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REVIEW

Muscle Mitochondrial Function in Patients with Type 2 Diabetes Mellitus and Peripheral Arterial Disease: Implications in Vascular Surgery

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KEYWORDS
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Clinical outcome

Abstract *Objectives:* (1) To review the available information on mitochondrial function in type 2 diabetes mellitus (T2DM) and peripheral arterial disease (PAD) obtained by non-invasive phosphor magnetic resonance spectroscopy (^{31}PMRS), near-infrared spectroscopy (NIRS) *in vivo* and respirometry on mitochondria isolated from muscle biopsies *in vitro* (2) to evaluate the usefulness of such data in the diagnosis, treatment and prognosis of these patients.
Design: Review.
Methods: Search strategy: PubMed (http://www.ncbi.nlm.nih.gov/PubMed) and manual literature search.
Main results: Fifty-three articles were retrieved, which included ^{31}PMRS, 15, NIRS, 11, Combined, 1 and Respirometry, 2 and background literature, 24.
Conclusion: Muscle mitochondrial function is impaired in both T2DM and PAD patients, but differently. Patients suffering from both pathological conditions will display more serious impairment of the mitochondrial function. Mitochondrial function and the degree of ischaemic disease as evaluated by ^{31}PMRS and NIRS are well correlated. The NIRS technique appears to determine the degree of PAD better than ^{31}PMRS. It is argued that systematic testing of mitochondrial function may be a useful prognostic tool with PAD and T2DM, but clinical studies are needed.

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The muscle mitochondria serve as the energy-generating organelle in the majority of cells of the body, including muscle cells. Patients affected by T2DM (type 2 diabetes mellitus) and PAD (peripheral arterial disease) have some

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degree of mitochondrial dysfunction, which may have serious consequences for the muscle function and the mobility of these patients. Mitochondrial function in cellular energy metabolism is concerned with the processes of fatty acid and pyruvate oxidation, resulting in the formation of acetyl-CoA, which is subsequently oxidised in the TCA cycle. When combined, these processes generate reduced coenzymes, which, through the respiratory chain of the inner membrane, deliver electrons to oxygen to form water. The whole process of fat and carbohydrate oxidation is strongly exergonic and the normal mitochondrion conserves the major part of this energy in the form of ADP phosphorylation to ATP. This dependence on oxygen is critical in skeletal muscle, which, under normal circumstances, has the capacity to increase its energy turnover by some 50-fold, making the transition from rest to maximal exercise.[1] Efficient oxygen delivery, therefore, becomes of paramount importance for normal mitochondrial function.

Thus, in all diseases involving the circulatory system, either the pumping function and/or the vascular bed, symptoms of abnormal mitochondrial function will eventually arise. T2DM and PAD are two such illnesses with vascular involvement. Patients suffering from PAD have a decreased blood flow to the legs due to arteriosclerosis, making less oxygen available to the mitochondrias. Type 2 diabetics have a high occurrence of PAD, but the arteriosclerosis is located more distally and differently distributed in the arterial wall (media sclerosis).

As diagnostic measures, it is logical to choose methods that evaluate the mitochondrial energy transformation, either directly as with ^{31}phosphorous magnetic resonance spectroscopy (^{31}PMRS) or indirectly as with near-infrared spectroscopy (NIRS), due to its ability to monitor the tissue oxygen level. Both techniques are non-invasive and, in particular, the NIRS technique is unique by its simplicity and portability. In addition, mitochondrial function may be evaluated *in vitro* by respirometry on relatively small (30—50 mg) muscle biopsies.

^{31}PMRS

The ^{31}PMRS is a non-invasive method used for determination of relative concentrations of metabolites involved in muscle energy metabolism *in vivo* (i.e., PCr (phosphocreatin), P_i (inorganic phosphate) and ATP (adenosine triphosphate)).[2] From these data, free ADP (adenosine diphosphate) and pH may be calculated as well as the anaerobic and aerobic ATP turnover.[3,4] The ^{31}PMRS is a dynamic method with a time resolution of down to 5—10 s, which can be used at rest and during exercise by placing the patient in the magnet and the magnetic resonance (MR) probe on the relevant muscle.[5—19] The re-synthesis of PCr, which is deprived during exercise and ischaemia, is an indirect measure of aerobic mitochondrial function (i.e., rate of maximal ATP synthesis).[3]

