INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 14: 1065-1071, 2004

Molecular characterization of human gastric mucosa by HR-MAS magnetic resonance spectroscopy

V. TUGNOLI[1], A. MUCCI[2], L. SCHENETTI[2], C. CALABRESE[3], G. DI FEBO[3], M.C. ROSSI[4] and M.R. TOSI[5]

[1]Dipartimento di Biochimica 'G. Moruzzi', via Belmeloro 8/2, I-40126 Bologna; [2]Centro SCS, Dipartimento di Chimica, Università di Modena e Reggio Emilia, via G. Campi 183, I-41100 Modena; [3]Dipartimento di Medicina Interna e Gastroenterologia, Università di Bologna, via Massarenti 9, I-40138, Bologna; [4]Centro Interdipartimentale Grandi Strumenti, Università di Modena e Reggio Emilia, via G. Campi 213/A, I-41100 Modena; [5]ITOI-CNR, Sez. di Bologna, c/o IOR, via di Barbiano 1/10, I-40136 Bologna, Italy

Received August 2, 2004; Accepted September 27, 2004

Abstract. The present study was aimed at identifying the molecular profile characteristic of the healthy human gastric mucosa. *Ex vivo* HR-MAS magnetic resonance spectroscopy performed at 9.4 Tesla (400.13 MHz for ^{1}H) on gastric specimens collected during endoscopy, permits the identification of more than forty species giving a detailed picture of the biochemical pattern of the gastric tissues. These preliminary data will be used for a comparison with gastric preneoplastic and neoplastic situations. Moreover, the full knowledge of the biochemical pattern of the healthy gastric tissues is the necessary presupposition for the application of magnetic resonance spectroscopy directly *in vivo.*

Introduction

The neoplasms of the human gastrointestinal tract are an important public health problem due to their high incidence (1) and the high rates of recurrence and metastasis after resection, the outcome of treatment being highly dependent on correct and early diagnosis. The detection of early lesions, without lymph node involvement or distant metastases, will result in a reduction of mortality (2). Thus, achieving a better understanding of the molecular features of gastric and colorectal tumors and the development of reliable methods for early detection is highly desiderable.

In vivo magnetic resonance spectroscopy (MRS) is a molecular investigation method well suited for attempting to correlate the biochemical composition of tissues with their diseases with particular regard to neoplasms (3). The clinical impact of MRS in medicine has been widely reviewed by Smith and Steward (4) and is currently showing promise as method to complement routine diagnostic tools such as endoscopy and histopathology. It provides biochemical information, and is thus in a position to detect early, premorphological molecular changes in tissue. MRS of cultured cells and of biopsy specimens from humans have demonstrated great sensitivity to the onset of malignancy by comparing human healthy and neoplastic tissues (5). Whereas organs, such as the brain (6-10), prostate (11,12) and breast (13-15) have been widely studied, and MRS is used routinely in clinical medicine to analyse them, MRS in the evaluation of neoplasms of human gastrointestinal mucosa is in its infancy.

Recently, an *ex vivo* ^{1}H MRS study of human gastric carcinomas was reported (16). Notwithstanding this work was aimed to assess the clinical importance and usefulness of the MRS in the evaluation of human gastric carcinoma, we believe that a better precision in the metabolite assignments is desiderable.

In vivo MRS can be used when the molecular markers of the tissues are well established by means of a detailed biochemical picture. This is performed, in a first stage, by the spectroscopic analysis on the extracts obtained from the tissues (*in vitro* MRS) or directly on tissue specimens (*ex vivo* MRS). High resolution magic angle spinning (HR-MAS) *ex vivo* MRS is a powerful analytical tool for human tissues potentially bridging the divide between *in vitro* and *in vivo* MRS. The first applications of HR-MAS MRS on human tissue specimens date back to 1997 (17,18) and have become possible thanks to the commercial diffusion of MRS probe-heads capable of studying the samples in rapid rotation around an axis 54.7˚ ('magic angle') tilted with respect to that of the static magnetic field. These probe-heads drastically reduce contributions from dipolar couplings and chemical shift anisotropy providing high resolution spectra from semi-solid samples such as tissues (19-26). The quality of the obtained spectra is comparable to that obtained from aqueous extracts (*in vitro* MRS), and the acquisition techniques which is possible to utilize are the same, with the advantage of carrying out the measurements on intact tissue specimens, without any pretreatment.

Correspondence to: Dr V. Tugnoli, Dipartimento di Biochimica 'G. Moruzzi', via Belmeloro 8/2, I-40126 Bologna, Italy
E-mail: vitaliano.tugnoli@unibo.it

Key words: gastric mucosa, *ex vivo* HR-MAS MRS, biochemical characterization

The ability of the *ex vivo* HR-MAS MRS analysis is to relate the biochemical composition with quantitative histopathologic findings from the same tissues to determine the metabolic profiles associated with functional, benign and malignant tissues. Correlations between some metabolites and proliferative markers to gain insight into the relationships between cellular proliferation and the metabolic changes associated with the presence and aggressiveness are thus possible.

