

Application of Network Pharmacology to Elucidate The Potential Mechanism of Finger Citron Against Obesity

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Methodology

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Abstract

Background: Finger citron (FS) is one of many traditional Chinese herbs that have been used to treat obesity. However, the active components and potential targets of FS in improving obesity remain unclear.

Methods The aim of this study was to elucidate the pharmacological effects and mechanisms of FS on obesity using network pharmacology analysis. We used network pharmacology to determine the active components, potential targets and mechanisms in the treatment of obesity.

Results We identified 25 active ingredients of FS such as diosmetin, hesperidin and sitosterol- α 1 with important biological effect. A total of 258 key targets were screened, containing TNF- α , NOS2, MAPK8 which were found to be enriched in 27 signaling pathways, such as apoptosis, TNF, PPAR and AKT1, and Insulin resistance signaling pathways. Moreover, molecular docking analysis showed that the main ingredients were tightly bound to the core targets, further confirming the effects of weight loss.

Conclusion: Based on network pharmacology and molecular docking analysis, our study provides insights into the potential mechanism of FS in ameliorating obesity after screening for associated key target genes and signaling pathways. These findings further provide a theoretical basis for further pharmacological research into the potential mechanism of FS in treating obesity.

1. Introduction

The rising prevalence of obesity has been described as a global pandemic recently. It seriously affects people's health because it substantially increases the risk of various chronic comorbidities, such as diabetes, cardiovascular diseases, and cancers, thus contributing to a decline in both quality and expectancy of life [1–3]. Therefore, the prevention and treatment of obesity are extremely important. Several anti-obesity drugs have been marketed in the last few years but have been successively withdrawn because of serious adverse effects. Only a few drugs are currently approved useful for the long-term management of weight loss with prominent side effects [4, 5]. Herbal remedies are recognized as an important roles in human health care as they have few adverse outcomes [6]. Therefore, there is a growing interest in herbal remedies and functional foods [4, 5]. However, low bioavailability is a concern, as it may limit or even interfere with the effectiveness of herbal remedies and remains a major challenge in the development of clinically useful functional foods and medicines. The development of appropriate extraction methods to increase bioavailability and the contents of effective compounds may offer a solution.

Traditional Chinese medicine (TCM) Finger citron (FS) is one of the homology of medicine and food (Fig. 1). FS has been broadly used in food and daily necessities with utilization prospects. Because of its health benefits, FS has attracted increasing attention. Studies revealed that active constituents of FS which were isolated from the herb could be divided into volatile oils, flavonoids, polysaccharides, amino acids, vitamins, and some essential elements [7–10]. Studies in vitro and in vivo have suggested that extracts of finger citron exert various beneficial bioactivities, such as anti-microbial [10, 11], antioxidant [12,

13], anticancer[10], anti-inflammatory[13, 14], anti-dyslipidemia[13, 15] anti-obesity[8] and regulate immune function[11] activities.

Network pharmacology is a new analytical method used to uncover the complex mechanisms of traditional Chinese medicine on diseases [16]. Although traditional Chinese herbs often exert clinical effects with multiple biological targets, their complex components impede the identification of active ingredients and pharmacological mechanisms [17, 18]. Most literatures only analyzed the pharmacological effects, but without further discussion of the active components and potential targets of finger citron in improving obesity remain unclear. Therefore, we constructed a network pharmacology-based method [19, 20] to predict biological targets of the anti-obesity mechanisms of FS. Furthermore, the anti-obesity effects of FS involving glycolipid, low-inflammation, liver function, peroxidation and TGR5 mechanisms.

2. Materials And Methods

2.2 Active chemical composition of FS

The active components and target proteins of finger citron were selected from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, <http://lsp.nwu.edu.cn/tcmsp.php>) which is a unique platform for the analysis of active ingredients of Chinese herbal medicine and their mutual effect [21]. Based on the ADME (absorption, distribution, metabolism, and excretion) model[22], We obtained the 4 key active components of finger citron when oral bioavailability (OB) $\geq 30\%$ [23] and drug likeness (DL) ≥ 0.18 [24], Meanwhile, other 21 components were replenished from relevant literature. The species of target proteins were set to "Homo sapiens" and the predicted target proteins were normalized using the official symbol of their names and submitted to the UniProt protein sequence resource (<http://www.uniprot.org/>) and the whole gene information including gene name, gene ID, gene symbol was obtained.

