Early changes in the skin microcirculation and muscle metabolism of the diabetic foot

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Summary

Background Changes in the large vessels and microcirculation of the diabetic foot are important in the development of foot ulceration and subsequent failure to heal existing ulcers. We investigated whether oxygen delivery and muscle metabolism of the lower extremity were factors in diabetic foot disease.

Methods We studied 108 patients (21 control individuals who did not have diabetes, 36 patients with diabetes who did not have neuropathy, and 51 patients with both diabetes and neuropathy). We used medical hyperspectral imaging (MHSI) to investigate the haemoglobin saturation ($S_{HSI}O_2$; % of oxyhaemoglobin in total haemoglobin [the sum of oxyhaemoglobin and deoxyhaemoglobin]) in the forearm and foot; we also used ^{31}P-MRI scans to study the cellular metabolism of the foot muscles by measuring the concentrations of inorganic phosphate and phosphocreatine and calculating the ratio of inorganic phosphate to phosphocreatine (Pi/PCr).

Findings The forearm $S_{HSI}O_2$ during resting was different in all three groups, with the highest value in controls (mean 42 [SD 17]), followed by the non-neuropathic (32 [8]) and neuropathic (28 [8]) groups (p<0·0001). In the foot at resting, $S_{HSI}O_2$ was higher in the control (38 [22]) and non-neuropathic groups (37 [12]) than in the neuropathic group (30 [12]; p=0·027). The Pi/PCr ratio was higher in the non-neuropathic (0·41 [0·10]) and neuropathic groups (0·58 [0·26]) than in controls (0·20 [0·06]; p<0·0001).

Interpretation Our results indicate that tissue $S_{HSI}O_2$ is reduced in the skin of patients with diabetes, and that this impairment is accentuated in the presence of neuropathy in the diabetic foot. Additionally, energy reserves of the foot muscles are reduced in the presence of diabetes, suggesting that microcirculation could be a major reason for this difference.

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Introduction

Changes in large vessels and microcirculation of the diabetic foot have a central role in the development of foot ulcers and their subsequent failure to heal. Initial studies using laser flow measurements showed that the maximum hyperaemic response of the foot skin microcirculation to heating or minor trauma was impaired in patients with diabetes compared with healthy controls.[1] Subsequent studies from our unit have indicated that both endothelial and vascular smooth muscle function are impaired and that these abnormalities are present not only in the foot microcirculation but also in forearm skin, an area that is not usually affected by diabetes-related somatic peripheral neuropathy.[2,3] Furthermore, nerve function has an important role in microcirculation since the nerve axon-related vasodilation (Lewis triple-flare response) that is present in areas adjacent to skin injury is equal to a third of the maximum vasodilatory capacity in healthy individuals, whereas it is absent in the feet of patients with diabetic neuropathy.[4]

Despite the clinical effect of diseases of the macrocirculation and microcirculation, little information is available about the effect of blood-flow changes in oxygen delivery and tissue metabolism of the lower extremity. Early clinical studies have reported increased venous oxygen saturation in the feet of patients with

diabetic neuropathy, and attributed this effect to increased arteriovenous shunting and reduced oxygen delivery to foot tissues.[5] Similarly, the effect of diabetes and neuropathy on metabolism of the foot muscle has also not been explored.

In this study, we have used a novel technique, medical hyperspectral imaging (MHSI), to measure oxygen delivery and oxygen extraction of skin tissue on the basis of pixel-by-pixel measurements of oxyhaemoglobin and deoxyhaemoglobin, with laser doppler flow measurements to assess changes in skin microcirculation in the foot and forearm of patients with diabetes with or without peripheral neuropathy. We also measured foot muscle metabolism by non-invasively imaging and quantifying the ^{31}P cellular metabolites with a rapid acquisition MRI method.

Methods
Patients

The study included three groups: healthy individuals with no diabetes, patients with diabetes but without neuropathy, and patients with both diabetes and neuropathy. The diagnosis of type 1 or 2 diabetes was established according to the recommendations of the American Diabetes Association (ADA) Expert Committee.[6] Participants with open lesions on the studied foot, peripheral vascular disease (ankle-brachial

index <0·75, claudication, or both), heart failure that resulted in lower extremity oedema, or any other serious chronic diseases needing active treatment were excluded. The study protocol was approved by the Beth Israel Deaconess Medical Center institutional review board. All participants gave written informed consent.

Procedures

Medical history assessment included age, sex, weight, height, body-mass index, history of alcohol consumption, type and duration of diabetes, and presence of other microvascular and macrovascular complications.

