Insights into human sperm metabolism by ^{13}C magnetic resonance spectroscopy

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Rationale:
Poor sperm quality contributes significantly to the infertility of couples yet our knowledge of many basic aspects of sperm physiology remain unknown. Human sperm can produce ATP through the metabolic processes of glycolysis and/or oxidative phosphorylation. However, much of the research into this has varied due to species dependency and there remains considerable debate about which of these pathways is more important for the various aspects of human sperm function during their post-ejaculatory life. The use of ^{13}C-labelled molecules and magnetic resonance spectroscopy can identify differing metabolic pathways even if the end product is the same.

Objectives:
1. Which ^{13}C substrates implicated in human sperm energy metabolism can be detect by ^{13}C MRS?
2. What the kinetics of such reactions are?
3. Do sperm populations of higher and lower motility utilise these substrates differently?

Methodology:
Human sperm were extracted from their seminal plasma using Percoll density gradient centrifugation to yield either unfractionated sperm or sperm split into 'high' and 'low' motility populations. Sperm were then incubated with ^{13}C-labelled substrates ($^{13}C_u$-glucose, $^{13}C_u$-fructose, $^{13}C_1$-pyruvate, $^{13}C_3$-lactate, $^{13}C_{2,4}$-D-3-hydroxybutyrate, $^{13}C_1$-butyrate, $^{13}C_5$-glutamate, $^{13}C_2$-glycine, $^{13}C_u$-galactose) for at least 4 hours at 37°C and then frozen prior to MRS analysis. In a subset of experiments live sperm were incubated with ^{13}C-substrate ($^{13}C_u$-glucose, $^{13}C_u$-fructose, $^{13}C_1$-pyruvate) whilst acquiring spectra in the MRS scanner to determine the rate constant of metabolism.

Findings:
Sperm samples (n=8) consistently metabolised glucose, fructose and pyruvate into lactate and to a less extent to bicarbonate (an indicator of OxPhos). The rate constant for pyruvate to lactate was ~3 time faster than for glucose or fructose to lactate. Lactate was also observed to be converted to pyruvate and acetate. Other notable products of metabolism were 3-D-hydroxybutyrate's conversion to acetoacetate and butyrate conversion to glutamate. Motile sperm (n=15 samples) with lower motility converted more glucose to lactate than higher motility sperm, $p<0.0055$ (Mann-Witney U-test).

Conclusions:
Significantly more lactate was produced from $^{13}C_u$-glucose by vital or motile sperm recovered from the 'low' motility population compared to those from the 'high' and this could not be accounted for by differences in the number of non-sperm cells present in these two populations.^{13}C-MRS can provide information on the underlying metabolism of multiple pathways in live sperm. Dysfunction in sperm metabolism as a result of either impaired enzymes of lack of metabolisable substrate could be detected in sperm by a non-destructive assay, potentially offering new treatment options to improve overall sperm quality and outcome for reproduction.