COMPARATIVE CHANGES IN PLASMA, URINE AND SWEAT DURING ENDURANCE EXERCISE USING NMR-BASED METABOLOMICS

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Abstract

Objective: The purpose of this study was to compare the relative metabolic profile of human biofluids before, during, and after strenuous endurance exercise. *Method:* Urine, blood, and sweat samples were collected from eleven healthy endurance trained cyclists at the beginning of, during, and immediately after a two hour cycle ergometer ride at 65% VO_2peak. The exercised-induced metabolic changes in the sampled biofluids were followed using nuclear magnetic resonance (NMR) spectra recorded on a 700 MHz NMR spectrometer. Within the biofluids, group separation was accomplished based on relative changes in the distribution of metabolites based on their concentrations using a combination of principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Specific metabolites in the NMR spectra could be identified from their characteristic peak positions. *Results:* Urine profiles showed significant pre- vs post-exercise differences based on relative metabolite concentration changes. The significant contributors to this difference were creatinine and acetoacetate, which increased following exercise, and glycine, citrate and alanine which decreased. The differences seen in blood plasma were almost exclusively due to the substantial changes in glucose and lactate levels. The most novel of the biofluids, sweat, interestingly showed pre-, mid-, and post- exercise differences due to significant increases in lactate and to a lesser extent, changes in pyruvate and glycerol. In several instances plasma and sweat showed contrasting metabolite concentration changes across the exercise regimen. *Conclusion:* The metabolic profile (metabolome) of plasma, sweat, and urine all show significant, yet differing responses to strenuous endurance exercise.

Key words: Biofluid, blood plasma, urine, Nuclear Magnetic Resonance spectroscopy, metabolome

1. Introduction

Presently, it is thought the metabolic profile of sweat generally reflects that of the plasma (Taylor *et al.*, 1994). Nevertheless, while plasma metabolite concentrations are routinely measured for health and physiological (investigative) purposes, those of sweat have received much less attention.

Sweating occurs, primarily, to assist thermoregulation, but sweat rates and composition will vary according to factors such as: body core temperature, age, gender, training status, physical activity, sympathetic stimulation, and anatomical position (Shibasaki and Crandall, 2010). Since sweat, once appearing on the epidermis, has minimal reabsorption (Sawka *et al.*, 2001) sweat-based metabolites should be considered lost to the body and thus could impact upon nutritional requirements beyond water needs.

The greatest rates of sweat production in healthy individuals occur during exercise where body core temperature rises, and rates of sweat production may exceed 1.8 $mg.min^{-1}.cm^{2}$ (Fortney *et al.*, 1981) . In these situations, significant losses in sweat-based metabolites may occur. Yet, we know little about what these metabolites are and the extent to which they might change. Extrapolation of plasma metabolite concentrations during exercise to sweat may therefore be inaccurate.

For example, durations of 90 min or more at a moderate exercise intensity are associated with increases in circulating free fatty acids, glycerol (Havel *et al.*, 1964), β-hydroxybutyrate (Hickson *et al.*, 1977), and selected

amino acids (Ahlborg *et al.*, 1974) whereas, plasma concentrations of glucose and lactate will decrease (Ahlborg *et al.*, 1974). Yet, for sweat, information regarding changes in the concentrations of these metabolites during exercise is scarce. If indeed sweat was to reflect the metabolic profile of the plasma, we would expect to see parallel changes in the profiles of both.

Metabolomics in the wider context of bio-analytical techniques is a relatively new "cousin" to genomics and proteomics. Specifically, metabolomics involves high through-put characterization of small molecule metabolites found in an organism. It offers a unique opportunity to look at genotype-phenotype as well as genotype-envirotype relationships. By applying metabolomics experimenters are not limited to analysis of individual assays of specific individual metabolites or metabolite groups. Metabolomics allows for an unbiased view of exercise metabolism and indeed may allow identification of new biomarkers associated with a particular exercise intervention.

To our knowledge, only one study to date has applied the metabolomics method to sweat. That study, performed on resting subjects and which involved only a single sample showed relatively high concentrations of lactate, serine, glucose, and glycerol (Harker *et al.*, 2006). Comparison with plasma was not made. Pechlivanis et al. 2010 have applied NMR-based metabolomics to urine collected before and after exercise. The results showed significant changes in lactate, hypoxanthine, pyruvate, alanine and intermediates of the TCA cycle (Pechlivanis *et al.*, 2010). The present study was designed to investigate the changes in the circulating metabolites in blood and if

they are reflected by changes in the composition of sweat before and after exercise. Further, we also aimed to compare these humoral metabolic profiles with that of the urine.

2. Methodology

2.1 Participants: Eleven trained male cyclists were recruited from the local cycling club. Their age, body mass (BM), peak aerobic capacity (VO_2peak) and peak heart rate (HRpeak) were 37.8 ± 12.4 y, 74.9 ± 8.0 kg, 63.3 ± 6.8 ml/kg/min and 176.8 ± 8.7 bpm (mean ± SD) respectively. The study was approved by the University's Human Ethics Committee.

