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Effect of Diabetes Mellitus on Muscle Size and Strength in Patients Receiving Dialysis Therapy

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• ***Background:*** **Diabetes mellitus (DM) is a potential contributor to the muscle abnormalities and poor physical functioning of the dialysis population.** ***Methods:*** **Thirty-three dialysis patients without DM (non-DM group) were compared with 25 dialysis patients with DM (DM group). Measures were made of cross-sectional area and composition of the leg muscles by using proton T_1-weighted magnetic resonance imaging; body composition by means of dual-energy X-ray absorptiometry; leg muscle strength by means of isokinetic knee extension; isometric dorsiflexor maximum voluntary contraction by means of force plate; physical activity by means of 3-dimensional accelerometry; and functional capacity by using various functional tests.** ***Results:*** **The DM group was older, weaker, more atrophic, and had a greater amount of intramuscular fat compared with the non-DM group. However, when the overall analysis was adjusted for age and sex, there were no differences between the 2 groups with respect to muscle cross-sectional area, leg strength, or physical activity. To further account for sex and age differences, a paired analysis was performed after matching patients by age (within 5 years) and sex (N = 16/group). In the matched analysis, only intramuscular fat and leg adipose tissue were different between the 2 groups.** ***Conclusion:*** **DM is associated with more fat within muscles of dialysis patients, but is unrelated to muscle size or strength. Demographic differences between the DM and non-DM groups on dialysis therapy likely are responsible for the general perception that patients with DM are more debilitated.** ***Am J Kidney Dis*** **xx:xxx.**

INDEX WORDS: Dialysis; diabetes; muscle; strength; intramuscular fat; physical activity; magnetic resonance imaging.

AQ: 2

RENAL FAILURE IS a catabolic disease and has been associated with atrophy in skeletal muscles of patients with end-stage renal disease (ESRD).[1,2] Muscle atrophy in this population has been linked to weakness and poor performance of the physical tasks required for activities of daily living.[3,4] Although inactivity may explain a significant degree of the observed atrophy in patients with ESRD, other factors also appear to be important.

Diabetes mellitus (DM) is an increasingly important cause of ESRD and a potential added contributor to the muscle abnormalities and poor physical functioning of the ESRD population. DM continues to account for the greatest number of new ESRD cases. However, incident rates for patients with a primary diagnosis of DM have been level since 2000, at 145 to 147 cases per million population (40% of incident patients with ESRD).[5] Patients with ESRD with DM are more likely to be malnourished[6] and may have neuropathy or myopathy related to DM[7] that could predispose this population to have a greater degree of muscle atrophy and more impaired functional capacity.[8] Studies of elderly populations showed that DM was associated with poor physical functioning.[9] However, to our knowledge, the potential impact of DM on muscle quality and function in patients with ESRD has not been investigated.

Accordingly, we endeavored to determine whether patients with ESRD with DM have more severe muscle abnormalities than patients with ESRD alone. Goals of the current study are to: (1) quantitatively assess morphological character-

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istics of the leg muscles (calf and thigh) in patients with renal failure secondary to DM and patients with other causes of renal failure, and (2) determine whether differences in muscle content among these groups are associated with differences in physical performance. We hypothesized that patients with DM would have more severe muscle atrophy and, as a consequence, diminished functional abilities compared with patients without DM with renal failure.

METHODS

Study Subjects

Fifty-eight dialysis patients were recruited from the University of California, San Francisco–affiliated dialysis units, including the San Francisco Veterans Affairs Medical Center, University of California-Mt Zion Dialysis Center, and the University of California Renal Center at San Francisco General Hospital. Entry criteria for dialysis patients included receipt of long-term hemodialysis for 3 months or more with adequate dialysis delivery (Kt/V $\geq$ 1.2). Patients were excluded if they had reasons for being in a catabolic state, such as human immunodeficiency virus infection, known malignancy, or infection requiring intravenous antibiotics within 2 months before enrollment. Patients were considered to have DM (DM group) if they carried this diagnosis in their medical records or were administered insulin or oral agents for control of DM, regardless of whether DM was thought to be the cause of ESRD.

