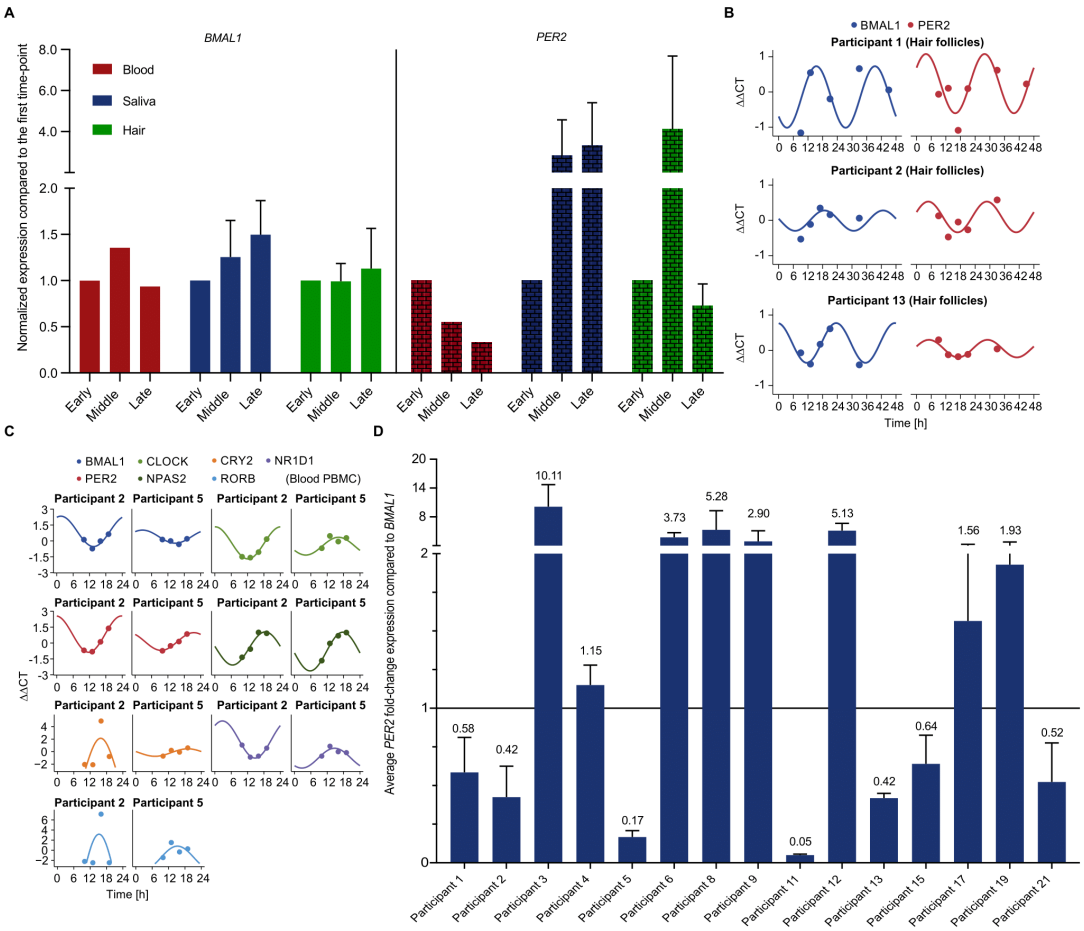
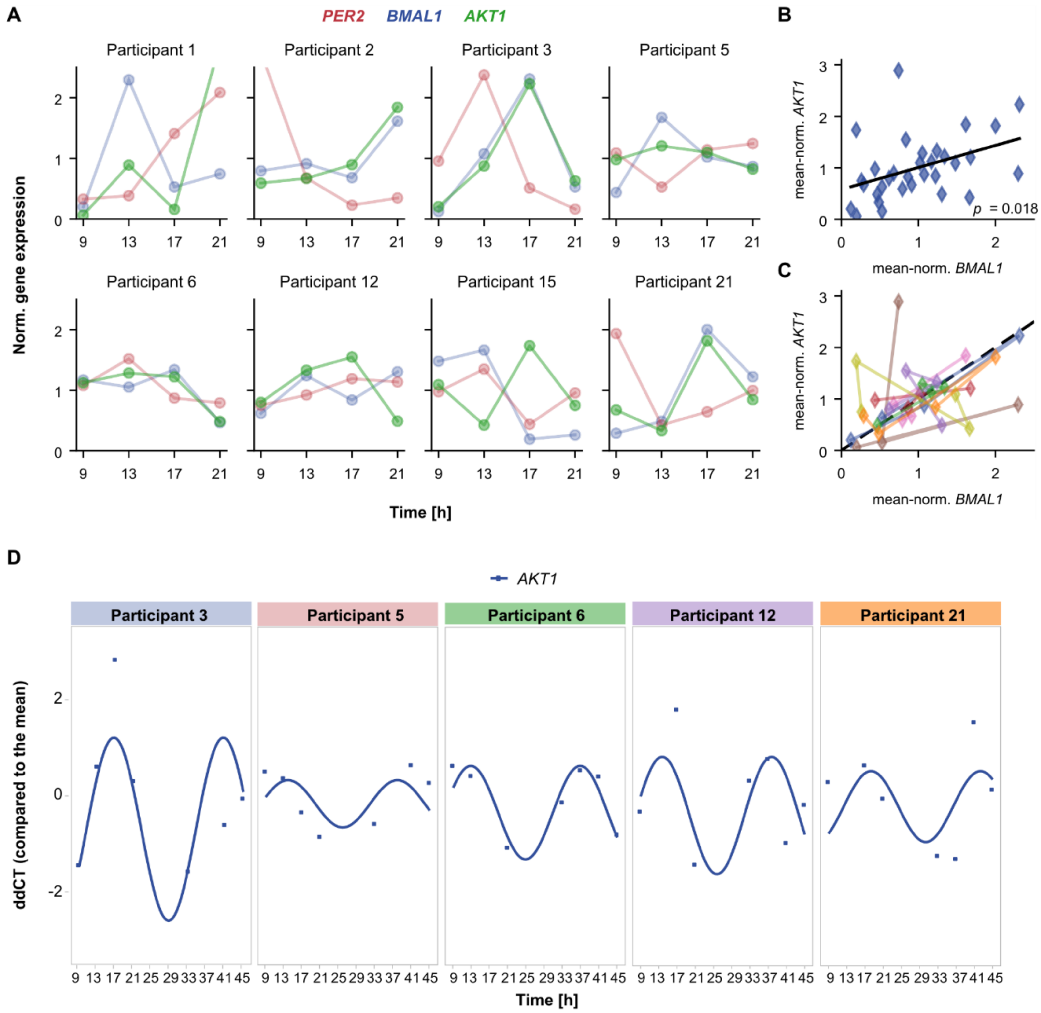
SUPPLEMENTARY MATERIAL

