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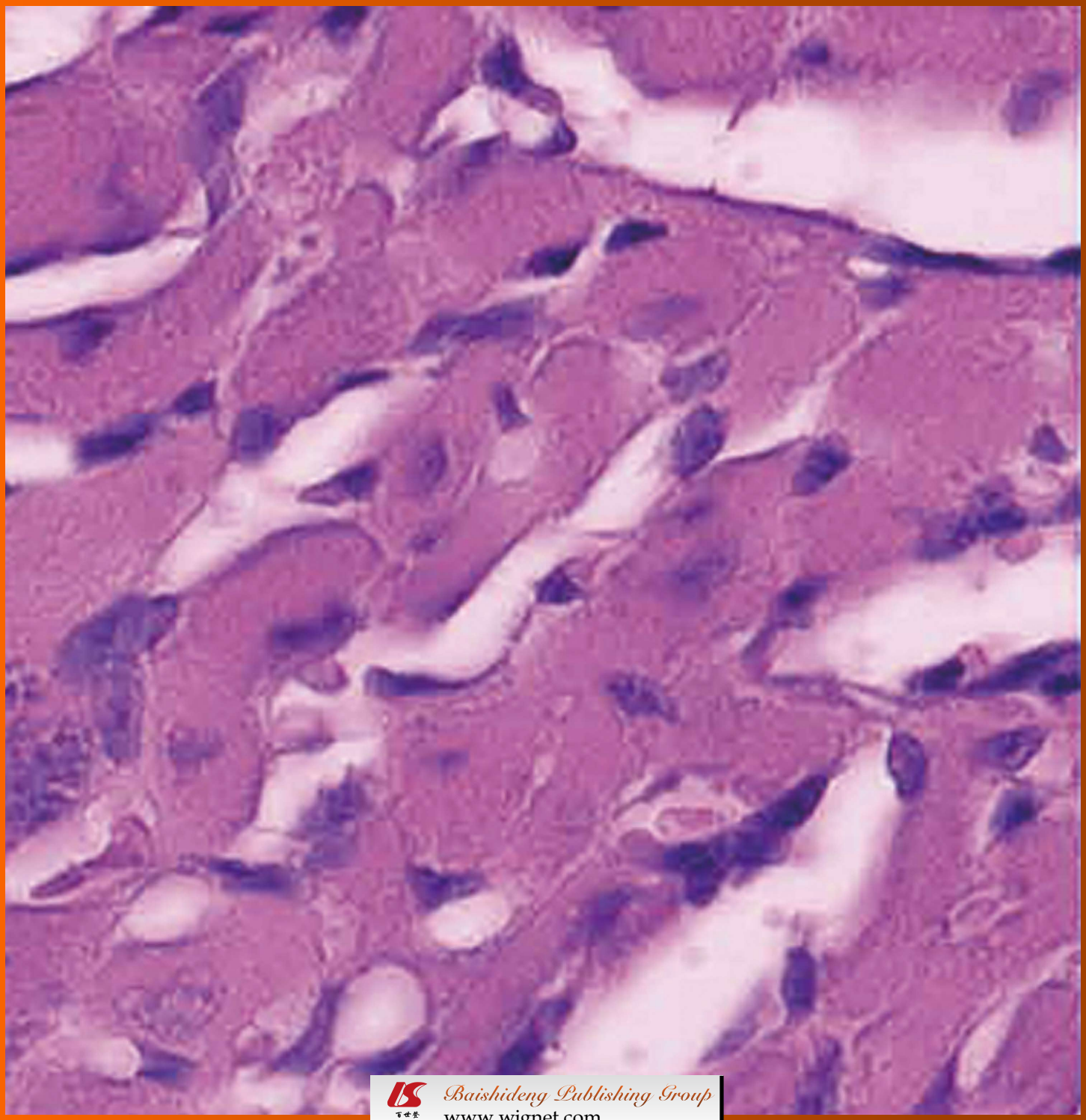

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MINIREVIEW

Modulation of immune response in experimental Chagas disease

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Abstract

Trypanosoma cruzi (*T. cruzi*), the etiological agent of Chagas disease, affects nearly 18 million people in Latin America and 90 million are at risk of infection. The parasite presents two stages of medical importance in the host, the amastigote, intracellular replicating form, and the extracellular trypomastigote, the infective form. Thus infection by *T. cruzi* induces a complex immune response that involves effectors and regulatory mechanisms. That is why control of the infection requires a strong humoral and cellular immune response; hence, the outcome of host-parasite interaction in the early stages of infection is extremely important. A critical event during this period of the infection is innate immune response, in which the macrophage's role is vital. Thus, after being phagocytized, the parasite is able to develop intracellularly; however, during later periods, these cells induce its elimination by means of toxic metabolites. In turn, as the infection progresses, adaptive immune response mechanisms are triggered through the TH1 and TH2 responses. Finally, *T. cruzi*, like other protozoa such as *Leishmania* and *Toxoplasma*, have numerous evasive mechanisms to the immune response that make it possible to spread around the host. In our Laboratory we have developed a vaccination model in mice with *Trypanosoma rangeli*, nonpathogenic to humans, which modulates the immune response to infection by *T. cruzi*, thus protecting them. Vaccinated animals showed an important innate response (modulation of NO and other metabolites, cytokines, activation of macrophages), a strong adaptive cellular response and significant increase in specific antibodies. The modulation caused early elimination of the parasites, low parasitaemia, the absence of histological lesions and high survival rates. Even though progress has been made in the knowledge of some of these mechanisms, new studies must be conducted which could target further prophylactic and therapeutic trials against *T. cruzi* infection.

Key words: *Trypanosoma cruzi*; Chagas disease; Innate and adaptive immune response

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INTRODUCTION

Trypanosoma cruzi (*T. cruzi*), the etiological agent of Chagas disease, affects nearly 2 500 000 people in Argentina and 18 million in Latin America. The parasite presents two stages of medical importance in the host, the amastigote, intracellular replicating form, and the extracellular trypomastigote, the infective form. That is why control of the infection requires a strong humoral and cellular immune response; hence, the outcome of host-parasite interaction in the early stages of infection is extremely important. In humans the disease presents different clinical and immunological periods: the acute period, characterized by the presence of trypomastigotes in the bloodstream, associated with immunosuppressive phenomena[1], which is asymptomatic in 95% of cases[2] and remits spontaneously, to enter in a second indeterminate phase, after 3

or 4 mo, which can last the rest of the host's life with no clinical signs. It is characterized by low parasitaemia and positive serology and, in later years, approximately 30% of infected people develop some degree of cardiac or digestive pathology in the chronic period of infection. This is attributed to direct action of the parasite, or to autoimmune reactions induced by *T. cruzi*. Gironès *et al*[3] critically reviewed the evidence in favour of and against autoimmunity through molecular mimicry as responsible for Chagas disease pathology from clinical, pathological and immunological perspectives. Also in this sense, Bonney *et al*[4] observed that vaccination with heat-killed *T. cruzi* induces the development of autoimmunity *via* molecular mimicry and other mechanisms and potentially fatal cardiomyopathy. Their results show that exposure to *T. cruzi* antigen alone is sufficient to induce autoimmunity and cardiac damage, yet additional immune factors, including a dominant TH1/TH17 immune response, are likely required to induce cardiac inflammation.

Immune response to *T. cruzi* is highly complex and involves many components, both effectors and regulators. The unspecific immunosupression that occurs during the first stage of the infection and *T. cruzi*'s capacity to adapt and evade this response allow it to invade cells and spread, which means that the parasite may remain indefinitely in the host's tissues because it is not completely eliminated[1].

The process in which trypomastigotes enter the host's cells involves several stages: initial parasite-cell contact, trypomastigote adhesion, early induction of immune response, which causes modifications to the membrane proteins. The parasite has been claimed to enter the host' s cell using a variety of mechanisms: (1) it enters professional phagocytic cells by phagocytosis; (2) the cellular membrane emits pseudopodia, modifications are produced in the actin filaments, and protein tyrosine kinases such as PI-3 are activated; this process culminates with the formation of a parasitophorous vacuole and soon after lysosomes and endosomes are recruited[5]; (3) it enters non phagocytic cells by means of endocytosis but there is no emission of pseudopodia in the host's cell; and (4) another mechanism involves direct penetration by the parasite in the cell by means of membrane invagination, with an important intake of energy[6].

After entering, the parasite lodges in the cytoplasmic vacuolar compartment where a gradual differentiation process occurs from trypomastigote to amastigote[7,8], and the latter divide by means of binary fission, to then become trypomastigotes once again; they leave the cell to spread *via* lymph and blood, and infect other cells in which they once again go through the replication cycle. *T. cruzi* primarily infects cells belonging to the reticuloendothelial system, nerve and muscle tissue, including cardiac fibres[9].

In order to progress with regard to knowledge of the immune response set off by *T. cruzi* infection and to analyze whether it is possible to modulate this complex response, several experimental models have been developed. A model for vaccinating mice with *Trypanosoma rangeli* (*T. rangeli*), a parasite closely related to *T. cruzi*, but nonpathogenic to humans[10-12], has been designed in our laboratory[13]. *T. rangeli* shares areas of geographical distribution, epidemiological characteristics, and antigenic and immunogenic components with *T. cruzi*. Specific diagnosis becomes difficult by means of classical serological methodologies because it induces a response of crossed antibodies. Moreover, both parasites cannot be morphologically differentiated[14-16]. The antigenic similitude between *T. rangeli* and *T. cruzi* has been shown by means of different methods by numerous research groups[17-20].

T. rangeli presents an enzyme, sialidase, with neuraminidase activity which is fundamentally expressed in the epimastigote stage and, unlike *T. cruzi*, does not present transialidase. Recent studies show that the sialidase system is very complex and can take on different expressions in different strains of the parasite, owing to genetic mutations[21]. In addition, it induces a complex modulation of the immunological mechanisms of the infected vector (*Rhodnius* genus) causing a reduction in the production of soluble mediators such as nitric oxide, oxygen free radicals, and the inhibition of phagocytosis as well as humoral response, among others, which favours the development of the parasite and results in the death of the vector[11].

The strategy of vaccinating with a parasite that is nonpathogenic to humans is based on the fact that, in the event of the future development of a vaccine for human use, and accepting the role played by autoimmune mechanisms in the pathology of Chagas disease, the possible induction of auto-aggression due to vaccination must be avoided[3,4].

