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# Triglyceride-lowering effect of rice protein due to the regulation of fatty acid uptake and transport of triglyceride in rats fed normal/oil-enriched diets

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ABSTRACT

Dysregulation of fatty acid uptake and triglyceride transport can induce excess triglyceride accumulation. We propose that rice protein might suppress fatty acid uptake and/or triglyceride transport. To elucidate potential mechanisms, expressions of cluster determinant 36 (CD36), microsomal triglyceride transfer protein (MTP), fatty acid transport protein-2 (FATP-2), fatty acid-binding protein-1 (FABP-1), lipoprotein lipase (LPL) and Niemann-Pick C1-like 1 (NPC1L1) were investigated in growing and adult male Wistar rats fed with caseins and rice proteins under normal and oil-enriched dietary conditions. After two weeks of feeding, rice protein depressed the gene and protein expressions of CD36, MTP, FATP-2, FABP-1 and NPC1L1, whereas rice protein up-regulated those of LPL. As a result, rice protein significantly reduced the concentrations of triglyceride and fatty acid in the plasma and liver ($P < 0.05$) as well as the deposit of perirenal, epididymal and mesenteric fat ($P < 0.05$). The present study demonstrates an association between the depression of fatty acid uptake and triglyceride transport and the triglyceride-lowering effect of rice protein.

## 1. Introduction

Fatty acid (FA) uptake and triglyceride (TG) transport are the important processes in managing TG concentration (Ademović et al., 2023; Berriozabalgoitia et al., 2022). Dysregulation of FA uptake and TG transport can induce excessive TG accumulation, which is associated with various diseases, such as hypertriglyceridemia (Chait, 2022). Hypertriglyceridemia is a major risk factor for the development of cardiovascular disease (Ademović et al., 2023; Chait, 2022; Rygiel, 2018; Santos-Baez & Ginsberg, 2020). Therefore, effective regulation of FA uptake and TG transport is extremely important to reduce TG concentration so as to prevent the occurrence of hypertriglyceridemia.

Some transport proteins facilitate FA uptake, including FA transport protein (FATP), FA-binding protein (FABP) and cluster determinant 36 (CD36) (Dourlen et al., 2015; Makowski & Hotamisligil, 2005; Pepino et al., 2014). FATP and FABP are key actors in FA uptake and transport. As FA translocase, CD36 mediates the uptake of long-chain FA (Abumrad et al., 1999; Glatza et al., 2022). Thus, to prevent excessive TG accumulation leading to hypertriglyceridemia, the molecular mechanisms governing FA uptake should be taken into account.

In addition to FA uptake, CD36, FATP and FABP also serve as facilitators for promoting TG transport (Dourlen et al., 2015; Febbraio et al., 2001; Makowski & Hotamisligil, 2005; Pepino et al., 2014). It has been shown that hepatic very-low-density lipoprotein (VLDL)-TG secretion can by regulated by CD36, FATP and FABP (Abumrad et al., 1999). Moreover, as a major transport protein, microsomal TG transfer protein (MTP) participates in TG transport (Hussain & Bakillah, 2008; Hussaina et al., 2008). It is evident that MTP exerts triacylglycerol transfer activity and VLDL secretion to regulate the TG concentration (Tietge et al., 1999; White et al., 1998). Thus, to comprehensively explore the TG-lowering mechanism, the expressions of FATP, FABP, CD36 and MTP involved in the regulation of TG transport should also be emphasised.

Rice is a major plant source of energy and protein for human nutrition and increased intake of rice protein might reduce the risk of some metabolic diseases (Yang et al., 2007; Yang, Chen, Lv, et al., 2012). Our previous studies reported that RP could reduce TG concentrations (Yang et al., 2007; Yang, Chen, Lv, et al., 2012). Moreover, RP could up-regulate lipolysis and down-regulate lipogenesis to inhibit TG accumulation in growing rats (Yang, Chen, Lv, et al., 2012). Furthermore, the depression of hepatic total TG and VLDL-TG output by RP was observed

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in the perfusion study, suggesting that RP could suppress TG secretion to reduce TG concentration (Yang & Kadowaki, 2009). However, to date, the precise molecular mechanism by which RP regulates FA uptake and TG transport involved in TG-lowering action is not fully established.

TG concentration can be affected by dietary oil. The consumption of high-level dietary oil increases FA uptake, thereby excess amounts of TG accumulation in the plasma (Luna-Castillo et al., 2022; Packard et al., 2020; Sahin et al., 2022). Thus, normal and oil-enriched diets were utilised in this study. Thus, this study aimed to determine whether there is an association between TG-lowering action and modulations of FA uptake and TG transport associated with RP. Further, if there is a difference in regulatory effects of RP on CD36, MTP, FATP and FABP driving FA uptake and TG transport between normal and oil-enriched diets.

## 2. Materials and methods

### 2.1. Animal experiment

RP and casein (CAS) were used as dietary protein sources. RP was extracted from *Oryza sativa* L. cv. *Longjing* 20 (Rice Research Institute of Heilongjiang Academy of Agricultural Sciences, Jiamusi, China) using the alkaline method (Wang et al., 2016; Yang, Chen, Zhang, et al., 2012). CAS, as a control, was purchased from Gansu Hualing Industrial Group (Gansu, China). Using RP and CAS as dietary protein sources, animal diets were prepared for growing and adult rats in this study according to the formula recommended by the American Institute of Nutrition (AIN-93) (Reeves et al., 1993).

Two animal experiments were conducted. In Experiment 1, all animals were fed experimental diets with normal dietary oil level (according to the standards of AIN-93); 7-week-old (growing) male Wistar rats (body weight 210–230 g, purchased from the Vital River Laboratory Animal Technology Co. Ltd., Beijing, China) were fed *ad libitum* CAS (CAS-G) and RP (RP-G) with a dietary protein level of 20 % (as crude protein [CP] according to AIN-93G) for 2 weeks, while 20-week-old (adult) male Wistar rats (body weight 380–400 g, purchased from the Vital River Laboratory Animal Technology Co. Ltd.) were fed *ad libitum* 14 % (as CP, according to AIN-93 M) dietary proteins of CAS (CAS-A) and RP (RP-A) for 2 weeks. In Experiment 2, all animals were fed oil-enriched diets, in which extra 10 % soybean oil was added to increase dietary oil level; 7-week-old (growing) male Wistar rats (body weight 210–230 g, purchased from the Vital River Laboratory Animal Technology Co. Ltd., Beijing, China) and 20-week-old (adult) male Wistar rats (body weight 380–400 g, purchased from the Vital River Laboratory Animal Technology Co. Ltd.) were fed *ad libitum* CAS (CAS-G, CP 20 %; CAS-A, CP14 %) and RP (RP-G, CP 20 %; RP-A, CP 14 %), respectively, for 2 weeks. Altogether, four groups (CAS-G, RP-G, CAS-A and RP-A) were respectively arranged in Experiment 1 and 2. Each group consisted of six animals with similar body weight.

The experiments were approved and conducted in conformity with the Guidelines of the Committee for the Experimental Animals of the Harbin Institute of Technology (IACUC-2020009, Harbin, China). The growing and adult rats were individually housed in metabolic cages with a maintained temperature of 22 °C ± 2 °C under a 12-h light/dark cycle (07:00–19:00 for light). All rats were allowed free access to commercial pellets (Vital River Laboratories, Beijing, China) for 3 days. After acclimatisation, the rats were assigned into experimental groups with similar body weight and fed experimental diets for 2 weeks. During the feeding period, food consumption and body weight were recorded daily in the morning before replenishing the diet.

