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Comparison of immunohematological profile between endurance and power-oriented elite athletes

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Title: Comparison of immunohematological profile between endurance and power-oriented elite athletes

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Abstract

There is general perception that elite athletes are highly susceptible to changes in immunohematological profile.

Purpose: to compare immunohematological parameters of elite athletes of different aerobic and muscular strength sports and analyze changes over 2-months.

Methods: 16 judoists and 14 swimmers were evaluated 2-months before (M1) and prior to competition (M2). Hemogram and lymphocytes subpopulations were assessed with automatic counter and flow cytometry, respectively.

Results: judoists had higher neutrophils, and lower monocytes and eosinophils percentages than swimmers at M1 and M2. At M2 judoists had lower red blood cells (RBC), hemoglobin, and hematocrit than swimmers. At M2 judoists' hematocrit, and CD16 decreased while swimmers' hemoglobin and hematocrit increased.

Conclusions: neither sports characteristics nor intense training seem to displace the athletes' immunohematological profile out of the clinical range, despite the possibility of occurrence of microlesions that may stimulate production of leukocytes and reduction of RBC in judoists.

Keywords: Judo; Swimming; Training; Immune System; Hematology, Red blood cells, White blood cells, Lymphocytes.

Introduction

There is a general perception that elite athletes are more susceptible to suffer from changes in immunohematological profile with possible health consequences due to high intensity training sessions. However, to date, the associations between intense exercise and health problems have not been conclusive. Prolonged intense exercise sessions may induce “hemodilution and/or immunesuppression”; nevertheless, these situations have been shown to be reversible with adequate periods of rest (Gleeson 2006). Some reports of infections and anemias in exercising population tend to favour a higher incidence of health problems among athletes, if compared to sedentary or moderately exercising individuals (Shaskey and Green 2000; Malm 2004; Mercer and Densmore 2005; Gleeson 2006; Banfi et al. 2011; Rama et al. 2013). Health problems may affect athletes differently as a consequence of specific sports characteristics such as: intensity, frequency and duration of the task, the quality and duration of the resting periods between training sessions, the phase of training program during the period under investigation, as well as environmental factors (Schumacher et al. 2002; Malm 2004). Stability of immune and hematological parameters over long-lasting periods is crucial for athletes in order to maintain optimal performance (Malm 2004; Mercer and Densmore 2005; Gleeson 2006). Nevertheless, none of the aforementioned investigations focused on the effects of an endurance or a power-oriented sport on the immune and hematological variables. In fact, most common findings refer mainly the hematological differences in athletes when comparing with non-athletes. Some authors have found that athletes have higher white blood cells (WBC), decreased red blood cells (RBC), hemoglobin (Hb), and hematocrit (Ht) levels or a dilutional pseudoanemia, caused by plasma volume (PV) expansion (Shaskey and Green 2000; Schumacher et al. 2002; Banfi et al. 2006; Mørkeberg et al. 2009; Banfi et al. 2011; Broadbent 2011), while others have observed no differences between athletes and non-athletes or lower WBC in athletes (Banfi et al. 2006; Saygin et al. 2006). In endurance cyclists, Corsetti and colleagues (2012) reported (data also supported by Mørkeberg et al. (2009) but contrary to data from Saris et al.(1998)) a decrease of Hb values during the first part of a stage race (until day 12 out of 22). Authors

inferred that the decrease in Hb and, consequently, in Ht and RBC, were expected and assigned the phenomenon to hemodilution typical of heavy exercise, as there was an increase of 1.6 % in PV during this first phase. In the second part of the race (from day 12 to day 22), these authors verified that Hb stabilised and PV % changes showed modest increases. Anemia as a result of decreased RBC, Hb, and Ht has proved to have negative effects on physical exercise performance (Schumacher et al. 2002), while dilutional pseudoanemia is not considered a pathological state and normalises with training cessation after a short period of time (3 to 5 days) (Shaskey and Green 2000; Mercer and Densmore 2005). Corsetti and colleagues (2012) reinforce that the time course of Hb decrease should also be established, since some authors observed a decrease as soon as after 2 or 3 days (Hue et al. 2006; Schumacher et al. 2008; Garvican et al. 2010) while, for others it was not yet evident after 4 days (Konig et al. 2003).

