DEFERIPRONE EVALUATION IN PAEDIATRICS

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under Grant Agreement n° 261483

MRI Analysis of the Relaxation Data and Hidden Surprises

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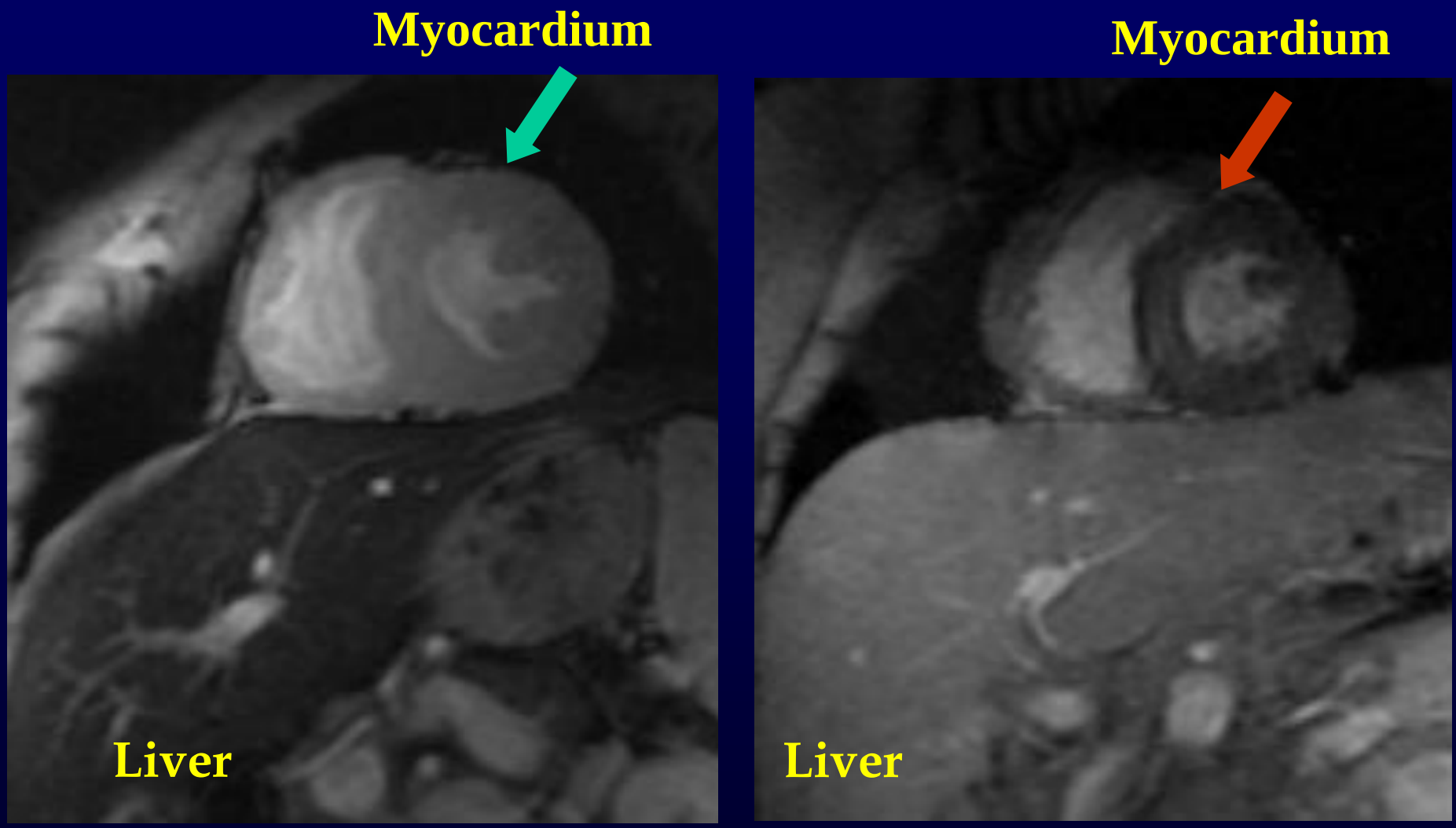
Topics to be discussed

1. **Iron storage in the tissues**
2. **Relaxation times T_2 and T_2* and relaxation rates R_2 and R_2***
3. **Relationship between R_2 και R_2* and with iron concentration**
4. **Techniques for calculating T_2***
5. **FERRISCAN vs. R2***
6. **Quantitative evaluation of fat infiltration (hidden surprise)**

Why MRI?



(Gotsis et al.)

Steps in MRI for Iron overload

1. Good data acquisition (patient cooperation, proper protocol, good experimental setup

2. Determination of relaxation parameters (proper fitting equation, avoidance of areas with vessels and motion artifacts, etc.)

3. Interpretation of data (what kind of ferritin-hemosiderin mix? Fat infiltration? Fibrosis? Inhomogeneity of iron distribution in the organs?

The only FDA-approved method for measuring liver iron concentration (LIC) is FERRISCAN. The scientific community however has accepted the use of R2* as a method of equal value and its ease of use has made R2* measurements very common for all organs of interest.

Before we get to these methods let us review how iron is stored in the organs.

1. **Free iron in the body is very toxic, even in very small concentrations. Thus nature has arranged for iron to be carried "around" in the body "hiding" in the core of ferritin and excess iron is being stored in the liver (Kupffer cells).**

2. **Every ferritin molecule can be loaded with up to 4000 iron atoms in its central core of radius 15 A. The total magnetization of the ferritin molecule exceeds the sum of the magnetization of each individual iron atom (super-paramagnetic effect).**

3. **Ferritin is water-soluble thus it can circulate easily in the blood. Tissue water molecules can come close to the hydrophilic ferritin and tissue water relaxation rate is enhanced (is relaxation time is shortened) via chemical exchange.**

4. When LIC exceeds about 7 mg/g dwt (my own experience out of thousands of examination in the past 15 years) part of ferritin degenerates into hemosiderin, a molecule with higher capacity for storing iron (up to 5000 iron atoms in its central core).

5. Hemosiderin is hydrophobic and cannot circulate freely as ferritin does. Thus it precipitates wherever is being formed, often in clusters mixed with ferritin. Tissue water cannot approach the hydrophobic hemosiderin and chemical exchange cannot take place, thus in a sense it is being "invisible" to the R2 mechanism. It affects however the R2* mechanism through the magnetic susceptibility mechanism.

The relaxation rate R_2 we measure in MRI is a weighted average between free tissue water molecules (the bulk) and coordinated water (bound to the paramagnetic center via coordination bonds for a short period of time of the order of 10^{-9} sec) to ferritin. Therefore,

$$R_2 = k[Fe]$$

The water molecules must approach very closely the paramagnetic center (it is a dipole-dipole interaction and is distance-dependent) and chemical exchange transfers the magnetization effects of ferritin to the bulk water. Therefore the R2 mechanism exploited by FERRISCAN recognizes directly only ferritin-stored iron (hydrophilic molecule) and not hemosiderin (hydrophobic).

How does hemosiderin-stored iron is being recognized by FERRISCAN?

By the lack of linearity in the R2 versus LIC calibration curve in FERRISCAN!!!

The relationship between R_2 και R_2^* is given by the equation:

$$R_2^* = R_2 + R_2^{mag.inh} + R_2^{mag.sus}$$

$$R_2 = \frac{1}{T_2} \qquad R_2^* = \frac{1}{T_2^*}$$

mag.sus = Magnetic susceptibility
magn.inh = magnetic inhomogeneity

The contribution of magnetic homogeneity is small in well-shimmed magnets as compared to the magnetic susceptility of the paramagnetic ferritin and hemosiderin.

THE RELATIONSHIP BETWEEN FERRITIN AND HEMOSIDERIN IN RABBITS AND MAN*

By ARNE SHODEN, BEVERLY WESCOTT GABRIO, and CLEMENT A. FINCH

(From the Department of Medicine, University of Washington School of Medicine, Seattle, Washington)

(Received for publication, February 2, 1953)

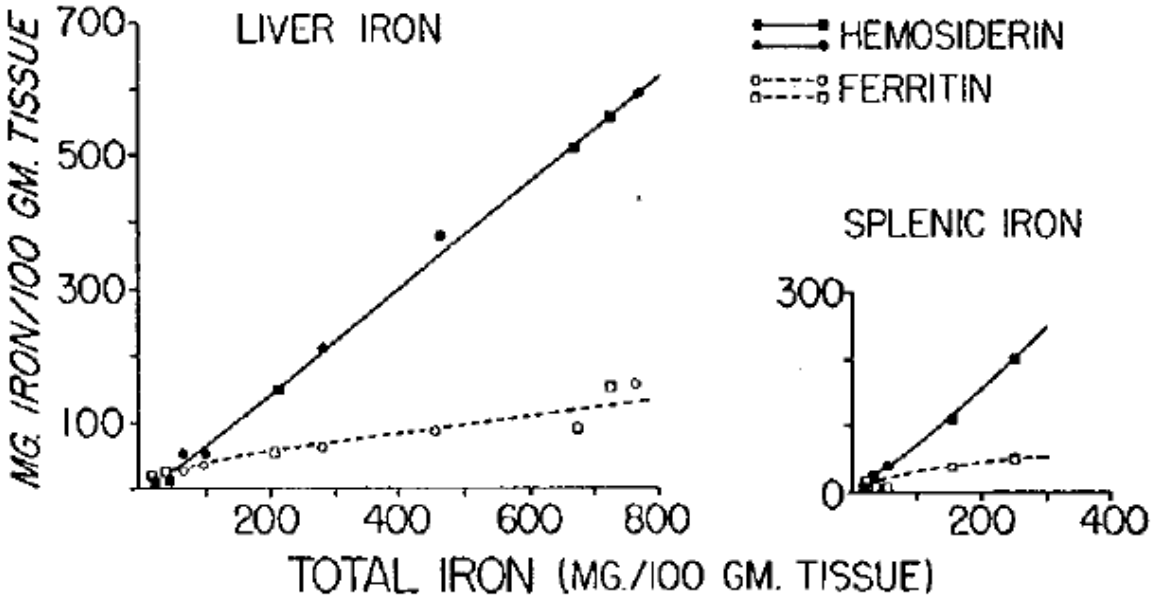
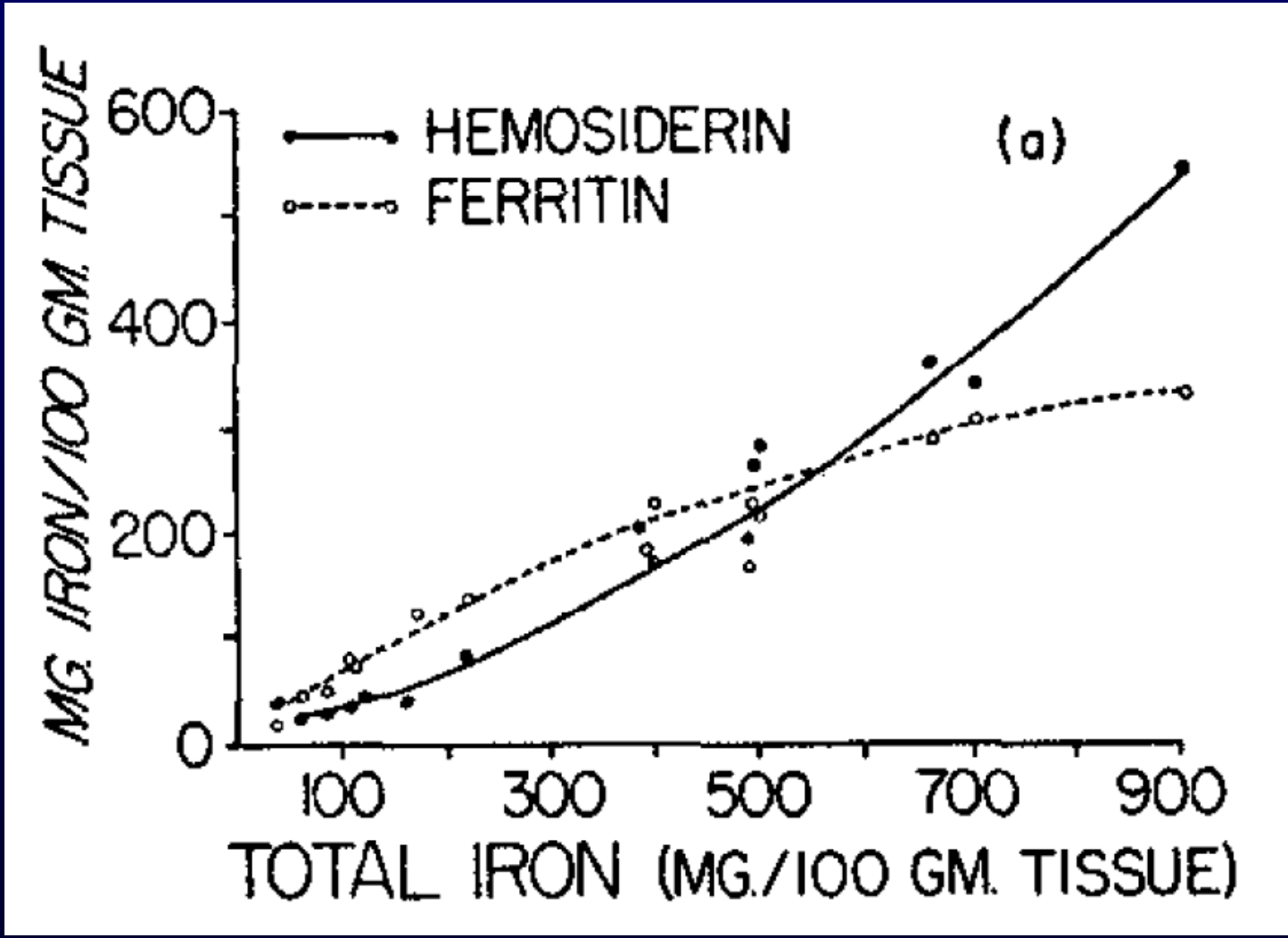


FIG. 3. Fractionation of liver and splenic iron in man. Each set of points (ferritin and hemosiderin) represents the fractionation of one tissue. The squares refer to subjects in which storage fractions of both liver and spleen are graphed.

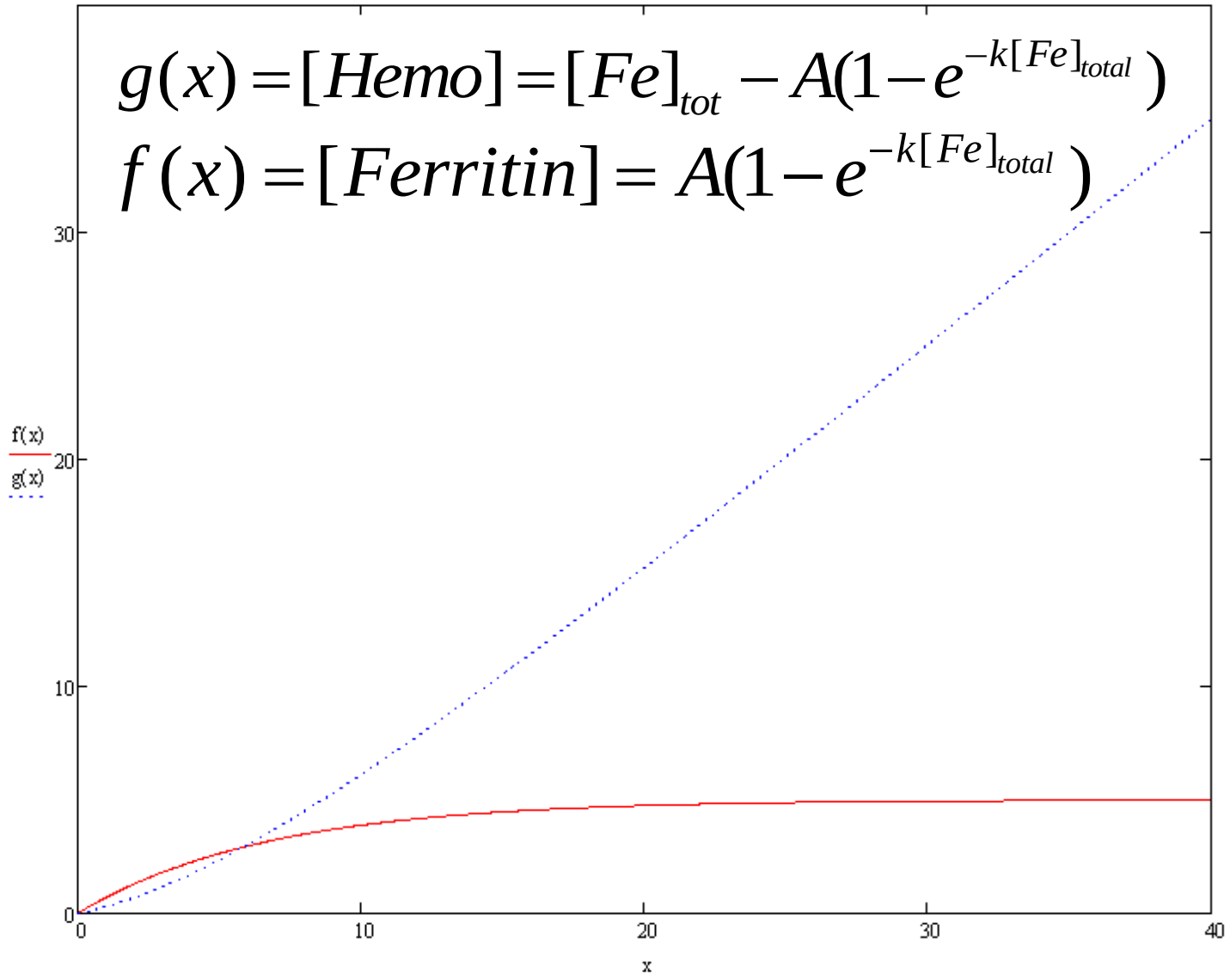
Iron overload of rabbit following injections of iron



