 and *APOE* (apolipoprotein E) [51]. Recent GWASs have identified new variants of cytokines
396 such as *IL- 6* in the Japanese population and *TREM2* (triggering receptors expressed by
397 myeloid cells 2) in African American women [52]. It is reported that differences in allele
398 frequencies of high- risk gene variants in question, linkage disequilibrium, effect size, and
399 biological adaptations may influence the inflammatory status of individuals of different ethnic
400 groups [53]. While pathway- based analysis may enable better understanding of the
401 biochemical mechanism of systemic inflammation in diverse population groups, such research
402 is expensive and far between. Our study identifies ethnic differences in CRP concentrations
403 in the Malaysian population and suggests dietary modulation to alleviate risk of disease in
404 those at risk due to high CRP concentrations.

405 It is important to highlight that, no significant difference was found in the protein intake per day,
406 as well as the intakes of other macronutrients including carbohydrate and fat when
407 comparative analyses were done between overweight and obese individuals with low and
408 elevated inflammatory status. However, when the effect of gene variants was included in the
409 model of nutrient- gene interaction analysis, it was evident that individuals in the third tertile
410 PRS had significantly lower CRP concentrations when consuming ≥14% energy from protein
411 per day, compared to those consuming <14% energy from protein per day, even after adjusting
412 for covariates including BMI. Such effects were not found in the first tertile and second tertile
413 PRS. Therefore, it can be inferred that the presence of excess fat related multiple risk alleles
414 of certain genes may have cumulative risk in raising the inflammatory status of an individual
415 and that such individuals may benefit from dietary intake of certain nutrients over and above

416 the general dietary recommendations. In case of the current study, dietary protein (≥14%
417 energy from protein per day) may have modulated CRP concentrations towards a positive
418 metabolic profile of our study participants particularly in those who are genetically predisposed.
419 There is evidence that food sources of protein may have differential effects on different
420 biomarkers of inflammation. Dietary intake patterns that are rich in plant-based proteins (e.g.
421 whole grains, vegetables, nuts, and legumes) have been associated with lower inflammatory
422 status, whereas animal-based proteins (e.g. fat and processed meat) were associated with
423 higher inflammatory status [54–56]. A 4- year longitudinal randomized clinical trial also
424 reported that increase in soy protein consumption significantly reduced the serum
425 concentrations of CRP in type 2 diabetic patients with nephropathy compared to the control
426 group [57].

427 In the current study, our study participants consumed an average of 15% energy from protein
428 per day (Table 3), this intake is as per the recommendation of the Ministry of Health, Malaysia
429 [25]. Based on our data, we found (not presented here) that primarily on an average, dietary
430 protein was sourced from poultry, fish, tofu, lentils, pulses and whole grains, with less reliance
431 on red meat and processed meat. The Framingham Heart Offspring Study Cohort, reported
432 that dietary protein particularly from plant-based sources, rather than animal- protein, was
433 inversely associated with inflammation and oxidative stress score [16]. Plant protein sources
434 may contain high concentrations of polyphenols and a host of other anti-inflammatory
435 constituents, which may contribute to the lower inflammatory status and oxidative stress that
436 was observed in our research and some other researches. [58,59]. Further, future research
437 analyzing plant and animal protein separately on the inflammatory status of study participants
438 is warranted. For causal inference, biological pathway analysis and metabolomic studies are
439 necessary to elucidate the underlying mechanisms of nutrient- gene interaction on
440 inflammatory markers.

441 This is an observational study. Due to the nature of the study design, drawing causal inference
442 on the association between dietary protein, PRS and CRP concentrations was not possible.

Thus, it is acknowledged that the current study is exploratory. However, with the association and interaction analyses, we have generated a hypothesis, and paved the path for future large-scale research to confirm the findings presented in this study. Other limitations include the assessment of body composition using bioelectrical impedance analysis (BIA) as BIA estimates are influenced by many factors including the food and fluid intake, environment, ethnicity, and phase of menstrual cycle. Moreover, there is the possibility of unmeasured or residual confounding including individual's mental health and socioeconomic indicators, although we have carefully controlled several lifestyle and dietary factors which were previously found to be associated with CRP concentrations. These factors may have contributed to the variations in the observed effect of nutrient- gene interactions on CRP concentrations in our study participants. As in any nutritional research, measurement errors may occur in self-reported dietary intake and physical activity status. Moreover, our study participants were recruited from a specific geographical location (Beranang, Jalan Broga and Bandar Kajang) and they were predominantly women. These factors may have influenced the study outcome and hence the findings of current experiments may not be generalizable to the Malaysian population.

