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在体法评价琥珀酸对骨骼肌收缩特性的影响

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摘要:旨在建立在体测定骨骼肌收缩特性的方法,用于评价琥珀酸对C57BL/6J小鼠胫骨前肌收缩性能的影响。试验以C57BL/6J小鼠为研究模型,制作离体胫骨前肌、在体坐骨神经-腓肠肌和坐骨神经-胫骨前肌样本,对比不同方法、不同周龄和不同肌肉最大张力和张力半衰时间差异。随后,选取12只6周龄雌性小鼠,按体质量随机分为2组($n=6$),分别在饮水中添加0和1%琥珀酸盐,单笼饲养,自由采食及饮水,12 h循环光照,每周记录采食量及体质量。饲养结束后,采用所建立的在体法检测胫骨前肌最大张力、张力半衰时间,以及肌肉中葡萄糖、乳酸含量和乳酸脱氢酶、琥珀酸脱氢酶活性。在体法检测胫骨前肌收缩持久性显著高于离体法。用在体法评价不同日龄、不同部位肌肉发现,腓肠肌的最大张力和张力半衰时间均极显著高于胫骨前肌($P<0.01$),8周小鼠胫骨前肌的最大张力半衰时间极显著短于20周小鼠($P<0.01$),说明已成功建立在体测定肌肉收缩特性的方法。随后的饲养试验表明,虽然饮水添加1%琥珀酸盐对小鼠生长性能无显著影响,但采用在体测定肌肉收缩特性发现,琥珀酸盐显著提高了小鼠胫骨前肌的张力半衰时间和收缩后肌肉中琥珀酸脱氢酶的活性($P<0.05$),显著降低了收缩后肌肉中的乳酸含量($P<0.05$)。实验成功建立了在体检测骨骼肌收缩特性的评价方法,并发现饮水添加琥珀酸能增强骨骼肌抗疲劳性。研究结果对于完善骨骼肌类型转化评价方法,研制改善骨骼肌运动机能和畜禽胴体品质的新型营养素提供依据。

关键词:在体骨骼肌;琥珀酸;肌纤维类型;收缩特性

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In vivo Evaluation of the Contract Properties of Skeletal Muscle Induced by Succinate

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Abstract: The main purpose of this paper is to establish an in vivo method to determine the contraction properties of skeletal muscle and evaluate the effect of succinate on the contraction of tibialis anterior muscle in

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C57BL/6J mice. In this experiment, in vitro and in vivo models of isolated tibial anterior or gastrocnemius muscles of C57BL/6J mice of different ages were used to test the maximum muscle tension and half-life time. Then, twelve 6-week-old female mice were randomly divided into two groups according to their body weights ($n=6$). 0 and 1% of succinate were added into the drinking water respectively. The mice were housed in single cages with 12 h dark/light cycle, and free access to feed and drinking water. Their feed intake and body weights were recorded every 2 weeks. At the end of the experiment, the maximum tension, half-life time of tension, the content of glucose and lactic acid, the activity of lactate dehydrogenase and succinate dehydrogenase in the tibialis anterior muscle were measured in vivo. The contraction durability of the tibialis anterior muscle measured by in vivo method was significantly higher than that measured by ex-vivo method. The contraction properties of different muscles were compared with in vivo method and it was found that the maximum tension and half-attenuation time of the gastrocnemius muscle were significantly higher than that of the anterior tibial muscle ($P<0.01$). In addition, the maximum tension half-life time of the anterior tibial muscle was significantly shorter in 8-week mice than that in 20-week mice ($P<0.01$). This data demonstrated that in vivo method was successful to evaluate muscle contraction characteristics. The subsequent experiment showed that although the addition of 1% succinate to drinking water had no significant effect on the growth performance of mice, succinate significantly increased the tension half-life of the tibialis anterior muscle and the succinate dehydrogenase activity, while significantly reduced the lactic acid content in the muscle after contraction ($P<0.05$). This experiment successfully established an in vivo method to evaluate skeletal muscle contraction. Based on this model, it was found that succinate significantly enhanced the fatigue resistance of skeletal muscle. This paper sheds further light on the novel methods to evaluate skeletal muscle type transition. These data are also of great value for developing new nutrients to increase skeletal muscle function and carcass quality.

