^{1}H- and ^{31}P-magnetic resonance spectroscopy and imaging as a new diagnostic tool to evaluate neuropathic foot ulcers in Type II diabetic patients

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Abstract

Aims/hypothesis. We studied 36 Type II (non-insulin-dependent) diabetic patients without occlusive arterial diseases in the lower extremities and 12 age-matched and sex-matched non-diabetic subjects to clarify the association between diabetic polyneuropathy and foot ulcers using ^{1}H- and ^{31}P-magnetic resonance spectroscopy and imaging.

Methods. The 36 diabetic patients consisted of 12 patients with superficial foot ulcers and 24 patients free from this disease. We measured fat to water and phosphocreatine to inorganic phosphate (PCr:Pi) ratios and calculated the intracellular pH of resting plantar muscles by depth-resolved surface-coil spectroscopy using an ^{1}H-^{31}P double tuned coil. Furthermore, foot vasculature, fat and PCr contents of plantar muscles were visualised by phase-contrast angiography, T_1-weighted spin-echo imaging and ^{31}P-chemical shift imaging.

Results. The 12 foot ulcer patients showed a reduced PCr to Pi ratio ($p < 0.001$) and peripheral nerve functions ($p < 0.01$–0.001) but an increased fat to water ratio ($p < 0.001$) and intracellular pH ($p < 0.001$) compared with the 24 patients without ulcers. From stepwise multiple regression analyses, motor nerve function as well as severity of nephropathy was associated with both fat to water and PCr to Pi ratios. When these patients were categorised into three groups based on their level of motor nerve function, the frequency of foot ulcers of the lowest group was higher than that of the highest group.

Conclusion/interpretation. Our findings indicated that motor nerve dysfunction in diabetic patients was closely associated with impaired energy metabolism, fatty infiltration and increased intracellular pH of plantar muscles and high frequency of foot ulcers. These new techniques could contribute to help clarify the predisposing factors for foot ulcers. [Diabetologia (2000) 43: 165–172]

Keywords Magnetic resonance, spectroscopy, imaging, neuropathic foot ulcers, fat, phosphocreatine, intracellular pH.

Diabetic foot ulcers are frequently severe at the plantar aspect of the first metatarsal head, and mechanical forces on the sole of the foot while standing and walking contribute to the development and progression of the wound [1, 2]. Sensory neuropathy associated with loss of protective sensation allows abnormal mechanical forces to cause painless injury including skin wound and asymptomatic bone fracture [3]. Furthermore, plantar muscles play an important part in protecting the foot against these mechanical forces. Therefore, weakness or atrophy of plantar muscles may lead to diabetic foot ulcers although no study concerning abnormalities in these muscles has yet been reported.

A combination of various factors, such as metabolic disorders associated with diabetes [4], peripheral neuropathy [5] and occlusive arterial diseases [6] possibly contribute to the impairment of energy metabolism in skeletal muscles. Ultrasonography can detect focal lesions but technical limitations hamper the global depiction due to the compact bone structure of the foot [7]. Measurements of plethysmography, thermography and transcutaneous O_2 tension ($TcPO_2$) [8] are sometimes useful to estimate skin blood flow in the foot, which is influenced by both occlusive arterial diseases and impaired microcirculation. These techniques, however, provide little information on subcutaneous tissues or muscles of deep structures in the foot. Magnetic resonance techniques are not affected by bone structure and show excellent resolution of the tissue contrast. Therefore, these techniques allow global depiction of foot vasculature, flow volume measurement and waveform analysis of the peripheral artery [9] as well as quantification and visualisation of fat [10, 11] or high-energy phosphate metabolites in foot muscles [6, 12].

This study was designed to assess vascular and muscular foot lesions with newly developed ^{1}H- and ^{31}P-magnetic resonance spectroscopy and imaging. We quantified flow volume of popliteal artery, fat and high-energy phosphate contents of plantar muscles in diabetic patients. These were analysed in terms of abnormalities associated with diabetic polyneuropathy and foot ulcers. Furthermore, we present images of the foot vasculature, fat and phosphocreatine contents of the plantar muscles in a representative case of a diabetic patient with superficial foot ulcers.