We defined the presence of diabetic peripheral neuropathy according to principles of the San Antonio Consensus criteria.[7] For this assessment, we measured the neuropathy symptom score, neuropathy disability score, and vibration perception threshold with a biothesiometer (Biomedical Instruments, Newbury, OH, USA); we also measured the cutaneous pressure perception threshold with Semmes-Weinstein mono-filaments as previously described.[8]

Endothelium-dependent vasodilatation in the cutaneous microcirculation was measured by iontophoresis of acetylcholine. The measurement was done on the dorsum of the foot and flexor aspect of the forearm as described elsewhere.[9] In brief, a MIC1 iontophoresis system (Moor Instruments, Millwey, UK) and a laser doppler perfusion imager (Lisca PIM 2.0, Lisca Development AB, Linkoping, Sweden) were used.

MHSI data were obtained with a HyperMed Visible MHSI System (HyperMed Inc, Watertown, MA, USA) as previously described.[10] In brief, MHSI is a method of imaging spectroscopy, based on local chemical composition.[11] MHSI uses a spectral separator to vary the wavelength of light admitted to a digital camera to provide

a spectrum for every pixel—a spectral image. Tissue spectra were compared with standard spectra for oxyhaemoglobin and deoxyhaemoglobin, and total tissue haemoglobin determined for every pixel. Oxyhaemo-globin values represent OxyHbHSI units, which are the measurement of oxyhaemoglobin in the standard tissue volume reported by MHSI. Deoxhhaemoglobin values represent DeoxyHbHSI units, which are the measure-ment of deoxyhaemoglobin in the standard tissue volume reported by MHSI. A 30-s tissue image was obtained at a 12-inch focal distance and converted to a calibration image by use of a HyperCal-1 calibrator (HyperMed Inc). The spatial resolution of the MHSI images was 60 mm.

Participants were analysed while sitting in a standard reclining chair. MHSI images were obtained from the same forearm and foot dorsum sites as for laser doppler flow measurements both before and after iontophoresis. We analysed data offline using decomposition, image processing, and image registration techniques. MHSI images were reduced to give average values for oxyhaemoglobin and deoxyhaemoglobin, taking all spatial points higher than a 75% threshold to account for regions of the skin where iontophoresis of the drug was effective. $S_{HSI}O_2$ values, a measurement of haemoglobin saturation (% of oxyhaemoglobin in total haemoglobin [the total defined as the sum of oxyhaemoglobin and deoxyhaemoglobin]), were calculated as described elsewhere.[10]

[31]P-metabolite MRI data was acquired on a GE 3T whole-body magnetic resonance scanner (General Electric Medical Systems, Milwaukee, WI, USA). Participants were placed supine on the scanning bed, and one foot was placed into a birdcage radio-frequency coil designed to image the forefoot.[12] Two reference samples containing a 75 mM solution of phosphocreatine and a 75 mM solution of inorganic phosphate were placed into the coil adjacent to the participant's foot.[13] A transaxial imaging plane was prescribed through the metatarsal head region of the foot. Separate images of the cellular ^{31}P-metabolites were acquired with the MRI pulse sequence known as rapid acquisition with relaxation enhancement (RARE), which had been modified to have chemical selective capabilities.[14] The acquisition of each metabolite image lasted 4 min. After ^{31}P imaging, standard T2-weighted (T2-W) proton (^{1}H) spin-echo MRI was done to acquire detailed images of the anatomy. Spatial resolution of the images of phosphocreatinine and inorganic phosphate was 0·47 cm×0·47 cm×2·5 cm (voxel volume=0·55 mL), and that of T2-W images was 0·06 cm×0·06 cm×0·25 cm (0·0009 mL). Each metabolite image was acquired over 4 min and the T2-W image over 6 min and 24 s.

For every participant, we calculated ^{31}P-metabolite concentrations in the metatarsal head region as previously described.[13] A map was generated by division of the inorganic phosphate image by the phospho-creatine image (Pi/PCr), and was registered to the T2-W