Keywords: in vivo skeletal muscle; succinate; muscle fiber type; contractile characteristics

骨骼肌由大量的肌纤维组成,是动物体最具可塑性的组织之一。肌纤维可根据肌肉颜色、代谢酶活^[1]、肌球蛋白重链(Myosin Heavy Chain, MyHC)和收缩特点^[2]等划分为不同类型,也受部位、年龄、营养、运动和神经内分泌调控的影响而发生改变^[3]。肌纤维类型研究不仅在治疗骨骼肌萎缩,提高人类运动成绩具有重要的意义^[4],而且对于改善畜禽肉品质同样具有重要应用价值^[5]。不同类型的骨骼肌具有典型的收缩特点。骨骼肌的最大收缩张力可以反映肌肉的爆发力,而张力半衰时间、乳酸含量和乳酸脱氢酶等则代表骨骼肌的耐力和抗疲劳性^[6-7]。通常慢肌纤维比例较高肌肉的抗疲劳性优于快肌纤维比例高的肌肉,但后者的爆发力强于前者。目前,研究骨骼肌收缩特性主要采用离体法进行评价,但离体时骨骼肌供血不足,易疲劳和失活。也有研究团队尝试在体法测定肌肉的收缩特性^[8],但在体法通常对仪器设备要求较高。

琥珀酸(succinate, SUC),又称为丁二酸,是常见的短链脂肪酸,广泛存在于多种动植物的组织中。研究表明,SUC能通过琥珀酸受体引起细胞钙离子动员^[9],进而调节钙离子依赖性信号通路调节相关基因,参与骨骼肌纤维类型转化^[10]。但目前尚不清楚日粮中添加琥珀酸对动物骨骼肌收缩特性有何影响。因此,本文基于生物实验机能系统,建立了在体骨骼肌收缩能力测定法。并深入探究琥珀酸对骨骼肌在体收缩能力的影响。研究结果将对完善骨骼肌类型转化评价方法,研制改善骨骼肌运动机能和畜禽胴体品质的新型营养素提供依据。

1 材料与方法

1.1 试验材料

液体石蜡(河南万山化工产品有限公司)、水合氯醛(武汉远成共创科技有限公司,纯度为99%)和琥珀酸钠(生工生物工程股份有限公司,纯度为98%)。

1.2 试验动物与饲养管理

试验用鼠均是购自广东省实验动物中心(广东佛山)的C57/BL小鼠。12只6周龄雌性小鼠,按体质

量随机分为2个组($n=6$),分别在饮水中添加0和1%琥珀酸盐。小鼠单笼饲养,自由采食及饮水,12 h循环光照,试验过程中每周记录小鼠采食量及体质量。饲养试验结束后,分别测定琥珀酸处理、在离体检测方法、日龄对小鼠胫骨前肌收缩特性的影响及胫骨前肌和腓肠肌收缩特性的区别。

1.3 腓肠肌及胫骨前肌样本的制作

按每100 g腹腔注射1 mL的水合氯醛溶液(浓度为5%)对小鼠进行麻醉后,俯卧并固定于手术板上,制作离体胫骨前肌标本、在体坐骨神经-腓肠肌标本和坐骨神经-胫骨前肌标本,用约8 cm手术线缚于腓肠肌和胫骨前肌肌腱处,离体肌肉标本从两端剪断,在体肌肉标本一端与骨骼相连。在待测肌肉上滴加两滴液体石蜡为肌肉保湿,并将保温垫调至37℃覆盖于待测肌肉上,为其保温。