It has been demonstrated that immune and hematological variables are highly sensitive to exercise and could display seasonal adaptations, depending on training programs and competition schedule of the athletes (Gleeson et al. 1999; Schumacher et al. 2002; Banfi et al. 2006; Morkeberg et al. 2009; Banfi et al. 2011; Broadbent 2011). Data comparing these variables in different sports as well as within different training intensities and levels of performance are scarce and inconclusive.

Considering that swimming is an endurance and predominantly aerobic sport characterized by low impact and that judo is a predominant power and strength sport with a high level of physical impact where injuries and traumas are frequent and sometimes serious, this study aim was to compare immune and hematological parameters of elite athletes of two sports with different aerobic and muscular strength characteristics (endurance – swimming / power – judo) and analyze the changes occurring over a 2-month training period.

Materials and methods

Participants

Thirty elite Portuguese male athletes comprising 16 judoists and 14 swimmers were recruited for the study. Top-level judo athletes were recruited from the Lisbon region with the help of the Portuguese judo federation, and swimmers were recruited from the three most representative swimming teams of the Lisbon region.

All judoists were national medallists, several were international medallists, and some participated in European and/or World Championships or in the Olympic Games.

All swimmers were medallists at the national level and several participated in the European and/or World Championships.

After receiving detailed information about the aim of the study and the possible risks of the investigation, either the subjects or their parents, as appropriate, provided their written informed consent to participate. All procedures were approved by the Ethic Committee of the Faculty of Human Kinetics, University of Lisbon and were conducted in accordance with the declaration of Helsinki for human studies (World Medical Association 2008).

Study design

This study used a longitudinal approach: the baseline testing (M1) and the competitive-period assessment (M2) that occurred approximately two months apart. Baseline period (M1) corresponds to middle september and october, while M2 corresponds to November/December, depending on the baseline assessment and also depending on judo individual competitions or swimming competitions.

Judoists training included ~2 h in the morning and ~ 2 h in the evening, 6 days a week. From those, twice a week the morning sessions aimed to improve cardiorespiratory fitness and strength while the other sessions consisted of judo specific skills, drills and randori (fighting practice).

Swimmers undertook ~ 13-15 h of pool training and ~ 4 h of dry-land training per week. Swimming training macrocycle was characterized by an aerobic training predominance with the progressive increase of training volumes and intensities from M1 to M2 period assessment.

At each moment of evaluation, all measurements were performed on the same day after a 12-hour fast, with no alcohol or stimulant beverage consumption or exercise participation taking place within the last 15 hours. During the period of observation athletes were asked not to take dietary supplements, nor any kind of medication other than that prescribed for episodes of acute illness.

Anthropometric Measures

Height (cm) and body weight (kg) were assessed to the nearest 0.1 cm and 0.01 kg respectively (SECA Scale and Stadiometer, Hamburg-Germany), and body mass index (BMI; $\text{kg} \cdot \text{m}^{-2}$) was calculated.

Immune and hematological parameters

Blood samples were collected by venipuncture from the antecubital vein into tubes containing EDTA and analyzed in the next hour.

Hemogram quantification

Whole blood was analyzed in an automatic cell counter (Coulter LH 750 Beckman) for total white blood cells (WBC), neutrophils, monocytes, lymphocytes, eosinophils and basophils, red blood cells (RBC), hemoglobin (Hb) and hematocrit (Ht) quantification.

Lymphocytes assessment

Total lymphocytes and subsets CD3 (total T cells), CD4 (T helper), CD8 (T cytotoxic), CD16 56 (NK cells) and CD19 (B cells) were counted by flow cytometry, using the FACS Calibur Becton, Dickinson and Company (Biosciences 2000) cytometer equipped with two laser beams at 635 e 488 nm. This cytometer was linked to a Macintosh computer that uses the software Multiset to analyse the

cytometry results, according to standard procedures (Burtis and Ashwood 1999; Radbruch 2000; Goldsby et al. 2003).

Statistical analysis

Descriptive statistics was applied to characterize the sample. All variables were checked for normality using Shapiro-Wilk test.

