Magnesium, Sodium, and Potassium Content and [^{3}H]Ouabain Binding Capacity of Skeletal Muscle in Relatives of Patients With Type 2 Diabetes: Effect of Dexamethasone

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Theoretically, disturbancies in sodium (Na) and potassium (K) homeostasis and a magnesium (Mg) deficit could be possible factors in the development of obesity, type 2 diabetes, and hypertension. Therefore, we measured electrolyte content and [^{3}H]ouabain binding capacity of skeletal muscle in 20 relatives of type 2 diabetic patients and in 20 controls before and after glucose infusion and before and after treatment with dexamethasone, which decreases insulin sensitivity. Muscle electrolyte content and [^{3}H]ouabain binding capacity did not differ between groups. Infusion of glucose increased muscle Na content 25%, decreased muscle potassium content 9%, and muscle Mg content 5%. Muscle potassium/Mg ratio decreased only in relatives. Treatment with dexamethasone increased muscle Na content 15% and decreased muscle Mg content 7%, whereas muscle potassium/Na ratio decreased 17%. Dexamethasone increased muscle [^{3}H]ouabain binding capacity by 42% in both groups. Basal and 1-hour intramuscular glucose content correlated inversely with basal muscle potassium/Na ratio in relatives only. In conclusion, persons who were predisposed to the development of type 2 diabetes exhibited an increased interdependency between glucose, Na, and potassium handling in skeletal muscle. Muscle Na content and [^{3}H]ouabain binding capacity increased during treatment with dexamethasone, and muscle potassium/Na ratio decreased. Intravenous (IV) glucose injection decreases muscle Mg content, as does a decrease in insulin sensitivity, without any differences between relatives and controls.

BOTH IN TYPE 1 diabetes[1-3] and type 2 diabetes,[4] a decreased content of magnesium (Mg) has been found in skeletal muscle. A Mg deficit has been related to the development of type 2 diabetes,[5] atherosclerosis,[6-10] hypercholesterolemia,[11] hypertriglyceridemia,[12-14] and hypertension[15] and probably to the development of some of the diabetic late complications and components of the metabolic syndrome. In a recent publication, we found that an increase in whole body Mg content lead to decreased insulin-sensitivity.[16] As both insulin[17] and glucose[18] increases renal Mg excretion, a feed-back mechanism regulating both Mg and glucose homeostasis might be postulated.[16] Therefore, it is of interest to study the effect of reduced insulin sensitivity on muscle Mg content. If the postulated feed-back mechanism partly regulating Mg and glucose homeostasis[16] exists, a reduction in insulin sensitivity should lead to a compensatory decrease in muscle Mg content.

There seems to be a linear relationship between the content of Mg and both the number of sodium (Na), potassium (K)-adenosine triphosphatase (ATPase)[19] and the K content[19,20] in skeletal muscle. It has been shown that the activity of K-channels influences insulin sensitivity,[21,22] and treatment with dexamethasone increases the content of some K-channels.[23] Likewise, Na metabolism seems to be associated with insulin sensitivity.[24,25] It has been established that glucose/insulin increases intracellular K content.[26,27] However, apart from a small study in which a nonsignificant tendency towards a decrease in muscle K content and a tendency towards an increase in muscle Na content were found in a control group,[28] we have not been able to find any study in which muscle K content has been measured before and after the infusion of glucose.

In a recent study, we found a decreased Na,K-ATPase content in skeletal muscle of type 2 diabetic patients,[28] and the decrease in Na,K-ATPase number correlated negatively with an increase in waist/hip ratio,[28] which is a measure of central obesity and a known risk factor for the development of type 2 diabetes. An increased Na,K-ATPase number has been found in muscles of both type 1 and type 2 diabetic patients in 1 study,[29] but the mean Na,K-ATPase number in the control group was extremely low.[29] In animal experiments, the concentration of Na,K-ATPase is decreased in skeletal muscle of diabetic animals when they are untreated, but the number of Na,K-ATPase in skeletal muscle is normalized[30] or increased above normal[29] when the animals are treated with insulin. Insulin is known to acutely regulate the amount of Na/K-ATPase subunits in the plasma membrane in response to insulin.[31] In humans, both the number and function of the Na,K-ATPase seems to be decreased in erythrocytes from diabetic patients.[32,33]

