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Efeito dos ácidos linoleico e oleico na regeneração do músculo gastrocnêmio de ratos após laceração

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RESUMO

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Avaliou-se o efeito do tratamento com os ácidos linoleico e oleico (0,44g por Kg de peso corpóreo) por quatro semanas sobre a regeneração do músculo gastrocnêmio lacerado em ratos. Os efeitos dos ácidos graxos (100 μM) foram também avaliados em mioblastos, miotubos e fibroblastos em cultura para se determinar o mecanismo envolvido. A laceração provocou aumento da razão oleico/esteárico e palmitoleico/palmítico indicadores da atividade de dessaturase. Promoveu diminuição das forças isotônica específica e tetânicas absoluta e específica. Ocorreu queda da resistência à fadiga e aumento da área de tecido fibroso. Esses achados indicam regeneração incompleta e recuperação parcial da função contrátil do músculo lesionado. A suplementação com o ácido linoleico diminuiu a massa, a força isotônica específica, a resistência à fadiga e a área de secção tranversa das fibras dos músculos gastrocnêmios contralateral e lesionado, reduziu a força tetânica específica e elevou o conteúdo de *p-S6* e *Pax7* no músculo contralateral; aumentou a área de tecido fibroso e reduziu o conteúdo de *alfa actina* no músculo lesionado. O tratamento de fibroblastos com ácido linoleico diminuiu a expressão de mRNA de *PCNA*, colágeno e fibronectina. Por sua vez, a suplementação com o ácido oleico não modificou a massa e a área de secção tranversa das fibras do músculo gastrocnêmio; aboliu a diminuição da força isotônica específica e o aumento da área de tecido fibroso induzidos pela lesão; preveniu a queda das forças tetânica, absoluta e específica, e aumentou a resistência à fadiga e o conteúdo de vimentina nos músculos gastrocnêmios contralateral e lesionado. O tratamento com ácido oleico *in vitro* aumentou o conteúdo de mRNA de *MyoD* em mioblastos, de desmina em miotubos já diferenciados e inibiu a expressão de mRNA de *PCNA*, colágeno e fibronectina em fibroblastos. Concluímos que a suplementação com o ácido linoleico comprometeu a regeneração do músculo esquelético lesionado, causando redução da massa muscular e elevação de tecido fibroso, comprometendo a função contrátil. O ácido oleico, por sua vez, atenuou o quadro de reparo incompleto, otimizando a capacidade regenerativa e a função contrátil do músculo lesionado.

Palavras-chave: Laceração muscular. Músculo esquelético. Ácidos graxos. Função contrátil. Mioblastos. Miotubos e Fibroblastos.

ABSTRACT

ABREU, P. S. T. **Effect of linoleic and oleic acids on regeneration of gastrocnemius muscle after laceration in rats**. 2014. 156 p. Ph. D. thesis (Human Physiology) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2014.

The effect of treatment with linoleic and oleic acids (0.44g per kg body weight) for four weeks on regeneration of lacerated gastrocnemius muscle in rats was evaluated. The effects of fatty acids (100 μM) were also evaluated in cultured myoblasts, myotubes and fibroblasts to determine the mechanisms involved. The laceration itself raised the oleic/stearic acids and palmitoleic/palmitic acids ratios, suggesting increased activity of desaturase. Laceration promoted a reduction of specific twitch force and absolute and specific tetanic forces. Decreased resistance to muscle fatigue and increased area of fibrous tissue were also observed. These findings indicate incomplete regeneration and partial recovery of the contractile function of the injured muscle. The supplementation with linoleic acid reduced the mass, specific twitch force, the resistance to muscle fatigue and the cross-sectional area of fibers of the controlateral and injured gastrocnemius muscles, reduced the specific tetanic force and the contents of *p-S6* and *Pax7* in controlateral gastrocnemius muscle; increased the area of fibrous tissue and reduced the content of alpha actin in injured muscle. The treatment in fibroblasts with linoleic acid reduced the expression (mRNA) of PCNA, collagen and fibronectin. In turn, the supplementation with oleic acid did not change the mass and cross-sectional area of the fibers of the gastrocnemius muscle; abolished the decrease of the specific twitch force and the increase of the fibrous tissue area in injured gastrocnemius muscle; prevented the fall of absolute and specific tetanic forces, and increased the resistance to muscle fatigue and the content of vimentin in controlateral and injured gastrocnemius muscles. The treatment with oleic acid increased the contents (mRNA) of *MyoD* in myoblasts, desmin in differentiated myotubes and inhibited the expression of *PCNA*, collagen and fibronectin in fibroblasts. We concluded that supplementation with linoleic acid impaired the regeneration of injured skeletal muscle, as indicated by reduced mass of the muscle and increase of fibrous tissue, leading to decresed contractile function. In turn, oleic acid attenuated the incomplete repair, optimizing the regenerative capacity and contractile function of the injured muscle.

