

Návrh projektu

MEZIOBOROVÉ VÝZKUMNÉ PROJEKTY

GAMU - Program podpory výzkumu
2018

Název projektu: Novel sampling and analysis methods for optimization of athletes' training load.		
Období řešení projektu	od: 1.3.2018	do: 31.12.2020
Hlavní řešitel	Doc. RNDr. Petr Kubáň, Ph.D. (CEITEC MU)	
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Spoluřešitelé: <i>(tj. klíčové osoby ze zapojených pracovišť, ke každému spoluřešiteli uveďte do závorky HS)</i>	Prof. RNDr. Viktor Kanický, DrSc (PřF MU)	
	Doc. Mgr. Martin Zvonař, Ph.D. (FSpS MU)	
	Prof. MUDr. Jan Nobvotný, CSc.	
	Mgr. Jan Cacek, Ph.D.	
<i>Hodnotící kritérium 1 (životopisy)</i>		
Anotace projektu (max. 300 slov)		
<p>Intense sports training, as well as any other high intensity physical activity is characterized by a high complexity and large number of performance tests are commonly executed. Among the most common tests VO₂ max test, substrate utilization test or lactate threshold are used to evaluate athlete's performance parameters before, during and after training. Monitoring of various performance parameters helps to increase the effectiveness of training. It relies on recording of changes on an athlete during various stages of training or under the influence of main elements of sport activities. Usually these tests require extensive experience and access to a biochemical laboratory with specialized instrumentation and mostly rely on invasive acquisition of blood specimen. This complicates the sampling procedure and restricts the frequency at which the samples can be taken. The tests need to be performed in a laboratory setting that is often different from the real competition and present apparent practical limitations.</p> <p>We propose to develop and optimize non-invasive sampling methods of alternative athletes' body fluids, such as exhaled breath condensate, saliva and sweat. These samples are much simpler to be acquired, can be taken as often as needed and are much easier sampled outside the laboratory, being more realistic and closer to the real competition conditions. We aim at screening for the most relevant biomarkers and metabolites in these samples using state of the art analytical techniques with regard to optimization of performance, estimation of exhaustion and assessment of muscle damage. By identifying the most relevant metabolites, we aim to develop quicker and simpler monitoring methods, allowing to decrease the cost of performance evaluation and make the personalized training plan more effective. Further, by identifying the metabolite changes during the personalized training we assume to gain new insights into personalized metabolomics that will lead to the expansion of the basis for further correlation studies with genotype/phenotype data.</p>		

Popis projektu (max. 3000 slov)

Introduction

Modern system of elite sportsmen's training is characterized by its intrinsic complexity. Every one of its various structural components can substantially influence individual person's result. Sports training continues to be more intensive and complex and requires among others high aerobic abilities of athlete's organism, formation of which takes significant amount of time [1]. For this period of time the athletes master rational techniques and tactics of training, increase motor skills and improve organism's functional systems.

Current performance tests

Currently, a large number of performance tests are executed [2] such as VO₂ max test, substrate utilization test, lactate threshold test in blood, sweat rate and sweat electrolyte test, to name a few. Many of these tests are instrumentally rather complicated, include invasive sampling, and are time consuming and costly. Moreover, they can only be performed under supervision of a trained personnel and evaluation of the results requires extensive experience and access to a biochemical laboratory with specialized instrumentation. Further, the training needs to be interrupted during sample acquisition, which may inconveniently disrupt the training activity. Additionally and importantly, the number of invasive samples that can be taken during the test is limited. For instance it is not realistic to take the blood samples with very short time intervals and thus measurement of the correlation of blood metabolites with on-line monitoring tests is only approximate.

