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## Assessing the evidence: exploring the effects of exercise on diabetic microcirculation

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### Abstract

Diabetes mellitus (DM) is associated with cardiovascular complications. Impairment of glycemic control induces noxious glycations, an increase in oxydative stress and dearrangement of various metabolic pathways. DM leads to dysfunction of micro and macrovessels, connected to metabolic, endothelial and autonomic nervous system. Thus, assessing vascular reactivity might be one of the clinical tools to evaluate the impact of harmful effects of DM and potential benefit of treatment; skin and skeletal muscle microcirculation have usually been tested. Physical exercise improves vascular dysfunction through various mechanisms, and is regarded as an additional effective treatment strategy of DM as it positively impacts glycemc control, improves insulin sensitivity and glucose uptake in the target tissues, thus affecting glucose and lipid metabolism, and increases the endothelium dependent vasodilation. Yet, not all patients respond in the same way so titrating the exercise type individually would be desired. Resistance training has, apart from aerobic one, been shown to positively correlate to glycemc control, and improve vascular reactivity. It has been prescribed in various forms or in combination with aerobic training. This review would assess the impact of different modes of exercise, the mechanisms involved, and its potential positive and negative effects on treating patients with Type I and Type II DM, focusing on the recent literature.

Keywords: Diabetes, exercise, aerobic training, resistance training, high intensity interval training, microcirculation, laser Doppler fluxmetry

## 1. Introduction

Diabetes mellitus (DM) is a chronic endocrine and metabolic disorder, the prevalence and incidence of which is increasing all over the world: according to the World Health Organisation (WHO), in 2014, 422 million of people were suffering from DM [1]. DM induces, due to its pathophysiological mechanisms, a series of complex metabolic and vascular changes, associated with neuropathies, which subsequently compromise vascular function, and lead to cardiovascular complications and increased morbidity and mortality [2].

Type 2 Diabetes Mellitus (T2DM) is the most frequent type of DM, accounting for 90 % of cases and affecting older population, and related to age, to unhealthy diet habits and lifestyle. T2DM is mainly characterised by insulin resistance, associated with impaired insulin-mediated glucose uptake resulting in systemic hyperglycemia. In early phases, there may be compensatory hyperinsulinemia produced by compensatory increased pancreatic insulin secretion, which progressively turns into insulin deficiency due to pancreatic exhaustion and dysfunction [2-6]. Due to insidious onset and progression, the disease may be diagnosed several years after the onset, when complications have already been established. Another concerning fact is that even younger adults could be affected. In addition, T2DM patients often present with other comorbidities aggravating the symptoms of DM such as high blood pressure (BP), dyslipidemia, obesity and physical inactivity [7-9].

It is a well-known fact that the progression of T2DM could successfully be delayed with suitable lifestyle modifications, i.e. diet and exercise training programmes [3, 9-18].

On the other hand, Type 1 DM (T1DM), is much less frequent, and is characterised by an absolute deficiency of insulin due to pancreatic insufficiency of yet unknown cause; the most probable cause being autoimmune disorder. As such, insulin substitution therapy is unavoidable and it is yet to be determined if and to what extent its progression could be altered by lifestyle modifications [15].

The hallmark of both types is impairment of glycemic control, with subsequent hyperglycemia leading to noxious glycation of the plasma and vessel wall proteins and increased generation of reactive oxygen species (ROS) as well as derangement of various metabolic pathways, aggravating serum lipid profile, markers of inflammation and hemostasis, dysbalance of the autonomic nervous system, all of which favour accelerated development of atherosclerotic vascular changes [6, 10, 19]. In this respect, DM leads to the dysfunction of macro- and micro-vessels, with this being related to metabolic, endothelial and autonomic nervous system dysfunction, which are in turn linked to altered vascular reactivity [2, 20-22].

