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Metabolic load comparison between the quarters of a game in elite male basketball players using sport metabolomics

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Abstract

Purpose: A basketball match is characterized as highly intermittent, thereby relying extensively on both aerobic and anaerobic metabolic pathways with four 10-min quarters. Here, we aimed to compare the metabolic fluctuations between the quarters of high-level basketball games using metabolomics analyses.

Methods: 70 male basketball players with at least 3 years of experience in the Iran national top-league participated. Before and after each quarter, saliva samples were taken for subsequent untargeted metabolomics analyses, where Principal component analysis (PCA) and Partial least squares-discriminant analysis (PLSDA) were employed for statistical analysis.

Results: Quarters 1 and 3 showed similar metabolic profiles, with increased levels of ATP turnover (higher Lactate, Pyruvate, Succinic Acid, Citric Cid, Glucose and Hypoxanthine), indicating more

reliance on anaerobic energy systems than quarters 2 and 4. In comparison, Quarters 2 and 4 showed a reduction in Valine and Lucien and an increase in Alanine, Glycerol, Aceto-Acetic Acid, Acetone, Succinic Acid, Citric Acid, Acetate and Taurine that was not present in quarters 1 and 3, indicating greater reliance of aerobic energy contribution, fat metabolism and gluconeogenesis. **Conclusion**: Our data demonstrates that the higher intensity of movements in the first quarter, where players are more rested; induce a increase anaerobic energy contribution. This seems to be the case also for the third quarter that follows 15 minutes of rest, whereas the accumulated fatigue and reduction of high-intensity movements in the second and fourth quarters also reduces the speed of energy production and players relay more on aerobic energy.

Keywords: internal load, external load, team sport

Introduction

Modern basketball is characterized by intermittent high-intensity activities, relying extensively on both anaerobic and aerobic metabolic pathways (1). The match is divided into four 10-min quarters, with a 15 min break between the second and third quarters and the only a 2-minutes break between the others. Previous studies have examined movement demands of basketball, showing that distance traveled during entire basketball matches ranging from 4404 to 7558 m (2). Here, the relative activity frequencies varied between 21.2 and 56.9 movements per min across studies, including various types of activities/movements. The most used activity is standing/walking, representing 23.4–66.3% of the time, complemented by 5.6–36.3% jogging, 4.5–33.2% running, 0.3–8.5% sprinting, 2.1–14.7% low-intensity shuffling, 6.5–19.8% moderate-intensity shuffling, 0.4–9.3% high-intensity shuffling, 0.6–2.3% jumping and 1.2–10.6% dribbling (2). The large variations in the relative use of these activities are mainly influenced by playing positions, playing levels and geographical locations (2). In addition, when approaching the end of the halves/game movement type tend to change due to the level of player's fatigue. Consequently, these different movement demands result in different metabolic responses in different quarters.

Currently, the difficulties in performing metabolic measurements during real-life games have limited the measurements to heart rate (HR) and blood lactate (2). Average game HR and blood lactate concentrations have been reported, ranging between 89–95% of maximal HR and lactate

levels of 5.2±2.7 mmol L^{-1} (3, 4). However, these measures have clear limitations and do not provide a detailed understanding of how the aerobic and anaerobic metabolic pathways interact to provide energy for displacements, accelerations, decelerations, sprints, continuous changes of direction, jumping, and specific technical skills (5, 6). In addition, a key factor for good performance throughout the basketball game is the ability of players to sustain high power output in all quarters of the game (2), in which aerobic metabolic pathways play a key role for lactate clearance (3) and phosphocreatine regeneration (3, 7).

Due to the different movement demands across quarters, it is also likely that the four quarters of a basketball match would have different metabolic demands. For example, Abdelkarim et al. showed the higher value in the work to rest ratio in the first half compared to the second half (8). In addition, another study by Abdelkarim et al. reported that the first and third quarters were played at a higher intensity than the second and fourth quarters (9). Conversely, no significant effect of game timing (first half vs. second half) was observed on HR and blood lactate concentration in the women's basketball game (10). Investigations of the more detailed metabolic changes during a real game, including the possible changes across quarters, would provide us with important information, both for understanding the demands of the sport and for designing sport-specific training programs (11). However, the complexity and integrated nature of the exercise response warrants global unbiased systems approach to illustrate the response and adaptation caused by exercise (12). Omics technology has contributed to solve this problem, aiming for collective characterization and quantification of pools of biological molecules underpinning exercise responses and adaptations (13). In this context, metabolomics has an advantage as it provides "functional" information and represents the final "omics" level in a biological system (14). Different biological samples, including plasma, serum, saliva and muscle tissue have been used in exercise metabolomics studies (15). However, saliva is considered a non-invasive method and its collection requires neither laboratory facilities nor skilled healthcare professionals. Still, it contains both body as well as oral bacteria metabolomes (16). Taken together, researchers have suggested that the use of saliva provides particular valuable information in exercise metabolomics studies due to its advantages (15-20).

In the current study, we characterized the metabolic response in each quarter through a metabolomics approach where we compared metabolic responses and performance across quarters of a basketball match among elite male basketball players. We hypothesized that the first quarters

of each half would rely more on anaerobic whereas the second and fourth quarters would demand more aerobic metabolism.

Material and Methods

Participants

All teams, which were participated in the 2017-2018 Iranian national basketball league, were informed about the study and invited to participate in the introduction meeting. After this meeting, fourteen teams were agreed to participate in the study, which their starters (5 starters per team, total 70 players) had at least three years of league experience (inclusion criteria). Participants' demographic properties were as follows: age: 24.2±5.3 years, body height: 192.4±12.3 cm, body mass: 88.7±6.4 kg, body fat percentage: 10.9±1.3, BMI: 24.1±0.8 kg/m^2. Participants with physiological disorders were excluded from the study to avoid physiological interference. Participants were requested not to use supplements during the study period. They were also asked to follow a pre-competition diet that included at least 7 $g.kg^{-1}.d^{-1}$ of carbohydrates. The obligation to this diet was confirmed by an eating diary. These diaries were also used to calculate nutrition intake by a nutrition software (PCN software; Cesnid, Spain). The day before games, participants consumed 3986±305 kcal, which consisted of 67.2% carbohydrate (2678.2 kcal), 20% (797.20 kcal) protein and 12.8% fat (510.60). The training load was decreased by 50% three days prior to the beginning of the study. This study was approved by the Research Ethics Committee of the University of Tehran (IR.UT.SPORT.REC.1398.007).

