ORIGINAL CONTRIBUTION

Application of ^{31}P MRS to the Differential Diagnosis of Hepatic Disease

Kumiko HIRAISHI, Isamu NARABAYASHI, Takae MATSUOKA,
Kojiro TABUCHI, Yasuo UESUGI, Takeshi KAWAI

Department of Radiology, Osaka Medical College
2-7 Daigaku-cho Takatsuki, Osaka 569

Localized phosphorus-31 magnetic resonance spectroscopy of liver by DRESS (depth resolved surface coil spectroscopy) technique[1] was performed on fifty patients (twenty-eight malignant hepatic neoplasms (seventeen hepatocellular carcinomas, four cholangiocarcinomas and seven metastases from a variety of primary tumors), eighteen liver cirrhosis, four hemangiomas) and eleven healthy volunteers. The tumors selected for this study were larger than ¢ 8cm to minimize contamination from surrounding non-tumor tissues.

A marked increase in peak area ratio of PME/β-ATP and PME/PDE in malignant tumors compared with controls, which is consistent in previous reports[2)~6)], and a decrease in PDE/β-ATP in liver cirrhosis were observed. Hemangiomas were disposed to show high Pi peaks. In metabolite quantification and identification, ^{31}P MRS may contribute greatly to our understanding of the metabolic changes that occur in human liver disease. Combined use of PME/β-ATP and PDE/β-ATP ratios, eventually PME/PDE ratios may prove to be a good non-invasive marker in differentiating hepatic disease.

INTRODUCTION

Phosphorus-31 magnetic resonance spectroscopy (^{31}P MRS) allows non-invasive determination of phosphate-containing compounds *in vivo* and subsequent characterization of the energy state of organs. ^{31}P MRS has proved to be a useful method for studying hepatic metabolism in perfused organs and in the living subjects[7)~26)]. In order to determine the clinical feasibility and applicability of ^{31}P MRS and to assess its potential for characterization of human hepatic tissue, ^{31}P MR spectroscopy of liver was performed on patients suffered from a variety of hepatic disease and healthy volunteers.

The aim of this study is to assess whether

Keywords ^{31}P MRS, liver, hepatic disease, PME, PDE

^{31}P MRS contributes to differentiating hepatic disease.

METHODS

Combined MRI/^{31}P MRS examinations were performed on a G. E. SIGNA 1.5T superconducting system equipped with spectroscopy accessory. We employed a 3-inches circular surface coil, which acted both as radiofrequency transmitter and receiver. The coil was placed under the prone subject so as to cover the lesions best and to suppress respiratory motion of abdominal wall. The imaging studies were performed in all patients to determine the depth of interest and to reduce contamination from surrounding tissues. The homogeneity of the field was adjusted in all cases by shimming on the proton resonance from tissue water to the goal below 20Hz at half maximum height. Phosphorus-31 spectra were obtained from a 25mm slice thickness using the depth resolved surfacecoil spectroscopy (DRESS) technique[1]. The center frequency we used was 25.85MHz. for phosphorus. repetition time was 2s, dwell period and sampling time was 1.5ms and number of excitation was 256. The ^{31}P MRS data were processed with base line correction, exponential filtering (15Hz), zero filling, Fourier transformation and manual phase correction. A broad signal underlying the PDE region was removed by convolution difference before each spectrum was plotted. The peak areas were measured by pure Gaussian curve fitting. All of these steps were performed on a spectroscopy data station Nicolet 1280 computer (G.E. NMR Instruments, Fremont, Calf.). The relative amounts of PME (Phosphomonoesters), Pi (inorganic phosphate), PDE (Phosphodiesterters) and β-ATP (Adenosinetriphosphate) were quantified by measuring the ratios of the peak areas. Statistical difference between means were calculated by the Student's t-test for unpaired data. Localized ^{31}P MRS was measured in 17 hepatocellular carcinoma, 4 cholangiocarcinoma, 7 metastatic liver tumor, 18 liver cirrhosis and 4 hemangioma patients. And 11 volunteer subjects with no known history of liver disease were examined. The tumors selected for this study were superficial and larger than 8cm in diameter to minimize contamination from surrounding non-tumor tissues. All subjects fasted for 6 hours before examination. For diet can greatly affect the inorganic phosphate peak[7].

