Simultaneous analysis of neopterin, kynurenine and tryptophan by amine-HPLC shows minor oxidative stress from short-term exhaustion exercise

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Abstract: Introduction: Neopterin, kynurenine and tryptophan can be used to measure activation of monocytes and macrophages during immunological events such as exercise inducing inflammation. Endurance exercise and high-impact sports have shown significant increases in these biomarkers. Measurement is typically conducted by high-performance liquid chromatography (HPLC) using C18 or SCX columns. However, kynurenine and tryptophan are not measured simultaneously to neopterin using these separation systems. Here we have used an amine column for separation and simultaneous determination of neopterin, kynurenine and tryptophan.

Methods: Optimization and validation for the amine-HPLC method was conducted using plasma from 43 participants subjected to a short maximal exercise bicycling regime or rest period. The order of exercise and rest was randomized and separated by a 3.5 week washout period.

Results: Using an amine column developed with ammonium acetate formic acid (33%) and acetonitrile (72%) provided optimal separation and run time for analysis. Neopterin increased significantly post-exercise and subsided to baseline by 30 minutes. Total neopterin remained elevated until 60 minutes following exercise.

Conclusion: Amine-HPLC can be used for simultaneous determination of kynurenine, tryptophan and neopterin in plasma. Short intense exercise causes a significant increase in plasma neopterin suggesting a prolonged activation of monocytes and macrophages.

Keywords: Neopterin; biomarker analysis; high-performance liquid chromatography; indoleamine 2,3-dioxygenase; immune activation.

Introduction

Clinical and experimental research is often limited by the inability to quantify compounds of interest in a convenient, simple and cost-effective manner. Neopterin, kynurenine and tryptophan are frequently used as plasma biomarkers of immune system activation in clinical research. These compounds have been analyzed in plasma, serum and urine as biomarkers of oxidative stress and inflammation, with measurements traditionally being made using HPLC with a C18 or SCX column utilizing reverse phase or ion exchange mechanisms, respectively, and fluorescence detection [1–4]. However, these methods have not been suitable for simultaneous detection of all three biomarkers.

Neopterin is a product of oxidative stress and inflammation, which has led to it being studied in many fields of research, including exercise [5, 6], atherosclerosis [7] and surgery [8], amongst others. Neopterin is an oxidation product of its parent compound, 7,8-dihydroneopterin, which is a monocyte and macrophage synthesized antioxidant, produced from guanosine triphosphate metabolism (GTP) by GTP cyclohydrolase-I (GTPCH-I) upon stimulation by interferon-γ (IFN-γ) [9, 10]. As 7,8-dihydroneopterin does not have strong absorbance or fluorescent characteristics, it is typically oxidized to neopterin for detection. The neopterin measured following chemical oxidation of
a sample is termed “total neopterin” and represents neopterin plus 7,8-dihydroneopterin [11, 12].

Kynurenine is a product of tryptophan metabolism by indoleamine 2,3-dioxygenase (IDO), which is found on human dendritic cells and represents the rate limiting and regulatory step of the kynurenine pathway. Like GTPCH-I, IDO is activated by IFN-γ, as well as, tumour necrosis factor-α (TNF-α). IDO activation is associated with increased inflammation and disease progression [13, 14]. Moreover, plasma or serum kynurenine/tryptophan ratios alongside neopterin concentrations are often used to monitor, or make diagnostic predictions, of many illnesses which involve an inflammatory component [15 - 17]. Reverse phase-HPLC can be used for detection of either neopterin [18], or kynurenine and tryptophan together [19]. However, combined in a single HPLC method, these three compounds would provide a simple and practical quantitative analysis for oxidative stress and immune system activation.

Alternatively to plasma or serum measurements, HPLC coupled with a strong cation (SCX) exchange column has been used and validated for measurement of urinary neopterin and total neopterin in high-impact and physically demanding exercise studies. The results indicated that moderate to high levels of physical activity produce a significant increase in urinary neopterin and total neopterin [5, 6]. SCX-HPLC has proven to be very useful for urinary analysis [6], with plasma samples there can be problems with fully resolving the compounds of interest, especially at the low concentrations observed. Recently, an amine column method has been developed for the determination of multiple pteridines in urine using tandem mass spectrometry [20]. While MS/MS is the ideal analytical tool for compound determination due to low detection limits, it adds considerable cost and complexity compared to fluorescence detection.