	Control group (n=21)	Non-neuropathic group (n=36)	Neuropathic group (n=51)	p
Age (years)	47 (17)	55 (12)	54 (8)	NS
Sex (male/female)	11/10	19/17	33/18	NS
Body-mass index*	26·4 (5·9)	34·7 (10·4)	31·9 (9·3)	0·015
Diabetes type (1/2)	..	15/21	28/23	NS
Diabetes duration (years)†	..	15 (13)	23 (14)	0·012
Haemoglobin A1c (HbA1c) test result*	5·6 (0·4)	7·6 (1·7)	8·1 (1·9)	0·006
Systolic blood pressure (mm Hg)*	115 (34)	135 (17)	139 (21)	<0·0001
Diastolic blood pressure (mm Hg)	73 (10)	77 (10)	74 (8)	NS
Total cholesterol (mmol/L)‡	5·51 (0·54)	4·34 (1·01)	4·84 (0·98)	0·024
LDL (mmol/L)‡	3·36 (0·7)	2·17 (0·85)	2·61 (0·78)	0·005
HDL (mmol/L)	1·50 (0·39)	1·34 (0·44)	1·37 (0·36)	NS
Ankle-brachial index	1·14 (0·13)	1·13 (0·17)	1·10 (0·20)	NS
Neuropathy symptom score*	0 (1)	4 (3)	4 (3)	<0·0001
Neuropathy disability score§	0 (0)	3 (3)	12 (7)	<0·0001
Vibration perception threshold (V)§	11 (9)	15 (8)	35 (15)	<0·0001
Semmes-Weinstein filaments§	3·8 (0·7)	4·2 (0·4)	6·2 (0·9)	<0·0001

Data are mean (SD) unless stated otherwise. NS=not significant. Semmes-Weinstein filaments are expressed as the mean of logarithmic values of applied pressure (ie, mean log force). Both non-neuropathic and neuropathic groups consist of individuals with diabetes. *Control group vs non-neuropathic and neuropathic groups. †Non-neuropathic group vs non-neuropathic group. ‡Non-neuropathic group vs control and neuropathic groups. §Control and non-neuropathic groups vs neuropathic groups.

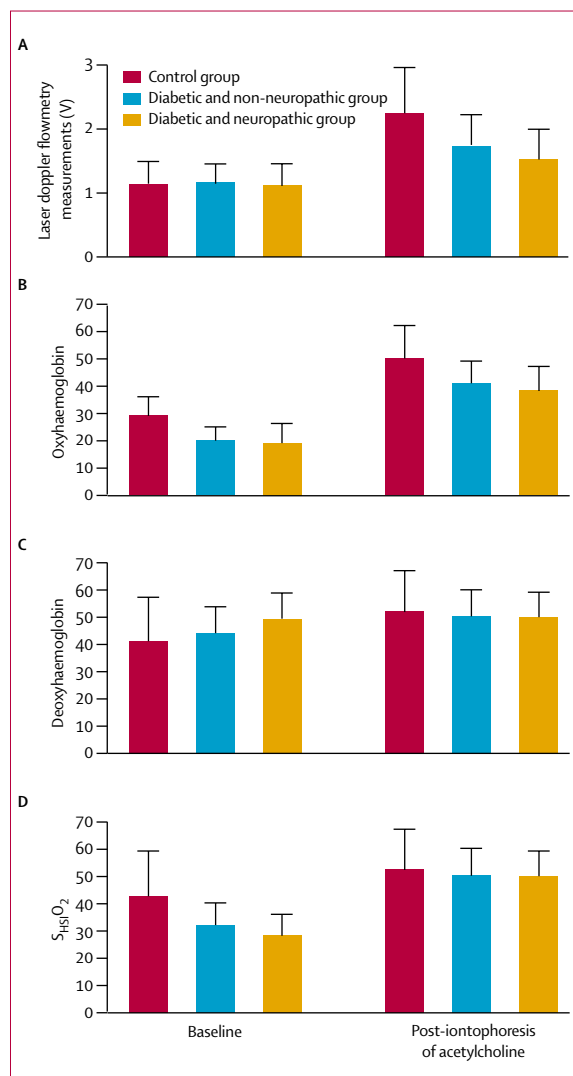*Table 1:* Characteristics of study participants

Figure 1: Analysis at the forearm at baseline (resting) conditions and post-iontophoresis of acetylcholine
Error bars=SDs. (A) Laser doppler flow measurements. (B) MHSI measurements of oxyhaemoglobin. (C) MHSI measurements of deoxyhaemoglobin. (D) MHSI measurements of $S_{HSI}O_2$.

anatomical images to identify the muscle beds that had abnormal ^{31}P-metabolite ratios.

Statistical analysis

The Minitab statistical package (version 14·2, Minitab, State College, PA, USA) for personal computers was used for statistical analysis. For parametrically distributed data, we used the ANOVA test, followed by the Fisher test to identify differences between the various groups. Correlation between variables was tested by use of Pearson correlation analysis.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or

writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

108 individuals participated in the study; 21 control individuals, 36 with diabetes but without neuropathy, and 51 with both diabetic neuropathy. All groups were matched for age and sex. Table 1 shows further details about the participants' characteristics.