NIRS

The NIRS can measure the state of oxygen saturation in haemoglobin and myoglobin in blood and muscle at a given time and a given location, thereby providing an indirect measure of the muscle perfusion vs. oxygen consumption. The method works by placing the NIRS probe on the skin overlying the relevant muscle, on which oxygenation is estimated. Light at specific wavelengths in the near-infrared spectral region may penetrate the muscle tissue and the absorption at specific wavelengths (typically 760, 800 and 900 nm) allows the calculation of the tissue oxygenation (0—100%).[20—31]

Respirometry

Respirometry is an *in vitro* technique that quantifies mitochondrial oxygen consumption under specific circumstances with regard to substrate supply, with and without specific inhibitors.[32—35] The method is invasive since it requires the sampling of a muscle biopsy (50—100 mg (wet weight) of tissue).[32,34] The sarcolemma of the muscle fibres is removed with saponin revealing the mitochondrias in the cytoplasma of the muscle cell. The biopsy is examined in chambers with oxygen electrodes for measuring the oxygen consumption with different substrates added.[32—35]

Methods

Article search limits

Inclusion criteria: Articles addressing NIRS, ^{31}PMRS and respirometry in combination with either PAD, T2DM, peripheral bypass surgery or all of them were included. Literature was found using the search function in PubMed (http://www.ncbi.nlm.nih.gov/PubMed) and by manual search. The search was conducted using words such as NIRS, Respirometry, ischaemic muscle, magnetic resonance spectroscopy, diabetes (type 2), claudication, mitochondrial function, graft patency and peripheral bypass surgery.

Exclusion criteria: Case reports and articles describing aortic aneurism surgery were excluded.

Additional literature was found in the above process.

Results

Mitochondrial function expressed as oxidative recovery after exercise is related to the degree of disease of both T2DM and PAD.[12,22,23] The results are therefore presented according to the degree of ischaemic disease (as defined by the TASC II consensus report[36]) and with and without T2DM.

Patients with functional ischaemia

At rest, muscle metabolism and tissue viability are only slightly affected in this group of patients as determined by ^{31}PMRS (245 patients in 12 studies (range: 7—56 patients)) and the NIRS (481 patients in 11 studies (range: 6—153)).[5—16,20—31]

During exercise, however, ^{31}PMRS shows an increased PCr splitting, correspondingly higher P_i (increased P_i/PCr ratio) and eventually a drop of pH by the end of exercise, indicating that anaerobic metabolism has commenced. Furthermore, an increased recovery time of the PCr, ADP concentrations and pH is seen. This applies to patients with both moderate and critical ischaemia independent of the

choice of training protocol (isotonic or isometric) (245 patients, $p < 0.001-0.05$).[5–16]

The NIRS measurements in the gastrocnemius muscle during exercise show a large drop in the oxygen saturation in the muscle and an increased oxygenation recovery time after exercise when compared to age-matched controls[10,20–25,28,29] (339 patients, $p < 0.009-0.045$). The observed drop in oxygen saturation is abnormal, even when compared to normal controls with tourniquet-restricted blood flow.[22] An increased drop in the oxygen saturation at the beginning of exercise has been observed by Bauer et al.[20] This suggests a decreased blood supply in patients with PAD due to their impaired blood-flow response during exercise. On the other hand, histological and EM examinations have paradoxically identified more type I muscle fibres containing a high amount of mitochondria in the gastrocnemius muscle of claudication patients compared to controls. Furthermore, an increased percentage of type I fibres was correlated with the severity of PAD.[37]

Chronic critical limb ischaemia

In patients at rest, ^{31}PMRS examination showed a higher P_i/PCr ratio and higher intracellular pH compared to controls (45 patients, $p < 0.005-0.02$).[17–19] Similar examinations with NIRS have been conducted by Eiberg et al. during and after bypass surgery.[30,31] These measurements indicated decreased oxygen saturation during surgery, with return to supernormal levels as expected after completion of the operation. Like most other NIRS measurements, only oxygenation changes are reported, and there is no information about actual oxygen consumption.

