Relationship of Skin Thickness to Duration of Diabetes, Glycemic Control, and Diabetic Complications in Male IDDM Patients

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Skin thickness is primarily determined by collagen content and is increased in insulin-dependent diabetes mellitus (IDDM). We measured skin thickness in 66 IDDM patients aged 24–38 yr and investigated whether it correlated with long-term glycemic control and the presence of certain diabetic complications. With univariate analysis, skin thickness was increased and significantly related to duration of diabetes ($P < .001$), previous glycemic control ($P < .001$), retinopathy ($P < .001$), cheiroarthropathy ($P < .001$), and vibration-perception threshold ($P < .05$). There was a negative correlation between forced expiratory volume at 1 s ($P < .05$) and vital capacity ($P < .05$) with duration of diabetes. Neither skin thickness nor ankle arteriomedial wall calcification correlated with abnormal autonomic function tests. When corrected for duration of diabetes, there was a weak correlation between skin thickness and glycemic control ($P < .05$) but no correlation with retinopathy, cheiroarthropathy, and vibration-perception threshold. This study confirms that there are widespread connective tissue changes in diabetes mellitus, although the biochemistry needs further elucidation. *Diabetes Care* **12:309–12, 1989**

Diabetes mellitus is accompanied by widespread biochemical, morphological, and functional abnormalities of collagen and elastin (1–3). Nonenzymatic glycosylation (NEG), involving various proteins, occurs in diabetes, and glycosylated hemoglobin and glycosylated serum proteins are routinely used as indices of glycemic control (3–5). Collagen is the most studied protein regarding advanced NEG, because of the ease with which it can be examined in skin biopsies, and because of its importance as a protein that is present in several tissues subject to complications in diabetes, e.g., vascular basement membrane, arterial wall, and lung (6–10).

Skin thickness (epidermal surface to dermal fat interface), which is primarily determined by collagen content, is greater in insulin-dependent diabetes mellitus (IDDM) patients who have been diabetic for >10 yr (11,12). This possibly reflects increased collagen cross-linkage and reduced collagen turnover (2,3).

The aims of this study were to investigate whether the increase in skin thickness related to long-term glycemic control and correlated with microangiopathic complications, cheiroarthropathy, and abnormalities in respiratory and autonomic function.

MATERIALS AND METHODS

Subjects. Sixty-six male IDDM patients routinely attending the clinic (mean ± SD age 32.6 ± 2.8 yr [range 24–38 yr] and mean duration of diabetes 13.0 ± 7.3 yr [range 1–29 yr]) and 21 healthy male volunteers of comparable age (30.1 ± 2.8 yr, range 26–36 yr) took part in the study. Retinopathy was assessed ophthalmoscopically after mydriasis. Thirty-one (47%) had no retinopathy, whereas 18 (27%) had background changes, 5 (8%) had exudative retinopathy, and 12 (18%) had proliferative retinopathy. All patients had palpable peripheral pulses. None had plasma creatinine levels >130 μM, but 10 diabetic patients' urine tested Albustix positive. Ankle arteriomedial wall calcification was assessed by X ray with a standard lateral view and was

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graded as either positive or negative (13). Nineteen patients (28%) and 1 control subject had ankle arteriomedial wall calcification.

Glycemic control was assessed by glycosylated hemoglobin (HbA_1) measured by electrophoresis with commercially available agar plates, the normal range being 6–8% (14). The mean of at least 12 HbA_1 results from the routine clinic visits over the previous 4 yr allowed retrospective analysis of glycemic control.

Skin thickness (epidermal surface to dermal fat interface) was measured with an ultrasound A scan system (Dermal depth detector, Cutech, Slough, UK) (15–16). Skin sites measured were the flexor surfaces of both forearms (10 cm proximal to the wrist crease), the medial aspects of the upper arms (10 cm proximal to the antecubital fossa), and the middorsal area of both feet.

Limited joint mobility, as a measure of cheiroarthropathy, was evaluated by the prayer maneuver described by Rosenbloom et al. (17). Of the 66 diabetic patients, 25 (38%) had mild cheiroarthropathy, 6 (9%) had moderate, and none had severe cheiroarthropathy. The findings were confirmed by two examiners.

Vital capacity (VC) and forced expiratory volume at 1 s (FEV_1) were measured with a bellows spirometer (Vitalograph modal S, Maids Morton, Bucks, UK). The highest value for each volume from three maneuvers was taken (18). All subjects had an FEV_1-VC ratio >75% at the time of the study, and none had a history of atopy or recent respiratory tract infection. Because of the possible confusing effects of smoking, smokers and ex-smokers were excluded from this part of the study, leaving data from 38 diabetic patients and 19 control subjects for analysis (10,19). Venous carboxyhemoglobin measured (IL 282 Co-Oximeter) at the time of study was <1% in all nonsmokers.

A standard battery of five cardiovascular autonomic nerve function tests (i.e., heart-rate responses to Valsalva's maneuver, deep breathing, and standing up and blood pressure responses to standing up and sustained hand grip) was performed and graded as previously described (20). Twelve diabetic patients did not undergo Valsalva's maneuver because of retinopathy, and in 1 diabetic patient no heart-rate tests were performed because of an arrhythmia. Pupil cycle time (PCT) was measured by timing 100 successive pupil oscillations induced by a slit-lamp beam (21). It was not possible to induce pupil cycling in 7 diabetic subjects. Vibration-perception threshold was measured with a standard technique as an assessment of large nerve fiber involvement (22).

Informed consent was obtained from each participant, and the protocol was approved by the local hospital advisory ethical committee.

