Chapter 4

Multinuclear Magnetic Resonance Spectroscopy of Human Skeletal Muscle Metabolism in Training and Disease

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Additional information is available at the end of the chapter

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Abstract

In this chapter, techniques and application of multinuclear (^{1}H, ^{13}C, and ^{31}P) *in vivo* magnetic resonance spectroscopy (MRS) for the assessment of skeletal muscle metabolism in health and disease are described. Studies focusing on glucose transport and utilization, lipid storage and consumption, handling of energy rich phosphates, and measurements of newly emerging noninvasive biomarkers, i.e., acetylcarnitine and carnosine are summarized. Muscle metabolism connections to exercise physiology and the development as well as possible treatment of metabolic diseases, such as obesity and diabetes are also discussed. Taken together, multinuclear *in vivo* MRS on humans helped to uncover defects in skeletal muscle metabolic pathways in insulin-resistant conditions; and to discover links between defects in mitochondrial activity/capacity and lipid metabolism, as well as defects in whole-body and/or muscle glucose metabolism. There is also to mention that several of the MR-derived readouts are affected by both training status and metabolic disease in a specific way, and thus could serve as potential markers of training status and metabolic flexibility.

Keywords: magnetic resonance spectroscopy, skeletal muscle, energy metabolism, training status, pathophysiology, glucose, lipids, diabetes mellitus, obesity, exercise

1. Introduction

Skeletal muscle is the key human tissue responsible for the body weight bearing and movement and plays a central role in whole-body energy metabolism. Even in the resting conditions

skeletal muscle accounts for ~30% of metabolic rate of human body [1]. In particular, as the main target of insulin activity, skeletal muscle effectively regulates the glucose uptake, while serving also as a glycogen storage [2]. The extent to which skeletal muscle fulfills these roles is affected by many physiological and pathophysiological factors, which can change over time. Several diseases largely manifesting in skeletal muscle pathology have a rapidly increasing socioeconomic impact, as they start to affect not only the elderly, e.g., sarcopenia, but also young productive, population, e.g., insulin resistance and type 2 diabetes mellitus (T2DM). Insulin resistance and T2DM are rapidly reaching epidemic proportions worldwide and the associated treatment costs of T2DM also continue to grow. The cost of diabetes (with over 85% attributable to T2DM) was in 2012 over £1.5 milion an hour or 10% of the entire National Health Service budget for England and Wales [3]. In order to improve the understanding and clinical management of such disorders, it is vital to be able to assess muscle function and metabolism *in vivo* noninvasively, to support their diagnosis, monitor changes in tissue status during disease progression and interventions, and above all, to establish robust markers that can be used in disease prevention [4].

Magnetic resonance spectroscopy (MRS) represents an advanced noninvasive technology that allows for assessment of tissue metabolism in the healthy as well as diseased conditions [5]. In particular, MRS techniques are able to noninvasively monitor intramyocellular storage and turnover of important energy storage pools, namely lipids and glycogen. In addition, MRS is uniquely suited to quantitatively assess adenosine-triphosphate (ATP) production reactions in the muscle, i.e., mitochondrial oxidative phosphorylation, glycolysis, and creatine kinase activity. Among other things, proton (^{1}H) MRS is best suited to quantify intramyocellular lipid (IMCL) storage, carbon (^{13}C) MRS is optimal for glycogen reserves measurements and phosphorus (^{31}P) is ideal for investigations of ATP metabolism. This chapter briefly describes the basic principles and availability of these measurements, and further focuses on applications of MRS techniques for studying functional properties of skeletal muscle in health and disease. Obesity, type 2 diabetes mellitus, and skeletal muscle insulin resistance serve as good model for pathologic conditions, while the summary of MRS observable adaption to training is brought as positive control or contrast to aforementioned circumstances.

While most of the described methods and measurements have been introduced at lower field strengths 20–30 years ago [6–8], recent development in MR technology, namely the transition towards ultra-high field MR systems ($B_0 \geq 7$ T), meant significant improvements to *in vivo* MRS [9–13]. Next to the linear gain in signal-to-noise ratio (SNR), which can be translated to significantly shorten data acquisition time [12] or improved signal localization [14], higher field strength also provides improved spectral resolution, reducing metabolite overlapping, and thus, improving quantification accuracy. The increase in SNR is of particular importance to nonproton MRS, which is limited mainly by SNR in its applicability [15]. ^{31}P MRS benefits from additional increase in SNR per unit of time due to shortening of the T_1 relaxation of ^{31}P metabolites at 7 T [11]. MR systems ($B_0 \geq 7$ T) equipped with multinuclear broadband capabilities hold great potential for investigations of the not yet well-understood mechanisms of tissue metabolism.

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2. Methods of magnetic resonance spectroscopy

2.1. ^{1}H MRS

The main application of ^{1}H MRS that is used in exercise and nutrition research, just as often as in studying the etiology of insulin resistance and T2DM, is the quantification of intramyocellular lipids (IMCL) [16–18]. Among other typical uses of ^{1}H MRS belong: (a) detection of lactate (Lac) formation during exercise [8, 19–21]; (b) measurement of total creatine (Cr) content [22–24]; (c) assessment of muscle fiber orientation using dipolar coupling [25]; (d) measurement of intramyocellular metabolite diffusion [26]; and (e) the dynamic measurement of tissue (de)oxygenation using the signal of deoxymyoglobin (DMb) [27–29]. Furthermore, detection of resting muscle carnosine [30, 31] and acetylcarnitine (AcC) [32, 33] has been recently promoted as a promising use of ^{1}H MRS. An example of high resolved *in vivo* acquired ^{1}H MR spectrum of skeletal muscle is given in **Figure 1**.