Only two studies have been performed that applied respirometry on permeabilised human muscle fibres in this patient group. Both the studies found a reduced mitochondrial respiratory rate (nano-atoms of oxygen per minute; 34 patients (35 legs)) in the gastrocnemius muscle compared to controls.[32,33]

In one of the studies, the reduced respiratory rate was located specifically to enzyme complexes I, III and IV of the respiratory chain. In this study as well, a dysfunctional capacity of anti-oxidative enzymes in both mitochondria and cytosol in PAD muscles was found, implying that the decreased activity of complexes I, II and IV may be due to reactive oxygen species (ROS)-generated damage.[32] Consequently, PAD patients may not gain from the increased amount of mitochondrias as they are dysfunctional.

T2DM and PAD

Patients suffering from both PAD and T2DM are associated with higher mortality than PAD alone (47% vs. 36%, 6-year observational period), and the prospects after re-vascularisation are low[38] despite the presence of similar graft patency rates after bypass surgery.[39] Prolonged healing of ischaemic tissue lesions after bypass surgery is also seen within this group.[40]

Patients with T2DM have a well-documented impaired ability to exercise. Muscle weakness is often experienced, characterised by reduced strength and endurance.[41] Patients with PAD and T2DM have a reduced ability to exercise when compared to patients with PAD alone in spite of similar ankle-brachial indices (ABIs).[42] The reason for this phenomenon is related to the pathology and complications of T2DM. The insulin resistance may impair muscle metabolism by reducing the substrate supply. The lipid accumulation seen in the muscle cell has been shown to cause muscle weakness in T2DM.[41] A group of particular high risk is type 2 diabetics with neuropathy, which have serious consequences for both muscle function and development of foot ulcers.[43]

Type 2 diabetics have a decreased amount of type I muscle fibres, which contain a larger amount of mitochondrias when compared to type II muscle fibres.[44] Rabøl et al.[45] confirmed this by a reduced mitochondrial content in muscle biopsies from type 2 diabetics.

^{31}PMRS measurements of the capacity for aerobic ATP formation in the same patient group correlate with this finding.[46] Muscle fibre transition is also seen in other muscles in both human and animal models. Atrophy of type I muscle fibres is seen together with an increased amount of type II fibres, which are decreased in size. The remaining type I fibres are increased in size and mitochondrial content. Both diabetes and neuropathy can cause this fibre-type transistion.[41,47] The altered fibre-type composition could explain the impaired endurance and strength.[48] This, however, is not the whole explanation since T2DM is associated with co-morbidity of a complex pathology.[49]

In a small patient serie, an increased accumulation of phosphate monoesters is seen after exercise in patients with chronic ischaemia and diabetes using ^{31}PMRS measurements when compared to patients with chronic ischaemia and no diabetes.[16] No other ^{31}PMRS study has addressed patients with chronic ischaemia and diabetes. The NIRS measurements in the gastrocnemius muscle in patients with diabetes and PAD have shown a better determination of the degree of ischaemia when compared to measurement of the ABI.[23] Furthermore, a decreased micro-vascular response during exercise has been shown in the legs of diabetics. The micro-vascular blood flow in patients with PAD increases to supernormal levels, probably in order to compensate for the lack of blood flow from the greater arterial vessels. In this sense, diabetics are not able to use this compensatory mechanism where even the normal response to exercise is impaired.[26]

The muscle fibre transitions for patients with combined PAD and T2DM are not yet characterised.

The articles are summarised in Table 1.

Peripheral bypass surgery, angioplasty, graft patency and mitochondrial function

^{31}PMRS has been used in two studies to measure the effect on muscle metabolism of peripheral bypass surgery and angioplasty. The study done by Schunk et al.[13] examines 31 patients before and after vascular therapy; of which 23 patients were treated with percutaneous transluminal angioplasty and eight with vascular surgery (four bypass and four thrombectomies). The severity of their symptoms was classified according to the modified Fontaine classification.[13] The majority of the patients were claudicants with different walking distances (modified Fontaine stage II_{a-c}).