Supplementary Figures



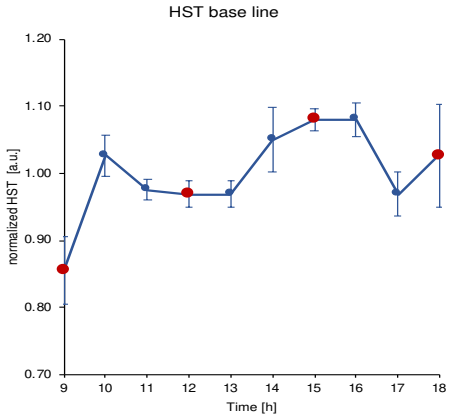
Supplementary figure 1. *BMAL1* and *PER2* expression display variation during the day in human blood, hair and saliva samples. **(A)** Three time-point comparison of *BMAL1* and *PER2* expression for the averaged data of all participants in **figure 1**. Expression data is compared to the first time-point (Early). For hair and saliva data Early, Middle and Late time-points represent 9h, 17h and 21h, respectively. For PBMCs data Early, Middle and Late time-points represent 10h, 16h and 19h, respectively. Depicted are mean + SEM. **(B)** Time-course RT-qPCR measurements normalised to the mean of all time points (ΔΔCT) of *BMAL1* (blue) and *PER2* (red) of Participant 1, 2, and 13 with a fitted linear sine-cosine function (period = 24h). For Participant 1, we collected one additional sample at 21h on the 2nd day. Harmonic regression best *p*-values for tested periods (20-28h): Participant 1; *BMAL1* (0.517, period = 21.4h), *PER2* (0.353, period = 24h). Participant 2; *BMAL1* (0.038, period = 20h), *PER2* (0.276, period = 28h). Participant 13; *BMAL1* (0.014, period = 20h), *PER2* (0.086, period = 21.4h). **(C)** Time-course RT-qPCR measurements of human PBMCs normalised to the mean of all time points (ΔΔCT) of *BMAL1* (blue), *CLOCK* (green), *NPAS2* (dark green), *PER2* (red), *CRY2*

(orange), *NR1D1* (purple), and *RORB* (light blue) of Participant 2 and 5 with a fitted linear sine-cosine function (period = 24 h). Harmonic regression best *p*-values: Participant 2; *BMAL1* (3.05E-01, period = 20h), *CLOCK* (6.31E-02, period = 28h), *NPAS2* (1.67E-01, period = 20h), *PER2* (4.78E-04, period = 20.8h), *CRY2* (7.17E-01, period = 20h), *NR1D1* (1.48E-01, period = 28h) and *RORB* (7.58E-01, period = 20h). Participant 5; *BMAL1* (5.56E-01, period = 20h), *CLOCK* (6.81E-01, period = 28h), *NPAS2* (9.75E-02, period = 28h, *PER2* (1.23E-01, period = 28h), *CRY2* (5.40E-01, period = 28h), *NR1D1* (6.43E-01, period = 28h) and *RORB* (7.73E-01, period = 28h). **(D)** Average *PER2* expression compared to *BMAL1* using saliva time-course data for each participant (mean + SEM).

