Exercise can be pyrogenic in humans

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Exercise increases mean body temperature ($\bar{T}_{\text{body}}$) and cytokine concentrations in plasma. Cytokines facilitate prostaglandin (PG) production via cyclooxygenase (COX) enzymes, and PGE$_2$ can mediate fever. Therefore, we used a COX-2 inhibitor to test the hypothesis that PG-mediated pyrogenicity may contribute to the raised $\bar{T}_{\text{body}}$ in exercising humans.

**Methods:** In a double-blind, cross-over design, 10 males (age: 23 yr (SD 5), VO$_2$ max: 53 ml·kg$^{-1}$·min$^{-1}$ (SD 5)) consumed Rofecoxib (50 mg·d$^{-1}$; NSAID) or placebo (PLAC) for 6 d, 2 wk apart. Exercising thermoregulation was measured on day 6 during 45-min running (~75% VO$_2$ max) followed by 45-min cycling and 60-min seated recovery (28°C, 50% rh). Plasma cytokine (TNF-$\alpha$, IL-10) concentrations were measured at rest and 30-min recovery. **Results:** $\bar{T}_{\text{body}}$ was similar at rest in PLAC (35.59°C) and NSAID (35.53°C) and increased similarly during running, but became 0.33°C (SD 0.26) lower in NSAID during cycling (37.39°C versus 37.07°C; P=0.03), and remained lower throughout recovery. Sweating was initiated at $\bar{T}_{\text{body}}$ of ~35.6°C in both conditions but ceased at higher $\bar{T}_{\text{body}}$ in PLAC than NSAID during recovery (36.66°C (SD 0.36) versus 36.39°C (SD 0.27); P=0.03). Cardiac frequency averaged 6 min$^{-1}$ higher in PLAC (P<0.01), whereas exercising metabolic rate was similar (505 versus 507 W·m$^{-2}$; P=0.56). A modest increase in both cytokines across exercise was similar between conditions. **Conclusions:** COX-2 specific NSAID lowered exercising heat and cardiovascular strain and the sweating (offset) threshold, independently of heat production, indicating that PGE$_2$ mediated inflammatory processes may contribute to exercising heat strain during endurance exercise in humans.

**Keywords:** Body temperature regulation, sweating, inflammation, fever, cyclooxygenase inhibition
INTRODUCTION

Humans live with a core temperature within ~5°C of heat-induced death. Body temperature is thus tightly and powerfully regulated. This is achieved using a proportional control system in which thermoeffectors such as sweating and behavior are activated in response to thermal perturbations of cutaneous and deep tissues. Exercise is the most prevalent, intense thermal perturbation facing humans in contemporary societies; hard exercise can generate sufficient heat to elevate body temperature by 1°C in four minutes. Fever also elevates body temperature, commonly associated with a systemic immune response to trauma, infection or inflammation, possibly via cytokines acting on thermoregulatory structures such as the preoptic anterior hypothalamus (29, 30). However, exercise also causes cytokinemia and has other effects that might result in pyrogenically-mediated elevation of body temperature. We therefore investigated whether pyrogenic effects of exercise might contribute to exercise-induced hyperthermia in humans.

The notion of a fever response to exercise in humans has several lines of support. First, there is a post-exercise elevation in body temperature which is defended, and this is not fully attributable to post-exercise unloading of baroreceptors (28). Second, there are similarities in the cytokine response between exercise and fever, albeit with a smaller magnitude in exercise (39, 40). Third, endotoxemia can occur during running and triathlon events (4, 5, 25). This may be associated with an exercise-induced splanchnic vasoconstriction (42) and tissue heating (36) allowing gut bacteria derived lipopolysaccharide (LPS) endotoxins – a commonly used fever inducing agent - to leak into circulation and trigger an inflammatory response. Finally, perhaps the strongest support comes from early research by Canon and Kluger (7, 8). They showed that plasma drawn from humans after exercise produced a fever in rats, whereas pre-exercise plasma did not, indicating an exercise-induced accumulation of endogenous pyrogens in circulation (7, 8). They subsequently examined exercise effects on body temperature and
thermal behavior of a poikilotherm (iguana: (7)), and observed a post-exercise rise in preferred body temperature, which contrasted to a post-exercise drop when an anti-pyretic drug was administered before exercise.