Each subject gave informed consent to the investigation beforehand.

RESULTS

The typical six resonance peaks (PME, Pi, PDE, α-ATP, γ-ATP and β-ATP) were detected from the liver in all cases (Fig.1). Although we were hampered somewhat by muscle contamination, significant trends can be appreciated. The relative amounts were calculated as the ratios of the peak ares. The mean metabolite ratios and standard deviation in control and each of the groups are shown in Table 1. The PME peak in the ^{31}P MRS was significantly higher in many of the patients with malignant hepatic neoplasms than in controls. Among malignant tumors,

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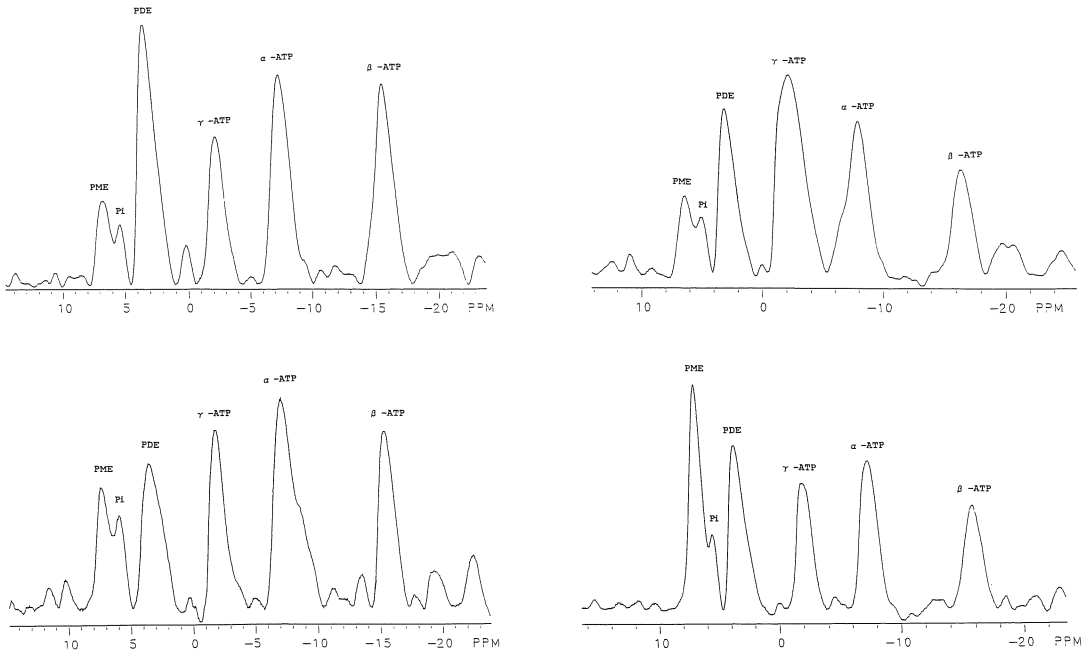

a | b
c | d

Fig.1. P-31 MR spectra from normal human liver (a), hemangioma (b), liver cirrhosis (c), hepatocellular carcinoma (d) demonstrated the typical six renonance peaks. Although there were some Pcr resonance from muscle contamination, significant trend can be appreciated. Note the significant elevation in PME peak observed in malignant hepatic tumor (d), whereas the spectrum of hemangioma (b) appeared normal. The characteristic of cirrhotic liver (c) is the decrease in PDE height compared with normal control, wihtout much elevation of PME.

Table 1. ^{31}P MRS quantitative data human liver

	N	PME/ β-ATP	PDE/ β-ATP	PME/PDE
Normal	11	0.454±0.186*	1.448±0.340	0.327±0.141
Benign	4	0.435±0.193	1.948±0.269	0.240±0.101
Cirrhosis	18	0.623±0.140	0.934±0.281	0.728±0.261
Malignant	28	1.326±0.611	1.160±0.486	1.223±0.593
HCC	17	1.326±0.563	1.207±0.563	1.265±0.625
CC	4	1.415±0.594	0.738±0.189	1.558±0.482
Meta	7	1.276±0.718	1.289±0.187	0.954±0.429

