ความสัมพันธ์ระหว่างการบริโภคไข่และโปรตีนในเลือดที่เกี่ยวข้องกับกลไกทางเมแทบอลิคในกลุ่มเพศชายที่มีภาวะอ้วน

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บทคัดย่อ

ข้อมูลจากการศึกษาทางด้านระบาดวิทยา ยังไม่สามารถสรุปแน่ชัดในประเด็นความสัมพันธ์ของการบริโภคไข่และอุบัติการณ์การเกิดโรคหัวใจและหลอดเลือด การศึกษานี้เป็นการศึกษาแรกที่ใช้เทคนิคโปรติ-โอมิกส์ เพื่อวิเคราะห์รูปแบบของโปรตีนในเลือดและกลไกทางด้านเมแทบบอลิคที่สัมพันธ์กับปริมาณการบริโภคไข่ โดยทำการศึกษาเปรียบเทียบในกลุ่มควบคุมที่มีดัชนีมวลกายปกติ (จำนวน 116 คน) และกลุ่มที่มีภาวะอ้วน (จำนวน 217 คน) มีการประเมินความถี่การบริโภคอาหาร การวิเคราะห์ทางชีวเคมีและรูปแบบของโปรตีนในเลือดโดยใช้เครื่องมือ LC-MS/MS ผลจากการเปรียบเทียบระหว่างกลุ่มควบคุมที่มีการบริโภคไข่ในระดับต่ำกับกลุ่มคนอ้วนที่มีการบริโภคไข่ในระดับสูงโดยพิจารณาจากค่า relative fold changes (log 2) พบว่ามีความแตกต่างกันของโปรตีน ได้แก่ Tumor necrosis factor receptor superfamily member 18, NF-kappa-B-activating protein, Complement C1s subcomponent และ Collagen alpha-6 (VI) chain การวิเคราะห์เพิ่มเติมในส่วน protein-protein interaction ของโปรตีนที่พบเฉพาะในกลุ่มคนอ้วนที่มีการบริโภคไข่ในระดับสูง จำนวน 238 ชนิด พบความสัมพันธ์กับกลไก mTOR, AMPK, HIF-1, และ insulin signaling pathway โดยสรุปพบว่าการเปลี่ยนแปลงของตัวชี้วัดทางคลินิกและชีวเคมีในเลือดของกลุ่มคนอ้วน มีความสัมพันธ์กับรูปแบบของโปรตีนในเลือด ที่เชื่อมโยงกับกลไกที่เกี่ยวข้องกับการเปลี่ยนแปลงดังกล่าว ผลที่ได้จากการศึกษาครั้งนี้ จะเป็นข้อมูลพื้นฐานเพื่อการพิจารณาแนวทางการให้คำแนะนำการบริโภคไข่สำหรับกลุ่มคนสุขภาพดีและกลุ่มที่มีความเสี่ยงต่อการเกิดโรคทางเมแทบอลิค

คำสำคัญ: โปรติโอมิกส์ การบริโภคไข่ ภาวะอ้วน กลไกระดับโมเลกุล

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Egg Consumption Impacts Serum Proteome Profiles and Metabolic Pathways in Obese Men

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Abstract

There are inconclusive data from epidemiological studies linking egg consumption to an increased prevalence of cardiovascular disease. This is the first study using the proteomic approach to analyze the serum proteome profile and potential metabolic pathways related to difference egg intake, compared between the control with normal body mass index (N=116) and the obese groups (N=217). Clinical assessment, food frequency questionnaire, biochemical analysis were performed and serum proteomic was analyzed by LC/MS-MS. Significant findings from comparison between the controls with low egg intake and the obese with high egg intake indicated relative fold changes of log 2 of upregulated and downregulated proteins, including Tumor necrosis factor receptor superfamily member 18, NF-kappa-B-activating protein, Complement C1s subcomponent, and Collagen alpha-6(VI) chain. Further analysis for protein-protein interaction of unique proteins (N=238) from obese group with high egg intake demonstrated association with mTOR, AMPK, HIF-1, and insulin signaling pathway. In conclusion, changes in clinical and biochemical markers in obesity were linked to potential pathways identified from proteomics and these results provided data for consideration in the development of dietary guidelines for egg consumption among healthy and metabolic risk groups.

Keywords: Proteomics, Egg intake, Obesity, Molecular pathways

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Introduction

Obesity is a major public health epidemic worldwide, especially in Thailand. According to the findings of the 5th Thai National Health Examination Survey (NHES) in 2014, the prevalence of obesity (defined as Asian people's BMI of 25 kg/m2) has increased from the 4th survey in 2009. Obesity prevalence in women ascended from 40.7% in 2009 to 41.8%, while it increased in males from 28.4% to 32.9%[1]. Obesity is characterized by abnormal or excessive fat accumulation. Adipose tissue is an endocrine organ that expansion of these tissues results in more productions of free fatty acids, adipokines, and pro-inflammatory cytokines, subsequently with chronic low grade inflammation, insulin resistance, dys-lipidemia nonalcoholic fatty liver disease and obesity-related metabolic diseases [2]. Dietary approaches are used as a first line for obesity management through caloric restriction together with limiting carbohydrate or fat consumption (a low-carbohydrate diet or a low-fat diet). In addition, a normal or high-protein diet was required to prevent the breakdown of muscles as well as promote satiety [3-4]. An egg is a good source of complete protein that contains essential amino acids and several vitamins and minerals. The number of nutrition studies investigate components of eggs (especially in a chicken egg) and explored the bioactive compounds including proteins, derived hydrolytic peptides, lipids, vitamins and antioxidants such as zinc, selenium, vitamin E, lutein, and zeaxan-thin[5-7]. Moreover, proteins in chicken eggs, including ovalbumin, contain thiol (-SH) that regulate redox reactions and have the ability to bind metal ions; ovotransferrin binds with iron against superoxide anions similarly as SOD (superoxide dismutase), and lysozyme inhibit glycation – the process that induces ROS production[8-9].