Procedure

70 players were analyzed in 7 games (10 players in each game). Each team starters played 40 minutes and no substitutions were allowed. Games consisted of 4 quarters of 10 minutes with 15 minutes rest between second and third quarters and 2 minutes between others. Players were asked to come to the stadium at 8:00 am. to eat normal breakfast of 400 calories (240 calories of carbohydrate, 120 calories of protein and 40 calories of fat). Players drank 200 ml water before the game and during each half break to avoid dehydration (9). The water was pure and didn't include electrolytes and glucose. Salivary flow rate (dividing the volume of saliva by the collection time) and body mass were measured to assess hydration status. No differences in body mass or saliva flow rate from pre-game to post-game were observed within any of the games, suggesting

that the athletes were able to maintain an appropriate hydration status during all games (21). All the games were started at 11:00 pm. Players started to warm up 30 minutes before the game, which was consisted of running, dribbling, shots and, passes. Before (10 minutes after water drinking) and immediately after each quarter (without any rest interval) saliva samples were collected. Players spit in the 15 ml Falcon until at least 3 ml saliva was included in each sampling. This process remained the same for all seven games. The samples were placed on liquid nitrogen and kept at -80°c until analysis (9).

Metabolomics

Hydrogen nuclear magnetic resonance (HNMR) was used to compare the metabolic changes between quarters. One-dimensional HNMR was performed using a Bruker DRX-500 spectrometer (Bruker company, Madison, USA). equipped with a BBFO probe with z-axis gradients maintained at 298K. Each spectrum was acquired using the Carr-Purcell-Meiboom-Gill (CPMG) pulse, with 154 scans over a spectrum width of 8389.26 Hz, and acquisition time of 2 s. CPMG reduces wide protein resonances and helps increase the resolution of low molecular weight metabolites. All spectrum referred to methyl lactate (1.33 ppm). After sampling, the samples were placed at ambient temperature to be liquefied. They were then centrifuged for 20 minutes (10000 rpm, 4°c) to remove supernatant. Thereafter, 450 microliters of the samples were dissolved in 150 microliters of D2O, transferred to 5mm high-quality tubes, and put in HNMR device. We used D2O as internal standard, which can be considered as our calibration method. The samples were analyzed one by one using the HNMR device. Each analysis took about 20 minutes to complete. Unfortunately, we did not confirm our analyses by using 2D HNMR.

Spectra processing, Statistical analysis and metabolites identification

Baseline correction, phase correction, normalization, and the water peak deletion were performed using MestreNova (MasterLab company, Santiago De Compostela, Spain). Then, all data entered into Excel (each sample consisted of 408 numbers). Principal component analysis (PCA) and Partial least square discriminant analysis (PLS-DA) were used to identify the different bins using metaboAnalyst (www.metaboanalyst.ca)(22). After that, the bins with 2 fold-changes considered as significantly different and entered to the human metabolome database (http://www.hmdb.ca) to identify associated metabolites.

Video-based Time motion analysis (VBTMA)

All games were recorded with 2 static cameras (Basler A602FC; Basler Vision Technologies, Germany). Each camera was mounted aligned with the middle of half-court at height (~15 m) and distance (~9 m) to capture all activity within one half of the court. Players were recorded for entire matches (including all stops) and their activity was analyzed during live match time. Mean frequency and distance (m) were calculated for each quarter. Recordings were analyzed by Dartfish 10 Pro (Dartfish company, Fribourg, Switzerland). Dartfish 10 pro is 3D software which has a basketball court template matched with the court in our recordings. To avoid inter-observer variability, a single experienced observer analyzed all games. Before the study, the observer analyzed two quarters of the same game 1 month apart, reporting high test-retest reliability (r = 0.85).

Statistics

PCA (unsupervised method) and PLS-DA (supervised method) were used to identify the different bins in spectrums as mentioned. In PCA, the spectra are categorized only by frequency, and regardless of the sample conditions (e.g. quarters). PCA produce principal components (PCs) that explain the variance of data, derived from the linear combination of variables (metabolite signals) obtained from NMR. PLSDA, requires data with specified conditions (e.g. specified quarter) and is develop a model regarded effective for predicting physiological conditions and to detect subtle differences between similar samples (23). Descriptive statistics (mean±SD) were calculated for each variable of VBTMA. To assess normality, the Kolmogorov–Smirnov test was conducted and supported use of parametric analyses. A repeated measures ANOVA was used to study the differences between quarters, followed by a Bonfferoni post-doc test.

Results

Players' movements frequency and distance were calculated in each quarter (Table 1). Repeated measure ANOVA showed significant differences between the quarter's movements frequency and distance (both p=0.00), with post hoc tests localizing differences in frequency between the first and second quarters (p=0.01), first and fourth quarters (p=0.00), third and second quarters (p=0.02) and third and fourth quarters (p=0.00). The specific differences in movement distance were between the first and second quarters (p=0.02), first and fourth quarters (p=0.03), third and second

quarters (p=0.00), and third and fourth quarters (p=0.00). Players movements details can be found in appendix 1.

Spectra measurements done before and after each of the quarters were compared using PCA and PLS-DA (Figures 1). PCA shows some differences in the first quarter while PLSDA shows significant differences (Figures 1a and 1b). Specifically, significant increases in Lactate, Pyruvate, Succinic Acid, Citric Acid, Glucose, and Hypoxanthine are responsible for these differences, which all show more than 2-fold changes.

