WAGENINGEN
UNIVERSITY & RESEARCH

2020

SUPERVISOR: DR A.G. NIEUWENHUIZEN
CHAIR: HUMAN AND ANIMAL PHYSIOLOGY
COURSECODE: YNH-80312

Potential benefits dietary protein on mitochondrial protein synthesis after endurance exercise

WAGENINGEN UNIVERSITY AND RESEARCH
BACHELOR NUTRITION & HEALTH
F.W. HOOGSTEDE - 990914361060

ABSTRACT

The effect of protein supplementation on exercise-induced muscle adaptations, especially mitochondrial adaptations, and ultimately endurance performance is still relatively unexplored. The aim of this literature research is to review the current published data on the relation between dietary protein and mitochondrial protein synthesis after endurance exercise. Endurance exercise increases mitochondrial biogenesis by upregulating the master regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1α) expression and activity. Enhanced mitochondrial protein synthesis is an indication of increased endurance performance because it will delay the onset of muscle fatigue, increase utilization, and create more oxidative capacity. It is established that dietary protein enhances the expression of PGC-1α in rest, through interference with the mammalian target of rapamycin complex 1 (mTORC1) pathway. Still, it is inconclusive whether exercise-induced changes in PGC-1α expression due to mTORC1 result in enhanced mitochondrial biogenesis. Studies did find an increase in endurance performance after protein ingestion, but it is not clear whether this effect can be attributed to enhanced oxidative capacity, since the influence of dietary protein on mitochondrial protein synthesis after endurance exercise and its mechanism is still undetermined in humans. Further research should be conducted to uncover the potential effects of protein supplementation on mitochondrial protein synthesis after endurance exercise.

TABLE OF CONTENTS

1. BACKGROUND

Exercise has profound effects on the body. Prolonged endurance exercise depletes fuel storages, damages muscle protein and causes dehydration (Moore et al., 2014). During recovery refuelling, rehydration and repair takes place. Dietary adjustments can affect the recovery process. For example, post-exercise carbohydrate intake positively influences the replenishing of glycogen stores after endurance training (Burke et al., 2004). Dietary protein is important for the repair of damaged muscle during recovery. Studies on the effect of protein supplementation on skeletal muscle protein synthesis is often performed in combination with resistance training regimes. Endurance training was thought to have no effect on skeletal muscle protein synthesis because studies were contradictory about this potential effect. Until a systematic review from Konopka & Harber (2014) revealed that acute and chronic endurance training does induce skeletal muscle protein synthesis. Skeletal muscle protein can be myofibrillar and mitochondrial, and both are positively affected by endurance training. Increased mitochondrial protein suggests an increase in mitochondrial size and number. Mitochondrial adaptations are important for endurance athletes because it may enhance performance by delaying the onset of muscle fatigue and increase the oxidative capacity. Mitochondrial biogenesis, the process of mitochondria production, increases oxidative capacity by enhancing the production of ATP in the oxidative phosphorylation. The master regulator of mitochondrial biogenesis, PGC-1α, plays an important role in multiple exercise-induced adaptations like angiogenesis and increased fat oxidation, but most importantly mitochondrial biogenesis and oxidative phosphorylation enzymes are affected by changes in PGC-1α activity (Baar, 2014). PGC-1α activity increases after a single bout of endurance exercise (Baar et al., 2002; Calvo et al., 2008; Daussin et al., 2012; Konopka et al., 2014; Ventura-Clapier et al., 2008). One of the many regulators of PGC-1α activity is mTORC1. mTORC1 activity is nutrient-dependent, making its pathway a potential target for nutrient intervention in mitochondrial biogenesis. So far, studies mainly looked at the effect of dietary protein on skeletal muscle protein synthesis after endurance exercise but did not distinguish the different muscle protein fractions.

1.1. RESEARCH QUESTION

In this literature review, research is conducted on the effects of dietary protein on mitochondrial protein synthesis after endurance exercise, and whether this effect may be a possible pathway for enhancing endurance performance.

1.2. METHODS

The literature research started by splitting up the research question in three concepts and finding synonyms for each concept. These concepts were inserted in PubMed to search for articles with all three concepts. The concepts were mitochondrial biogenesis, protein supplementation and endurance exercise. In the selected articles, the reference list was used for further selection of relevant papers.

2. MITOCHONDRIA

Mitochondria are most famous for its role as the energy houses of the human body. They generate ATP from macronutrients, which is used in numerous cellular activities. Mitochondria are semiautonomous considering mitochondrial DNA (mtDNA) codes for only 13 subunits of the respiratory chain. All other proteins are encoded in nuclear DNA (Scarpulla, 2002). These proteins can be imported into the mitochondria through a protein import machinery. The nuclear DNA and mtDNA act jointly in the process of mitochondrial biogenesis. mtDNA expression is dependent on transcription and regulatory factors that are produced in the cell. An important factor is the mitochondrial transcription factor A (TFAM). After production, TFAM binds with the regulatory D-loop region of mtDNA, activating mtDNA transcription and replication (Scarpulla, 2002; Wu et al., 1999).

In this review the mitochondrial focus will be on the oxidative phosphorylation. In the inner mitochondrial membrane, oxidation of NADH and $FADH_2$ and phosphorylation of ATP is facilitated by the electron transport chain. Electron carriers work together in transferring electrons from NADH and $FADH_2$ to O_2, creating a proton gradient that is necessary for ATPsynthase to produce ATP from ADP and P_i. The coupling of these processes is determinant in the efficacy of oxidative phosphorylation. Uncoupling results in the production of energy in the form of head instead of ATP which is an undesirable effect in athletes since it will not attribute in enhancing exercise performance (Holloszy, 1967).

2.1. MITOCHONDRIAL BIOGENESIS AND DYNAMICS

Mitochondria are extremely adaptable to environmental cues like cellular stress. Mitochondrial adaptations to enhance oxidative capacity can be either quantitative of qualitative and both processes can occur at the same time. Mitochondrial quality remains adequate by disposing damaged components. This process is regulated by mitochondrial fusion and fission protein (Konopka et al., 2014). Mitochondrial fusion is the process where

multiple mitochondrial membranes merge to exchange damaged segments. Mitochondrial fission proteins package these damaged components in compartments to be removed from the mitochondria (Konopka et al., 2014). When mitochondrial fusion and fission is not adequate, mitochondria will perform suboptimal. Mitochondrial proliferation, or so-called mitochondrial biogenesis, is a quantitative adjustment. The formation of additional mitochondria will result in an increase of the body's capacity to generate ATP. Both quantitative and qualitive changes improve resistance to muscle fatigue and thus improve aerobic performance (Hood et al., 2006; Margolis & Pasiakos, 2013). Mitochondrial proliferation is regulated by two classes: transcription factors and transcriptional co-activators and changes in their mRNA expression precedes biogenesis (Baar, 2004). PGC-1α is an important transcriptional co-activator, a protein that increases transcription without directly binding to it, and the nuclear respiratory factors (NRFs) and TFAM are important transcription factors (Baar, 2014; Scarpulla, 2002). NRFs, mainly NRF-1, regulate the expression of TFAM, but TFAM is not completely depended on the expression of the NRFs (Scarpulla, 2002).

