

EFFECT OF SMOKING AND SMOKING CESSATION ON MUSCLE AND VASCULAR
FUNCTION-A Pilot study

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Abstract

Smoking is a preventable risk factor for the development of chronic diseases in the lungs, brain, heart and kidneys, but smoking can also affect vascular and muscle function. It has been observed, for instance, that the smokers suffer from an earlier onset skeletal muscle fatigue that may at least partly be caused by toxins, such as carbon monoxide (CO), in the blood. The impact of smoking and smoking cessation on skeletal muscle and vascular function however are unknown.

Methodology. After obtaining ethical approval and informed written consent, we investigated the impact of smoking and 2 weeks smoking cessation on muscle and arterial function in 13 participants (8 smokers (S); 5 controls (C)). We measured blood CO with a smokelyser and nicotine using an ELISA. In the right femoral artery (FA) we measured flow and vasodilation induced by contractile activity and a short occlusion with 2D US. The muscle strength, fatigability of the right Vastus lateralis muscle were determined with a dynamometer. **Results.** The blood CO (S: $4 \pm 0.9\%$; C: $0.0 \pm 0.0\%$, $P < 0.003$) and cotinine levels (179 ± 24 vs 27 ± 16 ng·ml⁻¹, $P < 0.001$) were significantly higher, whereas maximal torque (208 ± 21 vs 259 ± 14 Nm, $P < 0.03$), flow (548 ± 42 vs 714 ± 80 ml·min⁻¹ $P < 0.01$) and FA diameter (6.8 ± 0.3 vs 7.6 ± 0.2 mm, $P < 0.01$) were significantly lower in smokers than non-smokers. After 2 weeks of smoking cessation, CO decreased to $0.3 \pm 0.2\%$ ($P < 0.01$) and cotinine to 30 ± 13 ng·ml⁻¹ ($P < 0.02$) while there were no significant changes in FA diameter, flow, maximal torque or fatigue resistance.

Conclusion Our data suggests that the reduced muscle and arterial function in smokers does not revert to normal after a short cessation period, although CO/Cotinine levels decline and may require a longer cessation period, supplemented with antioxidants to stabilize, improve or recover function.

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Abbreviations

ATP = Adenosine triphosphate

C = Non-smokers

CFA = Common femoral artery

CO = Carbon monoxide

COHb = Carboxyhaemoglobin

COPD = Chronic obstructive pulmonary disease

CVD = Cardiovascular disease

ED = Endothelial function

ELISA = Enzyme-linked immunosorbent assay

FI = Fatigue index

FMD = Flow mediated dilation

FT = Fatigue test

HRP = Horseradish peroxidase colorimetric conjugate (protein) used

IMT = Intima media thickness LA = Linear array

LFA = Left femoral artery

MFT = Muscle function test

MVC = Maximum voluntary contraction

NRT = Nicotine replacement therapy

OFR = Oxygen free radicals

PES = Percutaneous electrical stimulations

QF = Quadriceps Femoris

RFA = Right femoral artery

S = smokers while smoking

SC = Smokers on cessation

TQ = Torque

USG = Ultrasonography

VA = Voluntary activation

VFT = Vascular function test

VL = Vastus lateralis

1. Introduction and literature review

Tobacco use is a devastating public health problem. According to World Health Organisation, 1 billion men and 250 million women worldwide smoke cigarettes daily. Smoking is the single most preventable cause of disease and accounts for 5 million deaths per year globally. The top three smoking-related killer diseases are cardiovascular disease, lung cancer and chronic obstructive pulmonary disease. Although a few approved pharmacological therapies of smoking cessation have claimed to be successful, the majority of smokers struggle to quit smoking (Siu, 2010).

Cigarette smoking is often associated with low levels of physical activity and poor nutrition (Mesquita et al., 2015). A growing body of evidence suggests that smoking reduces exercise tolerance and increases muscle fatigability (Prior et al, 2004; Larsson et al, 1988; Wüst et al, 2008). An impaired lung function and oxygen delivery to the muscle are distinct consequences of smoking and may contribute to the increased fatigability of the muscles of the smokers, but also muscle atrophy and a slow-to-fast fibre type transition during smoking may impair muscle fatigue resistance and exercise tolerance (Wüst and Degens, 2007). Before discussing smoking-induced muscle dysfunction, it is important to understand the basic muscle and arterial structure and function. (*see appendix 1*). This will be followed by a literature review of the effects of smoking on vascular (section 1.1) and muscle (section 1.2) function.

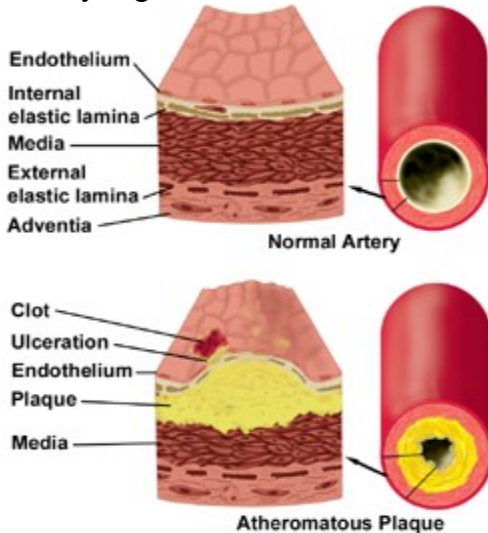
1.1 Smoking and vessel function

1.1.1 Common Femoral Artery (CFA) Diameter in health

The vastus lateralis muscle is considered as the largest part of the quadriceps femoris muscle in the thigh. It is supplied by the common femoral artery and its branches. There are very few studies related to the CFA size and diameter in normal subjects and even fewer that compare the effect of smoking or smoking cessation on CFA in relation to the contractile activity of the muscle. However, a preliminary study has shown that CFA diameter decreases with age, which may be a consequence

of reduced sensitivity to and or impaired release of nitric oxide (NO) by the endothelium (Trinity et al, 2012)

Dysregulation in the balance of endothelium-secreted vasodilators and vasoconstrictors can be a



result of endothelial dysfunction (ED), characterized by a compromised dilation of the vessel in response to vasoactive substances such as NO, but also other vasodilators such as endothelium-derived hyperpolarizing factor (EDHF). ED is commonly present in individuals with chronic diseases such as atherosclerosis, hypertension, type II diabetes, and in smokers (Barton, 2011). Fig (Left) Cross-section of a normal artery and

damaged by J Norah yoursurgery.com

1.1.2. Vascular supply to the skeletal muscle fascicle

Apart from increasing the blood viscosity, plasminogen, platelet and fibrinogen levels in the blood, cigarette smoke reduces the capacity of blood to deliver oxygen to the muscle cells and cause atherosclerosis (Celermajer,1993; Ambrose and Barua, 2004). The NO-dependent endothelial function test, called flow mediated dilation (FMD), was devised by Celermajer 20 years ago (Celermajer, 1993) and may show differences depending on the artery tested, placement of the occlusion cuff and duration of cuff occlusion (Trinity, 2012).

The skeletal muscle function depends on oxygen and nutrients for its contraction and the blood flow to the muscle is thus critical for the overall muscle performance (Prior et al., 2004). This is evident by the increase in the capillary-to-fibre ratio, the diameter of the vessels and flow capacity in the capillary bed of regularly exercised muscles. Further, the capillary bed that supplies a particular

muscle group might increase in the number of capillaries (angiogenesis) to match the energy requirements of that muscle (Prior et al., 2004). Likewise, because of disuse, the loss of muscle mass may be accompanied with a reduction in the number of capillaries to that muscle (Degens & Alway, 2006).

Pitcher & Miles (1997) examined the influence and mechanism of increased muscle blood flow on the prevention of muscle fatigue during isometric exercises of the hand muscles. Others found and suggested that the occlusive reduction of blood flow, low oxygen levels and formation of inflammatory free radicals may contribute to the diminished muscle endurance in smokers (Degens et al, 2015). Another study reported that the blood flow modulation mechanism to the muscle, was located in the vessel wall and that it facilitated the insulin-mediated glucose uptake by muscle fibres, which was dependent on capillary recruitment (Clark et al., 2003).

It is well established that type I fibres have a significantly higher oxidative capacity than type II fibres. In this regard, Laughlin and Armstrong (1982) noted that the blood flow to type I fatigue resistant fibres during a constant and graded exercise was four times more than that to type II fibres. Further evidence for the importance of blood flow for fatigue resistance comes from the observation that muscle fatigue is increased dramatically after abolishing flow to the muscle (Wust et al., 2008) and the fact that increasing perfusion pressure increases muscle fatigue resistance (Petrofsky & Hendershot, 1984).

1.1.3 Effects of smoking on vascular function

Blood flow to the muscle can be increased by vasodilation. Vascular diameter and vascular tone is the result of a fine balance between vasoconstriction and vasodilation. Endothelial cells have surface receptors that can sense stress-mechanic forces by sending out vasodilator signals for relaxation. Endothelial cells also respond with NO release to various stimuli like ADP, bradykinin, and calcium. Smoking related structural and mechanical damage to the endothelium, by substances in

smoke, such as carbon monoxide, nicotine and cotinine, can interfere also with the acetylcholine-induced vascular dilation (Mayhan, 1998; William, 1999; Hosokawa, 2008; Mayhan and Sharpe, 1998, 1999; Hosokawa et al., 2008). The core feature of ED commonly seen in chronic smokers is indeed impaired bioavailability of NO and altered response to ACh, which reflects as a disease state, with increased vascular rigidity and flow disturbances (Celermajer et al., 1993; Hosokawa et al., 2008). Smoking has also been reported to affect the microcirculatory network of tissues, causing permanent distal tissue damage that was attributed to drop in oxygen tension and increased lactate accumulation (Lehr, 2000; Luu, 2013). The observed degenerative necrosis of cells was attributed to nicotine and proportional to the dose and duration of exposure and reductions in tissue blood flow (Lehr, 2000). Others, however, observed that nicotine caused “narrowing and widening” of the red cell column, as in the healthy and stated that if there was a drop in capillary blood flow/decline in smokers, it was not related to the nicotine content or level in cigarette (Richardson, 1987).

Any alterations to normal arterial diameter parameters per se may have functional consequences in tissue function (Pohl, 1986). In a study of brachial artery endothelium already as smoking as little as one cigarette diminished effective arterial vasomotion for 60-90 minutes, while repeated smoke exposure reduced lumen and the tissue blood flow and may over time precipitate cardiovascular events such as stroke or heart attack (Lekakis et al., 1998).

The lowered flow (51%) in chronic smokers was associated with lowered muscle glucose uptake and metabolism, which could be overcome by insulin infusion (Ronnemaa et al., 1999). This suggests that lowered blood flow in smokers was also directly and simultaneously linked to the lowered muscle glucose uptake in muscle that could contribute to an early muscle fatigue (Ronnemaa, 1998). Thus, firstly it is suggesting that the overall flow reduction to the muscle and the associated reduction in glucose uptake is reversible and suggests that also muscle fatigability in smokers is readily reversible by smoking cessation..

1.1.4. The effects of smoking cessation on vessel function.

Even a low dose of smoke exposure, such as that experienced during passive smoking, impairs endothelial function in healthy young children (Kallio et al., 2009). Another study concluded that the nicotine and CO levels, apart from increasing the blood viscosity and propensity to blood clotting, could also greatly decrease micro perfusion and tissue oxygenation (Lee et al., 2013). In smokers, the L arginine-NO pathway was impaired and resulted in a reduced NO production (Takajo et al, 2001). These changes were exclusively attributable to the higher nicotine dose exposure, in heavy smokers and were reversed after two weeks of smoking cessation (Morita et al., 2006). Further, the platelet aggregation was later enhanced again by re-initiation of regular smoking. This suggests that smoking-induced cellular platelet aggregation; vessel dilation and flow malfunction was associated, partly with the blood nicotine levels and oxidative stress. Further, oxidative stress and muscle endurance could be restored and enhanced by high dose of antioxidants, suggesting a vital role for antioxidants in the muscle fatigue reversal process (Kallio, 2007; Meyer et al., 2013).