One sample t-test was used to compare group means with the upper or lower limits of the reference interval provided by the National Health Institute Doctor Ricardo Jorge (INSA) (Lewis et al. 2006) to verify if participants were within the “clinically normal” values associated with each variable.

Repeated measures ANOVA was used for the assessment of specific sports characteristics: aerobic and strength (between-subjects factor – Swimmers vs. Judoists) and training (within-subjects factor - M1 vs. M2) on the parameters of interest. Sphericity assumptions were evaluated with the Mauchly’s test. If the assumptions of the ANOVA for repeated measures were not met, or of if there was a significant interaction between the specific sports characteristics and training effects, analysis of the effect of training were performed in each group separately by paired sample T-test (or Wilcoxon test when normality was not observed) and differences between groups were assessed using independent samples T-test for parametric variables (or Mann-Whitney test when a non-normal distribution was observed).

Data were analyzed with PASW for Windows version 21.0 (SPSS Inc., an IBM Company, Chicago).

For all tests, statistical significance was set at $p < 0.05$.

Results

Participants’ characteristics, immune and hematological parameters of elite judoists and swimmers are displayed in Table 1 for M1 and M2. BMI was higher in judoists than in swimmers.

At both moments, all parameters were within the clinical reference interval associated with each variable.

Judoists showed a higher percentage of neutrophils ($F=11.550$; $p=0.002$), and lower percentages of monocytes ($F=24.293$; $p<0.001$) and eosinophils ($F=5.866$; $p=0.022$) than swimmers at both moments of evaluation. At the competitive period judoists presented lower values of RBC ($p=0.025$), Hb ($p=0.021$), and Ht ($p<0.001$).

The competitive period significantly differed from baseline, Ht ($p<0.001$) and CD16 lymphocytes count ($p=0.029$) decreased in judoists while RBC ($p<0.001$), Hb ($p=0.003$), and Ht ($p=0.006$) increased in swimmers.

Table 1 about here

Discussion

Studies addressing the question how exercise affects immune and hematological parameters regarding different sports disciplines are scarce (Horn et al. 2010). Few studies were conducted regarding only swimmers (Morgado et al. 2012; Rama et al. 2013) and judoists (Yamamoto et al. 2008; Shimizu et al. 2011; Garatachea et al. 2012), nevertheless, to our knowledge, our study is the first concerning judoists and swimmers.

We wish to remark that all immune and hematological mean values were within the normal clinical reference range. Thus, our results do not support the common idea that elite athletes are more prone to infectious diseases or anemia (Malm 2004; Gleeson 2006).

Regarding sports disciplines, our results showed no differences in total white blood cells counts (WBC) between judoists and swimmers. However, judoists presented higher percentages of neutrophils and lower percentages of monocytes and eosinophils at both moments of evaluation. Additionally, judoists showed lower RBC counts, Hb, and Ht, at the competitive period, than swimmers.

Low neutrophil counts (neutropenia) seem to occur commonly among athletes (Miura et al. 2005; Horn et al. 2010). It has been shown that more aerobically oriented sports tend to have lower WBC and

neutrophil counts (Horn et al. 2010), which is in accordance with the observed results in our study. The reasons for the aerobic exercise-induced low neutrophil counts are unclear (Barreda et al. 2004; Horn et al. 2010). The mechanisms involved in this line of defense against invading microorganisms result in the death of both the microorganism and the neutrophil (Miura et al. 2005; Segel et al. 2011). This cell loss is compensated for by the ability to produce neutrophils at a prodigious rate and to have them accumulate in the tissues (Segel et al. 2011). Although neutrophils are an important cell population in the innate defense system, they have a dichotomous role as both perpetrator of tissue damage and initiator of repair. Neutrophils infiltrate injured tissues but can also be present after no injurious exercise (Butterfield et al. 2006). These cells have both specific and nonspecific defensive immune system functions that can cause tissue damage in isolation or as sequela to another tissue injury. It might seem that limiting the action of neutrophils would be clinically beneficial, but these cells are also responsible for initiating the reparative process that is later managed by monocytes and macrophages (Butterfield et al. 2006).

Our results show higher mean percentage of neutrophils and lower mean percentage of monocytes in judoists. This may be a consequence of the high impact strength training of these athletes, frequently involving contusions that may result into microlesions in the muscular tissue. In theory, these microlesions may stimulate the production of neutrophils from the bone marrow and simultaneously the migration of monocytes into the damaged tissue differentiating into macrophages in order to phagocyte damaged cells, as suggested above.