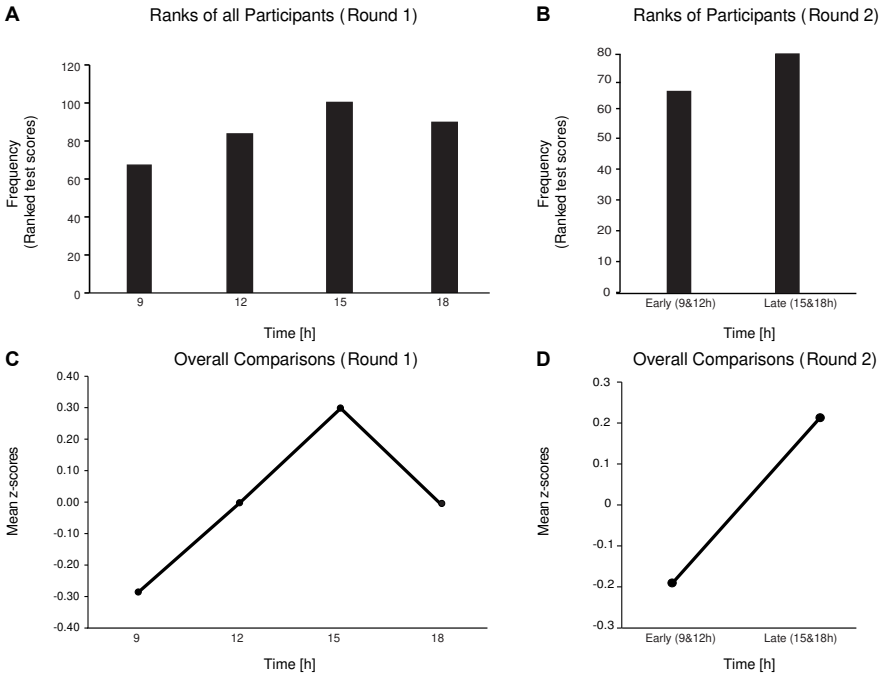


Supplementary figure 2. Gene expression of *BMAL1* and *AKT1* covary. **(A)** Mean-normalized gene expression profile for the three participants for whom the gene *AKT1* was measured besides *BMAL1* and *PER2*. The two days were treated as repetitions. The diurnal variation of *AKT1* follows *BMAL1*. **(B)** The data points from the mean-normalized time-series of *BMAL1* and *AKT1* correlate, linear regression with $p = 0.018$. **(C)** The same data points as in (B), but with a line connecting the different time points for each individual participant

(color-coded) shows that a large proportion of the lines align with the dashed diagonal, suggesting a one-to-one relation of the mean normalized *BMAL1* and *AKT1*. Pearson's correlation coefficient calculated on the mean-normalized expression levels of *BMAL1* and *AKT1* when concatenating all participants evaluates to 0.41. **(D)** Harmonic regression plots for the participants with at least 5 time points. Depicted values are based on individual best fitting period (20h – 28h, **Supplementary table 10**).