5. Conclusion

Our study revealed that elevated CRP concentrations were significantly associated with higher body weight, BMI, WC, WHR, fat mass and percent body fat in our study participants. Highest tertile PRS was significantly associated with increased odds of elevated CRP concentrations, suggesting that CRP concentrations were higher in individuals with higher number of obesity-related risk alleles. Furthermore, higher proportion of Malaysian Indians were categorized under third tertile PRS compared to Malays and Chinese. Our findings also revealed that individuals in the third tertile PRS were associated with lower CRP concentrations with intake of ≥14% energy from dietary protein per day compared to those consuming <14% energy from protein per day, suggesting that higher protein intake may have alleviated the inflammatory status and reduced CRP concentrations systemically in our study participants.

Acknowledgment

The authors would like to express their gratitude to Mr. Sunil Rao from the Diagnostic Centre for his assistance with the blood collection and analysis.

Sources of Support

This study was funded by an internal grant from the University of Nottingham Malaysia (UNM) (UNHB0008).

Author Contributions

Pui Yee Tan: Methodology, Investigation, Project Administration, Data Curation, Formal Analysis, Visualization, Writing-Original Draft and Writing-Review and Editing. **Farahnaz Amini**: Supervision, Methodology and Writing-Review and Editing. **Soma Roy Mitra**: Conceptualization, Methodology, Project Administration, Supervision, Validation, Writing-Review and Editing and Funding Acquisition.

Author Declarations

None.

References

[1]. Naidu BM, Yusoff MFM, Abdullah S, Musa KI, Yaacob NM, Mohamad MS, et al. Factors associated with the severity of hypertension among Malaysian adults. PLoS One 2019;14:e0207472. doi: 10.1371/journal.pone.0207472.

[2]. Rocha VZ, Libby P. Obesity, inflammation, and atherosclerosis. Nat Rev Cardiol 2009;6:399-409. doi: 10.1038/nrcardio.2009.55.

[3]. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm 2010;2010:289645. doi: 10.1155/2010/289645.

[4]. Yousuf O, Mohanty BD, Martin SS, Joshi PH, Blaha MJ, Nasir K, et al. High-sensitivity

C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? J Am Coll Cardiol 2013;62:397-408. doi: 10.1016/j.jacc.2013.05.016.

[5]. Ji P, Zhu J, Zhong Z, Li H, Pang J, Li B, et al. Association of elevated inflammatory markers and severe COVID-19: a meta-analysis. Medicine (Baltimore) 2020;99:e23315. doi: 10.1097/MD.0000000000023315.

[6]. Devaraj S, Singh U, Jialal I. Human C-reactive protein and the metabolic syndrome. Curr Opin Lipidol 2009;20:182-9. doi: 10.1097/MOL.0b013e32832ac03e.

[7]. Rodríguez-Hernández H, Simental-Mendía LE, Rodríguez-Ramírez G, Reyes-Romero MA. Obesity and inflammation: epidemiology, risk factors, and markers of inflammation. Int J Endocrinol 2013;2013:678159. doi: 10.1155/2013/678159.

[8]. Blaha MJ, Rivera JJ, Budoff MJ, Blankstein R, Agatston A, O'Leary DH, et al. Association between obesity, high-sensitivity C-reactive protein ≥2 mg/L, and subclinical atherosclerosis: implications of JUPITER from the multi-ethnic study of atherosclerosis. Arterioscler Thromb Vasc Biol 2011;31:1430-8. doi: 10.1161/ATVBAHA.111.223768.

[9]. Pfützner A, Forst T. High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. Diabetes Technol Ther 2006;8:28-36. doi: 10.1089/dia.2006.8.28.

[10]. Ellulu MS, Patimah I, Khaza H, Rahmat A, Abed Y, Sci AM. Obesity and Inflammation : the linking mechanism and the complications. Arch Med Sci 2016;13:851-63. doi: 10.5114/aoms.2016.58928.