2.3 Related targets of obesity and prediction of potential targets of FS against obesity

Using "obesity," "fat," and "adiposis" as keywords, we conducted searches on the GeneCards (<https://www.genecards.org>), Online Mendelian Inheritance in Man (OMIM, <http://www.omim.org>), DisGeNET (<https://www.disgenet.org>), and DrugBank (<https://www.drugbank.ca>)[25] databases for obesity-associated target proteins. We combined the search results from each database to eliminate duplicate results.

2.4 Identifying obesity-related targets in FS

The obesity-related targets of finger citron were imported into the VENN (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) online tool to generate a Venn diagram. Obesity-related genes targeted by FS were determined, and a database of target genes of compounds for treating obesity was constructed by extracting the intersecting target genes. Components and disease target

genes unrelated to obesity were removed. Finally, the disease target genes of FS were obtained, and a common target database was established.

2.5 Network construction of the protein interaction

We using the STRING11.0 (<https://string-db.org>) platform to further elucidate the interactions of the common target genes. The protein species was set to "Homo sapiens." The minimum interaction score was set to "Medium Confidence (0.400)." The rest are set to default values. Enhanced interaction between targets while ensuring a positive rate, exported the protein-protein interactions (PPI) network after removing the discrete genes. Finally, the PPI network graph was visualized. We collected the targets of each ingredient and the repeating targets were removed to obtain the FS related targets. The PPI network diagram shown the common key targets of FS and obesity.

2.6 Construction of the network modules and analysis

CytoScape3.7.1 software was used to construct a component-disease, target-path network diagram to analyze network topology parameters, including degree of degree, betweenness, and closeness of active components and targets. We determined the core target and the main active ingredient of FS that exerts the anti-obesity effect according to the network topology parameters.

2.7 Pathway enrichment analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) Bioinformatics Resources 6.8 platform (<https://david.ncifcrf.gov>) was used to perform enrichment analysis on the biological functions and molecular signaling pathways of the common targets identified. After the PPI network was successfully constructed, co-targets were imported into R software and the cluster profile. Bio-conductor package was used to perform the GO and KEGG enrichment analysis, to obtain the biological processes, cellular components, molecular function and key signaling pathways. Only functional annotations with enrichment p values smaller than 0.05 were chosen for further analysis.

3. Results

3.1.1 Screening FS targets for obesity

We screened 258 potential target proteins of 25 active ingredients [10, 26] of finger citron (includes diosmetin, hesperidin and sitosterol-alpha1) by searching the TCSMP online database and supplementary documents, eliminated duplicate target proteins. We retrieved 951 obesity targets through screening as well as deleting the duplicate disease targets from the GeneCards, OMIM, DisGeNET, and DrugBank databases. The intersection of 258 herbal targets and 651 obesity targets were finally refined to 61 common therapeutic targets as shown in Table.1 and Fig. 2.

3.1.2 Constructing the PPI network of shared targets

To further explore the potential mechanisms of FS on obesity, we constructed a Protein-protein interactions (PPI) network diagram by importing the 61 common therapeutic targets into the STRING software platform, as shown in Figure 3. A total of 56 interaction proteins and 326 number edges were found between the FS components and obesity.

3.1.3 Identification of candidate targets network for FS against obesity

The interaction proteins were imported into the CytoScape3.8.0 software. We constructed a network containing 27 signaling pathways, including insulin resistance, HIF-1, TNF and AMPK signaling pathway. Table2 explains the identification numbers in figure 4. Some targets/pathways were play critical roles in obesity.

3.1.4 Enrichment analysis of candidate targets for FS against obesity

As shown in Fig. 5, the 61 common target genes were analyzed using the DAVID database and mapped for an enrichment analysis chart of the gene ontology terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways. Specifically, KEGG analysis demonstrated that non-alcoholic fatty liver disease and the G-protein coupled receptor, insulin, adipocytokine, c-AMP, and TNF signaling pathways all played a significant role in the pathogenesis of different diseases. Based on these data, we postulated that finger citron components interact in the pathogenesis of glycolipid utilization, inflammation reduction, liver function improvement, and anti-lipid peroxidation related to obesity. Therefore, we performed exploration in vitro to validate the effects of FS in the treatment of obesity.

