BBA - Molecular and Cell Biology of Lipids 1862 (2017) 1250–1259

Contents lists available at ScienceDirect

BBA - Molecular and Cell Biology of Lipids

journal homepage: www.elsevier.com/locate/bbalip

Review

Triglyceride metabolism in exercising muscle☆

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ARTICLE INFO

Keywords:
Exercise
Skeletal muscle
Triglyceride
Adipose triglyceride lipase
Metabolism
Fatty acid

ABSTRACT

Triglycerides are stored within lipid droplets in skeletal muscle and can be hydrolyzed to produce fatty acids for energy production through β-oxidation and oxidative phosphorylation. While there was some controversy regarding the quantitative importance of intramyocellular triglyceride (IMTG) as a metabolic substrate, recent advances in proton magnetic resonance spectroscopy and confocal microscopy support earlier tracer and biopsy studies demonstrating a substantial contribution of IMTG to energy production, particularly during moderate-intensity endurance exercise. This review provides an update on the understanding of IMTG utilization during exercise, with a focus on describing the key regulatory proteins that control IMTG breakdown and how these proteins respond to acute exercise and in the adaptation to exercise training. This article is part of a Special Issue entitled: Recent Advances in Lipid Droplet Biology edited by Rosalind Coleman and Matthijs Hesselink.

1. Introduction

Exercise invokes a requirement for an enormous increase in ATP production, which is fuelled in the first few seconds by substrate level phosphorylation (*i.e.* phosphocreatine and adenosine triphosphate (ATP) degradation), and thereafter, by the breakdown of carbohydrates and fatty acids to produce NADH and $FADH_2$ for oxidative phosphorylation. Fatty acids are the predominant metabolic substrate used at rest, and during low and moderate intensity exercise (< 65% VO_2 max), and fatty acid oxidation decreases at higher exercise intensities [1]. The fatty acids used for energy production are derived from adipose tissue lipolysis, which is increased above resting rates by ~3-fold during moderate-intensity exercise [2], from triglycerides contained in circulating lipoproteins [3] and from intramyocellular triglycerides (IMTG), which are stored within lipid droplets that are often localized to the mitochondria within the myocytes (Fig. 1). This close localization of organelles is postulated to facilitate the efficient transfer of fatty acids from bulk storage in the lipid droplet to energy producing sites in the mitochondria. While there has been some debate regarding the contribution of IMTG to energy production during exercise, owing to technical limitations [4], recent developments in confocal microscopy and magnetic resonance spectroscopy approaches to measure IMTG support earlier findings demonstrating a significant contribution of IMTG-derived free fatty acids to energy production during prolonged moderate-intensity exercise.

This review aims to (1) provide the reader with an understanding of the quantitative importance of IMTG as a metabolic substrate during exercise, (2) to provide an update on the understanding of the cellular and molecular regulators of IMTG metabolism, with a special focus on triglyceride lipases and their co-activators and repressors, and (3) describe how this lipolytic machinery is regulated during exercise and adapts to exercise training.

1.1. Rates of IMTG utilization during exercise

IMTG-derived fatty acids make a significant contribution to ATP production, both at rest and during exercise, and their contribution is quantitatively most important during prolonged moderate-intensity exercise. These claims are supported by studies that demonstrated a reduction in skeletal muscle triglyceride content after exercise, when compared with before exercise, using either chloroform: methanol extraction of muscle tissue and biochemical determination of triglyceride [5,6] or by evaluating the number and density of lipid droplets by Oil Red O staining [7] (Fig. 2). Interestingly, lipid depots located in the subsarcolemmal area of the skeletal muscle are utilized to a greater extent than the more centrally located depots [7] and triglyceride utilization occurs at higher rates in type 1 muscle fibers (*i.e.* oxidative, slow twitch) compared with type 2 muscle fibers (*i.e.* glycolytic, fast

Abbreviations: AC, adenylate cyclase; AMPK, AMP activated protein kinase; ATGL, adipose triglyceride lipase; CGI-58, comparative gene identification 58; DAG, diglyceride; ERK, extracellular regulated kinase; G0S2, G0/G1 switch gene 2; HSL, hormone sensitive lipase; PKA, protein kinase A; PLIN2, perilipin 2; PLIN5, perilipin 5

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http://dx.doi.org/10.1016/j.bbalip.2017.06.015
Received 2 May 2017; Received in revised form 15 June 2017; Accepted 20 June 2017
Available online 24 June 2017
1388-1981/

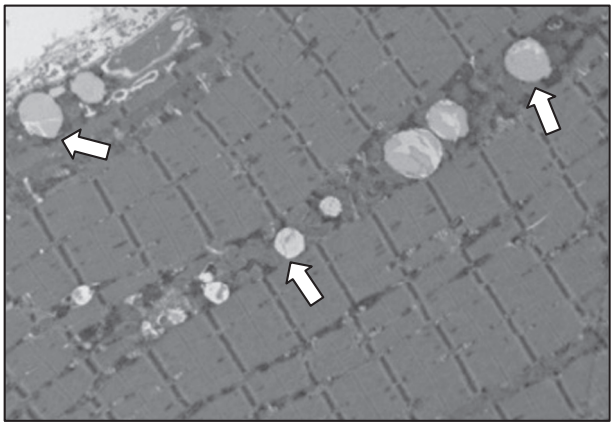

Fig. 1. Electron micrograph showing the presence of lipid droplets in human skeletal muscle. The white arrow highlights lipid droplets in close proximity to mitochondria. Image courtesy of Prof. Mark Tarnopolsky (McMaster University).

