In vivo MR spectroscopic imaging of the prostate, from application to interpretation

Nassim Tayari*, Arend Heerschap, Tom W.J. Scheenen, Thiele Kobus

Department of Radiology and Nuclear Medicine (766), Radboud University Medical Center, P.O. Box 9101, Nijmegen, The Netherlands

Abstract

Proton magnetic resonance spectroscopic imaging (1H MRSI) enables non-invasive assessment of certain metabolites in the prostate gland. Several studies have demonstrated that this metabolic information, in combination with anatomical information from T2-weighted MR imaging significantly improves prostate cancer detection, localization and disease characterization. The technology of 1H MRSI is continuously evolving with improvements of hardware and acquisition methods. Recently, 31P and 13C MRSI of the prostate have regained new interest after a dormant period of decades.

This review focuses on recent technical progress of in vivo 1H MRSI of the prostate, in particular those that enhance clinical applicability at 3T with respect to commonly used techniques to examine the prostate. These developments consist of higher magnetic field strengths, and better MR coils and acquisition techniques. Besides the improvements for 1H MRSI, the developments and opportunities for 31P and 13C MRSI for the prostate are reviewed. Finally, we briefly review 13C MRS of the prostate, in particular the new possibilities with hyperpolarized substrates.

© 2017 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

MR imaging is increasingly being used in the clinical management of prostate cancer such as for the detection and localization of cancer tissue, and to assess the stage and aggressiveness of the disease. This is currently performed by multi-parametric MR imaging (mpMRI) of the prostate, consisting of T2-weighted imaging (DWI) and dynamic contrast enhanced imaging (DCE-MRI). For the interpretation of mpMRI in cancer detection a reporting system called PI-RADS has been developed, which assesses the likelihood of clinically significant disease. As MR spectroscopic imaging (MRSI) has been demonstrated to be valuable in the diagnosis, localization and characterization of the disease it was included in the original PI-RADS, but not in the most recent version. PI-RADS 2.0, due to the low practicality of current 1H MRSI methods in routine clinical use. In particular non-standardized and sometimes rather long examinations, the need for in-house expertise, lack of standardized automated processing and adequate display limit the application of prostate MRSI mainly to clinical research.

There are many recent developments that might lead to a more prominent role for MRSI in prostate cancer management such as improved radiofrequency (RF) coils, faster and more robust acquisition schemes with a higher sensitivity, and more dedicated automatic processing software. In addition, important advances on ultra-high field 7T MR systems enable to achieve higher spatial resolutions in 1H MRSI and to perform 3D 31P MRSI of the entire prostate. Finally, the introduction of in vivo 13C MR spectroscopic imaging of the human prostate using hyperpolarized compounds opens new possibilities for the characterization of prostate cancer.

Acknowledging the previously published review articles on clinical 1H MRSI of prostate cancer, here we will highlight (i) the recent technical developments in 1H MRSI, (ii) recent results on 1H and 31P MRSI at 7T MR systems, and (iii) 13C MRS applications in prostate cancer.

2. 1H MRSI of the prostate

Since the first acquired 1H MR spectra of the prostate in 1990, substantial progress has been made in the methodology with higher field strengths and improved coils and acquisition techniques. These improvements make it possible to acquire MR spectra

* Corresponding author.

E-mail address: Nassim.Tayari@radboudumc.nl (N. Tayari).
of voxels with sizes in the order of 0.5 cm³ with sufficient SNR and spectral resolution to detect metabolites throughout the entire prostate in a clinically feasible measurement time.

3. MR hardware

3.1. Field strength

The first in vivo ¹H spectra of the prostate were obtained at a field strength of 2T [10]. Nowadays instruments with field strengths of 1.5T and 3T are commonly used, along with some initial experiments at 7T. Higher magnetic field strengths offer increased spectral resolution and higher signal-to-noise ratio (SNR), but are challenged by the absorption of RF by the tissue, and by increased susceptibility variations causing faster signal attenuation of the free induction decay, adversely affecting SNR and spectral line widths. A comparison for MRSI between 1.5T and 3T showed an SNR improvement by a factor of 2 [11]. This increase in SNR enables a higher spatial and/or temporal resolution. At higher spatial resolution SNR is relatively enhanced due to reduced intra-voxel dephasing [11]. No comparison in SNR or spatial/temporal resolution between 3T and 7T in prostate is available yet, but such studies of the brain suggest a more accurate assessment of metabolites levels at higher field strength [12,13].

The increased frequency dispersion at higher fields improves the separation between metabolite resonances, but also requires pulses with more RF power deposition, reaching or exceeding the maximum specific absorption rate (SAR) limit. To stay within SAR-limits, an increase in repetition time (TR) might be necessary, which results in longer acquisition times. Clever acquisition schemes to deal with these challenges will be discussed below.

3.2. RF coils

MR(S) of the prostate is generally performed with an integrated body coil for excitation together with external phased array coils and/or endorectal coil for signal reception. The use of an endorectal coil is recommended for MRSI at 1.5T, but optional at 3T, due to its proximity to the prostate. The spectral quality of MRSI data at 1.5T with endorectal coil is comparable to that at 3T without endorectal coil except for the voxels close to the endorectal coil, which have a higher SNR [14]. At 3T a mild but significant improvement in prostate cancer localization using ¹H MRSI was observed when an endorectal coil was used compared to no endorectal coil [15]. However, no difference in cancer localization performance was observed at 1.5T between both coil configurations [16].