*Mean±SD
HCC : Hepatocellular carcinoma
CC : Cholangiocarcinoma
Meta : Metastatic tumor

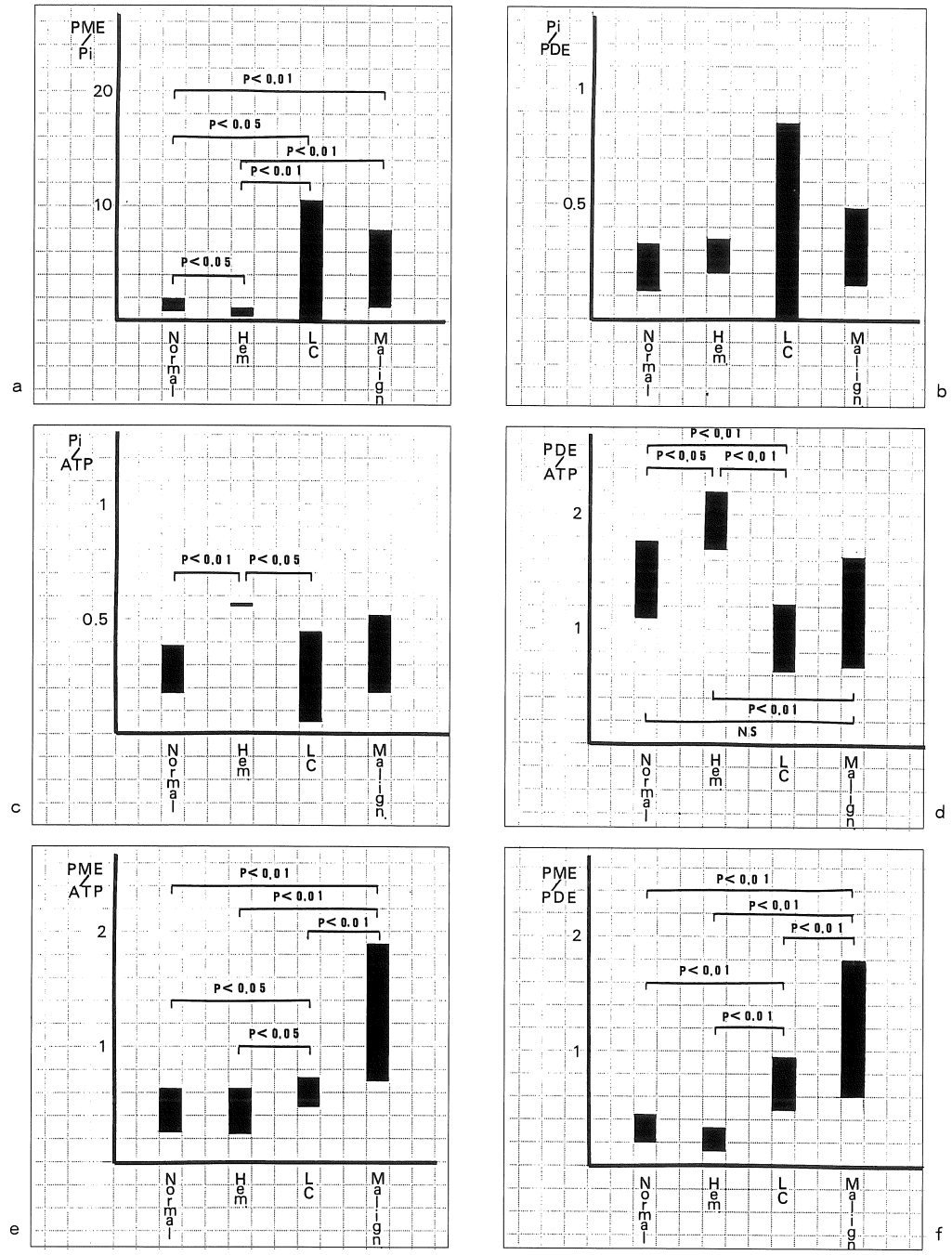

Fig.2. Statistical difference between each hepatic disease were calculated by the Student's t-test for unpaired data. The combined use of PME/β-ATP and PDE/β-ATP, in substitution PME/PDE ratio may prove to be a non-invasive characteristic index to differentiate hepatic disease.

though cholangiocarcinoma (CC) trend to show lower PDE/β-ATP ratio than hepatocellular carcinoma (HCC) and metastatic tumor (Meta), the tumorous pathology on the basis of spectral characteristics and metabolic ratios between HCC and Meta from various primary neoplasms could not be differentiated. So HCC, CC and Meta were lumped as malignant tumor group and the statistical difference were calculated among four groups each other— malignant hepatic tumors (Malign.), benign hepatic hemangioma (Hem.), liver cirrhosis (LC) and healthy controls (Normal), as shown in Fig.2.