Table 1 Summation of the biochemical and physiological changes of PAD and T2DM in the reviewed articles

	Functional ischaemia	Reference	Critical ischaemia	Reference	PAD and diabetes type 2	Reference
^{31}PMRS	At rest: no effect on muscle metabolism. During exercise: Increased PCr degradation, higher P_i concentration (Increased P_i/PCr ratio) and a lower end exercise pH. Increased recovery time for PCr, ADP and end exercise pH. Improvement in ^{31} PMRS parameters (intracellular pH, P_i/PCr ratio, PCr recovery) during exercise after peripheral bypass surgery.	Di marzo et al.[5]; Greiner et al.[6]; Hands et al.[7]; Keller et al.[8]; Kemp et al.[9,10]; Pipinos et al.[11]; Schunk et al.[12,13]; Tsuchiba et al.[27]; Von Melchert et al.[14]; Wahl et al.[15]	At rest: higher P_i/PCr ratio and higher intracellular pH. During exercise: Increased PCr degradation, higher P_i concentration (Increased P_i/PCr ratio) and a lower end exercise pH. Increased recovery time for PCr, ADP and end exercise pH. Delayed improvement in ^{31}PMRS parameters (intracellular pH, P_i/PCr ratio, PCr recovery) during exercise after peripheral bypass surgery.	Hands et al.[17,18]; Zatina et al.[19]	Correlation of muscular oxidative capacity to a decreased amount of type I muscle fibres in diabetics. Increased accumulation of phosphate monoesters is seen after exercise in patients with chronic ischaemia and diabetes compared to patients with chronic ischaemia and no diabetes.	Praet et al.[46]; Zatina et al.[19]
NIRS	At rest: no effect on muscle metabolism During exercise: A large drop in the oxygen saturation in the muscle and an increased recovery time after exercise. Increased drop in oxygen saturation at the beginning of exercise.	Bauer et al.[20]; Comerota et al.[21]; Egun et al.[22]; Kemp et al.[10]; Komiyama et al.[23]; Kooijman et al.[24]; McCully et al.[25]; Watanabe et al.[29]; Ubbink and Koopman[28]	Decreased oxygen consumption at rest. Improved muscle oxygenation after peripheral bypass surgery.	Eiberg et al.[30,31]	Better determination of the degree of ischaemia when compared to measurement of the ABI. Decreased micro vascular-response during exercise in the legs of diabetics.	Komiyama et al.[23]; Mohler et al.[26]
Respirometry			Muscle mitochondrial dysfunction has been found as a reduced oxidative capacity	Pipinos et al.[32,33]; Rabøl et al.[45]	Reduced amount of mitochondrias in patients with type 2 diabetes and hereby reduced oxidative capacity	Rabøl et al.[45]

(*continued on next page*)

Table 1 (*continued*)

	Functional ischaemia	Reference	Critical ischaemia	Reference	PAD and diabetes type 2	Reference
Biochemical and Histological Findings in muscle biopsies.	Structural changes in the calf muscles: Altered fiber type composition. Mitochondrial damage. Larger amount of type I muscle fibres. Altered enzyme activities.	Makitie et al., 1977[37]; Pipinos et al.[32]; Sun et al.[41]	Dysfunction of complex I, III and IV in the respiratory chain and a decrease in the antioxidants defence.	Pipinos et al.[32]	Decreased amount of type I muscle fibres in them. vastus lateralis and other muscles in patients with type 2 diabetes. Increased amount of type II muscle fibres, which are diminished in size	Oberbach et al.[44]; Sun et al.[41]; Dolan et al.[42]; Klueber et al.[47]

Only two patients had resting pain before treatment. After surgery, an improvement in symptoms and haemodynamic parameters (ABI) was seen in all patients studied, this was also reflected in an improvement in the ^{31}PMRS parameters (intracellular pH, P_i/PCr ratio, PCr recovery) during the conducted exercise protocol (isometric and isotonic) in the rectus femoral muscle indicating reversibility of the mitochondrial dysfunction. The post-treatment ^{31}PMRS measurements were obtained 25 ± 6 days after treatment.

Zatina et al.[19] obtained different results although not significant. The post-treatment results in this study indicated a prolonged recovery of the ^{31}PMRS parameters of up to several months after treatment even though the haemodynamic parameters had recovered. These results indicate impaired oxygen consumption, which is not due to post-treatment ischaemia.