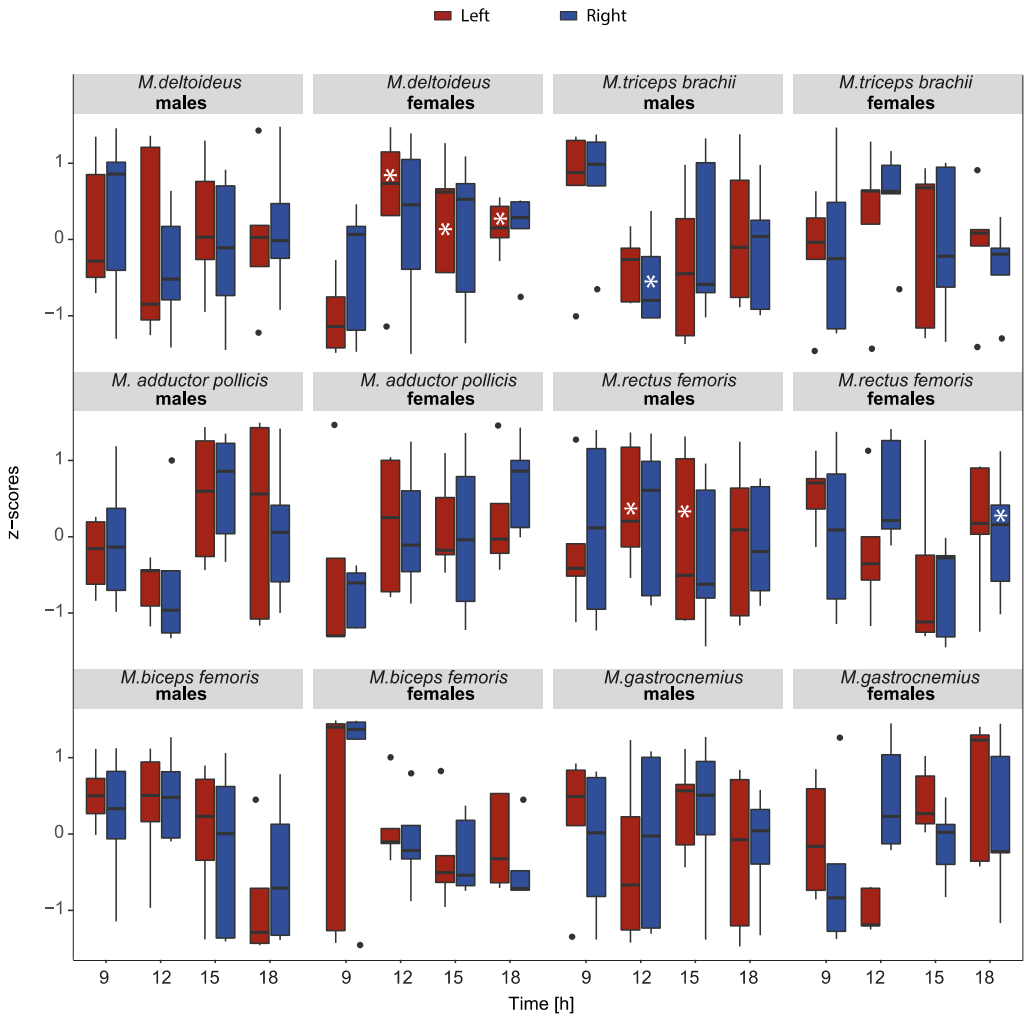


Supplementary figure 3. HST base line measurements. Depicted are mean values for three participants (9h-18h in one-hour intervals, N = 3, mean ± SEM). The HST measurements were randomly distributed across three measurement days with one day break in between. The red full circles represent the time point chosen for the subsequent exercise sessions.