Training efficiency monitoring

The use of blood metabolite monitoring during sports training is of high importance, because knowledge about the pathways that lead to the formation of a specific metabolite, its transformation and subsequent elimination are important for the estimation of training load, estimating the possible muscle damage, rate of exhaustion, aerobic and anaerobic thresholds and so on [1,2]. Among the commonly studied metabolites, are *for instance* lactate, pyruvate, urea, uric acid, creatine, creatinine, creatinine kinase, inorganic ions (ammonia, chloride, sodium, magnesium, calcium and potassium), amino acids/proteins (myoglobin), trace metals (zinc, copper, iron, chromium, nickel, lead, etc), oxidative stress markers and immunosuppression markers. Each of the studied analytes has a specific indicating function during training and their levels are suggestive of the processes taking place in the body. Extensive information is available on few metabolites and current performance evaluation is mainly based on the monitoring of selected compounds in blood. For instance, blood lactate, is frequently used to determine the contribution of anaerobic glycolysis in energy production during exercise [3]. From the time-point of monitoring, lactate is meant to be used immediately during training sessions, while other metabolites, such as urea characterize the influence of prolonged aerobic exercise and serves as an index of recovery process [4]. This is true not only for endurance athletes, but also for instance for sportsmen training in power disciplines. Inorganic ions and metals may indicate the dehydration/ionic content loss during training, while creatinine kinase is an indicator of rhabdomyolysis and muscular dystrophy. It is important to know what metabolic processes the chosen metabolite represent, what its limitations are and how to interpret the results. As the main aim of sport training is to increase performance level, the event-specific performance tests must have a constant place in training monitoring programs.

However, because sports training influences an athlete in a wide way, starting with changes at molecular level and ending up with changes in functioning of different organs, one should not concentrate only on few biochemical tests and make deep conclusions according to their results. Also, because each person's metabolism may differ greatly, personalized training and corresponding personalized biochemical analysis comes into play. The details of training monitoring depend on the goals of the

concrete training monitoring process. As monitoring is a purposeful process performed with the aim to increase the effectiveness of training guidance and is based on recording of changes on an athlete during various stages of training or under the influence of main elements of sport activities (training sessions, competition, microcycle or mezocycle of training) the aim of the monitoring determines the frequency and the choice of markers. Training monitoring is a specific process depending on sport event, performance level of an athlete and age/gender peculiarities, health/injury status. Therefore, the methods for training monitoring should be chosen depending on the specificity of a sport event and athlete's characteristics.

Sampling of body fluids

The plethora of analytes related to the athlete's performance and their exact function is rather complex and it would be experimentally extremely demanding to analyze the complete profile of blood in a reasonably short time with the results obtained within a usable time frame for training and recovery processes optimization. The sampling typically involves "invasive" mode, such as venipuncture during the blood sampling. This procedure requires special equipment, trained medical personnel and leads to the necessity to interrupt the training for an extended time period. Even if the sample is taken from the finger prick or ear lobe, the sampling is non-trivial and repeated sampling during short intervals may lead to excessive load on the athlete organism.

As the human body is an enclosed entity, the biomarkers and metabolites will be distributed throughout various body fluids, although the absolute concentration level may be significantly different among the fluids. One clear advantage of sampling alternative body fluids, such as exhaled breath condensate, saliva, or sweat is the possibility to get the sample completely non-invasively without any significant interruption of the training activity, thus allowing the semi-continuous or continuous monitoring of selected performance parameters. The non-invasive sampling and sample analysis however has not been extensively used. The reason maybe that the sampling techniques are not standardized (as is for instance blood sampling) and that more sensitive and selective analytical methods are required for these samples. In this project we propose three innovative, non-invasive, sampling methods to acquire body fluid samples from athletes:

Exhaled breath condensate (EBC)

EBC is the liquid obtained upon cooling and condensation of exhaled air. Exhaled breath is saturated with water vapors, which will condense by breathing through a cooling or freezing system. Although the condensate consists mostly of water vapor, it also contains aerosol particles or respiratory fluid droplets. These droplets are released from the lung epithelial lining fluid, in which the aerosolized particles contribute to the non-volatile constituents, such as inorganic ions, small organic molecules and proteins. Additionally EBC may contain water soluble volatile gases as well. EBC was first reported as human body fluid in 1980 by Sidorenko et al. [5] but has not been extensively studied in the sports research. EBC, however, is an attractive sample, because it may reflect the composition of the circulating blood, as the O₂/CO₂ exchange takes place in the alveoli of the human lungs and the inhaled/exhaled breath comes into close contact with the bloodstream. In a few previous studies [6], including one of the principal investigator [7], for instance lactate could be monitored in EBC during and after exhaustive exercise with tight relation to the measured blood lactate [6]. We assume that other constituents of blood that are increased during training and post-training sessions will be traceable in EBC. The sampling method and samplers for EBC were developed recently in the laboratory of the principal investigator [8], securing the necessary expertise in the field.

