A sportomic follow-up of a muscle injury succeeded by acetaminophen hepatotoxicity

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Abstract

Introduction
The aim of this study is to communicate the diagnosis followed by a successful treatment and rapid evolution from a silent liver injury due to the use of self-medication with large doses of acetaminophen, without a stop in training or a loss of performance.

Materials and methods
We measured the VO2max and analysed a sportomic profile of four national elite cyclists and diagnosed a liver injury caused by pharmaceutical abuse in one athlete. We suggested that the injured athlete decrease both training intensity and volume by 30% to 40% while simultaneously increasing resting and sleeping time. We discontinued the use of acetaminophen and started a high intake of dietary methionine and cysteine together with N-acetyl-cysteine daily.

Results
After 2 weeks of following our regimen and dietary recommendations, the athlete reported pain relief. This change was corroborated by biochemical analysis, which showed that the amounts of creatine kinase and gamma-glutamyltransferase in blood were less than 20% and 70% of pre-treatment levels, respectively. As a referral of our treatment, the team won third place in an 800-km cycling competition.

Conclusion
This study has shown that collecting and analysing physiological data during training can give important information about an athlete’s clinical condition as well as the degree of performance. In this particular case, we have shown that 2 weeks of reduced training, combined with dietary changes, can promote liver recovery. The importance of this report is that we were able to diagnose and treat a silent liver injury and maintain an athlete’s performance during both training and competition.

Introduction
We previously proposed sportomics as an approach to mimic both the real challenges and the conditions faced during sports situations1,2. Sportomics is the use of ‘-omics’ sciences together with classic clinical laboratory analyses to understand sport-induced modifications3,4. It can also be used for observational research. Generally speaking, our study is holistic and top-down, treating data in a systematic way and generating a large amount of data with a large computational effort5.

Back pain is one of the most prevalent injuries in high-level cyclists6-8. It is a critical factor for athletes’ performance because it renders them unable to effectively train or compete. Modern management of acute lower-back pain emphasizes self-care, and the use of acetaminophen (N-acetyl-p-aminophenol (APAP)) in relieving pain is widely accepted. APAP therapy involves N-acetyl-cysteine (NAC) treatment compared to Met as a control1. This approach allows us to follow and distinguish hepatocyte damage from muscle injury caused by exercise.

APAP-induced hepatotoxicity is proposed to be caused by mitochondrial damage, and reducing agents such as N-acetyl-cysteine (NAC) can be used as an antidote to minimize hepatotoxicity and prevent liver failure and death10. In addition, carbohydrates and some amino acids intake, such as methionine (Met) or cysteine (Cys), have been proposed as a way to decrease and treat APAP poisoning11. Currently, the superiority of NAC treatment compared to Met as a way to decrease APAP-induced hepatotoxicity16 is uncertain. In fact, the use of Met combined with APAP has been proposed and defended as ‘safe’ APAP17.

Here, we describe the use of sportomics methodology combined with a wide sports data bank to diagnose, produce an intervention and follow the evolution of hepatotoxicity caused by a large APAP dose to treat back pain. This approach allowed...
us to prescribe and help an injured athlete in his recovery and return to training and competition within a 2-week time frame.

Materials and methods

Subjects

Four national cycling elite men athletes participated in this case, following the same training and nutritional program in the previous 8 months and with similar performances based on ergospirometry (Figure 1).

This study was conducted according to the guidelines dictated by the Declaration of Helsinki. All of the procedures involving human subjects were approved by the Ethics Committee for Human Research at the Federal University of the State of Rio de Janeiro (117/2007, renewed in 2011) and met the requirements regulating research on human subjects (Health National Council, Brazil, 1996). The nature of the study and the procedures involved were described to all of the subjects, and written informed consent was obtained from all of the subjects.

Methodology

We performed a sportomic evaluation of the athletes, and their VO_{2max} \textsuperscript{18} and haematological, biochemical and enzymatic profiles were evaluated twice, with an interval of 15 days between the first and the second evaluation.

Data analysis

The data collected were analysed and compared for identifying the differences between injured and healthy athletes, which were expressed as mean with standard deviation.