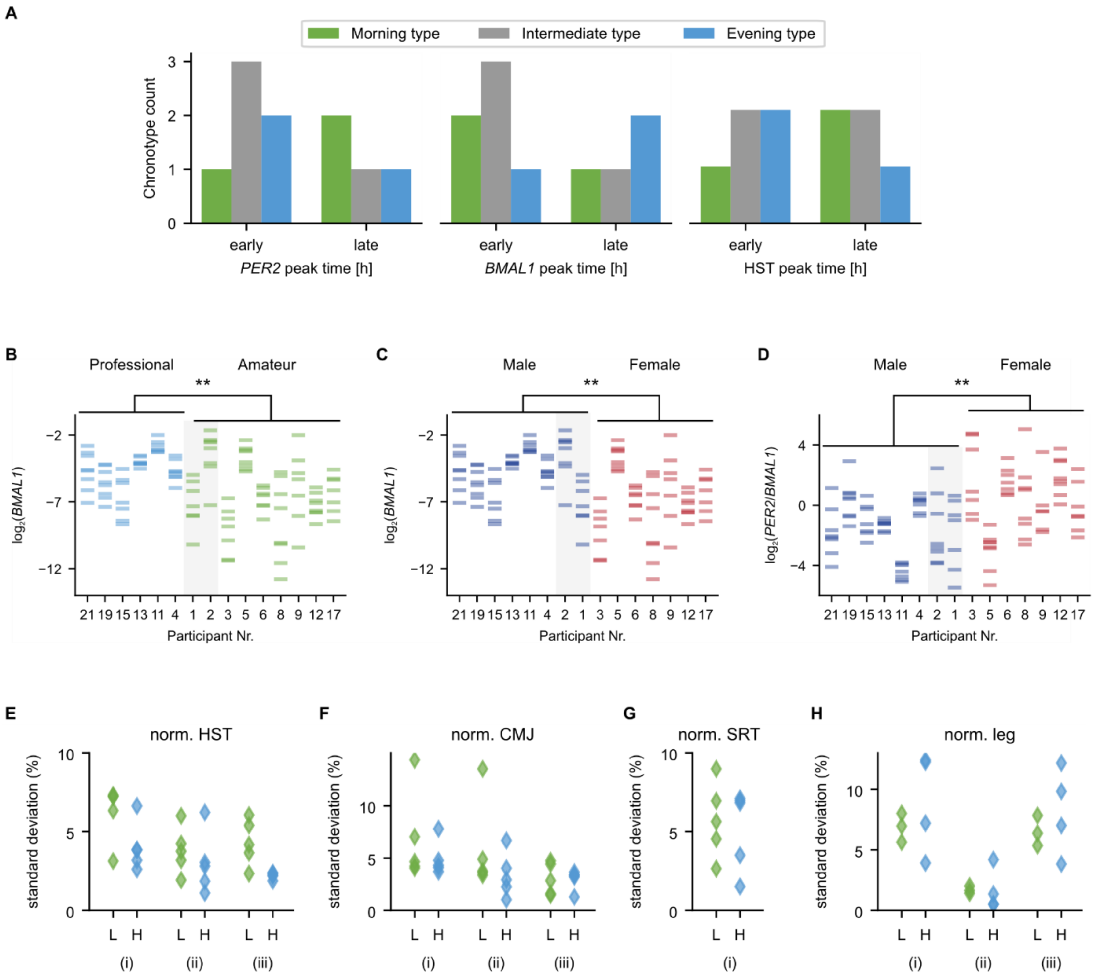


Supplementary figure 4. Cumulative ranking scores for all participants and exercise **(A)** for participants (N=11) who took part in first round of athletic performance tests and completed

all exercise sessions (Total scores per exercise session: 9h - 69, 12h - 83, 15h - 97, 18h - 84) **(B)** for participants (N=8 in total, 4 of the participants completed all exercise sessions) in second round of athletic performance tests (Total scores per exercise session: early (9h &12h) - 66, late (15h &18h) - 78 **(C)** Mean of z-scores for participants (N=11) who took part in first round of athletic performance tests, **(D)** Mean of z-scores for participants (N=8) who took part in second round of athletic performance tests.



Supplementary figure 5. Myotonometric analysis shows daily variation in muscle tone (frequency, F) for female and male participants. Only participants who completed all exercise sessions were included in the MyotonPRO measurements (N=12). Mean of normalized scores for the myotonometric parameter frequency [Hz] for each exercise session (T1-9h, T2-12h, T3-15h, T4-18h) and each muscle: *M. Deltoideus*, *M. Triceps Brachii*, *M.adductor pollicis*, *M. rectus femoris*, *M. biceps femoris, M. gastrocnemius.* The measurements were carried out from top to bottom on the right (R - blue line) and the corresponding left (L - red line) side of the body. Corresponding statistics for intrapersonal variation between each time points can be found in **Supplementary Table 9**. * $p < 0.05$, compared to time point 9h.



Supplementary figure 6. (**A**) For the group of ten participants with exercise data and genetic data, the chronotype distributions based on the Morningness/Eveningness Questionnaire are comparable for the subgroups with early versus late peak time for *PER2*, *BMAL1* and *HST*, respectively. (**B**) For each participant with genetic data, the expression values of *BMAL1* are plotted for all timepoints. Participants with a professional background (light blue) have a significantly higher *BMAL1* expression compared to participants without a professional background (amateurs, green) (Welch's t-test, $p < 0.0001$). The grey background marks male amateurs. **(C)** Significantly higher *BMAL1* expression is also observed when comparing males (dark blue) with females (red) (Welch's t-test, $p < 0.0001$). For this comparison, the male amateurs marked with a grey background switch groups compared to (B). (**D**) The separation between male and female expression values becomes visually more prominent when considering the ratio of *PER2* and *BMAL1* (Welch's t-test, $p < 0.0001$). (**E-H**) Standard deviations of normalized exercise and muscle tone data (L: group with low *BMAL1*, H: group with high *BMAL1*). Mean standard deviation calculated on the normalized exercise performance and the normalized muscle tone data for different (i) repetitions and timepoints, (ii) timepoints, (iii) repetitions (for details see Methods). **(E)** HST, **(F)** CMJ, **(G)** SRT (no repetitions were measured, thus the standard deviation (i) over all data is the same as (ii) over timepoints), **(H)** muscle tone of the leg muscles *(M. rectus femoris*, *M. biceps femoris*, *M. gastrocnemius*).

