摘 要

目的：探讨游泳对自发性高血压(SHR)大鼠血压和心功能及心肌AGEs/RAGE-p38 MAPK-NF-kB 通路的影响。

方法：选用雄性 WKY 大鼠 24 只，随机分为 3 组，分别是 0 周正常对照组(C-0 组，n=8)、4 周正常对照组(C-4 组，n=8)、8 周正常对照组(C-8 组，n=8)。雄性 SHR 大鼠 56 只，随机分为 7 组，分别是 0 周安静对照组(S-0 组，n=8)、4 周安静对照组(S-4 组，n=8)、8 周安静对照组(S-8 组，n=8)、4 周 30 分钟运动组(SE-4 组，n=8)、4 周 60 分钟运动组(LE-4 组，n=8)、8 周 30 分钟运动组(SE-8 组，n=8)、8 周 60 分钟运动组(LE-8 组，n=8)。正常对照组、安静对照组不运动，运动组运动方式：游泳；运动强度（尾部负荷）：0%bw；运动时间：SE-4 组和 SE-8 组时间设为 30min/d，5d/w；LE-4 组和 LE-8 组时间设为 60min/d，5d/w。持续周数：SE-4 组和 LE-4 组持续 4w，SE-8 组和 LE-8 组持续 8w。进行连续 4 周、8 周的游泳训练。采用尾套法测定大鼠 SBP、DBP；超声测量心脏射血分数（EF）、短轴缩短分数（FS）、左心室质量（LVM）、左心室舒张末期容积（LVEDV）、左心室收缩末期容积（LVESV）、每搏输出量（SV）；心肌 HE 染色观察心肌形态变化；碱水解法测定心肌羟脯氨酸含量；免疫组化法测定心肌组织晚期糖基化终末产物（AGEs）、晚期糖基化终末产物受体（RAGE）、p38 丝裂原活化蛋白激酶（磷酸化 p38 MAPK）、核转录因子 kB（NF-kB）表达。

结果：⑴血压：与同周龄 WKY 大鼠相比较，SHR 安静对照组大鼠 SBP、DPB 均显著升高；不同周龄 SHR 安静对照组的 SBP、DPB 随周龄增长均显著升高；与同周龄 SHR 安静对照组相比较，运动组 SBP、DPB 均显著降低；与同周龄 SHR 大鼠 30min 运动组相比较，LE-4 组 DPB 显著降低。

⑵心功能：与 C-0 组比较，S-4 组 EF、FS 显著性下降，S-8 组 LVM 显著性升高；与 S-0 相比，S-4 组 LVM、LVESV 显著性增加，S-8 组 LVM 显著性升高，SE-4 组 SV 升高，SE-8 组 LVEDV 升高，LE-8 组 SV 升高；与同周龄 WKY 大鼠相比较，SHR 安静对照组大鼠 S-4 组 LVESV 显著性升高，S-8 组 LVM 显著性升高；与 S-4 组比较，SE-4 组的 EF、FS 显著性升高，LE-4 组 FS 显著性升高，LVESV 显著性下降；与 SE-4 组比较，SE-8 组 FS 显著下降。

⑶心肌 HE 染色：WKY 对照组心肌排列较为整齐，心肌细胞大小正常；与对照组相比，SHR 大鼠心肌纤维横径增粗、排列紊乱、细胞较肥大、部分肌丝断裂；运动组心肌排列相对整齐，结构较为清晰，心肌细胞肥大有一定的减小。

⑷羟脯氨酸：与 WKY 大鼠组比较，S-8 组羟脯氨酸显著性上升；与同周龄 SHR 大鼠安静组相比较，运动组中 LE-4 组、SE-8 组、LE-8 组羟脯氨酸显著性下降。

⑸心肌 AGEs 和 RAGE 含量：与同周龄 WKY 大鼠相比较，SHR 安静对照组大鼠中 S-4 组、S-8 组 AGEs、RAGE 含量极显著性升高；不同周龄 SHR 安静对照组比较，S-4 组、S-8 组 AGEs、RAGE 含量极显著性升高；与同周龄 SHR 安静对照组相比较，运动组中 LE-4 组、SE-8 组、LE-8 组 AGEs、RAGE 显著性降低，SE-4 组 AGEs 显著性降低；与同周龄 SHR 大鼠 30min 运动组相比较，LE-8 组 RAGE 含量降低；SHR 大鼠不同周数运动组相比较，LE-8 组比 LE-4 组 RAGE 含量降低。

