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Doctoral thesis abstract

The Kinetics of Cell-Free DNA in Response to External and Internal Factors

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BACKGROUND AND OBJECTIVES

To assess the inflammatory response or muscle damage after exercise, researchers commonly use numerous physiological markers such as creatine kinase (CK), interleukins, and a reduction in muscle function (Souglis et al., 2015; Kamandulis et al., 2021). However, the sensitivity of most of these markers is inadequate for detecting various physiological events such as fatigue, overtraining, muscle damage, adaptation, acute or delayed inflammation. Cell-free DNA (cfDNA) stands as a potential biomarker for assessing exercise load and ensuring training regimes are appropriately tailored to an athlete's capacity. By monitoring cfDNA, practitioners can identify individuals at risk of overtraining and implement interventions to prevent chronic fatigue and address recovery needs. cfDNA in sports science is in its infancy, yet its short half-life and high sensitivity make it an attractive biomarker (Fatouros et al., 2006; Tug et al., 2017). The general aim of the dissertation was to determine the impact of different internal and external instances, such as 3-week sprint interval training (SIT), increased rectal temperature, and muscle-damaging exercise, on cfDNA kinetics.

METHODS

Three studies were conducted. *Study 1* included nine sessions (three sessions per week over three weeks) of 4–6 cycling bouts (Wingate tests) lasting for 30 s, with 4 min recovery intervals between bouts. In *Study 2*, each participant completed exertional heating (cycling at 60% $\dot{V}O_2$max until rectal temperature (T_{re}) reached 39°C) and exogenous heating (lower-body immersion in hot water until T_{re} reached 39°C) in a randomised cross-over design. There were three groups in *Study 3*, where each group performed a different number of drop jumps (DJ) (50, 25, 10) at intervals of one every 20 s. All measurements were performed as follows:

- **Study 1 (subjects: young healthy men (n = 10), elderly healthy men (n = 9):** Blood samples for cfDNA and lactate were taken, and neuromuscular testing was done after the first and last session. Measurements were done before, post-exercise, post 1 h, and post 24 h.
- **Study 2 (subjects: young healthy men (n = 12):** Every increase in T_{re} of 0.5°C up to 39°C and down to 37.5°C, parameters were documented, including subjective sensation, cardiovascular status, T_{re}, and blood samples were collected.
- **Study 3 (subjects: young healthy men (DJ-50 group = 14, DJ-25 group = 7, DJ-10 group = 4):** Measurements (blood samples, neuromuscular testing, assessment of muscle pain) were done starting from 15 min pre-exercise until several time points to 96 h post-exercise.

RESULTS

In *Study 1*, only young participants exhibited a significant increase in $\dot{V}O_2$max after three weeks of SIT ($p = 0.0039$). The plasma cfDNA concentration increased significantly ($p < 0.001$) during exercise in each group. There was a significant decrease between the pre-exercise cfDNA values of the 1st compared with the 9th SIT session in the old group ($p = 0.043$).

In *Study 2*, the time until a certain T_{re} was similar between the modalities (no significant interaction effect was detected ($F(6, 120.93) = 1.194$, $p = 0.314$). The increase in cfDNA concentration was greater in response to exertional heating than to exogenous heating (T_{re} × heating modality, $F(6, 135.95) = 7.51$, $p < 0.001$). In both heating modalities, cfDNA level increased steadily until T_{re} reached 39°C (T_{re} effect, $p < 0.01$). Norepinephrine response differed between heating methods (heating modality effect, $p < 0.001$) and was larger during exertional heating.

In *Study 3*, plasma CK activity increased after DJ exercise (time effect: $p < 0.001$) in the DJ-50 and DJ-25 groups. cfDNA values increased acutely after 50 DJs ($p < 0.01$) and normalised 90 min post-exercise ($p > 0.05$). A significant increase in the cfDNA concentration was also noticed after 6 h ($p < 0.01$), 12 h ($p < 0.01$), 48 h ($p < 0.05$), and 72 h ($p < 0.01$) in the DJ-50 group. There was a significant increase in cfDNA values immediately post-exercise in the DJ-25 group ($p = 0.012$).

CONCLUSIONS

The present findings advance the understanding of cfDNA as a biomarker in sports medicine, particularly concerning its responsiveness to different exercise modalities and its potential applications in monitoring physiological stress and adaptation. In *Study 1*, along with a diminished amount of cfDNA in the elderly, the results report that three weeks of SIT is too short for inducing adaptation in this group. There are, however, signs of an anti-inflammatory response that occurs in the elderly, but future studies are needed to determine these insights. Results from *Study 2* suggest that incremental T_{re} elevation via exogenous heating gradually increases plasma cfDNA concentration in young healthy men. However, cfDNA release appears to be greater following exertional heating than exogenous heating, even at similar T_{re} and subjective sensation. *Study 3* shows that exercise without an accumulation of metabolites can lead to distinct time-course changes in cfDNA levels. Additionally, cfDNA levels respond to muscle-damaging exercise without an accumulation of metabolites in a dose-dependent manner. Considering that blood markers such as CK appear elevated in the blood only after several hours or days after a single bout of muscle-damaging exercise, cfDNA may provide advantages over CK as a marker of inflammation responding to acute and secondary inflammatory processes in healthy subjects.

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