Previous report from Korean adults aged 19 yrs and older (N=23,993) investigated association between egg consumption and metabolic syndrome (MetS). When compared to people who consumed eggs less frequently than monthly, egg consumption of 4-6 times per week and 1 time per day was significantly associated with a lower prevalence of MetS (Odds ratio (OR)=0.82; 95% Confidence interval (CI)=0.71-0.95 for 4-6 times per week, and OR=0.83; 95% CI=0.69-0.99 for 1 time per day) [10]. The good beneficial effect may due to a nutrient-dense food containing high-quality protein, vitamins, minerals and several bioactive components in egg resulting in regulations of lipid absorption, hepatic lipid metabolism and help to increase HDL-C levels[11]. The cross-sectional study data from the China Health

and Nutrition Survey (1991-2009) found that after multivariate adjustment, total egg consumption (>1 egg/d) increased risk of MetS (odds ratio [OR] 1.20, 95% CI 1.06 to 1.37; P trend = .001) compared with consumption of ≤1/2 egg/d [12]. Effects of egg consumption with Thai food in hyperlipidemic patients (N=71) revealed that individuals with one or three eggs/day consumption were significantly increases serum total cholesterol and LDL-C levels as com-pared to period of no egg consumption[13]. Based on different effects of egg consumption in various populations, further investigation for the relationship between egg consumption and MetS and explore possible mechanisms of action are needed. Proteomic in experimental study has been used to identify candidate proteins and underlying mechanisms of MetS and comorbidities for over a long time. However, the proteomics employ for uncovering the molecular mechanisms of metabolic changes related to dietary egg intake were limited. Therefore, this is the first study using the proteomic approach among Thai obese men to analyze the serum proteome profile and potential metabolic pathways in normal BMI or control and obese groups with different egg intake levels.

Materials and Methods

Participants

The current study is a part of the Electric Generating Authority of Thailand (EGAT) cohort study conducted in 2018. The study details and protocols of the EGAT cohort study have been previously described [14]. This study is a cross-sectional design among 333 males between 45 and 60 years old. The participants were divided into two main groups, including the normal BMI group (defined as BMI < 23 kg/ m^2) and the obese group (defined as BMI ≥ 25 kg/ m^2). The obese group was classified into an obese group and obese group with MetS. MetS criteria includes waist circumference over 40 inches (men), blood pressure over 130/85 mmHg, fasting triglyceride (TG) level over 150 mg/dl, fasting high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl (men) and fasting blood sugar over 100 mg/dl. Participants diagnosed with cancer, chronic disease during drug treatment, and abnormal of liver and kidney function were excluded from the study. A comprehensive set of information was obtained from the EGAT database, which was compiled using data from self-reported questionnaires, interview-administered questionnaires, and physical examinations. The weight and height were collected, then calculated into a BMI by dividing the weight in kilograms by the square of the height in

meters. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded as the means of three blood pressure assessments made using an automatic device while in a seated position, after a rest period of at least 15 minutes. Waist circumference measurements were performed by trained medical staff in a standing position midway between the lowest ribs and the superior border of the iliac crest. The semi-FFQ was used to estimate the egg intake amount and was evaluated by trained staff. For dietary assessment with egg intake, there were three categories: low (1-2 eggs per week), moderate (3-4 eggs per week), and high (≥5 eggs per week) based on previous food intake within 6 months.

Biochemical analysis

Venous blood samples were collected in the morning after an overnight fast of 12 hours, and serum samples were separated and stored at −80° C for subsequent analysis. Glycated hemoglobin (HbA1c), fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total protein, albumin, uric acid, blood urea nitrogen, creatinine, AST, and ALT were measured using automated methods (Cobas-Mira, Roche, Milan, Italy).

Proteomics Analysis

Protein concentration of all serum samples was determined by Lowry assay using BSA as a standard protein [15]. Tryptic peptide samples were resuspended in 0.1% formic acid before injection into an Ultimate3000 Nano/Capillary LC System (Thermal Scientific, UK) coupled to a hybrid quadrupole Q-TOF impact II™ (Bruker Daltonics) equipped with a nanocaptive spray ion source. Peptides were enriched on a μ-precolumn (300 μm i.d. ×5 mm with C18 PepMap 100, 5 μm, 100 A, Thermo Scientific, UK) and separated on a 75 μm I.D. ×15 cm Acclaim PepMap RSLC C18 (2 μm, 100Å, nanoViper, Thermo Scientific, UK). Solvents A and B contain 0.1% formic acid in water and 0.1% formic acid in 80% acetonitrile, respectively. A gradient of 5–55% solvent B was used to elute the peptides at a constant flow rate of 0.30 μL/min for 30 min. Electrospray ionization was carried out at 1.6 kV using the CaptiveSpray. Mass spectra (MS) and MS/MS spectra were obtained in the positive-ion mode over the range (m/z) 150–2200 (Compass 1.9 software, Bruker Daltonics). The LC-MS analysis of each sample was done in triplicate.

MaxQuant 1.6.1.12 was used to quantify the proteins in individual samples using the Andromeda search engine to correlate MS/MS spectra with the UniProt

Homo sapiens database. The following parameters were used for data processing: a maximum of two miss cleavages, a mass tolerance of 20 ppm for the main search, trypsin as the digesting enzyme, carbamidomethylation of cysteines as a fixed modification, and the oxidation of methionine and acetylation of the protein N-terminus as variable modifications. Only peptides with a minimum of seven amino acids, as well as at least one unique peptide, were required for protein identification. Only proteins with at least two peptides and at least one unique peptide were considered to be identified and used for further data analysis.

Then, all differentially expressed proteins were analyzed for their intersections among the different sample groups using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). Gene ontology annotation, including biological processes and molecular function, was performed using Panther (http://www.pantherdb.org). The identified proteins are simultaneously submitted to The Search Tool for Interacting Chemicals (STITCH) (http://stitch.embl.de) to search for understanding of cellular functions and interactions between proteins and small molecules.

Ethical Considerations

The present study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University (protocol number: COA. MURA2022/460). All the participants were informed with respect to the objective, process, benefits, and potential risks. Then, written informed consent was obtained before their participation.

Statistical Analysis

The statistical analyses were performed with the SPSS for Windows Software Package, version 23.0 (SPSS Inc., Chicago, IL, USA). Data is presented as mean±SD for continuous variables and frequency (%) for categorical variables. The differences between groups were analyzed by an independent t test for continuous variable and chi-square test for categorical variables with p significant values < 0.05.

