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Minireview

# Application of Magnetic Resonance Spectroscopy in metabolic research☆

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ABSTRACT

The etiology of metabolic disease in humans is far from understood, and even though potential pathways are identified in animal models and cell studies, it is often difficult to determine their relevance in humans, as the possibilities of tissue sampling are limited. The application of non-invasive imaging techniques can provide essential metabolic information and this mini review focuses on the opportunities of Magnetic Resonance Spectroscopy (MRS) to add to our understanding of the metabolic processes during health and disease. MRS is a volatile technique that can give us information about the concentrations of endogenous metabolites in a completely non-invasive way. In this mini review we discuss the opportunities that MRS is giving us by describing how the investigation of ectopic fat depots has gained a lot of attention and has really taken off after $^{1}$H-MRS for quantification of lipid content became widely available. We furthermore discuss how other MRS techniques, such as $^{31}$P-MRS and $^{13}$C-MRS can add valuable information and especially highlight the strength of MRS to be applied dynamically and therefore monitor metabolic changes during physiological challenges such as exercise or meal tests.

## 1. Introduction

Animal research and cell studies have provided us with a wealth of valuable mechanistic knowledge on metabolic changes in health and disease, however translation to humans can be challenging and often human relevance remains unclear. This is at least in part due to the fact that tissue sampling is generally needed for quantification of metabolites, and such invasive procedures are not easily performed in humans. For muscle and adipose tissue, a biopsy is feasible, however for other tissues, non-invasive procedures are warranted.

Magnetic Resonance Spectroscopy (MRS) yields chemical information in a non-invasive way in vivo and can therefore contribute to our understanding of metabolism in humans. A big advantage of MRS is that it can be used to gain dynamic information and therefore can determine the response to physiological challenge. In contrast to other non-invasive imaging techniques, like for instance, Positron Emission Tomography (PET), MRS does not expose subjects to ionizing radiation. Furthermore, MRS does not require injection with a contrast agent (e.g., gadolinium), which otherwise can be limiting in patients with renal disease. MRS is based on the same physical principles as Magnetic Resonance Imaging (MRI), which is widely used in the clinical setting to gain anatomical information. With MRS, instead of generating an image, a spectrum is generated, in which various peaks can be discriminated and attributed to different chemical groups and metabolites, enabling noninvasive metabolite quantification.

The MR signal of various nuclei can be detected, and in metabolic research, $^{1}$H-, $^{13}$C- and $^{31}$P-MRS are most commonly used, each method unraveling different aspects of cellular metabolism.

## 2. Ectopic lipid accumulation, investigated by $^{1}$H-MRS

The storage of fatty acids in tissues other than adipose tissue is better known as ectopic fat accumulation. This altered storage of lipids in ectopic tissue has been implicated in the development of insulin resistance. Hence, monitoring these ectopic lipid stores is of great value in metabolic research.

### 2.1. Skeletal muscle

After the first reports of a positive correlation between ectopic fat

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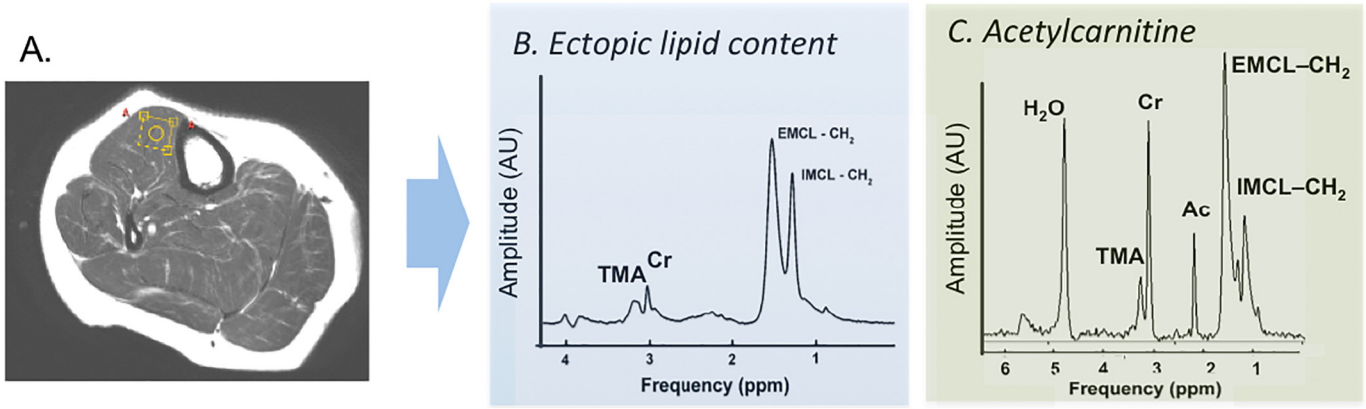