Sweat

Sweat (or perspiration) is a fluid excreted by the sweat glands of the skin. Sweat contains primarily water (99%), but also salts and metabolic waste products—primarily sodium chloride ((0.9 g/l), urea, lactic acid (0.2 g/l), calcium (0.015 g/l), and magnesium (0.0013 g/l) ions , but also minor compounds in trace amounts such as zinc (0.4 mg/l), copper (0.3–0.8 mg/l), iron (1 mg/l), chromium (0.1 mg/l), nickel (0.05 mg/l), lead (0.05 mg/l). The volume of water lost in sweat daily is highly variable, ranging from 100 to 8,000 ml/day. While sweat rate is generally linked to size (larger sportsmen typically sweat more) and gender (males sweat more than females), the concentration of electrolytes and metabolites in sweat is uncorrelated. Two similar athletes can have different sweat rates and sweat composition and the electrolyte loss can vary by up to 200-fold. Also, an extensive comparison of analytes in sweat and blood provides inconclusive correlation evidence, a primary compound studied being lactate. While some studies show a decrease of lactate in sweat compared to blood samples, other studies show exactly the opposite. We believe that part of the confusing results may be due to the variable sweat dilution caused by the increased sweat production, without proper standardization of sampling. Another important aspect, electrolyte depletion, can lead to gradual and subtle loss of performance. As electrolyte depletion becomes more extreme, debilitating muscle cramps can suddenly set in. For every person, the point at which performance decreases or muscle cramps start is different. Athlete sweat testing and sweat composition analysis is the only way to know the body specific needs. Information on the amount and loss of minerals, especially in endurance athletes (runners, cyclists etc) is critical for diagnosing the developing hypohydration and hyponatremia. Therefore sweat testing is critical for optimizing performance, the supply of water mineral and energy and minimizing the possible injuries.

Saliva

Saliva is produced in the mouth of humans and most animals and is composed of 98% water, while the other 2% consists of electrolytes, mucus, glycoproteins, enzymes, and antibacterial compounds. In relation to sport analysis, sampling saliva has a significant importance as the concentration levels of some specific compounds reflect their concentration in the blood. Another possible aspect is the indirect indication of immunosuppression during heavy training load (for instance in endurance athletes, swimmers etc.) that could be indicated by the amount of antibacterial compounds. Saliva offers a possibility of non-invasive, stress-free and real-time repeated sampling where blood collection is either undesirable or difficult. This facilitates the development and introduction of screening tests that can be performed by athletes during the training. Analysis of saliva can offer a cost-effective approach for the screening of large number of samples.

The goals and aims of the proposal

1. Novel sampling methods

We propose to **develop and optimize novel and original non-invasive sampling methods of alternative athletes' body fluids, such as exhaled breath condensate, saliva and sweat**. These types of samples have not obtained such research interest, probably because the metabolite levels are not as well defined in these samples as they are in the blood. The samples of alternative body fluids are however much simpler to be acquired, can be taken as often as needed and are much easier taken outside the laboratory, being more realistic and closer to the real competition conditions.

The sampling methods would allow the following:

- (i) decrease the sampling time and complexity,
- (ii) allow for multiple and repeated sampling during the athlete training period,
- (iii) allow sampling from multiple sites, i.e. legs vs. arms, to target specific muscle groups,
- (iv) decrease the cost for such an performance evaluation,

2. Screening for biomarkers

We aim at **screening for the most relevant biomarkers and metabolites** in these samples using state of the art analytical techniques with regard to optimization of performance, estimation of exhaustion and assessment of muscle damage. By identifying the most relevant metabolites, we aim to develop quicker and simpler monitoring methods, allowing to decrease the cost of performance evaluation and make the personalized training plan more effective. Further, by identifying the metabolite changes during the personalized training we assume to gain new insights into personalized metabolomics that may set the basis for further correlations with genotype/phenotype data.

These further goals are:

- (v) find suitable and most representative biomarkers of interest in the samples of exhaled breath condensate, sweat and saliva,
- (vi) find representative concentration levels of these biomarkers to allow personalized training support,
- (vii) perform the analysis of some selected, most important, biomarkers on-site with no need for sample transportation to the analytical laboratory.
- (viii) form a basic research knowledge on the metabolic pathways during the performance optimization, estimation of exhaustion and assessment of muscle damage and thus pave the new research avenues towards personalized metabolomics and possible future correlation studies with genotype/phenotype data.

