长期负重跑对老龄大鼠脂肪细胞焦亡相关基因表达的调控作用

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摘要 目的:探究长期负重跑对老龄大鼠脂肪组织中NLRP3介导的细胞焦亡相关基因表达的影响。**方法:**将35只8月龄SD大鼠分为基线组(适应性喂养3 d后取材)、对照组(饲喂32周,不运动)和运动组(饲喂32周,期间进行3次/周的负重跑台训练),最终每组10只(5只死亡)。干预后测试大鼠体质量、体成分、肾周和皮下脂肪组织湿重和血脂;HE染色观察脂肪细胞形态,统计脂肪细胞横截面积;RT-PCR测试ASC、NF-κB、IL-1β、GSDMD、NLRP3和Caspase1 mRNA相对表达水平;免疫荧光染色观察ASC、IL-1β、GSDMD、NLRP3和Caspase1免疫阳性表达,统计积分光密度(IOD)值。**结果:**① 干预后,对照组大鼠体质量高于基线组($P<0.05$);运动组大鼠体质量低于对照组($P<0.05$),体脂率分别低于对照组和基线组($P<0.05$);对照组大鼠肌肉含量百分比低于基线组($P<0.05$),运动组大鼠肌肉含量百分比高于对照组($P<0.05$)。② 干预32周后,对照组大鼠血浆甘油三酯、总胆固醇、高密度脂蛋白胆固醇、低密度脂蛋白胆固醇和游离脂肪酸水平均高于基线组($P<0.05$),运动组甘油三酯和游离脂肪酸水平均低于对照组($P<0.05$)。③ 干预32周后,对照组大鼠肾周和皮下脂肪湿重百分比均高于基线组($P<0.05$);运动组大鼠肾周脂肪湿重百分比分别低于对照组和基线组($P<0.05$),皮下脂肪湿重百分比低于对照组($P<0.05$);运动组大鼠肾周和皮下脂肪细胞横截面积均分别小于基线组和对照组($P<0.05$)。④ 干预32周后,对照组大鼠肾周脂肪中ASC、NF-κB、IL-1β、NLRP3和Caspase1 mRNA相对表达水平均高于基线组($P<0.05$),皮下脂肪中NF-κB和IL-1β mRNA相对表达水平均高于基线组($P<0.05$)。运动组大鼠肾周脂肪中ASC、NF-κB、IL-1β和NLRP3 mRNA相对表达水平均低于对照组($P<0.05$),且NF-κB和NLRP3 mRNA相对表达水平均低于基线组,Caspase1 mRNA相对表达水平显著高于基线组($P<0.05$);皮下脂肪中NF-κB和IL-1β mRNA相对表达水平均低于对照组($P<0.05$),且ASC、NF-κB和Caspase1 mRNA相对表达水平均高于基线组($P<0.05$)。⑤ 干预32周后,对照组大鼠肾周脂肪中NLRP3、Caspase1、GSDMD和IL-1β的IOD值均高于基线组($P<0.05$),运动组大鼠肾周脂肪中Caspase1、GSDMD和IL-1β的IOD值均高于基线组($P<0.05$),NLRP3、Caspase1和GSDMD值均低于对照组($P<0.05$);对照组大鼠皮下脂肪中NLRP3、Caspase1和IL-1β的IOD值均高于基线组($P<0.05$),运动组大鼠皮下脂肪中ASC、NLRP3、Caspase1、GSDMD和IL-1β值均低于对照组($P<0.05$)。**结论:**长期负重跑训练能够降低老龄大鼠体脂率和血脂水平,其作用机制可能与NLRP3介导脂肪细胞焦亡有关。

关键词 衰老;负重跑;脂肪组织;NLRP3炎性小体;细胞焦亡

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衰老是指机体对环境的生理和心理适应能力进行性降低、逐渐趋向死亡的现象。衰老不可避免地导致与年龄相关的疾病发病率增加。在衰老的过程中脂肪组织质量、分布及功能不同程度地发生变化,进而影响人体整体健康水平[1]。无论健康与否,衰老机体中常见脂肪量的堆积[2],脂肪会随年龄增长而重新分布,表现为内脏脂肪增多和下肢皮下脂肪减少[3],然而内脏脂肪堆积会增加机体代谢异常的风险[2];白色脂肪组织作为脂质储存和内分泌器官,衰老可增加其免疫反应,呈现慢性炎症状态[4],是衰老过程中炎性细胞因子的主要来源,因此研究脂肪组织的状态改变对促进老年机体健康具有重要意义[5]。细胞焦亡是一种促炎性程序性细胞死亡方式,其特点是在质膜上形成微孔并释放大量促炎细胞因子[6]。细胞焦亡主要由炎性小体介导,如NOD样受体热蛋白结构域相关蛋白3(NOD-like receptor thermal protein domain associated protein 3,NLRP3)炎性小体,其复合物由NLRP3、凋亡相关斑点样蛋白(apoptosis-associated speck-like protein containing a CARD,ASC)和半胱天冬酶1前体(pro-cysteinyl aspartate specific proteinase-1,pro-Caspase 1)组成,激活可促进形成有活性的Caspase1,使白细胞介素-1β与-18(Interleukin -1β and -18,IL-1β and -18)成熟,细胞焦亡执行分子消皮素(Gasdermin D,GSDMD)裂解,导致细胞穿孔,释放内容物,激活核因子κB(nuclear factor kappa-B,NF-κB)等炎症信号通路[7]。细胞焦亡参与肥胖及相关疾病的发病进程[8],但脂肪细胞焦亡是否参与衰老相关的脂肪质量、分布和功能改变还鲜有研究。

衰老使体力活动水平降低,导致脂肪含量增加和肌肉含量降低[9]。规律的运动训练,尤其是抗阻训练不仅增加衰老机体肌肉质量和功能,还可以降低体脂含量,调节血脂水平,其作用机制是否与调控脂肪组织中的细胞焦亡水平有关尚不明确。因此,本研究以8月龄大鼠为研究对象,探究抗阻训练对大鼠脂肪质量和分布的影响;从脂肪细胞免疫功能入手,探究该训练对内脏和皮下脂肪细胞焦亡相关基因的影响,以明确长期抗阻训练促进老龄机体脂肪功能改善的可能机制,为老年人运动处方的制定提供理论参考。

