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在线全文

四川黑茶药膳配方通过重塑肠道菌群和短链脂肪酸代谢改善肥胖小鼠肾脏脂质紊乱*

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【摘要】 **目的** 探讨以四川黑茶为基础的药膳配方（元清）对高脂饮食诱导的肥胖小鼠的肾脏保护作用并探究其具体机制。**方法** 将雄性C57BL/6小鼠随机分为对照组、模型组和治疗组，每组8只。对照组饲喂普通维持饲料和纯净水，另外两组给予高脂饲料喂养12周以建立肥胖模型。此后模型组继续饲喂高脂饲料，治疗组同时给予元清12周，期间每周记录小鼠体质量。12周后处死小鼠，取血清检测三酰甘油（TG）、总胆固醇（TC）、天冬氨酸转氨酶（AST）、丙氨酸转氨酶（ALT）及白蛋白水平以评估肝功能，提取肾脏脂质检测肾脏TG及TC含量，过碘酸-雪夫（Periodic Acid-Schiff, PAS）和油红O染色评估肾脏病理损伤。Western blot检测肾脏组织中的磷酸化AMPK（pAMPK）/AMPK比值。PCR及Western blot检测肾脏组织中调控脂肪酸氧化蛋白乙酰辅酶a羧化酶1（acetyl-CoA carboxylase 1, ACC1）、肉毒碱酰基转移酶1（carnitine acyltransferase 1, CTP1）、过氧化物酶体增殖物激活受体γ（peroxisome proliferators-activated receptor γ, PPARγ）、过氧化物酶体增殖物激活受体γ辅助激活因子-1α（peroxisome proliferator-activated receptor gamma coactivator-1 alpha, PGC1α）及脂肪酸合成关键分子胆固醇调节元件结合蛋白-1（sterol-regulatory element binding proteins, SREBP-1）、脂肪酸合成酶（fatty acid synthase, FASN）、硬脂酰辅酶a去饱和酶1（stearoyl-CoA desaturase 1, SCD1）的表达水平。16SrRNA及代谢组学分析其肠道内容物中肠道菌群及其代谢产物。**结果** 与对照组相比，模型组小鼠肝质量（P=0.000 3），血清ALT（P<0.000 1）、AST（P=0.000 1）水平，肾脏TC（P=0.019 1）、TG（P=0.010 1）水平升高，肾脏脂质沉积。与模型组相比，治疗组有效降低小鼠肝质量（P=0.031 6），改善血清AST（P=0.001 2）、ALT（P=0.002 7），肾脏TC（P=0.020 0）、TG（P=0.049 9）异常水平，同时显著改善肾脏脂质沉积。治疗组与模型组相比，pAMPK/AMPK比值升高。与对照组相比，模型组小鼠肾脏中脂质合成相关基因和蛋白（SREBP-1、FASN、SCD1）的表达上调，而脂肪酸氧化相关基因和蛋白中，ACC1表达升高，CPT1A、PPARγ、PGC1α表达相应降低，而治疗组以上变化均得到改善。治疗组肠道菌群稳态得到改善，盲肠内容物中短链脂肪酸，尤其是异戊酸和丙酸含量也得到恢复。**结论** 四川黑茶药膳配方可通过调节肠道菌群和短链脂肪酸含量、改善肾脏脂质代谢，从而保护肥胖相关肾损伤，异戊酸和丙酸可能是其调节肠道微生物群的关键代谢物。

【关键词】 高脂饮食 肥胖 肾脏脂质代谢 慢性肾脏病 四川黑茶

Sichuan Dark Tea-Based Medicated Dietary Formula Improves Obesity-Induced Renal Lipid Metabolism Disorder in Mice by Remodeling Gut Microbiota and Short-Chain Fatty Acid Metabolism

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【**Abstract**】 **Objective** To investigate the renoprotective effects of a Sichuan dark tea-based medicated dietary formula (alternatively referred to as Qing, or clarity in Chinese) on mice with diet-induced obesity (DIO) and to explore the specific mechanisms involved. **Methods** Male C57BL/6 mice were randomly assigned to three groups, a control group, a DIO group, and a Qing treatment group, or the Qing group, with 8 mice in each group. The mice in the control group were given normal maintenance feed and purified water, and the other two groups were fed a high-fat diet for 12 weeks to establish the DIO model. After that, high-fat diet continued in the DIO group, while the Qing group was given Qing at the same time for 12 weeks, during which period the weight of the mice was monitored and recorded every week. The mice were sacrificed after 12 weeks. Serum samples were collected and the levels of triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin were measured to evaluate liver function. In addition, renal lipids were extracted to determine the levels of TG and TC in the kidney and periodic acid-Schiff (PAS) and oil red O stainings were performed to evaluate kidney pathological injury. Western blot was performed to determine the phosphorylated AMPK (pAMPK)/AMPK ratio in the kidney tissue. RT-qPCR and Western blot were used to determine the expression of proteins related to fatty acid oxidation, including acetyl-CoA carboxylase 1 (ACC1), carnitine acyltransferase 1 (CTP1), peroxisome proliferators-activated receptor γ (PPARγ), peroxisome proliferators-activated receptor-1 α (PPAR1α), sterol-regulatory element binding proteins (SREBP-1), and key proteins related to lipid