1.4 骨骼肌收缩特性测定

肌肉标本的制作完成后,将张力换能器一端连接生物机能实验系统(成都泰盟科技有限公司,BL-420F)的输入口通道,另一端连接结扎线,调整换能器并固定。打开电源,将刺激电极搭在待测肌肉上。刺激参数设置为波宽1 ms,延迟100 ms,连续单刺激,刺激频率2 Hz,刺激强度从零开始逐渐递增,寻找引起骨骼肌最大收缩的最小刺激强度,用该强度与阈刺激强度之差的中值强度测定疲劳性。收缩特性指标包括待测肌肉最大张力,0,30,60,120 s时的张力,计算张力衰减百分比和张半衰时间。

1.5 代谢物和代谢酶活性检测

待上述肌肉标本收缩试验结束后,剪取肌肉,放入离心管内,投入盛装液氮的保温瓶中,液氮急速冷冻。采样完成后,将装有肌肉样品的离心管放入负80℃低温冰箱中保存,等待后续检测疲劳相关生化指标。乳酸含量、葡萄糖含量、乳酸脱氢酶活性及琥珀酸脱氢酶活性,试验中所采用的测试盒均购自南京建成生物研究所,并用多功能酶标仪(美国Bio TeK)进行检测。

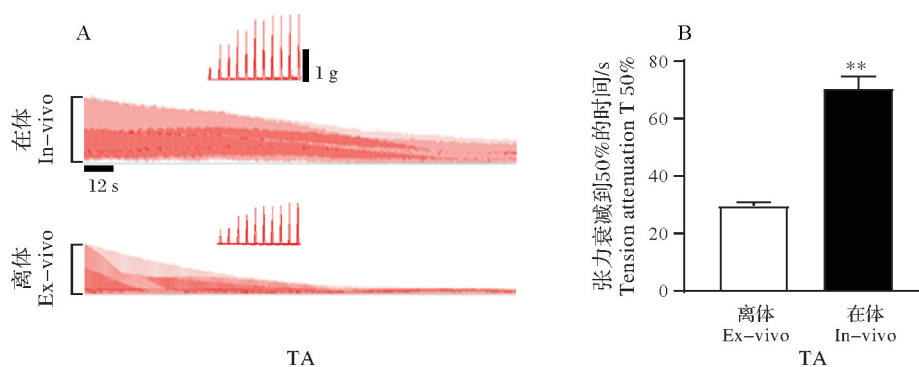
1.6 数据处理与统计分析

试验结果用平均数±标准误表示,采用GraphPad Prism 8.0.2软件对数据进行 t 检验, $P<0.05$ 表示显著差异。

2 结果与分析

2.1 离体与在体法测定胫骨前肌收缩特性

由图1可知,胫骨前肌在体检测张力半衰时间极显著高于离体检测张力半衰时间($P<0.01$)。说明在体法测定肌肉收缩特性优于离体法。



*表示组间差异显著($P<0.05$, Duncan's法),**表示 $P<0.01$ 。TA为胫骨前肌;A为离体和在体胫骨前肌收缩图;B为胫骨前肌离体和在体检测张力半衰时间

