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Astragalus polysaccharide (APS) improves the immune function in diet-induced obese (DIO) mice

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Abstract: Obesity is a major health hazard, suppressing the immune system and complicating inflammatory symptoms treatment. Traditional Chinese medicine emphasizes holistic principles and syndrome-based diagnosis/therapy. Its primary focus is on enhancing overall well-being, rather than solely aiming for weight loss. *Astragalus* polysaccharide (APS), extracted from *Astragalus membranaceus*, has demonstrated promising effects in enhancing the health status of obese individuals. Therefore, this study employed DIO mouse model to explore the immunomodulatory effects of APS in obese mice. The findings revealed a dose-dependent effect of APS on obesity prevention in DIO mice. Specifically, a 4% concentration of APS significantly reduced body weight, whereas a 2% concentration tended to increase it. Furthermore, APS effectively modulated blood glucose and lipid profiles, demonstrating varying degrees of improvement in blood glucose and blood lipid-related factors. Notably, APS also facilitated the reactivation of suppressed immune function in obese mice, regulating a range of immunological variables associated with obesity and thereby maintaining homeostasis. In conclusion, the functional benefits of APS were dose-related, with a 4% concentration demonstrating promising results in obesity prevention and immune system modulation. These findings provide a potential reference for treating inflammatory conditions associated with obesity, contributing academic understanding of obesity management and immunomodulation.

Keywords: *Astragalus* polysaccharide, immunosuppression, diet-induced obese mice.

INTRODUCTION

The incidence of obesity has rapidly increased due to changes in people's food habits and improved living standards, and it has now become a severe threat to human health (Jensen *et al*., 2013; Kyle *et al*., 2016) The complications of obesity, such as hypertension, dyslipidaemia, type 2 diabetes, and fatty liver, cause great harm to human health (Kim *et al*., 2016; Lavie *et al*., 2016). According to recent research, obesity alters DNA methylation in blood leukocytes and results in extensive alterations in gene expression across several organs. These changes impair immunity and interfere with the body's metabolism and immune system (Shirakawa and Sano 2021; Farhana and Rehman 2023). Studies have demonstrated that immunological problems exist and that obese individuals have variable degrees of immune function suppression (Lin Min, 2014). Obesity can impair the function of leptin (LEP), peroxisome proliferator-activated receptor–γ (PPAR-γ) and cause the Th2-type inflammatory response to convert to the Th17-type inflammatory response (Bapat *et al*., 2022). Obesity may trigger autoimmune diseases linked to the interaction between IgM and apoptotic inhibitor of macrophages (AIM) (Arai *et al*., 2013). Moreover, numerous immune-related factors, including interleukin (IL), interferon (IFN), and tumour necrosis factor (TNF), are closely associated with obesity (Gregor and Hotamisligil 2011; Foratori-Junior *et al*., 2021; Cai and Chen 2021; Ververs *et al*., 2021). Modifying the level of immune-related factors may help improve the health of obese individuals and reverse the suppression of their immune function. Moreover, it is vital to conduct research on healthcare products that affect the immune systems of obese people.

Substantial and in-depth research on the aetiology, diagnosis and treatment of obesity in traditional Chinese medicine (TCM) has been conducted since ancient times (Huang Huili, 2018; Xu Chunhua *et al*., 2021). Compared with surgical weight loss, TCM emphasizes individualized treatment based on syndrome differentiation and customized therapy and it offers the benefits of overall regulation, multiple targets, safety and economy. *Astragalus* polysaccharide (APS) is a major active ingredient in the roots of the traditional Chinese herbal medicine *Astragalus membranaceus* (Zheng *et al*., 2020). Studies indicate that APS can ameliorate fatty acid metabolism disorders and lipotoxicity in diet-induced obese (DIO) mice and increase glucose metabolism, insulin sensitivity and the serum levels of IL-1 and IL-6 (Mao *et al*., 2009; Zhang *et al*., 2009; Cheng *et al*., 2011). APS can reduce the levels of blood lipids and plasma cholesterol in DIO mice by activating the SIRT1-PGC-1α/PPARα-FGF21 signalling pathway (Gu *et al*., 2015). In addition, APS has a bidirectional effect on blood glucose control. APS has no significant effect on blood

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glucose in normal mice, but it significantly decreases blood glucose in mice after glucose loading (Li Cunliang, 2011). Previous studies on APS have shown that it can play a good regulatory role in the inflammatory response. This study investigated the effect of APS on the suppression of immune function in obese mice, which may provide a reference for treating inflammation or improving obesity-related diseases in obese patients.