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synthesis, including fatty acid synthase (FASN) and stearoyl-coenzyme A desaturase 1 (stearoyl-CoA desaturase) in the kidney tissue. 16SrRNA and metabolomics were applied to analyze the gut microbiota in the intestinal contents and its metabolites. **Results** Compared with those of the control group, the levels of liver mass (P=0.000 3), serum ALT (P<0.000 1) and AST (P=0.000 1), and kidney TC (P=0.019 1) and TG (P=0.010 1) of the DIO group were significantly increased and there was lipid deposition in the kidney. Compared with those of the DIO group, mice in the Qing group showed effective reduction in liver mass (P=0.031 6) and improvements in the abnormal serum levels of AST (P=0.001 2) and ALT (P=0.002 7) and kidney TC (P=0.020 0) and TG (P=0.049 9). In addition, mice in the Qing group showed significant improvement in lipid deposition in the kidney. Qing group showed increased pAMPK/AMPK ratio in comparison with that of the DIO group. In comparison with those of the control group, mice in the DIO group had upregulated expression of lipid synthesis-related genes and proteins (SREBP-1, FASN, and SCD1). As for the fatty acid oxidation-related genes and proteins, DIO mice showed upregulated expression of ACC1 and downregulated expression of CPT1A, PPARγ, and PGC1α in comparison with those of the control group. In the Qing goup, improvements in regard to all these changes were observed. The Qing group demonstrated improvement in the disrupted homeostasis of the gut microbiota. Short-chain fatty acids in the cecal contents, especially isovaleric acid and propionic acid, were also restored. **Conclusion** Sichuan dark tea-based medicated dietary formula may improve renal lipid metabolism by regulating gut microbiota and the levels of intestinal short-chain fatty acids, thereby protecting obesity-related kidney injury. Isovaleric acid and propionic acid may be the metabolites key to its regulation of gut microbiota.

【Key words】 High-fat diet　Obesity　Renal lipid metabolism　Chronic kidney disease　Sichuan dark tea

肥胖在世界范围内的发病率逐年升高，已成为全球性的健康问题[1]。肥胖显著增加代谢性疾病及心血管疾病的发生风险，也是慢性肾脏疾病（chronic kidney disease, CKD）的独立危险因素[2]。长期高脂饮食（high-fat diet, HFD）可引发胰岛素抵抗、血脂异常等一系列问题，增加肾脏负担[2]。研究已证实肾脏脂质累积可导致严重的细胞功能障碍和肾损伤[3]。生理条件下，近端肾小管细胞摄取游离脂肪酸进行线粒体β氧化，多余的脂肪酸与甘油酯化形成三酰甘油，以脂滴的形式沉积并储存在细胞中[4]。HFD降低AMP活化蛋白激酶（AMP-activated protein kinase, AMPK）活性，抑制脂肪酸β氧化途径，增加肾脏脂肪生成和脂质积累[4]。HFD诱导的CKD模型中，脂肪酸氧化减少导致的能量缺乏可能是小管间质纤维化和疾病进展的重要因素[5]。

肠道菌群及肠道代谢物与遗传、环境影响，以及宿主饮食模式等多种因素相关[6]。长期高脂饮食会扰乱肠道微生物群落的稳态，破坏肠上皮细胞紧密连接的完整性[6]。肠道菌群结构异常和肠道屏障破坏共同导致CKD患者血清尿毒症毒素的累积，进一步加剧肾纤维化和肾小管损伤[7]。四川黑茶产于中国四川雅安[8]，具有预防心血管疾病、减重、缓解代谢综合征和调节肠道微生物群的作用[9]。然而，国内外对四川黑茶的研究较少。HE等[10]证实四川黑茶能通过调控小鼠肠道菌群改善肥胖。目前，临床上尚缺少针对肥胖相关肾损害的治疗药物，他汀类药物作为高胆固醇血症的一线用药，被用于治疗CKD患者血脂异常，然而他汀类药物存在肌痛、肝功能损伤等副作用[11]。因此，人们倾向于从植物药中寻找其他改善脂质代谢的方法。与传统中药和化学药物相比，“药食同源”的中药因患者依从性高、副作用少、可综合干预、应用灵活等特点逐渐展现出潜在的优势[12]。本研究采用四川黑茶与多种中草药的药膳配方（配方名为元清），用该方水提取物饲喂饮食介导的肥胖（diet-induced obesity, DIO）小鼠，探究其调节肠道菌群、改善肾脏脂质沉积的功能和机制。

1　材料与方法

1.1　元清水溶液的制备

本研究使用的四川黑茶配方（元清）由雅安藏茶与决明子、桑叶等十余种中药配伍而成（四川科瑞欣医药科技有限公司，食品生产许可证号SC10351012401028），成分均符合《中华人民共和国药典》的要求。精准称取相应药材后，加入药材总质量10倍的纯净水，煎煮3次，每次3 h。合并滤液，在−0.085 MPa、65 ℃下干燥粉碎，过80目筛后，得干粉约250 g。测量治疗组（具体分组见1.3）每只小鼠的每日饮水量，在其饮用水中添加一定量元清，使小鼠口服元清量达到400 mg/(kg·d)。

1.2　主要材料和试剂

Mouse Anti-CPT1A antibody（ab128568），Rabbit Anti-PGC1α antibody（ab54481）和Rabbit Anti-GPR43 antibody（ab124272）购自美国 Abcam 公司。Rabbit Anti-AMPK antibody和Rabbit Anti-phospho-AMPK antibody购自美国 Cell Signaling Technology公司。Rabbit Anti-