The beneficial effects of regular exercise to treat DM and postpone its complications have been well-documented and advocated in large, cross-sectional, studies, reviews and meta-analyses [13-16, 23]. However, the optimal and most favourable exercising modality in terms of type, volume and intensity, frequency and duration and its effect on improving vascular dysfunction on one side and the least negative outcomes on the other has yet to be established [3, 9, 11, 12, 17, 18, 24]. While studies mostly focused on elucidating potential

mechanisms in animal models, or assessing human macrocirculation and cardiovascular events, there is surprisingly little information on microcirculation. In the light of this, we undertook a review on the currently available and most-recent (e.g. 2000-onwards) literature on the impact of different exercise modalities and training protocols on various aspects of human microcirculation, assessing their strengths and weaknesses, and also suggest some future directions in establishing appropriate measures in terms of physical activity.

## 2. Diabetic-induced changes in the human microcirculation

DM is associated with structural and functional vessel alterations, including generalized endothelial dysfunction of both, macro- and micro-vasculature [6, 22], as well as altered responsiveness of vascular smooth muscle cells (VSMC) [25], predisposing an increased vasoconstrictor response. The mechanisms affecting endothelial function in DM include: altered metabolism due to hyperglycaemia and associated increased ROS and protein kinase C (PKC) activation [19, 26, 27], dyslipidaemia and elevated levels of fatty acids [4, 29], hyperinsulinaemia and insulin resistance [4, 6, 27, 29], activation of the sympathoadrenal system [8, 30], altered cytokine release and increased inflammatory activation [31-33], the induction of a prothrombotic diathesis predisposing to hypercoability [34] and finally an impaired angiogenesis [8, 27, 35].

Most frequently-used methods for the dynamic *in vivo* assessment of microcirculation include laser Doppler fluxmetry (LDF) and its successor laser Doppler imaging (LDI) [36, 37], (contrast-enhanced) ultrasonography (CEUS) [38], or dynamic capillaroscopy [39, 40]. *In vivo* human studies have confirmed a decrease in endothelium-dependent vasodilatation of skin microvessels in T2DM patients [20, 21, 34, 41, 42]. This decrease appears to correlate in most [41, 42] but not all cases [20] with macrovascular dysfunction.

A large body of evidence confirms a reciprocal relationship between insulin resistance and endothelial dysfunction [29]. In physiological concentrations, insulin favours the generation of nitric oxide (NO) by endothelial NO synthase (eNOS) via the activation of Akt-dependent pathway [4, 6, 35]. Using LDF to assess microcirculation in skin and skeletal muscles, physiological hyperinsulinaemia has been shown to increase cutaneous microvascular perfusion, and augment human skeletal muscle microvascular recruitment and vasomotion in humans [40]. On the other hand, insulin is also involved in the generation of endothelial vasoconstrictor endothelin via MAPK/ERK kinase whose action is attenuated by high levels of AMP kinase which could be stimulated by adiponectin [43]. The levels of adiponectin, in turn, have been shown to be decreased in DM [6, 27]. The dysbalance of vasoconstrictors and vasodilators in DM is thought to contribute to deranged vascular tone. Concomitant neuropathy and sympathoadrenal dysbalance might aggravate the microvascular dysfunction [21]. Additionally, in the settings of decreased insulin sensitivity, high glucose levels also decrease the availability of NO, either by directly inhibiting eNOS through the activation of PKC or, independently, favouring oxidation and uncoupling of eNOS [25]. Apart

from reduced bioavailability of NO, increased production of cytochrome P450 (CYP)- derived vasoconstrictor metabolites, 20-hydroxy-eicosatetraenoic acids (20-HETE) have also been implicated in the dysbalance of vascular tone [44]. Hyperglycemia concomitantly with deranged metabolic pathways of lipids and mitochondrial dysfunction may lead to intracellular changes in the redox state increasing the formation of ROS [5]. In addition, the increased production of advanced glycation end products and increased activity of the polyol pathway induced by hyperglycemia increase ROS production via NADPH oxidase [5, 6]. Therefore, the overproduction of ROS, through their NO-scavenging effect, is involved in T2DM-induced vascular dysfunction.

