

Spectroscopy for Body MR

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Introduction: In this lecture we describe the current and potential role of magnetic resonance spectroscopy (MRS) and magnetic resonance spectroscopic imaging (MRSI) in organs of the body with an emphasis on the technical aspects of applications in cervical, prostate and breast cancer, and diseases of the liver. In contrast to anatomical magnetic resonance imaging (MRI), which detects changes in the relaxivity or density of bulk tissue water, spectroscopy detects small molecular weight metabolites within the cytosol of cells or within extracellular spaces such as glands or ducts. The addition of spectroscopy has been shown to improve the ability [i.e., sensitivity, specificity, and accuracy(1)] of conventional MRI to detect and stage prostate and breast cancer (2-4) and has shown promise in the evaluation of primary and metastatic liver tumours (5) and other liver diseases (6,7). Spectroscopy can often measure changes in multiple metabolic markers within the same spectra that correlate with disease state, cancer aggressiveness, and therapeutic response (8-10). The clinical use of spectroscopy as an adjunct to MRI has expanded dramatically over the past several years. This has been due to both the need to answer clinically relevant questions and recent technical advances in hardware and software that have provided improvements in the spatial and time resolution of the spectral data and have resulted in the incorporation of this technology on commercial MR scanners. These breakthroughs have allowed the routine addition of spectroscopy sequences to clinical MRI exams, and have led to spectroscopy being factored into the clinical decision process.

Historically, ^{31}P and ^1H have been the nuclei of choice for in vivo MRS in the body and each has distinct advantages and disadvantages. The major advantage of ^1H spectroscopy is its high sensitivity, which is necessary to achieve high spatial resolution spectroscopic data ($<1\text{ cm}^3$) in a clinically reasonable amount of time. Because the sensitivity of ^{31}P is only 6.6% that of ^1H , much larger voxel sizes (typically $>8\text{ cm}^3$) must be used to achieve the same sensitivity in the same amount of time. ^{31}P MRS also suffers from long T_1 and short T_2 relaxation times relative to ^1H . The inherently low sensitivity of ^{31}P MRS can be improved by broadband ^1H decoupling (e.g., WALTZ) during the acquisition, which sharpens signals by collapsing multiplets and produces a large nuclear Overhauser enhancement (NOE)(11), and through the use of higher magnetic field clinical MR scanners. The major advantages of ^{31}P MRS are that no water or lipid suppression is needed and there is less spectral overlap because a relatively small number of metabolites are dispersed over a large spectral window ($\sim 25\text{ ppm}$). Both ^1H and ^{31}P spectroscopy require additional hardware, software, and post-processing and display tools, much of which can now be purchased in the form of spectroscopy packages from the major MR scanner manufacturers.

Detectable Metabolites by MRS: Several metabolites are present in high enough concentrations ($>1\text{ mM}$) to be detected by ^1H or ^{31}P MRS, although many have not yet been detected or fully exploited in vivo in the body. The ^1H MR spectrum spans a frequency range of about 10 ppm and is centered around water at 4.8 ppm. To date, the resonances upfield of water have received the most attention, including lactate (Lac, $\delta = 1.33, 4.12\text{ ppm}$), alanine (Ala, $\delta = 1.48, 3.78\text{ ppm}$), glutamine/glutamate (Glx, $\delta = 2.04, 2.11, 2.35, 3.76\text{ ppm}$), taurine (Tau, $\delta = 3.26, 3.43\text{ ppm}$), myo-inositol (mI, $d = 3.28, 3.54, 3.60, 4.05\text{ ppm}$), scyllo-inositol (sI, $d = 3.35\text{ ppm}$), creatine/phosphocreatine (Cr/PCr, $\delta = 3.04, 3.93\text{ ppm}$), the choline containing compounds choline (Cho, $\delta = 3.21, 3.55, 4.07\text{ ppm}$), phosphocholine (PC, $\delta = 3.23, 3.62, 4.18\text{ ppm}$) and glycerophosphocholine (GPC, $\delta = 3.24, 3.68, 4.34\text{ ppm}$), and the ethanolamine containing compounds ethanolamine (Eth, $d = 3.15, 3.80\text{ ppm}$), phosphoethanolamine (PE, $d = 3.22, 3.99\text{ ppm}$), and glycerophosphoethanolamine (GPE, $\delta = 3.30, 4.12\text{ ppm}$). Healthy prostate tissue is unique in that citrate ($d = 2.55, 2.71\text{ ppm}$)(12) and polyamines (predominantly spermine, $\delta = 3.11, 2.09, 1.78\text{ ppm}$)(13) are also present in very high concentrations and can be readily observed by ^1H MRS. There is also much interest in observing glucose (3.43, 3.80, 5.23 ppm) and uridine diphosphate (UDP) sugars (5.5 to 6.1 ppm), which resonate very close to water, because of the role of increased glycolysis in cancer(14). It has also been demonstrated that amide proton transfer from the downfield exchangeable amide protons of proteins and peptides (~ 7.8 to 8.8 ppm) to water can improve sensitivity by several orders of magnitude and provide a novel imaging mechanism (15).