In our experimental model, two groups of mice were used, one vaccinated with *T. rangeli* (at least $n = 6$ in each experiment) and then challenged by *T. cruzi*, and another group of control animals ($n = 6$), which were only infected with *T. cruzi*. A fixed number of 1500 virulent parasites were used to infect and the starting time of the infection was determined.

We observed that previously vaccinated mice showed very low parasitaemia, high survival rates and an absence of histological and autoimmune lesions, while mice that were only infected showed high parasitaemia, high mortality and severe histopathological alterations in the heart, skeletal muscle, spleen and liver[13,22,23]. For histological studies, mice from each group: vaccinated with *T. rangeli* and afterward challenged with *T. cruzi* (V) ($n = 6$) and non-vaccinated but infected with *T. cruzi* (I) ($n = 6$), were killed with ether anesthesia. Hearth, spleen, liver and skeletal muscles from the quadriceps were immediately removed from each mouse, fixed in buffered, 10% formalin (pH 7.0), and embedded in paraffin wax. One-half of each organ was cut into 5-μm-thick sections, and they were stained with haematoxylin-eosin. At least 20 areas from each section were checked for parasites and histopathology under a 40-x objective in a blind study.

The Figures 1 and 2 show a representative experi-

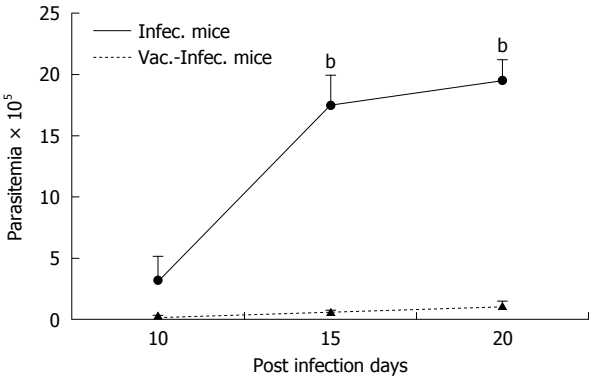

Figure 1 Parasitemia levels (geometric mean ± SE) in *Trypanosoma cruzi* infected mice (I) and in mice previously vaccinated with *Trypanosoma cruzi* and challenged with *Trypanosoma cruzi* (V-I). The differences in parasitemia levels were evaluated by *t*-test: $^{b}P < 0.001$.

ments. Similar results were obtained with two strains of *T. rangeli* from different origins, isolated in Colombia and Brazil, which revealed that the capacity to protect mice against lethal infection by *T. cruzi* is a characteristic common to different strains of *T. rangeli*. This result represents a clear advantage for the future preparation of possible vaccines for animal or human use[24].

On the other hand, it was demonstrated[25] that, in the acute period of experimentally infected mice, *T. cruzi* induces a response that presents different patterns in each different immune system compartment, splenomegaly, lymphoid subcutaneous tissue expansion, persistent polyclonal activation of lymphocyte T and B, and at the same time, thymus and mesenteric node atrophy.

A critical event during early stages of the infection is the innate immune response, in which the macrophage' s role is vital. Thus, after being phagocytized, the parasite is able to develop intracellularly; however, during later periods, these same cells induce its elimination by means of toxic metabolites. In turn, as the infection progresses, adaptive immune response mechanisms are triggered through the TH1 (cellular) and TH2 (humoral) responses.

INNATE IMMUNE RESPONSE

Soluble mediators and cells

Early in the infection, *T. cruzi* induces an intense inflammatory response, which plays a crucial role in the disease's pathogenesis. In experimental models, some of the immunological events that take place during the first few hours after infection are known. Indeed, it has been observed that *T. cruzi* antigens induce activation of the natural killer (NK) cells prior to expansion of T lymphocytes[26]. During this stage the macrophages induce a cascade of cytokines: initially they produce interleukin (IL)-12, which acts on NK cells to induce the production of interferon (IFN)γ, which in turn increases the production of IL-12, tumor necrosis factor (TNF)α and NO in the macrophage, thus contributing to the elimination of the parasite[27]. At the same time, both types of cells synthesize regulatory cytokines such as IL-10 and IL-4 to reduce the harmful effects associated with excess stimulation of the immune system[28]. In very early stages of the infection, components of *T. cruzi*, including its DNA and membrane glycoconjugates, trigger the innate response through their interaction with Toll like Receptors: TLR2, TLR4 and TLR9 in macrophages and dendritic cells. After activation, both cells secrete cytokines and chemokines, and increase the expression of their co-stimulatory molecules, inducing endocytosis and intracellular death. As mentioned above, an adequate production of proinflammatory cytokines such as IFNγ, TNFα, IL-1, IL-12, IL-6 and IL-18 is essential for controlling infection by intracellular parasites[29]. Meanwhile, it has been observed that IL-17 might regulate the recruitment of inflammatory cells and the differentiation of TH1 in heart tissue[30]. Therefore, in order to resolve the *T. cruzi* infection, a balance is necessary between the immune response mediated by TH1 and by TH2[31]. TH1 cells are responsible for the production of inflammatory cytokines, while TH2 cells have an anti-inflammatory function and are involved in the antibody mediated response. IL-12 and IL-18 produced by dendritic cells and macrophages promote the development of TH1 cells that produce IFNγ, while IL-4 induce the expansion of TH2 cells and of high amounts of IL-10. As a result of this process, regulation of the cellular response occurs due to a reduction in the activation of dendritic cells and in the macrophage's microbicidal activity. In addition, IL-4 takes part in inducing transforming growth factor (TGF)β which regulates the activity of the antigen presenting cells and enhance the susceptibility of infection by *T. cruzi*[32].

In this sense, we observed in our experimental model[31,33] (n = 6 for each group and each experiment performed) that vaccinated animals had a significant increase of IL-12, down regulation of the proinflammatory cytokines, IL-6, IFNγ, TNFα, and increase of soluble TNF receptors sTNFIR and sTNFIIR, which inhibit the deletereous activity of TNFα, in accord with Camargo *et al*[27]. Also Chandrasekar *et al*[34] detected proinflammatory cytokine production (IL-6, TNF-α, IL-1β) in the myocardium of *T. cruzi* infected mice, which suggests the probable involvement of the production of these molecules *in situ* in the injury of the myocardial function. The diminished production of proinflamatory cytokines in the immunized group of our model, associated with higher survival rates, suggests that both, IL-6 and TNF-α, are probably involved in the fatal outcome of the infected mice.

The high IFN-γ production during acute *T. cruzi* infection has been also widely demonstrated[35,36]. This finding is generally associated with protective effects since IFN-γ enhances trypanocidal activity of the macrophages *via* a nitrogen oxide mediated mechanism[37,38]. In this sense, we have observed a high production of IFN-γ in both vaccinated and control experimental groups. This finding is in agreement with Reed *et al*[39], who detected high IFN-γ levels in both susceptible and resistant mice. Moreover, in our mentioned works it was observed that in immu-

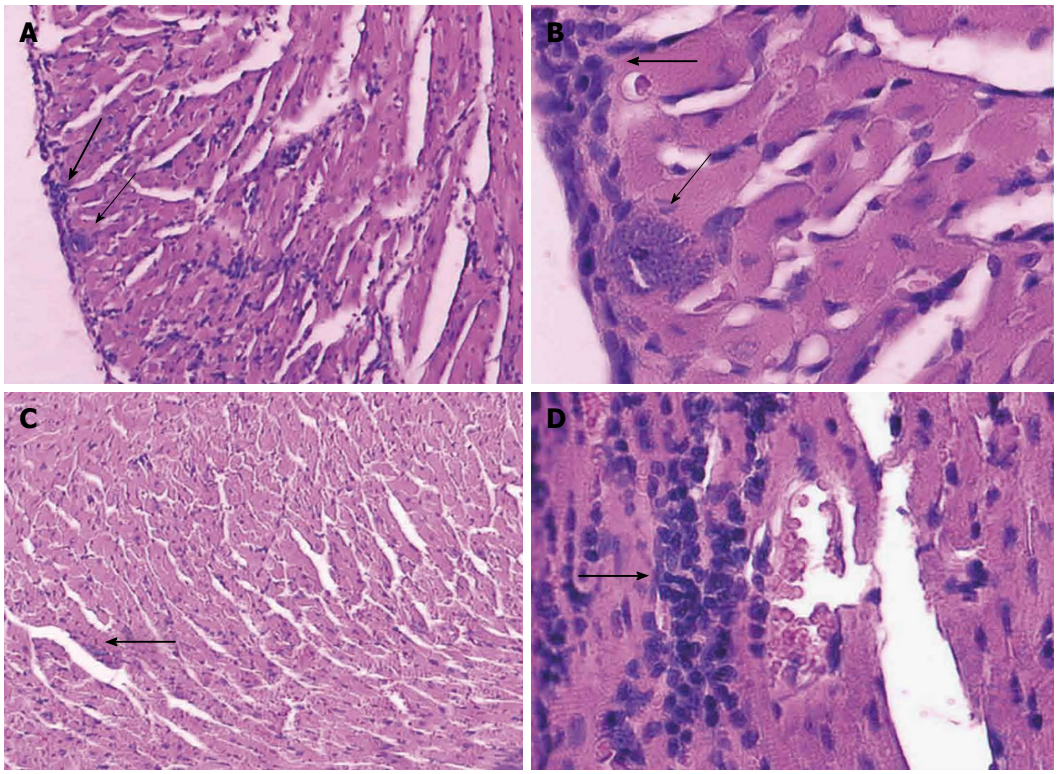

Figure 2 Histological studies: all sections were stained with hematoxylin and eosin. Histological sections of heart. A, B: Control groups show nests of amastigotes (thin arrows) and mononuclear cell infiltration (thick arrow); C, D: Representative sections from mice vaccinated with *Trypanosoma cruzi*. Infected mice show focal mononuclear cell infiltrates (thick arrows). No amastigote nest was observed (A, C: 100 ×; B, D: 400 ×).

nized animals, the ratio IFN-γ/IL-10 was greater than in the non immunized-infected mice. Taken together, these results suggest that, in vaccinated - infected animals, the action of protective IFN-γ could be more effective, without the antagonist action of IL-10. On the other hand, in all the experiments performed, the serum concentration of IL-10 correlated with parasitemia levels. These results are consistent with those of Reed *et al*[39], who detected a lower IL-10 production in resistant mice compared to the susceptible ones. Furthermore, Abrahamsohn *et al*[40] observed a lower number of parasites and higher IFN-γ production in IL-10 KO mice than in the wild type ones.