2.2 Aerobic Capacity Test: The peak aerobic capacity test and familiarisation consisted of a continuous incremental exercise test on an electronically braked cycle ergometer (Lode B.V., Groningen, The Netherlands). The oxygen cost of cycling at a series of submaximal intensities was determined from the same test. The protocol consisted of four 5 min blocks starting at 150 W and increasing to 300 W in 50 W increments. Expired respiratory gases were captured in Douglas bags for the last 60 sec of each incremental block. The participant was then actively rested for approximately 5 min before beginning the VO_2peak section, which was a linear ramp protocol. Workload began at 100 W and increased by 30 $W.min^{-1}$. After the respiratory compensation point, expired respiratory gases were captured in Douglas bags for adjacent 30-40 sec periods until exhaustion. The contents of each Douglas bag were analysed for composition and volume using a dry gas meter and oxygen and carbon dioxide gas analysers. The rate of oxygen consumption was then calculated. A mathematical relationship between workload and VO_2 at the submaximal

intensities was developed, from which the workload corresponding to 65% VO_2peak could be determined.

2.3 Sweat Collection: Sweat samples were obtained by sticking an approx. 10 cm x 10 cm patch of polyethylene onto the participant's back after it had been cleaned with an ethanol wipe and allowed to dry. The sweat collected inside the patch and was removed using a Pasteur pipette at exercise time = 0, 60 min, and 120 min.

The experimental trial consisted of a fixed-intensity exercise bout lasting 2 hrs at the workload calculated to elicit 65% of the participant's VO_2peak. All experimental sessions were preceded by 24 h of dietary and physical activity control using a Food and Physical Activity Diary. On arrival to the laboratory, participants voided with urine being collected (mid-stream), and body weight was measured. The participants were placed on the cycle ergometer and allowed to warm-up while wearing a polyethyelene vest to initiate the onset of sweating. After a sufficient amount of sweat had collected in the patch (~2 ml) a sample was collected using a Pasteur pipette and the first 5 ml blood sample was taken via venepuncture (antecubital vein, BD Vaccutainer, Serum Tube). This was immediately followed by the commencement of exercise. Further blood and sweat samples were collected at the half-way point (t = 60 min) and on completion of the exercise bout (t = 120 min). Participants were stopped to allow for fluid sample collection, which took approximately 5 min. On completion of the exercise and sample acquisition the participant was immediately removed from the cycle ergometer and their final body weight and urine sample was taken. The participant was allowed to ingest up to

2.1 L of water. The session was completed in thermo-neutral conditions (18 ^{o}C) in the presence of a moderate-speed fan which facilitated convective and evaporative cooling. Heart rate was monitored throughout the protocol and noted at t = 0, 60 and 120 min.

2.4 Sample Prep: All samples were stored in a -80 $^{\circ}$C freezer. To remove any potential particulate contamination all thawed samples were centrifuged (6 min @ 6000rpm). Plasma samples were diluted 50:50 with buffer (75 mM sodium phosphate ; pH 7.4; ~80% Milli-Q (Millipore MA, USA)-H_2O/20%D_2O, 0.04% NaN_3, 0.08% d4- Trimethylsilyl propionic acid (TSP)).Urine samples were diluted 85:15 with phosphate buffer (1.5 M sodium phosphate, pH 7.4; 100% D_2O, 0.13% mM NaN_3, 0.1% d4- (TSP). Sweat samples were mixed 33:67 with the same buffer as used for plasma apart from TSP, which was reduced by 75% to avoid its peak dominating the NMR spectrum. 600 µL aliquots of the resulting solutions were then transferred to 5 mm NMR tubes.

2.5 NMR Analysis: All spectra were recorded using a Bruker Avance 700 MHz NMR spectrometer (Bruker, Rheinstetten, Germany) equipped with a standard four channel inverse detection probe. The temperature for all measurements was 300 K, calibrated using the residual ^{1}H signals from a standard sample of d4-methanol. For sweat and urine samples 1D NOESY spectra were recorded using the standard Bruker "noesygppr1d" sequence with a mixing time of 0.1 s, A spectral width of 11.16 kHz and 800 or 128 scans and 50k or 32k points for sweat and urine respectively. For blood samples the broad signals from high molecular weight components (proteins / lipids etc.) were partially filtered out using the 1D CPMG experiment with the standard Bruker "cpmggppr1d"

sequence with a spectral width of 9.76 kHz and 64k points. The CPMG pulse train had an inter-pulse delay of 0.3 ms and was applied for 38.4 ms. Signals were averaged for 256 scans. 2D J-resolved (JRES) spectra were also recorded for plasma samples using the standard Bruker "jresgpprqf" pulse program. Spectral widths of 11.7 kHz and 60 Hz digitized using 16k and 40 points were used in the direct and indirect dimensions respectively. Each row was averaged for 8 scans. All spectra used a recycle delay of 2 s.

