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| 論文題目   | Studies on endurance exercise training adaptation and endurance performance in mice under different pharmacological, physiological, and dietary conditions<br><br>(薬理的・生理学的処理と種々の飼料条件がマウスの持久運動トレーニングに対する適応および持久運動能力に及ぼす効果に関する研究) |    |                      |
| (論文内容の要旨)  |  |    |                      |
| <p>Endurance training leads to the changes in whole-body metabolism and phenotype and the improvements in endurance ability through the activation of contraction-induced signals. Downstream to these signals are transcription factors that control genes regulating cellular metabolism and overall adaptation to increased energy demand brought about by exercise. As these transcription factors are also activated by non-contraction signals, e.g. pharmacological metabolic activators and nutrients such as fatty acids and carbohydrates, endurance training together with pharmacological or dietary manipulation could influence downstream adaptations. Likewise, genetic background which could influence the chemical milieu of cells, particularly the muscle, could also affect training-induced adaptations. In this doctoral thesis, adaptations that occur in training with pharmacological, genetic or physiological, and dietary environment were investigated.</p> <p>[Chapter 1] Pharmacological agents that activate the AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor delta (PPAR<math>\delta</math>) have been considered as exercise mimetics. Their individual use causes changes on muscle physiology and endurance performance that are somewhat similar to those exercise training causes. However, their simultaneous administration with endurance exercise training have not been investigated. The author determined the effects of their combined pharmacological activation with endurance exercise training. Four weeks of daily administration of the AMPK activator AICAR and the PPAR<math>\delta</math> selective agonist GW0742 with training on alternate days greatly improved performance than training alone. This could be attributed to increased mRNA expression of pyruvate dehydrogenase kinase 4 (PDK4), which has a critical role for switching substrate</p> |  |    |                      |

utilization to fat oxidation during exercise, and eventually resulted in muscle glycogen accumulation. AMPK activation with training also improved endurance but modestly relative to training. Increased citrate synthase indicated mitochondrial biogenesis. Similarly, muscle glycogen was spared with AICAR administration. These imply that while pharmacological activation of AMPK can potentiate endurance, greater potentiation could be achieved with combined pharmacological activation of AMPK and PPAR $\delta$ .

[Chapter 2] The uptake of long-chain fatty acids (LCFAs) in cells are mediated by different fatty acid transporters on cell membranes. The major fatty acid transporter in the muscle is CD36. While it has been general knowledge that LCFAs are utilized for energy production, their significant role in fatty-acid mediated signaling by the PPAR family has been recognized in the past decades. Because fatty acid uptake is increased by muscle contraction during training, this could also influence the exercise-training adaptations by influencing PPAR-mediated signaling. The author determined how CD36 influences exercise training adaptations and endurance in chronically trained mice. Exercise training failed to ameliorate endurance in CD36 knock-out (KO) mice, despite having similar adaptations in mitochondrial biogenesis and intact albeit lower glycogen accumulation with training as wild-type (WT) mice. Likewise, exercise training-induced whole-body metabolism changes at rest and during exercise, as well as transcriptional increases in PPAR $\alpha$  and PPAR target genes observed in WT mice were absent in KO mice. These findings show the importance of CD36 not only in endurance but also in exercise training-induced adaptive changes in substrate metabolism and PPAR-related transcriptional regulation.

[Chapter 3] Different fat sources contain varying composition of fatty acids. Fatty acids classified broadly on chain length have been demonstrated to influence metabolism differently. Medium-chain fatty acids (MCFAs) are easily metabolized in cells due to their lack of dependence on fatty acid transport proteins unlike LCFAs. Increased endurance in swimming exercise in mice fed with diet supplemented with purified MCFAs was observed in comparison to LCFAs. Also, MCFAs increase mitochondrial biogenesis in the muscle. However, the influence of MCFAs on training

adaptations to a treadmill running, particularly on endurance, whole-body metabolism, substrate handling, and transcriptional changes in the muscle and liver remained unexplored. Also, the use of coconut oil as a source of MCFAs has not been compared with the diets with different fat content. The author compared the effects of low-fat diet and those of medium-fat diets containing coconut oil and soybean oil on exercise training adaptations. Coconut oil modestly improved endurance, which was attributed to increased fatty acid oxidation markers in the muscle. With training, all diet groups increased endurance probably through increased mitochondrial functions and exercise efficiency. Despite this, coconut oil showed inhibition of training-induced increase in transcription of PPAR $\delta$  and its targets in the muscle. In the liver, coconut oil was thought to increase fatty acid oxidation markers through PPAR $\alpha$  activation. Furthermore, while glycogen accumulation was inhibited by coconut oil with training, compensatory mechanisms by glycogen sparing through ketogenesis and ketolysis possibly prevented impairment of endurance in this group. These findings show that training improves endurance by generally improving muscle mitochondrial biogenesis, although transcriptional and metabolic adaptations in the muscle and liver are diet-dependent.