As 7T MR-systems lack an integrated body coil, local coils are required that can both transmit and receive RF-signals. The first ¹H MR spectra of the prostate at 7T were obtained with a transmit/receive endorectal loop-coil [17,18]. A loop coil provides high sensitivity for adjacent tissue of interest, but suffers from transmit field (B₁⁺) and receive field (B₀) inhomogeneity. To compensate for RF field inhomogeneity, adiabatic RF pulses have been applied [16], providing a uniform RF field over the region of interest (see pulse sequence section). Others have developed an external transmit/receive 8-channel phased array coil for prostate imaging at 7T [19]. As the ¹H wavelength is short at 7T, destructive interferences can occur. To generate an homogeneous and constructive B₁⁺-field in the prostate with this setup, B₁⁺-shimming is required [19]. During B₁⁺-shimming, the phase of each transmit-channel is optimized in order to have a constructive B₁⁺ in the prostate. Next to receiving with an external array coil for ¹H MRSI, these 8-channel coils can also be combined with a receive-only endorectal coil [20,21].

The use of an endorectal coil has some issues: the positioning is time consuming, demands a level of expertise, and can be uncomfortable for patients. Most often, an endorectal coil with an inflatable balloon is used, which brings the coil close to the prostate. With a dual-channel inflatable coil the SNR and image quality is increased compared to common single loop endorectal coil [22]. Note however, an inflatable balloon changes the shape and size of the prostate significantly, which can cause difficulties if the images are used for subsequent treatment [23]. Recently, a rigid reusable dual-channel endorectal coil became available that provided an increased SNR and image quality up to 3 cm from the coil compared to the single loop inflatable coil [24].

4. Acquisition

4.1. Pulse sequence

Initially, merely the field of view of the endorectal coil was used to localize prostate spectra [10]. Subsequently, in vivo single voxel MRS of the prostate was performed [25,26]. Due to the multi-focal nature of prostate cancer, single voxel MRS is inadequate for prostate applications and MR spectroscopic imaging was introduced [27–31]. Using phase-encoding in three directions and weighted elliptical k-space sampling, spectra of the entire prostate were obtained in 8–15 min with nominal voxel sizes down to 0.4 cc at a field strength of 1.5T [31].

Volume localization in the prostate was first performed using point-resolved spectroscopy (PRESS) [25] and stimulated echo acquisition mode (STEAM) [26], of which the former is commonly used in the clinic to acquire prostate spectra (Fig. 1A). The interpulse timing and magnetic field strength affect the spectral appearance of some compounds detectable in prostate spectra [7], which will be discussed in more detail in the section on metabolites.

In 2009, the use of adiabatic pulses for localization by adiabatic selective refocusing (LASER) was introduced for prostate MRSI [17,32]. They have better slice profiles, reducing outer volume signal contamination, are less sensitive to B₁ inhomogeneities, and have larger bandwidths, thus diminishing chemical shift displacement artifacts. However, the pulses are RF power-demanding and need to be played out in pairs to achieve a homogeneous phase distribution over the selected slice. To lower RF power deposition, GOIA (gradient-modulated offset independent adiabaticity) pulses [33] are used that require less RF to reach adiabaticity [34], and slice-selective excitation is performed by a single conventional 90° excitation pulse: the semi-LASER (sLASER) sequence (Fig. 1B) [35,36]. At 3T, the measurement time for 3D MRSI using sLASER with GOIA pulses can be reduced to about 5 min without an endorectal coil with a nominal voxel resolution of 7 × 7 × 7 mm³ [37].

The transmit/receive endorectal coil used for 3D ¹H MRSI at 7T has a very inhomogeneous B₁⁺. This can be addressed by LASER sequences which are insensitive to B₁ inhomogeneities [17]. As the excitation pulse in the sLASER sequence is non-adiabatic, sequences were introduced using either a composite adiabatic slice-selective excitation (cLASER) or a non-slice-selective adiabatic excitation (nSLASER), allowing for shorter TE’s, whilst maintaining the adiabatic spin excitation [38,39]. However, long repetition times were required due to high RF power deposition and SAR limitations, leading to long measurement times. These SAR-issues were addressed in a feasibility study at 7T using external phased array coils and a double spin-echo with asymmetric slice-selective excitation pulses and a pair of spectral-spatial pulses. The spectral-spatial pulses excite and refocus only the metabolites of interest and eliminate the need for additional water or lipid suppression pulses [20]. The potential of 3D MRSI at 7T in prostate cancer requires further research.
As prostate MRS measurements may suffer from movement artifacts, motion reduction is essential. This can be tackled by several approaches such as limiting bowel movement using antiperistaltic drugs and the application of a navigator [40]. MRSI data can be measured faster by simultaneous sampling in spatial and spectral dimensions, e.g. by traversing k-space in several short spiral trajectories within one read-out period [41,42]. Besides the time-gain of this approach, the spiral readouts start at the center of the k-space, which enables correcting for motion induced phase variations [42]. Spiral k-space acquisitions are an attractive flexible alternative to a Cartesian sampling grid for prostate MRSI [43].