The patients and their corresponding clinical stage and type of treatment are not described in the article for the data obtained after treatment, which complicates the interpretation. The ^{31}PMRS data were obtained in the gastrocnemius muscle, which is situated more distal than the rectus femoral muscle studied by Schunk et al., which may have serious consequences for the muscle metabolism parameters in patients with PAD.[16]

Graft patency studies have never been conducted with ^{31}PMRS measurements and respirometry before. Eiberg et al. have conducted two NIRS studies,[30,31] where graft patency was considered, one in the foot and the other in the gastrocnemius muscle in a mixed patient population according to clinical stage, graft material and type of bypass operation. Both studies were done in small patient groups and showed immediate recovery of the haemodynamic parameters measured with NIRS after completion of the bypass operation. This correlate well with the studies of Zatina et al.[19] and Schunk et al.,[13] where the haemodynamic parameters measured as ABI also recovered fast after bypass surgery. No haemodynamic flow characteristics measured with NIRS were able to predict graft patency in the studies done by Eiberg et al.[29,30]

Graft patency is shown in several studies to depend on several factors, including choice of graft material, the type of surgical technique, ABI < 0.40 and other co-morbidity risk factors.[50,51]

Discussion

The available data indicate that the mitochondrial function is well correlated by the degree of PAD. This correlation has, however, not been shown in patients with T2DM separately. Is mitochondrial damage/dysfunction a relevant predictor of functional outcome of re-vascularisation in patients with T2DM and PAD?

The most important predictors of a poor functional outcome of bypass surgery in PAD with and without diabetes have been identified by Taylor et al.[38] to be:

(1) impaired ambulatory ability at the time of presentation, (2) failure to eventually ambulate (walk), (3) loss of independent living and (4) dementia. This indicates that for a patient with bad mobility before re-vascularisation the chance of extensive improvement is low, although it might have helped in salvaging a leg and healing the wounds. The

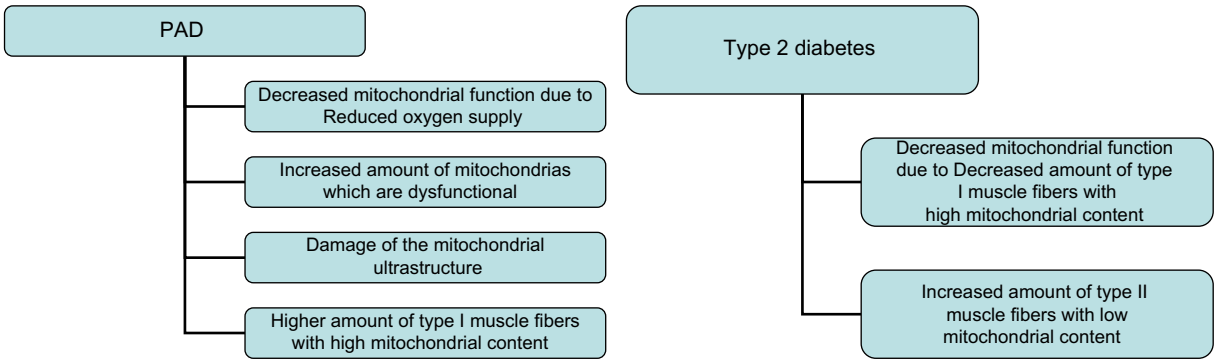

Figure 1 Changes in the mitochondrial amount and function in patients with PAD and T2DM.

diabetic patients fall in to this group because the pathophysiology of the disease impairs the muscle function and thereby the mobility, as explained earlier. Patients with diabetes have a higher mortality rate, higher frequency of failed ambulation and loss of independent living.[38] This poor functional result amongst the diabetics is probably a reflection of several (many accumulated) independent risk factors such as a higher frequency of cardiac disease and renal failure. Whatever the reason of the impaired mobility, the level of oxidative ATP synthesis (mitochondrial function) in the muscle will properly correlate with mobility, as indicated in the studies reviewed above. This suggests that muscle oxidative metabolism, and thereby mitochondrial function, could be a predictor of functional outcome. Obviously, further prospective studies of both functional outcome and muscle metabolism are needed to build a strong argument for this claim.

