LIBM Scientific Day

30/11/2020

Role and modulation of eryptosis in sickle cell anemia : impact of red blood cell microparticle release in vascular function

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Introduction: Chronic hemolysis, enhanced oxidative stress and decreased nitric oxide (NO) bioavailability promote vasculopathy in sickle cell anemia (SCA). Oxidative stress and NO are known to modulate eryptosis in healthy red blood cells (RBCs), but the impact on the genesis of RBC-derived microparticles (RBC-MPs) is poorly described. RBC-MPs could play a role in vascular dysfunction in SCA. The aim of this study was to evaluate the roles of oxidative stress and NO in eryptosis and RBC-MPs release, and to determine whether RBC-MPs are involved in vascular dysfunction in SCA.

Methods: Markers of eryptosis (RBC phosphatidylserine (PS) externalization, Ca^{2+}, reactive oxygen species level (ROS) and glucose uptake) were evaluated in SCA patients and healthy individuals (AA). RBC deformability, hemolytic level; systemic oxidative stress (AOPP) an RBC-MPs plasma concentration were also measured. Macrovascular function was assessed by carotido-femoral pulse wave velocity (PWV) measurement. The *in vitro* effects of anti-oxidant (N-Acetyl-cysteine, NAC), pro-oxidant (Cumene Hydroperoxide) and NO donor (Sodium Nitroprusside, SNP) drugs were tested on eryptosis and MPs emission in SCA. Finally, RBC-MPs from SCA patients and AA were incubated with human aortic endothelial cells (HAEC) with and without a TLR4 inhibitor (TAK-242) and their consequences on HAEC phenotype and cytokines release were assessed by flow cytometry.

Results: Eryptosis, RBC-MPs, systemic oxidative stress and arterial stiffness were increased in SCA. NAC increased RBC deformability and decreased eryptosis and RBC-MPs, while cumene did the opposite. SNP increased RBC deformability and limited eryptosis, but had no effect on RBC-MPs. Arterial stiffness was increased in SCA and correlated with RBC MPs concentration. Correlations between RBC MPs, hemolysis level, oxidative stress were also observed. Compared to AA, SCA RBC-MPs increased adhesion molecules expression (ICAM-1and E-Selectin) and the production of cytokines by HAEC, while TLR4 inhibition alleviated these effects.

Discussion: Together, our results show that RBC-MPs, that are released during enhanced eryptosis, could play a crucial role in macrovascular dysfunction in SCA patients, and that enhanced oxidative stress is a strong modulator of eryptosis and subsequent RBC-MPs release. RBC-MPs could exert deleterious properties on endothelial cells through the activation of TLR4 which could contribute to vascular dysfunction. Further investigations are required to identify the specificity of SCA RBC-MPs at the origin of TLR4 activation, but this study opens new perspectives to understand the underlying mechanisms of vascular dysfunction in SCA. It also points toward new therapeutic targets focusing on preventing eryptosis and/or TLR4 activation in SCA

Obstructive sleep apnea is associated with increased coagulation activity and autonomic nervous system activity imbalance in sickle cell disease.

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Introduction: Despite the fact that obstructive sleep apnoea (OSA) is highly prevalent in patients with sickle cell disease (SCD), studies focusing on the impact of OSA on SCD pathophysiology are scarce. OSA is one of the most common conditions responsible for hemoglobin desaturation. The nocturnal hemoglobin desaturation occurring in some sickle cell disease (SCD) patients with OSA could trigger hemoglobin S polymerization and red blood cell (RBC) sickling, leading to further blood rheological alterations, hence increasing the risks for acute vaso-occlusive complications. Moreover, OSA is accompanied by impaired autonomic nervous system (ANS) balance and increased coagulation activity in the general population, which increase the risks for cardiovascular disorders. Indeed, the presence of OSA in SCD could increase the clinical severity of patients by modulating blood rheology, coagulation activity and ANS activity. The aim of this study was to compare different biomarkers and the frequency of vaso-occlusive like events between SCD patients with mild or moderate-to-severe OSA to those without.

Methods: Thirty-eight patients suffering from homozygous SCD, aged from 15 to 50 yrs old, followed at the Hospice Civils de Lyon hospital were recruited. They all underwent a complete polysomnographic exam associated with heart rate variability measurement (an indicator of the Autonomic Nervous System (ANS) activity) and blood exams in the morning in order to perform hematological, hemorheological and coagulation exams. The presence of OSA was defined as an Apnea Hypopnea Index (AHI) > 5: AHI between 5 and 15 = mild OSA and AHI > 15 = moderate-to-severe OSA. The rate of vaso occlusive complication (hospitalized vaso-oclusive crisis (VOC) and Acute Chest Syndrome (ACS) events) was calculated for each patient over a two years period before inclusion.

Results: Fourteen patients had no OSA, 15 had a mild form and 9 had a moderate-to-severe OSA. The most severe OSA group was characterized by a lower prothrombin time and activated partial thromboplastin time, and greater ANS activity imbalance than patients with no OSA. No difference was observed between the three groups for the different hematological parameters, fibrinogen and d-dimer levels, red blood cell deformability and aggregation, blood viscosity. The frequency of patients having developed VOC or ACS events in the previous 2 yrs was not different between the three groups.

Discussion: OSA seems to promote coagulation through both the stimulation of the extrinsic and intrinsic pathways and to further increase ANS imbalance by rising the activity of the sympathetic system. While no association was observed with the frequency of vaso-occlusive like events, these alterations could participate in the modulation of the clinical severity and the development of chronic complications.

Blood viscosity and its determinants in the highest city in the world

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Introduction: Chronic mountain sickness (CMS) is a condition characterised by excessive erythrocytosis (EE). While EE is thought to increase blood viscosity and subsequently to trigger CMS symptoms, the exact relationship between blood viscosity and CMS symptoms remains incompletely understood. We assessed the effect of living at high-altitude on haemoglobin, haematocrit and haemorheological parameters (blood viscosity and red blood cell aggregation), and investigated their relationship with CMS in highlanders living in the highest city in the world (La Rinconada, Peru, 5,100 m).

Methods: Ninety-three men participated in this study: 10 Caucasian lowlanders, 13 Andean highlanders living at 3,800 m and 70 Andean highlanders living at 5,100 m (35 asymptomatic, CMS score ≤5; 15 with mild CMS, CMS score between 6 and 10; 20 with moderate-to-severe CMS, CMS score >10). Blood viscosity was measured at native and corrected haematocrit (40%).

Results: Haemoglobin concentration and haematocrit increased with the altitude of residency. Blood viscosity also increased with altitude (at 45 s^{-1}: 6.7±0.9 mPa·s at sea level, 14.0±2.0 mPa·s at 3,800m and 27.1±8.8 mPa·s at 5,100; $p<0.001$). At 5,100 m, blood viscosity at corrected haematocrit was higher in highlanders with moderate-to-severe CMS (at 45 s^{-1}: 18.9±10.7 mPa·s) than in highlanders without CMS (10.2±5.9 mPa·s) or with mild CMS (12.1±6.1 mPa·s) ($p<0.05$).

Discussion: Blood viscosity may contribute to CMS symptomatology while the increased blood viscosity in CMS patients cannot solely be explained by the rise in haematocrit.

Role of hydroxyurea as a nitric oxide donor in modulating erythrocyte deformability in patients with sickle cell disease

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Introduction: Sickle cell disease is characterized by a decrease in the deformability of the red blood cell (RBC) and by chronic vascular dysfunction, leading to acute complications (e.g. vaso-occlusive attacks) and multisystemic degenerative damage. The pathophysiology remains complex and varied, at the centre of which the nitrogen monoxide (NO) signalling pathway may play a major role. Recent work in healthy subjects has shown that RBC has a functional and active NO-synthase (NOS) capable of producing NO and modulating the rheological properties of RBC. Hydroxyurea (HU) remains the current reference treatment for this disease, but apart from its undisputed effect on foetal haemoglobin (HbF) production, its mechanism of action remains little known but could involve the metabolism of NO. The aim of the study was therefore to compare the deformability of red blood cells and the production pathway of erythrocyte NO between healthy subjects and SS sickle cell patients treated or not treated with HU.

Methods: Twenty-eight patients with sickle cell disease (including 16 on HU) and 14 control subjects were recruited during their annual clinical consultation. Patients under transfusion protocol, recently transfused or who have had a recent complication within the last 2 months were not eligible. A tube of heparinized blood was drawn to perform various tests: measurement of RBC deformability by ektacytometry, determination of HbF level and mean corpuscular volume (MCV), determination of intraerythrocyte NOS activity, measurement of plasma and red blood cell nitrite, and evaluation of the impact of a NO donor such as sodium nitropusside (SNP) on RBC deformability.

