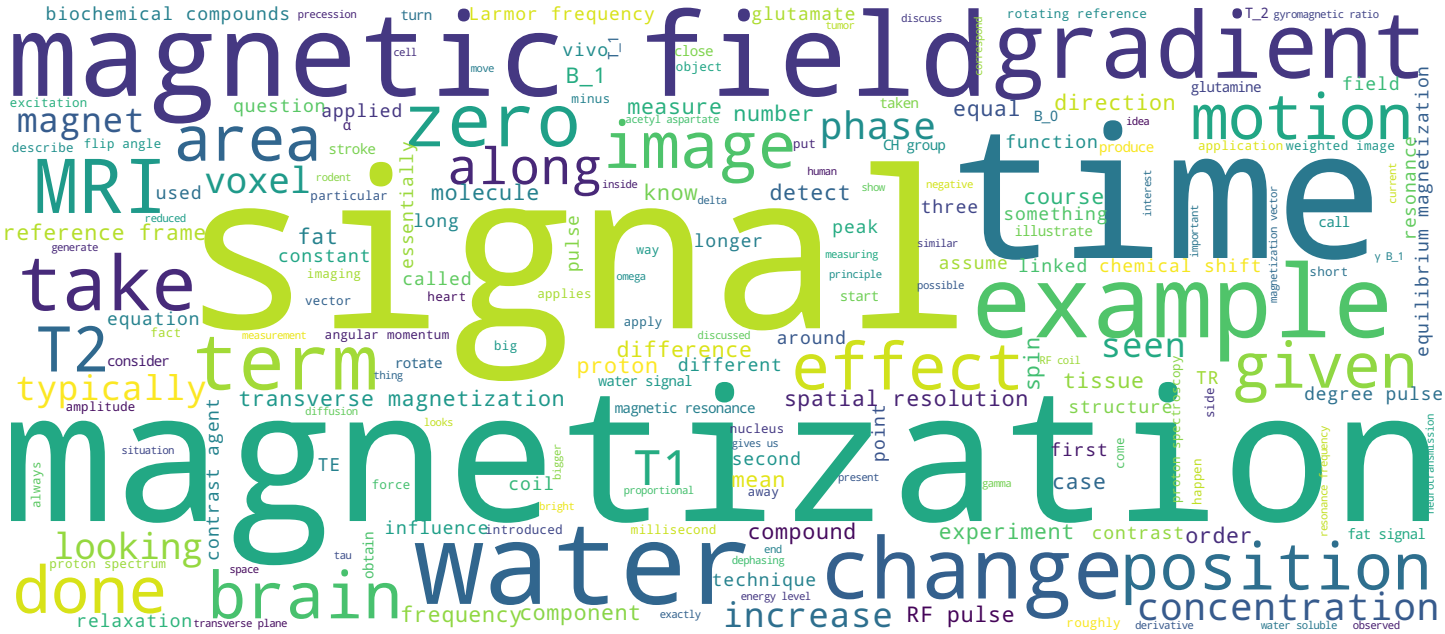
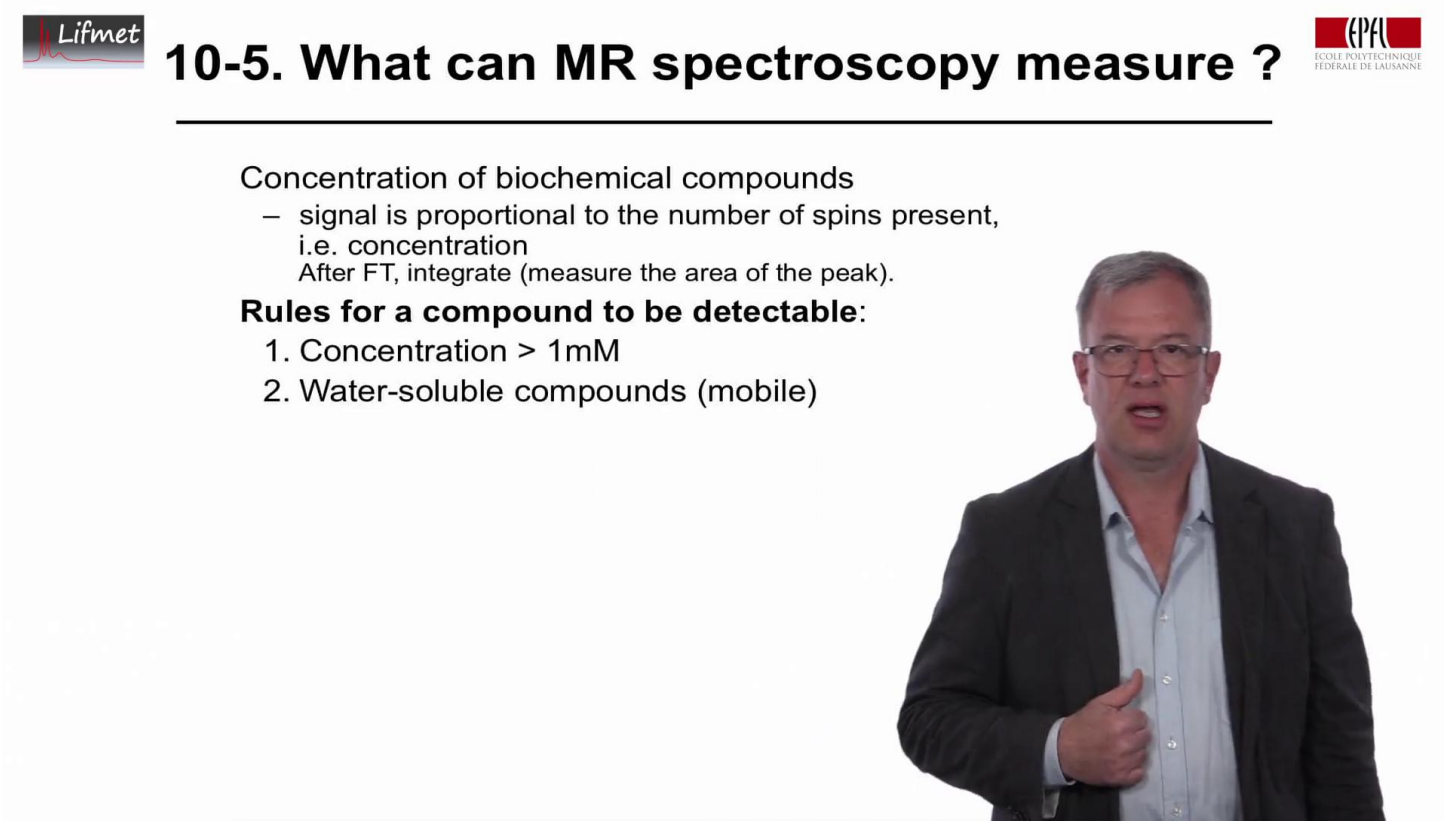
10.5 What spectroscopy measures & Examples