Acetyl-CoA Carboxylase antibody(ET1609-77), Rabbit Anti-SREBP-1 antibody(ER1917-19)和Rabbit Anti-Fatty Acid Synthase antibody(ET1701-91)购自中国华安生物技术有限公司。Rabbit Anti-PPARγ antibody(AF6284)和Rabbit Anti-SCD1 antibody(DF13253)购自美国Affinity Biosciences 公司。生化仪检测试剂盒由深圳迈瑞医疗国际有限公司提供。ELISA试剂盒购自南京建成生物工程研究所。

1.3 动物模型

8 ~ 10周龄雄性SPF级C57BL/6小鼠, 体质量约25 g, 均购于江苏集萃药康生物科技股份有限公司。实验前, 将所有小鼠于温度23 ℃、湿度50% ~ 60%、日夜节律12 h的环境中饲养1周, 允许小鼠自由获取食物和水。本研究的动物实验符合中国实验动物学会的标准, 并经四川大学动物保护与利用委员会批准。

根据2017年ARIFIN提出的资源方程法(Resource Equation Approach)进行动物实验的样本量计算[13]。假定ANOVA中可接受自由度(degree of freedom, DF)的误差范围在10 ~ 20之间。组间比较ANOVA的推导公式为: n= DF/k+1。n为每组受试动物数量, k为组数。计算可得, n(max)= 7.7, 取整可得8, 则每组受试动物8只, 3组总受试动物数量为24只。本研究将24只小鼠随机分为3组(对照组、模型组和治疗组), 每组8只。对照组饲喂普通维持饲料和纯净水, 另外两组给予脂肪占比60%的高脂饲料喂养12周, 建立DIO模型。此后, 模型组(DIO组)继续饲喂HFD+纯净水, 治疗组则饲喂HFD+元清(400 mg/(kg·d)12周。饲养期间没有动物死亡, 每周记录小鼠的体质量、摄食量和饮水量。12周后, 处死小鼠并收集血清、盲肠内容物和肾脏样本。

1.4 血清和肾脏组织生化指标的检测

取小鼠血液样本, 立即3 000 r/min离心15 min后得到上层血清。使用全自动生化仪 (BS-240, Mindrary, 中国深圳)及相应试剂盒检测血清三酰甘油(triacylglycerol, TG)、总胆固醇(total cholesterol, TC)、天冬氨酸转氨酶(aspartate aminotransferase, AST)、丙氨酸转氨酶(alanine aminotransferase, ALT)和白蛋白 (albumin, ALB)水平。使用ELISA试剂盒检测肾脏组织TC和TG水平。

1.5 肾脏组织病理检查

肾脏组织经体积分数10%甲醛溶液固定24 h后进行冲洗及脱水, 石蜡包埋。包埋好的组织切片厚度为4 μm。脱蜡和水洗后, 切片进行过碘酸-雪夫(Periodic Acid-Schiff, PAS)染色, 切片风干后用中性树胶封片。封片后显微镜观察并拍照。冰冻肾脏组织包埋于OCT溶胶中, 使用恒温箱冷冻切片机进行切片, 切片厚度4 μm和8 μm。冰冻切片贴附于载玻片上, 固定、冲洗、晾干后, 使用配制好的油红O工作液染色5 ~ 10 min, 甘油封片后显微镜下观察。

1.6 实时荧光定量聚合酶链反应(RT-qPCR)和免疫印迹实验(Western blot)

按照前人研究的方法进行定量PCR和免疫印迹实验[14]。Western blot检测肾脏磷酸腺苷活化蛋白激酶(AMPK)及磷酸化-磷酸腺苷活化蛋白激酶(pAMPK)的表达。PCR及Western blot检测肾脏组织中调控脂肪酸氧化蛋白乙酰辅酶a羧化酶1(acetyl-CoA carboxylase 1, ACC1)、肉毒碱酰基转移酶1 (carnitine acyltransferase 1, CTP1)、过氧化物酶体增殖物激活受体γ(peroxisome proliferators-activated receptor γ, PPARγ)、过氧化物酶体增殖物激活受体γ辅助激活因子-1 α (peroxisome proliferator-activated receptor gamma coactivator-1 alpha, PGC1α)及脂肪酸合成关键分子胆固醇调节元件结合蛋白-1(sterol-regulatory element binding protein 1, SREBP-1)、脂肪酸合成酶(fatty acid synthase, FASN)、硬脂酰辅酶a去饱和酶1 (stearoyl-CoA desaturase 1, SCD1)的表达水平。以磷酸甘油醛脱氢酶(GAPDH)为内源对照, 以$2^{-\Delta\Delta Ct}$为mRNA相对表达量。以β-actin或GAPDH为内源性对照, 由Image J软件测得条带灰度值, 将目的蛋白的灰度值除以内参蛋白的灰度值, 得出相对蛋白表达量。

1.7 肠道菌群16S rRNA基因测序及数据处理

采集3组小鼠盲肠内容物样本用于16S rRNA基因测序分析。使用E.Z.N.A. Stool DNA Kit试剂盒(Omega Bio-tek, 美国)提取小鼠肠道微生物群的基因组DNA。配置PCR反应体系, 正向引物341F(5′-CCTACGGGNGGCWGCAG-3′)和反向引物805R(5′-GACTACHVGGGTATCTAATCC-3′)扩增细菌16S rRNA基因的V3-V4区DNA片段。使用AMPure XT beads试剂盒(Beckman-Coulter, 美国)纯化PCR产物, Qubit试剂盒(Thermo Fisher Scientific, 美国)用于PCR产物定量。随后, 在Illumina Miseq平台上对扩增子文库进行测序, 获得原始读取数据, 并对其进行进一步处理以生成高质量的clean标签。然后利用分裂扩增子去噪算法(DADA2)进行聚类, 构建扩增子序列变异(amplicon sequence variants, ASVs)特征表与物种注释表。特征表和序列最终通过α多样性分析和β多样性分析评估物种丰度、分布和结构。