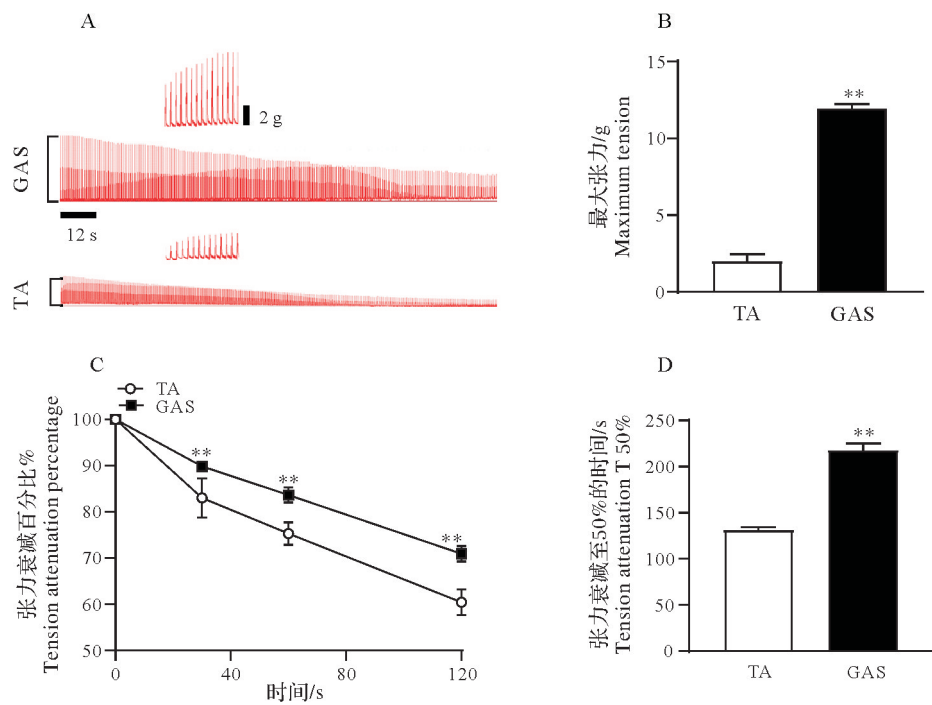
* indicates significant difference between groups ($P<0.05$, Duncan's method), and ** indicates $P<0.01$. TA is the tibialis anterior muscle; A is the ex vivo and in vivo tibialis anterior muscle contraction map; B is the tibialis anterior muscle ex vivo and in vivo detection of tension half-life time.

图1 离体与在体法测定胫骨前肌收缩特性的比较

Fig.1 Comparison of ex-vivo and in vivo measurement of contractile characteristics of tibialis anterior muscle

2.2 在体法比较腓肠肌与胫骨前肌收缩特性

由图2可知,腓肠肌的最大张力极显著大于胫骨前肌($P<0.01$),腓肠肌的张力在30 s、60 s和120 s时的衰减程度均极显著低于胫骨前肌($P<0.01$)。相应地,腓肠肌的张力半衰减时间也极显著长于胫骨前肌($P<0.01$),说明腓肠肌的抗疲劳性强于胫骨前肌。

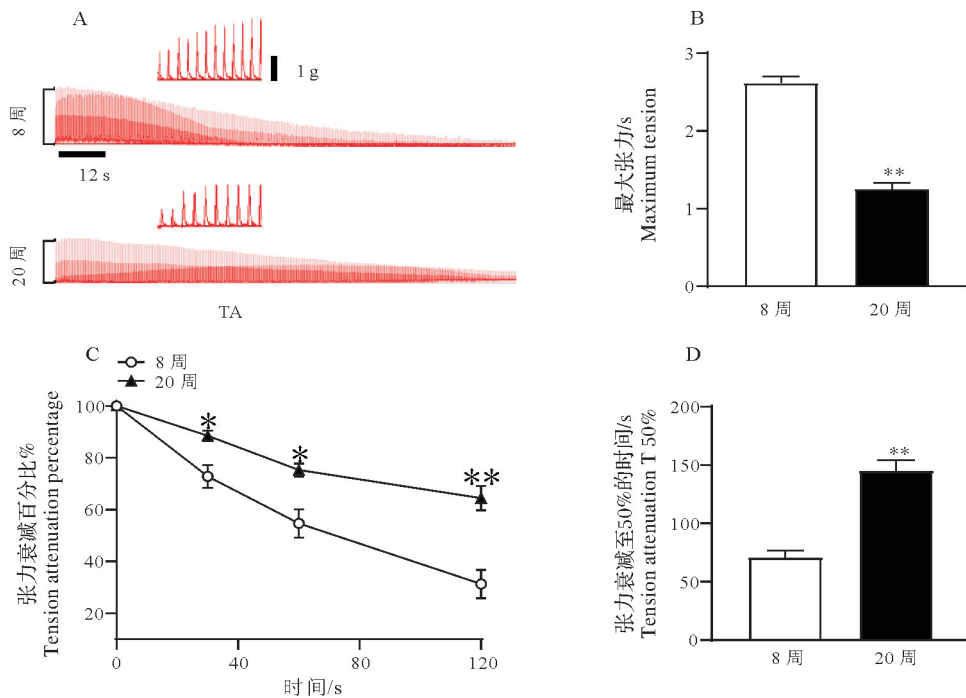


*表示组间差异显著 ($P<0.05$, Duncan's 法), **表示 $P<0.01$ 。GAS 为腓肠肌;TA 为胫骨前肌;A 为腓肠肌和胫骨前肌肌收缩图;B 为肌肉最大张力;C 为不同时间点肌肉张力衰减百分比;D 为肌肉张力半衰时间