The Figure 3 shows the levels of IL-6, IL-10, IFN-γ and TNF-α in a representative experiment, in different groups of mice. From the results obtained in mice treated only with saponina adjuvant and after infected, the effect of the adjuvant alone became evident. In this group of animals a delay in the increase of parasitemia was detected, but the systemic production of IL-6 and TNF-α and the mortality rate were very similar to those in non vaccinated-infected group. This could be due to the unspecific action of the adjuvant on the immune system, which is not sufficient to help control the infection. Additionally, the results of these experiments suggest that, in this experimental model, the levels of IL-6 and TNF-α seem to be earlier markers of fatal outcome than the parasite load[33].

Likewise, the vaccinated group had very low levels of IL-10, which allowed IFNγ to maintain its protective activity, activating macrophages, essential for the elimination of parasites, unlike the control group, which showed high levels if IL-10, which blocks activation of the macrophages and their microbicidal function[31,33]. It was not possible to detect IL-4 or IL-5 in either group with the methodology used. Taken together, these results show that vaccination with *T. rangeli* made it possible to induce a profile of the cytokines different from that of the non immunized-infected mice, with a delicate balance between TH1 and TH2, suitable for overcome the infection. This modulation of the synthesis and liberation of cytokines and soluble receptors was also observed during the acute period of natural human infection[41].

On the other hand, during the process of invading the host cell, *T. cruzi* interacts with different receptors of the macrophage to induce its own phagocytosis. Different molecules of the family of Toll type receptors recognize different molecular patterns associated with bacteria, viruses, fungi and protozoa. As a result, innate immune response mechanisms and the development of the subsequent adaptive response are triggered[42].

With regard to NO, it is considered to be the most important early soluble mediator produced by immune system cells. Macrophages recognize antigen microorganisms through their different receptors (Toll-like, NLRs and RIG-like) and trigger the production of inflammatory mediators inducing the activity of the inducible Nitric Oxide synthase enzyme. This enzyme is produced by the antigen presenting cells and may inhibit the expression

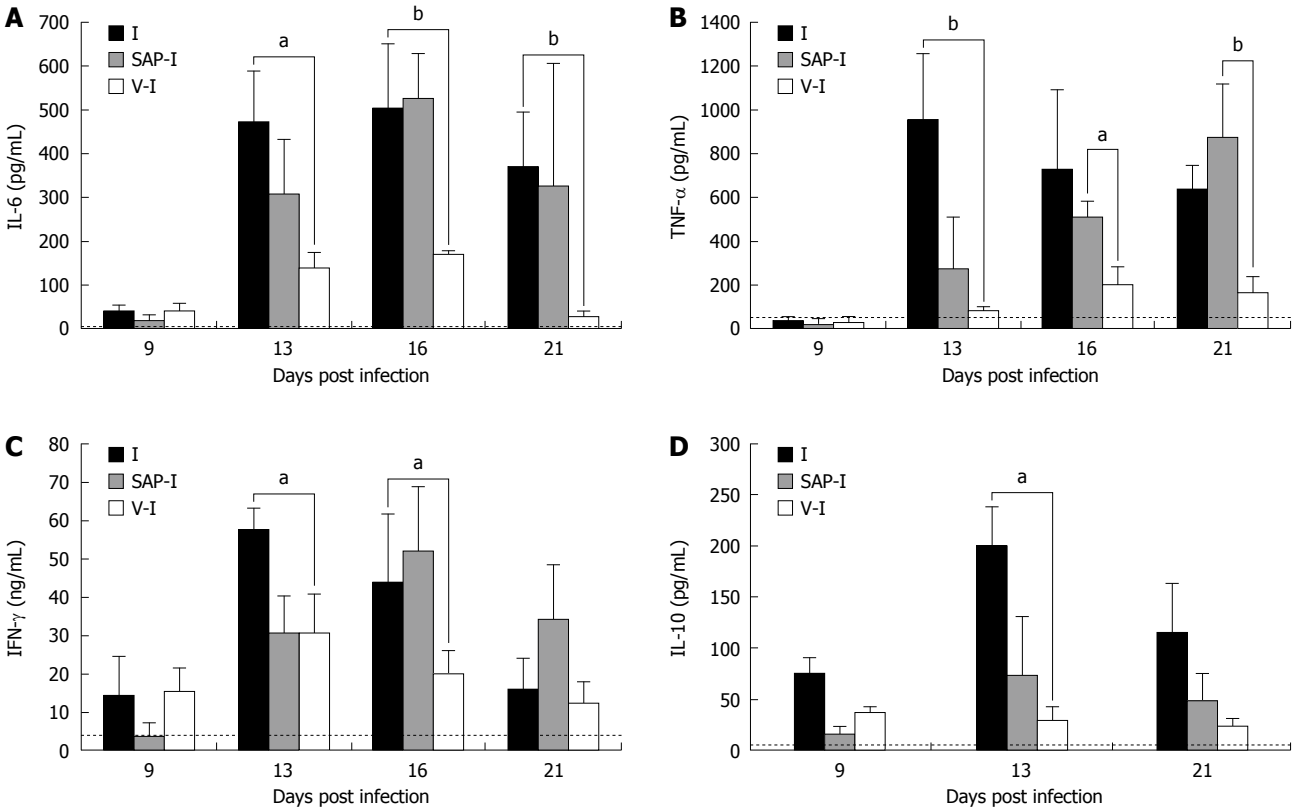

Figure 3 Kinetic of circulating cytokine levels in mice infected with *Trypanosoma cruzi* (A), in mice treated with saponin adjuvant and infected and in mice vaccinated with *Trypanosoma cruzi* and after infected (A-D). mean ± SE cytokine are represented. The area below the pointed line indicates the 95% confidence limit of each cytokine observed in plasma from uninfected mice. A: Interleukin (IL)-6; B: Tumor necrosis factor (TNF)-α; C: Interferon (IFN)-γ; D: IL-10. The differences in cytokine levels among I and SAP-I groups with respect to V-I group were evaluated by *t*-test: $^{a}P < 0.05$, $^{b}P < 0.01$.

of class II histocompatibility molecules. However, when effector cells are activated by inflammatory stimuli, important amounts of NO are synthesized, causing modifications in the cellular microenvironment[43].

At high concentrations, NO inhibits the synthesis of IL-12 and apoptosis, contributing to regulating the TH1/TH2 balance since TH1 cells are more susceptible to this process than TH2 cells[44]. In addition, this favours the proliferation of regulatory T cells during the acute experimental infection and inhibits the expression of molecules involved in adhesion and migration of cells. NO exerts its cytotoxic function on *T. cruzi*, affecting growth factors, for example by nitrosylation of the haem group, reducing the availability of iron. It is also the most important mediator in the destruction of intracellular amastigotes[45]; however, it has been shown that an excess of NO has harmful effects on the host's tissues[45,46]. In this sense, the results obtained in our studies are in agreement with these authors. In fact vaccinated animals revealed a modulation of NO levels, and the subsequent absence of lesions in the host, unlike the control group, which showed significantly increased levels of this metabolite[31].

Meanwhile, with respect to the cells, macrophages play an indispensable role in the primary response to pathogens but also take part in the resolution process of the inflammation and homeostasis of tissues. The function of macrophages is polarized towards an inflammatory or a regulatory profile, depending on the microenvironment they are in[47]. This cell population can be activated by classical way (type 1) dependent on IFNγ and TNFα, or by an alternative way (type 2) stimulated by IL-4 and IL-13[48]. Classically activated ones are currently grouped in M1, alternatively activated ones in M2a, those that polarize towards a TH2 response in M2b, and those whose stimulation is mediated by glucocorticoids and TGFβ in M2c. Therefore the different types of macrophages are involved in different response to pathogens, tumours and autoimmune diseases, showing markers exclusively associated with the function they play[49]. There is an important consumption of oxygen during the process of phagocytosis. The respiratory burst caused by macrophages and neutrophils is regarded as a powerful microbicidal mechanism. Oxygen free radicals are toxic to pathogens and prevent colonization by microorganisms in tissues. However, most of M1 macrophages' microbicidal activity is put down to NO and its derivatives like peroxynitrites. NO is produced by activation of iSON, whose substrate is L-arginine. In macrophages, this enzyme is induced by proinflammatory cytokines like TNFα, IFNγ and IL-12.

During the acute phase of the infection by *T. cruzi* and other protozoa like *Leishmania sp*[50] and *Plasmodium sp,* induction to the inflammatory response is necessary to be able to control parasitaemia[51]. However, if the classi-

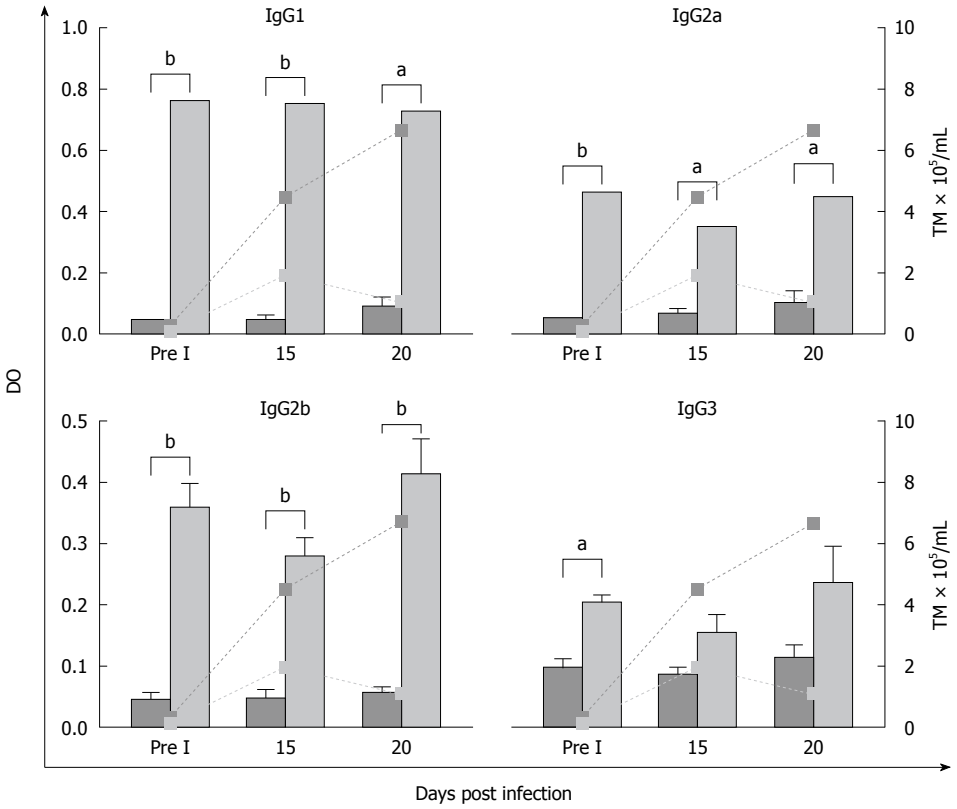

Figure 4 Specific IgG isotype levels in peritoneal fluid obtained from vaccinated, (light gray bars) or control mice, (dark gray bars) before and after infection determined by enzyme-linked immunosorbent assay (optical density mean ± SE). The doted line is the parasitemia level at different time of infection. Significant differences between both groups evaluated by Student's *t*-test ($^{a}P < 0.05$, $^{b}P < 0.001$).

cal activation of the macrophages is not regulated, it can cause severe harm to the host's tissue, which is why the production of IL-4, IL-10 and TGFβ is very important, because they modulate the action of NO, oxygen reactive metabolites and proinflammatory cytokines. In the early stages of the infection by *T. cruzi*, the action of soluble mediators like IL-12, IL-18, IFNγ and NO is crucial to inhibit the replication of the parasite.