1 材料与方法

1.1 实验动物

35只SPF级8月龄雄性SD大鼠,购于斯贝福(北京)生物技术有限公司[SCXK(京)2019-0010]。饲养于北京体育大学动物实验室[SYXK(京)2021-0053]。饲养期间各组大鼠自由饮水,饲喂普通饲料。昼夜各半循环照明12 h,相对湿度恒定40%~70%,温度控制在22~25 ℃。所有操作均符合北京体育大学运动科学实验伦理学要求(审批号2018012A)。

1.2 主要实验试剂与设备

异氟烷(河北一品制药股份有限公司,26675-46-7);多聚甲醛(Sercivebio公司,G1101);戊巴比妥钠(Merck,P8410);RNA提取试剂盒(TAKARA公司,9767);逆转录试剂盒(天根公司,KR106-01);NLRP3抗体(Abcam,ab4207)、IL-1β(Novus Biologicals,NB600-633)、ASC抗体(Proteintech,10500-1-AP)、Caspase1抗体(CST,2225)、GSDMD抗体(Santa,sc-393656);全自动生化分析仪(Beckman Coulter,AU480);双能X射线骨密度仪(GE,iDXA);小动物呼吸麻醉机(莱艾特,LAT-10-0500);PCR仪(Bio-rad,T100);石蜡切片机(LEICA,2245);倒置显微镜(LEICA,DMI 4000B);激光共聚焦显微镜(LEICA,TCS SP8);动物跑台(杭州段氏,PT-204);组织破碎仪(Nextadvance,BBY24M)。

1.3 实验方法

1.3.1 实验动物分组及训练 将8月龄的35只雄性大鼠按照随机数字表法分为基线组(n=10)、对照组(n=12)和运动组(n=13),实验期间意外死亡5只,最终每组10只。适应性喂养3 d后,基线组取材(8月龄);对照组大鼠不进行运动,自由饮食32周;运动组进行负重跑训练32周,2组同时取材(16月龄)。

运动组训练方案:进行坡度为35°的负重跑训练。大鼠身着30%体质量负重袋(钢珠填充,专利号:201921178505.1),每跑15 s休息30 s,进行4次为1小组,小组间休息3 min,进行3小组作为1个大循环,大循环间休息间歇为10 min,每天训练2个大循环。隔天训练1次,3次/周[10]。

1.3.2 体成分测试和取材 干预期间记录大鼠的体质量和体成分(异氟烷麻醉后,双能X射线吸收法测量),并记录体成分(脂肪和肌肉含量);取材前各组大鼠禁食12 h,腹腔注射戊巴比妥钠溶液(45 mg/kg体质量)麻醉,腹主动脉取血处死,取大鼠双侧肾周脂肪组织和腹股沟处的皮下脂肪组织,称量湿重,分别置于4%多聚甲醛固定和-80 ℃超低温冰箱保存。各组指标同时进行检测。

1.4　观察指标

1.4.1　血脂测试　使用全自动生化分析仪(Beckman Coulter,AU480)分析大鼠血浆甘油三酯、总胆固醇、高密度脂蛋白胆固醇、低密度脂蛋白胆固醇和游离脂肪酸(试剂盒购于中生北控生物科技股份有限公司)。

1.4.2　苏木精-伊红(hematoxylin-eosin,HE)染色观察脂肪细胞形态　脂肪组织固定 24 h 后,经过浸蜡、包埋、切片后,进行二甲苯脱蜡、梯度乙醇浸泡、Harris 苏木素和 0.5% 伊红分别染色、梯度乙醇浸泡、封片,显微镜下观察并拍照。Image Pro Plus 6.0 软件统计脂肪细胞横截面积。

1.4.3　RT-PCR 检测细胞焦亡基因 mRNA 表达　脂肪组织匀浆研磨,提取总 RNA,检测 RNA 浓度,使用 cDNA 合成试剂盒对所提取的 RNA 进行反转录。用 Oligo 7 软件设计引物,GENEWIZ 公司合成引物,序列为 ASC,F:CTGGACGCTCTTGAAAACTTG,R:CTTCCTCATCTTGTTTTGGTT;NF-κB,F:GGACTTCTGGTGCATTCTGAC,R:CTGGCGTTTCCTCTGTACTTC;IL-1β,F:CAGCAGCATCTCGACAAGAG,R:CATCATCCCACGAGTCACAG;GSDMD,F:CCAGTGCCTCCATGAATGTGT,R:CATCTTCTTCCGGCTTTGGTG;NLRP3,F:AGATTACCCACCCGAGAAAGG,R:CAAACCTATCCACTCCTCTTC;Caspase1,F:GATTGCTGGATGAACTTTTAGA;R:GATAATGAGGGCAAGACGTGTA;β-Tubulin,F:GCGGCAACTATGTGGGGGACT;R:CATGATGCGGTCAGGGTACTC。配制 PCR 反应体系,进行 PCR 扩增标准程序:95 ℃变性 30 s,循环扩增 95 ℃ 30 s→72 ℃ 30 s(循环 30 次),添加溶解曲线。琼脂糖凝胶电泳检测 RNA 表达量。采用$^{\triangle\triangle}$Ct 法表示目的基因的相对表达,β-Tubulin 为内参基因。

1.4.4　免疫荧光染色检测细胞焦亡蛋白免疫阳性表达　脂肪组织石蜡切片后过梯度乙醇,蒸馏水冲洗后进行抗原修复、自发荧光淬灭、血清封闭、孵育 ASC、NLRP3、Caspase1、GSDMD 和 IL-1β 蛋白一抗过夜、孵育荧光二抗 50 min,封片后镜检拍照,统计蛋白质荧光强度,用积分光密度(integrated option density,IOD)值表示。