[11]. Faucher G, Guénard F, Bouchard L, Garneau V, Turcot V, Houde A, et al. Genetic contribution to C-reactive protein levels in severe obesity. Mol Genet Metab 2012;105:494-501. doi: 10.1016/j.ymgme.2011.11.198.

[12]. Best LG, Balakrishnan P, Cole SA, Haack K, Kocarnik JM, Pankratz N, et al. Genetic

analysis of hsCRP in American Indians: the Strong Heart Family study. PLoS One 2019;14:e0223574. doi: 10.1371/journal.pone.0223574.

[13]. Mitra SR, Tan PY, Amini F. Effect of FTO rs9930506 on obesity and interaction of the gene variants with dietary protein and vitamin E on C-reactive protein levels in multi-ethnic Malaysian adults. J Hum Nutr Diet 2018;31:758-72. doi: 10.1111/jhn.12593.

[14]. Sun Y, Sun J, Wang X, Yang M. Variants in the fat mass and obesity associated (FTO) gene are associated with obesity and C-reactive protein levels in Chinese Han populations. Clin Investig Med 2010;33:405-12. doi: 10.25011/cim.v33i6.14592.

[15] Julia C, Assmann KE, Shivappa N, Hebert JR, Wirth MD, Hercberg S, et al. Long-term associations between inflammatory dietary scores in relation to long-term C-reactive protein status measured 12 years later: findings from the Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) cohort. Br J Nutr 2017;117:306-14. doi: 10.1017/S0007114517000034.

[16]. Hruby A, Jacques PF. Dietary protein and changes in biomarkers of inflammation and oxidative stress in the Framingham Heart Study Offspring cohort. Curr Dev Nutr 2019; 3:nzz019. doi: 10.1093/cdn/nzz019.

[17] Aycart DF, Acevedo S, Eguiguren-Jimenez L, Andrade JM. Influence of plant and animal proteins on inflammation markers among adults with chronic kidney disease: a systematic review and meta-analysis. Nutrients 2021;13:1660. doi: 10.3390/nu13051660.

[18] Aquilani R, Zuccarelli GC, Maestri R, Boselli M, Dossena M, Baldissarro E, et al. Essential amino acid supplementation is associated with reduced serum C-reactive protein levels and improved circulating lymphocytes in post-acute inflamed elderly patients. Int J Immunopathol Pharmacol 2021;35:1-12. doi: 10.1177/20587384211036823.

[19]. Mitra SR, Tan PY, Amini F. Association of ADRB2 rs1042713 with obesity and obesity-related phenotypes and its interaction with dietary fat in modulating glycaemic indices in Malaysian adults. J Nutr Metab 2019;2019:1-10. doi: 10.1155/2019/8718795.

[20]. Mitra SR, Tan PY. Effect of an individualised high protein, calorie-restricted diet on anthropometric and cardio-metabolic parameters in overweight and obese Malaysian adults: a 6-month randomised controlled study. Br J Nutr 2019;121:1002-17. doi: 10.1017/S0007114519000345.

[21]. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;363:157-63. doi: 10.1016/S0140-6736(03)15268-3.

[22]. WHO. Waist circumference and waist-hip ratio report of a WHO Expert Consultation. World Health. 2008;(December):8–11.

[23]. Loy SL, Marhazlina M, Nor Azwany Y, Hamid Jan JM. Development, validity and reproducibility of a food frequency questionnaire in pregnancy for the Universiti Sains Malaysia birth cohort study. Malays J Nutr 2011;17:1-18.

[24]. Suzana S. Atlas of food exchanges & portion sizes. Second edition. Kuala Lumpur: MDC Publishers Sdn Bhd; 2009.

[25]. National Coordinating Committee on Food and Nutrition. Recommended nutrient intakes for Malaysian 2017: a report of a technical working group on nutritional guidelines. Malaysia: Ministry of Health; 2017.

[26]. Tee ES. Nutrient composition of Malaysian foods: Malaysian food composition database programme. Fourth edition. Malaysia: Ministry of Health; 1997.

[27]. Goldberg G, Black A, Jebb S, Colte T, Murgatroyd P, Coward W, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. derivation of cut-off limits to identify under-recording. Eur J Clin Nutr 1991;45:569-81.

568 [28]. Ainsworth BE, Haskell WIL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al.
569 Compendium of physical activities: an update of activity codes and MET intensities.
570 Med Sci Sports Exerc 2000;32:498-504. doi: 10.1097/00005768-200009001-00009.