Gotsis mathematical model assuming degeneration of ferritin to hemosiderin

$$g(x) = [Hemo] = [Fe]_{tot} - A(1 - e^{-k[Fe]_{total}})$$

$$f(x) = [Ferritin] = A(1 - e^{-k[Fe]_{total}})$$



Speciation of Tissue and Cellular Iron with On-Line Detection by Inductively Coupled Plasma–Mass Spectrometry

Lidija Stuhne-Sekalec, Sonny X. Xu, Joel G. Parkes, Nancy F. Olivieri, and Douglas M. Templeton[1]
Department of Clinical Biochemistry, University of Toronto, 100 College Street, Toronto, M5G 1L5, Canada

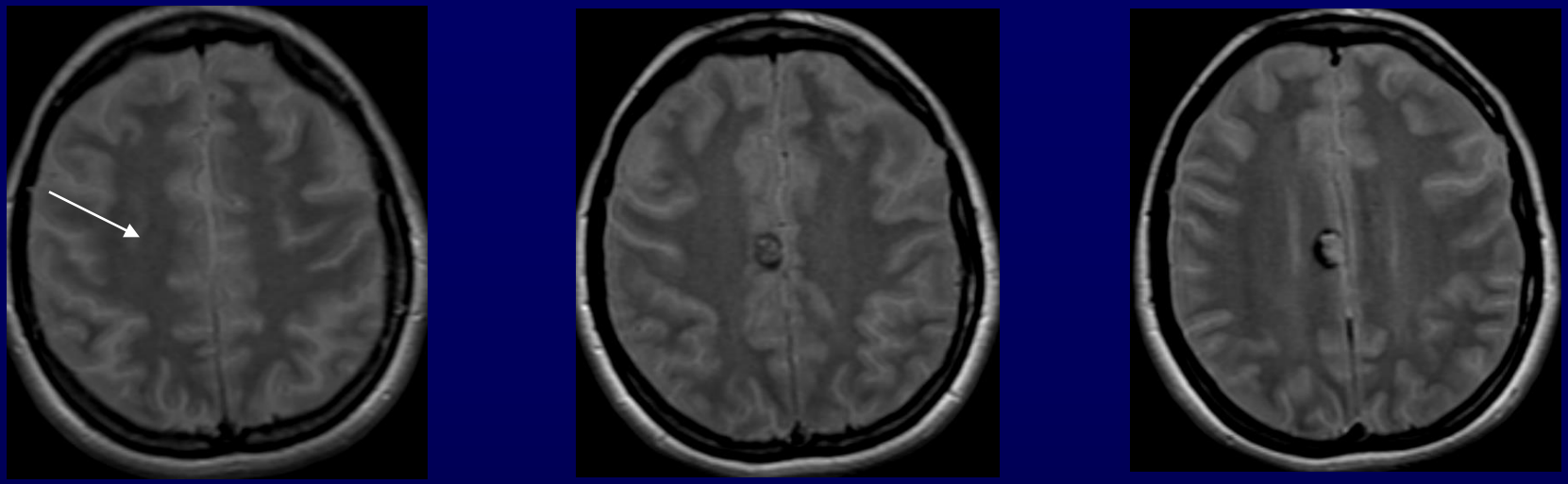
TABLE 5

Fe Content of Human Tissue

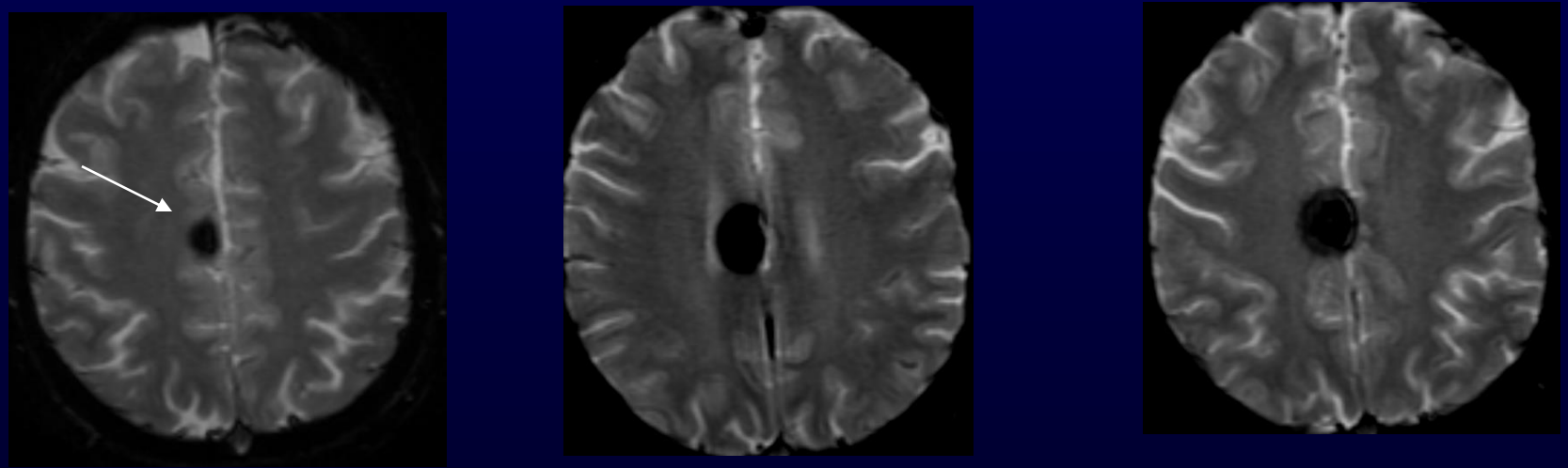
Fraction	Fe content (%)	
	ICP–MS	ET-AAS
Transferrin		
Liver	0.60 ± 0.29 (0.07–1.3)	1.22 ± 0.92 (0.45–3.9)
Heart	1.8	3.1 ± 0.2
Ferritin		
Liver	21.9 ± 18.0 (6.3–62.3)	21.6 ± 13.7 (4.8–50.1)
Heart	8.35 ± 1.25	10.2 ± 0.7
Hemoproteins		
Liver	3.78 ± 3.26 (0.37–9.2)	0.88 ± 0.45 (0.20–1.9)
Heart	1.7 ± 0.5	1.2 ± 0.3
Hemosiderin		
Liver	73.7 ± 19.3 (33.5–92.1)	76.3 ± 13.8 (46.7–93.4)
Heart	88.2 ± 1.8	85.6 ± 0.8

Note. Values are expressed as the mean ± SD (range) of Fe measured in 15 liver biopsies and the average of two samples from a heart, by both ICP–MS and ET-AAS. All samples were from patients with thalassemia. The mean Fe content of the samples was 6.58 ± 3.47 (1.47–13.3) mg/g fresh weight. Values for transferrin and hemoproteins differ by the two methods at $P = 0.02$ and $P = 0.002$, respectively.

T_{2w} and T_{2w}*- images of brain cavernoma with hemorrhage

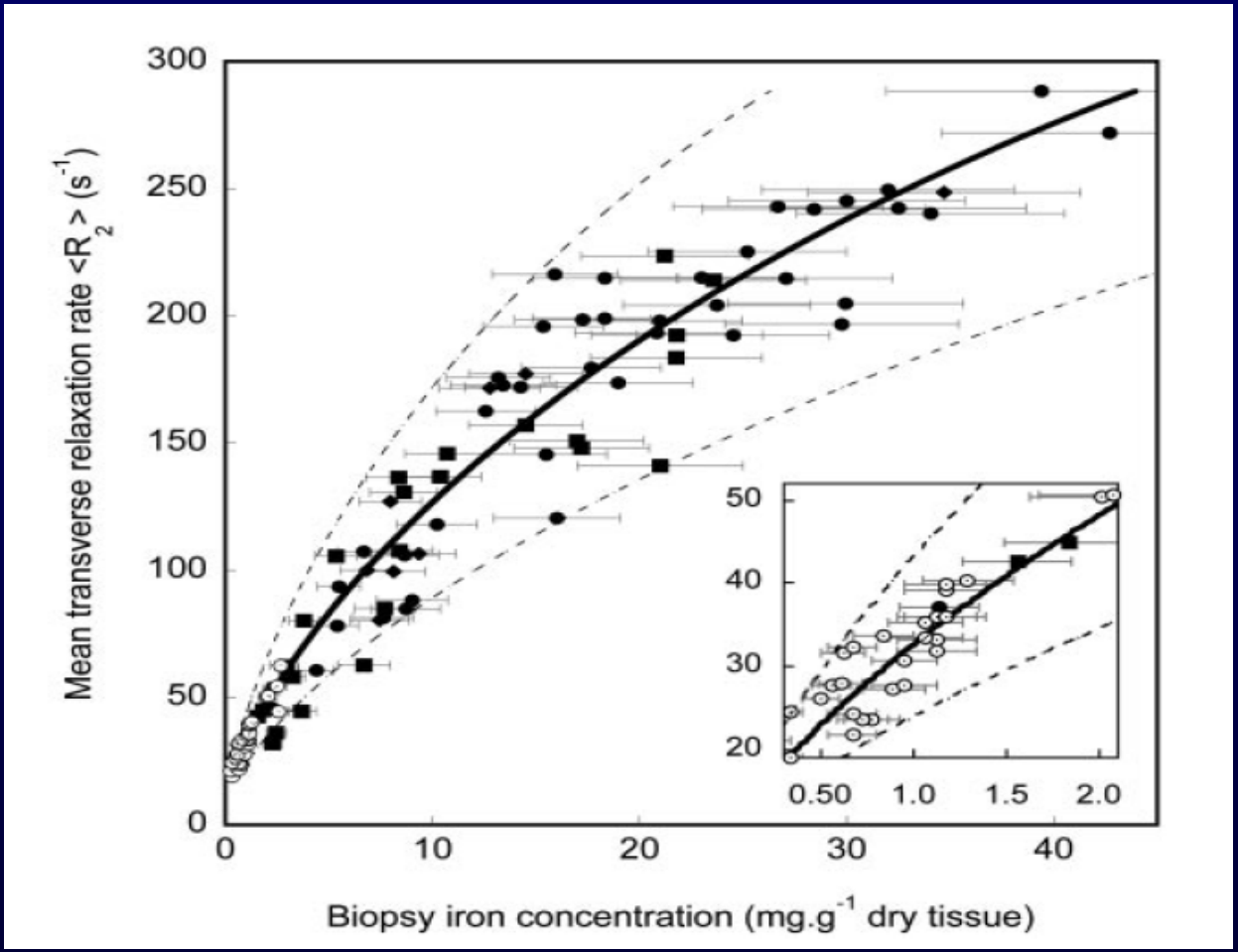


Spin echo T_2-weighted images

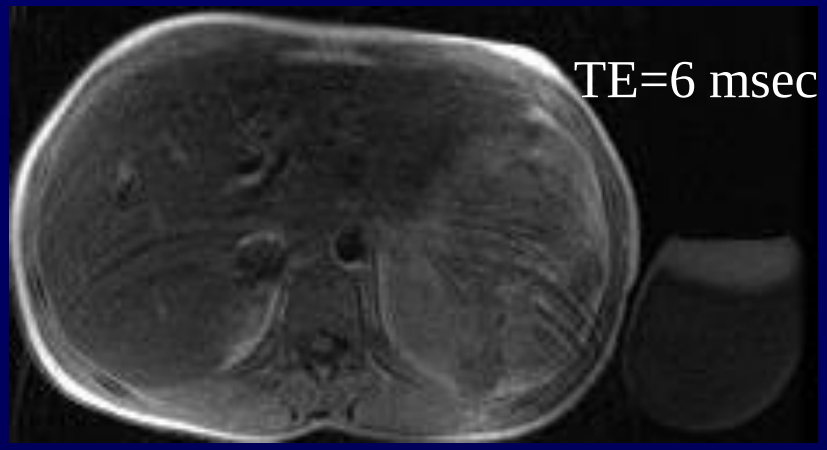


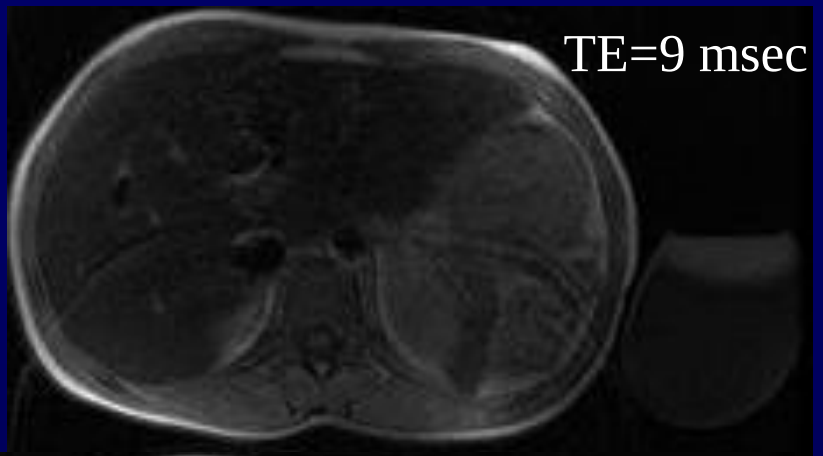
Gradient-echo T_2*-weighted images

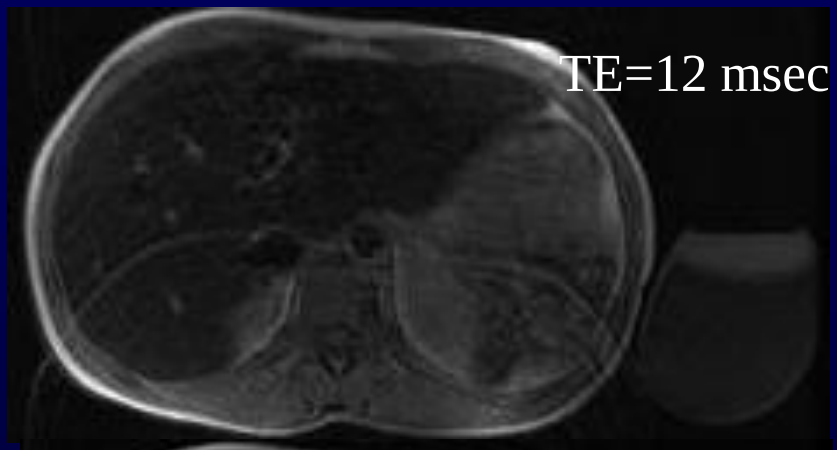
Calibration of Liver R_2 vs. Liver Concentration (Tim St Pierre et al., Blood, 2005)

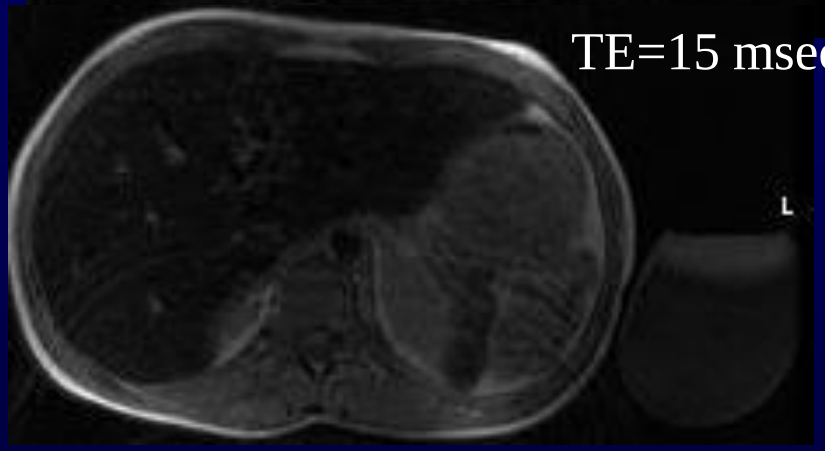


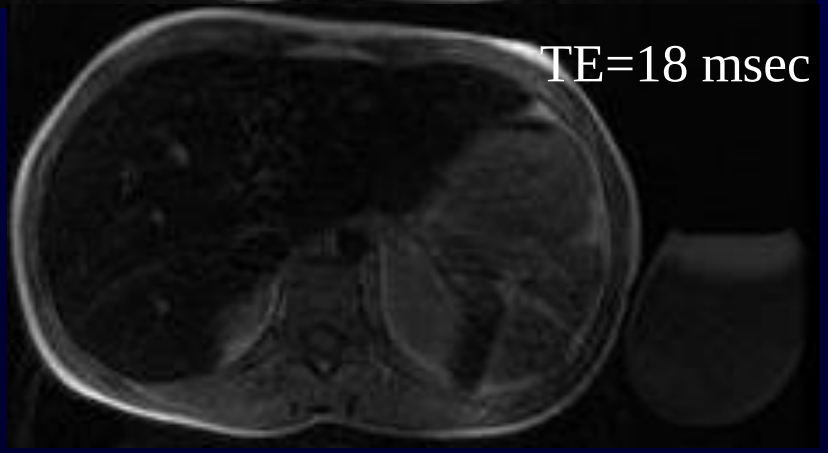
$$[\mathrm{Fe}]_{\mathrm{R2\text{-}SP}} = \left(29.75 - \sqrt{900.7 - 2.283R2}\right)^{1.424}$$











ΜΕΘΟΔΟΣ **FERRISCAN**
11 slices centered in the liver with TR=1000 msec and TE=6, 9, 12, 15, 18 msec. Every dataset is acquired separately. Total acquisition time is 10 min.