2.1.1. Static examinations by ^{1}H MRS

Next to water, lipid accounts for the strongest signals in a ^{1}H spectrum of skeletal muscle at rest. However, even with optimal tissue selection, not all lipid signals in the spectrum are intramyocellular (IMCL). Fortunately, it is possible to differentiate between IMCL and extramyocellular

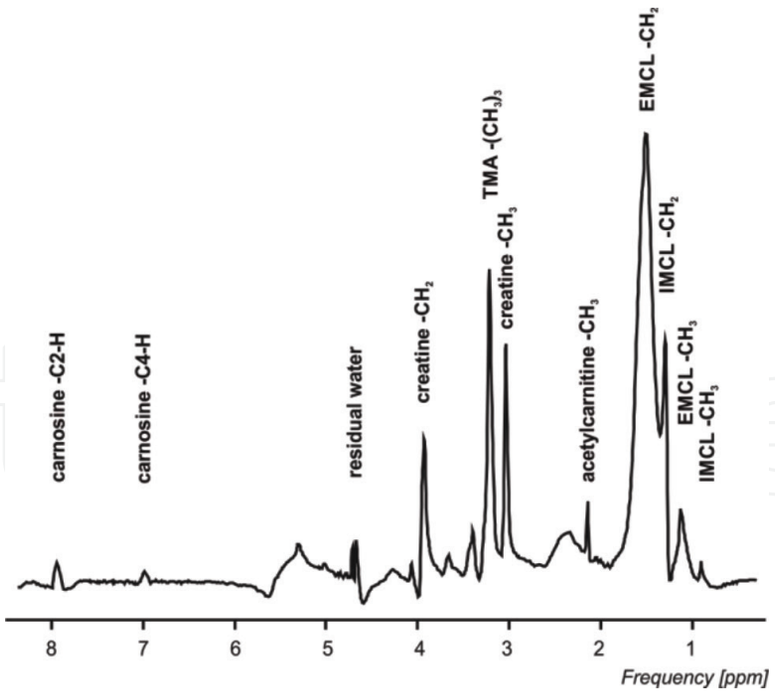

Figure 1. A representative ^{1}H-MRS spectrum from an athlete acquired from the vastus lateralis muscle at 7 T showing intramyocellular (IMCL) and extramyocellular (EMCL) lipids [0.9 and 1.1 ppm (CH_3 groups) 1.5 and 1.3 ppm (CH_2 groups)], AcC at 2.13 ppm, Cr at 3.03 and 3.9 ppm, trimethyl ammonium (TMA) groups of carnitine, AcC, and choline at 3.20 ppm, residual water peak at 4.7 ppm, removed in postprocessing, and carnosine spectral lines at 7 and 8 ppm.

lipids (EMCL). Inside myocytes, lipids form small droplets in the cytoplasm, whereas EMCLs are found layered between myocytes along the main muscle orientation, and are tubular in shape. This difference in spherical versus cylindrical geometry influences the bulk magnetic susceptibility of these lipid compartments making the differentiation possible [34, 35]. The IMCL/EMCL peak separation depends on the angular orientation of EMCL to the external magnetic field as a result of anisotropy effects [36] which results into maximum of 0.2 ppm frequency shift in case of fully parallel orientation [4], as is the case in tibialis anterior [25, 37].

In general, to maximize the acquired signal, MRS sequences with short echo time (TE) are often used for IMCL quantification [38–40]. This requires suppression of water signal and can also lead to broad resonances of various shapes and strong IMCL/EMCL overlap, which can cause inaccurate quantification of IMCLs [41, 42]. Contamination from subcutaneous adipose tissue or bone marrow can make this even more challenging. Moreover, if the water signal is to be used as an internal concentration reference, additional acquisition of water signal is necessary. Better separation of EMCLs and IMCLs and improved fitting of lipid resonances was suggested and observed when using an MRS acquisition with longer TEs [10, 42, 43]. This improved separation is a result of the different T_2 relaxation times of IMCL and EMCL resonances and the line width narrowing effect [10]. Thus, the long-TE acquisition has a major advantage in increased spectral resolution [10, 34] and provides the possibility to omit water suppression, reducing energy deposition in tissues. On the other hand, absolute quantification from the long-TE MR spectra requires precise T_2 relaxation correction, which can be inaccurate especially for signals with short T_2, i.e., water signal [10, 44]. Thus, an ideal acquisition combines a short TE measurement of water signal with long-TE detection of lipids [14].

Another muscle metabolite that greatly benefits from long-TE acquisition is acetylcarnitine (AcC). This relatively low concentrated metabolite fulfills a major role in translocation of long-chain fatty acids from cytosol to the mitochondrial matrix [45] and in maintaining pyruvate dehydrogenation activity [46], and is, therefore, of high interest in skeletal muscle research. The straight forward detection and quantification of AcC is challenging, due to the strong overlap of the 2.13 ppm line with lipid resonances, and the fact that the line at 3.20 ppm represents a combination of the trimethylammonium (TMA) groups of carnitine, AcC, and choline. Fortunately, the differences in T_2 relaxation times of AcC and lipids allow the detection of the 2.13 ppm line at rest, using long-TE ^{1}H MRS [32, 33].

The downfield region of the ^{1}H spectrum, i.e., left to the water signal, gets often overlooked as the detectable signals belong to low concentrated metabolites, e.g., carnosine, and can be easily mistaken for noise. This is very unfortunate, as carnosine is a pH-buffering metabolite that can be manipulated externally [47, 48]. The concentration of carnosine is mainly determined by muscle fiber type composition, with fast-twitch glycolytic fibers containing up to twice as much carnosine as slow-twitch oxidative fibers [49, 50]. In addition, chemical shift of carnosine is sensitive to pH, and thus, carnosine signal can be also used to assess intramyocellular pH [51, 52]. While it is possible to detect carnosine using clinical systems [30, 51], the increased SNR of ultra-high fields, provides high repeatability [31].

2.1.2. Dynamic examinations by ^{1}H MRS

While most of the metabolite signals can be observed in basal resting conditions, metabolic adaption to stress induces by exercise and/or ischemia may alleviate the visibility of specific

resonance lines. Of particular interest has been the formation of lactate (Lac) during exercise challenge or ischaemia [8, 19, 20, 53, 54], because lactate is the end-product of anaerobic metabolism and a source of free H^+, and thus, it plays an important role in skeletal muscle metabolism and pH regulation. Although ^{1}H MRS measurements of Lac were shown to be in good agreement with tissue extracts analysis [53], due to overlapping lipid signals, dipolar coupling and relaxation effects, quantification of Lac levels in skeletal muscle *in vivo* is extremely challenging, and thus, prone to inaccurate estimation [4].

It can often be unclear whether the measured results reflect real change in skeletal muscle metabolism or just manifest inadequate oxygenation state of the muscle. This query can be also answered by ^{1}H MRS, which can serve to noninvasively monitor the (de)oxygenation state of human skeletal muscle under stress through the measurement of deoxymyoglobin (DMb) [29]. Very low concentration of DMb is not an obstacle, as DMb resonates substantially downfield away from the typical spectral range, securing no overlap with other metabolites and has extremely short T_1 [55].

Formation of AcC during strenuous exercise and its slow decay after exercise has also been under investigation using ^{1}H MRS [56, 57]. While at lower fields, it is only the 2.13 ppm resonance line that gets resolved after strenuous exercise [56], 7T allows direct observation of split in resonance lines of AcC and carnitine in the TMA region, providing the option to quantify their ratio. Besides, the ^{1}H signal of AcC at this resonance is twice as strong, improving sensitivity of the measurement [57].