Subjects and methods

Diabetic patients and control subjects. We admitted 36 Type II (non-insulin-dependent) diabetic patients ranging in age from 50 to 69 years and 12 age-matched and sex-matched non-diabetic subjects consecutively to our hospital between July 1997 and March 1998. All of the 36 patients were admitted for strict glycaemic control or assessment of diabetic complications and 12 of these patients had superficial foot ulcers with no apparent soft tissue necrosis or inflammatory signs in laboratory testing and the other 24 patients were free from these diseases. For comparison we studied 12 non-diabetic subjects, who were diagnosed according to the WHO criteria based upon a 2-h plasma glucose concentration of less than 11.1 mmol/l by a 75-g oral glucose tolerance test [13] and who had neither a personal history of hypertension nor dyslipidaemia. Patients who had heart failure, liver cirrhosis, severe nephropathy (serum creatinine more than 133 μmol/l or with foot oedema), alcohol abuse, occlusive arterial diseases in the lower extremities, non-diabetic neurological disorders, myopathies and acute illness were excluded from the study. All patients were fully mobile and were neither athletic nor sedentary with disuse atrophy of foot muscles following immobilisation. Patients with foot ulcers needed strict glycaemic control, local wound care and proper footwear, but antibiotics, debridement, bed rest or use of crutches to limit weight bearing were not needed for them.

The study was approved by the ethics committee of our institution and informed consent was obtained from all patients before the examinations which were done during their stay in hospital.

Clinical evaluation. Fasting plasma glucose (FPG) and glycated haemoglobin (HbA_{1c}) were examined to assess the glycaemic control. Neurological assessments were done by motor nerve conduction velocity (MCV) of the posterior tibial nerve and sensory nerve conduction velocity (SCV) of the sural nerve were examined using electromyography (Medelec MS-25, San-ei, Tokyo, Japan) and the coefficient variation of the R-R interval (CV_{R-R}) during deep breathing monitored on an electrocardiogram (Cardimax FX-3301, Fukuda Denshi, Kyoto, Japan) to evaluate autonomic function. Transcutaneous O_2 tension ($TcPO_2$) on the dorsal aspect of the first intermetatarsal space of patients at rest in the supine position was measured at 44 °C using a cutaneous PO_2 monitor (Cutaneous PO_2 Monitor 8000, Kohken Medical, Tokyo, Japan) [8]. Ankle-brachial systolic blood pressure index (ABI) was examined by a handheld ultrasound Doppler (ES-1000SP, Nihon Kohden, Tokyo, Japan) to assess occlusive arterial diseases in the lower extremities [14]. Patients with an ABI < 0.90 and/or abnormal waveform assessed by flow analysis of the popliteal artery as described below were excluded from the study [9]. A trained ophthalmologist carried out fundus ophthalmoscopies and defined diabetic patients as no retinopathy, having simple retinopathy corresponding to levels 21–53 or proliferative retinopathy to levels 60–80 of the modified Airlie House System [15]. Furthermore, diabetic patients with normoalbuminuria, microalbuminuria and overt proteinuria were defined as having a urinary albumin excretion rate of less than 15, 15–199 and greater than 200 μg/min by a 24-h urine collection in our university hospital.

Magnetic resonance spectroscopy and imaging. A magnetic resonance imaging scanner at 1.5 Tesla (Signa Horizon, GE Medical Systems, Milwaukee, Wis., USA) was used for the following experimental protocols to quantify and visualise the peripheral arterial circulation of the lower extremities and contents of fat and high-energy phosphorus compounds in the plantar muscles.

Quantitative blood flow measurement and waveform analysis at the level of the popliteal artery were done by axial two-dimensional cine-mode phase-contrast magnetic resonance imaging with peripheral gating, 80 cm/s velocity encoding, 5 mm scan thickness and 256×256 acquisition matrix [9]. Evaluation of morphologic findings of the foot artery with no contrast medium was done in 60 slices by three-dimensional phase-contrast magnetic resonance angiography (MRA) with 5 cm/s velocity encoding, 2 mm partitions and 24 cm field of view using a quadrature head coil.