Results: SS patients on HU had higher HbF levels and increased MCV (reflecting better cell hydration) compared to AA subjects and untreated SS patients. The deformability of these same patients was improved, and the level of intraerythrocytic nitrite was higher. On the other hand, erythrocyte NOS activity was lower in HU patients. We observed a positive correlation between the HbF level and the level of nitrite in the RBC. Finally, incubation of sickle cell RBCs with a NO donor (SNP) improved the deformability of the erythrocytes.

Discussion: In addition to its action on HbF synthesis, HU appears to act as a NO donor on RBC, thus contributing to increase the deformability of sickle cell RBCs. The results of this study should lead to a better understanding of the mechanisms of action of HU; and guide the development of new therapeutics targeting the NO signalling pathway in sickle cell disease.

Myostatin gene invalidation inhibits a glucocorticoid-like response in skeletal muscle and liver of ApcMin/+ mice during cancer cachexia

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Introduction: Cancer cachexia (CC) results in a progressive loss of skeletal muscle that strongly reduces patients' quality of life and ultimately patients' lifespan. Glucocorticoids (GC) are multifaceted hormones exerting powerful metabolic effects on skeletal muscle and liver. If the metabolic actions of GC have been described in these tissues, the functional roles of these hormones on skeletal muscle and liver during CC are currently unknown. Here, we asked whether GC are molecular determinants of CC by coordinately regulating skeletal muscle mass and hepatic metabolism.

Methods: *Quadriceps* muscle, liver, hypothalamus, adrenal glands, and blood samples were removed from 13- (mild cachexia) and 23- (advanced cachexia) week-old *ApcMin/+* male mice and C57Bl/6J wild-type age-matched littermates.

Results: *ApcMin/+* mice recapitulated the main features of CC, i.e. loss of body weight, adipose tissue and skeletal muscle, together with a decrease in muscle force and an increase in muscle fatigue. This was associated with an imbalance in skeletal muscle proteostasis towards reduced proteosynthesis and increased proteolysis. A liver gene reprogramming was also observed in advanced cachectic mice as evidenced by an increased expression of gluconeogenic genes and a decreased expression of glycolytic, ketogenic and lipogenic genes along with a decreased glycaemia and an increased lactatemia. An activation of the hypothalamus-pituitary axis (HPA), as shown by an increased expression of genes involved in GC production in the hypothalamus and adrenal glands, and a reduction in the expression of liver GC detoxification genes are associated with an increase in the concentration of corticosterone (the main GC in mice) in serum, *quadriceps* muscle and liver of 23-week-old *ApcMin/+* mice. The transcriptional signature in *quadriceps* muscle and liver of 23-week-old *ApcMin/+* mice was almost completely reproduced in mice treated with dexamethasone, a GC analog, indicating that GC are critical molecular determinants of CC by coordinately regulating skeletal muscle loss and hepatic metabolism. Finally, *Myostatin* gene invalidation, which completely prevented the loss of skeletal muscle mass and increased survival of *ApcMin/+* mice, restored corticosterone levels and abolished skeletal muscle and hepatic metabolic gene reprogramming.

Discussion: Our data indicate that HPA and liver GC detoxification are coordinately regulated during CC in *ApcMin/+* mice to increase corticosterone action and that GC act systemically to drive a transcriptional program that coordinately regulates muscle mass loss and hepatic metabolism. The preventive effect of *Myostatin* invalidation on muscle mass loss and hepatic gene reprogramming during CC suggests the existence of a molecular dialog between skeletal muscle and liver that drives hepatic metabolic gene reprogramming, and that *Myostatin* may exert either direct or indirect transcriptomic effects on the liver.

Altered response to submaximal mono-articular exercise in the skeletal muscle of patients with sickle cell disease

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Introduction: Sickle cell disease is a group of haemoglobin disorders including a wide diversity of sickle genotypes, such as homozygous sickle cell anaemia (SS) and sickle cell-haemoglobin C disease (SC), with various clinical severities. SS patients exhibit skeletal muscle abnormalities such as microcirculatory remodelling, amyotrophy and decreased oxidative capacity [1] while muscle characteristics of SC are currently unknown. The muscle functional status of SS and SC patients are not described. The aim of this study was to characterize the muscle function of SS and SC patients during an acute submaximal mono-articular exercise.

Methods: Nineteen healthy African control subjects (AA) (10 M/9 F), 28 patients with homozygous sickle cell anaemia (14 M/14 F) and 18 patients with sickle cell-haemoglobin C disease (6 M/12 F) were recruited in this study. Maximal isometric torque of the quadriceps (Tmax) was measured before and after 4 repeated bouts of 20 knee extensions (tEnd) at 25% of Tmax interspaced by a 1-minute recovery. Electromyographic (EMG) activity of the *Vastus Lateralis* (VL) as well as VL tissue saturation index (TSI), in SS and AA, by near infrared spectroscopy of the VL were recorded. Rate of perceived exertion (RPE) was measured with the Borg Scale.

Results: Tmax decreased only in SS ($p < 0.001$) and SC ($p < 0.05$) groups after tEnd indicating skeletal muscle fatigue in these patients. The mean root mean squared EMG (RMS) value measured during Tmax decreased in SS ($p < 0.05$) but not in SC and AA. The median power frequency (MPF) was not modified by tEnd in all groups. Considering the repeated contractions, the RMS/RMSmax ratio increased over contractions of each bout in SS while it increased only in three of the four bouts in SC and in only one bout in AA. MPF significantly decreased over contractions in all bouts for both SS and AA and in 3 of 4 bouts in SC. RPE increased between bouts for the three groups and was higher in SS compared to AA in the last two bouts. At rest, TSI was lower in SS compared to AA ($p < 0.05$) but no difference in oxygenation kinetics was observed during and after tEnd.

Discussion: Altogether, these results suggest that SS and SC patients exhibit a greater skeletal muscle fatigability that AA subjects, with a different fatigue etiology between SS and SC. In SS, muscle fatigue seems to be the result of intramuscular modifications, independently of oxygenation, possibly triggering inhibitory feedbacks to the central nervous system as shown by the reduced EMG signal after tEnd and the increased RPE. Muscle fatigue in SC seems to be the consequence of intramuscular modifications decreasing the skeletal muscle capacity to generate strength.

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Association between monocytes phenotype and physical activity

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Introduction: Atherosclerosis is associated with low grade inflammation, involving circulating monocytes. It has been shown that the levels of classical and intermediate pro-inflammatory monocytes are associated with cardiovascular mortality [1]. It has been shown that physical activity (PA) decreases inflammation markers, incidence of strokes and mortality [2]. In this cross-sectional study, we tested the effect of PA on circulating monocytes phenotype rate.

Methods: 27 patients with a carotid stenosis were recruited. Levels of physical activity (MET.min/week) were measured by the GPAQ questionnaire and arterial sample of blood was collected to analyses monocyte phenotype (Classical:CM, Intermediate:IM and non-classical:NCM) assessed by flow cytometry.

Results: PA level groups were equivalent in complete blood count, risk factors and comorbidities but were significantly different for intensity. CM percentage was 51.31±15.95%, 34.96±11.64% and 52.56±18.84%; IM percentage was 5.02±1.01, 3.20±1.27 and 6.63±3.55% and NCM percentage was 47.64±10.98%, 61.80±12.24% and 40.83±19.31% respectively for group 1, 2 and 3.
To explain this unexpected results in group 3, we hypothesized that the beneficial effect of PA may be blunted with high intensity PA. We thus separated patients from groups 2 and 3 according their PA intensity: intense (8 METs) or moderate (4 METs). CM percentage was 35.27±14.57% and 55.43±13.58%, IM percentage was 4.53±3.48% and 4.98±2.21% and NCM percentage was 60.17± 16.39% and 39.57±14.53% respectively for moderate and intense PA groups.

Discussion: Decreases in CM and IM for the 1600-4500MET.min/week group of patients could be attributed to the positive effects of moderate PA. The increase in the rate of CM in the intense PA groups (vs. moderate intensity PA) may be explain by systemic pro-inflammatory and pro-oxidant effects of high intensity PA [3]. Intense PA is known to increase the inflammatory state through cytokines secretion [4-5]. Our results suggest that patients with carotid plaque should prefer moderate than high intense PA and could be put in perspective of previous results showing that over 4500MET.min/week, PA shows no further beneficial effect on cardiovascular mortality. Changes in monocyte phenotype has been shown to have distinct effects on plaque apparition, growth and vulnerability [6]. Thus, from a circulating monocytes perspective, completion of moderate PA should be encouraged in atherosclerotic patients.

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Muscle contraction and vibrational properties of the gastrocnemius muscle in response to a pendulum impact

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Introduction: During running, each impact between the foot and the ground causes a sudden deceleration, experienced by soft tissue packages. The '*muscle tuning'* paradigm suggests strategies to minimize these vibrations, namely changes in the damping properties of the soft tissues related to an increase in muscular activity [1-3]. However, the effects are subject [1,2,4] and muscle [5,6] specific. The purpose of this study, which takes part of a larger project evaluating soft tissue vibrations (STV) in running, was to quantify the natural vibration frequency and the damping coefficient of the gastrocnemius muscle after a pendulum impact for different levels of force. The overall objective of this project is to study "subject/body composition" effect on STV in a large population.