⑹心肌磷酸化 p38 MAPK、NF-kB 表达：与同周龄 WKY 大鼠相比较，S-4 组、S-8 组磷酸化 p38 MAPK、NF-kB 表达极显著性升高；不同周龄 SHR 安静对照组比较，S-4 组、S-8 组磷酸化 p38 MAPK、NF-kB 表达极显著性升高；与同周龄 SHR 安静对照组相比较，运动组的 SE-4 组、LE-4 组、SE-8 组、LE-8 组磷酸化 p38 MAPK 极显著性降低，SE-4 组、LE-4 组 NF-kB 表达极显著性降低。

结论：（1）SHR 大鼠增龄性血压升高，心功能下降，同时伴有心肌 AGEs、RAGE 表达增高。有氧运动可有效阻止 SHR 大鼠增龄性血压的升高，改善心功能，同时降低心肌 AGEs、RAGE 含量，说明有氧运动可能是通过减少心肌糖基化来减缓高血压发展和心功能障碍的发生。

（2）有氧运动能阻止 SHR 大鼠心肌磷酸化 p38 MAPK 活性上升，减少 NF-kB 核移位，表明有氧运动预防和改善高血压的机制可能与 AGEs/RAGE-p38 MAPK-NF-kB 通路有关。

（3）不同运动时间和持续周数的有氧运动，都能有效降低血压和改善心功能。除更长的运动时间和持续周数可使 RAGE 表达降低更显著之外，其余指标无显著性差异。表明有氧运动改善大鼠血压、心功能的效应与运动时间、持续周数不是递增关系。

关键词：SHR 大鼠；有氧运动；血压；心功能；晚期糖基化终末产物（AGEs）

目 录

Abstract

Objective: To investigate the effects of swimming on blood pressure, cardiac function and myocardial AGEs/RAGE-P38 MAPK-NF-kB pathway in spontaneously hypertensive rats (SHR).

Methods: Twenty four male WKY rats were randomly divided into three groups: 0-week normal control group (C-0 group, n=8), 4-week normal control group (C-4 group, n=8), and 8-week normal control group (C-8 group, n=8). 56 male SHR rats were randomly divided into 7 groups: the 0 week quiet control group (S-0 group, n=8), the 4 week quiet control group (S-4 group, n=8), the 8 week quiet control group (S-8 group, n=8), the 4 week 30 minute exercise group (SE-4 group, n=8), the 4 week 60 minute exercise group (LE-4 group, n=8), the 8 week 30 minute exercise group (SE-8 group, n=8), and the 8 week 60 minute exercise group (LE-8 group, n=8). The normal control group and the quiet control group did not exercise, and the exercise mode of the exercise group was swimming; Exercise intensity: 0% bw; Exercise time: the time of SE-4 and SE-8 groups was set as 30min/d, 5d/w; The time of LE-4 group and LE-8 group was set at 60min/d, 5d/w. Duration weeks: SE-4 and LE-4 lasted for 4 weeks, and SE-8 and LE-8 lasted for 8 weeks. Conduct swimming training for 4 and 8 weeks in a row. The SBP and DBP of rats were measured by tail cuff method; Cardiac EF, FS, LVM, LVEDV, LVESV and SV were measured by ultrasound; The morphological changes of myocardium were observed by HE staining; The content of hydroxyproline in myocardium was determined by alkaline hydrolysis method; The expression of AGEs, RAGE, phosphorylated p38 MAPK and NF-kB in myocardial tissue was determined by immunohistochemistry.

Results: (1) Blood pressure: Compared with WKY rats of the same age, SBP and DPB in SHR quiet control group were significantly increased; The SBP and DPB of SHR quiet control group at different ages increased significantly with the increase of age; Compared with the SHR quiet control group at the same age, SBP and DPB in the exercise group were significantly decreased; Compared with the same week-old SHR rats in the 30min exercise group, the DPB in LE-4 group was significantly reduced.

(2) Cardiac function: compared with C-0 group, EF and FS in S-4 group decreased significantly, while LVM in S-8 group increased significantly; Compared with S-0, LVM and LVESV were significantly increased in S-4 group, LVM in S-8 group, SV in SE-4 group, LVEDV in SE-8 group and SV in LE-8 group; Compared with WKY rats of the same age, LVESV in S-4 group and LVM in S-8 group were

significantly higher in SHR quiet control group; Compared with S-4 group, EF and FS in SE-4 group increased significantly, FS in LE-4 group increased significantly, and LVESV decreased significantly; Compared with SE-4 group, FS in SE-8 group decreased significantly.

