

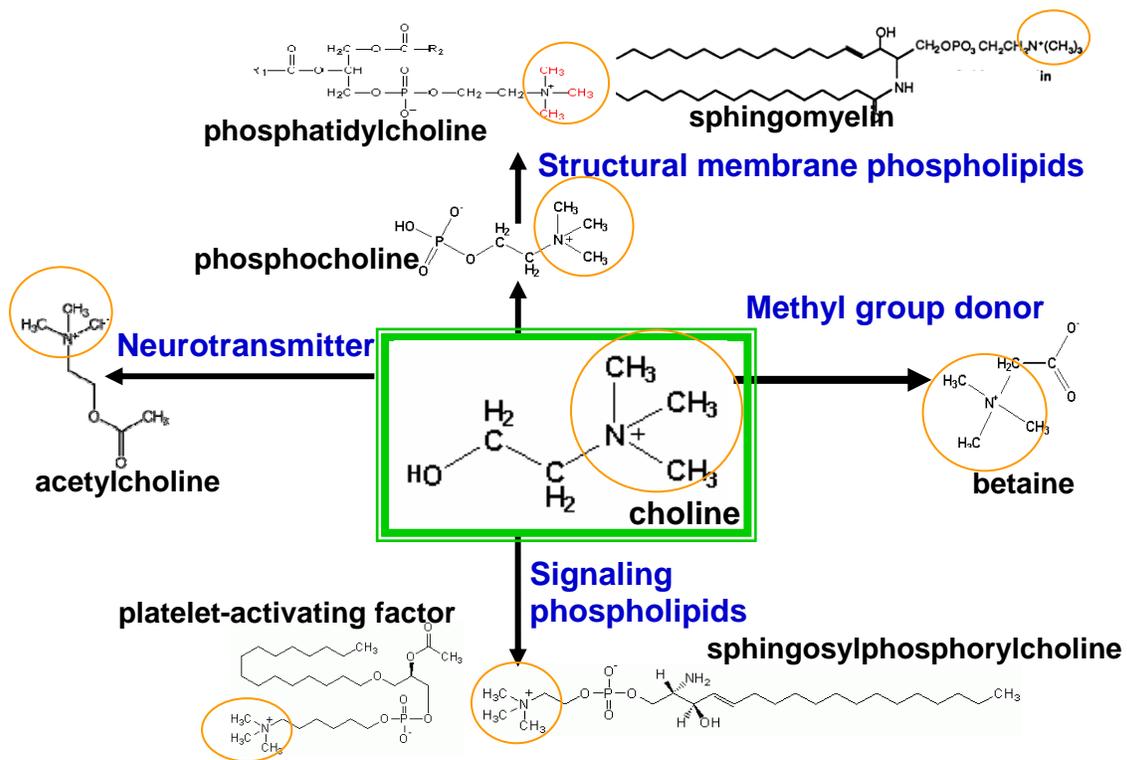
Choline Metabolism: Meaning and Significance

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Choline is an essential nutrient

Choline (Cho), a quaternary amine, is an essential nutrient supplied by the diet (1, 2). Choline uptake and intracellular metabolism during the prenatal period were shown to have a critical role in brain development and cognitive processes (3, 4). Choline is a precursor in several key biochemical pathways as demonstrated below in scheme 1.

Scheme 1: Metabolic Fate of Choline



Choline transport

The life-time of choline in the blood and its distribution in the body, as well as its transport into the cells are critical steps in the metabolic fate of choline. The transport is the rate limiting step for the synthesis of acetylcholine (5) and of PCho (6). The transporters of choline belong to the group of organic cation transporters (7,8). There are several transporter genes encoding for the following choline transporters:

1. The plasma membrane choline transporter CHT1 that mediates choline uptake with high affinity; a K_m of 1 to 3 μM . This transporter is a Cl^- and Na^+ dependent co-transporter (9). It is mainly expressed in cholinergic neurons, however, recent studies in our lab has shown that it is also expressed in human breast cancer cells.
2. The family of choline transporter-like proteins - CTLs. Knowledge about human CTLs is limited (8), however, findings in other species, particularly in the mouse, strongly suggest an important role in human physiology. mCTL1 expression was found inside and outside the nervous system. High levels of hCTL1 mRNA were found in colonic and lung epithelial cells (9).
3. The three electrogenic organic cation transporters OCT1, OCT2, and OCT3 which operate independently from Na^+ , Cl^- and H^+ ions (10). In species tested so far OCT1 was found to be mainly expressed in liver, OCT2 in kidney and OCT3 expression was relatively broad, in skeletal muscle, liver, placenta, kidney and heart. Low expression of OCT1 was also found in the mammary gland.