In this work we aimed to modify the amine-HPLC method presented by Allegri et. al. [20] for use in determination of plasma neopterin, tryptophan and kynurenine by fluorescence and absorption spectroscopy. This method employs a hydrophilic interaction chromatography separation mechanism (HILIC) with an amine column stationary phase. We aimed to develop and validate the method using a standardized clinical exercise test which investigated low levels of exercise and trauma. We hypothesize that these participants will experience a minor and measureable burst of oxidative stress and inflammation, as determined by changes in plasma neopterin, total neopterin, kynurenine and tryptophan concentrations. Urinary neopterin and total neopterin is also analyzed for comparison and contrast against high intensity high impact exercise.

Methods

Study design and population

Healthy adults, aged 18-30 years, were recruited to participate in a randomized, controlled, cross-over trial via advertising around Dunedin, and the University of Otago campus, and by social media. Exclusion criteria included: habitual smokers, those who are pregnant or lactating, amenorrhea or oligomenorrhea, anaemia, contraindications to exercise, diagnosis of major illnesses and use of prescription drugs (including oral contraceptive agents). A screening questionnaire was used to determine eligibility and collect anthropometric and lifestyle information, such as demographic data, smoking habits, alcohol intake, medical history, intake of any prescription medications, contraindications to exercise, and nutritional supplement use. Females were asked additional questions regarding pregnancy status and menstruation.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all relevant national regulation, institutional polices and in accordance the tenets of the Helsinki Declaration. The Human Ethics Committee (Health) of the University of Otago approved the study protocol (H16/117), and the trial was registered at www.anzctr.org.au (ACTRN12616001466437).

Exercise protocol

Enrolled participants (n = 43, 20 females and 23 males) completed two intervention sessions (exercise session and control session). The order of the two sessions was randomised using a computer-generated random-number sequence. Participants and researchers could not foresee assignment of the next participant as the randomisation and associated codes were held by an investigator who was not directly involved with participant recruitment and enrolment. The intervention sessions were separated by a 3 - 5 week wash out period.

For both exercise and control sessions, participants arrived into the clinic after an overnight fast of 10 hours. Participants were advised to abstain from exercise and alcohol intake for 24 hours prior to the intervention session. In the exercise session, participants completed a maximal exercise test using a continuous progression protocol on an ergometer bicycle (Duam Electronic,
Fürth, Bavaria, Germany). During the exercise test, heart rate was monitored by a Polar H7 heart rate sensor (Polar Electro, Kempele, Finland) and gas exchange was measured using a MetaLyzer 3B (Cortex Biophysik GmbH, Leipzig, Germany). Participants began the exercise test with a resistance of 100 W for males or 75 W for females; the resistance was increased by 50 W every 2.5 min until heart rate of 160 bpm, following which the resistance was increased by 25 W until voluntary exhaustion. During the control session, the participant remained seated during their clinic visit.

**Blood collection and preparation**

At the beginning of the intervention session, a cannula was inserted into an antecubital vein. A baseline blood sample was taken at least 5 minutes following the insertion of the cannula. The participants then completed either the maximal exercise test, or remained sedentary for 10 min, prior to the second blood collection. Three further blood collections were taken at 30, 60 and 120 min following the end of the exercise or 10 min resting period. Trace element tubes (K\textsubscript{2}EDTA; Becton-Dickinson, Franklin Lakes, NJ) were used for the analysis of neopterin, 7,8-dihydroneopterin, kynurenine and tryptophan. Within two hours of blood collection, plasma was separated from whole blood after centrifugation at 4°C for 10 min at 1200 \( \times \) g, and stored at –80°C until analysis.

**Plasma sample preparation and analysis**

Plasma samples were defrosted in the dark at room temperature. 200 µL of sample was vortexed with 200 µL of ice cold acetonitrile for protein precipitation before being centrifuged at 21,000 g for 5 minutes at 4°C. 100 µL of the supernatant was aliquoted into HPLC vials for simultaneous analysis of neopterin, kynurenine and tryptophan. 60 µL of acidic iodide was vortexed with the remaining 300 µL of plasma solution before being incubated in the dark at room temperature for 15 minutes to oxidize the 7,8-dihydroneopterin into neopterin. 30 µL of 0.6 M ascorbic acid (made fresh for each preparation) was added to the solution, followed by vortexing, to quench any remaining acidic iodide before being centrifuged at 21,000 g for 5 minutes. 100 µL of this solution was transferred into HPLC vials for total neopterin analysis.

