Review

α₁–Antitrypsin: Anti-inflammatory roles in exercise and atherosclerosis

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α₁-Antitrypsin, also known as serum trypsin inhibitor, is an acute phase protein that is upregulated in response to tissue damage and infection. More specifically this glycoprotein affords the host protection against enzymes that are released by immune inflammatory cells. The most notable of these enzymes is neutrophil elastase. Neutrophil elastase has the ability to damage vasculature and in doing so may contribute to atherosclerosis and other chronic diseases in which inflammation is an integral component of the pathology. Exercise has recently been defined as anti-inflammatory in nature, however, the complex mechanism underlying this beneficial effect is not fully understood. This paper provides an overview of the roles that α₁-antitrypsin may play in atherogenesis, summarises the findings from exercise studies in which α₁-antitrypsin was measured, and proposes that transient exercise induced elevations in α₁-antitrypsin may potentially contribute to the anti-inflammatory effect of exercise.

Key words: Protease inhibitor, exercise, cardiovascular disease, inflammation.

INTRODUCTION

α₁-Antitrypsin (AAT) is categorized as an acute-phase protein that is up-regulated in response to tissue necrosis and inflammation (Baumann and Gauldie, 1994; Gabay and Kushner, 1999; Lisowska-Myjak, 2004). It rarely increases more than 4-fold, and exhibits a 4.5 day half life (Lisowska-Myjak, 2004). The primary function of this protein is to protect the lower respiratory tract of lungs from proteolytic attack by neutrophil elastase and other neutrophil-derived proteinases (Brantly et al., 1988; Carrell et al., 1982). The majority of research conducted on AAT has focused on the deficiencies thereof, however, as will be discussed, AAT may be linked to a broader range of physiological functions many of which may be implicated in chronic disease that is inflammatory in nature.

CELLS SYNTHESISING α₁-ANTITRYPSIN

In keeping with its role as an acute-phase protein, AAT synthesis has been shown to occur within hepatocytes (Brantly, 2002; Bhan, 1976). However, Coakley et al. (2001)

reported, it can also be actively secreted by neutrophils, mononuclear phagocytes and enterocytes. In addition to the aforementioned secretory cells, it has also been shown that epithelial cells can synthesise leukocyte protease inhibitors (Marchand et al., 1997; Cichy et al., 1997). A study by Cichy et al. (1997) demonstrated that cells originating from the respiratory tract epithelium produced AAT, and those inflammatory mediators, such as interleukin-1, contributed towards this expression. Lu et al. (2006) have also implicated skeletal muscle in the expression of AAT. In this study, recombinant adeno-associated virus serotype 1 was injected intramuscularly into mice. Immuno-localisation demonstrated that the AAT transgene was expressed in the skeletal muscles, and that it formed a complex with neutrophil elastase in a dose dependant manner. Although not fully understood, this finding highlights the potentially important role that AAT may play in regulating skeletal muscle homeostasis.

Deficiencies of α₁-Antitrypsin

The major function of AAT is to inhibit proteinase activity (Talamo, 1975). This involves 'neutralising' the tissue damaging effects, particularly of human neutrophil elastase

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(HNE) (Luisetti and Seersholm, 2004; Sanford et al., 1999). HNE is packaged in neutrophil granules and is released by activated neutrophils as part of an inflammatory response (Coakley et al., 2001). In the lungs, the controlled release of HNE facilitates the movement of inflammatory cells through the extracellular stroma. However, excessive, uncontrolled production of HNE results in lung damage and destruction. The key regulatory protein of HNE in the lungs is AAT (Coakley et al., 2001). Therefore, a deficiency of AAT retards the effectiveness of the host to remove excessive HNE, leading to gradual destruction of connective tissue and loss of alveolar units. This damage manifests clinically as chronic obstructive pulmonary disease (COPD).

α_1-Antitrypsin and exercise

For some time now it has been accepted that exercise may retard the atherogenic process. Evidence supporting this includes findings that physical activity is associated with reductions in low-density lipoproteins and blood pressure, improved endothelial function, and increases in high-density lipoproteins (Stefanick et al., 1998; Schlager et al., 2011; Fagard, 2001, Leon and Sanchez, 2001). Strenuous, prolonged or unaccustomed physical activity has the capacity to elicit skeletal muscle damage (Clarkson and Trembley, 1988; Evans, 1987). It is well documented that an acute phase inflammatory response accompanies this damage and is characterised by the increased concentration of a number of plasma proteins and immune cells (Weight et al., 1991; Semple et al., 2004). Neutrophil elevations have been observed following moderate intensity physical activity (Avloniti et al., 2007). It has been proposed that the neutrophil elevations following acute exercise are linked to both muscle damage and oxidative stress (Tidball, 2005; Quindry et al., 2003). Although the precise role of neutrophils in muscle injury and repair processes requires clarification, it is generally agreed upon that AAT would play an important role in regulating neutrophil activity. Relatively few studies have investigated AAT responses to exercise. These are summarised in Table 1.