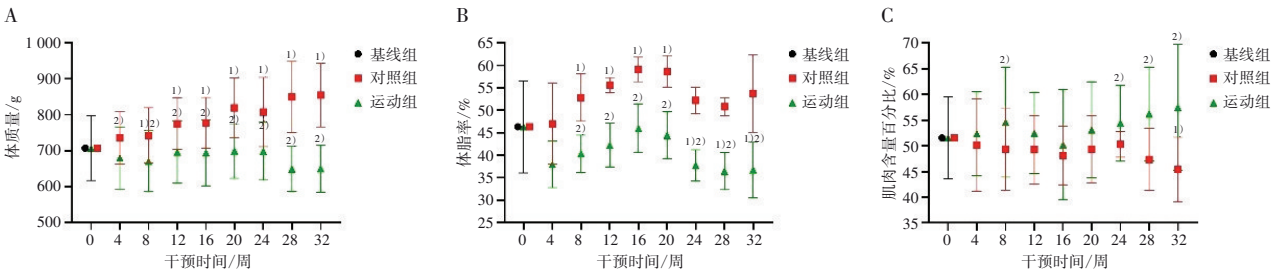
1.5　统计学方法

所有数据经 SPSS 19.0 统计分析,用 Graphpad 软件作图。数据符合正态分布采用($\bar{x}\pm s$)表示,体质量和体成分的比较采用重复测量方差分析;其他指标多组间比较采用单因素方差分析,方差齐用 LSD-t 检验,方差不齐用 Tamhane's $T2$ 检验。以 $P<0.05$ 表示差异有统计学意义。

2　结　果

2.1　3组大鼠体质量和体成分比较

干预期间对照组大鼠体质量整体呈上升趋势,从第 12 周起对照组大鼠体质量高于基线组($P<0.05$);运动组大鼠体质量整体呈下降趋势,从第 4 周起运动组大鼠体质量低于基线组($P<0.05$),且第 8 周体质量低于对照组($P<0.05$),见图 1A。干预第 8、12、16 和 20 周对照组大鼠体脂率(体脂含量/体质量)高于基线组($P<0.05$);从第 8 周起运动组大鼠体脂率低于基线组($P<0.05$),且从第 24 周起体脂率低于对照组($P<0.05$),见图 1B。干预第 32 周,对照组大鼠肌肉含量百分比(肌肉含量/体质量)低于基线组($P<0.05$),干预第 8、24、28 和 32 周,运动组大鼠肌肉含量百分比高于对照组($P<0.05$),见图 1C。



注:与基线组比较,1)$P<0.05$;与对照组比较,2)$P<0.05$。

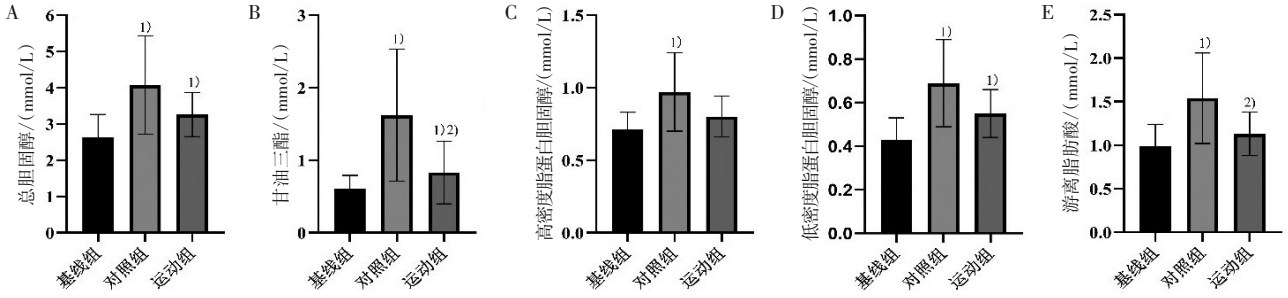
Note: Compared with the baseline group, 1) $P<0.05$; compared with the control group, 2) $P<0.05$.

图 1　干预期间 3 组大鼠体质量和体成分比较

Figure 1　Comparison of body weight and composition in three groups during intervention

2.2 3组大鼠血脂水平比较

干预32周后,对照组大鼠血浆总胆固醇、甘油三酯、高密度脂蛋白胆固醇、低密度脂蛋白胆固醇和游离脂肪酸水平均高于基线组($P<0.05$);运动组大鼠甘油三酯和低密度脂蛋白胆固醇水平均高于基线组($P<0.05$),运动组大鼠甘油三酯和游离脂肪酸水平均低于对照组($P<0.05$),运动组大鼠高密度脂蛋白胆固醇水平较对照组差异无统计学意义($P>0.05$)。见图2。



注:与基线组比较,1) $P<0.05$;与对照组比较,2) $P<0.05$。

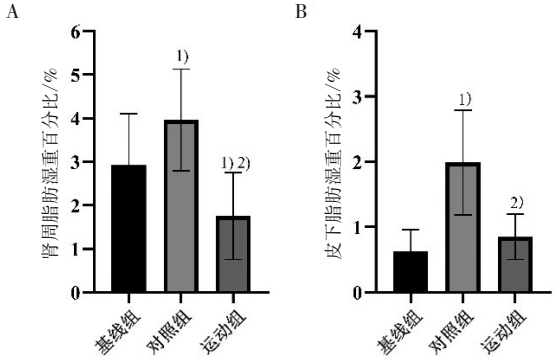
Note: Compared with the baseline group, 1) $P<0.05$; compared with the control group, 2) $P<0.05$.

图2 3组大鼠血脂水平比较

Figure 2 Comparison of blood lipid level of rats in three groups

2.3 3组大鼠脂肪组织湿重比较

干预32周后,对照组大鼠肾周和皮下脂肪湿重百分比均高于基线组($P<0.05$);运动组大鼠肾周脂肪湿重百分比分别低于对照组和基线组($P<0.05$),皮下脂肪湿重百分比低于对照组($P<0.05$)。见图3。



注:与基线组比较,1) $P<0.05$;与对照组比较,2) $P<0.05$。

Note: Compared with the baseline group, 1) $P<0.05$; compared with the control group, 2) $P<0.05$.