(3) Myocardial HE staining: The myocardium of WKY control group was arranged orderly and the size of myocardial cells was normal; Compared with the control group, the transverse diameter of myocardial fibers in SHR rats increased, the arrangement was disordered, the hypertrophy of cells, and the fragmentation of myofilament; In the exercise group, the myocardial arrangement was relatively neat, the structure was relatively clear, and the hypertrophy of myocardial cells was reduced to a certain extent.

(4) Hydroxyproline: Compared with WKY rats, hydroxyproline in S-8 group increased significantly; Compared with the quiet group of SHR rats of the same age, hydroxyproline in the exercise group was significantly decreased in the LE-4 group, SE-8 group and LE-8 group.

(5) The contents of AGEs and RAGE in myocardium: compared with WKY rats of the same age, the contents of AGEs and RAGE in S-4 and S-8 groups in SHR quiet control group were significantly increased; Compared with the quiet control group of SHR at different weeks of age, the contents of AGEs and RAGE in S-4 and S-8 groups were significantly increased; Compared with SHR quiet control group at the same age, AGEs and RAGE in LE-4 group, SE-8 group and LE-8 group in the exercise group decreased significantly, while AGEs in SE-4 group decreased significantly; Compared with the same week-old SHR rats in the 30min exercise group, the content of RAGE in LE-8 group decreased; Compared with the exercise group of SHR rats for different weeks, the content of RAGE in LE-8 group was lower than that in LE-4 group.

(6) The expression of phosphorylated p38 MAPK and NF-kB in myocardium: compared with WKY rats of the same age, the expression of phosphorylated p38 MAPK and NF-kB in S-4 and S-8 groups was significantly increased; The expression of phosphorylated p38 MAPK and NF-kB in S-4 group and S-8 group was significantly higher than that in SHR quiet control group at different weeks of age; Compared with SHR quiet control group at the same age, the phosphorylated p38 MAPK in SE-4 group, LE-4 group, SE-8 group and LE-8 group of exercise group was significantly decreased, and the expression of NF-kB in SE-4 group and LE-4 group was significantly decreased.

Conclusion: (1) In SHR rats, blood pressure increased with age, cardiac function decreased, and myocardial AGEs and RAGE increased. Aerobic exercise can effectively prevent the increase of aging blood pressure in SHR rats, improve cardiac function, and reduce the contents of cardiac AGEs and RAGE, indicating that aerobic exercise may slow down the development of hypertension and cardiac dysfunction by reducing myocardial glycosylation.

(2) Aerobic exercise can prevent the increase of phosphorylated p38 MAPK activity in myocardium of SHR rats and reduce NF-kB nuclear translocation, indicating that the mechanism of aerobic exercise to prevent and improve hypertension may be related to AGEs/RAGE-p38 MAPK-NF-kB pathway.

(3) Aerobic exercise with different exercise time and duration can effectively reduce blood pressure and improve cardiac function. There was no significant difference in other indexes except that longer exercise time and duration of weeks could significantly reduce the expression of RAGE. It indicates that the time and weeks of aerobic exercise may not have an increasing relationship with the improvement effect of blood pressure and cardiac function in rats.

Key Words： Spontaneously hypertensive rats; Aerobic exercise; Blood pressure; Cardiac function; AGEs

中英文名词缩略词表

缩写	英文全称	中文全称
WKY 大鼠	Wistar-Kyoto rats	京都种 Wistar 大鼠
SHR 大鼠	Spontaneously hypertensive rats	自发性高血压大鼠
AGEs	Advanced glycation end products	晚期糖基化终末产物
RAGE	Receptor advanced glycation end products	晚期糖基化终末产物受体
p38 MAPK	P38 mitogen-activated protein kinases	p38 丝裂原活化蛋白激酶
NF-kB	Nuclear transcription factor	核转录因子
SBP	Systolic blood pressure,	收缩压
DBP	Diastolic blood pressure	舒张压
EF	Eject fiction	射血分数
FS	Fractional shortening	短轴缩短分数
LVM	Left ventricle mass	左心室质量
LVEDV	Left ventricle end diastolic volume	左心室舒张末期容积
LVESV	Left ventricle end systolic volume	左心室收缩末期容积
SV	Stroke volume	每搏输出量
MG	Methylglyoxal	甲基乙二醛
3DG	3-deoxy-D-glucosone	3-脱氧-D-葡萄糖酮
GO	Glyoxal	乙二醛
CML	Nε-carboxymethyl-lysine	Nε-羧甲基赖氨酸
ALE	advanced lipid end products	脂质终产物
MDA	Malondialdehyde	丙二醛
MG-HL	Methylglyoxal-derived hydroimidazolone	甲基乙二醛衍生的氢咪唑酮
esRAGE	Endogenous secretory RAGE	内源性分泌 RAGE
sRAGE	Soluble RAGE	可溶性 RAGE
ICAM-1	Intercellular cell adhesion molecule-1	细胞间粘附分子-1
VCAM-1	Vascular cell adhesion molecule-1	血管细胞粘附分子-1
VEGF	Vascular endothelial growth factor	血管内皮生长因子
IL-1α	Interleukin-1α	白介素-1α
IL-6	Interleukin-6	白介素-6
TNF-α	Tumor Necrosis Factor-α	肿瘤坏死因子-α