PGC-1α is considered the master regulator of mitochondrial biogenesis. PGC-1α regulates both nuclear and mitochondrial gene expression and elevated levels of PGC-1α results in increased biogenesis, respiratory capacity and mitochondrial coupling (Daussin et al., 2012; Scarpulla, 2002; Wu et al., 1999). The mechanism behind these adaptations start with the interaction between PGC-1α and the NRFs. PGC-1α increases activation of the NRFs, who alter the expression of TFAM, uncoupling proteins (UCPs), and mitochondrial respiratory enzymes (Daussin et al., 2012; Scarpulla, 2002; Wu et al., 1999), resulting in increased mtDNA content (Wu et al., 1999), mitochondrial density (Wu et al., 1999), ATP synthase (β-subunit) (Puigserver et al., 1998) and 2- to 3-fold higher gene expression of cytochrome c oxidase subunits II and IV (COX II and COX IV) (Puigserver et al., 1998; Scarpulla, 2002; Wu et al., 1999).

PGC-1α activity is regulated by transcriptional and post-translation pathways. The transcriptional pathway affects PGC-1α protein levels with regulating the promotor binding activity. After translation, phosphorylation and acetylation affect the PGC-1α activity (Fernandez-Marcos & Auwerx, 2011). Phosphorylation increases the activity of PGC-1α and acetylation decreases its activity. PGC-1α can regulate its own promotor binding activity by acting on myocyte enhancer factor 2 (MEF2), forming a positive feedback loop (Handschin et al., 2003). PGC-1α expression is regulated by many factors. mTORC and p38 MAPK will be discussed later in this literature review since both factors are relevant for the research aim. mTORC activity is nutrient-dependent and p38 MAPK can be activated by exercise.

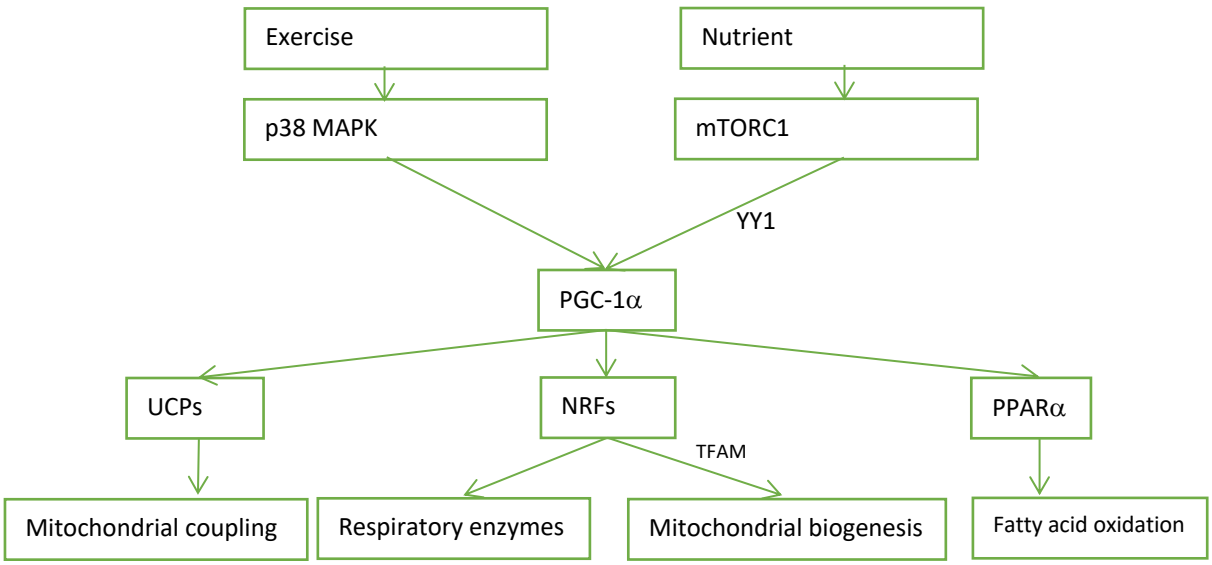

FIGURE 1. PGC-1α PATHWAY

In short, PGC-1α increases the activity of UCPs, NRF-1 and PPARα, that are respectively responsible for the increase in mitochondrial coupling; oxygen consumption, citrate synthase, respiratory enzyme complexes and general mitochondrial protein; and fatty acid oxidation.

3. ENDURANCE EXERCISE-INDUCED ADAPTATIONS

Endurance trained athletes have higher aerobic capacity than their sedentary counterparts. In the previous century, physiological differences were attributed to cardiovascular adaptations in response to training. However, not all could be explained by cardiovascular adaptations, for example lower blood lactate concentrations during rest and exercise. Therefore, further research was conducted, and different exercise adaptations were found in mitochondria and muscle mass. The extent of exercise-induced adaptations, both mitochondrial and skeletal muscle mass, is dependent on the frequency, intensity and duration of the exercise (Hood et al., 2006; Konopka & Harber, 2014). An increase of external loading in resistance exercise did not result in enhanced muscle protein synthesis and that PGC-1α mRNA, myofibrillar protein synthesis and mitochondrial protein synthesis levels are more elevated after a high-intensity endurance exercise compared to a low-intensity exercise (Di Donato et al., 2014; Egan et al., 2010; Mitchell et al., 2012). Therefore, Konopka & Harber (2014) suggested that high-volume, low external loading is the best applicable training program for both young and old sedentary people in order to increase muscle protein synthesis (Di Donato et al., 2014; Egan et al., 2010; Mitchell et al., 2012).

Resistance training regimes has its main focus on muscle hypertrophy, whereas aerobic training focuses on increasing mechanical efficiency and oxygen consumption. Consequently, research on the effects of exercise on muscle hypertrophy aims at resistance training. Nonetheless, endurance training does induce skeletal muscle protein synthesis, increasing both myofiber and whole muscle size, and improving muscle function (Konopka & Harber, 2014). Other adaptations to endurance exercise, that will be explained more elaborate below, is mitochondrial biogenesis and mitochondrial fusion and fission. Mitochondrial adaptations and muscle protein synthesis influence each other. Muscle protein synthesis is needed for mitochondrial biogenesis and at the same time mitochondrial adaptations can stimulate skeletal muscle protein synthesis. For example, additional mitochondria will produce more ATP during recovery, which can be used for the energy-costly process of muscle protein synthesis (Konopka & Harber, 2014).