Typical results report provided by FERRISCAN

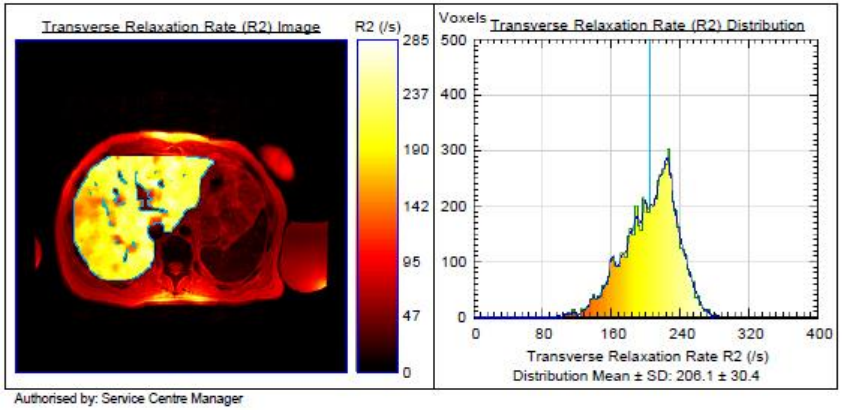
FerriScan®

Liver Iron Concentration Report

Report No: 10005272_S06		Scan Date:	12 May 2008
Patient ID:		Analysis Date:	14 May 2008
Name:		Referrer:	
Birth Date: 09 Oct 1971		MRI Centre:	Institute Euromedica - Encephalos, Greece

Average Liver Iron Concentration	**23.0 mg/g dry tissue**	(NR: 0.17-1.8)
	411 mmol/kg dry tissue	(NR: 3-33)

Normal range (NR) is taken from Bassett et. al., Hepatology 1986; 6: 24-29.



Authorised by: Service Centre Manager

Resonance Health Analysis Services Pty Ltd ABN: 11 092 813 244
Level 1, 216 Stirling Hwy, Claremont, WA 6010, AUSTRALIA. T: +61 (0)8 9286 5300 E: support@ferriscan.com Web: www.ferriscan.com

$$[Fe]_{R2\text{-}SP} = \left(29.75 - \sqrt{900.7 - 2.283R2}\right)^{1.424}$$

23.0 mg/g dwt

Correlation of R_2* with Total Hepatic Iron concentration (John Wood et al., 2005)

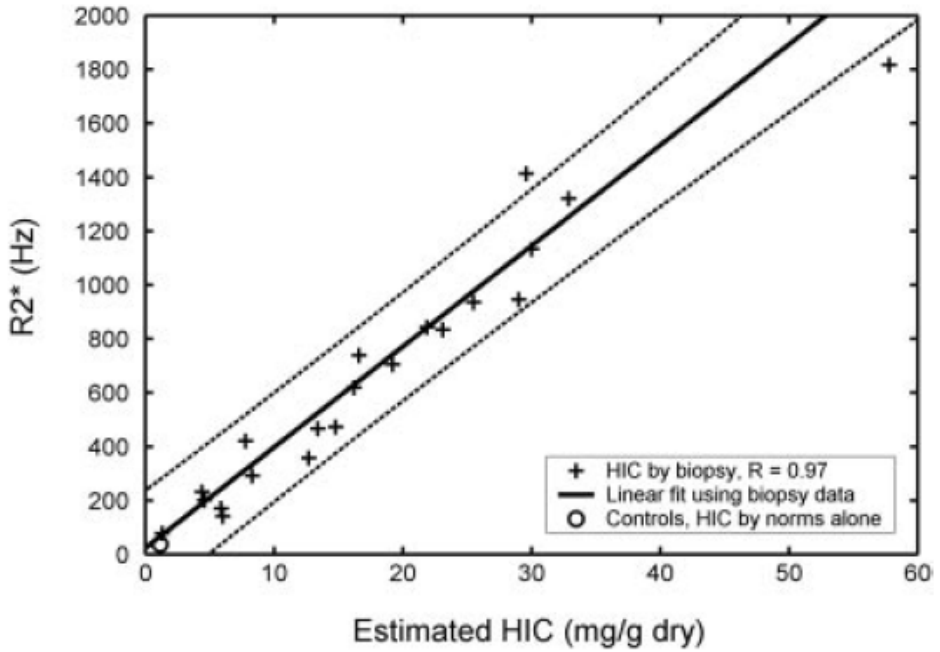
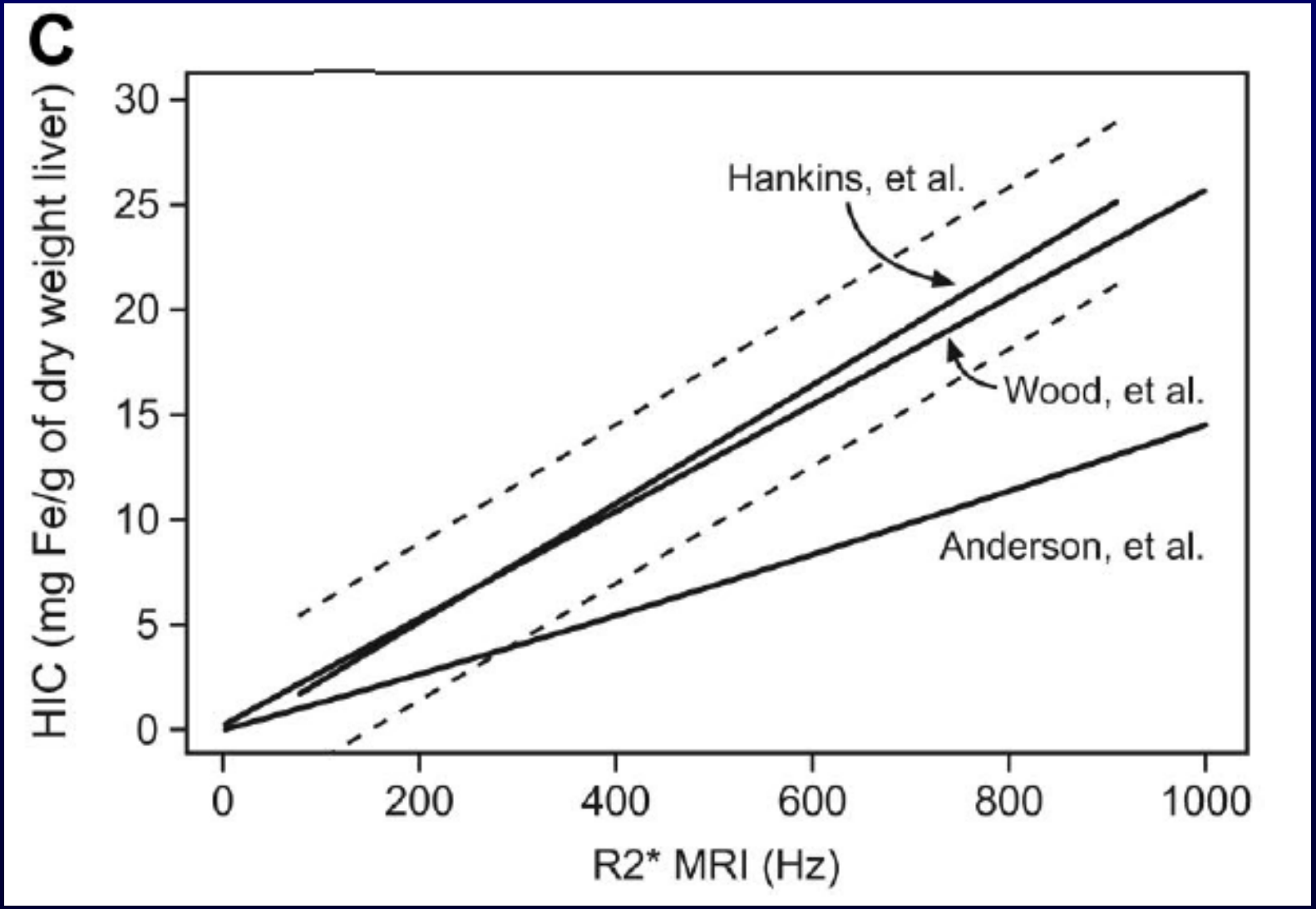


Figure 1. Plot of transverse relaxivity R2* (1/T2*) versus biopsied hepatic iron concentration (HIC) in 21 patients (23 biopsies). R2* has units of hertz and HIC has units of milligram per gram dry weight of liver. R value was 0.97, and dashed lines indicate 95% prediction intervals for the regression. Average R2* value for 13 healthy controls is shown for comparison ○, plotted using an HIC value estimated from normative data (no biopsy). Repeat MRI and biopsy examinations as well as control data were excluded from statistical calculations.

$$[Fe]_{R2*} = .0254 \times R2* + 0.202 \quad (2)$$

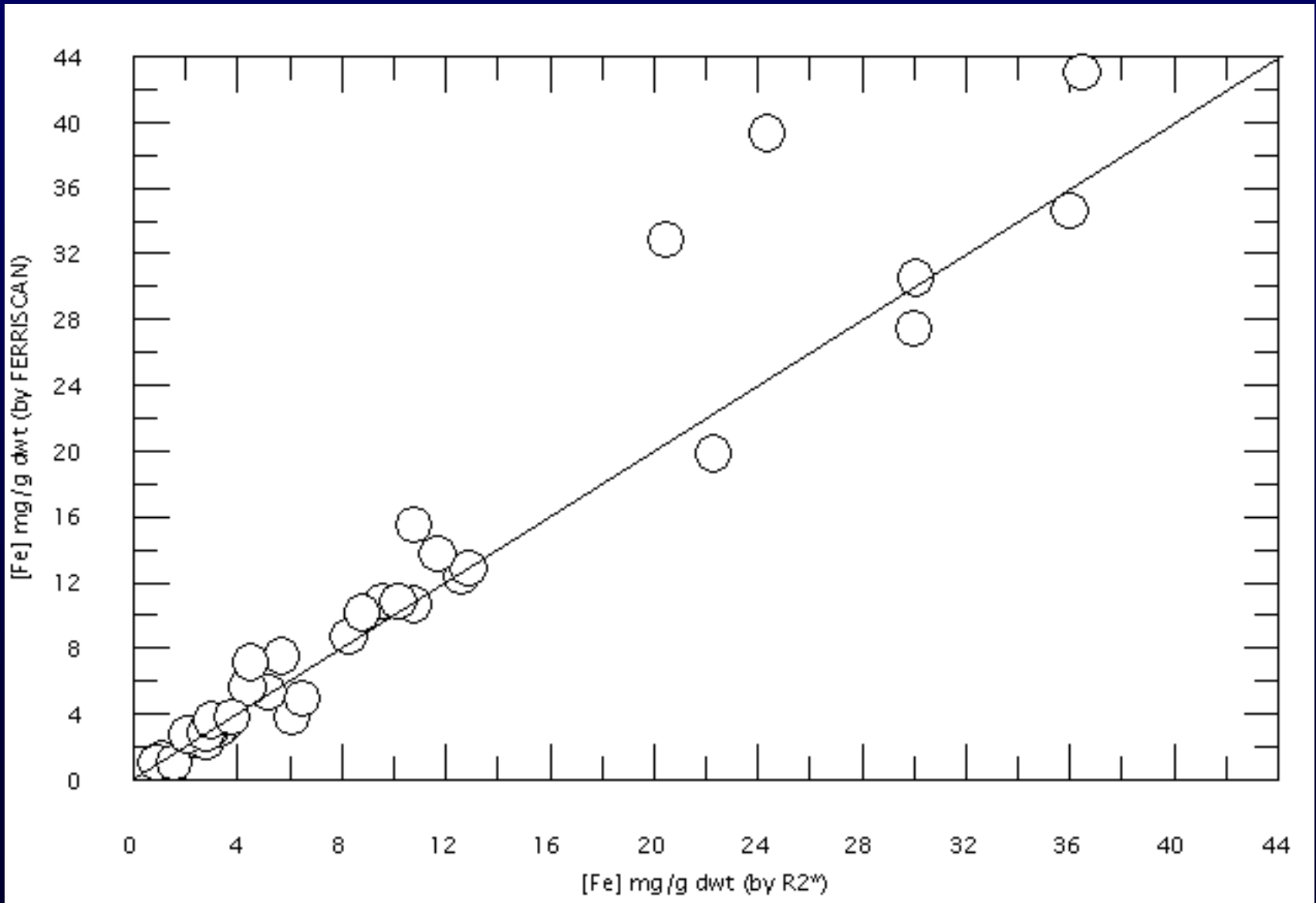
$$[Fe]_{R2\text{-}L} = 0.148 \times R2 - 6.51 \quad (3)$$

Comparison of R_2* with Total Hepatic Iron concentration (Jane S. Hankins et al., 2009)

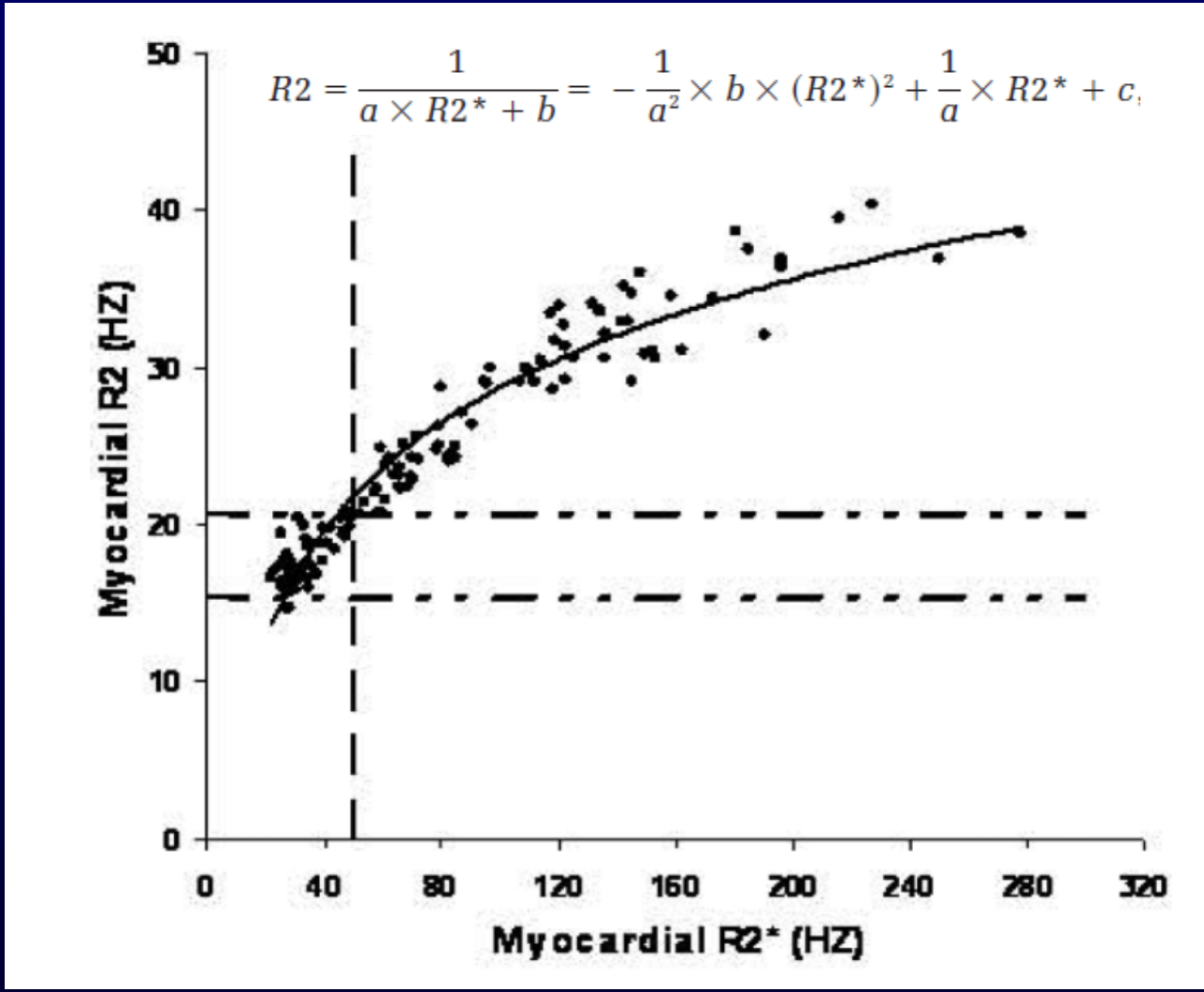


Good agreement between Hankins and Wood

Calculation of LIC by FERRISCAN and R2* (ED Gotsis, 2014, unpublished data)