All procedures involving the rats were performed in strict accordance with the ethical standards for animals. Sample collection followed the protocol established in previous studies (Yang et al., 2012a). At the end of the 2-week feeding period, the rats were fasted for 12 h and their final body weights were recorded. Anaesthesia was induced *via* intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). Criteria for confirmation of successful anaesthesia were absence of blink and tail reflexes, and pain response. Blood was withdrawn from the abdominal vein into a heparinised syringe, cooled on ice, and separated by centrifugation (12,000 ×*g*, 5 min). Plasma was frozen at −20 °C for analysis. Tissues, including the liver and fat, were excised, rinsed in saline, weighed after blotting on a filter paper, frozen in liquid nitrogen, and stored at −80 °C.

### 2.2. Measurement of triglyceride and free fatty acid contents

The contents of TG and free FA in the plasma and liver were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Animal samples were analysed in triplicate and then averaged ($n = 6$).

### 2.3. Quantitative real-time PCR

Total RNA was extracted from the individual livers of growing and adult rats after 2 weeks of feeding using the TRIzol Reagent Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNA was reverse-transcribed from 1 μg of total RNA using the PrimeScript™ First Strand cDNA Synthesis Kit (Takara Bio. Inc., Otsu, Shiga, Japan). For quantitative real-time PCR, cDNAs were analysed using the ABI 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) and SYBR Green (Takara Bio. Inc., Otsu, Shiga, Japan); this step was repeated three times of single extract to ensure technical consistency. The results were normalised to the level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The primer sequences used are presented in Table 1. In this study, the relative mRNA level in groups CAS-G and CAS-A was respectively set to 1.00.

### 2.4. Western blot analysis

As described in our previous studies, hepatic proteins were extracted and used for western blot analysis (Liang et al., 2019). The proteins with the 2 × SDS sample loading buffer were denatured by boiling for 5 min and separated *via* 10 % SDS-PAGE. The gel was transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). After blocking with 5 % fat-free milk in TBS at room temperature for 1 h, the membranes were incubated overnight at 4 °C with primary antibodies for CD36 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), MTP (Santa Cruz Biotechnology), FATP-2 (Proteintech, Wuhan, China), FABP-1 (Proteintech), lipoprotein lipase (LPL, Santa Cruz Biotechnology), Niemann-Pick C1-like 1 (NPC1L1, Santa Cruz Biotechnology), β-actin (Cell Signaling, Danvers, MA, USA) and GAPDH (Proteintech). Subsequently, the membranes were washed with TBST (TBS with 0.1 % Tween-20) three times and incubated at room temperature for 2 h with a second antibody (Santa Cruz Biotechnology). The protein bands were visualised using an ECL reagent (Beyotime). The amount of protein was quantified using the QuantityOne software (Bio-Rad, Hercules, CA, USA). Each assay was repeated in triplicate to ensure technical consistency. In this study, the relative protein expression in groups CAS-G and CAS-A was respectively set to 1.00.

### 2.5. Statistical analysis

Data are expressed as mean ± SEM. Differences between the groups were examined for statistical significance *via* one-way analysis of variance, followed by the least significant difference test. $P < 0.05$ was considered to indicate statistical significance.

## 3. Results

### 3.1. Body weight, food intake and liver weight

With the growing and adult rats having similar initial body weight,

**Table 1**
Sequences of primers for quantitative real-time PCR.

| Gene | Forward | Reverse |
|---|---|---|
| GAPDH | ACAGCAACAGGGTGGTGGAC | TTTGAGGGTGCAGCGAACTT |
| CD36 | TGCTGCACGAGGAGGAGAATGG | CACAGCCAGGACAGCACCAATAAC |
| MTP | TTCTGCCTACACTGGCTACG | TCTCCTCTCCCTCATCTGGA |
| FATP-2 | TGTGGCTCTGGCTGGGACTG | GTAGCAGAGACTTGGCACGGATG |
| FABP-1 | CCAGAAAGGGAAGGACATCAAGGG | TGGTCTCCAGTTCGCACTCCTC |
| LPL | CTGGTGAAGTGCTCGCACGAG | CTGCTTCTCTTGGCTCTGACCTTG |
| NPC1L1 | CCTGTTGTGATGCTGCTGTT | CCTCGCTGGAGAAGTTGAAG |

as shown in Fig. 1, although food intake was not significantly different between the CAS and RP groups ($P > 0.05$, Fig. 1A), the body weight gains were respectively reduced by RP-G (normal diets, −30.16 %; oil-enriched diets, −29.25 %) and RP-A (normal diets, −31.93 %; oil-enriched diets, −27.83 %) as compared with CAS-G and CAS-A, respectively ($P < 0.05$, Fig. 1B). The results suggest that RP could effectively control body weight gain under normal and oil-enriched dietary conditions, independent of the influence of the dietary oil level.

After 2 weeks of feeding, the liver weights were significantly reduced by RP-G and RP-A as compared with CAS-G and CAS-A, respectively, under normal and oil-enriched dietary conditions ($P < 0.05$, Fig. 1C), suggesting that the target of these dietary proteins is the liver.

### 3.2. Fat deposit

Under normal dietary condition, the deposits of perirenal, epididymal and mesenteric fat were respectively inhibited by RP-G (perirenal fat, −39.55 %; epididymal fat, −24.05 %; mesenteric fat, −11.64 %) and RP-A (perirenal fat, −13.44 %; epididymal fat, −23.00 %; mesenteric fat, −13.11 %) as compared with CAS-G and CAS-A, respectively ($P < 0.05$, Fig. 1D). Similarly, with oil-enriched diets, RP-G and RP-A also significantly reduced the fat levels ($P < 0.05$, Fig. 1D) in growing rats (perirenal fat, −46.61 %; epididymal fat, −28.08 %; mesenteric fat, −20.22 %) and adult rats (perirenal fat,−29.72 %; epididymal fat, −13.69 %; mesenteric fat, −15.66 %). The results indicate that RP could considerably depress fat deposit under normal and oil-enriched dietary conditions, independent of the influence of the dietary oil level.

### 3.3. Triglyceride concentrations

Under normal dietary condition, RP-G and RP-A considerably reduced plasma TG concentrations to 18.60 % in growing rats and to 25.35 % in adult rats compared with CAS-G and CAS-A, respectively ($P < 0.05$, Fig. 2A). Similarly, hepatic TG accumulations were distinctly depressed by RP-G to 35.09 % and by RP-A to 36.99 % ($P < 0.05$) as compared with CAS-G and CAS-A, respectively, under normal dietary condition (Fig. 2B). These findings are consistent with those of our previous studies (Yang et al., 2007).

With the addition of oil in diets, although the TG concentrations were higher in growing and adult rats fed oil-enriched diets than those fed normal diets, RP-G and RP-A still exerted TG-lowering effects under oil-enriched dietary condition. Compared with CAS-G and CAS-A, the plasma TG concentrations were significantly decreased by RP-G to 32.10 % ($P < 0.05$, Fig. 2A) in growing rats and by RP-A to 35.54 % ($P < 0.05$, Fig. 2A) in adult rats fed oil-enriched diets. Similarly, the hepatic TG contents were significantly decreased by RP-G to 45.33 % and by RP-A to 45.23 % in growing and adult rats as compared with CAS-G and CAS-A, respectively, under oil-enriched dietary condition ($P < 0.05$, Fig. 2B).