Results

Participants were 333 men (57.66 ± 1.38 years old) and they were divided into 2 groups as the controls with mean of BMI 21.39 ±1.04 kg/m^2 and the obeses with means of BMI ~28.16 ±3.18 kg/m^2, WC~ 99.83 ±8.41 cm. There were significantly higher levels of SBP (139.05 ±16.84 vs

126.36 ±5.32 mmHg) , DBP (80.12 ±9.74 vs 72.31 ±6.23 mmHg) , FPG (105.39 ±24.36 vs 92.4 ±12.95 mg/dL) , HbA1C (6.13 ±0.91% vs 5.02 ±0.47%) , TG (160.10 ±83.59 vs 108.59 ±49.63 mg/dL) but lower of HDL level (48.83 ±10.68 vs 58.73 ±14.45 mg/dL) among the obese group compared to the control group (all p values < 0.05), as described in Table 1. Based on dietary intake data from dietary questionnaire, participants were classified into three subgroups of egg intake/week as low egg intake (1-2 eggs/wk), moderate egg intake (3-4 eggs/wk) and high egg intake (≥5 eggs/wk). We found statistical diffferences in percentages of egg intake frequencies between both groups.

Table 1. General characteristics and biochemical parameters of the control and obese groups

Characteristics and biochemical parameters	**Total (N=333)**	**Control group (N=116)**	**Obese group (N=217)**
Age (years)	57.66 ±1.38	57.56± 1.31	57.73 ±1.41
BMI (kg/m^2)	25.81 ±4.17	21.39 ±1.04	28.16 ±3.18[a]
Waist circumference (cm)	93.98 ±10.09	82.77± 4.70	99.83 ±8.41[a]
Waist-hip ratio	0.94 ±0.05	0.89 ±0.04	0.97 ±0.04[a]
SBP (mmHg)	136.72 ±16.70	126.36 ±7.32	139.05 ±16.84[a]
DBP (mmHg)	78.58 ±9.53	72.31 ±6.23	80.12 ±9.74[a]
FPG (mg/dL)	100.51 ±21.95	92.4 ±12.95	105.39 ±24.36[a]
HbA1c (%)	5.92 ±0.83	5.02 ±0.47	6.13 ±0.91[a]
TC (mg/dL)	199.43 ±41.34	194.36 ±26.25	197.69 ±42.83
TG (mg/dL)	142.15 ±77.48	108.59 ±49.63	160.10 ±83.59[a]
LDL-C (mg/dL)	130.18 ±19.63	126.51 ±10.02	131.62 ±40.15
HDL-C (mg/dL)	52.28 ±12.99	58.73 ±14.45	48.83 ±10.68[a]
Egg intake, number/wk[b]			
- Low egg intake (1-2 eggs/wk)	87 (26.1%)	35 (30.2%)	52 (24.0%)
- Moderate egg intake (3-4 eggs/wk)	95 (28.6%)	24 (20.7%)	71 (32.7%)
- High egg intake (≥5 eggs/wk)	151 (45.3%)	57 (49.1%)	94 (43.3%)

[a] Significant difference (p<0.05) from control group (analysis by independent t-test)

[b] Significant difference between control and obese groups (analysis by chi-square test, p<0.05)

Further proteomic analysis based on egg intake, there were 55, 162 and 84 uniques proteins found in the conrol group with low, moderate and high egg intake, respectively (Figure 1). For overlaping proteins, 8 protiens were found in low and moderate egg intake, 22 proteins identified in moderate and high egg intake, 8 proteins identified in low and high egg intake, and only one protein found in all three groups. In addition, uniques proteins (N=84) found only in the control group with high egg intake were analyzed for molecular functions, including ATP dependent analysis, protein binding, catalytic activity, regulation of molecular function, molecular transduce activity, structural molecule activity, transcription regulatory activity, translation regulator activity, and transporter activity.

For the obese group, Venn diagram showed 190, 313 and 154 unique proteins identified in in this group with low, moder and high egg intake, respectively (Figure 2). There were 54 proteins found in low and moderate egg intake, 35 proteins identified in moderate and high egg intake, 35 proteins identified in low and high egg intake, and 33 protein found in all three groups. Similar to analysis in the control group, additional molecular functions of total unique protein (N=154) found in the obese group with high egg intake were cytoskeletal mortor activity, and moleculr adaptor activity.