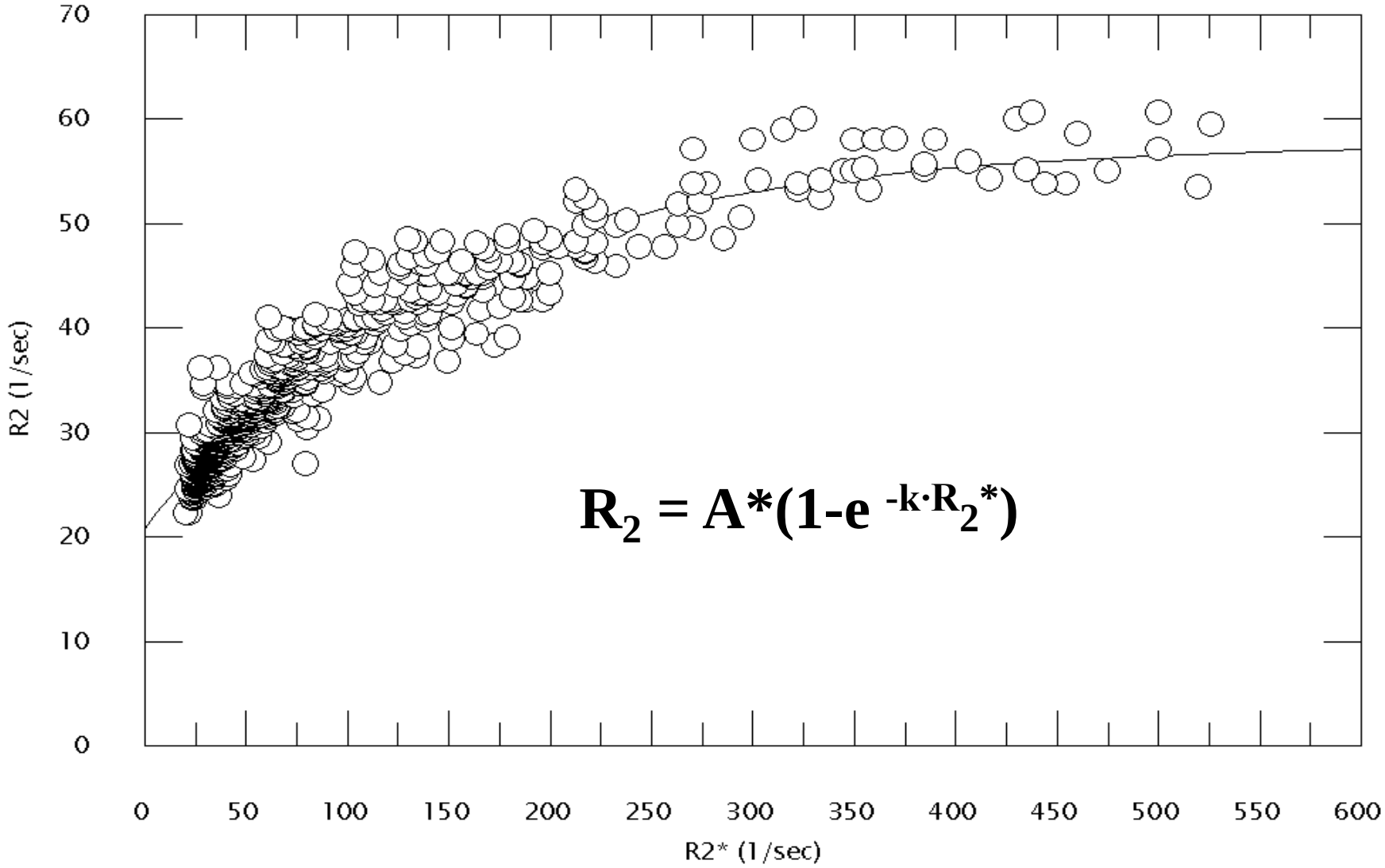


R_2 vs. R_2* for myocardium (fitted by a quadratic equation)



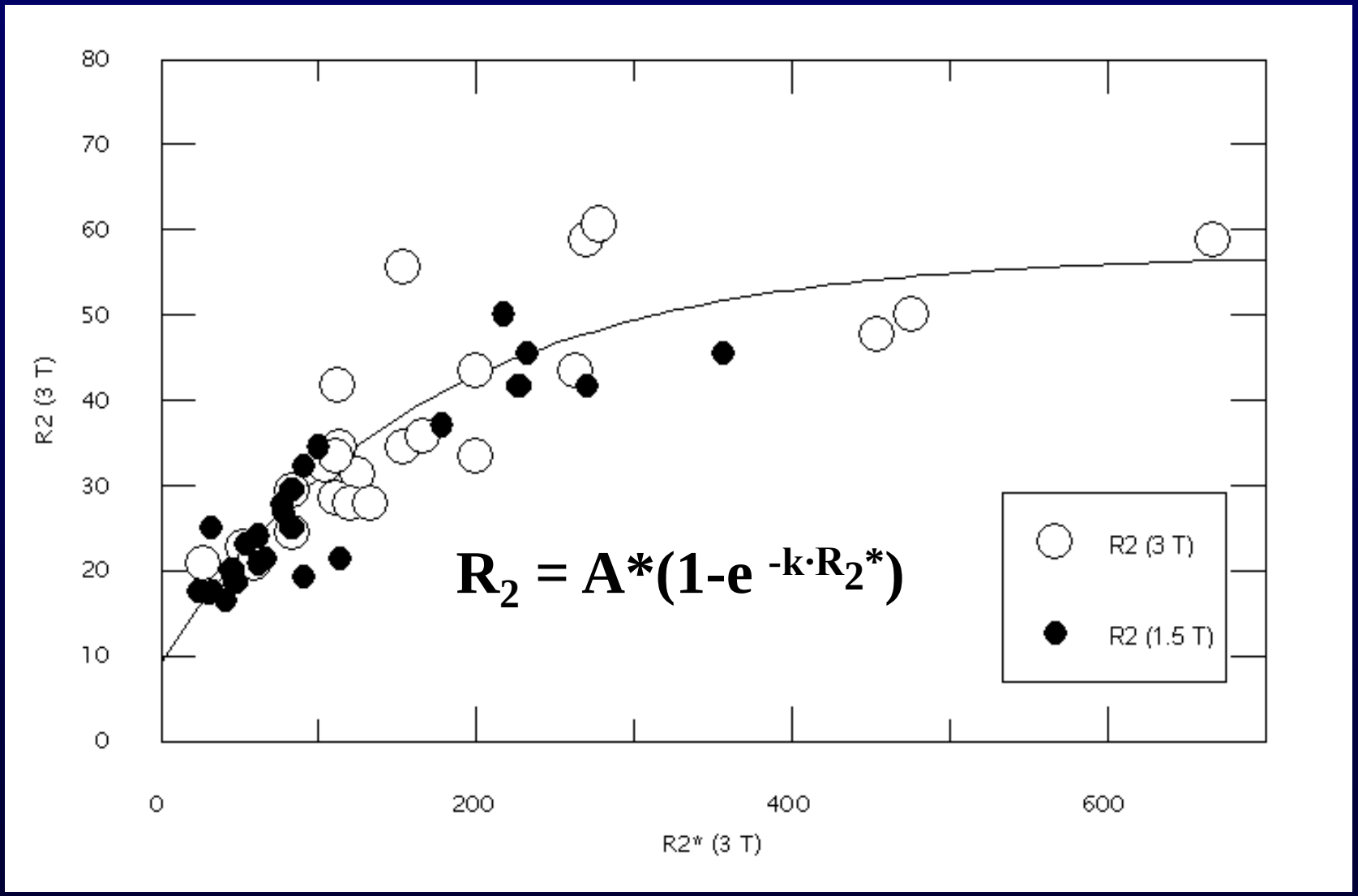
D. Pennell et al, 2009

R_2 vs. R_2* in Myocardium

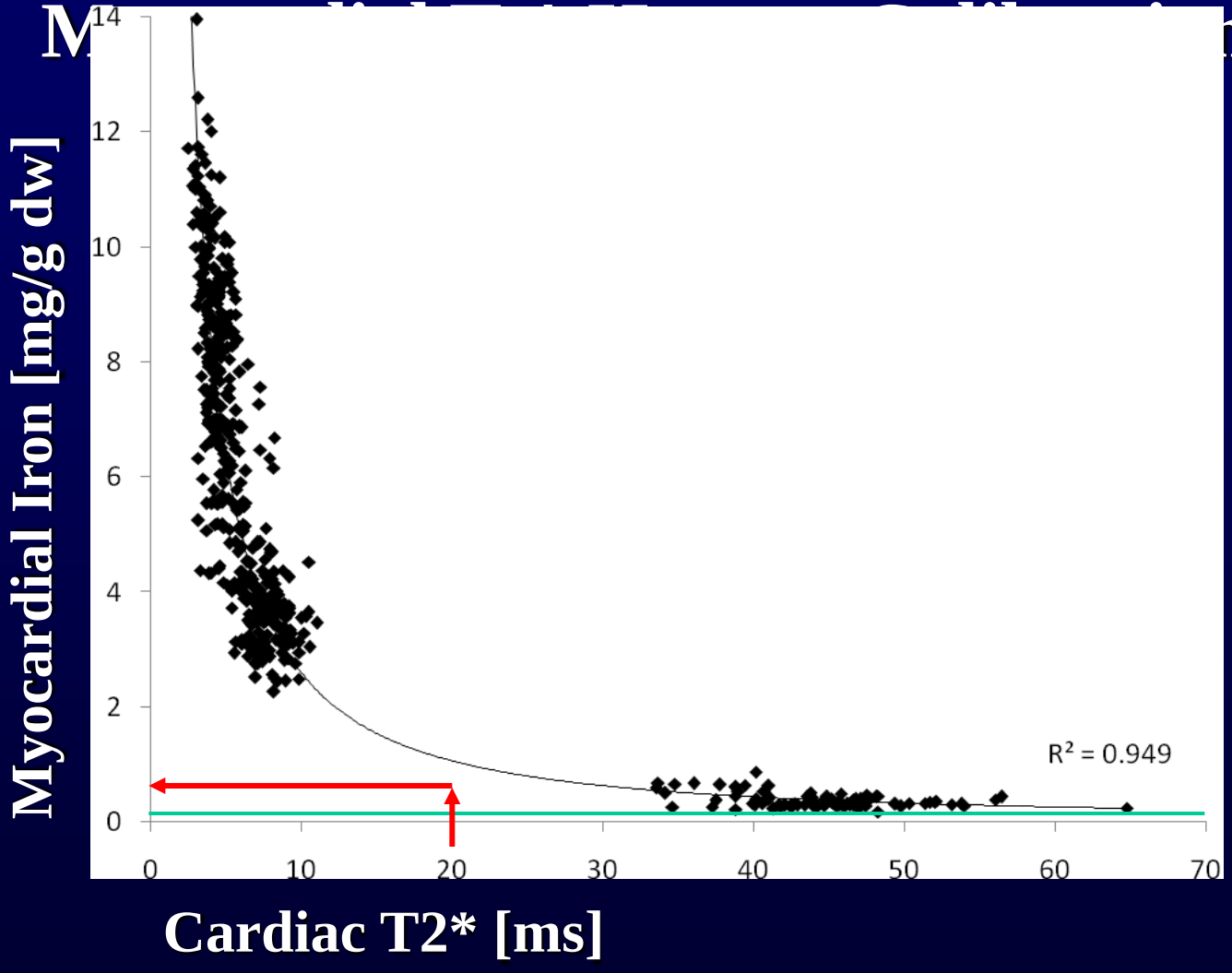


ED Gotsis et al., 2014 (manuscript in preparation)

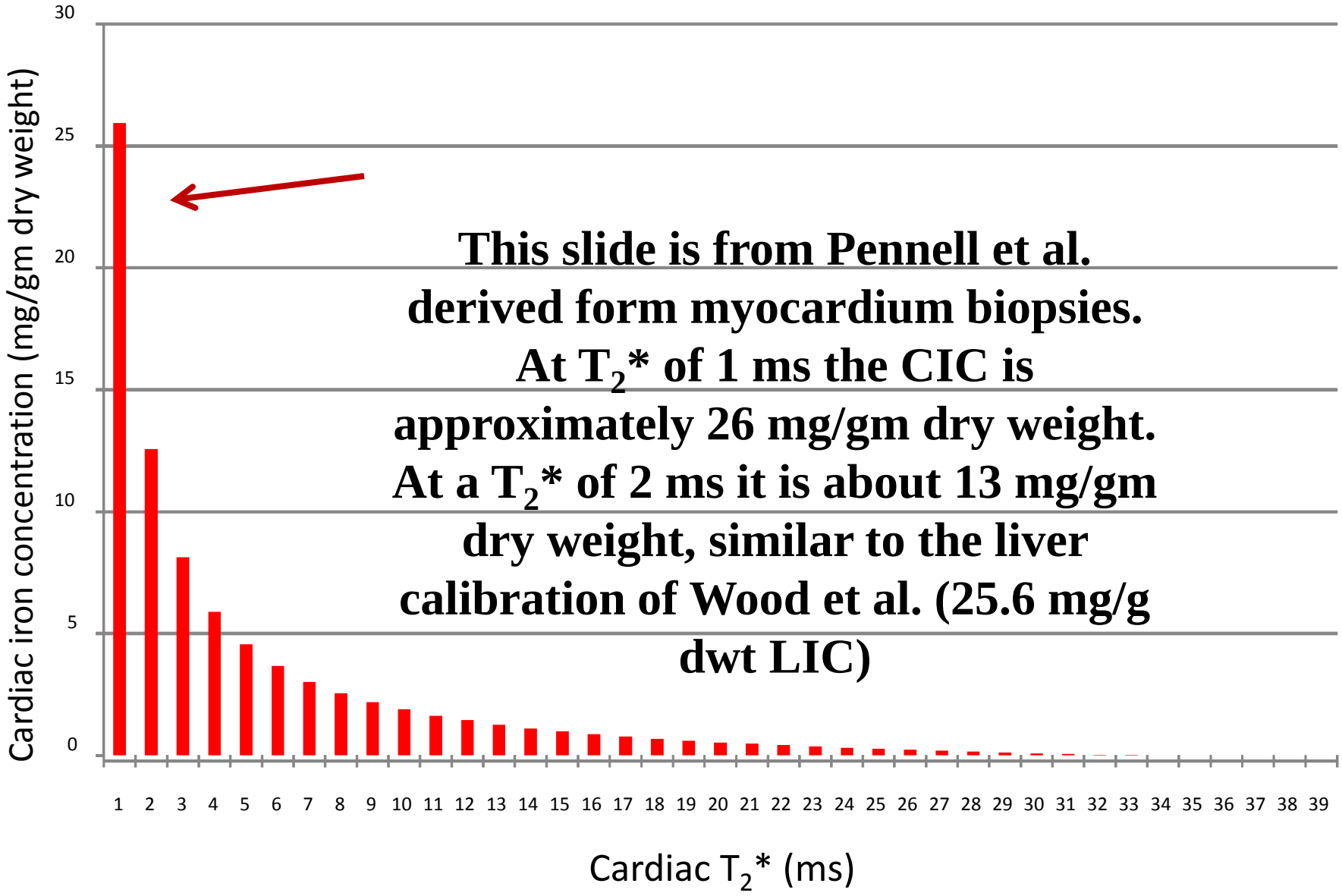
R_2 vs. R_2* for Myocardium at 1.5 and 3.0 Tesla