MATERIALS AND METHODS

Animal management and administration

The protocols used here were all in accordance with the guidelines issued by the Animal Care Advisory Committee of the Institute of Laboratory Animals of the Sichuan Academy of Medical Sciences (AE2020009).

Twenty-four male C57BL/6 mice were randomly divided into a blank group and a model group. Six mice in the blank group were fed a normal chow diet (NCD; D12450B; 10% kcal fat, 70% kcal carbohydrate and 20% kcal protein; Xiao Shu You Tai Biotechnology Co., Ltd., Beijing, China) without any APS treatment. Eighteen mice were used to establish a DIO mouse model and were fed a high-fat diet (HFD; D12492; 60% kcal fat, 20% kcal carbohydrate and 20% kcal protein). After one week of adaptive feeding, all 6-week-old male C57 mice were replaced with HFD and continued to be fed for 12 weeks, with a total age of 18 weeks. The criterion for successful modeling was that the DIO mice were 20% heavier than the mice on a normal chow diet (Jiang X *et al*., 2022).

DIO mice were divided into a control group (without APS), a low-dose group (2% APS: 20g APS /kg HFD) and a high-dose group (4% APS: 40g APS / kg HFD) (Li B *et al*., 2020; He Xuyun *et al*., 2016), with 6 mice in each group. After one week of adaptive feeding, each drug was administered once daily for 8 weeks (He Xuyun *et al*., 2016). All animals were maintained at the Sichuan Academy of Chinese Medicine Sciences, China, at a room temperature of 22-24°C, a relative humidity of 50%-55%, and a light-dark cycle of 12h with free access to water and food.

Experimental diets

The APS (with a purity of 80%) sample used in this study was a commercial product purchased from Shaanxi Ciyuan Biotechnology Co., Ltd. (China) and was mainly composed of glucose, mannose, arabinose, xylose, galactose, rhamnose and ribose (Koh GY *et al.*, 2009).

Measurement of biochemical indicators

Blood was collected from the heart and serum total cholesterol (TC), triglyceride (TG), blood glucose (GLUC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were detected by an automatic biochemical analyser (BS-420, Shenzhen Mindray Bio-Medical Electronics Co. Ltd. China) after centrifugation (Li L *et al.*, 2023).

Detection of immune-related indices by ELISA

Serum immunoglobulin-M (IgM), apoptosis inhibitor of macrophages (AIM) (Arai *et al*., 2013), leptin (LEP) (Zhang *et al*., 2017), interleukin-2 (IL-2), interferon-γ (IFN-γ), CD8+ T-lymphocytes, CD4+ T-lymphocytes, interleukin-4 (IL-4), interleukin-13 (IL-13) (Lord *et al*., 1998; Matarese 2000), peroxisome proliferator-activated receptor-γ (PPAR-γ) (Bapat *et al*., 2022), and tumour necrosis factor-α (TNF-α) (Noh *et al*., 2021) were measured by ELISA (Jiangsu Meibiao Biotechnology Co., Ltd., China). The relevant test procedures were performed according to the instructions of the ELISA kit.

STATISTICAL ANALYSIS

GraphPad Prism 8 was used to perform the statistical analysis. All the values are presented as the means ± SDs. Statistical analyses were performed using unpaired Student's t tests for two groups and one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) post hoc test for the groups larger than or equal to 3.

RESULTS

APS prevents body weight gain in DIO mice

Male C57 mice were fed either a normal chow diet (NCD) or a high-fat diet (HFD) with or without APS supplementation for eight weeks. Body weights were recorded every half week. The changes in the body weights of the mice during this period are shown in fig. 1. Overall, compared with the DIO group, 4% APS treatment group had a control effect on the weight gain of mice, especially at week 5 and 7. However, mice treated with 2% APS gained weight uncontrollably and were even heavier than the mice given blank doses. The trend of body weight changes indicated that although it was not noticeable in the first two weeks, there was a change by the third week, which made this an extremely intriguing product. This may be partially explained by the appetites of the mice. A high-fat diet given to mice over an extended period of time can decrease their hunger (Huili, 2018; Chunhua *et al*., 2021). And some studies have shown that low doses of APS may increase hunger in mice, causing them to eat more (Challis *et al*., 2003; le Roux and Bloom 2005). But in this study, higher doses of APS reduced body weight in mice more than it increased appetite and the overall trend was toward weight control in mice, a more common outcome in previous studies (Huang *et al*., 2017; Li *et al*., 2020). Further studies may be needed to confirm the different effects of different APS doses on body weight.