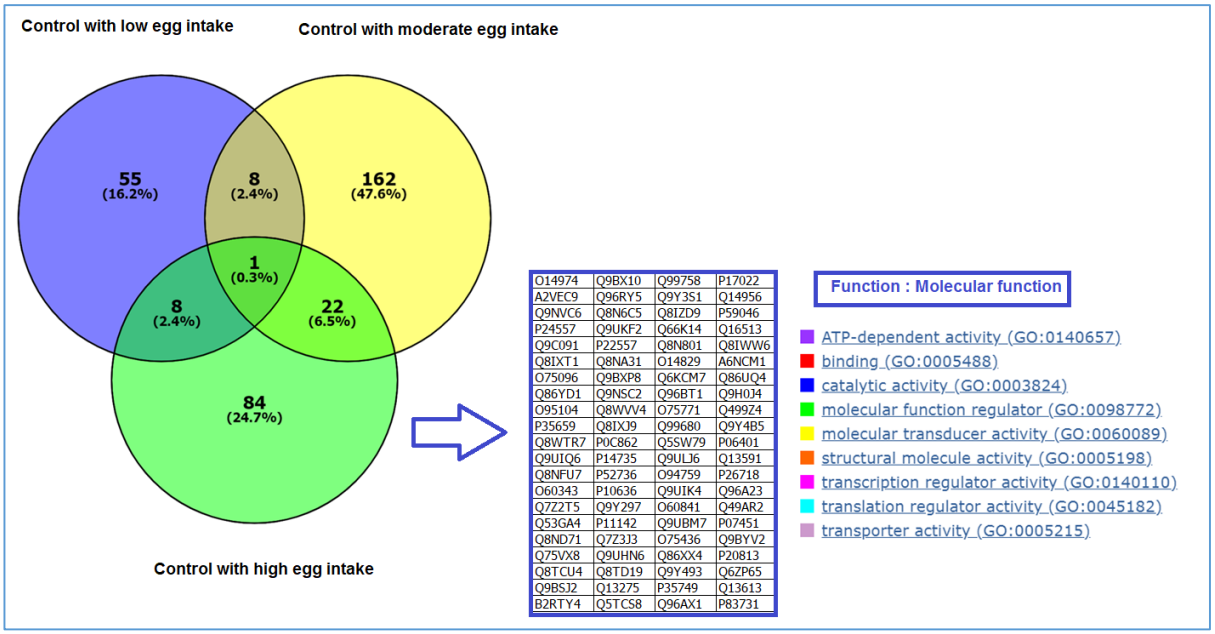
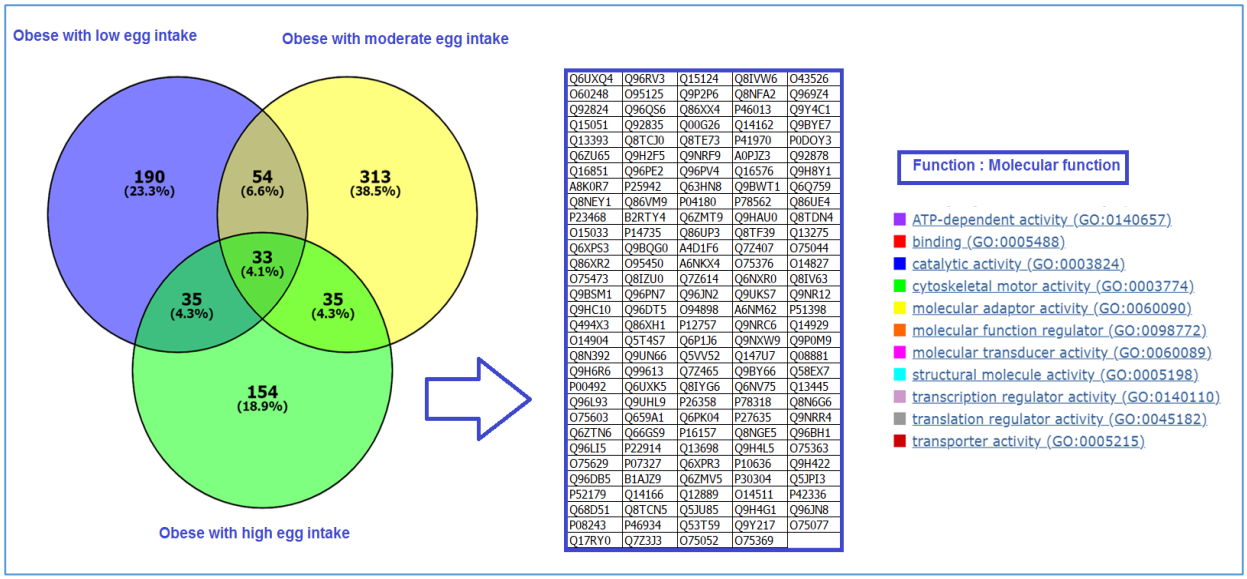


Figure 1. Venn diagram with unique and overlapping proteins in control group with different egg intake



Q6UXQ4	Q96RV3	Q15124	Q8IVW6	O43526
O60248	O95125	Q9P2P6	Q8NFA2	Q969Z4
Q92824	Q96QS6	Q86XX4	P46013	Q9Y4C1
Q15051	Q92835	Q00G26	Q14162	Q9BYE7
Q13393	Q8TCJ0	Q8TE73	P41970	P0DOY3
Q6ZU65	Q9H2F5	Q9NRF9	A0PJZ3	Q92878
Q16851	Q96PE2	Q96PV4	Q16576	Q9H8Y1
A8K0R7	P25942	Q63HN8	Q9BWT1	Q6Q759
Q8NEY1	Q86VM9	P04180	P78562	Q86UE4
P23468	B2RTY4	Q6ZMT9	Q9HAU0	Q8TDN4
O15033	P14735	Q86UP3	Q8TF39	Q13275
Q6XPS3	Q9BQG0	A4D1F6	Q7Z407	O75044
Q86XR2	O95450	A6NKX4	O75376	O14827
O75473	Q8IZU0	Q7Z614	Q6NXR0	Q8IV63
Q9BSM1	Q96PN7	Q96JN2	Q9UKS7	Q9NR12
Q9HC10	Q96DT5	O94898	A6NM62	P51398
Q494X3	Q86XH1	P12757	Q9NRC6	Q14929
O14904	Q5T4S7	Q6P1J6	Q9NXW9	Q9P0M9
Q8N392	Q9UN66	Q5VV52	Q147U7	Q08881
Q9H6R6	Q99613	Q7Z465	Q9BY66	Q58EX7
P00492	Q6UXK5	Q8IYG6	Q6NV75	Q13445
Q96L93	Q9UHL9	P26358	P78318	Q8N6G6
O75603	Q659A1	Q6PK04	P27635	Q9NRR4
Q6ZTN6	Q66GS9	P16157	Q8NGE5	Q96BH1
Q96LI5	P22914	Q13698	Q9H4L5	O75363
O75629	P07327	Q6XPR3	P10636	Q9H422
Q96DB5	B1AJZ9	Q6ZMV5	P30304	Q5JPI3
P52179	Q14166	Q12889	O14511	P42336
Q68D51	Q8TCN5	Q5JU85	Q9H4G1	Q96JN8
P08243	P46934	Q53T59	Q9Y217	O75077
Q17RY0	Q7Z3J3	O75052	O75369	

Figure 2. Venn diagram with unique and overlapping proteins in obese group with different egg intake

In this study, the proteome profiles related to obesity and egg intake were investigated to find out potential mechanisms linked between both status. Venn diagram in Figure 3 showed 55 and 238 unique proteins in the controls with low egg intake and obeses with high egg intake, respectively and 16 overlapping proteins were found in both groups. Further exploring functions based on biological process of unique proteins (N=238) in obeses with high egg intake indicated various protein functions involving biological adhesion, biological phase, biological regulation, cellular process, developmental process, growth, immune system process, localization, locomotion, metabolic process, multicellular organism process, reproduction, responding to stimuli and signaling pathways.