1.8 盲肠内容物样本短链脂肪酸测定

取盲肠内容物约20 mg放至1.5 mL离心管中, 管中提前加入研磨珠和250 mL异丙醇水溶液(1∶9配制)。使用

组织研磨仪50 Hz研磨2次, 每次30 s, 置于4 ℃离心机内12 000 r/min转速离心5 min。样品过滤后转移上清液到新离心管内, 加65 mL 20 mmol/L氢氧化钠溶液和200 mL氯仿, 涡旋均匀后再次12 000 r/min转速离心5 min。吸取上清液转移至离心管中进行衍生化反应。反应完成后, 加入75 mL正己烷萃取上清液, 采用气相色谱质谱联用仪(gas chromatography mass spectrometry, GC-M)进行检测。数据采集和分析在Mass Hunter (Version B.08.00)中进行。

1.9　统计学方法

用Image J软件统计分析图像表达。所有统计分析及图表制作均采用SPSS 26.0和GraphPad Prism 8软件进行。连续变量以$\bar{x} \pm s$表示。采用Spearman相关性分析获得差异菌群与差异菌群、差异代谢物与差异代谢物、差异代谢物与差异菌群之间的关系。采用Pearson相关性分析获得差异代谢产物与血清生化学指标之间的关系。两组间比较采用Student's *t* 检验, 3组及以上组间比较采用单因素方差分析(one-way ANOVA), 然后用Dunnett法进行两两比较。本研究属于探索性分析且涉及大量统计学检验, 为避免增加犯Ⅰ型错误的概率, 故于结果中报告具体*P*值, 不设置检验水准。

2　结果

2.1　小鼠生化指标和肾脏病理染色

结果如表1所示, 与对照组相比, DIO组小鼠肝质量(P= 0.000 3)、血清ALT(P< 0.000 1)、AST (P= 0.000 1)水平升高, ALB水平降低(P= 0.046 3)。治疗组可降低小鼠肝质量(P= 0.031 6), 改善HFD引起的AST(P= 0.001 2)、ALT(P= 0.002 7)、ALB(P= 0.038 4)异常。相比于DIO组小鼠, 治疗组血清TC、TG浓度未得到明显改善。DIO组小鼠肾脏TC (P= 0.019 1)、TG (P= 0.010 1)水平升高, 而治疗组小鼠肾脏TC (P= 0.020 0)、TG (P= 0.049 9)低于DIO组。肾脏组织PAS染色和油红O染色显示HFD引起的肾损伤和脂质沉积(图1A): PAS染色可见治疗组有效减少了小鼠肾脏组织糖原沉积, 抑制肾小球系膜增生; 油红O染色结果显示DIO组小鼠肾脏染色阳性面积显著增加, 红色脂滴主要聚集在肾小管上皮细胞胞质内, 治疗组小鼠肾小管细胞脂滴数量明显减少。以上结果提示四川黑茶药膳配方可有效改善HFD所致的肝功能障碍, 减轻肾脏脂质沉积。

2.2　小鼠肾脏脂质代谢相关基因和蛋白表达

3组相比, 肾脏总AMPK含量未见明显差异, 但治疗组与DIO组相比, pAMPK/AMPK比值升高(P= 0.028, 图1B)。PCR结果显示, 与对照组相比, DIO小鼠肾脏中*SREBP*-1 (P< 0.000 1)、*FASN* (P< 0.000 1)和*SCD*1 (P< 0.000 1)的表达上调。Western blot结果同样显示DIO小鼠肾脏中SREBP-1 (P= 0.000 9)、FASN (P= 0.001 1)和SCD1 (P= 0.003 6)的表达上调。元清下调肾脏组织中SREBP-1 ($P_{基因}$< 0.000 1, $P_{蛋白}$= 0.000 1)、FASN ($P_{基因}$< 0.000 1, $P_{蛋白}$= 0.021 1)和SCD1 ($P_{基因}$< 0.000 1, $P_{蛋白}$= 0.002 9)基因及蛋白的表达(图1C)。此外, 本研究检测了脂肪酸氧化过程调节蛋白的表达。DIO组小鼠肾脏ACC1表达升高($P_{基因}$< 0.000 1, $P_{蛋白}$< 0.000 1), CPT1A ($P_{基因}$= 0.000 5, $P_{蛋白}$= 0.001 9)、PPARγ ($P_{基因}$< 0.000 1, $P_{蛋白}$= 0.034 3)、PGC1α ($P_{基因}$< 0.000 1, $P_{蛋白}$= 0.015 3)表达相应降低, 而治疗组肾脏组织ACC1 ($P_{基因}$< 0.000 1, $P_{蛋白}$< 0.000 1)、CPT1A ($P_{基因}$< 0.000 1, $P_{蛋白}$= 0.001 8)、PPARγ ($P_{基因}$< 0.000 1, $P_{蛋白}$= 0.020 4)、PGC1α ($P_{基因}$< 0.000 1, $P_{蛋白}$= 0.001 3)变化均