On the longer time scale of few 10 minutes during prolonged submaximal exercise and following recovery decrease and replenishment of IMCL pool can be observed [58, 59].

2.2. ^{13}C MRS

The presence of carbon nuclei in almost every organic structure, the nonzero spin of carbon-13 (^{13}C) nuclei, and a very wide chemical shift range of up to 200 ppm have made ^{13}C MRS well-suited for studies of molecular structure and biochemistry in cellular and animal models since the early days of biochemical MRS. The dynamic assessment of biochemical pathways in particular, forms the basis for the current application of ^{13}C MRS in humans.

Due to the different magnetic properties of ^{13}C compared to protons, the resonance frequency of ^{13}C at a given magnetic field is approximately one-quarter that of ^{1}H MRS. Although the natural abundance of carbon nuclei is very high in living tissues, i.e., almost matching the abundance of protons, the ratio of MR visible ^{13}C to MR invisible ^{12}C is extremely low (approx. 1:99). Lower gyromagnetic ratio and consecutively lower intrinsic sensitivity of ^{13}C MRS, together with lower natural abundance of ^{13}C nuclei leads to inherently low SNR, and thus, hampers the spatial and temporal resolution of ^{13}C MRS experiments. Techniques to increase low SNR of ^{13}C MRS include: (a) increased volume of interests and/or averaging of the MRS signal using a high number of repetitions, (b) elimination of the spin-spin coupling interaction between ^{13}C-nuclei and its coupled protons by the ^{1}H decoupling pulses in the period of ^{13}C signal acquisition; (c) the utilization of the ^{1}H-^{13}C magnetic interaction with polarization transfer techniques; (d) the use of a higher field-strength MR apparatus; and (e) increasing the abundance of the ^{13}C isotope by systemic infusion of ^{13}C-enriched metabolic substrates.

2.2.1. ^{13}C MRS natural abundance studies

The use of ^{13}C MRS for *in vivo* studies of skeletal muscle without artificial isotope enrichment is essentially limited to measurements of metabolites present at high concentrations, in particular glycogen and triglycerides [4]. Despite its high molecular weight, the glycogen C-1 resonance line is 100% MR visible [60, 61] due to the high intramolecular mobility of its glucose residues. Skeletal muscle glycogen is present at approximately 80–120 mM concentrations, depending on the muscle and physiological conditions [62–64]. Good reproducibility of natural abundance muscle glycogen measurements by ^{13}C MRS [65] favors the use of dynamic experimental protocols to assess the depletion of glycogen during exercise (**Figure 2**) and its resynthesis over the course of several hours during post-exercise recovery [58, 59, 66, 67].

2.2.2. ^{13}C MRC labeling studies

To overcome the low SNR due to low natural abundance of ^{13}C nuclei and increase the measurement sensitivity, it is common to use an isotope enriched infusion in ^{13}C MRS studies [4]. After an infusion of ^{13}C-labeled glucose under steady-state conditions, glycogen synthesis in skeletal gastrocnemius muscle has been quantified and correlated with whole-body carbohydrate consumption [7, 69, 70].

Another exciting use of ^{13}C MRS *in vivo* is the quantification of the flux through the tricarboxylic acid (TCA) cycle, which serves as a surrogate for the rate of mitochondrial oxygen consumption by the cellular respiration that is vital for skeletal muscle function. The labeling of substrates in the TCA by infusing [2-^{13}C]-acetate and observing the enrichment of the C4 position of glutamate, has been performed in muscle. These measurements can easily be combined with experiments in which undirectional flux through the skeletal muscle ATP-synthase is measured by means of ^{31}P saturation transfer [4].

Alternative approach for further improvement of signal-to-noise and localization is the application of so called indirect ^{13}C measurements, where high sensitivity and low chemical shift displacement of ^{1}H MRS is used for signal excitation and detection and chemical specificity is introduced exploiting magnetic interaction with coupling ^{13}C atoms. Proof of the principle for this approach has been demonstrated the measurements of fatty acid composition of human subcutaneous tissue [71], while application of similar methodology with the sensitivity enhancement by concomitant ^{13}C label infusion has been demonstrated in the study focused on postprandial lipid partitioning in liver and skeletal muscle in prediabetic and diabetic rats [72].

2.3. ^{31}P MRS

Skeletal muscle was the first human tissue studied by ^{31}P MRS *in vivo*, mainly because of its high metabolic activity, physiological importance, and relatively simple access [6, 73, 74]. ^{31}P MR spectra of skeletal muscle typically depict five major resonances from inorganic phosphate (Pi), phosphocreatine (PCr), and adenosine-triphosphate (ATP).

Other detectable ^{31}P metabolites include cell membrane precursors, i.e., phosphomonoesters (PMEs) combined from—phosphocholine (PC) and phosphoethanolamine (PE) and cell membrane degradation products, i.e., phosphodiesters (PDEs) in particular

