Exercise-induced DNA damage: Is there a relationship with inflammatory responses?

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ABSTRACT

Both a systemic inflammatory response as well as DNA damage has been observed following exhaustive endurance exercise. Hypothetically, exercise-induced DNA damage might either be a consequence of inflammatory processes or causally involved in inflammation and immunological alterations after strenuous prolonged exercise (e.g. by inducing lymphocyte apoptosis and lymphocytopenia). Nevertheless, up to now only few studies have addressed this issue and there is hardly any evidence regarding a direct relationship between DNA or chromosomal damage and inflammatory responses in the context of exercise. The most conclusive picture that emerges from available data is that reactive oxygen and nitrogen species (RONS) appear to be the key effectors which link inflammation with DNA damage. Considering the time-courses of inflammatory and oxidative stress responses on the one hand and DNA effects on the other, the lack of correlations between these responses might also be explained by too short observation periods. This review summarizes and discusses the recent findings on this topic. Furthermore, data from our own study are presented that aimed to verify potential associations between several endpoints of genome stability and inflammatory, immune-endocrine and muscle damage parameters in competitors of an Ironman triathlon until 19 days into recovery. The current results indicate that DNA effects in lymphocytes are not responsible for exercise-induced inflammatory responses. Furthermore, this investigation shows that inflammatory processes, vice versa, do not promote DNA damage, neither directly nor via an increased formation of

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RONS derived from inflammatory cells. Oxidative DNA damage might have been counteracted by training- and exercise-induced antioxidant responses. However, further studies are needed that combine advanced –omics based techniques (transcriptomics, proteomics) with state-of-the-art biochemical biomarkers to gain more insights into the underlying mechanisms.

Key words: DNA damage, systemic inflammatory response, lymphocytopenia, muscle inflammatory responses, endurance exercise

INTRODUCTION

Due to extensive research in the past decades, the effects of exercise on the immune system are well documented [20, 33, 53]. However, researchers in this area are still puzzled by questions about the underlying molecular mechanisms of the observed immunological alterations [32, 33]. Extremely demanding endurance exercise has been shown to induce both a systemic inflammatory response [15, 42, 53, 71] as well as DNA damage [21, 36, 58, 62, 80]. Exercise-induced DNA damage in peripheral blood cells appear to be mainly a consequence of an increased production of reactive oxygen and nitrogen species (RONS) during and after vigorous aerobic exercise [58]. Besides oxidative stress, other factors such as metabolic, hormonal and thermal stress in addition to the ultra-structural damage of muscle tissue are characteristic responses to prolonged strenuous exercise, that can lead to the release of cytokines, acute phase proteins and to the activation or inhibition of certain lines of the cellular immune system [15, 29]. In addition to these effectors, exercise-induced modifications in DNA of immuno-competent cells have been hypothesised to be related with immune and inflammatory responses to prolonged intensive physical activity, either by playing a causative role and/or by resulting from exercise-induced inflammatory processes [21, 40, 44, 53]. Nevertheless, both experimental data as well as a more mechanistic understanding regarding this relationship are still incomplete.

The aim of this review is to outline the findings and current state of knowledge on potential associations between DNA modulations and inflammatory responses after exercise. In the first part of this article, a short description of the most commonly applied techniques to evaluate genome stability is provided. This is followed by a brief summary of studies that have investigated the effects of exercise on DNA in general. The latter issue has been presented elsewhere in detail with a focal point on methodology in an article by Poulsen et al. [58]. In the second part of this review the focus is on studies that have investigated *both*, certain endpoints of DNA damage *and* immuno-endocrine and inflammatory parameters in the context of exercise. Since apoptosis (programmed cell death) has been suggested to influence the regulation of leukocyte counts after exercise [53], we also addressed studies on this topic in the present review. Furthermore, we included the few investigations that examined exercise-induced DNA modulations and markers of muscle damage, since this issue might give some indirect evidence for inflammatory processes following exercise. Finally, data from our own study is presented, which aimed to get a broader and more thorough insight into oxidative [43], myocardial [28], skeletal muscular, inflammatory and immuno-endocrine

stress responses [42] as well as genome stability [62, 63] in a large cohort of Ironman competitors. By investigating a range of divergent parameters and by quantifying the resolution of recovery up to 19 days (d) after the Ironman race, the results specifically enabled us to verify potential interactions between several endpoints of DNA and chromosomal damage on the one hand and inflammation and muscle damage on the other hand.

Commonly Applied Techniques to Monitor DNA and Chromosomal Stability in Exercise

A number of different approaches have been used to evaluate DNA stability in exercise studies. The aim of this part of the present article is to give a brief overview on the principles of the most frequently applied methods, since this topic has been comprehensively reviewed in the scientific literature [8, 17, 26, 58].

Many studies in this context applied the single cell gel electrophoresis (SCGE or COMET) assay due to its sensitivity and simplicity [8]. This technique is based on the determination of the migration of damaged DNA out of the nucleus in an electric field, whereas the migrated DNA resembles the shape of a comet [21, 26]. The standard version (under alkaline conditions) enables the detection of DNA single and double strand breaks, and apurinic sites [77], while the use of the lesion specific enzymes endonuclease III (ENDO III) and formamidopyrimidine glycosylase (FPG) allows the detection of oxidized purines and pyrimidines, respectively [7, 8]. Regarding the interpretation of the results that are obtained by the SCGE assay it is important to bear in mind that endpoints are differently reported as tail lengths of the comets, percentage DNA in tail and tail moment [8].

Contrary to the SCGE assay, the cytokinesis block micronucleus cytome (CBMN Cyt) assay allows to assess persistent chromosomal damage [16, 21]. Endpoints of this precise method includes the formation of micronuclei (MN) resulting from chromosomal breakage or loss, nucleoplasmic bridges (NPBs) indicating chromosome rearrangements, and nuclear buds (Nbuds) that are formed as a consequence of gene amplification [16, 18]. The reliability of this MN in pathophysiological conditions has been substantiated by a recent study which has shown an association between MN frequency and cancer incidence [3].

In several exercise studies 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG), was investigated, which is formed through oxidative modification of guanine, and mainly detected in urine or in leukocytes [26]. Measurement of urinary 8-oxodG is thought to be the result of the repair of these lesions in DNA, excretion into the plasma and subsequently into urine [58]. Hence, it does not necessarily reflect the steady-state of un-repaired DNA damage [80]. Moreover, urinary 8-oxodG represents a general oxidative damage marker for the whole body, and consequently, is not specific to DNA damage in white blood cells [60, 80]. Attention should also be given in the interpretation of this biomarker due to methodological drawbacks and discrepancies among divergent approaches which are currently used to analyse 8-oxodG [26, 58].