4.2. Suppression of contaminating lipid and other signals

The prostate is surrounded by lipid tissue, which may cause large resonances in the ppm-range close to those of citrate, which is one of the important prostate metabolites. Great care should be taken to prevent lipid signal contamination in prostate spectra. To achieve this several techniques have been used: outer volume saturation (OVS), additional pulses for lipid and water suppression, frequency selective excitation or refocusing, and k-space apodization.

OVS slabs can be placed around the prostate (Fig. 2) to prevent saturation signals from peri-prostatic lipids. The signals of the excited spins are crushed with dephasing gradients. Conventional OVS bands are optimized to compensate for poor edge profiles, B1 field inhomogeneity and chemical shift errors [44]. Very selective saturation (VSS) pulses have a reduced B1 and T1 dependency [44]. The saturation slabs are usually positioned manually; however, in conformal voxel MRS, the assignment of spatial saturation planes is optimized by automatic placement, orientation, timing and flip angle setting of VSS pulses around the excitation volume based on the shape of the prostate [45,46]. To facilitate clinical use of prostate MRSI, automation of certain steps such as prostate volume segmentation, field of view and 3D volume selection and OVS placement are warranted.

Additionally, dual-frequency selective MEGA pulses have been incorporated in prostate MRSI to suppress lipid and water resonances (Fig. 1). The RF pulses selectively refocus water and lipid signals and are surrounded by crusher gradients to dephase the water and lipid spins while those of the metabolites of interest remain unaffected [47]. MEGA pulses are used in 3D 1H MRSI sequences such as PRESS [31], GOA-sLASE [38], clASER, nLASE [38].

Unnecessary excitation of peri-prostatic lipids and water can also be prevented by spectral-spatial selective RF pulses [11]. Dual band spectral-spatial RF pulses for prostate MRSI were specifically developed to fully excite the prostate metabolites and partially the water resonance [48]. The applicability of spectral-spatial pulses for prostate MRSI has been successfully demonstrated at 1.5T, 3T and 7T [11,20,48].

Next to robust suppression of lipid signals, signal contamination from neighboring voxels including lipids should be minimized. In conventional MRSI acquisitions, a standard Cartesian grid is sampled in k-space covering two or three spatial phase encoded dimensions. The limited number of k-space steps in MRSI results in a poor spatial response function (SRF) leading to inter-voxel signal contamination. Application of a suitable apodization filter in k-space can smooth the SRF to minimize signal contamination at the cost of a larger voxel size [49]. Combining this apodization with a weighted elliptical k-space sampling scheme results in a considerably shorter acquisition time with sustained sensitivity [31].

5. Prostate metabolites in 1H MRSI

Proton MR spectra of prostate tissue commonly contain signals from citrate (Cit), choline-containing compounds (tCho), creatine (Cr) and polyamines (PA) (Fig. 2). As metabolite signal intensities are used as biomarkers for prostate cancer or prostate abnormalities like benign prostatic hyperplasia (BPH), understanding the origin of these signals and the underlying mechanisms leading to metabolic changes is essential.

5.1. Citrate

In the normal human prostate epithelial cells secrete Cit in the luminal space, where it accumulates at high concentrations [50,51] (Fig. 3). In numerous studies it has been demonstrated that tissue levels of Cit are reduced in prostate cancer and thus may serve as an in vivo marker to discriminate cancer from normal prostate tissue [25,26,29,52]. The likely processes that contribute to this decrease are the lower secretion of Cit in the lumen and a reduced luminal space in prostate cancer (Fig. 3). As expected from its luminal accumulation, Cit levels are higher in glandular than in stromal tissue [53,54]. For this reason these levels vary between different zones of the human prostate [55–57] and may be increased in mixed tissue BPH compared to normal prostate tissue [53,54].

The protons of Cit resonate around 2.6 ppm, but the precise chemical shift and the scalar coupling of these protons depend on pH [58] and cation concentration [59]. Two magnetically equivalent methylene groups are present in Cit and the protons in these -CH2-groups are strongly coupled. Therefore, the spectral shape of Cit depends on inter-pulse timing including TE, pulse shape, and field strength. Several optimization studies have been conducted to optimize Cit detection at 1.5T and 3T [36,60–62], in which the inter-
pulse timing was optimized to invoke absorptive Cit signals in the spectrum. For double spin-echo techniques, two variables ($t_1$ and $t_2$) and for the sLASER sequence four delays ($t_1$ to $t_4$) can be changed at a constant TE to optimize the shape (Fig. 1A and B). TEs

---

**Fig. 2.** MR(S)I data of a patient with a Gleason score (3 + 4) prostate cancer in the left transition zone. The patient was measured with both the PRESS and GOIA-sLASER sequence at 3 T. On the T2w images with the spectroscopy grid (C) and (D) the location of the normal voxel (blue) and tumor voxel (red) is indicated. Representative spectra from normal appearing tissue acquired with PRESS (A) and GOIA-sLaser (E), and tumor tissue acquired with PRESS (B) and GOIA-sLaser (F) illustrate the differences in metabolite signal intensities between healthy and tumor tissue. The PRESS data was measured at TR/TE = 1020/145 ms and the GOIA-sLASER data was measured at TR/TE = 1020/88 ms. Indicated are the main prostate metabolites: citrate (Cit), choline (Cho), spermine (Spm) and creatine (Cr). Healthy spectra A and E have the same scaling, and tumor spectra B and F have the same scaling, showing the increase in SNR of the GOIA-sLASER sequence compared to PRESS. Note that because of their different distances to the endorectal coil, scaling between tumor and healthy voxel was different.
between 120 and 130 ms at 1.5T and between 75 and 145 ms at 3T are used by the major MR vendors [7]. Published T1 and T2 relaxation times for Cit at 1.5T and 3T are provided in Table 1, taking into account the changes in citrate shape with TE. As Cit has a relatively short T1, a short TR can be used, which minimizes acquisition times.