Positive correlation between the levels of glycated haemoglobin (HbA1c), and the endothelial dysfunction has been confirmed in many *in vivo* human studies which assessed microvascular reactivity and thus indirectly support the involvement of hyperglycemia in endothelial dysfunction [45, 46]. Interestingly, the endothelium-dependent, acetylcholine (ACh)-induced vasodilation has been shown to be readily impaired in non-diabetic women with insulin resistance, suggesting an important role of insulin in vascular homeostasis [47]. Endothelial function has also been related to increased levels of oxidative stress in diabetic patients [35, 48, 49]. Moreover, the skeletal muscle microvascular responses to insulin were abnormal in late-stage diabetes and in obesity [50, 51].

The use of LDF in T1DM patients belonging to a wide age-range (children, adolescents, and young adults) has shown decreased endothelium-dependent and independent vasodilation possibly due to poor glycemic control and T1DM duration [42, 52-55]. Moreover, a recent study by Tibirica et al [56] in young T1DM patients without other chronic co-morbidities, assessed by intravital video capillaroscopy, has shown significantly impaired skin capillary function in both fingers and toes. Additionally, their capillary density did not increase in either extremity during venous occlusion suggesting that their capillaries at rest are already maximally recruited [56].

Although the data on the involvement of VSMC in the progression of microvascular dysfunction are not consistent, indices suggest a decreased sensitivity of VSMCs to NO donors [25]. Similarly, Walther et al have shown decreased endothelium-dependent and independent vasodilation in macro- and microcirculation in T2DM patients, which was more pronounced in patients suffering also from metabolic syndrome [41]. This suggests the involvement of central abdominal fat and systemic inflammation in the pathogenesis of vascular dysfunctions in patients with metabolic syndrome and T2DM. This notion is supported by the fact that the metabolic signalling between adipose tissue and muscle in DM is altered [4], with *in vivo* human studies having confirmed a link between lowered adiponectin levels and microvascular function [57].

Other studies have demonstrated a decreased capillary density of skeletal muscles in patients with DM microcirculation, which additionally compromises the delivery of nutrients and insulin to skeletal muscle [58, 59] and aggravates the symptoms of the condition. In insulin-resistant patients and animals, capillary blood volume and capillary blood flow do not

increase normally in response to insulin, which may contribute to abnormal glucose homeostasis and microvascular complications [50, 51].

Insulin sensitivity determines also the level of capillarization as it is indicated in animal [60, 61] and healthy human studies. In humans, physiological hyperinsulinaemia increased the intramuscular microvascular reactive hyperaemia and vasomotion [40]. Insulin-mediated capillary recruitment has been shown to be largely NO-dependent [62] and since in DM, the bioavailability of NO is reduced, this might additionally contribute to microvascular dysfunction. Apart from functional alterations, structural changes of VSMCs in terms of remodeling of small resistance arteries have also been confirmed in DM [8, 63]. The angiogenic potential seems to be altered in DM, which might partly be associated with decreased levels of circulating endothelial progenitor cells (EPCs) shown in DM [35, 64, 65]. This data suggest a link between EPCs and T2DM-associated vasculopathies and glucose intolerance.

Therefore, endothelial and VSMC dysfunction may be critical targets for prevention and treatment of DM progression.

### 3. Impact of acute exercise in diabetic microcirculation

It is well-accepted that even a single bout of exercise, in both healthy and patient populations, elicits changes in microvascular responsiveness, particularly regarding endothelial function. However, the exact impact on diabetic populations is yet to be determined, as studies gave inconclusive results. This might partly be accounted for on the different modes of exercise applied and on different microcirculatory bed and parameters tested.

A single session of low intensity exercise (50 - 65% of maximal oxygen uptake;  $VO_{2max}$ ) has been shown to increase insulin sensitivity (as assessed by hyperinsulinemic-euglycemic clamp) for up to 72 hours after exercise cessation in healthy and obese patients [66]. This in turn might positively affect endothelial function due to reducing hyperglycemia as a consequence of increased glucose uptake in muscles.

Additional mechanisms of acute changes of endothelial function might reflect an increased eNOS expression and NO production, induced by increased shear stress which in turn augments the endothelium-dependent vasodilation. Other vasodilators may also be involved [67, 68].