2D phase sensitive TOCSY spectra were recorded for metabolite identification using the improved sensitivity of a cryoprobe. The experiment was performed using the Bruker "mlevesgpph" pulse sequence with a mixing time of 80 ms. A spectral width of 8.389 kHz with 2k and 256 points in the direct and indirect dimensions respectively. Each FID was averaged for 200 scans. All spectra were phased and baseline corrected using TOPSPIN (v. 2.5, Bruker, Rheinstetten, Germany) with urine and sweat referenced to the TSP peak at 0 ppm and plasma referenced to formate at 8.48 ppm before analysis.

2.6 Statistical Analyses: Statistical analysis was performed using MNova NMRsoftware (v. 9.0.0, Mestrelab Research, Santiago de Compostela, Spain) and SIMCA (v13, Umetrics, Umeå, Sweden). Each spectrum was divided into intervals (buckets) of 0.04 ppm over the range -0.5 – 10 ppm. The 4.15 – 5.15 ppm range, which includes the residual water signal, was excluded from all spectra, as was urea at 5.50-6.30 ppm and TSP reference at 0.00 ppm. The following additional ranges were excluded from plasma and sweat: 3.092-3.288, 3.610-3.685 ppm and urine: 3.24-3.32 ppm respectively. These include the signals corresponding

to EDTA anticoagulant (3.25, 3.65 ppm) in the plasma samples. The summed signal intensity of each bucket was normalized to the total intensity of its parent spectrum. Bucket intensities were then mean-centred and Pareto scaled for PLS-DA and OPLS-DA analysis. The effect of exercise on sweat lactate concentration was calculated using a 2-way ANOVA of exercise intervention and relevant lactate buckets (1.3365, 1.37643, 4.13118and 4.1711 ppm) from the sweat spectra. Permutation tests were used to determine the validity of all supervised multivariate models.

3. Results

3.1 The effects of exercise on plasma metabolomic profiles

PCA was performed on the plasma spectra classified as either taken before or after exercise. This resulted in a five component model with an R^2 of 1 and a Q^2 of 0.904. Visual inspection of the scores plot showed clustering of the samples with their assigned groups. A PLS-DA model was built using the same classification of samples resulting in a one component model with an R^2 of 0.677 and a Q^2 of 0.556. A clear separation of pre- and post- exercise samples was evident. This model was validated using a permutation test with 999 permutations; none of the R^2 and Q^2 values from the permuted models were higher than those for the original model. An OPLS-DA model was built to aid in the interpretation of the buckets contributing most to the group separation. The model consisted of one predictive component and four orthogonal components with an R^2 of 0.970 and a Q^2 of 0.873, separation of groups was clear across the predictive component (Fig. 1). The one dimensional loadings plot (Fig. 2) was used in combination with variable

importance on projection (VIP) scores for each column to determine the significant buckets. These buckets were used in conjunction with the original spectra and reference chemical shifts to determine the metabolites causing the groups separation.

Fig. 1 Plasma JRES OPLS-DA Scores Plot, differentiating pre-exercise (grey circles) and post-exercise (black triangles) samples