An important pyrogen in the development and maintenance of fever is prostaglandin E₂ (PGE₂) (48), which is formed in most cells from the cyclooxygenase-mediated metabolism of arachidonic acid (33). Non-steroidal anti-inflammatory drugs (NSAIDs) exert their effects by inhibiting cyclooxygenase (COX) enzymes and thus PGE₂ production (56). The COX-2 isoform is inducible and appears to produce PGs involved in febrile and inflammatory responses (33, 57), whereas the COX-1 isoform is constitutively expressed in most cells and catalyses production of PGs involved in homeostatic control and cell maintenance. Thus, a COX-2 selective NSAID may be effective in limiting possible febrile responses to exercise while also avoiding possible inflammatory effects of NSAID-induced degradation in gastro-intestinal epithelial integrity.

Anti-pyretic agents have previously been used to examine their effects on exercising heat strain in humans (10, 24). In those studies exercising core temperature was unchanged or slightly increased, but exercise intensity was low and the anti-pyretic agent used was sodium salicylate. Sodium salicylate does not exert its action via COX inhibition; an effect that is mediated by the acetyl group of acetyl salicylate. Therefore, the purpose of this study was to use COX inhibition to determine whether a PG-mediated pathway is involved in exercising thermoregulation in humans. We used a COX-2 selective NSAID for the reasons indicated above. We hypothesized that a fever-like response would develop in exercise, as evidenced by higher exercising body temperature and thresholds for heat loss activity following administration of placebo compared with the NSAID. We used recreationally active rather than highly fit individuals because they are overly represented in exertional heat stroke casualties, and, similarly, higher fitness was shown to cause insensitivity to NSAID (Indomethacin) mediated reductions in the core temperature of passively heat stressed sheep (47).
METHODS

A double-blind crossover design was used. Following fitness and familiarization sessions, participants consumed a course of COX-2 specific NSAID (Rofecoxib) or a placebo (PLAC; ascorbic acid), on the sixth day of which they undertook an exercising stress test. Drug consumption periods were separated by a two-week washout period. The NSAIDs were consumed as a standard, orally-administered pill, 25 mg twice a day, with the placebo taken likewise. The study was ethically approved by the University of Otago Human Ethics Committee in March 2003. Participants were first enrolled at that time, and data collection was completed in March 2004.

Participants

Ten recreationally-active males provided their written, voluntary consent of participation. Their mean age was 23 yr (SD 5), mass was 78.22 kg (SD 7.95), and maximal oxygen consumption ($\dot{V}O_2$ max) was 53.1 ml·kg$^{-1}$·min$^{-1}$ (SD 5.3). Participants were screened via questionnaire for cardiovascular, musculoskeletal and heat intolerance risks. None were heat acclimated.

Procedures

Fitness and Familiarization: Participants’ $\dot{V}O_2$ max was measured in an incremental treadmill test to exhaustion, with verbal support to encourage maximal exertion. The $\dot{V}O_2$ was calculated in real time from respiratory gases measured for O$_2$ and CO$_2$ content and for volume using calibrated transducers (Sensormedics 2900Z BXB, Sensormedics Corporation, Yorba Linda, California, USA). The $\dot{V}O_2$ max (highest 20-s mean) and the highest running speed were recorded and used to estimate an appropriate intensity that would elicit approximately 75% $\dot{V}O_2$ max on the treadmill during the exercising stress test. These intensities were subsequently confirmed during a familiarization session which
exposed participants to the full experimental protocol, measurements and exercise requirements.