Induction of arginase has been shown to inhibit the mechanisms involved in eliminating the parasite, among them the activation of T lymphocytes favouring their permanence in tissues. Therefore, evolution of the infection by *T. cruzi* depends on the magnitude of the TH1 - TH2 response and the macrophages activated classically *vs* those activated alternatively[52]. Moreover, it has been suggested that the increase in NK cells could act as a "bridge" between the innate and the early adaptive responses[53].

ADAPTIVE IMMUNE RESPONSE

To bring about protection against *T. cruzi*, CD4+ and CD8+ effector cells need to be generated, which are capable of migrating from lymph nodes to tissues and exert a strong immune response. As it was above mentioned, both types of cells secrete IFNγ, which activates the macrophage in order to exert trypanosomicide activity by means of NO. Antigen presenting cells like macrophages, dendritic cells and B lymphocytes play an essential role in generating effector T lymphocytes, which produce different cytokines involved in the polarization of the TH1 or TH2 immune response[32]. Despite this, *T. cruzi* is capable of surviving in the host for long periods, which contributes to the development of symptoms and chronic disease[54]. It initially produces immunosupression; however, during infection, large quantities of CD8+ are generated, which circulate towards places where the parasite persists in order to find the antigen; there they exert their effector functions, they acquire an activation phenotype and then become memory cells, responsible for perpetuating the immune response in the face of a second encounter with the antigen[55]. The importance of the function of CD8+ lymphocytes is based on the reduction of the parasite load observed in most of the tissues from which these cells are recruited. On the other hand, the presence of the parasite in tissues might be due to a lack of stimulation for the recruitment of CD8+ or to the fact that the functions of these cells might be inhibited by other populations like CD4+, CD25+ and producers of TGFβ[56].

At a second stage, the immune response mediated by antibodies is very important to control infection. Numerous experimental models with antibody or B cell deficiency have shown high parasitaemia and a low survival rate[57].

T. cruzi infection is known to induce polyclonal activa-

tion of B lymphocytes, and as a result hypergammaglobulinemia occurs. Recent studies have shown that most of the activated B lymphocytes do not synthesize specific antibodies during the first days of infection by *T. cruzi*[58], but they do produce a specific response at the end of the acute stage of the infection. However, this polyclonal activation may also be an important cause of the autoimmune phenomena mediated by autoreactive antibodies. In addition, the proliferation of B cell populations responding specifically to the antigen with poor polyclonal response is associated with resistant strains (C57Bl/6), with IFNγ production and a prevalence of the TH1 profile[59].

Different immunoglobulin isotypes, mainly IgG subclasses, are involved in the elimination of the parasite at the local and systemic levels by means of mechanisms such as complement fixation, agglutination and cytotoxicity. In this sense, Umekita *et al*[60] observed that IgG2 might contribute along with other mechanisms to the reduction of parasitaemia in mice infected with *T. cruzi* upon recognizing the parasitical antigen and acting as opsonin. We observed in our works, in accordance with these authors, that another factor involved in the clearance of the parasite might be associated with high levels of specific antibodies induced by vaccination, which might also act as an early mechanism for controlling the infection[61]. As it is shown the Figure 4, we observed the increase in IgG1 and IgG2 isotypes in peritoneal fluid (the site of the infection) related to different immune response patterns. The ratio IgG1/IgG2a in vaccinated group was 1.6 before infection, 2.1 at 15th *pi* and 1.6 at 20th *pi* day. In control mice the ratio was: 0.9; 0.8 and 0.9 respectively. These results showed the importance of TH2, related to antibody response, in vaccinated animals which, together with TH1 response observed through the patterns of cytokines, are both involved in the protection.

Brodskyn *et al*[62] could reveal the importance of the effector function at the infection site, induced before the challenge, contributing to the reduction of the parasite load. These results are in agreement with those obtained by Gruppi *et al*[63] who worked with an immunization model using exoantigens of *T. cruzi* and observed protection associated with increases of IgG1 and IgG2, with low levels of IgG3 at the systemic level. Other authors studying the acute period of the infection detected high levels of IgM, IgG and the isotypic variant IgG1 parallel to the reduction of parasitaemia[64,65]. In our work, IgM, responsible for the specific primary immune response, was high in both experimental groups, in both peritoneal fluid and in plasma. As was expected, the levels detected were always higher in vaccinated animals than in those belonging to the control group[61].

The protective role of the antibodies in the acute phase of the infection is mainly linked to the capacity to induce the elimination of the parasite from circulation, parallel to other cellular events, as we observed in our work, in agreement with others authors[60]. It has been shown that the specific IgG, particularly IgG2, recognizes an important number of parasitical antigens and is able to form microaggregates that fix complement, and increase opsonisation and cytotoxicity mechanisms[66]. In this sense, the neutralization studies developed in our research showed that antibodies and soluble mediators present in the peritoneal fluid of vaccinated mice might be involved in some of the mechanisms responsible for the lysis and reduction in the infectivity of trypomastigotes when these enter the host.

Our findings suggest that the immunogen used in this vaccination model induces an important modulation of the host's immune response, which are involved in the early clearance of the *T. cruzi* used in the challenge. Similar results were obtained by Paláu *et al*[67] and Zuñiga *et al*[68] when they immunized BALB/c mice with metacyclic trypomastigotes of *T. rangeli* and later challenged them with a virulent strain of *T. cruzi*, observing a reduction of the parasitaemia and of the severity of the progress of the infection, with high survival in relation to non immunized and infected mice.

There are also other modulators to the immune response, such as Actinomycetes. Treatment with these actinomycetes significantly reduces acute parasitemia, modifies cell infiltration during acute myocarditis and limits chronic myocarditis in comparison with the infected control group. Similar results were obtained for immunized pregnant mice and then challenged with live *T. cruzi*[69,70].

These findings are a stimulus to go further in the search for knowledge of immunological events, identifying target cells and molecules, with the goal of advancing in prophylaxis or immuno-intervention, directed towards the development of therapeutic approaches to Chagas disease.

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ORIGINAL ARTICLE

Effects of exercise on leukocytosis and blood hemostasis in 800 healthy young females and males

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Abstract

AIM: To investigate the effects of exercise on healthy individuals of both genders.

METHODS: This study lasted 6 years and involved about 800 healthy people. Individuals were divided into females and males and further sub-divided into two groups; in the first group individuals run (or skied in the winter time) and then rested for 3 h, whereas individuals in the second group intensely cycled for 5 min. The status of health was determined by measuring the sedimentation rate and the intensity of exercises by measuring the heart rate. Blood samples were collected before and after exercise.

RESULTS: We observed that in the first group a significant increase of the total white blood cells, segmented neutrophils, band neutrophils, eosinophils and to a lesser extent lymphocytes but not monocytes in the blood circulation. However, all cell types were increased in the circulation after 5 min intense exercise. No differences in the pattern of cell increase were observed among the genders. Activated partial thromboplastin time (APTT) and D-dimer were also measured in the blood of individuals who cycled intensely for 5 min to determine the coagulation and fibrinolytic activities in the blood. APTT is reduced and D-dimer values significantly increased after intense exercise. However, APTT was statistically lower in males than females, whereas no differences in the D-dimer values were observed among the genders.

CONCLUSION: Our results indicate that exercise whether leisure or strenuous affects leukocytosis and hemostasis in both genders. A major advantage of this study is the high numbers of individuals involved and the inclusion of both females and males values.

Key words: Exercise; Leukocytosis; Activated partial thromboplastin time; D-dimer

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INTRODUCTION

Recreational exercise is now part of everyday life style because it is important for maintaining cardiovascular fitness. Emphasis is on improving cardiac input and activity as well as manipulating the immune system for a better and prolonged life. Hence, several studies were dedicated to examine the blood hemodynamic and hemostasis after exercise whether being leisure, intense or strenuous. The

total number of white blood cells (WBCs) was increased during and immediately after exercise[1], and both leukocytosis and thrombocytosis occurred in the first 10 min of high-intensity exercise[2]. In 16 male volunteers (average age 30 years), all leukocytes except basophils and eosinophils were increased very early after resistance exercise but declined after 15 and 30 min with the exception of neutrophils[3]. About 5 h after exercise, there was a marked increase in the numbers of blood granulocytes and monocytes along with several inflammatory cytokines and chemokines for both well-trained and untrained runners[4]. Another study examined leukocytosis in 10 female soccer players and observed neutrocytosis but not lymphocytosis after one bout of intense exercise "at 75% maximal heart rate"[5]. Upon comparing the immunological parameters in age and gender different groups it was observed that exercise induced significant increases in total leukocytes and lymphocytes in 11 girls as compared to 13 boys studied[6].