571 [29]. National Coordinating Committee on Food and Nutrition (NCCFN). Malaysian Dietary
572 Guidelines 2010. Malaysia: Ministry of Health; 2010.

573 [30]. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care
574 2004;27:1487-95. doi: 10.2337/diacare.27.6.1487.

575 [31]. Yamada C, Moriyama K, Takahashi E. Optimal cut-off point for homeostasis model
576 assessment of insulin resistance to discriminate metabolic syndrome in non-diabetic
577 Japanese subjects. J Diabetes Investig 2012;3:384-7. doi: 10.1111/j.2040-
578 1124.2012.00194.x.

579 [32]. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density
580 lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin
581 Chem 1972;18:499-502.

582 [33]. Buckley DI, Fu R, Freeman M, Rogers K, Helfand M. C-reactive protein as a risk factor
583 for coronary heart disease: a systematic review and meta-analyses for the U.S.
584 preventive services task force. Ann Intern Med 2009;51:483-95. doi: 10.7326/0003-
585 4819-151-7-200910060-00009.

586 [34]. Namipashaki A, Razaghi-Moghadam Z, Ansari-Pour N. The essentiality of reporting
587 hardy-weinberg equilibrium calculations in population-based genetic association
588 studies. Cell J 2015;17:187-92. doi: 10.22074/cellj.2016.3711.

589 [35]. Yin X, Cheng H, Lin Y, Wineinger NE, Zhou F, Sheng Y, et al. A weighted polygenic
590 risk score using 14 known susceptibility variants to estimate risk and age onset of
591 psoriasis in Han Chinese. PLoS One 2015;10:e0125369. doi:
592 10.1371/journal.pone.0125369.

593 [36]. Marees AT, de Kluiver H, Stringer S, Vorspan F, Curis E, Marie-Claire C, et al. A tutorial
594 on conducting genome-wide association studies: quality control and statistical analysis.
595 Int J Methods Psychiatr Res 2018;27:e1608. doi: 10.1002/mpr.1608.

596 [37]. Reisberg S, Iljasenko T, Läll K, Fischer K, Vilo J. Comparing distributions of polygenic
597 risk scores of type 2 diabetes and coronary heart disease within different populations.
598 PLoS One 2017;12:e0179238. doi: 10.1371/journal.pone.0179238.

599 [38]. Zhang H, Wu J, Yu L. Association of Gln27Glu and Arg16Gly polymorphisms in beta2-
600 adrenergic receptor gene with obesity susceptibility: a meta-analysis. PLoS One
601 2014;9:e100489. doi: 10.1371/journal.pone.0100489.

602 [39]. Wrzosek M, Zakrzewska A, Ruczko L, Jabłonowska-Lietz B, Nowicka G. Association
603 between rs9930506 polymorphism of the fat mass & obesity-associated (FTO) gene &
604 onset of obesity in Polish adults. Indian J Med Res 2016;143:281-7. doi: 10.4103/0971-
605 5916.182617.

606 [40]. Kalantari N, Keshavarz Mohammadi N, Izadi P, Gholamalizadeh M, Doaei S, Eini-Zinab
607 H, et al. A complete linkage disequilibrium in a haplotype of three SNPs in fat mass and
608 obesity associated (FTO) gene was strongly associated with anthropometric indices
609 after controlling for calorie intake and physical activity. BMC Med Genet 2018;19:146.
610 doi: 10.1186/s12881-018-0664-z.

611 [41]. Daghestani MH, Warsy A, Daghestani MH, Al-Odaib AN, Eldali A, Al-Eisa NA, et al.
612 Arginine 16 glycine polymorphism in β 2-adrenergic receptor gene is associated with
613 obesity, hyperlipidemia, hyperleptinemia, and insulin resistance in saudis. Int J
614 Endocrinol 2012;2012:945608. doi: 10.1155/2012/945608.

615 [42]. Mansfield ER, Helms BP. Detecting multicollinearity. Am Stat 1982;36:158-60. doi:
616 10.2307/2683167.

617 [43]. Lin CC, Kardia SLR, Li CI, Liu CS, Lai MM, Lin WY, et al. The relationship of high

618 sensitivity C-reactive protein to percent body fat mass, body mass index, waist-to-hip
619 ratio, and waist circumference in a Taiwanese population. BMC Public Health
620 2010;10:579. doi: 10.1186/1471-2458-10-579.