5.2. Total choline (tCho): free choline, glycerophosphocholine and phosphocholine

Choline-containing compounds are involved in the biosynthesis and degradation of phospholipids that are essential elements of

---

**Fig. 3.** Citrate production in the prostate [50,51]. A) The hematoxylin and eosin (H&E) stained histopathological slice of the normal prostate on the right, shows acini, surrounded by epithelial cells and ducts. Citrate is present at high concentrations in the luminal space of these glandular structures [63,64]. Cho and Cr are mostly present in stromal tissue including smooth muscles. On the left, is a schematic overview of the metabolic pathways related to citrate productions in a normal epithelial cell. Citrate is synthesized in the mitochondria and limited in its further metabolization to isocitrate due to blockage of the pathway by the inhibition of aconitase (ACON) by zinc (Zn²⁺). The epithelial cells then secrete Cit into the luminal space where it accumulates to high concentration (blue arrow). B) On the right, a stained H&E slice of tumor tissue illustrates a reduced luminal space due to invasion of cancer cells. The altered citrate metabolism indicated on the left is caused by less inhibition of aconitase and Cit is mostly oxidized in the Krebs cycle rather than secreted out of the cell. *OAA: oxaloaceticacid, ACON: aconitase, AcCoA: Acetyl coenzyme A, a-KG: α-ketoglutarate.*
5.3. Polyamines/spermine

About 50–90% of the polyamines (PA) in the prostate is spermine (Spm). Like Cit, it is secreted in the luminal space by ductal cells [69–71] and known to decrease in prostate cancer [68,71,72]. Absolute tissue levels of spermine were quantified by in vivo single voxel MRS at short TE [72,73].

Spm is a coupled spin system containing two amine groups, two amide groups and ten methylene groups (CH$_2$). These methylene protons resonate in five sets of four magnetically equivalent protons at 1.81 ppm, 2.11 ppm, 3.13 ppm, 3.12 ppm, and 3.18 ppm [74]. Similar to Cit, the Spm spectral shape and dispersive components are affected by inter-pulse timing and field strength. Interestingly, by partial refocusing of coupled spins due to the frequency-selective refocusing scheme, the Spm signal in in vivo $T_2$ prostate spectra appeared unexpectedly large (Fig. 4) [17,20,75]. In retrospect, perhaps in some earlier studies at 1.5 and 3T the signal intensity of Spm has been underestimated to some extent because of overlapping Cr and tCho signals.

5.4. Creatine and phosphocreatine (tCr)

In $^1$H MR spectra of the prostate the methyl protons of tCr have a resonance at 3.03 ppm. Its methylene peak at 3.91 ppm is usually not seen in vivo. Stromal tissue in the prostate mainly consists of fibroblasts and smooth muscle cells [76], of which the latter probably contribute most to the Cr resonances in prostate MRSI. The total creatine (tCr) signal consists of resonances from free creatine and phosphocreatine (PCr), compounds that play a key role in storage and transfer of energy [77]. PCr supports adenosine triphosphate (ATP) levels in tissue by supplying phosphate to adenosine diphosphate (ADP) to form ATP [78]. In $^1$H HR-MAS spectroscopy studies, no significant difference in tCr levels between normal prostate and cancer tissue was observed [53,68], in agreement with similar values for the stromal component in normal and cancer tissue [8,79] although a small decrease of this component is observed with higher Gleason grades [80]. Only in very high-grade tumors or after hormonal deprivation therapy a decrease of the tCr signal is observed [81].

5.5. Other metabolites

Besides the four major metabolites visible in in vivo $^1$H MR spectra of the prostate, other metabolites with shorter T2 or J-coupled protons may become visible at echo times below about 100 ms. At TE $\approx$ 88 ms signals for myo-inositol and taurine are recognizable [82]. At still shorter TE, also signals for, scyllo-inositol and glutamine/glutamate become detectable in prostate spectra [45,73]. The value of these metabolites as biomarker for prostate cancer requires further investigation. Metabolite profiling in prostate tissue suggested myo-inositol as biomarker for cellular membranes. An increase in the tCho signal is observed in in vivo $^1$H MRSI of prostate cancer tissue [27,29], which is associated with neoplastic changes in cell membrane synthesis and degradation [65,66].

The choline-containing metabolites that contribute to the main peak at 3.2 ppm in in vivo proton MR spectra are free choline, glycerophosphocholine and phosphocholine. Ex vivo HRMAS studies indicate that all choline compounds contribute to the tCho increase in prostate cancer tissue [53,67,68]. In vivo differentiation between the tCho compounds by $^1$H MRS is difficult because of the small chemical shift difference in comparison to the broad spectral linewidth, hence the referral to the composite signal at 3.2 ppm as small chemical shift difference in comparison to the broad spectral linewidth, hence the referral to the composite signal at 3.2 ppm as...
localization of malignancy in the prostate [83]. Lactate is another metabolite of interest for cancer characterization and has been found in high concentrations in brain tumors [84]. However, no lactate signal was detectable in in vivo 1H MRSI of the prostate and it was concluded that its concentration is low (<1.5 mM) even in high-grade prostate cancer [85].