Higher mean percentage of eosinophils in swimmers may suggest a higher prevalence of allergies, possibly due to the environmental conditions in the pool facilities.

In an epidemiological study that investigated the RBC characteristics and iron metabolism in athletes at different levels of performance and in distinct sports, it was concluded that the specific type of exercise plays a major role in the hematological response, and that there are differences between endurance-trained athletes and athletes engaging strength training for RBC, Hb, and Ht (Schumacher et al. 2002).

In the aforementioned investigation neither judo nor swimmers were included in the sample, but categories were created based on sports type in, namely, endurance and power categories. Schumacher and colleagues (2002) reported lower values of these parameters for endurance training athletes (cycling, running, or other endurance sports), when compared to strength athletes (weight lifting, shot-put, hammer-throwing, javelin, wrestling or discus). Our data showed different results from this study, as our power-oriented athletes (judoists) presented lower values than endurance training athletes (swimmers) for RBC counts, Hb, and Ht at the competitive period. These divergent results may have occurred due to the fact that the sports performed by the strength-training group studied by these authors were mostly sports with low impact.

The investigation conducted by Corsetti and colleagues (2012) reinforce the need for hematological monitoring of athletes, especially in endurance sports, as it is important because of the occurrence of “sports anemia”. Although this may be a direct consequence of the hemodilution, linked to heavy physical exercise, other additional sources have been recognized. Among them, hemolysis is the most frequent, due to an important destruction of RBCs resulting from the impact with hard surfaces such as the ground (foot-strike hemolysis) or to their mechanical breakdown in contracting muscles.

We propose that the results obtained for RBC and Ht corroborate the suggestion anticipated for the results observed for neutrophils and monocytes, reinforcing the possible occurrence of microlesions with some intravascular hemolysis, as it has previously been suggested (Malczewska et al. 2000; Matias et al. 2008). Also, it has been proposed free Hb capture by Hp and removal of the Hp-Hb complex from circulation as cause of the low values of Hp observed in an investigation conducted in judoists (Matias et al. 2008) and in studies including runners (Schumacher et al. 2002) and cyclists (Corsetti et al. 2012). As Hp is an acute phase protein, its concentration increases with inflammation. One other possible mechanism to explain RBC and Hb decreases is the increase in the production of reactive oxygen and nitrogen species that occur during exercise. These species can attack RBCs, in particular their membranes, compromising their flexibility and functionality. Without mechanical stressors, these

senescent cells are mostly removed intact by the spleen and do not lyse or fragment in the general circulation, at least to any significant extent (Jollow and McMillan 2001). However, their increased removal from circulation can also contribute to the reduction of RBCs and Hb although this would not induce hypohaptoglobinemia.

Nevertheless Haptoglobin (Hp), other acute phase proteins, or other inflammatory or oxidation markers were not scrutinized in the present investigation.

A seasonal follow-up of the immunohematological response is essential for elite athletes in order to avoid or limit performance pitfalls. Our results show that at the competitive period, RBC, Hb and Ht increased in swimmers while RBC, Ht, and CD16 lymphocytes count decreased in judoists. Numerous studies demonstrated that immune and hematological variables are highly sensitive to the effects of exercise and can display seasonal adaptations, depending on training and competition schedule of the athletes (Gleeson et al. 1999; Schumacher et al. 2002; Banfi et al. 2006; Morkeberg et al. 2009; Banfi et al. 2011; Broadbent 2011). Literature regarding the variations of the immunohematological parameters along the training seasons is quite controversial. Our result showed that total WBC were highly stable in both sports during the period of investigation suggesting the nonappearance of viral and/or bacterial diseases (Schumacher et al. 2008). This is in accordance with the reports that intensive training during long-lasting periods does not cause significant modifications on immune parameters (Banfi et al. 2006; Ostojic and Ahmetovic 2009; Garatachea et al. 2012; Morgado et al. 2012). In swimmers, Morgado et al (2012) reported that the frequency and absolute numbers of the different cell populations in the blood did not significantly change during a season of training, with the exception of a decrease in the absolute numbers of monocytes in the first 7 weeks. Similar results were observed in judoists by Garatachea et al. (2012) who stated that the leukocyte profile did not change over a 7-week period of weight loss, with the exception of monocytes that increased from week 3 to week 7.