Baseline (resting) measurements of laser doppler flow were similar among control patients and those with neuropathy and without neuropathy (mean 1·13 V [SD 0·38] *vs* 1·12 V [0·34] *vs* 1·17 V [0·29]; non-significant difference; figure 1, A). The response to iontophoresis of acetylcholine was reduced in both individuals with
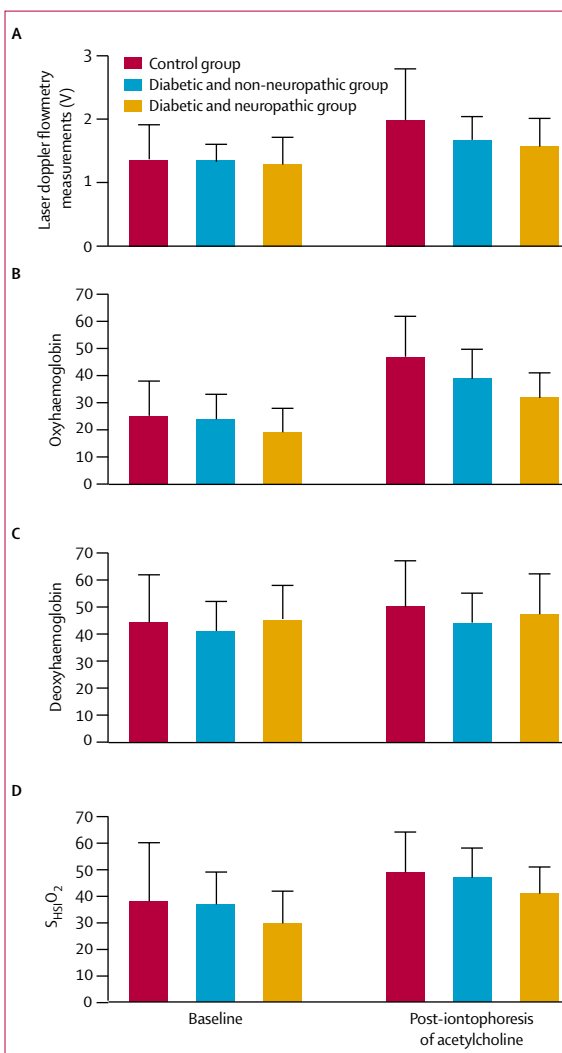

Figure 2: Analysis at the dorsum of the foot at baseline (resting) conditions and post-iontophoresis of acetylcholine
Error bars=SDs. (A) Laser doppler flow measurements. (B) MHSI measurements of oxyhaemoglobin. (C) MHSI measurements of deoxyhaemoglobin. (D) MHSI measurements of $S_{HSI}O_2$.
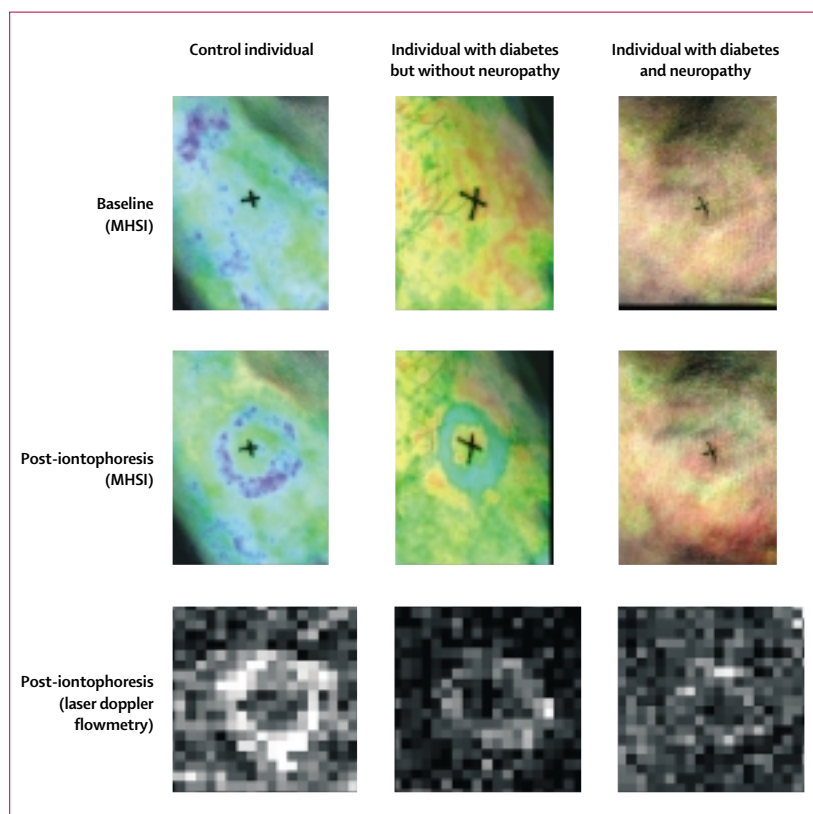
Figure 3: Comparison between MHSI and laser doppler flow measurements at the dorsum of the foot