Fundamentals of Biomedical Imaging

Prof. Rolf Gruetter





The question now arises, what can MR spectroscopy measure? I've just given you an example, phosphorus, out of historical interest. Essentially MR spectroscopy allows to assess the concentration of biochemical compounds in vivo. The signal is typically proportional to the number of spins present and in particular to the concentration. After the Fourier transform, what one does is measure the area, so integrate in this case. This is assuming that the effect of relaxation, such as T1, has been taken into account, but it's still proportional to the concentration, even if there is a flight of T1. Now what compounds can one detect? And there are three rules I want to share with you here. One is, one needs typically a concentration on the order of millimoles, millimolar. If you translate this into tissue concentration, since the density is roughly 1 g/mL this translates into typically 1 µM/g wet weight to give you an idea of the concentration that one needs to be able to see the signal. Then what one would need is typically a water-soluble compound. It doesn't have to be exactly-- but it is a good guess that if it is highly water-soluble then it is sufficiently mobile to be detected.

Notes

Summary

0m 04s

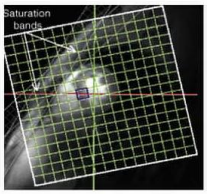
10-5. What can MR spectroscopy measure ?

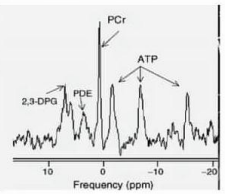
Concentration of biochemical compounds
- signal is proportional to the number of spins present, i.e. concentration
  After FT, integrate (measure the area of the peak).

Rules for a compound to be detectable:
1. Concentration > 1mM
2. Water-soluble compounds (mobile)
3. ^{1}H is most sensitive nucleus (gyromagnetic ratio)

Spatial Resolution
Voxel volume ~ 1/Signal

Water
(80M ^{1}H concentration)





10-13

Remember, T2 is very short for immobile signals. So the main requirement here is mobile but a good indication here is water-soluble compounds. We can, for example, measure vitamin C in our brains, we know it is 1.5 µM/g. The concentration of vitamin E which intercalates into the membranes in the lipid bilayers is not detectable. Third one to remember is the influence of the gyromagnetic ratio. So proton, the hydrogen nucleus of the proton, is the most sensitive nucleus. So taken together, if we are now looking at the spatial resolution that's achievable, we have to bear in mind that the voxel volume that one can, in a certain amount of time measure, is inversely proportional to the signal. The more signal we have, the smaller the voxel can be. And here's an example, this is phosphorus spectroscopy of the heart. Here's an MRI of the heart and here this little square is the voxel from which the phosphorus data has been taken. So if you look at this grid here, which gives you the size of the voxels in the heart, this grid has a course resolution that would not allow us to detect the individual anatomic features of the heart, so it's much bigger. And why is this so? Well, let's look at water.

Notes

Summary

1m 33s

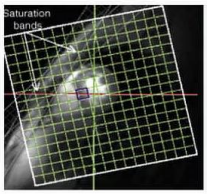
10-5. What can MR spectroscopy measure ?

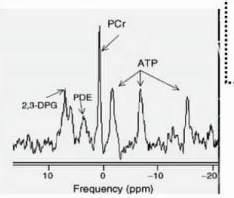
Concentration of biochemical compounds
- signal is proportional to the number of spins present, i.e. concentration
  After FT, integrate (measure the area of the peak).

Rules for a compound to be detectable:
1. Concentration > 1mM
2. Water-soluble compounds (mobile)
3. ^{1}H is most sensitive nucleus (gyromagnetic ratio)

Spatial Resolution
Voxel volume ~ 1/Signal

Water (80M ^{1}H concentration)	Biochemical compounds (~mM concentration)
~1mm (human)	~ cm (human)
~50µm (rodent)	~ mm (rodent)





10-13

The water protons in the tissue are at roughly 80M concentration. Biochemical compounds, on the other hand, are typically on the order of millimolar concentration. So we're looking at something that is roughly 10^5 times more concentrated when we image water compared to when we measure informational biochemical compounds. So one gets, as a good ballpark, a millimeter spatial resolution in humans. For water MRI and for biochemical compounds the spatial resolution is typically on centimeters in humans. If one looks at rodents, one can get 50 microns spatial resolution in rodents for water imaging and millimeter spatial resolution in rodents for the biochemical compounds. So these are some ideas. The main point here is, the difference between what one can see with MRI this gray-scale image versus what the biochemical measurements with spectroscopy, the main difference here has to deal with the fact that the concentration of most biochemical compounds of interest is on the order of millimolar.