Methods: 92 males (35.8 ± 11.5 yrs) and 62 females (37.9 ± 12.4 yrs) laid down in a prone position on plantar flexor ergometer. Vibrations were measured with a skin-mounted tri-axial accelerometer *(3273 Series, Dytran Instrument, USA, m=3g, sampling frequency=2000Hz, range=±50g, sensitivity=50mV/g)* sticked on the belly of gastrocnemius muscle. The isometric test included four maximum voluntary contractions (MVC). A homemade pendulum ensured a repeatable impact on the gastrocnemius, 5cm proximal to the accelerometer. Four strikes were performed at each force level (0%, 50% and 100% MVC). The dominant frequency f and the damping coefficient c were estimated by respectively using a fast Fourier transform and wavelet analysis [7].

Results

Table 1: Natural frequency for soft tissues during isometric contractions (*Values are means ± SD)*

	Natural frequency (Hz)
0% MVC	15.66 ± 5.07
50% MVC	48.59 ± 10.53
100% MVC	62.44 ± 10.96

The increase in force resulted in a ~300% of natural frequency. Damping coefficient are not yet processed but one of our preliminary study indicated that the increase in force resulted in a ~25% increase in damping coefficient.

Discussion: As expected, the muscle mechanical properties change during force production as more cross bridges are attached and muscle became stiffer. These results suggest that an increase of muscular force leads to an increase in natural frequency of the selected soft tissue mass as shown by previous results [1, 6]. Vibrations could be minimized by shifting the free vibration frequencies away from those of the impact forces in running. Further analysis should be done to see whether there is a link between these vibrational responses and anthropometric data (calf girth, triceps surae mass) also measured. Experiments now on a treadmill, will be done to determine functional runners' groups clustered by different vibrational responses and anthropometric features.

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Effect of endurance training on recovery after exercise in sickle cell disease patients

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Introduction: Sickle cell disease (SCD) is the most common genetic disease in the world [1]. A recent study has shown that endurance training is beneficial for muscle micro-vasculature and physical capacity of SCD patients [2]. However, the effects of training on recovery following exercise, have not been studied in SCD patients. The purpose of this study was to evaluate the effects of endurance training on heart rate (HR) during recovery following submaximal incremental exercises in patients with SCD.

Methods: Two groups of SCD patients were studied [2]. Trained patients (n = 15) were involved in an 8-week endurance training program while control patients (n = 15) did not change their everyday life. Each group performed a submaximal incremental test before and after the 8-week period (SIT1 and SIT2, respectively) [2]. When the blood lactate concentration ([lactate] b) exceeded 4 mmol/L, the test was stopped. SIT1 and SIT2 were followed by two minutes of active recovery at 20 or 30 W, for women and men respectively, and thereafter by at least 6 minutes of passive recovery. Heart rate (HR) was measured continuously. HR during passive recovery was fitted by the following equation: $HR(t) = A*\exp(B*t) + C$, where A refers to the difference between the starting and infinite HR values, B is the velocity constant and C refers to value to infinity. D (=A+C) denotes the starting value of HR.
Statistics: Values are means ± standard errors. Statistical analyses included repeated ANOVAs. The critical level of statistical significance was set at 5%.

Results: Table 1 reports HR and parameters of the equation during recovery of SIT1 and SIT2, their evolutions and their differences in the two groups and between the two groups.

Discussion: The statistical results indicate that training has no significant effect on HR during passive recovery. On the other hand, training had a significant impact on HR which decreased faster in the trained group.

Table 1 : HR values and time courses in the control and trained groups before and after the 8-week period. Comparison between the pre and post period values as well as between groups are reported.

	Control				Trained				p (control vs trained)
	SIT1	**SIT2**	**Delta**	**p**	**SIT1**	**SIT2**	**Delta**	**p**	
0	161 ± 23.40	154 ± 21.27	-6.52 ± 11.99	0.053772	156 ± 14.35	154 ± 16,20	-1.88 ± 6.96	0.31357	0.050954
2	139 ± 23.06	132 ± 19.30	-7.41 ± 12.81	0.041768*	132 ± 14.72	125 ± 10,13	-7.05 ± 10.52	0.02106 *	0.469184
% 0 - 2	86.60 ± 5.18	85.65 ± 4.99	-0.95 ± 7.16	1.000000	84.38 ± 5.06	81.07 ± 3.32	-3.31 ± 4.65	0.01532 *	0.015175*
A	40.08 ± 9.01	33.27 ± 10.75	-6.80 ± 8.93	0.01042*	38.49 ± 14.70	30.66 ± 9.15	-7.83 ± 12.62	0.03075 *	0.78162
B	-0.83 ± 0.30	-1.00 ± 0.20	-0.19 ± 0.30	0.02564*	-0.88 ± 0.37	-0.99 ± 0.40	-0.11 ± 0.44	0.34632	0.81750
C	100.53 ± 17.67	99.30 ± 15.90	-1.23 ± 11.96	0.70096	93.4 ± 9.93	95.13 ± 9.11	1.73 ± 11.93	0.58329	0.42223
D= A + C	140.60 ± 22.97	132.57 ± 19.39	-8.03 ± 14.34	0.04891*	131.89 ± 14.42	125.79 ± 10.41	-6.1 ± 10.15	0.03550 *	0.45525

*Values are means ± SD or p values. * Significant result.*

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Validation of a bedside ergometer dedicated to longitudinal evaluation of neuromuscular function in intensive care unit patients

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Introduction: A large proportion of patients admitted to intensive care unit (ICU) will experience severe neuromuscular (NM) dysfunctions (i.e., muscle wasting and weakness), known as ICU acquired muscle weakness (ICUAW), especially for muscles that have an important role in locomotion, e.g. quadriceps [1]. Early and longitudinal assessment of muscle deconditioning in ICU patients are needed to treat these symptoms as early as possible and to propose adequate rehabilitation protocols. However, early measurement of the NM function is difficult because of the inability for sedated patients to collaborate, i.e. to perform voluntary contractions, and because of the particular environment where ICU patients are, i.e. lying in bed. To allow the measurement of NM function as soon as possible, the use of peripheral nerve stimulation appears as an interesting strategy. The purpose of this study was to test in healthy subjects the feasibility and reliability of measuring evoked force and electromyographic (EMG) of quadriceps muscle (i.e. using both electrical [2] and magnetic[c] stimulations of the femoral nerve) thanks to an innovative ergometer directly placed at bedside.

Methods: Fifteen healthy volunteers (6 women) participated in 3 testing sessions on two separated visits. The first (H0) and second (H1) sessions were separated by one hour to assess intra-day reliability while a third session (H24) was performed 24h after H0 to assess inter-day reliability.
A bedside adjustable ergometer on wheels, which can be put on four stabilizers to limit motions during the stimulation, was designed. It encompassed two force transducers on a movable and gradable platform to fit the patient's morphology and prevent injuries.
Subjects were lying in the exact same bed that is used in ICU settings and the position was optimized. For each session (i.e. H0, H1 and H24), force and associated vastus medialis (VM), vastus lateralis (VL) and rectus femoris (RF) electromyographic (EMG) activity were recorded in response to stimulation of the femoral nerve using both electrical (ES) and magnetic (MS) stimulations. Evoked-force in response to single (Tw) and paired stimulations (Db10, Db100) were assessed while VL, VM and RF M-wave amplitudes were also recorded. Intra- and inter-day reliabilities were assessed using the intra-class correlation coefficients (ICC) and coefficients of variation (CV).

Results: There was no significant difference between the three sessions for either ES or MS or any type of stimulations (Tw, Db10, Db100) for both force and EMG ($p>0.05$). For the evoked-force parameters, the inter- and intra-days CVs ranged between 5.4–6.8% and 4.7–5.3% for ES and between 4.2-10.6% and 5.8-10.8% for MS. The ICC values ranged from 0.93 to 0.97, depending of the modality of stimulation. For the M-wave, the inter-and intra-days CVs ranged between 8.2–16.8% and 3.9–11.5% for ES and between 9.4-28.7% and 6.8-13.2% for MS. The ICC values ranged from 0.77 to 0.99, depending of the modality of stimulation.

Discussion: This study showed that measuring force and EMG at bedside in healthy volunteers using an innovative ergometer provided good-to-excellent intra- and inter-day reliability whatever the modality of stimulation in volunteers. These promising results should allow reliable measurements of muscle function, i.e. will allow to track deconditioning and quantify early NM function alterations in ICU patients during their stay. This should provide early, quantitative and longitudinal force measurements in sedated patients that would be of utmost importance to refine the diagnosis of ICUAW.