The spectra from benign hepatic hemangioma appeared almost normal, with somewhat higher Pi/β-ATP and PDE/β-ATP than normal. In liver cirrhosis, PME/β-ATP did not rise so much as malignancy but higher than normal, and PDE/β-ATP decreased significantly compared with controls. We can differentiate LC from normal and hemangioma by PDE/β-ATP, malignancy from the others by PME/β-ATP and hemangioma from normal by Pi/β-ATP and PDE/β-ATP. PME/PDE was proved to be a good characteristic but it concealed the difference between normal and hemangioma.

Fig.3 summarizes the main results of this study. Calculated PDE/β-ATP are plotted against PME/β-ATP for patients with malignant tumors, hemangioma and liver cirrhosis and for control subjects. The plot can be divided four sections as shown. The upper left sector contains hemangiomas, the middle left section indicates normal subjects, the lower left sector stands for liver cirrhosis and the right section represents malignant tumors.

In conclusion, the combined use of PME/β-ATP and PDE/β-ATP ratios, simply PME/PDE in substitution, may be good indi-

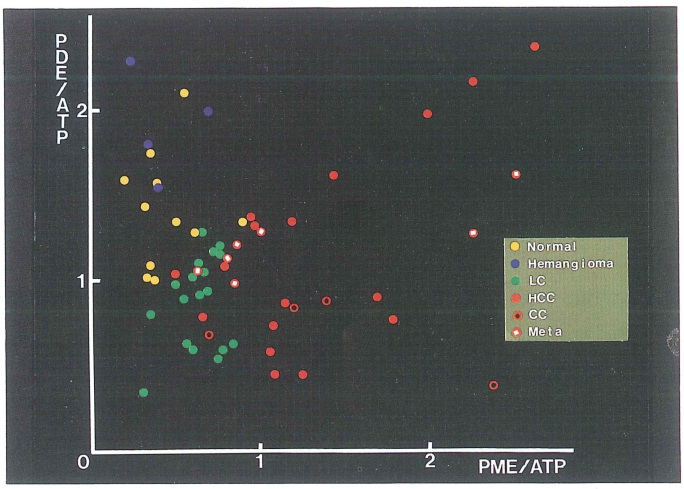

Fig.3. PDE/β-ATP ratios plotted against PME/β-ATP ratios. The plot can be divided to three sections as shown — upper left section is mainly occupied by normal and benign groups, lower left section contains cirrhotic group and right section represents malignant group.

cators in distinguishing hepatic diseases.

These results suggest that ^{31}P MR spectroscopy may be useful in the non-invasive assessment of liver disease.

DISCUSSION

^{31}P MR spectroscopy provides metabolic information in human liver cells non-invasively. Previous investigation have been reported intravenous fructose loading test for valuation of liver function[8)~10)]. Many of the authors have mentioned that PME peaks elevated above normal in malignant tumors relative to the other metabolites[7),11),23)~26)], but relatively increased PME levels are not specific indicator for malignancy.

Restricted to liver, increased PME is also seen in other hepatic diseases such as sclerosing cholangitis, Caroli disease, fatty liver and alcoholic hepatitis[18)~24)].

Previous researches have observed that phosphocholine (PC) and phosphoethanolamine (PE) are the prominent components of the PME peaks which are concerned with phospholipid biosynthesis, whereas glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE) are the major constituents of the PDE peak which are intermediates in phospholipid breakdown[26),27)].

The level of PME which represents the pool size of phospholipid precursors is pertinent valuation in the pre-membranation period. The PDE level which is consequent on the catabolism of membranous phospholipids exhibitis conspicuous valuation at later stages, corresponding to the period of vigorous biosynthesis of membrane components[28)]. It has suggested that changes in the relative concentrations of these compounds may reflect changes in rates of membrane synthesis, degeneration and modulation[23),26)].