4. Discussion

Network pharmacology has been widely used to analyze the potential biological mechanism of TCM in the treatment and prevention of various chronic diseases[22].To clearly illustrate the diversity of therapeutic targets and potential pharmacological mechanisms of FS against obesity, we used network pharmacology, a novel method to identify the active components of FS and predict its corresponding targets and mechanisms on obesity. The FS in our study which is enriched in diosmetin, hesperidin and cirsiolol has potent effect of weight loss. The enrichment and functional analyses we used further revealed that FS affected obesity-related physiology via the G-protein coupled receptor, insulin signaling pathway, adipocytokine, c-AMP signaling pathway, TNF signaling pathways and NAFLD. It is consistent with research results of Menichini, F. and Kim, K.-N.[13, 27]. Main mechanism of action involves AMPK phosphorylation. We found that FS is a potential candidate for the development of therapies for obesity and related diseases and provided new application methods for the further produce and apply of functional foods and nutraceuticals.

5. Conclusion

This study systematically expounds on the mechanism of FS in the treatment of obesity using network pharmacology. This effect might through molecular docking. In this study, 25 active ingredients, 61 main bioactive compounds and 27 main signaling pathways of FS effect on obesity are identified. In treating obesity, the pivotal mechanism includes Regulation of lipolysis in adipocytes, MAPK, Insulin resistance, etc.

6. Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request, unless there are legal or ethical reasons for not doing so.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

WZ and HXG conceived and designed the study. WY collected the data. WZ and YYJ performed the data analysis, WZ and HXG wrote the manuscript. All authors are responsible for reviewing data. All authors read and approved the final manuscript.

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Not applicable

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8. Tables

Due to technical limitations, table 1,2 is only available as a download in the Supplemental Files section.

Figures



Figure 1

Appearance of finger citron [*Citrus medica* L. var. *sarcodactylis*]. The left are ripened finger citron fruits and the right are traditional Chinese medicinal materials.