Table 1: Biochemical parameters of blood glucose and lipids in mice

Groups	Con	DIO	2% APS	4% APS
TC (mmol/L)	0.85±0.09	1.34±0.19^{d}	1.27±0.30^{A}	1.28±0.22^{A}
TG (mmol/L)	0.20±0.01	0.18±0.02^{a}	0.22±0.05^{A}	0.20±0.05
GLUC (mmol/L)	1.40±0.20	3.04±0.74^{b}	2.39±0.29	2.34±0.23
HDL-C (mmol/L)	0.73±0.08	1.11±0.16^{d}	1.07±0.25^{A}	1.09±0.18^{A}
LDL-C (mmol/L)	0.05±0.02	0.13±0.06^{a}	0.11±0.03	0.09±0.02

TC: total cholesterol, TG: triglyceride, GLUC: glucose, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol. The values are the means ± SDs (n=6). $^{a}p<0.05$, $^{b}p<0.01$, $^{d}p<0.001$, indicate significant differences compared with the Con group. $^{A}p<0.05$, significant difference compared with the DIO group.

Table 2: The levels of immune-related factors in mice

Groups	Con	DIO	2% APS	4% APS
IgM (μg/mL)	2101.33±183.82	4602.03±346.70^{e}	3772.03±333.54^{B}	2451.15±411.29^{E}
AIM (pg/mL)	211.12±62.60	624.39±47.94^{e}	491.24±32.70^{D}	311.94±60.74^{E}
LEP (ng/mL)	9.01±0.54	3.88±0.72^{e}	5.21±0.66^{B}	7.79±0.66^{E}
IL-2 (pg/mL)	177.24±36.03	455.86±29.14^{e}	380.34±40.51^{B}	243.10±43.59^{E}
IFN-γ (pg/mL)	463.06±64.43	1064.79±87.53^{e}	912.32±60.91^{B}	575.41±59.73^{E}
$CD8^{+}$ (ng/mL)	116.97±13.15	236.45±16.13^{e}	198.17±16.54^{B}	132.65±11.40^{E}
$CD4^{+}$ (ng/mL)	101.89±17.20	217.11±13.67^{e}	189.30±15.54^{B}	134.58±17.43^{E}
IL-4 (pg/mL)	42.54±2.65	17.45±2.34^{e}	20.69±4.94	35.34±3.54^{E}
IL-13 (pg/mL)	27.54±5.42	60.16±5.77^{e}	50.63±3.28^{B}	34.95±2.90^{E}
PPAR-γ (pg/mL)	693.07±70.82	1486.97±90.42^{e}	1236.04±70.03^{D}	905.81±90.93^{E}
TNF-α (pg/mL)	359.07±38.75	792.76±58.48^{e}	663.68±65.68^{B}	455.74±73.88^{E}

IgM: immunoglobulin-M, AIM: apoptosis inhibitor of macrophages, LEP: leptin, IL-2: interleukin-2, IFN-γ: interferon-γ, $CD8^{+}$: $CD8^{+}$ T-lymphocytes, $CD4^{+}$: $CD4^{+}$ T-lymphocytes, IL-4: interleukin-4, IL-13: interleukin-13, PPAR: peroxisome proliferator-activated receptor, TNF: tumour necrosis factor. The values are the means ± SDs (n=6). $^{a}p<0.05$, $^{b}p<0.01$, $^{d}p<0.001$, $^{e}p<0.0001$ indicate significant differences compared with the Con group. $^{A}p<0.05$, $^{B}p<0.01$, $^{D}p<0.001$, and $^{E}p<0.0001$ indicate significant differences compared with the DIO group.