To our knowledge, no studies have been reported in the international scientific literature dealing with the application of *ex vivo* HR-MAS MRS in the characterization of the biochemical composition of human gastrointestinal mucosa. From this perspective our research group will investigate the biochemical composition of normal human gastric mucosa by means of *ex vivo* HR-MAS MRS. As a part of a more comprehensive study on the biochemical characterization of human healthy and neoplastic tissue (5,27-40), the aim of this study is to characterize the molecular pattern of a healthy human gastric mucosa for the comparison with gastric adenocarcinomatous tissues which will be studied in a near future.

Materials and methods

Clinical materials

Selection of the patients. Five patients were enrolled and gave written informed consent to participate in the study which was approved by the local research ethics committee. For each eligible patient, 6 biopsies were taken (3 biopsies from the antrum and 3 biopsies from the gastric body) during endoscopy. Among them, 4 have been used for routine histologic evaluation (hematoxylin-eosin) and 2 were put in liquid nitrogen and stored at -85˚C. Endoscopy was performed using Olympus Instruments 140 series and biopsy forceps Olympus FB24Q, after sterilization in acetylacetic acid. The biopsies were obtained, on healthy tissues, in the subjects who do not present visible macroscopic alterations and the tissue was considered healthy only if the histological examination confirmed the macroscopic finding.

Tissue samples. The tissue samples were put in liquid nitrogen and stored at -85˚C until MRS analyses. Before MRS analyses all samples were flushed with D_2O prior to the insertion in MAS rotor (4 mm OD). Samples and instrument preparation take about 20 min.

Magnetic resonance spectroscopy. Proton HR-MAS spectra were recorded with a Bruker Avance400 spectrometer equipped with a $^1H/^{13}C$ HR-MAS probe, utilizing the sequences implemented in Bruker software. Samples were spun at 4000 Hz and three different types of 1D (monodimensional) proton spectra were acquired by using: i) a composite pulse sequence (zgcppr) (41) with 1.5 sec water-presaturation during relaxation delay, 8 kHz spectral width, 32 k data points, 32-64 scans; ii) a water-suppressed spin-echo Carr-Purcell-Meiboom-Gill sequence (42) (cpmgpr) with 1.5 sec water-presaturation during relaxation delay, 1 msec echo time (τ) and 360 msec total spin-spin relaxation delay (2nτ), 8 kHz spectral width, 32 k data points, 256 scans; and iii) a sequence for diffusion measurements based on stimulated echo and bipolar-gradient pulses (ledbpgp2s1d) (43) with Δ 200 msec, eddy current delay T_e 5 msec, δ 2x2 msec, sine-shaped gradient with 32 G/cm followed by a 200 μsec delay for gradient recovery, 8 kHz spectral width, 8 k data points, 256 scans. Two-dimensional $^1H,^1H$-COrrelation SpectroscopY (COSY) (44,45) spectra were acquired using a standard pulse sequence (cosygpprqf) and 0.5 sec water-presaturation during relaxation delay, 8 kHz spectral width, 4 k data points, 32 scans per increment, 256 increments. Two-dimensional (2D) $^1H,^1H$-TOtal Correlation SpectroscopY (TOCSY) (46,47) spectra were acquired using a standard pulse sequence (mlevphpr) and 1 sec water-presaturation during relaxation delay, 100 msec mixing (spin-lock) time, 4 kHz spectral width, 4 k data points, 32 scans per increment, 128 increments. Two-dimensional J-resolved (J-Res) spectra were acquired using the standard pulse sequence (lcjresprqf) (48) 1.5 sec water-presaturation during relaxation delay, 3.6 kHz spectral width in f_2, 61 Hz in f_1, 4 k data points, 16 scans per increment and 64 increments. Two-dimensional $^1H,^{13}C$-Heteronuclear Single Quantum Coherence (HSQC) (49) spectra were acquired using an echo-antiecho phase sensitive standard pulse sequence (hsqcetgp) and 0.5 sec relaxation delay, 1.725 msec evolution time, 4 kHz spectral width in f_2, 4 k data points, 128 scans per increment, 17 kHz spectral width in f_1, 256 increments.

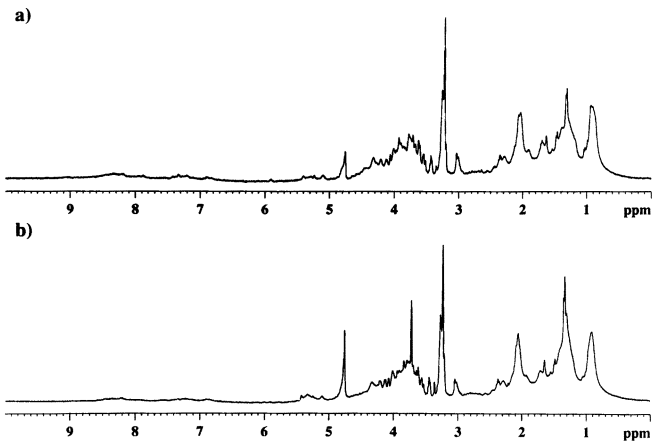

Figure 1. HR-MAS proton MR spectra of normal human gastric mucosa (a, sample 1, and b, sample 2).

Results and Discussion

The 1D proton spectra obtained from two representative samples of normal gastric mucosa (1 and 2), acquired at time = 0 by using a presaturation sequence with composite pulse (Bruker zgcppr), are reported in Fig. 1.