As Mg, Na, and K seem to influence insulin sensitivity, K stimulates insulin secretion, and the Na,K-ATPase content seems to be decreased in type 2 diabetes mellitus, and as insulin is known to stimulate Na,K-ATPase, the aim of the study was to examine electrolyte homeostasis in persons who had normal glucose tolerance, but a very high possibility for developing type 2 diabetes, to see if electrolyte handling is altered in

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persons heavily predisposed to the development of type 2 diabetes mellitus compared with persons without this predisposition. If such differences do exist, they may be related to the development of type 2 diabetes mellitus. As glucose and insulin induce fluxes of both Na and K across the cell membrane, all subjects were exposed to glucose loads. To assess the effect of decreased insulin sensitivity upon electrolyte homeostasis, all subjects were investigated before and after treatment with dexamethasone. As a secondary aim, the association between insulin concentration and [^{3}H]ouabain binding capacity was evaluated in relation to a genetic predisposition to the development of type 2 diabetes mellitus.

SUBJECTS AND METHODS

Subjects

First-degree relatives of type 2 diabetic patients were traced by questioning patients with verified type 2 diabetes. Twenty subjects with at least 2 first-degree relatives with type 2 diabetes or 1 first-degree relative and at least 1 second-degree relative with type 2 diabetes were included in the study. All of the relatives had normal oral glucose tolerance test (OGTT) and were without any medication. The relatives were matched according to age, sex, and body mass index to a group of normoglycemic control subjects without any family history of type 2 diabetes (Table 1).

Enough tissue to measure the muscle [^{3}H]ouabain binding capacity was not obtained in all subjects. Before treatment with dexamethasone, muscle [^{3}H]ouabain binding capacity could be determined in 19 relatives and 14 controls in the basal state and in 17 relatives and 18 controls after an intravenous glucose tolerance test (IVGTT). During treatment with dexamethasone, muscle [^{3}H]ouabain binding capacity could be determined in 20 relatives and 18 controls before an IVGTT and in 17 relatives and 18 controls after an IVGTT. There were not enough muscle biopsy specimens for determination of muscle electrolyte content in 1 relative after treatment with dexamethasone. Oral and written consent was obtained, and the study was approved by the local ethics committee.

Study Protocol

The design of the study and the measurement of metabolic variables have been described in detail elsewhere.[34,35] An OGTT and an IVGTT from which insulin sensitivity and glucose-mediated glucose disposal could be calculated were performed in all subjects before and after treatment with dexamethasone, 4 mg/d. The OGTT was performed after 4 days treatment with dexamethasone, and the IVGTT was performed after 5 days treatment. The IVGTT consisted of a 300 mg/kg body weight intravenous (IV) glucose load (max, 25 g), which was infused over 1 minute as a 25% solution, immediately followed by 50 mL isotonic saline. All blood samples were obtained through a polyethylene catheter in the antecubital vein. To obtain arterialization of the venous blood, the hand from which the blood was sampled was kept in a heated plexiglass box during the entire study day. The samples were analyzed for glucose, insulin, K, and Mg. During the OGTT, blood samples were obtained at -20, -10, 0 (basal period), 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 minutes. During the IVGTT, blood samples were collected at -30, -20, -10, -1 (basal period), 2, 3, 4, 5, 6, 7, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160, and 180 minutes. All of the glucose tolerance tests were preceded by a 10-hour overnight fast. Before and 60 minutes after the IVGTT, muscle biopsies were taken from vastus lateralis m. quadriceps femoris. This was found to be the time of maximum response in muscle electrolyte content upon glucose injection in a preliminary study of 7 supposedly healthy men with a median age of 31 years (range, 27 to 44 years). Biopsy specimens were obtained at 0, 30, 60, and 120 minutes during an IVGTT. It was found that muscle Na content increased and muscle K content decreased with the highest respective lowest values 60 minutes after the infusion of glucose. At 60 minutes, muscle Na content increased 29% (repeated-measures analysis of variance [ANOVA], $P < .05$) and muscle K content decreased 10% (repeated measures ANOVA, $P < .05$). Muscle Mg content followed the same pattern as muscle K content, but the change (-12%) in muscle Mg content did not reach statistical significance.