Taken together, after 2 weeks of feeding, the TG-lowering effects exerted by RPs were observed. Notably, the novel findings of this study indicated that RPs could reduce the TG concentrations in growing and adult rats fed normal and oil-enriched diets, independent of the influence of the dietary oil level.

### 3.4. Free fatty acid contents

After RP intake for 2 weeks, the downwards trend in the free FA contents were also observed in the RP groups, as the similar trend to the TG concentration in the plasma and liver. Under normal dietary condition, RP-G and RP-A significantly decreased the free FA contents in the plasma and liver compared with CAS-G and CAS-A, respectively ($P < 0.05$, Fig. 2C,D). Similarly, under oil-enriched dietary condition, the free FA contents were markedly decreased by RP-G and RP-A in the plasma and liver ($P < 0.05$, Fig. 2C,D).

As illustrated in Fig. 2, under normal and oil-enriched dietary conditions, the decreased concentrations of free FAs induced by RP-G and RP-A further suggested that RP could exert effective TG-lowering effects

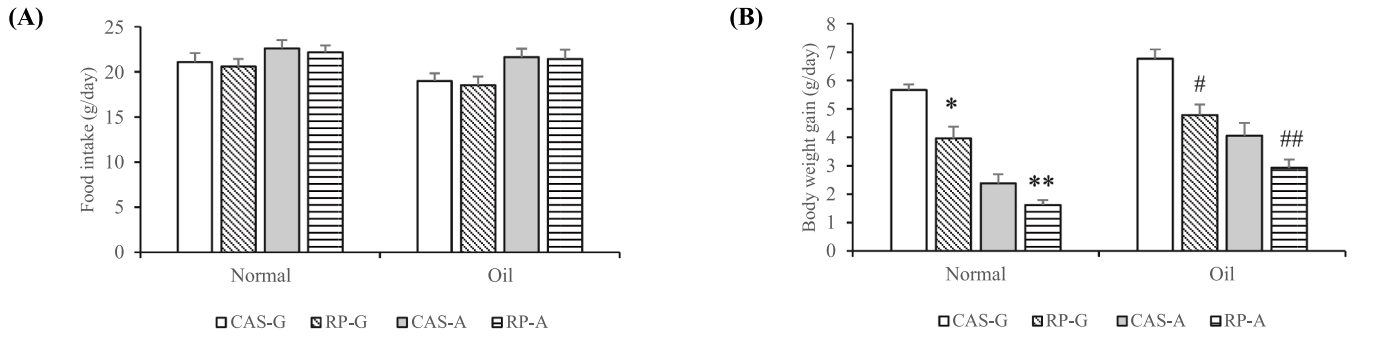


**Fig. 1.** Food intake (A), body weight gain (B), liver weight (C) and fay deposit (D) in growing and adult male Wistar rats after 2 weeks feeding. CAS-A, adult rats fed with casein at 14 % crude protein level; CAS-G, growing rats fed with casein at 20 % crude protein level; RP-A, adult rats fed with rice protein at 14 % crude protein level; RP-G, growing rats fed with rice protein at 20 % crude protein level. Values are the means ± SEM ($n = 6$). Bars marked with * are significantly different between CAS-G and RP-G under normal dietary condition ($P < 0.05$). Bars marked with ** are significantly different between CAS-A and RP-An under normal dietary condition ($P < 0.05$). Bars marked with # are significantly different between CAS-G and RP-G under oil-enriched dietary condition ($P < 0.05$). Bars marked with ## are significantly different between CAS-A and RP-An under oil-enriched dietary condition ($P < 0.05$).

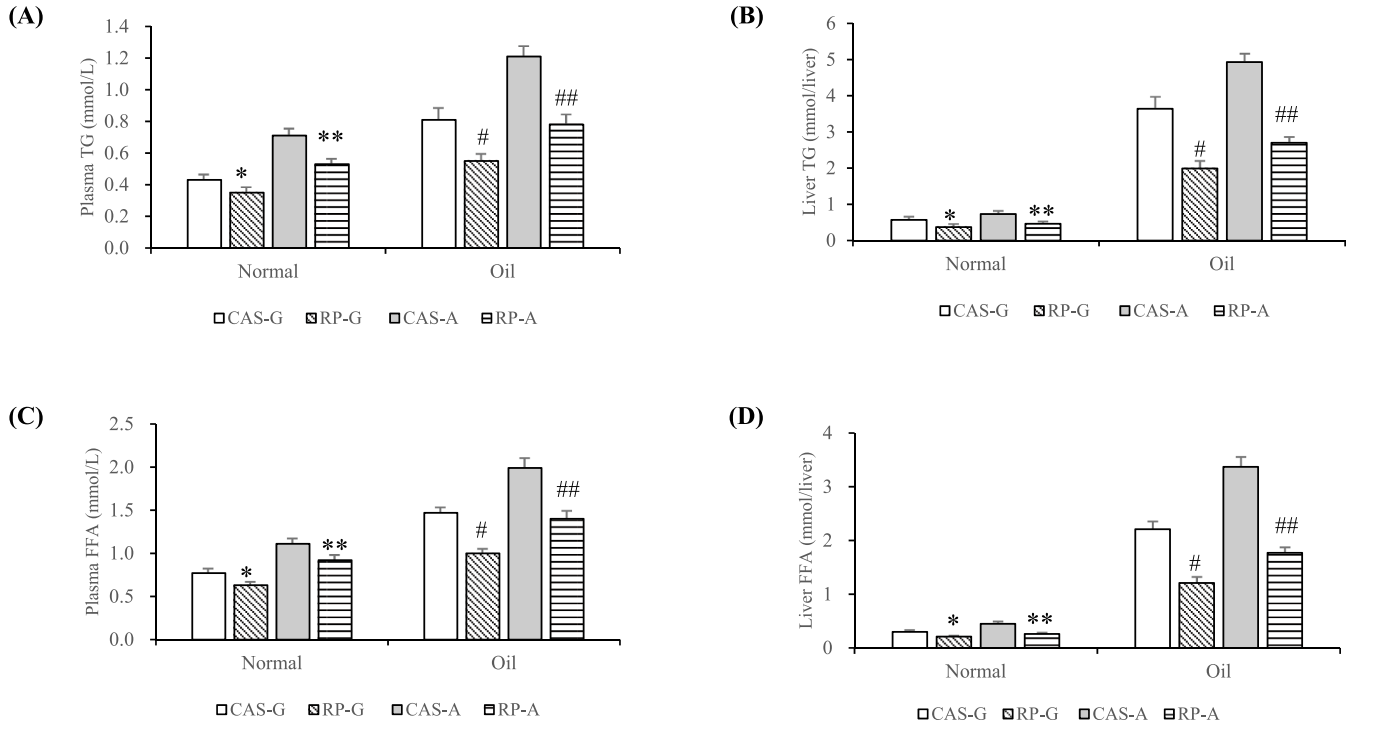


**Fig. 2.** Plasma and hepatic contents of TG and FFA in growing and adult male Wistar rats after 2 weeks feeding. (A) Plasma TG concentrations; (B) Hepatic contents of TG; (C) Plasma FFA concentrations; (D) Hepatic contents of FFA. Values are the means ± SEM ($n = 6$). Each measurement performed in triplicate as technical replicates. Bars marked with * are significantly different between CAS-G and RP-G under normal dietary condition ($P < 0.05$). Bars marked with ** are significantly different between CAS-A and RP-An under normal dietary condition ($P < 0.05$). Bars marked with # are significantly different between CAS-G and RP-G under oil-enriched dietary condition ($P < 0.05$). Bars marked with ## are significantly different between CAS-A and RP-An under oil-enriched dietary condition ($P < 0.05$). FFA, free fatty acid; TG, triglyceride.

on growing and adult rats despite the influence of dietary oil intake.