Before and after the second quarter, spectra comparison (both PCA and PLS-DA) show significant differences in the players metabolome (Figures 1c and 1d). Specifically, significant reduction in Valine and Lucien and increase in Alanine, Glycerol, Aceto Acetic Acid, Acetone, Succinic Acid, Citric Acid, Acetate and Taurine were detected.

As shown in the figure 1e, PCA does not show any difference in the players metabolome, while PLS-DA shows significant difference in the third quarter (Figure 1f). Specifically, increases in Lactate, Pyruvate, Hypoxanthine, Succinic Acid, Citric Acid, Glucose and Glycine are responsible for this significant difference.

Both PCA and PLS-DA show significant differences in the players metabolome in the fourth quarter (Figure 1g and 1h). Specifically, reduction in Valine, Lucien, and Methionine and increase in Alanine, Glycerol, Aceto Acetic Acid, Acetone, Succinic Acid, Citric Acid, Acetate, Taurine and 3-metyle histidine were the main changes in this quarter.

Table 2 shows the metabolic changes within each quarter along with their fold changes. Overall, by comparing the within-quarter changes, the implications are that more anaerobic energy system engagement is present in the first quarter compared to the other quarters. In contrast, the second quarter relay more on aerobic, brain chain amino acid (BCAA), and ketone body metabolism, whereas the third and fourth quarters have metabolic profiles that are very similar to first and second quarters, respectively. However, Glycine in the third quarter and 3-metylehistidine in the fourth quarter showed significant increases that were not present in the two first quarters. In addition, methionine was also reduced in the fourth quarter.

Discussion

In the current study, we used metabolomics to compare the metabolic load between different quarters of elite male basketball players in 40-minute basketball games where all players played entire game. Our main finding was that indicators of anaerobic metabolism such as Lactate, Pyruvate, Succinic Acid, Citric Cid, Glucose and Hypoxanthine showed an increase in the first and third quarters of the game that was not present in the two other quarters. In comparison, aerobic metabolism indicators such as Alanine, Glycerol, Aceto Acetic Acid, Acetone, Succinic Acid, Citric Acid, Acetate, and Taurine were increased in the second and fourth quarters, whereas Valine and Lucien showed a reduction that was not present in the two other quarters. In addition, Glycine and 3-methylhistidine were increased in the third and fourth quarters, respectively and methionine showed decreased in the fourth quarter only.

While players usually play about 30-minute in a real game, including several timeouts and substitutes, this study did not allow technical timeouts and substitutes. Consequently, only players playing the entire game were analyses and coaches needed to prepare their athletes to play entire game. Therefore, the metabolic responses in real games could be slightly different from the results presented here. Methodologically, Pitti et al. (16) and Ra et al. (17) studied soccer players salivary metabolome using HNMR and reported this method as a valid, non-invasive, unbiased and comprehensive approach to study metabolic changes during exercise training and sport games. These properties have made metabolomics more informative, sensitive and accurate compared to previous methods used study exercise physiology/metabolism in similar situations (24).

In this study, Lactate, Pyruvate, Succinic Acid, Citric Acid, Glucose and Hypoxanthine were increased significantly during the first quarter. Pyruvate and Lactate are well-known to increase during such intense exercise activities (7, 25-31), which can also explain why Citric Acid and Succinic Acid, two main mediators of the Krebs Cycle, increase in the first quarter (32). An increase in Krebs cycle activity is likely associated with restoration of phosphocreatine and lactate clearance following this intense activity (32). Pitti et al. also showed a significant increase of lactate and correlated metabolites, such as succinate and pyruvate, after a soccer game (16). Furthermore, this study and others (18, 19), report high correlation coefficients between lactate in blood and saliva during exercise. As expected, Glucose levels increased 3-folds in the first quarter as a sign of increased ATP demand (33). Increased ATP demand can result in increased Purine

cycle activity and its main enzymes adenylate deaminase and adenylate kinase which, in turn, can explain higher hypoxanthine concentration in the first quarter as shown here and in a previous study (30). Taken together, these changes indicate a high rate of ATP breakdown, consumption, and recovery; implying more anaerobic energy system engagement compared to the other quarters.

While our study uses a novel approach to study basketball metabolism, our findings are supported by previous findings of Abdelkarim et al. who showed higher lactate production in first than the second half of a basketball game (6, 8). Furthermore, McInnes et al. (34) and Matthew et al. (3) reported tendencies to decreased lactate production during a game time as a likely result of decreased movement intensity. Similarly, our data indicated that frequency, distance and duration of high intensity shuffling and movements with and without the ball were significantly higher in the first quarter compared to others. The activity profile (external load) will obviously lead to metabolic changes (internal load) and therefore the higher frequency, distance and duration of high intensity movement in the first quarter confirm the need for higher anaerobic system contribution in this quarter (35). This is further supported by Abdelkarim et al. (8), Delextart et al. (36) and García et al. (35) who all reported more high intensity movements in the first compared to other quarters. In contrast, Oba et al (37) and Scanlan et al (38) did not report any significant different in activity profile between the four quarters. This might be explained by methodological differences as we analyzed activity profiles in the live time (i.e. during the 40-min active part of the game), while Oba et al. (41) and Scanlan et al. (42) used total time (i.e. from start to end of the total game, including stopwatches). This should be taken into the account in the activity profile interpretation and comparisons.