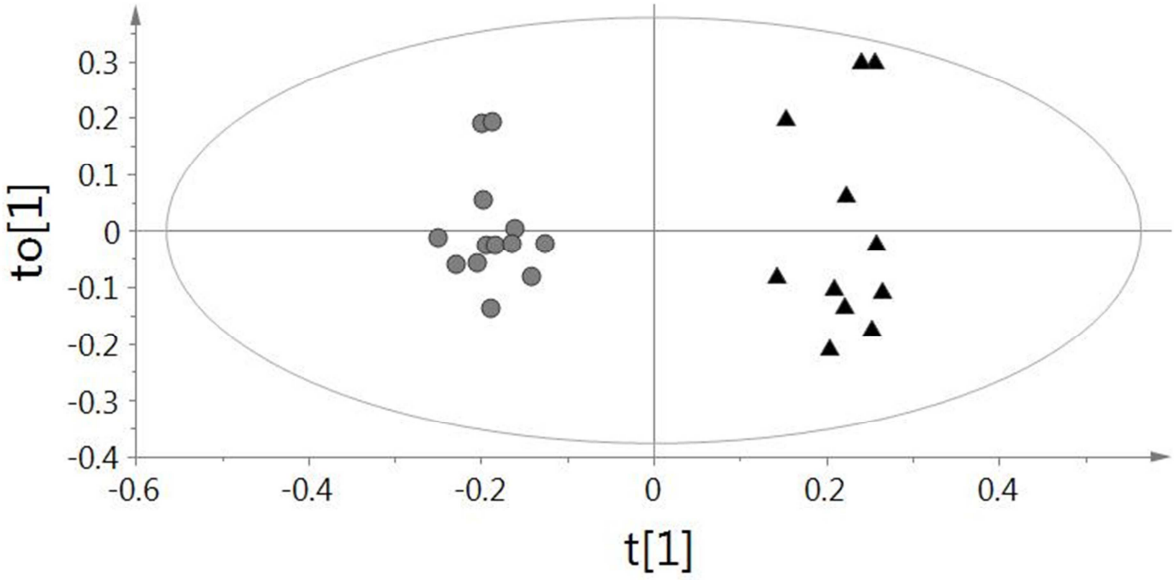
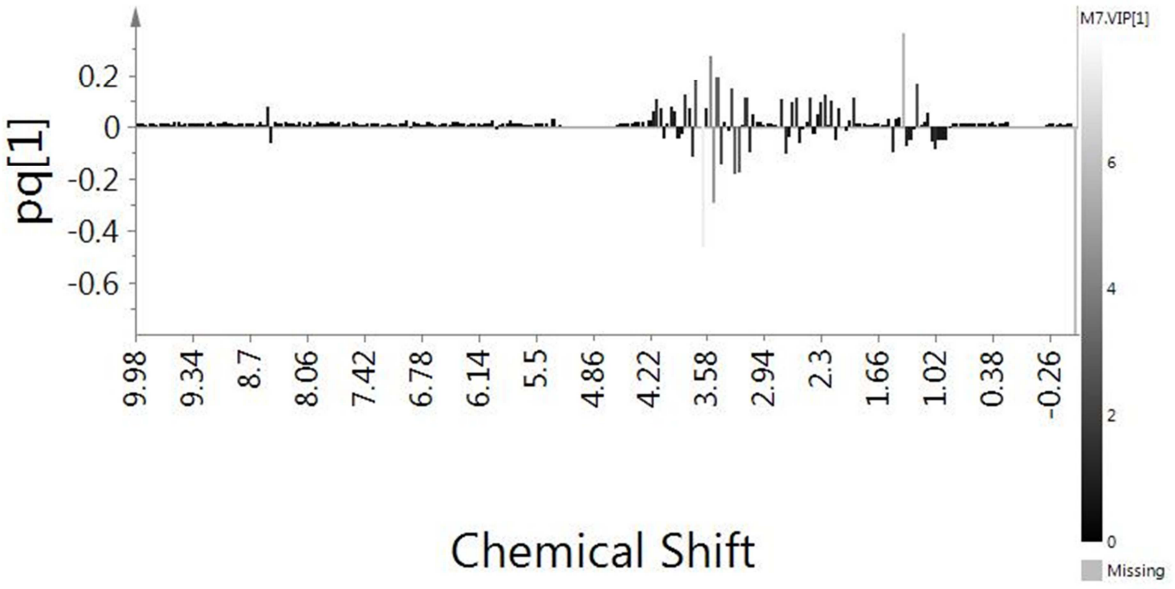


Fig 2. Plasma OPLS-DA Loadings Plot



3.2 The effects of exercise on sweat metabolomic profiles

Visual examination of the 1D JRES sweat spectra indicated that lactate had a much higher concentration than all other metabolites within each sample. It was decided to exclude the lactate signals from the multivariate statistical analysis and instead investigate it using two-way ANOVA. In this way the lactate concentration would not produce spurious variation in the other metabolites due to the effects of normalization to total intensity.

Two-way ANOVA of lactate spectra, based on exercise intervention, showed a significant effect ($p = 0.029$) of exercise on sweat lactate concentration with before and after bucket means of 17.195 ± 0.834 and 19.996 ± 0.580 respectively.

PCA of the sweat 1D JRES spectra before and after exercise produced a ten component model with an R^2 of 0.996 and a Q^2 of 0.965. Inspection of the scores plot showed clustering of the spectra into before and after exercise groups, though this clustering was not as distinct as that found in the PCA scores plot of plasma. The PLS-DA model built had two components with an R^2 of 0.819 and a Q^2 of 0.689, this model was validated using permutation testing as described above. The OPLS-DA model built to improve interpretation of the significant loadings consisted of one predictive component and five orthogonal components with an R^2 of 0.962 and a Q^2 of 0.770. The separation of pre- and post- exercise groups was large across the predictive component (Fig. 3). The one dimensional loadings plot (Fig. 4) was used in combination with variable importance on projection (VIP) scores for each column to determine the significant buckets. The metabolites

contributing to the group separation were identified as described above.