### 3.5. Effect of rice protein on FATP expression

In this study, RP-G and RP-A effectively regulated the expressions of FATP, which involved in FA transport.

As illustrated in Fig. 3, under normal and oil-enriched dietary conditions, the mRNA levels of FATP-2 were dramatically reduced by RPs in growing and adult rats ($P < 0.05$, Fig. 3A). Together with the strong down-regulation in gene expression, the protein levels of FATP-2 were also markedly decreased by RP-G and RP-A as compared with CAS-G and CAS-A, respectively, under normal and oil-enriched dietary conditions ($P < 0.05$, Fig. 3B). Thus, it is evident that RP can exert regulatory effects on FA transport under normal and oil-enriched dietary conditions, in which the suppression of FATP-2 expression was not attenuated by increased dietary oil level.

### 3.6. Effect of rice protein on FABP expression

Similar to the down-regulation of FATP-2, the protein and gene expressions of FABP-1 were also depressed by RP.

With the intake of RPs, the mRNA levels of FABP-1 were significantly decreased by RP-G and RP-A as compared with CAS-G and CAS-A, respectively, under normal and oil-enriched dietary conditions ($P < 0.05$, Fig. 4A). Along with a remarkable reduction in the FABP-1 mRNA level, RP-G and RP-A also dramatically decreased the FABP-1 protein expression as compared with CAS-G and CAS-A, respectively. Under normal dietary condition, FABP-1 protein levels were distinctly decreased by RP-G to 16.62 % and by RP-A to 19.80 % ($P < 0.05$, Fig. 4B)) as compared with CAS-G and CAS-A. Under oil-enriched dietary condition, FABP-1 protein levels were also markedly decreased by RP-G to 22.27 % and by RP-A to 21.58 % ($P < 0.05$, Fig. 4B) as compared with CAS-G and CAS-A. These results indicated that RP-G and RP-A exhibited regulatory capacities on FA transport, independent of the influence of the dietary oil level.

### 3.7. Effect of rice protein on CD36 expression

After 2 weeks of feeding, RP effectively depressed the protein and gene expressions of CD36 in growing and adult rats fed normal and oil-enriched diets.

The mRNA levels of CD36 were markedly decreased by RP-G and RP-A as compared with CAS-G and CAS-A, respectively, under normal and oil-enriched dietary conditions ($P < 0.05$, Fig. 5A). Similarly, RP-G and RP-A decreased the protein levels of CD36 in growing and adult rats. As illustrated in Fig. 5B, the hepatic protein levels of CD36 were dramatically decreased by RP-G to 19.66 % and by RP-A to 22.81 % under normal dietary condition ($P < 0.05$, Fig. 5B). With oil-enriched diets, the CD36 protein levels were also significantly reduced to 17.90 % by RP-G and to 18.72 % by RP-A ($P < 0.05$, Fig. 5B), despite the increased level of dietary oil.

The results indicated that CD36 expressions could be down-regulated by RP feeding under normal and oil-enriched dietary conditions, suggesting that the increase in dietary oil level could not attenuate the suppression of CD36 expression induced by RP.

### 3.8. Effect of rice protein on MTP expression

In this study, the regulatory effect of RP on MTP expression was determined after 2 weeks of feeding.

RP-G and RP-A dramatically decreased the mRNA levels of MTP as compared with CAS-G and CAS-A, respectively, under normal and oil-

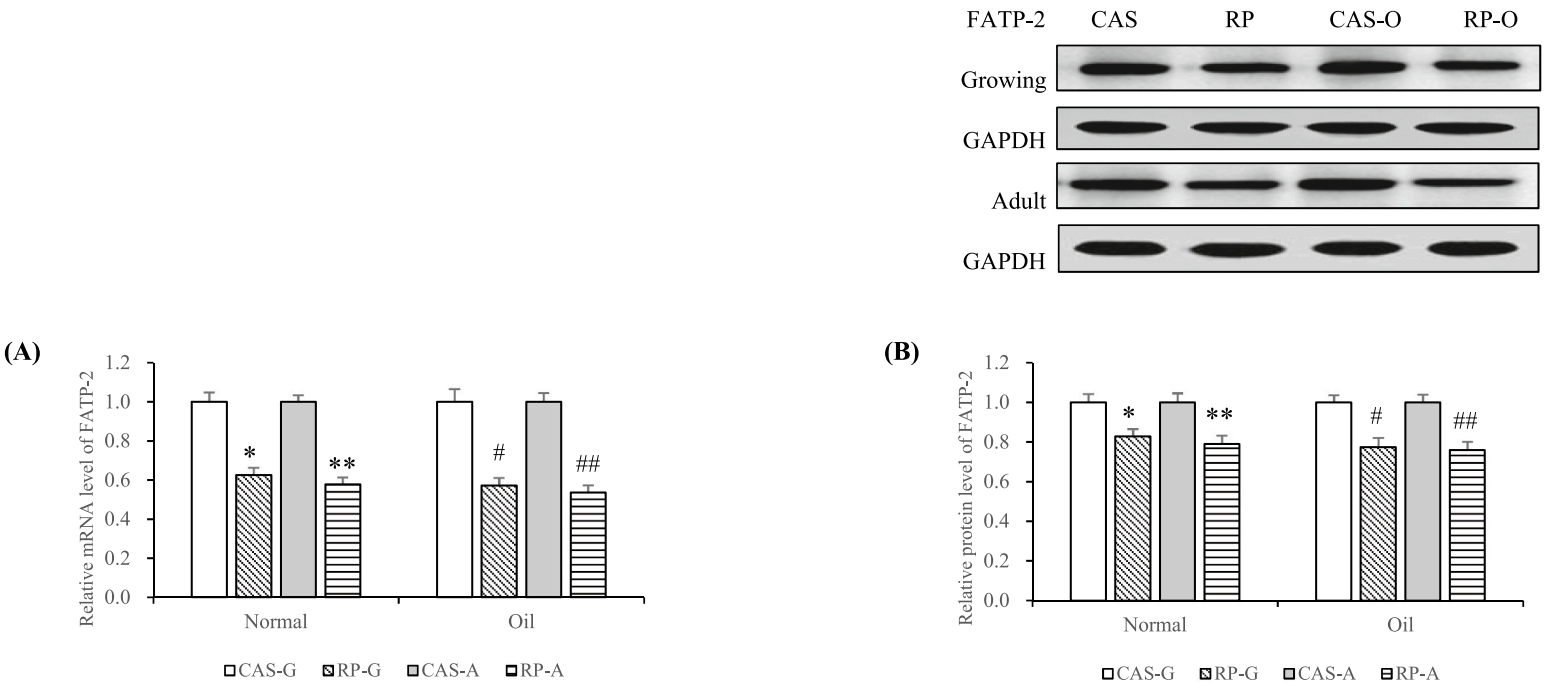


**Fig. 3.** Hepatic mRNA levels and protein expressions FATP-2 in growing and adult rats. (A) Hepatic mRNA levels of FATP-2; (B) Hepatic protein expressions FATP-2. Values are the means ± SEM (n = 6). Each measurement performed in triplicate as technical replicates. Bars marked with * are significantly different between CAS-G and RP-G under normal dietary condition ($P < 0.05$). Bars marked with ** are significantly different between CAS-A and RP-An under normal dietary condition ($P < 0.05$). Bars marked with # are significantly different between CAS-G and RP-G under oil-enriched dietary condition ($P < 0.05$). Bars marked with ## are significantly different between CAS-A and RP-An under oil-enriched dietary condition ($P < 0.05$). FATP-2, fatty acid transport protein-2.