Neutrocytosis may depend on the duration rather than the intensity of the exercise, which also depends on the release of adrenocorticotropic hormone[7]. Intensive short-term exercise resulted in increased leukocytosis which included lymphocytes, granulocytes and monocytes concomitant with alterations in plasma catecholamine levels[8]. A 30 min exercise in 8 male volunteers resulted in increased catecholamine and leukocytosis, whereas another round of exercise 3 h later also resulted in enhanced leukocytosis "neutrophils and lymphocytes" and increased cortisol levels[9]. It was proposed that catecholamine increases the number of circulating leukocytes, whereas cortisol which has a later effect maintains this increase in the vascular compartments[10]. Similarly, a short period of recreational vigorous exercise induced significant leukocytosis, which could be due to the release of adrenalin[11]. In contrast to these results it was reported that neither the total increase nor the subsequent decline in plasma cortisol concentration after exercise in 7 healthy male volunteers is important for leukocytosis[12].

Physical exercise also affects blood hemostasis since blood coagulation cascade was activated as demonstrated by shortening the activated partial thromboplastin time "activated partial thromboplastin time (APTT)"[13]. In 10 healthy adolescents, APTT is shortened by about 15% immediately after exercise which returned to normal value after 1 h concomitant with a high increase of fibrinolytic activity[14]. Similarly, activation of the coagulation cascade is detectable after acute physical exercise in 10 healthy subjects, which led to increased thrombin generation[15]. It was also reported that in 11 healthy male subjects irrespective of the type of exercise they performed, alterations in markers of thrombin and fibrin formation were pronounced after 1 h exercise. In this study of high impact, it was suggested that prolonged exercise is necessary for exercise-induced activation of coagulation resulting in thrombin and fibrin formation[16].

In 15 healthy individuals who performed strenuous exercise for 15, 45 and 90 s, it was observed that this exercise did not induce blood coagulation, whereas fibrinolysis, *e.g.*, generation of tissue-plasminogen activator "t-PA" was increased after 15 s and remained high through the duration of exercise. The release of t-PA might be due to increased catecholamine concentrations and blood sheer-stress, whereas no increase in D-dimer generation was observed[17]. In contrast, Gunga *et al*[18] observed that PT and APTT were both increased in 15 healthy individuals after 30 s exercise, and that t-PA and D-dimer levels were also elevated after the same period suggesting that short-time intensive exercise shifts the homeostasis system into a higher equilibrium. The influence of moderate exercise was also studied and it was observed that after 30 min exercise there was an increase in t-PA[19]. On the other hand, the levels of APTT or D-dimer were not significantly increased during 15 min short-term extreme exercise, but APTT was decreased and D-dimer increased after termination of the exercise[20].

Although these studies are informative, drawbacks include the small numbers of samples included in each study (not more than 30 individuals at best). Therefore, it was difficult to draw a reasonable conclusion from these studies regarding the effects of exercise. In the current study we combined monitoring blood leukocytosis and hemodynamic in about 800 healthy individuals, a study that lasted about 6 years and included comparison of both males and females.

MATERIALS AND METHODS

Physical exercise protocols

Healthy volunteers (age 19-44 years, average 23 years) were divided into two groups with different experimental designs. Individuals in the first group were instructed to run or go cross-country skiing for at least 1 h without any pauses. Blood samples were collected before exercise and 3 h after exercise, and all samples were analyzed immediately after blood withdrawal. Individuals in the other group were instructed to use an exercise bike for 5 min at high resistance and with as high rpm as they could manage. Blood samples were collected before exercise and immediately after exercise. An overview of the numbers of individuals involved and the sort of exercise they performed are shown in Table 1.

Hematological tests

Duplicates of total white blood cell counts before and after exercise were performed using coulter counter Z1 from Beckman Coulter (Miami, FL, United states). Full blood was diluted with an isotonic salt solution and mixed with Zap-Oglobin II (Beckman Coulter) to lyse red blood cells. Blood smears were made from each EDTA-tube and stained using color rapid set from Lucerna-Chem (Lucerne, Switzerland). Differential counts of at least 100 WBCs were done on these smears. Triplicates of hematocrit were measured before and after exercise to determine the changes in packed cell volume (PCV). Determination of hematocrit was done by centrifuging the hematocrit capillary tubes in hematocrit centrifuges and then mea-

Table 1 Overview of individuals involved in the current study

Blood examinations	Subjects	Exercise	Gender
SR	795	-	F
	506	-	M
WBCs	241	Running	F = 121/M = 120
	273	Cycling	F = 133/M = 140
Differential counting	241	Running	F = 121/M = 120
	273	Cycling	F = 133/M = 140
PCV	241	Running	F = 121/M = 120
	273	Cycling	F = 133/M = 140
APTT	291	Cycling	F = 132/M = 159
D-dimer	279	Cycling	F = 125/M = 154

SR: Sedimentation rate; PCV: Packed cell volume; APTT: Activated partial thromboplastin time; WBCs: White blood cells; F: Female; M: Male.

suring on a circular micro capillary reader (Damon IEC division).

Coagulation tests

Vacuette sodium citrate tubes from before and immediately after 5 min intensive exercise were centrifuged for 15 min at 2000 × *g* to obtain platelet free plasma. Plasma samples were then tested for APTT and D-dimer values. APTT was measured using DG-APTT kit and a Thrombotrack coagulometer (Axis-Shield PoC AS Oslo, Norway). D-dimer test was done using NycoCard D-dimer test kit and NycoCard READER II (Axis Shield PoC AS). The purpose of this test was to determine the levels of D-dimer as an indirect measurement of plasma t-PA. Citrated plasma (500 μL) from before and after exercise was added to 0.1 U/mL thrombin (Sigma-Aldrich, St. Louis, MO, United states) and incubated for 5 min. A positive test was done from 500 μL plasma mixed with 0.01 mg/mL actilyse "recombinant t-PA" from Boehringer Ingelheim (Ingelheim am Rhein, Germany) and 0.1 U/mL thrombin. D-dimer concentration was measured using NycoCard READER. In these tests, the coagulation was performed on an independent set of individuals performing only intense exercise.

Heart rate and sedimentation rate

Heart rate was measured before and after exercise at the same time blood was collected for the two different groups. For sedimentation rate (SR), blood was drawn in BD Vacutainer glass seditainer tubes, and SR was measured in a seditainer manual ESR stand (BD Diagnostics, Plymouth, United Kingdom) from all individuals involved in these experiments.

Statistical analysis

Data was collected over a period of 6 years from anonymous individuals reporting only gender and physical condition. All statistical analyses were determined utilizing Graphpad Prism program (San Diego, CA, United States), and significant values were determined using the two-tailed Student's *t* test. A P value < 0.05 was considered to be statistically significant.

RESULTS

Hematological evaluations

Individuals were divided into females and males and each were further sub-divided into two groups; individuals in one group run or skied for 1 h and then rested for 3 h, whereas those in the other group cycled at a maximum intensity for 5 min. Fitness levels ranking from 1-4 were investigated, where 1 indicates lack of training and 4 indicates athletic "training sports at a national level" (Figure 1). Only 4 subjects in total reported fitness level 1, and that group was not further analyzed. Blood was withdrawn before and after each exercise. A total number of 795 females were examined for SR, and those who showed 20 mm/h or more were excluded from the evaluation (Figure 2A). Heart rate measurement was important to determine whether individuals optimally followed the instructions, particularly with the intense exercise (5 min cycling). As can be seen pulse (heart rate) was higher after 1 + 3 h exercise and was more pronounced after the 5 min intense exercise (Figure 2A). There was no effect of 1 + 3 h exercise on PCV of females, but an increase was observed after the 5 min intense exercise. Exactly similar pattern was observed with males; those who had SR 15 mm/h or more were excluded from the evaluation. In addition heart rate difference between before (B) and after (A) exercise was more pronounced with the 5 min intense cycling. Similar to females, PCV was not increased after the 1 + 3 h exercise but increased after the 5 min intense exercise (Figure 2B).

Leukocytosis examination after 1 + 3 h exercise

A significant increase in the number of WBCs was observed in the blood of females who run (or skied) for 1 h and then rested for 3 h ($P < 0.0001$ when compared to the number of WBCs before exercise, Figure 3A). Both segmented and stab (band) neutrophils numbers were also significantly increased after this mode of exercise ($P < 0.0001$ for both cell types, Figure 3A). There was a significant increase in the number of lymphocytes ($P < 0.04$), albeit it was much lower than neutrophils. Monocytes number was not increased, whereas the number of eosinophils in the blood circulation was significantly increased after exercise ($P < 0.002$). Similar findings were observed with males blood where the numbers of total WBCs, segmented neutrophils, stab neutrophils, lymphocytes and eosinophils, but not monocytes were increased as compared to the resting state ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, $P < 0.03$, $P < 0.0001$, and not significant, respectively; Figure 3B).

Leukocytosis examination after 5 min intense exercise

WBCs numbers significantly increased in the blood circulation of females 5 min after intense exercise ($P < 0.0001$; Figure 3C). In this category, the numbers of granulocytes, lymphocytes, monocytes and eosinophils were also significantly increased after intense exercise ($P < 0.0001$ for all cell types). Similar results were observed with males

Table 2 Comparison of the exercise intensity levels among the various groups (mean ± SD)

Fitness level	Exercise form (*n*)	WBC increase ($\times 10^9$/L)	Change in heart rate right after 5 min cycling	APTT (after-before)(s)	D-dimer (after-before) (mg/L)
2	Running (78)	2.97 ± 0.24			
2	Cycling (130)	2.44 ± 0.20	59.82 ± 2.35	-2.11 ± 0.22	0.459 ± 0.22
3	Running (118)	4.93 ± 0.41			
3	Cycling (125)	3.54 ± 0.17	78.67 ± 2.34	-2.16 ± 0.19	0.631 ± 0.16
4	Running (31)	4.84 ± 0.27			
4	Cycling (25)	3.80 ± 0.41	86.80 ± 5.30	-2.05 ± 0.31	0.959 ± 0.67

Individuals were divided into groups according to physical activities ranging from 1 (no activity) to 4 (high intense activity). The numbers of increased WBCs after 1 h run and 3 h rest (Running group), and after 5 min intense cycling (Cycling group) are shown. Shown are changes in the heart rates among the various groups. Also shown are the differences in activated partial thromboplastin time (APTT) and D-dimer measurements. WBCs: White blood cells.