3. Personalized training optimization

As has been stated earlier, each human body is different and generalization of various biochemical parameters upon weight, sex, etc. does not necessarily correlate. Similarly to the concept of personalized medicine (in which even persons with the same illness may respond differently to the therapy) in sports training, we attempt to introduce a similar concept. Effective training is only possible upon good health and when the athlete is not overloaded or over-trained. Such condition will not occur immediately during training, but may occur afterwards. Individual diagnostics of these condition(s) is very important as proper identification may help avoid development of inner misbalance, oxidative stress, rhabdomyolysis and immunosuppression, to name a few examples. With this concept in mind we will introduce 2 types of monitoring of biomarkers in selected non-invasive samples during the course of the project.

(i) determination of basic biochemical parameters/still state status

The basic information on the athlete's biochemical profile (selected analytes that are related to the diagnosis of overtraining and overload) will be determined in the beginning of the training period and regularly checked during the training in still condition (typically in the morning). Other parameters will include basic screening for other relevant compounds.

(ii) determination of actual biochemical profile during active training

The information on status and changes of biochemical markers during active training (lactate threshold, ABR, metabolism of water, mineral content, loss of salts, saturation of muscles by oxygen) will be used for estimation of the actual athlete status, the reaction to the training load, the appropriateness of the current load and possible exhaustion etc.

Choice of the athlete groups

The selected cohort of athletes will include both elite and recreational sportsmen. We shall develop the model and test suitable sampling methods and biomarkers initially with a group of 10-15 top basketball players of the **mmcite Brno** that is the team in the NBL league. This group will be supplemented by a group of endurance sportsmen/women, such as swimmers, runners, triathlon athletes or badminton players. We shall start with a cohort of 10-15 basketball players initially and sequentially will extend the number of participants as needed after the first screening and evaluation period.

References

- [1] AA Viru, M Viru, Biochemical monitoring of Sport training, Human Kinetics, Inc, 2001.
- [2] J Hoffman, Physiological aspects of sport training and performance, Human Kinetics Inc. 2002.
- [3] ML Goodwin, JE Harris, ME Andres Hernandez, LB Gladden, J. Diabetes Sci Technol, 1 (2007) 558-569.
- [4] U Hartmann, J. Mester, Medicine & Science in Sports & Exercise 32 (2000), 209-215.
- [5] G.I. Sidorenko, E.I Zborovskii, D.I. Levina, Ter. Arkh. 52 (1980) 65-68.
- [6] E.M. Marek, J. Volke, I. Hawener, P. Platen, K. Muckenhoff, W. Marek, J. Breath Res. 4 (2010) 1752-1755.
- [7] P. Kuban, E-G. Kobrin, M. Kaljurand, J. Chromatogr. A 1267 (2012) 239-245.
- [8] M. Gregus, F. Foret, P. Kuban, Electrophoresis, 36 (2015) 526-533.

Metodologie projektu s uvedením příslušných pracovišť/nositelů expertízy (max. 500 slov)

Non-invasive sampling methods

The sampling will involve specially designed samplers for collection of EBC. The sampler was developed at the institute of the principal investigator (PI, Assoc. Prof. Kubáň, CEITEC, Bioanalytical Instrumentation) and can collect significant amount of EBC from few exhalations [8]. The sampling of saliva will be done by buccal swabs the volume of which will be standardized with malonic acid (not present in the saliva). Sweat will be sampled with pre-cleaned cotton swabs, weighed before and after collection for standardization purpose. All sampling expertise will be provided by the PI and his team (Mgr. Ďurč, Mgr. Lačná) who have a long term experience with sampling of biological fluids. The novel sampling approaches will be optimized and standardized for usage with athletes as there are specific demands that must be considered during the training sessions. The collection parameters will be studied on a cohort of elite and recreational athletes, recruited from the faculty of sport studies. The experimental work in this part of the project will involve the cooperation of CEITEC and FSpS research units. The sampling will involve selected group of elite athletes (basketball team of mmcite Brno) that is under supervision of Assoc Prof. Zvonař. The group will be subjected to approved ergospirometric and other tests (muscle oxygen saturation – MOXY; markers of fatigue; antioxidant capacity, spectral analysis of HR- DiANS8 variability) to provide a reference value and to find correlation between the measured parameters (Dr. Cacek, Prof. Novotný).