Notes

Summary

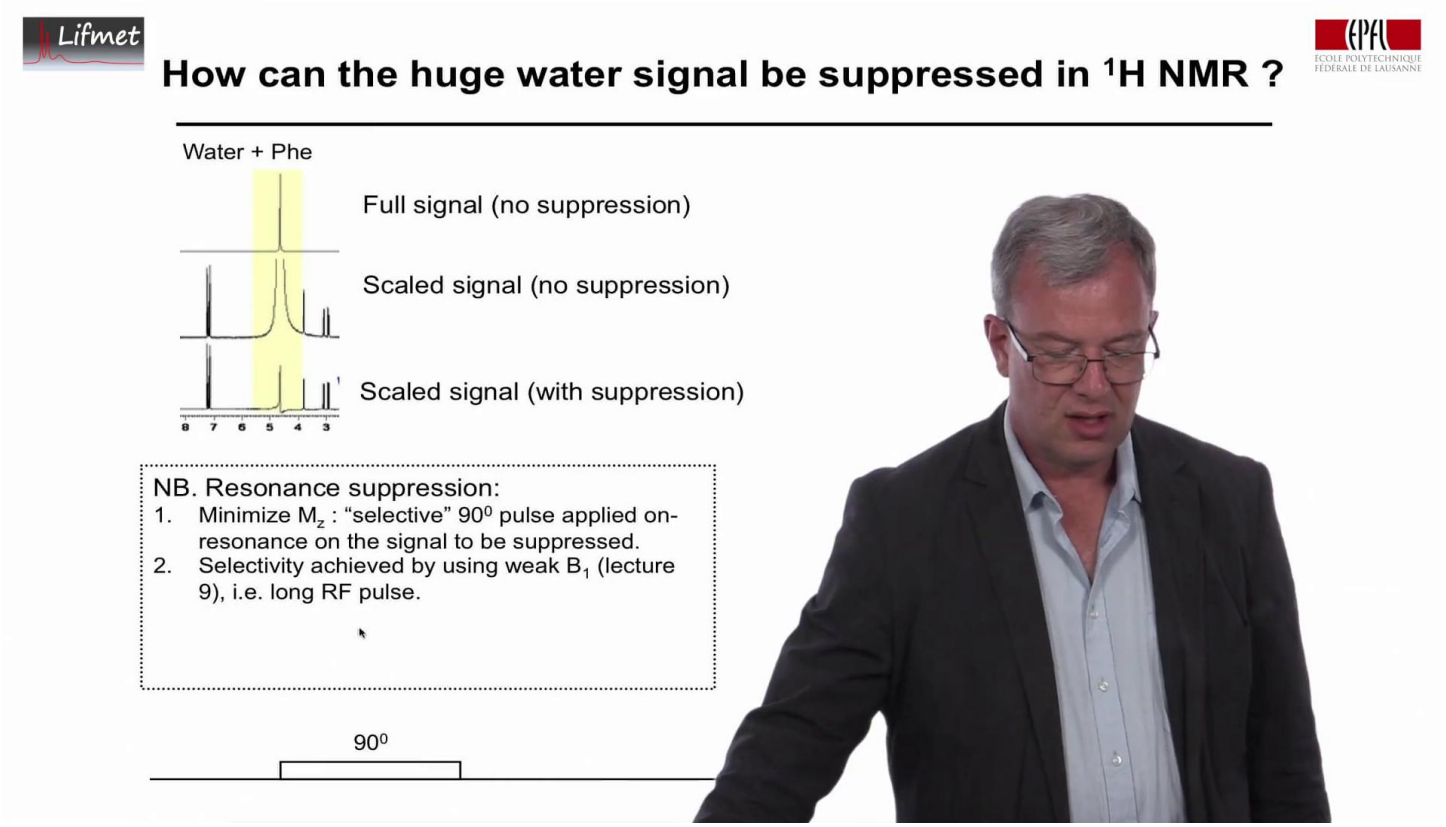
3m 06s

Now, there's a question: "Why is spatial resolution better for the rodent studies?" Think about it. Assume you have the same compound, same concentration and the same external magnetic field. Why do we get a better signal, a better spatial resolution in rodent studies? Well, the answer is, as we have seen, the RF coils which detect our signal are dictated to a large part by *B_0 sub r* which means that we want the coil to be as close to the organ as possible. And if you look at a mouse head, which is roughly the size here of my small finger, the entire dimension of the mouse head, corresponds to skull thickness and fat on my head, for example. So a RF coil that's designed to detect a signal from the mouse brain would not even see the signal in the human brain. And so because the detector, the RF coil, is further away from the organ of interest in humans, the spatial resolution is also lower. Now we have seen that there's a huge advantage in imaging the water. What if we're interested in measuring the signal from biochemical compounds which carry protons? We have to deal with the fact that we have a strong signal from water, which is response to concentration of 80M in order to be able to detect a signal of our compounds, which is on the order of millimolar.

Notes

Summary

4m 25s



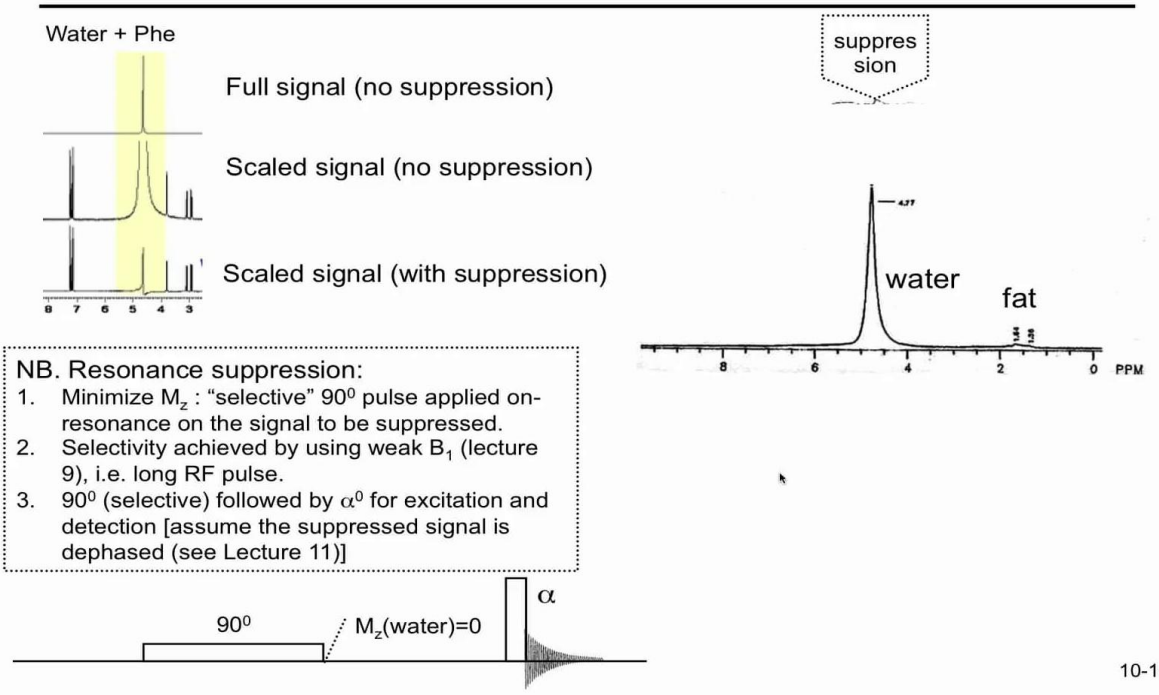
And here's an example. We have water and phenylalanine. This is just looking at the full signal of this mixture and here if one scales this up, now the vertical scale has been expanded, one can see the resonance is end up due to phenylalanine. And then here's the same thing shown again but now there has been something done to the water magnetization so the signal from water is much reduced. Now how can one suppress the resonance? How can one suppress the signal from a particular compound? And this is done with resonance suppression or with reducing the magnetization that is being detected relative to that resonance. And the simplest procedure here to describe is where one takes the z magnetization of, let's say, water and one takes the z magnetization and applies a so-called *selective* 90 degree pulse on the resonance of the water or on the signal to be suppressed here, particularly we'll stick with water. So the experiment to be done is a selective 90 degree pulse. This selectivity, remember, the selectivity is determined by *B_1*. We have said we can roughly excite a bandwidth that corresponds to $\gamma B_1/(2\pi)$ in frequency so one uses a weak *B_1*, a 90 degree pulse, so $\gamma B_1 \tau$ has to give us 90 degrees.