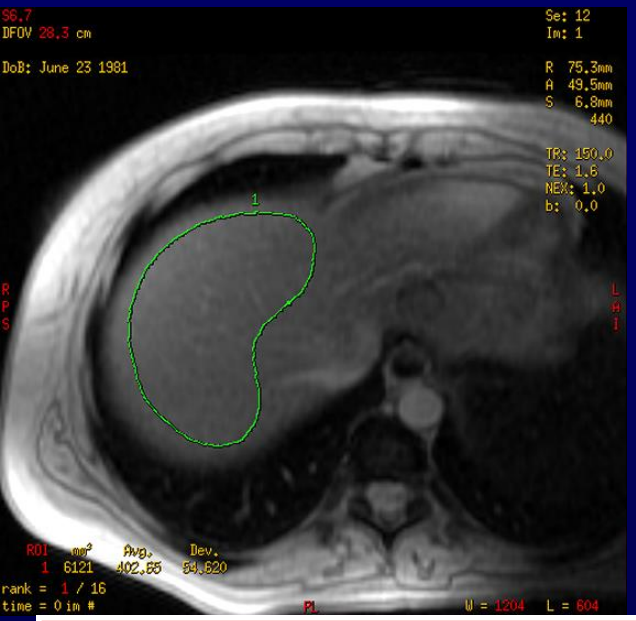


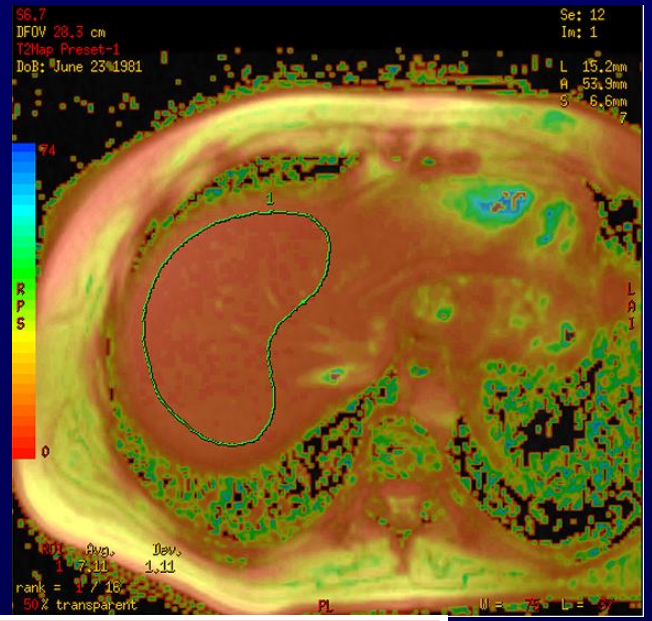
E.D. Gotsis, J. Seimenis, Ch. Economides et al., 2014, (manuscript in preparation)

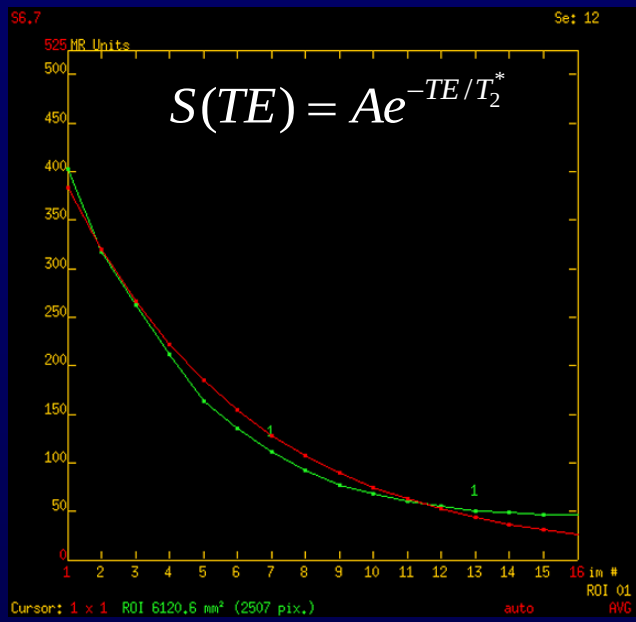


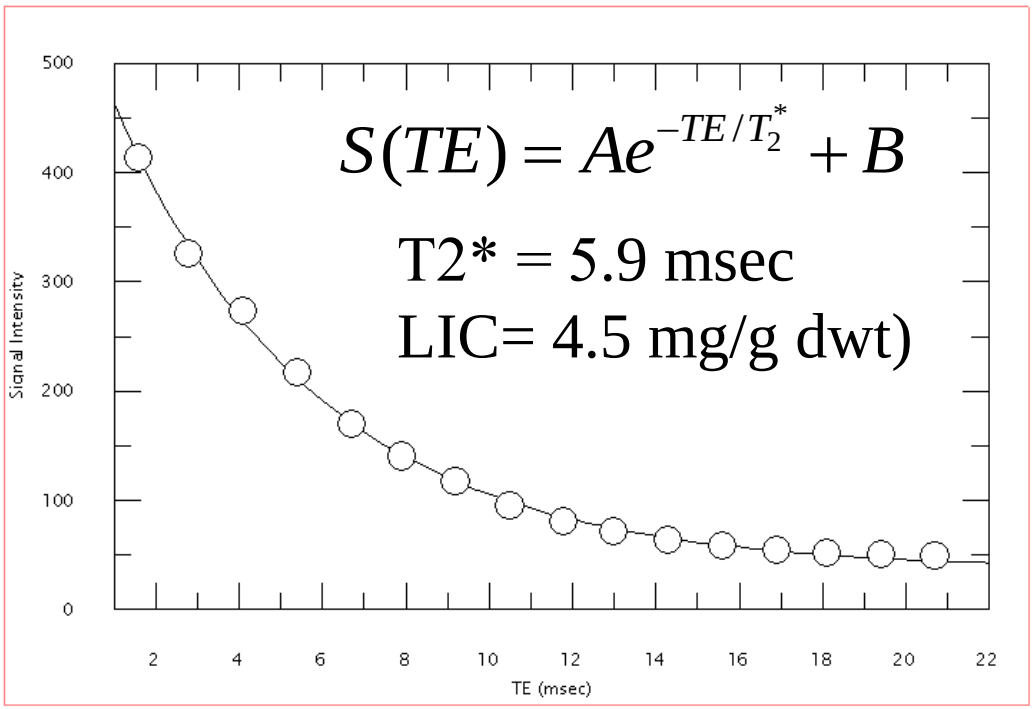
Pennell. 2009, NIH Grant: R01 DK066084-01


Cardiac iron concentration (mg/gm dry weight)
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
Cardiac T2* (ms)
This slide is from Pennell et al. derived form myocardium biopsies. At T2* of 1 ms the CIC is approximately 26 mg/gm dry weight. At a T2* of 2 ms it is about 13 mg/gm dry weight, similar to the liver calibration of Wood et al. (25.6 mg/g dwt LIC)









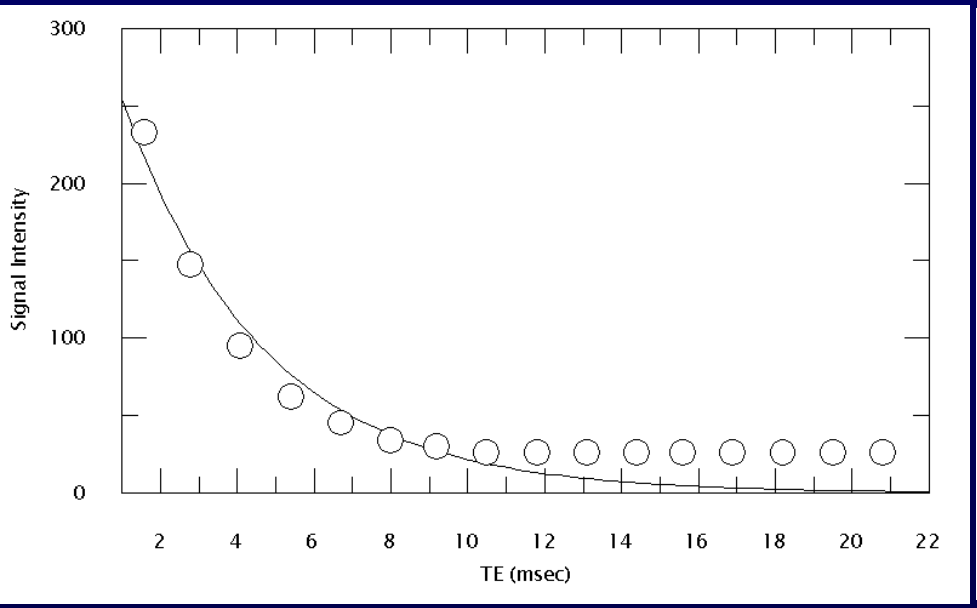
LSF από το MRI machine

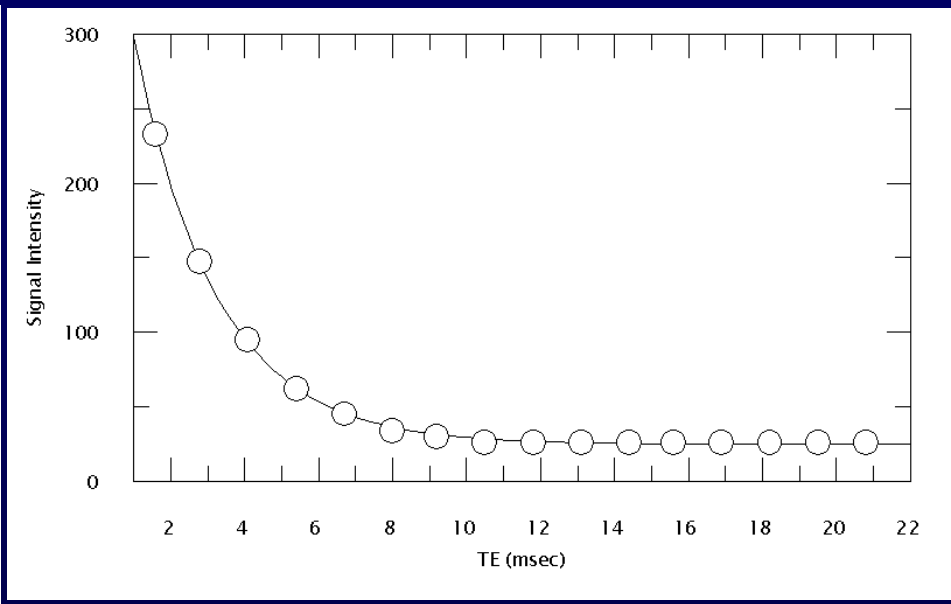
T2* = 7.1 msec

(LIC = 3.8 mg/g dwt)

LSF with the program **GRAFIT** and by using an **offset** for the electronic noise

Calculation of T_2* (16 echoes)





$y = Ae^{-TE/T_2*}$

T_2* = 3,65 msec

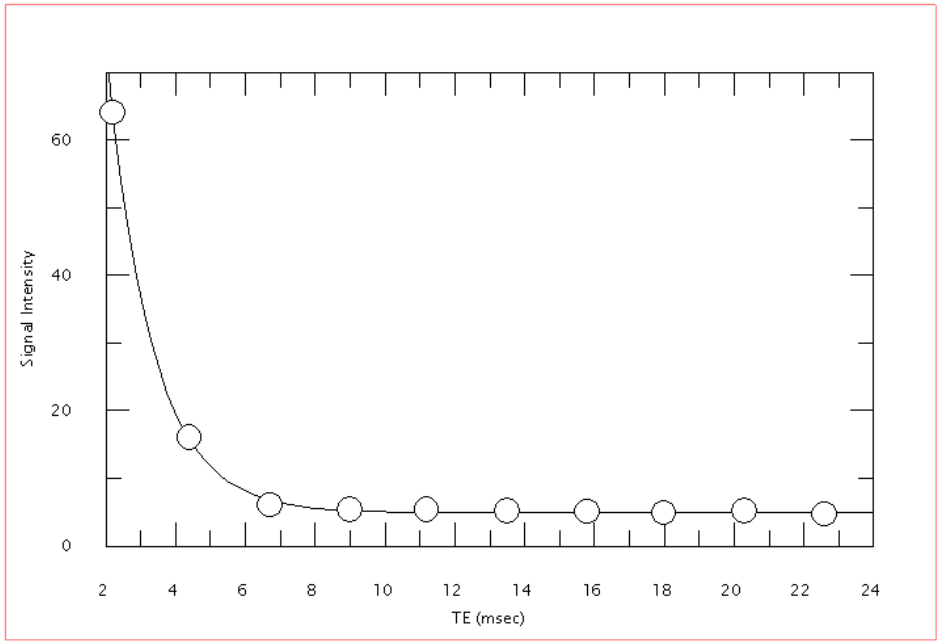
[Fe] = **7.2** mg/g dwt

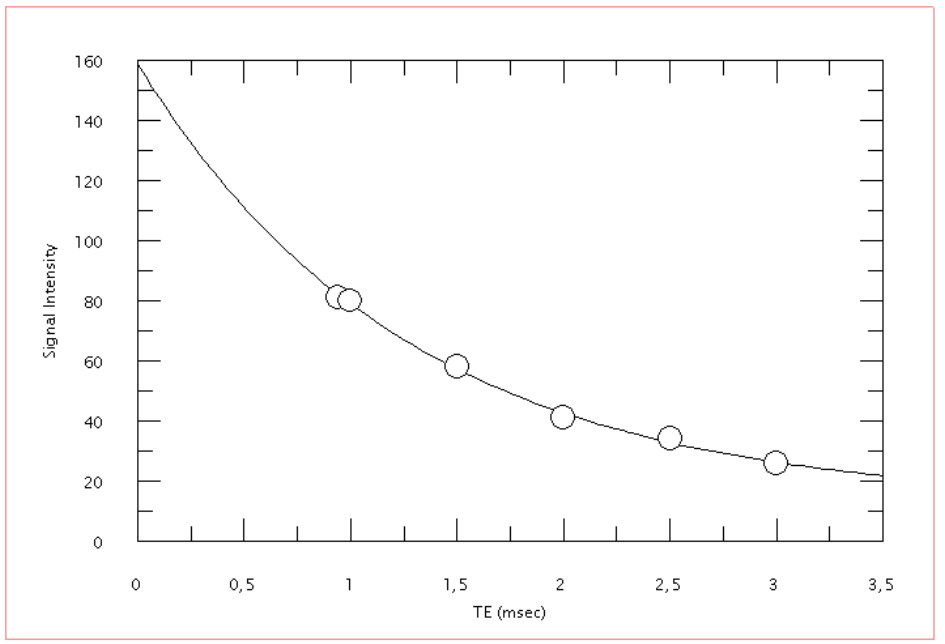
$y = Ae^{-TE/T_2*} + B$

T2* = 2.2 msec

[Fe] = **11.7** mg/g dwt

Calculation of T_2*





$y = \mathbf{A}e^{-TE/T_2^*} + B$

$T_2^* = 1{,}25$ msec

Single breath-hold sequence,
P-gating, 8 echoes in one breathhold

$y = \mathbf{A}e^{-TE/T_2^*}$

T2* = 1,26 msec

Multi-breath single-echo
1 echo/breath-hold

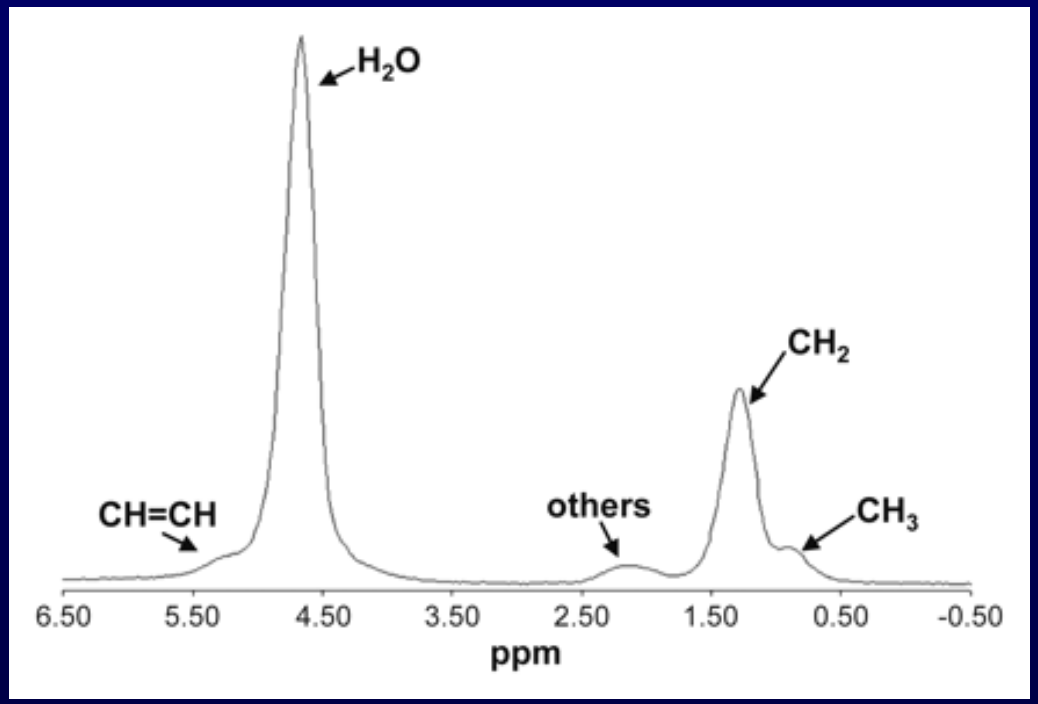
Liver Fat Infiltration

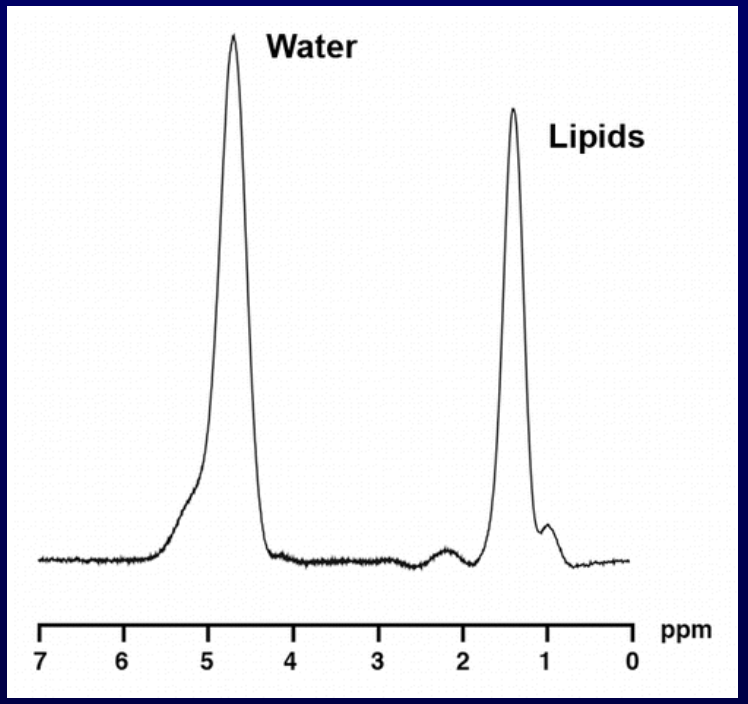
Fat Infiltration occurs in many pathological situations:

1. **Diabetes II**
2. **Alcoholism**
3. **High triglycerides**
4. **Obesity**
5. **HCV**
6. **Metabolic syndrome**
7. **…..**

Fat infiltration, depending on its severity, can lead to initial liver inflammation and if not reversed to edema, hepatomegaly (usually reversible if fat infiltration can be reduced). If not treated, fat infiltration may lead to fibrosis, cirrhosis, hepatic insufficiency and also cancer. Therefore is an additional risk factor to β-Thalassemia patients, some of which are diabetic, obese, etc.