Table 1. Clinical Characteristics of Study Subjects

	Relatives	Controls
M/F	12/8	12/8
Age (yr)	31.5	28.5
Median, range	19-40	18-41
Body mass index (kg/m^2)	25.1	25.1
Mean, 95% CI	23.0-27.1	23.1-27.0
HbA_{1c} (%)	6.2	6.1
Mean, 95% CI	5.9-6.4	6.0-6.3
Fasting plasma glucose (mmol/L)	5.4	5.2*
Mean, 95% CI	5.2-5.6	5.0-5.3
2-hour plasma glucose, OGTT (mmol/L)	5.5	5.5
Mean, 95% CI	5.0-6.0	5.0-6.0

Abbreviations: M, men; F, female; CI, confidence interval.
*$P < .05$ (t test).

Assays

The muscle electrolyte, water content, and the [^{3}H]ouabain binding capacity have been determined using freeze-drying and dissection of the biopsy specimens as described elsewhere.[36]

Potassium concentrations were determined using flame emission spectrophotometry, and Na and Mg concentrations were determined with atomic absorption (Perkin-Elmer 403, Bodenseewerk Perkin-Elmer & Co GmbH, Überlingen, Germany). Plasma glucose concentration was measured by the glucose oxidase method on a Glucose Analyzer (Beckman Instruments, Fullerton, CA). Insulin concentration was measured by a radioimmunoassay (RIA) (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden). Glycosylated hemoglobin (HbA_{1c}) was determined by high-performance liquid chromatography (HPLC).

Statistical Analysis

Statistical analysis was performed using the SPSS/PC+ package (SPSS, Chicago, IL).[37] After assuring that a Gaussian distribution could not be rejected using the Kolmogorov-Smirnovs test, all variables were examined for differences between groups and the effect of the infusion of glucose and treatment with dexamethasone using repeated measures ANOVA. The program automatically tests each question asked, that is the effect of the infusion of glucose, the effect of treatment with dexamethasone, the effect of glucose infusion during treatment with dexamethasone, and a comparison of the effect of glucose infusion during treatment with dexamethasone compared with before treatment with dexamethasone. The effect of glucose infusion or ingestion were evaluated as the area under the curve using the preceding basal value as zero. Differences between groups in a single variable have been evaluated using Student's t test. In the case of gender differences, gender has been included in the statistical analysis. All measurements were included. Relationships between variables have been evaluated using least square regression analysis. Apart from the associations described in the Results, associations have been sought between muscle Na and K content, muscle K/Na ratio on one side and basal intramuscular glucose content, 1-hour intramuscular glucose content, first phase in-

Table 2. Muscle Na, K, Mg, and Water Content Before and After Glucose Infusion and Before and After Treatment With Dexamethasone

		Before Dexamethasone		After Dexamethasone	
		Before IVGTT	After IVGTT	Before IVGTT	After IVGTT
Muscle Na (mmol/kg dry weight)	Relatives	165 (135 to 195)	213* (174 to 251)	204† (170 to 239)	208‡ (180 to 236)
	Controls	173 (148 to 197)	209 (181 to 236)	186 (156 to 215)	189 (160 to 218)
Muscle K (mmol/kg dry weight)	Relatives	309 (289 to 329)	271* (250 to 292)	283 (261 to 305)	287§ (270 to 304)
	Controls	301 (281 to 322)	286 (266 to 306)	295 (273 to 317)	293 (275 to 310)
Muscle Mg (mmol/kg dry weight)	Relatives	31.8 (30.4 to 33.3)	30.4‖ (29.2 to 31.6)	29.9¶ (28.1 to 31.7)	29.5 (28.1 to 31.0)
	Controls	31.7 (30.6 to 32.8)	30.0 (28.5 to 31.5)	29.4 (27.7 to 31.1)	29.2 (27.9 to 30.6)
Muscle water (%)	Relatives	76.2 (75.6 to 76.8)	76.3 (75.7 to 76.9)	76.4 (75.6 to 77.2)	76.5 (75.9 to 77.1)
	Controls	76.0 (75.4 to 76.6)	76.1 (75.2 to 77.0)	76.0 (74.9 to 77.1)	76.1 (75.4 to 76.8)

NOTE. Values are mean (95% CI). Electrolyte contents are mmol/kg dry weight, water content is percentage of wet weight. Repeated-measures ANOVA: *$P < .0005$ compared with before glucose; †$P < .05$ compared with before dexamethasone; ‡$P < .02$ compared with the response to glucose before dexamethasone; §$P < .005$ compared with the response to glucose before dexamethasone; ‖$P < .002$ compared with before glucose; ¶$P < .005$ compared with before dexamethasone.

sulin secretion, IV glucose tolerance, insulin sensitivity, and glucose-mediated glucose disposal on the other side. Only significant findings are reported. Differences between correlation coefficients have been evaluated as described in the Geigy Scientific Tables,[38] significance limit $c_{2\alpha}$. P values less than .05 were considered significant.