Fig. 3 Sweat (Excluding Lactate) OPLS-DA Scores Plot, differentiating pre-exercise (grey circle) and post-exercise (black triangle) samples

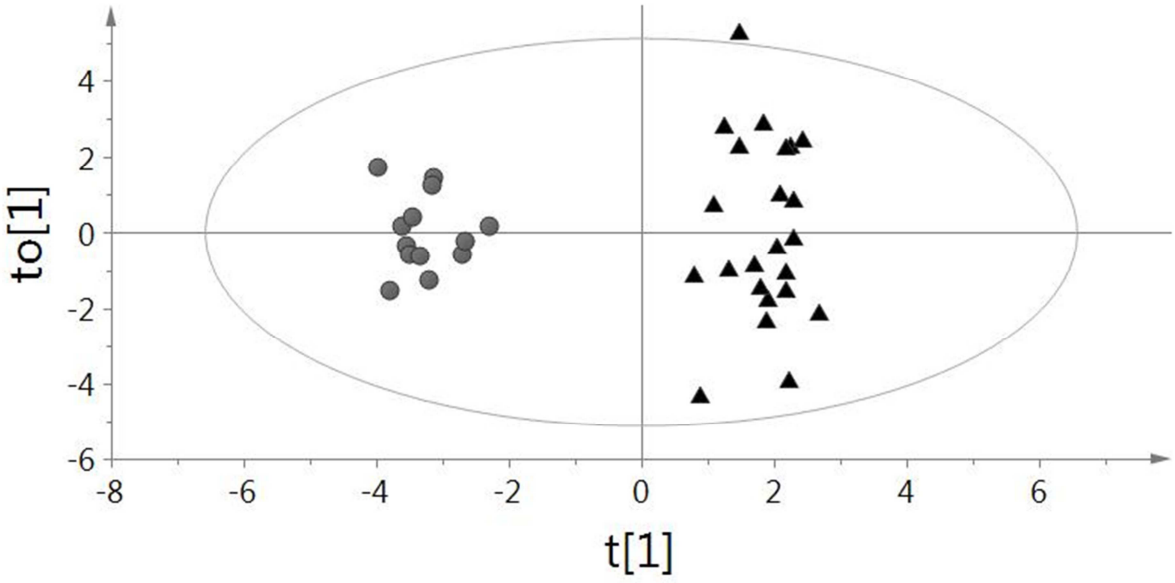
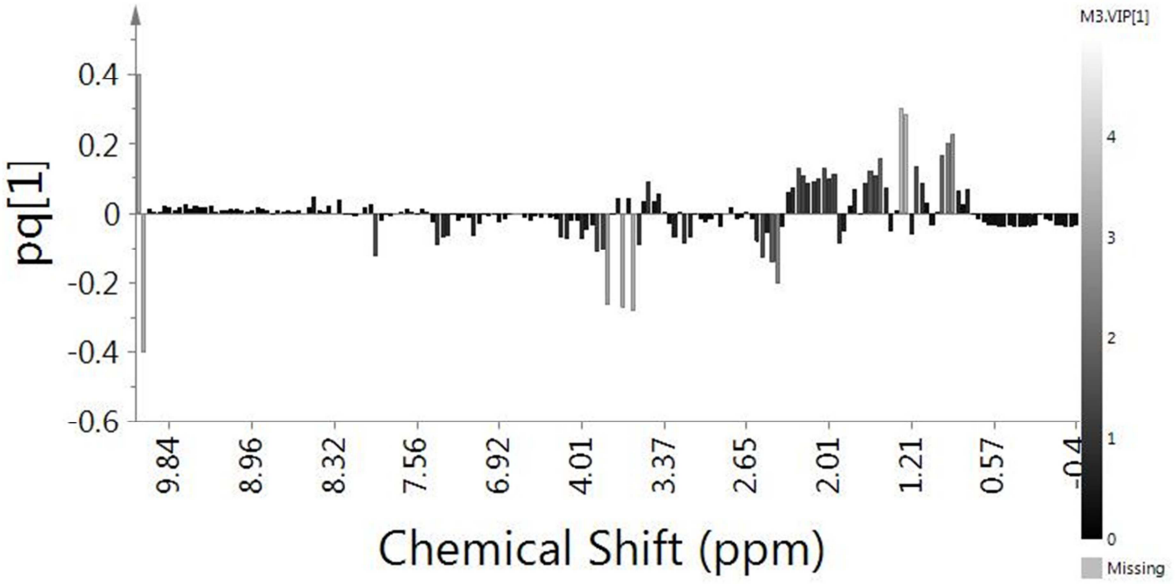


Fig. 4 Sweat (Excluding Lactate) OPLS-DA Loadings Plot



3.3 Comparison of sweat and plasma analysis

On comparison of sweat and plasma four of the major contributors were similar across fluids; lactate, pyruvate, glycerol and acetate. However, the nature of the changes in these metabolites varied with lactate increasing across both fluids, glycerol decreasing in both, and acetate and pyruvate increasing in the plasma but decreasing in the sweat (Table 1).