Overall, this thesis explored the complex interaction of non-contraction stimuli and contraction with exercise concerning different training adaptations in endurance, whole-body metabolism, and local adaptations in the muscle and liver. Results presented in this thesis show that in a healthy and genetically intact state, training primarily determines the endurance performance of mice. Furthermore, pharmacological compounds that alter metabolism as well as natural compounds commonly found in food can variably impact adaptations that occur during endurance exercise training. Data from this thesis may suggest a potential use of nutritional supplementation or alteration in the diet for the improvement of endurance of genetically compromised individuals predisposed with impaired metabolism.

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審査結果の要旨は日本語500～2,000字程度で作成すること。

(論文審査の結果の要旨)

持久運動トレーニングへの適応、即ち持久運動能力の増大は骨格筋での運動に関連する筋収縮信号とその下流の経路が関与するだけではなく、骨格筋がトレーニング中に曝される、種々の供給源に由来する化学環境にも影響される。本研究では、運動トレーニングへの適応に対する薬物の作用、骨格筋での脂質の取込みに関与する遺伝子の役割、および主として中鎖脂肪酸を含む脂肪摂取の効果について明らかとした。評価される点は以下の通りである。

1. AMP-activated protein kinase (AMPK) と peroxisome proliferator-activated receptor- $\delta$  (PPAR $\delta$ ) を各々活性化する薬剤 (AICAR と GW0724) を同時投与し、トレーニングを課したマウスでは、持久運動能力をさらに強化する効果が得られた。これらの効果は脂肪酸利用亢進、グルコース消費の節約、ミトコンドリアでのエネルギー産生能力の増大によると考えられ、トレーニング適応に対し相加的であった。
2. 脂肪酸輸送体である CD36 の遺伝子を欠損したマウスを用い、CD36 がトレーニングによる持久運動能力増大に必須であることを証明した。このマウスは野生型に比べて脂肪酸代謝能力の増強が見られず、PPAR $\alpha/\delta$  支配下にあるリポタンパク質リパーゼやピルビン酸デヒドロゲナーゼキナーゼ4 など脂肪酸酸化に関与する遺伝子発現に変化が観察されなかった。これらから、トレーニングへの適応には、CD36 によって運動を行うためのエネルギー源としての脂肪酸を取り込むことに加え、リガンドとして脂肪酸を核内受容体である PPAR $\alpha/\delta$  へ供給する作用が必要であることを明らかにした。
3. ココナツ油摂取は非トレーニング群骨格筋での脂肪酸酸化関連遺伝子発現を増大することを明らかとし、これが持久運動能力亢進の原因と考えられた。精製中鎖トリグリセリドに同様の作用があることが既に明らかとされているため、ココナツ油脂肪酸組成より、その機能的代替物として使用できることを示唆した。

以上のように、本論文は糖および脂質代謝の薬理的活性化がトレーニング効果をさらに増大し、CD36 欠損マウスにより脂肪がエネルギー源としてだけでなく PPAR リガンドとして持久運動への適応に重要であることを、また中鎖脂肪酸を多く含むココナツ油が脂肪酸酸化能力の亢進を介して持久運動能力を増大することを明らかとしたものであり、栄養化学、食品生理学、食品機能学の発展に寄与するところが大きい。

よって、本論文は博士 (農学) の学位論文として価値あるものと認める。

なお、平成30年1月16日、論文並びにそれに関連した分野にわたり試問した結果、博士 (農学) の学位を授与される学力が十分あるものと認めた。

また、本論文は、京都大学学位規程第14条第2項に該当するものと判断し、公表に際しては、当該論文の全文に代えてその内容を要約したものとすることを認める。

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要旨公開可能日： 年 月 日以降 (学位授与日から3ヶ月以内)