An 8-cm diameter surface coil double tuned to proton and phosphorus-31 (^{1}H-^{31}P) frequencies was set at the plantar aspect of the first metatarsal head of the patients. A slice of 25 mm thickness in the plantar muscles parallel to the plane of the surface coil was selected with depth-resolved surface-coil spectroscopy to avoid contamination of subcutaneous fat in the sole [10]. Findings from ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) for the composition of fat (-CH_2-) and water (H_2O) of plantar muscles were acquired with 5 s pulse repetition time and two excitations. The relative value for the fat to water ratio was calculated by each peak area. Using a 12.5 cm diameter surface coil tuned to ^{1}H frequency, the coronal T_1-weighted spin-echo image of the plantar muscles was obtained with 300 milli seconds pulse repetition time, 30 milli seconds echo time, 1 excitation, 5 mm slice thickness,

Table 1. Characteristics of diabetic patients with (DU group) or without (DC group) superficial foot ulcers and age- and sex-matched non-diabetic subjects (non-DM group)

	DU group	DC group	Non-DM group
n	12	24	12
Sex (Male/Female)	5/7	10/14	6/6
Age (years)	61.3 ± 6.3	58.6 ± 5.0	60.2 ± 8.0
Duration of diabetes (years)	16.4 ± 8.7	12.5 ± 6.6	–
Treatment (D/OHD/I)	0/4/8	1/14/9	–
FPG (mmol/l)	10.0 ± 5.7[b]	9.9 ± 3.0[b]	5.2 ± 0.5
HbA$_{1c}$ (%)	9.3 ± 1.7[c]	8.5 ± 1.7[c]	4.7 ± 0.5
ABI	1.13 ± 0.11	1.13 ± 0.17	1.13 ± 0.06
Flow volume (ml/min)	59.4 ± 25.8[a]	71.5 ± 31.5	90.3 ± 24.5
TcPO$_2$ (mmHg)	48.5 ± 16.8[b]	59.2 ± 13.5	68.1 ± 7.9
MCV (m/s)	33.2 ± 6.9[c, f]	40.9 ± 5.2	44.0 ± 2.8
SCV (m/s)	36.8 ± 5.2[b, d]	41.3 ± 5.0	43.8 ± 1.9
CV$_{R-R}$ (%)	1.48 ± 1.22[c]	2.39 ± 1.06[a]	3.53 ± 1.28
With retinopathy	12 (100 %)[e]	14 (58 %)	–
overt proteinuria	8 (67 %)[e]	4 (17 %)	–

Data are expressed as means ± SD. D, diet; OHD, oral hypoglycaemic drugs; I, insulin. The multiple comparison of significant differences among the three groups was analysed using one-way ANOVA followed by Scheffe's F test. Chi-squared test for 2 by 2 contingency table was used to compare the frequencies between two groups. [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ vs non-DM group; [d] $p < 0.05$, [e] $p < 0.01$, [f] $p < 0.001$ vs DC group

256×256 matrix and 24 cm field of view to analyse the fat distribution.

Using a ^{1}H-^{31}P double tuned 8-cm diameter surface coil, ^{31}P-MRS of phosphorus compounds in the plantar muscles was acquired with 1.5 s pulse repetition time and 512 excitations using a depth-resolved surface-coil spectroscopy sequence at the same location of ^{1}H-MRS. The relative value for the phosphocreatine to inorganic phosphate (PCr:Pi) ratio was calculated by each peak area. Intracellular pH was calculated using the following equation; $pH = 6.75 + \log[(\sigma\text{-}3.27)/(5.69\text{-}\sigma)]$, where σ is the chemical shift of Pi in parts per million from the PCr signal [6]. Using a ^{31}P tuned 12.5-cm surface coil, three-dimensional chemical shift imaging was carried out for PCr mapping of the plantar muscles with a 1.5 s repetition time, 8 excitations, 5 cm slice thickness, 24 cm field of view and 16×16 phase-encoding steps [12]. A 2.5-cm diameter tube that contained 50 mmol/l Pi was set between the hindfoot of the patient as a standard. Chemical shift images of PCr and Pi signals were constructed with a spatial resolution of 1.5×1.5 cm and a 5-cm slice thickness. Furthermore, the images of these two metabolites were superimposed to make a single image, in which the PCr signal was normalised by the intensity of the Pi reference signal.

Statistical analysis. Statistical evaluation was done using StatView 4.5 (Abacus Concepts, Berkeley, Calif., USA, 1989 and 1992) software for a Macintosh computer. The multiple comparison of significant differences among three groups were analysed by one-way analysis of variance (one-way ANOVA) followed by Scheffe's F test. The chi-squared test for 2 by 2 or Bonferroni test for 2 by 3 contingency table was used to compare the frequencies between two or among three groups. Simple linear or stepwise multiple regression analysis was done to evaluate the associations among fat to water or PCr to Pi ratio and various clinical characteristics of the subjects. P values < 0.05 were considered statistically significant. Data are expressed as means ± SD.

