Biochemical Changes in Skeletal Muscle in Patients with Uncontrolled Diabetes Mellitus

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The disturbances of water and electrolyte metabolism which may occur in patients with uncontrolled diabetes mellitus have received extensive investigation[1] by balance studies, and the occurrence of cellular dehydration[2,3] and potassium loss[4] in diabetic patients with ketosis suspected from such observations have been confirmed.[5,6]

With the object of seeking direct verification of the chemical changes occurring in tissues in such cases, Knowles and Guest[7] analyzed muscle samples removed from alloxan-diabetic rats which had been allowed to develop ketoacidosis. Their analytical data showed a reduction in the muscle contents of water and potassium. Values derived from the analytical data showed cellular dehydration and no change in the concentration of potassium in cell water. The present work was undertaken to determine whether similar changes occurred in the composition of muscle in humans with uncontrolled diabetes.

MATERIALS AND METHODS

Four adult male patients admitted consecutively to hospital for stabilization of their diabetes were selected for the study. None had vomited, none had previously received insulin and all had polyuria and polydipsia to variable degrees. The patient with the most severe degree of ketosis, (J.G.), was slightly drowsy, but there was no disturbance of consciousness in the other three. Brief summaries giving an indication of the clinical status of the patients are included in the appendix.

On the first day of admission to hospital and before the commencement of treatment, a sample of quadriceps femoris muscle was taken under local anesthesia and analyzed for water, neutral fat, nitrogen, sodium and potassium by the method of Litchfield and Gaddie.[8] Directly after the operation a sample of blood was withdrawn with minimal stasis from an antecubital vein and allowed to clot. The serum was separated and analyzed for water by drying to constant weight; for sodium and potassium using the Eel flame photometer; and chloride by the method of Schales and Schales.[9] The CO_2 combining power was determined on serum from blood drawn into an oxalated tube under paraffin by the method of Peters and Van Slyke.[10]

Blood glucose was determined by the method of Hagedorn and Jensen.[11]

Calculations: The results of the serum analyses have been calculated and expressed in terms of serum water since the serum of diabetic patients contains excessive amounts of fat.[12] The osmolar concentrations of serum sodium and the glucose present in excess of 100 mg./100 ml. serum have been calculated by the method described by Welt[13] and expressed also in terms of serum water.

The constituents of total muscle have been expressed per unit weight of dry fat free solids. Extracellular water was calculated assuming the distribution of chloride to be restricted to the extracellular phase and its concentration in that phase to be that existing in an ultrafiltrate of plasma. A Gibbs-Donnan factor of 0.95 was used. A further correction factor of 0.99 was applied when converting phase values from volumetric to gravimetric units since the water of the extracellular phase contains about 1 per cent of solids. The water content of the intracellular phase was estimated by subtracting extracellular water from total muscle water, and after correcting by subtraction for the amounts of sodium and potassium outside the cells, the intracellular concentrations of these ions were derived.

RESULTS

I. *Serum Analyses*: In the patients E.B., D.J., and R.P., the concentrations of serum water sodium were low, or low normal (table 1); in J.G., however, the sodium concentration was elevated to 168 mEq./L. serum water. The osmolar concentration of sodium + glucose was raised in this patient to 184 m.Osm. and within normal limits in the other three patients.

II. *Muscle Analyses.*

(a) *Water.* The total muscle water contents were reduced in all the samples except that of E.B. (table 2).

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TABLE 1

Serum values

Subject	H_2O gm./L. serum	Glucose gm./100 ml. serum water	Per liter of serum water: Na mEq.	Cl mEq.	K mEq.	CO_2 mEq.	Glucose mM.	Osm. conc. mOsm.
R.P.	843	540	144	105	5.9	—	30	159
J.G.	888	574	168	123	5.8	23.2	32	184
E.B.	939	353	136	113	5.1	27.6	19	143
D.J.	929	307	142	108	5.1	26.9	17	150

The normal range for serum water osmolarity in terms of sodium is 148-160 mOsm.

Intracellular water was reduced in R.P., J.G., and E.B. to 695, 690 and 702 gm./kg. cells respectively (table 3). In D.J., intracellular water was 719 gm./kg. cells (normal 731, S.D. 10.5) and was within normal limits.