1.1.4.1. Smoking Cessation

Nicotine is a major active component in tobacco smoke. It is not considered a pathological agent, although its presence in blood induces some inflammatory responses due to its metabolite constituents. However, upon smoking cessation, the abundance of foreign particles that cause inflammation and oxidative stress, and the presence of nicotine metabolites and carbon monoxide quickly return to undetectable levels in the blood. Given these observations, parallel reductions in oxidative stress and recovery of tissue function are to be expected to follow smoking cessation. In line with this, the negative effects of smoking on platelets were reversed after two weeks of

cessation. Additionally, Endothelial Dysfunction was reversible in just 24 hours of smoking cessation (Morita et al., 2006).

1.1.4.2. Effect on Mitochondrial dysfunction

Smoking-related mitochondrial dysfunction, reducing aerobic ATP production, has been suggested to underlie the increased fatigue and muscle dysfunction (Degens et al., 2015). Mitochondrial dysfunction has been seen in many muscle diseases. The increased circulating levels of CO from the inhaled smoke causes COHb formation and consequently a decline in tissue oxygenation (Lee et al., 2013). CO can also bind to components of the respiratory chain and has indeed been shown to reduce by 23% the mitochondrial respiratory chain /complex IV activity [cytc-ox activity] (Alonso et al, 2003). At cellular level in the skeletal muscles from smokers, reduced mitochondrial density, impaired mitochondrial respiratory chain complexes reaction and coupling are reported. These changes may well be reversible by smoking cessation (Meyer et al., 2013).

1.1.4.3. Voluntary activation of muscle

To produce maximum voluntary isometric contraction of muscle, concentration and effort is required to fully activate the muscle. It is possible that the activation of the muscle is impaired in smokers, but there is also a possibility that it is increased as increased levels of nicotine in blood stimulates acetylcholine receptors.

1.1.5. Biomarkers of smoking in blood:

1.1.5.1. Carbon monoxide-related hypoxia

Carbon monoxide (CO) formed from partial combustion of hydrocarbons in the cigarette smoke hinders oxygen delivery and its utilisation by mitochondria for aerobic ATP production. Part of the problem is related to the binding of CO to haemoglobin, forming carboxyhaemoglobin (COHb), thereby reducing the oxygen carrying capacity of the blood by up to 15% available oxygen from RBCs. Marketed as “safe” and “Organic” cigarettes produce 75 times more CO than traditional cigarettes, potentially even further reducing tissue oxygenation. Its rapid binding to Hb is 250 times stronger than oxygen, so that the smoke -inhaled CO in effect competitively displaces oxygen from Hb. The combination of CO with Hb also shifts the O₂ dissociation curve of the HbO₂ to the left, making the release of the bound oxygen more difficult and thereby adding to the hypoxic state of the tissue of smokers.

Given the above considerations CO can be thought off as a poisonous gas, due hindering the transport of oxygen to tissue by binding not only to Hb, but also myoglobin, and its inhibition of complex IV in the mitochondria (Gonzalez-Alonso, 2003; Gorman et al., 2003; Prockop and Chichkova, 2007). While many studies support the traditional CO “hypoxic” cytotoxicity theory others suggest rapidly progressive Nitrativ stress as a consequence of CO-related cell death (Thom et al., 1997, 2000; Patel et al., 2012) .

1.1.5.2. Nicotine

Biomarkers of smoking in blood may be indicative for the degree of smoke exposure of an individual. Twenty-one metabolites are elevated in serum of smokers and thus can act as potential biomarkers of smoking status. Smoking cessation both reduced these levels and the associated disease risk (Xu et al., 2015). One such marker is the breakdown product of nicotine: cotinine (Neunteuft et al, 2002). *Direct effects of nicotine have been documented.*

Long term, intense, repeated nicotine exposure induces desquamation damages, physically destroys endothelium and causes “junctional leaky holes” in the basal membrane that accelerates atherosclerosis (Villablanca, 1998; Lin et al, 1992; Lehr, 2000). This endothelial cell injury and loss of endothelium, increases selective permeability of the endothelium, and transport of macromolecules like LDL to and through the intercellular clefts, that is further accelerated by smoking-induced inflammation (.Lin et al, 1992). Apart from endothelial dysfunction and changes in endothelial structure, nicotine also selectively enhances noradrenalin-mediated constriction of deep arteries in bones that in effect results in chronic reduction in bone blood flow and function (Feitelson et al, 2002) and may underlie the reduced bone strength in smokers (Wust et al., 2010). It has been demonstrated that smokers have a decreased blood supply in conduit arteries (Celermajer et al., 2003) and suffer greater peripheral muscle fatigue than non-smokers (Wust et al., 2008). The decreased blood supply is considered as the main reason for this.

1.2. Smoking

1.2.1. Muscular effects

Smoking and muscle strength and mass

Smoking is also associated with a lower lean body mass that is associated with the lower muscle force (van den Borst et al., 2011). Smokers also have a reduced capillary to fibre ratio (Montes de Oca et al., 2008; Wust et al., 2008a). This lower capillary supply can result in reduced tissue oxygenation and contribute to the early muscle fatigue in smokers. Given these findings, it is likely that continued smoking causes progresses muscle dysfunction. Muscle wasting and weakness in smoking maybe reversible by smoking cessation

Some studies have reported that skeletal muscles of smokers have lower muscle force than healthy non-smokers, but it is not clear whether this weakness is due to smoking *per se* or due to inactivity in smokers. The consensus is, however, that smoking *per se* can induce muscle weakness (Barreiro et al., 2010; Saito, 2012; Degens et al, 2015). Part of the weakness is attributable to a lower muscle mass, that may be associated with up to 25% fibre atrophy (Montes de Orca et al., 2008). This supports the idea that smoking can cause muscle atrophy and subsequent weakness

1.2.2. Muscle damage and fatigue

Muscle Fatigue that is being suspected to occur in smoking is weakness, and is generally defined in terms of the loss of ability to generate force (Jones & Round, 1990). During exercise, fatigue develops where the individual is no longer able to perform exercise at a required level. Part of this inability to perform the exercise is thought to be due to the muscle fatigue, which may be caused by a progressive rise of cellular metabolites viz., H^+ , P_i , lactate, and pH decline because of glycolysis, within the locomotor muscles (Jones and Round, 1990).

Acrolein and acetaldehyde in cigarette smoke have been shown to induce muscle protein breakdown (Rom et al, 2013). In addition, cigarette smoke has high-level pro-oxidants, such as reactive nitrogen species, (RNS) that induce Nitrate and nitrosamine stress. Peroxynitrite in particular was involved in p38 MAPK phosphorylation during oxidative stress and caused down regulation of myosin heavy chain protein expression (Rom et al, 2012, Takajo et al., 2001). Smoking of even one cigarette can raise blood acetaldehyde level by 9.4 μ M and sustained elevated levels in smokers may contribute to the decline in muscle function observed in smokers. Similar to acetaldehyde, 25 μ M acrolein exposure of C2 myotubes induced rapid wasting and loss of myosin (REF). While other substances in smoke may also cause injury acrolein specifically is highly toxic to muscle and is 10 times more

abundant in saliva and breath of smokers than non-smokers and supports the notion that acrolein may cause muscle breakdown and muscle weakness in smokers (Rom et al., 2013).

Muscle weakness in smokers may also be related to decline in blood flow. Each inhaled smoke puff has nicotine, CO, CN and about 10^{15} free radicals and it is therefore no surprise that oxidative stress is common in smokers, that in turn leads to systemic inflammation. To make matters worse, systemic inflammation induces further production of Reactive oxygen species (ROS), Reactive Nitrogen Species (RNS), superoxide anions, which activate breakdown of proteins. It is thought that systemic inflammation and oxidative stress are two factors that contribute to the muscle protein breakdown and weakness in smokers (Takajo et al, 2001; Rom et al, 2013). It is interesting to note that mice exposed to cigarette smoke had a lower VL muscle mass that was strongly associated with the reduced force generation, which occurred by a parallel 51% decrease in femoral arterial flow (Montes de Oca et al., 2008; Ronnema, 1998).

1.2.3. Muscle fatigue resistance in smokers

Studies conducted by our group have previously shown that smoking causes a reduction in the skeletal muscle fatigue resistance independent of smoking history and physical activity level (Wust et al. 2008). This occurred in the absence of significant changes in muscle fibre type composition, fibre size, capillarisation and the activity of succinate dehydrogenase (SDH). It is not clear whether the reduced fatigue resistance is due to impaired oxygen delivery to muscles and/or due to damaged blood supply within the muscles. When matching age and activity, some studies have shown that muscle fatigability was associated with decline in aerobic metabolism in smokers (Barreiro et al., 2010; Degens et al, 2015).

It has been shown that CO inhalation that resulted in 6% carboxyhaemoglobin, as commonly observed in smokers, leads to similar reductions in muscle fatigue resistance (Morse et al., 2007) as

that observed in smokers, suggesting that fatigue in smokers was related to decreased oxygen supply to the muscle (Celermajer et al., 1994; Moreno et al., 1998). Indeed, an adequate blood flow is a critical factor for muscle performance and smoking has been shown to diminish peripheral blood flow and shear stress of the femoral artery in humans (Ronnemaa, 1998). To a certain degree, these factors were reversible by regular exercise (Anton et al., 2006) and smoking cessation for 8 weeks has been shown to restore venular and arteriolar function and endurance (Hashizume et al., 2000; Morita, 2006).

In exercising muscles the red type I fatigue resistant muscle fibres receive 4 times more blood flow than fatigue prone type II muscle fibres (Laughlin & Armstrong, 1982). This suggests that the high aerobic ATP production in type I fibres requires a constant oxygen supply, derived from the relatively large blood flow. This blood ultimately comes from the arteries and one could thus hypothesize that a decreased blood flow in smokers is one of the factors cause their reduced skeletal muscle fatigue resistance. Indeed, restriction of blood flow reduced fatigue resistance in hand muscles during isometric exercise performance significantly, independent of the aerobic capacity (Pitcher and Miles, 1997; Samra and Levine, 2015). Thus, decreased oxygen transport causing an impaired aerobic ATP generation and cardiovascular capacity was responsible for the accelerated onset of muscle fatigue during hypoxia. This indicates that as long as blood flow remained reduced or restricted, muscle endurance was impaired. Such a mechanism may be at work in smoker.

1.2.4 Oxygen supply and muscle function

Relatively less oxygen and more free radicals create an inflammatory response that may impair fibre performance (Degens et al, 2015). Some molecules in smoke could also damage muscle proteins and hinder protein synthesis, which could result in muscle weakness, while vascular

impairment may contribute to the earlier onset of muscle fatigue in smokers (Wust et al , 2008). Increased in vascular resistance, reduction in flow and hypoxia, commonly seen in smokers, appears to be dose related (Ronnemaa, 1998; Lehr, 2000; Celermajer, 1993)

1.2.4.1. Muscle loss and oxidative stress- pathways

The whole vapour phase of cigarette smoke promotes muscle catabolism, increases protein breakdown and reduces protein synthesis. The vapour phase of smoke, primarily with 10^{15} free radicals per puff, has CO, aldehydes and nitrogen oxides (Fielding, 2012) these substances induce intracellular inflammation and increased oxidative stress via oxygen free radicals and reactive nitrogen species stimulus. There are many cellular model pathways that when activated by inflammation affect muscle proteins. These include:

Nuclear Factor-kB (NF-kB) signalling pathway. The function of this pathway is to modulate inflammation and immune responses. In atrophic models, it has been shown that activation of this major pathway induces rapid degeneration of muscle and initiates myofibril death while its inhibition prevents atrophy. The aldehydes in the gas phase of cigarette smoke are volatile, toxic and soluble products that are carried by blood directly to muscle cells. C2C12 myotubes are directly affected by the vapour phase of tobacco smoke and show a reduced diameter and mass in proportion to smoke dose and exposure duration (Caron et al., 2013). Saturated acetaldehyde and unsaturated aldehyde acrolein in smoke is thought to be responsible for the direct structural and metabolic muscle breakdown. Pro-inflammatory cytokines disturb muscle energy pathways and stimulate the ubiquitin-proteasome system (Davidsen et al, 2014; Degens et al, 2015).

p38 MAPK is another pathway involved as a first step in muscle fibre loss is p38 MAPK activation. The P38 MAPK signalling pathway is activated by oxygen free radicals, such as those in cigarette

smoke, and stimulates in turn the muscle specific E3 ubiquitin ligases/- proteasome system that breaks protein resulting in the rapid muscle atrophy.