ANTI-INFLAMMATORY EFFECTS OF α_1-ANTITRYPSIN – POTENTIAL ROLES IN ATHEROSCLEROSIS

Atherosclerosis is a disease in which 'plaque' accumulates in between the layers that make up the walls of arteries. This process is referred to as atherogenesis, involves the progressive retention and oxidation of lipids within arteries such that the plaque mass or atheroma increases to a point where it can significantly occlude blood flow and/or result in thrombosis (Insull, 2009). The atherogenic process is now well defined as one in which immune system mediated chronic inflammation plays a central role in its pathophysiology (Perrins et al., 2010). Exercise plays an important role in dampening this process through its anti-inflammatory actions (Mathur and Pederson, 2008), some of which may be mediated by AAT.

Histamine is a major pro-inflammatory vasoactive amine that is released from mast cells when they bind to IgE (Janeway et al., 2001; Roitt, 2001). There is recent evidence showing that histamine can exacerbate atherosclerotic lesions (Rozenberg et al., 2010). He and Xie (2004) showed that AAT inhibited IgE dependant histamine release from mast cells by 36.8%. Thus, at inflammatory sites, the presence of AAT may serve to blunt the inflammatory response by inhibiting histamine release. In doing so it may reduce the amount of low-density lipoprotein that accumulates within the atheroma.

AAT has also been shown to regulate aspects of complement-mediated inflammation. The membrane proteinase, p57, cleaves C3, enhancing inflammation (Ollert et al., 1990). AAT inhibits the activity of p57, suppressing C3 activity limiting inflammation (Rodriguez-Lima et al., 1998).

Similarly, Nita et al. (2005) have highlighted the anti-inflammatory role of Prolastin (a preparation of purified human AAT used for augmentation therapy) in regulating cytokine release from human neutrophils and monocytes. The neutrophils and monocytes were stimulated with lipopolysaccharide (either alone or in conjunction with Prolastin) and the release of pro-inflammatory cytokines tumour necrosis factor- α (TNF-α), interleukin 1β (IL-1β) and interleukin-8 (IL-8) were determined. The Prolastin 'treated' monocytes showed a significant decrease in TNF-α and IL-1β production compared to controls. Prolastin significantly decreased IL-8 production by neutrophils. The authors concluded that Prolastin inhibits the effects of endotoxin (LPS) and thus reduces the inflammatory response. Endotoxins from bacteria found within atheroma's have been implicated in triggering inflammatory responses (Triantafilou et al., 2007).

Exercise induced AAT elevations – Heart/vascular health benefits

From the studies included in Table 1, it is apparent that in 50% of the studies, plasma AAT increased significantly following exercise. An additional study showed a substantial (32%), yet non-significant elevation. Two studies (20%) showed no changes, one showed a decrease, and in one it is unclear if the absolute plasma/serum concentration changed. From these results it seems as if the trend is for exercise to induce elevations in AAT. Its primary role may be to limit the potential damage associated with exercise induced leukocytosis and the accompanying damage inflicted by these activated neutrophils which release harmful enzymes (Correale et al., 2008). However, it is plausible

Table 1. Effects of exercise on α_1–antitrypsin.