Intervention

We suggested that the athlete decrease both training intensity and volume by 30% to 40% from the current levels and increase resting and sleeping time. We suspended the use of APAP and included a dietary plan that called for high carbohydrates (≥6 grams of carbohydrates kg\textsuperscript{-1} • day\textsuperscript{-1}) and high protein (≥2 grams of proteins kg\textsuperscript{-1} • day\textsuperscript{-1}). We recommended an increased intake of dietary Met and Cys and a daily dose of 3,600 to 4,200 mg of NAC.

Results

No difference could be found in the athlete’s VO_{2max} compared with the team’s average (Figure 1).

The athlete’s creatine kinase (CK), lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels were higher than the average value of the three teammates (375%, 25% and 140%, respectively). In addition to the muscle injury markers, we measured an increase in AST (35%), γGT (185%) and 350% in total bilirubin level in the injured athlete. We did not observe an increase in the levels of alkaline phosphatase (ALP) and ALT in our study subjects compared to their teammates. An analysis revealed that both direct and indirect bilirubin increased in the injured athlete. Indirect bilirubin increased almost 4.6-fold when compared to direct bilirubin (direct:indirect ratio = 1:4; Table 1).

After 2 weeks of following our intervention, we re-evaluated the injured athlete and teammates using the same sportomics protocol. The amounts of CK and γGT in blood were less than 20% and 70% of pre-treatment levels, respectively. We did not detect a change in the level of total bilirubin in blood; the injured athlete’s bilirubin level following treatment was still higher than the average value of his teammates. Nevertheless, we did detect an improvement in bilirubin conjugation by performing both direct and indirect bilirubin measurements (direct:indirect ratio = 1:1; Table 1).

Discussion

Here, we report the use of a sportomic analysis as a personalized medicine approach for a team of four athletes during their preparation for an 800-km non-stop race. During the first sportomic analysis, one athlete showed unexpected levels of muscle injury biomarkers and presented with much higher levels of inflammation markers than his teammates.

After a careful anamnesis, we found that the injured athlete had...
Table 1 Muscle and liver injury markers measured before and after treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Injured athlete (First analysis)</th>
<th>Healthy athletes</th>
<th>Injured athlete (Second analysis)</th>
<th>Healthy athletes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (U/L)</td>
<td>868.0 ± 182.7</td>
<td>190.3 ± 20.8</td>
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<tr>
<td>LDH (U/L)</td>
<td>383.0 ± 308.0</td>
<td>296.3 ± 53.0</td>
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<tr>
<td>AST (U/L)</td>
<td>57.3 ± 42.0</td>
<td>46.9 ± 11.4</td>
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<tr>
<td>ALT (U/L)</td>
<td>27.7 ± 25.3</td>
<td>35.2 ± 14.4</td>
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<tr>
<td>γGT (U/L)</td>
<td>47.1 ± 16.6</td>
<td>15.3 ± 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>54.0 ± 75.3</td>
<td>81.3 ± 16.8</td>
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<tr>
<td>CRP (mnol/L)</td>
<td>62.5 ± 25.9</td>
<td>12.7 ± 5.1</td>
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<tr>
<td>Total bilirubin (umol/L)</td>
<td>44.3 ± 9.7</td>
<td>18.1 ± 2.3</td>
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<tr>
<td>Direct bilirubin (umol/L)</td>
<td>9.6 ± 4.4</td>
<td>13.7 ± 4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect bilirubin (umol/L)</td>
<td>34.7 ± 5.3</td>
<td>4.3 ± 2.5</td>
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</tr>
</tbody>
</table>

Liver and muscle injury parameters were measured before and after team counselling and athlete treatment. Data for healthy athletes are presented as the average ± SD. CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; ALP, alkaline phosphatase; CRP, C-reactive protein.

experienced back pain in the 96 hours prior to the first evaluation. He attributed this pain to a previous training session during which a bike drift occurred, followed by the onset of severe lower-back pain. We suspected that muscle injury was the principal cause of high CK values. Consistent with this hypothesis, the athlete had higher levels of classical injury markers CK and LDH than his teammates, and these increases are consistent with our recent report of CK and LDH kinetics appearance in blood. Our hypothesis of muscle injury is further supported by the elevated CRP value, which indicates an acute-phase inflammatory response.