**Exercise Stress Test:** This test was performed on day six of drug ingestion, and at least one week after familiarization. The 6-d protocol was to permit time for attainment of steady state COX-2 inhibition (days 1-3) before first undertaking a preliminary assessment of resting thermoregulation (days 4-5; data not reported). Participants arrived at 7 am in a fasted state and without alcohol, caffeine or strenuous exercise during the preceding 24 hr. Their last drug ingestion was at 6:30 am. Participants had been instructed to drink before coming into the lab, but were also required to consume 500 mL of a carbohydrate and electrolyte beverage (8% carbohydrate, 20 mmol·L⁻¹ Na⁺) prior to instrumentation. Participants were then weighed (to accuracy of 20 g; Digi D1-10, Teraoka, Seiko Ltd, Tokyo, Japan) before they inserted a rectal thermistor 10 cm beyond the anus, to provide an index of core temperature (T<sub>core</sub>). Participants then rested while they were instrumented for measurement of cardiac frequency, skin temperature, sweat rate and forearm perfusion. Resting data, including a resting venous sample (~5 ml, without stasis, 30-min before exercise), were collected. Participants then entered a climatic chamber set at temperate conditions (28ºC, 50% relative humidity and 1.5 m·s⁻¹ air velocity) and rested for approximately 10 min while further resting measurements were taken. They then ran for 45 min on a treadmill (Q65 series 90, Quinton Instrument Co., Seattle, WA) at approximately 75% VO<sub>2</sub> max, followed by 45 min of cycling on an electromagnetically-braked cycle ergometer (Rodby Elektronik AB, Södertälje, Sweden). The two exercise modes were separated by a 5-min transition period to set the participant up on the cycle and draw a mid-exercise blood sample (5 ml). Cycling intensity was established for each participant during familiarization to equate with their perceived exertion during the previous running and to facilitate completion of the 45 min. The purpose of using this dual-mode exercise protocol was to elicit substantial strain using running (i.e. orthostatically and mechanically stressful), then maintain it with a mode of exercise that facilitated (a) measurement of forearm perfusion and blood pressure, (b)
continued exercise tolerance and (c) additional time for fever effects to become evident. Participants were partially rehydrated with 200 ml after 30 min running and 500 ml after 15 min cycling, with the carbohydrate and electrolyte beverage described above. This was done to minimize influences of hyperosmolality and hypovolemia on thermoregulatory control and exercising body temperature, to aid participant tolerance to the exercise regime, and to simulate common practice during prolonged exercise in warm conditions. The trial was stopped if participants requested termination or T<sub>core</sub> exceeded 39.5°C. Following the 90 min of exercise, participants rested in an upright seated position for 60 min while recovery data were collected. A further blood sample (~5 ml) was taken 30-min into this recovery period.

**Data Acquisition**

*Metabolic rate:* \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) were measured (Metalyser 3B, Cortex, Biophysik mbH, Leipzig, Germany) for 2-3 min at 10 and 25 min of both running and cycling.

*Temperatures:* Rectal temperature was measured from a flexible, sterile, and disposable thermistor (Mallinckrodt 400 general purpose, Mallinckrodt Medical Inc. St Louis, USA). Factory calibration (SD 0.1°C) was accepted, although participants used the same thermistor in both stress tests. Skin temperatures were measured using insulated skin thermistors (Type EU, Grant Instruments, Cambridge, England) at nine right-side sites, from which mean skin temperature (\( \overline{T}_{\text{skin}} \)) was calculated from standard area weightings (22) as: 0.07·forehead + 0.0875·chest + 0.0875·abdomen + 0.175·scapula + 0.14·dorsal forearm + 0.05·fifth finger + 0.19·anterior thigh + 0.15·calf + 0.05·dorsal foot. Temperatures were logged at 15-s intervals (resolution 0.05°C; Grant 1200 series, Grant Instruments Ltd, Cambridge, England) and subsequently downloaded to a PC computer for analysis.

*Sweat rate:* Sweat rate kinetics were measured using a ventilated capsule system, from two capsules (19 mm I.D.) glued to the forehead and lateral arm. Capsules were
ventilated at a known rate (flow sensor; Honeywell AWM5101, Illinois, USA) of ~0.5 L·min⁻¹ from bottled dry air. The post-capsular airstream was sampled for humidity (resistance hygrometer; Honeywell HIH 3605, Illinois, USA) and temperature (National Semiconductors LM35CAH, Hong Kong) using a custom-built system. Digital outputs from this system were obtained (PowerLab hardware 8e, ADI Instruments, Chain Hills, NSW, Australia) and recorded on a PC computer via data acquisition software (Chart 4.20, ADI instruments, Chain Hills, NSW, Australia). Sweat rates were calculated at 15-s intervals from standard equations, then represented as the mean from both capsules. Humidity sensor calibration was via saturated salt solutions. The 50 and 90% response times to an upward, full scale (0 to 100%) humidity step were 22 and 174 s, respectively.