* indicates significant difference between groups ($P<0.05$, Duncan's method), and ** indicates $P<0.01$. GAS is the gastrocnemius muscle; TA is the tibialis anterior muscle; A is the gastrocnemius and tibialis anterior muscle contraction; B is the maximum muscle tension; C is the percentage of muscle tension decay at different time points; D is the muscle tension half-life

图 2 在体法比较腓肠肌与胫骨前肌收缩特性

Fig.2 Contraction characteristics of gastrocnemius and tibialis anterior muscle in vivo



*表示组间差异显著 ($P<0.05$, Duncan's 法), **表示 $P<0.01$ 。TA 为胫骨前肌;A 为不同周龄胫骨前肌肌收缩图;B 为肌肉最大张力;C 为不同时间点肌肉张力衰减百分比;D 为肌肉张力半衰时间

* indicates significant difference between groups ($P<0.05$, Duncan's method), and ** indicates $P<0.01$. TA is the tibialis anterior muscle; A is the contraction diagram of the tibialis anterior muscle of different ages; B is the maximum muscle tension; C is the percentage of muscle tension decay at different time points; D is the half-life of muscle tension

图 3 在体法比较不同周龄小鼠胫骨前肌收缩特性

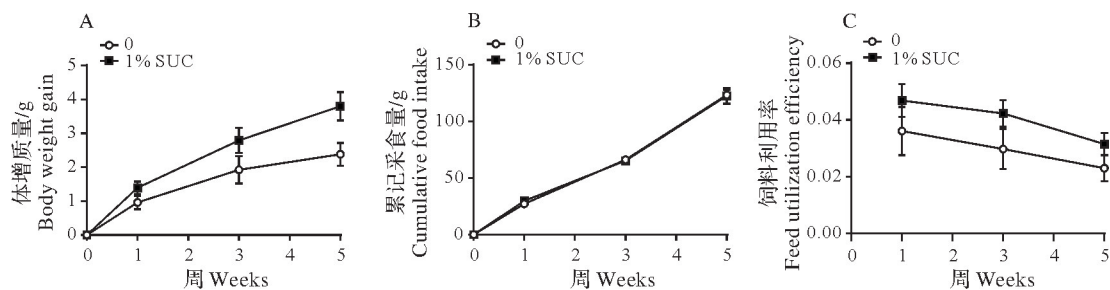
Fig.3 Contraction characteristics of tibial anterior muscle in mice of different weeks

2.3 在体法比较不同周龄小鼠胫骨前肌收缩特性

由图3可知,8周小鼠胫骨前肌的最大张力极显著大于20周小鼠($P<0.01$);8周小鼠胫骨前肌的张力在30 s、60 s时的衰减程度显著快于($P<0.05$)及在120 s时的衰减程度极显著快于20周小鼠($P<0.01$);8周小鼠胫骨前肌的张力半衰时间极显著短于20周小鼠($P<0.01$)。