621 [44]. Marques-Vidal P, Bochud M, Bastardot F, Lüscher T, Ferrero F, Gaspoz JM, et al.
622 Association between inflammatory and obesity markers in a swiss population-based
623 sample (CoLaus Study). Obes Facts 2012;5:734-44. doi: 10.1159/000345045.

624 [45]. Saijo Y, Kiyota N, Kawasaki Y, Miyazaki Y, Kashimura J, Fukuda M, et al. Relationship
625 between C-reative protein and visceral adipose tissue in healthy Japanese subjects.
626 Diabetes, Obes Metab 2004;6:249-58. doi: 10.1111/j.1462-8902.2003.0342.x.

627 [46]. Castro AM, Macedo-de la Concha LE, Pantoja-Meléndez CA. Low-grade inflammation
628 and its relation to obesity and chronic degenerative diseases. Rev Médica del Hosp
629 Gen México 2017;80:101-5. doi: 10.1016/j.hgmx.2016.06.011.

630 [47]. Petelin A, Bizjak M, Cernelic-Bizjak M, Jurdana M, Jakus T, Jenko-Praznikar Z. Low-
631 grade inflammation in overweight and obese adults is affected by weight loss program.
632 J Endocrinol Invest 2014;37:745-55. doi: 10.1007/s40618-014-0102-9.

633 [48]. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein
634 and inflammatory markers: a systematic review. J Am Coll Cardiol 2005;45:1563-9. doi:
635 10.1016/j.jacc.2004.12.077.

636 [49]. Ligthart S, Vaez A, Võsa U, Stathopoulou MG, de Vries PS, Prins BP, et al. Genome
637 analyses of >200,000 individuals identify 58 loci for chronic inflammation and highlight
638 pathways that link inflammation and complex disorders. Am J Hum Genet
639 2018;103:691-706. doi: 10.1016/j.ajhg.2018.09.009.

640 [50]. Fisher E, Schulze MB, Stefan N, Häring H-U, Döring F, Joost H-G, et al. Association of
641 the FTO rs9939609 single nucleotide polymorphism with C-reactive protein levels.
642 Obesity (Silver Spring) 2009;17:330-4. doi: 10.1038/oby.2008.465.

643 [51]. Okada Y, Takahashi A, Ohmiya H, Kumasaka N, Kamatani Y, Hosono N, et al.
644 Genome-wide association study for C-reactive protein levels identified pleiotropic
645 associations in the IL6 locus. Hum Mol Genet 2011;20:1224-31. doi:
646 10.1093/hmg/ddq551.

647 [52]. Reiner AP, Beleza S, Franceschini N, Auer PL, Robinson JG, Kooperberg C, et al.
648 Genome-wide association and population genetic analysis of c-reactive protein in
649 african american and hispanic american women. Am J Hum Genet 2012;91:502-12. doi:
650 10.1016/j.ajhg.2012.07.023

651 [53]. Menashe I, Maeder D, Garcia-Closas M, Figueroa JD, Bhattacharjee S, Rotunno M, et
652 al. Pathway analysis of breast cancer genome-wide association study highlights three
653 pathways and one canonical signaling cascade. Cancer Res 2010;70:4453–9. doi:
654 10.1158/0008-5472.CAN-09-4502.

655 [54]. Nettleton JA, Steffen LM, Mayer-Davis EJ, Jenny NS, Jiang R, Herrington DM, et al.
656 Dietary patterns are associated with biochemical markers of inflammation and
657 endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin
658 Nutr 2006;83:1369-79. doi: 10.1093/ajcn/83.6.1369.

659 [55]. Kostovcikova K, Coufal S, Galanova N, Fajstova A, Hudcovic T, Kostovcik M, et al. Diet
660 rich in animal protein promotes pro-inflammatory macrophage response and
661 exacerbates colitis in mice. Front Immunol. 2019;10:919. doi:
662 10.3389/fimmu.2019.00919.