The major contributors to the ^{31}P MR spectrum include inorganic phosphate (Pi: $\delta = 2.26\text{ ppm}$), phosphocreatine (PCr: $\delta = -2.89\text{ ppm}$), the phosphomonoesters (phosphocholine (PC) and phosphoethanolamine (PE): $\delta = 3.76$), the phosphodiester (glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE): $\delta = 0.11, 0.74\text{ ppm}$), diphosphodiester (e.g., UDP sugars: $\delta = -11.07, -12.76\text{ ppm}$), and nucleotide phosphates (e.g., adenosine triphosphate (NTP): $\delta = -5.24, -10.37, -19.02\text{ ppm}$) (16). ^{31}P chemical shifts are typically referenced to

either in vivo phosphocreatine (PCr) or external 85% H₃PO₄ (as listed here). It should be noted that when ³¹P chemical shifts are reported with PCr set to 0.00 ppm, the values are 2.89 ppm greater than the corresponding values referenced with H₃PO₄ set to 0.00 ppm. It is also widely believed that ¹³C spectroscopy using ¹³C labelled substrates (e.g., glucose, acetate, pyruvate), which to date has been primarily limited to the brain (17,18), will play a major role in the future of body MRS. The naturally low sensitivity of ¹³C spectroscopy be overcome using higher field human scanners (3 and 7 Tesla) that are becoming more widely available and due to the utilization of dynamic nuclear polarization techniques that can provide an ~4 orders of magnitude (10,000-fold) in sensitivity (120-121).

Previous MR studies on prostate, breast, and liver tumours have identified elevated levels of phosphomonoesters and phosphodiester (detected by ³¹P MRS)(5,16,20-24) and elevated levels of the composite choline resonance (detected by ¹H MRS) relative to normal healthy tissues (4,12,20,25-29). Although the *in vivo* ¹H signal that is attributed to choline contains contributions from Cho, PC, GPC, Eth, PE, GPE, Tau, mI, and sI, the choline head group contains nine equivalent protons; consequently, a small increase in concentration results in a large increase in signal intensity. In cancer, the observed increases in choline and ethanolamine containing compounds have been primarily attributed to increased cell membrane synthesis and degradation (24) associated with malignant transformation. However, changes in cell density and altered phospholipid metabolism with cancer evolution and progression may also contribute to the observed increase in phospholipid metabolites (27, 30).

In spectroscopy it is also very important to identify markers for healthy or normal tissue that can be used for quantitation purposes. In ³¹P MRS, metabolite ratios are often calculated relative to Pi, PCr, or NTP. In studies of cancer, before and after therapy, the phosphomonoester to phosphodiester ratio is particularly useful because it compares markers of proliferation (PC and PE) to markers of cellular breakdown or apoptosis (GPC and GPE). ¹H MRS has been highly successful in the prostate and brain because, in addition to increased choline, there are unique markers for healthy tissue that decrease in cancer (31). Consequently, the ratios of choline to citrate in the prostate and choline to N-acetyl aspartate (NAA) in the brain are significantly greater in regions of cancer compared to surrounding healthy tissues (2,32). Non-suppressed water can also be used to quantify choline levels particularly in tissues such as breast in which there are currently no other resonances within the spectrum to take a ratio with (73).

Techniques for Body MRS: The technology to perform body spectroscopy on clinical MR scanners is just becoming available and involves the following considerations.