Keywords: Muscle laceration. Skeletal muscle. Fatty acids. Contractile function. Myoblasts. Myotubes and Fibroblasts.

1 INTRODUÇÃO

O processo de regeneração muscular é desencadeado a partir de um estímulo lesivo que pode ser de natureza química (CONTE et al., 2008), mecânica (JARVINEN et al., 2014) ou térmica (SUGITA et al., 2012). O reparo do músculo esquelético, desencadeado após ruptura do sarcolema muscular e aumento da permeabilidade vascular, envolve eventos celulares e moleculares que se iniciam com o aumento do influxo de cálcio para o meio intracelular causando proteólise dependente desse cátion, necrose do tecido danificado e ativação da resposta inflamatória no local da lesão. Essa fase é seguida pela produção de proteínas de matriz extracelular, revascularização, concomitante à ativação de células miogênicas. Essas são células satélites mononucleadas e indiferenciadas localizadas entre a lâmina basal e o sarcolema da fibra muscular, que se proliferam, se diferenciam e se fundem levando à formação de uma nova miofibra e, assim, à reconstituição do aparato contrátil (CARLSON; FAULKNER, 1983; CHAKRAVARTHY et al., 2000; HANNAFIN et al., 1994; HORSLEY et al., 2003; HURME; KALIMO, 1992; JARVINEN et al., 2014; SERRANO et al., 2008). Vários grupos investigam estratégicas terapêuticas para acelerar o processo de reparo do músculo esquelético após lesão. Há evidências de que ácidos graxos (tais como linoleico, oleico que são abundantes nas dietas ocidentais) modulam a diferenciação de células musculares (BRIOLAY et al., 2013; HURLEY et al., 2006; LEE et al., 2009; MARKWORTH; CAMERON-SMITH, 2013; ZHANG et al., 2012). Contudo, os efeitos da suplementação com os ácidos graxos sobre a regeneração, função contrátil e expressão de proteínas das vias de sinalização do músculo esquelético não foram devidamente estudados. Esse foi o objetivo do presente estudo.

8 CONCLUSÕES

No presente estudo, concluímos que a suplementação com o ácido linoleico comprometeu a regeneração do músculo esquelético lesionado, causando redução da massa muscular, elevação de tecido fibroso, recuperação parcial da função contrátil. O ácido oleico, por sua vez, atenuou o quadro de reparo incompleto, otimizando a capacidade regenerativa e a função contrátil do músculo lesionado. O mecanismo de ação do ácido oleico envolve aceleração da expressão de *MyoD* em células-tronco satélites e de desmina em miotubos diferenciados.