表 1　四川黑茶药膳配方元清有效改善DIO所致血清生化指标异常

Table 1　Qing effectively improves DIO-induced abnormality in the levels of serum biochemical indicators

Index	Control group (n=8)	DIO group (n=8)	Qing group (n=8)	P^a	P^b
Liver mass/g, $\bar{x} \pm s$	0.844±0.089	1.607±0.410	1.150±0.314	0.000 3	0.031 6
Serum ALT/(U/L), $\bar{x} \pm s$	17.71±5.37	151.10±90.99	52.91±19.37	<0.000 1	0.002 7
Serum AST/(U/L), $\bar{x} \pm s$	116.70±40.35	265.70±86.91	140.50±27.94	0.000 1	0.001 2
Serum ALB/(g/L), $\bar{x} \pm s$	24.88±1.44	19.80±4.72	25.30±3.07	0.046 3	0.038 4
Serum TC/(mmol/L), $\bar{x} \pm s$	1.99±0.49	4.03±1.72	3.17±0.83	0.005 1	0.280 2
Serum TG/(mmol/L), $\bar{x} \pm s$	0.34±0.10	0.58±0.24	0.47±0.07	0.015 1	0.344 3
Kidney TC/(mmol/g prot.), $\bar{x} \pm s$	1.12±0.09	1.43±0.18	1.14±0.15	0.019 1	0.020 0
Kidney/TG (mmol/g prot.), $\bar{x} \pm s$	0.13±0.06	0.32±0.11	0.19±0.05	0.010 1	0.049 9

HFD: high-fat diet; DIO: diet-induced obesity; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALB: albumin; TC: Total cholesterol; TG: triacylglycerol. [a] DIO group vs. control group; [b] DIO group vs. Qing group.

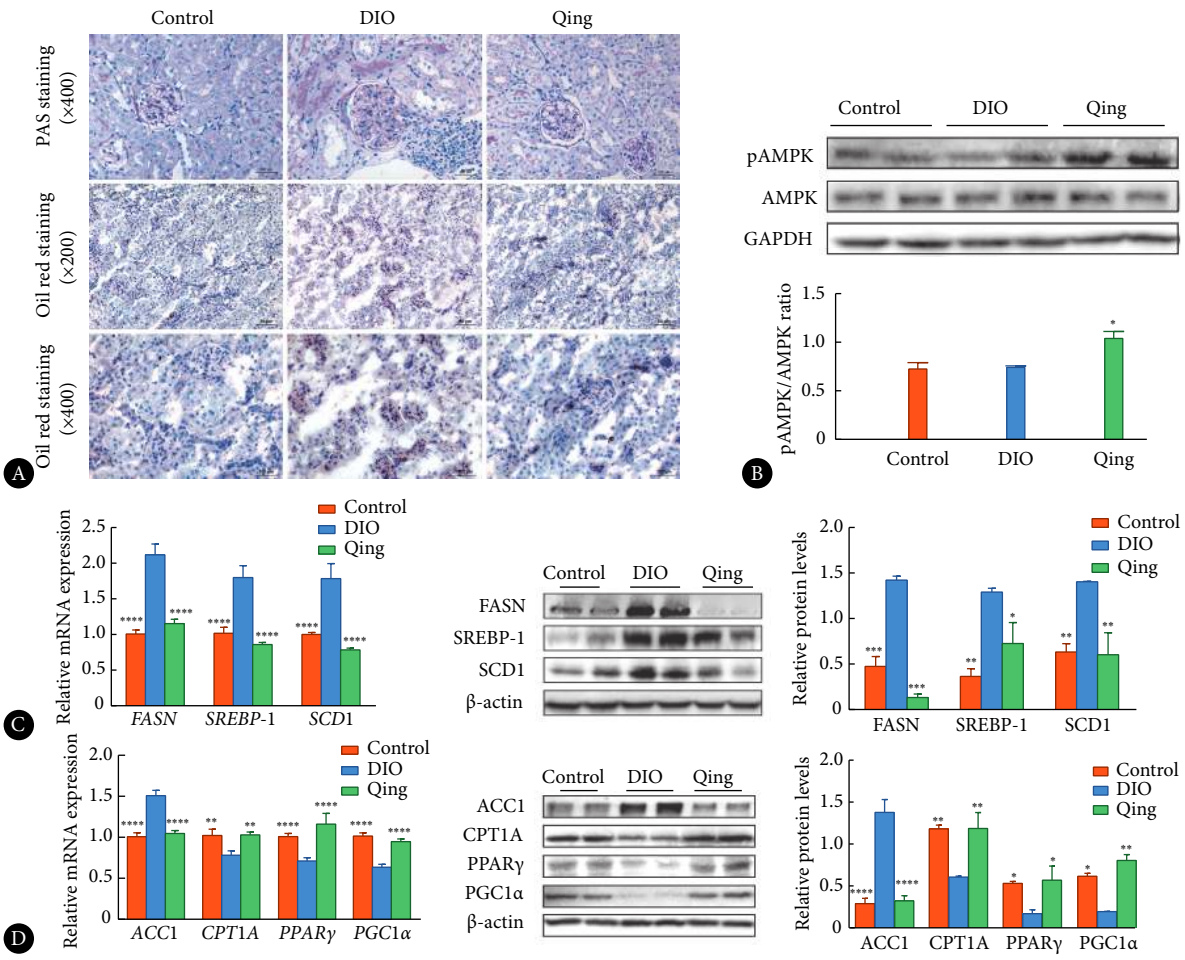

图 1 四川黑茶药膳配方元清改善DIO小鼠肾脏脂质代谢紊乱

Fig 1 Qing improves renal lipid metabolism disorders in DIO mice

DIO: diet-induced obesity. A, Histopathological analysis of kidney tissue; B, Western blot analysis of pAMPK/AMPK ratio in mice kidneys; C, the expression of mRNA and protein related to lipid synthesis in the kidney tissue; D, the expression of mRNA and protein related to fatty acid β-oxidation in the kidney tissue. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$, vs. the DIO group. n=8.