6. Processing and interpretation of 1H MRSI data

Intensity decreases of Cit and PA and increase in tCho signals can be used as a biomarker for malignancies in the prostate. For prostate cancer localization and characterization, generally a metabolite ratio, e.g. (tCho + PA + Cr)/Cit, is used instead of individual metabolite maps. With an endorectal coil, the individual metabolite maps suffer from B1 field inhomogeneity, because signal intensity of the coil drops towards the ventral parts of the prostate. Reconstruction of individual maps of tCho, PA and tCr maybe hampered by overlap of their resonances.

Calculations of absolute metabolite levels require an additional measurement (and thus more time) to acquire the water signal for internal referencing. Furthermore, in prostate MRSI usually long TEs are used, which should be accounted for in quantification. For these reasons absolute quantification of prostate metabolites is not frequently applied [28,56]. The use of a metabolite ratio as biomarker for prostate cancer solves many of these issues. To obtain ratios, the signals should be fitted or integrated. The many aspects to consider in fitting procedures are beyond the scope of this paper. For detailed information on post-processing of prostate spectra and interpretation of metabolite ratios, we refer to review papers that addressed these issues [4,8].

The (tCho + PA + tCr)/Cit ratio increases in cancer compared to normal prostate tissue due to a decrease in Cit (and Spm) together with an increase in tcho (Fig. 3). The decrease of Cit and Spm levels seen in 1H MR spectra of prostate cancer lesions may be caused by a remodeling of their metabolism by morphological changes in the gland leading to a decrease in luminal space in cancer tissue [86]. A substantial loss (>50%, depending on Gleason pattern [79,80,87]) of luminal space by dedifferentiating epithelial cancer cells results in less space for Cit and Spm to accumulate. Indeed, a significant correlation between the percentage area of luminal space and the (Cit + Spm + tCr)/tCho ratio has been observed [87].

The fact that the (tCho + PA + tCr)/Cit ratio is higher in cancer tissue compared to normal prostate tissue enables us to use the ratio for prostate cancer detection and localization [52,88,89]. The applicability of the (tCho + tCr)/Cit ratio to differentiate between tumor and normal prostate tissue has been demonstrated in a multicenter study [90]. Furthermore, the ratio correlates with the Gleason score, a histological score for the aggressiveness of the tumor [91,92]. Although the current role of 1H MRSI in clinical mpMRI is limited there is ample evidence it has added value. The performance of the metabolite ratio for a noninvasive in vivo aggressiveness assessment is comparable to the performance of the apparent diffusion coefficient obtained from DWI [91]. 1H MRSI outperformed DWI when assessing aggressiveness in transition zone (TZ) tumors [91]. When combining multiple modalities, 1H MRSI in combination with the wash-out, a parameter of DCE-MRI, gave the best performance for discrimination between low and high-grade tumors in the TZ [93].

7. 31P MRSI of the prostate

The first 31P MR spectra of the prostate were acquired at 1.5 and 2T in the early nineties using a 31P transmit/receive endorectal coil [94,95]. These spectra were unlocalized and the obtained metabolic information originated from the complete field of view of the coil. To study different parts of the prostate, the coil had to be repositioned between acquisitions [96]. Furthermore, the anatomical images were obtained in a separate session using a 1H coil. After these initial studies, interest in in vivo 31P MRS of the prostate dropped and only received new attention twenty years later with the introduction of ultra high field 7T MR-systems. The increase in SNR at this higher field strength enables 31P 3D MRI studies of the entire prostate in one session. Furthermore, the increase in spectral resolution makes it possible to better discriminate between metabolite signals. In this section we will discuss the information content and technical challenges of 3D 31P-MRSI at 7T.

8. Hardware

As ultra-high field MR systems lack an integrated body coil, local coils are required that can both transmit and receive RF-signals. For the acquisition of 3D 31P MRSI, two frequencies are of interest: the
$^1$H frequency for anatomical reference images and manipulation of $^1$H–$^{31}$P interactions, and the $^{31}$P frequency at which the spectra are acquired. Two different approaches have been presented to achieve this for $^{31}$P MRSI of the prostate at 7 T. For the first approach, an 8-channel $^1$H phased-array body coil was placed around the pelvic area of the subject and this was combined with a $^{31}$P transmit/receive endorectal [97]. Another approach is the use of an endorectal loop-coil tuned to both the $^1$H- and the $^{31}$P-frequency [98]. A challenge when using an endorectal coil for signal transmission is that the $\mathrm{B}_1^\ast$ drops at larger distances from the coil, which will be discussed in more detail in the acquisition section. The future might bring new set-ups involving external $^{31}$P coils or other combinations of transmit and receive coils.