Thus much of the recent interest in ^{31}P human spectroscopy surrounds the behavior of the PME and PDE peaks and definitive assignments of the resonances is obviously important.

Iles et al.[22)] have observed that PME increased and PDE decreased in regenerating liver after hepatectomy similar to the developing neonatal liver[29)]. On the contrary the elevation of PDE was found in growth retarded plancentae[30)]. Thus phospholipid breakdown products (PDE) reduction appears to correspond to rapid cell turnover including hepatic regeneration.

Our results were coincident with these reports — the levels of PME were significantly elevated in malignant hepatic tumors and the levels of PDE deteriorate in liver cirrhosis.

Concerning hemangiomas, the spectra appeared almost normal, with somewhat higher Pi/β-ATP and PDE/β-ATP than normal, which may be caused by contamination from 2, 3 diphosphoglycerate (DPG) in blood[16)]. Glazer et al.[24)] expressed that hemangioma showed very low signal to noise ratio (S/N) compared with spectra acquired from an identical depth in a normal volunteer, which could not be confirmed in our study.

In damaged liver, we guess that the decrease of PDE/β-ATP may arise in the first step to maintain function by regeneration of hepatocytes, and that according as the regeneration process advances to lose turnover rate, the value seems to return to normal. Theoretically if still more the damage progresses so far as to necrosis and so on, it exceeds the ability for compensation and

results in PDE/β-ATP increase because the decrease of β-ATP overcomes that of PDE. But we experienced no case of liver cirrhosis with PDE/β-ATP increase.

There might be much more complex reasons for the change of these values such as diminution of absolute PDE in quantity by infiltration of fat or collagen with preservation of β-ATP level to some extent. The decrease of ATP in hepatic disease may be due to decreased oxidative phospholylation induced by decreased oxygen consumption in the damaged liver[17]. Similarly a slight increase of PME/β-ATP leads to be a significant one by a decrease of β-ATP, as hepatic damage longstands, with the consequence that breeds HCC. Undoubtedly the increase of PME/β-ATP in malignant tumors is not only due to liver damage but also to abnormality in metabolism of memberaneous phospholipids. The increase in PME ratios in liver disease may reflect a rise in the hepatic concentrations of PME or fall in the concentrations of all the other phosphorus-containing metabolites relative to PME and/or both. The PME increase may be caused by an overproduction of abnormal phospholipid precursors which could not have been utilized for membraneous biosynthesis in an orderly manner. The β-ATP decrease may be due to exhaustion because of necrosis and so on.

And there is another possibility occured by saturation effect that may conceal real concentration change. TR of 2000msec. used in our study is not long enough and probably introduce some saturation effects. If T_1 relaxation time may be prolonred in diseased liver, the magnetization will not have fully recovered and thus the actual concentration will be understimated. It is to be desired that the interpulse delay is longre than about five times T_1[21),31)]. Thus variable saturation due to metabolite relaxation time differences will influence the relative peak area, particularly for long-T_1 metabolites. Though we did not caluculate in this study, the ratio at two different TRs (TR values) might be better indicators regardless of T_1 relaxation times of phosphorus containing metabolites.

Moreover in relative investigation, if the concentrations of compounds rise or fall together, the ratios may be unchanged. Meyerhoff et al. have described that absolute hepatic ^{31}P metabolite concentrations in patients suffering from alcoholic hepatitis and alcoholic liver cirrhosis were generally decreased by 20-40% compared to normal controls, whereas intensity ratios of metabolites in diseased liver did not differ significantly from those in normal liver[18),19)]. So it is necessary to invenstigate absolute value to confirm how the each value fluctuate. But the absolute metabolite concentration value was not calculated in this study because its measurement with surface coil requires a precise knowledge of the volume from which signal is collected and the degree of surface contamination. It was difficult to define by DRESS technique. And surface coil placement was also of key importance. A major disadvantage of the surface coil technique was that signal was received only from a limited area and depth and is dependent on the coil diameter. Only signal from the region within the coil diameter up to a depth of approximately one radius from the coil could be recieved. In DRESS technique we could only select the depth of interest without knowing how wide the area parallel to the surface coil spreaded. It suggested that there

were unexpected contamination and spectral degradation from abjacent liver tissue or other extrahepatic structures.