The spectra of these samples, which are representative of the all that analysed, show the presence of the resonances deriving from metabolites and macromolecules with short T_2, but not so short to be MRS invisible, and are characterised by signals with a resolution comparable to that obtained for solution spectra. Furthermore, it is to note that in stomach tissues the lipid content is very low in comparison with that of other metabolites, as can be derived from the low intensity ratio between signals at 1.3 ppm $(CH_2)_n$, and at 0.89 ppm CH_3

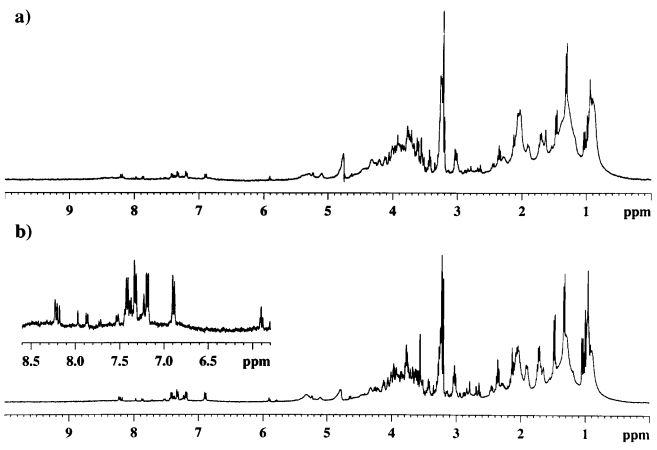

Figure 2. HR-MAS proton MR spectra of sample 1: (a), after 3 h and (b), after 6 h.

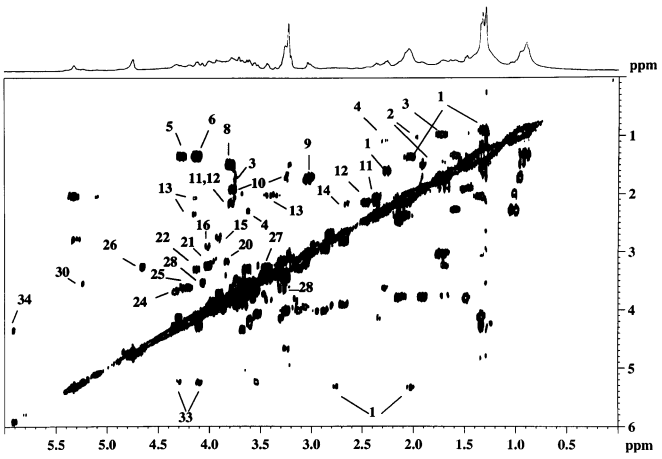

Figure 4. Partial COSY MR spectrum of sample 4. The identified metabolites are denoted with labels according to Table I.

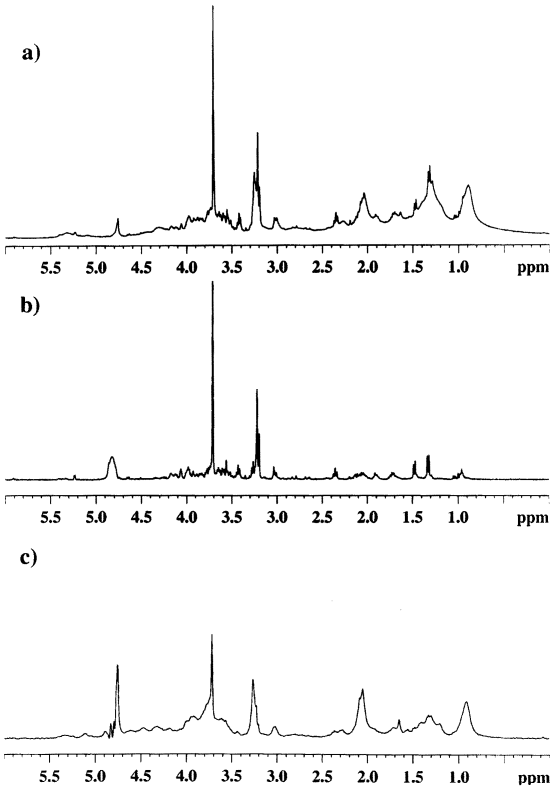

Figure 3. 1D proton MR spectra of sample 3: (a), conventional ^{1}H MR spectrum obtained with water-presaturation and composite pulse, (b) CPMG spectrum obtained with 360 msec total spin-echo time and (c) diffusion-edited spectrum obtained with Δ=200 msec, δ=4 msec and gradient strength of 32 G/cm.

(usually around 3:1). In order to evaluate the stability of the samples in the HR-MAS probe, the spectra were obtained at different times from their preparation. The spectra of sample 1 obtained after 3 h (trace a) and after 6 h (trace b) from the preparation are reported in Fig. 2. These spectra evidence a progressive increase of spectral resolution probably related to the increasing mobility of some metabolites, as a consequence of the partial disruption of subcellular structures. In any case, the lactate content does not appreciably change, whereas changes related to the mobility enhancement of amino acidic fractions are detected: an increase of glycine, alanine and other aliphatic and aromatic amino acids, and free choline is observed. We are not able to give a straightforward explanation of this behaviour; however we recall that the progastrin is cleaved at pairs of basic amino acids by a pro-hormone converter to form a glycine intermediate (G-Gly) that serves as a substrate for peptide-glycine α-amidating mono oxigenase (50). Since an overexpression of gastrin, known to promote the growth of several neoplasms, in human gastric carcinomas has been demonstrated (51), the presence of glycine could merit further investigations.