In larger conductance arteries, increased endothelium-dependent vasodilation after an acute bout of exercise has been confirmed in healthy [69], but the data on microvascular reactivity are scarce.

Acute changes in endothelial function might also be related to acute effects of exercise on glucose uptake and lipid profile which switches the metabolism and induces changes in capillary perfusion [70, 71]. As for the mode of exercise and glucose uptake, strength and resistance exercise have been shown to increase glucose uptake even more than aerobic exercise [Holten, Santos]. Postulations based on animal studies indicate that muscle

contraction induces glucose transporter (GLUT4) intracellular trafficking into the cell membrane and thus increases glucose uptake in skeletal muscle, independently of insulin action [72-74]. This may be an additional beneficial mechanism of decreasing hyperglycemia in diabetes on a longer-term basis [75]. Indeed, Jorge et al [76] have shown that insulin sensitivity depends on intensity and mode of exercise as they confirmed chronically-increased levels of insulin receptor substrate (IRS) in muscle biopsy samples after training. An interesting speculation on reducing hyperglycemia has been proposed by Metcalfe et al [77] who have suggested that high levels of glycogen depletion during strenuous exercise contribute to improvement in insulin sensitivity following high-intensity interval training (HIIT). For example, muscle glycogen availability has been shown to be inversely related to muscle GLUT4 content during insulin stimulation [78]. Furthermore, exercise has been shown to induce capillary growth: during muscle contraction, the level of vascular endothelial growth factor (VEGF) in interstitium increases and acts on capillary endothelium, thereby stimulating angiogenesis [79].

On the other hand, resistance and strength as well as aerobic exercise also increase ROS production, which in turn compromises endothelial function [80, 81]. To what extent the increased ROS affect endothelial function on acute basis depends on exercise intensity and the level of ROS removal [80]. The net effect on vascular reactivity thus depends on the balance between vasodilator and vasoconstrictor effects. It is obvious that there is a complex interplay of many factors that determines the final vascular response to exercise [67, 82, 83].

Responses to an acute exercise bout differ between healthy and diabetic populations: e.g. study by Simoes et al has shown that an acute bout of moderate-intensity aerobic exercise in T2DM patients lowered plasma NO and bradykinin concentrations and kallikrein activity as compared to healthy individuals [84]. Moreover, Womack et al have shown that DM patients with microvascular complications had lower skeletal muscle capillary recruitment and capillary volume in response to a single bout of exercise during low or high-intensity contractile exercise (25% and 80% maximal handgrip), assessed by CEUS as compared to DM patients without complications and healthy controls [58]. Unexpectedly, these abnormalities were independent of disease duration [58]: abnormalities in capillary recruitment may be related to abnormal hemorheology as patients exhibited elevated whole blood viscosity that correlated with plasma glucose and C-reactive protein (CRP).

Furthermore, Franklin et al have shown that flow-mediated dilation (FMD) of the large conductance (brachial) artery was lower in obese women compared to lean ones, reflecting diminished endothelial response to exercise [Franklin2014]. They also postulated that the sensitivity of VSMC to NO is diminished in obesity [85].

An interesting approach for evaluation of vascular function might be the determination of the EPCs level. Although the potential of acute (sub-maximal) exercise to stimulate and mobilize the EPCs has been shown to be blunted in T2DM patients as compared to healthy controls [65], Aoki et al [86] have proven an increment of EPCs during acute exercise load in diabetic patients, which seems an additional promising beneficial effect of exercise [65]. A

recent study, however, by Waclawovsky et al failed to show an increase of EPCs after an acute combined, aerobic and resistance training (ART) session in young T1DM patients in spite of increased response to postocclusive reactive hyperaemia (PORH), assessed by venous occlusion plethysmography [87]. In contrast, healthy controls did exhibit increased number of EPCs after exercise session [87]. The authors concluded that the unchanged number of EPCs in T1DM after exercise sessions might indicate a blunted endothelium regenerating capacity, revealing an early deterioration of the functional arterial characteristics that were not obvious after testing the response to PORH. Yet, due to small number of T1DM study participants, additional larger trials studies are needed.