Differential protein expression compared between obeses with high egg intake and the controls with low egg intake were demonstrated with relative fold changes of log 2. There were 12 up-regulated proteins with range of fold changes 0.92-4.07, including Immuno-globulin kappa constant (IGKC), Frizzled-6 (FZD6), Tumor necrosis factor receptor superfamily member 18 (TNFRSF18), NF-kappa-B-activating protein (NKAP), Integrin beta-3 (ITGB3), and others (Table 2). Four down-regulated proteins were Vitamin D-binding protein (GC), Complement C1s subcomponent (C1S), Collagen alpha-6(VI) chain (COL6A6), and Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta (PIK3C2B). Integration data of protein-protein or protein-small moleculs interaction by STITCH 4.0, a search tool for

interacting chemicals was applied in our study with unique proteins (N=238) in the obese group with high egg intake, as shown in Figure 4. Data from this analysis indicated interactions of these proteins linked to biological metabolism and many signaling cascades. Most of identified proteins were found with their functions in regulation of metabolic process (GO: 0019222) in the red circle in the Figure 4 and also further analysis showed link with KEGG pathways, including mTOR, AMPK, HIF-1, and insulin signaling pathway.

O60248	Q53GA4	Q9P2P6	Q8NDI1	Q16576	P00492	Q9UHL9	O94898	P41970	Q6NV75
Q92824	P25942	Q86XX4	Q8N9Z0	Q9BWT1	Q96L93	Q9HCK1	Q15031	O60308	P78318
Q15051	Q86VM9	Q00G26	Q6PK04	Q9Y2K3	Q96RV3	O75603	P12757	A0PJZ3	P27635
Q13393	B2RTY4	Q9Y297	P16157	P78562	O95125	Q6ZTN6	A8MZA4	Q7RTS5	Q8NGE5
Q6ZU65	P14735	Q8TE73	Q13698	Q9HAU0	Q96QS6	Q96LI5	Q6P1J6	P04035	P08476
Q16851	Q9BQG0	Q9NRF9	Q6XPR3	Q8TF39	Q92835	O75629	Q5VV52	O75369	Q9H4L5
A8K0R7	O95450	Q96PV4	P04114	Q9NYQ6	O60503	Q96DB5	Q7Z465	Q96JN8	P10636
Q8NEY1	Q8IZU0	Q63HN8	Q6ZMV5	Q7Z407	Q8TCJ0	P52179	P58546	Q96II8	P30304
P23468	Q96PN7	Q9ULJ1	Q5JU85	Q9UDR5	Q9H2F5	Q68D51	Q86TV6	Q8ND71	O14511
O15033	Q96DT5	P54829	Q12889	Q8WYB5	Q96PE2	P08243	P26358	O75445	Q9H4G1
Q6XPS3	Q9ULJ6	Q9UKU0	Q53T59	Q6NXR0	Q659A1	Q9BYE7	Q7RTV3	Q5T7N2	P41146
Q86XR2	Q96S86	Q6ZMT9	Q6EEV4	O75376	Q66GS9	Q14541	A9Z1Z3	Q7Z3J3	Q8TDN4
O75473	Q86TE4	Q86UP3	O75052	Q9UKS7	P22914	P0DOY3	Q9Y2M2	O94856	Q99504
Q5VW36	Q86XH1	A4D1F6	Q8IVW6	A6NM62	P01876	Q8IZF6	Q08881	Q13576	Q14512
Q9BSM1	Q9H329	A6NKX4	O75077	Q8IYE1	Q9NWS8	Q9ULB1	P13612	Q8N9G6	Q9BYJ1
Q9HC10	Q5T4S7	Q9Y3R4	P51532	Q9NRC6	P07327	Q92878	Q5BJF6	Q96BH1	P05186
Q494X3	Q92785	Q8IX29	Q8N6L0	Q9NXW9	Q09666	P01024	Q13509	O75363	Q13275
O14904	Q9UN66	A2RUR9	P02647	Q9BY66	Q6PIL6	Q9H8Y1	A0MZ66	Q9H422	O75044
Q8N392	Q99613	Q7Z614	Q8N6C5	Q147U7	A6NGA9	P01861	Q58EX7	Q53GL7	O14827
Q96HQ2	Q6UXK5	Q96JN2	Q14162	Q9ULX5	Q8TD19	Q6Q759	Q8N3Z6	Q52LW3	Q6P589
O43299	Q9P0M9	Q13445	Q8N6G6	Q9NRR4	B1AJZ9	Q86UE4	Q13206	Q9H706	Q8IV63
Q969Z4	Q14929	Q15047	P01859	Q07617	Q14166	Q4G148	Q96AE4	Q96JG9	Q99550
O43526	P51398	P42345	P0DOX5	Q8TEA7	Q8TCN5	Q8WZ42	Q9Y3V2	Q9Y2S2	Q6ZMW3
P42336	O9NR12	O9H2M3	O9Y4C1	O95782	O96MH2	O5VT06	O9Y217		

Figure 3. Venn diagram with unique and overlapping proteins between control group with low egg intake and obese group with high egg intake

Discussion

This study assessed the contribution of different egg intakes in non-obese and obese individuals and effects of these food group on serum protein profiles. Primary clinical and biochemical parameters found in obesity were in line with previous studies. Obesity-associated hypertension closedly associated with metabolic disorders including dyslipidemia, insulin resistance and diabetes mellitus, and inflammation. Potenial mechnisms have

been proposed, including activation of the renin–angiotensin–aldosterone system, increased sympathetic nervous system activity and increased renal tubular sodium reabsorption[16]. Meta-analysis to determine potentially association between obesity and hypertension as well as T2DM found that the pooled odds ratio (OR) between obesity and hypertension was 3.82 (95%CI: 3.39 to 4.25) and OR = 1.14 (95%CI: 1.04 to 1.24) for T2DM[17]. In addition, the potential contribution of inflammation in obesity caused the pathogenesis of T2DM have been suggeted with evidences from animal and human studies. Study in adipose tissue from four different rodent models of obesity and diabetes showed that high levels of Tumor necrosis factor-alpha (TNF-α) in adipose tissue infleunced on insulin sensitivity and glucose intolerance[18]. A cross-sectional study among healthy subjects and T2DM patients revealed that serum level of TNF-α in diabetic patients correlates with the level of insulin resistance and HbA1c[19]. The up-regulation of TNF-α induced insulin resistance in adipocytes and peripheral tissues by impairing the insulin signaling through serine phosphorylation and subsequently mediated the development of T2DM[20]. Our study also found high triglyceride and low HDL-C levels in obese group compared to the non-obese group. High level of free fatty acid (FFA) in obesity may result from the enlarged adipose tissue mass, impaired FFA clearance and insulin resistance-induced FFA dyshomeostasis[21]. These consequences of dyslipidemia is crucial for our understanding of how overweight and obesity increase the risk for CVD.