These pathways may thus underlie the decline in contractile function and the earlier onset of muscle fatigue in smokers.

1.2.4.2. Mechanism of oxidative stress [Oxidative Stress]

Smoking causes oxidative stress that increases with smoking intensity. This oxidative stress may underlie the endothelial dysfunction and impaired blood flow during exercise (Tanriverdi et al, 2006). People who quit smoking exhibit less oxidative stress due to increased antioxidant defence gained during the period of smoking. Thus, a major benefit of smoking cessation is to reduce oxidative stress (Lane et al., 1997).

1.2.5. Smoking Cessation

It is speculated here that smoking cessation would reverse many of the adverse effect of smoking on both skeletal muscle as well as vascular function. Some past cessation studies as mentioned below, have indeed reported an improved muscle force but it was not clear whether this improvement was clearly due to cessation alone or due to some other cardio-vascular factor in smokers, as also arterial function improved (Green et al., 2012; Caron et al., 2013).

A factor that might contribute to such an improvement is the potential reversal of systemic inflammation and oxidative stress (Rom et al, 2012). Furthermore, cessation increasing antioxidant and DNA reparative mechanism in tissue (Lane, 1997; Ishida, 2014). Other studies have suggested that muscle improvement after smoking cessation was linked to arterial remodelling and cardiac muscle adaptations (Benowitz, 2013).

1.3. Aims and objectives of the project

Considering that substances in cigarette smoke, such as nicotine, carbon monoxide (CO), cyanide (HCN), aldehyde and others may interfere with the delivery of, and ability to use, oxygen by the muscle, the aim of this study was to understand to which extent smoking cessation can reverse some of the detrimental effects of smoking on vascular and muscle function.

1.3.1. Objectives

1. To review the literature on the effects of smoking and smoking cessation on muscle and vascular function
2. To determine the levels of indicators of smoking habits in the blood
3. To assess muscle function before and after smoking cessation
4. To assess vascular function with Doppler ultrasound before and after smoking cessation

2.6 Hypothesis

Smoking results in reduced skeletal muscle fatigue resistance that is related to impaired vascular function. This cumulative smoking-induced impairment of the muscle and vascular function is reversible by two weeks of smoking cessation.

Our pilot study research will focus primarily on the muscle function (strength and fatigability) in relation to endothelial function in chronic smokers and after 2 weeks of smoking cessation.

3. Methodology

3.2 Recruitment and selection

Male and female smokers and non-smokers: (17 Participants in total) were invited by email, poster advertisement and in person to participate the smoking cessation study. Participant characteristics are given in table 1 .The advertisement flier briefly described the purpose and expected outcome of the research study (see appendix 2 for poster advert).

Inclusion criteria

Participants above 18 years of age and healthy as judged by their medical history and self-reported questionnaire and physical demography, were included in the study. The basic condition was that the smokers regularly smoked at least five cigarettes per day for at least three months preceding the study. Participants were familiarised with the laboratory set up. A written consent was obtained after the purpose, nature and outcome of the study and possible risks and hazards were explained to the participants. (See appendix 5 for consent form and description). The experimental protocols were approved by the Ethics Committee of the Manchester Metropolitan University.

Exclusion criteria

1. To minimize the effect of confounding factors of morbidity and functional outcome participants having any muscle, cardiovascular, lung, joint, or metabolic disease or reportedly taking any oral, injectable or inhalant medication were not included in the study. Pregnant women and people 60 years and older were excluded. In addition, ex-smokers and habitual smokers unwilling to refrain from smoking for two weeks were excluded from the study. One participant was found using a small dose oral contraceptive pill and another a regular high dose anti-histamine, both of which could affect blood vessels, and were excluded from participation.

2. A final group of 13 fit gender, mixed-race and age-matched participants were recruited; eight were smokers (five males, three females) and five were non-smokers (four males, one female).

3.1 Subject preparation

All subjects arrived at the lab after an overnight fast (of 6-8 hours), specifically with no alcohol, caffeine and fatty food intake the night before the lab visits. In the morning, they were allowed to consume a light non-fatty breakfast. Smokers were permitted to smoke as usual, such that their last cigarette smoked would be the one just before the sample of blood was taken before the 2-week abstinence.

At day 1 (D1) participants were classified as current smokers (S) and non-smokers controls (C). All measurements were carried in the environmentally controlled lab premises after allowing about to 5-20 minutes to reach hemodynamic stability, as verified by a stabilised heart rate (HR). All the muscle function tests (MFTs) were performed in the right Vastus lateralis muscle). On the left side, the flow mediated dilation (FMD) of the femoral artery (FA) was carried out with Doppler Ultrasonography (USG).

First, the baseline height and body mass were measured. HR, blood pressure (BP) and COHb were measured; then later with a Smokelyser. A venous blood sample was collected from the antecubital vein, 5-10 min after smoking one full cigarette (for smokers), by a certified phlebotomist. The samples were mixed, centrifuged at 3000 RPM for 10 min and serum separated and stored at -80⁰ C for analysis later. The entire measurement procedure took a maximum of two hours, starting with a smoking bout, blood collection and muscle, vessel and FMD measurements.

The test order, for both smokers and non-smokers was as follows:

1. (Pre) Resting vascular function in the right femoral artery RFA before muscle function – in this test the resting vessel Diameter, Velocity and flow were determined (≈10 min)

2. Muscle function in the right R Vastus Lateralis (VL) – maximal voluntary contraction force (MVC), voluntary activation (VA) and fatigue test (\approx 1 hour)
3. (Post MFT) Vascular function immediately after the fatigue test (same as 1) (2-3 min)
4. LFA Flow mediated dilation (FMD) (\approx duration 8 min)

To determine whether the decreased arterial blood flow in the smokers was responsible for the lowered muscle force (MVC) and fatigability, blood flow at before and after the muscle contraction test was performed. To test if short smoking cessation improved vasodilation by improving endothelial function, we conducted an FMD Test after 2 weeks of smoking cessation. To test if cessation-mediated improvement in smokers had improved both flow and muscle function, we also repeated the muscle function tests after 2 weeks of smoking cessation.

All tests were performed in the same sequence as described above before and after 2 weeks of smoking cessation. Vascular function before and after the fatigue test was determined in the R FA using a high-resolution ultrasound probe, in which the FA diameter (mm), blood velocity (cm/s) and blood flow (ml/min) were measured. The four muscle functions parameters assessed were isometric maximum voluntary contraction (MVC) (N), Torque (N.m), Voluntary activation (VA %) and the 2-min fatigue test (FT). A custom-made isometric dynamometer (Amsterdam chair, VU Amsterdam, the Netherlands) with standardised approved protocols was used. Lastly, an 8-minute FMD test was carried out on L FA, to test how vascular endothelial function is affected by smoking and smoking cessation. To test the level of smoking and whether smokers really abstained from smoking, COHb in the exhaled air, and serum Cotinine in blood by ELISA assays, was measured.

Under supervision, our team of three independent investigators assisted in the collection of muscle, vascular function and blood data. Each investigator at same time was responsible for one particular part of the experiment.

3.2 Experimental Tests

3.3 Biomarkers of smoking (Cotinine and COHb)

As a marker of smoke exposure, we determined the level of blood Cotinine, a metabolite of absorbed nicotine, by ELISA assay. On the arrival at the laboratory, on day 1 (D1) of the test, 5-10 minutes after smoking their chosen standard size cigarette, a sample of 3-5 mL venous blood was collected from antecubital vein in the forearm, in 10 ml sized vacutainer tubes in sterile condition.

Cotinine

An enzyme linked immunosorbent assay (ELISA) was used to measure cotinine in serum. Standards from the National Institute of Standards and Technology were used for comparison. Test serum sample of acute smoker and specimen serum sample of non-smoker control were taken. A ready-to-use cotinine HRP enzyme conjugate was added in duplicate to the selected wells coated with anti-cotinine polyclonal antibodies. Cotinine in the collected serum sample competes with a cotinine-HRP conjugate for binding sites. Unbound cotinine and cotinine enzyme conjugate was washed out by a series of washing steps. Upon the addition of substrate, the intensity of colour was inversely related to the concentration of cotinine in the collected samples. The standard curve was constructed relating the colour intensity to the concentration of the cotinine. All testing was done at room temperature (18-26⁰ C). Using a precision pipette, 10 µL of standard, controls and test specimen were pipetted. Further, 100 µL of enzyme conjugate was added to each well. The plate was then shaken for 10-30 seconds to ensure proper mixing. It was then incubated for 80 minutes at room temperature in the dark. The wells were washed six times with 300 µL-distilled water using an automatic plate washer. Care was taken not to cross-contaminate wells. Wells were inverted and vigorously slapped dry on the absorbent paper to ensure that all residual moisture was removed. This step was critical to ensure that the residual enzyme conjugate did not skew the results. Using

an automated system, we ensured that the final aspiration on the wash cycle was aspirated from either side of the well. Then 100 μ L of substrate reagent was added to each well and incubated for 30 minutes at room temperature in the dark. Finally, 100 μ L of stop solution was added to each well and the plate was shaken gently to mix the solution. The absorbance on ELISA reader was read at 450 nm within 15 minutes after adding the stopping solution. To construct the standard curve, we plotted the absorbance for cotinine standards (vertical axis) versus cotinine standards concentration (horizontal axis) on a linear graph paper. The best-fit polynomial trend line was drawn through the points. Equation and R^2 was displayed. The absorbance for controls and each unknown test sample was read from the curve.

COHb estimation

COHb estimation was carried out using Smokelyser (Micro 4 Smokelyser, Bedford scientific Ltd. Kent, and England). Five minutes after smoking a cigarette, the smoker participant was asked to hold the breath and breathe out slowly into the breathalyser, which analysed the CO in the expired air and digitally displayed the reading on the screen. The COHb in exhaled breath measure was given in %.

3.4 Cardiovascular function (Heart rate, Blood pressure)

To determine the effect of smoking on central cardiovascular parameters at rest before and after cessation, we measured heart rate, systolic blood pressure and diastolic blood pressure using an automated digital sphygmomanometer (Omron). Then cardiovascular parameters were calculated: mean arterial pressure, rate pressure product and pulse pressure. Peripheral vascular resistance, cardiac output and stroke volume was calculated using standard equations and formulae (see appendix).

3.4a Vascular function measurements

To determine if muscle fatigue was due to flow decline, femoral artery function was determined with Doppler-ultrasound with a multi-frequency LA 5-14 MHz transducer (Esaote MyLab-70, Netherlands; Figure 3.1).

3.41 Diameter, velocity and flow

We measured blood vessel diameter, blood flow velocity and flow in the femoral artery before and after smoking cessation with optimal predefined imaging pre-sets and output calculated automatically with inbuilt algorithms and installed software. 2D visualisation with B-mode pulsed wave US Doppler with spectral analysis, had four major advantages in that it was non-invasive, radiation safe and a reliable and validated method for characterizing flow dynamics in the femoral artery.

In the right thigh common femoral arterial segment that lies in the anatomical region of femoral point was surface marked at the junction of lateral 2/3rd point between the anterior superior iliac spine and the pubic symphysis. Female volunteers were always assisted by a female chaperon to access the lower abdominal region at the upper thigh regions. The medial region was covered with a towel and the transducer probe was held in place while measurements were made.