Supplementary tables

Mesor	qvals	pvals	Acrophase [h]	Acrophase [radians]	Amplitude	Period [h]	Condition
-0.50	0.49	0.18	15.83	4.14	1.22	23.6	BMAL1_Participant 5
0.42	0.60	0.42	25.32	6.62	0.92	26.2	PER2_Participant 5
-0.12	0.24	0.01	6.67	1.74	1.80	28	BMAL1_Participant 15
-0.03	0.70	0.54	6.60	1.72	0.58	28	PER2_Participant 15
-0.03	0.31	0.05	0.59	0.15	1.49	20.9	BMAL1_Participant 21
0.29	0.35	0.06	6.30	1.70	1.48	20	PER2__Participant 21
-2.03	0.52	0.23	15.08	3.94	4.38	25.2	BMAL1_Participant 8
-0.63	0.70	0.58	12.54	3.28	1.26	28	PER2_Participant 8
-0.71	0.21	0.01	18.33	4.79	3.85	22.9	BMAL1_Participant 9
-0.06	0.51	0.15	3.29	0.86	2.33	20	PER2_Participant 9
0.02	0.15	0.01	20.33	5.32	1.78	20.8	BMAL1_Participant 17
0.38	0.70	0.40	0.51	0.13	0.72	28	PER2_Participant 17
-0.01	0.48	0.14	21.05	5.51	0.28	22.1	BMAL1_Participant 13
0.05	0.56	0.15	24.33	6.36	0.11	28	PER2_Participant 13
-0.16	0.70	0.54	17.04	4.46	0.42	28	PER2_Participant 11
-0.16	0.73	0.63	15.67	4.10	0.34	28	BMAL1_Participant 11
-1.47	0.45	0.06	14.34	3.75	3.50	26.6	BMAL1_Participant 1
0.29	0.31	0.02	21.82	5.71	1.71	26.9	PER2_Participant 1
-0.85	0.21	0.02	16.80	4.39	2.65	23.5	BMAL1_Participant 3
-0.61	0.21	0.01	13.75	3.59	1.63	22.6	PER2_Participant 3
-0.12	0.10	0.03	13.36	3.49	1.43	20	BMAL1_Participant 19
0.38	0.56	0.07	27.44	7.18	0.76	28	PER2_Participant 19
0.17	0.88	0.58	4.98	1.30	1.07	20	BMAL1_Participant 2
0.02	0.69	0.29	11.28	2.95	0.50	20.3	PER2_Participant 2
0.00	0.60	0.31	21.15	5.53	0.56	23.4	BMAL1_Participant 4
-0.04	0.63	0.23	20.07	5.25	0.28	20.7	PER2_Participant 4
-0.01	0.67	0.28	1.88	0.49	0.71	20	BMAL1_Participant 12
-0.13	0.84	0.53	18.94	4.95	0.76	20	PER2_Participant 12
-0.60	0.46	0.09	12.22	3.19	1.46	26.7	BMAL1_Participant 6
-0.05	0.40	0.09	12.83	3.35	0.42	22	PER2_Participant 6

Supplementary Table 1 - Harmonic regression analysis (Figure 2)

			exercise tests			molecular tests			myotonometry
Participant #	**gender**	**MEQ**	**# exercise sessions**	**# round of tests**	**HST_long**	**saliva**	**hair**	**blood**	**MyotonPRO**
1	male	intermediate	-	-	-	Y	Y	-	-
2	male	intermediate	-	-	-	Y	Y	Y	-
3	female	intermediate	-	-	**Y**	Y	-	-	-
4	male	moderate morning	-	-	**Y**	Y	Y	-	-
5	female	moderate morning	4	1	Y	Y	-	Y	Y
6	female	moderate evening	4	1	-	Y	-	-	Y
7	female	intermediate	4	1	-	-	-	-	Y
8	female	intermediate	4	1	-	Y	-	-	Y
9	female	moderate evening	4	1	-	Y	-	-	Y
10	male	intermediate	4	1	-	-	-	-	Y
11	male	intermediate	4	1	-	Y	-	-	Y
12	female	intermediate	-	-	-	Y	-	-	-
13	male	intermediate	4	1	-	Y	Y	-	Y
14	male	intermediate	4	1	-	-	-	-	Y
15	male	intermediate	4	1	-	Y	-	-	Y
16	male	moderate evening	4	1	-	-	-	-	Y
5	female	moderate morning	3	2	-	-	-	-	-
8	female	intermediate	3	2	-	-	-	-	-
17	female	moderate evening	4	2	-	Y	-	-	-
18	male	intermediate	3	2	-	-	-	-	-
10	male	intermediate	4	2	-	-	-	-	-
19	male	moderate morning	3	2	-	Y	-	-	-
20	male	moderate evening	4	2	-	-	-	-	-
21	male	moderate morning	4	2	-	Y	-	-	-

Supplementary Table 2 - List of participants and tests (MEQ, Exercise tests, Molecular tests, Myotonometry) performed. Y = Yes, participant has carried out the test.

Time [h]	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
mean	0.86	1.03	0.98	0.97	0.97	1.05	1.08	1.08	0.97	1.03
SEM	0.05	0.03	0.02	0.02	0.02	0.05	0.02	0.03	0.03	0.08

Supplementary Table 3 - HST base line measurements (9h-18h in one-hour intervals, N = 3, mean ± SEM).

Exercise	Repetitions	Aim and muscle group used
Jogging – Forward and backwards	2 × 20 m Forwards 2 × 20 m Backwards	whole body warm up
"Butt kicks"	2 × 20 m	ischiocrucal muscles
High knees	2 × 20 m	lower limbs
Sidesteps	1 × 20 m	abductors
Cross-step exercise	1 × 20 m	foot coordination
Knee to chest	10 ×	stretching of the hamstrings (*M. biceps femoris*)
Foot inside pull	10 ×	stretching of the leg adductors
Lunge	10 ×	stretching of *M. psoas major*, activating the leg muscles and stability work
Caterpillar	5 ×	stretching the hamstrings (*M. biceps femoris*), core muscles activation and activation for the muscles of the upper limbs
Jogging – Forward and backwards	1 × 20 m Forwards 1 × 20 m Backwards	whole body warm up before sprinting
Intensity Sprints	30 % max speed 60 % max speed 90 % max speed 20 m sprint max speed	step by step preparation for explosive workout

Supplementary Table 4 - 15 min warm-up sequence.