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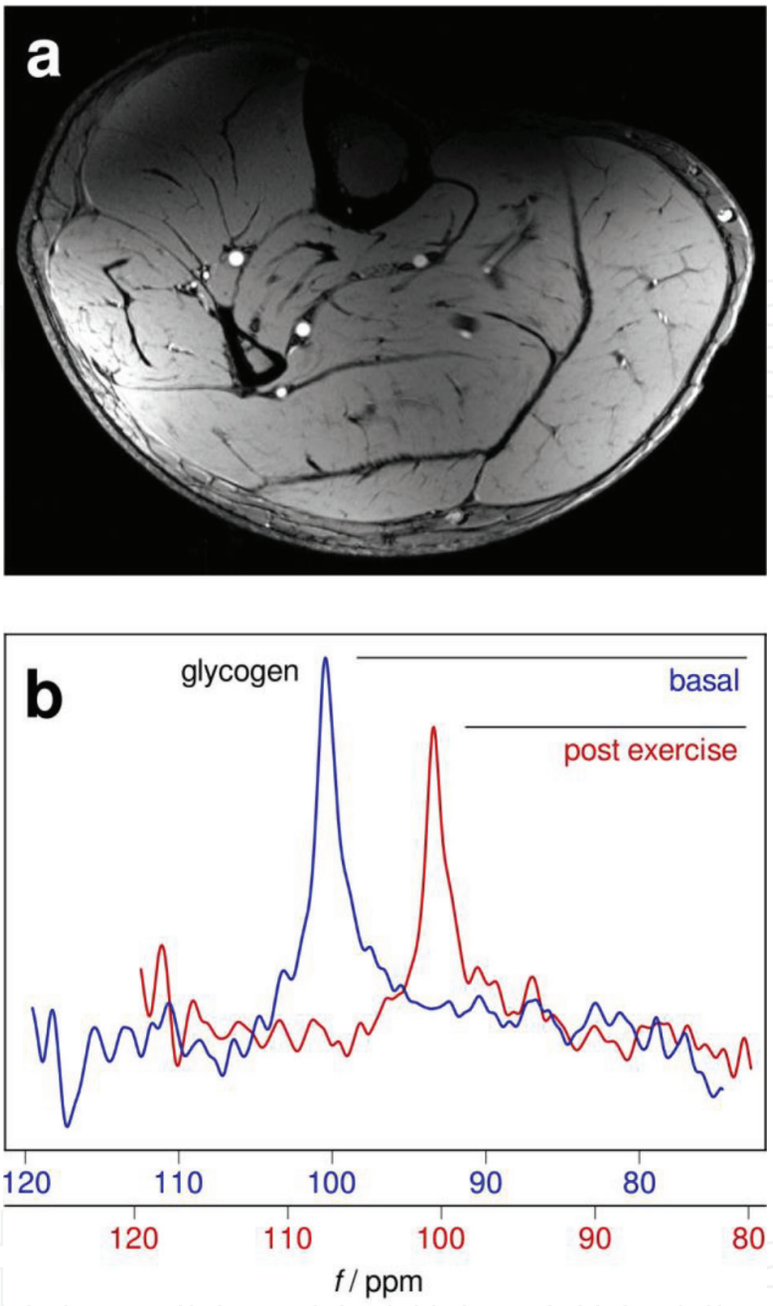

Figure 2. Transversal MRI of calf muscle (a) and natural abundance ^{13}C MR spectra acquired at 7T depicting glycogen signals from soleus and gastrocnemius muscle (b) (pulse-acquire block pulse MRS, acquisition time approx. 4 min). The glycogen signal is decreased after 90 s of toe raising exercise by approx. 30%. Adapted from Goluch et al. [68].

glycerolphosphocholine (GPC) and glycerol-phosphoethanolamine (GPE) [11] (**Figure 3**). Besides, using the chemical shift between PCr and Pi, intramyocellular pH can be calculated noninvasively [75].

Next to the analysis of resting ^{31}P MR spectra, for metabolite concentration determination, it is very frequent to obtain the ^{31}P MR spectra during exercise and consecutive recovery [6, 77]. Such dynamic ^{31}P MR experiments provide a measure of skeletal muscle oxidative metabolism, through quantification of mitochondrial capacity.

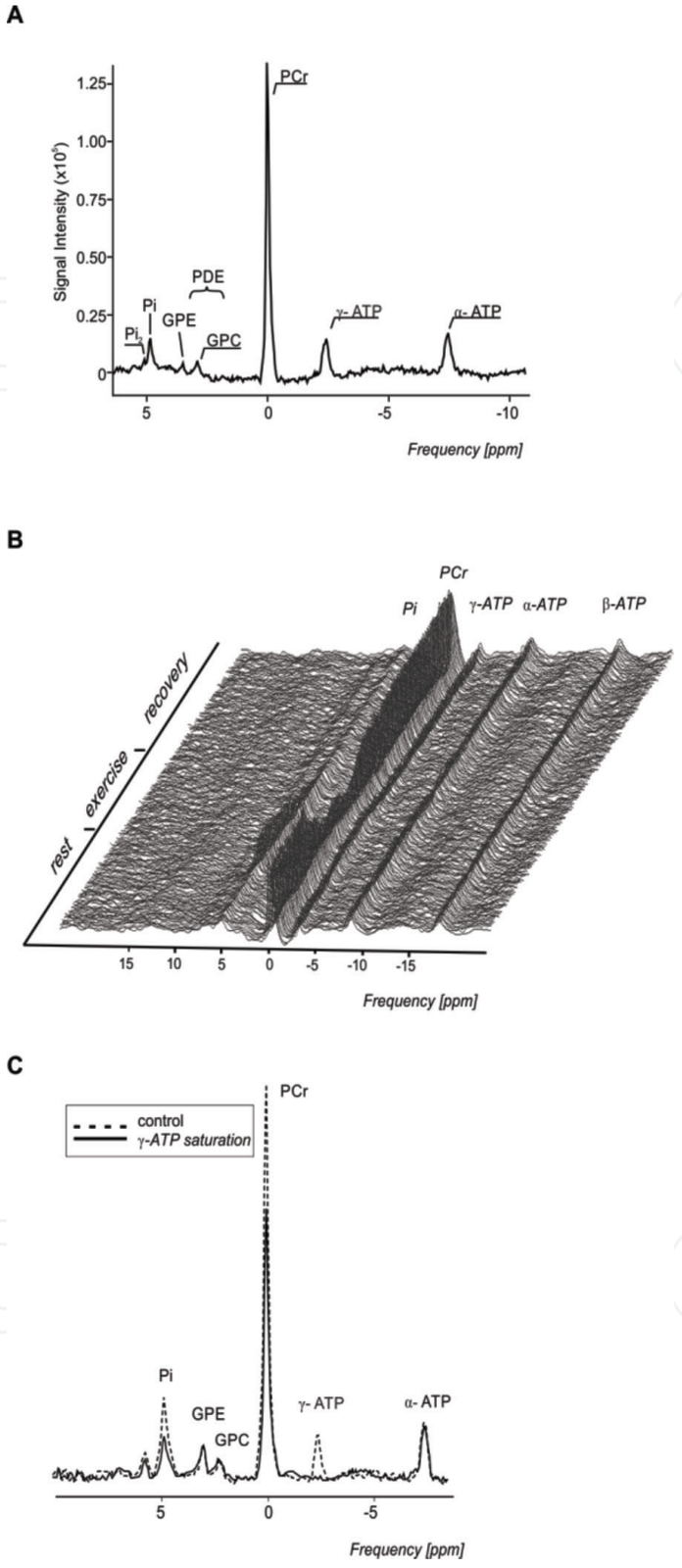

Figure 3. (A) A representative highly spectrally resolved static ^{31}P-MRS spectra acquired at 7T. (B) Time course of a ^{31}P MR spectra during a knee extension exercise with depicted depletion of the PCr signal and its subsequent resynthesis during the recovery period. (C) Saturation transfer spectra showing the effect of γ-ATP saturation, at approximately −2.48 ppm (solid line) on the Pi signal compared to the control experiment with saturation at approximately 12.52 ppm (dashed line). Adapted and reproduced from Klepochová et al. [76].