In order to separate the contribution of resonances due to macromolecules having short spin-spin relaxation times, spectra were acquired using a CPMG spin-echo sequence $[90^{\circ} - (\tau\text{-}180^{\circ}\text{-}\tau)_n]$, choosing τ and n in order to separate signals according to their different T_2. Signals from macromolecules, having short T_2 (or large linewidth) are attenuated leaving only the resonances due to mobile small molecules. Diffusion-edited spectra were also acquired choosing a diffusion delay (Δ) and a gradient-field pulse duration (δ) and strength in order to observe components with low diffusion rates, deriving from lipids, glycogen and small proteins. A conventional pre-saturated 1D spectrum with composite pulse (trace a), that highlights both lipids and small metabolites contribution, a CPMG spectrum (trace b), showing the enhanced resonances of metabolites, and the diffusion-edited spectrum (trace c) of the same stomach sample 3 are reported in Fig. 3.

Although some metabolites can be readily assigned by direct inspection of the ^{1}H MR spectra (by comparison with literature data), a complete assignment requires the acquisition of selected 2D experiments such as COSY (proton-proton COrrelation SpectroscopY) (44,45,52), TOCSY (TOtal Correlation SpectroscopY) (46,47), J-Res (J-resolved experiment) (48) and HSQC (^{1}H,^{13}C-Heteronuclear Single Quantum Coherence) (49).The COSY and the TOCSY spectra are very informative for the identification of hidden resonances: the COSY spectra enable the coupled proton-proton pairs to be found, whereas the TOCSY spectra permit to identify ^{1}H-^{1}H connectivities up to five or six bonds. The J-Res

Table I. List of ^{1}H and ^{13}C chemical shift (δ, ppm) of metabolites found in HR-MAS spectra of human healthy stomach tissue.[a,b]

Entry	Metabolite	δ ^{1}H	δ ^{13}C	
1	Fatty acids	0.89	14.6	CH_3
		1.31		$(CH_2)_n$
		1.62		CH_2-C-C=O
		2.02		CH_2-C=
		2.29	34.2	CH_2C=O
		2.78		=C-CH_2-C=
		5.32		CH=CH
2	Isoleucine	0.94(t)	11.7	δ-CH_3
		1.02(d)	15.5	γ-CH_3
		1.29, 1.48	25.1	γ-CH_2
		1.97		ß-CH
		3.69		α-CH
3	Leucine	0.95(d)	21.6	δ-CH_3
		0.97(d)	22.7	δ-CH_3
		1.70	24.8	γ-CH
		1.72	40.5	ß-CH_2
		3.75	[c]	α-CH
4	Valine	0.99(d)	17.3	γ-CH_3
		1.04(d)	18.7	γ-CH_3
		2.25		ß-CH
		3.61	[d]	α-CH
5	Threonine	1.33(d)	20.3	γ-CH_3
		4.27		ß-CH
		3.60	[d]	α-CH
6	Lactate	1.33(d)	20.3	CH_3
		4.11		CH
7	Unknown	1.37(t)		CH_3
		3.36(q)		CH_2
8	Alanine	1.48(d)	16.8	ß-CH_3
		3.79	51.3	α-CH
9	Lysine	3.04(t)	39.9	ε-CH_2
		1.73	27.3	δ-CH_2
		1.48	22.3	γ-CH_2
		1.91	30.6	ß-CH_2
		3.79	[c]	α-CH
10	Arginine	3.23	41.3	δ-CH_2
		1.69	24.9	γ-CH_2
		1.93	28.3	ß-CH_2
		3.78	[c]	α-CH
11	Glutamate	2.36(t)	34.3	γ-CH_2
		2.06, 2.15	27.6	ß-CH_2
		3.77	[c]	α-CH
12	Glutamine	2.48(td)	31.7	γ-CH_2
		2.14	27.1	ß-CH_2
		3.79	[c]	α-CH
13	Proline	3.43, 3.34		δ-CH_2
		2.01		γ-CH_2
		2.34, 2.07		ß-CH_2
		4.12		α-CH
14	Methionine	2.13(s)	14.9	SCH_3
		2.70		γ-CH_2
		2.25		ß-CH_2
		3.87		α-CH
15	Aspartatic acid	2.68, 2.82	37.5	ß-CH_2
		3.90		α-CH
16	Asparagine	2.85, 2.96		ß-CH_2
		4.01		α-CH
17	Creatine	3.04(s)		NCH_3
		3.92(s)		CH_2
18	Tyrosine	3.06, 3.20		ß-CH_2
		3.93	57.0	α-CH
		6.88	116.8	H*ortho*
		7.20	131.7	H*meta*
19	Phenylalanine	3.11, 3.28		ß-CH_2
		3.98		α-CH
		7.33	130.1	H*ortho*
		7.37		H*para*
		7.43	129.6	H*meta*
20	Ethanolamine	3.15(t)		CH_2
		3.82(t)		CH_2
21	Phosphoryl-ethanolamine	3.23	41.1	CH_2
		4.00	61.1	CH_2
22	Glycerophospho-rylethanolamine	3.30		CH_2
		4.12		CH_2
23	Free Choline	3.20	54.6	$N(CH_3)_3$
		3.53	68.2	NCH_2
		4.07	56.5	OCH_2
24	Glycerophos-phorylcholine	3.22	54.7	$N(CH_3)_3$
		3.68		NCH_2
		4.33		OCH_2
25	Phosphoryl-choline	3.22	54.7	$N(CH_3)_3$
		3.61	67.3	NCH_2
		4.21	59.0	OCH_2
26	ß-glucose	3.25		2-CH
		4.67(d)		1-CH
27	Taurine	3.26(t)	48.2	SCH_2
		3.42(t)	36.2	NCH_2
28	Myoinositol	3.29(t)	75.3	5-CH
		3.63(t)	73.2	4,6-CH
		3.53(dd)	72.0	1,3-CH
		4.06(t)	72.9	2-CH
29	Scyllo-inositol	3.35 (s)		
30	α-glucose	3.54		2-CH
		5.24(d)		1-CH
31	Glycine	3.56	42.3	CH_2
32	PEG	3.72	70.3	
33	Glycerol (in lipids)	4.10, 4.30		1,3-CH_2
		5.26		2-CH
34	Glycerols	3.57, 3.66	63.2	1-CH_2
		3.81	72.7	2-CH
35	UDPG	4.34		2-CHrib
		5.92		1-CHrib
		5.90		5-CHur
		7.87		6-CHur
36	Uracil	5.80		5-CHur
		7.53		6-CHur
37	Formiate	8.48		
38	NADH	8.21		
39	Tryptophane	7.73		4-CH
		7.19		5-CH
		7.29		6-CH
		7.52		7-CH
40	Adenine	8.18		8-CH
		8.21		2-CH
41	NAc	2.01÷2.12	23.0	NC=OCH_3
42	Acetate	1.92	24.8	CH_3C=O
43	Unknown	7.97		
44	Unknown	7.23÷7.15[e]		
		8.23÷8.02[e]		