There are conflicting results in the literature regarding the affinities of these transporters to choline; the Michaelis–Menten constants for the choline transport through OCTs vary from $\sim 20 \mu\text{M}$ to $600 \mu\text{M}$ (11-15). Both phosphorylation and rapid internalization and recycling of the transporters were suggested as potential regulating mechanisms (16). Choline can also cross the plasma membrane by a diffusion-like mechanism as was found for normal and cancerous epithelial mammary cells (17).

Recently we characterized the rates of transport and mRNA expression levels of choline transporters in five different human breast cancer cell lines and in normal primary cultures of human mammary epithelial cells. The expression of the transporters followed the order $\text{CTL1} > \text{OCT2} > \text{OCT1} > \text{CHT1}$ in both the normal and the cancer cells. We found that the transport rate was enhanced in all breast cancer cells as compared to that in the normal cells. This enhancement correlated with upregulation of the expression of the choline transporters CHT1 and OCT2 in the cancer cells as compared to the normal cells. In contrast to our results, Glunde et al reported that all choline transporter genes were expressed in [equal measure in](#) both spontaneous immortalized human mammary epithelial cells (MCF-12A), [as well as](#) MDA-MB-231 human breast cancer cells (18) This [finding](#) could [arise from the](#) induced expression of the transporters that occurred in the transformation of the normal MCF-12A cells to an immortalized line or from the limitation in the sensitivity of the method.

Choline Metabolism

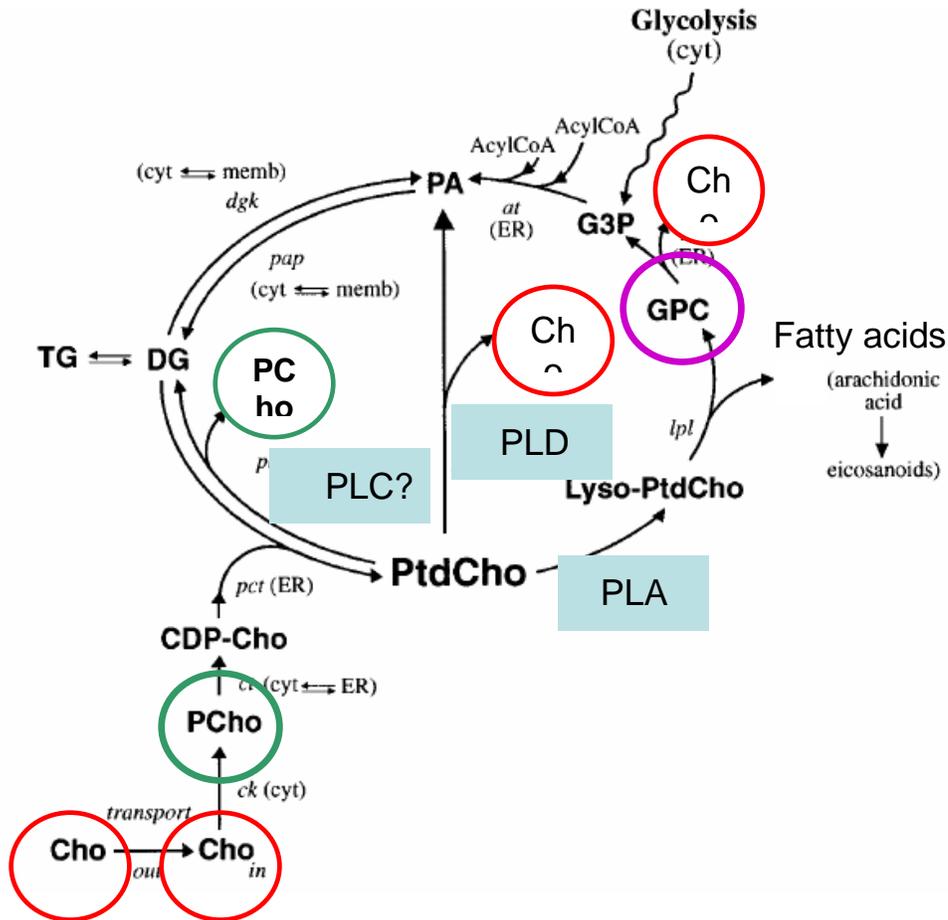
Choline is the precursor of various metabolites. The intracellular routing of choline to its various metabolic pathways, phosphorylation, oxidation, and acetylation is cell and tissue specific.