**Forearm Perfusion:** Venous Occlusion Plethysmography was used to measure forearm perfusion ($Q_{\text{forearm}}$) following standard procedures, thus providing a validated index of cutaneous vasomotor activity in the absence of arm exercise (27). Briefly, arterial blood flow to the hand is occluded at the wrist with a blood pressure cuff (~200 mm Hg) for 30 s before venous outflow from the arm is rapidly occluded (rapid inflation cuff, Hokanson Inc, Bellevue, WA). The $Q_{\text{forearm}}$ is then measured as the rate of forearm expansion, recorded from a mercury-in-silastic strain gauge (Hokanson Inc, Bellevue, WA) attached around the largest forearm circumference. Because the arm is positioned at eye level during measurement, venous return is facilitated upon cuff deflation. Chart software (4.20, ADI Instruments, Chain Hills, NSW, Australia) was used to regulate the inflation of the arm cuff to 50 mm Hg for 5 out of 13 s, four times at each sampling period: at rest and 5, 15, 30 and 43 min of cycling. Strain gauge and cuff pressure were recorded at 100 Hz via PowerLab hardware (8e, ADI Instruments, Chain Hills, NSW, Australia) and Chart software. The inflation slopes of strain were analyzed within the Chart software using least-squares regression, before being averaged across up to four samples. Blood pressure was measured immediately following each $Q_{\text{forearm}}$ measurement using sphygmomanometry with auscultation over the brachial artery.
Cytokines: Venous samples were analyzed for plasma concentrations of TNF-\( \alpha \) and IL-10, as markers of pro- and anti-inflammatory cytokine responses, respectively. Fresh venous samples were first transferred into tubes containing EDTA-anti-coagulant and immediately centrifuged at 4ºC and 3000 RPM for 10 min. The plasma was subsequently removed and stored in 1 ml aliquots at -80ºC until determination of cytokine concentrations using commercially available ELISA immunoassays (Quantikine High Sensitivity, R&D Systems, Minneapolis, USA).

Data Analysis

Calculations: Mean body temperature (\( \overline{T}_{\text{body}} \)) was calculated from \( T_{\text{core}} \) and \( \overline{T}_{\text{skin}} \) using a ratio of 4:1 (12). Thus, \( \overline{T}_{\text{body}} \) was used in the determination of sudomotor and vasomotor control because it more closely reflects their control than does \( T_{\text{core}} \) alone. The sweating onset and offset thresholds were calculated by plotting mean sweat rate against \( \overline{T}_{\text{body}} \) for each participant and visually identifying the \( \overline{T}_{\text{body}} \) at which sweating increased and continued to rise from baseline (onset), and the \( \overline{T}_{\text{body}} \) at which the sweating trace returned to baseline and showed no subsequent rises (offset). This was completed by two investigators, both of whom are familiar with these determinations. Forearm vascular conductance (FVC) was calculated as \( \dot{Q}_{\text{forearm}} / \text{MAP} \).

Statistics: We are unaware of previous research examining this issue in humans, but we used a sample size of ten because (a) a within-subjects design was used, (b) previous research showed statistically supported effects of NSAID on \( T_{\text{core}} \) responses to a passive heat stress in four unfit sheep (47), and to an exercising stress in seven iguana (7), and (c) we had previously obtained statistically supported effects of COX-2 specific inhibitors on renal function using twelve participants (1). Fully-repeated two-way ANOVA were used to examine the effects of NSAID administration on the relevant dependent variables. Specifically, there were two drugs (PLAC and NSAID) and either two time points (sweat onset and offset; metabolic rate in running and cycling; cytokines pre and post exercise)
or four periods (rest, run, cycle and recovery: \( \bar{T}_{body}, f_c, \dot{Q}_{forearm}, \) ratings). Degrees of freedom were corrected using a Huynh-Feldt adjustment, and a Bonferroni correction was used to control Type I error during the post hoc analyses of significant ANOVAs (SPSS 11.0 for Windows, SPSS Inc, Chicago, USA). A paired t-test was performed on simple comparisons between experimental conditions (i.e., ambient conditions and FVC: \( \bar{T}_{body} \) relationship). Statistical power (\( \phi \)) is reported for non significant results. The family-wise \( \alpha \) was 0.05. Data are reported as means with SD for n=10, unless otherwise stated.
RESULTS

All participants reported adhering to the drug ingestion protocol and dietary standardization, and they completed both stress tests. Dry bulb temperature was equivalent ($P=0.86$) between PLAC (28.13°C, SD 0.17) and NSAID (28.15°C, SD 0.20).