RESULTS

Basal Values

Before intervention, no differences were found between relatives and controls in muscle Na, K, Mg, or water content or in muscle [^{3}H]ouabain binding capacity (Tables 2 and 3). Basal plasma K and Mg concentrations did not differ between groups (Figs 1A, 2A, and 3A).

Effect of Glucose Infusion Before Treatment With Dexamethasone

Infusion of glucose increased muscle Na content and decreased muscle K content in both relatives and controls before treatment with dexamethasone (Table 2). In accordance with this, muscle K/Na ratio decreased due to the infusion of glucose (Table 4). The increase in muscle Na content induced by the infusion of glucose correlated negatively with the decrease in muscle K content in both relatives ($r = -.52$, $P < .02$) and control subjects ($r = -.45$, $P < .05$). Muscle Mg content decreased due to the infusion of glucose (Table 2), whereas the glucose load did not affect muscle [^{3}H]ouabain binding capacity (Table 3) or muscle water content (Table 2). Muscle K/Mg ratio decreased in relatives, but not in controls due to the infusion of glucose (Table 4). Plasma K and Mg concentrations decreased in response to the infusion of glucose in both relatives and controls (Figs 2A and 3A).

In the group of relatives, first phase insulin secretion correlated positively with the increase in muscle Na content and negatively with the decrease in muscle K content induced by the IVGTT before treatment with dexamethasone (Table 5). This resulted in a negative coefficient of correlation between first phase insulin secretion and the decrease in K/Na ratio (Table 5). An increased glucose-mediated glucose disposal has previously been reported in the relatives from this study.[34] As the handling of electrolytes also differed between groups, we looked at the association between glucose-mediated glucose disposal and the handling of electrolytes. In relatives only, glucose-mediated glucose disposal was associated with basal muscle Na content, relatives, $r = .54$; $P < .02$; controls, $r = .02$ (not significant [NS]).

A positive coefficient of correlation was found between muscle Na content and basal intramuscular glucose content in relatives ($r = .50$, $P < .05$), but not in controls ($r = .32$, NS). Basal intramuscular glucose content correlated negatively with basal muscle K content in both groups, in relatives, $r = -.64$; $P < .005$; in controls, $r = -.48$; $P < .05$. Basal muscle K content correlated positively with first phase insulin secretion

Table 3. Muscle Na,K-ATPase Concentration in Relatives of Patients With Type 2 Diabetes and Controls Before and After 5 Days Treatment With Dexamethasone and Before and After the IV Infusion of Glucose (0.3 g/kg body weight)

	Muscle [^{3}H]Ouabain Binding Capacity Before Dexamethasone		Muscle [^{3}H]Ouabain Binding Capacity After Dexamethasone		
	Before IVGTT	After IVGTT	Before IVGTT	After IVGTT	No.
Relatives (95% CI)					
Males	990.5 (834.7 to 1,146.4)	1,027.2 (802.1 to 1,252.2)	1,424.8 (1,191.8 to 1,657.8)	1,248.7 (1,082.4 to 1,415.0)	10
Females	707.3 (497.6 to 917.1)	701.3 (579.1 to 823.4)	1024.5 (826.8 to 1,222.2)	1054.9 (181.1 to 1,928.7)	4
Controls (95% CI)					
Males	976.5 (742.2 to 1,210.7)	1,014.6 (707.9 to 1,321.4)	1,377.0 (944.2 to 1,809.8)	1,311.2 (1,037.6 to 1,584.9)	7
Females	896.4 (425.3 to 1,367.4)	916.0 (596.0 to 1,236.0)	1,228.1* (984.5 to 1,471.8)	1,213.7 (587.0 to 1,840.4)	4

NOTE. Values are mean (95% CI). Muscle [^{3}H]ouabain binding capacity is nmol/kg dry weight. Only the individuals in which all measurements are available are shown, but all available specimens are included in the statistical analysis. Repeated-measures ANOVA, difference between sexes: $P < .05$; *$P < .0005$.