Effect of APS on blood glucose and lipids in DIO mice

Compared with those in the control group (Con), the levels of total cholesterol (TC), blood glucose (GLUC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) in the DIO group were significantly greater, and triglyceride (TG) level was significantly decreased, as shown in table 1. Compared with mice in the DIO group, the content of TC, GLUC, HDL-C, LDL-C in 2% APS treatment group showed a downward trend, and there were significant differences in TC and HDL-C. However, TG content increased significantly, which could be associated with the trend of weight gain in the 2% APS treatment group. The specific reasons still need to be further analyzed. In 4% APS treatment group, compared with mice in DIO group, TC, GLUC, HDL-C and LDL-C all showed a decreasing trend, while TG showed an increasing trend, and only TC and HDL-C showed significant differences. Both the 2% APS treatment group and the 4% APS treatment group exhibited comparable effects, effectively normalizing the biochemical parameters associated with blood glucose and lipids in DIO mice to a certain degree. The only difference was the change of TG content and the effect of 2% APS treatment group was more significant. Overall, APS treatment showed a tendency to reduce blood glucose and lipids in obese mice, mainly by reducing the levels of TC and HDL-C.

Effect of APS on immune-related factors in DIO mice

In addition to causing complications such as hypertension, hypercholesterolemia, certain cancers, and other issues, obesity also compromises the immune system. The change trend of immune-related factors in mice are shown in table 2. Compared with normal control mice (Con), the

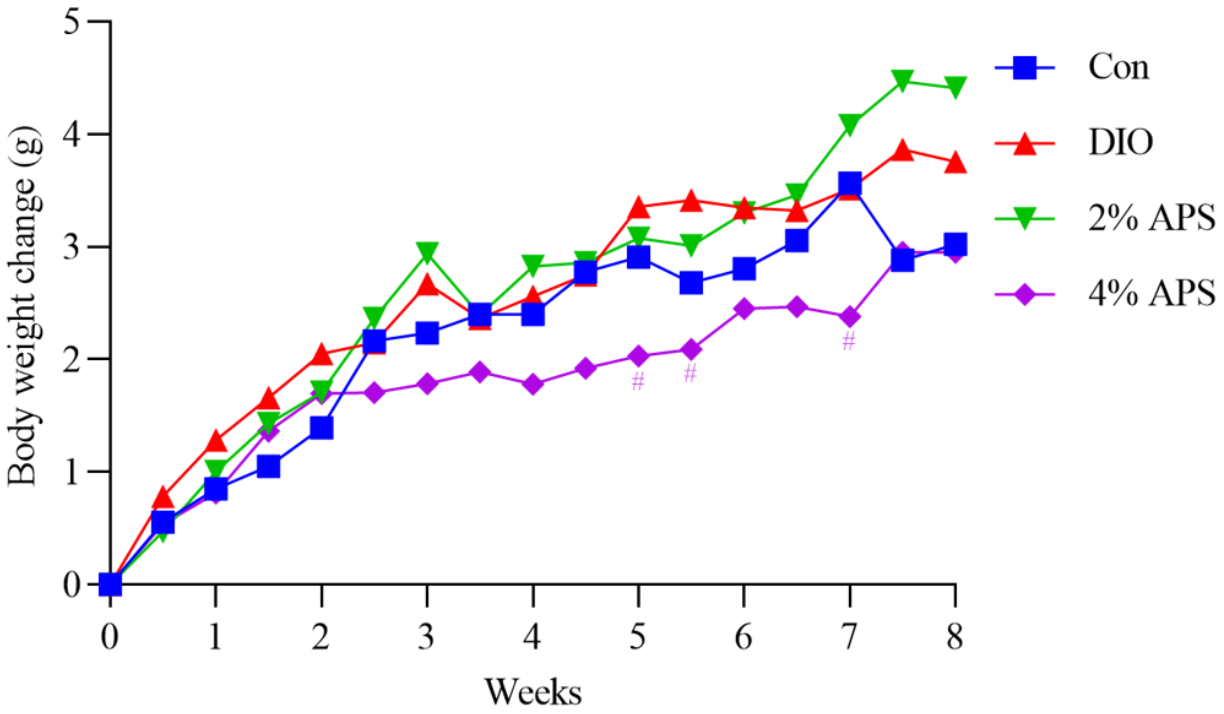

Fig. 1: Effect of APS on body weight changes in mice. One group of normal mice (Con) and three groups of DIO mice with or without APS supplementation (2% APS, 4% APS and DIO) were examined. Body weights were recorded every half week. The recorded values are the means ± SDs (n=6). $^{\#}p<0.05$, significant difference compared with the DIO group.