(b) *Potassium.* In the patient J.G., muscle potassium was reduced to 35.2 mEq./100 gm. fat-free solids; in the other three patients muscle potassium contents were within the normal range (table 2). The derived data (table 3) show that the intracellular concentrations of potassium were all within the normal range.

(c) *Nitrogen.* The amounts of noncollagen nitrogen (NCN) associated with 100 gm. fat-free solids were normal in the three samples in which this determination was made and the K/NCN ratios were within normal limits (table 2).

(d) *Sodium.* The amounts of sodium associated with 100 gm. fat-free solids were normal in J.G., E.B,. and D.J. (table 2) and probably high normal in R.P. (18.1 mEq./100 gm. fat-free solids).

TABLE 2

Muscle analyses: Analytical data per 100 gm. fat-free tissue solids

Subject	H_2O gm.	Cl mEq.	Na mEq.	K mEq.	NCN gm.	K/NCN
R.P.	310	9.2	18.1	40.3	12.8	3.15
J.G.	282	5.3	17.6	35.2	11.9	2.96
E.B.	329	10.0	15.7	42.7	—	—
D.J.	310	6.3	12.5	43.1	12.9	3.34
Normal* Mean	339	8.1	13.9	44.7	13.4	3.40
S.D.	11.0	1.4	1.9	2.5	1.1	0.29

NCN = noncollagen nitrogen.
*Normal values calculated from muscle analyses performed on twenty-three normal subjects.

DISCUSSION

The results of the present series of muscle analyses are in accordance with those of Knowles and Guest[7] in demonstrating depletion of intracellular water in the presence of hyperglycemia. The inspection of the present data suggests, furthermore, a possible correlation between the heights of the blood sugar levels and the degrees of cellular dehydration, but unfortunately the series is too small to permit statistical evaluation. On the other hand, the serum osmolarity was elevated only

TABLE 3

Muscle analyses: derived data

Subject	Gm./kg. fresh fat-free muscle: extracellular water	intracellular water	Gm./kg. cells: intracellular water	mEq./kg. intracellular water: K	Na	(K+Na)
R.P.	207	550	695	176.3	24.7	201.0
J.G.	119	607	690	158.2	46.5	204.7
E.B.	222	545	702	180.5	8.8	189.3
D.J.	139	618	719	169.4	16.8	186.2
Normal Mean	157	615	731	164.3	10.8	175.1
S.D.	25.5	25.8	10.5	6.3	6.5	7.1

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in J.G. who was the most distressed by thirst and in whom intracellular dehydration was greatest; in the other three patients serum sodium levels were low, resulting in apparently normal serum osmolarity despite the presence of hyperglycemia.

Explanations for the foregoing observations may be found in the works of Seldin and Tarail[14] and Tarail and associates[15] who showed that by raising the osmotic pressure of the extracellular fluid with the rapid infusion of hypertonic glucose solution, serum sodium concentration was depressed to an extent greater than could be accounted for by the administered solution, or by the renal excretion of sodium. It was concluded that since glucose does not readily enter cells[16] and that any which does is quickly metabolized, an osmotic gradient is set up across the cell membrane resulting in the transfer of water to the extracellular phase with consequent intracellular dehydration. The present work suggests that this new state of equilibrium may continue to exist with the persistence of hyperglycemia.

Conclusive evidence of deficiency of total muscle potassium was found only in J.G., and there was no reduction in the intracellular concentrations of potassium in any of the patients. Conversely in this patient who was the most thirsty and whose blood sugar level was the highest of the series, intracellular sodium was elevated to 46.5 mEq./kg. intracellular water. Although sodium is known to move into cells in states of potassium deficiency[17,18] this does not appear to have been the mechanism in the patients under present discussion. It seems more probable that hypertonicity of the extracellular fluid due to hyperglycemia induced this transfer as suggested by Elkinton and associates.[19] On the other hand, the extent to which the breakdown of cellular metabolism due to deficiency of insulin[20] played a part cannot at present be evaluated.

SUMMARY

Muscle analyses were performed on four male patients with uncontrolled diabetes mellitus.