Proton MR Spectroscopy in Liver Fat Infiltration



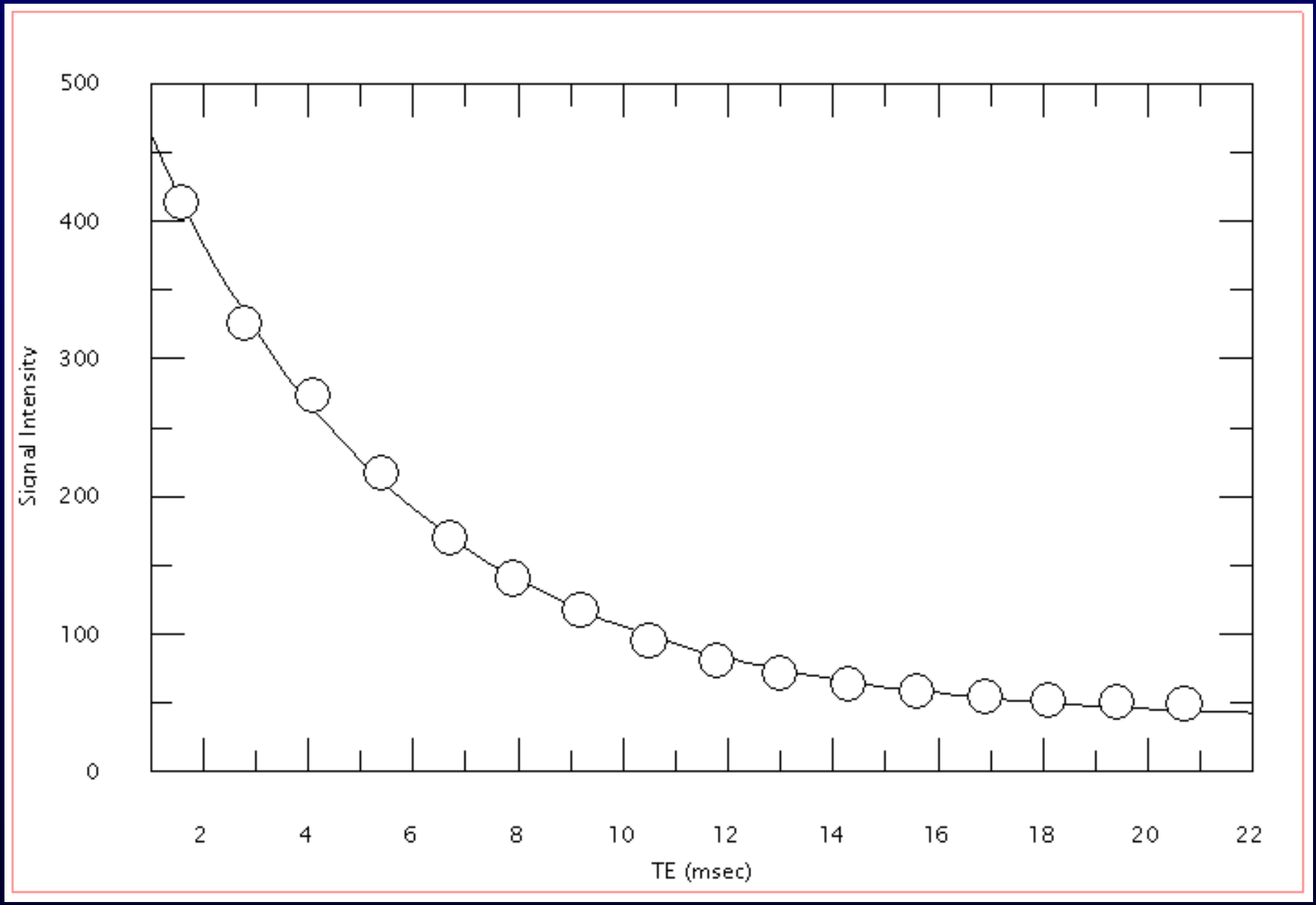


Fat Fraction ≈ 20%

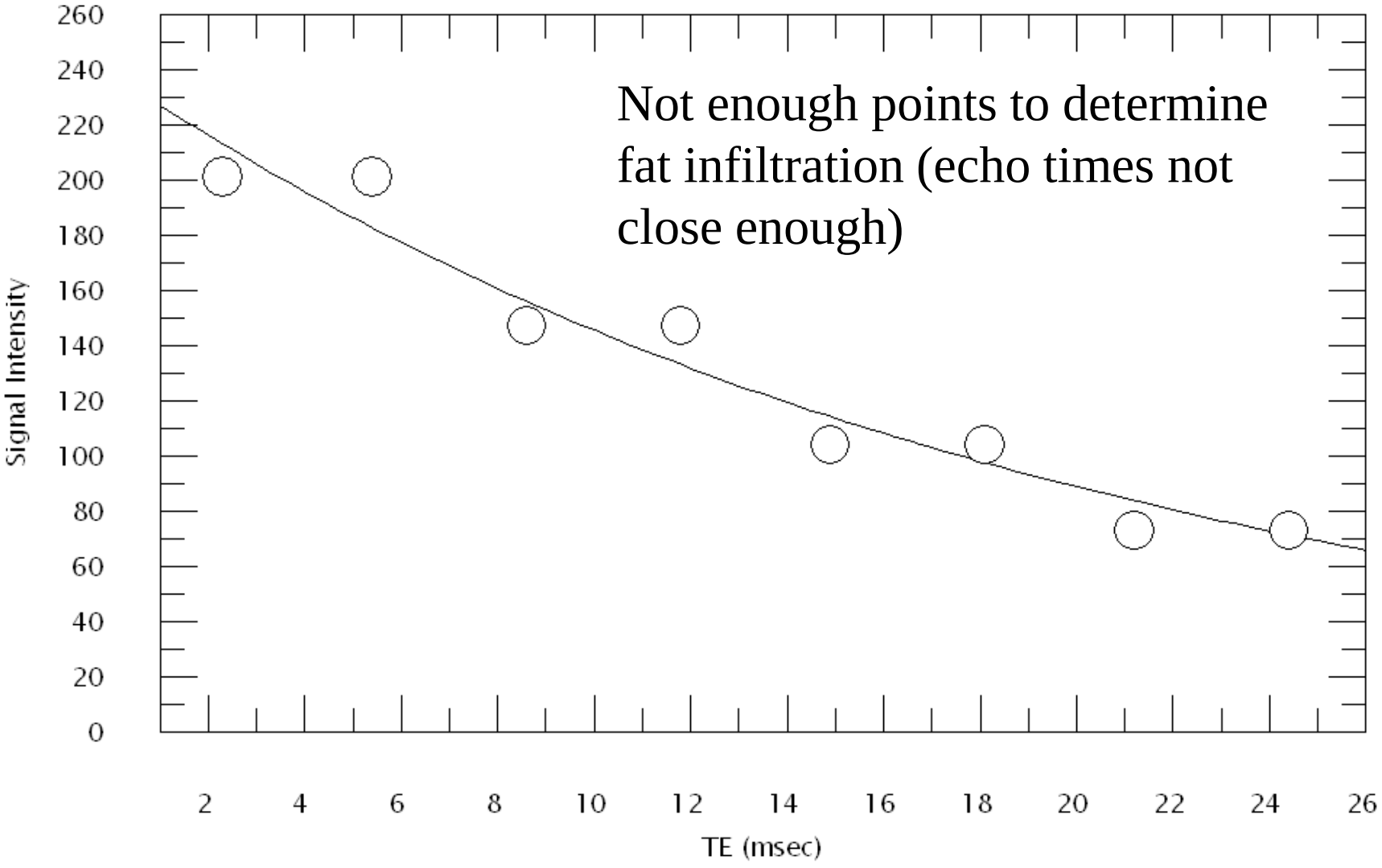
Fat Fraction ≈ 40%

Proton MR Spectroscopy is a very accurate way to determine liver fat infiltration. However Very few centers have a spectroscopy package and even if they have (eg., GE) the automatic water suppression prevents water signal estimation.

No Liver Fat Infiltration



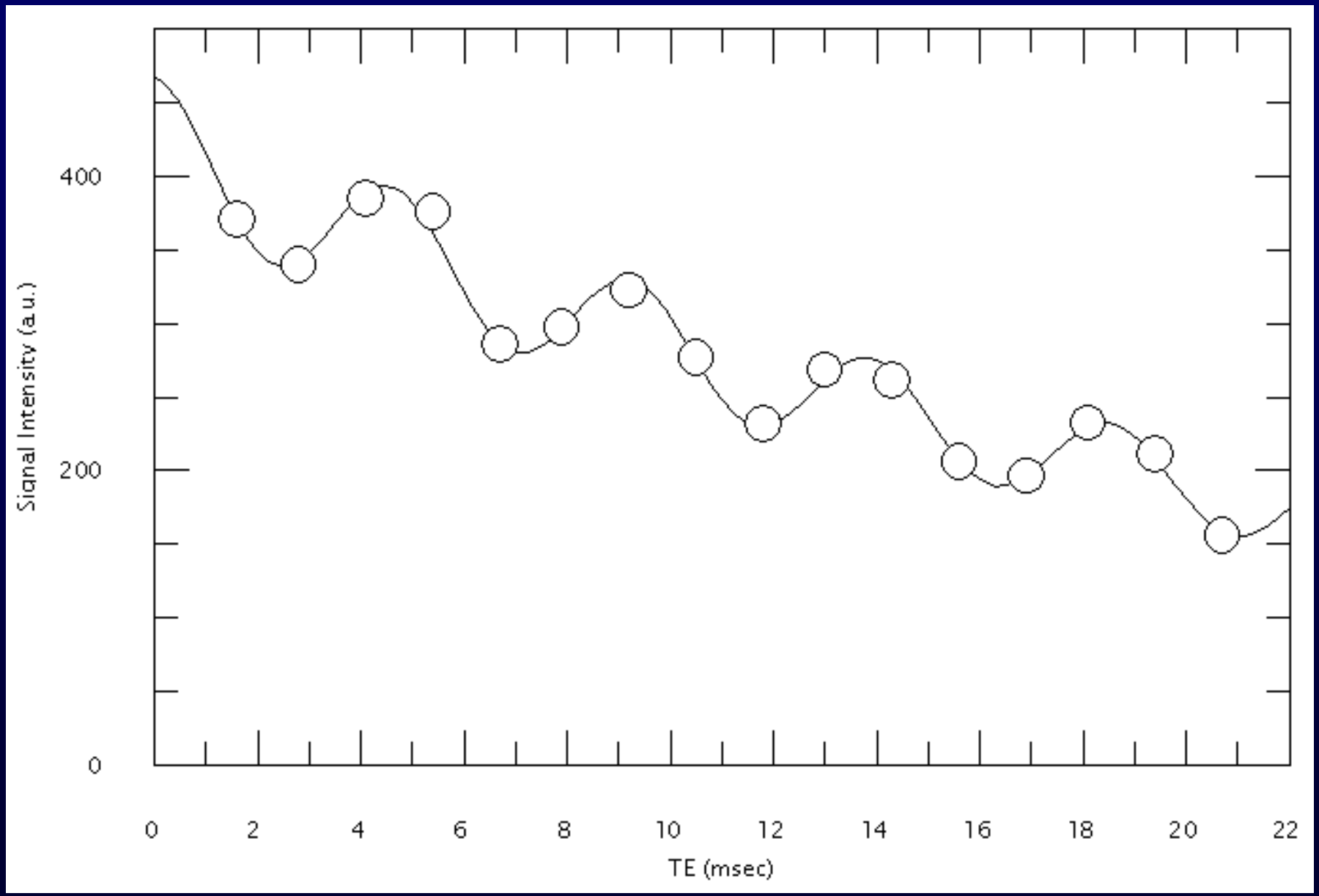
In phase-Out of phase in case of fat infiltration



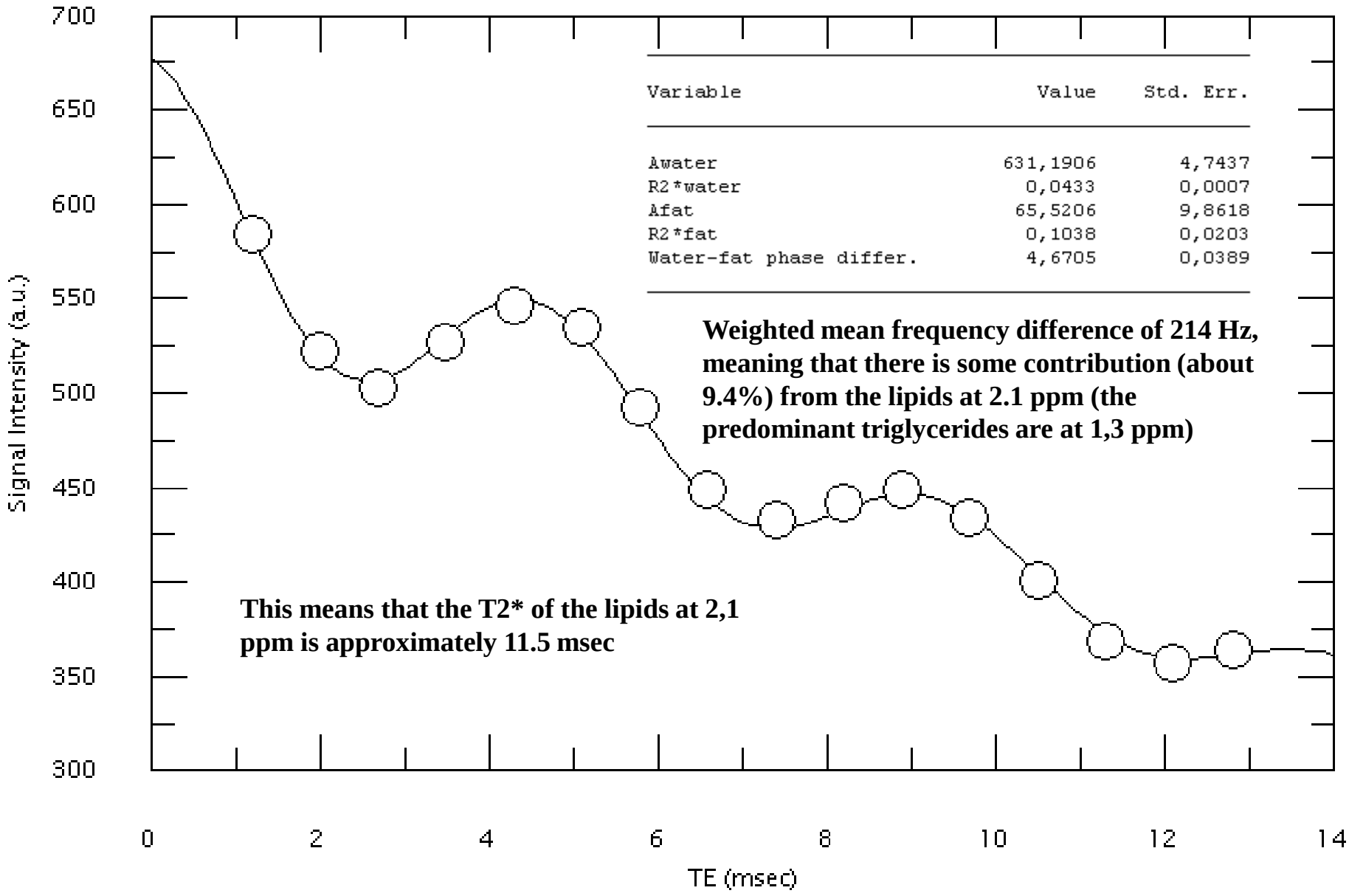
In phase-Out of phase and TE values

Echo number	TE (msec) 160x256 matrix	TE (msec) 128x128 matrix	In phase Out of phase TE values
********	**********	**********	**********
1	1.6	1.2	
2	2.9	2.0	**2.3**
3	**4.2**	2.7	**4.6**
4	5.4	3.5	
5	**6.7**	**4.3**	**6.9**
6	8.0	5.1	
7	**9.3**	5.8	**9.2**
8	10.6	**6.6**	
9	**11.9**	7.4	**11.5**
10	**13.2**	8.2	**13.8**
11	14.5	**8.9**	
12	**15.7**	9.7	**16.1**
13	17.0	10.5	
14	**18.3**	**11.3**	**18.4**
15	19.6	12.1	
16	**20.9**	12.8	**20.7**