[a] ^{1}H chemical shift are referred to alanine doublet at 1.48 ppm. [b] ^{13}C chemical shift are referred to alanine at 16.8 ppm. [c] Cα probably contributes to the 3.77, 55.1 ppm cross-peak. [d] Cα probably contributes to the 3.61, 61.1 ppm cross-peak. [e] Mobile pair of singlets.

experiments allow us to disentangle overlapped signals and to distinguish their multiplicity. $^{1}H,^{13}C$ HSQC spectra were performed in order to identify directly bonded carbon-proton correlations that make possible to assign the singlets (which do not give correlations in homonuclear COSY and TOCSY spectra) by comparison of both ^{1}H and ^{13}C data with those of literature, and to distinguish signals from different molecules having similar proton chemical shifts but diverse ^{13}C signals.

The whole of these experiments permits the complete and unambiguous identification of the metabolic pattern typical of the normal human gastric mucosa.

The analysis of the COSY spectrum (Fig. 4) evidences the presence of a pool of metabolites, especially osmolites, free amino acids and a fraction of mobile lipids, whose assignments are reported in Table I.

Some correlations, for example those due to glycerol (in lipids) protons at 5.24, 4.30 and 4.10 ppm are present in COSY spectra, but absent or attenuated in TOCSY spectra (Fig. 5). This difference can be explained by considering that in TOCSY spectra signals coming from large molecules relax during the mixing time and this, together with the characteristic chemical shifts, allows us to say that this glycerol moiety is not involved in small but belongs to larger lipidic species. The COSY spectra are particularly useful for the detection and assignment of α-hydrogens of several amino acids, whereas the presence of lysine and arginine is better evidenced in TOCSY spectra. Both COSY and TOCSY experiments permit to establish that two metabolites, lactate and threonine, contribute to the doublet at 1.33 ppm found in ^{1}H MR spectra.

A further insight into the assignment of metabolite signals can be gained exploiting ^{13}C chemical shift dispersion in the second dimension, with an HSQC experiment. The HSQC spectrum of sample 5 and relative assignments are reported in Fig. 6. Choline containing compounds, arginine and phosphorylethanolamine (PE) contribute all to the 3.2 ppm signal in the proton spectra but can be readily distinguished with HSQC due to the large difference in ^{13}C chemical shifts. The ^{13}C δ dispersion is also useful for the assignment of the signals of aliphatic amino acids (isoleucine, valine, leucine). The interpretation of the region between 3.4 and 4.0 ppm is more problematic, due to the presence of overlapped correlations around 62 ppm, presumably due to glycerols. All sample spectra show a strong resonance at 3.72 (s) ppm, attributable to the polyethylene glycol (PEG) used as an excipient in pharmaceuticals (confirmed by the ^{13}C resonance at 70.3 ppm).

The observed peaks seem to be principally related to the narrow components appearing in the CPMG spectrum (Fig. 3b), with the exception of signals due to N-acetyl groups (probably arising from sialic acid), which are instead evidenced in the diffusion-edited spectrum (Fig. 3c).