In the resting supine position after hemodynamic stability was achieved, the femoral area of the inguinal region, longitudinally the right common femoral artery (CFA) segment 1-2 cm above the bifurcation of deep Profunda Femoris artery was assessed for standardisation (Sandgren et al, 1999). The small segment, with anterior and posterior vessel wall clarity with luminal surfaces in between, was chosen for continuous 2D greyscale imaging. Its cross-sectional area was taken for reference to determine the maximum diameter of the lumen, since adequate image definition is also possible of its sidewalls. The diameter of the arteries using B mode, was measured with ultrasonic callipers

twice in the end-diastole and end-systole. The femoral artery diameter (D), velocity (V) and blood flow (VO) were measured in each participant on two occasions.

To ensure a good imaging contact with the skin and ultrasound transducer, a generous amount of ultrasound transmission gel was applied on the probe and securely held on the femoral point of the right thigh until steady coloured pulsation were clearly visible. The femoral artery region was specifically located on the US screen to visualize the arterial movement cross-sectionally. The good quality stable images were then captured in cross-section and longitudinal axis. First, the reference FA cross-section image was taken. The probe was later moved in transverse direction to record the clear anterior-posterior wall movement at the longitudinal section, two cm above the bifurcation of the profunda femoris artery. Using the basic and advanced image optimization techniques for ultrasound and with customized vascular pre-sets, the 2D real-time femoral artery images and clips were recorded. Automated optimization vascular pre-sets of frequency, depth, resolution and insonation angles were consistently used to collect still images and video flow clip data at a 29 frames/sec rate before and after muscle function tests (MFTs). A live clip over 20-40 beats in a steady state was also recorded after mid-arterial sample volume was adjusted to cover the width of the entire blood vessel and was taken continuously at a 60⁰-insonation angle. The velocity recording sample volume was 2 mm mid-arterial and corrected angle of 60⁰ with cursor parallel to the vessel lumen. The blood flow parameters were measured in supine position and recorded as stable spectral trace as Timed Average Velocity, blood flow and blood vessel diameter. From the recorded images, 3-4 best recorded vessel segments of the 10 cardiac cycles was chosen for the measurement and analysis (Harris et al 2010). The end-diastole in the R wave and the peak T wave end-systolic diameter are larger and were used for better assessment of vasodilation effect and diameter changes in relation to smoking status. Blood flow parameters were automatically calculated from the best quality segment, using the principle of instantaneous mean blood velocity over the cardiac cycle,

using analysis of waveform of the Doppler-shift effect, beat-by-beat flow seen as a time velocity integral profiles.

3.5 Muscle functions

Maximum voluntary contraction (MVC)

To test the maximum ability to generate muscle force of the extensor muscles, the volunteers wore loose shorts, comfortable enough to access the upper part of the shaved thigh skin surface over the four knee extensors. Two electrodes were placed at the ends of QF. The Participants initially were asked to do three small repetitive isometric contractions as a warm-up. Familiarization with machine and muscle function recording, strapping, testing with PES and fatigue testing, USG (before and after) test requirement, timing, FMD was carried out on a separate occasion before the actual data collection.

The Participants were asked to sit on a custom-made Amsterdam chair with their arms across their shoulders. The Amsterdam chair is a computer assisted Isokinetic dynamometer setup that tests and measures contractile performance of muscles groups (e.g. Knee extensors). It is used here to examine strength and fatigability of knee extensors. A shin strap below the knee and an abdominal harness above the upper limit of the femoral region was applied to stabilize the upper torso for maximal force generation in knee extensors and prevention of extraneous movement. After the warm-up session, the lever length was measured from the lower end of the femur lateral condyle to the top of the connection with the force transducer. A computer screen showed the muscle force and participant were asked to put in maximal effort. While visual feedback of the muscle force was provided, an additional verbal motivation was given. To ‘push’ against the shin restraint to extend the knee, as strongly as possible, to contract the quadriceps muscle maximally three trial with a small rest in between, were performed and the maximal value recorded as MVC in Newtons (N).

Voluntary activation (Interpolated twitch technique)

Voluntary activation levels were determined using the interpolated twitch technique with a doublet pulse. The current used to test the activation was determined using single pulses with the participants in a relaxed state, starting from 100 mA, increasing the current until no further increase in torque was observed. The first doublet was applied with the participants in a relaxed state, and the second one was delivered during the plateau phase off the MVC. The ratio of interpolated and resting doublet was used to provide an index of activation as given by the formula:

Voluntary activation (%) = $100 \times (1 - (\text{superimposed doublet torque} / \text{resting doublet torque}))$,

where the superimposed doublet torque is the additional torque during the MVC caused by the doublet.

For this stimulation the anode was placed over the proximal skin region of the quadriceps muscle and the cathode over the distal tour of the upper leg. Percutaneous electrical stimulation (PES) (square wave, pulse width 50 μ s, DSV Digimeter stimulator, Digimeter Ltd., Herts, UK) was applied using carbon rubber pads (76 mm x 127 mm, Versastim, Conmed Corporation, New York, USA). The current was increased until the doublet stimulus (separated by 10ms) induced a torque equivalent to $\geq 25\%$ MVC.

The Fatigue test (Fatigability)

To measure the VL muscle fatigability, the muscle was intermittently electrically stimulated. To distinguish peripheral fatigue from central fatigue, muscle fatigue was induced by controlled automated 2 minute electrical stimulation, as PES bypasses both the central motivation and the neural causes of fatigue, and the contractions thus induced, reflect only the input of local elements beyond the motor neuron junction to muscle.

The test consisted of 30 Hz, 60 stimuli of 1sec duration, separated by 1 sec rest interval [a duty cycle of 0.5 = 50%] for 2 minutes. This intermittent stimulation protocol allowed relaxation and

hence metabolic recovery, between contractions. The test was well tolerated; all participants, as visually and verbally instructed throughout the test, remained relaxed (i.e. without knee muscle voluntary contraction), and completed the protocol. A fatigue index was calculated as the force at the end of the test divided by the peak force during the test.

3.6 Vascular endothelial function (Ultrasound assisted FMD (US-FMD))

Due to a sudden surge of blood flow (reactive hyperaemia), the resultant increase in shear stress induces the endothelium to release NO that in turn mediates a cascade resulting in arterial dilation. This FMD % is not only an index of vascular endothelium health, but also an indicator of the presence of any underlying circulating or systemic environmental stimulus. Any compromised dilation, is apparent in all arteries of smokers (Tinken et al, 2010)

To measure this functional change an 8-minute US-FMD test was performed. After taking baseline arterial diameter readings using US, a cuff was strapped and inflated to 50 mmHg above resting systolic BP to block flow (Harris et al. 2010). The cuff was released 4.5 minutes later, which induces a sudden flow mediated dilation. The probe scan recorded increased flow and sudden rapid dilation (Diameter in mm) over the next 45- 75 seconds (Pyke, 2010). The two components of vascular shear stress are automatically assessed by the algorithms of US as the mean shear stress, which is made of summated positive antegrade and negative retrograde stress components and related to the laminar blood flow through, and causing dilation in, the femoral artery. After cuff deflation, a continuous scan was taken with 10-s intervals, for 3 minutes to record all changes in diameter dilation and return to rest values.

3.7 Statistical analysis

The muscle and arterial function test data differences between smokers and non-smokers and between pre and post smoking cessation were statistically evaluated and compared with SPSS.

Multiple regression analysis using repeated measures ANOVA with post hoc Bonferroni's test was used to determine the predictors of functional improvement in luminal diameter change in response to 2-week cessation. Between and within groups comparisons were carried out. Significance was accepted at $P < 0.05$. Reliability tests were performed in terms of pre (at Day 1) - and 2 weeks post measurements in non-smoker controls. All measured values were deemed reliable.

3.8 Definitions

Cessation (SC) was considered successful if cotinine levels were less than 20 ng.mL^{-1} and COHb of 0% at the 2-week time-point. Current Smoker (S) was defined as current smoking on enrolling for study or blood level of cotinine $>20 \text{ ng.mL}^{-1}$.

4. Results

4.1 Participant characteristics

There were no significant differences in age, body height, and body mass and body mass index between the smokers and non-smokers (Table 1). As expected, none of the other parameters in our study changed significantly in the controls (non-smokers) over 2 weeks. This indicates that our measurements were reproducible and that any changes detected in smokers are due to smoking cessation.

Variable	C n=4 m=3,f=1	S n=8 m=5,f=3	SC n=6 m=5,f=1
Age (yrs.)	24.5 ± 0.9	31.8 ± 3.4	31.8 ± 3.4
Height (m)	1.79 ± 0.02	1.72 ± 0.03	1.72 ± 0.03
Body mass (kg)	76.6 ± 4.9	70.1 ± 2.8	70.0 ± 3.5
BMI kg.m ⁻²	23.8 ± 1.4	22.6 ± 1.0	22.4 ± 0.9

Table 1. Participants' Characteristics C: combined pooled mean of control values taken on Day 1 and Day 14. S: Smokers values taken before and after smoking cessation (SC). n= number of Participants, m=men, f=women .BMI: Body mass index. All values are expressed as mean ± SEM.

4.2 Biomarkers of smoking (Cotinine and COHb)

To verify that smokers abstained from smoking we measured both COHb and cotinine. Both cotinine and COHb levels were higher in smokers compared to non-smokers (Fig. 1A, B; $P < 0.0001$). The Cotinine (Fig. 1A) and COHb (Fig. 1B) levels were significantly reduced after smoking cessation ($P < 0.001$). However, as expected, two habitual smokers were not able to maintain smoking cessation for the full 2 weeks and were therefore excluded from further analysis concerning the effects of smoking cessation.

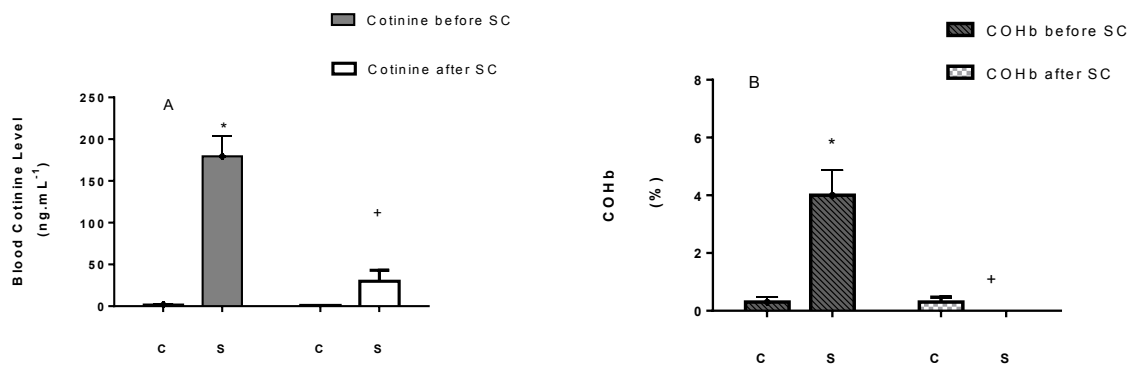


Fig 1 The effect of smoking and smoking cessation on - A) Blood Cotinine (ng.mL⁻¹) and (B) on blood carboxyhaemoglobin COHb (%) in Non-smoking controls (C), and smokers (S) before and after smoking cessation (SC) . * = significantly different from control at $P < 0.0001$ and + = significantly different from S at $P < 0.001$. The values are means \pm SEM.

4.3 Cardiovascular characteristics

There were no significant differences in systolic BP, diastolic BP, pulse pressure, stroke volume, cardiac output and total peripheral resistance between the smokers and non-smokers (Table 2).

The resting heart rate was 21 % higher in smokers compared to non-smoking controls ($P < 0.001$) and was decreased by 14 % after 14 days smoking cessation ($P < 0.002$, Fig.2A).