In the second quarter, Valine and Lucien were decreased, whereas Alanine, Glycerol, Aceto Acetic Acid, Acetone, Succinic Acid, Citric Acid, Acetate, and Taurine were increased. Since no previous study has assessed these metabolites in sport, we discuss these metabolic changes in response to previous studies examining bouts of exercise training. Increased Alanine in the second quarter indicates increased transfer of BCAAs induced Ammonia to the liver (32), a process called Alanine cycle (32), which can be considered as an justification for increased Alanine. In addition, glucose reduction and Epinephrine, Glucagon, Growth Hormone and Cortical enhancement can stimulate Alanine Amino transferase converting Pyruvate to Alanine which is Gluconeogenesis precursor and therefore can increase Gluconeogenesis (31). Also, several previous studies reported increased

Alanine after acute bouts of exercise (7, 12, 17, 25, 27, 28, 30, 39-41), which indicates an increase BCAAs metabolism and Gluconeogenesis. Furthermore, Fitti et al. (REF) detected significant changes in aromatic amino acid levels in a soccer game using salivary metabolomics, which was in line with that observed using blood analyses. The increased Glycerol during the second quarter represents an increase in both Gluconeogenesis (as a Gluconeogenesis precursor) and Lipolysis (as a byproduct of 3-acyl glycerol breakdown), which are pathways activated because of glycogen depletion (32). This is supported by increases of the two ketone bodies, AcetoAcetic Acid and Acetone, most likely due to Glycogen depletion in the second quarter (42).

The increase of Taurine, an organic acid to control osmotic pressure (27) and the main component of bile (42), can be explained by increased ROS production which might be started in the first quarter and reached significant levels in the second quarter. In addition, Ra et al. (REF) reported Taurine increase after three consecutive soccer games (17). Finally, Acetate showed a significant increase in the second quarter. Acetate is a fatty acid precursor, Glycerol is the product of triglyceride breakdown and ketone bodies are by products of fatty acids (32), indicating more focus on fat metabolism in the second quarter. In total, second quarter metabolic changes suggest an aerobic metabolism predominance compared to the first quarter, with increased utilization of fatty acid, ketone body and BCAAs metabolism, as well as gluconeogenesis and oxidative stress. These indicators can be a result of increased fatigue, decreased glycogen and increase in the waste metabolic products and in line with research showing that central and peripheral fatigue indicators continue to increase throughout a game with players having to reduce the movement intensity (35). Our activity profile confirm that movements at high intensity was decreased in the second quarter, although frequency, distance and duration of low intensity shuffle and movements (with and without ball) was increased.

Similar to the first quarter, Lactate, Pyruvate, Hypoxanthine, Succinic Acid, Citric Acid, and Glucose showed a significant increase in the third quarter. These data are in line with the higher frequency, duration and distance of high intensity movements in the third compared to second and fourth quarter. It seems that 15 minutes of rest between halves recovered subjects metabolically and allowed players to perform more high intensity movements. However, Glycine was also increased significantly in the third quarter indicating a greater level of fatigue than the first quarter. Glycine is the simplest and essential amino acid (43) and has a critical role in Glutathione, Certain

and purine nucleotide production (43) that help to renew phosphocreatine, act as an important antioxidant and have a critical role in cell energy charge, respectively (44).

In the same way, the fourth quarter showed similar metabolic and activity profiles as the second quarter but the amount of changes were greater which seems to be the result of accumulated fatigue, leading to a decrease movement intensity (35). In addition, 3-metylehistidine and methionine also showed significant increase and decrease, respectively, in the fourth quarter. 3-metylehistidine is considered as a common muscle damage index, since free 3-metylehistidine cannot contribute to protein resynthesizes. This is likely explained by the high impacts during the game, in which muscle damage might start in the first quarter but reach significant levels first in the fourth quarter. Methionine reduction can also be explained by the need to increase Gluconeogenesis towards the end of the game. Gluconeogenesis is suppressed by Methionine through PGC1α suppression (45), and Methionine reduction in the fourth quarter therefore led to increased gluconeogenesis (40).

Conclusion

Our data demonstrates that the higher intensity of movements in the first quarter, where players are more rested, seems to induce a greater anaerobic energy contribution. This was the case also for the third quarter that follows 15 minutes of rest, whereas the reduction of high-intensity movements in the second and fourth quarters also reduces the speed of energy production and players relay more on aerobic energy contribution. This is coincided with clear signs of accumulated fatigue in the second and fourth quarters. Overall, our data provides novel understanding of the metabolic demands of basketball games that could be used as a benchmark values for future studies. The implications for coaches include increased awareness of the changed demands across quarters of a game, which could contribute to better specifying training programs according to athletes' specific development areas.

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Declaration of interest statement

No potential conflict of interest was reported by the authors.

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Appendix 1

Appendix table1. Frequency (#), duration (s), and distance (m) of seven movements' categories in different quarters during elite male basketball games analyzed by video-based Time motion analysis (n=70, mean±SD).

