Title: Center of Excellence in CardioRespiratory Health of high-level athletes: Evaluation of long-term effects of training.

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1. BACKGROUND

Eleven National Sports Teams are located in the Quebec Metropolitan Area. In these groups, many athletes are aiming for Olympic medals in Beijing 2008 and Vancouver 2010 Olympic Games. A consultation of the coaches of these National Teams revealed that although a large proportion of these athletes present various cardio-respiratory symptoms, they do not have a rapid access to a systematic medical evaluation and follow-up. Furthermore, little is done in regard to prevention and optimization of treatment of pulmonary and cardiac conditions in the elite athlete’s population of the Quebec area.

Cardiorespiratory problems are therefore commonly found in high-level athletes. However, these pathologies are not well characterized in athletes and the associated symptoms often not well perceived. These problems can be serious and it is important to detect them before they appear while setting up a systematic medical follow-up. Health professionals should monitor health of the young athletes and help to reduce the risks associated with high level exercise. The following project is an evaluation and follow-up program of high-level athletes, aiming at gathering key-information on long-term effects of high-level training on cardio-respiratory and metabolic parameters.
2. CARDIORESPIRATORY HEALTH OF ATHLETES

Although musculo-skeletal injuries are undoubtedly the most frequent problems observed in high-level athletes, others such as asthma, cardiorespiratory disorders, chronic fatigue, exhaustion, recurring respiratory infections and metabolic disorders are also frequently encountered. Female athletes also have various specific health problems such as amenorrhea, osteoporosis and anorexia\(^2\).

2.1 Respiratory problems

Asthma is a complex disease which is the interaction between genetic and environmental factors. The prevalence of asthma has been increasing in the world in the last twenty years (particularly in children and young adults)\(^3\), which is about 5 to 10% in the general population. High-level athletes have the highest prevalence of asthma varying between 9 to 55%\(^4\). Among the risk factors considered to contribute to the development of asthma are the hygiene hypothesis with a reduced exposure to microbial agents, allergy with particularly indoors exposure and pollutants, obesity and intense exercise\(^4-7\).

2.1.1 Exercise-induced asthma

Exercise-induced asthma (EIA) is defined as an intermittent narrowing of the airways, generally occurring about 5 to 15 minutes after intense exercise. It is characterized by respiratory symptoms such as wheezing, dyspnea, chest tightness and cough\(^8\). It is particularly high in winter sports athletes and in swimmers, and its prevalence has been reported to be increasing\(^9\). Respiratory symptoms, associated or not with asthma, are frequently noted during training or competing periods and can interfere with the athlete’s performance. The prevalence of exercise-induced
asthma has been reported to be high in female athlete\textsuperscript{10-13}. This effect of gender may be link with hormone or morphologic differences\textsuperscript{14}.

\subsection*{2.1.2 Treatment of asthma in athletes}

Furthermore, asthmatic athletes seem often refractory to current asthma medications, including the frequently used inhaled $\beta_2$ agonist. This medication does not seem to have performance enhancing effects when used at dose required to prevent or treat exercise-induced bronchoconstriction, their use has been regulated\textsuperscript{15-18}. To ensure that $\beta_2$ agonist are used in confirmed case of asthma, the International Olympics Committee-Medical Commission (IOC-MC) has established criteria for a positive diagnosis of asthma. This criteria include a significant bronchodilator response, or a positive bronchial provocation challenge, that is a fall in FEV\textsubscript{1} of at least 10\% from pre-challenge measures being required for exercise or eucapnic voluntary hyperventilation\textsuperscript{19}. Accordingly, Dickinson \textit{et al.}\textsuperscript{20} looked at the difference in the prevalence of asthma in the 2000 and 2004 British Olympic Team. A similar prevalence of asthma was found; 21.2\% in 2000 and 20.7\% in 2004. However, 7 athletes without previous diagnosis of asthma tested positive to the bronchoprovocation test and 21\% previously diagnosed with asthma did not meet the IOC-MC criteria. This suggest that some athletes take an asthma medication without indication and expose them to sanctions, as inhaled corticosteroids and $\beta_2$ agonists are on the prohibited substances list (World Anti-Doping Agency). Indeed, athletes need a medical exemption and tests to prove they really need these medications. Athletes and coaches may use $\beta_2$ agonists to improve their physical performances, but studies demonstrated that this medication, inhaled at current doses, does not enhances performance in well trained non-asthmatic athletes\textsuperscript{21,22}. 
Asthma in the athletes population is probably an heterogeneous entity and some athletes may have an asthma-like condition. Specific studies on the prevalence of asthma, airway hyperresponsiveness, exercise-induced bronchoconstriction, inflammatory states and mechanisms are needed. At this time, little is known about the effects of long term high level training on respiratory health. Therefore, not only do we need to know more on the long-term effects of training, but the optimal management of these problems in high-level athletes with or without asthma need to be the subject of a specific respiratory monitoring.