The results showed reductions of intracellular water to 695, 690 and 702 gm./kg. cells in the patients with blood sugar levels of 540, 574 and 353 gm. glucose/100 ml. serum water respectively. In the patient D.J. who had the lowest blood sugar level of 307 gm./100 ml. serum water cellular hydration was within normal limits. Reasons are given for attributing these findings to hyperosmolarity of the extracellular phase due to the presence of abnormally large amounts of glucose.

There was no change in the intracellular concentrations of potassium although in the most severely affected patient the whole muscle content of potassium was reduced to 35.2 mEq./100 gm. fat free tissue solids. No definite explanation for the increased amount of intracellular sodium found in this patient can be given, but the results of the above work are generally concordant with those of other authors using balance technics.

SUMMARIO IN INTERLINGUA

Alterationes Biochimic in Musculo Skeletic de Patientes con Nonstabilisate Diabete Mellite

Analyses de musculo esseva effectuate in quatro masculos con nonstabilisate diabete mellite.

Le resultato del analyses monstrava reductiones del aqua intracellular usque al nivellos de 695, 690, e 702 g per kg de cellulas in le tres patientes con nivellos de sucro de sanguine de 540, 574, e 353 g de glucosa per 100 ml de aqua seral, respectivemente. In le patiente D.J., qui habeva le plus basse nivello de sucro de sanguine (307 g per 100 ml), le hydratation cellular con aqua seral esseva intra le limites del norma. Es presentate rationes que justifica attribuer iste constatationes al hyperosmolaritate causate in le phase extracellular per le presentia de anormalmente grande quantitates de glucosa.

Esseva notate nulle alteration del concentration intracellular de kalium, ben que in le plus severmente afficite patiente, le contento de kalium in le musculo total esseva reducite a 35,2 mEq per 100 g de non grassose solido histic. Nulle explication definite pote esser formulate pro le augmento del natrium intracellular in le caso de iste patiente, sed le resultatos del presente investigationes se trova de accordo general con le resultatos obtenite per altere autores per medio de technicas de balancia.

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APPENDIX

Clinical Summaries of the Four Patients Studied

R.P., aged nineteen, male. For two months before admission he complained of increasing thirst and polyuria. During the same period he had frequent nocturnal cramps in the legs. He had lost about forty pounds in weight. On admission on Oct. 26, 1955, a moderate degree of dehydration was shown by dryness of the mouth and diminished tissue turgor; the blood pressure was 130/80 mm. Hg. The urine contained about 2 per cent of sugar and a moderate amount of acetone.* Control of his diabetes proceeded without difficulty and intravenous fluids were not required.

J.G., aged twenty, male. For two months this man complained of excessive thirst and polyuria before being seen in consultation; he had lost about fifty pounds in weight. On admission on Nov. 30, 1955, he was markedly dehydrated, and early peripheral circulatory failure was present. The blood pressure was 112/70 mm. Hg. The patient was drowsy and air hunger was present. The urine contained over 2 per cent of sugar and the urine test for acetone was strongly positive. He was given two liters of normal saline intravenously during the first three hours, following which he was stabilized by conventional methods.

E.B., aged forty-six, colored male. The patient complained of thirst and polyuria of two months' duration; he had lost weight, but to what extent was unknown. On admission on Dec. 13, 1955, slight dryness of the mouth was present, but other clinical evidence of dehydration was absent; the blood pressure was 120/75. The urine contained 2 per cent of sugar and no acetone. No difficulty was experienced during control.

D.J., aged twenty-eight, male. The history of progressive thirst and polyuria was of six weeks' duration and the patient had lost about forty pounds in weight. He was admitted to hospital on Jan. 2, 1955, at which time there was no clinical evidence of dehydration; the blood pressure was 120/80 mm. Hg. Respiration was normal. The urine contained 2 per cent of sugar and the urine test for acetone was positive. Intravenous fluid therapy was unnecessary and control was carried out without complications.

Although an accurate comparison of the severity of the metabolic disturbances in these four patients cannot be made on clinical grounds alone, it is possible to say that J.G. was the most dehydrated and that in D.J. clinical signs of dehydration were lacking. R.P. and E.B. were slightly dehydrated and, of the four patients studied, E.B. was the only one in whom ketosis was absent. Thirst appears to have been the most severe in R.P. and J.G.

*Urine sugar was estimated by the Clinitest (Ames) reagent and acetone by the Acetest (Ames) reagent.

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