**Fig. 1.** Skeletal muscle $^{1}$H-MRS.
Static measurement of intra-myocellular lipid content (IMCL) in a volume of interest (VOI) in skeletal muscle. The planning of the voxel is depicted in panel A, where the VOI is positioned in the tibialis anterior muscle. Measuring with $^{1}$H-MRS with water suppression results in the identification of 2 partially overlapping peaks at respectively ~1.5 ppm extra-myocellular lipids (EMCL) and 1.28 ppm intramyocellular lipids (IMCL). This allows (relative) quantification of skeletal muscle lipid accumulation. In panel C a $^{1}$H-MRS measurement with a TE of 350 ms is depicted where the acetylcarnitine peak at 2.13 ppm can be identified, as well as the creatine peak at 3.03 ppm. Measurements were performed with spin-echo sequences at a 3 T system.

storage in muscle cells (intramyocellular lipids, IMCL) and insulin resistance [1,2], the phenomenon of ectopic fat storage gained a lot of interest. It was demonstrated that $^{1}$H-MRS can be used to differentiate the signal from lipid droplets in muscle cells (IMCL) from the signal of adipose tissue that is typically 'marbeling' skeletal muscle [3,4], thereby providing a non-invasive method for IMCL quantification.

In a typical $^{1}$H-MR spectrum of skeletal muscle, the most prominent peaks originate from lipids (e.g. $CH_2$ and $CH_3$ resonances of the fatty acid chains), creatine (Cr), trimethylammonium compounds (TMA) containing carnitine and choline and water ($H_2O$) (see Fig. 1B and C). Mostly, an internal reference metabolite, such as creatine or water, is used to normalize signal intensities and to convert them to concentrations in mmol. Previous studies validating this measurement [5,6], showed good agreement between skeletal muscle lipid content measured in tissue biopsies and in vivo measurements with MRS with a high sensitivity of this measurement in vivo.

Next to the fact that IMCL content is elevated in insulin resistance, it also became apparent that highly trained athletes also store a lot of IMCL as a substrate source and this increased storage of IMCL in a metabolically healthy state, as is the case in trained athletes is not associated with insulin resistance. On the contrary, these individuals are highly insulin sensitive. This phenomenon is also referred to as the "athletes paradox" [7]. Importantly, intramuscular lipid content is decreased after prolonged exercise [8,9], showing that IMCL functions as an intramuscular substrate store which, very similar to intramuscular glycogen, is addressed during periods of exercise.

In line with the use of substrate during exercise, IMCL stores increase during endurance training as an adaptive response to habitual exercise. While such training-induced increase in IMCL is paralleled by improvements in insulin sensitivity, [8,10], other interventions that increase IMCL, such as high fat diet (SCHR-HI) and lipid infusion [11,12], decrease insulin sensitivity. Thus despite a similar increase in fat storage, the outcome on insulin sensitivity is markedly different. Hence, IMCL is not a straightforward marker of metabolic health, but rather has a u-shaped relationship with insulin sensitivity and should be interpreted within the context of changes in energy demand [13].

Next to the spectral region downfield of the water which is mostly considered in metabolic spectroscopy, also the upfield region can give valuable information. When focusing on these frequencies, the histidine resonances of muscle carnosine can be detected in skeletal muscle with $^{1}$H-MRS [14]. Carnosine is a dipeptide that may attenuate acidosis in exercise training, by acting as a pH buffer and may also improve excitation-contraction coupling and defence against reactive oxygen species [15]. Studies have shown that the chronic oral ingestion of β-alanine can elevate carnosine content of human skeletal muscle, which improves performance in high-intensity exercise in both untrained and trained individuals [16–19]. However, additional and thorough investigation is needed to investigate the potential effects and side-effects of supplementation of carnosine [15].

### 2.2. Liver

A few years after the widely accepted IMCL determination by $^{1}$H-MRS, this method was also successfully applied to the liver by Longo et al. in 1993 [20] and Thomsen et al. [21] in 1994. This technique makes it possible to non-invasively monitor the lipid content of the liver [5,22,23].

The liver is a central organ for lipid metabolism. The liver cannot only store fat, but is also the organ responsible for cholesterol synthesis, liponeogenesis, the production of triglycerides and (the bulk of) lipoproteins. When applying $^{1}$H-MRS in the liver, a spectrum is generated in which the dominant resonances come from lipids ($CH_2$ and $CH_3$ groups) and water ($H_2O$) (see Fig. 2B). Like in the skeletal muscle, the $CH_2$ resonance is usually normalized to the water resonance (expressed as a percentage of the water resonance), allowing relative quantification of the intra-hepatic lipid content. This technique was validated and showed a good agreement with measurements in tissue biopsies in the liver [5].

Normally the hepatic lipid content is low (below 5% of fat by wet weight). When hepatic lipid content exceeds this value, this is described as hepatosteatosis or a fatty liver. Alcohol abuse used to be the most common cause of hepatosteatosis, though with the increasing prevalence of obesity and type 2 diabetes, the incidence of non-alcoholic fatty liver disease is rapidly increasing [24]. Eventually, fatty liver disease may convert into fibrosis and cryptogenic cirrhosis, which may lead to liver failure and the development of a hepatocellular carcinoma [25].

$^{1}$H-MRS is considered the gold standard for non-invasive determination of liver fat and the possibility to monitor hepatic lipid content non-invasively on a large scale has resulted in new insights. For instance it was shown, that the insulin resistant [26–28] and diabetic state [29] are characterized by an increased liver fat content.