2.4 饮水添加琥珀酸对小鼠生长的影响

如图4所示,饮水添加1%琥珀酸盐对小鼠体增质量、采食量和饲料利用效率均无显著影响($P>0.05$)。

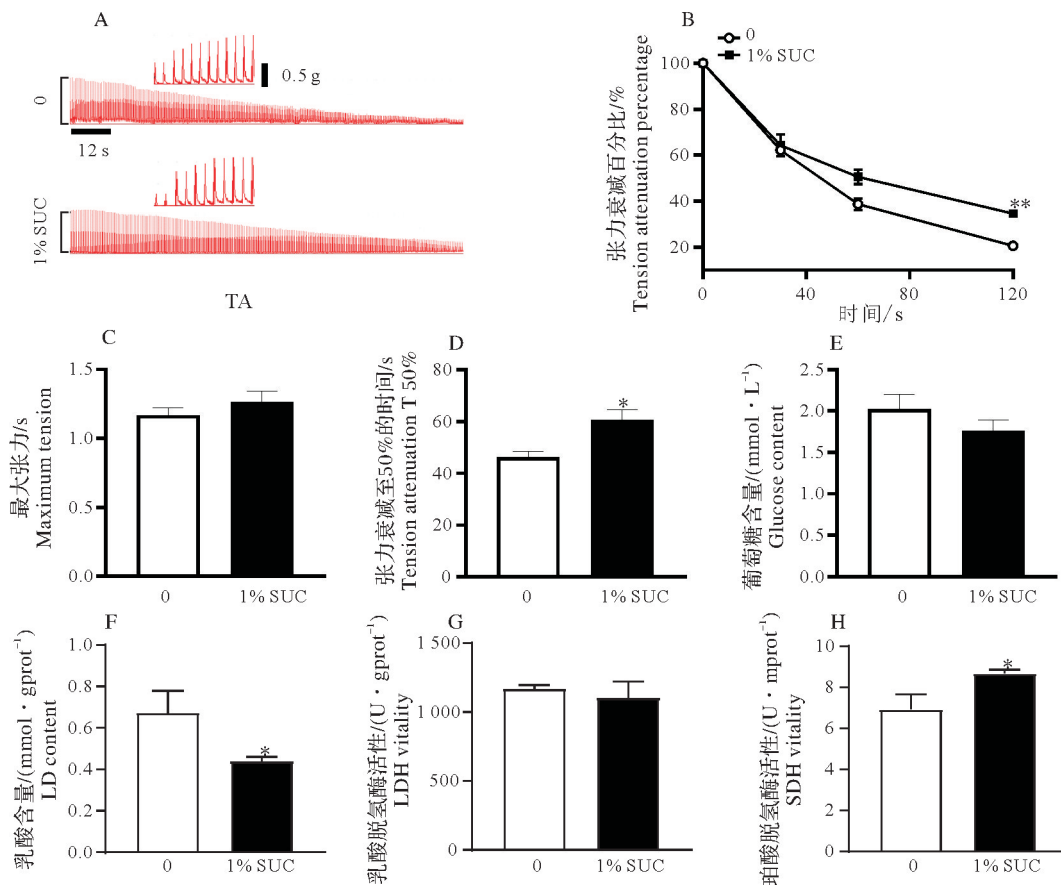


A为体增质量;B为累计采食量;C为饲料利用率

A is body weight gain; B is cumulative feed intake; C is feed utilization rate

图4 饮水添加琥珀酸对小鼠生长的影响

Fig.4 Effects of succinate in drinking water on the growth of mice



*表示与对照组差异显著($P<0.05$, Duncan's法), **表示 $P<0.01$ 。TA为胫骨前肌;A为对照组和1%琥珀酸组的胫骨前肌收缩图;B为不同时间点肌张力衰减百分比;C为肌张力半衰时间;D为肌肉最大张力;E为葡萄糖含量;F为乳酸含量;G为乳酸脱氢酶活性;H为琥珀酸脱氢酶活性

* indicates significant difference from the control group ($P<0.05$, Duncan's method), and ** indicates $P<0.01$. TA is the tibialis anterior muscle; A is the contraction of the tibialis anterior muscle of the control group and 1% succinic acid group; B is the percentage of muscle tension decay at different time points; C is the half-life of muscle tension; D is the maximum tension of muscle; E is glucose Content; F is lactic acid content; G is lactate dehydrogenase activity; H is succinate dehydrogenase activity

图5 饮水添加琥珀酸对小鼠胫骨前肌收缩特性的影响

Fig.5 Effects of succinate on contraction of tibial anterior muscle in mice

2.5 饮水添加琥珀酸对小鼠胫骨前肌收缩特性的影响

由图5可知,与对照组相比,饮水添加1%琥珀酸盐显著延长了小鼠胫骨前肌的张力半衰时间($P<0.05$),并极显著减少了肌张力在120 s时的衰减程度($P<0.01$);饮水添加1%琥珀酸盐显著降低了收缩后肌肉中乳酸的含量($P<0.05$),并显著提高了收缩后肌肉中的琥珀酸脱氢酶的活性($P<0.05$)。