$(1·54$ V $[0·47])$ and without neuropathy $(1·75$ V $[0·48])$ compared with controls $(2·24$ V $[0·74];$ p=0·004). During MHSI measurements, oxyhaemoglobin at baseline and after iontophoresis was reduced in patients with (baseline 19 [7], post-iontophoresis 38 [9]) and without neuropathy (20 [5], 41 [8]) compared with controls (29 [7], 50 [12]; both p<0·0001; figure 1, B). However, baseline and post-iontophoresis values of deoxyhaemoglobin did not differ significantly between control individuals (baseline 41 [16], post-iontophoresis 52 [15]), and those with (49 [10], 50 [9]) or without neuropathy (44 [10], 50 [10]; figure 1, C). The $S_{HSI}O_2$ values at resting differed between all three groups (control group, 42 [17]; non-neuropathic group, 32 [8]; neuropathic group, 28 [8]; p<0·0001; figure 1, D). But after iontophoresis, $S_{HSI}O_2$ measurements were lower in

the neuropathic group (43 [7]) than in controls (49 [10]; p=0·004).

For the dorsum of the foot, measurements of flow measurements at baseline were similar in all the groups (control, 1·35 V [0·57]; non-neuropathic, 1·35 V [0·26]; neuropathic, 1·31 V [0·41]; non-significant difference; figure 2, A). However, the post-iontophoresis response was reduced in both the non-neuropathic (1·67 V [0·38]) and neuropathic groups (1·58 V [0·44]) when compared with controls (1·98 V [0·82]; p=0·028). At baseline, oxyhaemoglobin was reduced in the neuropathic group (19 [9]) when compared with the non-neuropathic (24 [9]) and control groups (25 [13]; p=0·025; figure 2, B), although the post-iontophoresis measurements were different in control individuals and those with and without neuropathy (47 [15] *vs* 32 [9] *vs* 39 [11]; p<0·0001). For deoxyhaemoglobin values, no differences were seen among any of the groups at rest (control, 44 [18]; non-neuropathic, 41 [11]; neuropathic, 45 [13]; non-significant difference; figure 2, C) and in response to iontophoresis (50 [17], 44 [11], 47 [15]; non-significant difference). $S_{HSI}O_2$ at baseline and at post-iontophoresis was higher in the control (baseline, 38 [22]; post-iontophoresis 49 [15]) and non-neuropathic groups (37 [12], 47 [11]) than in the neuropathic group (30 [12], 41 [10]; p=0·027 and p=0·019, respectively; figure 2, D). Figure 3 shows MHSI and laser doppler images at the dorsum of the foot; MHSI images can detect changes at rest between healthy individuals and individuals with neuropathy, whereas both laser doppler flow measurements and MHSI can detect differences in post-iontophoresis vasodilation.

A subset of the study group underwent MRI measurements of ^{31}P metabolites: eight control individuals, seven with diabetes but no neuropathy (ie, from the non-neuropathic group), and five with both diabetes and neuropathy (ie, from the neuropathic group). We recorded no differences in any of the participants' characteristics between the entire group and those undergoing MRI measurements. Furthermore, no differences were seen in the exercise habits between the three groups, which we assessed by a simple questionnaire.

Significant differences in phosphocreatine measurements were recorded in all three groups, with the highest in the control group and lowest in those with diabetic neuropathy (table 2); the inorganic phosphate was higher in the non-neuropathic group than in the neuropathic group; and the Pi/PCr ratio was higher in both diabetes groups compared with controls. Figure 4 compares the muscle metabolite measurement between a control individual and a patient without neuropathy, indicating a shift to an increased Pi/PCr ratio in the patient. In this small sample, no correlations were seen between ^{31}P-metabolite measurements and flow or MHSI measurements in the foot before or after the iontophoresis of acetylcholine.