The presented literature addressing the muscle tissue viability in T2DM and PAD describes a theoretical disadvantage for diabetics in the fibre-type composition of the muscles, which is in contrast to patients with PAD only. This is because an increased amount of type I muscle fibres is seen, which probably is a compensating mechanism due to chronic ischaemia. Data are only available for patients with either T2DM or PAD (Fig. 1). Muscle biopsies of the combined disease are not currently available.

Surprisingly few studies have been conducted addressing the combined effect on muscle metabolism of T2DM and PAD. Measurements with the three methods mentioned above and after bypass surgery have only been conducted

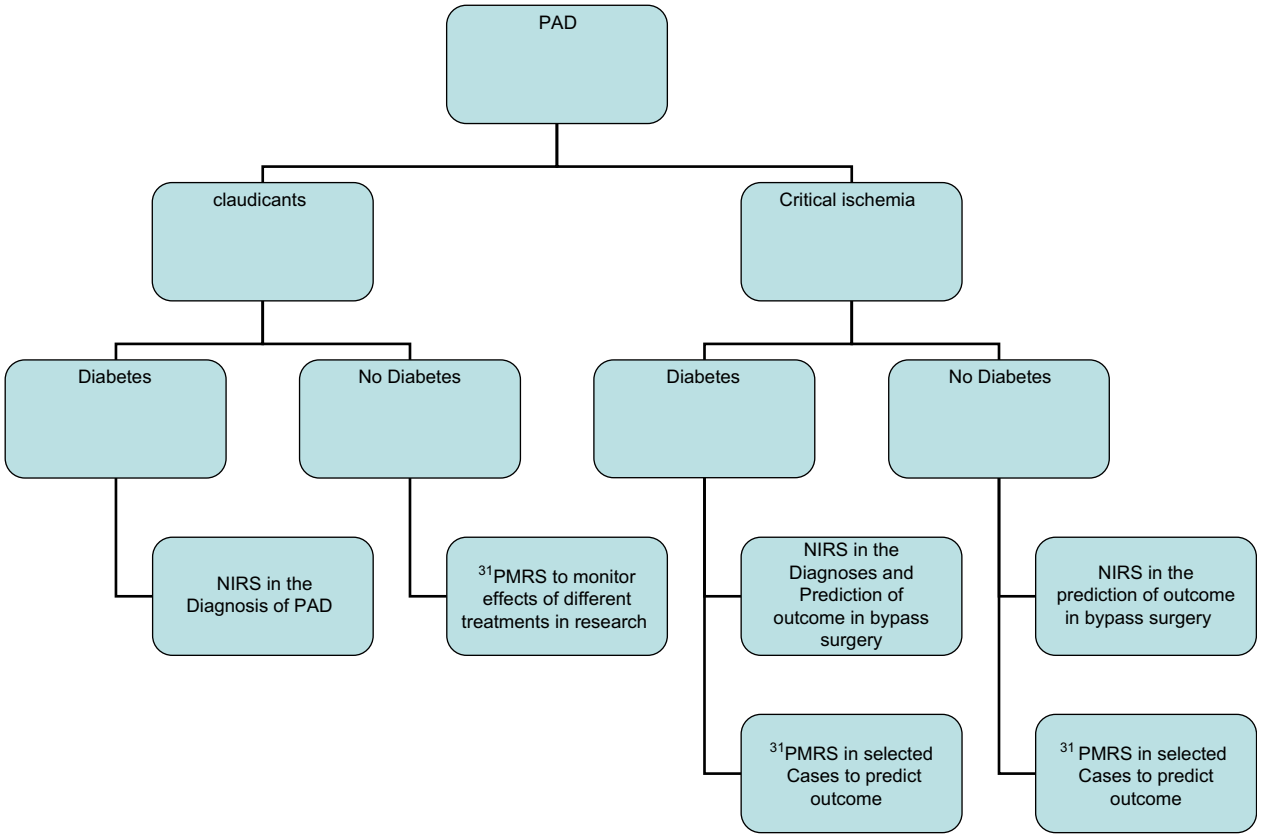

Figure 2 Suggested future use of ^{31}PMRS and NIRS in different patient groups with PAD.