Patient with fat infiltration and proper protocol



LSF of fat infiltrated liver data in short TE range (1.1-12.8 msec)



Liver Fat Infiltration

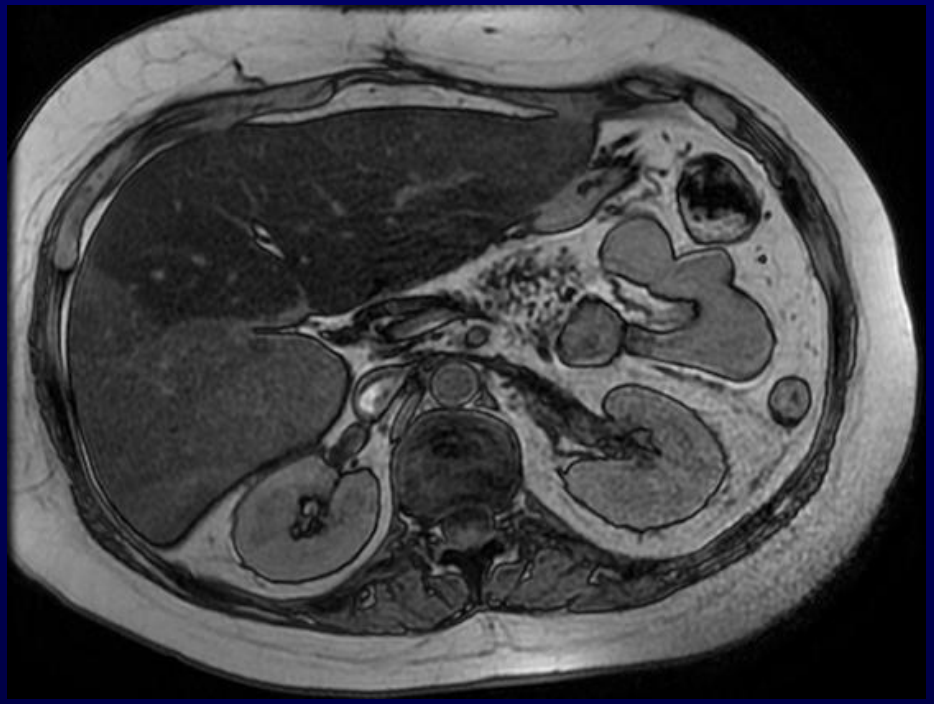
Dixon in1984 introduced the term fat fraction and he made measurements with proton spectroscopic imaging[1]

If water signal is S_w and that of fat is S_f, because of resonance frequency differences between water and fat in the order of 3,5 ppm (Δf = 3,4*63,87 Hz = 217 Hz at 1.5 Tesla). The inverse of this is 4,6 msec. Every n*4,6 msec the signals of water and fat are in phase and every n*(1/2)*4,5 msec = 2,3 msec the water and fat signals are out of phase. The total signal is:

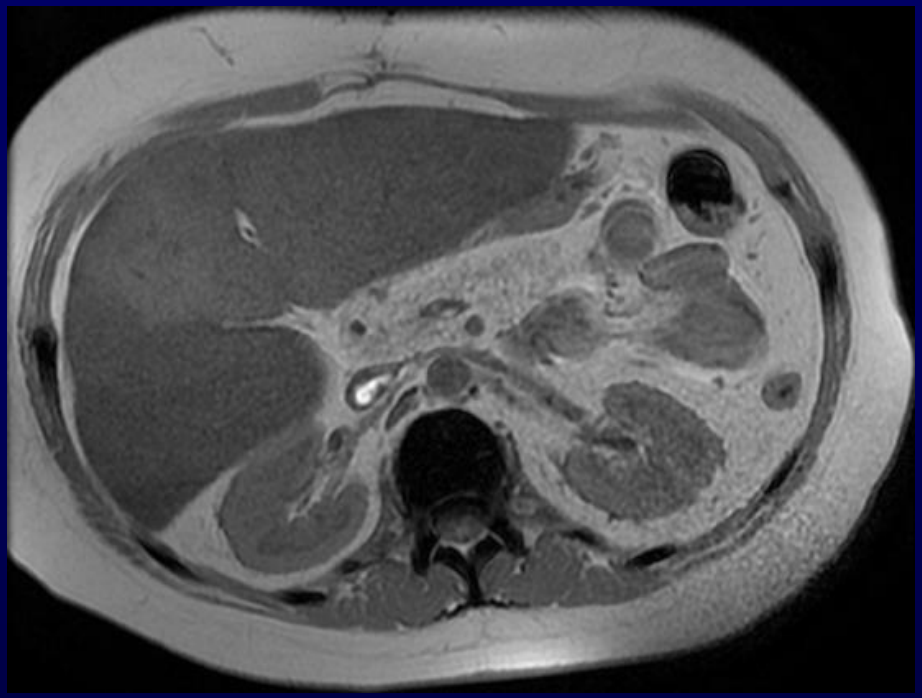
$S_{total} = S_w + S_f$ and Fat Fraction = $FF = \frac{S_f}{S_f + S_w}$

1. WT Dixon, "Simple proton spectroscopic imaging", Radiology 1984; 153:89

Quantitative Liver Fat Infiltration by Dual Echo

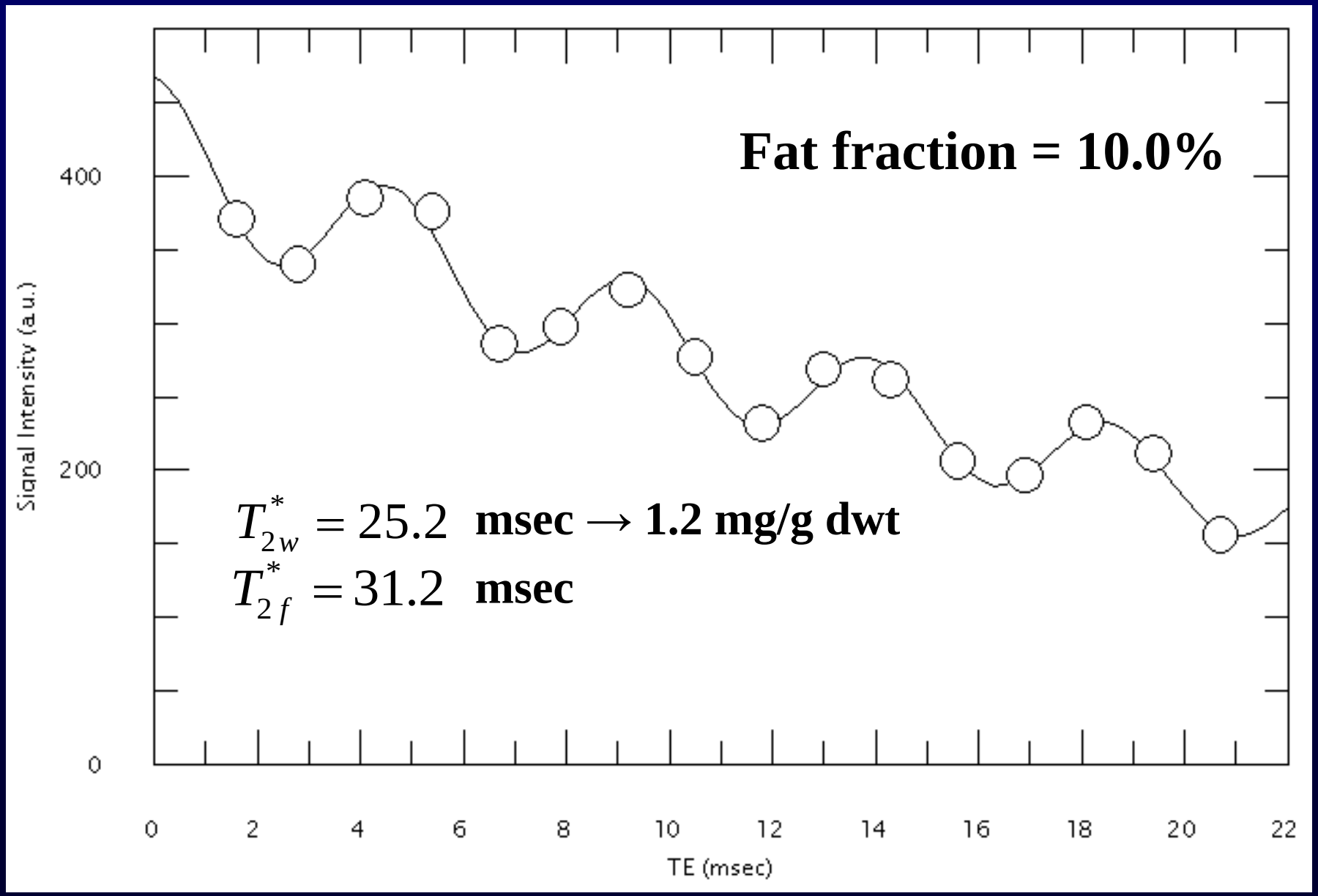


Out of phase image
TE=2,25 msec

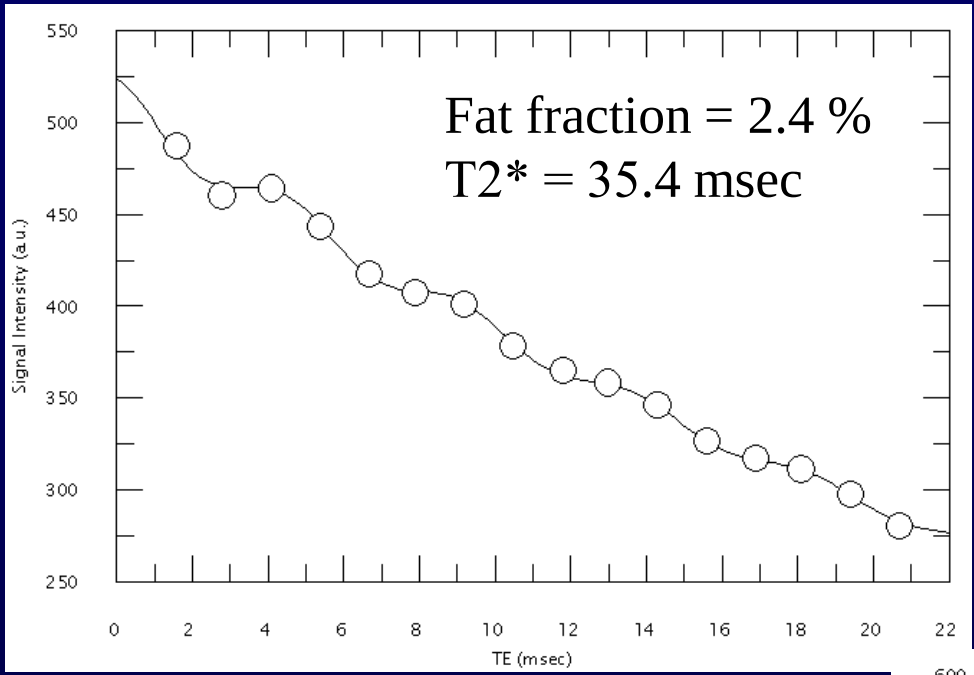


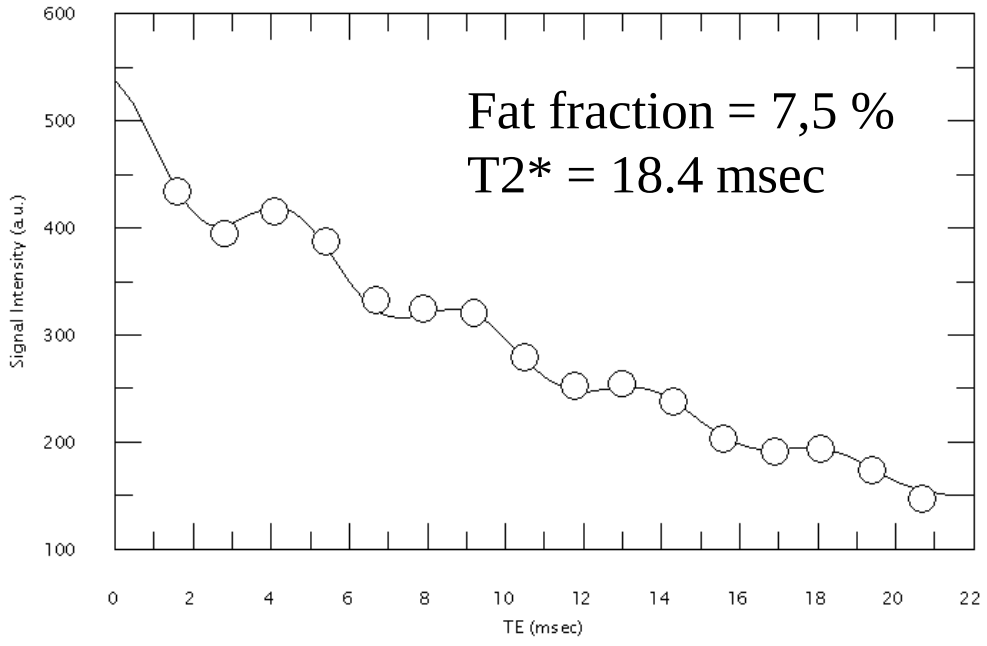
In phase Image
TE=4,5 msec

Patient without liver iron overload with fat infiltration

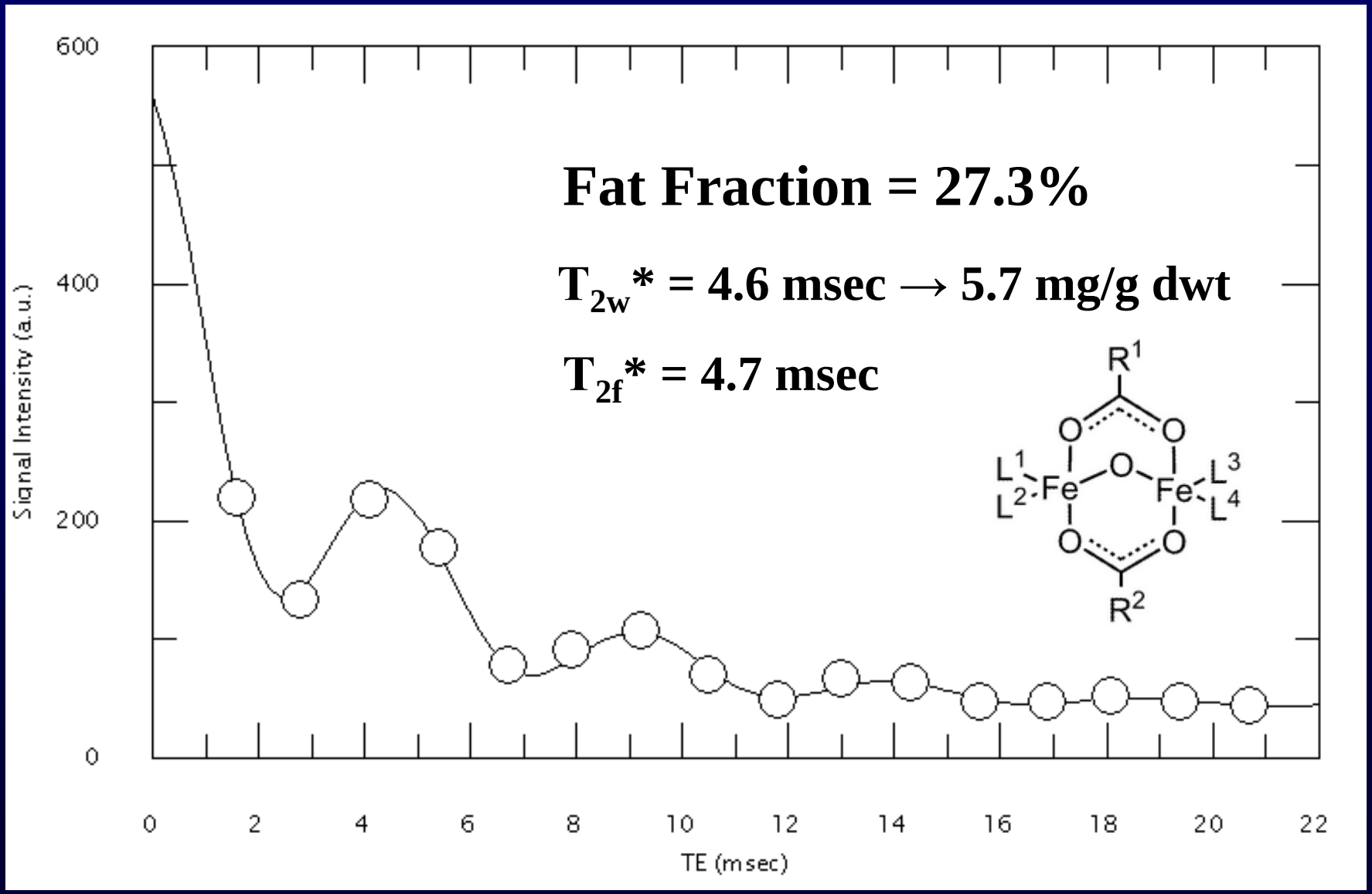


Patient without liver iron overload with fat infiltration





Patient with mild to moderate liver iron overload and fat infiltration



Conclusions

1. Both **FERRISCAN** (R_2) and **R_2*** are dependable methods for estimating iron overlaod

2. **FERRISCAN** costs approximately **200 euro** in addition to MRI that has to be performed anyway, and requires 10-25 additional min, depending on the protocol used (TR=2500 or TR=1000 msec)