levels of IgM, AIM, IL-2, IFN-γ, $CD8^+$, CD4+, IL-13, PPAR-γ and TNF-α in DIO mice were significantly increased. LEP and IL-4, however, were considerably reduced. The immune-related factors of mice treated with APS had different normalization trends, and the effect was more obvious at 4% dose of APS. Compared with mice in the DIO group, the levels of IgM, AIM, IL-2, IFN-γ, $CD8^+$, $CD4^+$, IL-13, PPAR-γ and TNF-α in the 2% APS group were significantly decreased, while the levels of LEP were significantly increased and IL-4 showed an increasing trend, but there was no statistical significance. The data change in 4% APS treatment group was similar to that in 2% APS treatment group. Similarly, the levels of IgM, AIM, IL-2, IFN-γ, $CD8^+$, $CD4^+$, IL-13, PPAR-γ and TNF-α were significantly decreased, while the level of LEP was significantly increased. However, the therapeutic effect of the 4% APS treatment group was better than that of the 2% APS treatment group, especially the effect on IL-4 was more obvious. In the 4% APS treatment group, the level of IL-4 was significantly increased compared with that of the DIO group, which was not observed in the 2% APS treatment group.

Changes in AIM and IgM are correlated, and together they play a synergistic role in autoimmune diseases in obese patients (Arai *et al*., 2013). PPAR-γ plays an important role in immune function conversion from Th2 type to Th17 type in obese patients, with knock-on effects on $CD4^+$, IL-4 and IL-13 levels (Bapat *et al*., 2022). Obese patients exhibited reduced LEP levels, with impacts on the multi-effector levels of IL-2, IFN-γ, $CD8^+$, IL-4, IL-13 and $CD4^+$ (Lord GM *et al*., 1998; Matarese G, 2000). Changes in levels of TNF-α, $CD8^+$, and $CD4^+$ are associated with a shift from M1-type inflammation to M2-type inflammation in obese patients (Noh JW *et al*., 2021). The above immune pathways are complex and have overlapping parts, so further studies are still needed to find out the specific mechanisms of APS on each immune pathway. Certainly, it is evident from the overall trend in table 2 that after APS treatment, the inflammatory state and immune function of DIO mice were improved and the therapeutic effect of 4% APS was more prominent.

DISCUSSION

APS is a macromolecular polysaccharide extracted from the dried root of *A. membranaceus* (Zheng *et al*., 2020). Understanding how APS affects changes in blood lipids, blood glucose and body weight in DIO mice can aid in the therapeutic application of APS. In previous studies, APS was shown to regulate stress-induced weight gain (Huang *et al*., 2017) and food-induced obesity (Mao *et al*., 2009) and to modify metabolic function in obese mice (Li *et al*., 2020). In addition to mice, dogs fed an APS diet do not gain weight and maintain good health (Luo *et al*., 2023). In this study, the data on body weight changes are of great interest, as we compared the effects of various APS doses.

The weight gain curve of the obese mice was considerably lower than that of the DIO control group, indicating that high-dose APS may in fact regulate their weight gain. Conversely, low-dose APS had the opposite effect, making the mice gain weight. We speculate that this may be related to appetite in mice, since studies have shown that a prolonged high-fat diet reduces hunger in mice (Challis *et al*., 2003; le Roux and Bloom 2005), and low-dose APS may slightly increase appetite. Therefore, it is concluded that different doses of APS have different effects on weight gain in obese mice; of course, more research is needed to confirm this notion.

However, weight gain not only leads to obesity, but also a series of complications, such as hyperglycaemia and hyperlipidaemia. These are the main risk factors for diabetes, atherosclerosis, cardiovascular and cerebrovascular diseases, fatty liver disease and stroke (Cheng *et al*., 2011). For this reason, it is critical to treat and avoid the development of hyperglycaemia and hyperlipidaemia in obese individuals. Currently available medications that effectively treat hyperglycaemia and hyperlipidaemia have long-term side effects, including gastrointestinal dysfunction and damage to the liver and kidneys (Mao *et al*., 2009). Therefore, finding effective medications with minimal toxicity and adverse effects is essential. Because it is derived from nature, this traditional Chinese medicine has fewer harmful side effects than synthetic medications (Zheng *et al*., 2020). *A. membranaceus* is a focal point for research (Wang *et al*., 2009; Alcalá *et al*., 2010). In this study, both the 2% APS treatment and the 4% APS treatment exhibited comparable effects, effectively normalizing the biochemical parameters associated with blood glucose and lipids in DIO mice to a certain degree. The only difference was the change of TG content and the effect of 2% APS was more significant. This outcome is comparable to our earlier findings in dogs (Han Dong *et al*., 2022). In addition, APS can improve insulin resistance and regulate lipid metabolism in obese mice (Huang *et al*., 2017; Ma *et al*., 2023). In general, APS is anticipated to be an effective component for the control of blood glucose and blood lipids in obese individuals.