Measurements of neopterin kynurenine, tryptophan and total neopterin were made using a Shimadzu 20A HPLC with a LD20A pump, CTO-20A column oven, Sil-20A autosampler, RF-10A\textsubscript{FR} fluorescence detector and Nexera X2 SPD-M30A photo diode array absorbance detector. Separation of the compounds was achieved using a HILIC separation mechanism facilitated by a Luna 5 µm NH\textsubscript{2} 100 Å 250 x 4.6 mm column stationary phase. The mobile phase was run isocratically at 1 ml/min and included 28 % 10 mM ammonium acetate with 0.3% (v/v) formic acid mixed pre-pump with 72 % acetonitrile. Tryptophan was quantified by fluorescence detection at 365 nm emission and 285 nm excitation, before switching wavelengths 7.5 minutes into the run to quantify the neopterin elution using 438 nm and 353 for emission and excitation, respectively. Kynurenine was quantified by absorbance 360 nm. Cation exchange using a SCX column was developed with a mobile phase of 20 mM ammonium phosphate pH 2.5 mixed pre-pump with 10% acetonitrile at 1 mL/min. The preparation of the plasma samples was identical for both HPLC methods. Peak analysis and result quantification was conducted using Shimadzu Lab Solutions version 5.86.

**Urine sample preparation and analysis**

Analysis of urinary neopterin was conducted cation exchange chromatograph using a SCX column [6]. Urine samples were defrosted in the dark at room temperature. 5 µL of urine was vortexed with 195 µL of 20 mM ammonium phosphate (pH 2.5) before 100 µL was transferred into HPLC vials for neopterin analysis. For analysis of total neopterin, 20 µL of acidic iodide was then added to the remaining 100 µL of diluted sample, before being vortexed and left in the dark at room temperature. After 15 minutes, 10 µL of 0.6 M ascorbic acid was added, followed by vortexing. 100 µL of this solution was then transferred into HPLC vials for total neopterin analysis. Compound separation was achieved with a Luna 5 µm SCX 100 Å 250 mm \( \times \) 4.6 mm column, using 20 mM ammonium phosphate pH 2.5 as a mobile phase being pumped at 1.0 ml min\(^{-1}\).

Specific gravity (SG) was measured using an ATAGO N-20 refractometer, and calculated, using the formula described below, which is based on the normal population with a SG\(_{1.020}\) [21]. Before measurement, samples were brought to room temperature to remove and temperature-dependent density variation bias [22].

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\text{[neopterin]}(\text{nM/SG}_{1.020}) = \frac{(\text{SG}_{1.020} - 1)}{(\text{SG}_{sample} - 1)} \times \text{[neopterin]}(\text{nM})
\]
Statistical analysis

Descriptive data were expressed as mean ± SD, and outcome measures were described as mean ± 95% confidence intervals (CI). Differences between baseline values of intervention sessions were analysed using paired t-tests. Period, sequence and carryover effects were investigated by paired or independent t-tests. Change in plasma neopterin, tryptophan and kynurenine concentrations were calculated by subtracting values at subsequent time points from baseline values. Differences of change between sessions for each participant were calculated by subtracting change values in the exercise session from change values in the control session. The outcomes, differences of the changes in plasma neopterin, tryptophan and kynurenine, were assessed by repeated-measures ANOVA for complete sets of data. Normality, sphericity and residuals were checked for compliance with model assumptions. Greenhouse-Geisser corrects were used when Mauchly’s sphericity test returned p<0.05.

Results

Amine-HPLC separation of neopterin, tryptophan and kynurenine

Ammonium acetate concentrations of 10 mM plus 0.3% formic acid was found to give optimal separation and retention of the compounds of interest. Methanol was found to give poor retention of the compounds on the amine column so varying concentrations of acetonitrile were trialed. Increasing or decreasing acetonitrile concentration from 72% would increase or decrease the neopterin retention time by approximately 0.5 minutes per 1% acetonitrile change. This effect was less dramatic with tryptophan and kynurenine, with peaks retention time being moved by approximately 0.3 minutes per 1% acetonitrile. The optimal mobile phase conditions for the separation and detection of neopterin, tryptophan and kynurenine in a single HPLC run were determined to be 28% 10 mM ammonium acetate with 0.3% formic acid and 72% acetonitrile. Standard curves produced are linear response (Fig. 1) with R² of 0.9999, 0.9996 and 0.9993 for neopterin, tryptophan and kynurenine, respectively. These standard curves were produced using 3 different amine column with less than 5% variation in peak area. The separation time between tryptophan (6.5 minutes) and neopterin (8.8 minutes) allows for the fluorescence detector to switch excitation and emission wavelengths at 75 minutes from 285 nm and 365 nm, respectively, for tryptophan, to 353 nm and 438 nm for neopterin. These wavelengths were chosen based on what has previously been reported as optimal [11, 19].