Though there are many problems, so far as relative phase, PME/β-ATP ratios can be used to differentiate malignant hepatic tumors from the other diseases, and PDE/β-ATP ratios can be used to differentiate liver cirrhosis from the other groups respectively. Therefore the combined use of PME/β-ATP and PDE/β-ATP ratios is useful in distinguishing hepatic diseases non-invasivaly. Quantitative ^{31}P MR spectroscopy provides important information about metabolic abnormalities associated with human liver disease. We are confident that the distinct advantage of being able to directly follow phosphate metabolism in the liver will prove valuable in detecting liver disease.

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REFERENCES

1) Bottomley PA, Foster TB, Darrow RD : Depth-resolved surface coil spectroscopy (DRESS) for *in vivo* 1H, 31P, and 13C NMR. J Magn Reson, 59: 338-342, 1984.

2) Oberhaensli RD, Hilton JD, Bore PJ, Hands LJ, Rampling BP, Radda GK : Biochemical investigation of human tumors *in vivo* with phosphorus-31 magnetic resonance spectroscopy. Lancet, 2 : 8-11, 1986.

3) Degani H, Horowitz A, Hzchak Y : Breast tumors : Evaluation with P-31 MR spectroscopy. Radiology, 161 : 53-55, 1986.

4) Semmler W, Gademann G, Bachert-Baumann P, Zable HJ, Lorenz WJ, Kaick G : Monitoring human tumor response to therapy by means of P-31 MR spectroscopy. Radiology, 166 : 533-539, 1988.

5) Semmler W, Gademann G, Schlag P, Bachert-Baumann P, Zabel HJ, Lorenz WJ, Kaick GV : Impact of hyperthermic regional perfusion therapy on cell metabolism of malignant melanoma monitored by ^{31}P MR spectroscopy. Magn Reson Imaging, 6 : 335-340, 1988.

6) Dewhirst MW, Sostman HD, Leopold KA, Charles HC, Moore D, Burn RA, Tucker JA, Harrelson JM, Oleson JR : Soft-tissue sarcomas : MR imaging and MR specteroscopy for prognosis and therapy monitoring. Radiology, 174 : 847-853, 1990.

7) Cox IJ, Sargentini J, Calam J, Bryant DJ, Iles RA: Four dimensional phosphorus-31 chemical shift imaging of carcinoid metastases in the liver. NMR Biomed, 1 : 56-60, 1988.

8) Oberhaensli RD, Galloway GJ, Tailor DJ, Bore PJ, Radda GK : Assessment of human liver metabolism by phosphorus-31 magnetic resonance spectroscopy. Br J Radiol, 59 : 695-699, 1986.

9) Oberhaensli RD, Rajagopalan B, Tatlor DJ, Radda GK : Study of hereditary fructose intolerance by use of ^{31}P magnetic resonance spectroscopy. Lancet, 24 : 931, 1987.

10) Terrier F, Vock P, Cotting J, Ladebeck R, Reichen J, Hentschel D : Effect of intravenous fructose on the P-31 MR spectrum of the liver : Dose response in healthy volunteers. Radiology, 171 : 557-563, 1989.

11) Cohen SM : Application of nuclear magnetic resonance to the study of liver physioligy and disease. Hepatology, 3 : 738-749, 1983.

12) Iles RA, Stevens AN, Griffiths JR, Morris PG :

Phosphorylation status of liver by ^{31}P NMR spectroscopy, and its implications for metabolic control. Biochem J 229 : 141-151, 1985.