Analysis methods

The analysis of the collected samples of aforementioned biological fluids will be performed by the plethora of techniques available in respective departments. Capillary electrophoresis, HPLC, ICP-OES/MS, AAS. These will be provided by the CEITEC (Dr. Greguš, Assoc. Prof. Kubáň) and PŘF (Prof. Kanický, Dr. Holá, Mgr. Šimoníková, Dr. Bittová). Inductively Coupled Plasma Mass Spectrometry (ICP-MS) will be used for determination of trace elemental contents in samples, whereas Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) or Atomic Absorption Spectrometry (AAS) will be employed for minor and major elemental constituents. Appropriate sample preparation technique will be employed. The collaboration with faculty of sport studies will be inevitable to provide feedback, test the athletes, evaluate the models etc. Statistical model for data evaluation will be prepared by the experts from the PŘF Dr. Bittová, Dr. Holá, Prof. Novotný and will be tested in the practice.

Personalized data plan

Models for individual screening that will include personalized data for each individual athlete that will contain the necessary biomarkers in the non-invasive samples to estimate the risk of training overload, muscle damage and overtraining will be developed. Second the real-time metabolite monitoring will be used for training planning, the correct execution of the training plan and estimation of its effectivity.

Podstata mezioborovosti projektu a přidaná hodnota (max. 200 slov)

The proposed project is based on collaboration of several specialists; in the field of sampling and separation science (Assoc. Prof. Kuban), the field of optical analytical methods (Prof. Kanicky) and sport training, spiroergometry and muscle strenght measuring (Assoc. Prof. Zvonar, Prof. MUDr. Novotny, Mgr. Cacek). The PI, Assoc. Prof. Petr Kubáň (CEITEC MU), will bring his expertise and equipment in the field of non-invasive sampling and capillary electrophoresis (Mgr. Greguš, Mgr. Lačná, Mgr. Ďurč). The field of HPLC and HPLC-MS will be saturated by Dr. Bittova, while trace metal analysis by involvement of Prof. Kanicky, Dr. Hola, and Mgr. Šimoniková (Faculty of Science). The inseparable unit of the proposed project is the part from the faculty of Sport studies (Asoc. Prof. Zvonař, Prof. MUDr. Novotný and Dr. Cacek), which will allow the access to elite athletes and the state of art current sports testing method (Ergospirometry, MOXY, etc.). The thoroughly assembled team of experts has an extremely promising potential to solve the proposed project, while without their joint effort it would be impossible to find an appropriate solution. There is a significant potential for keeping the initiated collaborations fully working even after finishing the project.

Předpokládané výstupy projektu

As demonstrated by the extensive publication and citation performance of the PI and the co-applicants, their scientific record warrants the extensive publication output from the proposed project. For instance, relevant to the research topic of non-invasive sampling, the PI has published in last 3 years 6 papers in the Q1 journals (Analytical Chemistry) such as J. Chromatogr. A (IF 3.98) , Talanta (IF 4.16), Anal. Chim Acta (IF 4.95). **MARTIN, VIKTOR – máte nějaké publikace k doplnění – i obecně jen časopis-** The results will be published in similar impacted, prestigious international analytical journals (e.g. Analytical Chemistry, Anal. Chim. Acta, J. Chromatogr. A, Anal. Bioanal. Chem., The Analyst as well as in the international high impact sports journals, such as Journal of Sports Science and Medicine, Journal of Sport and Health Science, International Journal of Sports Medicine, International Journal of Sports Physiology and Performance. We anticipate to publish at least **5 research papers** **STAČÍ ?** in the high IF journals. The results will be also presented on international symposia in the forms of lectures and posters. As the topic of athlete training optimization is very relevant, this area of research has a good publication potential.

Komentář k rozpočtu

I. VS Františka Foreta, CEITEC MU (579 tis./rok 2018, 647 tis./rok 2019, 640 tis./rok 2020).

Mzdy 296.5 tis./1. rok, další roky 346 tis./rok. Hlavní řešitel- Doc. Kubáň 10% úvazek (54.5 tis/rok v roce 2018 - koordinace odběrů plánování experimentů, příprava publikací) , Dr. Greguš 40% úvazek (128 tis/rok v roce 2018 - odběry vzorků tělních tekutin, příprava odběrných zařízení, optimalizace metodik stanovení, analýzy pomocí kapilární elektroforézy, spektrofotometrie). Studenti doktorského studia DPP– Mgr. Lačná, Mgr. Ďurč,– (celkem 50 tis./rok (odběry vzorků tělních tekutin, zpracování dat), Odvody + FKSP (35% mzdových výdajů) (64 tis./rok v roce 2018, další roky 77 tis. /rok). Cestovné: 30 tis./rok – účast na konferencích s tematikou separačních metod a vzorkování. DHM/DNM: 60 tis./rok v roce 2018, 80 tis. další roky. Zakoupení nebo upgrade nezbytného vybavení a spotřebního materiálu pro vývoj vzorkovacího zařízení pro neinvazivní vzorkování potu, EBC a slin (35 tis./rok), elektronické součásti (25 tis/rok). Kancelářský a ostatní spotřební materiál: (70 tis./rok 2018, další roky 60 tis.) - chemikálie a standardy (30 tis/ rok 2018), spotřební materiál a náhradní díly (30 tis./rok 2018), laboratorní pomůcky (10 tis./rok 2018). Služby: 5 tis./rok, servis stávajícího zařízení využívaného k projektu (CE), konferenční poplatky, poštovné, kopírovací a reprodukční práce. Režie 20,22%