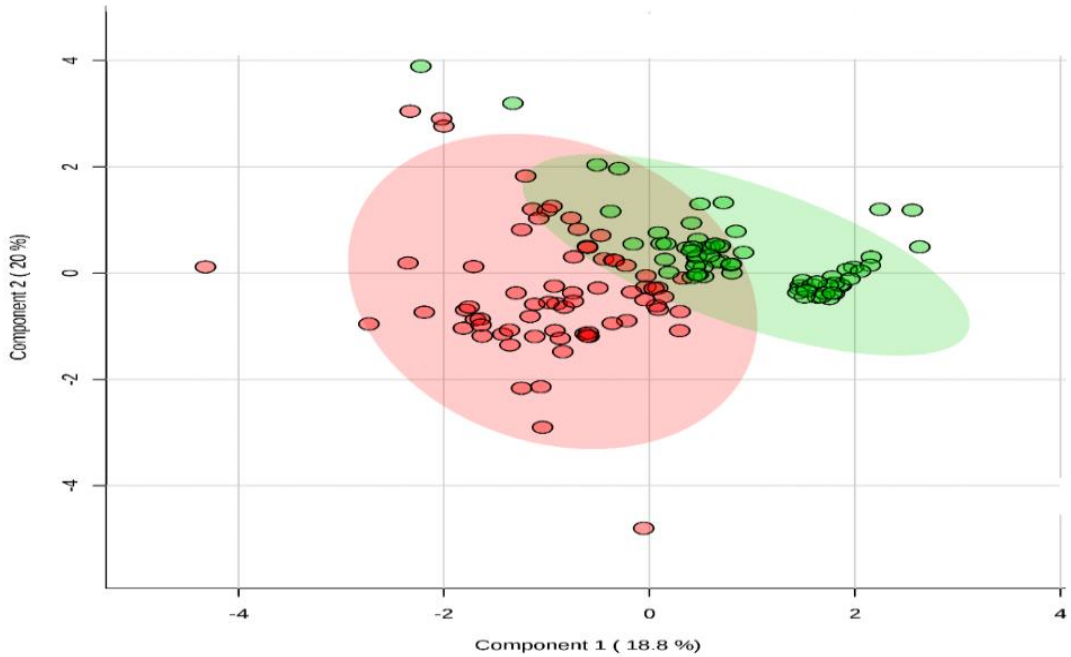
Motion variable	**Quarter**	**High intensity running with ball**	**High intensity running without ball**	**Low intensity running with ball**	**Low intensity running without ball**	**High intensity shuffle**	**Low intensity shuffle**	**Jump**
Frequency	First	12±4	30±6	12±3	45±9	15±4	8±3	14±4

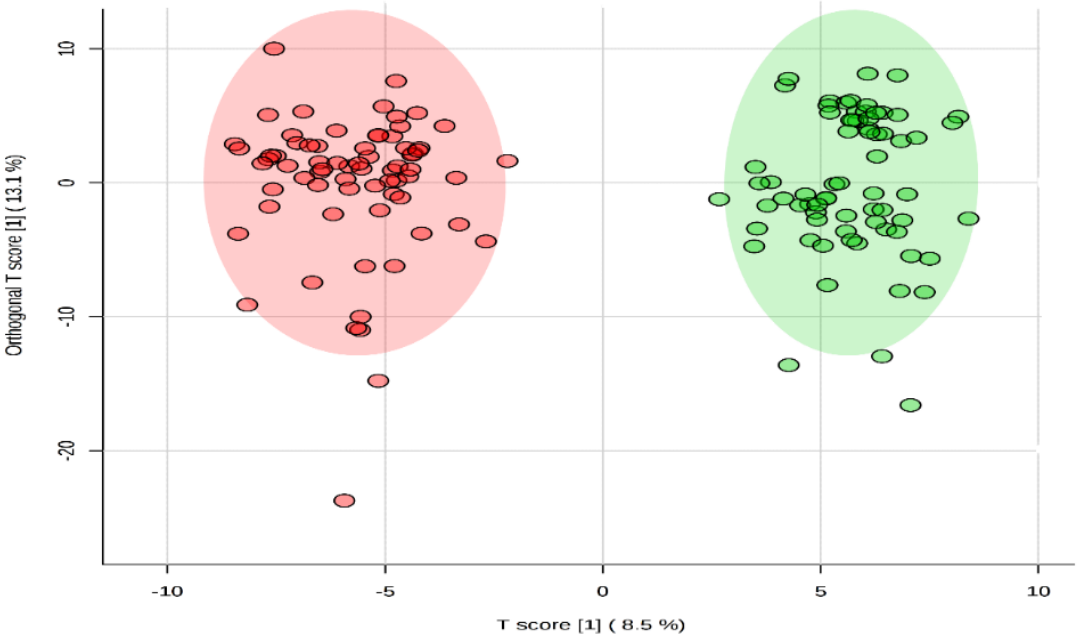
	Second	8±3	19±5	16±6	85±12	10±4	15±5	9±3
	Third	10±3	21±7	15±4	58±13	11±3	10±4	12±5
	Fourth	7±3	15±5	20±8	95±16	5±2	20±8	7±3
Duration	First	50±10	120±19	55±12	310±35	40±7	25±6	-
(s)	Second	25±7	71±8	73±73	369±36	17±4	41±8	-
	Third	40±7	95±9	56±8	341±59	33±6	36±7	-
	Fourth	15±4	40±8	80±9	412±38	12±5	45±8	-
Distance	First	185±61	621±58	73±12	425±76	63±12	25±8	-
(m)	Second	138±35	401±43	183±6	721±72	41±7	40±6	-
	Third	193±43	583±57	85±8	520±63	52±9	37±5	-
	Fourth	85±14	460±36	193±18	796±85	26±8	48±11	-

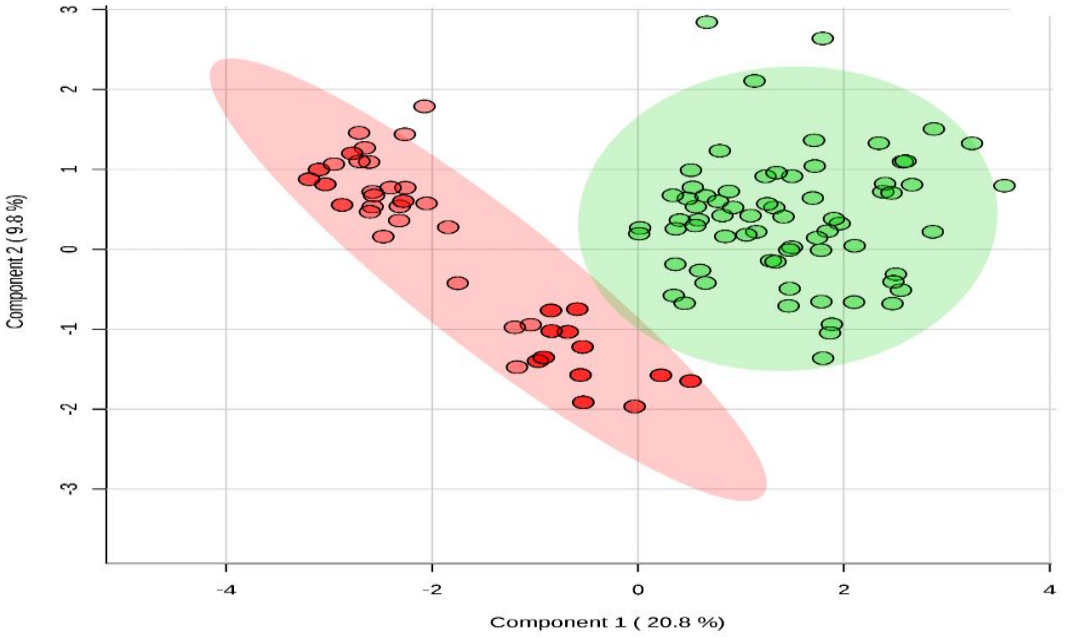
The movement classification was based on maximal player's speed (running or shuffling) using Dartfish Pro10 software (Appendix table 2).