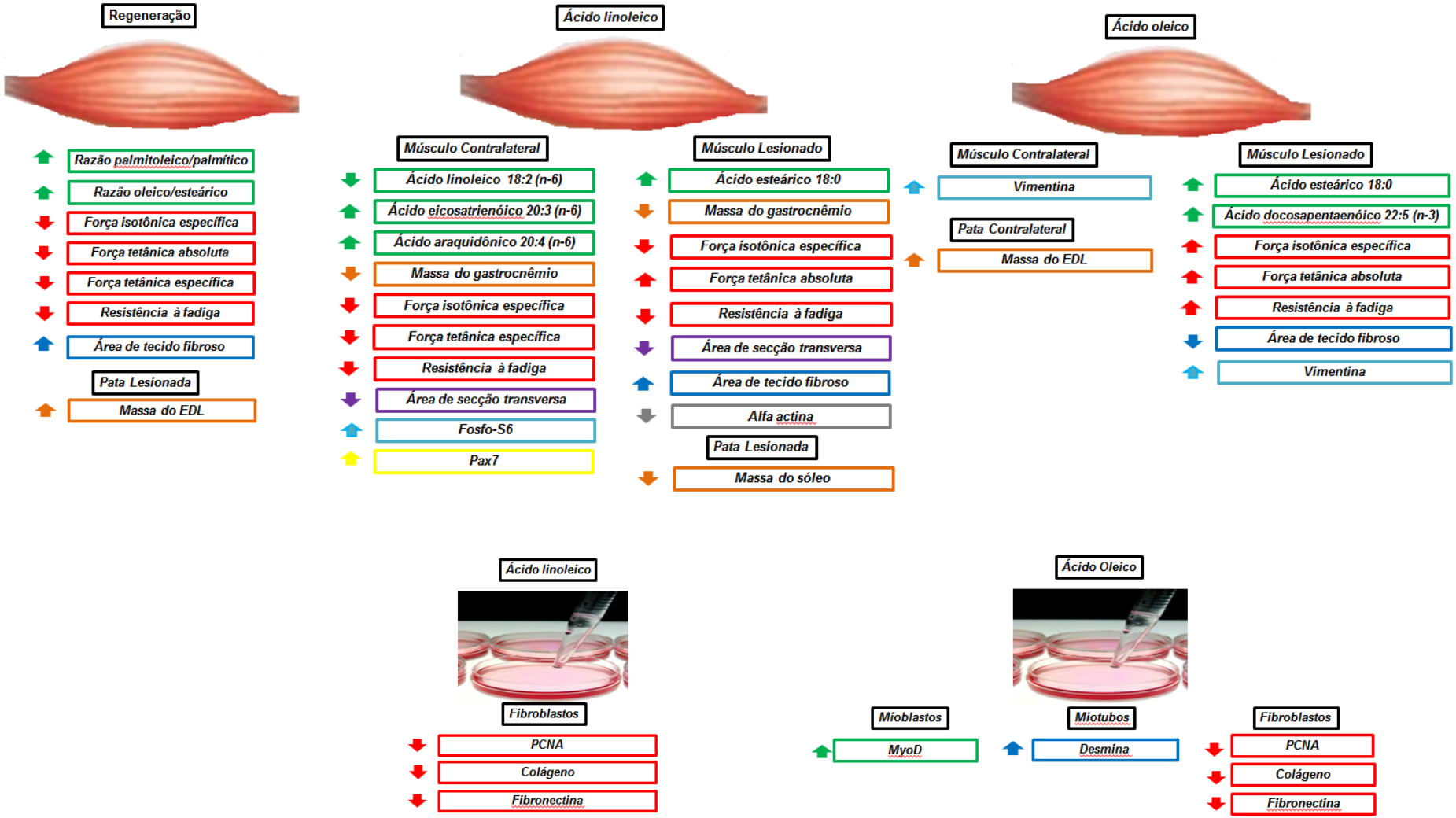

Figura 31: ***Representação esquemática dos resultados obtidos.*** Estão indicadas as alterações que ocorreram no processo de regeneração *per se* e os efeitos dos tratamentos com os ácidos linoleico e oleico *in vivo*. Os efeitos *in vitro* dos ácido graxos nos mioblastos, miotubos e fibroblastos também estão indicados. Proteína de determinação mioblástica *(MyoD);* Fator miogênico 6 *(Myf6); Tripartite motif-containing protein (TRIM32);* Antígeno nuclear de proliferação celular *(PCNA);* Extensor digital longo *(EDL); Paired box 7 (Pax7)*

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