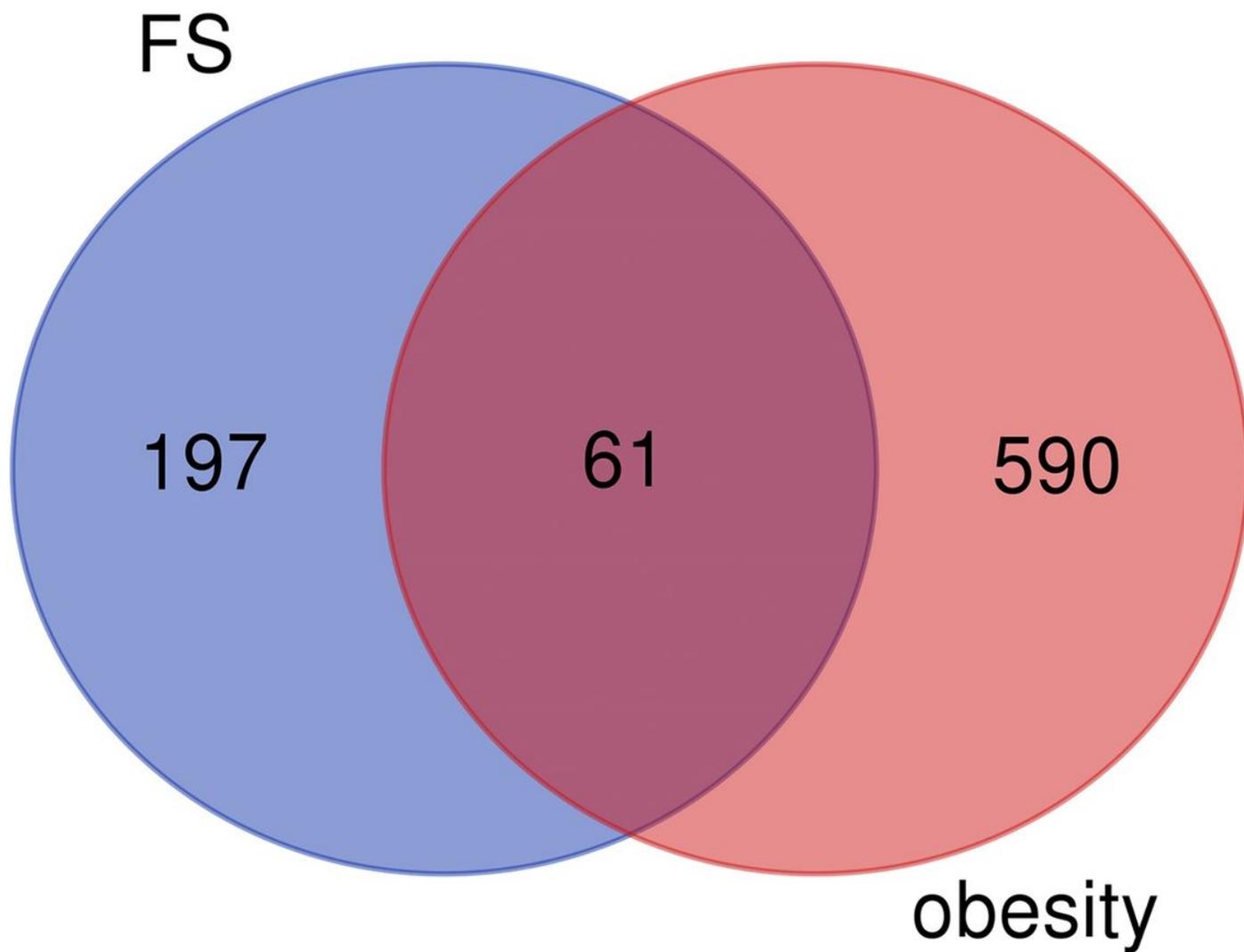


Figure 2

Venn diagram of drug-disease intersection target of FS in the treatment of obesity. The intersection of 258 herbal targets and 651 obesity targets were finally refined to 61 common therapeutic targets