Notes

Summary

5m 58s

How can the huge water signal be suppressed in ^{1}H NMR ?



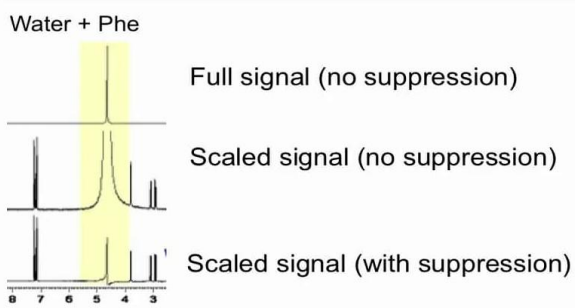
If *B_1* is weak the pulse has to be long, so it's a long RF pulse. After that pulse, the z magnetization of the water is zero but that of the other compounds has not been affected because the B effect is along z. And then one applies, for example, an *α* degree pulse for excitation of a detection. We'll assume here that something has been done between the 90 degree pulse on water and this excitation pulse such that the transverse magnetization of the water does not generate a measurable signal. You can assume it has been decayed by T2. We'll discuss, in next week's lecture, some of the measures that allow us to destroy the transverse magnetization that is by dephasing. But for now we'll just assume this has been done. So our signal here then will contain only the signals of interest and the water is no longer there. So let's take an example from muscle here. This is the water signal, here we have the lipid signals, water and fat. And now one applies such a 90 degree selective pulse on the water signal and here is the signal that is being observed. Not very interesting because at this scale we can roughly see some bump here, so one can vertically expand this scale.

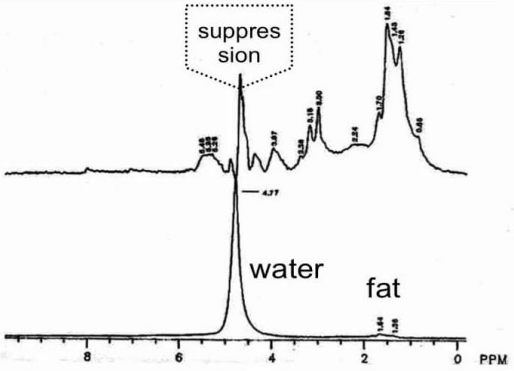
Notes

Summary

7m 35s

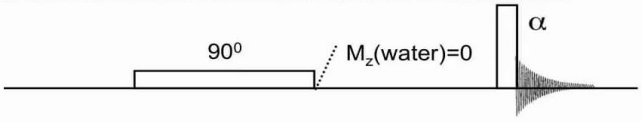
How can the huge water signal be suppressed in ^{1}H NMR ?

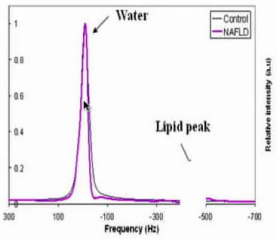




NB. Resonance suppression:

1. Minimize M_z : "selective" 90^0 pulse applied on-resonance on the signal to be suppressed.
2. Selectivity achieved by using weak B_1 (lecture 9), i.e. long RF pulse.
3. 90^0 (selective) followed by α^0 for excitation and detection [assume the suppressed signal is dephased (see Lecture 11)]





10-14

And now we can see all the resonances linked to the fat signal, monounsaturated fatty acids, polyunsaturated fatty acids and all sorts of different chemical environments of the proton nuclei and the water signal here is substantially reduced. That allows to see those details. So this technique is being used also in MRI for producing Fat-Sat scans, fat suppressed scans. So we'll first take the technique, we'll have here the signal from water and from the lipid or fat and if one applies now a selective pulse on the water, the water signal has been suppressed and one has only the fat signal to contend with. Conversely, if the resonance frequency of this long, weak RF pulse is adjusted to be on the resonance with the fat then the fat signal is being saturated, that is the z magnetization of the fat magnetization is set to zero and one obtains a water-only signal or more precisely a Fat-Sat scan.