II. PřF MU: Laboratoř atomové spektrochemie a Laboratoř separačních metod, obě součásti Ústavu chemie: 319 tis./rok 2018, 473 tis./rok 2019, 436 tis./rok 2020.

Mzdy 120 tis./2018, 144 tis./2019 a také 2020. Řešitel za PřF prof. Kanický 5% úvazek (koordinace, konzultace, příprava publikací). Spolupracovníci: Mgr. Markéta Holá, Ph.D. odměna 30tis./2018, 36 tis./2019 a také 2020 (příprava metodiky analýzy vzorků technikou ICP-MS a ICP-OES, výzkumné experimenty v plazmové spektrometrii, interpretace výsledků, analýzy ICP-MS); Mgr. Miroslava Bittová, Ph.D., odměna 30 tis./2018, 36 tis./2019 a také 2020 (optimalizace a validace metodiky stanovení organických markerů metodou HPLC, analýzy vzorků); Mgr. Lucie Šimoníková, 10% úvazek (Příprava a rozklad vzorků, analýzy ICP-OES). Mzdové prostředky na úvazky zahrnují příslušnou tarifní a nadtarifní (výkonnostní příplatek) složku. DPP: 1 student, 30 tis./2018, 36 tis./2019 a také 2020. (Odpovídající odvody + FKSP). Cestovné v roce 2019 a 2020 vždy 30 tis. – účast na konferencích s tematikou separačních metod, případně metod atomové/hmotnostní spektrometrie. DHM: Poměrný díl nákladů na ty náhradní díly pro kapalinovou chromatografii a plazmovou spektrometrii, které nejsou považovány za spotřební materiál (použití déle než 1 rok): zmlžovač pro ICP spektrometr, kolona pro HPLC. Spotřební materiál: plyny pro plazmovou spektrometrii (ICP-OES, ICP-MS – argon, hélium, dusík), rozpouštědla pro kapalinovou chromatografii, chemikálie a standardy, drobný laboratorní materiál, spotřební materiál použitý pro servis a údržbu ICP spektrometrů a kapalinového chromatografu. Služby: konferenční poplatky, poměrný díl nájmu na zásobník kapalného argonu pro ICP, poměrný díl nákladů na servis (opravy a údržbu). Režie 18,48%.

III. FSs MU (637 tis./rok 2018, 618 tis./rok 2019, 618 tis./rok 2020).

Mzdy – odměna 5 tis./měsíc pro řešitele doc. Zvonař, dr. Cacek, prof. Novotný (doc. Zvonař – organizace měření vrcholových basketbalistů a volejbalistů, zpracování dat pro publikace, dr. Cacek – organizace měření vrcholových fotbalistů, atletů a boxerů, zpracování dat pro publikace, prof. Novotný – srovnání parametrů ze spiroergometrie a chemické analýzy koncentráту). Odvody – 35% z mezd. DPP – 5tis./měsíc pro 2 studenty Ph.D. - realizace výzkumných měření a primární zpracování dat. Cestovné 2018-2020 vždy 40 tis./rok. Spotřební materiál – 260 měření/rok x 100 Kč spotřební materiál – 26 tis./rok

(2018-2020), 3xturbínka pro spiroergometrii 3 ks a 25 tis. – 75 tis/rok 2018. Služby 2018-2020 – publikační náklady. Režie 25,72%

Navrhovatel projektu	Titul, jméno a příjmení Doc RNDr. Petr Kubáň, Ph.D. Datum: 27.10.2017 Podpis:
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Přílohy

- Životopisy navrhovatele a spoluřešitelů (max. 5 životopisů)
- Čestné prohlášení hlavního navrhovatele
- Rozpočet projektu z ISEP
- Evidenční záznam z ISEP