All patients gave informed consent for study participation. The study was approved by the Committee on Human Research at the University of California San Francisco and the Research and Development Committee of the San Francisco Veterans Affairs Medical Center. Some data for a subset of these patients were presented previously.[4]

Clinical Measurements

Patients were studied on 2 separate days at the San Francisco Veterans Affairs Medical Center (Magnetic Resonance Unit) and San Francisco General Hospital (General Clinical Research Center). Height and weight were recorded with patients wearing only a hospital gown, and body mass index was calculated as weight in kilograms divided by the square of height in meters. Dialysis patients were weighed after a dialysis session. Routine monthly laboratory results were recorded for dialysis subjects, including serum albumin, hemoglobin, and predialysis glucose and glycosylated hemoglobin A_{1c} (HbA_{1c}) levels and single-pool Kt/V calculated from predialysis and postdialysis blood urea nitrogen measurements.

Magnetic Resonance Imaging

Magnetic resonance images were obtained on a nondialysis day. Proton T_1-weighted magnetic resonance imaging (MRI) was used to visualize the cross-sectional area of the right lower leg muscles (calf) using a 1.5-Tesla whole-body Siemens Magnetom Vision system (repetition time, 510 milliseconds; echo time, 14 milliseconds; field of view, 210 mm^2; 33 slices; 4-mm thickness), using a 31-cm diameter extremity coil, as performed previously.[4,10] The same MRI system was used for visualizing the cross-sectional area of the right thigh muscles using the body coil (repetition time, 510 milliseconds; echo time, 14 milliseconds; field of view, 210 mm^2; 15 slices; 8-mm thickness).

AQ: 3

Muscle and Fat Measurements

A customized software program written in Interactive Data Language (Interactive Data Language Research Systems Inc, Boulder, CO) allowed for the separate quantification of muscle (contractile), fat (noncontractile), and miscellaneous (connective tissue, fascia) components of the total cross-sectional area of the muscle compartment of the leg (excluding subcutaneous adipose tissue and bones) based on variations in signal intensity.[4,10] MRI slices with the largest cross-sectional area in the calf and thigh were analyzed for each subject. Each slice was measured 3 times, and the average value was obtained. Subcutaneous adipose tissue was quantified as the area below the skin and above the muscle fascia on the same MRI slice used to quantify muscle size and percentage of muscle content. Absolute muscle contractile cross-sectional area was used in statistical analyses because it was expected to be related to muscle strength. Intramuscular fat is expressed as a percentage because percentage of fat varies less with body size and is a better indicator of muscle "quality" than absolute muscle fat content.[10]

Physical Performance

Patients were timed to the nearest hundredth of a second once while walking 20 feet at their usual pace and once while walking as fast as possible. Patients also were timed while walking up 1 flight of stairs (22 stairs) and while rising from a chair 5 times.

Physical Activity

Physical activity was measured by using 3-dimensional accelerometers (TriTrac R3D; Professional Products Inc, Madison, WI), worn for 1 week as previously described.[11]

Body Composition

Body composition was assessed immediately after dialysis, except when dialysis finished after 8:00 PM, in which case, measures were performed the following morning while fasted (n = 3). Dual-energy X-ray absorptiometry was used to measure lean body mass and fat mass in kilograms by using a whole-body scan, as previously described.[12]

Muscle Strength

Isometric maximum voluntary contraction (MVC) force was recorded from the ankle dorsiflexor muscles of the right leg with patients in a seated position.[4] The signal from the force transducer was amplified, converted to a digital signal, and displayed using Labview Software (National Instruments, Austin, TX). Three MVCs (3 to 5 seconds each) were performed, with 2 minutes of rest between contractions. The greatest force from the 3 MVCs was recorded.

Muscle strength during isokinetic knee extension was tested by using a computerized dynamometer (Cybex Inc, Ronkonkoma, NY). Patients were positioned in the chair, and the knee joint was aligned with the axis of rotation of the dynamometer. Patients performed 5 maximal leg extension repetitions with the right leg at 90°/s, and peak torque was recorded for each leg.