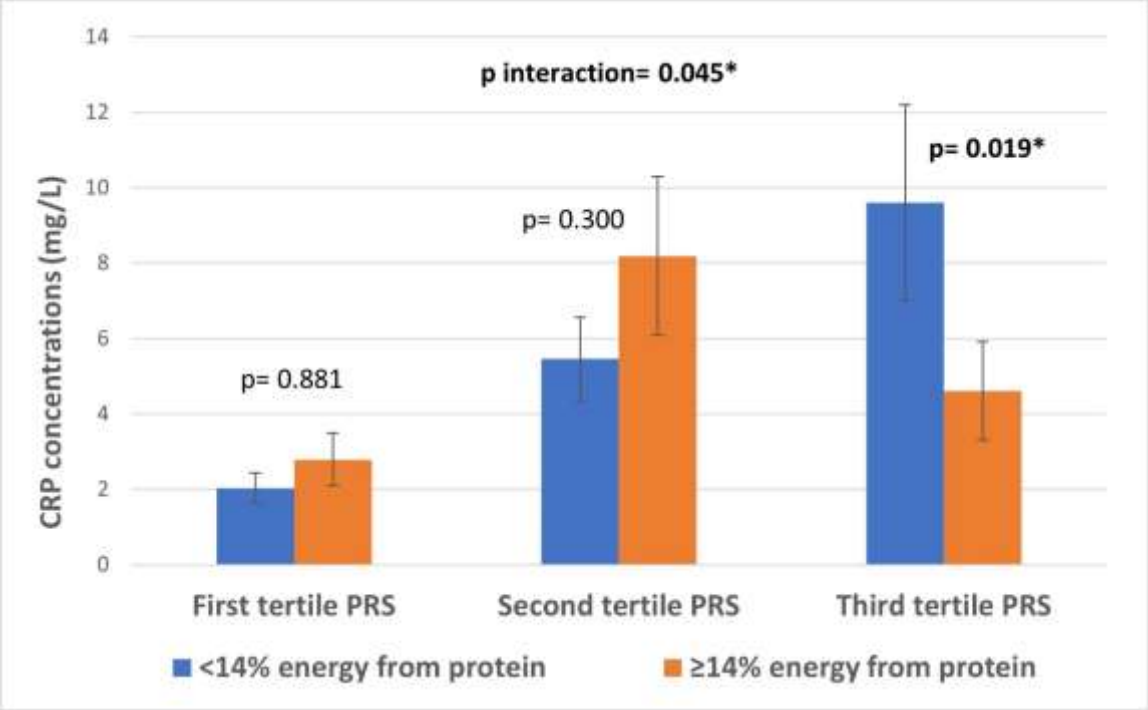
663 [56]. Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and
664 biomarkers of low-grade inflammation: a systematic literature review. Nutr Rev 2013;
665 71:511-27. doi: 10.1111/nure.12035.

666 [57]. Azadbakht L, Atabak S, Esmaillzadeh A. Soy protein intake, cardiorenal indices, and
667 C-reactive protein in type 2 diabetes with nephropathy. Diabetes Care 2008;31:648-54.
668 doi: 10.2337/dc07-2065.

669 [58]. Landberg R, Sun Q, Rimm EB, Cassidy A, Scalbert A, Mantzoros CS, et al. Selected
670 dietary flavonoids are associated with markers of inflammation and endothelial
671 dysfunction in U.S. women. J Nutr 2011;141: 618-25. doi: 10.3945/jn.110.133843.

672 [59]. González R, Ballester I, López-Posadas R, Suárez MD, Zarzuelo A, Martínez-Augustin
673 O, et al. Effects of flavonoids and other polyphenols on inflammation. Crit Rev Food Sci
674 Nutr 2011;51:331-62. doi: 10.1080/10408390903584094.

675 **Legends to Figures:**



676

677 **Figure 1: Effect of the interaction between PRS and percent energy from protein on**
678 **CRP concentrations in the overweight and obese Malaysian adults**

679 Nutrient- gene interaction was evaluated by using general linear model after adjusting for age,
680 gender, ethnicity, physical activity status, BMI, fat mass, percent body fat, and total energy
681 intake. Percent energy from dietary protein was dichotomized into 2 groups by using the
682 median value of 14% per day (for all participants), for analysis. Individuals in the third tertile
683 PRS had significantly lower CRP concentrations when consuming ≥14% energy from protein
684 per day, compared to those consuming <14% energy from protein per day, even after adjusting
685 for covariates.

CRP concentrations were expressed as mean ± standard error.