	C	S	SC
	n=4	n=8	n=6
Cardiovascular Function	m=3,f=1	m=5,f=3	m=5,f=1
BP-S (mmHg)	128.6 \pm 7	134 \pm 6	120 \pm 6
BP-D (mmHg)	68 \pm 3.4	74 \pm 2.7	65 \pm 4.2
PP (mmHg)	60 \pm 4.2	60 \pm 6.1	55 \pm 8.1
SV (ml)	105 \pm 7.3	106 \pm 110	97 \pm 14.2
CO (Lmin ⁻¹)	6.6 \pm 0.7	8.5 \pm 0.9	6.5 \pm 0.8
TPR mmHgL ⁻¹ min ⁻¹	.014 \pm 0.0	.012 \pm 0.0	.013 \pm 0.0

Table 2. Cardiovascular functions. C: mean of controls, S: Smokers values taken before and after smoking cessation (SC). n= number of Participants, m=men, f=women .BP-S systolic blood pressure; BP-D diastolic blood pressure; PP-Pulse pressure; SV: stroke volume; CO: Cardiac output; TPR : Total peripheral resistance . All values are expressed as mean \pm SEM.

The mean arterial pressure was higher in smokers than in non-smoking controls ($P < 0.001$) and was decreased after smoking cessation ($P < 0.002$; Fig.2B). The rate pressure product was higher in smokers than in non-smoking controls ($P < 0.001$) and returned to normal levels after smoking cessation ($P < 0.002$; Fig.2C).

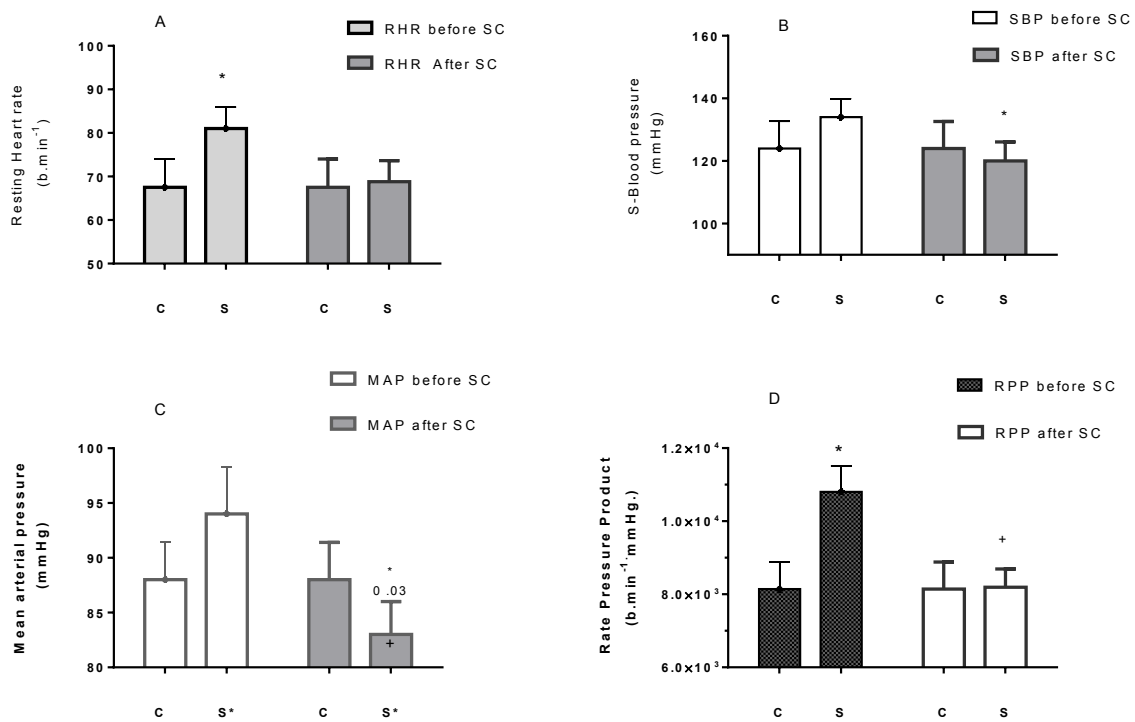


Figure 2: The effect of smoking and smoking cessation on- (A) Resting Heart rate (beats.min-1) (B) on Systolic Blood pressure (mmHg) (C) on mean arterial pressure (mmHg) . (D) On Rate pressure product (RPP in beats.min-1.mmHg) in Non-smoking controls (C), and smokers (S) before and after smoking cessation (SC) * = significantly different from control at $P < 0.05$ and + = significantly different from S at $P < 0.001$. The values are means \pm SEM.

There were no significant differences in systolic BP, diastolic BP, pulse pressure, stroke volume, cardiac output and total peripheral resistance between the smokers and non-smokers (Table 2). The resting heart rate was 21 % higher in smokers compared to non-smoking controls ($P < 0.001$) and was decreased by 14 % after 14 days smoking cessation ($P < 0.002$; Fig.2A). The mean arterial pressure was higher in smokers than in non-smoking controls ($P < 0.001$) and was decreased after smoking cessation ($P < 0.002$; Fig.2B). The rate pressure product was higher in smokers than in non-smoking controls ($P < 0.001$) and returned to normal levels after smoking cessation ($P < 0.002$; Fig.2C).

4.4 Muscle functions

Table 3: The effect of smoking and smoking cessation on muscle function. m=men, f=women, n= number of Participants. MVC = Maximum Voluntary Contraction; VA = Voluntary Activation; C: Mean of controls ; S: Smokers ; SC : Smoking cessation. The values are expressed as Mean \pm SEM.

Muscle Function	C	S	SC
	n=4 m=3,f=1	n=8 m=5,f=3	n=6 m=5,f=1
MVC (N)	730 \pm 33	655 \pm 54	697 \pm 61
VA (%)	92 \pm 3.3	89 \pm 4.1	89 \pm 3.1

Muscle strength: (Maximum Voluntary Contraction)

The MVC torque in smokers was significantly lower than non-smokers ($P < 0.02$; Fig 3A). When correcting for the lever arm the MVC force tended to be lower in the smokers than non-smokers (P

=0.09;Fig.3B).There were no significant difference in voluntary activation and fatigability scores between smokers and non-smokers (Table 3).

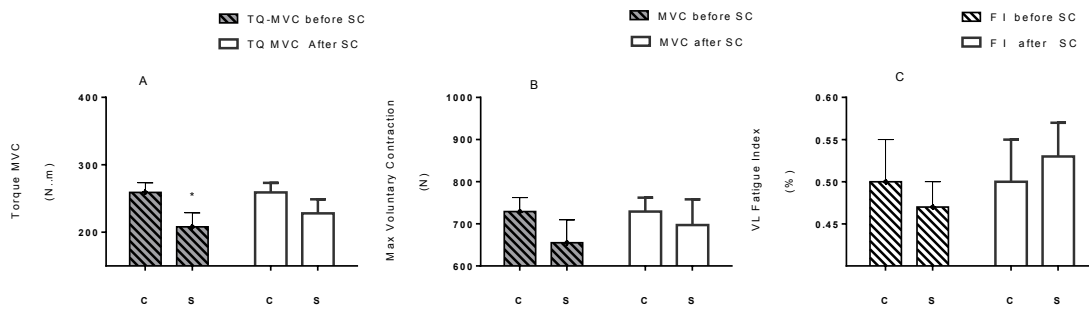


Fig 3 : The effect of smoking and smoking cessation in smokers before (S) and after smoking cessation (SC) - A) on Right Vastus lateralis Torque (N.m) (B) on maximal voluntary contraction (MVC in Newtons) (C) on Fatigue Index (%) C: non-smoking controls; F I = Fatigue Index; * = significantly different from control at $P < 0.01$ and + = significantly different from S at $P < 0.001$. The values are mean \pm SEM.

4.5 Femoral artery vasomotor function

Table 4 The effect of smoking and cessation on Vascular function C: pooled mean of values of controls taken on day 1 (D1) & day 14 (D14), S : Smokers values on Day 1 before cessation , SC : Smokers scores on Day 14 of cessation, m: men, f :women, n:number of Participants, L & R FA (Left & Right Femoral Artery), Pre and post-test vascular function scores of R FA artery in Participants before and after muscle function test, D:Diameter, Vel:Velocity, FMD : flow-mediated dilation in L FA.The values are expressed as the Mean \pm SEM .

Vascular Function	C n=4 m=3,f=1	S n=8 m=5,f=3	SC n=6 m=5,f=1
Pre-test RFA- Vel (cm.s⁻¹)	20 ± 2.0	17.8 ± 0.78	18.9 ± 1.04
Pre-test RFA- Flow rate (ml.min⁻¹)	516 ± 39.0	436 ± 50.3	409 ± 45.0
Post-test RFA-D (mm)	7.5 ± 0.29	6.9± 0.24	6.9 ± 0.36
Post-test RFA Vel (cm. s⁻¹)	24.5 ± 4.4	24.0± 1.6	25.0 ± 1.8
LFA FMD (%)	10.3 ± 3.2	10.0 ± 2.4	13.3 ± 2.4

Diameter

R FA mean pre-test diameter at day 1 was significantly lower (9%) in smokers (S1) than controls (C), $P < 0.02$, Table 4). There was no significant change after 2 week smoking cessation ($P = 0.06$, Fig 4 A) function test in smokers was significantly lower (21%) compared with controls (C) ($P < 0.01$, Fig 4 B), the flow rate increased significantly (5 % = 31 ml.min⁻¹) after muscle function test in smokers after 2 week cessation compared with controls (C) $P < 0.03$, Fig 4B)

Velocity

Rt F A blood velocity before and after muscle function tests in smokers, before and after 2 week smoking cessation was not significantly different when compared with controls (C) ($P < 0.09$)(Table 4)

The degree of FMD expressed as change in diameter was examined in C, S and SC. A reduction in the degrees of vasomotion was evidenced in smokers in comparison with control. After cessation of smoking this was increased. (Fig 5 A, B)

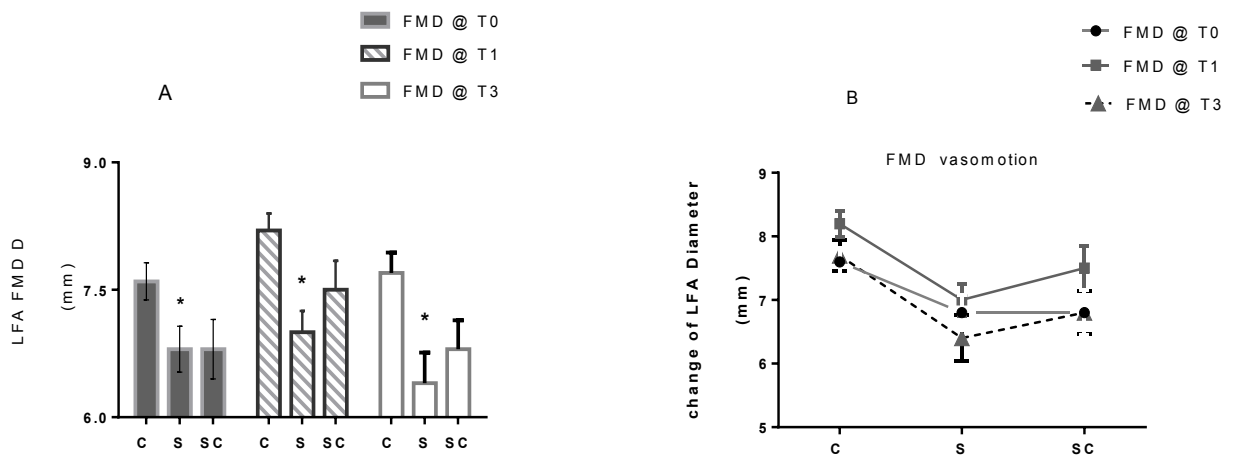


Figure 5 : The effect of smoking and smoking cessation on the degree of FMD Vasomotion expressed as change in L FA Diameter - A) on FA Diameter change (mm) in Non-smoking controls (C), and smokers (S) before and after smoking cessation (SC) . (B) On Arterial Vasomotion changes over 3 minutes time (T₀-T₃) T₀ = Baseline before occlusion cuff release T₁ = within 1 minute T₃=After 3 minutes of occlusion release. * = significantly different from control at P < 0.001 and + = significantly different from S at P < 0.01. The values are means ± SEM .