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Neuromuscular fatigue during repeated sprints assessed with an innovative ergometer.

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Introduction: Repeated sprint ability (RSA) is paramount in intermittent exercises as team sports. To date, there is no consensus on the respective contribution of the central and peripheral neuromuscular fatigue (NMF) in the limitation of such exercise, partly due to methodological considerations, as the time required between exercise cessation and fatigue evaluation (30 to 180 s) [1], or the lack of individualization in exercise modalities (e.g. a given number of sprints inducing various fatigue levels between subjects). To circumvent this limitation, we developed an instrumented cycle-ergometer allowing NMF to be assessed with no delay after a sprint. This study aimed to evaluate fatigue development and its etiology during and immediately after a cycle repeated sprint exercise performed until a given fatigue threshold.

Methods: Healthy males (n=11, 24±6 yr, 71±10 kg) realized a RSA test on a custom semi-supine bike (10-sec sprint / 28-sec recovery) until a 30% decrease in sprint mean power (Pmean). Maximum voluntary contraction of the quadriceps (MVC), central fatigue [voluntary activation (VA)] and peripheral fatigue [high-frequency doublet (Db100), twitch (Pt)] were evaluated before (pre), immediately (i.e. no delay) after each sprint and 0, 3 and 5 min after the test. For each RSA test, sprints were expressed as a percentage of the total number achieved. Individual data were then extrapolated at 20, 40, 60 and 80% of the test completion to be compared between subjects. Repeated measure ANOVAs and Holm correction for post-hoc tests were performed.

Results: Subjects realized 9.7 ± 4.7 sprints with a maximal Pmean of 585±89 W. MVC was decreased from 20% to 60% and then plateaued (pre: 344±57 N, 20%: 307±63 N, 60% 261±53 N, 100%: 247±55, $F_{(7,70)}$=21.7, $p<0.001$). Db100 and Pt decreased from 20% and plateaued after 60% ($F_{(7,70)}$=45.2, $p<0.001$, pre-60% = -32.4±9.2%) and 40% ($F_{(7,70)}$=44.0, $p<0.001$, pre-40% = -43.3±8.2%), respectively. VA was not significantly affected by RSA test until 80% and the end of the RSA test (pre-post = -4.9±5.7%, $F_{(7,70)}$=3.62, p=0.035). Unlike peripheral parameters, VA recovered significantly within the 3 to 5 min after the RSA test ($F_{(3,27)}$=5.64, p=0.003).

Discussion: During a RSA tests, the decreases in Pmean and MVC were first concomitant to peripheral disturbances from the beginning to 60% of the exercise and central fatigue was only observed in the final part of the test while peripheral fatigue plateaued. The distinct recovery kinetics in central versus peripheral components of fatigue further confirm the necessity to reduce traditional delays in NMF assessments after such type of exercise.

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Neuromuscular origin of chronic fatigue among patients with multiple sclerosis.

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Introduction: Multiple sclerosis (MS) is an autoimmune chronic disease targeting myelin of the central nervous system (CNS). Among the manifestations caused by the disease, fatigue is the most common and disabling symptom, being reported by 55% to 78% of MS patients [1]. Causes of the MS-related fatigue remain poorly understood but an impaired neuromuscular function could contribute to this phenomena. In fact, a higher fatigability in MS patients could lead to a greater reduction in functional capacity and over time, the repetition of the activity of daily living could induce fatigue accumulation [2]. This study aims to characterize the causes of chronic fatigue in MS patients using a multifactorial assessment, focusing primarily on neuromuscular function.

Methods: Thirty participants with relapsing-remitting multiple sclerosis (RRMS), and 15 healthy controls will be recruited. MS patients will be assigned in 2 groups (high fatigue (HF) and low fatigue (LF) group) based on the response to fatigue questionnaire (FSS and MFIS). Participants will take part in three visits in the laboratory. The main outcome of this study comes from an incremental fatiguing exercise until exhaustion on a home designed cycling-ergometer. Maximal voluntary contraction (MVC), central (voluntary activation, motor evoked potential, silent period) and peripheral fatigue (resting twitch) using transcranial magnetic as well as peripheral nerve stimulation techniques was assessed on quadriceps before, during and after the fatiguing task. The two other visits will allow to assess other potential mechanisms of fatigue (sleep quality, maximal oxygen uptake (VO_2max), heart rate, muscle volume (MRI) and metabolic fatigue (P31 NMR).

Results: Based on preliminary data from only 8 MS patients (6 fatigued and 2 non-fatigued), MVC torque seemed decrease after the fatiguing task to greater extent for the HF group than LF group (-43.5%±10.7 vs -25.7%±5.8%), probably due to a higher loss of peak twitch torque (-54.7±10.2% vs -30.1±27.7%).

Discussion: A better understanding of the etiology of chronic fatigue will permit to propose an adapted rehabilitation treatment. This second phase of rehabilitation will be offered to patients as a follow-up of the present study.

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Disparate kinetics of change in responses to electrical stimulation at the thoracic and lumbar level during fatiguing isometric knee extension

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Introduction: Assessing spinal excitability using lumbar stimulation when measuring responses in lower limbs has been suggested as an alternative method which could circumvent the issues associated with thoracic stimulation. Both the lumbar evoked potential (LEP) and thoracic motor evoked potential (TMEP) are suggested to be brought about through activation of descending corticospinal axons and monosynaptic connections with lower limb motoneurons [1-2]. Thus, it seems important to investigate whether the behaviour of the two responses is similar during interventions known to modulate motoneuronal excitability, such as fatiguing submaximal isometric contractions. The present study compared the fatigue-induced change of matched-amplitude TMEP and LEP following electrical stimulation.

Methods: Ten participants performed a 3 × 3 min isometric knee extension contraction separated by 4 min recovery at the level of EMG required to produce 50% maximal voluntary contraction (MVC) force at baseline. The TMEP and LEP were evoked during the on-going contraction at baseline and every minute into the fatiguing protocol and during recovery. Both responses were also assessed during a transcranial magnetic stimulation (TMS) evoked silent period to elicit a TMS-TMEP and TMS-LEP in order to assess responses without the confounding influence of descending drive. Differences between the level of discomfort associated with both types of stimuli were assessed through visual analogue scales.

Results: The results displayed disparate kinetics of the TMS-TMEP and TMS-LEP throughout the fatiguing protocol. The TMS-TMEP was reduced at all time-points during exercise ($P < 0.001$), whereas the TMS-LEP was reduced at 2 min into set 1, and 1 min into sets 2 and 3 ($P \leq 0.04$). TMS-LEPs were higher than the TMS-TMEPs at most time-points ($P \leq 0.04$). No change was observed in the TMEP or LEP. Both the stimulation intensity required to evoke responses and the level of discomfort were significantly lower for the LEP compared with the TMEP ($P < 0.001$).

Discussion: When evoked during the silent period, the reduction in TMEP is greater than the LEP during fatiguing isometric exercise. The disparate kinetics of change suggest that differential mechanisms are responsible for evoked responses to thoracic and lumbar stimulation. More research is required to identify the mechanisms responsible for the TMEP and LEP before precise inferences can be made on what fatigue-induced changes in these variables reflect.

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Occurrence and magnitude of extra force during neuromuscular electrical stimulation with and without superimposition of tendon vibration

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Introduction: The conventional form of neuromuscular electrical stimulation (NMES), with relatively narrow pulses, generates contractions due to the direct activation of motor axons. The use of NMES with wide pulses and high frequency (WPHF) may enhance the central contribution to motor unit recruitment through the depolarization of sensory axons, inducing a so-called "extra-force" (EF) [1]. The combination of tendon vibration (TV) and wide-pulse low-frequency (WPLF) NMES has also been reported to elicit considerable EF [2]. The aim of the present study was to investigate the occurrence and magnitude of EF during WPLF and WPHF NMES associated or not with TV.

Methods: NMES-induced isometric plantar flexion force was recorded in 28 healthy young adults. Stimulation trains were delivered through electrodes placed over the *triceps surae* muscles. The protocol consisted in 3 stimulation trains of 20 s randomly delivered with wide pulses and either low or high frequency [WPLF (1 ms, 20 Hz) and WPHF (1 ms, 100 Hz)]. Trains were delivered either with or without Achilles TV (100 Hz, 1-mm amplitude) applied from 2 s after NMES onset to the end of the stimulation period. Stimulation intensity was set to evoke an initial force level corresponding to 10% maximal voluntary contraction (MVC). The increase in evoked force from the beginning to the end of each stimulation train was measured [1]. The magnitude of EF was further calculated as the mean ratio between the real and the theoretical force-time integral [3]. Based on this, we used a k-means cluster analysis classifying subjects as responders or non-responders to determine EF occurrence.