**Body temperatures:** The $T_{\text{body}}$ response to exercise differed between drug conditions ($F_{[2.2,19.6]} = 11.67; P<0.01$), in that it was similar during the 45-min run ($P=0.33$), but became higher in PLAC during cycling (by 0.33°C, SD 0.26; $P=0.03$) and remained this much higher during recovery (0.34°C, SD 0.26; $P<0.01$; Figure 1C). This response was observed in nine participants, with the remaining one showing no difference. Mean skin temperature remained equivalent between drug conditions ($F_{[3,27]} = 0.92; P=0.44; \phi=0.23$) (Figure 1B), so the differential $T_{\text{body}}$ response was attributable to a differential $T_{\text{core}}$ response to exercise between drug conditions ($F_{[1.7,15.1]} = 16.91; P<0.01$; Figure 1A).

**Thermoeffectors:** No sweat rate response differences were observed at the forehead or arm between drug conditions, thus the unweighted mean of both sites was used. The $T_{\text{body}}$ thresholds for sweating responded differently between the two drug conditions ($F_{[1,8]} = 7.24; P=0.03; n=9$). Whereas sweating was initiated at a similar $T_{\text{body}}$ in both drug conditions, the elevation in sweating cessation was 0.19°C (SD 0.22) greater in PLAC than in NSAID (Figure 2A). That is, the post-exercise $T_{\text{body}}$ threshold for sweating cessation was elevated above the onset threshold, as expected, but the administration of NSAID reduced this effect. This attenuation by NSAID was evident in seven of the nine participants for whom sweating data were obtained (Figure 2B). Similar thresholds for FVC were not calculated because the running exercise prevented $\dot{Q}_{\text{forearm}}$ measurement during the initial heat stress period. Therefore, the change in FVC from rest to cycling exercise was used and normalized to the change in $T_{\text{body}}$. The FVC response was significantly higher in NSAID than in PLAC (0.13 versus 0.10 ml·100 ml tissue$^{-1}$).
1·min⁻¹·mm Hg⁻¹·ºC⁻¹, both SD 0.05; \( P=0.01 \). This indication of higher FVC sensitivity in
NSAID was evident in all eight participants for whom data were available.

**Cytokines:** The plasma TNF-\( \alpha \) and IL-10 responses to exercise indicated a mild
cytokinemia that was equivalent between drug conditions. Specifically, the TNF-\( \alpha \)
concentration increased by an average of 41\% (SD 36; \( F_{[1,9]} = 6.23; P=0.03 \)) from pre to
post exercise (2.96 to 4.17 pg·mL⁻¹, SD 2.07 and 3.25), with no statistical interaction
(\( F_{[1,9]} = 0.47; P=0.51 \)). The IL-10 concentration increased by 110\% (SD 112; 2.43 to 5.10
pg·ml⁻¹, SD 2.17 and 4.45; \( F_{[1,7]} = 6.97; P=0.03; n=8 \)), also with no statistical interaction
(\( F_{[1,7]} = 0.97; P=0.36 \)).

**Cardiac frequency:** In a similar manner to \( \bar{T}_{\text{body}} \), the progression of cardiac frequency in
exercise differed between drug conditions (\( F_{[3,27]} = 3.26; P=0.04; \) Figure 3), and whilst it
appeared to become progressively lower in NSAID relative to PLAC, the source of
significant difference was not identifiable from post hoc testing. A main effect of drug
was evident, with cardiac frequency being 6 min⁻¹ (SD 3) higher in PLAC than in NSAID
(124 versus 119 min⁻¹, both SD 14; \( F_{[1,9]} = 36.52; P<0.01 \)). This effect was observed in all
ten participants.

**Metabolic rate:** Metabolic rate was similar between drug conditions (\( F_{[1,4]} = 0.00; \)
\( P=0.99; n=5; \varphi=0.05 \)), averaging 547 W·m⁻² (SD 72) in PLAC and 549 W·m⁻² (SD 102)
in NSAID during running (\( P=0.88 \)), which was 75\% (SD 6) of \( \dot{V}O_2_{\max} \). Metabolic rate
was lower during cycling (64\% of \( \dot{V}O_2_{\max} \), SD 4), but was also similar between drug
conditions: 463 W·m⁻² (SD 56) and 465 W·m⁻² (SD 62).