	Control group (n=8)	Non-neuropathic group (n=7)	Neuropathic group (n=5)	p
Age (years)	47 (10)	60 (9)	54 (8)	NS
Phosphocreatine (mmol/L)*	24·0 (4·5)	15·0 (2·3)	5·9 (5·9)	<0·0001
Inorganic phosphate (mmol/L)†	4·7 (1·4)	6·0 (0·5)	3·1 (2·9)	0·027
Pi/PCr ratio‡	0·20 (0·06)	0·41 (0·10)	0·58 (0·26)	<0·0001

Data are mean (SD) unless stated otherwise. *Control group *vs* non-neuropathic *vs* neuropathic. †Non-neuropathic group *vs* neuropathic group. ‡Control group *vs* non-neuropathic and neuropathic groups.

Table 2: Characteristics of individuals with ^{31}P-metabolite measurements

Discussion

Our main findings have indicated that there are changes in oxygen delivery or extraction as manifested by MHSI measurements of oxygen saturation in the skin of the forearm and foot of patients with diabetes, with or without neuropathy. These changes are present both under resting conditions, in which no changes are seen in skin blood flow, and after induction of vasodilation by acetylcholine iontophoresis. Furthermore, measurements of ^{31}P metabolites in the foot muscle indicate a significantly higher Pi/PCr ratio in patients with diabetes (with or without neuropathy) than in control individuals. These findings indicate that skin oxygenation and muscle metabolism are impaired in the diabetic foot and could be a major contributing factor for the impaired wound healing seen in diabetic foot ulcers.

Previous studies with laser doppler flow measurement have shown that changes in the resting skin blood flow in either foot or forearm are not associated with diabetic microvascular disease, but instead with impaired vasodilation in response to heating or the iontophoresis of vasodilators such as acetylcholine or sodium nitroprusside.[2,3,15] We recorded similar results in the present study. By contrast, MHSI measurements showed that oxyhaemoglobin was reduced in patients with diabetes at rest and that these changes still existed after the induction of endothelium-dependent maximum vasodilation. Furthermore, deoxyhaemoglobin measurements were similar in all groups. As a result, $S_{HSI}O_2$, as an index of oxygen saturation, was significantly affected by diabetes in the forearm and by neuropathy in the foot.

Notably, in individuals with diabetes, $S_{HSI}O_2$ was lower in those with neuropathy than in those without neuropathy in the forearm, an area that is usually not affected by clinical somatic neuropathy, as well as the foot, where neuropathy is often clinically present and additional macrovascular disease can take place. Although the exact mechanisms are not completely understood, these findings accord with previous findings that have shown reduced oxygen saturation, as measured by spectroscopy, in the sural nerve microcirculation of patients with diabetes and neuropathy.[16] Therefore, our results are compatible with the hypothesis that changes in the microcirculation are associated with nerve dysfunction in diabetes.[17,18]