Alike ^{13}C MRS, ^{31}P MRS also has a lower gyromagnetic ratio in comparison to protons, and thus, suffers from lower intrinsic sensitivity. Therefore, SNR enhancing approaches, e.g., ^{1}H decoupling, at lower fields, or benefit from the SNR boost of higher magnetic fields are utilized [11, 12, 78].

2.3.1. ^{31}P MRS of resting muscle

The quantification of static ^{31}P-MR spectra was repeatedly exploited in the past to gather information about skeletal muscle fiber composition using the PCr/Pi ratio, however, the observed scattering in metabolite content is large and the final conclusions vary [79–83], thus severely limiting the reliability of these measurements [15].

On the other hand, ^{31}P MRS of skeletal muscle can provide valuable information about whole-body training status, metabolic health, and/or muscle integrity. In particular, the concentration of phospholipids-phosphodiesters seems to provide a valuable surrogate of metabolism or systemic muscle damage [82, 84–90]. At ultra-high fields (i.e., 7 T), or by using ^{1}H decoupling, the signal of main PDE in human skeletal muscle—GPC—can be separated and used directly rather than the total PDE signal [86]. Another very recent approach for the determination of skeletal muscle oxidative metabolism from resting ^{31}P MR spectra that profits from the increased spectral resolution of the ultra-high field systems (i.e., 7 T), is the assessment of alkaline pool of Pi signal (Pi_2) [91]. Based on its chemical shift (~5.1 ppm), relatively short T_1, and small contribution of extracellular space to skeletal muscle signal, the mitochondrial matrix has been recognized as the likely origin of this pool [91]. As such, it should be able to provide direct information about changes in mitochondrial density in response to training or defects of mitochondrial metabolism [15]. Thus far, Pi_2/Pi ratio was showed to be increased in the quadriceps of the trained subjects [92] and decreased in sedentary subjects [86] in comparison to normals, thus, supporting this hypothesis.

^{31}P MRS can also assess the reaction kinetics of energy metabolism through a technique called saturation transfer (ST). ST exploits the transfer of magnetization between nuclei that are in direct chemical exchange, thus estimating the unidirectional exchange rates and fluxes under steady-state conditions [4]. Unfortunately, ST experiment in skeletal muscle does not yield a net oxidative flux, as the measured flux contains a major glycolytic component and both turnover reactions operate close to equilibrium, i.e., the net rates of both glycolytic and oxidative ATP synthesis are low at rest [93]. On the other hand, the resting fluxes were correlated with parameters of oxidative metabolism [94, 95], and follow changes of oxidative metabolism observed in disease [96].

2.3.2. Dynamic ^{31}P MRS during exercise-recovery challenge

^{31}P MRS measured during muscle contraction and recovery, i.e., dynamic ^{31}P-MRS, can be used to observe the kinetics of intramyocellular pH and of the cytosolic concentrations of PCr, Pi, and ADP during perturbations of metabolic equilibrium. These measurements offer understanding of pH homeostasis, as well as insight into the oxidative ATP synthesis regulation driven by ATP demand. In short, to preserve stable ATP concentration, hydrolyzed ATP is resynthesized from PCr, causing PCr levels to decrease and Pi levels to increase during exercise. After the

challenge, the PCr buffer is restored primarily through oxidative phosphorylation allowing assessment of mitochondrial function [97]. The fitted PCr time recovery rate constant provides a good estimate on its own, however, it is pH dependent [98]. Using the calculated intracellular pH and consecutively the free ADP concentration [99], maximal oxidative capacity can be estimated providing a more robust parameter of mitochondrial capacity [15].

Unlike in static investigations, it is common to use only single spectral transient in dynamic examinations due to the high temporal resolution required (on the order of seconds) to sufficiently sample the PCr recovery time course. To boost the SNR for these experiments, highly sensitive surface receive coils are deployed and ^{31}P signal is "localized" by their restricted sensitivity volume. However, this type of localization does not allow to differentiate signals that originate from different anatomic and/or morphologic compartments, nor between muscle groups that are recruited differently in the performed exercise (e.g., soleus and gastrocnemius during plantar flexion [100–103]). Quantification of combined signal from differently active muscles significantly skews the measurement of mitochondrial capacity [103–105]. Over the last few years, many different localization techniques have been developed for dynamic ^{31}P MRS [103, 105–107], but as localization decreases available tissue volume and consecutively SNR, they are mainly used at ultra-high fields, i.e., 7T.

Examinations of skeletal muscle metabolism provide not only important information about muscle physiology, but can also be used to observe the effects of aging [108, 109] and/or to help define the training status [86, 110]. In addition, dynamic ^{31}P MR examinations can identify mitochondrial defects in muscular diseases and can uncover decreased oxidative metabolism of skeletal muscle.

3. Muscle MRS and training

Skeletal muscle demonstrates remarkable plasticity in functional adaptation and remodeling in response to contractile activity, i.e., exercise. Training-induced adaptations are reflected by changes in metabolic regulation, intracellular signaling, transcriptional responses and contractile protein and function [111]. Muscle mitochondrial density increases along with concomitant changes in organelle composition in just after 6 weeks of exercise training. Overall, the major metabolic consequences of the adaptations of muscle to endurance exercise are a slower utilization of muscle glycogen and blood glucose, a greater reliance on fat oxidation, and less lactate production during exercise of a given submaximal intensity [112]. Many of the named changes in skeletal muscles caused by exercise may be explored, identified, and potentially quantified by MRS (**Figure 4**). The effect of exercise can be studied from three angles: (i) direct comparison of differently trained subjects; (ii) exploration of acute exercise challenge effects; and (iii) longitudinal studies involving exercise intervention. The effect of dietary interventions on muscle metabolism and the role of MRS will also be discussed.