Volume Localization: The most common localization schemes for single voxel spectroscopy are image selected in vivo spectroscopy (ISIS)(33), stimulated echo acquisition mode (STEAM)(34), and point resolved spectroscopy (PRESS)(35). ISIS consists of a series of selective inversion (i.e., 180°) pulses, which are turned on and off according to an 8-step encoding scheme, in the presence of magnetic field gradients. Because the magnetization remains along the "z" axis prior to the read pulse, ISIS is relatively insensitive to T₂ relaxation, and therefore has historically been popular for ³¹P MRS. However, ISIS is particularly sensitive to motion because the eight transients must be added and subtracted to achieve spatial localization. Consequently, STEAM and PRESS, which are capable of three dimensional (3D) localization in a single acquisition, are preferred for ¹H MRS studies in the body.

The STEAM rf pulse sequence can be represented as 90° – t₁ – 90° – t₂ – 90° – t₁ – acquire, where t₁ and t₂ are inter-pulse delay times. STEAM generates three FIDs, four spin echoes, and one "stimulated echo" at distinct temporal positions, depending on t₁ and t₂, and the unwanted coherences are removed by applying crusher gradients. The desired stimulated echo appears at time 2xt₁ + t₂ and corresponds to the signal from the volume of interest. The PRESS rf pulse sequence can be represented as 90° – t₁ – 180° – t₁ + t₂ – 180° – t₂ – acquire, where t₁ and t₂ are inter-pulse delay times. When the first 180° pulse is applied after time t₁, a spin echo forms at time 2xt₁. When a second 180° pulse is applied after time t₂, the spin echo formed at 2xt₁ is refocused into a second spin echo at time 2xt₁ + 2xt₂, producing the signal that corresponds to the volume of interest. As in STEAM, crusher gradients are used in PRESS to remove the unwanted coherences.

Although STEAM can be used with shorter echo times for the observation of short T₂ metabolites, PRESS offers a factor of two times greater signal to noise, is less sensitive to motion and diffusion, and is not susceptible to the effects of multiple quantum coherence (36). The use of longer echo times with PRESS also improves water and lipid suppression, but with improved gradient technology, echo times of the PRESS sequence can be reduced to that of STEAM to exploit the increased chemical information that can be obtained at shorter echo times. Recently, modified PRESS sequences for single voxel MRS and spectroscopic imaging (MRSI) with very short echo times have been described using asymmetric radio frequency pulses as well as optimised design and timing of the PRESS sequence (37).

Improved volume selection and outer volume suppression (OVS): Spectroscopy studies in the body are critically dependent on accurate volume selection, since the region of interest is often adjacent to regions of lipid or air-tissue interfaces, which can significantly impair spectral quality. A recent technical advance for ¹H MRS has

been the substitution of optimally shaped rf pulses, e.g., Shinnar-Le Roux pulses (38), in place of conventional sinc-shaped pulses for improved volume selection in PRESS acquisitions. Although low tip angle pulses can produce reasonably good slice profiles, optimised pulses are essential for 90° and especially 180° excitations (38). Water saturation performance can also be improved using shaped pulses; however, due to the imperfect excitation profiles of the PRESS spin echo pulses, even with Shinnar-Le Roux pulses, significant contamination from lipids outside the PRESS selected region can still occur. Several groups have used outer volume suppression (OVS) sequences to better conform the volume of interest (39-43). These sequences utilized optimised pulses or special excitation schemes to shape the excitation volume to the region of interest. However due to the non-rectangular suppression profiles of these pulses, residual unsuppressed water and lipid signals at the band edges often rendered large portions of the spectral array unusable.

Quadratic phase pulse designs, e.g., very selective suppression (VSS) pulses (44), can provide excellent spatial selectivity, high effective bandwidths, and improved B₁ and T₁ insensitivity compared to conventional OVS pulses. VSS pulses can be inserted just before the PRESS excitation pulses and are used to better define the edges of the PRESS box. Additional VSS pulses can also be graphically prescribed in order to shape the selected volume to the region of interest to exclude regions of lipid or air tissue interfaces. Because of the imperfect PRESS excitation profile, the effects of chemical shift misregistration and the fuzzy edges of the PRESS selected volume can be dramatically reduced by over-prescribing the PRESS selection by ~20 to 30% and applying the VSS pulses to define the desired dimensions of the box. Graphically placed VSS pulses can subsequently be used to shape the rectangular PRESS volume to match the shape of the region of interest.