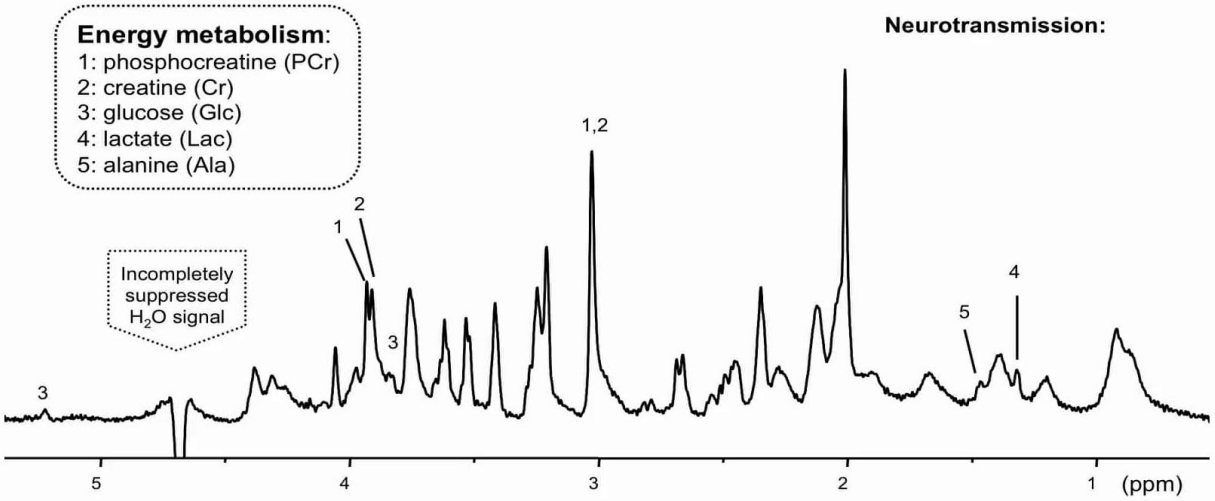
Notes

Summary

9m 04s

Ex. Proton spectroscopy of the brain

Biochemical compounds detectable in vivo



10-15

So now I want to give an example of proton spectroscopy of the brain. So we'll plot the chemical shift axis, that's in parts per million, and this is a proton spectrum of the brain. One will notice here, one of the signals that is negative, this is actually the incompletely suppressed water signal so water suppression, as we have discussed before, has been applied and this is the information that we can obtain. We have resonances linked to energy metabolism such as phosphocreatine and creatine. They are here. So phosphocreatine and creatine, whether phosphate is present or not does have an influence on the chemical shift of the methylene proton in creatine. One has the signal from glucose, that's this tiny little guy here, I have lactate at this position and alanine which is nearby. Lactate and alanine share a metabolic reaction that's alanine aminotransferase. Compounds that are linked to neurotransmission in the brain are glutamate, which has multiple resonances, there's two CH2 groups and a CH group in it; glutamine which is structurally very similar, so compound number 7 is close to compound number 6; gamma-aminobutyric acid, which has multiple resonances as well; and N-acetyle-aspartyl-glutamate, a dipeptide involved in neurotransmission.

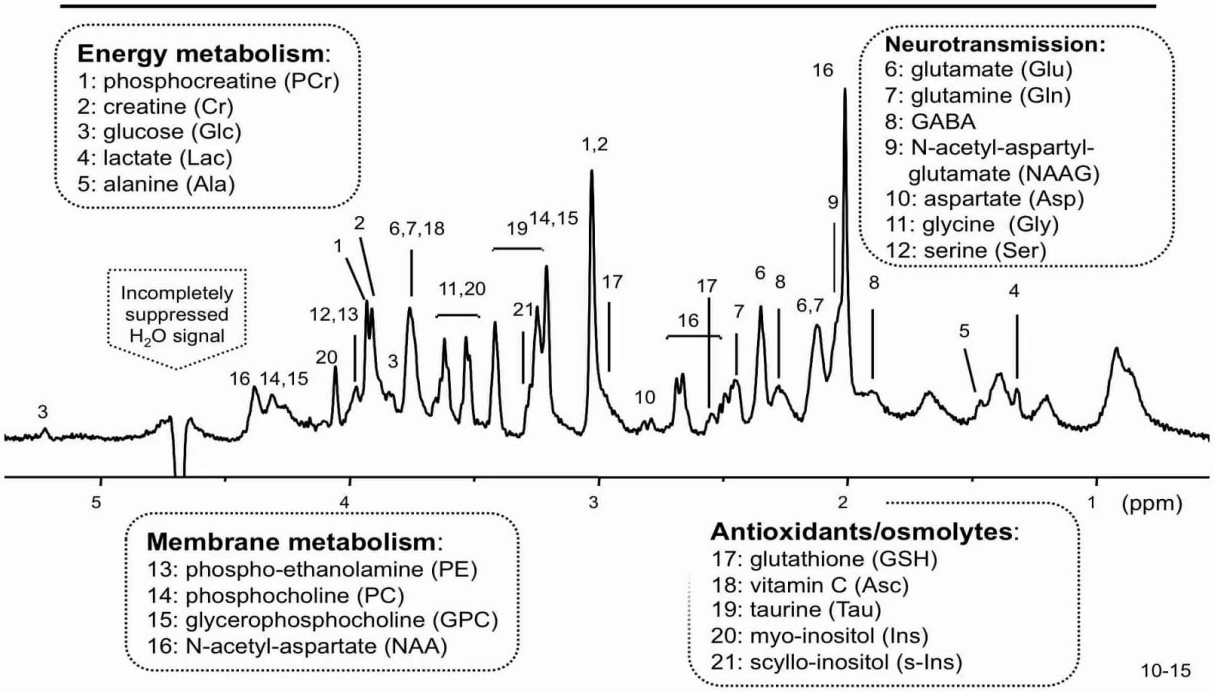
Notes

Summary

10m 18s

Ex. Proton spectroscopy of the brain

Biochemical compounds detectable in vivo



We have aspartate that's also been linked to the processes in neurotransmission, and glycine as well. And we have also serine contributing to the spectrum. We go on with compounds that are typically linked to membrane metabolism. Phosphoethanolamine and glycerophosphocholine, they are here, and here, and here. And N-acetyl-aspartate, which has the aspartyl group, is here, and here is the acetyl group, this prominent signal here. So these are the compounds linked to membrane metabolism. And finally there are some compounds linked to either antioxidative defenses, such as glutathione, vitamin C, ascorbate, taurine, and myo-inositol linked to taurine and myo-inositol have mostly been linked to osmoregulation but in the case of taurine, there's most likely multiple roles in the brain. And finally we have an isoform that is scyllo-inositol that contributes, as well, to the proton spectrum of the brain. So it's a wealth of chemical information on a number of important biochemical processes that can be measured in vivo with proton spectroscopy, and we'll briefly discuss on how these measurements are done and then I will give some examples and illustrate the measurements that are possible.