3.1. Metabolic differences in training status

Increased IMCL content has been reported in endurance-trained muscle indicating the switch to higher utilization and efficiency of fat oxidation, as during long-lasting exercise, IMCL stores are

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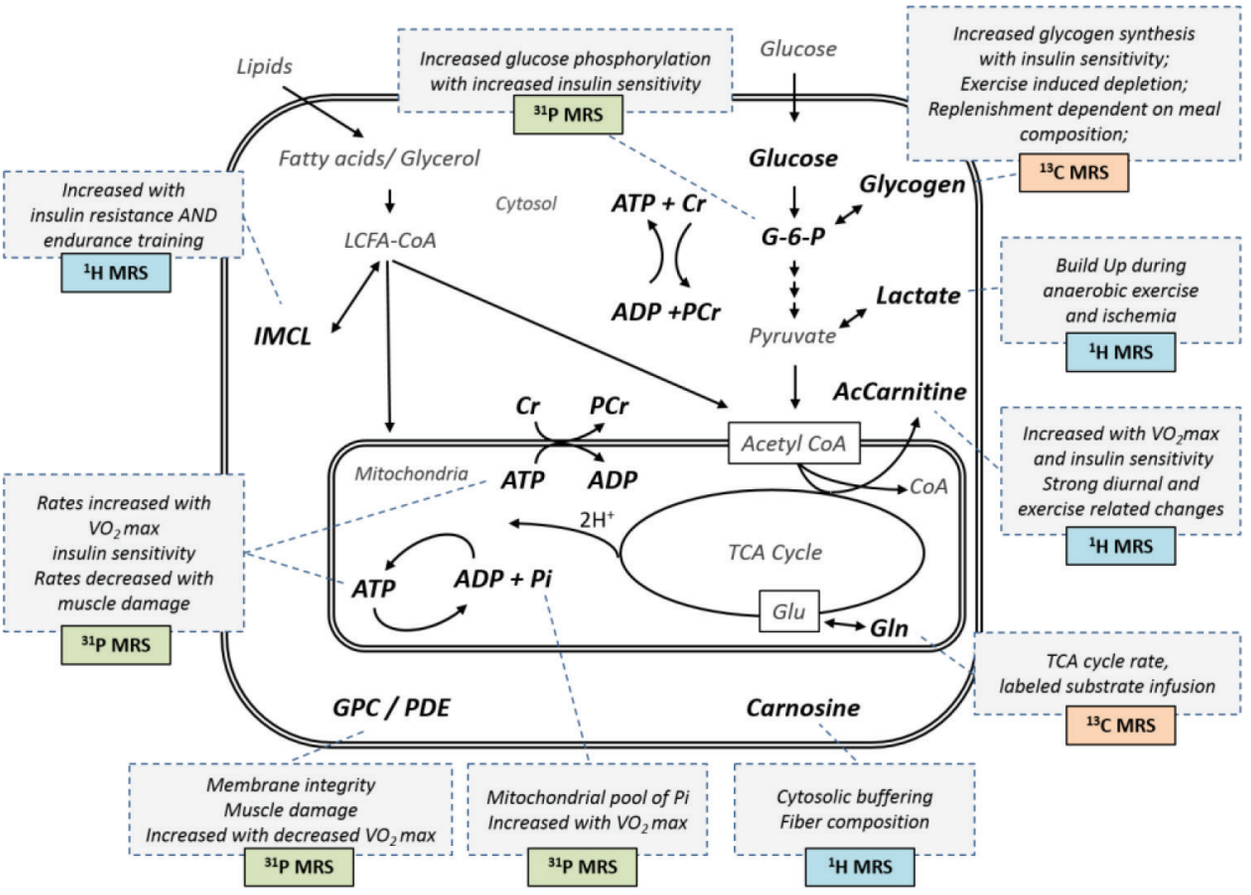

Figure 4. Summary of skeletal muscle metabolic processes exploitable by MRS. Linked in-figure legends denote observable effects, correlations with whole-body metabolic readouts, suggested mechanism in healthy trained, systemic metabolic disease or skeletal muscle myophaties/dystrophies and respective nucleus for MRS. Please note that several of the readouts are affected by both training status and metabolic disease and thus could serve as potential markers of training status and metabolic flexibility. Metabolites are abbreviated as follows: LCFA-CoA, long-chain fatty acid coenzyme A; IMCL, intramyocellular lipids; Cr, creatine; PCr, Phosphocreatine; ATP, adenosine triphosphate; ADP, adenosine diphosphophosphate; Pi, inorganic phosphate; GPC, glycerophosphocholine; PDE, phosphodiester; Glu, glutamate; Gln, glutamine; TCA cycle, tricarboxyacid cycle; AcCarnitine, acetylcarnitine; Acetyl CoA, acetyl coenzyme A; G-6-P, glucose 6 phosphate.

utilized as an energy source, similarly to glycogen [113, 114]. The use of these substrates depends heavily on the exercise intensity, and both are replenished in the recovery phase post-exercise. Similarly to IMCL, glycogen levels are also elevated in endurance-trained subjects, which promote their fatigue resistance [115, 116]. The phenomenon of increased IMCL was also termed athlete's paradox, because increased IMCL observed in obese, sedentary subjects are indicative of insulin resistance [17]; however, insulin sensitivity is not impaired in endurance-trained people [18]. IMCL content differs between individual muscle groups, depending on muscle fiber composition. In particular, lower IMCL content has been found in predominantly glycolytic, fiber type II-tibialis anterior, gastrocnemius, and vastus lateralis compared to the predominantly oxidative, fiber type I-soleus and vastus intermedius muscles [117–119]. As the concentration of carnosine is also fiber composition dependent [48, 49], it is no surprise that explosive athletes have 30% higher carnosine levels in gastrocnemius muscle compared to a reference population, whereas it is 20% lower than normal in typical endurance athletes [120]. No significant difference has been reported in acetylcarnitine (AcC) concentration between endurance-trained and

untrained lean sedentary or obese sedentary volunteers [32, 121]. A recent study performed in trained and normally active subjects showed significant differences between AcC concentrations measured after overnight fast or after lunch [33]. This makes the comparison difficult and emphasizes the need for strict standardization of measurement time, dietary conditions, and physical activity (explained below) for the measurement of AcC/carnitine.

Endurance-trained athletes also have a higher volume of mitochondrial density, and, therefore, faster oxidative metabolism which is mirrored by faster PCr resynthesis following submaximal exercise [122]. Faster PCr resynthesis has been demonstrated in comparison to untrained [123–125], and even sprint-trained athletes reflecting superior oxidative metabolism function of endurance-trained subjects [122, 126]. Gradually decreasing training status is also mirrored in decreasing ^{31}P MRS derived measures of mitochondrial capacity and Pi_2/Pi ratio when comparing endurance-trained, lean sedentary and overweight-to-obese sedentary volunteers [86, 92, 110]. Sedentary lifestyle, if accompanied by overweight, type 2 diabetes mellitus or in connection to different muscle specific disease, gives also rise to higher PDE levels in skeletal muscle [84, 86]. Increased PDE levels, although to a much lesser extent, have been also reported in professional cyclists in comparison to normally trained men [85] and in long-distance runners compared to sprinters [82]. These increased PDE levels in highly trained or pathology hampered subjects can potentially indicate persistently damaged (and actively remodeling) muscles as the result of their training or disease. As yet, the connection of PDE to oxidative metabolism and/or muscle integrity is not completely understood.