Mitochondrial composition and size can adapt due to endurance training. Strenuous endurance training increases numerous mitochondrial factors like oxygen consumption (Morgan et al., 1971), citrate synthase (Daussin et al., 2012), respiratory enzyme complexes (Calvo et al., 2008; Fink et al., 1977; Gollnick et al., 1972; Holloszy, 1967; Konopka et al., 2014; Morgan et al., 1971), and general mitochondrial protein (Churchward-Venne et al., 2019; Holloszy, 1967; Morgan et al., 1971; Philp et al., 2015). The fact that both mitochondrial protein and respiratory enzyme complexes are elevated suggests increased enzyme protein concentration (Holloszy, 1967). ATP capacity will also rise due to endurance exercise under the prerequisite that the oxidative phosphorylation remains tightly coupled to ATP synthesis, which is the case after endurance exercise. Holloszy (1967) found no increase in the P:O ratio between the sedentary and exercising group, meaning that the coupling remained the same, although higher oxidative phosphorylation indicators were found.

Most exercise-induced mitochondrial adaptations can be explained by its effect on the PGC-1α - NRF-1 pathway. PGC-1α increases NRF-1 activation which is involved in the expression of mitochondrial respiratory chain enzymes causing an increase in electron transport chain activity (Wu et al., 1999). Increased PGC-1α and NRFs mRNA and protein are found after one bout of endurance exercise (Baar et al., 2002; Calvo et al., 2008; Daussin et al., 2012; Konopka et al., 2014; Ventura-Clapier et al., 2008). In addition, increased levels of TFAM, a factor that is involved in this pathway of mitochondrial biogenesis, is found after aerobic exercise (Bengtsson et al., 2001; Calvo et al., 2008). Elevated levels of PGC-1α after short-intense and prolonged endurance exercise ultimately increases exercise capacity and performance (Calvo et al., 2008). PGC-1α activity is affected by endurance training through both the transcriptional and post-translational pathway (Margolis & Pasiakos, 2013). An example is the exercise-induced increase of p38 MAPK activity, a potent stimulator for PGC-1α (Akimoto et al., 2005; Boppart et al., 2000; Wright et al., 2007). p38 MAPK interacts with PGC-1α directly, by phosphorylating PGC-1α, which increases its activity (Egan et al., 2010). Another possible mechanism involved in increasing the effect of PGC-1α on mitochondrial protein synthesis does not involve post-translation pathways but transcriptional. PGC-1α has multiple isoforms and the type of promotor that is activated determines the produced isoform. PGC-1α1 is present in all tissue whereas PGC-1α2-4 is mainly present in brown fat and muscle tissue. The activity of the alternative promotor, producing PGC-1α2-4, increases after endurance exercise while the activity of the promotor for PGC-1α1 remains similar (Baar, 2014). This could be a possible an explanation for the increased activity of PGC-1α. PGC-1α2-4 have shorter mRNA variants without the exon 8 (Baar et al., 2002). Exon 8 is suggested to have an inhibitory effect on the production of NRFs (Vega et al., 2000). If this part is not present on the mRNA, the protein will be 40-fold more active according to in vitro studies (Baar, 2004).

A different pathway involved in the adaptation of mitochondrial functioning to endurance exercise is the interaction of PGC-1α with peroxisome proliferator-activated receptor (PPAR). PGC-1α increases PPARα activity and PPARα is involved in the change of metabolic substrate preference from carbohydrate to lipid by inducing the expression of enzymes of fatty acid oxidation (Hood et al., 2006; Scarpulla, 2002).

Not only mitochondrial quantity is affected by endurance training. Mitochondrial quality with mitochondrial fusion and fission proteins as indicators, improves after aerobic exercise. Konopka and colleagues (2014) studied the effect of endurance training on mitochondrial fusion protein (MFN1, MFN2 and optic atrophy protein-1) and fission protein (FIS1) and found elevated levels of MFN1, MFN2 and FIS1 in young and old men, suggesting an increase in mitochondrial quality due to endurance training.

In conclusion, endurance exercise increases PGC-1α activity and expression transcriptional and post-translational. p38 MAPK activity increases after endurance exercise which enhances the PGC-1α activity by phosphorylation of the protein. Transcriptionally, the binding of the alternative promotor is responsible for the increase in PGC-1α activity. Enhanced PGC-1α activity results in increased aerobic performance by increasing the muscle oxidative capacity and delay the onset of muscle fatigue.

4. PROTEIN SUPPLEMENTATION

Endurance training disturbs the net protein balance during exercise and recovery. During exercise amino acid oxidation rises to provide an alternative fuel source, especially branched-chain amino acid leucine oxidation increases (Moore et al., 2014; Tarnopolsky, 2004). Leucine can induce mitochondrial biogenesis by increasing PGC-1α gene expression, consequently amino acid oxidation is not beneficial for mitochondrial biogenesis (Sun & Zemel, 2009). Exercise-induced adaptations like enhanced capillarization and muscle protein synthesis require protein which enhances the protein need during recovery (Knuiman et al., 2018). Logically, additional protein availability would be beneficial for recovery processes. In a study from Howarth et al. (2019) significant higher levels of skeletal muscle protein synthesis were observed in the intervention group consuming protein and carbohydrate compared to the control group with an isocaloric carbohydrate beverage. No distinction was made between the different muscle fractions, but the study did encourage further research looking into the effect of dietary protein on myofibrillar and mitochondrial protein synthesis. However, these relatively new studies have not reached a consensus on the potential beneficial effects dietary protein has on mitochondrial protein. In the next section, a theoretical mechanism for dietary protein interference with mitochondrial protein synthesis will be presented and relevant papers are discussed.

4.1. THEORETICAL MECHANISM FOR DIETARY PROTEIN INTERFERENCE

The building blocks from protein, amino acids, influence muscle protein synthesis through translation initiation mammalian targets of rapamycin complex 1 (mTORC1) (Rowlands et al., 2011). mTORC1 is a protein complex made from mTOR and distinguishes itself from mTORC2 with the protein raptor. mTORC1 activity is nutrient sensitive whereas mTORC2 is not. Intracellular amino acid levels regulate mTORC1 activation through their effect on the formation of the Rheb-GTP complex. This complex can directly activate mTORC1 by binding to it (Avruch et al., 2009). Branched-chain amino acids (BCAAs), especially leucine, are most potent in increasing mTORC1 activation (D'Antona et al., 2010; Proud, 2007). mTORC1 is involved in increasing mitochondrial biogenesis in resting cells by interacting with PGC-1α and transcription factor yin-yang 1 (YY1) (Cunningham et al., 2007). YY1 binds with promotors of mitochondrial genes and recruits PGC-1α to target promotors, positively influencing mitochondrial gene expression. Inactivation of mTORC1 by rapamycin disrupts the phosphorylation of YY1, which is a necessity to form the PGC-1α-YY1 interaction, leading to lower levels of mitochondrial genes expression and oxygen consumption in mice and rates (Blattler et al., 2012; Cunningham et al., 2007).