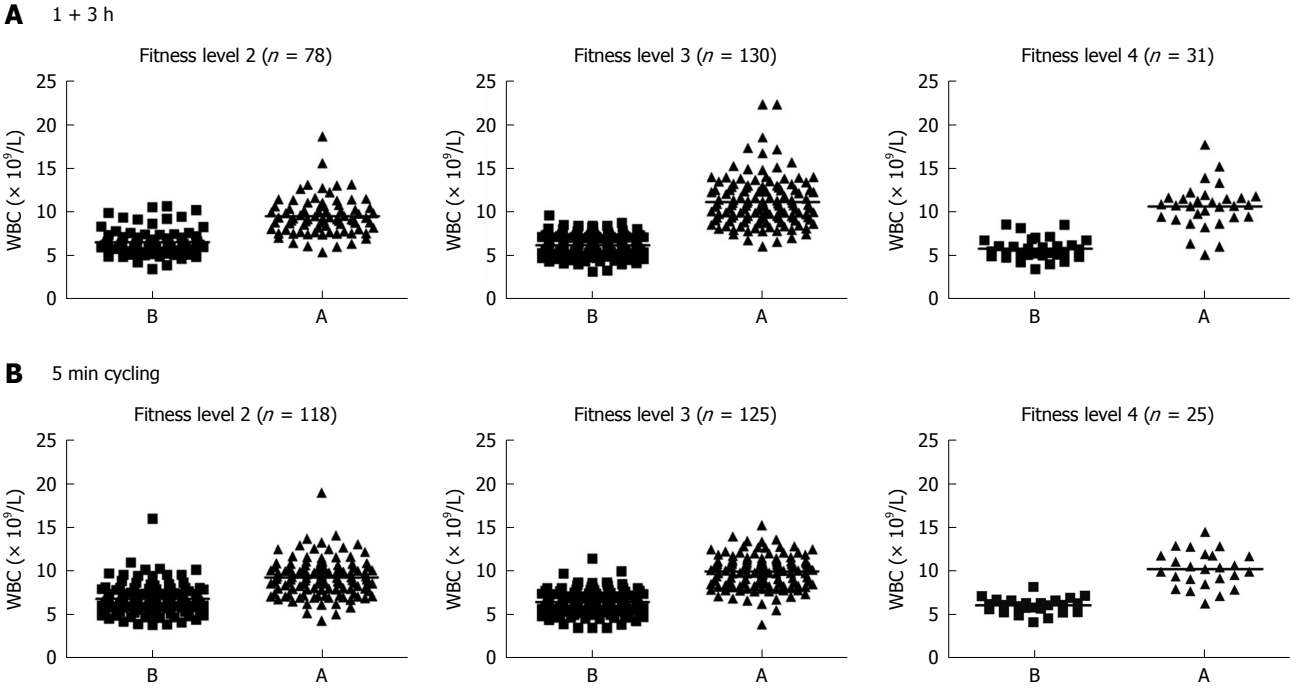


Figure 1 Fitness levels of individuals involved in this study. The numbers of white blood cells (WBCs) were counted according to the fitness levels of individuals (levels 1-4 from low to high intensity) in each group (those that run for 1 h and then rested for 3 h, and those that cycled for 5 min). The numbers of individuals in each category are shown. Also shown the numbers of WBCs before and after exercise. B: Before; A: After.

blood where the numbers of total WBCs, segmented granulocytes, stab neutrophils, lymphocytes, monocytes and eosinophils were increased when compared to the numbers of these cells in the blood of individuals at resting state ($P < 0.0001$ for all cell types; Figure 3D). Fold increases in the numbers of WBCs after both bouts of exercise are shown in Figure 4.

It should also be mentioned that data based on fitness levels showed no significant differences among the various groups on leukocytosis either after running or after cycling (Table 2). The only significant difference observed was the lower number of WBCs circulating at resting situation between members of group 4 as compared to those in group 2 (mean 5.8 × 10^9/L for group 4 and 6.6 × 10^9/L for group 2) and also a larger change "after-before" numbers of leukocytosis for members in group 4 (Table 2). For those who cycled significantly higher changes in heart rate was observed after exercise for those in groups 3 and 4 as compared to those in group 2. No significant differences were found when APTT and D-dimer were analyzed based on the fitness levels (Table 2). Further analysis showed that there was a significant ($P < 0.0001$) relationship between the number of WBC's and heart rate, *i.e.*, increased heart rates are correlated with increased leukocytosis (Figure 5).

Hemostasis shifts into a higher equilibrium after 5 min intense exercise

Next, we investigated the influence of short-term exercise on the hemodynamic of blood coagulation system. Both female and male healthy individuals cycled for 5 min at high intensity. Blood samples were withdrawn before and after exercise and APTT as well as D-dimer values were measured. Results shown in Figure 6A dem-

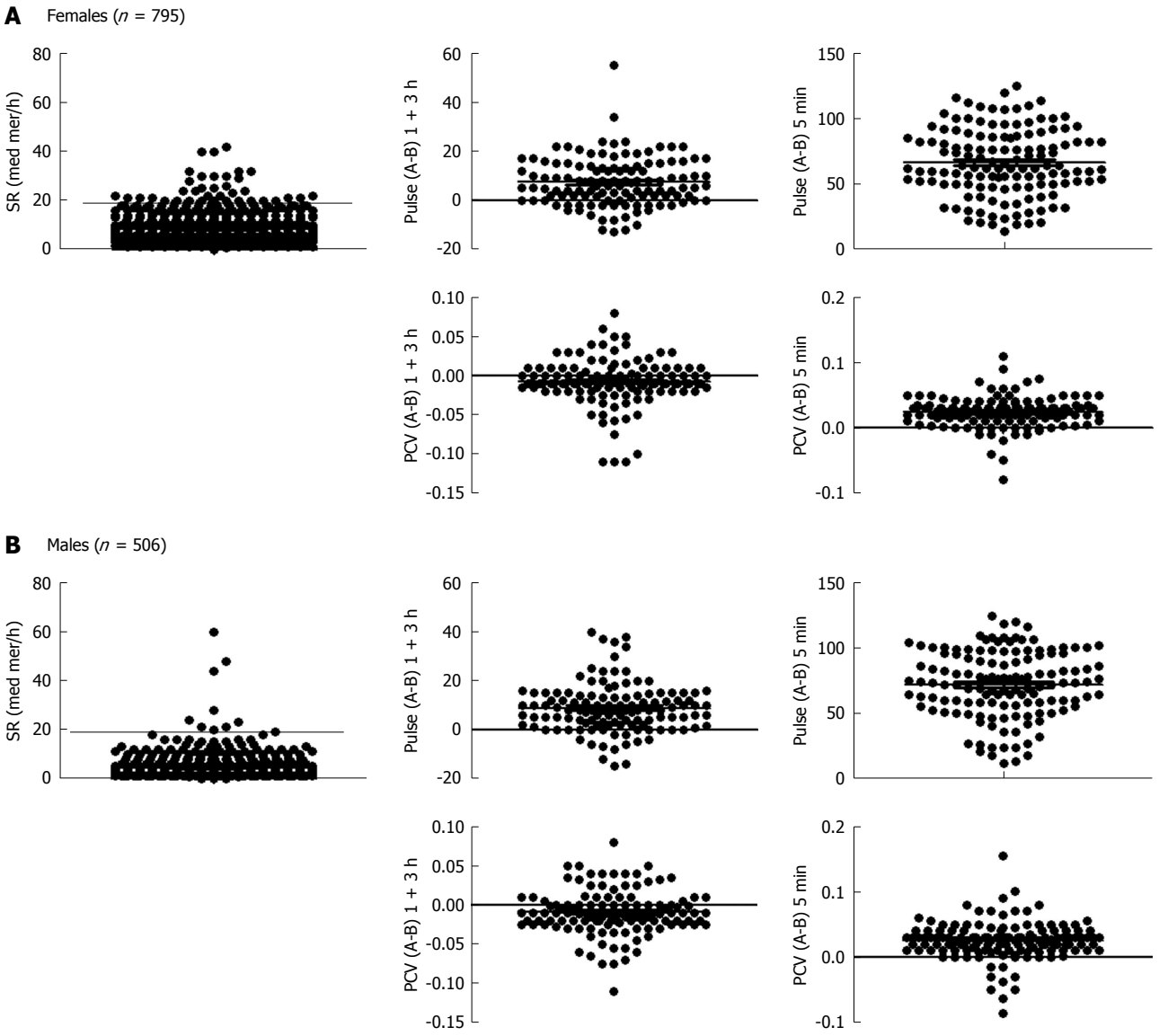
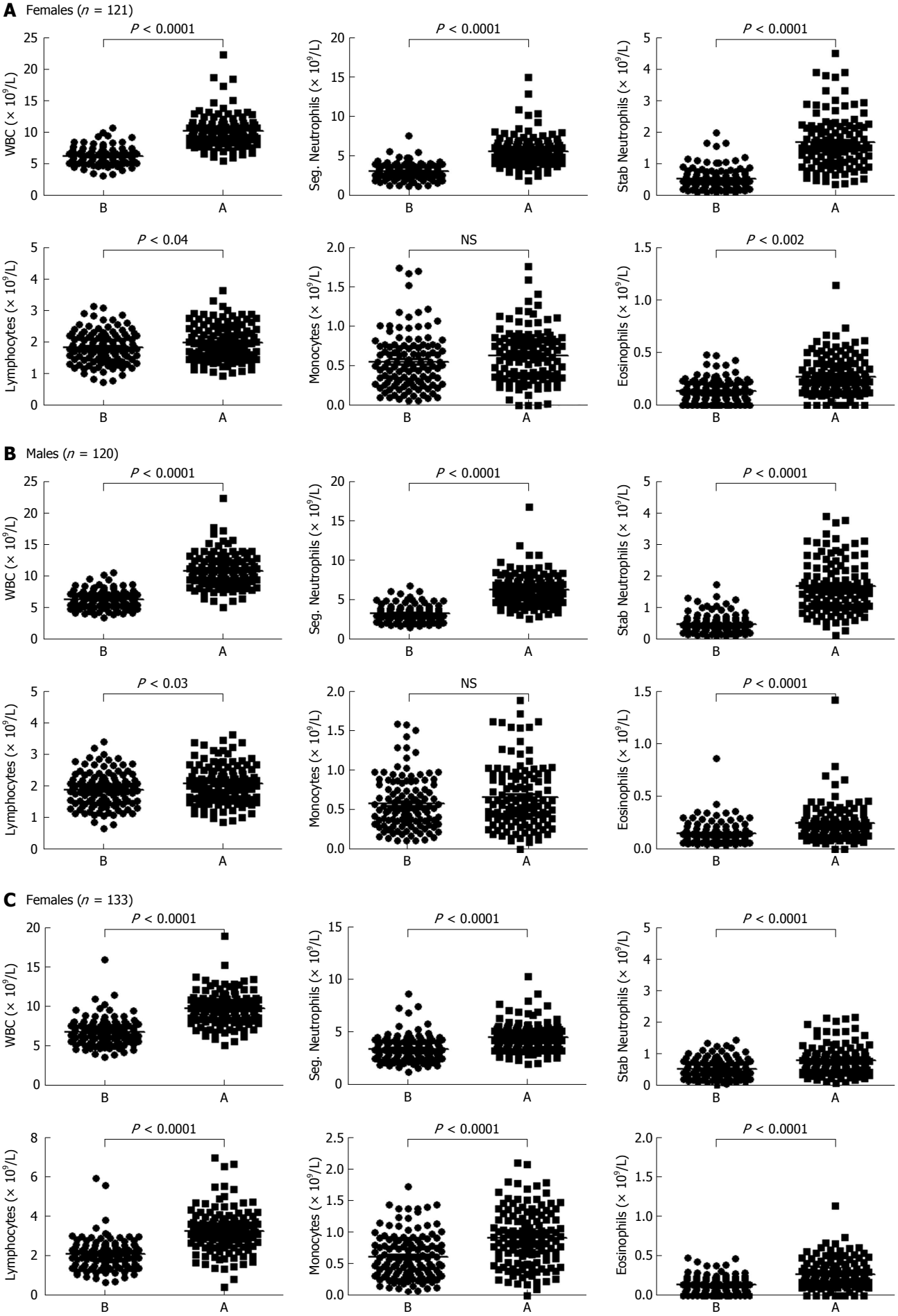