#### 4. The effect of chronic exercise on diabetic microcirculation: effect of type and duration

Current literature suggests that many potential mechanisms might play a role in chronic exercise adaptations. In healthy populations, exercise training unequivocally exerts complex beneficial effects, affecting vascular function and structure and decreasing the risk for cardiovascular events: exercise increases the endothelium-dependent vasodilation, induces the upregulation of ROS metabolising enzymes, increases the mobilization of EPCs, affects the autonomic nervous system balance in decreasing arterial BP, and improves proinflammatory cytokines and adiponectine profile to list just some of the effects [88]. As most of those mechanisms are compromised in DM, they all appear to be potential targets for an exercise intervention.

Most studies are focused on studying macrovessels whereas relatively little is known about the potential effects and mechanisms on exercise-induced adaptation in human microcirculation. Apparently, the effects not only depend on the type, intensity, frequency and duration of exercise but also on the vascular bed studied. It seem reasonable to speculate that vascular beds more intensively engaged in exercise such as skeletal muscle microcirculation would be mostly affected.

Since insulin exerts metabolic and vascular effects, applying measures to increase insulin sensitivity might indirectly affect vascular tone. Indirect evidence of positive effects of exercise might be deduced from the study of Sonne et al who have shown that 10-days bed rest diminished the insulin-induced increase in blood flow in the brachial artery as assessed by venous occlusion plethysmography in T2DM patients as well as in healthy volunteers, pointing to beneficial effects of physical activity on insulin sensitivity [89]. A recent review by dos Santos et al discussed the effect of physical exercise on some known epigenetic modifications on GLUT4 and mitochondrial proteins that lead to impairment of skeletal muscle glucose uptake and DM2 development, and might also be linked to vascular effects [90]. The effects seem to be most pronounced in the vessels of tissues mostly engaged in exercise, such as skeletal muscles; on the other side, increasing glucose uptake and affecting metabolic pathways in skeletal muscle might reduce systemic hyperglycemia and thus indirectly impact also other vessels, such as skin microcirculation. Indeed, Meijer et al have recently shown that insulin induces capillary recruitment in skin and muscles which is related

to increased glucose uptake [91]. Potential impact of insulin on glucose uptake in skin microcirculation is only speculative as there are no available data on the expression of GLUT4 in human skin microcirculation.

Skin microcirculation is a good model to study microvascular changes as it is easily accessible and has been speculated to reflect generalized vascular function [92]. Moreover, it has, in particular its nutritive capillaries, often been compromised in DM patients, which predisposes to ischemia, and finally the development of detrimental ulcerations and limb amputation. Consequently, exercise might be a valuable intervention to postpone diabetic complications.

In terms of adaptation to exercise, skin microcirculation is essentially involved in exercise as it is the main organ for thermoregulation. Taken these information into consideration, it is tempting to speculate that exercise might induce changes also in diabetic skin microcirculation. There are only few available studies that assessed the effects of different training regimes on cutaneous microvascular function, and the results are inconclusive. As previously noted, different responses might partly be explained by differences in the protocols used (e.g. differences in mode, volume and duration of exercise), or by the selection of patients (e.g. differences in age-groups, DM duration, medication etc) included. It is also common for interventions to include lifestyle modifications in the form of caloric restriction and diet apart from physical activity. Therefore, the observation (or not) of an effect might be attributed to the combination of more than one parameters.