The above suggested mechanism describes the role of mTORC1 in one of the PGC-1α pathways for increasing mitochondrial protein synthesis in rest. Whether this theoretical effect is present after endurance training is still open for discussion. Philip and colleagues found enhanced mitochondrial protein synthesis after endurance exercise in mice while mTORC1 is suppressed, suggesting that mTORC1 does not play a role in the exercise-induced increase of mitochondrial protein synthesis (Philp et al., 2015). In humans, elevated levels of mTOR phosphorylation are found after endurance training, which indicate an increase in activity (Mascher et al., 2007). Masscher et al. did not measure PGC-1α or other transcription factors related to mitochondrial biogenesis, nor did they measure endurance performance. Therefore, the only conclusion that can be drawn from this study is that mTOR activity increases after one bout of endurance exercise. Further research should be conducted in humans to clarify the role of mTORC1 in mitochondrial adaptations to endurance training.

4.2. RELEVANT PAPERS

Although the research area is relatively new, some relevant papers are published on the effect of dietary protein in combination with endurance exercise on mitochondrial adaptations (Table 1). D'Antona et al. investigated the effect BCAA has on mitochondrial biogenesis in muscle tissue in middle-aged mice. The used BCAA mixture improved numerous mitochondrial biogenesis biomarkers like PGC-1α. This effect was higher in exercised mice compared to their sedentary counterpart, suggesting a positive relation between dietary protein and mitochondrial protein synthesis after exercise . Soon, studies in humans followed exploring this potential interaction (Breen et al., 2011; Churchward-Venne et al., 2020; Coffey et al., 2011; Hansen et al., 2020; Knuiman et al., 2019).

4.2.1. SHORT-TERM STUDIES

Studies looking into the effect of protein ingestion on mitochondrial protein synthesis after one single bout of exercise did not observe an increase (Breen et al., 2011; Churchward-Venne et al., 2020; Coffey et al., 2011). Breen and colleagues investigated the effect that carbohydrate-protein co-ingestion has on mitochondrial protein synthesis rates. Subjects performed two similar trials with a 90 minute high-intensity, steady state cycling exercise and immediately consumed a beverage with either 25.4g carbohydrates (CHO) and 10.2g proteins (PRO) or only 25.2g CHO. Muscle biopsies were collected at 5- and 240-minutes post-exercise. Breen et al. established higher levels of mTOR phosphorylation in the PRO+CHO group compared with the control but no difference in mitochondrial protein synthesis rates was found between the treatments. Coffey and colleagues used a different type of exercise, namely sprint-exercise. They performed a randomized double-blind, cross over study with a relatively small simple size of eight endurance-trained males. Every subject performed two repeated sprint exercise session with two weeks of recovery. During these two weeks subjects maintained their habitual level of physical activity. Before each exercise session, subjects ingested either a non-nutrient beverage (control) or a nutrient beverage containing 24g whey protein, 4.8g leucine and 60g maltodextrin. Muscle biopsies were taken 60 minutes pre-exercise (resting biopsy) and 15- and 240-minutes post-exercise. They found a significant increase in mTOR phosphorylation after 15 minutes but not after 240 minutes post-exercise. However, no difference in mitochondrial protein synthesis was found between the interventions in both post-exercise muscle biopsies. Although, sprint-exercise do increase mRNA of important mitochondrial protein like cytochrome C (Coffey et al., 2011), it is different from prolonged endurance training. Sprint-exercise is more leaning to the middle of the resistance-endurance exercise spectrum. Therefore, Churchward-Venne et al. looked into the effect of continuous endurance exercise instead of sprint-exercise. Participants exercised ones for 90 minutes at 60% of their previously measured W_{max} and received one of four treatments afterwards: 45g CHO (control), 45g CHO + 15g PRO, 45g CHO + 30g PRO or 45g CHO + 45g PRO. Muscle biopsies were taken directly, 180- and 360-minutes post-exercise. Researchers found no significant increase in mitochondrial protein synthesis, but they did detect significant amounts of dietary protein-derived amino acids to be incorporated into new synthesized mitochondrial protein. This incorporation was dose-dependent, meaning that higher intake of dietary protein resulted in more incorporation. Not only the increase in incorporation follows a dose-dependent pattern, also the net protein balance increased more with growing levels dietary protein intake. This suggests that a higher dietary protein intake would be more beneficial compared to a lower intake which is also advocated by Kato and colleagues who found an estimated average requirement of 1.65-1.83 g/kg/d when following an endurance training regime (Kato et al., 2016).

All three studies investigating mitochondrial protein synthesis after one single bout of exercise did not observe a significant effect of supplementation. However, in two of the three mentioned studies that did not find an increase in mitochondrial protein synthesis after dietary protein ingestion, baseline measurements were not taken properly. They compared the mitochondrial protein synthesis rates between the different treatments and no difference was found, but it cannot be excluded that this is the result of between-subject variability or insufficient exercise intensity to induce mitochondrial adaptations. This could be a factor that may have altered the outcome. Yet, all three studies find non-significant results, strongly indicating that dietary protein intake does not further enhance mitochondrial protein synthesis after one bout of endurance exercise. Whether a training regime could enhance mitochondrial protein synthesis was out of the scope of these studies.

4.2.2. LONG-TERM STUDIES

Knuiman et al. and Hansen et al did look into the effect on mitochondrial protein synthesis after long-term protein supplementation. To date, only Hansen et al. (2020) found evidence supporting that long-term dietary protein results in an increase in mitochondrial protein synthesis in humans after endurance exercise. In this study subjects exercised 5-7 times per week and ingested pre- and post-exercise beverages within 10 minutes from every workout. In the protein group the pre-exercise beverage contained 0.3g PRO/kg bodyweight and the post-exercise 0.3g PRO/kg + 1.0g CHO/kg. The control group received 0.3g CHO/kg pre-exercise and 1.3g CHO/kg post-exercise. Muscle biopsies were taken two days prior to the treatment and two days after. Cytochrome C (Cyt C) levels changes were significantly higher in the protein group and although other mitochondrial proteins did not reach significancy, they followed a similar pattern. The researchers also investigated the effect of the supplementation on PGC-1α, but they did not find an increase in PGC-1α mRNA expression between the resting and post-exercise muscle biopsy. The activation of the transcription factor genes and PGC-1α gene is in between 1-4h after recovery (Moore, Tang, et al., 2009; Wilkinson et al., 2008). Post-exercise muscle biopsies were taken two days after the last exercise, which could explain the absence of increased PGC-1α mRNA expression.

