**Relationship between lactate integral and sperm/non-sperm concentration**

Previously we have shown how the lactate signal observed in the MRS spectrum was dependent on the concentration of sperm present (Calvert *et al.*, 2019). However, since some non-sperm cells that passed through the washing process could potentially contribute to the metabolism observed, this was examined for lactate using a linear regression model of the form:

IntLac = k + a.Concsperm + b.Concnon-sperm 1

(where Concsperm and Concnon-sperm are the sperm and non-sperm concentrations and IntLac the observed lactate integral. The symbols: a, b, k, are parameters to be fitted)

This model was fitted to each population (80N, 40N, 80A, and 40A) for all incubated 13C-substrates (including those used in combination) using a stepwise regression (Matlab, steplm), where a Bonferroni corrected p value < 0.025 was considered as a significant fit to the model (Table LRM1).

Generally, 13Cu-glucose and 13Cu-fructose containing incubations showed a significant linear correlation to sperm concentration only. However, in some cases the model also included some contribution from non-sperm cells (Table LRM1). Generally, lactate derived from 13C1-pyruvate incubations fitted poorly (r2 <0.34) to the model or were not significant (p > 0.025). There is the possibility of a self-correlation as non-sperm concentration was calculated from the ratio of non-sperm:sperm multiplied by the total sperm concentration (WHO, 2010). However, only three significant correlations (p < 0.05) were found between sperm vs non-sperm cell concentration for 13Cu-glucose (40N), 13Cu-fructose (40A) and 13Cu-fructose+13C1-pyruvate (40A) cohorts (see supplementary Table LRM2).

Table LRM1: Results of a series of multiple linear regression of models of observed lactate integral (IntLac) as the outcome and the sperm (Concsperm) and non-sperm concentrations (Concnon-sperm) as the predictor/explanatory variables (see main text).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Substrate | Group | Concentration  n (outliers) | Stepwise linear regression | |  |
| Model | r2 | p-value |
| 13Cu-glucose | 40N | Vital, n =27 (4)  Motile, n =27 (4) | k+Concsperm  k+Concsperm | 0.67  0.62 | <0.0001  <0.0001 |
| 80N | Vital, n =29 (2)  Motile, n =27 (4) | k+Concsperm  k+Concsperm | 0.40  0.58 | <0.001  <0.0001 |
| 40A | Vital, n =12 (0)  Motile, n =12 (0) | k+Concsperm+Concnon-sperm k+Concsperm+Concnon-sperm | 0.85  0.83 | <0.001  <0.001 |
| 80A | Vital, n =10 (2)  Motile, n =10 (2) | k  k+Concsperm | –  0.44 | 0.047  0.035 |
| 13Cu-fructose | 40N | Vital, n =30 (2)  Motile, n =29 (3) | k+Concsperm+Concnon-sperm k+Concsperm+Concnon-sperm | 0.50  0.57 | <0.0001  <0.0001 |
| 80N | Vital, n =31 (1)  Motile, n =30 (2) | k+Concsperm  k+Concsperm | 0.42  0.75 | <0.0001  <0.0001 |
| 40A | Vital, n =12 (0)  Motile n =12 (0) | k+Concnon-sperm  k+Concsperm+Concnon-sperm | 0.66  0.90 | 0.0013  <0.0001 |
| 80A | Vital, n =11 (1)  Motile, n =12 (0) | k  k | –  – | 0.085  0.042 |
| 13C1-pyruvate | 40N | Vital, n =28 (3)  Motile n =28 (3) | k+Concnon-sperm  k+Concnon-sperm | 0.22  0.22 | 0.012  0.012 |
| 80N | Vital, n =31 (0)  Motile, n =14 (2) | k  k | –  – | <0.0001  <0.0001 |
| 40A | Vital, n =12 (0)  Motile, n =12 (0) | k  k+Concsperm | –  0.34 | 0.017  0.047 |
| 80A | Vital, n =10 (0)  Motile, n =10 (2) | k  k | –  – | 1.0  1.0 |
| 13Cu-glucose & 13C1-pyruvate | 40N | Vital, n =16 (0)  Motile n =14 (2) | k+Concsperm  k | 0.87  – | <0.0001  <0.001 |
| 80N | Vital, n =14 (2)  Motile, n =16 (0) | k+Concsperm  k+Concsperm | 0.81  0.90 | <0.0001  <0.0001 |
| 13Cu-fructose & 13C1-pyruvate | 40N | Vital, n =14 (2)  Motile, n =14 (2) | k+Concsperm  k+Concsperm+Concnon-sperm | 0.90  0.78 | <0.0001  <0.001 |
| 80N | Vital, n =16 (0)  Motile, n =15 (1) | k+Concsperm  k+Concsperm | 0.81  0.86 | <0.0001  <0.0001 |

The algorithm reports the linear regression model that most closely fits the data (see Equation 1), where ‘k’ represents no fit to the 13C1-lactate integral. Outliers were removed from both independent and dependent variables using the interquartile range multiplied by 1.5. A stepwise linear regression (function steplm, Matlab) was used to determine the correlation coefficient r2 and significance (A Bonferroni correction of p < 0.025 (0.05/2) was regarded as being significant).

Table TXI: Pearson correlation (r2, p) of sperm concentration vital, motile) vs non-sperm concentration†. p < 0.05 was regarded as being significant.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Substrate | Normozoospermia | | Athenozoospermia | |
| ‘40%’ | ‘80%’ | ‘40%’ | ‘80%’ |
| 13Cu-glucose | **0.23, 9.1x10-3** | 0.04, 0.30 | 0.24, 0.1 | 0.00, 0.97 |
| 13Cu-fructose | 0.06, 2.0x10-1 | 0.00, 0.90 | **0.50, 0.01** | 0.28, 0.09 |
| 13C1-pyruvate | 0.04, 3.3x10-1 | 0.06, 0.19 | 0.26, 0.09 | 0.01, 0.73 |
| 13Cu-glucose & 13C1-pyruvate | 0.25, 6.8x10-2 | 0.11, 0.25 | – | – |
| 13Cu-fructose & 13C1-pyruvate | **0.39, 1.7x10-2** | **0.35, 0.02** | – | – |

† Outliers for sperm, non-sperm and lactate removed, as appropriate, for values outside of the interquartile range multiplied by 1.5.

**References**

Calvert SJ, Reynolds S, Paley MN, Walters SJ, Pacey AA. Probing human sperm metabolism using 13C-magnetic resonance spectroscopy. *Mol Hum Reprod* 2019; **25**: 30-41.