SCX-HPLC determination of neopterin and total neopterin in human plasma produced peaks that have considerable co-elution with other compounds (Fig. 2 A and B). With the developed amine-HPLC method, good separation of neopterin and total neopterin from other compounds in plasma samples was achieved (Fig. 2 B and C), while also allowing determination of tryptophan and kynurenine without co-elution (Fig. 2 E and F).

Stability of 7,8-dihydroneopterin in human plasma

Stability of 7,8-dihydroneopterin and neopterin in plasma was tested using 5 random samples from participants in the study. There was a significant (p<0.001) reduction in total neopterin (Fig. 3 A) by 6 hours at all temperatures assessed (4, 21 and 37°C), which continued at 12 hours. The largest degradation was at 37°C, with a total neopterin reduction of 83.2% after 12 hours. Neopterin (Fig. 3 B) did not change significantly at any of the assessed temperatures over the 12 hours.

Amine-HPLC determination of neopterin, total neopterin, tryptophan and kynurenine from low demand exercise

Of the study cohort, mean age was 22 ± 3.4 years. Rate of oxygen consumption (VO₂) peaked during exercise at 2.48 ± 0.87 L/min (36.3 ± 9.58 mL/min/kg), with an average heart rate peak of 176.0 ± 10.4 bpm and peak work rate of 217.9 ± 82.5 W.

The effectiveness of the amine-HPLC method to measure plasma neopterin, total neopterin, tryptophan and kynurenine was accessed in a clinical study of low demand exercise. Plasma neopterin concentrations (Fig. 4 A) from the exercise group were elevated compared to the control group immediately post-exercise by 1.4 nM with 95% CI (p<0.05) before returning to baseline levels by 60 minutes following exercise cessation. Plasma total neopterin concentrations (Fig. 4 B) were elevated, compared to control, post-exercise (5.8 nM with 95% CI, p<0.05) and 30 minutes (3.5 nM with 95% CI, p<0.05)
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Figure 1: Chromatograms and standard curves of neopterin (A and B), tryptophan (C and D) and kynurenine (E and F). Retention times are 8.8 and 6.5 minutes for neopterin (100 nM) and tryptophan (5 µM), respectively and are measured by fluorescence. Kynurenine (5 µM) elutes at 6.1 minutes and is measured by absorbance (360 nm). All standard curves are in triplicate produced over 3 separate amine columns and demonstrate linear curves with high R² values. Standard curve data is presented as mean with SD.
Figure 2: Chromatograms of neopterin (A) and total neopterin (B) measured by SCX-HPLC. Chromatograms of neopterin (C), total neopterin (D), tryptophan (E) and kynurenine (F) measured by amine-HPLC. SCX-HPLC fails to adequately separate neopterin from other compounds for both neopterin and total neopterin samples. Amine-SCX can separate neopterin and total neopterin samples from all other peaks. The tryptophan peak is considerably larger than neopterin. Kynurenine elutes separately to other peaks.
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Following exercise (p<0.05). Detection limits for neopterin and total neopterin were approximately 1 nM in plasma samples with a standard 10 µl injection volume. Total neopterin over neopterin (TNP/NP) ratios were calculated to show the oxidative stress and 7,8-dihydroneopterin production dynamics, and is displayed in Fig. 4 C. The control TNP/NP ratio remains relatively constant over time, whereas, there was a trend towards higher TNP/NP ratios at 30-120 minutes following exercise.

Plasma levels of tryptophan and kynurenine (Fig. 5 A and B) were not significantly altered by the exercise protocol. Plasma kynurenine over tryptophan (Kyn/Tryp) ratios were calculated (Fig. 5 C) but did not demonstrate an observable or significant change. Using this method, detections limits for kynurenine and tryptophan in were 1 µM and 0.2 µM, respectively, with a standard 10 µl injection volume.