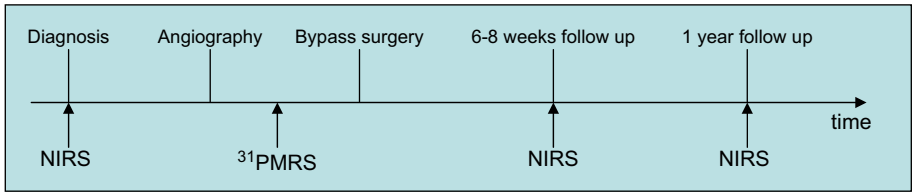

Figure 3 Suggested use of NIRS and ^{31}PMRS in bypass surgery treatment and follow up. Time line showing placement of the individual tests in a typically patient case.

in a very limited amount. There are no studies which examine the number of patients required for clinical use. Performing a study of this scale is difficult and perhaps even ethically questionable if muscle biopsies need to be included. The ^{31}PMRS and NIRS studies could be used in large series to obtain graft patency because of their non-invasive nature, although extensive effort on developing a standardised protocol should be emphasised.

No study has determined whether it is possible to design a protocol that will provide data based on ^{31}PMRS or NIRS measurements, which explicitly define poor functional outcome of bypass surgery although it is likely to be the case.

Ekert and Scnackerz[52] determined ^{31}PMRS values in human muscle tissue with acute irreversible ischaemia. In this study, the optimal time for re-vascularisation was defined as the time point just prior to the PCr concentration reaching its minimum. The end point where re-vascularisation no longer is possible is defined as the point in time where the ATP concentration is no longer detectable.

The situation is different in chronic limb ischaemia where the muscles of the leg are going through repeating cycles of ischaemia and reperfusion. In critical ischaemia, we hope that ^{31}PMRS values could be used in detecting the time point where the mitochondrial damage has reached an irreversible level, where no gain in muscle function is achievable by re-vascularisation. These results could be related to NIRS measurements, which are more feasible in a clinical setup. This leaves the open question: Is mitochondrial dysfunction reversible? If not, are there some patients who would benefit functionally from bypass surgery where others will not?

If mitochondrial function is irreversible at some point, are there some patients who should be operated earlier? Shunk et al. showed reversibility of mitochondrial function in patients with mild PAD symptoms measured with ^{31}PMRS; therefore, at early stages of PAD, mitochondrial function show reversibility. The limit where the severity of PAD progresses to a level where mitochondrial function is irreversible is not known presently. However, graft patency should also be considered.

Is it possible to use a combination of PMRS, NIRS and respirometry in practical clinical work?

In some grafts, flow may be observed, but without apparent effect on muscle function. In other words, there will be a certain number of cases with patent grafts but no gain in leg function. Therefore, we speculate that by actually testing the status of oxidative metabolism prior to surgery, the outcome of surgery, including mobility of the patient, would be more predictable. In the reviewed papers covering the use of ^{31}PMRS, NIRS and respirometry methods, no direct evidence of a poor clinical outcome in patients with impaired mitochondrial function has been obtained, but long-term follow up studies has not been conducted. Although evidence of good correlation of the degree of ischaemia and muscle metabolism parameters exists,[12,22,23] it is likely that impaired muscle metabolism is a predictor of poor clinical outcome similar to what have been learned from other studies, where the most severely affected ischaemic limb measured as an ABI below 0.40 will be a predictor of poor clinical outcome.[51] However, the use of ABI as a predictor in the diabetic group is problematic as the prognosis could be unreliable. [53] We therefore suggest that the use of NIRS in this patient group could be a promising alternative.

In future studies, respirometry could perhaps be reserved for small groups of patients to determine more detailed nature of the mitochondrial dysfunction. Such results could subsequently be related to ^{31}PMRS and NIRS.

A scheme of such future examination programme is shown in Figs. 2 and 3.

Conclusion

Muscle mitochondrial function is impaired in both T2DM and PAD, but differently. Patients suffering from both pathological conditions will display more serious impairment of the mitochondrial function. Mitochondrial function and the degree of ischaemic disease as evaluated by ^{31}PMRS and NIRS are well correlated. The NIRS technique appears to determine the degree of PAD better than the ^{31}PMRS. It is argued that systematic testing of mitochondrial function may be a useful prognostic tool with PAD and T2DM, but clinical studies to confirm this suggestion are needed.

Conflict of Interest/Funding

None declared.

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