Results

The occurrence of EF was significantly higher with WPHF than WPLF NMES (41.1% vs. 12.5%; $p<0.001$), without any effect of TV ($p=0.669$) (Fig. 1A). The magnitude of EF was only analysed in responders and is presented in Figure 1B. Mean force increased from 7.8±1.5 to 16.0±3.9% of MVC (i.e. +8.3±4.0% MVC) throughout WPHF trains. Throughout WPLF trains, mean force increased from 10.5±2.9 to 18.4±4.2% of MVC (i.e. +8.0±1.9% MVC). Magnitude of EF was not significantly different between WPLF and WPHF ($p=0.341$) and was not influenced by TV ($p=0.977$).

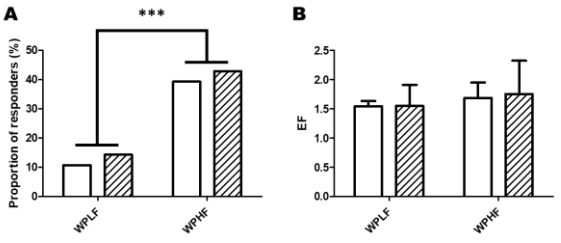

Fig. 1. Occurrence (A) and magnitude of EF in the responder group (B) during WPLF and WPHF NMES without (white bar) or with (hatched bar) superimposition of TV.

Discussion: The occurrence and magnitude of EF with WPHF NMES reported in the present study agree with the literature [4]. In contrast, although the magnitude of EF during WPLF was similar to WPHF in responders, its occurrence was poor, even when combined with TV. While this may contrast with previous findings [2], differences in vibration parameters or a population selection bias in the aforementioned study may likely explain such discrepancies. In conclusion, our results support the use of WPHF rather than WPLF to optimize EF generation, and do not support the addition of TV, at least using the tested characteristics, to maximize EF of the plantar flexors.

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Impact of running an ultramarathon on neuromuscular fatigue: effects of sex and distance.

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Introduction: Only a few studies have assessed sex differences in neuromuscular fatigue after prolonged running despite preliminary results suggesting that, compared to men, women exhibit fatigue in knee extensor (KE) muscles and less peripheral fatigue in plantar flexor muscles (PF) after a mountain ultramarathon [1]. The purpose of the present study was to further characterize sex differences in fatigue by examining various running distances.

Methods: Thirty-six runners completed the study: 18 men (36 ± 8 years, 72.5 ± 9.6 kg) and 18 women (36 ± 8 years, 59.1 ± 5.8 kg). Men and women were matched by relative performance (i.e. percent of winning time of their sex category). Participants completed various races of the Ultra-Trail du Mont-Blanc® ranging from 40 km with 2,300 m of positive elevation change to 170 km with 10,000 m of positive elevation change. One month before the race, participants visited the lab to be familiarized with electrical stimulation on both KE and PF muscles. Neuromuscular function was tested before (PRE) and after (POST) each race. The testing protocol consisted of a standardized warm-up followed by the assessment of maximal voluntary contractions (MVC), maximal voluntary activation (superimposed 100 Hz doublet) and contractile properties (potentiated 100 Hz doublet (Db100), potentiated 10 Hz doublet and potentiated single twitch (Pt)). For analysis, participants were further subdivided into two groups of 18 runners by distance of race completed (SHORT < 100 km vs LONG ≥ 100 km). Repeated-measures ANOVA for time (PRE, POST) with distance and sex as between subject factors was used for the analysis. Newman–Keuls post hoc test was used when the ANOVA revealed significant interactions.

Results: The analysis revealed a significant time-sex interaction in MVC for both KE and PF muscles ($p<0.01$ and $p<0.05$, respectively). Women lost less force than men in both KE (-27 ± 15% vs -36 ± 17%, respectively) and PF (-27 ± 19% vs -35 ± 12%, respectively). However, no time × sex × distance interaction was observed in MVC. On PF, women displayed greater decrease in Pt compared to men in LONG (-31 ± 13% vs -13 ± 13%, $p<0.05$). Despite men seemed to exhibit a greater decrease in VA than women in KE (-35 ± 12% vs -27 ± 19%, respectively), neither sex nor distance effects were observed in maximal voluntary activation for either muscle group.

Discussion: For runners of similar level of performance, our results showed that women lost less muscle strength than men, i.e. seemed globally more resistant to fatigue. Contrary to our hypothesis, this better resistance to fatigue seems to be independent on the running distance. Contrary to our previous findings, the present study displayed greater peripheral fatigue in women compared to men after LONG races yet men seemed more prone to central fatigue. It could be interesting to examine pacing strategies to better understand fatigability differences between sexes. Although we were able to answer the main question, the statistical power of our study may not have been high enough to clearly identify the mechanisms responsible for sex differences in fatigability induced by trail and ultra-trail running races.

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Physiological Differences Between Elite Male Trail vs Road Runners

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Introduction: The demands of training and competing for trail running-specific conditions may require specific physiological adaptations compared to road running, improving the athlete's ability to run on hilly terrain. The purpose of the study was to determine the physiological differences between elite road vs trail runners. It was hypothesised that trail runners would have a more force-oriented force-velocity profile (FVP), higher energy cost of running (Cr) on level but lower Cr on uphill, and a greater lower limb maximal strength compared to road runners.

Methods: 18 male elite athletes (11 trail and 7 road runners) were recruited for this study. All measurements where taken during the pre-season training camps organized by the Federation Française d'Athlétisme. FVP was measured using a two-sprint test against friction loads of 0.5 and 0.7 N per kilogram of body mass, on a cycle ergometer. The theoretical maximal force (F_0) and maximal velocity (V_0) were calculated as the intercepts with the ordinate and abscissa axes when plotting the force-velocity relationship of the two sprints. The theoretical maximal power (P_{max}) was calculated as $F_0*V_0/4$. Cr was calculated by measuring the O_2 consumption and CO_2 production while running at 10 and 14 km/h at 0% slope, and at 10 km/h on a 10% slope on a treadmill. Strength was assessed by measuring isometric maximum voluntary contraction of the knee extensors, knee flexors and hip extensors, normalised to body mass.

Results: F_0 was higher for Trail vs Road runners (11.2 vs 9.9 N/kg, $p<0.05$). V_0 was not significantly different, for Trail vs Road (22.5 vs 23.5 rad/s, $p>0.05$). The slope of FVP was significantly steeper for Trail (-0.50 vs -0.43, $p<0.05$). Trail had higher P_{max} compared to Road runners (10.7 vs 9.9 W/kg, $p<0.05$). Cr was higher for Trail compared to Road runners on flat at 10 (4.34 vs 4.10 J/kg/m) and 14 km/h (4.31 vs 4.06 J/kg/m, $p<0.05$), while it was not different during uphill running. No differences in maximal strength were found.

Discussion: F_0 was higher for Trail compared to Road runners, showing a trend to a more Force-oriented FVP. Road runners had a lower Cr while running on flat ground at high but not low speed. This may be explained because trail runners tend to train and compete at slower speeds, but on hillier terrain compared to road runners. The lack of difference in Cr in uphill between trail and road runners may be due to the fact that 10% was not steep enough.

The influence of physical activity on quadriceps muscle performance and biological characteristics of very old adults

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Introduction: The influence of physical activity (PA) on skeletal muscle performance in very old men and women (>80 yr) remains understudied. Yet, PA could influence muscle phenotype, capillarization and enzymes activity in ageing, and could be related to differences in muscle performance. The present study aimed to evaluate how PA influences knee extensors (KE) performance (i.e. isometric strength and fatigability) in healthy very old men and women. Muscle structural, microvascular and metabolic properties were further assessed using muscle biopsy in the vastus lateralis (VL) muscle.

Methods: Thirty very old adults (82±1 yr, 15 women) performed an isometric quadriceps intermittent fatigue (QIF) test [1] for the assessment of KE maximal force, fatigability and total work (index of absolute performance at the QIF test). Muscle biopsies from the VL were collected to assess muscle fiber type and morphology (myosin heavy chain isoform immunolabeling), microvasculature (capillary density and tortuosity, CD31 labeling) and enzymes activity (by spectrometry). Correlation analyses were used to investigate the relationships between KE performance, PA (steps.day^{-1}, actimetry) and biological data for each sex separately. Principal component analysis (PCA) was performed to detect the main components explaining variability between sexes.

Results: All participants were considered as active (men: 8841±2042 steps.day^{-1}; women: 9312±2089 steps.day^{-1}, P=0.53). Men showed greater maximal KE force and total work at the QIF test than women. Total work was correlated with PA level only for women (R=0.73, P=0.011). Fatigability and total work were not correlated to biological measurements for both men and women. PCA evidenced that cross-sectional area and capillarization of type I and IIA muscle fibers and oxidative enzymes activity explained most of the histological variability between sexes (44%). The component derived from these variables was lower in women than men and correlated with KE maximal force in this group (R=0.67, P=0.017).