第 1 章　前言

1.1　研究的背景

高血压是危害人们身体健康的最常见的心血管疾病之一，中国高血压发病率不断增加，根据中国慢性病及危险因素监测室的调查显示，2018 我国成年居民高血压患病率为 27.5%（95%CI:26.6%～28.4%）[①]。高血压会引起脑、心脏、肾脏和视网膜等器官发生病变，是导致心力衰竭、主动脉瘤、广泛性动脉粥样硬化、心肌梗塞等心血管疾病的高风险因素[②]，最终使患者的残疾和死亡风险提高，给个人、家庭和社会带来严重负担，消耗巨大的医疗和社会资源。高血压是威胁我国居民健康的一大问题，我国高血压患者有2.7亿人，在《健康中国行动（2019—2030）》中提到，缺乏身体活动是高血压等慢病的危险因素之一，其防治应包含适量的体育运动等措施[③]。原发性高血压病因不明，一些影响心血管的因素如晚期糖基化终末产物（advanced glycation end products，AGEs）交联沉积可能在高血压的病理生理学中发挥作用，导致高血压和心功能下降[④⑤]。AGEs 是蛋白质、脂质等与糖类发生非酶促反应后形成的一种聚合物，除了可以直接与细胞壁上的蛋白交联降低血管顺应性，还可以与晚期糖基化终末产物受体（receptor advanced glycation end products，RAGE）结合，激活 p38 丝裂原活化蛋白激酶（p38 mitogen-activated protein kinases，p38 MAPK），诱导核转录因子（NF-kB）的激活和迁移,促进内皮素-1（Endothelin-1，ET-1）、细胞间粘附分子-1(Intercellular cell adhesion molecule-1，ICAM-1)、血管细胞粘附分子-1(Vascular cell adhesion molecule-1，VCAM-1)等因子表达[⑥]，进而影响血压和心功能。减轻体内 AGEs 的损伤效应可能成为防治高血压和心脏疾病的途径之一。大量研究表明运动可以预防和治疗高血压，改善心功能，但较少人关注运动对高血压体内 AGEs 及其受体的影响。

1.2　研究目的

（1）考察 AGEs/RAGE 在有氧运动改善 SHR 大鼠增龄性血压升高，心功能下降过程中扮演的角色。

（2）从 p38 MAPK/NF-kB 通路的角度分析有氧运动降低 AGEs/RAGE，改善血压和心功能的可能机制。

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（3）探讨不同时长和持续周数的有氧运动对血压、心功能及心肌AGEs/RAGE-p38MAPK/NF-kB影响的差异，为高血压最佳运动处方提供理论和实践依据。

1.3 研究意义

1.3.1 理论意义

运动可能通过改变AGEs或通过影响AGEs/RAGE-p38 MAPK-NF-kB来影响血压和心功能。通过检测不同运动时间对SHR心脏相关指标的变化，来反映不同运动时间对SHR心脏AGEs及其受体的影响及机制，为有氧运动改善原发性高血压提供理论基础和实验依据。

1.3.2 实践意义

近年来 AGEs及其受体在高血压、动脉硬化等心血管疾病中的影响逐渐成为研究的切入点，而很少有人研究运动对SHR心脏AGEs及其受体的影响。本研究还探讨了不同时间的游泳运动对SHR心脏AGEs及其受体的影响及其作用机制，阐明不同时间的运动训练对高血压 AGEs及其受体影响的程度，为运动预防和辅助治疗高血压及相关疾病提供依据。

第 2 章　文献综述

2.1　AGEs

2.1.1　AGEs 的形成

AGEs 是蛋白质、脂质、核酸等大分子物质的碳基，与葡萄糖或其他还原糖的羰基发生不可逆的非酶促糖基化和氧化反应，在组织中形成结构稳定的多种物质的统称，糖尿病和衰老会使体内非常容易形成 AGEs。AGEs 在体内的过度积累会影响多种细胞的结构和功能，如内皮细胞、巨噬细胞、平滑肌细胞等等[①]。AGEs 的形成途径包括内源性生成和外源性积累两种方式，其中内源性生成又可以分为三种，分别是糖基化、脂质过氧化和糖酵解[②] (如图 2-1 所示)。