Supplementary Table 5 - Exercises: HST, CMJ, SRT. (provided as excel file). For overall and gender wise comparisons.

Test	Time point	T1	T2	T3	T4
HST	T1	NA			
HST	T2	0.642	NA		
HST	T3	0.642	0.894	NA	
HST	T4	0.894	0.642	0.642	NA
CMJ	T1	NA			
CMJ	T2	0.496	NA		
CMJ	T3	0.034	0.005	NA	
CMJ	T4	0.416	0.784	0.005	NA
SRT	T1	NA			
SRT	T2	0.0003	NA		
SRT	T3	0.0008	0.659	NA	
SRT	T4	2.739e-07	0.027	0.011	NA

Test	Gender	Time point	T1	T2	T3	T4
HST	Male	T1	NA			
HST	Male	T2	0.879	NA		
HST	Male	T3	0.879	1	NA	
HST	Male	T4	0.879	1	1	NA
HST	Female	T1	NA			
HST	Female	T2	0.373	NA		
HST	Female	T3	0.373	0.789	NA	
HST	Female	T4	0.514	0.514	0.514	NA
CMJ	Male	T1	NA			
CMJ	Male	T2	0.681	NA		
CMJ	Male	T3	0.015	0.005	NA	
CMJ	Male	T4	0.681	1	0.005	NA
CMJ	Female	T1	NA			
CMJ	Female	T2	0.805	NA		
CMJ	Female	T3	0.805	1	NA	

CMJ	Female	T4	0.622	0.805	0.805	NA
SRT	Male	T1	NA			
SRT	Male	T2	0.017	NA		
SRT	Male	T3	0.034	0.550	NA	
SRT	Male	T4	0.0003	0.098	0.034	NA
SRT	Female	T1	NA			
SRT	Female	T2	0.481	NA		
SRT	Female	T3	0.481	0.893	NA	
SRT	Female	T4	0.481	0.827	0.827	NA

Supplementary Table 6 - Statistics corresponding to Figure 4. Friedmann test was used for determining the pairwise intrapersonal variations between different exercise times.

Supplementary Table 7 - Biomechanical parameters from data collected for the five measured MPs (MP1 - *M.adductor pollicis*, MP2 - *M. rectus femoris*, MP3 - *M. biceps femoris*, MP4 - *M. gastrocnemius*) (provided as excel file)

Muscle_Name		T1	T2	T3	T4
m_deltoideus_L	**T1**	NA			
m_deltoideus_L	**T2**	0.131	NA		
m_deltoideus_L	**T3**	0.060	0.629	NA	
m_deltoideus_L	**T4**	0.060	0.707	0.786	NA
m_deltoideus_R	**T1**	NA			
m_deltoideus_R	**T2**	0.655	NA		
m_deltoideus_R	**T3**	1	0.655	NA	
m_deltoideus_R	**T4**	0.655	1	0.655	NA
m_triceps_brachii_L	**T1**				
m_triceps_brachii_L	**T2**	0.718	NA		
m_triceps_brachii_L	**T3**	0.718	0.766	NA	
m_triceps_brachii_L	**T4**	0.718	0.792	0.792	NA
m_triceps_brachii_R	**T1**	NA			
m_triceps_brachii_R	**T2**	0.585	NA		
m_triceps_brachii_R	**T3**	0.649	0.649	NA	
m_triceps_brachii_R	**T4**	0.585	0.792	0.720	NA
m_add_pollicis_L	**T1**	NA			
m_add_pollicis_L	**T2**	0.674	NA		
m_add_pollicis_L	**T3**	0.008	0.012	NA	
m_add_pollicis_L	**T4**	0.062	0.113	0.255	NA
m_add_pollicis_R	**T1**	NA			
m_add_pollicis_R	**T2**	1	NA		
m_add_pollicis_R	**T3**	0.051	0.051	NA	
m_add_pollicis_R	**T4**	0.028	0.028	0.699	NA
m_rectus_femoris_L	**T1**	NA			
m_rectus_femoris_L	**T2**	1	NA		