Discussion: PA level positively influenced the capacity of very old women to perform a fatiguing test, but not maximal force production capacity of the KE. However, stronger women possessed greater type I and IIA fibers surface area, capillarization and oxidative enzymes capacity. PA level of very old men was not correlated to muscle performance. We suggest that very old women could be at higher risk of autonomy loss and would benefit from increasing their PA level by combining walking and reconditioning exercises.

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Does accelerometer measure muscle vibrations?

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Introduction: Soft-tissues vibrations have been widely investigated for various purposes for decades. Some studies aimed to assess the vibration characteristics such as amplitude, frequency, and damping, in order to better understand the muscular activity tuning in response to impact characteristics (i.e., the muscle tuning paradigm [1]). Currently, the influence of fatigue on the soft tissue vibrations [2] and the assessment of the efficiency of sport equipment to reduce vibrations are the most on-trend topics about vibrations [3]. However, vibrations are quantified with accelerometers attached to the skin. Although using these devices is the most ecological method for assessing soft-tissues vibrations, accelerometers record accelerations of all of the soft tissues located below the measurement area (i.e., the skin, the subcutaneous fat, and the muscles). Considering that muscle vibration is the most important phenomenon that needs to be characterised, one may wonder if the vibration behaviour differs between the different soft-tissues components, and if the muscle vibration estimation is not biased by the superficial tissues. The purpose of this study was to assess whether skin mounted accelerometer reflects muscle vibrations measured with ultrafast ultrasonography.

Methods: For 15 participants, vibration characteristics initiated on the vastus lateralis muscle by an impactor were compared when assessed with accelerometry and ultrasonography. Continuous wavelet transforms and Statistical Parametric Mapping (SPM) were used to identify discrepancies in vibration power over time and frequency between the two devices. Furthermore, linear regressions were performed between the relative differences in the vibration characteristics and the subcutaneous fat thickness of the participants.

Results: SPM analysis revealed that accelerometer underestimated the muscle vibration power above 50 Hz during the first 0.06 seconds post impact ($p<0.05$). Furthermore, accelerometer overestimated the muscle vibration power under 20 Hz, from 0.1 seconds after the impact ($p<0.05$). Linear regression revealed that the thicker the subcutaneous fat localised under the accelerometer, the more the muscle vibration frequency and damping were underestimated by the accelerometer ($p<0.01$).

Discussion: Skin mounted accelerometer allowed only a salient characterisation of the muscle vibration. However, to eliminate some artefacts caused by the superficial tissues and to better assess the muscle vibration with accelerometer, it is suggested 1) to high-pass filter the acceleration signal at a 20 Hz cut-off frequency and 2) to include participants with low fat thickness. Future works could focus on developing correction model to better estimate the muscle vibration with accelerometer.

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Intrarater reliability and agreement of a modified Closed Kinetic Chain Upper Extremity Stability Test [1]

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Introduction: The Closed Kinetic Chain Upper Extremity Stability Test (CKCUEST) is a functional test developed by Goldbeck and Davies [2] to assess upper limb muscle capacity and neuromuscular control. The participant adopts a push up position, with hands on two lines spaced at 91.4 cm. Then, the participant touches alternatively as fast as possible, the floor with a hand crossing over the supporting hand during three sets of 15 s with a 45-s recovery. The relevancy of the CKCUEST among participants presenting various body dimensions may be however questioned [3]. Normalizing the hand spacing may be an alternative to address such issue. CKCUEST is also though to assess the upper extremity muscle endurance [4]. The 45-s recovery between sets was however designed to limit fatigue originally. Performing additional sets when reducing recovery may be an interesting way to explore upper limb muscular endurance. The aim of this study was to assess the reliability and agreement of a modified procedure of CKCUEST.

Methods: Twenty-seven athletes (22 ± 3 years; 177 ± 7 cm; 79 ± 9 kg) performed two sessions of modified CKCUEST (m-CKCUEST) one week apart (CPP n°2018-A03013-52). The test consisted of performing three maximal sets (sets 1 to 3), with 45-s recovery, completed with a fourth 1-min set (sets 4 to 7), performed after 15-s recovery following the third set. An examiner counted the number of touches during each 15-s set. The m-CKCUEST score was determined by considering the number of touches during sets 1, 2 and 3. The muscular endurance index (MEI) was based on number of touches from sets 4, 5, 6 and 7 expressed in reference to the m-CKCUEST score. For both the m-CKCUEST score and MEI, intra- and intersession reliability and agreement assessments were based on the intra-class coefficient of correlation ($ICC_{3,k}$), standard error of measurement (SEM) and minimal detectable change at 95% confidence level ($MDC_{95\%}$). The coefficient of variation (CV) was calculated to assess the consistency between repeated measurements.

Results: The best reliability and agreement were obtained for m-CKCUEST score (Table 1) when averaging the numbers of touches of second and third sets, and for MEI (Table 1) when computed by dividing the one-half number of touches counted during the last 30 s of 1-min set (sets 6 and 7) by m-CKCUEST score.

Table 1. Intersession reliability and agreement for outcome measures from m-CKCUEST

	Mean ± SD	ICC	SEM (touches)	$MDC_{95\%}$ (touches)	CV (%)
m-CKCUEST score	23.1 ± 2.3	0.92	0.68	1.87	4.07
MEI	0.7 ± 0.1	0.86	0.06	0.15	10.03

Discussion: The m-CKCUEST allowed the production of two reliable outcome measures, which assessed the upper limb muscle capacity and endurance. Such outcomes may be then used by sport coaches and clinicians in order to diagnose upper extremity impairments, assess the effects of rehabilitation on upper extremity functions, or follow-up upper extremity abilities.

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Predictive validity of the S-STARTS composite score in athletes after shoulder stabilization surgery by Latarjet procedure: A preliminary study

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Introduction: Anterior glenohumeral instability occurs frequently in contact sports, especially in young athletes [1]. Shoulder stabilization surgery is often recommended in young athletes presenting with recurrences [2]. The Shoulder–SanTy Athletic Return To Sports (S-STARTS) provides a composite score out of 21 points based on psychological and physical tests, which has been proposed to help the decision-making for returning to sport after shoulder stabilization surgery by Latarjet procedure. Although the S-STARTS score has been validated statistically [3], its validity to predict the return to sport remains to be explored. The aim of this study was therefore to assess the validity of the S-STARTS composite score to predict the athlete's ability to return to sport at 1-year postoperatively.

Methods: Forty patients (Age: 24.4 ± 6.5 years; height: 176.3 ± 8.1 cm; Mass: 75.7 ± 14.8 kg) who performed S-STARTS at 4.8 ± 0.7 months after a shoulder stabilization surgery by Latarjet procedure answered a questionnaire by phone at 1-year postoperatively including the following question : "Are you currently practicing a sport?". ROC curve, negative and positive likelihood ratios were used for data analysis.

Results: Thirty-four (85%) patients returned to sport at 1-year postoperatively. The ROC curve (Figure 1) indicated that the best compromise of sensitivity (77%) and specificity (83%) was found for a cut-off at 11.5 points. The area under the curve was 0.814 (excellent discrimination; p = 0.015). Positive and negative likelihood ratios were 4.58 and 0.28, respectively.

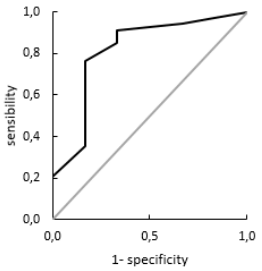

Figure 1. Receiver-operator characteristic (ROC) curve for the S-STARTS score composite

Discussion The main findings of this study were that a S-STARTS score higher than 11.5 is found 4.6 times more often among athletes who returned to sport at 1-year postoperatively than those who did not. By contrast, a S-STARTS score lower than 11.5 was observed 3.6 times more often in athletes who did not return to sport. Additionally, with a cut-off of 11.5 points, only 17% of false positive cases were denoted meaning the ability of the score to discriminate patients who could return to sport. Despite our sample presented with similar return to sport rate than that reported in the literature [4], these findings demand to be confirmed based on a larger sample size. The cut-off of 11.5 points for S-STARTS score may nevertheless be used as a primary benchmark by sport coaches and clinicians to help the return to sport care after shoulder stabilization surgery by Latarjet procedure.

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Sensory integration in young elite handball players. Which implications for ACL injuries ?

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Introduction: Anterior cruciate ligament injuries (ACL) are frequent in handball especially in young female players. Sensory integration was recently evocated as a potential important risk factor. Muscle vibration is a reliable tool to evaluate proprioceptive reweighting and sensory integration during postural control. Therefore, the aim of this study was to assess proprioceptive plasticity and muscular activity during postural control among healthy young handball players. This in order to better understand sensory integration as a possible risk factors for ACL injuries.