It has been proposed that endurance training can cause chronic liver injury. Given the high CK values in this athlete, we initially thought that he might be suffering from overtraining syndrome (OTS). However, analysis of the athlete’s VO2max test showed him as performing at a level that is similar to the performance level of his teammates. Because the VO2max test is widely accepted as the gold standard for OTS diagnosis, we excluded this possibility.

Following our sportomics approach, we re-interviewed the athlete and discovered that he started a large daily dose of APAP to reduce back pain. The athlete indicated that he has been using 7,000 to 9,000 mg of APAP per day to relieve muscle soreness. Different studies have linked excessive doses of APAP to hepatotoxicity. We have previously shown that it is possible to separate muscle and liver injuries using ALP and γGT as hepatocyte integrity markers. In this study, the athlete had a γGT level nearly twice as high as his teammates while following the same training schedule, and his AST level showed a slightly smaller increase. These findings support the hypothesis of a liver injury. After ruling out diseases such as hepatitis and cirrhosis, we hypothesized that the athlete had suffered a muscle injury, after which his use of APAP resulted in hepatocyte toxicity.

Muscle injury diagnosis can be made through the variations of enzyme levels detected in blood, especially CK and LDH. However, ALT and ALP elevation are also observed in non-liver-injury conditions and in apparently healthy athletes. γGT is an abundant enzyme in the liver, and abnormal γGT levels can be used to diagnose various diseases. In exercise biochemistry, γGT is used as a specific marker of liver injury to differentiate the location of damage.

Although we cannot ignore the reported influence of endurance training on chronic liver injury, we believe that the hepatotoxic effect of APAP was responsible in this case. Therefore, we concluded that the athlete was suffering from acute back pain caused by muscle injury and that subsequent APAP overdose resulted in hepatotoxicity exacerbated by exercise.

On the basis of these analyses, we suggested that the athlete decrease both training intensity and volume by 30% to 40% of current levels and increase resting and sleeping time. We suspended the use of APAP and included a dietary plan that included high amounts of carbohydrates (26 grams of carbohydrates kg⁻¹ • day⁻¹) and high amounts of protein (22 grams of proteins kg⁻¹ • day⁻¹).

NAC is a popular antioxidant because of its ability to minimize oxidative stress. The mechanism of NAC involves the synthesis of hepatic glutathione, preventing oxidative damage. NAC and other antioxidants have been used as APAP antidotes. To reinforce dietary protection, we recommended a high intake of dietary Met, Cys and a daily dose of 3,600 to 4,200 mg of NAC, an APAP antidote widely used in clinical treatment.

After 15 days of following dietary and training recommendations, a second evaluation was conducted. CK and CRP levels in the athlete had fallen within the range observed in his teammates. The effectiveness of
treatment was corroborated by biochemical analysis, which showed that the amounts of CK and γGT in blood were less than 20% and 70% of pre-treatment levels, respectively. The level of γGT had decreased by 38% from the level measured at the first evaluation, but it was still higher than the levels of his teammates. We did not, however, detect a change in the level of total bilirubin in blood; the injured athlete’s level following treatment was still higher than the average value of his teammates. Nevertheless, we did detect an improvement in bilirubin conjugation by performing both direct and indirect bilirubin measurements (Table 1). These results suggest that our diagnosis was correct and that the intervention that we developed may be effective in treating mild APAP toxicity in athletes. After 2 weeks of following our training and dietary recommendations, the athlete reported that the pain had been relieved. Moreover, the team was able to perform the race in 23 hours and won third place.

Conclusion
This study has shown that collecting physiological data during training can provide important information about an athlete’s clinical condition as well as the degree of OTS and performance. Here, we describe the use of sportomics to diagnose, produce an approach allowed us to prescribe and help an injured athlete in his recovery and return to training and competition within a 2-week time frame.

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References
23. Silva LA, Silveira PC, Pinho CA, Tuon T, Dal Fizol F, Pinho RA. N-acetylcysteine supplementation and oxidative damage