Isotonic knee extension strength was measured by using ankle weights adjusted in 1-lb increments, with the patient seated in an upright position and the knee and hip flexed at 90°. A 3-repetition maximum was determined as the maximum weight that could be lifted 3 times with good form. A 3-repetition maximum was used rather than 1 repetition maximum because of concerns about tendon injuries in this population.[13]

Statistical Analysis

Group data are presented as mean ± SD. Unpaired *t*-tests were used to compare groups for continuous normally distributed variables; chi-square, for categorical variables; and Mann-Whitney *U* test, for non–normally distributed variables. Muscle characteristics were compared by using analysis of variance without covariates, with age and sex as covariates, and with age, sex, and the natural log of physical activity as covariates. Physical activity was log transformed because of its nonnormal distribution. As an additional means of adjusting for differences in age and sex, a paired analysis was performed. Patients were matched by sex and age (within 5 years), and groups were compared by using paired *t*-tests or Wilcoxon matched-pairs analysis, as appropriate.

To determine whether muscle area was related to recent hyperglycemia, rather than diagnosis of DM per se, patients with DM were divided into 2 groups, 1 group with adequate glycemic control according to recent American Diabetes Association guidelines[14] ($HbA_{1c} < 7.0$ mg/dL) and 1 group with less well-controlled DM ($HbA_{1c} \geq 7.0$ mg/dL), and muscle characteristics were compared between the 2 DM groups by using analysis of variance with adjustment for age and sex. Finally, multiple regression analysis was performed to test whether HbA_{1c} level was associated with muscle cross-sectional area (adjusted for age and sex).

To determine whether muscle contractile or fat areas were related to physical performance, linear regression analyses were performed with muscle characteristics as predictor variables and physical performance tests as outcome variables. All analyses were performed using Statistica software (StatSoft Inc, Tulsa, OK). Statistical significance was established for *P* less than 0.05.

RESULTS

Characteristics of the study population are listed in Table 1. Briefly, 33 patients (22 men, 1 woman) without DM were recruited as the non-DM group. Twenty-five dialysis patients with DM (13 men, 12 women) were recruited as the DM group. Average age of patients in the DM group was older ($P = 0.07$) than that of the non-DM group, and the non-DM group had more male subjects. Dialysis-related parameters showed satisfactory dialysis dosing for both groups.[15] Both dialysis groups were well nourished and had controlled anemia before the study. Lean body mass and blood chemistry analyses were similar in both dialysis groups (Table 1). The non-DM group was more active than those with DM. Only 4 patients (16%) from the DM

Table 1. Subject Characteristics

Variable	Non-DM	DM	*P*
No. of patients	33	25	
Age (y)	52 ± 14	58 ± 12	0.07
Sex (M/F)*	22/11	13/12	0.19*
Height (m)	1.65 ± 0.13	1.65 ± 0.10	0.96
Weight (kg)	70.4 ± 18.5	71.5 ± 17.8	0.82
Body mass index (kg/m^2)	25.8 ± 5.7	26.1 ± 5.6	0.86
Lean body mass (kg)	46.3 ± 14.2	45.6 ± 9.0	0.83
Biochemical measurements			
Kt/V	1.45 ± 0.35	1.47 ± 0.25	0.77
Months of dialysis†	26 (13, 54)	36 (12, 51)	0.71†
Albumin (g/dL)	3.94 ± 0.45	3.92 ± 0.36	0.84
Hemoglobin (g/dL)	12.0 ± 1.4	11.6 ± 1.5	0.25
HbA_{1c} (mg/dL)	Not applicable	6.9 ± 1.3	Not applicable
Physical activity (arbitrary units)†	62.6 (43.7, 111.6)	38.5 (22.5, 67.8)	0.03

NOTE. Data expressed as mean ± SD unless noted otherwise. To convert albumin and hemoglobin in g/dL to g/L, multiply by 10.

*Fisher exact test.

†Data expressed as median (25th percentile, 75th percentile) because data are not normally distributed; comparison is by Mann-Whitney *U* test.