3.4 The effects of exercise on urine metabolomic profiles

A three component PCA model constructed from the NOESY spectra of the urine samples showed no clustering based on collection time in reference to exercise. The model's R^2 value was 0.986 and its Q^2 was 0.965. However, when a PLS-DA model was produced using the collection time labels it was found to cluster the samples well. The model had three components with an R^2 value of 0.881 and its Q^2 was 0.577. The model was validated as described above; all permuted Q^2 values were lower than that of the model while the R^2 of the model was higher than the majority of the permuted values. This still allows for the model to be described as valid. An OPLS-DA model (Fig. 5) was built to simplify the interpretation of the loadings plot. The model was constructed from one predictive component and five orthogonal components, and had an R^2 value of 0.968 and a Q^2 of 0.728. Using the loadings plot (Fig. 6), the original spectra and reference values the significant contributors to the group separation were identified; creatinine and acetoacetate increased following exercise while glycine, citrate and alanine decreased.

Fig. 5 Urine OPLS-DA Scores Plot, differentiating pre-exercise (grey circle) and post-exercise (black triangle) samples

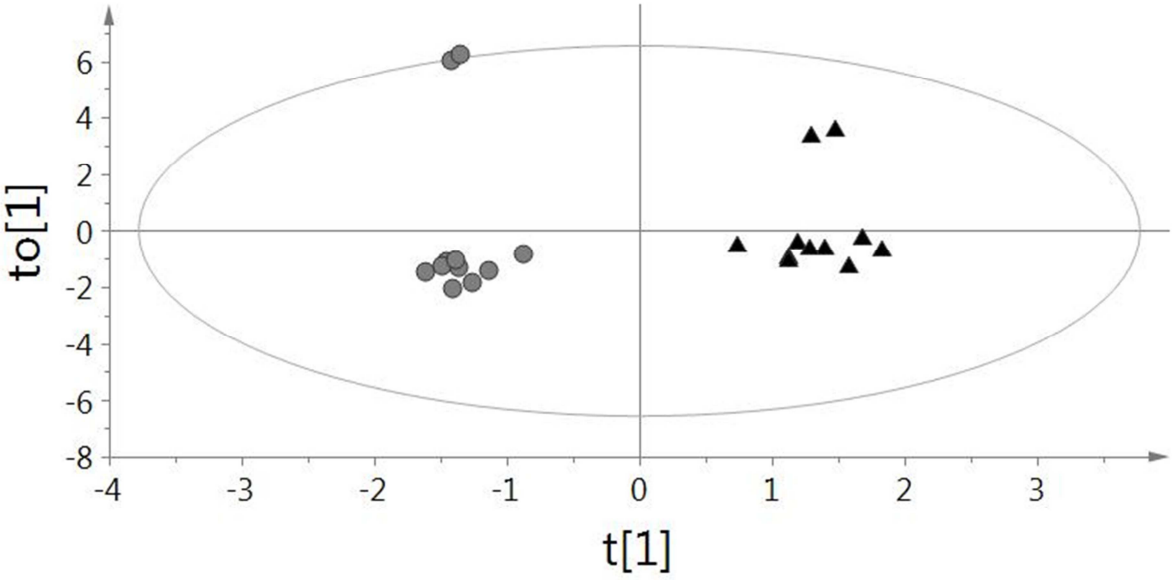


Fig. 6 Urine OPLS-DA Loadings Plot

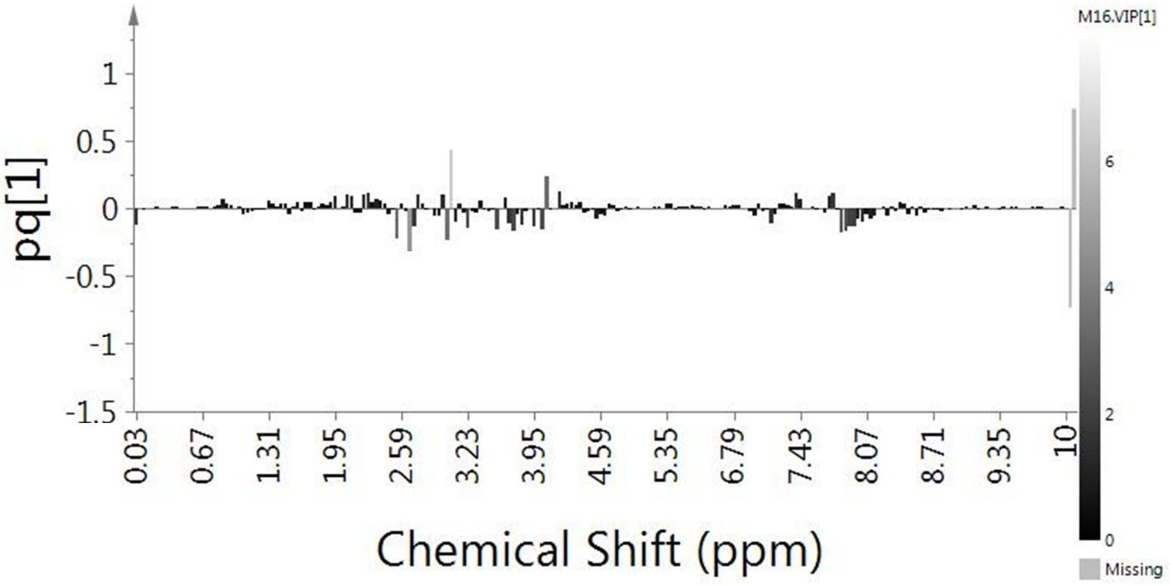


Table 1. Significant Metabolites and their Variable Importance Indices.