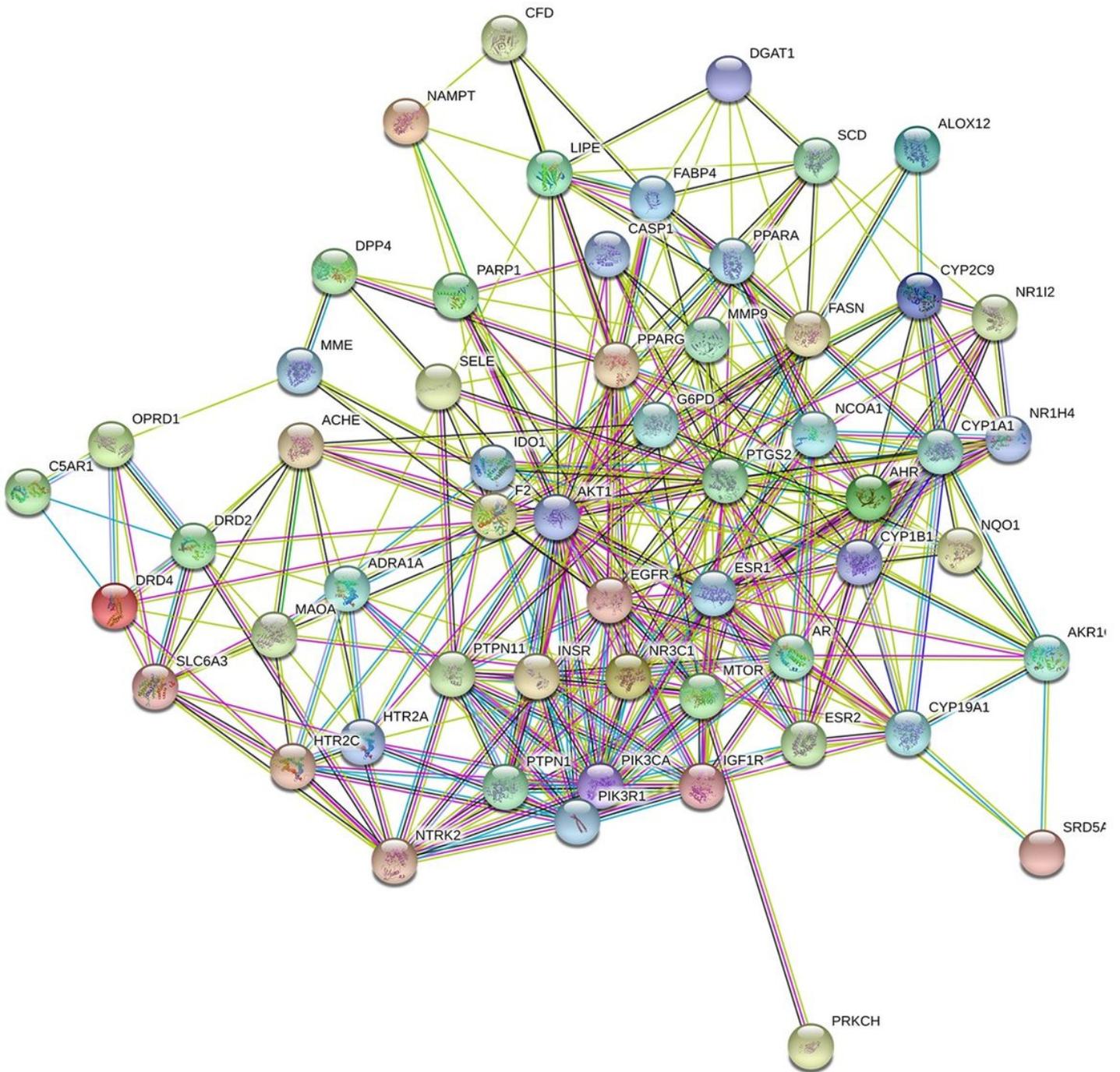


Figure 3

Protein-protein interactions PPI network diagram of finger citron (FS) and obesity. PPI network diagram contains 56 common therapeutic targets and 326 number edges. Key genes have a higher degree and are positively correlated with node size and color depth, such as AKT1, PTGS2, PIK3CA and PPARG.

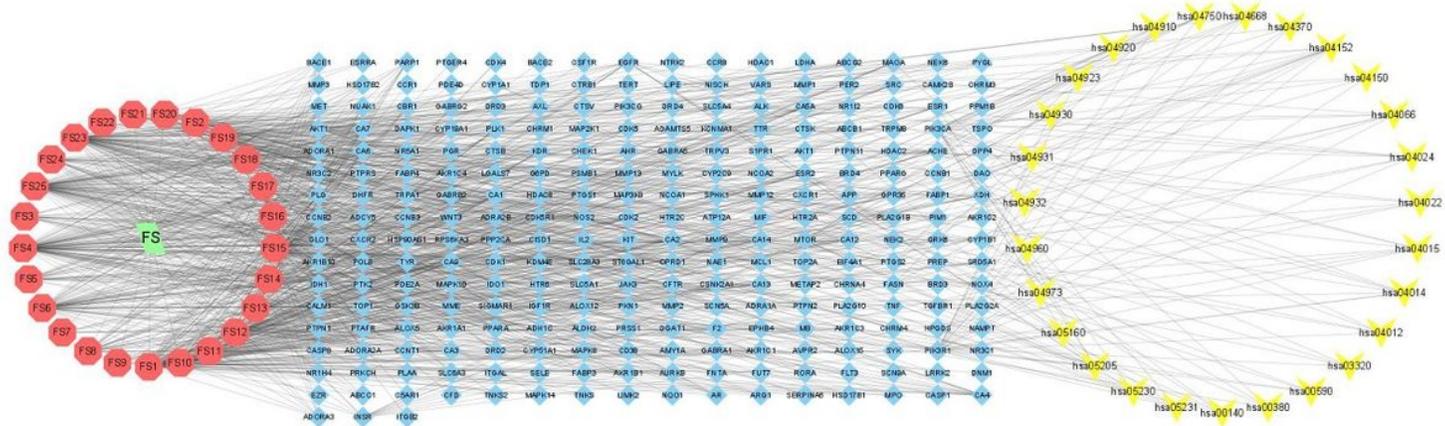


Figure 4

Construction of candidate-target network for FS against obesity. The red circles mean active components of FS—the blues represent target proteins—while the yellow show signaling pathways.