**Body mass:** There was a tendency for baseline body mass to be slightly lower in PLAC
(77.82 kg, SD 7.84) than in NSAID (78.49 kg, SD 7.95; \( P=0.14 \)). The net mass loss was
similar between drug conditions (1.62 kg (SD 0.36) and 1.61 kg (SD 0.34), respectively;
$P=0.93$), thereby indicating that sweat losses were equivalent and caused dehydration of $2\%$ of body mass.
DISCUSSION

To the best of our knowledge, this study provides the first direct evidence that a supplemental fever-like elevation in body temperature can occur during non-exhaustive exercise in humans. Administration of a COX-2 selective inhibitor lowered core and mean body temperatures during exercise and ensuing recovery, relative to the temperatures obtained following administration of a placebo. This was independent of endogenous heat stress or thermal environment. More importantly in relation to the notion of an exercise-induced fever, administration of a COX-2 specific inhibitor was associated with an unchanged $T_{body}$ at which sweating was initiated but a relative lowering of the $T_{body}$ at which sweating ceased in recovery from exercise, and a greater increase in FVC relative to $T_{body}$ in the latter portion of exercise. The post-exercise elevation in the $T_{body}$ threshold for sweating cessation observed in both conditions was expected due to effects of non-thermal factors, particularly the baroreceptor unloading due to residual vasodilation of skeletal muscle (28). These data therefore support our hypothesis and confirm, in humans, the work using animal models (7, 8, 47), that a pyrogenic effect can develop during exercise.

The inhibition of COX-2 enzymes in this study served our aim of identifying a fever-like effect during exercise, but the intervention has limitations. Fever is complex, likely inducible via numerous pyrogens and pathways, some of which remain unknown (3, 31, 52). Of those proposed, PGE$_2$ has been suggested as a common mediator in raising the thermoregulatory set point (61). However, even though COX-2 enzymes have been repeatedly associated with the production of PGE$_2$ largely responsible for fever and inflammation (23, 33, 49, 55), there is evidence for COX-1 having a regulatory role and for non-COX pathways (2, 58). Thus, we are unaware whether the difference of 0.3°C (~20% of end exercise elevation in $T_{core}$) seen between the two groups was the entire pyrogenic response. These issues could be addressed using non-selective NSAIDs (COX-1 & 2 inhibition) or other anti-pyretic drugs such as sodium salicylate or acetaminophen.
(paracetamol) which have anti-pyretic actions independent of COX inhibition. Equally, whether any fever-like effect would develop in non-exhaustive exercise of similar duration and intensity in endurance trained individuals is unknown.

This has been examined in sheep exposed to passive heat stress (47). The approximately 0.3°C attenuation in a 1.7°C hyperthermia in the present study (Figure 1A) was of similar magnitude to that observed in unfit sheep exposed to passive heat stress (~0.3°C in 1.3°C; (47)). In that study, no decrease in T\textsubscript{core} was seen in fit sheep, who possessed a higher splanchnic perfusion during heat stress that matched the baseline perfusion of the unfit sheep. Whether aerobically conditioned humans would similarly show no effect is unclear, but several factors indicate that such effects might occur. In most exercise settings they undergo higher absolute volumes of stress in accordance with their higher fitness. They can also suffer heat stroke. They have an anti-LPS IgG elevation that is proportional to their training volume and is diminished during exhaustive, competitive exercise (5, 25). Despite their larger splanchnic vessels (13) and perfusion (47), a pronounced splanchnic vasoconstriction still develops at exercise intensities similar to that used in this study (42). The regional competition for perfusion becomes especially pronounced during upright, dehydrating exercise in the heat (16, 17).

Fever appears to have two major pathways; neural and humoral. Neural pathways such as vagal or cutaneous sensory nerves have been implicated in the rapid transport of a febrile message to the CNS causing initiation of the fever response (38, 46). For instance, rats injected with 10-100 μg·kg\textsuperscript{-1} LPS showed a T\textsubscript{core} rise within 10-20 min, peaking initially by 45-60 min (44, 45); apparently preceding LPS-induced changes in circulating cytokines (2). The humoral pathway, involving transport of cytokines or PGs to the brain to elevate the thermoregulatory set point, seems more likely to be responsible for the maintenance of a fever response (2). The extent to which cytokines can cross the blood-brain barrier and act directly or indirectly on thermoregulatory neurones is unclear, but endurance exercise seems to impair the integrity of this barrier, at least in hot conditions.
It should also be considered that NSAIDs readily cross this barrier, thus local COX inhibition may attenuate PG production in response to stimuli of local, humoral or afferent neural origin. The time course of a NSAID-associated attenuation of Tcore in the present study (Figure 1A) unfortunately coincided with changing of exercise mode and the slight reduction in metabolic heat production. Nonetheless, the time course seemed to be more indicative of a humorally mediated fever (44, 45), which would also be consistent with the mechanism of intervention, i.e., COX-2 inhibition of PG production.