2.1.3 Exercise role in the development of asthma

Exercise may induce a beneficial effect on the control of asthma when practiced at mild to moderate level in usual condition\(^1,2\). However, it is possible that very intense and repeated exercise, particularly when performed over prolonged periods (years), could contribute to respiratory health problems such as recurrent upper respiratory track infection, rhinitis and asthma.

Helenius \textit{et al.}\(^23\) showed that the risk of developing asthma was reported to be increased by 6 in endurance athletes and 3.5 in strength-speed athletes. A Norwegian epidemiologic study, also showed that the risk of developing asthma in elite athletes was higher that in the general population\(^24\).

Indeed, high-level athletes have an increased prevalence of asthma, airway hyperresponsiveness and exercise-induced bronchoconstriction, and it has been suggested that intense and chronic training in various environments could lead or promote the development of airway diseases such as asthma \(^4,5\). Weiler \textit{et al.}\(^25,26\) showed that the prevalence of asthma was higher in Nagano Winter Olympics Games, with 22.4% compare to summer games with 16.7%. Athletes agreeing to complete a questionnaire, reported that they used medications for asthma, had a diagnosis of asthma, or both. The prevalence of asthma also seems to be higher for endurance sports, possibly
due to the high ventilatory demand required for prolonged time periods\textsuperscript{5,27,28}. The inhalation of large volumes of air to humidify and warm, may result in an osmotic and thermic stress on the airway\textsuperscript{29}. Hyperventilation can also cause a mechanical stress on the airway\textsuperscript{30-33}. Indeed, with a ventilation over 30L/min, most of the inhaled air is directly inhaled in the lung, bypassing the nose\textsuperscript{4}, and therefore the humidifying process. Dehydration may cause an osmotic stress and induce airway inflammation\textsuperscript{30,34-39}. This airway inflammation is associated with a vascular clogging and oedema and may be responsible of exercise-induced asthma\textsuperscript{29}.

2.1.4 Cold air environment in athletes

According to Freed \textit{et al.}\textsuperscript{37} the dryness of cold air may be involved in the high prevalence of asthma-like symptoms reported by athletes who train in cold air\textsuperscript{6,40,41}. Many reports looked at the prevalence of those conditions in athletes exercising in cold air. Mannix \textit{et al.}\textsuperscript{41}, found that 43 out of 124 skaters (35\%) had a fall in FEV\textsubscript{1} > 10\% within the first minutes after the exercise routine. Leuppi \textit{et al.}\textsuperscript{40} studied the prevalence of airway hyperresponsiveness (AHR) in athletes aged 17 to 35 years old and found a positive response to a methacholine challenge in 9 of 26 Swiss hockey players (35\%) in comparison with 5 of 24 basketball players (21\%, \textit{p} < 0.05), while the prevalence is estimated to be 7\% in the Swiss population. Larsson \textit{et al.}\textsuperscript{6} also showed that 33 of 42 (79\%) elite cross-country skiers had an increased airway responsiveness, as defined by a fall of 20\% in FEV\textsubscript{1} and in asthma symptoms whereas only one of the control subjects had a comparable response.
2.1.5 Swimmers in indoor pools

Previous studies showed that, among athletes training in various environments, swimmers have the highest prevalence of asthma\textsuperscript{42}. Indeed, Langdeau \textit{et al.}\textsuperscript{32} showed that swimmers had the highest prevalence of bronchial hyperresponsiveness compared to others sports in different training environments. This higher prevalence is probably attributable to chlorine-devised compounds, the main disinfectants used in pools. Chlorine and organic matter interaction results in the formation of chloramines, such as monochloramines, dichloramines and nitrogen trichloride. This last is a volatile compound with powerful upper respiratory tract irritant properties\textsuperscript{43}. Lévesque \textit{et al.}\textsuperscript{44} showed that symptoms associated with airway irritation during training were reported more frequently by young swimmers than by indoor soccer players. They also observed a link between the occurrence of these symptoms and chloramine concentrations, in the ambient air of indoor pools. This phenomenon is probably more marked in elite swimmers who train many hours a week at high intensity with high ventilation rate in this environment.