Interventional studies have demonstrated that interventions that decrease liver fat content, as measured by $^{1}$H-MRS, normalize insulin resistance and disturbed glucose metabolism. For instance, the effect of weight loss due to a low-calorie diet [30] or dietary counseling

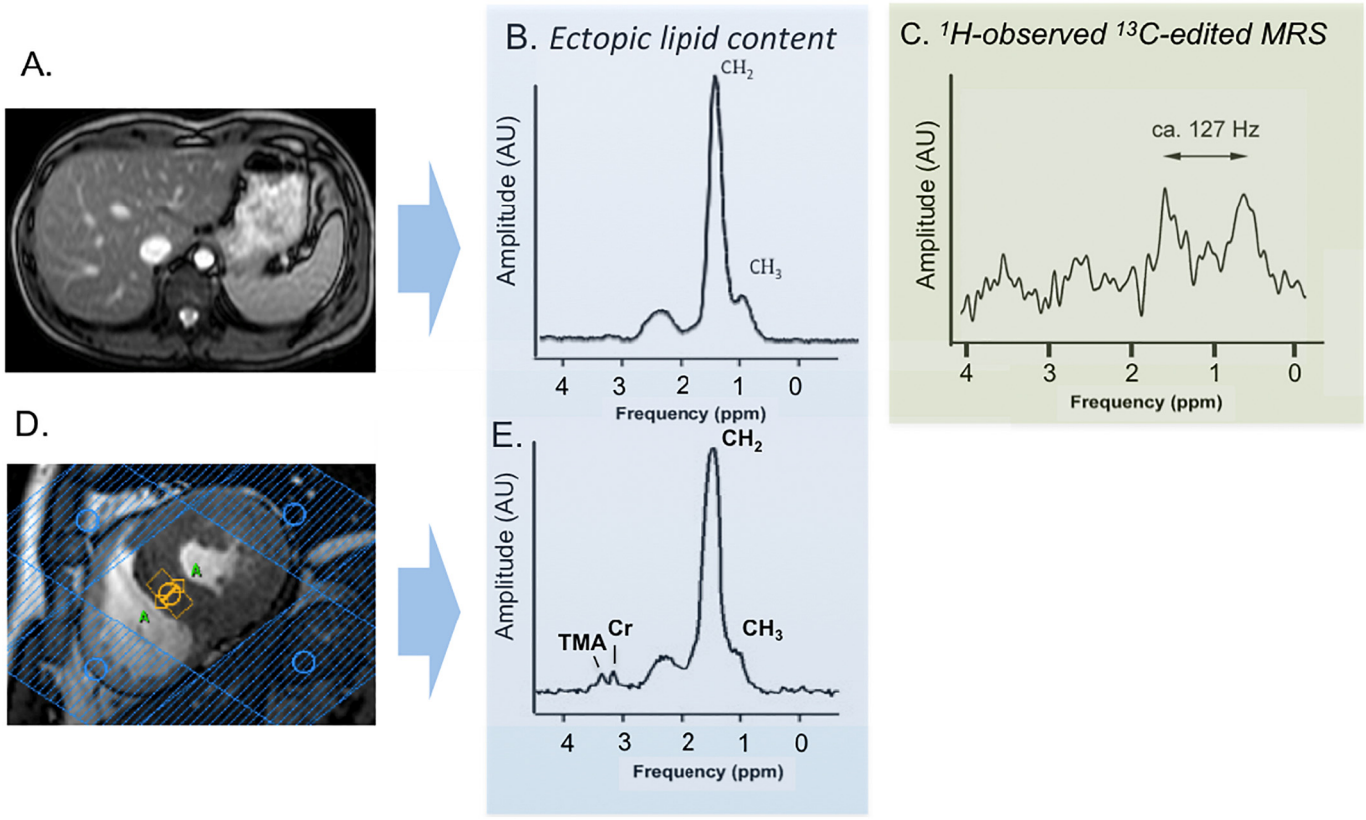


**Fig. 2.** Hepatic and cardiac $^{1}$H MRS and $^{1}$H-observed $^{13}$C-edited MRS of the liver.
Similar to skeletal muscle, $^{1}$H-MRS measurements with water-suppression in a volume of interest can be performed in liver (A) and heart (yellow box) (D). This results in the measurement of ectopic lipid accumulation in liver, where the relative amplitude of the $CH_2$ resonance at 1.3 ppm (1.5 T) can be used to relatively quantify liver lipid accumulation (B) or cardiac lipid accumulation (E). These techniques are more challenging compared to skeletal muscle, as due to breathing and cardiac motion, artifacts can occur. Also, more field inhomogeneities are present during these measurements. Besides these static measurements, dynamic measurements of lipid uptake can be performed with $^{13}$C-labbelled fatty acids. As the sensitivity of $^{13}$C-MRS is relatively poor, other techniques like for instance $^{1}$H-observed $^{13}$C-edited MRS can be performed (C). Here, the $^{13}$C-edited $^{1}$H-lipid spectrum is depicted, acquired with the ge-HSQC (heteronuclear single quantum coherence) sequence. As no decoupling was applied, two lipid $CH_2$ peaks are visible, which are separated by approximately 127 Hz. These peaks can be used for quantification of the absolute concentration of $^{13}$C lipids taken up by the liver [81].

combined with increased physical activity [31] reduced hepatic steatosis and insulin resistance. Weight loss through gastric bypass also leads to dramatic changes in liver fat content and can even normalize liver fat levels [32]. Furthermore, exercise training has been shown to reduce intrahepatic lipid content, in both non-NAFLD, and in NAFLD patients measured with $^{1}$H-MRS [33] and improve insulin sensitivity.