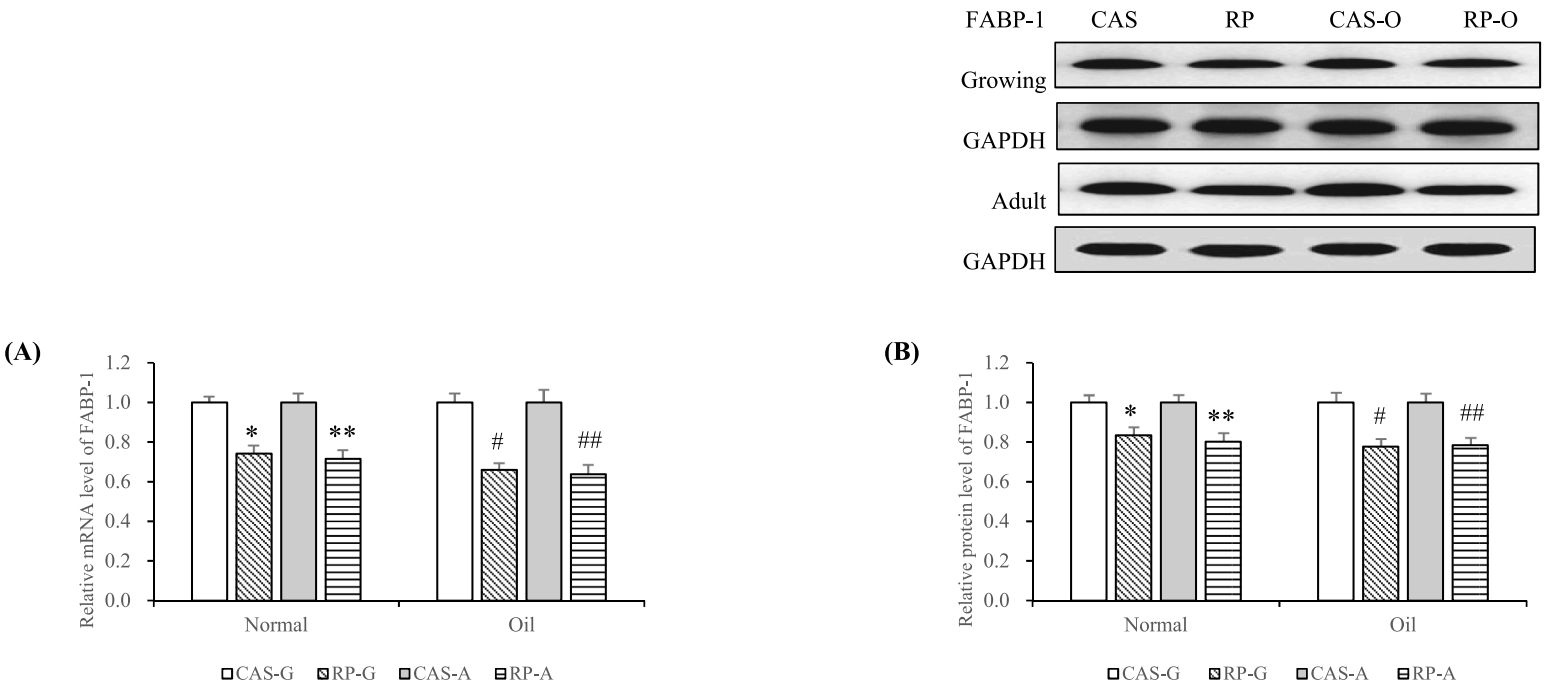


**Fig. 4.** Hepatic mRNA levels and protein expressions of FABP-1 in growing and adult rats. (A) Hepatic mRNA levels of FABP-1; (B) Hepatic protein expressions of FABP-1. Values are the means ± SEM (n = 6). Each measurement performed in triplicate as technical replicates. Bars marked with * are significantly different between CAS-G and RP-G under normal dietary condition ($P < 0.05$). Bars marked with ** are significantly different between CAS-A and RP-An under normal dietary condition ($P < 0.05$). Bars marked with # are significantly different between CAS-G and RP-G under oil-enriched dietary condition ($P < 0.05$). Bars marked with ## are significantly different between CAS-A and RP-An under oil-enriched dietary condition ($P < 0.05$). FABP-1, fatty acid-binding-1.

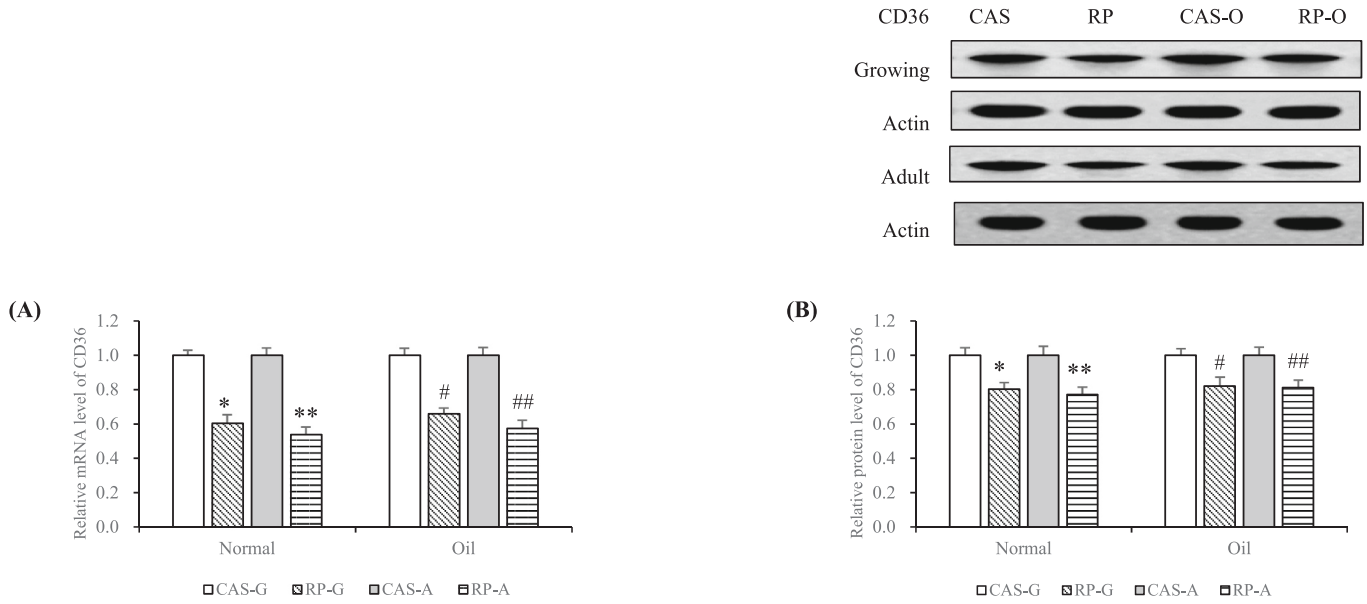


**Fig. 5.** Hepatic mRNA levels and protein expressions of CD36 in growing and adult rats. (A) Hepatic mRNA levels of CD36; (B) Hepatic protein expressions of CD36. Values are the means ± SEM (n = 6). Each measurement performed in triplicate as technical replicates. Bars marked with * are significantly different between CAS-G and RP-G under normal dietary condition ($P < 0.05$). Bars marked with ** are significantly different between CAS-A and RP-An under normal dietary condition ($P < 0.05$). Bars marked with # are significantly different between CAS-G and RP-G under oil-enriched dietary condition ($P < 0.05$). Bars marked with ## are significantly different between CAS-A and RP-An under oil-enriched dietary condition ($P < 0.05$). CD36, cluster determinant 36.

enriched dietary conditions ($P < 0.05$, Fig. 6A). Similarly, the protein levels of MTP were significantly decreased by RP-G to 14.13 % and by RP-A to 13.25 % under normal dietary condition ($P < 0.05$, Fig. 6B). With the intake of oil-enriched diets, the protein levels of MTP were also markedly decreased by RP-G to 16.20 % and by RP-A to 15.28 % as compared with CAS-G and CAS-A, respectively ($P < 0.05$, Fig. 6B). The results indicated that the increase in dietary oil level could not attenuate the regulatory effect of RP on MTP expression under oil-enriched dietary condition.