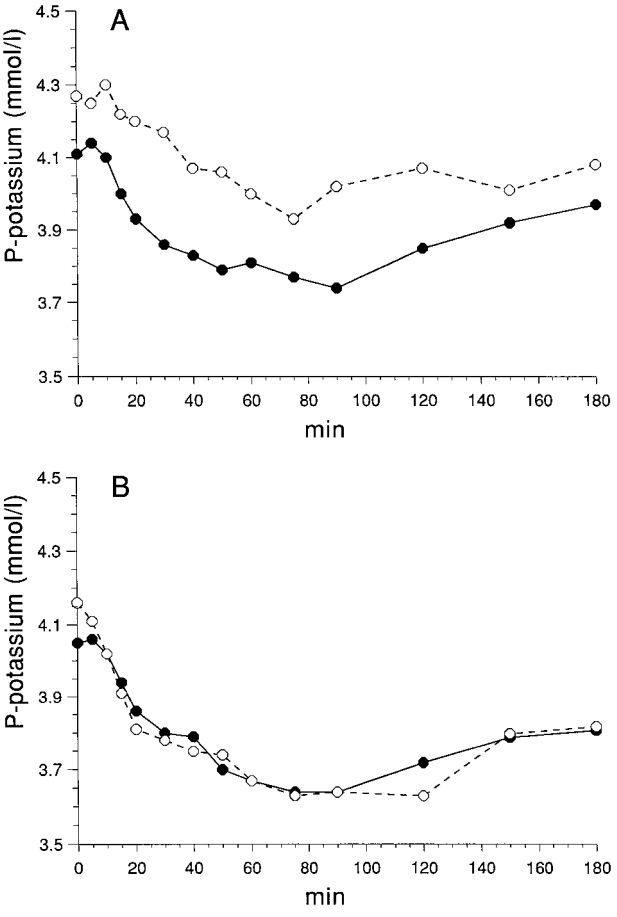

Fig 1. Plasma K concentration during an OGTT (A) before and (B) after treatment with dexamethasone for 4 days. Closed symbols represent relatives and open symbols represent controls. Repeated-measures ANOVA: effect of glucose, $P < .0005$ and the change differed after treatment with dexamethasone compared with before treatment, $P < .01$.

in relatives, $r = .57$; $P < .01$, but not in controls, $r = -.10$, NS. The associations differed between groups, $P < .05$. Looking at the K/Na ratio, a negative coefficient of correlation was found with both basal ($r = -.63$, $P < .005$) and 1-hour intramuscular glucose content ($r = -.47$, $P < .05$) in relatives. The corresponding coefficients of correlation in the control group were $r = -.39$ and $r = -.17$, respectively. Both were nonsignificant.

As the associations between intramuscular glucose content and both muscle Na and K content differed between groups, the associations between the increase in intramuscular glucose content and the change in muscle Na and K content and K/Na ratio as a function of the infusion of glucose were evaluated. The increase in intramuscular glucose content was associated with the decrease in muscle K content in both relatives ($r = -.52$, $P < .02$) and controls ($r = -.49$, $P < .05$).

The Effect of Treatment With Dexamethasone

Treatment with dexamethasone induced a 106% increase in fasting plasma insulin concentration, a 5% increase in plasma glucose concentration, and a 46% increase in the 2-hour plasma glucose concentration during an OGTT without any difference between groups as reported elsewhere.[35] Insulin sensitivity was highest in the control group before treatment with dexamethasone, whereas it was the same in the 2 groups during treatment.[35]

Both relatives and controls increased their [^{3}H]ouabain binding capacity of skeletal muscles during treatment with dexamethasone (Table 3). However, despite this increase in muscle [^{3}H]ouabain binding capacity, muscle Na content increased and muscle K/Na ratio decreased during treatment with dexamethasone (Tables 2 and 4).

Treatment with dexamethasone decreased muscle Mg content (Table 2) and plasma K and Mg concentrations (Figs 2 and 3). Overall, muscle K/Mg ratio was unchanged by treatment with dexamethasone, but the response to dexamethasone treatment differed between groups (Table 4).