图3 3组大鼠肾周和皮下脂肪组织湿重比较

Figure 3 Comparison of pWAT and sWAT wet weight of rats in three groups

2.4 3组大鼠脂肪细胞形态和CSA统计结果比较

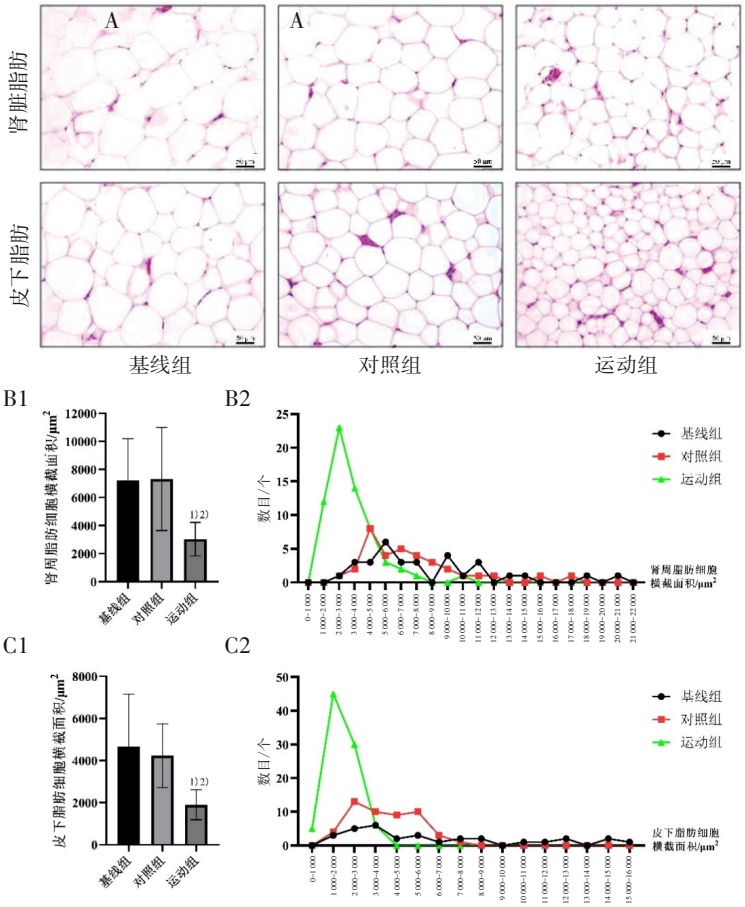
干预32周后,3组大鼠肾周和皮下脂肪细胞形态见图4A(×200)。统计脂肪细胞横截面积,对照组与基线组比较差异无统计学意义($P>0.05$),运动组大鼠肾周和皮下脂肪细胞横截面积均分别小于对照组和基线组($P<0.05$)。见图4B和4C。

2.5 3组大鼠脂肪组织细胞焦亡关键基因的mRNA表达比较

干预32周后,对照组大鼠肾周脂肪中ASC、NF-κB、IL-1β、NLRP3和Caspase1 mRNA相对表达水平均高于基线组($P<0.05$),对照组大鼠皮下脂肪中NF-κB和IL-1β mRNA相对表达水平均高于基线组($P<0.05$)。运动组大鼠肾周脂肪中ASC、NF-κB、IL-1β和NLRP3 mRNA相对表达水平均低于对照组($P<0.05$),且NF-κB和NLRP3 mRNA相对表达水平均低于基线组、Caspase1 mRNA相对表达水平显著高于基线组($P<0.05$);运动组大鼠皮下脂肪中NF-κB和IL-1β mRNA相对表达水平均低于对照组($P<0.05$),且ASC、NF-κB和Caspase1 mRNA相对表达水平均高于基线组($P<0.05$)。见图5。

2.6 3组大鼠脂肪组织细胞焦亡关键蛋白的免疫阳性表达比较

干预32周后,3组大鼠肾周脂肪中ASC、NLRP3、Caspase1、GSDMD和IL-1β免疫荧光染色见图6A~6C,对照组大鼠肾周脂肪中NLRP3、Caspase1、GSDMD和IL-1β的IOD值均高于基线组($P<0.05$),运动组大鼠肾周脂肪中Caspase1、GSDMD和IL-1β的IOD值均高于基线组($P<0.05$),运动组大鼠肾周脂肪中NLRP3、Caspase1和GSDMD值均低于对照组($P<0.05$),见图6D。3组大鼠皮下脂肪中ASC、NLRP3、Caspase1、GSDMD和IL-1β免疫荧光染色见图6E~6G,对照组大鼠皮下脂肪中NLRP3、Caspase1和IL-1β的IOD值均高于基线组($P<0.05$),运动组大鼠皮下脂肪中ASC、NLRP3、Caspase1、GSDMD和IL-1β值均低于对照组($P<0.05$),见图6H。

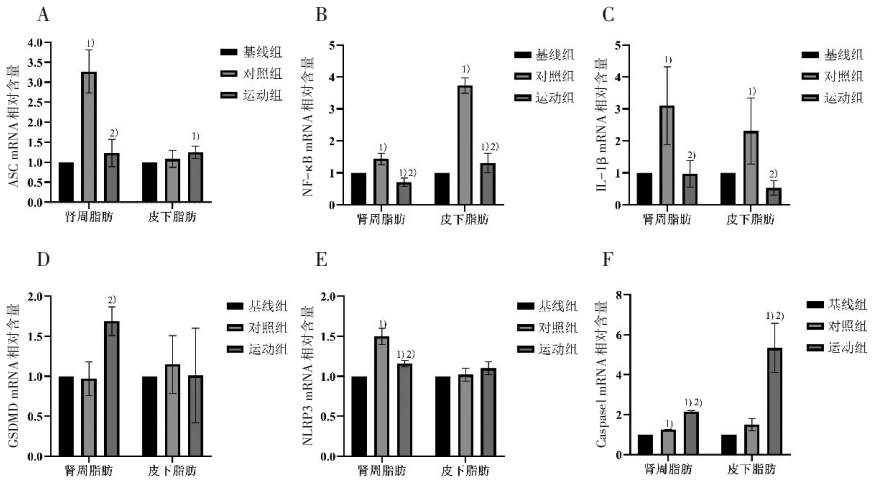


注：与基线组比较，1）$P<0.05$；与对照组比较，2）$P<0.05$。

Note: Compared with the baseline group, 1) $P<0.05$; compared with the control group, 2) $P<0.05$.