The phosphorylation by choline-kinase is the first step in the Kennedy pathway which is responsible for the biosynthesis of choline phospholipids such as phosphatidylcholine (PtdCho) (18-20). PtdCho is required for the build-up and maintenance of cell membranes. It also serves as a precursor of diacylglycerol (DAG), that has an important role in regulating cell growth, differentiation and death.

The biosynthesis of PtdCho (see scheme 2) occurs on the cytosolic side of the endoplasmic reticulum membrane, through a cascade of three enzymatic steps consisting of: 1) choline phosphorylation by choline-kinase; 2) PCho conversion to CDP-choline by CTP:PCho cytidylyltransferase (CCT) ; and 3) PCho transferase (PT)-mediated PtdCho

Scheme 2: Phosphatidylcholine Cycle

Adapted from Podo F, NMR Biomed, 12: 413, 1999 (19)



Choline and choline metabolites can be re-generated by controlled breakdown of choline phospholipids through several pathways. The main pathways for PtdCho mediated hydrolysis occur via Phospholipases D (PLDs) that produce choline and phosphatidic

acid, and phospholipases A A (PLAs) that generate free fatty acids and glycerol 3-phosphocholine (GPC). The subsequent hydrolysis of GPC into glycerol 3-phosphate and choline is catalyzed by a GPC phosphodiesterase.

PtdCho is the predominant component of cellular membranes but other choline phospholipids such as sphingomyelin, choline plasmalogens (21) and lysophosphatidylcholine are also involved in maintaining the structural integrity and the signaling functions of cellular membranes.

Choline is also oxidized in the mitochondria to betaine. The methyl groups of betaine are used to re-synthesize methionine from homocysteine, thereby providing methionine for protein synthesis and transmethylation reactions .

Choline metabolism and malignant transformation

Choline metabolism and choline-derived metabolites can undergo extensive alterations as a result of a malignant transformation. The level of PCho in human breast cancer cells was found to be markedly higher than in normal human mammary epithelial cells (22-24). Both choline transport and phosphorylation were found to be augmented in human breast cancer cells relative to their normal counterpart. The level of PCho correlated with the maximal rate of choline transport in the normal and cancerous cells, but it did not correlate with the tumorigenicity and invasiveness of the cancer cells (unpublished results). Progression of human mammary epithelial cells from a normal to a malignant phenotype was shown to be associated with a reversion in the balance, as well as an overall increase in the content of PCho and GPCCho (24). A similar trend was also exhibited by human prostatic epithelial cells (25). High levels of phosphomonoesters, including PCho, were detected in human breast cancer biopsies and patients (26-28). Ras transformed cells, and multi oncogenic transformed cells have also exhibited an increase in PCho content (29-31). Choline-kinase activation was shown to be critical for the proliferation of primary human mammary epithelial cells and breast tumors progression (32). Indeed, cessation of PCho synthesis by novel choline kinase inhibitors exhibited antitumor activity (33-35). We have recently characterized mRNA expression levels of the two choline kinase isoforms, choline-kinase α and β . The expression level of the α form increased by several folds in all breast cancer cells relative to that in normal mammary cells whereas the β form remained about the same in both the cancer and normal cells.

Choline is a precursor of choline derived phospholipids, but can be also recovered as a product of their hydrolysis. The synthesis and degradation of phospholipids may be induced by growth factors that play a major role in malignant transformations (36, 37). Several studies have demonstrated regulation of PtdCho metabolism by a receptor tyrosine kinase cascade, downstream of the ras/raf interaction (38, 39). In turn, molecules derived by the breakdown of choline-containing-phospholipids, such as diacylglycerol, ceramide, and PCho can act as second messengers in mitogenic signal transduction pathways (40-43).

MRS of Choline in living cells, cell extracts, tumor specimens and tumors in animal models.

Choline and choline metabolites can be traced in cells and tissues, *in vitro* and *in vivo*, by means of multinuclear magnetic resonance spectroscopy (MRS). Proton spectra of choline and its freely tumbling metabolites exhibit a strong single signal of the three methyl groups at ~3.2 ppm and multiplets of the two coupled methylene groups. In high resolution proton spectra of cells and cell extracts, it is possible to separate between the signals of the various choline metabolites, however, in spectra recorded *in vivo* the signals usually overlap. ³¹P spectra exhibit separate signals of the phosphorylated choline metabolites such as PCho – at ~ 4.2 ppm (pH dependent) and GPC at 0.48 ppm.

It is also possible to label choline nuclei with ¹³C or deuterons (²H) and monitor the incorporation of the label to the various choline metabolites (17,18, 44-48). Labeling with ¹³C of [1,2-¹³C]-choline, makes it possible to achieve a separation of 3-100 ppm between choline metabolites whereas the proton signals of the different choline metabolites span a small range of ~0.1 ppm and are difficult to resolve.