Endurance exercise may be capable of inducing a sub-clinical pyrogenic response, which, acting in concert with other fatigue processes, is usually self limiting before the development of exertional heat stroke (19-21). The source and identity of pyrogenic factors could potentially include pro-inflammatory cytokines (e.g., IL-1, TNF-α, IL-6), mainly from muscle, liver, leukocytes, adipose tissue or the central nervous system itself, LPS leaking across the gut wall, and/or cellular constituents from within muscle or other stressed tissue. These factors might act humorally, via cytokines, or neurally.

A probable source of a pyrogenic response is increased LPS from the GI tract in response to prolonged splanchnic vasoconstriction and elevated tissue temperature (4, 8, 14, 15, 18-21, 47). LPS leakage is normally low enough for hepatic detoxification and anti-LPS antibodies to keep pace. However, epithelial permeability and LPS leakage increase with a variety of factors, including local temperature (36, 50) and ischemia (14, 15). Increased LPS leakage can deplete antibodies and saturate hepatic clearance, increasing the LPS load and activating mediators of the non-specific immune system (cytokines, interferon), vascular reactivity (PGEs) and coagulation (Factor XII), initially causing inflammatory and fever-producing effects. In advanced stages this appears to underlie exertional heat stroke (20). However, data obtained from animal models indicates that even lower (i.e. sub-lethal) endotoxic loads may reduce heat tolerance (19), particularly if endurance fitness is limited (47).
Interleukin 6 (IL-6) release is stimulated by TNF-α, but also inhibits production of TNF-α, and in this respect has been proposed to have an important anti-inflammatory role as well as a pro-inflammatory role. It is now known to be released mostly from active muscle in endurance exercise, and seems to lower self-selected exercise intensity (43). Because of the pluripotential role of IL-6, we used TNF-α as a marker of the inflammatory cytokine response, but saw little change across exercise, and no differential response with Rofecoxib compared to placebo. Thus, our cytokine measurements provide no explanation for our findings, but we do not consider IL-6 release from active muscle as the most likely source of pyrogens.

Finally, responses to muscle tissue damage may also drive an exercise-induced inflammatory response. However, this effect has only been evidenced from higher exercising T\text{core} during exercise performed many hours after the damaging stimulus (6, 34), and may depend on prior migration of activating agents such as neutrophils, macrophages, and PAF (41, 51). The exercise undertaken in this study was not to exhaustion and none of the subjects reported difficulty or injury as a consequence of the exercise schedules. Even so, this study design does not allow us to exclude a potential acute effect of muscle damage on the pyrogenic response observed.

Recent studies using passively-induced hyperthermia have demonstrated that heat-related impairment of force development is attributable to both central and peripheral effects (35, 54). Thus, high body temperature itself can impair exercise tolerance independently of its effects in elevating cardiovascular, metabolic and neuroendocrine strain. Minimizing the fever component of any such hyperthermia would therefore seem beneficial.

The use of NSAIDs during exercise, presumably for musculoskeletal injuries, appears to be widespread. Seventy two percent of runners in an ultra-endurance race reported consuming NSAIDs, and, interestingly, the inflammatory cytokine profile of those runners was higher than that of non-users following the 100-mile race (37). Also
widespread is the belief that COX-2 selective NSAIDs avoid some dangers of non-selective NSAIDs, such as hyponatremia. However, COX-2 selective inhibition has been found to reduce free water clearance during and following endurance exercise to an extent similar to non-selective COX inhibition (1), along with the potential for a significant reduction in renal blood flow and glomerular filtration rate. Thus, even if attenuation of hyperthermia was known to be useful in a given athletic or occupational endeavor, and COX-2 selective inhibition was shown effective in that setting, the effect of ~0.3°C magnitude reduction in $T_{core}$ would be realistically achieved by a strategy focusing on adequate hydration, aerobic training, heat acclimatization, weight control, cooling, and possibly dietary modification, rather than the use of COX-2 inhibitors. In at-risk individuals, COX-2 inhibition as well as non-selective COX inhibition may carry an increased risk of cardiovascular events (26). The potential risk of COX-2 inhibitor associated cardiovascular event in athletes is unknown, but in light of the above discussion, we would recommend caution in the use of these agents in moderate to severe exercise.