Results

In Figs. 1 and 2 two representative cases are shown. A 60-year-old diabetic woman who had superficial foot ulcers without walking disturbance and inflammatory signs in laboratory testing (case DU) and an age-matched and sex-matched non-diabetic subject (case NC) were compared for peripheral arterial circulation of the foot, fat deposition and high-energy phosphate metabolism of plantar muscles. Coronally reconstructed phase-contrast angiography of case NC (Fig. 1 a) and case DU (Fig. 1 b) clearly show the vasculature of the branches of the anterior and posterior tibial arteries, such as the dorsalis pedis, medial and lateral plantar arteries and plantar arch in the foot. Coronal T_1-weighted spin-echo images, however, showed that case DU (Fig. 1 d) had neither apparent soft tissue necrosis nor inflammation in the foot but diffusely brighter plantar muscles than those of case NC (Fig. 1 c). The ^{31}P-chemical shift imaging of the foot in case NC (Fig. 1 e) was characterised by homogeneous PCr distribution in the plantar muscles and the images corresponding to toes and the calcaneus of the hindfoot were deficient. Phosphocreatine mapping of case DU (Fig. 1 f) was characterised by diffuse reduction of the PCr content, which was the background level. Only the signal of the Pi reference was observed. The ^{1}H spectrum of the plantar muscles in case NC (Fig. 2 a) showed two peaks of fat (-CH$_2$-) and water (H$_2$O). Case DU (Fig. 2 b) showed a relative increase in the fat component compared to that of case NC, indicating that bright muscles on T_1-weighted spin-echo image reflected fatty infiltration. The ^{31}P spectrum of the plantar muscles in case NC (Fig. 2 c) showed Pi, PCr and three high-energy phosphates (γ-, α-, β-) of ATP peaks. Case DU (Fig. 2 d)
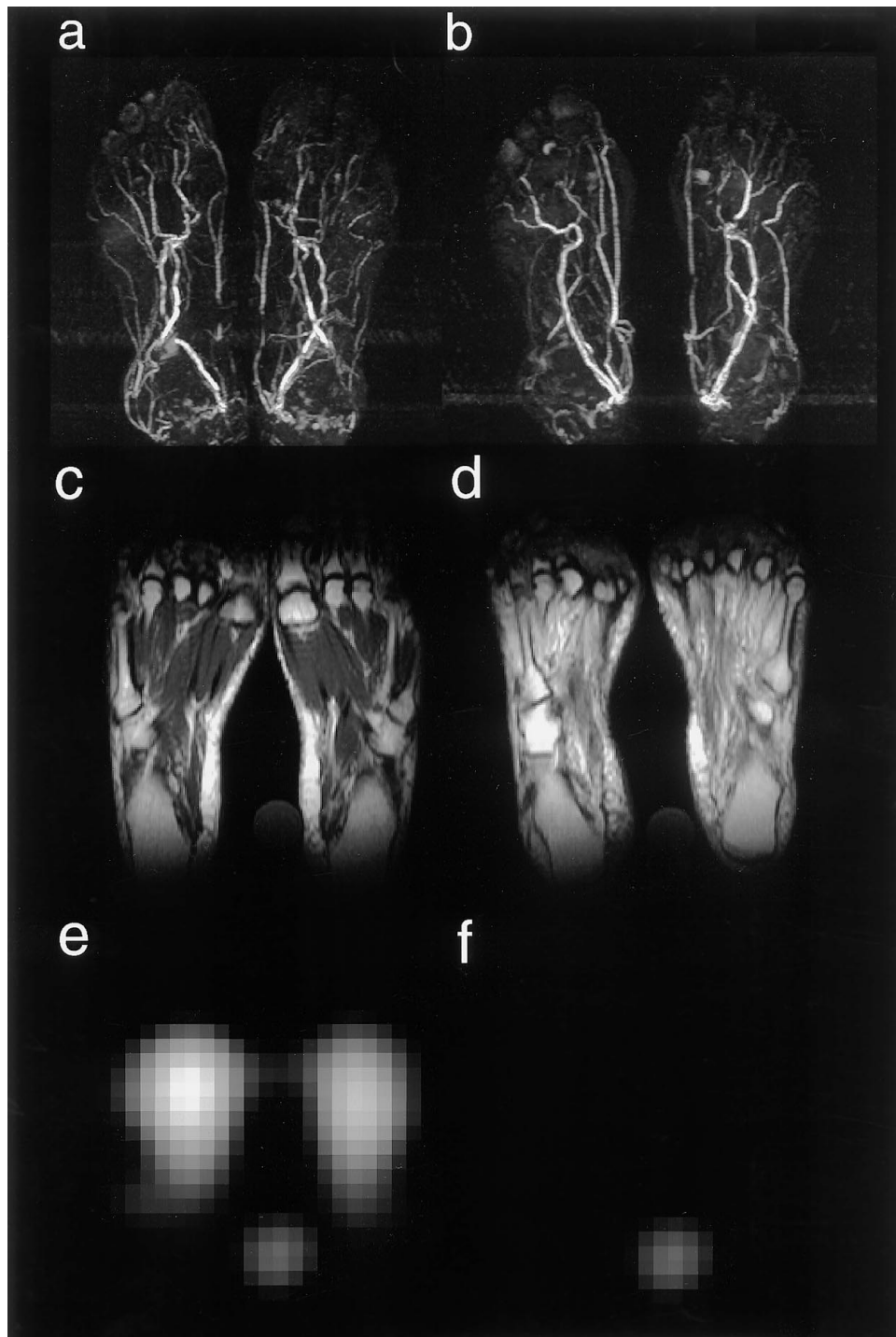
Fig. 1a–f. Coronally reconstructed magnetic resonance phase-contrast angiography (**a**, **b**), T_1-weighted spin-echo imaging (**c**, **d**) and ^{31}P-chemical shift imaging (**e**, **f**) show the foot vasculature, fat and phosphocreatine mapping of the plantar muscles in a 60-year-old diabetic woman with superficial foot ulcers (**b**, **d**, **f**) and of an age- and sex-matched non-diabetic subject (**a**, **c**, **e**)