Figure 2 Evaluation of blood and hearts. A: Sedimentation rate (SR) was measured in 795 female students (age 19-44 years, average 23 years old). Females with SR more than 20 mm/h were excluded from the evaluation (above the thick line). Packed cell volume (PCV) and heart rate (pulse) changes were calculated after exercise minus before exercise in 120 females who run or skied for 1 h and then rested for 3 h (1+3 h). PCV and pulse were also measured in about 135 females who exercised for 5 min at a maximum intensity; B: This is similar to Panel A except that 506 males were examined for SR; those that have more than 15 mm/h were excluded (above the thick line). Lines in the other panels show the PCV or pulse after minus before exercise. Each dot represents one individual. B: Before; A: After.

onstrate that APTT was decreased in females, *i.e.*, shortened time after this type of exercise. APTT is decreased after exercise and was significantly lower than before exercise ($P < 0.0001$). From the same blood, plasma levels of D-dimer showed increased values after exercise ($P < 0.04$). A high D-dimer level was also observed when the plasma from females was supplemented with thrombin and recombinant t-PA. Similar pattern was observed with the blood of healthy males, where APTT was significantly shortened ($P < 0.0001$), and D-dimer significantly increased ($P < 0.007$) after 5 min intense exercise (Figure 6B). Curiously, the decrease in APTT found in males was significantly higher than those found in females (Figure 6C), whereas no significant difference was observed when D-dimer was compared among the genders (Figure 6C).

DISCUSSION

The numbers of granulocytes, lymphocytes and monocytes have been reported to be increased after exercise[1-12], but the numbers of samples in these studies were too small to come up with a consensus. In addition, effects of exercise on gender differences are surprisingly unclear. Avloniti showed that there is a significant increase in total WBCs of elite female national-team soccer players as well as increased their heart rates after exercise[5]. Also, a great weight loss occurred after running in both males and females[21]. However, no differences in the post-exercise systolic blood pressure or heart rates were detected among healthy young males and females after exercise[22]. These results were supported by the study of Fernandez-Fernandez *et al*[23], showing no differ-


A Females (n = 121)
P < 0.0001
WBC (× 10⁹/L)
Seg. Neutrophils (× 10⁹/L)
Stab Neutrophils (× 10⁹/L)
P < 0.04
NS
P < 0.002
Lymphocytes (× 10⁹/L)
Monocytes (× 10⁹/L)
Eosinophils (× 10⁹/L)
B Males (n = 120)
P < 0.0001
P < 0.03
NS
P < 0.0001
C Females (n = 133)
P < 0.0001
B
A

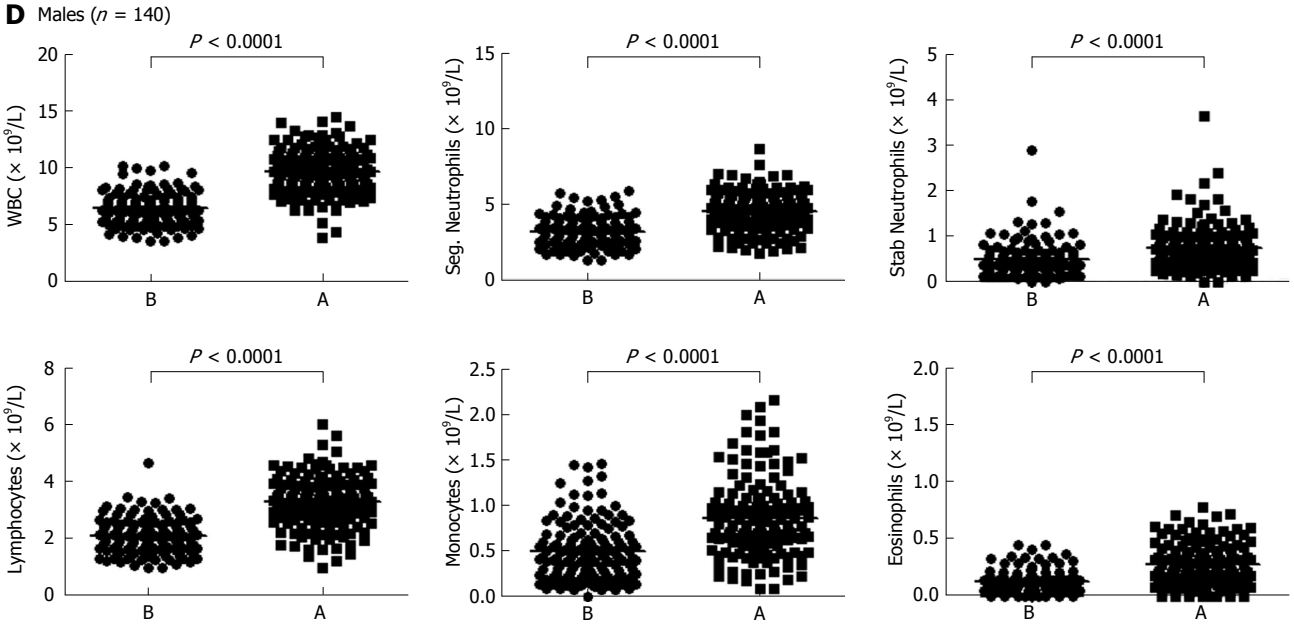


Figure 3 Evaluation of leukocytosis before and after leisure exercise. A: Total numbers of white blood cells, segmented (seg.) neutrophils, stab (band) neutrophils, total lymphocytes, monocytes and eosinophils were calculated from 121 healthy females before and after 1 h run (or ski) and 3 h rest. Lines indicate the mean values. *P* values compared the differences in the numbers between after and before exercise; B: This is similar to panel A, except that 120 healthy males were examined. Each dot represents one individual; C: Total numbers of white blood cells, segmented neutrophils, stab (band) neutrophils, total lymphocytes, monocytes and eosinophils were calculated from 133 healthy females before and after 5 min of intense exercise. Lines indicate the mean values. *P* values compared the differences in the numbers between after and before exercise; D: This is similar to panel A, except that 140 healthy males were examined. Each dot represents one individual. B: Before; A: After; WBC: White blood cell.

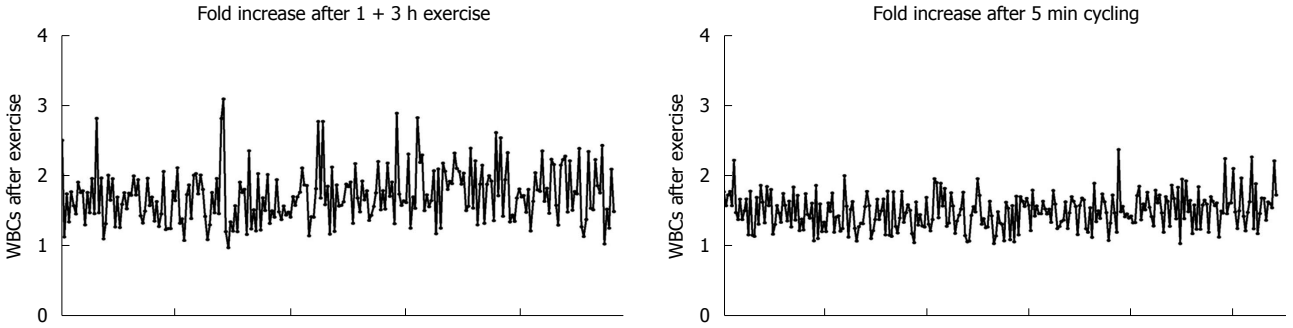


Figure 4 Fold increase in the numbers of white blood cells after exercise. Each dot represents one Individual. The increase in the numbers of leukocytes after exercise varies between 1-3 times. WBC: White blood cell.