In addition to body fat deposition and excess FFA metabolism contribute to metabolic disoders and CVD risks, diet with high cholesterol such as eggs has been debated for a long time. Current meta-analysis investigated the potential dose-response association of egg consumption with risk of mortality from all causes in the general population (N=2,216,720 partic-ipants)[22]. The significant finding with 2% and 4% increased risks of all-causes and cancer mortality, respectively were dose-dependent with an increased intake of 1 egg per week. These findings suggest that eggs be consumed in low to moderate amounts (≤1 egg/d) as part of a healthy diet[22]. Contrast to another meta-analysis (17 datasets from 14 studies conducted on CVD), there was an inconclusive evidence on moderate weekly egg consumption compared to no intake caused risk or protective effect on CVD[23]. Future studies with advance techologies may strengthen the evidence for more conclusive evidences of risk associated with high regular egg consumption.

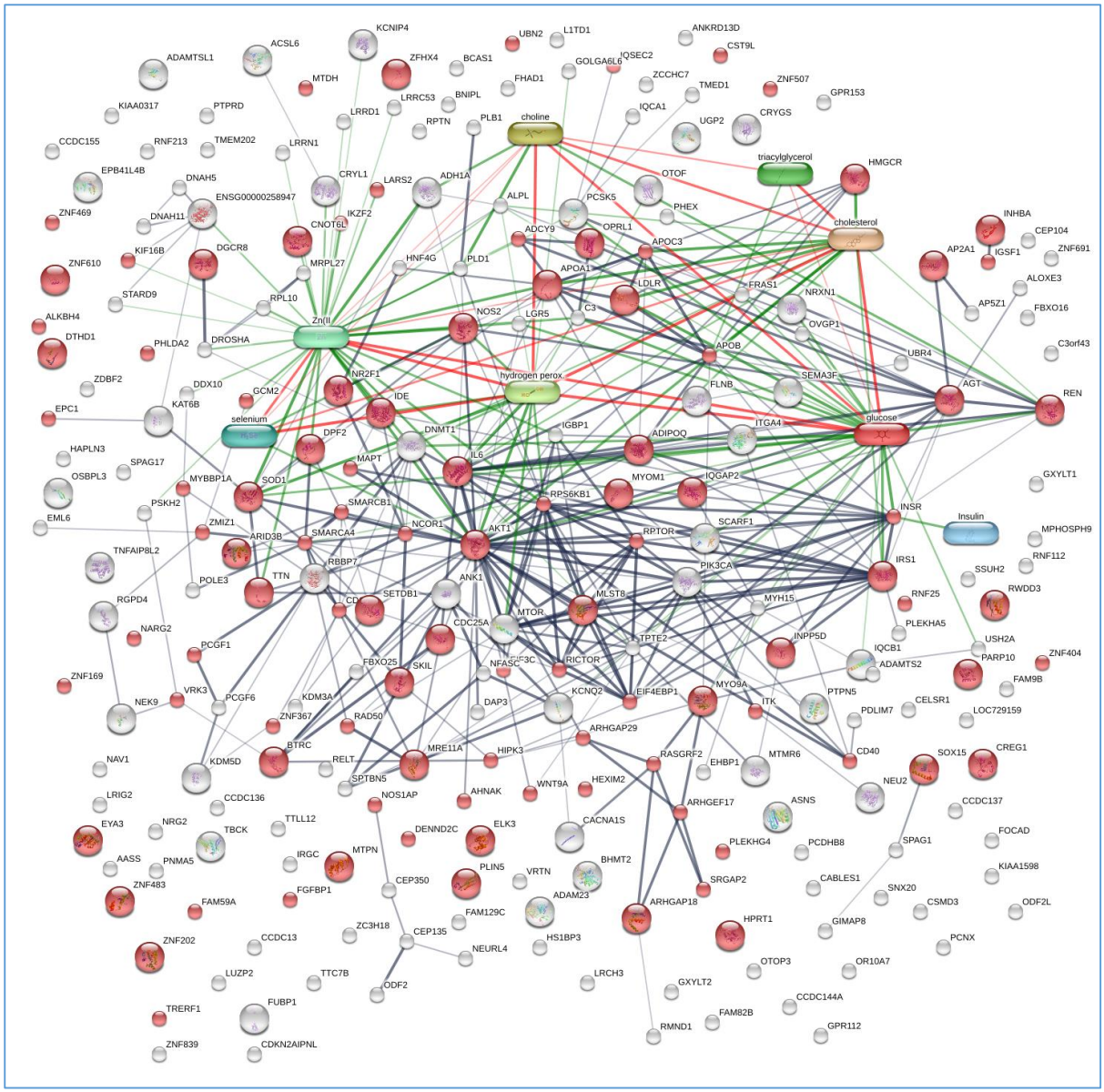


Figure 4. Protein-protein interaction of unique proteins (N=238) from obese group with high egg intake. (Stronger associations are represented by thicker lines. Protein-protein interactions are shown in grey, chemical-protein interactions in green and interactions between chemicals in red).

Our findings indicated different protein profiles with low, moderate and high egg intake bith in the conctrol and obeses group. We emphasized on the high egg intake and identified proteins with mechanism related to CVD risks such as hypertension, T2DM, and dyslipidemia. The unique proteins found in the control group with high egg intake compared to low and moderate intake (N=84) included thromboxane-A synthase (P24557) which played role in the conversion of prostaglandin H2 (PGH2) to Thromboxane A2 (TXA2), a potent inducer of blood vessel

constriction and platelet aggregation[24]; Leucyl-N-exopeptidase, LNPEP (Q9UIQ6) also known as angiotensin IV receptor (AT4R) found on endothelial and smooth muscle cells to mediate blood flow and involved the underlying mechanisms of the signaling pathways associated with the renin-angiotensin system (RAS)[25]; Methyl-cytosine dioxygenase (Q8NFU7) has been reported with an essential role of this enzyme-dependent demethylation in epigenetic regulation during pancreas specification and β-cell identity[26]; TBC1 domain family member 4 (O60343) involved in the mechanism of coupling the phosphorylated proteins to vesicle traffic appears to be dependent on linking to small GTPase of the Rab family, subsequently, increasing glucose uptake via promotes insulin-induced glucose transporter SLC2A4/ GLUT4 translocation at the plasma membrane[27].