showed a pronounced reduction of the PCr content compared to that of case NC.

The characteristics of the three groups including the 36 diabetic patients with (DU group, $n = 12$) or without (DC group, $n = 24$) superficial foot ulcers and 12 age-matched and sex-matched non-diabetic subjects (non-DM group, $n = 12$) are summarised in Table 1. In the two diabetic groups, FPG and HbA_{1c} were higher and CV_{R-R} was lower than in those of the non-DM group. Among the three groups the ABI was matched. Flow volume, $TcPO_2$, MCV and
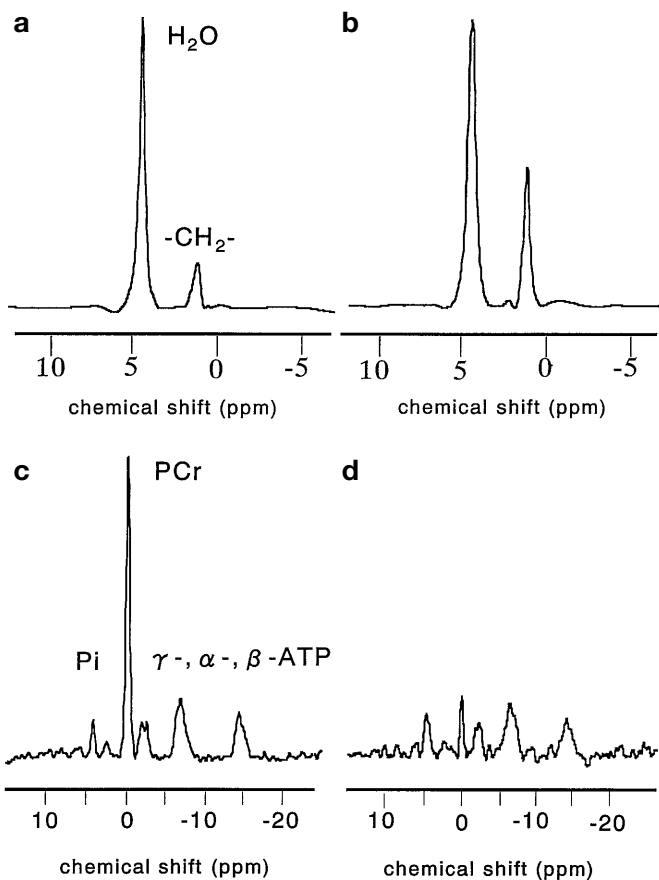
Fig. 2a–d. ^{1}H- and ^{31}P-magnetic resonance spectroscopic analyses show the contents of fat and phosphorus compounds in the plantar muscles in a 60-year-old diabetic woman with superficial foot ulcers (**b**, **d**) and an age- and sex-matched non-diabetic subject (**a**, **c**)
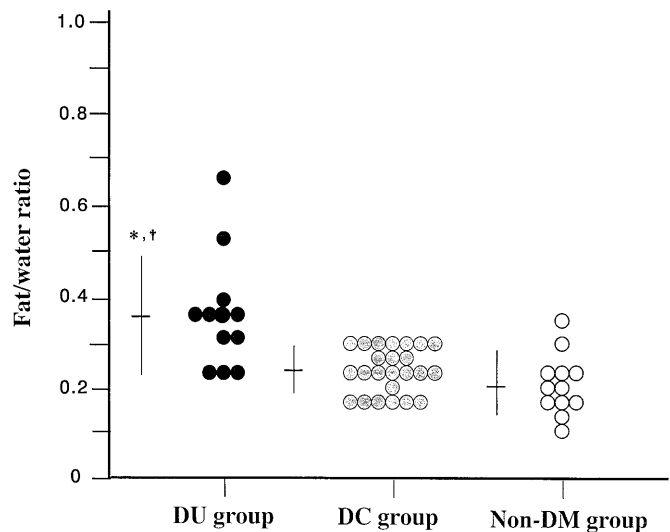