蛋白质糖基化是内源性生成 AGEs 最主要的方式。葡萄糖或还原糖的羰基与蛋白质的氨基，缓慢发生美拉德反应（Maillard reaction），在几个小时内形成希夫碱（Schiff base），希夫碱会发生分子内重排，产生更稳定的阿马多里产物（Amadori products），该产物在缓慢氧化过程会产生活性羰基化合物，如甲基乙二醛（methylglyoxal，MG）、3-脱氧-D-葡萄糖酮（3-deoxy-D-glucosone，3DG）和乙二醛（glyoxal，GO），并在数周到数月内进一步形成 AGEs，如戊糖苷（pentosidine）、Nε-羧甲基赖氨酸（Nε-carboxymethyl-lysine，CML）和葡糖苷（glucosepane）等晚期糖基化终末产物[③④]。

AGEs 内源性生成的途径还有脂质过氧化和糖酵解。脂质过氧化时，活性氧（ROS）将脂质转化为活性羰基化合物，形成 AGEs 或脂质终产物（advanced lipid end products，ALE），例如丙二醛（Malondialdehyde，MDA）[⑤]。细胞内糖酵解时，醛糖还原酶将葡萄糖转化为山梨糖醇（Sorbitol），然后在山梨糖醇脱氢酶的作用下转化为果糖（Fructose），果糖代谢转变为活性羰基化合物，活性羰基化合物再和蛋白质发生反应，最终形成如甲基乙二醛衍生的氢咪唑酮(methylglyoxal-derived hydroimidazolone，MG-HL)等晚期糖基化终末产物[⑥⑦]。外源性 AGEs 积聚在体内的途径主要来自饮食和吸烟两种方式。饮食是 AGEs 进入

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体内循环的主要外源性来源方式，尤其是食用那些在高温条件下制备并长期储存或使用食品添加剂的食物，如焦糖、烘焙的咖啡和面包等高温制备的食物，工业化生产的苏打水和果汁中也会添加美拉德反应的一些产物[①]。一般来说糖尿病或衰老会导致组织积聚 AGEs，但有学者发现在非糖尿病的个体中，吸烟者体内的晶状体纤维细胞和冠状动脉中，AGEs 和相关物质水平均高于非吸烟者[②]，说明除了糖尿病和衰老等内因，食用某些高温制备或工业添加剂的食品、吸烟这两个外因也会造成体内 AGEs 过多。

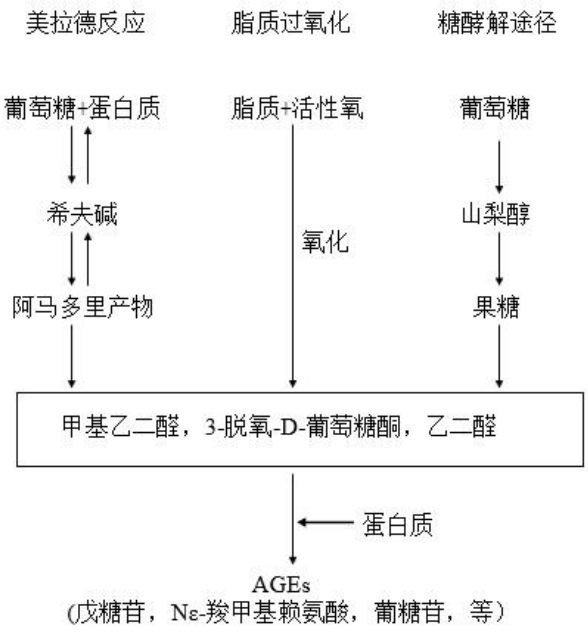

图 2-1　AGEs 内源性生成的途径

Fig.2-1　Endogenous generation pathways of AGEs

2.1.2　AGEs 的代谢

AGEs 代谢的基本途径包括受体依赖性和非受体依赖性两种方式。AGEs 的某些受体可以使其分解，然后排出体内，比如清道夫受体 A 类 II 型（MSR-AII）和 B 类 I 型（MSR-BI，CD36）以及 AGE 受体 1，2 和 3（AGE-R1，-R2 和-R3），他们都可以使 AGEs 分解后从体内排出[③]。研究发现，在牛绒毛细血管内皮细胞中，清道夫受体稳定蛋白-1（Stabilin-1）和稳定蛋白-2（Stabilin-2）可能参与了 AGEs 内吞[④]，说明清道夫受体稳定蛋白-1、稳定蛋白-2 参与了 AGEs 分解和代谢的过程。

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