Another limitation of using the COX-2 selective inhibitor to examine whether exercise can induce a fever response is that the drug may have a confounding influence on exercise thermoregulation. Whilst we believe that our hypothesis is supported primarily by virtue of the lower sweat offset threshold for NSAID compared with PLAC in the absence of a shift in MAP, the reduction of cardiovascular strain that was evident with NSAID usage in this study (cardiac frequency lower by ~6 min$^{-1}$; Figure 3) is harder to explain. A direct $Q_{10}$ effect chronotropically should only drop the frequency by ~2 min$^{-1}$, and whilst circulating catecholamine levels might further contribute, that should have been evident in measures such as the respiratory exchange ratio and MAP (both of which were equivalent). However, there may also be a direct effect of prostanoids on cardiac frequency (53), as evidenced from mice lacking TP and FP receptors (for the prostanoids thromboxane A$_2$ and prostaglandin F$_{2a}$, respectively). Relative to wild-type mice, the gene-deleted mice showed inhibition of inflammatory tachycardia, apparently via direct
action of these prostanoids on cardiac pacemaker cells rather than effects on sympathetic activity. Thus, use of a COX-2 inhibitor in our study could possibly have attenuated PGF$_{2\alpha}$ production and exerted a direct negative chronotropic effect despite the low dosage we used.

Regarding other possible cardiovascular effects, inhibition of COX-2 mediated PGE$_2$ production has been found, with higher drug dosages, to attenuate exercise hyperemia in endurance-exercised connective tissue (32), and prostacyclin (PGI$_2$) has been shown to impact on maintenance of resting forearm blood flow (11). In contrast, non-selective COX-inhibition with Aspirin had no effect on resting forearm blood flow but did reduce vasodilation following isometric contraction of the forearm muscles at 60% MVC (60). Whether such effects could have impacted on cutaneous perfusion in this study is unknown, but we also note that there seems to be considerable redundancy among vasodilatory pathways (e.g., involving prostaglandins and prostacyclins, acetylcholine, nitrous oxide and potassium; (9, 11, 32, 60)), such that non-thermoregulatory effects on exercise hyperemia might have been negligible. Finally, since we used a 5-d ingestion protocol (to attain steady state COX-2 inhibition before first assessing resting thermoregulation), we cannot preclude the possibility of a short-term adaptive response to COX-2 inhibition.

In conclusion, sub-maximal endurance exercise can induce a fever-like response in humans which causes a supplementary increase in mean body temperature, possibly via the effects of COX-2 mediated PGE$_2$ production on the thermoregulatory set point.
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GRANTS

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Figure Captions

Figure 1. Rectal temperature (A), mean skin temperature (B) and mean body temperature (C) during 90-min exercise and 60-min recovery, following 6-d administration of a COX-2 selective inhibitor (NSAID; ●) or placebo (PLAC; □). Data are means ±SD for ten males. *P<0.05, significantly different between NSAID and PLAC. To aid visual interpretation, symbols are illustrated intermittently with respect to data and are horizontally offset.

Figure 2. Sweat rate responses to 90-min exercise and 60-min recovery, following 6-d administration of a COX-2 selective inhibitor (NSAID; ●) or placebo (PLAC; □). Top panel: mean sweat rate as a function of mean body temperature (n=9). Bottom panel: individuals’ mean body temperature at which sweating was initiated (Onset) and ceased (Offset) in each drug condition. *P<0.05, significant interaction between NSAID and PLAC for onset and offset. ‡P<0.05, significantly different between onset and offset. Statistics shown on top panel only. To aid visual interpretation in the upper panel, symbols are illustrated intermittently with respect to data and are horizontally offset.

Figure 3. Cardiac frequency during 90-min exercise and 60-min recovery, following 6-d administration of a COX-2 selective inhibitor (NSAID; ●) or placebo (PLAC; □). Data are means ±SD for ten males. *P<0.05, significantly different between NSAID and PLAC. To aid visual interpretation, symbols are illustrated intermittently with respect to data and are horizontally offset.