Fig. 3. Distribution of the fat to water ratio in two groups of diabetic patients with (●, DU group, $n = 12$) or without (◐, DC group, $n = 24$) superficial foot ulcers and one age- and sex-adjusted non-diabetic group (○, non-DM group, $n = 12$). Each bar indicates means ± SD. *$p < 0.001$ vs the non-DM group, $^\dagger p < 0.001$ vs the DC group
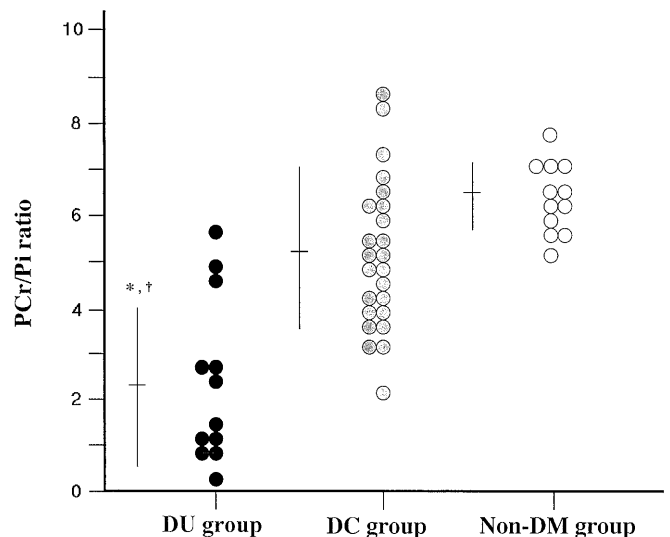

Fig. 4. Distribution of the phosphocreatine to inorganic phosphate ratio in two groups of diabetic patients with (●, DU group, $n = 12$) or without (◐, DC group, $n = 24$) superficial foot ulcers and one age- and sex-matched non-diabetic group (○, non-DM group, $n = 12$). Each bar indicates means ± SD. *$p < 0.001$ vs the non-DM group. $^\dagger p < 0.001$ vs the DC group

SCV of the DU group were reduced compared with those of the non-DM group. Furthermore, MCV and SCV of the DU group were lower than those of the DC group although duration of diabetes, FPG, HbA$_{1c}$, flow volume, TcPO$_2$ and CV$_{R-R}$ were similar between them. The prevalence of retinopathy and overt proteinuria in the DU group were higher than in the DC group. In the DU group, an increased fat to water ratio (Fig. 3) and reduced PCr to Pi ratio (Fig. 4) were observed compared with those of the DC and non-DM groups. Furthermore, intracellular pH in the DU group (7.29 ± 0.15) was increased ($p < 0.001$) compared with the DC (7.14 ± 0.07) and non-DM groups (7.12 ± 0.05).

Clinical characteristics affecting fat to water and PCr to Pi ratios in 36 diabetic patients were statistically analysed (Table 2). Simple linear regression analyses of the association between either the fat to water or PCr to Pi ratio and various possible risk factors in these patients showed that the variables were correlated with the duration of diabetes, MCV, SCV and CV$_{R-R}$, but not with age, FPG, HbA$_{1c}$, ABI, flow volume or TcPO$_2$. From stepwise multiple regression analyses, MCV as well as the severity of nephropathy were identified as significant independent variables for both fat to water and PCr to Pi ratios in these patients. Furthermore, simple linear regression analysis between PCr to Pi and fat to water ratios showed a negative correlation ($r = -0.540$, $p < 0.001$).