得到改善(图1D)。上述结果提示元清通过促进肥胖小鼠肾脏脂肪酸氧化和抑制脂质合成,调节脂质代谢和能量代谢,从而起到肾脏保护作用。

2.3 小鼠肠道菌群的结构

α多样性分析显示,代表样本的物种丰富度的Chao1指数在DIO组较对照组下降,在治疗组较DIO组升高,表明群落的物种丰富度得以恢复 (图2A)。同时,主坐标分析(principal coordinates analysis, PCoA)结果显示,3组样本肠道菌群结构分布存在显著差异(图2B),对照组与DIO组小鼠的肠道微生物结构呈明显各自聚类,治疗组则介于两者之间。随后,使用LEfSe(LDA Effect Size)分析比较各组间微生物丰度有差异显著的物种,并显示了组间微生物分类特征(图2C)。在门(Phylum)水平上,DIO小鼠肠道菌群中厚壁菌门(Firmicutes)、浮霉菌门(Plantomycetes)、芽单胞菌门(Gemmatimonadetes)和绿弯菌门(Chloroflexi)的丰度与对照组相比显著增加,并在元清治疗后得到有效调节。在属(Genus)水平上,拟杆菌门(Bacteroidetes)的*Alloprevotella*属,厚壁菌门的*Eisenbergiella*属,变形菌门(Proteobacteria)的*Pseudomonas*属在3组之间的丰度差异显著(图2D)。上述结果表明,元清能有效调节HFD引起的肠道菌群多样性减少和结构变化。

2.4 四川黑茶药膳配方元清调节DIO小鼠血清短链脂肪酸含量

根据GC-MS检测结果,SCFAs与血清生化指标Pearson相关性分析显示异戊酸、乙酸、丙酸含量与血脂水平(TG、TC)、肝功能指标(ALT、AST)呈显著负相关,而与血清ALB水平呈正相关(图3A),提示元清诱导的SCFAs含量改变与脂代谢相关。本研究进一步对SCFAs与测序获得的肠道优势菌群进行Spearman相关性分析。结果表明乳球菌属与异戊酸和丙酸呈负相关,而异戊酸与拟普雷沃氏菌属(*Alloprevotella*)、拟杆菌属和