	Participants	Intervention	Observation
Haralambie (1970)	Ten physically active, medical students (22 to 26 y)	2 h seated ergometer exercise performed between 140 to 150 W.	AAT increased significantly (p < 0.001) from 284 ± 33.4 mg / 100 ml before the exercise to 313 ± 40.2 mg / 100 ml after the exercise.
Liesen et al. (1977)	Eight well trained males (24 ± 2 y)	Prolonged (3 and 2 h) runs spaced 2 weeks apart. A smaller group partook in a 9 week endurance training program.	Moderate elevations in AAT were observed following the runs. The athletes exhibited 'elevated' resting levels of AAT following the endurance training.
Gleeson et al. (1995)	Eight healthy, untrained adults (28 ± 8 y)	40 min bench stepping exercise at 15 steps per minute on a 47cm bench.	AAT exhibited no significant changes compared to pre exercise concentrations.
Fallon et al. (2001a)	Elite female netball (n = 14) and soccer (n = 18) players	Routine moderate and heavy training weeks.	Compared to resting concentrations AAT showed significant (p < 0.05) elevations following routine moderate intensity training in soccer and netball players.
Fallon (2001b)	Seven males and one female (47 ± 7 y)	6-day ultra-marathon track race.	No significant changes observed for AAT at any of the time points. However increases up to 32% during the race from resting (pre-race) concentrations.
Dufaux and Order (1989)	Healthy, moderately trained males (n = 8; 20-28 y)	2.5 h treadmill running. The subjects covered a distance between 25 to 33 km. The mean running speed was 80.1 ± 6.5% of the speed at which blood lactate reached 4 mmol.l^{-1}	AAT was significantly (p < 0.01) elevated 1h into the run, as well as immediately after and at 1 h and 3 h after the run.
Petibois et al. (2001)	Elite rowers (n = 13; 24 ± 4 y)	AAT was measured at rest and during exercise (18 km of rowing, 80% of VO$_2$max) weekly from the 1st –8th week, and then after 10, 13, 15, 18, 20, 23, 28, 33, 37, 41 and 47 weeks.	No significant (p > 0.10) changes in AAT were observed at rest and during exercise for the entire study period.
Mila-Kierzenkowska et al. (2010)	Elite / Olympic female kayakers (n = 9; 23 ± 3 y)	Ten day intense combination training (strength, endurance and water sessions)	AAT was significantly lower after day six (p < 0 .01) and day 10 (p < 0.01) compared with pre training levels.
Zhang et al. (1993)	Rheumatoid arthritis patients (n = 5; 35-77 y)	Participants walked as "briskly as possible" for 10 min.	Synovial fluid AAT activity significantly (p < 0.05) decreased following the exercise. Plasma AAT concentration was significantly higher than that found within the synovial fluid.
Semple et al. (2006)	Professional cyclists (n = 17; 28 ± 1 y)	Two prolonged stages (194 and 164 km) of a cycling tour.	AAT was significantly (p < 0.02) elevated after the 194 km stage.

that a secondary, 'spill over effect' is to indirectly improve vascular or heart health by limiting endothelial damage. Ikari et al. (2001) have shown that AAT is a critical factor in protecting the extracellular matrix of vascular smooth cell from being degraded by proteinases and also inhibits the caspase activation and thereby functions as an antiapoptotic factor. These antiprotease and antiapoptotic functions are two anti-inflammatory mechanisms whereby AAT may impart heart health benefits by protecting the vasculature.

Additional mechanisms are those linked to regulating cytokine responses. AAT inhibits gene expression of the potent inflammatory cytokine tumor necrosis factor-α (Subramaniyam et al., 2008) and induces the release of interleukin-1 receptor antagonist from macrophages (Tilg et al., 1993). The majority of evidence in the literature supports the anti-inflammatory actions associated with AAT despite the fact that there is evidence that oxidised AAT may contribute to or exacerbate inflammation (Scott et al., 1999). It is unclear whether or not acute or chronic exercise may affect the levels of oxidised/modified AAT and thereby impact on the inflammatory processes. Similarly the opposing mechanism whereby AAT may impart or modulate atherogenic processes requires elucidation as authors have proposed conflicting theories (Correale et al., 2008).

In summary, AAT is the most abundant proteinase inhibitor. It exhibits pronounced anti-inflammatory actions and is integrally involved as an acute-phase protein in modulating aspects of the inflammatory response. As with many immune/biochemical proteins it is arguable that the exercise induced changes may be mode, duration, intensity and population dependant. Together these factors make it difficult to precisely describe and quantify the AAT response to exercise. Despite this, it does seem as if exercise elevates AAT. It is unclear however if transient exercise induced elevations in plasma translate into increases in intravascular AAT where inflammatory pathologies manifest in the form of atherosclerosis. If so this protein may be a crucial role player in combating cardiovascular disease. Lack of evidence also makes it difficult to determine if persistent up or down regulation of ATT accompanies chronic training or if the response is purely transient following each session. The latter, which seems more plausible based on the available evidence, would suggest that AAT benefits are only imparted if individuals exercise regularly (that is, on a daily basis), a concept upon which current exercise guidelines and recommendations are based in order for individuals to derive optimal health benefits. Recently, exercise has received considerable attention as an anti-inflammatory 'modality'. The aim of this paper was to highlight the potential roles that AAT may play in this complex phenomenon which to date has only focussed on selected immune cells and proteins. Strong evidence implicates the fact that AAT modulates inflammation; however, the mechanism whereby exercise induced elevations in AAT may create an impact on inflammatory disease remains speculative.

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