2.1.6 Swimmers in indoor pools

It has been previously shown that deep inspiration avoidance and time intervals between inhalation and measurement of FEV\textsubscript{1} influence methacholine challenges in normal subjects but not in asthmatic nor in obese subjects. We would like first to verify if doing the eucapnic hyperventilation test before the methacholine challenge could influence the FEV\textsubscript{1} fall after the methacholine. Secondly, we would like to verify if deep inspiration avoidance before inhalation could enhance the fall in FEV\textsubscript{1} after the methacholine challenge, as previously reported.
2.2 Cardiovascular problems

In regard to cardiovascular disorders observed in athletes, the most common include hypertension, arrhythmia and congenital malformations. These pathologies, although less frequent, have serious consequences on athletes’ health. They can be asymptomatic and they can be detected only by a systematic evaluation. A medical evaluation and an adequate long-term follow-up are necessary to detect these problems and to minimize their effects.
2.2.1 Cardiovascular effects of training

Trained endurance athletes have been noted to have profound bradycardia, which probably results from an increased cardiac vagal tone\textsuperscript{45-47}. Sacknoff \textit{et al.}\textsuperscript{48} showed that chronic exercise affect heart rate variability. Heart rate variability is an index of cardiac autonomic modulation through the measurement of instantaneous beat-to-beat variations in R-R interval length\textsuperscript{48}. Low heart rate variability, which probably results from increased sympathetic modulation and diminished parasympathetic modulation has been associated with increased mortality after myocardial infarction\textsuperscript{49-52}. Long-term physical training may add to structural changes in athlete’s heart distinct from normal subject\textsuperscript{53}. What is sometimes called \textit{athlete’s heart (AH)}, includes increased left-ventricular wall thickness and end-diastolic volume, sinus bradycardia at rest, a systolic murmur, audible third and fourth heart sounds, and cardiomegaly (heart weight generally $> 500\text{g}$) on chest radiograph\textsuperscript{54,55}. Electrocardiographic abnormalities may be seen in up to 40\% of competitive athletes and likely result from electrophysiological changes remodeling associated with physical training\textsuperscript{56}. These changes are less common in female athletes\textsuperscript{57}. Increased cardiac mass is present in athletes who train isometrically (weight lifting) and isotonically (running, swimming)\textsuperscript{58-62}. A study with professional cyclists, showed an increased in left atrial and ventricular dimension and ventricular wall after long-term isotonic exercise (approximately by 14\% over controls)\textsuperscript{61}.

2.2.2 Cardiac abnormalities in athletes

According to Burke \textit{et al.}\textsuperscript{63} athletes under the age of 35 years are most likely to die of hypertrophic cardiomyopathy and coronary artery abnormalities\textsuperscript{64,65}. Hypertrophic cardiomyopathy is characterized by cardiomegaly, atrial dilation, small or normal size left ventricular cavity, asymmetric septal hypertrophy, and myofiber disarray within the septum, involving at least 5\% of
muscle mass. Hypertrophic cardiopathy was the second most frequent cause of death (18%) and coronary artery atherosclerosis was the most frequent (30%)\textsuperscript{54}. In a study by Maron et al.\textsuperscript{66} the most frequent cause of death was also hypertrophic cardiomyopathy (36%) and the second most frequent finding was aberrant coronary arteries (13%). Of note, 3% died from right ventricular dysplasia. Others causes of cardiac death during exercise include myocarditis, floppy mitral valve, aortic stenosis and dissections and sarcoidosis induced arrhythmias, but these conditions do not seem to be more prevalent in athletes\textsuperscript{54}. Idiopathic left ventricular hypertrophy represents a variant of hypertrophic cardiomyopathy, results from undiagnosed systemic hypertension, or represents a distinct form of idiopathic hypertrophy\textsuperscript{54}. Right ventricular dysplasia is a cardiomyopathy characterized clinically by abnormalities of conduction, repolarization and depolarization, and ventricular arrhythmias\textsuperscript{54}. For athletes older than 35 years, coronary atherosclerosis is the most common cause of cardiac sudden death (78%) and hypertrophic cardiomyopathy and right ventricular cardiomyopathy are uncommon (3% and 1%)\textsuperscript{54}.