Another long-term study looking into the effect of supplementation on mitochondrial protein synthesis compared protein intake with an isocaloric carbohydrate intake (Knuiman et al., 2019). 44 active males ingested a beverage with either 29g casein protein or CHO every night and post-exercise. Subjects cycled 60 minutes continuously 2-3 times per week and after every session each subject received 56g CHO. Muscle biopsies were taken 3-4 days prior to the start of the intervention (resting biopsy) and in week 6 and week 12. The researchers did not find a significant difference between treatments in an increase of CS during intervention and CytC did not increase significantly compared to rest. The short half-life time of CytC and the late muscle biopsy could explain the lack of CytC. Researchers also looked at the effect of supplementation on endurance performance and found a significantly higher increase of VO_{2max} in the protein group compared to the controls. This is in line with previous studies that found an increase of VO_{2max} after six weeks of post-exercise protein intake (Koopman et al., 2004) and after four weeks of post-exercise protein and carbohydrate intake (Robinson et al., 2011) compared to the group with only carbohydrate intake and exercise.

Up to this point, these are the only two studies looking into the effect of dietary protein on mitochondrial protein synthesis after endurance training. The conflicting results can be attributed to differences in training and nutrition schedules. Both studies used an isocaloric control, but Hansen et al. added protein to an amount of carbohydrates, whereas Knuiman et al. replaced carbohydrates completely with proteins. Minimal carbohydrate intake (0.5g/kg/h) inhibits the exercise-induced activation of PGC-1α and other transcription factor genes, which might blunt mitochondrial biogenesis (Pilegaard et al., 2005). The found difference in outcome could indicate that replacing carbohydrates completely with proteins is not beneficial for mitochondrial protein synthesis. Additionally, subjects in the Hansen trial exercised more frequently than in the Knuiman trial. Mitochondrial adaptations dependent on the frequency, duration and intensity of exercise which could explain the difference in significancy between the two long-term studies.

TABLE 1. RELEVANT PAPERS ON DIETARY PROTEIN EFFECTS ON MITOCHONDRIAL PROTEIN SYNTHESIS AFTER ENDURANCE EXERCISE

Study	Subjects	Intervention	Control	Timing ingestion	Training schedule	Muscle biopsy	mTOR	Outcome
Coffey et al., 2011	8 endurance-trained males	24g whey protein, 4.8g leucine and 60g maltodextrin	Non-nutrient	30 minutes pre-exercise	Habitual	30 minutes pre-exercise (rest) + 15- and 240-minutes post-exercise	220% increase (P<0.01) after nutrient	Non-significant
Breen et al., 2011	10 endurance-trained males	25.4g CHO and 10.2g PRO	25.2g CHO	Immediately post-exercise	Habitual	5- and 240-minutes post-exercise	0.91±0.09 vs 1.05 ±0.05 (between treatments P<0.05)	Non-significant
Churchward-Venne et al., 2020	48 endurance-trained males	45g CHO and 15/30/45g PRO	45g CHO	Immediately post-exercise	-	0, 180- and 360-minutes post-exercise	Not measured	Non-significant (P=0.09)
Hansen et al., 2020	22 males and 2 females, endurance trained (18-50y)	0.3g PRO/kg pre-exercise + 0.3g PRO/kg and 1.0 g CHO/kg post-exercise	0.3g CHO/kg pre- and 1.3g CHO/kg post-exercise	-	5-7 workouts per week	2d prior (rest) and 2d after intervention	Not measured	CytC significant (P=0.04)
Knuiman et al., 2019	44 recreationally active males	29 casein protein	Isocaloric CHO	Immediately post-exercise and pre-night	3 workouts per week	3-4d prior to intervention (rest) + week 6 and week 12	Not measured	CytC and CS non-significant

DISCUSSION

In this literature review, the potential effect of dietary protein on mitochondrial protein synthesis after endurance exercise is studied. Due to the lack of sufficient relevant papers and the contradictions found in these papers, further research was conducted looking into the mechanism behind exercise- and nutrient-induced mitochondrial biogenesis. The master regulator of biogenesis, PGC-1α, activity is enhanced after endurance exercise and amino acid intake. mTORC1 is thought to be an important nutrient-dependent regulator of PGC-1α. In rest, PGC-1α activity is influenced by mTORC1 activity, which is regulated by amino acid concentration, especially BCAAs are potent in activating mTORC1. Yet, to my knowledge no studies are performed in humans measuring both mTORC1 and PGC-1α activity after endurance exercise.

The relevant papers on dietary protein supplementation can be divided into long-term and short-term studies. In the short-term studies, one bout of exercise did not reach significancy in increasing mitochondrial protein synthesis, suggesting that dietary protein did not enhance mitochondrial adaptations. However, in a study from Hill et al. (2013) PGC-1α mRNA activity increase after one bout of prolonged endurance exercise was higher in the intervention groups that consumed carbohydrates and whey protein compared to the control who only consumed carbohydrates. These findings suggest that exercise-induced mitochondrial adaptations require a certain amount of time which is in alignment with the observation that mitochondrial state 3 respiration is only achieved after a minimum of six to ten days (Pilegaard et al., 2000). This could also explain the increases found in the long-term supplementation study (Hansen et al., 2020)

In both short- and long-term studies active subjects were recruited. It could be a possibility that mitochondrial protein synthesis in endurance trained subjects was already at maximal level. Experienced athletes have a blunted response of exercise-induced AMPK activity, which is an important energy-sensing protein that positively regulates PGC-1α activity (Wilkinson et al., 2008). The possible increased effect that dietary protein has on mitochondrial protein synthesis may be blunted as well. Research in resistance exercise demonstrate that protein intake exceeding 20g does not enhance mitochondrial protein synthesis significantly further (Moore, Robinson, et al., 2009; Witard et al., 2014). This plateau could play a role in endurance athletes as well, but no research has been conducted in this area so far.

In conclusion, endurance exercise increases PGC-1α activity through transcriptional and post-translation pathways. Enhanced PGC-1α activity results in increased aerobic performance by increasing the muscle oxidative capacity and delay the onset of muscle fatigue. The PGC-1α mechanism involves increased activity of UCPs, NRF-1 and PPARα. This results in the following exercise-induced adaptations: increase in mitochondrial coupling, respiratory enzymes, mitochondrial biogenesis and fatty acid oxidation. The exercise-induced increase of PGC-1α mRNA activity enhances further after co-ingestion with protein and carbohydrates. This additional PGC-1α increase after one bout of exercise did not directly lead to an increase in mitochondrial protein synthesis. Possible, an accumulation of PGC-1α increase is needed to raise mitochondrial protein synthesis rates to such a level that is significantly detectable.

RECOMMENDATION

The amount of relevant papers was limited, indicating that there is still a relatively large unexplored research area. Studies often did not analyse muscle protein fractions, which is crucial in answering the research question. Since, the timetable of PGC-1α and mitochondrial protein synthesis is still relatively unknown it is important that more research will be performed. Otherwise, researchers might miss potential effects due to incorrect timing of muscle biopsies. There is an indication that PGC-1α activity will be elevated in the early-recovery phase (±4h) and in the late-recovery phase (24-48h mitochondrial protein synthesis rates will be higher (Di Donato et al., 2014; Little et al., 2011). Due to these limitations and others, I designed an ultimate experiment that would be able to answer all questions.