图 4　3 组大鼠脂肪细胞形态和 CSA 统计结果比较

Figure 4　Comparison of morphology of adipose tissue and CSA in three groups

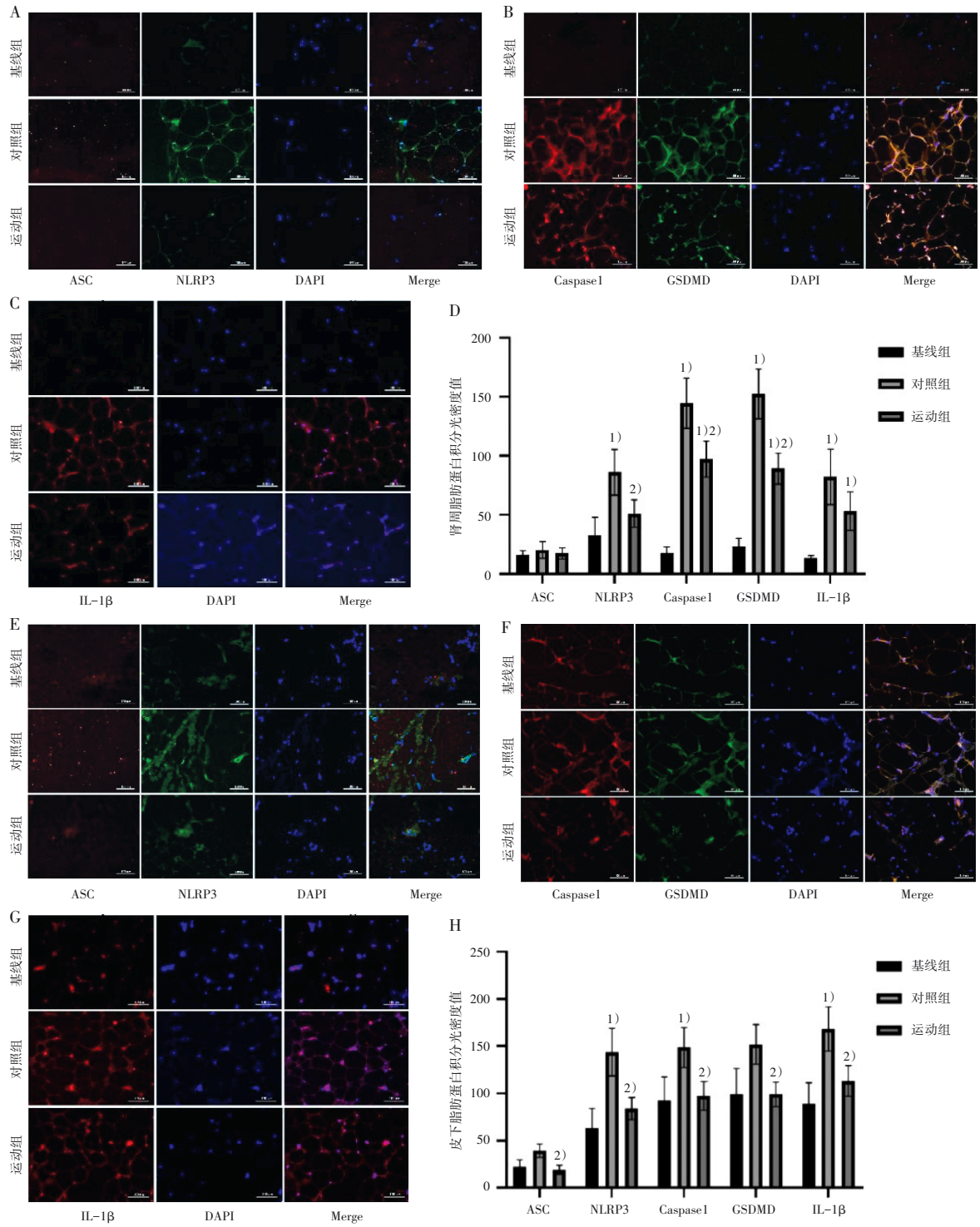


注：与基线组比较，1）$P<0.05$；与对照组比较，2）$P<0.05$。

Note: Compared with the baseline group, 1) $P<0.05$; compared with the control group, 2) $P<0.05$.

图 5　3 组大鼠脂肪细胞焦亡关键基因 mRNA 表达比较

Figure 5　Comparison of pyroptosis key genes mRNA expression of rats adipose tissue in three groups



注:A～D为肾周脂肪免疫荧光染色图和各指标积分光密度值;E～G为皮下脂肪免疫荧光染色图和各指标积分光密度值;DAPI染色为蓝色,代表细胞核;Merge为融合图。与基线组比较,1) $P<0.05$;与对照组比较,2) $P<0.05$。

Note: A-D are immunofluorescence (IF) diagrams and IOD of all the proteins in pWAT; E-G are immunofluorescence (IF) diagrams and IOD of all the proteins in sWAT; DAPI is dyeing chromatin nucleus, blue. Compared with the baseline group, 1) $P<0.05$; compared with the control group, 2) $P<0.05$.

图6　3组大鼠脂肪组织免疫荧光染色图和IOD值比较(×400)

Figure 6　Comparison of immunofluorescence staining of rats adipose tissue and IOD in three groups (×400)

3 讨 论

脂肪组织作为最大的能量储存和内分泌器官，在能量和代谢稳态中发挥重要的作用。随着年龄的增长，机体内分泌功能失调，脂肪组织缓冲多余营养物质的能力降低，使得老年人易发生肥胖等相关疾病[11]。

衰老致使脂肪组织的关键结构和功能改变，从而影响脂肪细胞因子分泌，不仅会引起脂肪的堆积和分布的改变，还会导致骨骼肌质量和功能的降低[12]。本研究中，老龄大鼠的体质量呈增龄性增长，体脂率也均高于基础值，肌肉含量百分比呈增龄性下降。衰老所致的脂肪再分布表现为内脏脂肪的优先增加。一般而言，皮下脂肪的增加对代谢具有积极的作用，而内脏脂肪的增加会导致代谢异常[13]，特别会增加胰岛素抵抗、糖尿病和心血管疾病的风险[14]。本研究中，干预32周后大鼠内脏脂肪（肾周脂肪）和皮下脂肪湿重均显著增加，但脂肪细胞CSA没有显著改变，说明衰老引起的脂肪堆积主要由脂肪数量的增加所致[15]。此外，衰老大鼠血脂水平均显著增加。衰老会降低脂肪代谢灵活性，主要是指脂肪细胞调节脂肪酸储存和释放的能力降低，导致血脂水平增加，这与炎症反应有关[16]。