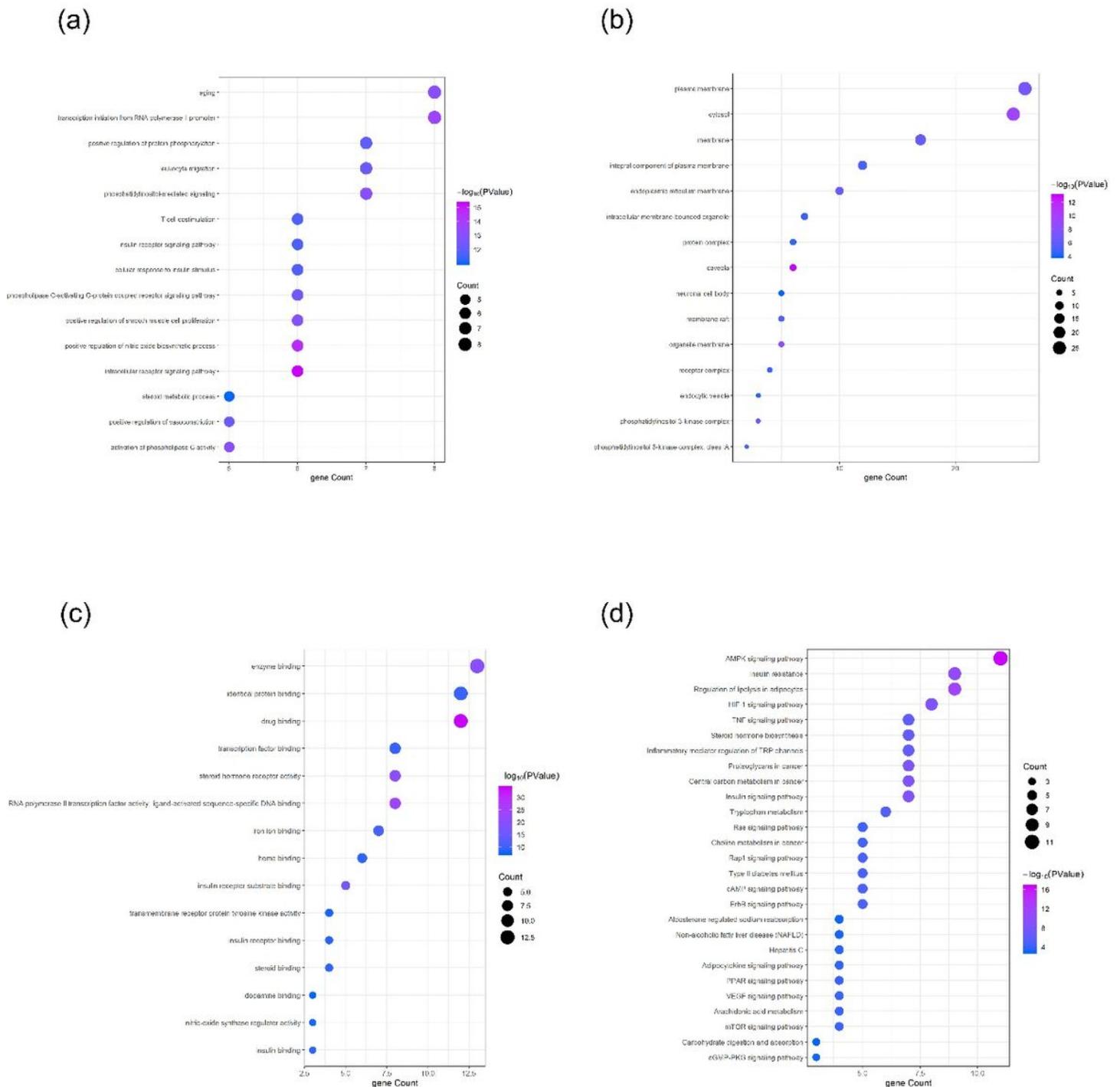


Figure 5

The GO and KEGG enrichment analysis of genes of FS in weight loss. The ontology covered three domains: biological process (a), cellular component (b), and molecular function (c), KEGG enrichment analysis chart of 27 signaling pathways (d). Size and color of dots represent the relevance of GO enrichment analysis.

Supplementary Files

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- [Table1.JPG](#)
- [Table2a.JPG](#)
- [Table2.JPG](#)