Recent epidemiological studies identified genetic variants that lead to liver fattening, though without the development of insulin resistance and therefore show that hepatic lipid content is not necessarily causally related to insulin resistance. However, the life-style induced fat accumulation remains by far the most important cause of a fatty liver and it is still a very sensitive marker of metabolic health in the vast majority of people.

Next to simply determining the quantity of hepatic fat, recently more attention is also given to the qualitative aspects [34–37]. Using $^{1}$H-MRS, it was suggested that exercise relatively increases the unsaturated fatty acid content in the liver [34], however, the classification into saturated and unsaturated fatty acids remains difficult due to low abundance of the unsaturated resonances and inadequate water suppression. Applying robust water suppression and analyzing the data by using maximal prior knowledge of the lipid resonances in terms of relative frequencies and spin-spin couplings can improve the measurement very much and in fact it was shown that the differentiation into saturated, monounsaturated and polyunsaturated fatty acids is possible [38].

When performing hepatic $^{1}$H-MRS, combining this measurement with other measurements of other metabolites, for instance $^{31}$P-MRS and/or $^{13}$C-MRS, allows getting more metabolic information on the interplay between lipid storage and for instance ATP levels and other high-energy metabolites or glycogen concentrations (see below, section dynamic measurements and meal responses).

### 2.3. Heart

$^{1}$H-MRS in the heart generates a spectrum in which, upon water suppression, the dominant signal comes from the triglycerides resonance signals ($CH_2$ and $CH_3$, see Fig. 2E). Furthermore a creatine resonance signal (Cr) and water ($H_2O$) resonance signal can be distinguished. This allows quantification of myocardial lipid stores relative to the water resonance. However, this technique is more challenging when compared to $^{1}$H-MRS of the liver and skeletal muscle. The anatomy of the heart does not offer large volumes of homogenous tissue, further challenging signal to noise ratio in the cardiac spectra. Also, as the heart is a continuously moving organ, cardiac MR spectroscopy is more sensitive to phase distortions and signal losses. Furthermore, adequate shimming and water suppression are more challenging. Therefore, ECG triggering and respiratory gating during acquisition of the spectra is essential [39]. Studies have shown that using a navigator to gate the measurement to the respiratory motion of the diaphragm, vastly improves the signal quality and hence is recommended for these measurements [40]. Using these techniques, a good reproducibility and sensitivity in measuring cardiac lipid content

can be established [41].

MRS of the heart has been employed in several studies showing that myocardial triglyceride is increased by twofold in type 2 diabetes, when compared to normoglycemic controls. Here, the increase in cardiac lipid accumulation was paralleled by a reduced systolic function [42] and impaired diastolic function [43]. Cardiac lipid content also increases with age, whereas cardiac function tends to decline with age [44]. Hence, the study of change of myocardial lipid content in the light of decreased cardiac function has gained interest over the last years [45,46]. Recent studies have shown that the myocardial lipid pool is dynamic and can change rapidly. However, some intervention studies also dissociated functional outcomes from cardiac fat accumulation; changes in myocardial lipid content during elevated free fatty acid levels during fasting and exercise were observed, which were not directly paralleled by acute effects on cardiac function [47]. Even more so, an acute, low caloric diet in type 2 diabetic patients did result in a decreased cardiac lipid content, but was initially paralleled by a decreased diastolic function [48], illustrating that the direct link between cardiac lipid content and myocardial function remain poorly understood.

As mentioned above, also creatine can be quantified in the heart by $^{1}$H-MRS. Creatine is taken up actively by the cardiomyocyte and plays an important role in the production of PCr. Creatine has a relatively low myocardial concentration, and the resonance frequency overlaps with the neighbouring Trimethylammonium (TMA) peak and the residual water peak. Hence, these factors complicate the measurement of creatine in the $^{1}$H-MRS spectrum. Nonetheless, this technique has been used to show that in cardiac failure cardiac creatine content dropped by 60%, causing a simultaneous drop in ATP [49–52], challenging energy provision of the heart.

A great opportunity when performing $^{1}$H-MRS of the heart is that it can be combined with the clinical MRI protocols for cardiac function and also with $^{31}$P-MRS to determine the PCr/ATP ratios in the myocardium, thereby gaining information cardiac energy status of the myocardium (see Fig. 4). PCr/ATP ratios were shown to be decreased in heart failure [51,53,54] and to have prognostic value for heart failure patients [55,56]. Studies combining $^{1}$H-MRS and $^{31}$P-MRS showed that in fact, creatine, PCr and ATP all are decreased in the failing heart when PCr/ATP ratio falls [55].