Futhermore, we also focused on roles of unique protins in the obese group with high egg intake intake compared to low and moderate intake (N=154) and possible link to CVD risks. Various proteins were identified such as Phospholipase D1 (Q13393) involved in the signaling lipid phosphatidic acid (PA) and has been known to mediate proliferation signal in vascular smooth muscle cells (VSMCs)[28]. In addition, it played a critical role in neointima through promoting the production of ROS. Therefore, inhibition of PLD1 may be used as a therapeutic approach to suppress neointimal formation in atherosclerosis and restenosis after angioplasty[28]; UTP-glucose-1-phosphate uridylyltransferase (Q16851) which functioned in the conversion of glucose-1-phosphate into UDP-glucose, a crucial precursor for the production of glycogen. Experimental in a mouse model of neonatal diabetes showed that hyperglycaemia resulted in marked glycogen accumulation, and increased apoptosis in β-cells[29]; Apoptosis-resistant E3 ubiquitin protein ligase 1 (O15033) was initially identified as a suppressor of p53-induced apoptosis and further study showed that it associated with TNF-induced necroptosis via the ubiquitination of Metaxin 2 (a components of the protein complex in mitochondria machinery)[30]; Protein Wnt-9a (O14904) functions in the canonical Wnt/beta-catenin signaling pathway. Dysregulation of protein-members in the Wnt canonical pathway caused cardiovascular inflammatory damage, alter cellular plasticity, cause intracellular cholesterol accumulation, and lead to atherosclerosis and vascular aging[31]; Palmitoyltransferase ZDHHC6 (Q9H6R6) controls cytoskeleton-linking membrane protein 6 which they fuction in the morphology of the ER,

and ER stress is an important patho-physiological roles in obesity-induced adipose tissue dysfunction[32]. Phospha-tidylinositol 3,4,5-trisphosphate 5-phospha-tase 1 (Q92835) play role in regulation of PI3K (phosphoinositide 3-kinase) pathways mediated insulin signaling pathway, and promotes multiple cellular processes by targeting a plethora of regulatory proteins that control glucose and lipid metabolism[33].

Compared between the obese with high egg intake and the control with low egg intake found both the unique proteins and overlapping proteins (Table 2). These changes of protein expression related to high egg intake in obese individuals have been explored to propose possible mechanisms underlying MetS. There were 12 proteins with up-regulation (with relative fold changes 0.92-4.07), including Immunoglobulin Kappa Constant (P01834, IGKC) involved in adaptive immune response, B cell receptor signaling pathway, and classical pathway of complement activation. Previous study of molecular characteristics by metabolomic, proteomic and peptidomic profiling among healthy, obesity and MetS group found that proteins in immune system such as complement factor D, IGKC and complement factor C3 were altered in MetS with implication in the importance of lipid metabolism and immune response in MetS [34]. Frizzled-6 (O60353) is a receptor for Wnt proteins. In the canonical pathway, Wnt ligands bind to the frizzled receptor and LRP-5 or 6 co-receptors and subsequently downstream metabolic changes such as activating LDL uptake through Clathrin mediated endocytosis, inhibiting LDL and triglyceride (TG) synthesis and excretion via activation of TCF7L2 transcription factor, increasing glucose uptake via the insulin receptor (IR) and inhibition of vascular smooth muscle cell (VSMC) proliferation[35]. TNF receptor superfamily member 18 (Q9Y5U5) is a member of the TNF-receptor superfamily and functions in TNF signaling pathway. TNF is a classical pro-inflammatory cytokine or adipokine which mediates in metabolic dysregulation in obesity. Activation of this signaling contributes to obesity-associated metabolic disease through influence on transcriptional regulation of key metabolic genes and crosstalk with insulin signalling involving IRS1 serine kinases, such as JNKs and inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ)[36].

Table 2. Up-and down- regulated proteins compared between control group with low egg intake and obese group with high egg intake

No.	Uniprot Accession Number	Protein name	Gene name	Funxtion	Fold change (log2)
1	P01834	Immunoglobulin kappa constant	IGKC	B cell receptor signaling pathway, complement activation, classical pathway	4.07
2	O60353	Frizzled-6 (Fz-6) (hFz6)	FZD6	Canonical Wnt signaling pathway	3.18
3	Q9Y5U5	Tumor necrosis factor receptor superfamily member 18	TNFRSF18	Tumor necrosis factor receptor activity, regulation of apoptotic process	2.50
4	Q8N5F7	NF-kappa-B-activating protein	NKAP	TNF and IL-1 induced NF-kappa-B activation	2.29
5	P05106	Integrin beta-3	ITGB3	Vascular endothelial growth factor receptor 2 binding	2.29
6	P02766	Transthyretin	TTR	Thyroid hormone binding,	2.20
7	Q8NFA2	NADPH oxidase organizer 1	NOXO1	Extracellular matrix disassembly, superoxide metabolic process	2.19
8	Q8NCU7	C2 calcium-dependent domain-containing protein 4A	C2CD4A	Regualtion of inflammatory process, cell architecture and adhesion	2.05
9	A0A0U1RRG3	beta-ketoacyl-[acyl-carrier-protein] synthase I	FASN	Fatty acid biosynthesis and lipid metabolism	1.05
10	K7ELZ7	Metallophosphoesterase 1	MPPE1	Lipid metabolic process	1.05
11	Q53EP0	Fibronectin type III domain-containing protein 3B	FNDC3B	Regulator of adipogenesis	0.97
12	P04180	Phosphatidylcholine-sterol acyltransferase	LCAT	cholesterol metabolic process, very-low-density lipoprotein particle remodeling	0.92
13	P02774	Vitamin D-binding protein	GC	Vitamin D transport and storage, inflammation and macrophage activation	-0.11
14	P09871	Complement C1s subcomponent	C1S	Classical pathway of the complement system	-0.11
15	A6NMZ7	Collagen alpha-6(VI) chain	COL6A6	cell adhesion	-0.14
16	O00750	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta	PIK3C2B	EGF and PDGF signaling cascades	-0.23