### 3.9. Effect of rice protein on NPC1L1 expression

In this study, the regulatory effect of RP on NPC1L1 expression, which is involved in lipid absorption, was also determined after 2 weeks of feeding.

The protein levels of NPC1L1 were significantly decreased by RP-G to 15.26 % and by RP-A to 20.51 % compared with CAS-G and CAS-A, respectively, under normal dietary condition ($P < 0.05$, Fig. 7A). With the intake of oil-enriched diets, the protein levels of NPC1L1 were also

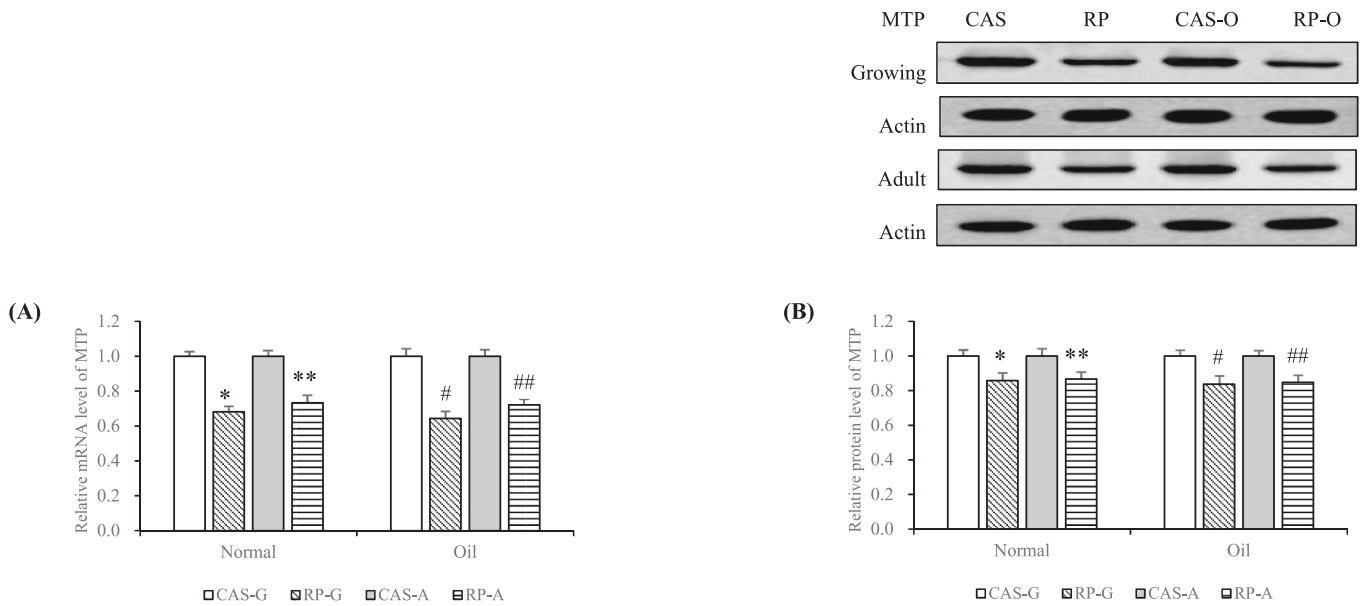


**Fig. 6.** Hepatic mRNA levels and protein expressions of MTP in growing and adult rats. (A) Hepatic mRNA levels of MTP; (B) Hepatic protein expressions of MTP. Values are the means ± SEM (n = 6). Each measurement performed in triplicate as technical replicates. Bars marked with * are significantly different between CAS-G and RP-G under normal dietary condition ($P < 0.05$). Bars marked with ** are significantly different between CAS-A and RP-An under normal dietary condition ($P < 0.05$). Bars marked with # are significantly different between CAS-G and RP-G under oil-enriched dietary condition ($P < 0.05$). Bars marked with ## are significantly different between CAS-A and RP-An under oil-enriched dietary condition ($P < 0.05$). MTP, microsomal triglyceride transfer protein.