We were able through the use of suitable one- and two-dimensional experiments to assign the MRS signals from more than forty species present in healthy human gastric mucosa. Among them only few components can to be confidently assigned by direct inspection of the ^{1}H MR spectrum, namely, a doublet at 1.48 ppm is for alanine, a triplet at 2.36 ppm is for glutamic acid, whereas a triplet at 3.43 ppm is for taurine. Instead, the doublet at 1.33 ppm, usually attributed to lactate, is due in our samples to the overlapping of lactate and threonine resonances, with the second species prevailing in the same

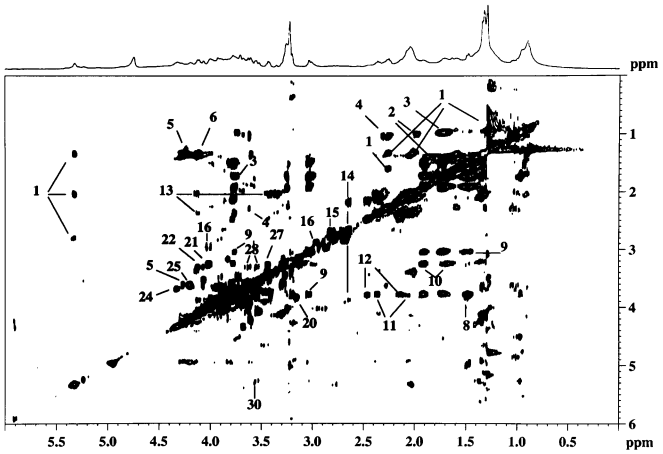

Figure 5. Partial TOCSY MR spectrum of sample 4. The identified metabolites are denoted with labels according to Table I.

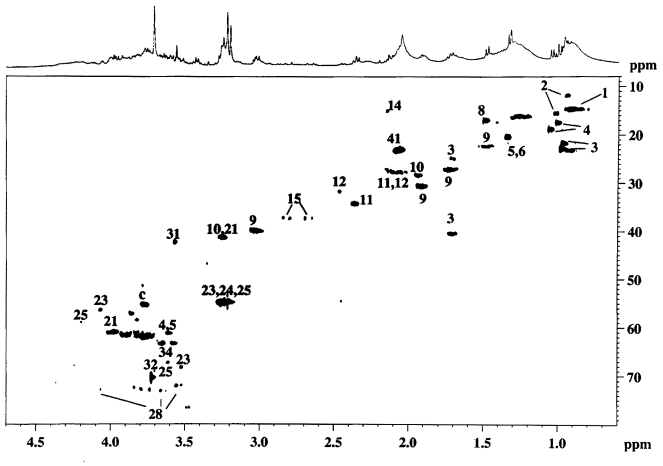

Figure 6. Partial $^{1}H,^{13}C$-HSQC spectrum of sample 5. The identified metabolites are denoted with labels according to Table I.

cases. The presence of lysine is normally well evident in the one-dimensional ^{1}H spectrum from the triplet at 3.04 ppm (over-lapped to the singlet of creatine), while it is harder to distinguish the triplet of arginine from those of taurine and phosphorylethanolamine which are found very close in one-dimensional ^{1}H spectrum (and partially hidden under the choline containing compounds singlets).

In conclusion, this report represents the first study, to our knowledge, aimed at elucidating the metabolic profile of normal human gastric mucosa by *ex vivo* HR-MAS MRS. All the samples analysed display the same resonances with only slight differences in the relative abundance. The 1D and 2D MRS techniques allow for the identification of several species. We have characterized the molecular pattern typical of the healthy gastric human mucosa: these biochemical data will be at the bases of the comparison with gastric neoplastic situations. Moreover, the full knowledge of the biochemical pattern of the healthy gastric tissues is the necessary presupposition for the application of magnetic resonance spectroscopy directly on the patient.

Acknowledgements

The Fondazione Cassa di Risparmio di Modena is greatly acknowledged for the financial support given for the acquisition of the Bruker Avance400 Spectrometer and the Centro Interdipartimentale Grandi Strumenti of the University of Modena and Reggio Emilia is greatly acknowledged for the use of it. This study was supported by grants of Murst (ex 60% and ex 40%) and Regional Grants A.I.R.C.