### 2.4. Pancreas

Monitoring of ectopic fat accumulation of the pancreas has been suggested to be related to the development of beta-cell failure. The first measurements of pancreatic lipid accumulation came from the group of Lingvay et al. in 2009 [57]. However, measuring pancreatic lipid content with $^{1}$H-MRS remains challenging, as the pancreas is not encapsulated and is highly infiltrated with adipose tissue. The interpretation of these results as purely parenchymal lipid accumulation of pancreatic cells therefore remains problematic and is highly inter racially and inter ethnic variable [58,59].

## 3. Dynamic measurements

A great strength of Magnetic Resonance Spectroscopy in metabolic research is that it allows getting dynamic information and therefore monitors the response to a physiological stimulus.

### 3.1. Monitoring changes during exercise

A classical example of such dynamic measurement is the application of $^{31}$P-MRS to skeletal muscle during and after exercise. By acquiring a time series of spectra, the relative changes of ATP, PCr and Pi can be monitored in the resting muscle, and during exercise (see Fig. 3). Creatine kinase (CK) catalyses the transfer of a phosphate group between creatine and ATP. The equilibrium constant of the CK reaction strongly favors ATP over PCr by a factor 100 [60]. Thus, in case ATP demand out-weighs ATP production of the mitochondria, PCr concentrations will fall, while ATP concentrations will be maintained through the CK constancy, supporting the notion that ATP in skeletal muscle is stable and allows the use of ATP as an internal concentration reference (61, 62). Indeed, it was shown that in healthy human skeletal muscle the muscular ATP concentration is stable and is approximately 5.5 mmol/kg wet weight. The PCr pool is recovered by resynthesis of PCr through exchange of Pi from ATP produced in the mitochondria (by mitochondrial creatine kinase). Indeed, PCr is well known to decrease at the beginning of exercise and to be very rapidly resynthesized during recovery from exercise. In the postexercise state, PCr resynthesis is driven almost purely oxidatively [63] and the kinetics of resynthesis (e.g. the half-time of recovery) reflects oxidative capacity and therefore can be used as a parameter of in vivo mitochondrial function [64] (see Fig. 3). Indeed, PCr resynthesis kinetics strongly correlated with ex-vivo measurements of mitochondrial function [65,66] and PCr recovery after exercise is strongly correlated with the maximal aerobic capacity (VO2max) and is delayed in mitochondrial disorders [67], confirming its value in the assessment of derangements of mitochondrial function in health and disease. It was suggested that not only the dynamic measurement, but also static $^{31}$P-MRS of muscle may give some information related to mitochondrial function: two distinct Pi-pools, separated by a small frequency shift at high magnetic field (7 T) were reported in resting muscle and the smaller pool was correlated with dynamically measured oxidative flux, but further investigation is needed to elucidate in more detail how this Pi pool is related to oxidative metabolism [68].

Much more dynamic information is accessible during and after exercise by MRS. As touched upon earlier, intramuscular substrate reserves can be monitored such as glycogen (by $^{13}$C-MRS) and IMCL (by $^{1}$H-MRS) stores, but also exercise-induced acidification can be followed in time, either by pH determination based on $^{31}$P-MRS [69,70] or by determination of lactate concentrations by $^{1}$H-MRS [71,72].

More recently, it was shown that acetylcarnitine can also be detected by MRS, more specifically long echo time or T1 edited $^{1}$H-MRS [73] (see Fig. 1B). Acetylcarnitine is formed from the corresponding acetyl-CoA via the action of carnitine acetyltransferase (CrAT), a bi-directional mitochondrial matrix enzyme. Therefore, acetylcarnitine is conceived as a buffer for acetyl-CoA. As acetyl-CoA has many regulatory functions, the tight regulation of acetyl-CoA concentrations is very important. The capacity to buffer acetyl-CoA may therefore depend on the capacity to form acetylcarnitine. Interestingly, a recent study showed that skeletal muscle acetylcarnitine concentration in the resting state correlate with insulin sensitivity in a population covering a wide range of metabolic health [74]. The ratio of carnitine and acetylcarnitine concentrations is not fixed, but in fact, changes very rapidly in response to changes in metabolic demand, such as exercise. Carnitine is acetylated during exercise in an intensity-dependant manner and at high intensity exercise, almost the total pool of carnitine will become acetylated [75,76]. Therefore, determining acetylcarnitine after high-intensity exercise may provide a mean to determine free carnitine availability. Also, the kinetics of acetylcarnitine may provide information on metabolic health, as the kinetics of acetylcarnitine recovery after exercise was shown to be different between trained and untrained subjects [77]. The relevance of these differences still needs further investigation.

Most studies on exercise-induced changes in metabolism focus on skeletal muscle, as metabolism in this tissue undergoes very pronounced changes upon muscle contraction.

However, acute exercise also affects other organs and changes in lipid content and phosphorous metabolites were also documented for the liver [78] and the heart [47], also see Fig. 4.