Effects of Different Kinds of Exercise on DNA

Epidemiological as well as empirical data indicate protective effects of physical activity on site-specific cancer risk [58, 64, 76]. However, similarly to the con-

cerns about ultra-endurance exercise and cardiovascular health [27], Poulsen et al. hypothesised a U-shaped curve relationship between exercise and health particularly in the context of oxidative DNA modifications [58]. Data are available now on the effects of acute bouts of very prolonged (ultra-endurance) exercise on genome stability, which will also be presented in the following overview. According to the literature [10], ultra-endurance is defined as exercise lasting more than 4 hours (h).

Ultra-endurance Exercise (> 4 h)
Increased DNA instability as detected by the SCGE technique [36, 63] or with the CBMN Cyt assay [62] or by analysis of urinary 8-OHdG concentrations [37, 60] were found after an Ironman triathlon [62, 63] and ultra-marathon races [36, 37, 60]. Importantly, changes regarding the SCGE assays as well as urinary 8-OHdG were only temporary [36, 37, 60, 62, 63] and endpoints of DNA damage measured with the CBMN Cyt assay even decreased in response to an Ironman race and declined further 19 d post-race [62]. These responses are discussed later in detail within the scope of our own observations.

Competitive Endurance Exercise (< 4 h)
Data regarding competitors of endurance races with a duration of less than four hours are partly inconclusive, albeit in most studies increased DNA migration was detected in SCGE assays after a half-marathon [44], a marathon [80] or a short-distance triathlon race [21]. On the contrary, neither changes in the levels of strand breaks nor in the FPG-sensitive sites, but increased ENDO III sites were observed after a half-marathon- and a marathon [4]. However, the subjects of the latter study were monitored only immediately post-race, while other investigations demonstrated that major DNA modulations were sustained until 5 d post-race in six short-distance triathletes [21] and for even 14 d following a marathon [80]. Nevertheless, based on the finding of an unaltered frequency in MN, Hartmann et al. [21] concluded that intense exercise with a mean duration of 2.5 h does not lead to chromosome damage.

Submaximal and Maximal Exercise Under Laboratory Conditions
Several studies conducted submaximal aerobic exercise protocols under laboratory conditions to investigate DNA effects. DNA damage was neither seen after intense treadmill running in male subjects of different training status [82] nor in well-trained endurance athletes [54]. In addition, Sato et al. showed that acute mild exercise as well as chronic moderate training does not result in DNA damage, but rather leads to an elevation in the sanitization system of DNA damage [66]. Interestingly, in an experiment that aimed to examine the influence of a downhill run before and after supplementation with vitamin E, no effect was found on the levels of leukocyte 8-OHdG in both 16 young and 16 older physically active men [65]. However, it has to be mentioned that DNA responses were not followed until at least 1 d post-exercise in most of these studies [54, 65, 82].

Conflicting findings were reported when maximal exercise protocols, i.e. tests until exhaustion, were conducted under laboratory conditions. Increased levels of DNA strand breaks were observed after exhaustive treadmill running in subjects of different training status [22, 45]. Moller et al. [38] demonstrated DNA strand breaks

and oxidative DNA damage after an maximal cycle ergometer test under high-altitude hypoxia, but not normal (normoxic) conditions. In another study, elevated levels of MN were reported after exhaustive sprints; however, the six subjects were of divergent training levels and gender and included one smoker [67]. On the contrary, Pittaluga et al. [56] detected no effects of a maximal exercise test on MN in 18 young subjects with different training status, but the authors noted chronic cellular stress including higher MN levels at rest in the athlete group. Furthermore, there were no differences in urinary 8-OHdG concentrations before and after supplementation with β-carotene within the 3 d following a cycle ergometer test to exhaustion [70].

Periods of intensified training

A few studies have examined whether periods of intensified training affect genome stability. Increased urinary 8-OHdG levels were observed in 23 healthy males in response to a vigorous physical training programme (about 10 h of exercise for 30 d) [57] and in male long-distance runners throughout a training period for 8 d compared to a sedentary period [47]. However, in a longitudinal study no differences in urinary excretion of 8-OHdG between a group of long-distance runners and a sedentary control group were observed [55]. In two separate studies that comprised a similar group of male triathletes, Palazzetti et al., reported either no [48] or increased DNA damage [49] after 4 weeks (wk) of overload training as detected by the SCGE assay, probably due to inter-individual differences.

In conclusion, there is growing evidence that strenuous exercise can lead to DNA damage that with few exceptions [36] is predominantly observed not before 24 h after the resolution of exercise [21, 44, 45, 80]. However, the diversity of methods and endpoints used to assess DNA modifications and different study designs (i.e. divergent exercise protocols and sampling time-points) make it difficult to determine the exact circumstances under which DNA damage occurs. Crucially, in addition to the aforementioned factors, the heterogeneity of study cohorts (varying in gender, age and training status) most likely contributes to inconsistencies among the studies on this topic. Nevertheless, results of the few studies that have examined the effects of ultra-endurance exercise on genome stability indicate that adaptations of endogenous protective antioxidant and/or repair mechanisms prevent severe and persistent DNA damage in well-trained athletes [36, 37, 45, 60, 62]. Thus, a clear dose-response relationship regarding the level of exercise that could be detrimental cannot yet be established. Currently, there are no indications that exhaustive endurance exercise increases the risk for cancer and other diseases via DNA damage. However, it remains to be clarified whether perturbances of the genomic stability of immuno-competent cells are involved in the post-exercise temporary dysfunction of certain aspects of immunity, which may increase the risk of subclinical and clinical infection [15, 20, 53].

Findings on Exercise-induced DNA Damage and/or Apoptosis and Inflammatory Responses

Table 1 summarizes the small number of studies that have examined the effects of exercise on DNA and/or apoptosis on the one side *and* inflammatory responses on the other. As one of the earlier works in the context of the effects of particularly competitive endurance exercise on DNA damage, Niess et al. [44] found that neu-

trophil counts 1 h after a half-marathon run correlated with DNA damage in leukocytes, assessed 24 h post-race. Without examining markers of oxidative stress, the authors could only speculate that RONS released by neutrophils might have been responsible for the formation of DNA strand breaks. However, their results led them to suppose that the observed DNA damage might be the key mechanism for the modifications in the immune cell counts [44]. On the contrary,

Table 1. Studies investigating exercise-induced DNA damage and/or apoptosis and inflammatory/immune parameters