Water and lipid suppression: In order to detect the resonances of biological interest, the 110 molar water resonance must be suppressed by approximately 1,000 – 10,000 fold and spectral contamination from lipids outside the volume of interest must be minimized as much as possible. Good shimming is absolutely essential for water and lipid suppression. Although automated shimming routines are often adequate and should be used as a starting point, it is well worth the extra time to manually shim and visually assess the shape of the water resonance and the FID. The ability to obtain a narrow water line-width (<10 Hz) is also dependent upon the proper placement of the PRESS or STEAM box. When there are large differences in magnetic susceptibility, which can be caused by air-tissue or bone-tissue interfaces or the presence of radioactive seeds, it may be impossible to obtain adequate shim. If an adequate shim cannot be obtained in less than five minutes, the volume of interest should be re-prescribed and shimmed again, or the spectroscopy exam should be aborted. Prior to starting the MRS sequence, the water and lipid suppression pulses should be turned on in the pre-scan window to ensure that adequate suppression is being achieved.

Techniques for water suppression have usually involved frequency selective saturation pulses (e.g., CHESS)(45). Lipid suppression can be achieved by the selective excitation of a region of interest or by a combination of frequency and/or spatially selective pulses and selective inversion (STIR) (46). These approaches require optimization for each individual study and often demonstrate inadequate water and lipid suppression, resulting in baseline artefacts and difficulty in quantifying metabolites. Recently, band selective inversion with gradient dephasing (BASING) pulses have been developed for combined water and lipid suppression, and have demonstrated suppression factors over 100 times greater than CHESS and STIR (47).

Spectral-spatial pulses: Spectral-spatial echo-planar spin-echo (EPSE) pulses simultaneously excite a selected frequency range and a selected volume, with the added advantage of reducing the chemical shift dependence of the PRESS volume (48). Water and lipid suppression are not needed since their frequency ranges are simply not excited. Schricker and co-workers recently developed dualband spectral-spatial pulses which completely exclude lipid but allow for a partially excited water resonance for phase and frequency referencing (48). This partial water signal can also be used to distinguish the absence of detectable metabolites due to therapy or atrophy from a technically failed study.

Magnetic Resonance Spectroscopic Imaging: Single voxel MRS studies are often sufficient for assessing diffuse disease or focal disease in which the region of interest can be defined by MRI such as for dynamic contrast imaging of the Breast cancer. However, in cases of focal disease, in which MRI cannot accurately define the regions of interest for spectroscopic evaluation, or when there is a need to assess the spatial extent of disease, a multi-voxel spectroscopic approach is necessary. Specifically, for a heterogeneous, multifocal disease like prostate cancer, the exact choice of voxel size and position has a critical impact on the spectra acquired and their interpretation. To overcome the limitations of single voxel techniques, 3D phase encoding techniques have been added to PRESS or STEAM localization schemes (49,50) to provide localized spectra from arrays of contiguous voxels from throughout the region of interest. These techniques are referred to interchangeably as chemical shift imaging (CSI) or magnetic resonance spectroscopic imaging (MRSI).

Surface and Endocavity Coils: While body coils can provide uniform excitation for ^1H spectroscopy, they are often too far from the organs or lesions of interest buried deep within the body to acquire sufficient spectroscopic signal to noise. Consequently, surface coils have been developed for signal reception, and for ^{31}P MRS applications, both transmission and reception. For both prostate (122) and cervical (123) spectroscopy, endocavity coils are essential to achieve the necessary sensitivity and to reduce motion. One problem with air inflated endocavity coils is the increased susceptibility introduced by the air pocket. This can be resolved by inflating these coils with susceptibility matched fluids (127). For other organs, larger arrays of coils are typically placed over the torso or abdomen. These arrays include rigid frames of coils that are placed anteriorly and posteriorly, flexible coils which can be moulded to the body allowing closer placement to regions of interest, and specialized breast coil arrays which circle the breasts and extend into the axilla for the supine patient. Many surface coils are now commercially available from a number of commercial vendors.

While surface coils and phased arrays of surface coils can provide several times the sensitivity of the body coil, their sensitivity varies with position and decreases with increasing distance from the coil. If metabolite ratios are to be used then the inhomogeneous reception profile of the surface coil can generally be neglected. However, the absolute amplitudes of individual metabolite peaks cannot be compared without taking into account the surface coil reception profiles. Fortunately, spectra (like MR images) can be corrected for inhomogeneous reception profiles by numerical evaluation of the Biot-Savart law (51). Simplistically, the coil is modelled as a series of finite elements of various lengths from which a theoretical reception profile is calculated and the spectra are then divided by the theoretical profile. In practice, such correction algorithms need to take into account the anatomical location of the coil with respect to the location of the voxels to be corrected. An alternative approach is to acquire proton density weighted images, which demonstrate the sensitivity profile of the coil, and use these to correct the data (52).