Methods: Thirty-five young healthy elite handball players (21 males and 14 females 15 ±1 year) participated in this cross-sectional study. Proprioceptive strategy during postural control was evaluated using muscle vibration (80 Hz) of the triceps surae (TS) and Lumbar Paravertebral Musculature (LPM) during bipedal stance in two conditions, firm and foam surface. Postural parameters and electromyographic (EMG) activity of the Gluteus Medius (GM) semitendinosus (ST) and fibularis Longus (FL) were recorded during PRE (10s), POST (20s) and VIBRATION period (20s). Data were compared using T test and analysis of variance (ANOVA) statistical analysis.

Results: Ankle proprioception was predominantly used on firm while lumbar cues where used on foam support in the overall population. However, two opposite behaviors were observed. *rigid* individuals increased their reliance on ankle cues when standing on unstable surface, whereas *plastic* individuals shift their proprioception reliance to a more proximal control ($p<.001$). Those subjects show more efficient balance recovery ($p<.05$). ST was more recruited during POST vibration in *rigid* individuals who additionally did not return to the PRE vibration level ($p<.05$) compare to *plastic*. Female players were significantly more *plastic* than males.

Discussion: The ability to dynamically reweight proprioceptive signals according to surface stability appears to be widely different among athletes. When comparing the two proprioceptive profiles, *plastic* individuals displayed both postural and neuromuscular parameter that could be considered more efficient for lower limb control. This neuroplasticity during postural control could be linked to injury risk factors. Further studies are needed to establish the link between proprioceptive plasticity and biomechanics during sport tasks.

Influence of alpine skiers level on the line-strategy based on turn switch events timing

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Introduction: In alpine ski racing, different line choices can drastically effect turn or sectional performance [1]. The transition phase between two turns is the main phase in a race where skier can gain speed, open his trajectory, or reduce their length path [2]. In the literature, this phase draws attention mainly in the form of a variety of kinematic and kinetic methods applied to separate one turn from another by detecting the timing of the turn-switch (TS) [3]. Beyond understanding methodological variability, these event markers might represent distinct biomechanical events and comparison of their timing could provide insight into athlete techniques, strategy and performance. This research aimed to examine various turn-switch events, and whether their timing is related to strategy used and the interaction with performance level.

Method: A mixed level population of male skiers (N=17), skied a timed giant-slalom course while equipped with specialized force-plates and GNSS collecting synchronized normal ground reaction force and position-time data, respectively. Five events were calculated for each turn (N=13, per athlete). The moment of edge taking (Fbeg), releasing (Fend), and minimum force (Fmin) were extracted from force signal. The turn radius passing above and below 30 meters (Traj>30 & Traj<30) were extracted from the positional data. Time of straight line between events Traj>30 & Traj<30 and time of edge switch between Fbeg and Fend are computed to assess the interaction between line strategy and performance. Speed increase and altitude decrease during the straight line are also computed in order to assess the interaction between straight line characteristics (i.e. associated with line strategy) and time of the straight line.

Results: Better skiers typically displayed less lag between Traj>30 and Fmin (r=-0.64, p=0.005), and a tendency for shorter time distance between Traj>30 and Traj<30 (r=-040, p=0.11). Shorter lag between Fend and Traj>30 is also link with the performance increase (r=-0.68, p=0.005). Other tests between TS event and time performance did not present significant correlation. No significant correlation was found between increase of speed between turn and course time

Discussion: Smaller lag between $Traj_{>30}$ and F_{min} could corroborate the hypothesis than high level athletes continue to turn later and spend more time during the turn. High level skier can perform faster the turn switch specifically by delating the end of the turn. This result could be explained by higher technical and physical capacity to push with lower limb at the end of a turn in order to project skiers' body faster in the straight part between turns.

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Effect of force, velocity and power exercise conditions, force-velocity-power endurance profile and endurance capacities on strength-endurance

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Introduction: Repetitive near-maximal efforts are frequent in daily life, in which successful performance depends on the production and maintenance of high mechanical power by the lower limbs over a series of repetitions. The maximal power production capabilities are described by the power-velocity relationship. The ability to maintain power output over time (*i.e.* strength-endurance; SE) is characterized by the power-time relationship, expressed relatively to P_{max} ($\%P_{max}$). The same $\%P_{max}$ can be developed in high force-low velocity conditions or in low force-high velocity conditions (force-velocity ratio conditions; R_{Fv}). However, the effect of force-velocity (*F-v*) condition on strength-endurance is not clear in the literature [1]. Also, for the same $\%P_{max}$,]he maximal power capability at this specific velocity ($\%P_{max}v$; the power reserve) is not similar according to the *F-v* conditions, potentially affecting SE [2]. Moreover, the effects of individual force-velocity-power endurance profile (P_{FvE}) and endurance capacities (*End*) are not clear on SE.

Methods: 14 athletes performed 5 loaded squat jumps to determine *F-v-P* relationships in squat jump, from which the assessment of SE was individualized in 10 steady-state *F-v-P* conditions in repeated jump test to exhaustion. SE was quantified by the maximal number of repetitions (SJ_{Rep}) completed in each repeated jumping condition. Intra and inter-day reliability was tested in one of the 10 conditions. The effects of *F-v-P* conditions on SJ_{Rep} were tested with i) a stepwise multiple linear regression with ($\%P_{max}$) and ($\%P_{max}v$), and R_{Fv} as independent variables, and with ii) two-way ANOVAs to test the effect of $\%P_{max}$, $\%P_{max}v$ and R_{Fv} on SJ_{Rep}. P_{FvE} was computed as the ratio of SJ_{Rep} obtained in high force-low velocity and low force-high velocity condition at $85\%P_{max}v$. Endurance capacities index (*End*) was computed as the mean SJ_{Rep} in three different *F-v-P* conditions at $85\%P_{max}v$. The effect of P_{FvE} and *End* on SJ_{Rep} obtained in 3 *F-v* conditions at $85\%P_{max}v$ were tested with i) three stepwise multiple linear regressions.

Results: SJ_{Rep} were dependent on $\%P_{max}v$ and R_{Fv} ($R^2 = 0.975$; RSME = 0.242; $p < 0.05$) with the effect of R_{Fv} increasing with decreasing $\%P_{max}v$ (*p* value of interaction = 0.03). SJ_{Rep} exhibited acceptable intra- and inter-day reliability (ICC = 0.94 and 0.86, respectively). Also, SJ_{Rep} in the 3 *F-v* conditions was dependent on individual P_{FvE} and *End* ($R^2 = 0.823$-0.965; RSME = 0.120-0.25; $p < 0.05$). The proportion of variance in SJ_{Rep} explained by P_{FvE} and *End* was not similar in the 3 *F-v* conditions at $85\%P_{max}v$. SJ_{Rep} in high force-low velocity conditions was more depend on P_{FvE}, whereas SJ_{Rep} in high force-low velocity conditions was more depend on *End*.

Discussion: At an individual level, SE was shown to depend at ~ 98 % on $\%P_{max}v$ and R_{Fv}, which were both defined relatively to the individualized force-velocity-power relationships. SE was higher when $\%P_{max}v$ was lower (*i.e.* higher power reserve) and when the balance between force and velocity to generate power was rather oriented toward low force-high velocity. However, the same *F-v-P*, differences in SE between individuals can be explained by P_{FvE} and *End*.

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Force output in giant-slalom skiing: a model of effectiveness

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Introduction: Alpine ski racers require a diverse range of physical capabilities to succeed at a high level. Since the application of force is the primary means performing skiing movements, the relationship between force production and performance is interesting. Enhanced force production is generally considered a key physical component for high-level skiers [1], however limited and convoluted data on its relevance during skiing highlights a more complicated relationship [2]. The aims of this study were 1) to clarify the association between skiing performance and reducing path length and/or maximizing velocity, 2) to test the importance of radial force in skiing performance, and 3) to further explore the importance of force magnitude and/or orientation of force-output in skiing turn performance.

Methods: We measured force output and positional data using ski-specialized force plates and a sports GNSS system in mixed-ability skiers (N=15) while they performed a race-paced giant slalom course. Data were separated into 14 turns using the point of trajectory inflection. For each turn, velocity, velocity normalised energy dissipation, radial force, total force (for both inside and outside limbs) and the ratio between radial and total force were calculated per turn and then averaged for a final course value. A stepped correlational (a) and regression (b) analysis procedure was created to examine the relative contribution of trajectory and speed-based parameters to course time (2), to contextualise the importance of radial forces (a and b; radial force vs. course time and energy dissipation), the contributors to radial force (b; total force and the ratio between radial and total force), and subsequently the balance of force output between the limbs to total force production (b).

Results: Better performance was associated with less normalized energy dissipation (R^2=0.708, p<0.001), rather than path length. Athletes with lower course times and lower dissipation of energy tended to display greater values of radial force (R^2=0.682 and 0.518, respectively, p<0.002), which itself was strongly predicted (in order) by both the capability to apply total force on the ski and an enhanced ability to apply a larger proportion of the force in the radial direction (model R^2=0.986; standardized β=0.939 and 0.739, respectively, p<0.001). Athletes who applied more force did so through both greater output to the outside limb and a more balanced production between the limbs (model R^2=0.995; standardized β=1.079 and -0.657, respectively, p<0.001).