Young adults with symptoms such as palpitations, syncope, or ventricular tachycardia should be investigated by echocardiography, right contrast ventriculography, and programmed electrical stimulation studies\textsuperscript{62}. The most common coronary abnormality is a misplaced coronary ostium in which the left main and the right coronary artery arise from the sinus of Valsalva. There is strong evidence that this anomaly precipitates sudden death during exercise\textsuperscript{54}. As there is frequently an history of previous syncope, any child or young person who had evidences of cardiac ischemia or exercise-induced syncope should be investigated angiographically\textsuperscript{54}, mostly young elite athletes, because of the high-level exercise performed.
2.2.3 Sudden death in athletes

The International Olympic Committee (IOC) stresses that the phenomenon of sudden death is underestimated in athletes. Cardiac lesions constitute the main cause of nontraumatic sudden death in high-level athletes. Two athletes out of 100 000 die each year of sudden death of cardiac origin compared to 0.7 person out of 100 000 in the general population. In 90% of cases, sudden death is primarily related to a preexistent cardiac abnormality. Other causes of sudden death, include asthma and other lung diseases, heath shock, cerebral embolism, sickle cell crisis, cerebral aneurysmal rupture, cranial traumatism, rachidian traumatism, doping and drug-addiction\textsuperscript{53,67}.

Most epidemiological studies have shown that persistent exercise is beneficial and prolongs life. Exercise may, however increase the risk of sudden death during or immediately after exertion in individual who have some cardiac conditions. It is therefore necessary to understand better what is predisposing athletes to sudden death\textsuperscript{54}.

The mechanisms of sudden death in athletes dying with hypertrophic cardiomyopathy include tachyarrhythmia arising in the malformed muscle mass or in ischemic areas of small vessel disease\textsuperscript{68,69}. The value of echocardiographic screening in asymptomatic young athletes is questionable, but it may distinguish with efficiency mild forms of hypertrophic cardiomyopathy from exercise-induced cardiac hypertrophy (athlete’s heart)\textsuperscript{70}. The value of a cardiac workup, including echocardiography, is indicated for athletes who have symptoms of syncope, arrhythmias or family history of sudden cardiac death\textsuperscript{54}. A genetic basis for familial hypertrophic cardiomyopathy has also been suggested in the form of a mutation of the gene coding for beta cardiac myosin heavy chain, located on chromosome 14q1\textsuperscript{71}. However, the clinical impact of these genetic findings in the athletes population needs to be investigated further.
Table 1. Common cardiovascular causes of sudden death in athletes

<table>
<thead>
<tr>
<th>Cardiomyopathies</th>
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<tbody>
<tr>
<td>Hypertrophic cardiomyopathy</td>
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<tr>
<td>Arrhythmogenic right-ventricular dysplasia or cardiomyopathy</td>
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<tr>
<td>Dilated cardiomyopathy</td>
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<tr>
<td>Idiopathic left-ventricular hypertrophy</td>
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<tr>
<td>Congenital malformation of coronary arteries</td>
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<tr>
<td>Coronary artery aberrancies and abnormalities</td>
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<tr>
<td>Intramural coronary artery (myocardial bridging)</td>
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<td>Coronary artery disease</td>
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<tr>
<td>Myocarditis</td>
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<tr>
<td>Aortic rupture</td>
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<td>Marfan’s syndrome</td>
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<td>Coarctation of aorta</td>
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<td>Valvular heart disease</td>
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<td>Aortic stenosis</td>
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<td>Mitral valve stenosis</td>
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<td>Arrhythmias ans conduction system abnormalities</td>
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<tr>
<td>Long QT syndrome</td>
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<tr>
<td>Wolf-Parkinson-White syndrome</td>
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<tr>
<td>Idiopathic ventricular tachycardia</td>
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<td>Illicit drugs</td>
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<td>Anabolic steroids</td>
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<td>Human growth hormone</td>
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<td>Amphetamine</td>
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<tr>
<td>Ma Huang and ephedra alkaloids</td>
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<td>Cocaine</td>
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2.3 Metabolic problems

Diet and exercise may increase body iron losses. Adequate iron stores are important to the athlete to provide adequate oxygen transport (haemoglobin), muscle aerobic metabolism (Kreb’s cycle enzymes) and cognitive function\(^{72}\). Inadequate calcium intake is also common in athletes, but sufficient dietary calcium is essential for normal bone growth, prevention and healing of stress fractures\(^{72}\).