**Urinary neopterin and total neopterin levels**

SCX-HPLC was employed to quantify the change in urinary neopterin and total neopterin levels with concentration standardization by specific gravity. The results (Fig. 6) showed no change in urinary neopterin or total neopterin levels between the exercise and control protocols from samples pooled over 24 hours post-exercise.
Figure 4. Plasma neopterin (A) and total neopterin (B) were measured using amine-HPLC and subtracted from baseline values. Total neopterin over neopterin (TNP/NP) ratio (C) was calculated. Exercise neopterin is elevated over the control group post-exercise. Total neopterin is elevated over control at post and 30 minutes. Both biomarkers increase following exercise. The TNP/NP ratio is not statistically significant but is higher for the exercise group at 30, 60 and 120 minutes after exercise. Results are presented as means and 95% CI. *p<0.05.

Figure 5: Plasma kynurenine (A) and tryptophan (B) were measured using amine-HPLC and subtracted from baseline values. Kynurenine over tryptophan (Kyn/Tryp) ratio (C) was calculated from unaltered plasma values. There is a small, non-statistically significant, increase in exercise plasma kynurenine versus control at 30 minutes post exercise. Tryptophan decreases for both groups over the study period. The Kyn/Tryp ratio increases for both groups. Data is presented as means with 95% CI.
Discussion

The presented data show that amine-HPLC allows for determination of low levels of plasma neopterin and total neopterin, while simultaneously measuring kynurenine and tryptophan using fluorescence and absorption spectroscopy. With this HILIC separation method we have shown that short-term maximal exhaustion exercise generates an increase in plasma neopterin and total neopterin concentrations, while having a minimal effect on kynurenine and tryptophan levels. This exercise regimen did not increase urinary levels of neopterin and total neopterin over the control.

With the inclusion of 72% acetonitrile in the mobile phase, the amine column separates neopterin and tryptophan (fluorescence detection) by 2.3 minutes (Fig. 1). This allows time to change emission and excitation wavelengths between the two compounds, thus allowing their detection at maximum fluorescence potentials. The standard curves and data from repeatedly injecting standards throughout each run of clinical samples demonstrated a low degree of intra- and inter-day variation, as well as was very limited variation between identical columns and a low margin of error (5%). Small changes in the mobile phase acetonitrile concentration had significant impacts on the elution time for neopterin which is beneficial when peaks are co-eluting. Importantly, the problems with peak shape and co-elusion found with the SCX method were ameliorated with the use of the amine column (Fig. 2). There was minimal carry over from previous samples, as determined by injections with a 30 minute run time. This allowed optimization of an 11 minute run time with carryover being negligible past the breakthrough point of the next sample. Moreover, percentage recovery (Table 1) indicates that there is no significant loss of any analyte during separation or detection while using an amine column. Detection limits for neopterin, total neopterin, kynurenine and tryptophan were lower than typical concentrations found in plasma samples. In the cases where accurate quantification of neopterin or total neopterin could not be obtained due to a low signal to noise ratio (<2.5), a larger injection volume was used with the calculations being altered accordingly. The ability to easily manipulate injection volume with HPLC greatly assists in these participants with naturally lower neopterin levels.

The presented method allows for simultaneous detection and quantification of two independent biochemical processes which represent immune cell activation; IDO [23] (kynurenine and tryptophan) and GTPCH-I [24] (neopterin and total neopterin). As our method was based off an HPLC-MS/MS method [20], we believe that with minimal, if any, modification and the use of MS/MS detection the presented amine-HPLC method may be able to detect 7,8-dihydroneopterin, which eluted 1.5 minutes after neopterin when tested using standards (data not shown), thus removing the need for an additional sample preparation and additional analysis for total neopterin. HILIC chromatography has also recently been shown to be able to separate many of the metabolites of the kynurenine pathway so enabling the simultaneous detection of tryptophan, kynurenine, kynurenic acid and quinolinic acid in human plasma [25].

7,8-Dihydroneopterin is known for being labile, forming 7,8-dihydroxanthopterin over time in air-equilibrated solutions [26]. We investigated the stability of both 7,8-dihydroneopterin and neopterin in human plasma samples (Fig. 3). 7,8-Dihydroneopterin was oxidized for measurement as total neopterin and shows a significant decrease over 12 hours, with the largest loss being at 37°C. The rate of loss slows from 6 to 12 hours which may be a result of measuring 7,8-dihydroneopterin as total neopterin. Given that the neopterin is not degraded under the same conditions (Fig. 3 B) and total neopterin contains not only oxidized 7,8-dihydroneopterin but also native neopterin, there will always be an amount of neopterin present from time zero which is not lost over time. Thus, the percent of 7,8-dihydroneopterin lost will not be reduced below the amount of neopterin already present in the sample, causing a plateauing effect. Nevertheless, these...
results indicate the importance of freezing plasma samples as soon as possible when 7,8-dihydrolepineopterin is to be analysed. Lastly, the lack of change in neopterin indicates that the lost 7,8-dihydrolepineopterin is oxidized into non-neopterin product(s), possibly 7,8-dihydroxanthopterin [26].