3 讨论与结论

3.1 在体检测骨骼肌收缩特性方法的建立

骨骼肌收缩能产生张力或缩短,收缩效能取决于它的收缩能力。目前,研究骨骼肌的收缩能力可在离体和在体条件下刺激所支配的神经或肌肉,观察肌肉的收缩情况。离体法操作简单,设备要求低,目前多数研究团队采用离体法测定骨骼肌的收缩特性^[11]。相比离体法,在体法测定系统需要特殊仪器设备,操作要求较高。本文采用生物实验机能系统,对比了离体和在体两种方法,发现在体法测得骨骼肌张力半衰时间明显长于离体法测得的张力半衰时间,这与先前报道一致^[12-14],说明在体研究模型可能最大程度避免组织缺氧和缺血对肌肉收缩特性的影响^[15]。

骨骼肌肌纤维具有多种类型,且受到年龄、性别和环境等因素的影响^[16]。腓肠肌和胫骨前肌的肌纤维均为混合型肌纤维,腓肠肌肌纤维以SO型纤维为主,收缩缓慢而不易疲劳,而胫骨前肌以II型纤维为主^[17]。试验结果中,与腓肠肌相比,胫骨前肌的最大张力更小,且更易疲劳,这可能与腓肠肌的体积更大和I型纤维比例多于胫骨前肌等有关。此外,不同年龄人和动物骨骼肌的运动单位、横切面积及有氧和无氧代谢等方面都有影响。年龄越大肌肉张力有所降低,I型纤维比例增加,而II型肌纤维比例减少^[18]。同时,年龄增加还会增加非收缩肌肉组织。本试验中用在体法评价肌肉收缩特性也发现,8周小鼠胫骨前肌的最大张力极显著大于20周小鼠,但8周小鼠胫骨前肌的张力半衰时间极显著短于20周小鼠。综上所述结果均说明本文所建立的在体评价肌肉收缩方法切实可行。

3.2 饮水中添加琥珀酸对小鼠生长性能和胫骨前肌收缩特性的影响

SUC是三羧酸循环中的一种重要物质。现有大量研究表明,SUC可以作为生物活性信号分子,在细胞代谢、成骨、血压调节等方面发挥作用。本实验室前期研究还发现,SUC可以促进体内外骨骼肌蛋白质的合成,并激活调控蛋白质合成的相关信号通路,抑制蛋白质降解的相关通路。有研究表明,骨骼肌的收缩可增加线粒体的数量,使快肌纤维向慢肌纤维的转变,同时也增加了血液SUC的含量。SUC可作用于琥珀酸受体(GPR91),增加细胞内 Ca^{2+} 含量,这可能SUC调节肌纤维类转化,增加运动耐力的机制之一。此外,SUC在线粒体基质中也是复合物II的底物,在传递电子和稳定线粒体氧化呼吸中发挥重要作用^[19]。由此推测日粮中添加琥珀酸可能促进骨骼肌抗疲劳性。为验证这一假设,本文采用建立的在体肌肉收缩评价装置进行比较,结果发现,虽然饮水添加1%琥珀酸盐的小鼠胫骨前肌张力半衰时间和琥珀酸脱氢酶活性都有明显提高。而且琥珀酸对小鼠体增质量、采食量和饲料利用效率均无显著影响,但明显降低了运动后肌肉中乳酸含量和乳酸脱氢酶的活性,表明饮水添加琥珀酸盐能明显改善肌肉的收缩机能,缓解肌肉疲劳,增强肌肉的耐力型运动。

本试验成功建立了骨骼肌收缩特性在体评价方法。基于此方法研究发现,饮水添加琥珀酸能明显改善肌肉的收缩机能,提高其抗疲劳性。

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