Reference	Experimental protocol and sampling time-points	Subjects	Endpoints of DNA damage and/or apoptosis	Inflammatory and immune parameters (plasma)	Correlations between DNA damage/apoptosis and inflammation	Main other results
Ultra-endurance exercise (> 4 h)						
Mastaloudis et al. 2004 [35, 36]	Ultra-marathon (50km) Pre-, mid-, 2 h post-, daily for 6 d post-race (supplementation with vitamins C and E versus placebo)	11 ♀and 11 ♂ runners	SCGE (LEU) ↑ mid-race, returned to baseline by 2 h post-race, ↓ by 6 d post-race	IL-6, TNF-α ↑ mid-race until 2 h post-race, CRP ↑ 1 – 2 d post-race	No significant correlation reported	F_2-IsoPs (plasma) ↑ immediately post-race only in placebo group
Reichhold et al. 2008 [62, 63], Neubauer et al. 2008 [42, 43]	Ironman triathlon (3.8km swimming +180km cycling +42km running) 2 d pre-, immediately, 1 d, 5 d and 19 d post-race	20 and 28 well-trained ♂ triathletes randomized for the CBMN Cyt and the SCGE assays, respectively	SCGE (LYM) ↑ immediately and 1 d post-race, back to baseline 5 d post-race, ↓ 19 d post-race, FPG ↔, ENDO III ↑ 5 d post-race compared to 1 d post-race, back to baseline 19 d post-race, MN ↓ immediately post-race, ↓ further 19 d post-race, Nbuds ↑ 5 d post-race, back to baseline 19 d post-race, NPB ↓ 19 d post-race, Apoptotic and necrotic cells: ↓ post-race	Immediately post-race: Total LEU counts ↑, MPO ↑, PMN-elastase ↑, CK ↑, myoglobin ↑ ,IL-6 ↑ IL-10 ↑, hs-CRP ↑ (all alterations persisted 1 d post-race) 5 d post-race: CK ↑, myoglobin ↑, IL-6 ↑, hs-CRP ↑ 19 d post-race: MPO ↓, PMN ↓, hs-CRP ↑, myoglobin ↑	Links between IL-6 and necrotic cells immediately post-race, no further significant correlations	Immediately post-race: Cortisol ↑, testosterone ↓, CD ↑, AOPP ↑, oxLDL ↓, oxLDL:LDL ↔, Plasma antioxidant capacity (TEAC, FRAP, ORAC) ↑, 1 d post-race: MDA ↑, CD ↑, AOPP ↑, plasma antioxidant capacity, Negative correlation between ORAC and ENDO III
Competitive endurance exercise (< 4 h)						
Niess et al. 1998 [44]	Half marathon Pre-, 1, 24 h post-race	12 moderately trained ♂	SCGE (LEU) ↑ 24 h post-race	CK ↑ 1 h and 24 h post-race, PMN count ↑ 1 h post-race, LYM count ↓ 1 h post-race	Significant correlation between PMN count 1 h post-race and DNA migration 24h post-race	
Mooren et al. 2004 [40]	Marathon Pre-, immediately, 3 h, 24 h post- race	17 high- to low-trained ♂ marathon runners	% of apoptotic cells (LYM) ↑ 3h post-race, ↓ 24 h post-race	LEU counts ↑ 3 h and 24 h post-race	No significant correlation reported	
Briviba et al. 2005 [4]	Marathon and half-marathon 10 d pre-, immediately post-race	10 half-marathon runners, 12 marathon runners (♂ and ♀)	After both races: SCGE (LYM) ↔ FPG ↔, ENDO III ↑, ↓ resistance of DNA to oxidative damage % of apoptotic cells ↓	After half-marathon: ↑ NK cell number and cytotoxicity, GRA oxidative burst ↑ After marathon: GRA and MON oxidative burst ↑, IFN-γ ↑	No significant correlation reported	After both races: Plasma antioxidant capacity ↔
Submaximal and maximal exercise under laboratory conditions						
Steensberg et al. 2002 [69]	2.5 h treadmill running at 75 % $VO_{2\,max}$. Pre-, 0.5 and 2 h post-exercise	11 healthy ♂ subjects	2 h post-exercise: % of apoptotic LYM ↑, total number of apoptotic cells ↔	LYM counts ↑ 0.5 h post-exercise, ↓ 1 – 4 h post-exercise	No significant correlation reported	F_2-IsoPs (plasma): ↑ immediately post-exercise, Cortisol: ↑ during exercise
Peters et al. 2006 [54]	2.5 h treadmill running at 75% $VO_{2\,max}$. Pre-, immediately, 3 h post-exercise	8 well-trained ♂ endurance athletes	% of apoptotic LYM ↔, SCGE (LYM) ↔	Immediately post-exercise: ↑ total LEU counts, ↑ LYM counts	No significant correlation reported	Cortisol (plasma) ↑ immediately post-exercise

Niess et al. 1996 [45]	Treadmill test until exhaustion Pre-, 15 min, 24 h post-exercise	6 trained and 5 untrained ♂	SCGE (LEU) 24 h post ↑	CK ↔ post-exercise, Total LEU counts ↑ 15 min post-exercise	No significant correlation reported	MDA (plasma) ↔ post-exercise
Mooren et al. 2002 [39]	1.) Exhaustive treadmill test at 80% $VO_{2\,max}$ 2.) Treadmill test at 60% $VO_{2\,max}$ with identical running time Before, immediately, 1 h post-exercise	12 healthy volunteers (7 ♂ and 5 ♀)	After exhaustive test: % of apoptotic LYM ↑ immediately post-exercise After test at 60% $VO_{2\,max}$: % of apoptotic LYM ↔,	After exhaustive test: ↑ LYM counts, ↑ LEU counts After test at 60% $VO_{2\,max}$: ↑ LYM counts, ↑ LEU counts	No significant correlation reported	

♂ = male subjects, ♀ = female subjects, ↑ = significant increase, ↓ = significant decrease, ↔ = no changes, LEU = leukocytes, LYM = lymphocytes, SCGE = single cell gel electrophoresis assay, FPG = formamidopyrimidine sensitive-sites, ENDO III = endonuclease-sensitive sites, CBMN Cyt = cytokinesis block micronucleus assay, NPBs = neoplasmic bridges, Nbuds = nuclear buds, MN = micronuclei, IL = interleukin, TNF-α = tumor necrosis factor α, (hs)-CRP = (high sensitive) C-reactive protein, MPO = myeloperoxidase, PMN-elastase = polymorphonuclear elastase, PMN = polymorphonuclear cells, NK = natural killer cells, GRA = granulocytes, CK = creatine kinase activity, F_2-IsoPs = F_2-Isoprostanes, CD = conjugated dienes, AOPP = advanced oxidation protein products, oxLDL = oxidized LDL, MDA = malondialdehyde, TEAC = trolox equivalent antioxidant capacity, FRAP = ferric reducing ability of plasma, ORAC = oxygen radical absorbance capacity

they found no correlation between changes in DNA migration in the SCGE assay and leukocyte counts in the 24 h after an exhaustive treadmill test [44], possibly also because the extent of the inflammatory response was relatively low following their exercise protocol. Although no immune and inflammatory parameters were measured in the study by Hartmann et al. [21], their explanations have further stimulated debate on a relationship between the activation of inflammatory cells and the occurrence of secondary tissue and DNA lesions. Based on their observations in short-distance triathletes (no indications for oxidative DNA modifications immediately post-race, but highest values within the standard SCGE assay 3 d after the competition), they suggested that DNA damage might occur as a consequence of exercise-induced injury of muscle tissue rather than acute oxidative stress during exercise [21]. The authors hypothesised that inflammatory reactions in the course of this initial muscle damage could be responsible for the transient DNA damage [21]. Indeed, there is evidence that activated neutrophils and macrophages infiltrate damaged muscle [68, 78]. Although this seems to be a beneficial response in terms of muscle repair and also muscle adaptation [33, 78], it may trigger further inflammatory processes and damage [25], in part through an enhanced formation of RONS [29].