Exhaustive exercise generates excess of free radicals followed by increased lipid peroxidation and oxidative damages of other biomolecules\(^{73}\). Prolonged high-grade oxidative stress caused damage
and it is recognized as playing an important role in the pathogenesis of several disorders, including cardiovascular disease. However, it has been suggested that regular adequate physical activity might maintain and promote the antioxidant defense capacity against oxidative stress. Recent findings have shown that high-sensitive C-reactive protein (hs-CRP), the inflammatory marker associated with higher risk of coronary heart disease, is decreased with regular physical exercise. However, there is not enough data indicating the effect of long-term intensive training on the oxidative stress status and the antioxidant defense capacity as well as on the hs-CRP level.

3. OBJECTIVES

3.1 General objectives

3.1.1 Establish a long-term program of systematic evaluation and follow-up of cardiorespiratory health and performance of high elite athletes.

3.1.2 Evaluate the prevalence of respiratory, circulatory and metabolic problems among high-level athletes.

3.1.3 Evaluate the effects of treatments on cardiorespiratory conditions and exercise performance in athletes who need asthma medication.

3.2 Specific objectives

3.2.1 Determine seasonal and long-term changes in airway caliber, airway responsiveness to methacholine and inflammation in high-level athletes involved in long-term intense training.

3.2.2 Evaluate the perception of respiratory symptoms after various airway challenges and evaluate if there is discordance between subjective and objective assessment.

3.2.3 Assess the efficacy of asthma medications in asthmatic athletes.
3.2.4 Assess R-R variability in high-level athletes overtime.

3.2.5 Assess cardiac activity in high-level athletes.

3.2.6 Evaluate maximal aerobic capacity in high-level athletes.

3.2.7 Evaluate the effect of deep inspiration avoidance and airway response to methacholine in athletes.

4. HYPOTHESES

4.1 Cardiorespiratory problems are frequent and often unrecognized in high-level athletes.

4.2 A systematic evaluation of cardiorespiratory function in athletes may improve their condition and sports performance in recognising undiagnosed cardiorespiratory problems who would benefit from.

4.3 Long-term intense training is associated with an increase in airway responsiveness, asthma, and structural change of heart (hypertrophy).

4.4 Response to asthma therapy is poor in asthmatic athletes, compared to non-athletes, suggesting different underlying pathophysiologic mechanisms.

4.5 Deep inspiration avoidance before methacholine inhalation enhances the fall of FEV$_1$ in athletes.

5. METHODS

5.1 Study design

Visits will be done by athletes to the laboratory on 2 occasions each year over a period of at least 3 years and field-tests at 2 times per year according to the annual planning schedule of each team, including 1 testing during competing season and 1 in the resting period. (For example, speed skaters begin their season in September and their resting period is in the month of April.) On the
first visit, consent form will be explained by the investigator or his/her delegate and signed by the subject. At the first visit, physical examination, medical questionnaire regarding their health condition, their family history of disease, their medication and their history in practicing their sport will be performed. Also, respiratory questionnaire, blood sample, eucapnic hyperventilation test, allergy prick skin test, methacholine challenge with Borg score and sputum analysis will be done, as well as the Holter. At the second visit maximal aerobic capacity test and MAPA will be done. At the third visit, spirometric field testing will be performed for each sports. Visit 4, 5 and 6 will be the same as visit 1, 2 and 3, except for allergy test which will be done only on the first visit.

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<tr>
<th>Visits</th>
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<td>Anthropometric parameters</td>
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<td>VO2max test with ECG</td>
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<td>Respiratory questionnaire</td>
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<td>Eucapnic hyperventilation test</td>
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<td>Allergy tests</td>
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<td>Methacholine test + Borg score</td>
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<td>Induced sputum</td>
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<td>Holter (heart rate variability)</td>
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<td>MAPA (24hr blood pressure monitoring)</td>
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In addition, volunteer athletes will have three supplementary visits during the summer season, inside 10 days (visit 7, 8 and 9). These three visits will include different methacholine challenge protocols. The first methacholine challenge (visit 7) will be the same than in the first visit of the excellence protocol (see Table above, standard Juniper protocol), but no
eucapnic voluntary hyperventilation challenge will be performed before. During the visit 8, a single-dose methacholine will be performed without previous deep inspiration during 20 minutes before the first inhalation. During the visit 9, a single-dose methacholine will be performed without deep inspiration avoidance. These protocols were previously used in asthmatic, control and obese subjects (Simard et al. 2005, Boulet et al. 2005).