In heteronuclear and high field (e.g., 3T to 7T) MRS, combined transmit/receive surface coils are required and inhomogeneous excitation profiles become a major issue. Specifically, an inhomogeneous excitation profile results in different locations experiencing different nutation (flip) angles, which causes poor or inadequate spatial selection and chemical shift misregistration. One way of overcoming problems associated with inhomogeneous excitation profiles is to excite with a much larger surface coil than that used for signal reception. Another way to overcome the problems with inhomogeneous excitation profiles of surface coils is to use adiabatic rf pulses, such as B1 insensitive rotation (e.g., BIR-4) pulses (53). Adiabatic pulses produce a uniform flip angle, typically 90° or 180° , across a region of interest despite variations in B1, provided that the rf power is above a minimum threshold value. Adiabatic pulses are highly versatile and can be exploited for spatial localization [e.g., localization by adiabatic selective refocusing (LASER)(54)], as well as water and outer volume suppression (e.g., B₁-insensitive train to obliterate signal (BISTRO)(43,55).

Motion: Respiratory and peristaltic motion can also be major problems in body spectroscopy. In the prostate, peristaltic motion is reduced by the use of an inflatable endorectal coil. Because the prostate is directly beneath the bladder, additional motion may be caused by the bladder filling up during the course of the examination. To minimize this, patients are asked to refrain from drinking (especially caffeinated beverages) for two to three hours prior to the exam. In the breast, motion is reduced by having the patient lay supine on a breast coil, supported by the chest, resulting in the breasts remaining relatively free of respiratory motion. Nonetheless, one study showed that in 20 breast and abdominal tumours, 30% moved 6-23 mm, while the diaphragm and fatty tissues of the gut typically moved ~15-20 mm(56). Recently, it has been reported that breathheld MRS can significantly reduce phase and frequency shifts and outer voxel contamination due to respiratory motion (57), while the use of navigator echoes can aid with retrospective motion correction (58).

Higher Magnetic Fields ($\geq 3\text{T}$): There are substantial benefits but also challenges to moving to higher field MR scanners [124-126]. Specifically, preliminary studies of prostate cancer patients have demonstrated increased SNR, spectral resolution and spatial resolution [125-126] at 3T prostate as compared to 1.5T. However, the successful acquisition of proton spectra at 3T requires the development of methods to address the key problems of 3T vs 1.5T prostate MR studies which include: 1) new rf coils for the higher frequency, 2) specific rf pulses for addressing the larger frequency range and reduced peak power; 3) much greater magnetic susceptibility effects; 4) and for prostate spectroscopy the more problematic j-modulation effects of citrate and polyamines.

Data Processing and Display: Single voxel MRS data can often be processed and displayed using commercial software packages designed for conventional NMR spectroscopy, provided that the header can be interpreted or removed. Basic data processing involves Lorentzian and/or Gaussian apodization of the FID to enhance resolution and/or signal-to-noise, baseline correction, zero-filling, and Fourier transformation of the data. Phasing and frequency referencing are then often performed manually on the resulting spectra, although there exist a number of automated approaches. Because MRSI data may contain hundreds of useable spectra, completely automated and robust data processing algorithms are essential. MRSI data has historically been processed offline using research

software, however, more recently commercial packages for processing MRSI data right on the MR scanner are becoming available. In addition to the basic data processing steps just described for single voxel spectroscopy, MRSI data must be reconstructed to correctly reproduce the spatial dependence of the data (59) and can also be spatially zero filled. After the spectra have been Fourier transformed, automated baseline, phase, and frequency corrections can then be applied using water as a reference or by using prior knowledge of the approximate relative positions of the major peaks in the spectrum. Peak areas may be estimated by integration across fixed frequency ranges, by fitting baseline subtracted data as a sum of components with particular lineshapes (60-63), or using linear combinations of model in vitro spectra (64).