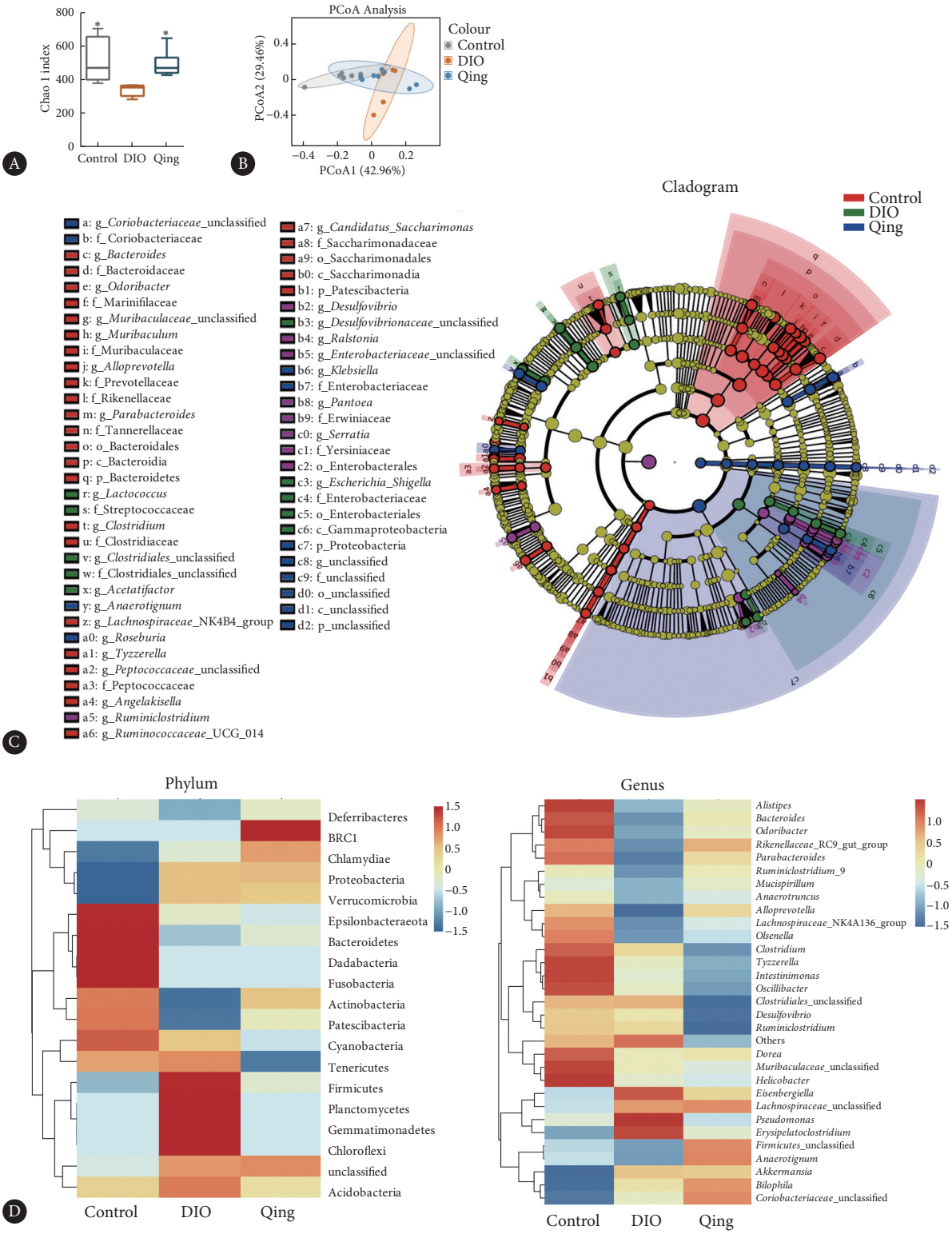

图 2 四川黑茶药膳配方元清调节DIO小鼠肠道菌群稳态

Fig 2 Effects of Qing on gut microbiota homeostasis in DIO mice

DIO: diet-induced obesity. A, Microbial α diversity by Chao1 index; B, microbial β diversity; C, the cladogram of LDA Effect Size analysis in the control, the DIO, and the Qing groups. In the cladogram, the circles radiating from the center to the outside represent taxonomic levels. The size of the nodes represents the relative abundance of species. The nodes of the species showing no significant difference among groups are uniformly colored yellow. The colors of the remaining nodes correspond to the designated group colors in the figure. For example, the red nodes indicate that the taxon has significant differences among groups and highest abundance in the Control group. D, Heatmap of bacterial abundance clustering at the phylum and genus levels. The values on scale placed on the right side represent the relative abundance values after Z-score normalization of the data. $^{*}P<0.05$, vs. the DIO group. n=8.

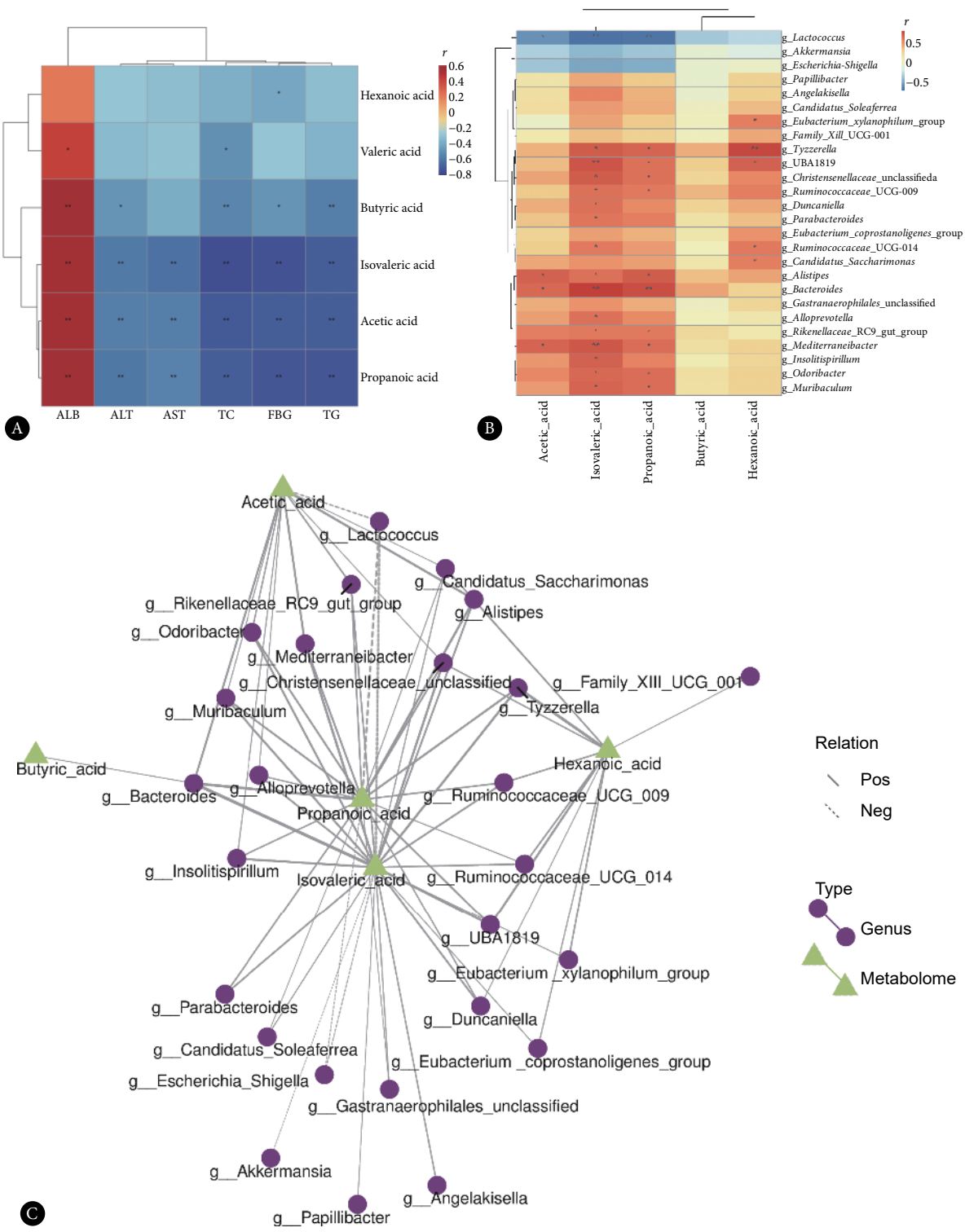

图 3 四川黑茶药膳配方元清调节肠道内短链脂肪酸含量

Fig 3 Effects of Qing on the content of short-chain fatty acids in the intestines