m_rectus_femoris_L	**T3**	0.241	0.241	NA	
m_rectus_femoris_L	**T4**	1	1	0.241	NA
m_rectus_femoris_R	**T1**	NA			
m_rectus_femoris_R	**T2**	0.127	NA		
m_rectus_femoris_R	**T3**	0.214	0.014	NA	
m_rectus_femoris_R	**T4**	1	0.127	0.214	NA
m_biceps_femoris_L	**T1**	NA			
m_biceps_femoris_L	**T2**	0.777	NA		
m_biceps_femoris_L	**T3**	0.250	0.190	NA	
m_biceps_femoris_L	**T4**	0.003	0.003	0.059	NA
m_biceps_femoris_R	**T1**	NA			
m_biceps_femoris_R	**T2**	0.780	NA		
m_biceps_femoris_R	**T3**	0.017	0.025	NA	
m_biceps_femoris_R	**T4**	0.017	0.017	0.780	NA
m_gastr_cm_L	**T1**	NA			
m_gastr_cm_L	**T2**	0.005	NA		
m_gastr_cm_L	**T3**	0.474	0.0009	NA	
m_gastr_cm_L	**T4**	0.775	0.007	0.388	NA
m_gastr_cm_R	**T1**	NA			
m_gastr_cm_R	**T2**	0.436	NA		
m_gastr_cm_R	**T3**	0.518	0.518	NA	
m_gastr_cm_R	**T4**	0.518	0.518	0.792	NA

Supplementary Table 8 - Statistical analysis with Friedmann test corresponding to **Figure 5**.

M. Deltoideus_Left (Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.038	NA		
T3	0.038	1	NA	
T4	0.047	0.902	0.902	NA
M. Deltoideus_Right (Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.629	NA		
T3	0.629	0.797	NA	
T4	0.797	0.670	0.629	NA
M. Triceps Brachii_Left (Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.807	NA		
T3	0.807	0.807	NA	
T4	0.807	0.807	0.807	NA
M. Triceps Brachii_Right(Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.741	NA		
T3	0.741	0.741	NA	
T4	1	0.741	0.741	NA
M. Rectus Femoris_Left(Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.922	NA		
T3	0.922	0.964	NA	
T4	0.922	1	0.964	NA
M. Rectus Femoris_Right(Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.210	NA		
T3	0.434	0.539	NA	
T4	0.049	0.210	0.142	NA
M. Biceps Femoris_Left(Females)	**T1**	**T2**	**T3**	**T4**

T1	NA			
T2	0.441	NA		
T3	0.377	0.712	NA	
T4	1	0.441	0.377	NA
M. Biceps Femoris_Right(Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.516	NA		
T3	0.516	0.488	NA	
T4	0.790	0.516	0.516	NA
M. Adductor Pollicis_Left(Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.797	NA		
T3	0.670	0.629	NA	
T4	0.629	0.629	0.797	NA
M. Adductor Pollicis_Right(Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.473	NA		
T3	0.185	0.333	NA	
T4	0.185	0.392	0.773	NA
M. Gastrocnemius_Left(Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.263	NA		
T3	0.197	0.038	NA	
T4	0.197	0.038	1	NA
M. Gastrocnemius_Right(Females)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.382	NA		
T3	0.589	0.382	NA	
T4	0.382	0.589	0.589	NA
M. Deltoideus_Left (Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.963	NA		
T3	0.963	0.963	NA	
T4	0.963	0.963	1	NA
M. Deltoideus_Right (Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.481	NA		
T3	0.600	0.719	NA	
T4	0.601	0.650	0.792	NA
M. Triceps Brachii_Left (Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.458	NA		
T3	0.466	0.689	NA	
T4	0.534	0.534	0.689	NA
M. Triceps Brachii_Right (Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.037	NA		
T3	0.203	0.203	NA	
T4	0.203	0.203	1	NA
M. Rectus Femoris_Left(Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.652	NA		
T3	0.039	0.047	NA	
T4	0.219	0.129	0.360	NA
M. Rectus Femoris_Right(Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.203	NA		
T3	0.203	0.067	NA	
T4	1	0.203	0.203	NA
M. Biceps Femoris_Left(Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.637	NA		
T3	1	0.637	NA	
T4	1	0.637	1	NA
M. Biceps Femoris_Right(Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.798	NA		
T3	0.798	0.798	NA	

T4	0.798	0.798	0.798	NA
M. Adductor Pollicis_Left(Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	1	NA		
T3	0.801	0.801	NA	
T4	0.076	0.076	0.118	NA
M. Adductor Pollicis_Right(Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.605	NA		
T3	0.605	0.605	NA	
T4	0.605	0.605	0.605	NA
M. Gastrocnemius_Left(Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	0.228	NA		
T3	0.584	0.228	NA	
T4	0.228	0.584	0.421	NA
M. Gastrocnemius_Right(Males)	**T1**	**T2**	**T3**	**T4**
T1	NA			
T2	1	NA		
T3	0.740	0.740	NA	
T4	0.740	0.740	0.740	NA

Supplementary Table 9 - Statistical analysis for **Supplementary Figure 4**, pairwise comparisons (Friedmann test).

Participant	qvals	pvals	Acrophase [h]	amplitude	Period [h]
Participant 3	0.249	0.178	18	1.506	28
Participant 5	0.061	0.017	12	1.042	28
Participant 6	0.011	0.001	11	1.068	26.6
Participant 12	0.696	0.229	14	1.067	20
Participant 21	0.249	0.167	14	1.386	28

Supplementary Table 10 - Harmonic regression results of AKT1 for the best fitting period corresponding to **Supplementary Figure 2**.