In this study, both male and female should be represented. Oestrogen receptors play an important role in inducing mitochondrial biogenesis and females have a very different hormonal regulation, hence the importance of including both sexes. Next to, subjects should be recreationally active but not endurance trained. The study will be a double-blind block-randomized controlled intervention trial with three arms, with all isocaloric beverages. Subjects will receive either pre-exercise 15g PRO + 10g CHO and post-exercise 30g PRO + 30g CHO, pre-exercise 25g PRO and post-exercise 30g PRO + 30g CHO, or pre-exercise 25g CHO and 60g post-exercise CHO. Due to the potential benefits of branched-chain amino acid leucine, leucine will account for 10% of the protein beverage.

All subjects will follow a training schedule with 5 workouts per week for the duration of 6 weeks. Each workout consists of bicycling on an ergometer for sixty minutes. Assuming that the expected timetable on mitochondrial protein synthesis is correct, two days prior to resting muscle biopsies all forms of exercise is denied. 4-, 24- and 48h after completion of the trial muscle biopsies are taken. From these muscle biopsies data on mitochondrial protein fraction, mTORC1 phosphorylation and PGC-1α can be drawn. Since the research aim involves a certain muscle protein fraction, it is important to analyse the muscle biopsy accordingly. Citrate synthase and complex I activity show the strongest association with mitochondrial content and therefore will be used as biomarker (Larsen et al., 2012). Before and after the intervention, all subjects will perform a stimulated 10-km time trial on a cycle ergometer as endurance exercise performance test and VO_{2max} will be examined using an incremental cycle-test to exhaustion.

REFERENCES

Akimoto, T., Pohnert, S. C., Li, P., Zhang, M., Gumbs, C., Rosenberg, P. B., Williams, R. S., & Yan, Z. (2005). Exercise stimulates Pgc-1α transcription in skeletal muscle through activation of the p38 MAPK pathway. *Journal of Biological Chemistry*, *280*(20), 19587–19593. https://doi.org/10.1074/jbc.M408862200

Avruch, J., Long, X., Ortiz-Vega, S., Rapley, J., Papageorgiou, A., & Dai, N. (2009). Amino acid regulation of tor complex 1. *Am J Physiol Endocrinol Metab*, *296*, 592–602. https://doi.org/10.1152/ajpendo.90645.2008.-TOR

Baar, K. (2004). Involvement of PPARγ co-activator-1, nuclear respiratory factors 1 and 2, and PPARα in the adaptive response to endurance exercise. *Proceedings of the Nutrition Society*, *63*, 269–273. https://doi.org/10.1079/pns2004334

Baar, K. (2014). Nutrition and the Adaptation to Endurance Training. *Sports Medicine*, *44*(1), 5–12. https://doi.org/10.1007/s40279-014-0146-1

Baar, K., Wende, A. R., Jones, T. E., Marison, M., Nolte, L. A., Chen, M., Kelly, D. P., & Holloszy, J. O. (2002). Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *The FASEB Journal*, *16*(14), 1879–1886. https://doi.org/10.1096/fj.02-0367com

Bengtsson, J., Gustafsson, T., Widegren, U., Jansson, E., & Sundberg, C. J. (2001). Mitochondrial transcription factor A and respiratory complex IV increase in response to exercise training in humans. *Pflugers Archiv European Journal of Physiology*, *443*, 61–66. https://doi.org/10.1007/s004240100628

Blattler, S. M., Verdeguer, F., Liesa, M., Cunningham, J. T., Vogel, R. O., Chim, H., Liu, H., Romanino, K., Shirihai, O. S., Vazquez, F., Ruegg, M. A., Shi, Y., & Puigserver, P. (2012). Defective Mitochondrial Morphology and Bioenergetic Function in Mice Lacking the Transcription Factor Yin Yang 1 in Skeletal Muscle. *Molecular and Cellular Biology*, *32*(16), 3333–3346. https://doi.org/10.1128/mcb.00337-12

Boppart, M. D., Asp, S., Wojtaszewski, J. F. P., Fielding, R. A., Mohr, T., & Goodyear, L. J. (2000). Marathon running transiently increase c-Jun NH2-terminal kinase and p38γ activities in human skeletal muscle. *Journal of Physiology*, *526*(3), 663–669. https://doi.org/10.1111/j.1469-7793.2000.00663.x

Breen, L., Philp, A., Witard, O. C., Jackman, S. R., Selby, A., Smith, K., Baar, K., & Tipton, K. D. (2011). The influence of carbohydrate-protein co-ingestion following endurance exercise on myofibrillar and mitochondrial protein synthesis. *The Journal of Physiology*, *589*(16), 4011–4025. https://doi.org/10.1113/jphysiol.2011.211888

Burke, L. M., Kiens, B., & Ivy, J. L. (2004). Carbohydrates and fat for training and recovery. *Journal of Sports Sciences*, *22*(1), 15–30. https://doi.org/10.1080/0264041031000140527

Calvo, J. A., Daniels, T. G., Wang, X., Paul, A., Lin, J., Spiegelman, B. M., Stevenson, S. C., & Rangwala, S. M. (2008). Muscle-specific expression of PPARγ coactivator-1α improves exercise performance and increases peak oxygen uptake. *Journal of Applied Physiology*, *104*(5), 1304–1312. https://doi.org/10.1152/japplphysiol.01231.2007

Churchward-Venne, T. A., Pinckaers, P. J. M., Smeets, J. S. J., Betz, M. W., Senden, J. M., Goessens, J. P. B., Gijsen, A. P., Rollo, I., Verdijk, L. B., & van Loon, L. J. C. (2020). Dose-response effects of dietary protein on muscle protein synthesis during recovery from endurance exercise in young men: a double-blind randomized trial. *The American Journal of Clinical Nutrition*, *112*(2), 303–317. https://doi.org/10.1093/ajcn/nqaa073

Churchward-Venne, T. A., Pinckaers, P. J., Smeets, J. S., Peeters, W. M., Zorenc, A. H., Schierbeek, H., Rollo, I., Verdijk, L. B., & Van Loon, L. J. (2019). Myofibrillar and Mitochondrial Protein Synthesis Rates Do Not Differ in Young Men Following the Ingestion of Carbohydrate with Whey, Soy, or Leucine-Enriched Soy Protein after Concurrent Resistance- and Endurance-Type Exercise. *Journal of Nutrition*, *149*(2), 210–220. https://doi.org/10.1093/jn/nxy251

Coffey, V. G., Moore, D. R., Burd, N. A., Rerecich, T., Stellingwerff, T., Garnham, A. P., Phillips, S. M., & Hawley, J. A. (2011). Nutrient provision increases signalling and protein synthesis in human skeletal muscle after repeated sprints. *European Journal of Applied Physiology*, *111*, 1473–1483. https://doi.org/10.1007/s00421-010-1768-0