Statistical analysis. Results are expressed as means ± SD or median (range) as appropriate. Student's *t* test or χ^2-tests were used for comparison between control and diabetic groups, and multiple linear regression was used to investigate the relationship between skin thickness (the dependent variable) and other factors. With regression analysis, patients were categorized into groups depending on whether they had indications of various complications (i.e., retinopathy, cheiroarthropathy, ankle arteriomedial wall calcification, and neuropathy). The Mann-Whitney test was used to compare PCT in diabetic and control subjects because in some diabetic individuals the pupils did not cycle, and therefore it was not possible to record a value.

RESULTS

Skin thickness was greater in diabetic patients than in control subjects at all sites tested (Table 1). FEV_1 (4.0 ± 0.5 vs. 4.5 ± 0.61, $P < .005$) and VC (4.9 ± 0.7 vs. 5.4 ± 0.91, $P < .05$) were reduced in the 38 nonsmoking diabetic patients when compared with control subjects. However, there was no detectable difference in the FEV_1-VC (82.2 ± 6.3 vs. 83.4 ± 5.8%) ratio between the two groups. Fifty-six patients (85%) had normal cardiovascular autonomic function tests, whereas 6 (9%) had early abnormalities and 4 (6%) had severe changes. There was no statistical difference in PCT between diabetic and control groups (median 1000 vs. 960 s). The diabetic group, however, had increased vibration-perception thresholds in both the upper (4.6 ± 1.7 vs. 2.9 ± 0.6 U, $P < .0001$) and lower (9.2 ± 5.5 vs. 5.6 ± 2.3 U, $P < .005$) limbs.

With univariate analysis, skin thickness was correlated with duration of diabetes ($r = .53$, $P < .001$), previous glycemic control ($r = .36$, $P < .001$), retinopathy ($P < .001$), ankle arteriomedial wall calcification ($P < .001$), cheiroarthropathy ($P < .001$), and vibration-perception threshold ($P < .05$). There were too few patients with clinical nephropathy for this to be included in the statistical analysis. There was a negative correlation between FEV_1 ($r = -.27$, $P < .05$) and VC ($r = -.26$, $P < .05$) and duration of diabetes. There was no significant relationship between skin thickness and auto-

TABLE 1
Skin thickness at different sites in arms and legs in diabetic and control subjects

Site	Groups	
	Diabetic	Control
n	66	21
Upper arm		
L	1.19 ± 0.24	0.96 ± 0.09
R	1.21 ± 0.25	0.97 ± 0.16
Forearm		
L	1.19 ± 0.17	0.96 ± 0.10
R	1.16 ± 0.21	0.98 ± 0.12
Foot		
L	1.53 ± 0.28	1.31 ± 0.22
R	1.52 ± 0.27	1.25 ± 0.20

Results are expressed as means ± SD in millimeters. $P < .001$ for all comparisons.

nomic neuropathy or between abnormal autonomic tests and ankle arteriomedial wall calcification. When duration of diabetes was taken into account with multiple regression analysis, there was a weak but significant correlation between skin thickness and previous glycemic control (partial correlation coefficient 0.29, $P < .05$). Although after correction for duration of diabetes skin thickness was not correlated with retinopathy, ankle arteriomedial wall calcification, or cheiroarthropathy, these factors were strongly associated with duration of disease ($P < .001$). When the group of patients with duration of diabetes >15 yr ($n = 30$) was analyzed separately, there was a significant correlation between skin thickness and HbA_1 (partial correlation coefficient 0.54, $P < .01$). However, in this subset there was an insufficient number of patients without retinopathy, ankle arteriomedial wall calcification, or cheiroarthropathy to assess the correlation between these parameters and skin thickness.

DISCUSSION

This study confirms that skin thickness is increased in IDDM patients and correlates with both duration of diabetes and glycemic control (12,23). After correction for duration of diabetes, there was no correlation between skin thickness and the diabetic complications assessed. However, the presence of these complications, including reduced FEV_1 and VC, correlated with duration of diabetes. Only a few patients had significant abnormalities in cardiovascular autonomic function tests, but because of the limitation of the small numbers with abnormalities, we found no correlation between autonomic function abnormalities and ankle arteriomedial wall calcification. This reinforces our previous clinical impressions and does not confirm a recent study (13).

There appear to be two possible pathophysiological mechanisms by which hyperglycemia leads to irreversible tissue damage. A major consequence of hyperglycemia is excessive NEG of proteins (2,3). The initial step in NEG is rapid and reversible and results in the formation of a Schiff base. This is followed by a slow rearrangement to a more stable ketoamine linkage (the Amadori rearrangement). With further reactions and rearrangements beyond the Amadori product, advanced glycosylation end-products accumulate causing increased cross-linkage of collagen, appearance of brown fluorescent pigments, decreased susceptibility to in vivo and in vitro proteolysis, increased stiffness, and thermal stability (24–27). Collagen browning is more intense in patients with more severe retinopathy, arterial wall stiffness, and joint stiffness (25). The exact role of browning in the etiology of diabetic complications remains unknown, and it may simply reflect duration and severity of hyperglycemia (28–30).

Another consequence of hyperglycemia is that it can induce altered steady-state levels of intracellular metabolites. The polyol pathway has been implicated in the pathogenesis of peripheral neuropathy and acute diabetic cataracts (31,32). Increased polyol pathway activity results in decreased levels of NADPH, glutathione, and *myo*-inositol (32). In addition, polyol accumulation is associated with increased collagen hydration and stiffness of connective tissue (33,34).

Collagen determines skin thickness and is the most abundant protein in the body (11). The increase in skin thickness occurring in association with diabetic complications may indicate that there is a common etiological link in their development or possibly that both are time-dependent processes. Although additional investigation is required into the biochemistry of NEG and the polyol pathway and the links between these processes and the development of diabetic complications, this study provides further insight into the widespread changes in connective tissue in patients with diabetes mellitus.

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