Table 2. Muscle Composition and Function

Variable	Non-DM (n = 33)	DM (n = 25)	*P* Unadjusted	*P* Adjusted for Age and Sex	*P* Adjusted for Age, Sex, and Physical Activity (N = 42)
Calf measurements					
Muscles CSA (cm^2)	55.5 ± 14.8	46.8 ± 9.5	0.01	0.10	0.24
EMCL (%)	11.7 ± 7.2	17.9 ± 8.5	0.004	0.02	0.21
SAT (cm^2)	13.0 ± 6.0	13.9 ± 8.4	0.62	0.74	0.76
Thigh measurements					
Muscles CSA (cm^2)	103.8 ± 29.	91.3 ± 19.1	0.07	0.39	0.95
EMCL (%)	9.7 ± 4.2	13.1 ± 4.6	0.005	0.04	0.03
SAT (cm^2)	75.1 ± 37.7	85.3 ± 65.2	0.46	0.64	0.64
Strength measurements					
TA MVC (N)	189.6 ± 54.9	153.0 ± 54.5	0.05	0.13	0.07
Knee extension 3RM (lb)	16.2 ± 7.2	14.6 ± 6.9	0.45	0.90	0.17
Isokinetic knee extension at 90°/s	45.0 ± 25.1	29.2 ± 12.4	0.008	0.05	0.24
Functional measurements					
Gait speed (cm/s)	107.0 ± 30.5	94.2 ± 29.5	0.11	0.63	0.46
Stair climbing time (s)	9.7 ± 7.4	11.3 ± 5.8	0.43	0.66	0.21
Sit to stand (s)	15.3 ± 8.7	17.4 ± 7.8	0.37	0.74	0.56

NOTE. Data expressed as mean ± SD. Muscle composition of the right thigh and calf was assessed by using MRI.

Abbreviations: CSA, cross-sectional area; EMCL, extramyocellular lipid content (referring to lipid deposition within the muscle compartments); SAT, subcutaneous adipose tissue; RM, repetition maximum.

group had type 1 diabetes mellitus. Seventeen patients with DM (68%) were treated with insulin, and 5 patients with DM (20%) were treated with an oral hypoglycemic agent (Glipizide). Three patients with DM were under diet control (12%). Generally, patients with DM had reasonable glycemic control. Twelve patients had an HbA_{1c} level of 7% or less (mean, 6.0% ± 0.2%), and 11 patients had an HbA_{1c} level greater than 7% (mean, 7.9% ± 0.9%). Two patients did not have HbA_{1c} data available.

Results of the MRI analysis for the entire study population are listed in Table 2. The DM group had reduced muscle cross-sectional area of both calf and thigh muscle groups, but those differences were no longer significant when the analysis was adjusted for age and sex or for age, sex, and physical activity level (Table 2). Overall, there were increased intramuscular fatty deposits (extramyocellular lipids) among the DM group. For the lower leg muscles, there was significantly more extramyocellular lipid content in the DM group without adjustment and after adjustment for age and sex, but not after additional adjustment for physical activity level. In the thigh, there was more extramyocellular lipid content among patients with DM before and after adjustment for age, sex, and level of physical activity. The amount of subcutaneous adipose tissue in the thigh and calf area was not different between the 2 groups.

The DM group was divided into those with adequate glycemic control based on recent practice guidelines (n = 12) and those with HbA_{1c} levels greater than the recommended target[14] (n = 11), and there was no significant difference between these groups in calf muscle area (43.2 ± 8.5 cm^2 for the group with $HbA_{1c} < 7\%$ versus 51.7 ± 9.4 cm^2 for the group with $HbA_{1c} \geq 7\%$; $P = 0.16$) or thigh muscle area (87.5 ± 26.1 versus 93.1 ± 19.2 cm^2; $P = 0.85$) after adjustment for age and sex. Furthermore, there was no significant correlation between HbA_{1c} level and calf muscle area ($r = 0.23$; $P = 0.34$) or between HbA_{1c} level and thigh muscle area ($r = 0.19$; $P = 0.36$).

The non-DM group appeared stronger on 2 of the strength measures, but this did not persist after adjustment for age and sex or age, sex, and physical activity (Table 2). There was no difference between groups in knee extension 3-repetition maximum or physical function.

Sixteen age- and sex-matched pairs could be assembled from the larger study cohort; the re-