CRP, C-reactive protein; PRS, polygenic risk scores.

*p< 0.05 was considered significant.

Table 1: The odds (ORs) of obesity associated with gene variants

Gene	SNP	Risk allele	RAF	ORs (95%CI)	Natural log (ORs)
FTO	**rs9930506** [13]	G	0.37	2.87 (1.14-7.19)	1.05
	rs9930501 [a]	G	0.37	3.03 (1.23-7.49)	1.11
	rs9932754 [a]	C	0.37	3.04 (1.22-7.59)	1.11
ADRB2	**rs1042713** [19]	G	0.13	1.38 (0.08-23.93)	0.32

Logistic regression was conducted to determine the ORs (with 95% CI) of obesity associated with each SNP after adjusting for confounding factors including age, gender, physical activity status, smoking status and alcohol consumption. The homozygous non-risk allele of each SNP was used as the reference group.

CIs, confidence intervals; *FTO*, fat mass and obesity-associated; *ADRB2*, beta- 2 adrenergic receptor; SNP, single nucleotide polymorphisms; RAF, risk allele frequency; ORs, odd ratios.

[a] Unpublished data

Table 2: General characteristics between the overweight and obese Malaysian adults with low and elevated inflammatory status

Variables		Total (n= 128)	CRP ≤3 mg/L (n= 63)	CRP >3 mg/L (n= 65)	p value
Age (y)		44.0 ± 1.0	46.0 ± 1.3	46.8 ± 1.4	0.664
Gender					
	Female	114 (89%)	53 (46%)	61 (54%)	0.157
	Male	14 (11%)	10 (70%)	4 (30%)	
Ethnicity					
	Malays	58 (45%)	30 (51%)	28 (49%)	0.088
	Chinese	20 (16%)	14 (71%)	6 (28%)	
	Indians	50 (39%)	19 (37%)	31 (63%)	
Physical activity status					
	Physical inactive	120 (94%)	55 (46%)	65 (54%)	**0.019***
	Physical active	8 (6%)	8 (100%)	0	

Data were expressed as mean (±SE) or number (percentage).
Elevated inflammatory status was defined as CRP concentrations >3 mg/L.
p value based on Chi-square test and Independent t-test for categorical and continuous variables, respectively.
*p<0.05 was considered as significant.
CRP, C- reactive protein.

Table 3: Differences in anthropometric, blood biochemical and dietary parameters between the overweight and obese Malaysian adults with low and elevated inflammatory status

Variables	Total (n= 128)	CRP ≤3 mg/L (n= 63)	CRP >3 mg/L (n= 65)	p value
Anthropometric parameters [1]				
Height (cm)	158.0 ± 0.7	157.9 ± 1.0	155.1 ± 0.9	0.146
Weight (kg)	73.9 ± 1.4	69.7 ± 1.5	73.2 ± 1.6	**0.025***
BMI (kg/m^2)	29.5 ± 0.4	28.0 ± 0.6	30.4 ± 0.6	**0.003***
WC (cm)	93.8 ± 1.1	89.6 ± 1.4	97.1 ± 1.4	**<0.001***
WHR	0.92 ± 0.01	0.89 ± 0.01	0.94 ± 0.01	**0.033***
Muscle mass (kg)	23.4 ± 0.4	23.0 ± 0.7	22.0 ± 0.5	0.744
Fat mass (kg)	31.1 ± 0.9	27.8 ± 1.0	32.7 ± 1.1	**0.001***
Fat- free mass (kg)	42.8 ± 0.7	41.9 ± 1.0	40.5 ± 0.8	0.980
Percent body fat (%)	41.6 ± 0.6	39.7 ± 0.9	44.3 ± 0.8	**<0.001***
Systolic blood pressure (mmHg)	122.7 ± 1.3	125.0 ± 2.0	123.3 ± 2.6	0.696
Diastolic blood pressure (mmHg)	80.9 ± 0.9	82.1 ± 1.5	81.2 ± 1.5	0.974
Pulse rate (bpm)	76.5 ± 1.0	79.6 ± 1.9	78.6 ± 1.4	0.442
Blood biochemical parameters [2]				
Fasting glucose (mmol/L)	5.2 ± 0.2	5.4 ± 0.2	5.5 ± 0.4	0.737
Fasting insulin (uU/mL)	10.1 ± 0.9	9.9 ± 1.6	10.9 ± 1.7	0.894
HOMA- IR	2.5 ± 0.3	2.8 ± 0.7	2.7 ± 0.4	0.689
TC (mmol/L)	5.5 ± 0.1	5.5 ± 0.2	5.4 ± 0.2	0.679
Triglyceride (mmol/L)	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	0.556
HDL- C (mmol/L)	1.5 ± 0.04	1.5 ± 0.1	1.5 ± 0.1	0.305
LDL- C (mmol/L)	3.4 ± 0.1	3.4 ± 0.1	3.3 ± 0.1	0.454
TC / HDL- C	3.7 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	0.737
CRP (mg/L)	5.8 ± 0.7	1.6 ± 0.1	9.8 ± 1.1	n.a.
Dietary parameters [3]				
Total energy intake (kcal)	2009 ± 30	1961 ± 41	2022 ± 43	0.201
Protein intake (g)	74.8 ± 2.1	73.5 ± 3.2	73.8 ± 3.2	0.854
Fat intake (g)	82.7 ± 1.9	81.8 ± 2.7	87.7 ± 3.6	0.362
Carbohydrate intake (g)	253.7 ± 5.7	245.7 ± 8.3	247.8 ± 7.8	0.515
Percent energy from protein (%)	14.8 ± 0.4	14.8 ± 0.7	14.5 ± 0.6	0.814
Percent energy from fat (%)	36.7 ± 0.6	36.9 ± 0.9	38.4 ± 1.1	0.382
Percent energy from carbohydrate (%)	47.0 ± 0.7	46.9 ± 1.2	45.2 ± 1.4	0.347