Regarding lymphocytes subpopulations, Shimizu and colleagues (2011) reported a decrease in lymphocytes CD3, CD4, and CD8, in judoists. In judoists from our study, a decreased in CD16 was

observed during the period of investigation. Similar results were observed by Rama and colleagues (2013) in swimmers, where CD16 cells decreased from a baseline to a period coincident with an increased training load period. These cells are considered unspecific defenses, as they act without sensitization against infection (Gleeson 2006). Changes of immune cells counts may reflect adaptation to the metabolic and mechanical stressors evident in sports (Pyne 1994), rather than an underlying pathological response and have been reported before in several sports, especially at periods of high training volume (Degoutte et al. 2006; Yamamoto et al. 2008; Shimizu et al. 2011; Garatachea et al. 2012; Morgado et al. 2012; Rama et al. 2013). As in other studies, this decrease in CD16 may be an indication that the innate immune system may be more susceptible to periods of great physical demand than the adaptive immune system.

Regarding RBC system, the true prevalence of anemia in athletic populations remains unknown (Shaskey and Green 2000; Mercer and Densmore 2005), however, it is currently accepted that alterations of the RBC system truly influence physical performance (Schumacher et al. 2002). Literature reviews indicated that numerous studies have examined the prevalence of “anemia” in athletic populations (Shaskey and Green 2000; Mercer and Densmore 2005). Authors suggested that possibly anemia does not occur, but instead a “dilutional pseudoanemia” seems to be the response to exercise. Dilutional pseudoanemia can be easily explained in elite athletes as it is a benign temporary condition that is developed secondary to training-induced plasma expansion and hemodilution (Shaskey and Green 2000; Mercer and Densmore 2005). This condition is very different from sports anemia, that results as a pathologic cause of RBC deficiency in athletes, intravascular hemolysis and iron deficiency (Mercer and Densmore 2005). Thus, Hb and Ht have been pointed out as poor indicators of the RBC system in athletes as they are easily influenced by exercise (Schumacher et al. 2002). However, nutritional anemia probably occurs more frequently in athletes than the so-called “dilutional pseudoanemia”, specifically in judoists or other sports where body weight restriction is placed at a premium (Shaskey and Green 2000).

326 In our swimmers, mean values of RBC, Hb and Ht increased at the competitive period, possibly
327 associated with an improvement in endurance performance, as described elsewhere (Schumacher et al.
328 2002), while in judoists Ht decreased reinforcing the already proposed possibility of occurrence of
329 microlesions with intravascular hemolysis.

330 Despite the clinical normal values observed in all parameters, it would be interesting to reinforce that
331 swimmers engage a taper period prior to competition while judoists frequently undergo weight
332 reduction. This weight reduction can be achieved due to intracellular water decrease, which has been
333 proven to be related to an increase in the cell membrane fragility (Matias et al. 2015). Taper periods aim
334 to promote recovery of fatigue indicators and to allow the athlete to compete at their best.

335 In this study we observed higher levels of neutrophils in judoists than in swimmers and lower levels of
336 hematological parameters at M2, suggesting some exercise-induced trauma in judoists. For this reason,
337 caretakers of athletes enrolled in judo or other sports with high and repeated body contact and impact,
338 should carefully monitor iron metabolism. In addition, at M2 not only hematological parameters had
339 decreased in judoists but also CD16 cells, suggesting a decrease of the innate immune response. As all
340 the values are within the reference limits established for the general population, discipline-adapted
341 immunohematological reference values are improbable to arise. Thus, individual profiles should be
342 established in order to allow the identification of increased susceptibility periods and avoid or limit
343 performance pitfalls or the development of illness.

344 We reinforce the need to adopt preventive strategies in order to avoid the onset of fatigue or health
345 issues that might compromise attendance to training sessions or health, especially during periods of
346 heavy training loads.