Several different approaches have been used to display the information from multi-dimensional localized spectra and to correlate spatial variations in metabolites with the anatomy (50,65-68). These include superimposing a grid on the MR image and plotting the corresponding arrays of spectra, and calculating images of the spatial distribution of metabolites to overlay on the corresponding MR images. These formats provide an excellent summary of the spatial distribution of different metabolites enabling rapid identification of regions of suspected abnormal signal and facilitating correlation with the anatomy. Additionally, since 3D volume MRI and MRSI data are collected, the data can be viewed in any plane (axial, coronal or sagittal), and the spatial position of spectroscopic voxels can be selected retrospectively via "voxel-shifting", using the appropriate mathematical weighting of the raw data based upon the translation property of the Fourier transform (69,70). This method of interactive analysis will be the way that MRI/MRSI data is used in the future and should reduce interpretative errors associated with the overlap of normal and abnormal tissues.

Data interpretation: Interpretation of spectroscopy and spectroscopic imaging data requires both knowledge of what constitutes a clinically interpretable spectrum and an understanding of the underlying biochemistry and morphology that result in the observed changes. ^1H spectra are considered clinically interpretable if they are not contaminated by insufficiently suppressed water or lipid and have resolvable metabolite peaks with peak area to noise ratios of greater than 5 to 1. Metabolic criteria must then be established to distinguish abnormal from normal metabolism and then validated using a pathologic "gold standard." In the prostate, the (choline+creatine)/citrate ratio discriminates prostate cancer from benign glandular tissues with high specificity (12). In the other organs of the body, similar metabolic criteria must still be established. *Ex vivo* high resolution magic angle spinning (HR-MAS) spectroscopy and quantitative pathologic analysis of intact surgical or biopsy tissues can aid in understanding the relationship between metabolism and tissue composition and thereby help with the identification of the appropriate metabolic criteria (71,117-118). Another confounding factor to spectroscopic interpretation is partial voluming of regions of disease with surrounding benign tissues. Partial-volume effects can be reduced by using higher magnetic field scanners that provide higher S/N thereby allowing for higher spatial resolution spectral data. Finally, the metabolic criteria used to identify residual or recurrent disease often change following therapy (8). Treatment effects typically result in an overall reduction in the signal to noise of all metabolites, which underscores the need to be able to distinguish an absence of metabolites (termed "metabolic atrophy") from a technically failed study.

Applications in the Prostate, Cervix, Breast, and Liver: To date, in vivo MRS has been applied to the cervix (116-119), prostate (12, 22), breast (4,25,72,73), liver (74,75), kidney (76,77), colon (78), heart (79-81), skeletal muscle(82-84), sarcomas(85,86), and non-Hodgkin's lymphomas (10,87,88). In the following sections, specific applications in the prostate, cervix, breast, and liver are described.

Prostate Cancer: The earliest MRS studies of the human prostate involved ^{31}P spectroscopy using a dual tuned ($^{31}\text{P}/^1\text{H}$) transmit/receive endorectal probe (22,89,90). The proton frequency was used to image the location of the coil and obtain a homogeneous field of view, prior to performing phosphorus spectroscopy. These studies demonstrated the ability of ^{31}P MRS to detect metabolic differences between normal, hyperplastic, and malignant prostate tissues. Specifically, the ^{31}P MR spectra taken from regions of prostate cancer were characterized by increases in the phosphomonoester to β -NTP ratio and decreases in the PCr to β -NTP ratio relative to healthy prostate tissues (22,89,90). Additional studies using murine models of prostate cancer also identified ^{31}P spectral characteristics that may be related to the hormone sensitivity, radiation sensitivity, and metastatic potential of the cancer (16). However these early ^{31}P MRS studies of the prostate were limited by coarse spectral localization and spatial resolution due to the inherent insensitivity of ^{31}P MRS. Therefore most of the clinical prostate studies performed to date have utilized a combination of high spatial resolution MRI and ^1H MRSI.

High resolution T_2 weighted imaging, using combined endorectal and pelvic phased array coils, has demonstrated good sensitivity but relatively poor specificity for identifying prostate cancer, because numerous other conditions, including prostatitis, benign prostatic hyperplasia, and treatment effects can all mimic cancer. Prior to therapy, the addition of 3D-MRSI to anatomical MRI has been shown to improve the localization (2) and staging (3) of prostate cancer, provide a measure of prostate tumour volume (91), and provide an assessment of prostate cancer

aggressiveness (92). The ability of combined MRI/MRSI to identify residual or recurrent prostate cancer after hormone deprivation (8,9) and radiation therapy (93,94) has also been described.