On the basis of these findings, researchers in this field questioned whether damage to cellular DNA in the course of vigorous exercise could also induce apoptosis and whether programmed cell death, in turn, might be related to the exercise-induced regulation of leukocyte counts and, particularly, lymphocyte trafficking and distribution [53]. A decline of the total lymphocyte concentration is characteristic after exercise of prolonged duration and/or high intensity [33, 53]. Although the mechanisms of exercise-induced lymphocytopenia are still not fully understood [33], it has been suggested that this effect may account, at least partly, for the post-exercise immune dysfunction [15]. Exercise-induced changes in corticosteroids and catecholamines are known to play a major role in characteristic post-exercise alterations of leukocyte subsets [20, 41] including leukocytosis [42] as well as lymphocytopenia [53]. Previous studies indicated that the glucocorticoid concentrations observed after submaximal exercise are sufficient to induce apoptosis [23]. These observations further support the assumption of a relationship between exercise-associated induction of apoptosis and lymphocy-

topenia [53]. In response to cellular stressors that lead to DNA damage, apoptosis is vital in preventing the propagation of severely damaged DNA and in maintaining genomic stability [30] and is regarded to be required for the regulation of the immune response [39].

Mars et al. were the first to describe apoptosis in lymphocytes after exhaustive exercise (treadmill running) that was paralleled by DNA damage [34]. However, in the latter study, cell death was only investigated in three subjects and the methodology (the TdT-mediated dUTP-nick end labelling or TUNEL method) has been criticized due to its insufficient specificity [40]. Nevertheless, by the use of flow cytometry and annexin-V to label apoptotic cells, Mooren et al. [39, 40] confirmed that either short maximal exercise (in untrained subjects) [39] as well as competitive endurance exercise (a marathon run) [40] has the potential to induce lymphocyte apoptosis. This phenomenon could be explained, to a certain extent, by an up-regulation of the expression of cell death receptors and ligands [40] and an exercise-induced shift to a lymphocyte population with a higher density of these (CD95-)receptors [39]. Nevertheless, the authors concluded that the changes in the proportions of apoptotic cells after exhaustive exercise were small and, if at all, might only partially account for the concomitantly observed significant decline of lymphocytes to below baseline levels [39]. An additional finding of Mooren et al. [40] was that apoptotic sensitivity was inversely related to the training status of the marathon runners, since analysis of subgroups revealed that programmed cell death occurred only in less well-trained, but not in highly-trained athletes. Recent research in this context suggests that intensive endurance exercise does neither automatically induce apoptosis in lymphocytes nor cause DNA damage (assessed immediately and 3 h post-exercise), provided that subjects are well-trained [54]. Since there was no correlation between the (non-significant) decrease in circulating lymphocytes and the percentage lymphocyte apoptosis after a 2.5 h treadmill run at 75% $VO_{2\,max.}$, Peters et al. [54] concluded that the characteristic post-exercise lymphocytopenia is not due to apoptotic regulation by the immune system. The latter results are consistent with another study which was conducted with a similar exercise protocol, but in untrained subjects [69]. Steensberg et al. [69] noted that the lymphocytes which left the circulation during the first 2 h post-exercise were characterised by not being apoptotic. Thus, mechanisms other than apoptosis seem to play a more important role in inducing lymphocytopenia after exercise, including a redistribution of lymphocytes and/or a lack of mature cells that can be recruited [53]. Moreover, contrary to previous findings [23], recent results imply that cortisol affects the cellular immune system more by other pathways than via apoptotic regulation [54]. Furthermore, the occurrence of DNA damage in the course of exercise does not necessarily implicate induction of apoptosis [40]. Alternative cellular outcomes to prevent the propagation of DNA damage include cell cycle arrest or DNA repair [30].

In general, there is strong evidence which suggests that enhanced DNA stability and, most likely in turn, the absence of a change in the levels of apoptotic lymphocytes after strenuous exercise [54] are associated with protective adaptations due to training. As mentioned above, Mastaloudis et al. [36], reported that DNA damage in leukocytes increased temporarily mid-race of an ultra-marathon, but returned to baseline 2 h after the competition and even decreased to below

baseline values by 6 d post-race. As probable causes for this decrease in the proportion of cells with DNA damage, the authors suggested enhanced repair mechanisms, increased clearance and/or a redistribution of damaged cells [36]. Noteworthy, plasma concentrations of inflammatory parameters, F_2-isoprostanes and antioxidant vitamins were investigated in the same subjects. Although acute oxidative and inflammatory stress responses were observed [35], the authors reported no correlations between either of these markers with DNA damage [35, 36]. Furthermore, supplementation with vitamins E and C prevented increases in lipid peroxidation [35], but had no noticeable effects on DNA damage, on inflammation and on muscle damage [36]. Interestingly, there were different responses regarding oxidative stress and DNA damage in male and female runners, highlighting the importance of studying both sexes [35, 36]. In general, these findings in ultramarathon runners indicate that the mechanism of oxidative damage is operating independently of the inflammatory and muscle damage processes [35, 36, 79].

There are only few studies on the issue of DNA damage and immune and inflammatory responses in the course of exercise. Briviba et al. [4] found oxidative DNA damage parallel to an increased oxidative burst ability of granulocytes and monocytes after both a half-marathon- and a marathon race, but no correlations were detected. Again, the authors could only speculate that the exercise-induced activation of phagocytes might have contributed to the increased RONS production, oxidative DNA damage and the high percentage of apoptotic lymphocytes [4]. Furthermore, it is notable that the monitoring period of this study probably was too short to detect possible interactions between DNA alterations and immune modifications.