347 In conclusion, our results suggest that neither sports discipline with these particular characteristics
348 (endurance vs power) nor intense training, from a baseline to a competitive period, seem to affect the
349 immunohematological profile of elite swimmers or judoists. Nevertheless, sports with a higher contact,
350 such as judo, can involve the occurrence of microlesions that may stimulate the physiological

production of neutrophils, the migration of monocytes into the damaged tissue and the reduction of RBCs.

As all variables remained within the clinically normal range during the period of investigation, further research should be performed in order to achieve a better and comprehensive knowledge about the implications of long-lasting training periods of sports with the prevalence of endurance or power characteristics on the immunohematological profile of athletes.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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478 **Table 1. Participant characteristics, immune and hematological parameters**

	Judo (n=16)		Swimming (n=14)		Clinical Reference (INSA 2011; Lewis et al. 2006)
	M1	M2	M1	M2	
Age (Yrs)	22.9 ± 2.7		17.9 ± 1.4		
Weight (kg)	72.3 ± 7.6	71.6 ± 7.0	71.3 ± 5.3	70.2 ± 5.2	
Height (cm) *	174.1 ± 4.2	174.6 ± 3.8	178.5 ± 5.1	179.0 ± 5.1	
BMI (kg/m ²) *	23.8 ± 2.2	23.5 ± 2.3	22.4 ± 1.7	21.9 ± 1.7	18.6 – 24.9
WBC (cells·10 ⁹ ·L ⁻¹)	7.0 ± 2.1	6.6 ± 1.5	7.0 ± 0.9	7.4 ± 1.5	4 – 10
Neutrophils (%) *	56.5 ± 10.9	53.7 ± 9.4	44.3 ± 9.1	46.9 ± 8.3	40 – 80
Lymphocytes (%)	35.7 ± 9.5	37.8 ± 8.5	38.9 ± 13.4	40.0 ± 8.5	20 – 40
Monocytes (%) *	5.3 ± 2.9	5.9 ± 2.1	10.0 ± 3.0	9.3 ± 2.9	2 – 10
Eosinophils (%) *	2.0 ± 1.6	2.1 ± 1.5	3.3 ± 1.4	3.3 ± 1.6	1 – 6
Basophils (%)	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.2	0.4 ± 0.1	< 2
RBC (cells·10 ¹² ·L ⁻¹)	4.8 ± 0.3	4.7 ± 0.2[§]	4.8 ± 0.2	5.0 ± 0.2[†]	4.5 – 5.5 x 10 ¹²
Hb (g·dL ⁻¹)	14.9 ± 0.9	14.7 ± 0.9[§]	14.8 ± 0.7	15.8 ± 0.8[†]	13 – 17
Ht (%)	44.4 ± 2.7	43.2 ± 2.6^{†;§}	43.5 ± 2.0	45.6 ± 2.2[†]	40 – 50
CD4 Lymph.	1193 ±	1109 ± 393	1150 ± 321	1121 ± 374	700-1100

(cells·μL ⁻¹)	611				
CD8 Lymph.	686 ± 370	659 ± 253	697 ± 220	695 ± 277	500-900
(cells·μL ⁻¹)					
CD4/CD8 ratio	1.9 ± 0.6	1.8 ± 0.6	1.7 ± 0.5	1.8 ± 0.5	1.0-1.5
(cells·μL ⁻¹)					
CD16 Lymph.	332 ± 189	237 ± 103[†]	383 ± 214	487 ± 273	200-400
(cells·μL ⁻¹)					
CD19 Lymph.	292 ± 204	323 ± 144	452 ± 200	402 ± 168	200-400
(cells·μL ⁻¹)					
Total Lymph.	2592 ±	2388 ± 754	2784 ± 588	2808 ± 836	1600-2400
(cells·μL ⁻¹)	1292				

479 Abbreviations: ^{M1}, baseline period; ^{M2}, competitive training period; ^{BMI}, body mass index; ^{WBC}, White Blood Cells; ^{RBC}, Red
480 Blood Cells; ^{Hb}, hemoglobin;
481 ^{Ht}, hematocrit; ^{Lymph}, Lymphocytes. * Different between judo and swimming at both moments of evaluation; [§] Different
482 between judo and swimming; [†] Different from M1, p<0.05.
483 Clinical reference interval values according to Lewis et al. (2006) and INSA (2011)
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