Treatment of inflammatory symptoms in obese people is hampered by their altered immune system function and complicated immunological state. On the one hand, obesity may cause autoimmune diseases, which are related to the combination of AIM and IgM (Arai *et al*., 2013). Fatty acids stimulate immune cells in obese mice fed a high-fat diet, producing a large amount of IgM that, when combined with AIM, may cause diabetes, atherosclerosis, and other diseases (Arai *et al*., 2013). The results of this study showed that the increase in AIM and IgM caused by obesity was significantly decreased in the APS treatment group, especially after APS treatment at a 4% dose and the levels of IgM were not significantly different from those in the healthy group. These findings suggested that APS may be able to treat autoimmune disorders caused by elevated IgM and AIM levels. On the other hand, obesity can cause a shift in immune function from Th2 to Th17, leading to immunological dysfunction. This can impact how obese individuals are treated for inflammation, a condition in which PPAR-γ plays a major role (Bapat *et al*., 2022). Consequently, we examined the levels of Th2 inflammatory components and PPAR-γ. Our findings revealed that APS treatment improved these parameters and that the levels of PPAR-γ, $CD4^+$, IL-4 and IL-13 tended to return to normal. It can be seen that APS can control the inflammatory response of obese mice from Th2-type to Th17-type to a certain extent, which is helpful for reducing immune function suppression and improving health.

More critically, APS may help to prevent obese mice from gaining more weight by regulating LEP. LEP can suppress appetite, reduce energy intake, increase energy expenditure and inhibit fat synthesis (Widiker *et al*., 2010; Cordero *et al*., 2011; Zhang *et al*., 2017). As shown in fig. 3, the DIO group mice had greatly reduced LEP levels, which led to further increases in obesity parameters in the already obese mice. In contrast, LEP levels were increased in the APS groups, which could better maintain the health of obese mice. Additionally, reduced LEP level induces a transition in the inflammatory response from Th2-type to Th1-type (Lord *et al*., 1998; Matarese 2000), primarily reflected in variations in IL-2, INF-γ, $CD8^+$, IL-4, IL-13 and $CD4^+$ levels. However, this study showed that the above indices tended to be normal in the APS treatment group, especially the level of $CD8^+$, which was not significantly different from that in the healthy group.

The effects of obesity on immune function are complex. An increase in the number of M1 pro-inflammatory macrophages can promote the proliferation of preadipocytes and further increase the number of posterior adipocytes (Weisberg *et al*., 2006). The number of M1 macrophages is positively correlated with obesity (Keophiphath *et al*., 2009), so promoting the transition of M1 pro-inflammatory macrophages to M2 anti-inflammatory macrophages is conducive to weight loss and keeping health (Noh *et al*., 2021). This can be partially confirmed by the changes in TNF-α, $CD8^+$, and $CD4^+$ levels shown in fig. 3. In general, APS can play a role in regulating the inflammatory state, maintaining immune homeostasis, reducing the incidence of diseases caused by obesity, and effectively improving the quality of life of obese patients.

CONCLUSION

Different concentrations of APS have different effects on the body weight in DIO mice. 4% APS can control the weight gain of mice, but 2% APS can make mice gain more weight. The specific mechanism of weight change was not explored in this study, and in future studies, researchers can pay more attention to the dual effect of

APS dose, which may play an important role in the field of drugs.

APS treatment showed a tendency to reduce blood glucose and lipids in obese mice, mainly by reducing the levels of TC and HDL-C. APS suppresses IgM and AIM elevations in DIO mice due to obesity, potentially managing autoimmune diseases triggered by their combination. It also decreases PPAR-γ levels, mitigating the shift of inflammatory response from Th2-type to Th17-type. APS enhances LEP levels, reducing energy intake and mitigating a transition from Th2-type to Th1-type inflammatory response. Furthermore, APS inhibits M1-related immune factors, preventing DIO mice from gaining excessive weight.

APS can enhance the immune function and improve the complex inflammatory state of DIO mice. However, in this study, the immune pathway was not explored in detail. In the future, scholars can specifically explore the immune mechanism effects of APS through *in vitro* experiments.

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