#### *4.1. Aerobic training*

The study of Middlebrooke et al [93] which included 22 patients (aged 62) with mean diabetes duration of 4 years, showed no improvement in the response to local heating, ACh or sodium nitroprusside (SNP) iontophoresis after six months (30 min-exercise set, three times a week, 70–80% of maximal heart rate) of aerobic training. No changes were observed either in glycemic control, insulin sensitivity or VO<sub>2</sub>max, implying insufficient quantity of exercise in inducing any, not only vascular changes. Similarly, in a group of 10 DM patients (aged 54.5 years), Colberg et al [94] failed to show any effect of a 10-week aerobic training (3-times weekly for 45-min up to 65% VO<sub>2</sub>max) on microvascular reactivity in dorsal foot skin after local heating nor any increase of NO levels. In contrast to the microcirculation, patients' VO<sub>2</sub>max and lactate threshold improved after training protocol. The authors concluded that the programme duration might not have been long enough to induce changes of microvascular reactivity. On the other hand, Hamdy et al [95] in a study involving DM patients in a 6-month intervention programme of aerobic exercise (three times per week, 30 min each, at 60–80% max heart rate) and diet failed to show any effect on cutaneous microvascular function (assessed by LDI) whereas they showed an improvement in macrovascular endothelial function as assessed by FMD, improved glycemic control, and decreased levels of markers of endothelial activation and coagulation (ICAM and plasminogen activator inhibitor-1 (PAI-1)). Taking into consideration the existing knowledge

[36], it is therefore possible that the lack of sensitivity of the method to assess microvascular reactivity could have affected the "no microcirculatory impact" finding, rather than an actual difference between macro- and micro-vascular function.

In contrast to the above noted studies, Krcma et al have shown that even moderate 4-week exercise in terms of solely walking induced changes in microvascular response to local heating and PORH [96]. However, they included eight DM patients who were completely sedentary before the training protocol started, and the exercise protocol consisted of everyday walking for at least one hour. Nevertheless, no other parameter tested (i.e. fasting plasma glucose, Hb-gly, or lipid profile, or BP) did not change after finishing the programme [97].

The effect of aerobic exercise on VO<sub>2</sub>max, NO levels, and oxidative stress, which all affect endothelial function, depends on training intensity; nevertheless, the responses significantly differ between DM patients and healthy [48, 83]. These differences might also account for variations in microvascular responses between healthy and diabetics.

#### *4.2. Resistance training*

As DM patients might have difficulties in performing aerobic exercises, specially those with diabetic food and skin ulcerations associated with comorbidities, resistance training might be an alternative.

Colberg [97] did not report any changes in NO levels nor in the responsiveness of cutaneous perfusion in response to local heating after 8 weeks of moderate resistance training either in DM individuals or in their matched controls. This is in contrast to the findings of Cohen et al [45], who have confirmed that resistance training of high intensity and involving large muscle groups semi-weekly improved both the ACh- and SNP-mediated vasodilation of forearm skin microvessels in 29 DM patients (mean age 60 years) that were negatively correlated with Hb1Ac levels; in addition, they showed a decrease in BMI and systolic arterial BP. Interestingly, the observed changes were only significant after a 14-month training period, which was not the case 2 months after, suggesting the importance of regular training on a longer term basis. It is also worth noting that in the study by Cohen, rather than just being localised, as previously suggested [98], vascular effects seem to be systemic [45]. In favour of a longer training period is also the study by Sixt et al, who showed improved endothelial function of coronary arteries after a 5-month- and not after a 4-week period [99].

Duration however, is also one factor affecting the extend of adaptations; intensity can also be a crucial factor. However, with no high intensity, resistance exercise training studies in diabetic populations, current evidence is limited, with only speculations existing on causing impaired cerebral autoregulation due to greater systemic oxidative stress [83, 100]. Nevertheless, this mode of resistance exercise training might be inappropriate for this population [18] due to it being related to extremely high (e.g. up to 400 mm Hg) increases in systolic BP [101].

### *4.3. Combined aerobic and resistance training*

Current physical activity guidelines favour combined activity (ART) over either aerobic or resistance training as it is considered to provide a combination of benefits. As for the effect of ART on skin microcirculation, Maiorana et al and Naylor et al confirmed improved endothelium-dependent and -independent vasodilation in older [102] and younger populations [103], pointing to beneficial effects irrespective of age already after 8- or 12-week exercising programme, respectively. Naylor did not find any changes in VO<sub>2</sub>max, body weight or BMI, which suggests that vascular effects are related to metabolic changes - this supports the notion of including ART in the lives of young T2DM patients, as early as possible. Hordern et al have shown that only 4 weeks of moderate-intensity ART improved glycemic control, VO<sub>2</sub>max, and caused a reduction in the resting heart rate and systolic BP [3].