In summary, the diameter and flow rate changes were observed with short cessation, but were not significant.

Discussion

The main findings of the present study are that smoking is associated with a reduction in vascular function and muscle force generating capacity that cannot be restored within two weeks of smoking cessation.

5.1 Nicotine and CO (Biomarkers)

To verify that the smokers were really smoking and had really stopped smoking during the smoking cessation study; we measured the COHb and cotinine levels in the blood. The levels of nicotine and CO in the smokers group were higher than controls and normalised after smoking cessation (Fig.1A, 1B).

5.2 Muscle function

Torque

In line with other studies (Orlander et al. 1979, Al-Obaidi et al. 2004), we found a lower maximal voluntary strength (MVC) in smokers. Such a decline can be a consequence of a smaller muscle mass, a reduced ability to activate the muscle voluntarily, increased activation of antagonistic muscles during a maximal contraction and/or smoking-induced muscle damage. Cellular damage by definition is understood as lasting alteration of cellular or subcellular parts that do not respond to intervention. A smaller muscle strength could be the consequence of lower levels of activity in smokers as observed previously (Orlander et al. 1979). In the present study smokers and non-smokers were matched for physical activity level and, similarly to Larsson et al. (Larsson 1984), we did not observe a difference in strength between smokers and non-smokers. Thus, the decrease in strength observed in previous studies is likely to be explicable by a reduced activity level in smokers, rather than an effect of smoking per se on maximal knee extensor strength or other determinants of strength (activation). Yet, it has been reported that hypoxia can cause damage to sarcomeres and sarcolemma and be associated with a delayed recovery (Mayer et al, 2014). Our data do suggest, however, that smoking does not induce, at least in the young people we recruited, the extensive muscle fibre damage and localised and persistence necrosis (Lehr.2000; Mayer et al,2014) observed by others. Similar to the previous observations (Luu, 2013; Feitelson et al, 2002)

muscle torque correlated positively with the resting femoral artery blood flow (Fig 6B). This suggests that there is some link between muscle and vascular function.

Fatigue

COHb also hampers the release of O₂ as reflected by the leftward shift of the O₂-Hb binding curve. Furthermore, the facilitated diffusion of O₂ by myoglobin within the muscle may be impaired by the formation of carboxymyoglobin (Wittenberg & Wittenberg 1987). It has been reported that the raised levels of COHb reduce O₂ extraction during the stimulated contractions in in-situ canine gastrocnemius, which was accompanied by a reduction in muscle tension (King et al. 1987).

Our study showed 4% hypoxia by an indirect measure of 4 % COHb. However, a reduced O₂ carrying capacity of the blood induced by breathing hypoxic air had no effect on fatigue resistance during the same test as employed in the present study (Degens et al. 2006), although others did observe an earlier muscle fatigues during hypoxia (Katayama, Amann, Pegelow, Jacques, & Dempsey, 2007; Romer et al., 2007). Whatever the cause of the discrepancy, it should be noted, however, that the tissue hypoxia might be more severe than expected from the increase in COHb, as the oxygen dissociation curve is shifter to the left, resulting in an impaired dissociation of oxygen from the Hb molecule.

In addition, there might be problems with the ability to use oxygen, as reflected by the lower mitochondrial enzyme activities (citrate synthase and 3-hydroxyacyl-CoA dehydrogenase) in the muscles of smokers than non-smokers and the 23% inhibition of COX (complex IV) activity in mitochondria of lymphocytes (Orlander et al. 1979; Alonso et al, 2003).

Although only two percent of the oxygen will be oxidized to an unstable reactive oxygen species (ROS-superoxide) in complex III and a 40-50 minutes of hypoxia increased ROS by 2.5 fold short time. The elevated ROS production during hypoxia and derived from cigarette smoke, may cause

activation of cell signalling pathways, that result in muscle protein breakdown, damage to muscle membranes and oxidised proteins (Degens et al., 2015; Hoppeler, 2003).

Indeed, in smokers it has been observed that mitochondrial function may be further impaired by oxidative peroxidation as observed in 'outer cell membrane of lymphocytes' in smokers and systemic inflammation (Alonso et al, 2003; Meyer et al, 2013). Such an inflammatory process, at the level of muscle myofibrils, may also be interlinked with disturbed glycogen stores and usage, and may play a vital role in the muscle energy metabolism, catabolism and efficiency.

Interestingly, this instability in metabolism was all reversed after as little as 24 hr smoking cessation and healthy ex-smokers did not show lower activities of enzymes of oxidative metabolism than non-smokers (Larsson, 1984). These and the observations in COPD patients who stopped smoking, who exhibited improved fatigue resistance, suggests that the smoking-induced deranged alterations in the activity of oxidative enzymes and muscle fatigue resistance are reversible (Degens et al., 2005). Such reversible changes may be related to the wash out of cyanide that by polarising the membrane decreases the electron flow in complex I and of CO that inhibits cytochrome c oxidase (complex IV) (Alonso et al. 2003; Fontaine et al, 1999), These are thought to alleviate some of the potential negative effects on muscle fatigue resistance.

Surprisingly, we did not see overall improved muscle function. Despite all the elevated COHb in our study and all the other potential mechanism discussed above we did not observe significant differences in muscle fatigue resistance between smokers and non-smokers, or an impact of smoking cessation on muscle fatigue resistance, similar to other studies employing voluntary contractions (Orlander et al. 1979, Larsson 1984). This is in contrast with our previous studies, where we did see a lower muscle fatigue in smokers, which may be due to a decline in contraction-induced blood flow in the peripheral vessels of the leg (Degens et al, 2015; Wüst et al., 2008).

One possibility for this discrepancy might be the larger number of participants in the study by Wüst et al., (2008) than in the present study, as suggested by the similar pattern of differences in fatigue resistance in our study. Another explanation could be that the low level of COHb as 6% COHb that is often observed in the smokers did result in a reduced muscle fatigue resistance (Morse et al., 2008). Such an assumption may be misleading. In contrast, some recent evidence suggests that even low level chronic CO acts as a vasodilator and increased coronary artery flow rate via membrane hyperpolarization by using the antioxidant stress Heme-Oxygenase -I / Carbon monoxide (HO-1/CO) pathway (Slebos, 2003). In addition, the acute neural stimulation effects of nicotine in cigarette smoke is well-established (Cryer et al. 1976). Mundel & Jones (2006) have demonstrated that nicotine has a beneficial effect on cycling endurance in muscle. In our study, it might thus be that the circulating nicotine more than compensated for the detrimental effects of COHb on muscle fatigability in our subjects.

It has been shown that the using of direct muscle stimulation, superimposed on nerve stimulation, has shown to cause neurotransmission failure that contributed to an earlier onset of fatigue during hypoxia (Zhu et al. 2006). Here we observed no such problem as the voluntary activation did not differ significantly between smokers and non-smokers, in line with our previous observations (Wüst et al., 2008).

Cardiovascular adaptations

Our study noted functional improvement in resting HR, MAP and RPP similar to those observed in long-term smoking-cessation studies involving six weeks and even 3 years of cessation (Asano, 2012; Bassareo, 2014). In another study, the damaging CVD effects were shown to have rapidly reversed on smoking cessation (Xu et al., 2013). In asthmatics, smoking cessation induced considerable improvement in lung function, decline in the airway inflammation and cutaneous

vasoconstrictor responses (Chaudhuri et al., 2006). This suggests that cessation has a beneficial effect on heart and lung function and reduces death rate in 10 weeks of cessation (Anthonisen et al, 2005). We used 2-week cessation only in our study.

Rationale for using two weeks of cessation

In previous cessation studies, 24 hours of cessation caused a rapid reversal of endothelial dysfunction and 2 weeks cessation had corrected platelet aggregability in high risk patients (Hirohiko Morita, Hisao Ikeda and Hiroyuki Eguchi, 2005). Four weeks of cessation had improved myocardial blood flow and coronary endothelial dysfunction in smokers (Morita et al., 2006) and 1 week cessation had a profound effect on the CV parameters and catecholamines (Minami et al., 1999). It is not known if two-week cessation would bring improved blood flow in CFA and improve fatigability in in Vastus lateralis muscle in smokers.

Changes in vascular function

Our 2-week cessation results complement and extend from other similar studies of smoking cessation involving one day and one-week duration that have also showed significant improvement in endothelial function (Moreno et al, 1998; Morita et al; 2006). While we observed benefits of smoking cessation on the femoral artery function, the benefits might be even more pronounced in the microcirculation. As it has been observed that vascular, changes first occur at the microcirculatory level (Saito, 2012; Lehr, 2000; Hosokawa, 2008).

Nicotine is unpredictable and can lead to both vasodilation or vasoconstriction depending on concentration and tissue site (Lehr,2000; Mundel and Jones, 2006). The normalisation of the resting femoral artery diameter after cessation may thus be due to a normalisation of the nicotine levels in

the blood. Circulating vasoactive constrictors and inflammatory agents in blood also decrease correspondingly on short-term cessation, additionally improving the local blood flow and distribution. Briefly, the distinct properties of the endothelium, such as tone, thickness, diameter, which are closely linked to the perfusion of muscle tissue are clearly impaired by smoking and can be potentially altered by a short or long-term abstinence.

General discussion/mechanisms

The majority of skeletal muscle and all advanced peripheral, coronary and endothelial dysfunction, that inhibited vasodilation, observed during smoking maybe due to the effects blood levels of substances present in the inhaled smoke that affect organelles directly (Lekakis,1998;Morita,2006;Naya,2011) and/or caused the disruption of cell signalling pathways (Degens et al,2015). For instance, a systemic review of 6-12 months random control trials showed that the CO, nicotine and hypoxia during smoking had caused disruption of the vital tubulin cytoskeleton that keeps cell functional and stable, in addition to increased blood platelet aggregation (Ludvig, 2005).

5.3 Vascular function

Diameter

While the arterial diameter (in vasodilation) reflects endothelial wellbeing and its decrease size in smoking and its persistence even after 2 week cessation had reflected ongoing dysfunction in arterial vasomotion. Many studies have shown that the conduit common femoral artery in healthy people does not dilate in response to passive leg movement or in exercise (Trinity, 2012). We observed that in smokers, a significant decrease in diameter (30%) had occurred as a result of

habitual smoking and a failure to dilate immediately after 2 week cessation and after muscle function test was evidently seen. Such observations confirm persistence of endothelial dysfunction, failure of adequate relaxation in large artery even after short cessation. In line with other studies, it confirms that it is not the larger vessels but the microcirculation and subcellular regions of muscle that are focal points of responsive action, that experience earliest damage and during smoke exposure and also they are the ones that first to revert to normalcy in abstinence (Clark et al, 2003; Mayer et al, 2014).

