# Metabolic Imaging of Cachectic Tumors

**M-F. Penet[1], S. Kakkad[1], D. Artemov[1], and Z. M. Bhujwalla[1]**

[1]Department of Radiology and Radiological Science, JHU ICMIC Program, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

**Introduction:** 'Cachexia' is derived from the Greek "kakos" meaning bad and "hexis" meaning condition. Clinically, cachexia is defined as an unintentional weight loss of 5% over a 3 to 6 month period (1). Cancer patients with an involuntary weight loss of 5% have a shorter median survival rate (1); death from cachexia occurs with a weight loss of 30% (2). The ability to arrest or reverse this condition would have a tremendous impact on improving quality of life, treatment outcome, and increasing life expectancy, allowing a patient to have recourse to currently available or newly developing treatment options. Understanding the metabolism of cachexia-inducing tumors may lead to the identification of new targets to arrest or reverse the condition.

**Methods:** In this preclinical study, we performed $^1$H MRS/I of tumors derived from cachectic (MAC16) and non-cachectic (MAC13) murine colon adenocarcinoma cell lines inoculated in the flank of male SCID mice. Cachectic MAC16 tumors induce extensive weight loss in tumor-bearing animals, unlike MAC13 tumors, which although histologically similar, do not alter body weight (3). These cell lines were originally obtained from Dr. Tisdale (Birmingham, UK). *In vivo* localized MRSI of tumors was performed on a 4.7T Bruker Biospec spectrometer using a solenoid coil placed around the tumor. Localized spectra from a 4 mm thick slice were acquired with FOV 16 mm; 4 scans per phase encode step; TE=120 ms; TR=1 s with VAPOR water suppression. Quantitative maps of total choline and lactate/lipid were generated using unsuppressed water signal as an internal reference, using in-house IDL programs. A dual-phase extraction method based on methanol/chloroform/water (1:1:1), was used to obtain lipid and water-soluble fractions from tumors (4). Fully relaxed $^1$H MR spectra of tumor extracts were acquired on an 11.7T Bruker Avance spectrometer using a 5-mm HX inverse probe and the following acquisition parameters: 30° flip angle, 6000 Hz sweep width, 12.7 s repetition time, time-domain data points of 32K, and 128 transients. Spectra were analyzed using Bruker XWIN-NMR 3.5 software (Bruker BioSpin). Integrals of the metabolites of interest were determined and normalized to the tumor weight. Metabolite concentrations were obtained from $^1$H spectra using an internal standard.

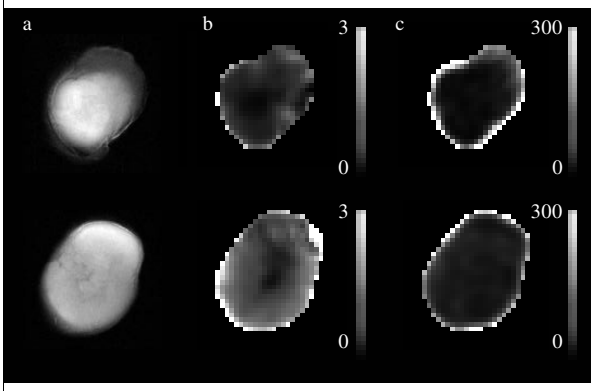


**Figure 1:** (a) $T_1$-weighted images, (b) total choline maps and (c) lipid/lactate maps of a MAC13 tumor (upper panel) and of a MAC16 tumor (lower panel). $T_1$-weighted images were acquired from a 4 mm slice corresponding to the MRSI slice using a spin-echo sequence with an echo time of 10 ms, a repetition time of 1 s, and an in-plane spatial resolution of 65 mm. Total choline and lactate/lipid maps were generated from the MRSI data shown in Figure 2 and normalized to the water signal to display concentrations in mM units. An increase of total choline is evident in the MAC16 tumor compared to the MAC13 tumor (compare images in b).

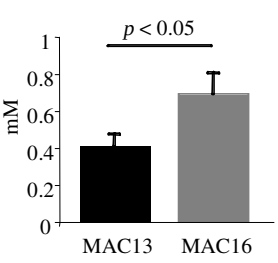


**Figure 2:** *In vivo* total choline concentration in MAC13 (n=6) and MAC16 (n=4) tumors. Values represent Mean +/- SEM.

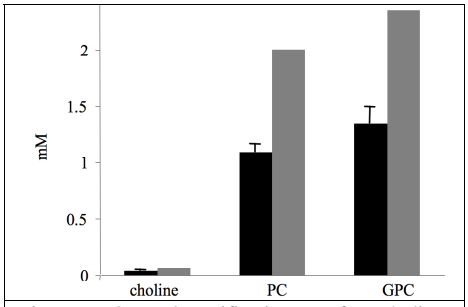


**Figure 3:** Quantification of choline, phosphocholine (PC), and glycerophosphocholine (GPC) in MAC13 and MAC16 tumors (n=3 and n=1 respectively). Both PC and GPC were higher in the cachectic tumor.

**Results and Discussion:** Our preliminary $^1$H MRSI studies revealed that the cachectic MAC16 tumors had a significantly higher total choline levels compared to non-cachectic MAC13 tumors (Figures 1 and 2). From high-resolution spectra of extracts it was apparent that the increase of total choline was due to higher phosphocholine (PC) and glycerophosphocholine (GPC) (Figure 3). There was a trend towards higher levels of lipid/lactate in non-cachectic tumors, but this trend was due to increased lipids at the tumor periphery, since $^1$H MRS of lipid-phase extracts demonstrated that tumor lipid levels were not significantly different between cachectic and non-cachectic tumors.

Since PC is a membrane precursor and GPC is a membrane breakdown product, the increase of both PC and GPC suggests a rapid synthesis and breakdown of membrane choline phospholipids in cachectic tumors. An increase of cellular PC and total choline-containing compounds has been consistently observed in cancer cells and tissue, and is closely related to malignant transformation, invasion, and metastasis. Choline kinase (Chk), a cytosolic enzyme that catalyzes the phosphorylation of choline to form PC by ATP in the presence of magnesium, is one of three enzymes which, along with phosphatydylcholine-specific phospholipase C (PC-PLC) and CTP:phosphocholine cytidylyltransferase, can lead to increased PC levels (5,6). Chk targeted therapy is currently being explored in preclinical models (7,8). The increased GPC observed in the cachectic tumors suggests an increase in the expression or activity of phospholipase A2 or lysophospholipase (9). The enzymes in the choline phospholipids metabolism therefore present potential molecular targets to arrest the tumor from inducing cachexia.

**References:** (1) Inui, A., CA Cancer J Clin, 2002. 52:72-91; (2) Loberg, R.D. *et al.,* CA Cancer J Clin, 2007. 57: 225-41; (3) Tisdale, M.J. *et al.,* Br J Cancer, 1986. 54: 601-6; (4) Glunde *et al.,* Neoplasia (2006) 8: 758-771; (5) Glunde, K. et al., Mol Pharm, 2006. **3**:496-506; (6) Podo, F., NMR Biomed, 1999. **12**: 413-39; (7) Rodriguez-Gonzalez, A. et al. Oncogene, 2003. **22**:8803-12; (8) Glunde, K., et al., Cancer Res, 2005. **65**:11034-43; (9) Aboagye, E.O. et al., Cancer Res, 1999. **59**: 80-4.

This work supported by NIH P50 CA103175. We thank Mr. Gary Cromwell for maintaining the cell lines and inoculating the tumors, and Ms. Flonne Wildes for technical assistance.