Cunningham, J. T., Rodgers, J. T., Arlow, D. H., Vazquez, F., Mootha, V. K., & Puigserver, P. (2007). mTOR

controls mitochondrial oxidative function through a YY1-PGC-1a transcriptional complex. *Nature*, *450*, 736–741. https://doi.org/10.1038/nature06322

D'Antona, G., Ragni, M., Cardile, A., Tedesco, L., Dossena, M., Bruttini, F., Caliaro, F., Corsetti, G., Bottinelli, R., Carruba, M. O., Valerio, A., & Nisoli, E. (2010). Branched-chain amino acid supplementation promotes survival and supports cardiac and skeletal muscle mitochondrial biogenesis in middle-aged mice. *Cell Metabolism*, *12*(4), 362–372. https://doi.org/10.1016/j.cmet.2010.08.016

Daussin, F. N., Rasseneur, L., Bouitbir, J., Charles, A. L., Dufour, S. P., Geny, B., Burelle, Y., & Richard, R. (2012). Different timing of changes in mitochondrial functions following endurance training. *Medicine and Science in Sports and Exercise*, *44*(2), 217–224. https://doi.org/10.1249/MSS.0b013e31822b0bd4

Di Donato, D. M., West, D. W. D., Churchward-Venne, T. A., Breen, L., Baker, S. K., & Phillips, S. M. (2014). Influence of aerobic exercise intensity on myofibrillar and mitochondrial protein synthesis in young men during early and late postexercise recovery. *American Journal of Physiology-Endocrinology and Metabolism*, *306*(9), E1025–E1032. https://doi.org/10.1152/ajpendo.00487.2013

Egan, B., Carson, B. P., Garcia-Roves, P. M., Chibalin, A. V., Sarsfield, F. M., Barron, N., McCaffrey, N., Moyna, N. M., Zierath, J. R., & O'Gorman, D. J. (2010). Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor γ coactivator-1α mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. *Journal of Physiology*, *588*(10), 1779–1790. https://doi.org/10.1113/jphysiol.2010.188011

Fernandez-Marcos, P. J., & Auwerx, J. (2011). Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. *American Journal of Clinical Nutrition*, *93*(4), 884S. https://doi.org/10.3945/ajcn.110.001917

Fink, W. J., Costill, D. L., & Pollock, M. L. (1977). SUBMAXIMAL AND MAXIMAL WORKING CAPACITY OF ELITE DISTANCE RUNNERS. PART II. MUSCLE FIBER COMPOSITION AND ENZYME ACTIVITIES. *Annals of the New York Academy of Sciences*, *301*(1 The Marathon), 323–327. https://doi.org/10.1111/j.1749-6632.1977.tb38210.x

Gollnick, P. D., Armstrong, R. B., Saubert, C. W., Piehl, K., & Saltin, B. (1972). Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. *Journal of Applied Physiology*, *33*(3), 312–319. https://doi.org/10.1152/jappl.1972.33.3.312

Handschin, C., Rhee, J., Lin, J., Tarr, P. T., & Spiegelman, B. M. (2003). An autoregulatory loop controls peroxisome proliferator-activated receptor γ coactivator 1α expression in muscle. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(12), 7111–7116. https://doi.org/10.1073/pnas.1232352100

Hansen, M., Oxfeldt, M., Larsen, A. E., Thomsen, L. S., & Christensen, B. (2020). Supplement With Whey Protein Hydrolysate in Contrast to Carbohydrate Supports Mitochondrial Adaptations in Trained Runners. *Journal of the International Society of Sports Nutrition*, *17*(46), 1–28. https://doi.org/10.1186/s12970-020-00376-3

Holloszy, J. O. (1967). Biochemical Adaptations in Muscle. *The Journal of Biological Chemistry*, *242*(9), 2278–2282.

Hood, D. A., Irrcher, I., Ljubicic, V., & Joseph, A. M. (2006). Coordination of metabolic plasticity in skeletal muscle. In *Journal of Experimental Biology* (pp. 2265–2275). https://doi.org/10.1242/jeb.02182

Kato, H., Suzuki, K., Bannai, M., & Moore, D. R. (2016). Protein requirements are elevated in endurance athletes after exercise as determined by the indicator amino acid oxidation method. *PLoS ONE*, *11*(6). https://doi.org/10.1371/journal.pone.0157406

Knuiman, P., Hopman, M. T. E., Verbruggen, C., & Mensink, M. (2018). Protein and the adaptive response with endurance training: Wishful thinking or a competitive edge? In *Frontiers in Physiology* (Vol. 9, Issue MAY). https://doi.org/10.3389/fphys.2018.00598

Knuiman, P., Van Loon, L. J. C., Wouters, J., Hopman, M., & Mensink, M. (2019). Protein supplementation elicits greater gains in maximal oxygen uptake capacity and stimulates lean mass accretion during prolonged endurance training: A double-blind randomized controlled trial. *American Journal of Clinical Nutrition*, *110*(2), 508–518. https://doi.org/10.1093/ajcn/nqz093

Konopka, A. R., & Harber, M. P. (2014). Skeletal muscle hypertrophy after aerobic exercise training. In *Exercise*

and Sport Sciences Reviews (pp. 53–61). https://doi.org/10.1249/JES.0000000000000007

Konopka, A. R., Suer, M. K., Wolff, C. A., & Harber, M. P. (2014). Markers of human skeletal muscle mitochondrial biogenesis and quality control: Effects of age and aerobic exercise training. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *69*(4), 371–378. https://doi.org/10.1093/gerona/glt107

Koopman, R., Pannemans, D. L. E., Jeukendrup, A. E., Gijsen, A. P., Senden, J. M. G., Halliday, D., Saris, W. H. M., Van Loon, L. J. C., & Wagenmakers, A. J. M. (2004). Combined ingestion of protein and carbohydrate improves protein balance during ultra-endurance exercise. *American Journal of Physiology - Endocrinology and Metabolism*, *287*, E712–E720. https://doi.org/10.1152/ajpendo.00543.2003

Larsen, S., Nielsen, J., Hansen, C. N., Nielsen, L. B., Wibrand, F., Stride, N., Schroder, H. D., Boushel, R., Helge, J. W., Dela, F., & Hey-Mogensen, M. (2012). Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. *Journal of Physiology*, *14*, 3349–3360. https://doi.org/10.1113/jphysiol.2012.230185

Little, J. P., Safdar, A., Bishop, D., Tarnopolsky, M. A., & Gibala, M. J. (2011). An acute bout of high-intensity interval training increases the nuclear abundance of PGC-1 and activates mitochondrial biogenesis in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*, *300*. https://doi.org/10.1152/ajpregu.00538.2010.-Low-volume