We used the present analytical process to examine potential changes in plasma biomarkers from participants in a short-term exhaustion exercise study. Prolonged exercise has been shown to increase neopterin [3, 5], total neopterin [27] and kynurenine [28] concentrations in plasma and urine [29]. In this study, plasma concentrations of neopterin, total neopterin, kynurenine and tryptophan at baseline and over a 2 hour time period following a short-term exercise exhaustion test were determined. The results show a statistically significant increase in neopterin over basal levels immediately following exercise, which returns almost to baseline after 30 minutes (Fig. 4). This indicates the exercise inflicted on the participants caused a transient oxidative burst. Total neopterin levels were also increased following exercise and remain significantly elevated until 60 minutes post exercise. The prolonged increase in total neopterin suggests that 7,8-dihydrolepineopterin is continuing to be generated after the event, which has been seen in other studies [6]. This is further supported by the total neopterin over neopterin (TNP/NP) ratio which gives an indication of monocyte and macrophage activation (total neopterin) versus oxidative stress (neopterin). The TNP/NP ratio remains elevated for the duration of the post-exercise samples and suggests that GTPCH-I activation continued for at least two hours after the initial oxidative burst. Moreover, immediately post-exercise there is a decrease in the TNP/NP ratio, demonstrating an increased level of neopterin with a decrease in 7,8-dihydrolepineopterin. This may be the result of a large portion of the cellular pool of 7,8-dihydrolepineopterin being consumed by an intense oxidative burst, caused by the exercise regime, faster than the GTPCH-I can be activated to replenish it, though this is highly speculative and requires further investigation.

Measurement of kynurenine and tryptophan (Fig. 5) did not produce statistically significant changes in their plasma concentrations following the current exercise protocol. Both groups presented with a decrease in plasma tryptophan over the measurement period, which may be due to minor IDO activation or natural circadian rhythmic variations seen throughout the day [30]. The kynurenine/tryptophan ratio increases immediately after exercise, suggesting minor activation of IDO, however, the lack of clear and statistical differences between the groups leave the results inconclusive. One study that found significant, but small changes in tryptophan (12% decrease) and kynurenine (6% increase) following exhaustion exercise testing, however, in contrast to the presented study, the protocol from the former study involved longer exercise times and trained athletes [31]. Combined, the aforementioned results suggest that plasma neopterin and total neopterin are sensitive biomarkers for short intense bursts of oxidative stress and inflammation, implying that GTPCH-I may be more readily induced by lower stress conditions than IDO.

Unlike high-impact and prolonged endurance exercise, which show large changes in urinary neopterin and total neopterin concentrations [2, 32], this exercise protocol showed no change in urinary neopterin levels from a 24 hour cumulative sample (Fig. 6). Macrophages are a key cell type involved in tissue destruction and remodeling following injury [33], which results in high levels of neopterin following extensive high-impact exercise and tissue damage [34]. As urinary 7,8-dihydrolepineopterin can be unstable over a long time period, it may make small changes in cumulative samples difficult to accurately analyse with the 7,8-dihydrolepineopterin having broken down [8]. It is also likely that the small increases seen in plasma neopterin and total neopterin from the exercise event were lost against the basal production of neopterin and 7,8-dihydrolepineopterin when measured in urine over 24 hours.

Conclusion

The presented method of amine-HPLC separation with fluorescence and absorption detection allows for simultaneous measurement of plasma neopterin, kynurenine and tryptophan. The method has been used to measure low levels of oxidative stress and inflammation which were not otherwise observable using urinary neopterin and total neopterin analysis. Short term exhaustion exercise causes a rapid and transient oxidative burst that is followed by activation of monocytes and macrophages, as measured by plasma neopterin and 7,8-dihydrolepineopterin production. Neopterin and 7,8-dihydrolepineopterin generation proved to be more sensitive to short-term exhaustive exercise stress than kynurenine and tryptophan, suggesting that GTPCH-I activation is more susceptible to low levels of exercise than IDO activation.

Conflict of interest: Authors state no conflict of interest
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