5.2 Subjects selection

High-level athletes who are members of one of the 11 National Teams in the Quebec area. This includes athletes with the mention of excellence, élite, relève or espoir.

5.2.1 Inclusion criteria

All subjects

1. All subjects will provide a written informed consent and the study will be approved by the institutional ethics committee.

2. Subjects will be aged from 18 to 45 years.

3. Athletes will be training for at least 10 hours per week.

5.2.2 Exclusion criteria

1. Subjects who are, in the opinion of the investigator, mentally or legally incapacitated thus preventing informed consent from being obtained.

2. Subjects unable to perform or with contraindications to the tests proposed. In regard to respiratory tests, no respiratory infection or unstable condition will be noted in the last 4 weeks.

5.3 Measured parameters
5.3.1. Measured parameters in pulmonary evaluation

5.3.1.1 Respiratory symptoms questionnaire

5.3.1.2 Forced expiratory volume in 1 second (FEV₁), forced expiratory flow (FEF₂₅₋₇₅), FEV₁/FVC ratio and forced vital capacity (FVC)

5.3.1.3 PC₂₀ methacholine

5.3.1.4 FVC and FEV₁ after eucapnic hyperventilation.

5.3.1.5 Respiratory symptom scores (baseline and at 20% fall in FEV₁ on methacholine and eucapnic hyperventilation challenges)

5.3.1.6 Induced sputum eosinophils/neutrophils and cytokines analysis

5.3.1.7 FVC and/or FEV₁ + perception of symptoms (Borg) after an intense training session (field-test).
5.3.2 Measured parameters in cardiovascular evaluation

5.3.3.1 $\text{VO}_2\text{ max}$

5.3.3.2 Significant clinical changes in electrocardiogram

5.3.3.3 Blood pressure response to exercise

5.3.1.4 24h monitoring of blood pressure

5.3.1.5 Heart rate variability

5.3.1.6 Anthropometric parameters by bioimpedance (percentage of body fat)

5.3.1.7 Other measures such as cardiac size, stroke volume and cardiac output (echography) are usually done in athletes for clinical purpose, these will be obtained for additional analysis.

5.3.3. Measured metabolic parameters

5.3.3.2 Significant and systemic markers of inflammation (C-reactive protein, fibrinogen)

5.3.3.3 Complete blood formula

5.3.3.4 Serum cholesterol (CHOL), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), apo B, apo A and glucose level.

5.3.3.5 Ferritin, serum iron, percentage of saturation, saturation capacity

5.3.3.6 Creatin kinase (CK), ions, urea, creatinin

5.3.3.7 Surfactant associated protein A or B (SP-A, SP-B)

5.3.3.8 Cytomegalovirus and Epstein-Barr virus
4.4 Description of the Methods (a summary of investigation can be found on appendix 1)

4.4.1. Pulmonary evaluation

**Questionnaires and asthma control assessment:** Apart from current subjects’ characteristics (age, sex, duration of asthma, etc.), current respiratory symptoms will also be reported according to The European Community Respiratory Health Survey (ECRHS)\textsuperscript{77}. Outdoor and indoor training duration will be also noted.

**Physical Examination:** A cardio-thoracic examination including blood pressure will be performed at the baseline visit.

**Allergy skin prick tests:** Atopy will be determined using skin prick tests to a battery of common aeroallergens. Normal saline and histamine will be used as negative and positive controls respectively. Skin wheal diameter will be recorded at 10 min as the mean of 2 perpendicular measurements. A positive response will be defined as a skin wheal diameter of 3 mm or more.

**Spirometry:** FEV\textsubscript{1}, FVC and FEF\textsubscript{25-75}\% will be measured from flow-volume curves performed according to the American Thoracic Society (ATS) specifications\textsuperscript{78}. Predicted values will be obtained from European Respiratory Society (ERS)\textsuperscript{79}. The baseline FEV\textsubscript{1} will be calculated as the best of three reproducible values (with maximum change of 5\%). Spirometry will be performed with an ATS approved spirometer.