Findings on Exercise-induced DNA Damage and Muscle Damage

As mentioned, given the scarceness of data regarding associations between DNA modulations and inflammation in the course of exercise, we included investigations that examined exercise-induced effects on DNA together with markers of muscle damage. These studies are summarized in Table 2. Though several major stressors are needed and the integrity of the organism has to be challenged (e.g. by extremely demanding endurance exercise) [29, 42, 53, 72] to induce a *systemic* inflammatory response, it has been shown that leukocytes can explicitly be mobilised in response to muscle damage [42, 51, 74], possibly due to activation of the alternative complement pathway [51, 74]. Therefore, these studies may also reveal whether muscle damage (induced by mechanical and/or metabolic stress [25, 75]) and subsequent repair and inflammatory responses [78] are associated with DNA damage. In one of the first studies on this issue, which comprised three subjects of different gender and training history, Hartmann et al. reported a parallel increase, but no correlation between the DNA migration in the SCGE assay and plasma creatine kinase (CK) between 6 and 24 h after intense treadmill running [22]. Likewise, applying the standard SCGE assay, Palazzetti et al. [48] observed signs of increased oxidative stress and muscle damage induced by a duathlon race after 4 wk of overload training, whereas no effects on leukocyte DNA were found, probably due to efficient DNA repair. Other studies on this topic predominantly measured 8-OHdG in urine, which reflects the average rate of oxidative DNA damages in all cells of the body [58]. Consequently, changes in urinary 8-OHdG excretion after muscle-damaging exercise might largely repre-

Table 2. Studies investigating exercise-induced DNA damage and muscle damage

Reference	Experimental protocol and sampling time-points	Subjects	Endpoints of DNA damage	Markers of muscle damage (plasma)	Correlations between DNA damage and muscle damage	Main other results
Ultra-endurance exercise (> 4 h)						
Radak et al. 2000 [60]	4 d-supra-marathon (93+120+56+59 km) Pre-race, after each race d	5 well trained ♂ runners	8-OHdG (urine, ELISA) ↑ after d 1, thereafter trend to decrease, back to baseline after d 4	CK ↑ after d 1, further increasing until d 3, and decreasing thereafter	No significant correlation reported	
Miyata et al. 2008 [37]	2 d-ultra-marathon race (42+90km) Pre-, 1 d, 2 d post-race	79 ♂ and 16 ♀non-professional runners	8-OHdG (urine, HPLC-ECD) ↑ 1 d post-race, back to baseline after 2 d post-race	CK ↑, myoglobin ↑ 1 and 2 d post-race	No significant correlation reported	
Competitive endurance exercise (< 4 h)						
Tsai et al. 2001 [80]	Marathon Pre-, immediately, 1, 3, 7, 14 d post-race	14 ♂ runners, 20 sedentary, healthy ♂	SCGE (LYM) ↑ 1 – 14 d post-race, FPG ↑ immediately 1 d post-race, ENDO III ↑ 1 – 7 d post-race, 8-OHdG (urine, ELISA) ↑ 1 – 14 d post-race	CK ↑ immediately – 7 d post-race	Significant correlations between ENDO III, 8-OHdG and CK	LPO (plasma): 1 – 14 d post-race, Significant correlations between LPO and all markers of DNA damage
Periods of intensified training						
Okamura et al. 1997 [47]	30±6km/d for 8 d Urine samples throughout the training period, 3 d control	10 ♂ long-distance runners	8-OHdG (urine, HPLC-ECD) ↑ throughout the training period compared to control period	After the training period: CK ↑, myoglobin ↑	No significant correlation reported	
	(sedentary) period, blood samples before and after the training period					
Palazzetti et al. 2003 [48]	4 wk of overloaded training	9 ♂ triathletes, 6 ♂ sedentary subjects	SCGE (LEU) ↔ after training period	After training period: myoglobin ↔, CK ↑	No significant correlation reported	After training period: TBARS ↔, GSH/GSSG ↔, Total antioxidant status ↓ (all plasma)
Submaximal and maximal exercise under laboratory conditions						
Umegaki et al. 1998 [82]	30 min treadmill running at 85% $VO_{2\,max}$. Pre-, immediately and 30 min post-exercise	8 untrained and 8 endurance-trained ♂ subjects	MN (LYM) ↔ immediately and 30 min post-exercise	CK ↔ immediately and 30 min after the test	No significant correlation reported	
Sacheck et al. 2003 [65]	45 min downhill running at 75% $VO_{2\,max}$. Pre-,immediately, 6 h, 1 d and 3 d post-exercise (before and after supplementation with vitamin E)	16 young and 16 older physically active healthy ♂	8-OHdG (LEU, HPLC-ECD, measured pre- and 1 d post-exercise) ↔ 1 d post-exercise	CK ↑ immediately post-exercise and peak: 1 d post-exercise	No significant correlation reported	MDA (plasma) ↑ immediately post-exercise, F_2-IsoPs (plasma): ↑ 3 d post-exercise, ORAC: ↓ 3 d post-exercise
Hartmann et al. 1994 [22]	1.) Treadmill test until exhaustion, 2.) Intense treadmill running	2 ♂, 1 ♀ healthy subjects (untrained – trained)	SCGE (LEU) After exhaustive test: ↑ 6 h, maximum 1 d post-exercise, after 3 d back to	CK ↑ between 6 h and 1 d after exhaustive test ↔ after 45 min of treadmill running	No significant correlation reported	
	(45 min) Pre-, 6 min, 6 h, 1 – 4 d post-exercise		initial levels, ↔ after 45 min of treadmill running SCE ↔ after both tests			

♂ = male subjects, ♀ = female subjects, ↑ = significant increase, ↓ = significant decrease ↔ = no changes, LEU = leukocytes, LYM = lymphocytes, 8-OHdG = 8-oxo-7,8-dihydro-2-deoxyguanosine, ELISA = enzyme linked immuno assay, HPLC-ECD = high performance liquid chromatography with electrochemical detection, SCGE = single cell gel electrophoresis assay, FPG = formamidopyrimidine sensitive-sites, ENDO III = endonuclease-sensitive sites, SCE = sister chromatid exchange assay, MN = micronucleus assay, polymorphonuclear elastase, PMN = polymorphonuclear cells, NK = natural killer cells, GRA = granulocytes, CK = creatine kinase activity, LPO = lipid peroxidation products, TBARS = thiobarbituric acid reactive substances, GSH/GSSG = reduced vs. oxidized glutathione, F_2-IsoPs = F_2-Isoprostanes, MDA = malondialdehyde, ORAC = oxygen radical absorbance capacity

sent DNA damage of skeletal muscles [60]. Radak et al. [60] and Miyata et al. [37] determined urinary 8-OHdG levels and markers of muscle damage in competitors of ultra-marathon events which lasted 2 [60] and 5 d [37], respectively. No propagation of oxidative DNA damage was observed after the first race d in both studies [37, 60]. Interestingly, 8-OHdG significantly decreased to levels below their peak values during the race on the second d [37], and on the fourth race d [60], respectively. Both research groups suggested that a rapid induction of antioxidant and repair systems occurred [37, 59]. In contrast, parameters for muscle damage continuously increased during the 2-d-race period [37] and until the third d of the 4-d-race [60], and no correlations were reported with 8-OHdG. Taken together, these data may show that, even if myofibrillar injury occurs, an adaptive up-regulation of repair and nucleotide sanitization mechanisms is capable of preventing further damage of DNA. Consistently, no correlations between biomarkers of DNA- and muscle damage were reported after a period of intensified training (despite that both 8-OHdG and muscle damage markers were found to be increased) [47] or downhill running on a treadmill [65]. However, given that 8-OHdG levels remained unchanged, but were measured only until 1 d post-race, the authors of the latter investigation noted that oxidative DNA damage probably had occurred in the period between the first and the third d after exercise, when some links amongst circulating oxidative stress markers and CK activity were observed [65].