Cardiovascular function

In our study raised RHR, SBP, MAP and RPP- the main central hemodynamic parameters were stabilised by short cessation. This rise in systolic BP overtime in smokers had reflected the progression of arterial wall pathological stiffness against flow, usually seen in atherosclerosis of smokers, as ED had progressed over duration of habitual smoking (Langham et al., 2015). While diastolic BP reflects coronary flow reserve and the perfusion of the myocardium. Heart rate increase, which was reflection of the pumping action of overstressed heart in smokers, was significantly reduced on cessation. As the resting heart rate changes are linked with lifestyle habits and independently predict cardio metabolic risk and mortality lowering it would cut these risk immensely (Palatini et al., 1999; Palatini, 2013). Raised systolic BP, predicts CVD risk and has two main components; pulse pressure (PP) and mean arterial pressure (MAP). PP is the pulsatile component of the BP, which is directly affected by LVEF and associated with MI, carotid stenosis and CVD death. The MAP is the steady component of blood flow through the aorta and its branches. It reflects the vital functions of LV contractility, heart rate and vascular resistance that is averaged over time. According to a study, a 10 mmHg rise in MAP above normal range, strongly correlated with 12% rise in relative risk of CVD risk being (1.48) in the young smokers less than 60 years

of age (Sesso, 2000). On cessation, a similar reduction in MAP in our study, suggestively reflects a strong resurgence of overall recovery in cardiovascular function, and an onset of possible vascular remodelling, that mirrors the initiation of clinical advantage in this young cessation cohort. Additionally, the lack of increase in PP suggests that the peripheral conduit artery, the femoral artery in our case, did not progress at least to stiffening, but was associated with lowered systemic vascular load and some increased coronary perfusion (Sesso,2000). These adaptations alone offer a great clinical advantage to smokers and this knowledge of rapid physiological recovery may be an incentive for smokers to adopt a long-term cessation or to choose quitting permanently

Blood velocity, the flow and the CFA 2 diameter dilation observed in cessation were not improved by 2-week cessation significantly, even as the endothelial functions remained inhibited. However, the blood flow function may be enhanced in cessation and on passive exercise of large muscles. Some studies show this. A study of an isolated passive muscle extension exercises of quadriceps , with NOS inhibition, had caused reduction in hyperaemic blood flow and vasodilation responses (by 80 %) in comparison to controls and these effects were independent of the relative exercise intensity (Trinity et al,2012). Suggesting that passive exercise had improved hyperaemic (flow) that had increased nitric oxide synthase (NOS) activation via the endothelial NG-monomethyl-L-arginine (L-NMMA).pathway and facilitated the bioavailability of arterial NO, such that NO alone had contributed to the amount of vasodilation and flow (Trinity et al, 2012). Applying this principle to our outcome This clearly indicates that our short smoking cessation had not improved the flow mediated NO release nor its availability ,nor had influenced the cellular pathway to any improvement in our smokers, as the baseline endothelial NOS inhibition dysfunctions had persisted and had not improved significantly as to see a change. There may be several possible explanations as to why no change had occurred in vessels and muscles as measured by us after 2 weeks of cessation.

5.4 Smoking cessation

It is known that smoking cessation improves the cellular and systemic environment as its antioxidant system washes out toxins from the body, leading to an adequate recovery. Above we have reported this as the restoration process of mitochondrial function seen in lymphocytes after 24 hours of smoking cessation. This is, however, may not the only effect of smoking cessation, as it is likely to boost antioxidants. It is also reported that DNA damages and arterial plaques were repaired because of smoking cessation, which would translate into clinical benefits such as vessel wall remodelling (Ishida, 2014; Lane, 1997; Lietz, 2013). The question however is, how long should this cessation be in order to realise these effects in smokers?

Duration of cessation

Although temporary cessation in smokers clearly mitigates some problems of smoking, but Lee et al (2013) has advocated a longer cessation for more specific muscle function benefits. Several animal and human studies have suggested that in order to see a verifiably significant reversal in muscle functions, the smoking cessation, in an oxygen rich environment, must at least be equal to, the time and duration of smoking exposure (Yen et al, 2008; Caron et al, 2013). In our study, most of the smokers were chronic smokers with a 3- to 10-year smoking history and our two-week cessation may thus have been inadequate to see substantial improvements in the muscle function.

Studies with longer duration of cessation explain some facts lacking in our study. Interestingly a study of six weeks of cessation, in asthmatics, had induced a considerable lung function improvement, coupled with a decline in the airway inflammation and a gross improvement in the cutaneous vasoconstrictor response (Chaudhuri et al., 2006). In another long cessation study, the damaging CV and ED effects were reversed only after 3 and 7 years long smoking cessation (Xu et

al., 2013; Hosokawa, 2008). These long cessation-induced function reversals studies were also associated with a reduced disease risk (Xu et al., 2013).

It has been observed that the magnitude of the vascular function response was related to the duration of cessation. Other studies have noted, however, that the duration with as little as 12 weeks of lifestyle intervention, including smoking cessation was insufficient to induce an increase in the resting arterial diameter and advocated a period of 6 months of cessation to see a complete arterial reversal (Spence et al., 2013; Bassareo et al., 2014). Some investigators suggest that a part of the discrepancy may be related to a different response times in different arteries (Spence et al., 2013). Our study shows, however, an encouraging data as we already saw a positive normalisation trend of vascular function and gain of some muscle strength after just 2 weeks smoking cessation. It is thus questionable, whether such long duration of smoking cessation is actually required.

Aids to smoking cessation

Although Nicotine Patches have a low dose of nicotine with some additional chemicals, from a public health perspective, it is still the first line to support for cessation, as it does not contain those 4000 active particles, chemicals and tar-like substances present in the mainstream cigarette smoke (Rom et al., 2013) NRT is helpful up to some extent in smokers . However, in the interest of safety, and possible CV endothelial dysfunction effects, clinicians are cautious to co-prescribe Nicotine patches with substances like Bupropion, as they are still chemical medications that may cross-react with prescribed drugs (Benowitz, 2013). Indeed, a study that investigated NRT as a cessation modality, conclusively discouraged its use, as it was reported unsafe and risky as it had worsened endocrine glucose metabolism and fatigue symptoms were precipitated and was associated with weight gain (Tonstad, 2009).

Two of our participants used nicotine patches or gum briefly, to keep the intense craving away especially at the onset of the second week of cessation. They were advised to use some Lemon juice

(Vit C- an anti-oxidant) instead, to keep the craving away. The details of gum use, duration and strength was not recorded, as it did not seem to help them. The transient usage of gum or patch did not possibly cause some physiological changes to a great degree but it appealed more so on psychology of these two participants, as they seem to feel well. It possible that nicotine may have helped these users in some way if they used it consistently and frequently in higher doses (2 mg). However, we know that Nicotine can also increase muscle endurance shown in other studies. Nicotine infusion was found to be vasodilatory (Benowitz, 2013) and we may thus have underestimated the impact of smoking on vascular function.

5.5 Implications

Among the 4000 substances present in the cigarette smoke, many may specifically have a detrimental effect on muscle fibre structure and function (Rom et al, 2013). Our knowledge of how smoking affects muscle and vascular function is limited and to what extent cessation reverses the detrimental effects of smoking is growing. However, the exact molecular mechanisms in effective fatigue reversal has remained elusive. vascular dysfunction - Impairment or damage?

Our study assumed that the vascular dysfunction in chronic smokers was an impairment. By definition, functional “impairment” is a temporary and reversible phenomenon while damage is lasting and not easily irreversible in a short time. Although we believe endothelial dysfunction in smokers is primarily a reflection of oxidant damage, they would potentially improve with long cessation and antioxidant support.

determination of muscle fatigue resistance. This is unlikely a minor problem, however, as we used electrically evoked contractions bypassing the central nervous system.

5.6 Conclusions

The findings of our study suggest that long-term smoking bears serious deleterious effects on both the vascular endothelium and force generating capacity of the VL. It was encouraging to see that despite the small sample size, there were significant normalisations of resting femoral artery diameter, resting heart rate, mean arterial pressure and rate pressure product; all these significantly indicating that a 2-week smoking cessation resulted in a rapid initiation of reduced strain on the heart and Cardiovascular system.

6. References

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7. Appendix

1. *Structure and function of muscle and blood vessel.*

1.1 -Structural and Functional organisation of muscle In the human body are 600 muscles arranged in pairs or groups that together comprise approximately 40% of the body mass. Almost all muscles attach to bones, and on receiving an electrical stimulus or action potential, they contract and initiate movement. A single muscle cell, also known as a muscle fibre, is a multi-nucleated cell, made up of myofibrils. These are arranged in contracting units called sarcomeres. The sarcomere is the smallest functional unit of myofibrils made of the contractile proteins actin and myosin. The light-dark banded sarcomere, measuring 2-2.5 microns (from Z to Z line), is made of parallel contractile and stabilising structural proteins (M line proteins, titin, nebulin, α -actinin, desmin, Spectrin, dystrophin). The alternate bands, light (I) band and central dark (A) band of the sarcomere, are evident, when seen through the Electron microscope. The A band is constant in width and contains the myosin and remains constant in length during contraction. The I band contains actin, but no myosin, and changes its length during contraction. The Central H zone is where actin and myosin overlap. Titin molecules connect both ends of Myosin to the Z line (Fig. 1; Jones and Round, 1990)

1.2 Actin Actin is a filament made of G-actin monomers and has a molecular weight of 42,000 Da. Each actin filament consists of two beaded protein strands twisted together and has points of attachments for myosin heads, the myosin binding sites. Each actin is surrounded by 3 myosins, which can interact with the actin filament. In the groove of the actin helix lays tropomyosin that in the resting state covers the myosin binding sites on the myosin molecule. Tropomyosin is associated with three troponin molecules (TnC (Ca⁺-sensitive), TnT and TnI). When Ca²⁺ released from the sarcoplasmic reticulum in response to depolarisation of the plasma membrane binds to the TnC subunit, it moves the tropomyosin protein to uncover the underlying binding sites for myosin heads to attach and the cross bridge cycle starts.

Myosin

Each myosin molecule (molecular weight of 200,000 Da) consists of a tail and two opposite head (S1) regions. In between the head and the tail is a flexible neck region (S2). In the centre, the orientation of the head changes in the opposite direction. The head has the binding site for actin to form a cross bridge, and the site to hydrolyse ATP.

Cross-bridge cycling

Actin [thin filaments] and Myosin [thick filaments] are arranged in parallel to each other. The sliding interaction between them, by cross-bridge formation, is responsible for muscle contraction. The sliding filament theory of muscle contraction states that the 2 sets of actin filaments, anchored on to the opposite

Z lines, are pulled inwards towards the centre of the sarcomere, by the myosin filaments. As a result, the sarcomere is shortened along with the I band. For this to happen, the myosin heads, energised by binding ATP, attach to the nearby actin filaments and form connecting cross bridges. The cross bridge attachments and hydrolysis of ATP lead to the initiation of power stroke that creates shortening, and a generation of force in the sarcomere. This cyclical process is followed by binding of ATP to the myosin head, causing detachment, and making the myosin ready for the next stroke of contraction. This cumulative generated force by a large number of cross-bridges in fibres is used for body movement tasks.

1.2 Muscle contraction function

When an action potential arrives at the neuromuscular junction it is transmitted to the t-tubuli, resulting in the release of Ca^{2+} from the SR. This Ca^{2+} then binds to TnC which in turn causes a conformational change in tropomyosin unblocking the myosin binding sites on actin. The hydrolysis of ATP results in cross bridge and the subsequent power stroke. The inward pull on the bilateral actin filaments by the myosin heads, results in shortening and force generation. This coupling between electrical excitation and subsequent contraction is called excitation–contraction coupling (Shorten, et al , 2007).

There are 2 types of contractions; Isometric and Isotonic; during an isometric contraction the muscle length remains constant, while during an isotonic muscle contraction the tone remains constant.