**Methacholine challenge:**
- Methacholine responsiveness will be measured using the “classical” tidal volume method described by Juniper. Briefly, concentrations of methacholine up to 128 mg/ml will be used. Response will be expressed as the PC<sub>20</sub> methacholine. Before each FEV<sub>1</sub> manoeuvre, the Borg score for perception of breathlessness will be recorded. FVC will also be noted at baseline and after the lowest post-methacholine FEV<sub>1</sub>.

- During the visit 1, the methacholine challenge is performed after the eucapnic voluntary hyperventilation challenge. During the visit 7, the same methacholine challenge is performed but without previous eucapnic voluntary hyperventilation challenge.

- During the visit 8, a single-dose methacholine test will be performed. Baseline FEV<sub>1</sub> will be measured in triplicate and the lowest baseline value will be retained to estimate the percentage fall of FEV<sub>1</sub>. There will be no saline inhalation. The dose of methacholine chosen for the test will be the final dose that will have induce a 20% fall in FEV<sub>1</sub> on methacholine of the visit 7. FEV<sub>1</sub> will be measured only at 3 and 4 minutes from the end of the inhalation. At the end of the inhalation, the patient will continue to avoid deep inspiration for three minutes after which the first FEV<sub>1</sub> will be obtained.

- During visit 9, the same test as in visit 8 will be performed, except that after baseline measures of FEV<sub>1</sub>, the inhalation of a single dose of methacholine will be preceded by 20 minutes of deep inspiration avoidance. During that period, the athletes will avoid any deep inspiration including sneezing, coughing, laughing or any abnormal respiratory movement for 20 minutes before the onset of the inhalation of the single dose of methacholine. To ensure that no deep inspiration will be taken, the respiratory volume will be checked with a pneumotachograph.
Eucapnic Voluntary Hyperventilation Test: The method described by Argyros et al. will be used\textsuperscript{82}. Briefly, eucapnic hyperventilation consist in hyperventilating dry air containing 5% of CO$_2$ at room temperature, during 6 min at 30 X baseline FEV$_1$, i.e. 85% of maximal voluntary ventilation. FEV$_1$ will be recorded before and 1, 3, 5, 10, 15 and 20 minutes after the test to see if bronchospasm appear. At 10% fall in FEV$_1$ the tests is considered positive. Before each FEV$_1$ manoeuver, the Borg score for perception of respiratory symptoms will be noted.

Spirometric field testing: FEV$_1$ and FVC will be measured before and 1,5,10,15 and 20 minutes after maximal exercise to see if bronchospasm appear. At 10% fall in FEV$_1$ the tests is considered positive. Before each FEV$_1$ manoeuvr, the Borg score for perception of respiratory symptoms will be noted. Exercise will be specific for each sport. Maximal exercise will be defined with maximal heart rate for each subject. The duration of exercise will be approximately 10 to 12 minutes.

Induced sputum analysis: Sputum will be obtained with hypertonic saline by the method described by Pin et al.\textsuperscript{83} and modified by Pizzichini et al.\textsuperscript{84} which involves inhaling increasing concentrations of saline (3, 4, and 5%) for seven minutes each through a mouthpiece without a valve or nose clip. Sputum processing will be performed as previously reported\textsuperscript{85}. Cytospins will be prepared. One cytospin will be dried and Wright-stained, and a 400 non-squamous cell differential will be performed. IS supernanant will be frozen in aliquots for delayed measurements of mediators and cytokines: VEGF, alpha-2 macroglobulin, ECP and MPO. Mucosal injury will be assessed by looking at the number of desquamated epithelial cells in the samples.

Assessment of efficacy of asthma medication: In the athletes who require a medication to treat asthma, we want to evaluate if the bronchodilatators and inhaled corticosteroids are effective, as
they are in normal asthmatic (non-athletes). We will collect preliminary data, by analyzing the evolution of the respiratory symptoms following the medication administration. Afterwards, we will see if it is pertinent to make a controlled randomized studies.