Healthy glandular prostate tissue demonstrates two unique metabolic markers, citrate and spermine, that are produced by highly specialized epithelial cells and secreted into the prostatic ducts that empty into the ejaculate. Both citrate and spermine are reduced or absent in regions of prostate cancer, due to both biochemical changes and a loss of the prostate's normal ductal morphology (95-98). Although the citrate resonance is completely resolved, its detection is complicated due to strong coupling. At 1.5 T, under good shimming conditions, polyamines are seen as a hump resonating between choline and creatine, while in regions of cancer, the reduction or loss of polyamines is observed as an increase in the discrimination between choline and creatine. The increased spectral resolution provided at 3T improves the ability to resolve choline from polyamines and creatine.

Spectroscopy is always performed in conjunction with high spatial resolution MRI. MRI is necessary for the selection of the region of interest, for purposes of spectral interpretation, and because it is the combination of MRI and MRSI findings that often provide the most accurate assessment disease location and extent. Specifically for prostate cancer, axial high resolution T₂-weighted images (3 mm slice thickness, no intersection gap) are used to select a PRESS volume (typically 50 to 100 cm³) that encompasses the entire prostate, but excludes periprostatic lipids, the seminal vesicles which contain very high levels of GPC, and the air tissue interface of the rectum. The PRESS box is over prescribed by 20 to 30% in all three dimensions and then six VSS pulses are used to define the desired edges of the PRESS box. Six additional VSS pulses are graphically prescribed to eliminate contamination from surrounding lipids. With a typical voxel size of ~7 mm on a side (0.34 cm³), 16x8x8 step phase encoding (112x56x56 mm³ field of view) is performed with a one second repetition time for a total acquisition time of ~17 minutes. The output of the combined MRI/MRSI exam consist of contiguous high spatial resolution T₂ weighted MR images through the prostate with the corresponding arrays of 0.3 cm³ proton spectra. Prostate cancer is most accurately when there is concordance of decreased T₂ signal intensity on MRI and an elevation of choline, and decreases in citrate and polyamines from the same region on MRSI.

The interpretation and utility of prostate MRSI can be complicated by the presence of chronic inflammation (prostatitis)(99), which appears metabolically similar to prostate cancer. Additionally, post-biopsy hemorrhage can persist for six to eight weeks or more after biopsy and demonstrates a reduction or absence of citrate and polyamines due to the disruption of prostatic ducts, and in worse cases results in a complete absence of all prostatic metabolites (100). Regions of hemorrhage are usually identified as bright areas, and less frequently dark areas, on axial T₁ weighted images and these can be used to exclude suspected regions from the MRSI data analysis. Following therapy, the time course of treatment-induced metabolic changes must be taken into account when interpreting the data. For example, hormone deprivation has a very fast impact on prostate metabolism and often results in a total loss of citrate and polyamines within sixteen weeks (8). Conversely, radiation therapy has a much slower impact on prostate metabolism and may take one to three years to achieve metabolic atrophy. In either case, the presence of elevated choline relative to creatine appears to be the best current indicator of recurrent prostate cancer.

Cervical Cancer: *Ex vivo* HR-MAS spectroscopic studies of cervical biopsies from women with normal cervix, cervical intraepithelial neoplasia (CIN) and cervical cancer have demonstrated that cervical cancer had significantly higher levels of triglycerides (-CH₂ and -CH₃) and choline as compared to the normal cervix and CIN (117-119). To date, *in vivo* spectroscopic studies of cervical cancer have typically utilized an endovaginal coil and single voxel proton spectroscopy. Clinically, combined MRI/MRS has been used for the preoperative assessment of cervical cancer (116-119), and in assessing tumor response to neoadjuvant chemotherapy prior to radical hysterectomy (116).

Breast Cancer: For breast cancer, one of the critical clinical questions is whether the lesion detected on screening is benign or malignant. About 75% of the breast lesions detected by mammography and about 50% of the enhancing lesions detected by contrast-enhanced MRI are pathologically benign (101). Sonographic classification of benign and malignant tumours has low specificity (about 30%) as well (102). Recent advances in contrast-enhanced MRI methodology and interpretation have greatly improved the ability to differentiate malignant from benign breast tumours. However, there still remains a clinical need for improved specificity (103,104), which spectroscopy may be able to provide (4).