At the foot level, we also recorded no differences in $S_{HSI}O_2$ between control individuals and patients with diabetes but no neuropathy, which accords with previous studies (in our unit and elsewhere) showing no differences in transcutaneous oxygen measurements between these two groups.[2,19] The mechanisms that are responsible for these findings are not well known, but are probably related to the fact that the endothelium-dependent and independent vasodilation in the foot is much lower than in the forearm in both control individuals and in patients with diabetes (with and
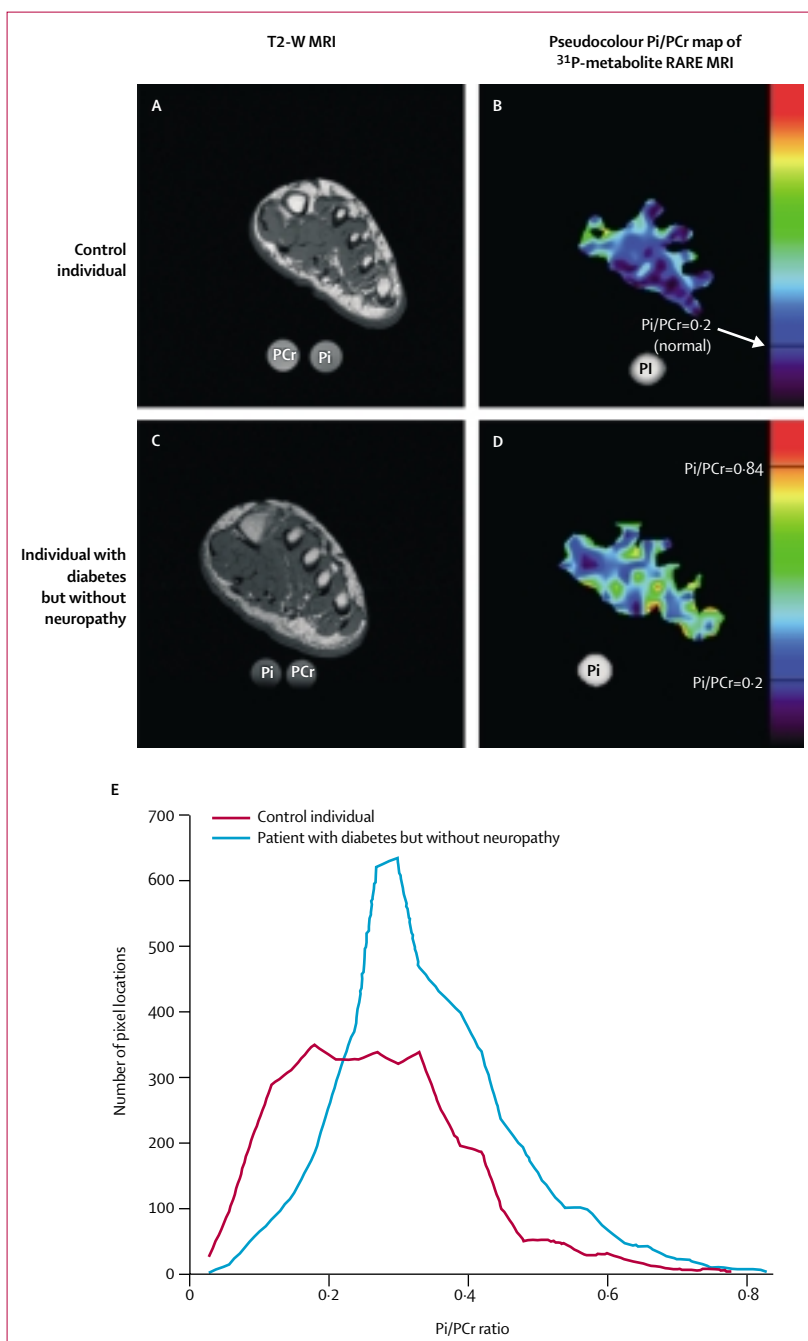

Figure 4: **MRI of muscle metabolism data from the foot of a control individual and a patient with diabetes but no neuropathy**

Images are acquired through the metatarsal head region of the foot. No obvious signs of pathology are seen in T2-W MRI scans of either (A) or (C). Only the muscle tissue appears in (B) and (D), since very low concentrations of phosphorus exist in the subcutaneous fat that are undetectable with the RARE MRI technique. (B) shows mostly healthy muscle tissue (colours ranging from violet to light blue), whereas (D) indicates that the patient with diabetes but no neuropathy has large areas of ischaemic muscle (green to red). (E) Histogram of Pi/PCr ratios also show a clear shift in the number of image pixels toward much higher Pi/PCr ratios in the patient with diabetes and no neuropathy than in the control individual, indicating regions of muscle tissue ischaemia.

without neuropathy).[9] Thus, the current thinking is that the reduced vasodilatory ability in the foot results in the obliteration of small differences seen in the forearm

between healthy individuals and patients with diabetes but without neuropathy.

MHSI cannot distinguish whether the recorded changes are related to reduced oxygen supply or to increased oxygen consumption. Although our findings are compatible with the hypothesis that reduced oxygen supply is the main reason for the development of tissue hypoxia and nerve dysfunction, we cannot exclude the possibility that increased oxygen consumption due to a more oxidative metabolism related to hyperglycaemia-induced chronic inflammation.

Tissue oxygenation has also been measured in the renal cortex and medulla by a blood-oxygenation-level-dependent (BOLD) technique of MRI.[20] Initial studies of this technique indicated reduced medullary oxyhaemo-globin during water diuresis in patients with diabetes, which is probably related to early impairment of adaptive vasodilation in the renal medulla.[21] Furthermore, subsequent studies showed an association between the kidney cortical oxygenation and the skin microvascular reactivity.[22] Thus, our results concur with previous studies of the kidney, and support the hypothesis that reduced tissue oxygenation is present in organs or tissues that are vulnerable to diabetes, such as the nerve, skin, and kidney.