This data suggests that the increased vasodilatory capacity could be attributed to endothelial and VSMC adaptations. Apart from NO, prostaglandins seem to play greater role in exercise-induced endothelial vasodilating capacity in DM [96]. As oxidative stress uncouples eNOS and reduces NO bioavailability, improved endothelium dependent vasodilation might also result from decrease in ROS induced by exercise that has been confirmed in DM patients [48]. Nojima et al [49] showed that 12-month 30 minutes at least thrice per week (either aerobic or ART) over a 12-month period improved glycemic control and attenuated oxidative stress as assessed by determination of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG).

Additional confirmation on the efficiency of combined ART on microvasculature comes from the study of Vinet et al [104] who assessed microvascular reactivity concomitantly with other DM markers after a lifestyle intervention program, including diet and 6-month ART. They showed an increased response of skin microvessels to iotophoresis of insulin along with improved vasomotion profile, improved glycemia, Hb1Ac, as well as thrombotic and inflammatory markers, active PAI-1, CRP, and a decrease of systolic BP after intervention. As patients underwent diet and exercising programme, each parameter cannot be evaluated separately and positive effects might have resulted from both, metabolic and vascular effects. This is partly in contradiction with Lucotti et al, who have shown that the effect of aerobic training was more efficient than ART in improving BP, insulin sensitivity and endothelial factors, adipokines and pro-inflammatory marker release [105]. Furthermore, combined ART did not prove to be more efficient than aerobic one on arterial stiffness of larger conductance arteries [106]. On the other hand, the improvement of insulin sensitivity as assessed by the expression of IRS seems to be more sensitive to ART and resistance training than to aerobic training alone [76].

Therefore, the contrasting findings confirm the need of additional, larger, longer-term studies in humans.

### *4.4. High intensity interval training*

It is important to make a note about high intensity interval training (HIIT) which recently has been widely discussed as a less time-consuming, benefit-inducing mode for exercise. The only available study in T2DM patients confirmed that HIIT was more effective in improving microvascular function, as assessed by inducing PORH, as compared to continuous training of lower intensity and adjusted to the same energy expenditure per exercise session [107]. In addition, HIIT was also more efficient in improving glycemic control, physical fitness, and macrovascular function, whereas the reductions in erythrocyte malondialdehyde and serum von Willebrand factor and increases in plasma glutathione peroxidase and NO were only shown after HIIT, and not after continuous training [107]. Similarly, Tjonna et al have shown that aerobic interval training vs. continuous moderate was more effective in increasing VO<sub>2</sub>max, enhancing endothelial function (assessed by FMD of the brachial artery), insulin signaling in fat and skeletal muscle, skeletal muscle biogenesis and excitation-contraction coupling, and reducing blood glucose and lipogenesis in adipose tissue in patients with metabolic syndrome [108]. Considering potential proinflammatory effect of DM on microvasculature, Balducci et al have also shown that HIIT or ART are most effective in exerting their anti-inflammatory effect [32]. On the other hand, regarding eNOS expression, both types of training seem to be comparable, at least in skeletal muscle microvessels of healthy [109]. However, when high intensity exercise is applied, previous clinical evaluation as well as follow-up is strongly recommended in patients. It should however be noted that this kind of modality may not be appropriate for DM patients with multiple comorbidities [110]. Further work however, is needed to establish benefits and explore its impact in different age-groups.

#### *4.5. Other aspects of regular training on diabetic microcirculation*

As microvascular dynamics depends on viscosity, which is altered in DM [58, 111], it is necessary to note the study of Simmons et al [112] who have shown improved viscosity, inflammation and pro-aggregation profile after 12 weeks of supervised treadmill walking at an intensity equivalent to the gas-exchange threshold (120min·semi- or thrice-weekly). As for blood viscosity, a 2-month lasting low intensity exercise pilot study of Brun et al has shown that aerobic capacity improved blood viscosity via an increase in red blood cell deformability and decrease in hematocrite, associated with weight loss [113]. Apart from improved vascular reactivity after training programme, training might also induce structural changes in terms of beneficial angiogenesis: exercise is known to increase the circulating levels of EPCs favouring the formation of new vessels [64, 114]. Yet, compared to healthy, the exercise-induced increase in EPCs was reduced in both T1DM [115] and T2DM patients [65].