抗阻训练是应对增龄所致肌肉减少、脂肪增加的非药物性的有效方法之一[17-19]。为了避免运动强度和运动量过大对老龄大鼠的损伤，本研究选择负重跑运动作为抗阻训练的方案[20]。结果显示，负重跑训练可降低老龄大鼠的体质量和体脂率，并增加肌肉含量百分比；降低内脏和皮下脂肪湿重和脂肪细胞CSA，以及血脂水平。研究发现，平均年龄为63岁的久坐少动女性进行16周抗阻训练可以降低血液中IL-6、瘦素和抵抗素的水平[21]；增龄所致肌肉减少性肥胖症患者进行抗阻训练可通过抑制促炎因子的释放，以促进骨骼肌蛋白质的合成和降低脂肪含量[22]。高强度间歇训练（high intensity intermittent training，HIIT）较中等强度持续训练（moderate intensity continuous training，MICT）更有效地抑制增龄大鼠内脏脂肪中衰老相关分泌表型（senescence-associated secretory phenotype，SASP），该过程与抑制脂肪中NF-κB的激活有关。提示运动训练可能通过调节老龄机体脂肪组织的炎症状态而抑制脂肪的堆积[23]。

白色脂肪组织中与增龄最相关的特征为免疫反应，且在中年时期就可观察到[24]。在衰老过程中，来自脂肪组织分泌的促炎细胞因子被认为是全身性慢性低度炎症的主要因素，且循环系统中30%的促炎因子来自于内脏脂肪[25-26]。本研究中，衰老显著增加了NLRP3介导的细胞焦亡相关基因和蛋白的表达，但并未提示这2种脂肪组织间的差异，有待进一步的观察研究。脂肪巨噬细胞含量已被证明与衰老呈正相关，巨噬细胞衍生的NLRP3炎症小体可能与活化的T细胞协同作用，从而诱导脂肪组织炎症的发展[27]。此外，本研究中增龄引起增高的低密度脂蛋白胆固醇和甘油三酯等指标也可激活NLRP3炎性小体，而NLRP3缺失可防止肥胖引起的脂质沉积[28-29]。增龄所致的脂肪组织炎症与NF-κB信号通路激活有关，后者可以促进脂肪组织巨噬细胞促炎极化。NF-κB是GSDMD的重要转录因子。当细胞外细胞焦亡相关信号激活NLRP3炎症小体［由核苷酸结合寡聚化结构域（nucleotide-binding oligomerization domain，NOD）、富含亮氨酸重复序列（leucin-rich repeat，LRR）、热蛋白结构域（pyrin domain，PYD）、ASC和Caspase1组成］，GSDMD随后被切割，导致GSDMD的N端释放，从而在细胞膜中形成孔隙，导致促炎物质释放和细胞肿胀。NLRP激活与多种年龄相关疾病有关[30-31]。

研究发现，运动训练对新陈代谢的影响伴随着Toll样受体（Toll-like receptor，TLR）和促炎细胞因子水平的降低[32-33]。考虑到TLR参与NLRP3炎症小体的激活[34]，我们推测运动可能通过抑制NLRP3的激活而抑制老龄大鼠脂肪细胞焦亡。本研究中，负重跑训练均显著降低了这2种脂肪组织中NLRP3介导的细胞焦亡关键基因和蛋白的表达。有研究证实了本研究的发现，8周50 min/d中等强度的跑台训练可抑制高脂膳食所诱导肥胖机体（内脏和皮下）脂肪组织中NLRP3的激活[34]。其可能机制在于在运动过程中，过氧化物酶体增殖物激活受体-γ辅激活因子-1α（peroxisome proliferator-activated receptor-gamma coactivator-1 alpha，PGC1α）和腺苷酸活化蛋白激酶（AMP-activated protein kinase，AMPK）从骨骼肌释放到血液中，通过调节巨噬细胞活性参与抑制脂肪组织炎症和改善脂代谢功能[35]。

综上，本研究发现32周负重跑训练可改善老龄大鼠体成分，降低血脂水平。其作用机制可能与抑制脂肪组织中NLRP3介导的细胞焦亡相关基因和蛋白的表达有关。

参考文献

[1] LÓPEZ-OTÍN C，BLASCO M A，PARTRIDGE L，et al. The hallmarks of aging［J］. Cell，2013，153(6)：1194-1217.

[2] RAGUSO C A，KYLE U，KOSSOVSKY M P，et al. A 3-year longitudinal study on body composition changes in the elderly：role of physical exercise［J］. Clin Nutr，2006，25(4)：573-580.

[3] DIRKS A，LEEUWENBURGH C. Apoptosis in skeletal muscle with aging［J］. Am J Physiol Regul Integr Comp Physiol，2002，282(2)：R519-R527.