Pos: positive; Neg: negative; DIO: diet-induced obesity; FBG: fasting blood glucose; ALB, ALT, AST, TC, and TG denote the same as those in Table 1. A, Heatmap of the correlation between SCFAs and serum biochemical parameters. Red represents a positive correlation, while blue represents a negative correlation, with the darker shade indicating the greater strength of the correlation. B, Heatmap of the correlation between the dominant gut microbiota and SCFAs. Red represents a positive correlation, while blue represents a negative correlation, with the darker shade indicating the greater strength of the correlation. C, Network regulation map of SCFAs and gut microbiota. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

*Mediterraneibacter*等多种菌群呈正相关(图3B)。此外,短链脂肪酸与肠道菌群的网络调控图更直观地展示了异戊酸(isovaleric acid, IVA)和丙酸位于调节网络的中心,提示可能是元清调节肠道微生物群的关键代谢物(图3C)。

3　讨论

近年来, “药食同源”理念在治疗代谢性疾病及心血管疾病等大健康领域逐渐展现出优势。黑茶因其在减重、降脂等方面的作用受到越来越多的关注[15-16]。本研究发现四川黑茶药膳配方元清具有调节肾脏脂质代谢、减轻高脂饮食所致肝肾损伤及内脏脂质沉积的作用。

研究表明, 长期过量摄入脂肪会导致肾脏脂质累积[17], 脂质代谢紊乱可诱导炎症、氧化应激, 导致肾脏损伤和功能障碍[18]。肠道微生物群及其代谢物可能在肥胖相关肾脏疾病中起重要作用[19]。本研究发现DIO小鼠肠道菌群多样性降低, 菌群结构发生改变。门水平上, DIO小鼠肠道中拟杆菌和放线菌显著减少, 而厚壁菌、疣微菌和变形菌显著增加。HILDEBRANDT等[20]报道, 肥胖小鼠中厚壁菌和变形菌数量增加, 拟杆菌数量减少, 与本结果一致。此外, 肥胖或超重人群的肠道微生物多样性降低, 变形菌[21]和厚壁菌[22]水平升高。据报道, 冠心病患者肠道拟杆菌显著减少, 口服活的拟杆菌可减轻小鼠动脉粥样硬化损害[23]。本研究和上述研究均表明拟杆菌有助肠道稳态和脂质代谢, 而厚壁菌和变形菌则不利机体代谢, 这与目前的主流观点一致。有报道称茯砖茶中的冠状芽孢杆菌逆转了肠道厚壁菌门/拟杆菌门的比例[24]。除此之外, 元清治疗显著调节*Muribaculaceae*的丰度, 该物种于2019年被命名, 并被证明具有降解碳水化合物的能力[25]。

肠道菌群发酵的SCFAs可作为能量底物参与葡萄糖和脂肪酸的合成[19]。有研究称, SCFAs通过激活小鼠白色脂肪组织中的GPR43促进糖脂代谢, 抑制脂质累积[26]。本研究显示, 元清增加DIO小鼠肠道内SCFAs, 尤其是丙酸和异戊酸的含量。同时, 元清在体内通过促进AMPK磷酸化调节能量代谢, 改善脂质代谢紊乱。越来越多的研究证实了肠道来源的SCFAs与肾脏疾病之间的关系。在HFD和链脲佐菌素诱导的糖尿病肾病小鼠中, 丁酸通过抑制氧化应激和NF-κB信号通路改善肾功能和胰岛素抵抗[27]。既往研究也揭示了丙酸对肥胖及心血管疾病的保护作用[28-29]。

本研究存在一定的局限性。异戊酸对肾小管细胞的保护作用局限于体外研究, 其体内疗效及肝脏保护作用还需进一步探索。此外, 本研究假设元清可能通过调节肠道菌群及其代谢产物来改善肥胖相关的肝肾损伤, 尚未讨论四川黑茶药膳配方的有效成分及直接作用。

本研究证实了四川黑茶配方能够减轻高脂饮食致肝肾损伤, 减少内脏脂质沉积。其肾脏保护作用主要通过调节肠道菌群的多样性和组成, 增加SCFAs的含量来实现。本研究首次揭示了四川黑茶配方改善肾脏脂质代谢的潜在机制, 为肥胖相关肾病的治疗提供了依据。

*　　*　　*

作者贡献声明　李卉负责验证、可视化和初稿写作, 张历涵负责数据审编和正式分析, 黄蓉双负责数据审编和研究方法, 任倩负责验证和初稿写作, 郭帆负责研究方法, 石敏和杨乐天负责经费获取, 于洋负责研究项目管理, 马良负责论文构思、经费获取、研究项目管理、审读与编辑写作, 付平负责研究项目管理和监督指导。所有作者已经同意将文章提交给本刊, 且对将要发表的版本进行最终定稿, 并同意对工作的所有方面负责。

利益冲突　所有作者均声明没有利益冲突

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