13) Malloy CR, Cunningham CC, Radda GK : The metabolic state of the rat liver *in vivo* measured by ^{31}P NMR spectroscopy. Biochimica et Biophysica Acta, 885 : 1-11, 1986.
14) Segebarth C, Grivegnee A, Luyten PR, den Hollander JA : ^{1}H image-guided localized ^{31}P MR spectroscopy of the human liver. Magn Reson Med Biol, 1 : 7, 1988.
15) Ross BD : The current state of clinical magnetic resonance spectroscopy with phosphorus-31 : A view from Hammersmith. Magn Resn Med Biol, 1 : 81-98, 1988.
16) Thompson RT, Schneeberger AL, Marsh GD, Driedger AA, Inculet RI : Serial investigation of hepatic metabolism using P-31 NMR spectroscopy (abstr). Book of abstracts : Soc Magn Reson Med, 1 : 400, 1988.
17) Moreau R, Lee SS, Soupison T, Roche-Sicot J, Sicot C : Abnormal tissue oxygenation in patients with cirrhosis and liver failure. J Hepatol, 7 : 98-105, 1988.
18) Meyerhoff DJ, Boska M, Thomas A, Twieg DB, Rockey D, Weiner MW, Decreased phosphorus metabolite concentration in acute alcoholic hepatitis and cirrhosis of the liver-P-31 MRS with ISIS and spectroscopic imaging (absrt). Soc Magn Reson Med, 2 : 621, 1988.
19) Meyerhoff DJ, Boska MD, Thomas AM, Weiner MW : Alcoholic liver disease : Quantitative image-guided P-31 MR spectroscopy. Radiology, 173 : 393-400, 1989.
20) Dixon RM, Rajagopalan B, Angus PW, Simpson KJ, Peters TJ, Radda GK : ^{31}P magnetic resonence spectroscopy of patients with alcoholic liver disease ; The phosphomonoester/inorganic phosphate ratio is related to severity of liver damage (abstr). Soc Magn Reson Med, 2 : 622, 1988.
21) Angus PW, Dixon RM, Rajagopalan B, Ryley NG, Simpson KJ, Peters TJ, Jewell DP, Radda GK : A study of patients with alcoholic liver disease by ^{31}P nuclear magnetic resonance spectroscopy. Clin. Sci, 78 : 33-38, 1990.
22) Iles RA, Cox IJ, Bell JD, Dubowitz LMS, Cowan F, Bryant DJ : ^{31}P mgnetic resonance spectroscopy of the human paediatric liver. NMR Biomed, 3 : 90-94, 1990.
23) Meyerhoff DJ, Karczmar GS, Matson GB, Boska MD, Weiner MW : Non-invasive quantitation of human liver metabolites using image-guided ^{31}P magnetic resonance spectroscopy. NMR Biomed, 3 : 17-22, 1990.
24) Glazer GM, Smith SR, Chenevert TL, Martin PA, Stevens AN, Edwards RHT : Image localized ^{31}P magnetic resonance spectroscopy of the human liver. NMR Biomed, 1 : 184-189, 1989.
25) Francis IR, Chenevert TL, Gubin B, Collomb L, Ensminger W, Andrews SW, Glazer GM : Malignant hepatic tumors : P-31 MR spectroscopy with one-dimensional chemical shift imaging. Radiology, 180 : 341-344, 1991.
26) Schilling A, Gewiese B, Berger G, Boese-Landgraf J, Fobbe F, Stiller D, Gallkowski U, Wolf KJ : Liver tumors : Follow-up with P-31 MR spectroscopy after local chemotherapy and chemoembolization. Radiology, 182 : 887-890, 1992.
27) Daly PF, Lyon RC, Faustino PJ, Cohen JS : Phospholipid metabolism in cancer cells monitored by ^{31}P NMR spectroscopy. J Biol Chem, 262 : 14875-414878, 1987.
28) Carpinelli G, Podo F, Giannini M, Granta F, Cibati M, Augusti Tocco G : ^{31}P NMR studies during the development of chick embryo nervous system (abstr). Book of abstracts : Soc Magn Reson Med, 1 : 501, 1988.
29) Murphy E, Rajagoplan B, Brindle K, Harington R, Radda GK : ^{31}P-MRS of regenerating rat liver (abstr). Book of abstracts : Soc Magn Reson Med, 2 : 626, 1988.
30) Raman J, Trupin SR, Roman BB, McFarlane B, Dawson MJ : ^{31}P-NMR spectral abnormalities of human placenta associated with specific pregnancy disorders (abstr). Book of abstracts : Soc Magn Reson Med, 1 : 346, 1988.
31) Cox IJ, Coutts GA, Gadian DG, Ghosh P, Sargentoni J, Young R : Satulation effects in phosphorus-31 magnetic resonance spectra of the human liver. Magn Reson Med 17 : 53-61, 1991.