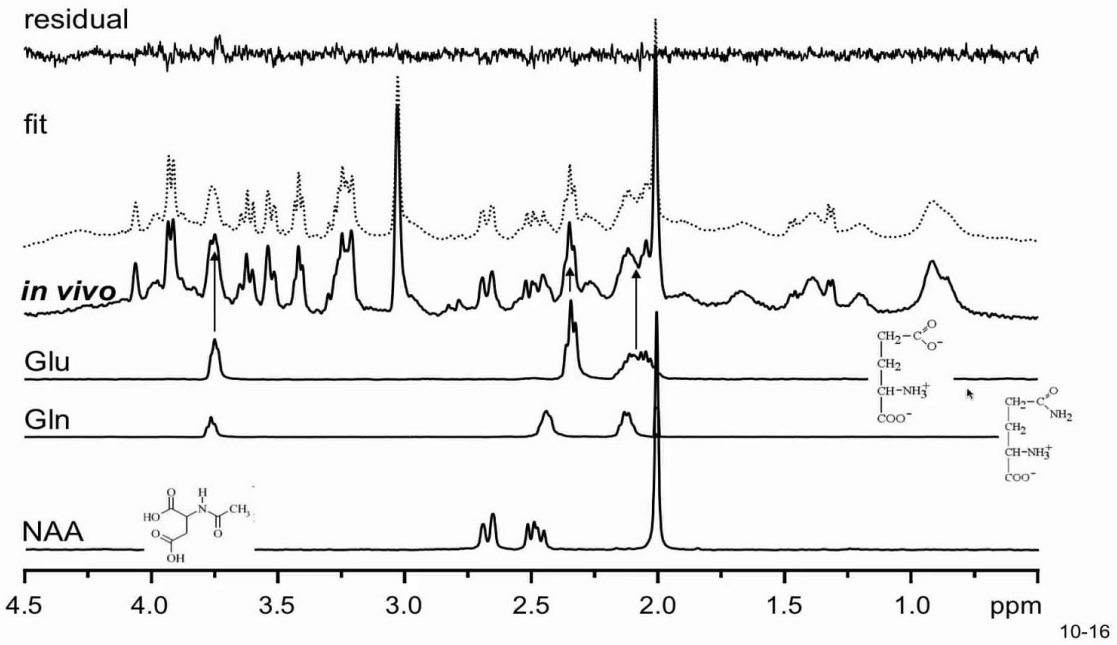
Notes

Summary

11m 54s

How can biochemical compounds be measured in vivo ?

Analysis of ^{1}H NMR spectroscopy of the brain



So we have introduced that the area under a peak is essentially proportional to its concentration and so how can we actually get biochemical concentrations in vivo? So we'll look at the proton spectrum here again, here is a measured example. And then what is done is a fit that's been made to that spectrum and one looks at the residual. And I just want to illustrate this principle here because for most compounds we know the ionic strength, we know the pH and we of course know the temperature. So one knows very precisely the exact resonance frequency, that is Larmor frequency, of the particular compounds and their groups. So we have N-acetyl-aspartate. We have the acetyl group here and the aspartyl group, which is here. Here's the chemical structure that we're looking at and these are the peaks that are recognizable here. We have glutamine, here's its structure and here are the peaks that are linked to the glutamine, two CH2 groups and one CH group. And finally we have glutamate. Here's its structure and glutamate has these resonances. Note this, the difference between glutamate and glutamine is this NH2 group that changes this group here to an NH2 group.

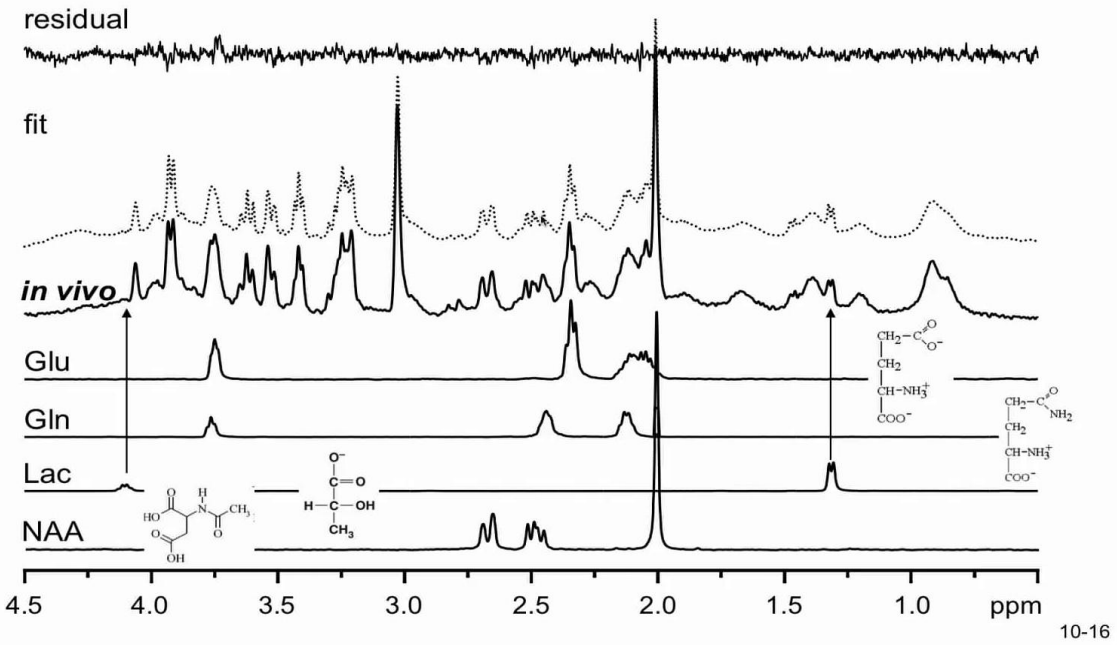
Notes

Summary

13m 31s

How can biochemical compounds be measured in vivo ?

Analysis of ^{1}H NMR spectroscopy of the brain



So for the resonances, the further away that we are from here, the more similar they are. So we have glutamine, glutamate here, fairly similar. Here it's almost the same chemical shift but in this group which is the closest to the carboxyl-terminal here is the biggest chemical shift at this position. That has to do with chemical proximity and the change in the electron cloud configuration. Last example here is lactate. We have the methyl peak here, and the CH group is here. Here's the molecule methyl peak here, and the CH group is here. And we see, very nicely, the doublet here that tells us that there's a CH group nearby. For measuring these concentrations, one measures an in vivo spectrum fixed with the model spectra obtained under in vivo conditions and this way allows us to deconvolute the number of concentrations and measure, therefore, up to 20 different neurochemical compounds in vivo.