Table 3. Age- and Sex-Matched Analysis

Variable	Non-DM (n = 16)	DM (n = 16)	*P*
Age (y)	57.7 ± 12.4	57.8 ± 13.4	0.85
Sex (M/F)	10/6	10/6	Not applicable
Calf measurements			
Muscle CSA (cm^2)	54.6 ± 17.4	48.5 ± 9.5	0.18
EMCL (%)	13.2 ± 10.8	19.1 ± 10.0	0.05
SAT (cm^2)	14.9 ± 6.8	10.5 ± 3.2	0.05
Thigh measurements			
Muscle CSA (cm^2)	99.1 ± 32.1	97.9 ± 17.3	0.88
EMCL (%)	10.1 ± 4.4	13.9 ± 5.0	0.0002
SAT (cm^2)	89.8 ± 44.7	70.8 ± 28.2	0.10
Strength measurements			
TA MVC (N)	172.1 ± 52.2	132.6 ± 48.1	0.13
Isokinetic knee extension at 90°/s	43.0 ± 28.1	31.5 ± 10.7	0.20
Functional measurements			
Gait speed (cm/s)	97.4 ± 21.9	102.5 ± 25.3	0.55
Stair climbing time (s)	10.1 ± 5.3	10.0 ± 4.5	0.91
Sit-to-stand (s)	16.6 ± 10.2	17.6 ± 8.6	0.75
Physical Activity (arbitrary units) (n = 11)*	53.1 (43.7, 66.8)	47.8 (30.4, 71.4)	0.72

NOTE. Data expressed as mean ± SD unless noted otherwise. Muscle composition of the right thigh and calf was assessed by using MRI.

Abbreviations: CSA, cross-sectional area; EMCL, extramyocellular lipid content (referring to lipid deposition within the muscle compartments); SAT, subcutaneous adipose tissue; RM, repetition maximum.

*Data expressed as median (25th, 75th percentile) and comparison is by Wilcoxon matched-pairs test because data are not normally distributed.

maining patients did not have counterparts in the other group of the same sex and within 5 years of age. The analysis is listed in Table 3. Eleven pairs had physical activity data available for both members of the pair, and in this subset, activity levels were similar in both groups. Results of the paired analysis were similar to those of the adjusted analysis of the entire study cohort. Specifically, there was no significant difference in muscle size or strength between the DM and non-DM groups. Excess extramyocellular lipid content again was shown in patients with DM, with trends for excess subcutaneous adipose tissue compared with patients without DM.

As expected, muscle size was related to muscle strength (Table 4). Muscle contractile area was associated with physical performance, but there was no relationship between muscle fat content and performance (Table 5).

DISCUSSION

In unadjusted analyses, patients with DM were weaker and had more atrophic muscles than those without DM, as hypothesized. However, adjusted analyses indicated that these differences were caused by the older age and lower activity level of patients with DM, as well as more women in the DM group. There did not appear to be a

Table 4. Correlation Between Muscle Composition and Strength

	Knee Extension, 3RM (n = 50)		Isokinetic Knee Extension, 90°/s (n = 50)		TA MVC (N = 37)	
Variable	*R*	*P*	*R*	*P*	*R*	*P*
Thigh muscle CSA (cm^2)	0.70	<0.001	0.66	<0.001		
Thigh fat CSA (cm^2)	0.26	0.07	0.16	0.28		
Calf muscle CSA (cm^2)					0.57	<0.001
Calf fat CSA (cm^2)					0.01	0.95

NOTE. Muscle composition of the right thigh and calf was assessed by using MRI.

Abbreviations: CSA, cross-sectional area; 3RM, 3-repetition maximum; TA, tibialis anterior.

Table 5. Correlation Between Muscle Composition and Functional Capacity

Variable	Gait Speed		Stair Climbing		Sit to Stand	
	R	*P*	*R*	*P*	*R*	*P*
Thigh muscle CSA (cm^2)	0.50	<0.001	−0.36	0.008	−0.28	0.04
Thigh fat CSA (%)	0.01	0.95	0.09	0.52	0.05	0.73
Calf muscle CSA (cm^2)	0.53	<0.001	−0.44	<0.001	−0.46	<0.001
Calf fat CSA (%)	−0.08	0.56	0.15	0.27	0.38	0.004

NOTE. Muscle composition of the right thigh and calf was assessed by using MRI.
Abbreviation: CSA, cross-sectional area.

specific effect of DM on muscle size or strength or physical performance. Thus, in this small cohort, we confirm the general belief that patients with DM are more debilitated than those without DM. However, contrary to our original hypothesis, we show that these effects are related more to age, sex, and physical activity than to DM per se.