The prolonged monitoring period after a marathon race in an investigation by Tsai and co-workers [80] might account for the observed significant correlations between peak levels of ENDO III-sensitive sites and urinary 8-OHdG on the one side and plasma parameters of muscle damage and lipid peroxidation on the other. In agreement with the conclusions of previous investigations [21, 44], the authors suggested that inflammatory cells infiltrating into injured skeletal muscle tissue and activated phagocytes were responsible for the increased production of RONS and consequently the delayed oxidative DNA damage during the reparative processes after the marathon [80]. This idea is supported by a study in rats, in which DNA damage in circulating white blood cells was closely related to muscle damage due to exercise [81]. Nevertheless, based on these findings it is not possible to draw a clear conclusion as to whether oxidative DNA modifications in peripheral immuno-competent cells are casually related with immune disturbances or whether DNA damage in leukocytes, in fact, results from oxidative stress that occurs through inflammatory processes after strenuous exercise.

Purpose of the Current Study in Ironman Triathletes

The data presented here are part of a larger study that aimed to comprehensively examine certain stress and recovery responses to an Ironman triathlon race. One primary aim of the study was to test the hypothesis whether there is a relationship between indices of muscle damage and/or inflammatory stress and endpoints of DNA damage in lymphocytes, which were assessed by the SCGE- and the CBMN Cyt assays for the first time in the course of competitive exercise of such duration. Furthermore, by concomitantly exploring oxidative stress markers and antioxidant-related factors, we aimed to particularize a potential interaction of oxidative stress between inflammatory and DNA responses.

MATERIALS AND METHODS

The study design has been described previously [28, 42]. Briefly, the study population comprised 48 non-professional, well-trained healthy male triathletes, who participated in the 2006 Ironman Austria. Forty-two of them (age: 35.5 ± 7.0 yr, height: 180.6 ± 5.6 cm, body mass: 75.1 ± 6.4 kg, cycling $VO_{2\ peak}$: 56.6 ± 6.2 ml kg^{-1} min^{-1}, weekly net endurance exercise time: 10.7 ± 2.6 h) completed the study and were included in the statistical analysis to investigate inflammatory and immuno-endocrine responses as well as muscle damage [42]. The physiological characteristics of the study participants (assessed on a cycle ergometer three weeks before the competition), information on their training over a period of six months prior to the race, their performance in the Ironman triathlon as well as the only moderate ("recovery") training thereafter have been presented in detail elsewhere [42, 43]. Of the entire study group 20 and 28 subjects were randomly selected for the CBMN Cyt and the SCGE assays, respectively [62, 63]. Consequently, these randomized subjects were included in the data analysis for the results that are exclusively provided within this report. All participants of the study did not take any medication or more than 100% of RDA of antioxidant supplements (in addition to their normal dietary antioxidant intake) in the six weeks before the Ironman race until the end of the study. The Ironman triathlon took place in Klagenfurt, Austria on July 16th 2006 under near optimal climatic conditions and consisted of 3.8 km swimming, 180 km cycling and 42.2 km running. Blood samples were taken 2 d pre-race, immediately (within 20 min), 1, 5 and 19 d post-race.

The samples were immediately cooled to 4°C and plasma separated at 1711 * g for 20 min at 4°C and aliquots for the measurement of biochemical parameters were frozen at –80°C until analysis. For the analysis of DNA and chromosomal damage in lymphocytes, blood samples were processed instantly as described previously [62, 63]. Blood samples were analysed for haematological profile, plasma creatine kinase (CK) activity, plasma concentrations of myoglobin, interleukin (IL)-6, IL-10, high-sensitivity C-reactive protein (hs-CRP), myeloperoxidase (MPO), polymorphonuclear (PMN) elastase, cortisol and testosterone (see [42]). All these values (except for the steroid hormones) were adjusted for exercise-induced changes in plasma volume [11]. As reported previously [62, 63], the SCGE and CBMN Cyt- assays were carried out according the methods described by Tice et al. [77] and Fenech [17], respectively. Within the SCGE-assay, oxidative DNA base damage was assessed on the basis of the protocols of Collins et al. [7], Collins and Dusinska [6] and Angelis et al. [1]. Analysed endpoints within the SCGE assay included: 1.) determination of DNA migration under standard conditions to measured single and double strand breaks (determined as percentage of DNA in the tail), and 2.) ENDO III and FPG to detect oxidized pyrimidines and purines, respectively. Biomarkers within the CBMN Cyt block included the number of 1.) MN, 2.) NPBs, 3.) Nbuds, and 4.) necrotic and apoptotic cells.

All statistical analyses were performed using SPSS 15.0 for Windows. Details of the data analysis has been presented previously [42, 62, 63]. For the additional correlation analysis that is reported in this article, Pearson ´s correlation was used to examine significant relationships. In case of observed trends or significant correlations, subjects were divided into percentile groups by the asso-

ciated variables (e.g. IL-6). One-factorial ANOVA *and post* hoc analyses with Scheffé´s test were then applied to assess whether differences in endpoints of DNA or chromosomal damage were associated with the percentile distribution. Significance was set at a P-value <0.05 and is reported P<0.05, P<0.01 and P<0.001.

RESULTS

Race Results

The average completion time of the whole study group was 10 h 52 min ± 1 h 1 min (mean ± SD). The estimated average antioxidant intake during the race was 393 ± 219 mg vitamin C and 113 ± 59 mg alpha-tocopherol. There were neither significant differences in the performance nor in the consumed antioxidants between the whole study group and the subgroups that were tested for genome stability.

