Detection of metabolic effects of fatty acid synthase inhibition by magnetic resonance spectroscopy

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Introduction Fatty acid synthase (FASN) is the key enzyme responsible for *de novo* synthesis of long-chain fatty acids (FA). In most normal cells it is expressed at low levels and FA are obtained from the diet. In contrast, FASN expression is significantly increased in cancer cells and is associated with poor prognosis, particularly in breast and prostate cancer (1). Furthermore, inhibition of FASN by siRNA or pharmacological inhibitors, such as orlistat (2) results in cell cycle arrest and apoptosis. We therefore became interested in using magnetic resonance spectroscopy (MRS) to identify pharmacodynamic markers of FASN inhibition. Importantly, FASN expression is modulated by PI3K and MAPK signaling. These pathways are central to cancer development and the targets of many novel therapies (1). We have previously shown that inhibition of MAPK and PI3K signaling results in MRS detectable metabolic changes, and in particular a drop in phosphocholine (PCho) (3,4). In light of the link between these pathways and FASN, we also questioned whether the effect on PCho of MAPK and PI3K inhibition could be mediated in part by FASN.

Methods PC3 human prostate cancer cells were routinely grown under standard conditions in DMEM/F-12 with 10% FBS. For metabolic and choline kinase activity analysis by MRS, FBS levels were reduced to 5% (to limit the amount of available extra-cellular FA). To monitor FA synthesis, glucose in the medium was reduced by half to 8.76 mM and replaced by 1-^{13}C labeled glucose. To monitor PCho and PtdCho synthesis, choline was replaced by 1,2-^{13}C labeled choline at its normal concentration of 64.1 μM. FASN inhibition and its effects were determined by incubating PC3 cells for 24 and 48 h with 30 μM orlistat (treated cells) or with the carrier DMSO (control cells). The effect of treatment on cell proliferation was determined by cell count. To measure the effect of orlistat treatment on FASN activity, ~ 5 x 10^6 cells were extracted and used in a spectrophotometric assay of NADPH oxidation (5). For metabolic analysis by MR, ~ 5 x 10^7 cells were extracted using the dual-phase extraction method (3,4,6). MR spectra were recorded on an Avance DRX500 Bruker spectrometer at room temperature: 30° flip angle, 5 s repetition delay for ^{1}H (with water suppression for aqueous samples); 30° and 3.5 s for ^{13}C and 30° and 4.5 s for ^{31}P with broad-band proton decoupling. Relative metabolite concentrations were determined by integration and normalization to both external reference (MDPA, ^{31}P; TMS, ^{1}H; $CHCl_3$, ^{13}C) and to cell number. To monitor choline kinase activity, ~ 15 x 10^6 cells were trypsinized after 48 h of treatment and their lysates assayed as previously described (7).

Results FASN activity decreased by 51±10% (n=3) at 24 h and 83±11% (n=2) at 48 h following treatment with orlistat. This was associated with inhibition of cell proliferation by 32±21% (n=3) at 24 h and 63±7% (n=3) at 48 h relative to control (data not shown). ^{13}C and ^{31}P MRS data indicated progressive inhibition of the *de novo* synthesis of FA, PtdCho and PCho at 24 h (n=3) and 48 h (n=2) followed by a progressive decrease in their total pools (Figure 1a-d). In addition, all membrane phospholipids decreased by 48 h (data not shown). Importantly, correlations were found between inhibition of FASN and inhibition of the *de novo* synthesis of FA (R^2=0.9955), PtdCho (R^2=0.8750) and PCho (R^2=0.9993). Furthermore, FASN inhibition correlated with total PCho levels (R^2=0.9987). Preliminary results also indicate that choline kinase activity was reduced by 64% (n=1) relative to control (data not shown).

Discussion and Conclusions As expected, inhibition of FASN by orlistat decreased *de novo* FA synthesis. FA are components of triacylglycerol, which in turn is a substrate for PtdCho synthesis. Thus, we hypothesize that the drop in *de novo* PtdCho is due to the reduced availability of its substrate. We further hypothesize that this led to the downstream inhibition in synthesis of another precursor of PtdCho, namely PCho, as evidenced by the drop in its *de novo* synthesis and its total pool as well as the drop in choline kinase activity. We show here that (i) the metabolic consequences of FASN inhibition on cellular metabolism and (ii) mechanistic information can be studied in detail by combining ^{1}H, ^{13}C and ^{31}P MRS. We also show that PCho levels are indeed affected by FASN.

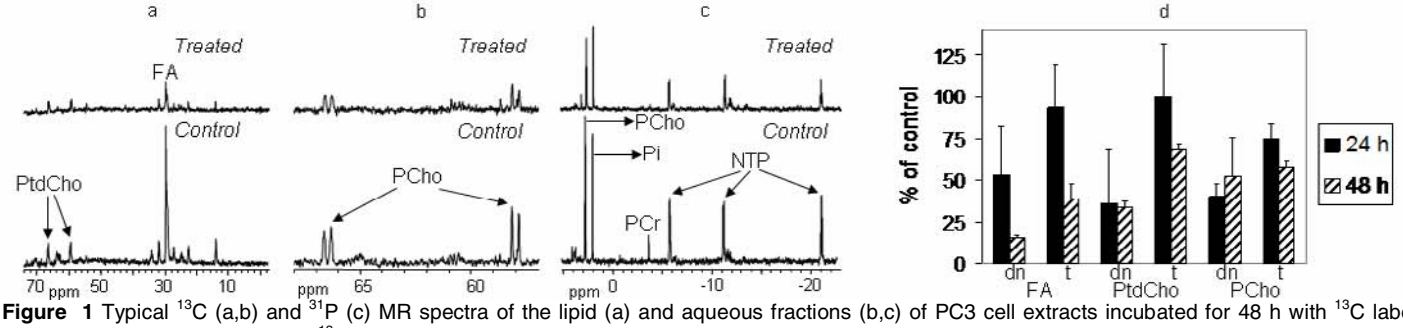

Figure 1 Typical ^{13}C (a,b) and ^{31}P (c) MR spectra of the lipid (a) and aqueous fractions (b,c) of PC3 cell extracts incubated for 48 h with ^{13}C labeled glucose and choline (Control) or ^{13}C labeled glucose and choline with 30 μM orlistat (Treated). Orlistat treatment inhibits *de novo* synthesis of FA and PtdCho (a) and *de novo* synthesis of PCho (b). Orlistat treatment decreases cellular levels of PCho and NTP (c). Labeled *de novo* (dn) and total (t) relative metabolite pools following 24 and 48 h of orlistat treatment relative to controls (d) showing an initial decrease in *de novo* FA, PtdCho and PCho synthesis followed by a later decrease in their total pools.

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