[4] CAMELL C D, SANDER J, SPADARO O, et al. Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing [J]. Nature, 2017, 550(7674): 119-123.
[5] OU M Y, ZHANG H, TAN P C, et al. Adipose tissue aging: mechanisms and therapeutic implications [J]. Cell Death Dis, 2022, 13(4): 300.
[6] FINUCANE O M, LYONS C L, MURPHY A M, et al. Monounsaturated fatty acid-enriched high-fat diets impede adipose NLRP3 inflammasome-mediated IL-1β secretion and insulin resistance despite obesity [J]. Diabetes, 2015, 64(6): 2116-2128.
[7] BURDETTE B E, ESPARZA A N, ZHU H, et al. Gasdermin D in pyroptosis [J]. Acta Pharma Sin B, 2021, 11(9): 2768-2782.
[8] PAN M, TAIR Q, LIUY R, et al. Pyroptosis: a possible link between obesity-related inflammation and inflammatory diseases [J]. J Cell Physiol, 2022, 237(2): 1245-1265.
[9] DE CARVALHO F G, JUSTICE J N, FREITAS E C, et al. Adipose tissue quality in aging: how structural and functional aspects of adipose tissue impact skeletal muscle quality [J]. Nutrients, 2019, 11(11): 2553.
[10] 翁锡全,林文弢,孟艳,等. 衰老大鼠跑台抗阻训练模型的实验研究[J]. 中国运动医学杂志, 2013, 32(3): 226-231, 235.
WENG X Q, LIN W T, MENG Y, et al. Experimental study on establishment of resistance training model for aged rats [J]. Chin J Sports Med, 2013, 32(3): 226-231, 235.
[11] OUCHI N, PARKER J L, LUGUS J J, et al. Adipokines in inflammation and metabolic disease [J]. Nat Rev Immunol, 2011, 11(2): 85-97.
[12] PELLEGRINELLI V, ROUAULT C, RODRIGUEZ-CUENCA S, et al. Human adipocytes induce inflammation and atrophy in muscle cells during obesity [J]. Diabetes, 2015, 64(9): 3121-3134.
[13] 代火祥,张欣,申军,等. 衰老过程中的脂肪组织炎症研究进展[J]. 中国全科医学, 2019, 22(32): 4020-4026.
DAI H X, ZHANG X, SHEN J, et al. Adipose tissue inflammation in aging [J]. Chin Gen Pract, 2019, 22(32): 4020-4026.
[14] GAVI S, FEINER J J, MELENDEZ M M, et al. Limb fat to trunk fat ratio in elderly persons is a strong determinant of insulin resistance and adiponectin levels [J]. J Gerontol A Biol Sci Med Sci, 2007, 62(9): 997-1001.
[15] KARAGIANNIDES I, TCHKONIA T, DOBSON D E, et al. Altered expression of C/EBP family members results in decreased adipogenesis with aging [J]. Am J Physiol Regul Integr Comp Physiol, 2001, 280(6): R1772-R1780.
[16] SPARKS L M, UKROPCOVA B, SMITH J, et al. Relation of adipose tissue to metabolic flexibility [J]. Diabetes Res Clin Pract, 2009, 83(1): 32-43.
[17] LUO L, LU A M, WANG Y, et al. Chronic resistance training activates autophagy and reduces apoptosis of muscle cells by modulating IGF-1 and its receptors, Akt/mTOR and Akt/FOXO3a signaling in aged rats [J]. Exp Gerontol, 2013, 48(4): 427-436.
[18] 杨星雅,李鹏飞,李良,等. 有氧运动和抗阻运动对肥胖大鼠脂肪组织内质网应激及炎症反应的影响[J]. 中国运动医学杂志, 2021, 40(2): 129-137.
YANG X Y, LI P F, LI L, et al. Effects of aerobic and resistance training on endoplasmic reticulum stress and inflammation of adipose tissue of obese rats [J]. Chin J Sports Med, 2021, 40(2): 129-137.
[19] 齐玉刚,王津,徐冬青. 有氧抗阻结合与单纯有氧运动减重干预的对比研究[J]. 天津体育学院学报, 2020, 35(5): 541-544.
QI Y G, WANG J, XU D Q. Comparison of the effects of aerobic-resistance and aerobic training on body weight control in female university students with obesity [J]. J Tianjin Univ Sport, 2020, 35(5): 541-544.
[20] 胡敏,邹亮畴,刘承宜,等. 大鼠抗阻训练模型的归类与分析[J]. 广州体育学院学报, 2009, 29(5): 91-96.
HU M, ZOU L C, LIU C Y, et al. Classification and analysis of progressive resistance training model of rats [J]. J Guangzhou Sport Univ, 2009, 29(5): 91-96.
[21] PRESTES J, SHIGUEMOTO G, BOTERO J P, et al. Effects of resistance training on resistin, leptin, cytokines, and muscle force in elderly post-menopausal women [J]. J Sports Sci, 2009, 27(14): 1607-1615.
[22] ALIZADEH P H. Exercise therapy for people with sarcopenic obesity: myokines and adipokines as effective actors [J]. Front Endocrinol (Lausanne), 2022, 13: 811751.
[23] 马松,李方晖,吴大帅,等. 高强度间歇训练对增龄大鼠脂肪组织衰老相关分泌表型的影响及其机制探讨[J]. 中国体育科技, 2022, 8(58): 1-9.
MA S, LI F H, WU D S, et al. Effects and mechanisms of high-intensity intervals training on senescence-associated secretory phenotype in adipose tissue of aging rats and mechanisms [J]. Chin Sport Sci Tech, 2022, 8(58): 1-9.
[24] SCHAUM N, LEHALLIER B, HAHN O, et al. Ageing hallmarks exhibit organ-specific temporal signatures [J]. Nature, 2020, 583(7817): 596-602.
[25] 徐唯. 炎性衰老、肌肉丢失与抗阻运动[J]. 中国老年学杂志, 2016, 36(23): 6035-6037.
XU W. Inflammatory aging, muscle loss and resistance exercise [J]. Chin J Gerontol, 2016, 36(23): 6035-6037.
[26] 林凯玲,薛艳,余惠珍. NLRP3炎性小体与慢性炎症相关疾病的研究进展[J]. 中国临床新医学, 2020, 13(7): 733-737.
LIN K L, XU Y, YU H Z. Research progress in inflammasome NLRP3 and chronic inflammatory related diseases [J]. Chin J New Clin Med, 2020, 13(7): 733-737.
[27] VANDANMAGSAR B, YOUM Y H, RAVUSSIN A, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance [J]. Nat Med, 2011, 17(2): 179-188.
[28] 贺润铖,黄芷棋,冯倩倩,等. NLRP3炎症小体在机体脂质代谢中的作用研究进展[J]. 中国药理学与毒理学杂志, 2021, 35(10): 784-785.
HE R C, HUANG Z Q, FENG Q Q, et al. Research progress on the role of NLRP3 inflammatory bodies in lipid metabolism [J]. Chin J Pharmacol Toxicol, 2021, 35(10): 784-785.
[29] JOHNSON A R, MILNER J J, MAKOWSKI L. The inflammation highway: metabolism accelerates inflammatory traffic in obesity [J]. Immunol Rev, 2012, 249(1): 218-238.
[30] CLAVIJO-CORNEJO D, MARTÍNEZ-FLORES K, SILVA-LUNA K. The overexpression of NALP3 inflammasome in knee osteoarthritis is associated with synovial membrane prolidase and NADPH oxidase 2 [J]. Oxid Med Cell Longev, 2016, 9: 1472567.
[31] TRIM W, TURNER J E, THOMPSON D. Parallels in immunometabolic adipose tissue dysfunction with ageing and obesity [J]. Front Immunol, 2018, 9: 169.
[32] 林小晶,汪燕,王凡,等. 有氧运动降低动脉粥样硬化大鼠血清