#### *4.6. The effect of exercise training in type 1 diabetes*

As the etiopathogenesis and the age-group mostly affected differ in T1DM, it should be treated as a separate entity, although existing data on microvascular function is even more limited. However, the available data suggests that exercise affects positively microvascular function in T1DM patients. A study by Roche et al with 29 adolescents and children with DM duration of at least two years, has shown a positive correlation between maximal skin microvascular vasodilation induced by local heating and VO<sub>2</sub>max [26]. On the other hand, no correlations were found between the level of Hb1Ac, glycemic control and microvascular reactivity, which seems to be worth of further investigation, considering the previously mentioned putative mechanisms. Nevertheless, they did not assess other markers such as oxidative stress or inflammation markers which also affect vascular function. Contrary to the observations of Roche et al, a four-week low intensity aerobic training programme in DM1 patients did not affect skin microvascular reactivity, as assessed by local heating, PORH and iontophoresis of ACh and SNP [39]. On the other hand, capillary density in response to PORH, assessed by dynamic capillaroscopy, was increased in the same group of patients [39]. We might assume that the most effective and apparent changes are triggered in nutritive capillaries, which could be evaluated by capillaroscopy. It seems plausible to consider that changes did not affect more deeper, larger resistance vessels or pre-capillary sphincters that are also captured with LDF, which, on the other hand, might not be sensitive enough to detect subtle changes. Speculatively, an increase of free fatty acids level, which are the preferential skeletal muscle fuel during low intensity exercise (below 40% of VO<sub>2</sub>max), can activate peroxisome proliferator-activated receptor beta or delta (PPAR- $\beta$  or PPAR- $\delta$ ), and PPAR- $\delta$  coactivator-alpha (PGC-1 $\alpha$ ), contributing to the expression of VEGF and in this respect, favour angiogenesis.

To the best of our knowledge, there are no studies on different modalities of exercise training in T1DM.

## 5. Limitations and perspectives

We limited our review on microcirculation. The main limitation of the studies included in this review, is the small number of participants, which considering the notable variability of the methods used to study microcirculation (e.g. LDF), might be accountable for the contrasting findings. Additionally, in many studies, no control group of healthy participants was included. A further problem might be the existence of comorbidities and medications which affect microvascular function and exercise adherence. Moreover, the assessment of human microcirculation is limited to superficial vascular beds or associated with invasive techniques, such as muscle biopsy.

Another limitation of studies in humans is that elucidating potential mechanisms is rather speculative and could only be deduced from animal model or *in vitro* studies. In this respect, final outcome *in vivo* is a complex interplay of many mechanisms and individual genetic susceptibility. Last but not least, all persons do not respond equally or fail to respond which might partly be explained by their genetic susceptibility [116, 117].

Therefore, it is fair to state that the outcome on an exercise intervention programme depends on many facts: age of the participants, the primary status of microvessels prior to starting the exercise programme, the disease duration, potential comorbidities and medication and patients adherence which should all be taken into consideration when designing the study. Consequently, comparing studies should happen with caution as well as drawing general conclusions.

## 5. Conclusions

In conclusion, the available data confirm the importance of regular exercise to prevent further deterioration of microvascular function in DM. Data also suggests that exercise of longer duration is needed to elicit significant alterations of endothelium-dependent and -independent vasodilation, contributing to a decrease of cardiovascular events. As for microvascular adaptations, the combination of ART is supposed to be most efficient. Nevertheless, an optimal protocol is yet to be agreed, and consequently, larger observational studies on a large cohort of patients are warranted to further elucidate the impact of exercise and different modalities on diabetic microcirculation.

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