Slow progressive proximal bilateral thigh weakness, frequently affecting the quadriceps and iliopsoas muscles, has been described in patients with DM.[16] In addition, uremia affects proximal muscles in dialyzed patients.[17] Uremic myopathy,[1] inactivity,[4] hyperparathyroidism, vitamin D deficiency,[18,19] acidosis, or malnutrition[20] could all contribute to atrophy among dialysis patients. In addition, the dialysis treatment per se is a catabolic event.[21] Therefore, one would expect that the combination of DM and renal failure would have a greater impact on skeletal muscles and thus physical function than either condition alone. However, results of our study suggest that the combination of DM and renal failure does not have an additive effect on muscle wasting beyond those of uremia and/or dialysis.

Patients with DM in this study showed greater extramyocellular lipid content than dialysis patients without DM. To our knowledge, this is the first study to show that DM may be independently associated with increased extramyocellular lipid content in patients on dialysis therapy. However, other researchers noted that high intramuscular lipid content in the thigh is associated with insulin resistance[22] and type 2 DM,[23] and our findings are consistent with these observations. The increased extramyocellular lipid content in patients with DM could be related to diabetes per se because of dysregulation of intramyocellular fatty acid metabolism, with decreased mitochondrial fatty acid oxidation.[24-26] Other factors, such as inactivity or obesity, also were associated with increased intramuscular lipid content.[27,28] However, in our study, body composition did not differ between the dialysis groups, making obesity an unlikely reason for the findings. Physical inactivity mitigated the excess extramyocellular lipid content in the calf, but not in the thigh muscles.

A decrease in muscle size or increase in muscle fat might be expected to have a negative effect on muscle function and thus on physical performance. However, we found no differences between patients with and without DM in tests of muscle strength or physical performance, which was consistent with the similar lean body mass and adjusted muscle cross-sectional area in the 2 groups. Although we found that physical performance was related to muscle size, cross-sectional area did not explain the majority of the variance in performance, suggesting other important determinants of physical performance in this population, such as age and physical activity.

A study of this size cannot rule out the possibility that small differences in muscle morphological characteristics or function occur as a result of DM. However, post hoc analyses suggested that we had sufficient power to detect clinically relevant differences between groups. For example, we had approximately 80% power to detect a difference of 8 cm^2 (or 16%) in calf muscle cross-sectional area between groups in the entire cohort, and 14 pairs would yield similar power in the paired analysis. Thus, it appears that our results are consistent with a true lack of additional effect of DM on muscle size and physical function in patients on hemodialysis therapy. This is in agreement with a recent report from the

Women's Health and Aging Study that examined the association between DM and lower-extremity function in a sample of disabled older women.[29] The study showed that women with DM had poorer lower-extremity function than those without DM. However, when the analysis was adjusted for the greater burden of comorbidity in those with DM, the difference in function was no longer present.

Although this study was limited by the relatively small sample size and that the groups were not matched a priori, having a smaller cohort allowed for quantitative measurement of physical activity, an important potential determinant of muscle size and fat content.[10] Differences in the age and sex distribution of the groups were handled by using statistical adjustment, and a matched analysis also was performed to further ensure that overadjustment for age and sex differences was not responsible for the lack of difference between groups. The close agreement between results in the entire cohort and the paired analysis strengthens the validity of the findings.

In this study, patients with DM had more atrophy of thigh and calf muscles. However, after adjusting for age and sex differences, dialysis patients with DM showed muscle size and strength similar to that of patients without DM. Patients with DM also had increased extramyocellular lipid content in the locomotor muscles of the leg, but these reductions did not significantly influence performance.

We conclude that although DM is associated with greater intramuscular fat content, there was no effect of DM on size or function of leg muscles. Observed differences between patients with ESRD with and without DM are related more to advanced age and inactivity among patients with DM than to DM per se.[30]

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES 1

AQ1— Please supply city for Northern California Institute.

AQ2— References need to be listed only once in reference list. Duplicates (original references 18, 23) were deleted and references were renumbered to reflect order cited in text. Please cite reference 30 (original no. 27) and renumber references to reflect order cited or delete from reference list.

AQ3— Please supply manufacturer's name and location (city and state or counrty) for Siemens Magnetom.

AQ4— Please supply manufacturer's name and location (city and state or counrty) for brand name Glipizide.

AQ5— "cm2" correct as added for calf and thigh muscle area unit of measure?

AQ6— In sentence beginning "Patients with DM also had increased. . . ," is "reductions" correct in second part of sentnece?

AQ7— Please supply page range or indicate abstract if appropriate and spell out journal name, reference 23.

AQ8— Please supply volume number and page range, reference 30.