Metabolite	Chemical shift (ppm)	Sweat (VIP)	Plasma (VIP)
Pyruvate	2.38s	↓ 2.84	↑ 1.75
Acetate	1.92s	↓ 1.17	↑ 1.77
Gycerol	3.58m, 3.67m, 3.79m	↓ 1.19/3.37	↓ 1.11
Lactate	1.33d, 4.12q	Set as Exclusion Baseline	↑ 5.81, 1.7

The values beside the metabolites are their variable importance on projection (VIP) values. The higher the VIP value for a bucket, the more important it is in terms of being able to classify the samples into the separate groups. Metabolites are often ranked based on their VIP values or their VIP range e.g. all those with VIP between 1-2 then the ones 2-3 etc. Included are all the buckets which have a VIP greater than 1 (these all have a greater than average impact on the classification) and which are not noise.

4. Discussion

The primary purpose of this study was to investigate the relationship between the metabolic profiles of blood, sweat and urine before and after prolonged exercise. The "broad brush" metabolomic approach we applied was useful in identifying and characterising the changes in concentration of metabolites previously undetected, particularly in sweat, and the relative changes in these as a result of prolonged exercise. The main finding was that compared to plasma, the metabolome of sweat saw similar group separation pre- and post-exercise intervention but the cause of the separation differed between the two bio-fluids. Urine however, failed to show any such comparison with either of the other biofluids.

The major metabolite classes identified in sweat were lactate, creatinine, sugars

and lipids; lactate being the dominant metabolite found across all sweat samples. This is in agreement with previous published work as the typical lactate concentration of eccrine sweat in exercising adult humans is approximately 10 mM (Taylor *et al.*, 1994). Lactate present in sweat is considered to be derived from blood glucose (Gordon *et al.*, 1971) and its concentration is often used as an index of metabolic activity (Wolfe *et al.*, 1970). The significant increase in lactate ($p < 0.05$) thus correlates with the increased metabolic activity expected during prolonged moderate intensity exercise (Horowitz and Klein, 2000), compared to the initial (warm-up) sample. Further, the activity of the heat-activated eccrine sweat glands require increased myoepithelia cell contraction to support the expulsion or secretion of sweat (Green *et al.*, 2004). The concurrent decrease in sweat and increased blood pyruvate supports the idea of increased glandular metabolism causing the higher lactate concentration over time rather than a simple transpose of what is occurring in the blood.

It is interesting to note that three of the four significant and comparable changes in sweat and blood involve H^+/ monocarboxylate transporters (MCT) (Lin *et al.*, 1998; Juel and Halestrap, 1999) which may suggest a pH regulatory mechanism for the changes in acetate. A possible relation could be the proton-dependent aquaporins that facilitate the transport of water molecules and which are also transporters of glycerol (Zeuthen and Klaerke, 1999), another of the comparable metabolites.

The detection of lipid as a major metabolite class in the human sweat samples confirms previous reports

detailing the occurrence of the lipids in eccrine sweat (Bunting *et al.*, 1948).

The most important metabolites responsible for a pre-post group separation within urine were creatinine, acetoacetate, glycine, citrate and alanine. The increase in serum creatinine, the dominant contributor, after prolonged heavy exercise is likely due to an increase in production and a lesser than proportional increase or even a decrease in urinary excretion (Refsum, H. E. & Strömme, S. B. 1974). The remaining four contributing metabolites are indicative of increased myocellular metabolism(Essén *et al.*, 1977). However, the urinary excretion is said to be correlated with age (Refsum and Strömme, 1974), which varied considerably in the present study (37.8 ± 12.4 y). Many of the contributing metabolites found in blood plasma were representative of increased rates of glycogenolysis and, mobilization of lipid for oxidative phosphorylation in the working skeletal muscle (Ormsbee *et al.*, 2007).

The comparative decrease of glycerol during exercise in sweat and plasma could either be related to an overall lower rate of lipolysis and greater reliance on IMTG as exercise progresses, or increased uptake and utilization of glycerol in the liver. It has previously been shown that during prolonged exercise both FFA and Glycerol concentrations decrease in endurance trained males (Hurley *et al.*, 1986). Whether the sweat glycerol concentration is linked to blood glycerol concentration remains to be seen. It is possible glycerol could be more like the comparable increases in lactate which were more specific to glandular metabolism rather than blood lactate.