Table 2. Simple linear or stepwise multiple regression analysis of clinical factors affecting the fat to water or phosphocreatine to inorganic phosphate (PCr:Pi) ratio in 36 diabetic patients

	Simple linear regression analysis		Stepwise multiple regression analysis	
	r value	p value	β value	F value
Fat to water ratio				
Age	0.191	NS	–	0.290
Duration of diabetes	0.407	< 0.05	–	2.863
FPG	0.035	NS	–	0.494
HbA$_{1c}$	– 0.022	NS	–	0.045
ABI	– 0.175	NS	–	3.573
Flow volume	0.089	NS	–	1.780
TcPO$_2$	– 0.065	NS	–	0.641
MCV	– 0.475	< 0.01	– 0.005	6.392
SCV	– 0.334	< 0.05	–	0.049
CV$_{R-R}$	– 0.427	< 0.01	–	0.511
Retinopathy			–	1.115
Nephropathy			0.036	4.099
r^2			0.288 ($p = 0.0014$)	
PCr to Pi ratio				
Age	– 0.098	NS	–	0.011
Duration of diabetes	– 0.325	NS	–	1.036
FPG	– 0.091	NS	–	0.576
HbA$_{1c}$	– 0.187	NS	–	0.613
ABI	0.017	NS	–	1.359
Flow volume	0.244	NS	–	1.301
TcPO$_2$	– 0.034	NS	–	0.001
MCV	0.561	< 0.001	0.126	9.135
SCV	0.495	< 0.01	–	0.692
CV$_{R-R}$	0.404	< 0.05	–	1.933
Retinopathy			–	3.941
Nephropathy			– 0.433	10.382
r^2			0.447 ($p < 0.0001$)	

F value used was set at 4.0 at each step. Retinopathy (none, simple, and proliferative) and nephropathy (normoalbuminuria, microalbuminuria, and overt proteinuria) were classified into three groups based on severity as described in the "Subjects methods" section

We assigned 36 diabetic patients to three groups based on their MCV levels (Table 3). Although age, sex, FPG, HbA$_{1c}$, ABI, flow volume and TcPO$_2$ were similar among the groups, SCV, CV$_{R-R}$ and the PCr to Pi ratio were decreased and the duration of diabetes, fat to water ratio and intracellular pH were increased in the lowest group compared with those of the highest group. The prevalence of foot ulcers in the lowest group was higher than that in the highest group, although prevalence of retinopathy and overt proteinuria was similar between the two groups.

Discussion

In this study, ^{1}H- and ^{31}P-magnetic resonance spectroscopic analyses of 12 diabetic patients with neuropathic foot ulcers showed considerable impairments of energy metabolism and higher fat content as well as intracellular pH of plantar muscles, while there

were no noticeable differences in indices of peripheral arterial circulation of the lower extremities compared with those of 24 patients free from these diseases.

The energy metabolism of skeletal muscles was affected by a variety of factors, such as training [16], ageing [17], metabolic disorders associated with diabetes [4], global ischaemia [6] or denervation from multiple causes including trauma [5]. Diabetic patients in this study were neither athletic nor sedentary with disuse atrophy of foot muscles following immobilisation. Age and indices of glycaemic control were matched between diabetic patients with and without foot ulcers. The energy metabolism of the skeletal muscle in global ischaemia was accompanied by reduction of PCr and ATP, a complementary increase in inorganic phosphate and reduction of intracellular pH [6]. The diabetic patients, however, who had neuropathic foot ulcers had an impaired energy metabolism and increased intracellular pH of plantar muscles, while indices of peripheral arterial circulation were similar to those of patients free from these diseases, indicating that global ischaemia was not evident in the ulcerated patients.