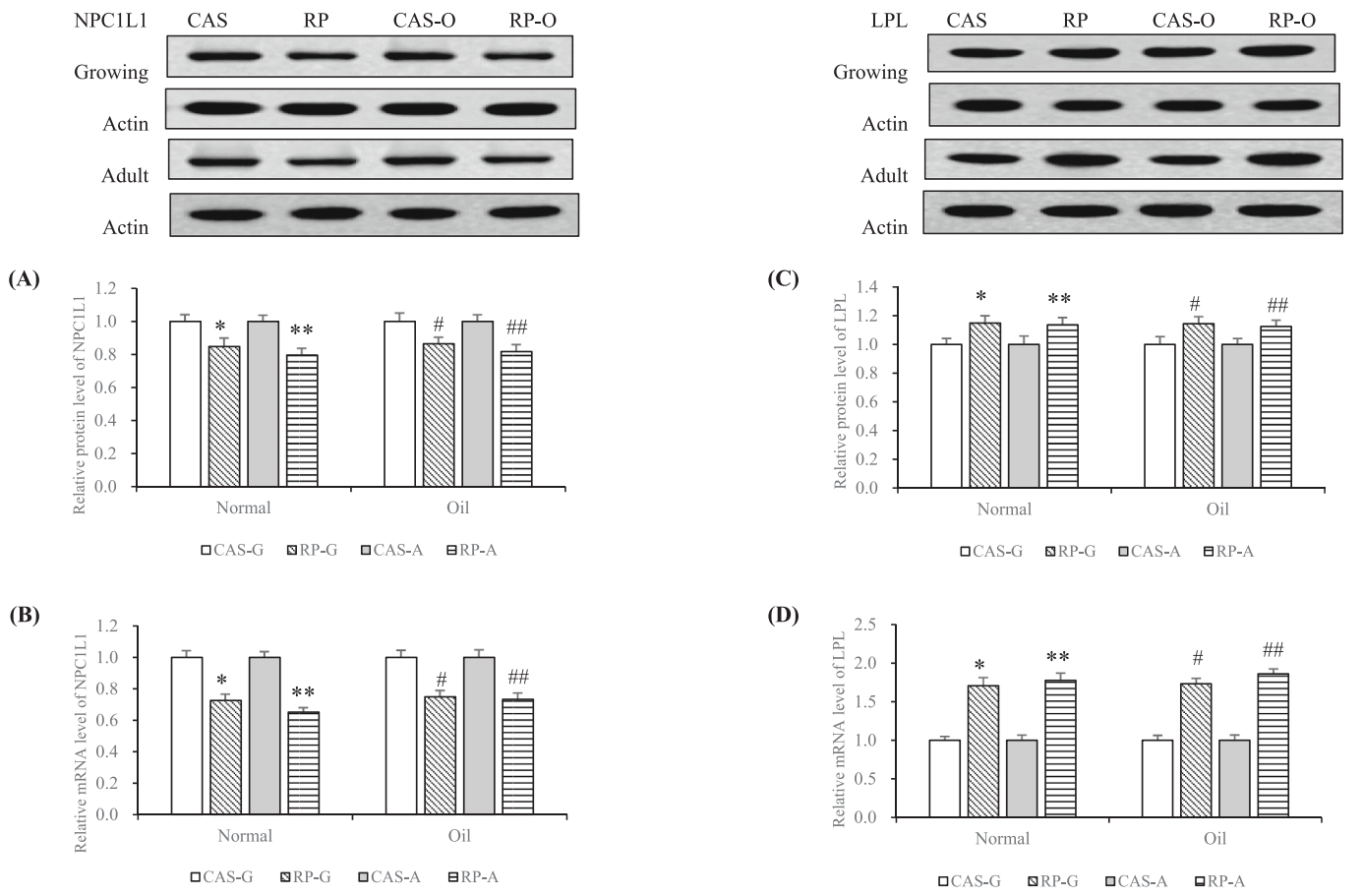


**Fig. 7.** Hepatic protein expressions and mRNA levels of NPC1L1 and LPL in growing and adult rats. (A) Hepatic protein expressions of NPC1L1; (B) Hepatic mRNA levels of NPC1L1; (C) Hepatic protein expressions of LPL; (D) Hepatic mRNA levels of LPL. Values are the means ± SEM (n = 6). Each measurement performed in triplicate as technical replicates. Bars marked with * are significantly different between CAS-G and RP-G under normal dietary condition ($P < 0.05$). Bars marked with ** are significantly different between CAS-A and RP-An under normal dietary condition ($P < 0.05$). Bars marked with # are significantly different between CAS-G and RP-G under oil-enriched dietary condition ($P < 0.05$). Bars marked with ## are significantly different between CAS-A and RP-An under oil-enriched dietary condition ($P < 0.05$). LPL, lipoprotein lipase; NPC1L1, Niemann-Pick C1 like 1.

significantly decreased by RP-G to 13.57 % and by RP-A to 18.25 % as compared with CAS-G and CAS-A, respectively ($P < 0.05$, Fig. 7A). Similarly, RP-G and RP-A dramatically decreased the mRNA levels of NPC1L1 under normal and oil-enriched dietary conditions ($P < 0.05$, Fig. 7B). The results indicated that the addition of dietary oil could not attenuate the depression of NPC1L1 expression by RP under oil-enriched dietary condition, further suggesting that RP could regulate TG transport, despite of the influence of dietary oil intake.

### 3.10. *Effect of rice protein on LPL expression*

In this study, the regulatory effect of RP on LPL expression was also determined after 2 weeks of feeding.

After the 2 weeks feeding, the remarkable up-regulation of the protein expressions of hepatic LPL were augmented by RP feeding. The LPL protein levels were distinctly increased by RP-G to 14.88 % and by RP-A to 13.57 % as compared with CAS-G and CAS-A, respectively, under normal dietary condition ($P < 0.05$, Fig. 7C). With the intake of oil-enriched diets, the protein levels of LPL were also significantly increased by RP-G to 14.38 % and by RP-A to 12.53 % as compared with CAS-G and CAS-A, respectively ($P < 0.05$, Fig. 7C). Similarly, the mRNA levels of LPL were markedly increased by RP-G and RP-A as compared with CAS-G and CAS-An under normal and oil-enriched dietary conditions ($P < 0.05$, Fig. 7D). These results indicated that RP could stimulate TG oxidation, independent of the influence of the dietary oil level.

## 4. Discussion

TG concentration depends on FA uptake, TG transport, TG synthesis and TG catabolism (Ademović et al., 2023). Our previous studies have reported that RP could reduce TG concentration by inhibiting TG synthesis and stimulating TG catabolism (Yang et al., 2012a). Therefore, to further elucidate the TG-lowering mechanism, the regulation of FA uptake and TG transport by RP was particularly emphasised in this study. Under normal and oil-enriched dietary conditions, the TG-lowering actions of RP were closely associated with the suppression of FATP, FABP, CD36 and MTP expressions. To our knowledge, this is the first study to provide convincing evidence on the association between the TG-lowering effect exerted by RP and the modulation of CD36, MTP, FATP and FABP expressions in growing and adult rats fed normal and oil-enriched diets.

Hypertriglyceridemia usually results from excessive TG accumulation in the plasma and impaired clearance of TG-rich VLDL in the circulation (Chait, 2022; Packard et al., 2020; Rygiel, 2018; Santos-Baez & Ginsberg, 2020). Thus, the key regulator involved in TG-rich VLDL secretion should be drawn attention in this study. MTP is a key protein in the assembly and export of TG-rich VLDL (Tietge et al., 1999; White et al., 1998). Hepatic MTP overexpression has been reported to increase the secretion of VLDL-TGs, whereas MTP inhibition decreases TG secretion, suggesting that MTP is rate-limiting for VLDL-TG secretion to regulate plasma TG concentration (Gordon & Jamil, 2000; Hussain

et al., 2003; Lemieux et al., 2005). In this study, RP markedly reduced the expressions of hepatic MTP, consistent with our previous findings indicating that RP effectively depressed the output of VLDL-TG to lower plasma TG concentration (Yang & Kadowaki, 2009). In addition to promoting VLDL export, MTP can facilitate TG transfer (White et al., 1998). Thus, MTP inhibition is preferable for reducing plasma TG concentration. Some studies have reported that hypertriglyceridemia is associated with increased MTP expression, whereas the down-regulation of liver MTP is a therapeutic intervention to lower plasma TG concentration, suggesting that a novel feasible mechanism for the anti-hypertriglyceridemic effect is to lower liver MTP expression (Lemieux et al., 2005; Pan & Hussain, 2007). In this study, RP markedly reduced hepatic MTP expression and plasma TG concentration, strongly confirming that the TG-lowering effect was attributed to MTP inhibition by RP. To support this view, a significant positive correlation between MTP expression and plasma TG concentration ($r = 0.7861$, $P < 0.05$) was observed in this study.

In addition to MTP, CD36 controls TG concentration by regulating TG secretion (Febbraio et al., 2001). Some studies have reported that CD36 deletion can suppress VLDL output to reduce TG secretion, suggesting that the absence of CD36 can decrease TG concentration (Campbell et al., 2004; Febbraio et al., 2002; Nassir et al., 2013). In light of these facts and our previous findings indicating that the TG-lowering effect of RP was attributed to the suppression of hepatic VLDL-TG secretion (Yang & Kadowaki, 2009), we hypothesised that the decreased CD36 expressions by RP might be associated with its TG-lowering action. Notably, a significant positive correlation was observed between CD36 expression and plasma TG concentration ($r = 0.7955$, $P < 0.05$), which validated our hypothesis. Taken together, the TG-lowering mechanism exerted by RP could be partly attributed to the suppression of MTP and CD36 expressions, which was pivotal for regulating TG transport.

As FA is stored as TG in the body, hypertriglyceridemia is associated with the increase in FA uptake (Chait, 2022; Rygiel, 2018; Santos-Baez & Ginsberg, 2020). Thus, to elucidate the TG-lowering mechanism exerted by RP, some regulators involved in FA uptake were particularly investigated in this study.