The present study progressed from that of Harker et al (2006) by including an

intervention (exercise) and higher concentration samples. The comparative increase in concentration and resolution allowed for better separation of groups and identification of lower concentration metabolites which are potentially relevant under more in-depth analysis and would otherwise be missed. During further analysis this would allow to progress further down the hierarchy of metabolic changes.

There is no pre-existing sweat pool in resting humans (Lloyd, 1959), implying that the metabolome of sweat should be closely related to that of blood-borne metabolites during exercise. Buono (1999) displayed a direct correlation (r = 0.98) between sweat ethanol concentrations and those of co-existing blood values. When expressed as millimoles per litre (*mmol/L*) of water both biofluids were proportional to each other, suggesting rapid equilibrium of ethanol across the sweat gland epithelium. This may be correct for ethanol as endothelia and phospholipid bilayers in general are very permeable to ethanol, but information on the permeability of cells to almost every other metabolite is poorly known. Buono (1999) also provides evidence that coefficients could potentially be applied to metabolites that are not proportional in both blood and sweat. Thus, once coefficients have been determined for major metabolites calculating their concentrations from sweat can be done with relative easy.

To date there are only a handful of instances where this method has been applied to research relating to sport and/or exercise (Harker *et al.*, 2006; Kuhl *et al.*, 2008; Yan *et al.*, 2009; Pechlivanis *et al.*, 2010). These studies reveal the potential benefits of metabolomics in sport and exercise by comparing results against those obtained with “traditional” techniques

such as biochemical assays. For example, Pechlivanis et al. 2010 compared the effect of two different exercise sessions on the NMR detected metabolic fingerprint of human urine. To change the metabolic profile, subjects completed two exercise sessions of 3 sets of 80 m runs separated by either 10 seconds (group A) or 1 min (group B) with subsequent urine collection. The urine of group 'A' had a higher lactate concentration than group B because of reduced time to metabolise the lactate in the first two bouts of exercise. However, when lactate was suppressed from the analysis, separation between A and B was due to pyruvate and citrate. It is this ability to distinguish a hierarchy of separation between conditions (e.g. pre- and post-exercise) through which metabolomics can help derive new knowledge in the discipline of exercise science.

The results obtained in the present study confirm the beneficial application of NMR-based metabalomics to the metabolite profiling of human biofluids and in particular human sweat samples. High-resolution ^{1}H NMR spectroscopy allowed acquisition of spectra from the biofluid samples, in which a large array of metabolites could be readily identified by comparison of the data with spectra from authentic reference standards of common endogenous metabolites (Lindon *et al.*, 2000).

5. Conclusion

Determining the biochemical significance of the results from each of the samples has the potential for further discoveries. Results for individual fluids were in accordance with previously reported data, but differences between fluids indicate some interesting and

novel findings. The opposing changes, during prolonged exercise, of specific metabolites existing within sweat and plasma suggest sweat constituents are not a direct replica of what is circulating in the blood but rather the result of a far more complex system of cellular and glandular metabolism. Future studies should seek to reduce intra-group variance of the biofluid profiles by use of a controlled diet in the lead up to the trial and the recruitment of participants within a smaller range of age and fitness (37.8 ± 12.4 y, VO2peak = 63.3 ± 6.8 ml/kg/min for the participants in the present study). Nevertheless, the present study confirms that NMR-based metabolomics is a viable means of analysing biochemical changes caused during exercise.

Key Points
• Sweat can be used as a non-invasive measure of exercise related biomarkers which could be used to determine specific metabolic activity.
• Metabolomics provides an efficient means of comparing the metabolic status of individuals by simultaneously detecting changes in concentrations of multiple metabolites, offering some distinct advantages over traditional targeted analysis.
• Changes in metabolite concentrations in sweat are not directly related to those in the blood plasma.

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Abbreviations:

NMR	Nuclear Magnetic Resounace
PCA	Principle Component Analysis
PLS-DA	Partial Least Squares Discriminant Analysis
OPLS-DA	Orthangonal Partial Least Squares Discriminant Analysis
TCA	Tricarboxcyclic Acid Cycle
VO_2peak	Peak Aerobic Capacity
BM	Body Mass
HR	Heart Rate
Hr_{peak}	Peak Heart Rate
TSP	Trimethylsilyl Propionic Acid
H_2O	Water
NaN_3	Sodium Azide
D_2O	Deuterium Oxide
NaH_2PO_4	Sodium Dihydrogen Phosphate
M	Molar
^1H	Hydorogen
JRES	J-Resolved
ANOVA	Analysis Of Variance
EDTA	Ethylenediaminetetraacetic Acid
VIP	Variable Importance On Projection
IMTG	Intramuscular Trigyceride
FFA	Free Fatty Acid

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