### 3.2. Monitoring meal responses

With MRS endogenous concentrations of metabolites can be

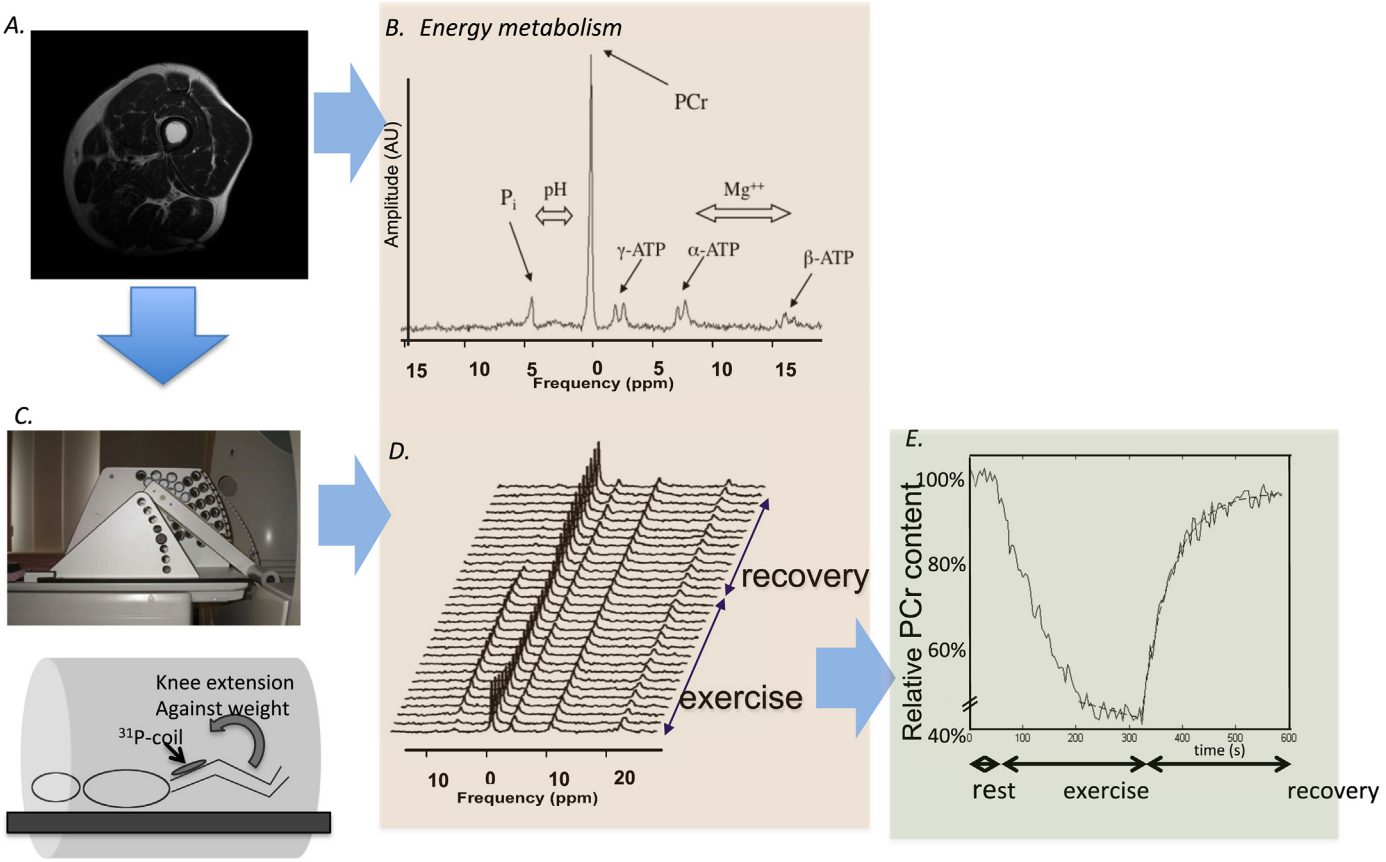


**Fig. 3.** Skeletal muscle $^{31}$P-MRS; static and dynamic evaluation of muscle ATP metabolism.
Planning of the volume of interest of the measurement of skeletal muscle high energy phosphates in the m. vastus lateralis with $^{31}$P-MRS is depicted in panel A. Static measurements yield a $^{31}$P-MRS spectrum (B) with the resonances of inorganic phosphate (Pi) at 5.3 ppm, Phosphocreatine (PCr) 0 ppm and three resonances of the ATP (gamma, alpha and delta peaks, at 2.3, 7.5, and 15.8 ppm, respectively). Changes in Pi can be monitored in the spectrum, as changes in the PH will cause a (chemical) shift of the Pi peak relative to the PCr peak. Also, the concentration of Magnesium (Mg) can cause a chemical shift in the ATP peaks. Dynamic measurements can be performed in the scanner through leg-extension, as depicted in panel C. Here, a patient performs one leg extension lifting up a weight attached to the leg-extension apparatus within the repetition time (Tr = 4 s) of the measurement. The upper leg is fixated to avoid displacement of the volume of interest in the m. vastus lateralis muscle. By doing this repetitive measurement with continuous leg extension exercise in the MRI scanner, a depletion in PCr can be observed and an increase in Pi (disintegration product of PCr) can be seen in the spectrum. Once the exercise is ended, PCr will be resynthesized and PCr and Pi concentrations in the muscle will normalize in the recovery phase. These dynamic changes are depicted in panel D; the measurement of these high energy phosphates is depicted here every 4 s during exercise and recovery showing the initial decrease and the final recovery of the PCr signal. The amplitude of the PCr peak during the measurement is plotted in a curve (panel E). From this curve, the halftime or rate-constant of the PCr recovery can be calculated. This parameter reflects the oxidative capacity of the mitochondria and can be used to assess mitochondrial function.