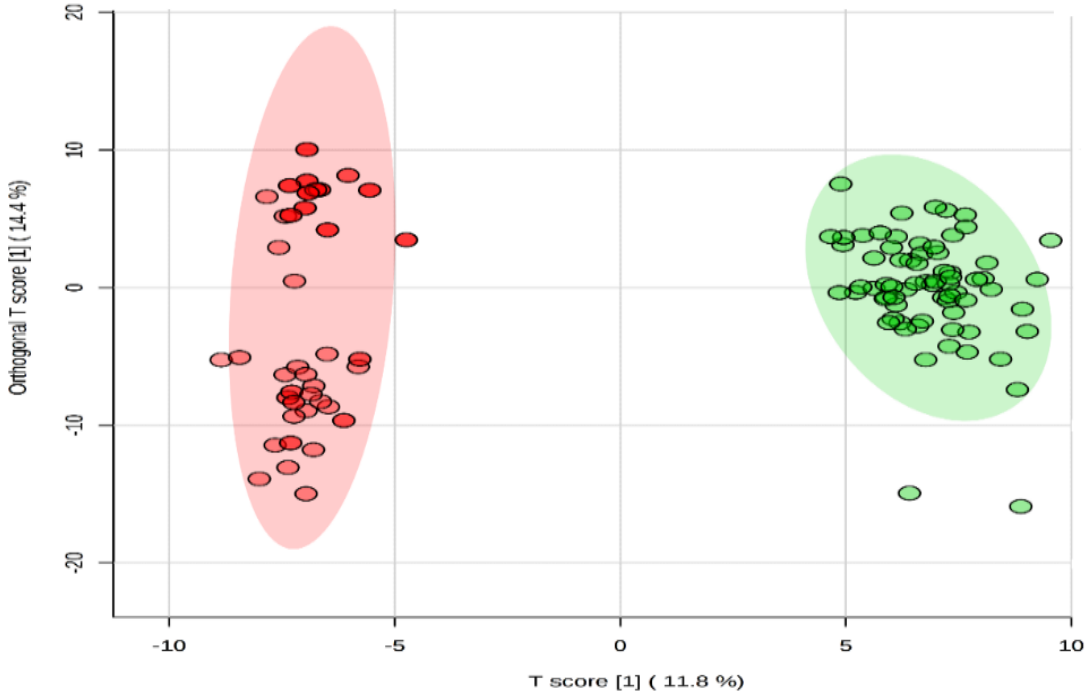
Appendix table 2. Seven movement categories (based on each player's maximal speed).

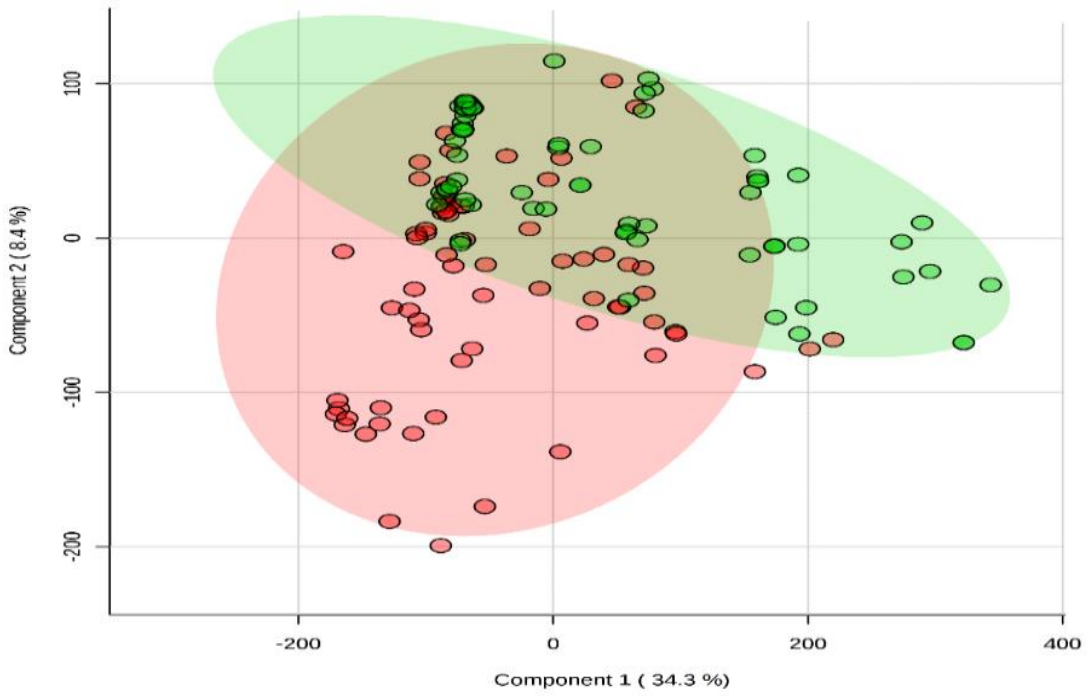
Movement	Speed
Low intensity running without ball	≤70% player's maximal speed
Low intensity running with ball	≤70% player's maximal speed
High intensity running without ball	>70% player's maximal speed
High intensity running with ball	>70% player's maximal speed
Low intensity shuffling	≤70% player's maximal shuffle speed
High intensity shuffling	>70% player's maximal shuffle speed
Jump	-