9. Acquisition

The use of an endorectal loop-coil, essential to address the lower sensitivity of the $^{31}$P nucleus has both advantages and disadvantages for $^{31}$P MRSI. Since the $\mathrm{B}_1^\ast$ drops further from the coil, the transmit and receive fields are inhomogeneous. To ensure that the flip angle is the same over the entire prostate, adiabatic pulses can be used [97,99]. To stay within SAR-limits, only a limited number of adiabatic pulses can be applied or the TR (and thus the measurement time) should be increased. On the other hand, as the field of view of the endorectal coil just exceeds the volume of the prostate, the entire field of view of the coil can be phase encoded, so unlocalized adiabatic excitation can be used. An example of an adiabatic excitation pulse is the BIR-4, of which the flip angle can be adjusted by the values of the phase shifts within the pulse [100]. Although the flip angle of the BIR-4 pulse is constant over the entire field of view, the excitation bandwidth decreases at larger distances from the coil with the drop of $\mathrm{B}_1^\ast$. With the knowledge of the T1 relaxation times of the metabolite spins, the best combination of flip angle and TR can be selected [99].

Lateral to the prostate, muscle tissue is present. As muscle contains large amounts of PCr, it is important to minimize contamination from neighboring voxels. To this end, similar to $^1$H MRSI, weighted k-space sampling with a Hanning filter can be applied to decrease signal contribution from muscle tissue. Furthermore, the spatial resolution should be as high as possible. At this time, an effective voxel size of 3.0 cm$^3$, after weighted sampling, is the smallest that could be obtained with sufficient SNR for $^{31}$P MRSI of prostate with an enrorectal coil at 7T [101].

There are several techniques to enhance the sensitivity of $^{31}$P spectra. For instance by $^1$H irradiation the effects of heteronuclear ($^1$H–$^{31}$P) can be eliminated. With this technique the sensitivity of certain $^{31}$P signals can be increased by merging triplets, caused by $^1$H–$^1$H–$^{31}$P heteronuclear coupling, into a single peak. The $^1$H–$^{31}$P coupling constant of the metabolites of interest is 6–7 Hz. As the line width that can be obtained with shimming of the prostate at 7T is about 15–20 Hz, the effect of merging the triplets hardly improves the line width of the $^{31}$P resonances. More interesting is exploiting the Nuclear Overhauser Effect (NOE), where water protons are saturated, which leads to signal enhancement of the $^{31}$P signals through dipolar interactions. In the prostate, most metabolites showed positive NOE-enhancements, although the enhancement was variable [99].

10. Prostate metabolites in $^{31}$P MRSI

There are compounds with $^{31}$P-atoms that reside in the prostate at MR measurable concentrations. These metabolites are important for energy and membrane phospholipid metabolism.

10.1. Phosphocreatine and adenosine triphosphate

Both PCr and ATP play an essential role in energy metabolism. Basically, when energy is needed, ATP is hydrolyzed into ADP and inorganic phosphate (Pi). PCr serves as an buffer by rephosphorylating ADP into ATP when energy demand is high. PCr is a single resonance assigned to a chemical shift of 0 ppm. Maps of PCr show high PCr intensities in the muscles lateral to the prostate and low PCr levels within the prostate (Fig. 5). The T1 relaxation time at 7T for PCr is 4.1 s, which is comparable to the T1 relaxation times observed in muscle [99,102].

ATP contains three $^{31}$P atoms ($\gamma$, $\alpha$ and $\beta$) that resonate around $−2.5$ ($\gamma$), $−7.5$ ($\alpha$) and $−16.3$ ($\beta$) ppm, but their exact positions are dependent on physiological conditions like pH and Mg$^{2+}$ content. The $^{31}$P-atoms are coupled with a coupling constant around 16 Hz. To excite all ATP resonances, pulses are required to have a large bandwidth. If the bandwidth is not sufficient to fully excite the $\beta$-ATP (and $\alpha$-ATP) resonance, the $\gamma$-ATP intensity is used to estimate ATP-levels. $\gamma$-ATP maps show great variation among patients, which cannot be explained only by the coil profile, and no consistency was observed in ATP-levels in cancer tissue [101]. The T1 relaxation time at 7T for $\gamma$-ATP is 3.0 s [99]. In skeletal muscle, the PCr/ATP ratio is between 4 and 5 [103]. This ratio is much lower in smooth muscle tissue (104) and similar to the PCr/ATP ratio observed in prostate: 1.3–1.5 [96,99], which indicates that most of the PCr arises from stromal smooth muscle tissue in the prostate.

10.2. Phosphomonoesters and diesters

Next to the energy balance related metabolites, $^{31}$P MR spectra of the prostate contain resonances of compounds participating in phospholipid metabolism. These resonances belong to the phosphomonoesters (PME), e.g. phosphocholine (PC) and phosphoethanolamine (PE), and their glycerol derivatives, the phosphodiester (PDE) glycero phosphocholine (GPC) and glycerylphosphoethanolamine (GPE). They are involved in signalling pathways, lipoprotein metabolism and the synthesis of phosphatidylcholine and phosphadylethanolamine, two major cell membrane compounds [104]. The metabolites are of interest in oncology as membrane phospholipid metabolism is altered in cancer.

PC, PE, GCP and GPE resonate around 5.6, 6.8, 2.8 and 3.2 ppm, respectively. The $^{31}$P-atoms in these molecules are hetero-nuclear coupled to the protons in adjacent methylene group with a coupling constant around 6–7 Hz [105]. At lower field strengths without $^1$H decoupling, the two PMEs and two PDEs peaks are not resolved, but with the increased spectral resolution of 7T they can be distinguished from each other. The T1 relaxation times of these metabolites at 7 T vary between 5.9 and 8.3 s [99].