accurately measured and the responses upon a meal in specific target tissues can be monitored. As discussed before, hepatic fat content can be monitored by $^{1}$H-MRS and changes in the postprandial state were investigated. It was shown that 2–3 h after a high-fat, high-energetic meal, hepatic fat content is increased by 13–20% [79,80] and remained elevated even at 5 h after the meal [80]. In contrast to skeletal muscle lipid content, which remained unchanged in the postprandial state. Although $^{1}$H-MRS does allow monitoring total lipid content in the organs, it does not provide information on the source of the lipids. To investigate the latter, $^{13}$C-labelled substrate can be used.

As 99% of the fatty acids in our environment consist of $^{12}$C isotopes, the use of $^{13}$C-labelled fatty acids can in principle enable the tracking of external fatty acids from a meal and follow their postprandial storage. When providing a meal with $^{13}$C-labelled fatty acids, the $^{13}$C in enrichment of the lipids in the liver reflects the storage of meal-derived fatty acids. However, the accurate detection of $^{13}$C fatty acid signal, e.g. in the liver is very challenging due to the low sensitivity of $^{13}$C-MRS (when compared to $^{1}$H-MRS). Hence, specialized MRS sequence development is necessary to achieve this. With an indirect $^{13}$C-method (proton-observed, carbon edited sequence, also see Fig. 2C) based on quantum coherence spectroscopy, it was shown to be possible to follow the fatty acids from a mixed liquid meal to the liver and to determine the accumulation of dietary exogenous fatty acids in the liver in the first 3–5 h after a meal both in lean as well as in overweight to obese subjects [81]. Although very promising for future development and proof of principle experiments, this method may not be rapidly employed on a large scale due to the technical challenges and the relatively high costs of $^{13}$C-labelled tracers.

In response to a meal, insulin strongly stimulates the conversion of meal-derived glucose into glycogen and interestingly, the dynamics of glycogen storage and glycogen hydrolysis seem to be disturbed in chronic metabolic disease. Glycogen concentrations can be determined by $^{13}$C-MRS and have been measured in liver and in skeletal muscle before and after meals, allowing determination of net post-prandial glycogen storage. These studies showed an increase of 17% in muscle glycogen stores in healthy volunteers after a series of three meals. In contrast in type 2 diabetic patients; the glycogen concentrations remain unchanged, illustrating a disturbed glycogen storage in muscle in the diabetic state [82]. Also during clamps, glycogen increase was diminished in muscle of diabetic patients [83]. Combining these measurements with $^{31}$P-MRS to monitor concentrations of glucose-6-phosphate led to the insight that the transport of glucose (and not its intracellular