We also found that changes in the phosphorus-metabolite concentrations occur in the foot muscle of patients with diabetes but without neuropathy or peripheral arterial disease. The energy needed for muscle contraction and other cellular functions is produced by the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate. Phosphocreatine provides the phosphate moiety used in the conversion of ADP to ATP, and is the energy reservoir that maintains the ATP concentration needed for adequate cellular function. If the oxygen supply to the tissue is insufficient, the Pi/PCr ratio increases.[23] Previous studies of human skeletal muscle under ischaemic conditions have shown an increase in the Pi/PCr ratio in the resting muscle of patients with peripheral vascular disease.[24,25] These studies, among many others, have also provided ample evidence that the Pi/PCr ratio, as measured by MRI spectroscopy, indicates the energy reserve of the muscle and corresponds to the tissue oxygenation.[26–28]

We show an increased Pi/PCr ratio in patients with diabetes but without neuropathy or peripheral arterial disease. Our results, combined with those of the skin microcirculation, suggest that changes in the microcirculation could result in reduced oxygen supply to the muscle and reduce its energy reserves. Our finding that both phosphocreatine and inorganic phosphate were drastically reduced in the patients with diabetes and neuropathy is noteworthy. These changes are mainly related to the muscle atrophy that is associated with diabetic neuropathy and are consistent with previous findings that showed reduced concen-trations of total phosphorous in patients with diabetes, with or without clinically present neuropathy.[29] Despite the drastic reduction in ^{31}P metabolites in the patients with diabetes and neuropathy, the Pi/PCr ratio was similar in both diabetic groups and much higher than that of the control group. These results further emphasise that this ratio indicates the energy reserves of the muscle and not the possible changes in muscle size due to atrophy, and also agrees with other findings showing that inorganic phosphate and phosphocreatine at resting are not related to age or regular exercise.[26] Therefore, we believe that neither the difference in age nor slight differences in exercise habits could affect our results.

Currently, insufficient data are available regarding muscle metabolism in the diabetic foot. A preliminary study in five patients with diabetes with lower extremity ischaemia reported lower concentrations of high-energy metabolites than five control individuals at the skin of the dorsum of the foot.[27] A subsequent study reported an increased Pi/PCr ratio in patients with diabetic foot ulceration but no differences between controls and patients with diabetes but no foot ulceration.[28] We believe that the difference between our results and other studies pertains largely to differences in the spatial resolution that we have achieved using ^{31}P RARE MRI (in this study 0·55 mL needing a scan time of 4 min vs previous work with 11·25 mL needing a scan time of more than 50 min).

MHSI measurements can provide relevant physiological information in systemic, regional, and local settings. Forearm data can potentially measure the systemic microvasculature, since the forearm is traditionally not differentially afflicted by microvascular or macrovascular disease to the extent of the lower extremities. Measurements at the dorsal-foot surfaces, in turn, provide regional information, can be indicative of both microvascular and macrovascular changes associated with atherosclerotic disease in large vessels exacerbated by diabetes, and can differentiate the stages of this damage on either the right or left lower extremity. Finally, the technique can be used to investigate the foot tissue for local information that could be associated with risk of ulceration, or the tissue surrounding an ulcer. Additionally, the MRI technique can provide valuable information about the metabolism of lower extremity muscles. Use of both techniques, either alone or in combination, could have important clinical applications since they can help to identify patients at risk of developing foot problems.

In summary, our results indicate that $S_{HSI}O_2$ is reduced in the skin of patients with diabetes, and that this impairment is accentuated in the presence of neuropathy in the foot. Furthermore, energy reserves of the foot muscles are reduced in the presence of diabetes, suggesting that microcirculatory changes could have a major role. These changes could underlie the

development of foot ulceration and, especially could preclude the healing of existing ulcers. Early diagnosis and interventions that can address these abnormalities can have important effects on clinical management of the diabetic foot.

Contributions
R L Greenman participated in the design and execution of MRI studies; acquisition, processing, analysis, and interpretation of MRI data; and writing of the report. S Panasyuk participated in the design and execution of MHSI studies; acquisition, processing, analysis, and interpretation of MHSI data; and writing of the report. X Wang participated in the processing of MRI data and measurement of phosphorous-metabolite concentrations. T E Lyons participated in the design and execution of the studies, data analysis, and writing of the report. T Dinh participated in the design and execution of the studies, data analysis, and writing of the report. L Longoria acted as clinical coordinator, and participated in the execution of study, data analysis, and the writing of the report. J M Giurini participated in the design and execution of the studies, data analysis, and writing of the report. J Freeman participated in the design and execution of MHSI studies; acquisition, processing, analysis, and interpretation of MHSI data; and writing of the report. L Khaodhiar participated in the design and execution of the studies, data analysis, and writing of the report. A Veves was the principal investigator of the study; developed the hypothesis; was responsible for the initial concept of the study design; and supervised the execution of the study, data collection, and data analysis; he also wrote the first draft of the report and had main responsibility for the introduction and discussion.

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