Margolis, L. M., & Pasiakos, S. M. (2013). Optimizing intramuscular adaptations to aerobic exercise: Effects of carbohydrate restriction and protein supplementation on mitochondrial biogenesis. In *Advances in Nutrition* (Vol. 4, Issue 6, pp. 657–664). https://doi.org/10.3945/an.113.004572

Mascher, H., Andersson, H., Nilsson, P.-A., Ekblom, B., & Blomstrand, E. (2007). Changes in signalling pathways regulating protein synthesis in human muscle in the recovery period after endurance exercise. *Acta Physiologica*, *191*(1), 67–75. https://doi.org/10.1111/j.1748-1716.2007.01712.x

Mitchell, C. J., Churchward-Venne, T. A., West, D. W. D., Burd, N. A., Breen, L., Baker, S. K., & Phillips, S. M. (2012). Resistance exercise load does not determine training-mediated hypertrophic gains in young men. *Journal of Applied Physiology*, *113*(1), 71–77. https://doi.org/10.1152/japplphysiol.00307.2012

Moore, D. R., Camera, D. M., Areta, J. L., & Hawley, J. A. (2014). Beyond muscle hypertrophy: Why dietary protein is important for endurance athletes. In *Applied Physiology, Nutrition and Metabolism* (Vol. 39, Issue 9, pp. 987–997). https://doi.org/10.1139/apnm-2013-0591

Moore, D. R., Robinson, M. J., Fry, J. L., Tang, J. E., Glover, E. I., Wilkinson, S. B., Prior, T., Tarnopolsky, M. A., & Phillips, S. M. (2009). Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *American Journal of Clinical Nutrition*, *89*(1), 161–168. https://doi.org/10.3945/ajcn.2008.26401

Moore, D. R., Tang, J. E., Burd, N. A., Rerecich, T., Tarnopolsky, M. A., & Phillips, S. M. (2009). Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *Journal of Physiology*, *587*, 897–904. https://doi.org/10.1113/jphysiol.2008.164087

Morgan, T. E., Cobb, L. A., Short, F. A., Ross, R., & Gunn, D. R. (1971). Effects of Long-Term Exercise on Human Muscle Mitochondria. In *Muscle Metabolism During Exercise* (pp. 87–95). https://doi.org/10.1007/978-1-4613-4609-8_8

Philp, A., Schenk, S., Perez-Schindler, J., Hamilton, D. L., Breen, L., Laverone, E., Jeromson, S., Phillips, S. M., & Baar, K. (2015). Rapamycin does not prevent increases in myofibrillar or mitochondrial protein synthesis following endurance exercise. *The Journal of Physiology*, *593*(18), 4275–4284. https://doi.org/10.1113/JP271219

Pilegaard, H., Ordway, G. A., Saltin, B., & Neufer, P. D. (2000). Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. *American Journal of Physiology - Endocrinology and Metabolism*, *279*(4), E806-814. https://doi.org/10.1152/ajpendo.2000.279.4.e806

Pilegaard, H., Osada, T., Andersen, L. T., Helge, J. W., Saltin, B., & Neufer, P. D. (2005). Substrate availability and transcriptional regulation of metabolic genes in human skeletal muscle during recovery from exercise. *Metabolism: Clinical and Experimental*, *54*, 1048–1055. https://doi.org/10.1016/j.metabol.2005.03.008

Proud, C. G. (2007). Amino acids and mTOR signalling in anabolic function. *Biochemical Society Transactions*,

1187–1190. https://doi.org/10.1042/BST0351187

Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., & Spiegelman, B. M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell*, *92*(6), 829–839. https://doi.org/10.1016/S0092-8674(00)81410-5

Robinson, M. M., Turner, S. M., Hellerstein, M. K., Hamilton, K. L., & Miller, B. F. (2011). Long-term synthesis rates of skeletal muscle DNA and protein are higher during aerobic training in older humans than in sedentary young subjects but are not altered by protein supplementation. *The FASEB Journal*, *25*(9), 3240–3249. https://doi.org/10.1096/fj.11-186437

Rowlands, D. S., Thomson, J. S., Timmons, B. W., Raymond, F., Fuerholz, A., Mansourian, R., Zwahlen, M. C., Métairon, S., Glover, E., Stellingwerff, T., Kussmann, M., & Tarnopolsky, M. A. (2011). Transcriptome and translational signaling following endurance exercise in trained skeletal muscle: Impact of dietary protein. *Physiological Genomics*, *43*(17), 1004–1020. https://doi.org/10.1152/physiolgenomics.00073.2011

Scarpulla, R. C. (2002). Transcriptional activators and coactivators in the nuclear control of mitochondrial function in mammalian cells. *Gene*, 81–89. https://doi.org/10.1016/S0378-1119(01)00809-5

Sun, X., & Zemel, M. B. (2009). Leucine modulation of mitochondrial mass and oxygen consumption in skeletal muscle cells and adipocytes. *Nutrition & Metabolism*, *6*(26). https://doi.org/10.1186/1743-7075-6-26

Tarnopolsky, M. (2004). Protein Requirements for Endurance Athletes. *European Journal of Sport Science*, *4*(1), 1–15. https://doi.org/10.1080/17461390400074102

Vega, R. B., Huss, J. M., & Kelly, D. P. (2000). The Coactivator PGC-1 Cooperates with Peroxisome Proliferator-Activated Receptor α in Transcriptional Control of Nuclear Genes Encoding Mitochondrial Fatty Acid Oxidation Enzymes. *Molecular and Cellular Biology*, *20*(5), 1868–1876. https://doi.org/10.1128/mcb.20.5.1868-1876.2000

Ventura-Clapier, R., Garnier, A., & Veksler, V. (2008). Transcriptional control of mitochondrial biogenesis: The central role of PGC-1α. In *Cardiovascular Research* (Vol. 79, Issue 2, pp. 208–217). Oxford University Press. https://doi.org/10.1093/cvr/cvn098

Wilkinson, S. B., Phillips, S. M., Atherton, P. J., Patel, R., Yarasheski, K. E., Tarnopolsky, M. A., & Rennie, M. J. (2008). Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *The Journal of Physiology*, *586*(15), 3701–3717. https://doi.org/10.1113/jphysiol.2008.153916

Witard, O. C., Jackman, S. R., Breen, L., Smith, K., Selby, A., & Tipton, K. D. (2014). Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *The American Journal of Clinical Nutrition*, *99*(1), 86–95. https://doi.org/10.3945/ajcn.112.055517

Wright, D. C., Han, D. H., Garcia-Roves, P. M., Geiger, P. C., Jones, T. E., & Holloszy, J. O. (2007). Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1α expression. *Journal of Biological Chemistry*, *282*(1), 194–199. https://doi.org/10.1074/jbc.M606116200

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R. C., & Spiegelman, B. M. (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*, *98*, 115–124. https://doi.org/10.1016/S0092-8674(00)80611-X