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Simultaneous plasma and interstitial profiles of hormones and metabolites using URHYTHM: a novel ambulatory collection device

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Increasing the sampling resolution when examining plasma hormone and metabolite profiles will more accurately describe diurnal/circadian rhythms and expose previously undiscovered ultradian rhythms that underpin metabolic physiology(1). In human research studies this must be tempered by the practical, ethical and safety concerns of repeated cannulation/blood draws. Here we examine the correlation between hormone and metabolite profiles present in interstitial fluid microdialysate(2), and plasma to help solve this dilemma.

Hormonal markers of circadian phase (melatonin, cortisol) and metabolites will show similar profiles in plasma and interstitial fluid.

Compare time-series hormone and metabolite profiles collected in interstitial fluid using a novel ambulatory microdialysis collection device (U-RHYTHM) with simultaneously drawn plasma samples.

All study protocols were reviewed by Health Sciences Faculty Research Ethics Committee, University of Bristol. Fasted healthy male volunteers aged 18-35 (n=3) were fed a standardised breakfast (08:00), lunch (13:00), dinner (19:00) and snack (22:00) (2225kCal [83g protein, 273g carbohydrate, 83g fat, 27g fibre]). Participants remained on the study bed, lights off/sleep occurred between 23:00-07:00 (<4 lux).

A 20kDa cutoff 30 mm linear microdialysis membrane was placed in periumbilical subcutaneous tissue, perpendicular to the midline. The membrane was perfused at 1µl/min and the microdialysate collected every 20 minutes for 25 hours into discrete samples using a portable fraction collector (URHYTHM) worn around the waist. Time-matched blood samples were obtained from an antecubital fossa canula.

All samples were stored at -80°C prior to analysis. Hormones and metabolites were measured via targeted UPLC-MS/MS metabolomics, for the metabolites the AbsoluteIDQ® p180 kit (Biocrates Life Sciences AG)) was used with 10µL of plasma(3) and 15µL of microdialysate.

Simultaneous time-courses of n = 24 metabolites were detected in plasma and interstitial microdialysate: alanine, arginine, asparagine, citrulline, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, trans-4-Hydroxyproline, taurine, carnitine and acetylcarnitine.

Spearman rank cross correlation identified the time shift that was required to reach highest correlation between the plasma and microdialysate profiles. Excluding taurine, microdialysate profiles were delayed by 32.3 minutes (range 0-130) and 12.9 minutes (range 0-50) for participant 1 and 2, respectively ($p < 0.05$), only 3/20 metabolite profiles were correlated for participant 3. Trans4-hydroxyproline had the strongest correlation (r_s 0.890, 0.814 and 0.808), with only participant 3 displaying a time shift (50 minutes). Taurine had the weakest correlation in all 3 participants. (r_s 0.096, 0.182, 0.325).

These data demonstrate that our novel approach can be used to comprehensively detect hormone and metabolite profiles in interstitial fluid. Thus, the U-RHYTHM would serve as a useful tool, not only to increase sampling resolution in controlled laboratory studies but would prove particularly useful for examining circadian/ultradian profiles for chronobiology and nutrition studies in free-living individuals.

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