1.3 Vascular structure and function

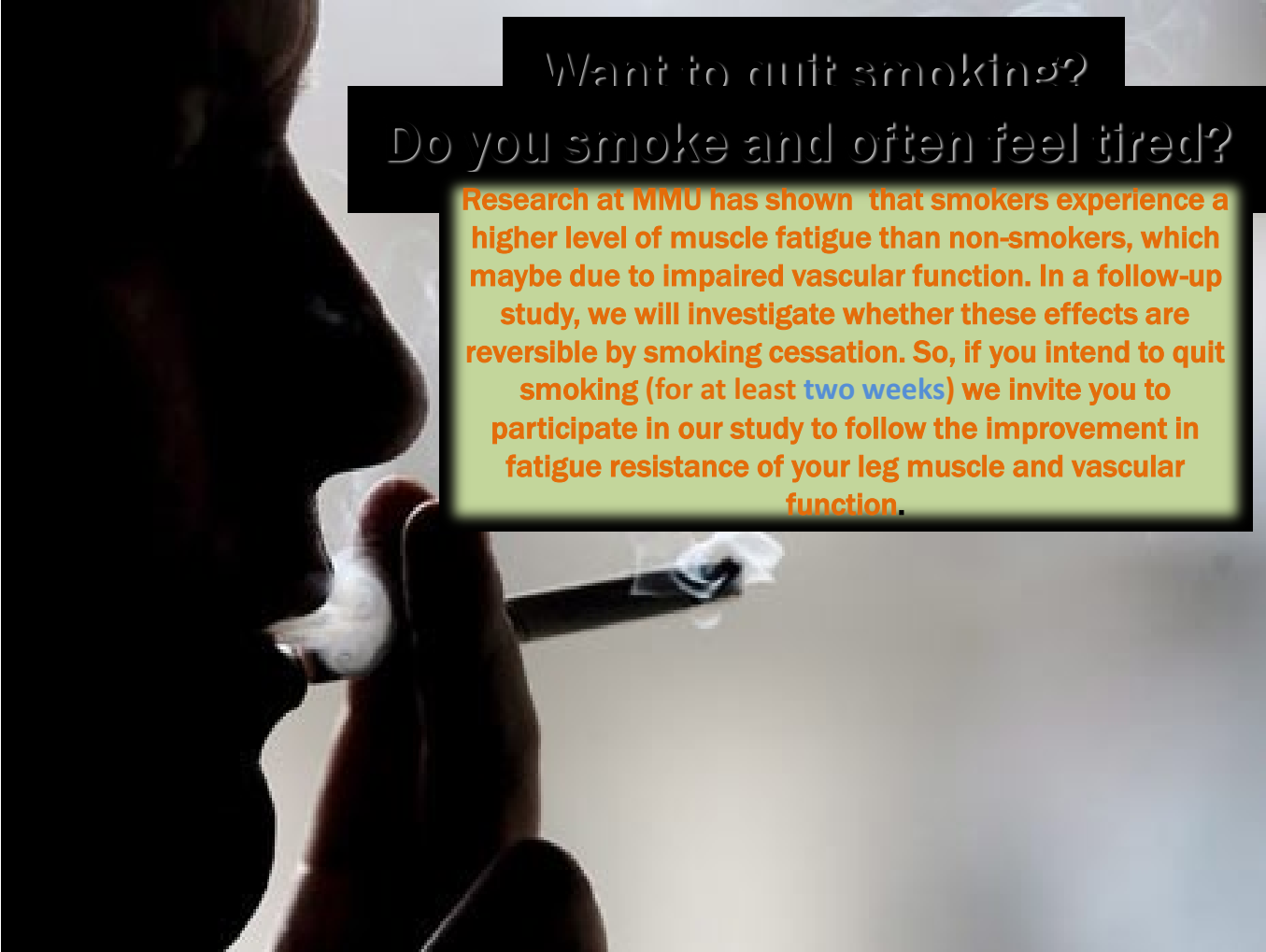
Vascular endothelium. The innermost lining is the vascular endothelium, which is present in the arteries, veins and capillaries. In terms of its surface area it is the largest organ in the body, with a surface area equivalent to that of 10 football fields! The vascular endothelium is composed of endothelial cells that serve as a permeable barrier between the surrounding tissue (including vascular tissue in larger blood vessels) and the blood. In arteries, we have from the inside of the vessel to the outside first the endothelium, then the tunica intima, then the tunica media, consisting of vascular smooth muscle cells, and then the tunica adventitia, consisting of fibrous coating, houses nerves and blood vessels. The vascular endothelium is critical for the adequate functioning of the cardiovascular system. It regulates vascular tone, the selective transport of substances into the cells, as well as platelet aggregation, coagulation and fibrinolysis (Wheatcraft et al, 2003).

1. Endothelium in health Normally, endothelium-induced relaxation of smooth muscle cells in the arteries and arterioles expands the lumen in arteries; vasodilation. Acetylcholine induces decreased vasomotor tone in arteries caused by the release by the healthy endothelium of nitric oxide, which spreads to the underlying smooth muscle cells and excites calcium efflux by a cyclic guanosine monophosphate-dependent pathway, resulting in smooth muscle relaxation. (Mays, 1998).The increased arterial diameter results in an increase in flow, improving tissue perfusion, increasing oxygen delivery, and thereby the removal of toxic waste products and heat. It is therefore no surprise that during increased contractile activity, muscle blood flow is elevated, and paralleled by a rise in VO_2 (Richards et al, 2012).

In addition, one can expect a reduced muscle fatigue resistance in the presence of an impaired ability to elevate blood flow during contractile activity.

1.4. Arterial Function The blood vessel regulates myogenic flow by constricting and dilating in response to a series of vasoactive molecules derived from the endothelium. The most important vasodilators are nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarising factor (EDHF). On the other hand, angiotensin II, endothelin-I and thromboxane A2 are amongst the main vasoconstrictors. An intricate homeostatic balance between the secretion of the vasodilators and vasoconstrictors achieves the vascular tone. The simultaneous constriction of some vessels and dilation of others allows for the redistribution of blood to different tissues during feeding, exercise and maintenance of body heat (Barton, 2011). Endogenous release and use of NO also has a dominant role in vascular health and blood homeostasis because of its antiplatelet, antithrombotic and anti-inflammatory properties (Ludvig, 2005). A major functional role of NO is vasorelaxation that results in increased diameter of blood vessels and increasing blood flow.

2..Poster advert



Want to quit smoking?

Do you smoke and often feel tired?

Research at MMU has shown that smokers experience a higher level of muscle fatigue than non-smokers, which maybe due to impaired vascular function. In a follow-up study, we will investigate whether these effects are reversible by smoking cessation. So, if you intend to quit smoking (for at least two weeks) we invite you to participate in our study to follow the improvement in fatigue resistance of your leg muscle and vascular function.

The Brief Tobacco Intervention Training Program

Fagerstrom Test for Nicotine Dependence

PLEASE TICK (✓) ONE BOX FOR EACH QUESTION

How soon after waking do you smoke your first cigarette?	Within 5 minutes	<input type="checkbox"/>	3
	5-30 minutes	<input type="checkbox"/>	2
	31-60 minutes	<input type="checkbox"/>	1
Do you find it difficult to refrain from smoking in places where it is forbidden? e.g. Church, Library, etc.	Yes	<input type="checkbox"/>	1
	No	<input type="checkbox"/>	0
Which cigarette would you hate to give up?	The first in the morning	<input type="checkbox"/>	1
	Any other	<input type="checkbox"/>	0
How many cigarettes a day do you smoke?	10 or less	<input type="checkbox"/>	0
	11 – 20	<input type="checkbox"/>	1
	21 – 30	<input type="checkbox"/>	2
	31 or more	<input type="checkbox"/>	3
Do you smoke more frequently in the morning?	Yes	<input type="checkbox"/>	1
	No	<input type="checkbox"/>	0
Do you smoke even if you are sick in bed most of the day?	Yes	<input type="checkbox"/>	1
	No	<input type="checkbox"/>	0
Total Score			
SCORE	1- 2 = low dependence 3-4 = low to mod dependence	5 - 7= moderate dependence 8 + = high dependence	

Add up the scores from the questionnaire.

Information about scoring the Test is on the next page

4. Descriptive Informed Consent form

Appendix IV

Informed Consent Form (to be retained by the investigator)

Participant:

Name: Sex: Male / Female

Date of Birth:

Supervisors/Principal Investigator: Dr. Hans Degens; Dr May Azzawi , Miguel Diaz,/ Sujay Chavan

Investigator/Collaborators:

Ethics Committee Approval Number:

Project Title: Impact of smoking /cessation on muscular and vascular function

Purpose of study and brief description of procedures.

In previous studies we have found that fatigue is more pronounced in the skeletal muscle of smokers than that of non-smokers. The altered state of fatigue in muscles is possibly due to altered structure in blood vessel wall due to acute toxic load (Nicotine) in blood , leading to decreased blood supply and reduced ability of the surrounding tissue to take up oxygen for energy generation. The aim of the experiments is to explain whether changes in blood vessel mechanism do occur and cause decreased flow in muscle tissue of young healthy smokers of varied duration of smoke exposure (toxic load) . To prevent any bias related to the acute effects of cigarette smoke, you should refrain from smoking for at least 2 hours before each visit of the laboratory.

The study will be conducted in two parts, the first part of this study will consist of an assessment of Nicotine toxic load in blood at baseline ie 2 hours of abstinence .A 3 ml sample of blood will be drawn to know the toxic level. In the smokers group , the next step will be to allow them to smoke 1 cigarette in < 5 minutes and blood will be drawn and ultrasound scans of the blood vessel will be carried to check the immediate effect of toxic load on the vessels' tone . Some people may perceive the blood drawing as uncomfortable. However, the small stinging is only a small once with cannula is fixed on . In case you cannot stand you have the possibility to prevent any further action or withdraw from the study. Muscle study to see effect on strength of contraction and fatigue time During a second session at least 2 weeks of smoking cessation .after the first one, the blood test and ,scan test will be repeated same way. The US scan test of vessel is much less discomfoting (gel may feel sticky and cold) than in the blood drawing. All the stinging sensations disappear within 20-30s after release of the cannula and there are no long-lasting after effects.

As stated above, you are required to refrain from smoking at least for 2 hrs before each laboratory visit and two weeks after first test . We request that you keep a record of your smoking behaviour throughout the study and jot down any failure (so we could record it)

We will support any effort to quit smoking.

Participant Statement

I fully understand what is involved in taking part in this study. Any questions I have about the study, or my participation in it, have been answered to my satisfaction. I understand that I do not have to take part and that I may decide to withdraw from the study at any point without prejudice. I have had my attention drawn to the document 'Ethical Regulations for the Use of Humans in Research'. My concerns regarding this study have been answered and such further concerns as I have during the time of the study will be responded to. It has been made clear to me that, should I feel that these Regulations are being infringed or that my interests are otherwise being ignored, neglected or denied, I should inform the Chair of the Ethics Committee of the Department of Exercise and Sport Science, Manchester Metropolitan University, Hassall Road, Alsager, Cheshire, ST7 2HL who will undertake to investigate my complaint.

Signed Date

I certify that the details of this study have been fully explained and described in writing to and have been understood by him/her and that I consent to his/her participation in this study.

Signed Date

(Parental consent for minors only)

5. Equations OF muscle function AND NORMAL; VALUES

$$\text{MVC (force)} = (N)$$

$$\text{MVC (torque)} = \text{MVC} \times \text{Lever (N.m)}$$

$$\text{FI ratio} = F_1 / F_2$$

6. Equations OF CV FUNCTION

Primary measured haemodynamic (Normal values) data

Arterial blood pressure (BP) systolic (SP) = 90 - 140 mmHg

Diastolic (DP) = 60 - 90 mmHg

Mean arterial pressure (MAP): = 70 - 105 mmHg

Cardiac output (CO): $HR \times SV/1000 = 4.0 - 8.0 \text{ L/min}$

; where is the pulse pressure,

Derived haemodynamic data

Cardiac index (CI): $CO/BSA = 2.5 - 4.0 \text{ L/min/m}^2$

Stroke volume (SV): $CO/HR \times 1000 = 60 - 100 \text{ ml/beat}$

Systemic vascular resistance (SVR): $80 \times (MAP - RAP)/CO = 1000 - 1500 \text{ dyne s/cm}^5$

SVR: Systemic vascular resistance. Represents the load applied to the left ventricular muscle during ejection.

RPP Rate pressure product: to measure the workload—or oxygen demand—of the heart, and reflects hemodynamic stress. $RPP = \text{max HR} \times \text{max SBP}$