Monitoring the labeled choline and its incorporation to other metabolites were particularly useful when the kinetics and turn-over rates of phospholipids metabolism were investigated.

Recently progress has been made by applying high resolution magic angle spinning spectroscopy (HRMAS) to study *ex vivo* tissue specimens (49-57). The rapid spinning of the samples at 54.7° to the static magnetic field (the magic angle), around its own axis, reduces the broadening caused by restricted molecular motion and magnetic susceptibility. Consequently, the resulting spectra reveal the presence of numerous metabolites and make it possible to quantify their relative content. However, as tissue preparation and conditions during the recording of the spectra may cause substantial degradation and modify the metabolites profile, the results may not reflect the *in vivo* situation. This approach can, nevertheless, provide detailed biochemical information and lead to a better characterization of metabolic markers of cancer. For example Valonen et al (58) studied changes in the choline metabolites in rat glioma *ex vivo* during apoptosis induced by thymidine kinase-ganciclovir gene therapy. HRMAS was able to resolve the peaks of choline, glycerophosphocholine, phosphocholine, taurine and myo-inositol and show early increase in GPCho and PCho associated with induced apoptosis and long term effect of cell death associated with a decline in taurine.

Numerous multinuclear MRS studies of tumors in animal models revealed the presence of choline and choline metabolites (59-63). A large fraction of studies were devoted to monitor changes in the proton composite choline signal or ³¹P phosphomonoesters signal as a result of treatment in order to identify surrogate markers for response (64, 65).

1H and 31P MRS studies of choline and choline metabolites in cancer patients

MRS studies performed on different types of human cancers *in vivo* were first comprehensively reviewed by Negendank (66). Leach *et al* (67) summarized the finding from nine different ³¹P MRS studies *in vivo* on human breast cancer. In line with the results obtained in cellular systems and in tumors in animal models a large fraction of breast cancers exhibited a strong phosphomonoester signal, composed of both PCho and phosphoethanolamine. Breast ¹H MRS studies performed *in vivo* have also demonstrated the presence of a high proton signal of the N-methyl groups of choline, PCho and GPC

(composite choline signal). Katz-Brull et al (68) and Bolan et al (69) recently reviewed the clinical utility of ^1H MRS in differentiating malignant from benign breast lesions. High sensitivity and specificity were indicated in the early studies (70, 71) as well as in the most recent study performed at 4T (72). The choline signal in cancers was distinct, although a peak overlapping with choline was also detected in normal breast-feeding volunteers (73). Moreover, other, non choline metabolites such as taurine and myoinositol were found to overlap with the choline signal and cause misinterpretation of the peak at 3.2 ppm (73). Interestingly, in a subgroup of young women the sensitivity and specificity based on the presence of a composite choline signal approached 100% (68).

In the aforementioned ^1H MRS studies of the breast the differentiation was based on the presence or absence of the composite choline signal at 3.2 ppm. Quantitative approaches were recently applied, measuring the area of the choline peak in reference to an external standard or to the water signal (74, 76). The latter quantification also enabled monitoring changes in this peak in response to chemotherapy (76). Similar changes in the ^{31}P phosphomonoester peak also served to predict response to chemotherapy (67); response to breast cancer therapy was associated with a decrease in the ^{31}P signal of the phosphomonoesters, whereas non-responding patients demonstrated an increase in this peak.

The composite choline signal also served as a marker of malignancy in cancers other than those found in the breast, particularly to diagnose lesions in the prostate and brain (77). However, the delineation of malignancy was based on referencing the choline signal to other metabolites such as choline to citrate in the prostate or choline to NAA or to total creatine in the brain. The differences could, therefore, partially stem from changes in the peak used as a reference. Studies of cell lines and tumors in animal models indicated that malignant transformation, in general, is associated with increased choline metabolites' levels; however, comprehensive basic biochemical and molecular studies, as well as clinical evaluations are necessary for each malignancy.

Localized MRS is a challenging protocol and requires optimization of the scanner in terms of field and RF homogeneity. For localization of the lesions spectra were recorded from a specific region using different techniques such as DRESS and ISIS. To acquire long and short TE spectra within the sampling volume, PRESS and STEAM were applied. Detection of the relatively small choline signal in the background of the strong water and methylene lipid signals required the application of water suppression sequences and attenuation of the lipid signals. Recently MRSI sequences were successfully applied in the prostate and the breast (78, 79). In the future, with the development of high field scanners, new advanced coils and pulse sequences for improved frequency resolution; it might be possible to monitor the process of malignant transformation at a preventive stage by quantifying the various choline metabolites.

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