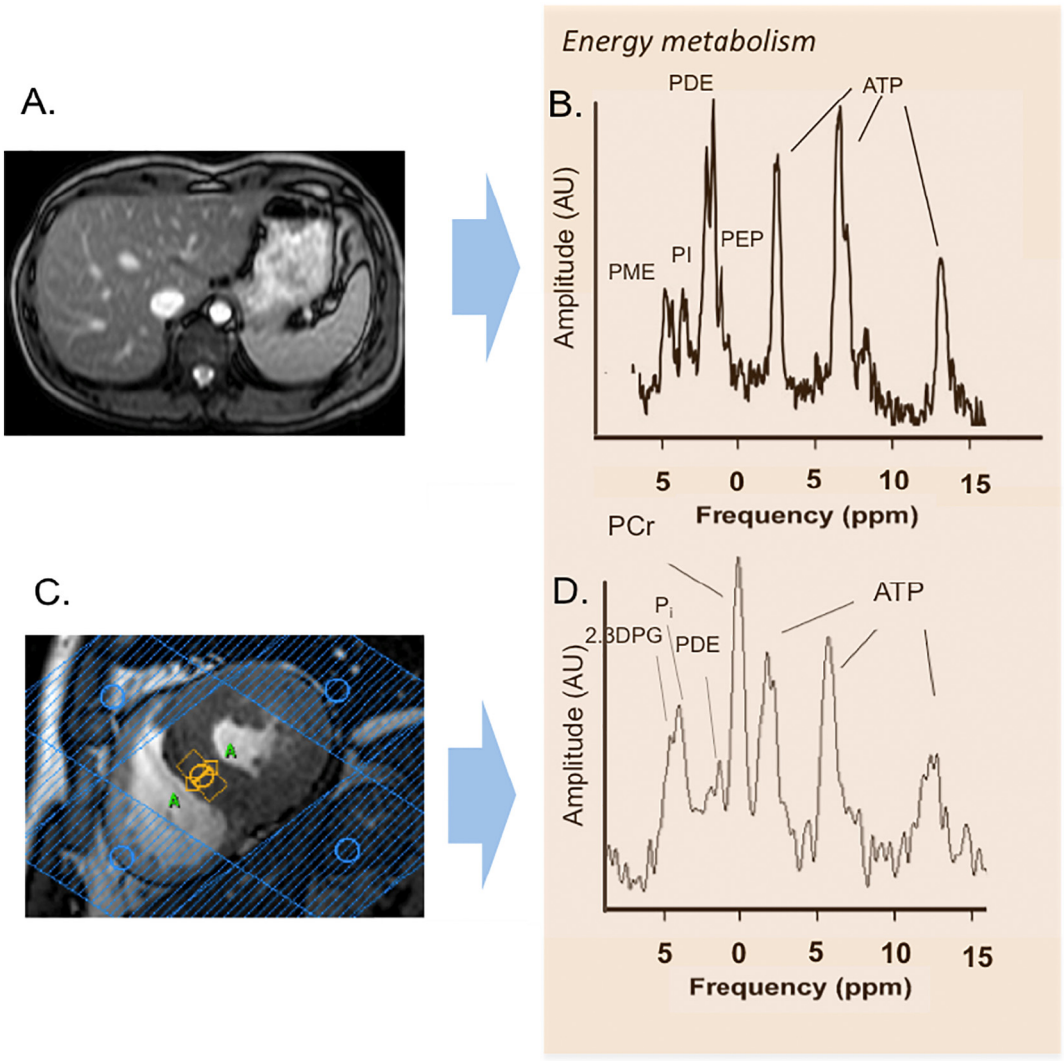


**Fig. 4.** Hepatic and cardiac $^{31}$P-MRS for the determination of high energy substrates.
In this figure a VOI is placed in the liver (A) or septum of the left ventricle of the heart (B) for the measurement of high energy phosphates with $^{31}$P-MRS. The spectrum obtained from the liver is slightly different from skeletal muscle, as the liver does not contain any phosphocreatine (PCr). Nonetheless, ATP phosphate groups (gamma 2.3 ppm, alpha 7.5 ppm and beta at 15.8 ppm), inorganic phosphate (Pi, 5.3 ppm), phosphomonoesters (PMEs; cell membrane precursors) and phosphodiesters (PDEs; cell membrane degradation products) can be visualised. This spectrum thus can be used for the absolute or relative quantification of hepatic ATP content. The spectrum obtained from cardiac muscle (D) is very similar to skeletal muscle, containing the resonances of PCr (0 ppm), ATP (gamma 2.3 ppm, alpha 7.5 ppm and beta at 15.8 ppm), 2.3DPG (derived from the erythrocytes 5.4 ppm) and PDE. As the differences in chemical shift of 2.3DPG and $P_i$ are very small, these cannot be measured separately. The static spectrum allows either absolute quantification of the metabolites (with phantom replacement techniques or reference measurements) or the measurement of the concentration PCr relative to the ATP concentration, also better known as the PCr/ATP ratio. This ratio has been suggested to be a surrogate marker for mitochondrial function. As the heart is working continuously, a dynamic measurement for the assessment of PCr recovery is not possible.

metabolism) is hampered in T2DM.

Net glycogen storage in the liver can also be determined after a single meal, and peak glycogen concentrations in the liver were found at 3–4 h after a meal [84] with higher glycogen increases after a meal with a high glycemic index [84]. The blunted increase in hepatic glycogen in diabetes and insulin resistance is also well-documented after single meal [85–87]. Performing glycogen measurements during hyperinsulinemic, hyperglycemic clamps has shed some more light on the possible etiology of hepatic insulin resistance and diminished insulin-stimulated glycogen accretion was reported in diabetes and insulin resistance. Here it has been suggested that the altered insulin-to-glucagon ratio may account for defective hepatic glycogen metabolism in type 2 diabetic patients [87–89].

Another well-known meal response is the modulation of ATP concentrations in the liver. A decrease in hepatic ATP is described in response to glucose uptake, and this decrease is even stronger in response to a fructose challenge [90]. The decrease in ATP is thought to reflect increased phosphate use in response to the very high phosphorylation rates of glucose and fructose when availability is high and to monitor such response in a standardized fashion may also be a source of information of hepatic postprandial metabolism and the capacity to handle glucose ad fructose challenges. However, earlier studies investigating hepatic ATP levels after fructose loads, showed contradicting results [91,92] and therefore, interpretation remains difficult.

## 4. Conclusion

Magnetic Resonance Spectroscopy offers a wide range of non-invasive techniques that give insights into metabolism encompassing lipid storage, glucose storage, oxidative metabolism that may be used to further investigate metabolic disturbances in health and disease. Especially valuable is the possibility to perform dynamic measurements

in a non-invasive fashion and therefore investigate the responses to physiological challenges, such as exercise and responses to a meal challenge.

## Transparency document

The Transparency document associated with this article can be found, in online version.

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