Data were expressed as mean ± standard error.
Elevated inflammatory status was defined as CRP concentrations >3 mg/L.
p value based on one-way ANCOVA, after adjusting for covariates in different models [1] age, gender, ethnicity, and physical activity; [2] model [1] + BMI, fat mass and percent body fat; [3] model [2] + total energy intake.
*p< 0.05 was considered as significant.
BMI, body mass index; HDL- C, high-density lipoprotein cholesterol; HOMA- IR, homeostatic model assessment- insulin resistance; CRP, C- reactive protein; LDL- C, low-density lipoprotein cholesterol; n.a., not applicable; TC, total cholesterol; WC, waist circumference; WHR, waist- hip ratio.

Table 4: Association between PRS and the odds of elevated inflammatory status in the overweight and obese Malaysian adults

PRS	CRP ≤3 mg/L (n= 63)	CRP >3 mg/L (n= 65)	Unadjusted ORs (95% CI)	Unadjusted p value	Adjusted ORs (95% CI) [a]	Adjusted p value [a]
First tertile (PRS 0 to 0.64)	28 (73%)	10 (27%)	1	-	1	-
Second tertile (PRS 0.65 to 3.58)	16 (34%)	31 (66%)	**5.18 (1.67-16.09)**	**0.004***	**7.56 (1.98-28.80)**	**0.003***
Third tertile (PRS 3.59 to 8.18)	19 (44%)	24 (56%)	**3.49 (1.15-10.62)**	**0.028***	**3.87 (1.10-13.60)**	**0.035***

ORs with 95% (CIs) were estimated for each tertile using logistic regression. The first tertile PRS was used as the reference group.
Elevated inflammatory status was defined as CRP concentrations >3 mg/L.
[a] adjusted for age, gender, ethnicity, height, BMI, fat mass, percent body fat, physical activity status and total energy intake.
*p< 0.05 was considered significant.
CIs, confidence intervals; CRP, C- reactive protein; PRS, polygenic risk scores; ORs, odds ratio.

Table 5: Difference in the distribution of PRS between the overweight and obese Malaysian adults of three different major ethnic groups

Ethnicity	PRS			p value
	First tertile (PRS 0 to 0.64)	Second tertile (PRS 0.65 to 3.58)	Third tertile (PRS 3.59 to 8.18)	
Chinese (n= 24)	12 (50%)	7 (29%)	5 (21%)	**0.016***
Malays (n= 57)	20 (35%)	19 (33%)	18 (32%)	
Indians (n= 47)	6 (13%)	21 (45%)	20 (42%)	

*p< 0.05 was considered as significant based Chi- square test.
PRS, polygenic risk scores.