Neurological disorders in the lower limbs are associated with diabetes mellitus. Sensory neuropathy associated with an impaired protective sensation allows repetitive stress of walking to cause painless injury including skin wound and asymptomatic bone fractures [3]. Autonomic neuropathy results in abnormal opening of arterio-venous shunting [18], which could reduce capillary blood flow and impair oxygen delivery to the tissues, consequently diminishing arterio-venous oxygen differences [19]. Motor nerve involvement causes muscle weakness or atrophy with foot imbalance leading to clawing of the toes, a high arched foot or high pressure of the plantar surface [20]. Furthermore, muscular atrophy has been defined as histological replacement of normal muscle by fat cells caused by traumatic denervation [11]. Stepwise multiple regression analyses showed that motor nerve function was statistically significantly associated with both the decreased PCr to Pi ratio and increased fat to water ratio. Therefore, the fatty infiltration of plantar muscles in ulcerated patients was most likely associated with neurogenic atrophy following motor nerve dysfunction. It has been reported previously that patients with traumatic denervation in resting forearm muscles results in a reduced PCr to Pi ratio and an increase in intracellular pH [5]. We confirmed these alterations in resting plantar muscles of diabetic patients with neuropathic foot ulcers. The physiological and biochemical changes occurring in denerved muscle have been reported previously[21]. The exact mechanisms, however, by which the denervation and reinnervation processes affect energy metabolism and intracellular pH of the skeletal muscle remain unclear.

Table 3. Characteristics of three diabetic groups categorized by their motor nerve conduction velocity of the posterior tibial nerve

	Lowest (30.8 ± 2.8 m/s)	Intermediate (38.3 ± 2.2 m/s)	Highest (46.0 ± 3.0 m/s)
n	12	12	12
Sex (Male/Female)	5/7	5/7	5/7
Age (years)	60.6 ± 6.2	58.8 ± 4.7	59.2 ± 5.6
Duration (years)	18.2 ± 6.9[a]	13.3 ± 7.9	9.8 ± 5.3
Treatment (D/OHD/I)	0/6/6	1/6/5	0/6/6
FPG (mmol/l)	10.1 ± 5.1	9.2 ± 3.6	10.4 ± 3.2
HbA_{1c} (%)	9.2 ± 1.7	8.7 ± 1.9	8.6 ± 1.6
ABI	1.08 ± 0.13	1.19 ± 0.13	1.11 ± 0.19
Flow volume (ml/min)	58.7 ± 26.5	71.9 ± 28.8	71.8 ± 34.6
$TcPO_2$ (mmHg)	53.4 ± 17.9	60.0 ± 16.4	53.6 ± 11.1
SCV (m/s)	36.2 ± 4.6[b]	40.4 ± 5.7	42.8 ± 3.9
CV_{R-R} (%)	1.21 ± 0.70[b]	2.01 ± 1.01	2.59 ± 1.15
With retinopathy	12 (100%)	7 (58%)	7 (58%)
overt proteinuria	7 (58%)	3 (25%)	2 (17%)
foot ulcer	9 (75%)[a,d]	1 (8%)	2 (17%)
Fat to water ratio	0.35 ± 0.13[a,c]	0.25 ± 0.05	0.24 ± 0.06
PCr to Pi ratio	2.61 ± 1.78[b,c]	4.70 ± 2.03	5.31 ± 1.60
Intracellular pH	7.28 ± 0.16[a,c]	7.15 ± 0.06	7.15 ± 0.07

Data are expressed as means $\pm$ SD. D, diet; OHD, oral hypoglycaemic drugs; I, insulin. The multiple comparison of significant differences among three groups were analysed using one-way ANOVA followed by Scheffe's F test. The Bonferroni test for 2 by 3 contingency table was used to compare the frequencies among the three groups. [a] $p < 0.05$, [b] $p < 0.01$ vs the highest group. [c] $p < 0.05$, [d] $p < 0.01$ vs the intermediate group

In our study, statistical analysis showed that the severity of nephropathy was not only associated with fat deposition but also decreased PCr content of plantar muscles in diabetic patients. These findings suggest that progression of diabetic microangiopathy could partly be related to the plantar muscle dysfunction.

In conclusion, we developed [1]H- and ^{31}P-magnetic resonance spectroscopy and imaging for clinical use to evaluate the severity and extent of diabetic foot lesions. We found that motor nerve dysfunction in diabetic patients was associated with diffusely impaired high-energy phosphate metabolism of plantar muscles and a high frequency of foot ulcers, although no meaningful clinical signs of occlusive arterial disease in the lower extremities were observed. These patients were also characterised by diffuse fatty infiltration and increased intracellular pH of plantar muscles, indicating that these changes were not related to a mechanism causing ischaemia. These new techniques could contribute to clarify the predisposing factors for foot ulcers.

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