Component 2 (20 %)
4
2
0
-2
-4
-4
-2
0
2
4
Component 1 (18.8 %)

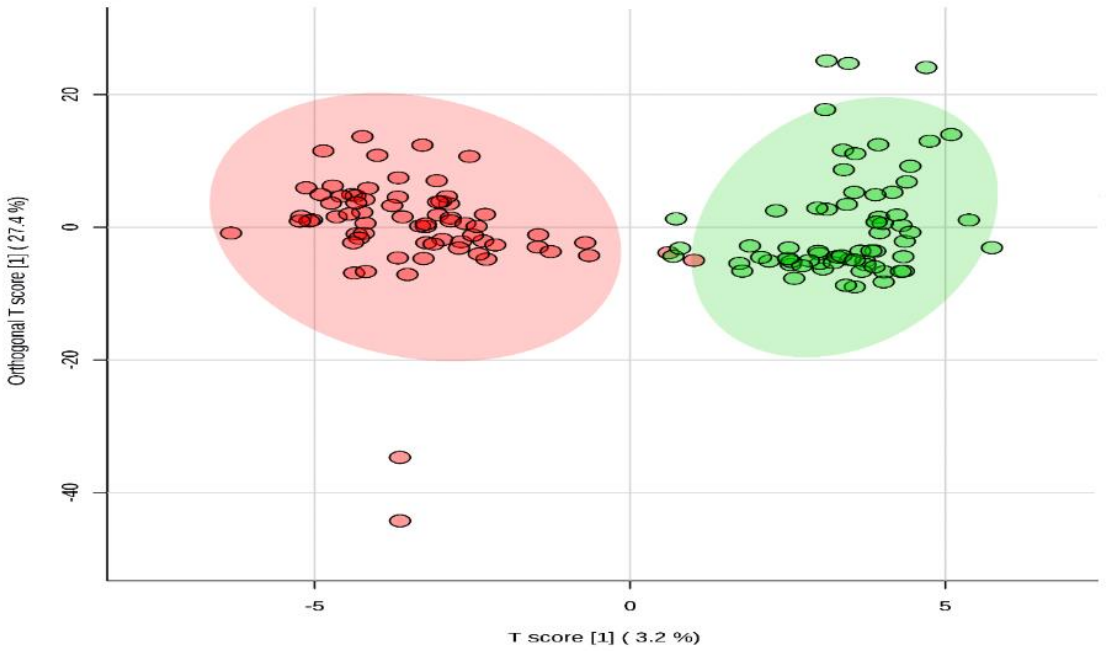

Orthogonal T score [1] (13.1 %)
10
0
-10
-20
-10
-5
0
5
10
T score [1] (8.5 %)