Effect of Glucose Infusion During Treatment With Dexamethasone

During treatment with dexamethasone, the infusion of glucose did not affect muscle electrolyte content or muscle

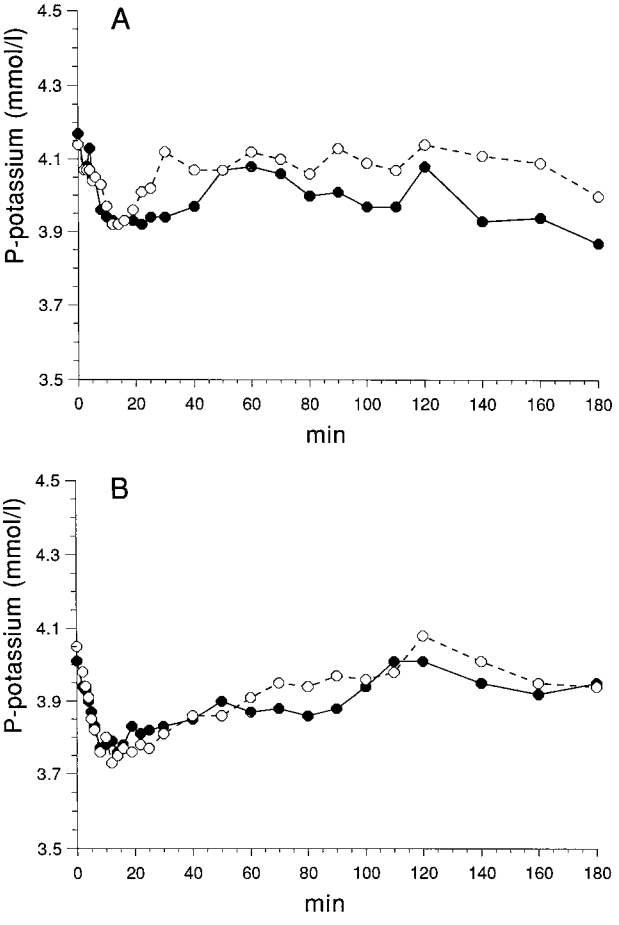

Fig 2. Plasma K concentration during an IVGTT (A) before and (B) after treatment with dexamethasone for 5 days. Closed symbols represent relatives and open symbols represent controls. Repeated-measures ANOVA: effect of glucose, $P < .0005$; effect of dexamethasone, $P < .05$.

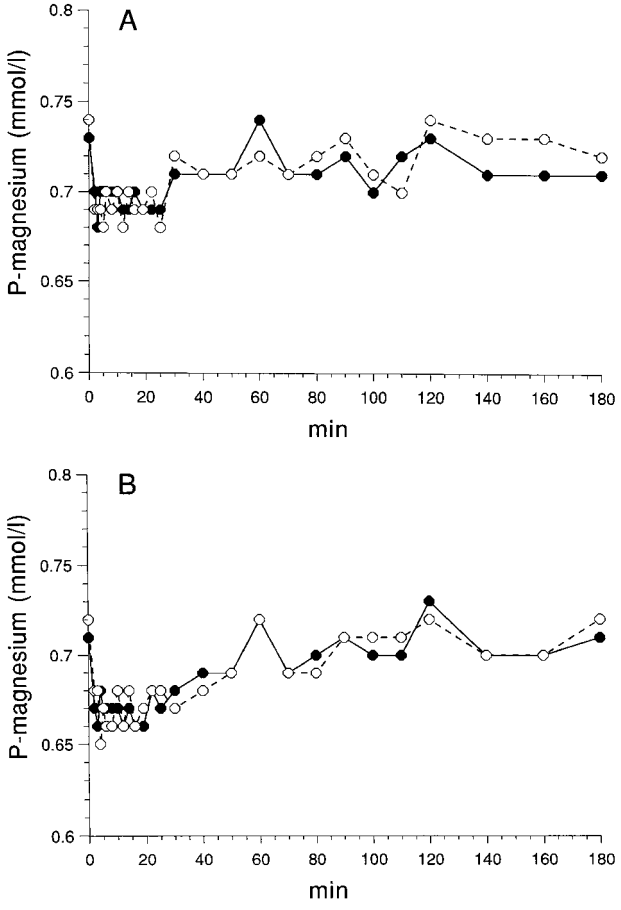

Fig 3. Plasma Mg concentration during an IVGTT (A) before and (B) after treatment with dexamethasone for 5 days. Closed symbols represent relatives and open symbols represent controls. Repeated-measures ANOVA: effect of glucose, $P < .0005$; effect of dexamethasone, $P < .05$.

[^{3}H]ouabain binding capacity (Tables 2, 3, and 4). However, plasma K and Mg concentrations still decreased in both relatives and controls just as before treatment with dexamethasone (Figs 2B and 3B).