3.2. Acute exercise challenge

From the metabolic point of view acute exercise challenge relates to changes of concentrations in energy storage pools, e.g., glycogen, lipids, or phosphocreatine, boost in the aerobic and anaerobic metabolism, lactate formation, following pH changes and effects on cell osmotic equilibrium.

From the MRS point of view: although carnosine concentration in gastrocnemius nor in soleus muscles could be influenced by a 1-h-long submaximal street run, the carnosine peak was shown to change in shape, demonstrating an exercise-induced change in pH [31]. The appearance of the second line of carnosine peak can potentially mirror the existence of two skeletal muscle compartments with different pH, possibly as a result of oxidative (slow-twitch) and glycolytic (fast-twitch) fiber composition. Acute exercise has been also shown to alter carnitine metabolism. Low-intensity exercise (below the individual's lactate threshold) does not cause significant changes in the MR detectable muscle carnitine pool, however, after only 10 min of high-intensity exercise, majority of muscle carnitine pool is redistributed to short-chain acylcarnitine. This redistribution is highlighted over a further 20 min of exercise and has long recovery period (over a 60-min) [45, 127]. Likewise, no changes in creatine (Cr) concentration were detected during exhaustive exercise, but a specific change in its methylene (Cr2) resonance line advocate for detection of compartmentation of Cr pool to bound and free sections [23].

Recently, high-intensity exercise challenge to the vastus lateralis muscle by performing squats continuously for 10 min also showed an increase in the AcC level and approximately 15 min after the cessation of exercise, AcC depletion or washout was observed [33]. Similar effect of increasing AcC levels was observed in trained and untrained subject after 30 min of cycle

ergometer exercise. While, during 40-min recovery period, the AcC signal decayed rapidly in the trained group, it continued to rise in the untrained group [121]. Exercise that results in muscle glycogen depletion are followed by adenine nucleotide loss and muscle fatigue [116, 128]. Later on, depending on the diet and exercise regimen during the recovery, glycogen super-compensation can be seen. Comparing trained cyclists with untrained subjects, it has been shown that endurance-trained subjects resynthesize glycogen faster and are able to accumulate more muscle glycogen during the super-compensation period [116].

IMCL depletion can be observed during prolonged submaximal (60–70% of VO_2max) running or cycling [38, 58, 129, 130], but not during the sprints or repetitive bouts of strenuous exercise [129, 131], supporting the notion that increased IMCL stores serve as important energy reserves for endurance athletes. Following the exercise, repletion of IMCL stores was shown to be dependent on the diet composition in recovery period [16, 58, 130, 132].

3.3. Training interventions

Interventional studies focused on endurance training show an increase in IMCL after the intervention period of 4–6 weeks [133, 134]. On the other hand, 12 weeks of high-intensity training does not seem to have a similar effect [135]. This is potentially due to relative increase of type I oxidative muscle fibers during endurance training and the fact that IMCL concentration is fiber dependent, as discussed earlier. A recent overview of effects of a varying periods and different training types on the carnosine content in the vastus lateralis muscle showed that in most of them carnosine levels did not change after training. Only 8 weeks of power-training led to an increase of muscle carnosine levels [136]. Examining muscle glycogen resynthesis rate and levels after a glycogen-depleting exercise before and after 10 weeks of endurance training exposed higher glycogen concentration as well as an accumulation rate in trained than in untrained state [128], what is in good agreement with studies directly comparing trained and untrained subjects [116]. Eight weeks of endurance training also leads to lower PCr depletion and increased pH levels after exercise [137]. Similarly, the PCr resynthesis rate and muscle mitochondrial capacity can be improved by regular exercise [138].

3.4. Dietary interventions

Alternative approach to alter muscle metabolism without changing the physical activity pattern of an individual is a dietary intervention. This includes calorie restricting diets, carbohydrate loading, as well as substrate supplementation studies. Even very short, but rigorous calorie restriction in obese sedentary subjects leads to decrease in IMCL stores [139]. Although one could expect an improvement in muscular oxidative metabolism to accompany the IMCL reduction, it has been demonstrated using biopsies that mitochondrial capacity is unaltered by diet alone and can be improved only if combined with exercise intervention [140]. Creatine supplementation is often advertised as a tool to increase body mass in body building and physical sports [141]. An increase in total creatine and PCr levels in the muscle can be demonstrated [22], however no improvement in PCr resynthesis has been found after creatine supplementation [22, 142], off-putting the effect on muscle oxidative metabolism. Still, creatine supplementation leads to an increase in glycogen super-compensation [143], and thus can potentially be considered an affective ergogenic aid [141].

Increase in skeletal muscle glycogen super-compensation by carbohydrate loading due to the preceding depletion exercise was also detected in longitudinal study applying ^{13}C MRS [144]. Similar study setup where carbohydrate loading yielded glycogen super-compensation and insulin-stimulated glycogen synthesis as well as glucose-6-phosphate (G-6-P) accumulation was measured by $^{13}C/^{31}P$ MRS during hyperinsulinemic-euglycemic clamp confirmed the hypothesis that glycogen limits its own synthesis through feedback inhibition of glycogen synthase activity, as reflected by an accumulation of intramuscular G-6-P, which is then shunted into aerobic and anaerobic glycolysis [145]. Sequential ^{13}C MRS measurement could also show that caffeine ingestion 90 min before prolonged exercise did not exert a muscle glycogen-sparing effect in athletes with high muscle glycogen content [63].

4. Muscle MRS in metabolic and skeletal muscular disease

Variations in skeletal muscle metabolism are not only connected to training, but are also indicative of many health conditions. Whole-body metabolic disorders, e.g., insulin resistance, T2DM and metabolic syndrome are accompanied by impaired skeletal muscle metabolism [17, 84]. Similarly, skeletal muscle myopathies effect the metabolic health of skeletal muscles [146, 147]. The usability of MRS to monitor these two major groups of diseases influencing muscle metabolism will be discussed now.