10.3. Inorganic phosphate/tissue pH

Pi is formed during the hydrolysis of ATP in ADP. It is involved in many metabolic processes and is essential in glycolysis and to synthesize nucleic acids, phospholipids and proteins.

The resonance frequency $\mathrm{Pi}$ in cell is around 5 ppm, but the exact frequency strongly depends on pH. As the pKa of Pi is about 6.9, which is close to the range of physiological pH values, the chemical shift of Pi measured relative to the PCr signal, can be employed to determine tissue pH. In $^{31}$P spectra of the prostate, one or two resonances in the 5.0-ppm region were observed [97–99,101], most likely both belonging to Pi. The observation of two Pi resonances could point towards two compartments in the prostate with different pHs, calculated to be $\sim$7.1 and $\sim$7.6 [99,101]. This hypothesis of two compartments is strengthened by the observation that both resonances have a different T1 relaxation time: 6.5 s for
the low pH and 4.6 s for the high pH resonance [99]. Interestingly, there was a trend towards a higher ratio of $\Pi$ (pH~7.6) over $\Pi$ (pH~7.1) for cancerous tissue compared to normal tissue. Further research is needed to unravel the underlying physiology of the two resonances and their value in prostate cancer characterization.

11. Processing and interpretation of $^{31}$P MRSI

Acquisition of $^{31}$P MRSI is commonly done by a pulse-acquire scheme, which simplifies post-processing of the spectra with respect to $J$-coupling evolution for coupled resonances. However, there is a small time delay between the pulse and acquisition to facilitate phase encoding, which requires a first-order phase correction. A detailed fitting procedure for quantification of the spectra in Metabolite Report (work-in-progress package of Siemens Healthcare) was described by Lagemaat [99]. Both Lorentzian (PCr, PC, PE, GPC, GPE) and Gaussian (ATP and Pi) lineshapes were used to fit the metabolites and heteronuclear coupling was incorporated for PMEs and PDEs.

There is limited information available on the interpretation of $^{31}$P MR prostate data. AT 1.5T an increase in the PME/PCr ratio was observed in cancer compared to non-cancer tissue [95,96]. However, these results could not be reproduced at higher field strength. As localization was poor in that initial study, these spectra might have suffered from signal contamination of PCr from adjacent muscle tissue in small prostates, leading to unrepresentative ratios. Results at 7T showed quite some overlap between the $^{31}$P metabolite levels in cancer and normal tissue, which may be due to partial volume effects as the MR spectroscopy voxels were relatively large (~5.1 cm$^3$) compared to the small size of most tumors. An increased spatial resolution is needed to fully evaluate the potential of these metabolites as biomarker for prostate cancer. Interestingly, GPC and GPE were nearly absent in the 7T $^{31}$P spectra of non-cancerous tissue, but more often observed in cancerous tissue [101].

12. $^{13}$C MRSI of the prostate

The first in vivo MR spectra of the human prostate were obtained by natural abundance $^{13}$C MRS [106]. At a field strength of 1.5 T, the authors obtained spectra that contained, among other signals, Cit. As the natural abundance of $^{13}$C is low (~1% of all natural carbon), 700 averages were needed to record this spectrum. With the increasing interest in $^{1}$H MRS, which also detects Cit and does not suffer from low natural abundance, $^{13}$C MRS of the prostate was abandoned. Recently, $^{13}$C MRS of the prostate has regained interest with the new technical developments in the field of hyperpolarization. The ability to increase the sensitivity of $^{13}$C-labeled substrates more than 10.000 fold using dynamic nuclear hyperpolarization (DNP) and to subsequently dissolve the substrate quickly to create a solution that can be injected, makes it possible to study fast dynamic metabolic processes in vivo [107]. After injection of the hyperpolarized $^{13}$C-labeled substrate, the conversion of the substrate into other metabolites can be observed with $^{13}$C MRS(I).

Similar to the acquisition of $^{31}$P spectra, coils dedicated to the $^{13}$C frequency are necessary to acquire $^{13}$C signals in combination with $^{1}$H coils for anatomical reference images. For the first in vivo study of the human prostate, a $^{13}$C volume transmit coil was combined with an endorectal $^{1}$H/$^{13}$C receive coil [108].

The acquisition of signals from labeled $^{13}$C-substrates is challenged by T1 relaxation by which the sample starts to lose polarization once dissolved and taken out of the polarizer. As polarization also decreases due to RF excitation typically low flip angle approaches are used combined with short TRs. To ensure that as much information as possible is obtained in the short imaging window, clever acquisition techniques are needed.

The substrates that can be measured with this technique are also limited by their T1-relaxation time. [1-1$^{13}$C]-pyruvate is a popular substrate as the quaternary carbon has a relatively long T1. It also is a key metabolite at the crossroad of metabolic pathways (i.e. glycolysis, citric acid cycle). The first human in vivo study using hyperpolarized $^{13}$C-labeled pyruvate demonstrated the safety and feasibility of the method and also indicated clinical potential as the ratio of lactate to pyruvate was elevated in regions of prostate cancer (Fig. 6) [108].