References

1. Boring CC, Squires TS and Tong MS: Cancer statistics. CA Cancer J Clin 44: 7-26, 1994.
2. Rudy DRA and Zdon MJ: Update on colorectal cancer. Am Fam Physician 61: 1759-1769, 2000.
3. Kwoch L, Smith JK, Castillo M, *et al*: Clinical applications of proton MR spectroscopy in oncology. Technol Cancer Res Treat 1: 17-28, 2002.
4. Smith ICP and Steward LC: Magnetic resonance spectroscopy in medicine: clinical impact. Prog Nucleic Magn Res Spect 40: 1-34, 2002.
5. Tosi MR, Fini G, Tinti A, Reggiani A and Tugnoli V: Molecular characterization of human healthy and neoplastic cerebral and renal tissues by *in vitro* ^{1}H NMR spectroscopy (Review). Int J Mol Med 9: 299-310, 2002.
6. Smith JK, Castillo M and Kwoch L: MR spectroscopy of brain tumors. Magn Reson Imaging Clin N Am 11: 415-429, 2003.
7. Kuztnesov YE, Caramanos Z, Antel SB, *et al*: Proton magnetic resonance spectroscopic imaging can predict length patients with supratentorial gliomas. Neurosurgery 53: 565-574, 2003.
8. Majòs C, Alonso J, Aguilera C, *et al*: Proton magnetic resonance spectroscopy (^{1}H MRS) of human brain tumors: assessment of differences between tumor types and its applicability in brain tumor categorization. Eur Radiol 13: 582-591, 2003.
9. Gruber S, Mlynàrik V and Mose E: High-resolution 3D proton spectroscopic imaging of the human brain at 3T: SNR issues and application for anatomy-matched voxel sizes. Magn Reson Med 49: 299-306, 2003.
10. Howe FA, Barton SJ, Cudlip SA, *et al*: Metabolic profiles of human brain tumors using quantitative *in vivo* ^{1}H magnetic resonance spectroscopy. Magn Reson Med 49: 223-232, 2003.
11. Swindle P, McCredie S, Russel P, *et al*: Pathologic characterization of human prostate tissue with proton MR spectroscopy. Radiology 228: 144-151, 2003.
12. Dhingsa R, Qayyum A, Fergus V, *et al*: Prostate cancer localization with endorectal MR imaging and MR spectroscopic imaging: effect of clinical data on reader accuracy. Radiology 230: 215-220, 2004.
13. Kim JK, Park SH, Lee HM, *et al*: *In vivo* ^{1}H MRS evaluation of malignant and benign breast diseases. Breast 12: 179-182, 2003.
14. Bolan PJ, Meisamy S, Baker EH, *et al*: *In vivo* quantification of choline compounds in the breast with ^{1}H MR spectroscopy. Magn Reson Med 50: 1134-1143, 2003.
15. Tse GM, Cheung HS, Pang LM, *et al*: Characterization of lesions of the breast with proton MR spectroscopic comparison of carcinomas, benign lesions and phyllodes tumors. Am J Roentgenol 181: 1267-1272, 2003.
16. Mun CW, Cho JY, Shin WJ, *et al*: *Ex vivo* proton MR spectroscopy (^{1}H-MRS) for evaluation of human gastric carcinoma. Magn Reson Imaging 22: 861-870, 2004.
17. Millis KK, Maas WE, Cory DG and Singer S: Gradient, high-resolution, magic-angle spinning nuclear magnetic resonance spectroscopy of human adipocyte tissue. Magn Reson Med 38: 399-403, 1997.
18. Cheng LL, Ma MJ, Becerra L, Ptak T, Tracey I, Lackner A and Gonzalez RG: Quantitative neuropathology by high resolution magic angle spinning proton magnetic resonance spectroscopy. Proc Natl Acad Sci USA 94: 6408-6413, 1997.
19. Millis K, Weybright P, Campbell N, *et al*: Classification of human liposarcoma and lipoma using *ex vivo* proton NMR spectroscopy. Magn Reson Med 41: 257-267, 1999.
20. Barton SJ, Howe FA, Tomlins AM, *et al*: Comparison of *in vivo* ^{1}H MRS of human brain tumors with ^{1}H HR MAS spectroscopy of intact biopsy samples *in vitro*. MAGMA 8: 121-128, 1999.
21. Garrod S, Humpfer E, Spraul M, *et al*: High-resolution magic angle spinning ^{1}H NMR spectroscopic studies on intact rat renal cortex and medulla. Magn Reson Med 41: 1108-1118, 1999.
22. Tate AR, Foxall PJD, Holmes E, *et al*: Distinction between normal and renal carcinoma kidney cortical biopsy samples using pattern recognition of ^{1}H magic angle spinning (MAS) NMR spectra. NMR Biomed 13: 64-71, 2000.
23. Sitter B, Sonnewald U, Spraul M, *et al*: High-resolution magic angle spinning MRS of breast cancer tissue. NMR Biomed 15: 327-337, 2002.
24. Taylor JL, Chin-Lee W, Cory D, *et al*: High-resolution magic angle spinning proton NMR analysis of human prostate tissue with slow spinning rates. Magn Reson Med 50: 627-632, 2003.
25. Wang Y, Bollard ME, Keun H, *et al*: Spectral editing and pattern recognition methods applied to high-resolution magic-angle spinning ^{1}H nuclear magnetic resonance spectroscopy of liver tissues. Anal Biochem 323: 26-32, 2003.
26. Swanson MG, Vigneron DB, Tabtabai L, *et al*: Proton HR MAS spectroscopy and quantitative pathologic analysis of MRI/3D-MRSI-targeted postsurgical prostate tissues. Magn Reson Med 50: 944-954, 2003.
27. Tugnoli V, Tosi MR, Barbarella G, Bertoluzza A, Ricci R and Trevisan C: *In vivo* ^{1}H MRS and *in vitro* multinuclear MR study of human brain tumors. Anticancer Res 16: 2891-2900, 1996.
28. Tugnoli V, Tosi MR, Barbarella G, Ricci R, Calbucci F and Bertoluzza A: *In vitro* and *in vivo* MRS study of human glioma metabolites. Int J Oncol 11: 319-324, 1997.
29. Tugnoli V, Tosi MR, Barbarella G, Ricci R, Leonardi M, Calbucci F and Bertoluzza A: Magnetic resonance spectroscopy study of low grade extra and intracerebral human neoplasms. Oncol Rep 5: 1199-1203, 1998.
30. Tosi MR, Tugnoli V, Bottura G, Lucchi P, Battaglia A, Giorgianni P, Ferri C, Mannini D and Reggiani S: *In vitro* MRS and HPLC studies of human renal cell carcinomas. Oncol Rep 7: 1355-1358, 2000.
31. Tosi MR, Tugnoli V, Bottura G and Lucchi P: Multinuclear magnetic resonance spectroscopy of human healthy and neoplastic renal tissues. Appl Spectr 55: 908-912, 2001.
32. Tugnoli V, Tosi MR, Tinti A, Trinchero A, Bottura G and Fini G: Characterization of lipids from human brain tissues by multinuclear magnetic resonance spectroscopy. Biopolymers 62: 297-306, 2001.
33. Tosi MR, Fini G, Taddei P, Tinti A and Tugnoli V: Magnetic resonance spectroscopy of human brain tissues. Recent Res Dev Mol Struct 1: 53-64, 2001.
34. Tosi MR, Bottura G, Lucchi P, Reggiani A, Trinchero A and Tugnoli V: Cholesteryl esters in human malignant neoplasms. Int J Mol Med 11: 95-98, 2003.
35. Tosi MR, Reggiani A and Tugnoli V: Are molecular features of a chromophobic cell renal carcinoma correlated with clinical findings? Int J Mol Med 11: 99-102, 2003.
36. Tugnoli V, Reggiani A, Beghelli R, Tomaselli V, Trinchero A and Tosi MR: Magnetic resonance spectroscopy and high performance liquid cromatography of neoplastic human renal tissues. Anticancer Res 23: 1541-1548, 2003.
37. Tugnoli V, Bottura G, Fini G, Reggiani A, Tinti A, Trinchero A and Tosi MR: ^{1}H and ^{13}C NMR lipid profiles of human renal tissue. Biopolymers 72: 86-95, 2003.
38. Tosi MR, Rodriguez-Estrada MT, Lercker G, Poerio A, Trinchero A, Reggiani A and Tugnoli V: Magnetic resonance spectroscopy and chromatographic methods identify altered lipid composition in human renal neoplasms. Int J Mol Med 14: 93-100, 2004.
39. Tugnoli V, Poerio A and Tosi MR: Phosphatidylcholine and cholesteryl esters identify the infiltrating behaviour of a clear cell renal carcinoma: ^{1}H, ^{13}C and ^{31}P MRS evidence. Oncol Rep 12: 353-356, 2004.
40. Sonnewald U, Westergaard N, Isern E, Mueller T, Schousboe A, Petrsen S and Unsgard G: MRS evaluation of brain metabolites in extracts from cell cultures, human tumors and normal tissue from brain: cholesteryl ester, choline containing compound and creatine as markers for development, differentiation and pathology. Int J Oncol 2: 545-555, 1993.
41. Bax A: A spatially selective composite 90˚ radiofrequency pulse. J Magn Reson 65: 142-145, 1985.
42. Meiboom S and Gill D: Modified spin-echo method for measuring nuclear relaxation time. Rev Sci Instrum 20: 688-691, 1958.
43. Wu D, Chen A and Johnson CS Jr: An improved diffusion-ordered spectroscopy experiment incorporating bipolar gradient pulses. Magn Reson Series A 115: 260-264, 1995.