4.4.2. Cardiac evaluation

**Blood sample:** A blood sample (46 mL) will be obtained for metabolic parameters described at section 4.3.3.

**Maximal aerobic capacity test:** Maximal oxygen consumption ($\text{VO}_2\text{max}$) will be evaluated using a progressive a maximal aerobic capacity test (RAMP). The RAMP protocol consists of an increase in work capacity at each second until the subject cannot continue. This protocol was selected, in order that each subject can reach its $\text{VO}_2\text{max}$ in approximately ten minutes. The $\text{VO}_2\text{max}$ will be considered when a plateau of $\text{VO}_2$ will be observed in spite of the increase in work capacity (unable to increase $\text{VO}_2$ by more than 150 ml/min with the increase in the capacity for work), the heart rate will not increase any more in spite of the rise in the intensity of the exercise and/or when $\text{RER} > 1.15^{86}$. This test will be carried out on a ergocycle or treadmill, depending of which one is closer to the athletes’ sports. During this test, each subject will breathe trough a mouthpiece connected to gas analyser and their nose will be occluded by using a nose-clip. The gas exchange will be collected by the gas analyser, making it possible to obtain for each breath, measurements of $\text{VO}_2$ and $\text{VCO}_2$, ventilation ($\text{VE}$), ventilatory equivalents in oxygen ($\text{VE}/\text{VO}_2$) and in carbon dioxide ($\text{VE}/\text{VCO}_2$), respiratory exchange ratio (RER) as well as the ventilatory threshold. A spirometry will also performed before and 1, 5, 10, 15, 20, 25 and 30
minutes after the tests to quantify bronchoconstriction. Heart rate and blood pressure will be continuously monitored.

**Heart rate variability:** The heart rate variability will be obtained from a 24-hours Holter recording for each participant. During this period, the patients will continue their daily activities normally. The parameters of the temporal and the frequency domain will be devised from the 24-hours Holter measures. Some of these parameters (r-MMSD, p-NN50 and high frequency) are indices of the activity of the parasympathetic autonomous nervous system. Other parameters such as the SDNN and the low frequency are indices of the activity sympathetic and parasympathetic nervous system autonomous. The 24-hours Holter recording will make it possible to determine if there is an imbalance between the activity of the autonomous nervous system cardiac sympathetic nerve and parasympathetic among subjects.

**Blood pressure monitoring:** Monitoring of 24-hours blood pressure. This recording will make it possible to assess in a noninvasive way the average blood pressure over 24 hours and the average blood pressure during the day and the night period for the subjects. Blood pressure will be measured every 15 minutes during the day and every 30 minutes during the night.
4.5 Sample size

As such study has never been done, we will recruited 100 subjects. There was no control group. Controls subjects will be include in under-studies.

4.6 Analysis

Descriptive statistics will be used to summarize the subjects’ clinical characteristics. Normally distributed data will be reported as the arithmetic mean and standard deviation. Non-normally distributed data such as sputum cell count will be reported as the median and interquartile range. Non normally distributed data will undergo the most appropriate transformation to be normalised. A Wilcoxon signed rank test will be used to compare the data that could not be normalised. Significance will be accepted at a level of 95%. The analysis will be performed using the SPSS 10.0 statistical package (Chicago IL).

5. RATIONALE AND PERSPECTIVES

Prevalence of asthma is increasing in the general population and also in athletes. Also, the increasing use of bronchodilators and the false diagnosis of asthma is of concern in olympic athletes. There is almost no data on athletes’ health in Canada and in the province of Quebec, in regard to sport’s related health problems, particularly in the field of pneumology and cardiology. These high-level athletes have specific problems and specific needs requiring investigation in order to understand which mechanisms are implicated, especially in asthma-like symptoms, and to prevent cardiac events, which are more frequent in athletes than general population. For example, previous studies in Europe showed evidence that established ECG and systematic medical evaluation is efficient to prevent fatal cardiac events in athletes. Our program will provide the Canadian athletes with these preventative measures and will help them to reach high standards while keeping staying healthy.
### Appendix 1

#### Cardiac evaluation (AHA recommendations)

<table>
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<th>Visits</th>
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<tr>
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<tr>
<td>Exertional chest pain</td>
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<tr>
<td>Heart murmur</td>
<td>X</td>
</tr>
<tr>
<td>Easy fatigability</td>
<td>X</td>
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<tr>
<td>Syncope</td>
<td>X</td>
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<tr>
<td>Exertional dyspnea</td>
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<td>Systemic hypertension</td>
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<tr>
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<tr>
<td>Heart disease (younger 50 years old)</td>
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<td><strong>Physical examination</strong></td>
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<tr>
<td>Blood pressure measurement</td>
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</table>
Reference List


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