The challenges of this technique include the way of polarization, substrate administration, dedicated acquisition methods, and data post-processing. For instance in post-processing, the effects of T1 decay and metabolic conversion and supply on signal changes has to be taken into account, which requires complex modeling to understand the physiology that underlie these spectral changes [109]. A detailed review of this promising technique is beyond the scope of this paper and more information can be found elsewhere [110, 111].
13. Outlook

In this paper recent developments in hardware and software concerning prostate MRSI is reviewed. A new hardware improvement involves the development of a dual channel endorectal receive coil with higher SNR in the dorsal part of the prostate compared to single channel receive coils. Both inflatable and rigid coil designs have been equipped with this technology. Whether further improvements can be made by adding more coil elements is questionable, as the penetration depth decreases for smaller coil elements. However, further optimization of the external phased array coils towards MR without endorectal coil is probably more of clinical interest as it results in shorter patient preparation times, improved patient comfort and MRSI options for individual metabolite maps with uniform $B_1$ over the prostate.

Another hardware advance is the increased availability of 7T systems. There is no information available yet whether the increased SNR and spectral resolution of this field strength will have added value for prostate cancer management. However, it can be expected that the increased SNR can be used for either higher spatial resolution or shorter measurement times. As discussed, these measurements are not straightforward yet; there is a lot of room for improvement and clever acquisition techniques with minimal SAR-deposition are required. Ultra-high field MR systems...

Fig. 6. Images were obtained from a patient with serum PSA of 9.5 ng/ml who was diagnosed with bilateral biopsy-proven Gleason grade 3 + 3 prostate cancer and received hyperpolarized $[^{1-13}C]$ pyruvate (0.43 ml/kg). The axial T2-weighted image shows a unilateral region of reduced signal intensity (red arrows), which is consistent with a reduction in the corresponding apparent diffusion coefficient (ADC). The $^1H$ spectral arrays supported these findings, with voxels with reduced citrate and elevated choline/citrate (highlighted in pink) on the right side of the gland and voxels with normal metabolite ratios on the left side. The $^{13}C$ spectral arrays show voxels with elevated levels of hyperpolarized $[^{1-13}C]$ lactate/$[^{1-13}C]$pyruvate (highlighted in pink) on both the right and left sides of the prostate. The location of colored regions in the metabolite image overlay had a ratio of $[^{1-13}C]$ lactate/$[^{1-13}C]$pyruvate greater than or equal to 0.6. Reprinted with permission from [107].
open the possibility to perform 3D $^{31}$P MRSI of the entire prostate in a reasonable measurement time. There are some indications that $^{31}$P MR spectra might contain interesting information for prostate cancer management, but more patients need to be evaluated, preferably with more aggressive and larger tumors.

Besides progress in hardware, the recent improvements in $^1$H MRSI acquisition are of interest, in particular for clinical applications. The sLASER sequence enables accurate volume selection of the prostate and has a minimal chemical shift displacement artefact resulting in a more robust acquisition with little lipid signal contamination. The sLASER sequence combined with a spiral readout, offers a flexible approach to acquire 3D $^1$H MRSI data of the prostate in 5–8 min without an endorectal coil at 3T. This measurement time approaches the acquisition time of techniques like DWI and DCE-MRI. Still, for a more widespread use of MRSI in an mpMRI examination, not only fast and robust acquisition is desired, but also display of the results in a reliable and easy digestible way. To this end, full automation in post-processing is needed. Further refinement of MRSI acquisition is possible, such as movement correction.

With fully automated post-processing and intuitive display in place, MRSI might be a good alternative for DCE-MRI in an mpMRI prostate exam. The use of gadolinium as contrast agent is under discussion, because of complications such as accumulation of residual gadolinium in the brain and bone, and nephrogenic systemic fibrosis [111–113]. Especially for clinical applications where MR-examinations are repeated over time, MRSI might be preferable over DCE-MRI. Several studies have shown the ability of prostate MRSI (in particular combined with DWI) to identify aggressive prostate cancers, suggesting an important role for MRSI in selecting patients for active surveillance programs [114, 115].

Lastly, the promising development in hyperpolarized $^{13}$C MRSI are discussed. So far, only $[1-^{13}$C$]$-pyruvate has been studied in the human prostate, but more substrates can be polarized to investigate metabolic processes. In contrast to $^1$H and $^{31}$P MRSI providing static levels of metabolites, hyperpolarized $^{13}$C MRSI monitors dynamic processes of metabolism. The true value of $[1-^{13}$C$]$-pyruvate and other substrates in cancer diagnosis needs more investigation.

A prostate MR examination is multi-parametric and at this moment, MRSI is often not included in this approach. However, with all recent advancements, a new evaluation of the complementary role of MRSI for prostate cancer management is in place.

Acknowledgment

This work was funded by the EU Marie Curie Fp7-PEOPLE-2012-ITN project TRANSACT (PITN-GA-2012–316679) and the European Research Council: FP7/2007–2013 ERC Grant agreement 243115. Furthermore, the authors thank Sjak J van Asten, Miriam W. Lagemaat and Isabell K. Steienseifer for their contributions to the manuscript.

References

[2] J.C. Weinreb, et al., PI-RADS prostate imaging - Reporting and data system: with all recent advancements, a new evaluation of the completeness of MRSI acquisition is possible, such as movement correction.