Another up-regulated protein was NF-kappa-B-activating protein (Q8N5F7) involved in the TNF and IL-1 induced NF-kappa-B activation. Overweight and obesity

induces activation of inflammatory mediators in adipocytes and signaling from cytokine receptors and Toll-like receptors on the cell surface, and ER stress can activate the IKK complex and NF-κB to activate expression of pro-inflammatory cytokines and interfere insulin signaling contributing to insulin resistance and the development of T2DM[37] . Integrin beta-3 (P05106) is an integral cell surface proteins and binds to extracellular matrix (ECM) proteins and facilitates anchorage of cells to the extracellular environment, and initiate diverse intracellular signaling. In obesity, there was an abnormal of adipose tissue expandability resulting in tissue inflammation, insulin resistance, and adipocyte death. These changes caused insulin resistance and accumulation of lipid in liver and kidney, subsequently development of systemic IR and MetS[38]. Transthyretin (P02766, TTR) is previously known as prealbumin and the liver is the principal site of synthesis of TTR. Previous study in high-fat diet (HFD)-induced obese mice found that TTR inhibited AMPK activity in skeletal muscle, which leaded to insulin resistance and reduced exercise-induced insulin sensitivity in obese mice[39]. NADPH oxidase organizer 1 (Q8NFA2, NOXO1) positively regulates NOX1 and NOX3. NOX1 is expressed in pancreatic β-cells and plays an important role for ROS production during glucose-stimulated insulin secretion and modulating glycogen biosynthesis[40]. In particular, increased NOX signaling implicated the pathologies through impairment of insulin signaling, inflammation, and vascular dysfunction[40].

There were 4 proteins with down-regulation in obese with high egg intake in our study, including Vitamin D-binding protein (P02774, DBP), Complement C1s subcomponent (P09871, C1S), Collagen alpha-6(VI) chain (A6NMZ7, COL6A6) and Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta (O00750, PIK3C2B). DBP is the transporter of circulating vitamin D and its functions in fatty acid binding and actin scavenging. Abnormal level or function of DBP might influenced the amount and activity of vitamin D, and subsequently effects on impairment of insulin secretion, β-cell dysfunction and glucose metabolism[41]. Previous meta-analysis demonstrated that the DBP polymorphism was moderately associated with increased susceptibility to T2DM in Asians [42]. C1S is a serine protease and play role in the pathway of the complement system which is an important part of innate immunity, involves in the recognition of pathogens and induction of inflammation, contributing to in cellular and tissue homeostasis[43]. An interaction between adipose tissue and the innate immune

system, which becomes altered in obesity, and which impacts on metabolic function of adipocytes, resulting in insulin resistance and diabetic complication[44]. Obesity-induced adipose tissue remodeling is closely associated with systemic insulin resistance. However, the mechanistic involvement of adipocyte-derived extracellular matrix proteins under pathophysiological conditions remains unclear. COL6A6 is a subtype of type VI collagen and the C-terminal propeptide is a hormone called endotrophin, associated with the many disease-pathophysiologies such as MetS, fibrogenesis by modulating cell–cell interactions, the proliferation of mesenchymal cells and prevent cell apoptosis[45]. Previous study revealed that Type VI collagen and its cleavage product, endotrophin, participated in regulation of the adipogenic and lipolytic capacity of adipocytes via MAPK signaling pathways, which could be an important metabolic effector in obesity-related metabolic diseases[46]. PIK3C2B is a PI3-kinases which PI3K activates AKT, and AKT phosphorylates downstream substrates that are involved in the regulation of diverse cellular functions, including apoptotic, metabolism and cell cycle progression. In addition, PI3K/AKT regulates glucose metabolism through FoxO1 and GSK-3 and lipid metabolism through mTORC1 and SREBP[47].

Regarding to protein-protein interaction in only obese group with high egg intake, our findings by analysis with KEGG Pathways indicated the first report in involvement of these identified proteins with mTOR, AMPK, HIF-1, and insulin signaling pathway. A large number of proteins interacted with key mediators in metabolic pathays, including glucose, insulin, IRS1, triaclyglycerol, cholesterol, choline and SOD. Obesity and overnutrition induce a chronic hyperactivation of mTOR activity in multiple tissues. In turn, mTOR signaling dysregulation may facilitate the development of insulin resistance and T2DM and mTORC1 also stimulates glycolysis and glucose uptake through modulating the transcription factor hypoxia-inducible factor (HIF1α)[48]. For AMPK pathway, it regulates various physiological events, including glucose transport, lipid and protein synthesis, mitochondrial function, and regulation of transcriptional activators related to IR, inflammation, oxidative and ER stress, and autophagy[49]. HIF-1α is a transcription factor whose activity is induced by hypoxia, and it has been used as an indicator of adipose tissue hypoxia. Previous study found that HIF-1α activity in adipocytes was induced by obesity-associated factors such as

preadipocyte differentiation, insulin, and hypoxia, and inhibited by the corepresor of HDAC3-silencing mediator for retinoic and thyroid hormone receptors (SMRT)[50]. For insulin signaling pathway, overall results mentioned in this study showed significant association between alterations of these proteins in obeseity-induced IR resulting from insulin signaling and/or mediated with other signalings such as PI3K/AKT contributing to control glucose and lipid metabolism[33].

This study's findings revealed information of protein profiles that were both supporting and contradictory to other investigations related to identified protein functions. However, overnutrition and overweight/obesity are more common than underweight. Further research should be conducted to investigate health benefits or risks related to egg consumption and other nutrient sources in various susceptible groups such as patients with MetS, elderly and low grade inflammation. Mass spectrometry-based plasma/serum proteomics is technological advances enhancing possibilities for the incorporation of proteomics-scaled assays into clinical practice.

Conclusion

Proteomic analysis revealed differential expressed proteins within and between the control and obese groups with low, moderate and high egg intakes. Overall, this study promotes the understanding of the molecular mechanisms involving proteins and cholesterol rich diet, as egg. Changes in clinical and biochemical markers in obesity are important factors that possible links to increase metabolic disorder in susceptible population such as obesity and prediabetes. In addition, association between high eggs intake and risk of MetS in obesity should be concerned for appropriate health benefit and considerations in the development of dietary guidelines and updates. Additional prospective studies will be necessary in the future to determine these associations, potential factors and specific biomarkers for egg intake.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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