twitch), particularly in endurance exercise trained individuals [8,9]. These data are supported by studies employing stable isotope fatty acid tracer methodology, which generally report a contribution of IMTG fatty acids to total energy expenditure at ~25% during low to moderate exercise intensities (40–60% VO_2 max), with smaller contributions as exercise intensity increases [10,11] (Fig. 2b). More recently, studies have employed nuclear magnetic resonance (NMR) spectroscopy methods, which have the advantage of quantitatively assessing spectra that separates the intramyocellular triglyceride signal from the extramyocellular triglycerides, which are located between muscle fibers. These studies have supported earlier biopsy and tracer studies and reported an ~20% decline in IMCL per hour of moderate-intensity exercise for both men and women [12,13]. IMTG are also used during resistance exercise, particularly by type 1 muscle fibers, although their contribution to energy production is thought to be less than during endurance exercise [14,15]. Finally, IMTG levels are increased following endurance exercise training [16], which is due to storage in a greater number of lipid droplets rather than an increase in lipid droplet volume [8,17]. This is presumably advantageous because the increased surface area to volume ratio would provide greater access for lipases to hydrolyze their substrates and increase fatty acid mobilization (see Section 2).

While it is clear that IMTG fatty acids are utilised as metabolic substrates during exercise, it is highly likely that their contribution is under-estimated by all of the techniques described above because of the concomitant uptake of adipose tissue derived free fatty acids and their storage into IMTG. Indeed, studies in cultured myotubes [19] and resting human skeletal muscle [20–22] show that a large proportion of the plasma-derived FFA (60–90%) is first trafficked into IMTG before they are released and enter the long-chain acylcarnitine pools for eventual oxidation. Supporting the likelihood of high esterification rates in skeletal muscle during exercise are the observations that IMTG is increased in non-exercising skeletal muscle after prolonged moderate-intensity exercise [23] and that IMTG degradation is increased upon pharmacological blockade of adipose tissue lipolysis during exercise [24,25]. Thus, it is likely that the IMTG contribution to total energy expenditure is significantly more than commonly reported and, accordingly, the IMTG contained in lipid droplets should be considered a central hub for regulating fatty acid flux in skeletal muscle.

2. Proteins controlling triglyceride metabolism in skeletal muscle

The understanding of triglyceride metabolism in skeletal muscle has advanced in parallel with the major discoveries in adipose tissue biology, albeit with a lag of several years and considerably less detail with respect to regulation during contraction/exercise. The contemporary view is that IMTG lipolysis is controlled by the integrated actions of the lipases, adipose triglyceride lipase (ATGL) and HSL, the co-activator protein comparative gene identification 58 (CGI-58), the inhibitory protein G0/G1 switch gene 2 (G0S2), and members of the perilipin (PLIN) family of proteins. While the functions and regulation of these proteins are relatively well established in resting muscle, their role during exercise is less clear. Herein, we will discuss the role of these lipolytic proteins and conclude the section with an integrated view of IMTG lipolysis during exercise.

2.1. Adipose triglyceride lipase (ATGL)

2.1.1. Background

The initiation of triglyceride lipolysis was thought to be exclusively controlled by HSL until studies in *HSL* knockout mice revealed HSL-independent triglyceride lipase activity and diglyceride accumulation in most tissues, pointing to the existence of previously unidentified triglyceride lipases [26,27]. In 2004, three independent groups identified an enzyme shown to hydrolyze triglyceride and named it ATGL [28], desnutrin [29] and calcium-independent phospholipase A2ζ [30]. Very soon after these initial reports, ATGL orthologous genes and

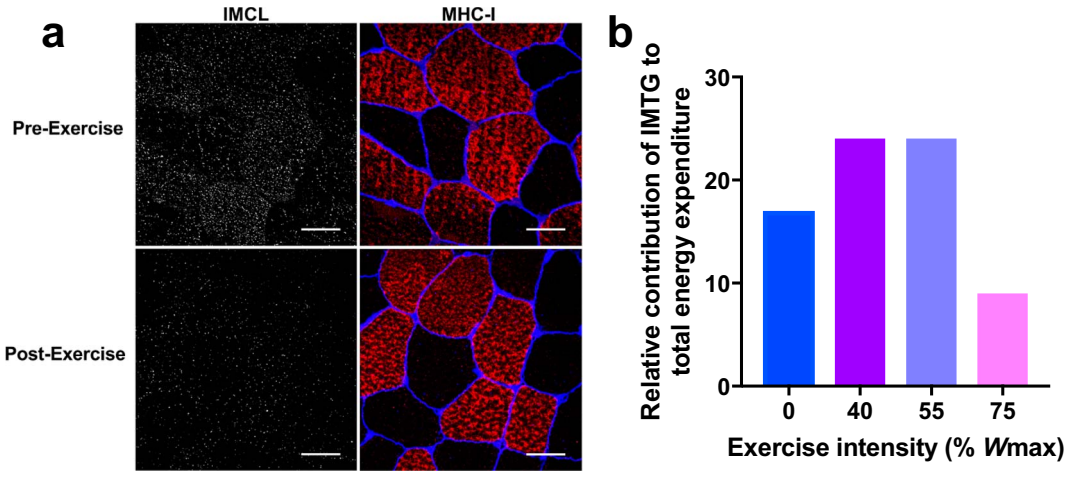

Fig. 2. a. Immunofluorescence images of skeletal muscle stained with bodipy (IMCL) and antibodies targeting myosin heavy chain type I (MHC-I) before and after 240 min of moderate intensity cycle exercise (56% $VO_{2\ max}$) in well-trained cyclists. Image courtesy of Dr. Sam Shepherd (Liverpool John Moores University). b. Influence of exercise intensity on the contribution of IMTG to total energy expenditure during cycle exercise in well-trained men. Results were calculated using isotope tracer methodology and the contribution of circulating triglycerides is considered negligible.
Adapted from van Loon et al. [11,18].