FATP is an important FA transporter for FA uptake (Abumrad et al., 1999; Dourlen et al., 2015). In this study, RP considerably suppressed FATP-2 expression under normal and oil-enriched dietary conditions to reduce the hepatic accumulations of FAs, showing a significant positive correlation between FATP-2 expression and hepatic FA concentration ($r = 0.7736$, $P < 0.05$). With FA uptake, FATP can promote TG storage to increase TG concentration, suggesting that FATP expression is associated with the regulation of TG concentration (Ademović et al., 2023). Consistent with this view, with the suppression of FATP-2 expression, RP markedly reduced the TG concentrations in the plasma and liver, suggesting that suppression of FATP-2 expression might contribute to the TG-lowering action exerted by RP. Notably, the results indicated the significant positive correlations between FATP-2 expression and plasma TG concentration ($r = 0.7640$, $P < 0.05$), as well as the accumulation of hepatic TG ($r = 0.7788$, $P < 0.05$).

Similar to the FATP, FABP can facilitate FA uptake for TG storage (Abumrad et al., 1999; Makowski & Hotamisligil, 2005). FABP-1 overexpression enhances FA uptake in the liver, whereas FABP-1 ablation inhibits FA uptake and reduces FA transport. Furthermore, some studies reported that elevated hepatic TG concentration is associated with up-regulated expression of FABP, whereas reduced hepatic TG content is due to down-regulation of FABP expression in fabp−/− mice (Furuhashi & Hotamisligil, 2008; Martin et al., 2005). Consistent with these findings, significant positive correlations were observed between FABP-1 expression and hepatic FA concentration ($r = 0.7718$, $P < 0.05$) as well as hepatic TG ($r = 0.7872$, $P < 0.05$) in this study. Moreover, with enhanced FA uptake, FABP can stimulate VLDL secretion to increase plasma TG concentration. It has been demonstrated that elevated VLDL secretion is associated with increased FABP expression. In contrast, fabp−/− mice exhibit decreased VLDL production (Sawicki et al., 2017). In light of this view, a significant positive correlation was observed between FABP expression and plasma TG concentration ($r = 0.7747$, $P < 0.05$) in this study, further supporting our previous findings indicating that RP could depress the output of hepatic VLDL-TG, which was associated with its TG-lowering action (Yang & Kadowaki, 2009). Taken together, the depression of FATP and FABP expression, which leads to the reduced net FA uptake both for TG storage and for VLDL-TG export, might be one of TG-lowing mechanisms exerted by RP.

As an important lipid transporter, CD36 also regulates FA uptake (Abumrad et al., 1999; Febbraio et al., 2001; Glatza et al., 2022; Pepino et al., 2014). It is clear that FA uptake is associated with CD36 expression, suggesting that CD36 plays a role in FA uptake to regulate TG concentration. Some studies have reported that the increases in hepatic FA uptake and TG accumulation are attributed to the stimulation of liver CD36 expression, whereas the absence of CD36 can protect FA uptake and reduce TG concentration (Campbell et al., 2004; Febbraio et al., 2002; Glatz & Luiken, 2018). The above findings are supported by the results of this study, which indicated the significant positive correlations between CD36 expression and liver FA concentration ($r = 0.7636$, $P < 0.05$) as well hepatic TG contents ($r = 0.8281$, $P < 0.05$). Thus, RP can indeed reduce TG concentration not only in the plasma but also in the liver due to the inhibition of FA uptake through the down-regulation of CD36 expression.

It is suggested that hypertriglyceridemia is exacerbated by an increase in dietary oil level (Luna-Castillo et al., 2022; Packard et al., 2020). However, this study unexpectedly found that the TG-lowering effect of RP could not be attenuated under oil-enriched dietary condition. Instead, substantial TG-lowering effects were clearly observed in growing and adult rats fed oil-enriched diets. Thus, this study should also discuss the influence of dietary oil on the TG-lowering action of RP. To explain this interesting phenomenon, we should emphasise the new finding indicating that the expressions of NPC1L1 and LPL were markedly depressed by RP-G and RP-An under normal and oil-enriched dietary conditions, suggesting that RP could inhibit TG absorption and stimulate TG oxidation, independent of the influence of the dietary oil level. Furthermore, a significant positive correlation was observed between NPC1L1 expression and plasma TG concentration ($r = 0.8752$, $P < 0.05$) as well as the weight of total fat ($r = 0.9244$, $P < 0.05$), whereas a significant negative correlation was observed between LPL expression and TG concentration in the plasma ($r = -0.8027$, $P < 0.05$) and liver ($r = -0.8236$, $P < 0.05$) as well as the total fat deposit ($r = -0.8104$, $P < 0.05$). More significantly, whatever the lipid-lowering mechanism exerted by dietary protein, the view that the amino acid composition is an important factor to affect lipid metabolism should be also taken into account to explain this interesting phenomenon. In our previous studies, we found that the lipid-lowering effect of RP was independent of the influence of the dietary cholesterol level, suggesting that the lipid-lowering effect of RP is dependent on its amino acid profiles (Yang et al., 2007, Yang et al., 2013). Furthermore, we have demonstrated that a higher level of some amino acids (arginine, etc) in RP might contribute to the lipid-lowering action (Yang et al., 2007; Yang et al., 2012b). In light of these facts, the insight that a higher arginine content in RP (RP, 87.8 μg/mg; CAS, 33.3 μg/mg) might represent a key role in TG-lowering action by regulating FA uptake and TG transport should be particularly emphasised in this study. A significant negative correlation was observed between arginine intake and plasma TG concentration ($r = -0.8387$, $P < 0.05$), which support our hypothesis. More significantly, significant negative correlations between arginine intake and the expressions of FATP-2 ($r = -0.8777$, $P < 0.05$), FABP-1 ($r = -0.9158$, $P < 0.05$), CD36 ($r = -0.8423$, $P < 0.05$), MTP ($r = -0.8827$, $P < 0.05$) and NPC1L1 ($r = -0.8120$, $P < 0.05$) were observed in this study. In contrast, a significant positive correlation was observed between arginine intake and the expression of LPL ($r = 0.8322$, $P < 0.05$). Accordingly, the fact that the TG-lowering effects of RP were found under normal and oil-enriched dietary conditions is convincing, which could

be attributed to the role of arginine in regulating FA uptake and TG transport. Clearly, additional studies are warranted to confirm this view.

## 5. Conclusion

The present study demonstrates the TG-lowering effects of RP owing to the modulation of CD36, MTP, FATP, FABP, NPC1L1 and LPL expressions in growing and adult rats fed normal and oil-enriched diets. It provides convincing evidence to confirm the association between the TG-lowering action and the depression of FA uptake and TG transport exerted by RP. Notably, the down-regulation of CD36, MTP, FATP, FABP and NPC1L1 and the up-regulation of LPL by RP to reduce TG concentration cannot be attenuated by the increase in dietary oil level. Not doubtfully, at present, the time has come for understanding the physiological functions of RP for human health. Thus, to provide more evidence confirming that RP exerts hypotriglyceridemic effect for application in food products, more detailed investigations are needed to investigate the precise mechanism exerted by RP to reduce TG concentration.

## CRediT authorship contribution statement

**Bingxiao Liu:** Formal analysis. **Zhengxuan Wang:** Formal analysis. **Mingcai Liang:** Formal analysis. **Lin Yang:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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