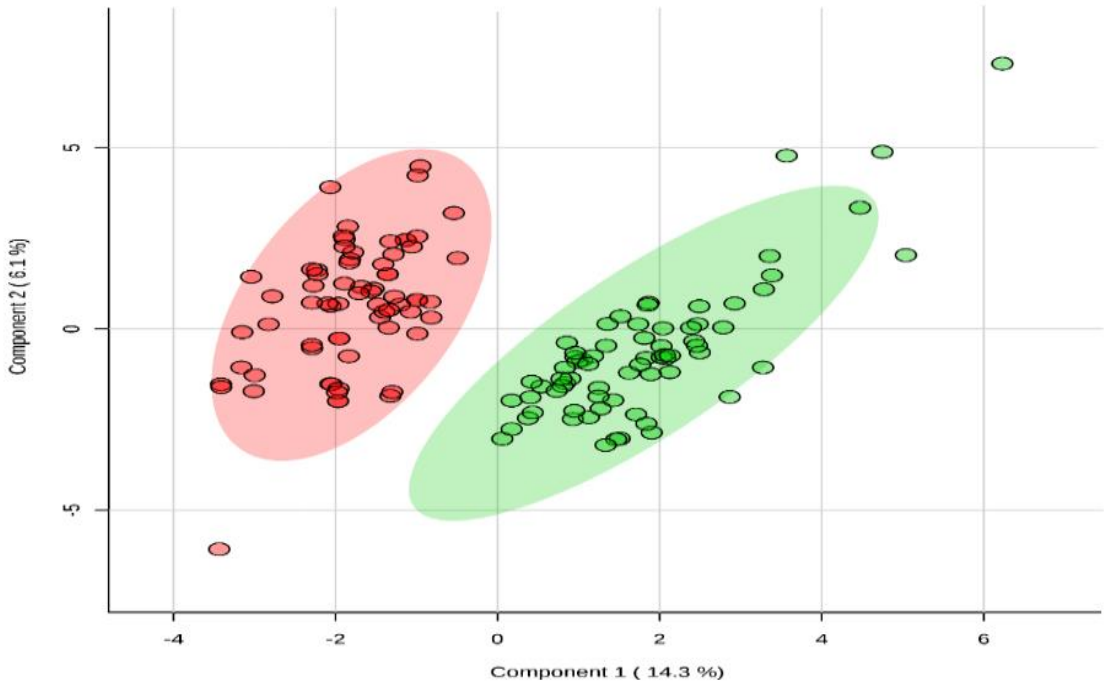

Component 2 (9.8 %)
Component 1 (20.8 %)
3
2
1
0
-1
-2
-3
-4
-2
0
2
4

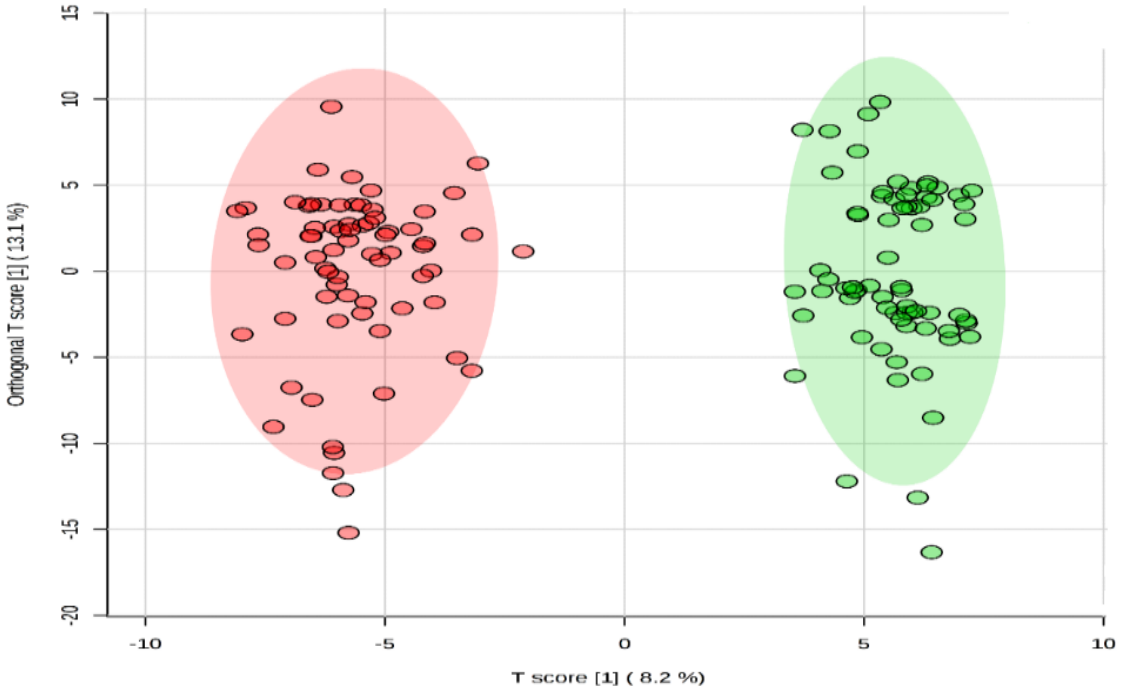

Orthogonal T score [1] (14.4 %)
T score [1] (11.8 %)
20
10
0
-10
-20
-10
-5
0
5
10


Component 2 (8.4 %)
100
0
-100
-200
-200
0
200
400
Component 1 (34.3 %)

Sc


Orthogonal T score [1] (27.4 %)
20
0
-20
-40
-5
0
5
T score [1] (3.2 %)


Component 2 (6.1 %)
Component 1 (14.3 %)
5
0
-5
-4
-2
0
2
4
6


Orthogonal T score [1] (13.1 %)
T score [1] (8.2 %)
15
10
5
0
-5
-10
-15
-20
-10
-5
0
5
10

Figures are in order.

Figure 1a. Comparison of players' metabolomes before and after the first quarter using principal component analysis. The result shows some difference between metabolomes. Green: before quarter, red: after quarter.

Figure 1b. Comparison of player's metabolome before and after the first quarter using partial least square discriminant analysis. The result shows significant difference between metabolomes. Green: before quarter, red: after quarter

Figure 1c. Comparison of player's metabolome before and after second quarter using principal component analysis. The result shows some difference between metabolomes. Green: before quarter, red: after quarter.

Figure 1d. Comparison of player's metabolome before and the after second quarter 2 using partial least square discriminant analysis. The result shows significant difference between metabolomes. Green: before quarter, red: after quarter.

Figure 1e. Comparison of player's metabolome before and after the third quarter using principal component analysis.

The result shows some difference between metabolomes. Green: before quarter, red: after quarter.

Figure 1f. Comparison of player's metabolome before and the after third quarter using partial least square discriminant analysis. The result shows significant difference between metabolomes. Green: before quarter, red: after quarter.

Figure 1g. Comparison of player's metabolome before and after the fourth quarter using principal component analysis. The result shows some difference between metabolomes. Green: before quarter, red: after quarter

Figure 1h. Comparison of player's metabolome before and after the fourth quarter using partial least square discriminant analysis. The result shows significant difference between metabolomes. Green: before quarter, red: after quarter.

Table 1. Frequency (#) and distance (m) of movements in the different quarters of elite male basketball games analyzed by video-based time motion analysis (n=70, mean±SD).

Variable / Quarter	Frequency	Distance (m)
First quarter	136±18	1392±135
Second quarter	162±23$^{*\$}$	1524±153$^{*\$}$
Third quarter	137±15	1470±126
Fourth quarter	169±24$^{*\$}$	1608±201$^{*\$}$

$^{\$}$Significant difference from the first quarter, *significant difference from the third quarter

Table 2. Metabolites with significant within-quarter changes.

First quarter	Second quarter	Third quarter	Fourth quarter
Lactate*	Valine$	Lactate*	Valine$
Pyruvate*	Lucien$	Pyruvate*	Lucien$
Succinic Acid*	Alanine*	Hypoxanthine*	Alanine*
Citric Acid*	Glycerol*	Succinic Acid*	Glycerol*
Glucose*	Aceto Acetic Acid*	Citric Acid*	Aceto Acetic Acid*
Hypoxanthine*	Acetone*	Glucose*	Acetone*
	Succinic Acid*	Glycine*	Succinic Acid*
	Citric Acid*		Citric Acid*
	Acetate*		Acetate*
	Taurine*		Taurine*
			3-metylehistidine*
			Methionine$

*indicates increase within the quarter, $indicates decrease within the quarter