DNA and Chromosomal Damage, Apoptosis and Necrosis

As previously reported [62, 63] and briefly discussed above, the results concerning DNA and chromosomal damage were as follows: Within the CBMN Cyt assay, the number of MN significantly (P<0.05) decreased immediately post-race, and declined further to below pre-race levels 19 d after the Ironman competition (P<0.01). There were no changes in the frequency of NPBs and Nbuds as an immediate response to the triathlon, but 5 d thereafter the frequency of Nbuds was significantly (P<0.01) higher than levels immediately post-race. However, 19 d post-race the frequency of Nbuds returned to pre-race levels, while the number of NPBs was significantly (P<0.05) lower than pre-race [62].

The overall number of apoptotic cells decreased significantly (P<0.01) immediately post-race, and declined further until 19 d after the race (P<0.01). Similarly, the overall number of necrotic cells significantly (P<0.01) declined immediately post-race, and remained at a low level 19 d after the Ironman. Within the SCGE assay, a decrease was observed in the level of strand breaks immediately after the race. One day post-race the levels of strand breaks increased (P<0.01), then returned to pre-race 5 d post-race, and decreased further to below the initial levels 19 d post-race (P<0.01). Immediately post-race there was a trend in ENDO III and FPG-sensitive sites to decrease. The ENDO III-sensitive sites significantly (P<0.05) increased 5 d post-race compared to 1 d post-race, but levels decreased until 19 d (P<0.05). No significant changes were observed in the levels of FPG-sensitive sites throughout the monitoring period [63].

Immune-endocrine and Inflammatory Responses, and Plasma Markers of Muscle Damage

Briefly, as described in details elsewhere [42], there were significant (P<0.001) increases in total leukocyte counts, MPO, PMN elastase, cortisol, CK activity, myoglobin, IL-6, IL-10 and hs-CRP, whereas testosterone significantly (P<0.001) decreased compared to pre-race. Except for cortisol, which decreased below pre-race values (P<0.001), these alterations persisted 1 d post-race (P<0.001, P<0.01 for IL-10). Five days post-race CK activity, myoglobin, IL-6 and hs-CRP had

decreased, but were still significantly ($P<0.001$) elevated. Nineteen days post-race most parameters had returned to pre-race values, with the exception of MPO and PMN elastase, which had both significantly ($P<0.001$) decreased below pre-race concentrations, and myoglobin and hs-CRP, which were slightly, but significantly higher than pre-race [42].

Associations between Endpoints of Genome Stability and Immuno-endocrine, Inflammatory and Muscle Damage Parameters

No significant correlations were found between all these markers at all time-points with the exception of a link between IL-6 and necrosis. Immediately post-race, the plasma concentration of IL-6 correlated positively with the number of necrotic cells ($r=0.528$; $P<0.05$). In addition, significant associations were observed on the basis of a group distribution into percentiles by the IL-6 concentrations immediately post-race. First, the numbers of necrotic cells increased with IL-6 across the percentiles, and the differences between all groups were $P=0.012$. Second, necrosis in lymphocytes was significantly ($P=0.017$) higher in the subject group with the highest IL-6 concentrations (top percentile) compared with the lowest IL-6 values (lowest percentile).

DISCUSSION

A major finding of the present investigation is that there were no correlations between different markers of DNA and chromosomal damage and parameters of muscle damage and inflammation in participants of an Ironman triathlon as a prototype of ultra-endurance exercise with the exception of a link between IL-6 and necrosis. The conclusions that can be drawn from these results are several. Overall, the current data indicate that DNA damage is neither causally involved in the initial systemic inflammatory response nor in the low-grade inflammation that was sustained at least until 5 d after the Ironman race [42]. Instead, based on several assessed relationships between leukocyte dynamics, cortisol, muscle damage markers and cytokines [42], the pronounced but temporary systemic inflammatory response was most likely induced by stressors other than DNA modulations. In fact, consistent with previous studies in this context, factors such as the initial ultra-structural injury of skeletal muscle [51, 74], changes in concentrations of cortisol [53] and IL-6 [71] apparently mediated leukocyte mobilization and activation [42]. Furthermore, although the temporary increased frequency of ENDO III-sensitive sites 5 d after the Ironman competition was found simultaneously with the moderate prolongation of inflammatory processes, correlations between hs-CRP and markers of muscle damage suggest that the latter phenomenon was rather related to incomplete muscle repair [42].

In addition, missing links between all these markers in the present study indicate that exercise-induced inflammatory responses *vice versa* do not promote DNA damage in lymphocytes. These results support those of Mastaloudis et al., who demonstrated that inflammatory and muscle damage responses, indeed, do not *directly* interact with the mechanisms of oxidative DNA damage [35, 36, 79]. Nevertheless, this does not rule out the possibility that inflammatory processes can trigger oxidative stress via oxidative burst reactions of circulating neutrophils

and an increased cytokine formation [15, 25, 29, 50, 73], which in turn might lead to secondary (oxidative) DNA damage in immuno-competent cells [80]. In fact, we observed correlations between markers of oxidative stress and inflammatory parameters (unpublished results) that might point to muscular inflammatory processes as a source of the moderate oxidative stress response 1 d after the Ironman triathlon. Nevertheless, we have recently demonstrated in the same study participants that training- and acute exercise-induced responses in the antioxidant defence system were able to counteract severe or persistent oxidative damage post-race. Despite a temporary increase in protein oxidation and lipid peroxidation markers immediately and 1 d post-race (except for oxidized LDL concentrations, which actually decreased), all these markers had returned to pre-race values 5 d post-race [43]. Concomitantly, there was an increase in the plasma antioxidant capacity following the Ironman triathlon (assessed by the trolox equivalent antioxidant capacity- (TEAC), the ferric reducing ability of plasma- (FRAP), and the oxygen radical absorbance capacity (ORAC)-assays) [43, 63]. These strong antioxidant responses most likely played a significant role in counteracting sustained oxidative stress post-race in the current study, while it seems that antioxidant defences in the study group of Tsai et al. [80] were not sufficient to confer protection against delayed oxidative damage to lipids and DNA due to reparative processes of muscular tissue. Whatever the reasons for these discrepancies in the oxidant/antioxidant balance are (differences in training-induced biochemical adaptations, antioxidant status and/or antioxidant intake during the race, etc.), this might be a major explanation for the inconsistencies between the findings of Tsai et al. [80] and ours [43, 64, 62]. In fact, the observed negative correlations between the ORAC and ENDO III-sensitive sites immediately and 1 d after the Ironman race suggest that an enhanced plasma antioxidant capacity might have prevented oxidative DNA damage [63]. These findings are in line with a recent animal study [2], which demonstrated the protective role of an enhanced serum antioxidant capacity in lymphocyte apoptosis. Taken together, whenever correlations between DNA damage in immuno-competent cells and inflammation [44] or muscle damage [80] were observed, RONS derived from inflammatory cells, appear to be the key effectors that link inflammation with DNA damage after vigorous exercise. Fig. 1 is a schematic illustration of the relationships between these stress responses to exhaustive endurance exercise. It may be argued that results from our study fit well into this picture insofar that antioxidant mechanisms neutralized an enhanced generation of RONS potentially resulting from inflammatory processes due to the injury of skeletal muscle tissue, and consequently were able to prevent lymphocyte DNA damage. It should also be noted that, similar to DNA effects, muscle inflammatory processes and related oxidative stress responses might be sustained for or appear days after muscle-damaging exercise [46]. Hence, potential links between these outcome measures might have been missed in investigations with shorter monitoring periods [4, 40, 54, 65, 69]. Beyond, it is important to note in this context that there is an additional difficulty in determining correlations between markers of oxidative DNA damage and other biomarkers of oxidative stress, partly due to differences in the biological sites where oxidative damage occurred [12].