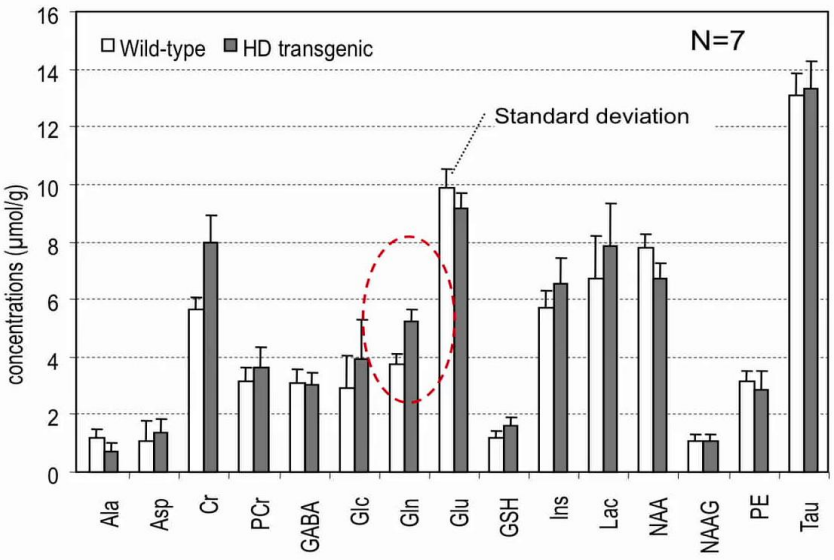
Notes

Summary

14m 53s

Ex. Metabolic phenotyping of transgenic animals

R6/2 mouse (human Huntingtin gene knockin) at wk 8



10-17

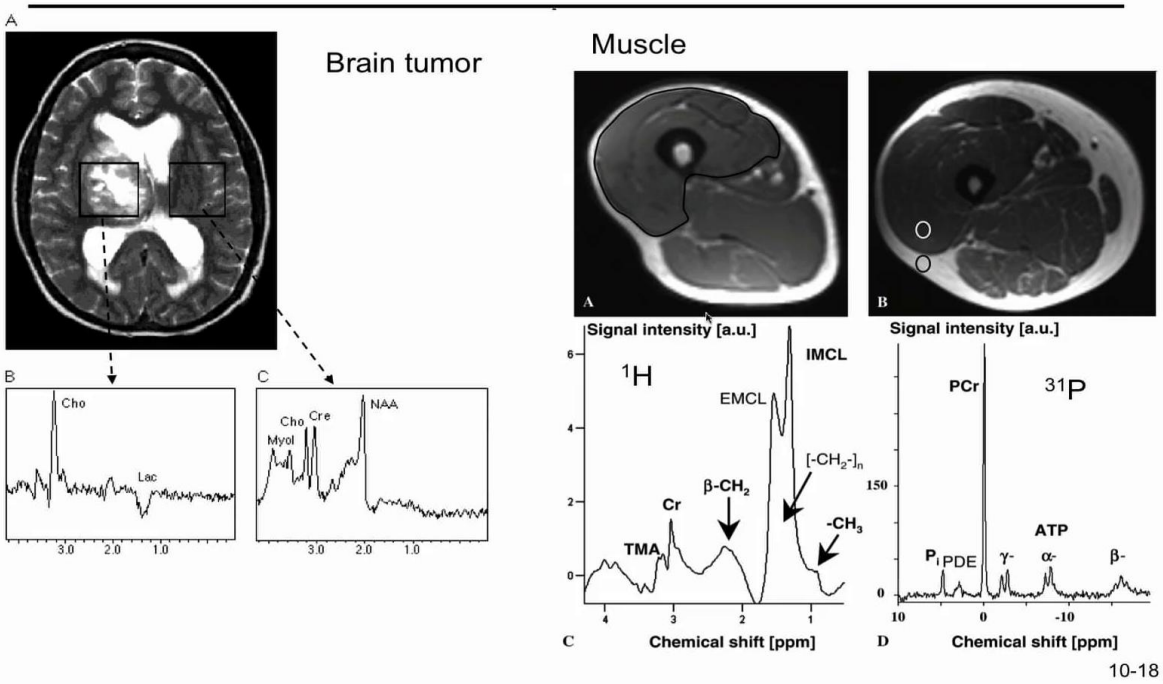
So here I want to give an example of metabolic phenotyping of transgenic animals. This is a historical study here. What one was looking at is wild-type animals and transgenic animals for Huntington's disease, it's a Huntingtin gene knock-in, the so-called *R6/2* mouse and in that particular study, over seven mice, what one saw is a difference in glutamine levels. Now the difference in absolute terms is not very big but the measurement with these types of analysis can be very precise so these error bars indicate standard deviation and it was, hence, already in the data, clear which animal had an abnormally high glutamine concentration compared to the others.