4.1. Insulin resistance, T2DM and substrate over-abundance

Insulin-resistant states are characterized by hampered reactions of skeletal muscle to increased peripheral serum insulin concentrations. Insulin signaling, glucose transport and/or phosphorylation, glycogen synthesis, and glycolysis rates are reduced. Many ^{13}C MRS studies have characterized the defects in skeletal muscle metabolism in insulin-resistant states, including experimental manipulations. These studies revealed a ~60% decrease of insulin-stimulated glycogen synthesis in overt T2DM patients [7], as well as a comparable impairment in their lean insulin-resistant offspring [62, 148] and in obese nondiabetic insulin-resistant volunteers [149]. Similar ^{13}C MRS approaches have shown decreased postprandial skeletal muscle glycogen synthesis under normal physiologic conditions after a standard carbohydrate rich mixed meal regimen in T2DM patients [64]. In combination with ^{31}P MRS measurement focused on glucose phosphorylation, i.e., the formation of intramuscular glucose-6-phosphate [148], ^{13}C MRS measurements of intra- and extracellular glucose demonstrated that the lowered glucose transport is one of the main defects effecting whole skeletal muscle glucose metabolism in T2DM [150]. Excellent time resolution of labeled ^{13}C MRS measurements of skeletal muscle resynthesis following a depleting exercise could reveal early insulin independent and subsequent insulin dependent phases of this process [151], from which the latter, insulin dependent, is impeded in insulin-resistant offspring of individuals with T2DM [62].

Combined ^{13}C and ^{31}P MRS has also been used to monitor the effect of lifestyle changes and pharmacological insulin-sensitizing therapy on skeletal muscle glucose metabolism. One

bout of aerobic exercise normalized insulin-stimulated glucose fluxes along with the normalization of whole-body insulin sensitivity in insulin-resistant offspring of T2DM patients [152], while troglitazone treatment improved the skeletal muscle glucose transport and the glycogen metabolism of patients with T2DM [70, 153].

Unlike in endurance-trained volunteers, where IMCLs act as an important energy source for prolonged exercise [113], accumulation of ectopic lipids inside muscle cells in untrained subjects is detrimental. Starting with obesity, through the insulin resistance toward T2DM, IMCL have an increasing tendency, showing a clear correlation between IMCL and insulin sensitivity in sedentary subjects [17], making IMCL a very good indicator of metabolic defect. However, due to the fact that endurance training also leads to increased IMCLs, i.e., due to the athletes-paradox, high IMCL levels cannot be used as a marker of metabolic disorder on their alone. Muscle acetylcarnitine (AcC) levels measured at rest could be potentially used to tip the scales in the right direction, as it has been shown that while T2DM subjects have low muscle AcC concentration, endurance-trained subjects have high stores of muscle AcC [32]. Unfortunately, as the AcC levels are dependent on dietary status and physical activity [33], more studies accounting for these dependencies are required to support these initial findings. Multinuclear MRS studies have also revealed a link between IMCL accumulation measured by ^{1}H MRS and skeletal muscle glucose metabolism [17, 118, 154] assessed by ^{13}C and/or ^{31}P MRS, which has also been studied in different states of insulin resistance and physical fitness [155].

The role of free fatty acids (FFA) and amino acids (AA) serum over-abundance on skeletal muscle glucose metabolism has been investigated in studies simulating the metabolic conditions of T2DM in young healthy men. An experimentally induced increase in plasma FA concentrations showed that substrate over-abundance decreased glucose transport and phosphorylation [156–158], and impaired skeletal muscle glycogen synthesis [156], which precedes the decrease in whole-body glucose uptake in a dose-dependent manner [157]. The observed effect of over-abundance also holds true in various conditions of insulinemia [156–158], as well as with depleted skeletal muscle glycogen [159]. Measuring skeletal glucose transport/phosphorylation and glycogen synthesis in the skeletal muscle of young healthy men during an experimental AA challenge showed a direct effect of AA on glucose transport or phosphorylation [160] and reduced skeletal muscle glycogen synthesis. Substrate over-abundance and defects in lipid oxidation can lead to increased lipid accumulation inside the skeletal muscle. Exchange kinetics between Pi and ATP, measured by ^{31}P MRS ST, are also decreased in T2DM in basal and glucose/insulin challenged conditions [161] as well as in the presence of increased serum FFA in healthy volunteers and hyperinsulinemic-euglycemia [162]. Slower PCr recovery rate after exercise and lower mitochondrial capacity also accompanies obesity [86] and insulin resistance [163, 164]. Similarly, increased muscle PDE levels were found in T2DM and shown to correlate with insulin resistance [84]. However, the PDE dependence on age [86, 165] has to be taken into account when using PDE to compare different metabolic groups.

4.2. Skeletal muscle myopathies

Skeletal muscle pathologies are often characterized by muscle pain, weakness, and defects in skeletal muscle energetic metabolism. From the MRS point of view, changes in relative ^{31}P

metabolite concentrations, i.e., drop in PCr and increase in Pi, were observed in patients with mitochondrial myopathy [97] and Duchenne dystrophy [166]. Increased levels of PDE measured at rest can be indicative of congenital lipodystrophy [87], fibromyalgia [90, 167], or various muscular dystrophies [166, 168, 169]. Slower PCr recovery and decreased mitochondrial capacity was found in patients with chronic fatigue syndrome [170], as well as in patients with lipodystrophy [87]. Pathologic defects in muscle trimethylamine compounds-to-creatine ratio were found in facioscapulohumeral muscular dystrophy already prior to macroscopic muscle fat infiltration [171]. Furthermore, analytic *in vitro* MRS could detect alteration of lipid metabolism in patients with muscular dystrophy in early phase of the disease [172].

5. Summary

Summarizing the knowledge gained from skeletal muscle magnetic resonance spectroscopic studies, we can say that the combination of ^{1}H, ^{13}C, and ^{31}P MRS: (i) can measure intramyocellular lipids deposition, which can be either utilized as a useful energy source in endurance-trained athletes, or is an indication of metabolic disorder (athletes-paradox); (ii) enables quantification of acetylcarnitine that may help to resolve the athletes-paradox; (iii) can improve the knowledge about buffering capacities of skeletal muscle by observing changes in lactate and carnosine metabolism; (iv) can measure glycogen metabolism and glycogenic substrate flux in the skeletal muscle under various conditions; (v) can assess oxidative and nonoxidative energy fluxes in basal and exercise challenged conditions. Taken together, it has helped to uncover defects in skeletal muscle metabolic pathways in insulin-resistant conditions; and to discover links between defects in mitochondrial activity/capacity and lipid metabolism, as well as defects in whole-body and/or muscle glucose metabolism. There is also to mention that several of the MR-derived readouts are affected by both training status and metabolic disease, and thus could serve as potential markers of training status and metabolic flexibility.

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Conflict of interest

None of the authors or authors' institutions have any conflicts of interest to disclose.

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