Discussion: Our results generally support normalized energy dissipation as a key discriminating performance factor in giant slalom in place of preferencing a more direct trajectory. In general, athletes who wish to increase the radial force applied to the ski may do so first by increasing total force application, and second by increasing their technical efficiency (applying a greater proportion of force in the radial direction). We theorise that increasing the ability to generate force-output on the skis, in combination with adopting techniques to improve force effectiveness, should feasibly raise the 'velocity barrier' [2] of athletes and allow them greater physical freedom to select advantageous trajectories. However, we cannot conclude whether our measurements correspond to the true physical capabilities or limits of the athletes, and further detailed analyses, including cross-examination of off-snow tests of physical output are needed.

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Using biofeedback and tDCS to prevent acute stress effects on executive functions and motor skill

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Introduction: The deleterious impact of acute stress on executive functions and motor performances is well-established. However, its potential impact on fine motor skills has received far less attention. This study not only aimed at characterizing in greater details the effect of stress on executive functions and motor skill, but also explored the potential benefits of transcranial direct current stimulation (tDCS) and cardiac coherence biofeedback (BFB) as *a priori* stress coping methods [1].

Methods: Seventy-two participants were randomly assigned to a CONTROL (n=20), BFB (n=20), tDCS (n=18) or tDCS + BFB (n=14) group. The experimental session was divided in 4 steps: anticipation stress (2 min), coping treatment (15 min), stress event (10 min, Trier Social Stress Tests) and a set of cognitive and motor tasks (Fig1, upper). Cardiac and respiratory parameters and self-report of perceived stress (Visual Analogous Scale, VAS) were collected during the experiment. Motor and cognitive performances were evaluated through explicit motor imagery (MI) and fine motor skills through trail making and 9 holes pegboard tasks. Executive functions were assessed with the Stroop test, the 3-back, the dual, and anagram tasks.

Results: Main results show that tDCS + BFB is the most effective method to decrease psychological anticipation stress (Fig1, coping treatment). No impact of stress or coping methods was found on fine motor skills: explicit MI ($\chi^2(3)=1.69$, p=0.64), trail making ($\chi^2(3)=1.58$, p=0.66) and 9 holes pegboard ($\chi^2(3)=3.91$, p=0.27). Data

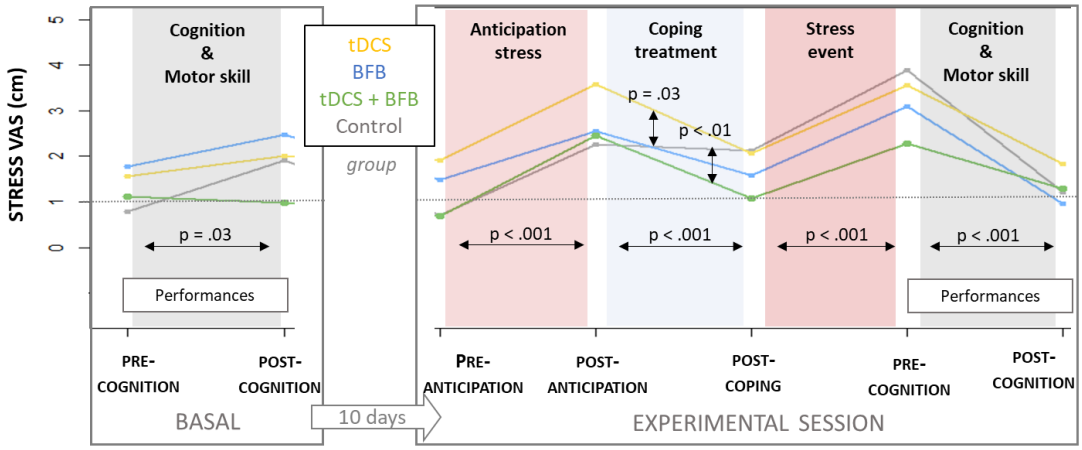

Fig 1. Experimental design and results of psychological stress. Linear mixed effects with a by-subject intercept models were used to analyze the effect of time on the dependent variables quantifying stress induction and performances. For performances, we entered TIME (BASAL, EXPERIMENTAL) and GROUP as fixed effects with interaction terms.

on executive functions are not presented here.

Discussion: Using a thorough test-retest procedure, data provide a deeper understanding of stress effects on motor skills, and practically support that manual dexterity and explicit MI are preserved under stressful conditions. Results somewhat challenge previous findings showing harmful stress effects in certain cases [2]. For instance, Noteboom et al. (2001) found that surgical performances were altered by an acute stress. However, they were interested in specific medical skills, allowing limited generalization. Finally, our research confirms previous data demonstrating that explicit MI performances [3] are not altered by stress.

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Physical and mental learning of sequential footstep movements

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Introduction: Sequential motor learning relies on a fast performance improvement during an acquisition session, followed by a sleep-consolidation enhancement. Although these effects have been extensively demonstrated using finger tapping movements, nothing is known for lower-limbs movements involving substantial displacements of the center of gravity (CoG) [1]. Motor imagery practice (MP; i.e. mental rehearsal of an action without physically executing it) benefits from sleep consolidation, such as physical practice (PP) [2]. This study aimed, therefore, to examine the acquisition and consolidation processes of a sequential footstep movements learnt by PP and MP.

Methods: Seventy-six adults were assigned into six groups according to the type of practice (MP; PP and no training CTRL) and the nature of consolidation (Night vs. Day). The footstep paradigm consisted in performing an 8-steeping sequence using alternatively the right and left foot over a mat, as fast and accurately as possible. All subjects performed a physical pre-test of 2 blocks of 30s. Then, subjects of the practice groups repeated the sequence using either PP or MP for 12 blocks of 30s. CTRL group did not practice during a similar time. Right after, all subjects performed a physical post-test, then a re-test (2 blocks of 30s each) following 10h of day or night consolidation period. Performance was analyzed using the number of correct steps and the distance traveled by the CoG.

Results: All groups showed an increase of correct steps (Fig.1), although PP showed a greater improvement, and a decrease of the distance traveled by the CoG following the training session (pre- vs post-tests). The magnitude of enhancement in the CTRL group remained lower relative to that in the PP and MP groups for the distance traveled by the CoG (see inset Fig.1). At re-test, only the MP Night group continued on increasing the performance, while all others groups showed a stabilization of performance.

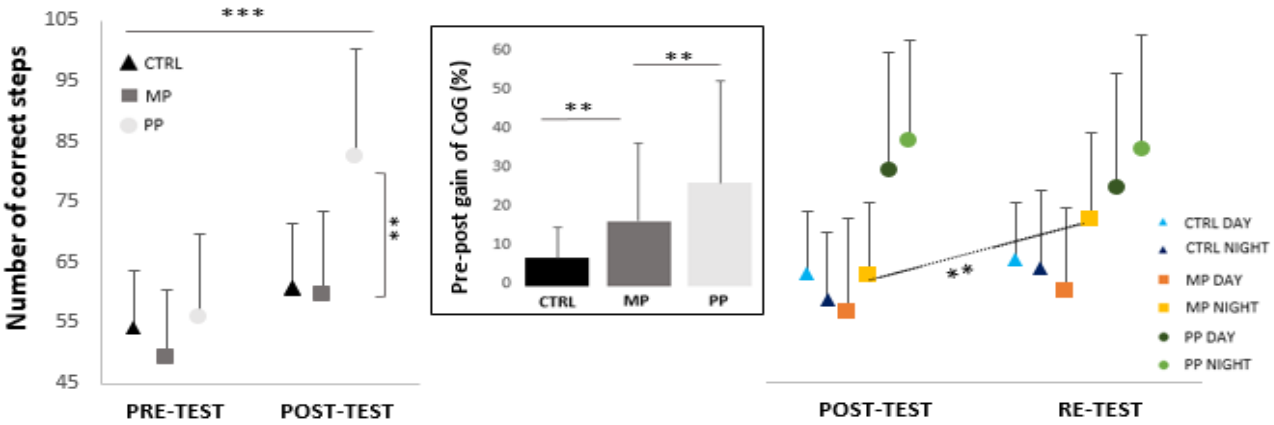

Fig1. Number of correct steps in each test for the MP, PP, and CTRL groups.

Discussion: This study highlights the positive effect of MP on sequential lower-limb learning associated with the displacement of CoG. Sleep-dependent consolidation was revealed for MP, which is in line with several studies that demonstrated similar effect using sequential finger tapping movements. Stabilization of performance for PP night after 10h of consolidation seems inconsistent with the literature. This motor paradigm might beneficiate from longer consolidation time (e.g. 24h rather than 10h here). Based on the balance and sleep issues in aging, it would be interesting to study the relationship between sleep and lower limb learning, both physically and mentally, in such population.

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