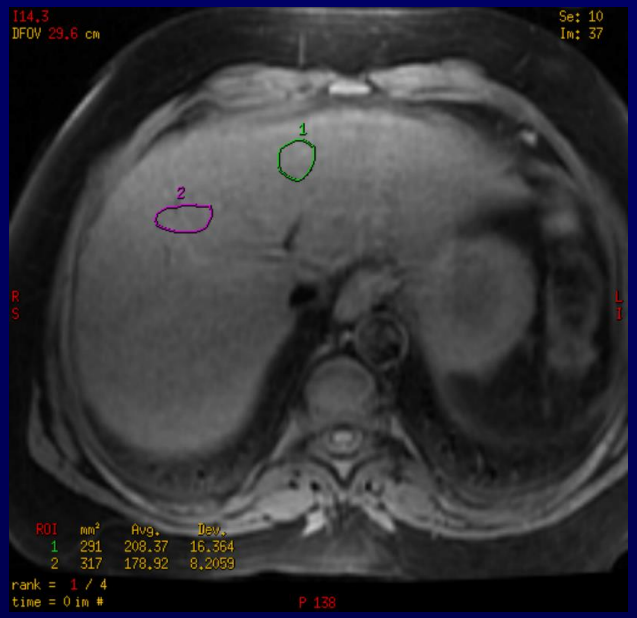
3. The multi-echo (16 echoes) gradient echo method can acquire the data rapidly in 1-4 breath-holds **and in addition can** determine fat infiltration quantitatively (hidden surprise)!

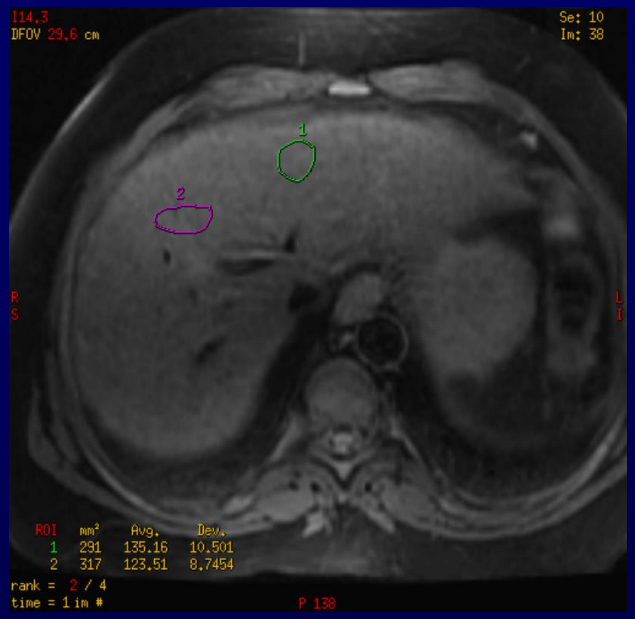
Conclusions

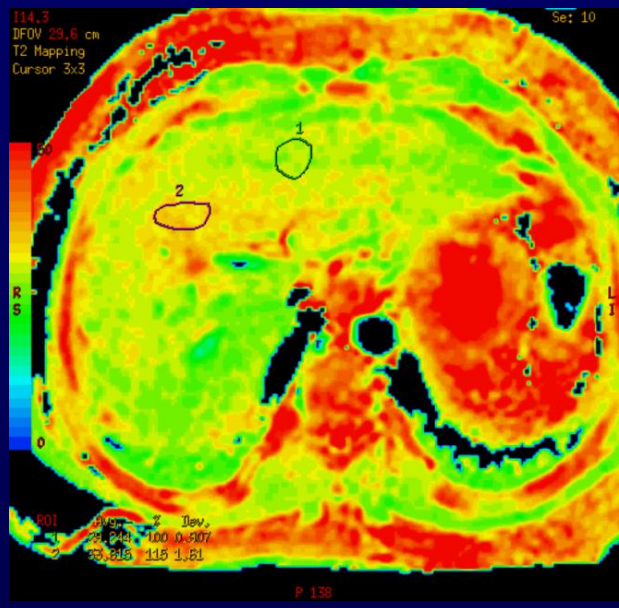
R_2 (**FERRISCAN but not only)** or R_2*;

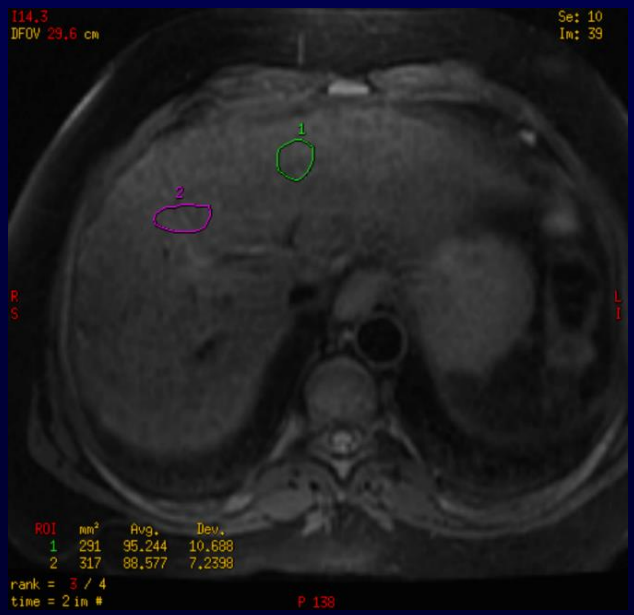
Dilemmas are not good for science! We should do both on each patient. Large deviations between R2 and R2* are due to hemosiderin as can be seen in the next two examples.

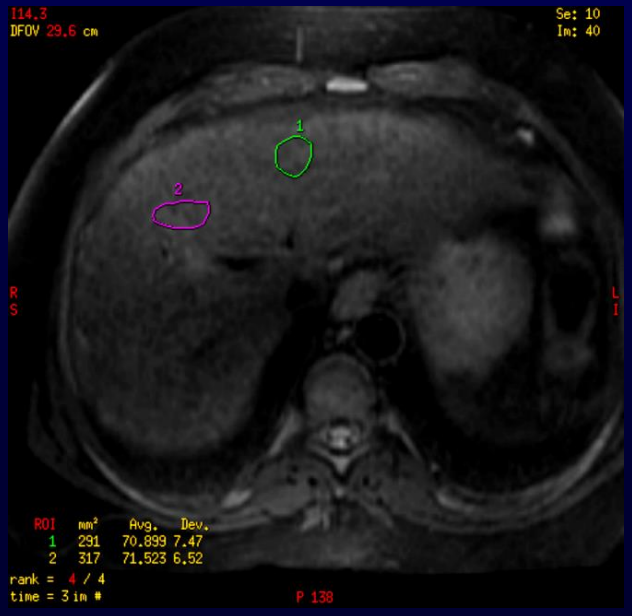
Example 1: T_2 = 34,9 msec (normal), T_2* = 11,0 msec (LIC = 2,5 mg/g dwt)

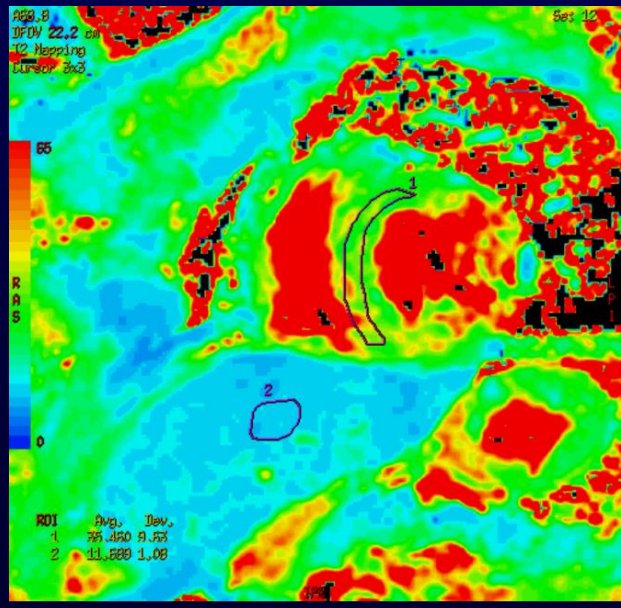




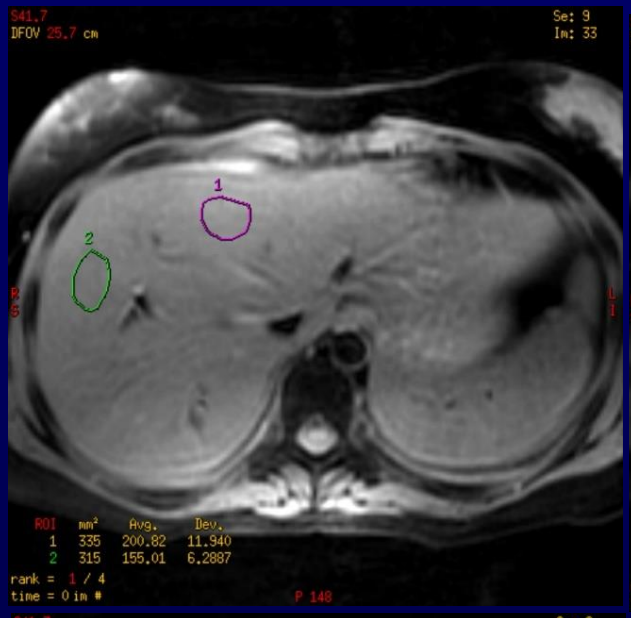


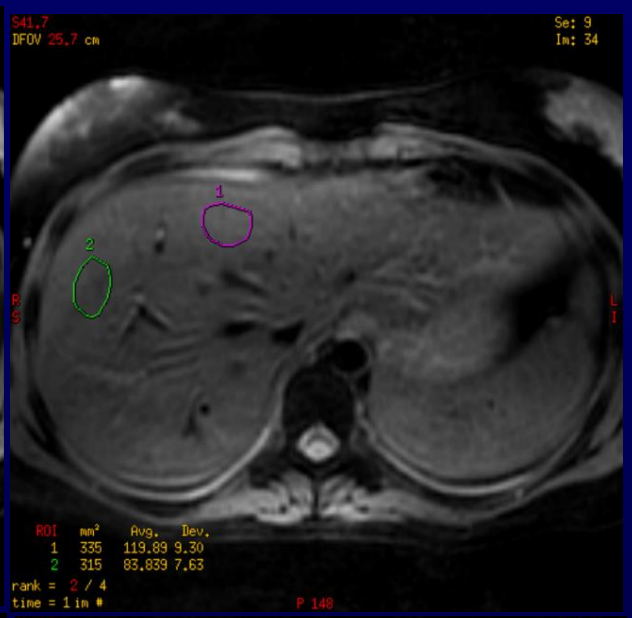


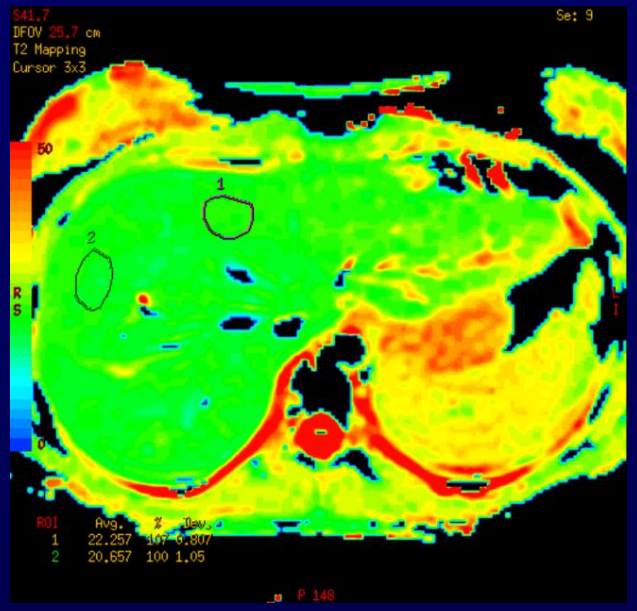


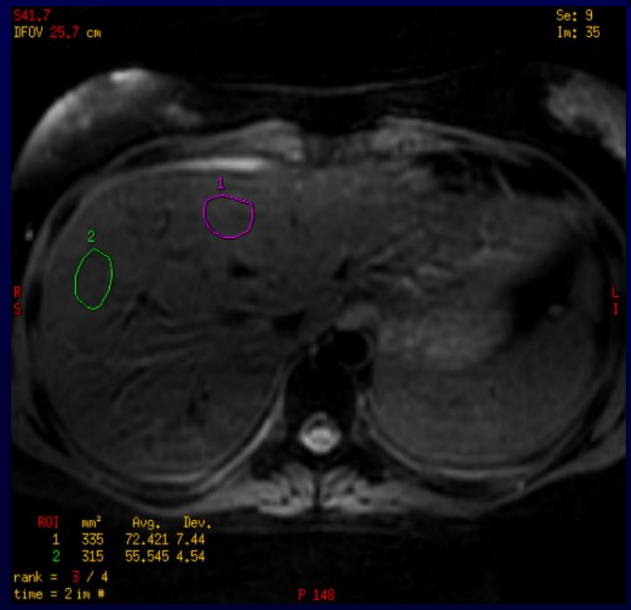


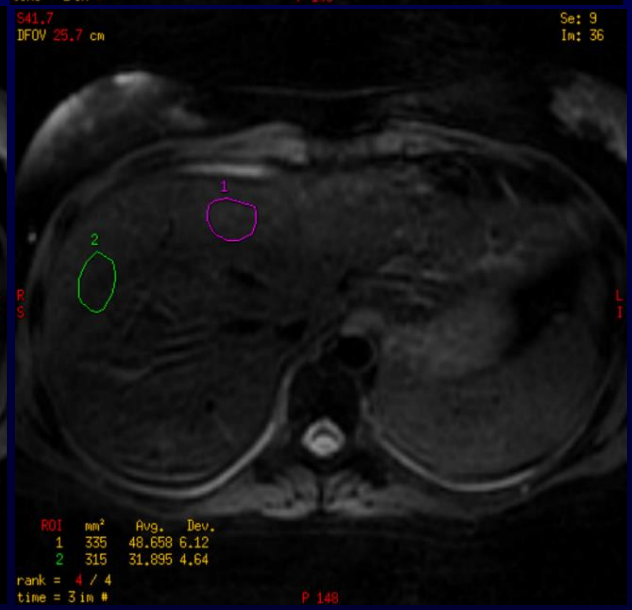
Example 2: T_2 = 23,6 msec, T_2* = 11,0 msec

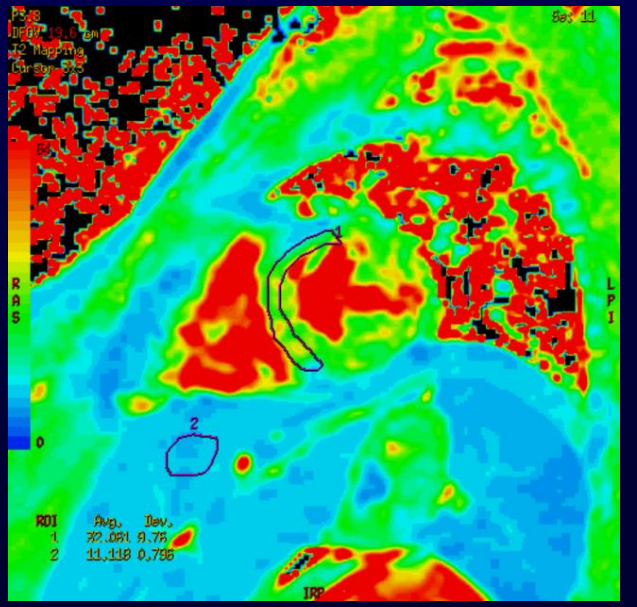












Thank you for your attention!