Breast spectroscopy is difficult because of the presence of mobile lipids and a lack of multiple metabolic markers. Early ³¹P MRS studies typically observed increased phosphomonoester, phosphodiester, and sometimes phosphocreatine levels in breast cancer versus normal tissues (105). However, because of the poor sensitivity of ³¹P MRS combined with the decreasing size of breast tumours due to early detection, ¹H MRS is now being primarily used. Typical breast MRS studies use a single voxel (~1 to 27 cm³) (4) localization technique such as STEAM or PRESS with the intent of selecting signals from the lesion of interest and excluding signals from the surrounding adipose tissue and normal parenchyma (4). However, due to the magnitude of lipid resonance in the breast, gradient induced sidebands of the lipid resonance can cause both positive and negative artefacts in the choline region of the

spectrum. Recently, a technique called “TE-averaging,” based upon oversampled 2D J-resolved spectroscopy (106), has demonstrated the ability to separate lipid induced sidebands and provide increased sensitivity for the study of small or irregularly shaped lesions (72).

Currently, the interpretation of breast ^1H MRS data is relatively simple and mainly involves determining whether choline is present (malignant) or absent (benign)(4). This observation is consistent with the high phosphocholine content of human breast cancer cells, which is 10-fold higher than that of normal human mammary epithelial cells(107). However, Stanwell and co-workers found that 20% of normal volunteers also demonstrated detectable choline, leading to an overall sensitivity and specificity of 80% and 86%, respectively, when choline presence alone is used to define cancer(108). Additionally, smaller tumours tend to be diagnosed as benign (false-negative) because of the lack of a detectable composite choline signal (4). Other complications in the interpretation of breast spectra have primarily been technical and associated with poor spectral quality (4,109). The utility and robustness of breast spectroscopy can be improved by increasing the choline signal-to-noise through the use of higher sensitivity coils, using unsuppressed water as a quantitation standard, and the use of improved pulse sequences and higher field human scanners.

MRS of the liver: The liver is the most biochemically complex organ in the human body, and in addition to being the second most common site of cancer metastasis (after lymph nodes) (110), the liver is subject to a variety of non-malignant diseases including inflammation, hepatitis, cirrhosis, and fatty liver disease. The majority of liver MRS studies have looked at fat content and have shown that proton MRS of liver fat correlates well with ex vivo liver fat measurements (111). Lipid levels have been shown to change with cancer(28,29), metabolism(112,113), and non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)(7,114,115).

Because of the problem of motion in the abdomen, liver MRS is typically performed as a single voxel technique, with a surface coil phased array used for reception. In the case of diffuse disease (e.g., fatty liver disease), where the location within the organ is not critical, the voxel should be placed at least 1 cm from all edges of the liver to reduce the chance of contamination from signals outside the liver. In the superior/inferior direction, the voxel should be placed such that it will remain in the liver throughout respiration. This can be ascertained by comparing breathheld images acquired at end-expiration to those at end-inspiration. During prescanning, the signal amplitude should also remain relatively constant from pulse to pulse. To account for small shifts in position over time, MR spectra can be saved individually before averaging. These individual MR spectra can then be phase and frequency aligned before summation to account for some respiratory motion.

Summary: MR spectroscopy and spectroscopic imaging are promising techniques for the metabolic assessment of cancer and other diseases in the body. However, spectroscopy in the body is more challenging than in the Brain due to both motion of and the deep location of many organs. The critical considerations when performing spectroscopy in the body include the choice of: nucleus (^1H is preferred at 1.5 T due to high sensitivity, but ^{31}P and ^{13}C may be valuable at higher field); single voxel versus multi-voxel (CSI or MRSI) approach; localization scheme (e.g., PRESS, STEAM, LASER); water and lipid suppression (e.g., BASING, CHES, STIR) and outer volume suppression techniques (e.g., VSS pulses); the use of surface and endocavity coils; and data display and analysis tools. Proper spectral interpretation based upon a pathologic "gold standard" and knowledge of the impact of therapy on metabolism are both critical for evaluating the clinical utility of new MRS applications in the body. The increased sensitivity provided by higher field MR scanners, improved pulse sequences and technology, and the ability to include additional metabolic markers and information from other functional imaging modalities (diffusion weighted and dynamic contrast imaging) will also have a major impact on the clinical potential of body spectroscopy in the future.

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