Muscle [^{3}H]Ouabain Binding Capacity and Insulin

Schmidt et al[29] have previously described an association between insulin dose and muscle [^{3}H]ouabain binding capacity in patients with diabetes mellitus. Due to these findings, we examined the possible associations between muscle [^{3}H]ouabain binding capacity and fasting plasma insulin concentration and the insulin response in relationship to the OGTT. In relatives, both fasting plasma insulin concentration and the insulin area under the curve during the OGTT correlated positively with [^{3}H]ouabain binding capacity, $r = .62$, $P < .005$ and $r = .46$, $P < .05$, respectively. Including sex in the calculation, the association between [^{3}H]ouabain binding capacity and fasting insulin concentration remained significant, $P < .05$, whereas the association with the insulin area under the curve became nonsignificant, $P < .07$. No association was found in the control group in which $r = .04$, NS and $r = -.27$, NS, respectively. Regarding the insulin area under the curve, the r values differed between relatives and controls, $P < .05$.

Table 5. Coefficients of Correlation Between the Difference In Muscle Na and K Content, and K/Na Ratio Induced by an IVGTT and First-Phase Insulin Secretion During the IVGTT Before Treatment With Dexamethasone

	Muscle Na	Muscle K	K/Na Ratio
First phase insulin secretion	0.68*/0.37	−0.58†/−0.25	−0.64‡/−0.33

NOTE. Indicated are the coefficient of correlation in the group of relatives before the slash and the coefficient of correlation in the control group after the slash. The electrolyte values are calculated as the value after minus the value before the IVGTT (n = 20).
*$P < .001$; †$P < .01$; ‡$P < .005$.

Plasma K and Mg During an OGTT

The OGTT decreased plasma K concentration, with the largest decrease after treatment with dexamethasone (Fig 1). Plasma Mg concentration was virtually unchanged.

DISCUSSION

Na, K, and Glucose

An increase in muscle Na content and a decrease in muscle K content induced by the infusion of glucose has not been described previously. The finding is surprising, because insulin and glucose infusion is used clinically to lower high plasma K concentrations, which also happened in the present study. The decrease in plasma K concentration has been ascribed to a transport of K into the cells due to insulin.[26,27] Using the insulin clamp technique, DeFronzo et al[27] have found that the decrease

Table 4. Muscle K/Na and K/Mg Ratios Before and After the Infusion of Glucose and Before and After Treatment With Dexamethasone

		Before Dexamethasone		After Dexamethasone	
		Before IVGTT	After IVGTT	Before IVGTT	After IVGTT
Muscle K/Na ratio	Relatives	2.2 (1.7 to 2.6)	1.5* (1.2 to 1.8)	1.6† (1.3 to 2.0)	1.5‡ (1.3 to 1.7)
	Controls	1.9 (1.6 to 2.3)	1.5 (1.3 to 1.7)	1.8 (1.5 to 2.0)	1.7 (1.4 to 2.0)
Muscle K/Mg ratio	Relatives	9.7 (9.2 to 10.2)	8.9§‖ (8.3 to 9.5)	9.5‖ (8.9 to 10.0)	9.8¶# (9.2 to 10.3)
	Controls	9.6 (8.8 to 10.3)	9.6 (9.0 to 10.2)	10.1 (9.5 to 10.6)	10.0 (9.5 to 10.6)

NOTE. Values are mean (95% CI). Repeated-measures ANOVA: *$P < .0005$ compared with before glucose; †$P < .05$ compared with before dexamethasone; ‡$P < .01$ compared with the response to glucose before dexamethasone; §$P < .05$ compared with before glucose; ‖difference in response between groups, $P < .05$; ¶$P < .05$ compared with the response to glucose before dexamethasone; #difference in response between groups, $P < .02$.