和组织Lp-PLA2水平及意义[J]. 中国体育科技,2019,55(1):28-36.
LIN X J,WANG Y,WANG F,et al. Inhibition of Lp-PLA2 in the serum and tissues of atherosclerotic rats through aerobic exercise and its significance [J]. Chin Sport Sci Tech, 2019, 55(1): 28-36.
[33] 刘恺麟. 运动对NLRP3介导的少肌性肥胖衰老小鼠的干预机制研究[D]. 上海:华东师范大学,2021:39-40.
LIU K L. Intervention mechanism of exercise on NLRP3-mediated in obesity sacropenia aging mice [D]. Shanghai: East China Normal University,2021:39-40.
[34] JAVAID H M A,SAHAR N E,ZHUGE D L,et al. Exercise inhibits NLRP3 inflammasome activation in obese mice via the anti-inflammatory effect of meteorin-like [J]. Cells, 2021, 10(12): 3480.
[35] RAO R R,LONG J Z,WHITE J P,et al. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis [J]. Cell,2014,157(6):1279-1291.

Regulatory Effect of Pyroptosis Related Genes Expression in Adipose Tissue of Aging Rats by Long-Time Weight-Bearing Running

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ABSTRACT **Objective:** To explore the effect of long-time weight-bearing running on the expression of NLRP3-mediated pyroptosis-related genes and proteins in adipose tissue of aging rats. **Methods:** A total of thirty-five 8-month-old SD rats were randomly divided into three groups: the baseline group (the samples were taken after 3 days of adaptive feeding); the control group (fed for 32 weeks, without exercise); and the resistance training group (fed for 32 weeks, with 3 times/week of weight-bearing running training), with 10 mice per group (5 mice died). After the intervention, the body weight, body composition, perirenal and subcutaneous adipose tissue (p/sWAT) wet weight and blood lipids were measured; the morphology of adipocytes were observed by HE staining, and the cross-sectional area (CSA) of adipocyte was calculated; the relative expression level of ASC, NF-κB, IL-1β, GSDMD, NLRP3 and Caspase1 mRNA were tested by RT-PCR; the immunopositive expression of ASC, IL-1β, GSDMD, NLRP3 and Caspase1 were observed by Immunofluorescence, and the integral optical density (IOD) value was calculated. **Results:** (1) After the intervention, the body weight of rats in the control group was higher than that in the baseline group, and the body weight of rats in the resistance training group was lower than that in the control group (P<0.05); the body fat percentage of the resistance training group was lower than those in the control and baseline groups (P<0.05), respectively; the muscle content percentage of rats in the control group was lower than that in the baseline group, and the muscle content percentage of rats in the resistance training group was higher than that in the control group (P<0.05). (2) After 32 weeks of intervention, the plasma levels of TG, TC, HDL-C, LDL-C and FFA of rats in the control group were higher than those in the baseline group, and the levels of TG and FFA of rats in the resistance training group were lower than those in the control group (P<0.05). (3) After 32 weeks of intervention, the wet weight percentages of pWAT and sWAT of rats in the control group were higher than those in the baseline group; the wet weight percentage of pWAT of rats in the resistance training group was lower than those in both the control and baseline groups, and the wet weight percentage of sWAT of rats was lower than that in the control group (P<0.05); the CSA of adipocyte of sWAT and pWAT of rats in the resistance training group was smaller than those in the control and baseline groups (P<0.05), respectively. (4) After 32 weeks of intervention, the relative expression levels of ASC, NF-κB, IL-1β, NLRP3 and Caspase1 mRNA in pWAT of rats in the control group were higher than those in the baseline group, and the relative expression levels of NF-κB and IL-1β mRNA in sWAT were higher than those in the baseline group (P<0.05). The relative expression levels of ASC, NF-κB, IL-1β and NLRP3 mRNA in pWAT of rats in the resistance training group were lower than those in the control group (P<0.05), and the relative expression levels of NF-κB and NLRP3 mRNA were lower than those in the baseline group, and the relative expression level of Caspase1 mRNA was higher than that in the baseline group (P<0.05).The relative expression levels of NF-κB and IL-1β mRNA in sWAT of rats in the resistance group were lower than those in the control group, and the relative expression levels of ASC, NF-κB and Caspase1 mRNA were higher than those in the baseline group (P<0.05). (5) After 32 weeks of intervention, the IOD values of NLRP3, Caspase1, GSDMD and IL-1β in pWAT of rats in the control group were higher than those in the baseline group (P<0.05). The IOD values of Caspase1, GSDMD and IL-1β in the resistance training group were higher than those in the baseline group (P<0.05), and the IOD values of NLRP3, Caspase1 and GSDMD were all lower than those in the control group (P<0.05). The IOD values of NLRP3, Caspase1 and IL-1β in sWAT of rats in the control group were higher than those in the baseline group (P<0.05), and the IOD values of ASC, NLRP3, Caspase1, GSDMD and IL-1β in the resistance training group were lower than those in the control group (P<0.05). **Conclusion:** The body fat percentage and blood lipid levels in aging rats are reduced by weight-bearing running training, via inhibiting the expression of NLRP3-mediated pyroptosis-related genes and protein of adipose tissue.

KEY WORDS aging; weight-bearing running; adipose tissue; NLRP3 inflammasome; pyroptosis
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