44. Jeener J: Pulse Pair Techniques in High Resolution NMR. Ampere International Summer School II, Basko Polje, Yugoslavia, 1971.
45. Aue WP, Bartholdi E and Ernst RR: Two-dimensional spectroscopy. Application to nuclear magnetic resonance. J Chem Phys 64: 2229-2246, 1976.
46. Braunschweiler L and Ernst RR: Coherence transfer by isotropic mixing: application to proton correlation spectroscopy. J Magn Reson 53: 521-528, 1983.
47. Bax A and Davis DG: MLEV-17-based two-dimensional homonuclear magnetization transfer spectroscopy. J Magn Reson 65: 355-360, 1985.
48. Aue WP, Karhan J and Ernst RR: Homonuclear broad band decoupling and two-dimensional J-resolved NMR spectroscopy. J Chem Phys 64: 4226-4227, 1976.
49. Bodenhausen G and Ruben DJ: Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy. Chem Phys Lett 69: 185-189, 1980.
50. Stepan V, Sugano K, Yamada T, Park J and Dickinson CJ: Gastrin biosynthesis in canine G cells. Am J Physiol 282: G766-G775, 2002.
51. Tang Z, Zhao M, Ji J, Yang G, *et al*: Overexpression of gastrin and c-met protein involved in human gastric carcinomas and intestinal metaplasia. Oncol Rep 11: 333-339, 2004.
52. Kumar A, Wagner G, Ernst RR and Wüthrich K: Studies of J-connectives and selective ^{1}H-^{1}H Overhauser effects in H_2O solutions of biological macromolecules by two-dimensional NMR experiments. Biochem Biophys Res Commun 96: 1156-1163, 1980.