The observed association between IL-6 concentration and the number of necrotic cells immediately post-race in the present study may indicate that lym-

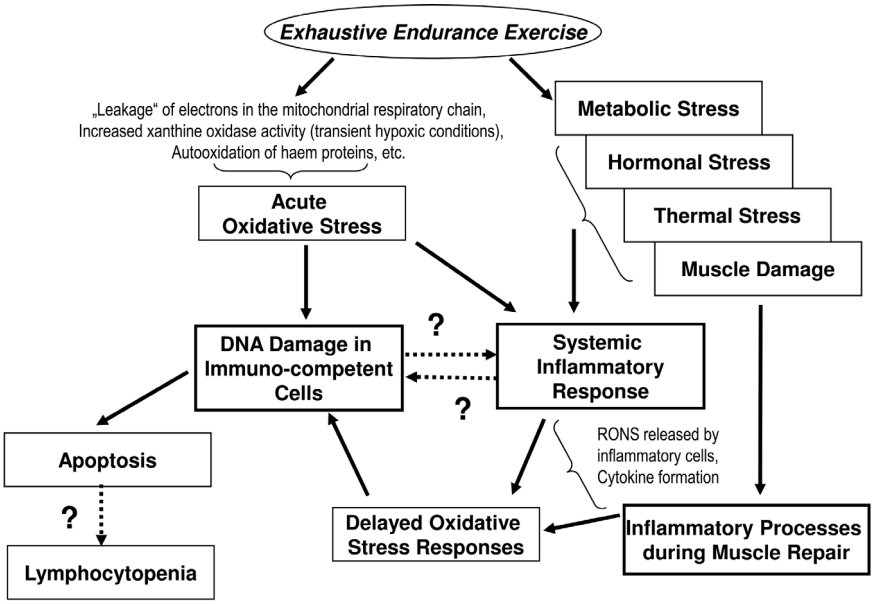

Fig. 1: Proposed model of exercise-induced DNA damage and inflammatory responses

phocytes partly undergo an unregulated cell death in athletes experiencing an overshooting inflammatory response. Based on recent research on the role of IL-6 in exercise [15, 19, 52], it is questionable whether IL-6, probably released by contracting muscles [19, 52], directly modulates necrosis in lymphocytes. In this case, plasma IL-6 concentrations may just serve as a marker for the pronounced initial systemic inflammatory response. However, the (patho-)physiological relevance of this association cannot be generalised based upon the present results, since the overall number of necrotic cells declined significantly to below pre-race values after the acute bout of ultra-endurance exercise, and remained at these levels at all time-points investigated [63]. Similarly, as to the decrease of necrosis, we demonstrated that levels of apoptosis also decreased immediately after the Ironman race, again remaining at these low levels throughout the whole monitoring period [63]. Crucially, our data revealed no link between apoptosis and post-race changes in lymphocyte counts. Mooren et al. [40] reported an initial increase in apoptotic cells in the whole group of marathon runners, but corresponding with the findings in the current study, lymphocyte apoptosis declined 1 d after the race. In agreement with the decrease of DNA damage after an ultra-marathon run [36], these findings might alternatively be explained by an overshooting removal of apoptotic leukocytes by phagocytic cells in order to protect tissue from overexposure to inflammatory and immunogenic contents of dying cells [31, 40]. Based on the concept that the phagocytic clearance of apoptotic immuno-competent cells plays a critical role in the resolution of inflammation [31, 83], this could be a further explanation for the lack of a link between inflammatory responses on the one hand, and DNA damage and/or apoptotic cell death on the other hand.

Finally, a reason that may also account for the lack of correlations within most of the few studies that have addressed this issue is that the majority of these

investigations have been conducted in trained individuals [21, 36, 37, 47, 48, 54, 60, 62]. Accumulating evidence points to adaptations in protective mechanisms due to (endurance) training - including improved endogenous antioxidant defences and enhanced repair mechanisms [59] - that appear to be responsible for maintaining genome integrity in immuno-competent cells in response to extremely demanding endurance exercise. While these protective mechanisms were suggested to prevent DNA damage and/or apoptosis in a number of studies [37, 40, 45, 48, 54, 60, 62], several other exercise-associated factors induce and mediate a systemic inflammatory response [15, 53]. This indirectly further implies that DNA damage in immuno-competent cells, if it occurs at all, might *not* be a major determinant of exercise-induced inflammation.

CONCLUSION

Thus far, there is only little evidence concerning a direct relationship between DNA damage and inflammatory responses after strenuous prolonged exercise. The most conclusive picture that emerges from the available data is that oxidative stress seems to be the main link between exercise-induced inflammation and DNA damage. Considering the very few studies in which markers of DNA damage were found to correlate with signs of inflammation or muscle damage, DNA damage in peripheral immuno-competent cells, indeed, most likely resulted from an increased generation of RONS due to initial systemic inflammatory responses or the delayed inflammatory processes in response to muscle damage (Fig. 1). The lack of correlations between these exercise-induced responses in most of the studies might also be explained by the fact that the monitoring period was too short. Hence, particular attention should be paid to the characteristic time-course of inflammatory and oxidative stress events on the one hand and DNA effects on the other hand. Though obvious differences exist in the manifestation and outcomes a comparable relationship is reported in patho-physiological conditions including carcinogenesis, where (chronic) inflammation induces DNA damage and mutations via oxidative stress [13]. However, there might be further mechanisms that link exercise-induced DNA modulations, inflammatory responses and RONS. It has been shown, that redox-sensitive signal transduction pathways including nuclear factor (NF) κB or p53 cascades are involved in inflammation as well as "cell stress management" in response to DNA damage [24, 30]. Recent explorations of the gene expression responses to exercise have already shed a light on hitherto unknown molecular mechanisms in exercise immunology [5, 9, 14, 61, 84, 85]. In the future, the combination of these powerful modern techniques *(transcriptomics, proteomics)* with state-of-the-art biochemical biomarkers should therefore enable researchers in this field to provide novel insights into potential further interactions between genome stability and inflammation.

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