Notes

Summary

16m 02s

Example: Human brain and muscle



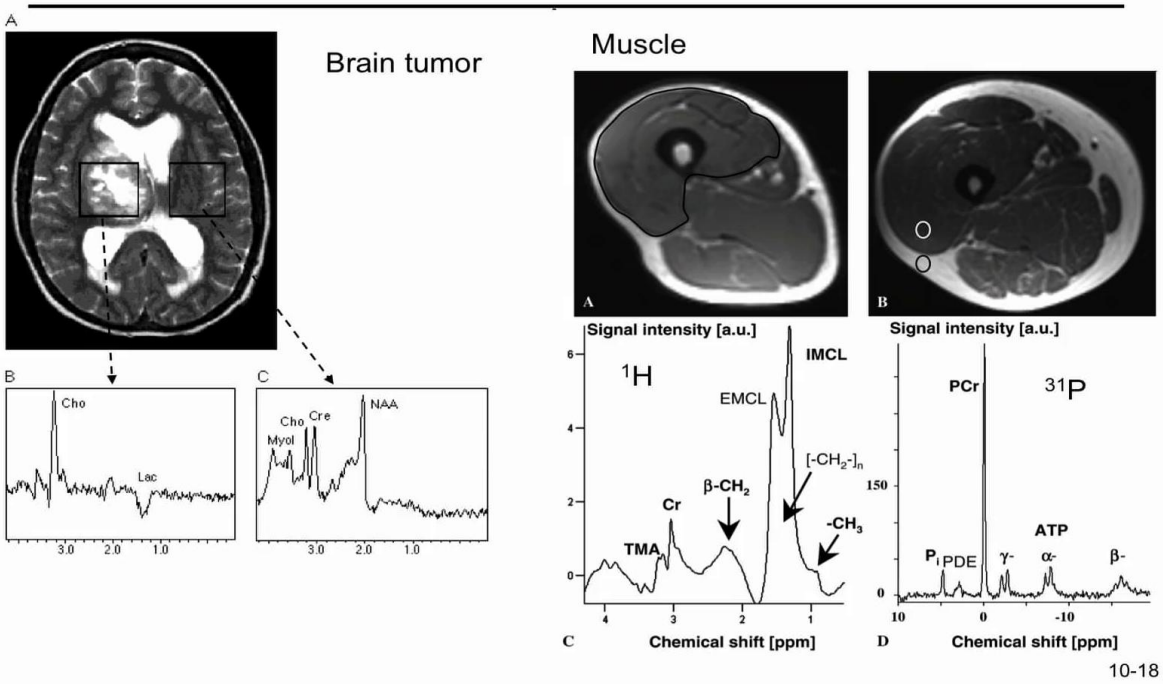
Now here I want to show some examples from the human side. This is a patient with a brain tumor. We have some changes in the MRI signal here. And now these squares here indicate where the signal has been taken. Here, this signal corresponds to this side, where the image intensity is abnormal. And here on the normal side, one sees a normal pattern for human patients. Now the knowledge of the biochemical composition of an area in the brain is illustrated here, it's advantage. The changes like these observed in vivo, they could mean that one has necrosis of the tissue, they could mean neoplastic tissue, they could also be consistent with edema. So there are a lot of cellular processes that influence the relaxation of water in the tissue. However, the biochemical information here which tells us that N-acetyl-aspartate is essentially not present in this area and there's a lot of choline-containing compounds, implies that this is indeed, to a large extent, tumorous tissue. In the muscle physiology, which has been an interesting area of application--this is a muscle here. The proton spectroscopy is taken here and here's the phosphorus spectroscopy, a lot of phosphocreatine relative to ATP, characteristic of skeletal muscle.

Notes

Summary

16m 54s

Example: Human brain and muscle



Now what is interesting here is for the proton spectra the fat signal is split into a signal that is attributed to intramyocellular lipid and extramyocellular lipid. The intramyocellular lipid is fat that's contained in vesicles that are bound to the mitochondria. This fat is what is being used for a marathon run. The extramyocellular fat, this signal here, is attributed to the white inclusion, so when you eat a steak you see the white stuff, that's the EMCL: extramyocellular lipid. The reason those two are distinguishable, research at the University of Bern have very nicely demonstrated it has to do with the magnetic field susceptibility, the magnetic field is slightly different between these two environments.

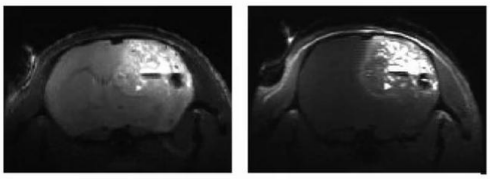
Notes

Summary

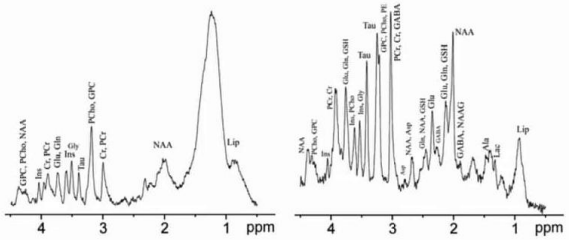
18m 27s

Ex. ^{1}H NMR spectroscopy in vivo

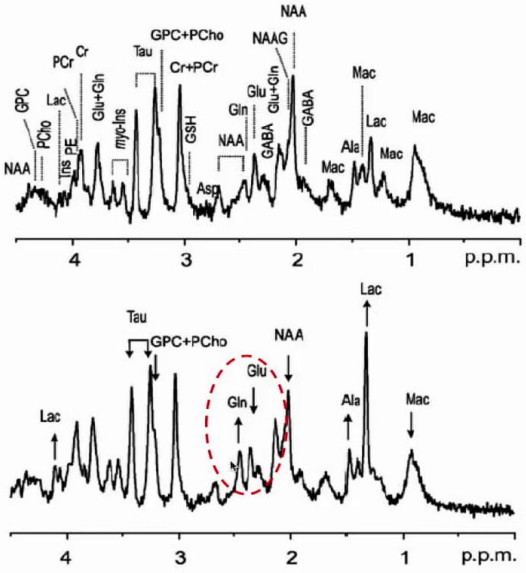
Mouse brain



Mouse model of human glioma



Mouse model of stroke



10-19

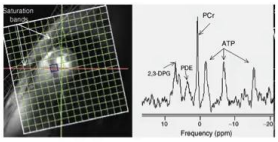
I'll show one last example. This is proton spectroscopy of the mouse brain. This is a tumor in a mouse. Here is the normal appearing spectrum and here is the side of the tumor. Now there are a lot of specific and detailed changes going on but in terms of seeing that there's something major going on with the biochemistry, we don't have to be magicians, this is fairly obvious that this is clearly different from what we see in the normal case. And here's an example of a mouse model of stroke. We have the normal proton spectrum here of a region that has not been subjected to stroke or before they've been subjected to stroke. And then three hours after the stroke one observes an increase in the glutamine signal and a decrease in the glutamate signal. Now, again, this is of course subjected to the type of analysis that I've illustrated before. But visually we can clearly see that this peak here has gone up and this one has gone down in relative terms so there is something going on in this area here.

Notes

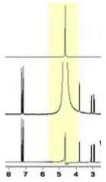
Summary

19m 18s

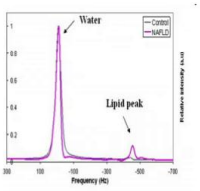
Credits



Metabolic imaging with cardiac magnetic resonance spectroscopy
Fig. 1 from Stefan Neubauer, Heart Metab. 2009; 44:17



Glenn Facey University of Ottawa
http://u-of-o-nmr-facility.blogspot.ch/2010/02/watergate.html



Newcastle Magnetic Resonance Centre
http://www.ncl.ac.uk/magres/research/liver/

And glutamine and glutamate share an important metabolic relationship in mediating neurotransmission. So this concludes the examples of NMR spectroscopy for this week. And next week we will discuss how in MRI one can reconstruct an image.

Notes

Summary

20m 28s