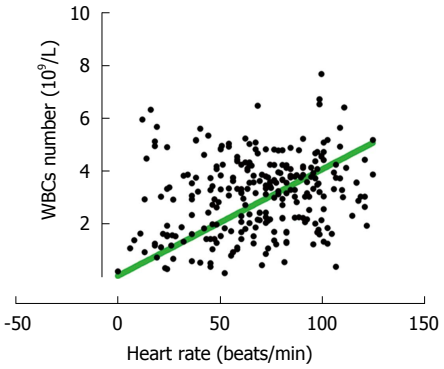


Figure 5 Correlations between the numbers of white blood cells and heart rate after intense exercise. The increase in the numbers of white blood cells accumulating in the blood after 5 min intense cycling is correlated with increased heart rate. WBCs: White blood cells.

ences among females and males heart rates during badminton match play. Here, we examined a large number of medical students whom we taught the blood course in the department of Physiology at the University of Oslo medical school between January 2007 and June 2012. The students were divided into two groups (males and females) and were further sub-divided into a group where individuals run (or skied during winter time) for 1 h and then rested for three h, whereas those in the other group cycled at a maximum intensity for 5 min. The intensity of the exercise was monitored by measuring the heart pulse after exercise and compared it to before exercise. PCV showed no difference in the group that run for 1 h and then rested for 3 h, but an increase in PCV occurred after 5 min exercise. This could be due to the pressure exerted on RBCs during intense exercise due to blood sheer

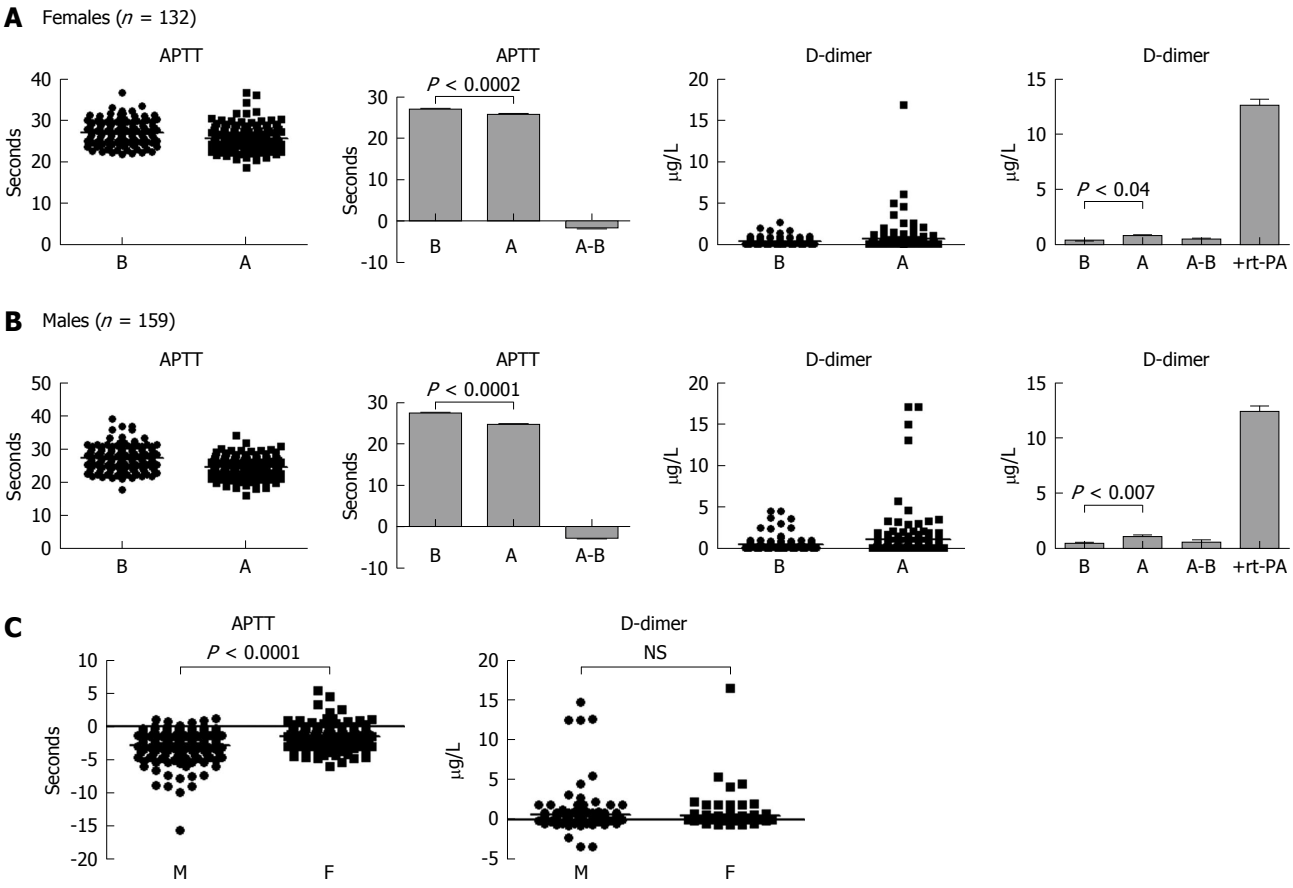


Figure 6 Evaluation of blood hemostasis. A: Activated partial thromboplastin time (APTT) and D-dimer were measured in 132 healthy females before and after 5 min intense exercise. *P* values show the difference between after and before exercise. In the last panel actilyse "recombinant tissue-PA = rt-PA" was added to the assay in the presence thrombin and plasma samples collected from these individual and represent positive control. Dot and column graphs are shown to indicate the differences between after and before exercise; B: This is similar to panel A except that plasma samples of 159 healthy males were examined; C: Comparison among males (M) and females (F) for differences in APTT or D-dimer values (before minus after exercise). B: Before; A: After; NS: Not significant.

stress and outward filtration of plasma from vasculature into tissue space, among other factors.

In the 1 + 3 h group, significant increases in the numbers of segmented neutrophils, band neutrophils and eosinophils were observed after this bout of exercise. Lymphocyte numbers were also increased, although to a much lower extent than the other cells, whereas no increase in the numbers of monocytes was observed. These results could be due to the recruitment of cells from the bone marrow. It was previously observed that CXCL1/interleukin (IL)-8 is increased in the blood circulation of female soccer athletes performing exercise[6]. This chemokine is also increased in the blood of 16 male volunteers 5 h after exercise[3]. Increased CXCL8/IL-8 concentration could explain the higher numbers of neutrophils observed after this bout of exercise. However, the numbers of eosinophils could not be explained by increased CXCL8/IL-8 levels, since these cells do not express CXCR1 or CXCR2, receptors that bind CXCL8/IL-8. Instead, the results may be due to increased cortisol levels which peaks after 3 h of exercise and which maintains cells in the vascular compartments, as shown by others[10]. Alternatively, the levels of eotaxin/CCL11 may also be increased which could be responsible for the recruitment of eosinophils into the circulation since these cells express CCR3 that binds this chemokine[24-26]. One can also exclude the possibility that MCP-1/CCL4 is released at this stage, since it is the major chemokine recruiting monocytes which express CCR2 that binds this chemokine[24-26]. Lastly, no differences were demonstrated in the patterns of cell accumulation in the blood circulation after 1+3 h exercise between females and males.

However, different patterns of cell accumulation in the blood circulation were observed after 5 min intense exercise. Blood samples collected from individuals that exercised intensely showed increased numbers of all cell types including segmented and stab (band) neutrophils, lymphocytes, monocytes, and eosinophils. This increase could be due to potentiating the sheer blood flow that occurs during this sort of exercise as previously described[17]. It could also be due to the release of catecholamine which was reported to be increased in plasma after intensive short-term exercise[8,11]. We also noted a similar pattern of cell accumulation in the blood circulation of males and females performing similar activity, suggesting that there are no gender differences in relation to neutrocytosis, lymphocytosis or monocytosis after this type of exercise. A correlation between increased heart rate and

enhanced leukocytosis was also observed.

Our work also combined leukocytosis with hemostasis. We observed that in both males and females a shift towards higher homeostasis occurred after 5 min intense exercise. Hence, both higher coagulation (shortened APTT) and D-dimer activation were observed. These results suggest that during exercise, the increase in coagulation is offset by enhanced fibrin degradation. To this end, we observed that increased coagulation was significantly higher in males than females, albeit no difference was observed in their D-dimer activity. This may suggest that males might be more prone to higher coagulation after intense short-term exercise than females. The impact of these findings on the exercise ability among genders has not yet been publicized but it might be important to distinguish the coagulation patterns among genders when one is planning exercise regiments. Collectively, our results support those of Gunga *et al*[18] where both coagulation and fibrinolytic cascades are increased in healthy individuals after short-term intense exercise.

D-dimer is increased in patients with deep venous thrombosis and pulmonary embolism[27]. In this regard, elevated plasma concentrations of von Willebrand factor, t-PA and PAI-1 could be predictors of myocardial infarction and stroke incidence in older or in cardiac patients[28]. However, fibrinolysis is increased during 2 h triathlon where it neutralizes the increase in thrombin generation[29], and increased D-dimer activity in healthy individuals is a marker of hydrolyzing the cross-linked fibrin by plasminogen which is generated from plasmin by t-PA.

The advantages of our study as compared to other published data are: (1) large numbers of samples were examined; (2) comparison was done among male and female healthy individuals performing two different kinds of exercise; and (3) both leukocytosis and hemostasis observations were included. Taken together, we observed that WBCs are accumulated in the blood circulation after 5 min intense exercise or after 1 h run plus 3 h rest and that the mechanism of accumulation is different among the two types of exercise. The results also suggest that in both types of exercise, changes in the hemodynamic of blood cells in the circulation took place, and the effects may not dampen the immune system but instead may potentiate it due to increased numbers of immune cells in the circulation. Hence, although exercise may resemble acute inflammation regarding recruitment of leukocytes, the effect of exercise could be beneficial since most cells are recruited into the blood circulation rather than into inflamed tissues such as what happens during infections. Regarding hemostasis, our results continue to support others demonstrating a shift of the hemostasis system into a higher equilibrium after exercise. It was previously suggested that changes in hemostasis during exercise may induced coronary ischemia and possibly sudden death[30]. However, based on the numbers of individuals examined in our study and their gender status, we support the consensus that short-term intense exercise or leisure exercise that may last up to 1 h is beneficial and should be continued particularly for healthy individuals.

COMMENTS

Background

Exercise is part of normal life. However, the effects of exercise in health and diseases are not clearly defined, basically due to the low numbers of individuals examined in most studies published in the literature.

Research frontiers

This is a large study that involves about 800 individuals. Consequently, the results can be regarded as standards for the effects of both intense and leisure exercises on the numbers of blood cells as well as blood coagulation and fibrinolytic components. In contrast to other reports which examined similar effects, this study included large sample size and showed comparison among healthy females and males. Further, this report combined leukocytosis and hemostasis in one study.

Applications

Although not all aspects of blood components were examined, the results may form a reference guide for any future study examining the effects of exercise on blood hemodynamic.

Terminology

Activated partial thromboplastin time and D-dimer are tests which measure blood hemostasis; one measures the time of blood coagulation and the other the degradation of fibrin. Hence, these tests are widely used to examine blood hemodynamic.

Peer review

The data from this paper strongly support the notion that exercise (independent of the intensity) affects leukocytosis and blood hemostasis in both genders. The present results are promising and very important to the research in this area of knowledge once this study includes a high number of individuals (both females and males). In general the paper is well written and the results are consistent, thus supporting the conclusions.

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