in plasma K during the first hour of the infusion of insulin and glucose could be ascribed to cellular uptake of K in the splanchnic region without any significant contribution from skeletal muscles. In insulin-deprived type 1 diabetic patients, a glucose load induces hyperkalemia,[39] probably confirming the efflux of K from skeletal muscle, found in the present study. The findings are also in accordance with the increased exchangeable Na found in both type 1[40] and type 2 diabetic patients.[41] The changes in muscle electrolyte content in relatives seemed to be both electrically and osmotically neutral as indicated also by the negative coefficients of correlation between muscle K and Na content. The findings of the present study might indicate the existence of either a Na-glucose cotransporter and/or a K-glucose countertransport. Na-glucose cotransporters are found in the eye,[42] kidney,[43] and intestine.[44] The results of the present study further indicate that the transport and/or utilization of glucose is associated with a change in muscle electrolyte handling in persons who are genetically predisposed to the development of type 2 diabetes compared with controls. Glucose injection did not induce the same magnitude of change in muscle K/Mg ratio in the control group as it did in the group of relatives. As muscle Mg content did not differ between groups, the effect of dividing muscle K content with muscle Mg content is partly to correct for the analytical variation associated with weighing and pipetting. Therefore, the difference in the change in the K/Mg ratio between groups, induced by the infusion of glucose, indicates a larger decrease in muscle K content in relatives compared with controls. The actual *P* value for this difference between groups in the decrease in muscle K content was .055. The difference between groups was small, but because both groups had normal glucose tolerance, one would not expect major differences between groups. Furthermore, even though glucose infusion induced changes in muscle Na and K content both in persons with and without a predisposition to type 2 diabetes, basal muscle Na and K contents and their ratio were associated with basal intramuscular glucose content in relatives, whereas only one of these associations reached statistical significance in the control group. Also the K/Na ratio was associated with 1-hour intramuscular glucose content in relatives only.

Treatment with dexamethasone induced an increase in muscle Na content and a decrease in muscle K/Na ratio in both groups. Muscle Mg content decreased and overall the K/Mg ratio was unchanged, but again a difference in response was found between relatives and controls, probably primarily due to a larger decrease in muscle K content in relatives compared with controls.

Possible Pathophysiologic Significance of the Na and K Results of the Present Study

A few studies lend support to suggest that Na and/or K metabolism might play a key role in determining insulin sensitivity.[21,22,24,25] Hansen et al[24] have shown that genetic polymorphism in the ATP-dependent K-channel Kir6.2 contributes to the variations in insulin sensitivity, and K-channels seem to be involved in insulin-mediated glucose transport in human skeletal muscle.[21] The intake of Na seems to influence insulin sensitivity,[24,25] and weight reduction increases muscle K content.[45] Insulin resistance has been found to be related to increased red cell Na and decreased red cell K,[46] consistent with a decreased Na,K-ATPase activity.[46] Reduction of insulin dose in type 2 diabetic patients promotes natriuresis,[47] and some transport systems, coupled with the influx of Na, are stimulated by insulin. However, because type 2 diabetes mellitus is characterized by reduced peripheral insulin sensitivity and a reduced insulin secretion, the pathways involved in regulating muscle Na and K content might not be primary factors in relationship to the development of type 2 diabetes, but rather, alternative pathways to insulin in utilizing glucose. These alternative pathways seems to be utilized more in individuals predisposed to the development of type 2 diabetes, and of course, a reduction in the function of the(se) carrier mechanism(s) will tend to decrease glucose tolerance. In this way, the present findings might contribute to the understanding of the development of type 2 diabetes mellitus. The associations between first phase insulin secretion and basal muscle K content and the changes in muscle Na, K, and K/Na ratio found only in relatives might indicate that the more this pathway is used, the better the glucose tolerance, if increased insulin secretion is a feedback reaction to insulin resistance. However, this was only the case in the group of relatives, and it was not reflected in the associations between muscle electrolyte content and glucose tolerance or insulin sensitivity. Also, the negative coefficient of correlation between the increase in intramuscular glucose and the decrease in the K content of the skeletal muscles could be a confirmation of the proposed coupling between the transport and/or utilization of glucose and the transport of electrolytes. As Na is gained[48] and K lost from skeletal muscle cells during exercise, the transport of glucose related to electrolytes might be part of the contraction-stimulated pathway of muscle glucose transport.[49] The increased transport of electrolytes across the membrane of the skeletal muscle cell, found in relatives in the present study, could also be associated with the increased glucose-mediated glucose transport, previously described in these relatives.[34] In favor of this is the association between glucose-mediated glucose disposal and muscle Na content found in relatives. No such association was found in the control group, probably indicating a genetic difference between relatives and controls concerning the interrelationships between glucose and electrolyte metabolism.

The pathway involved in the transport of glucose and electrolytes in skeletal muscle cells seems to be closed by dexamethasone, whereas it probably still works in the splanchnic area, as seen from the larger decrease in plasma K concentration induced by the OGTT during treatment with dexamethasone. Furthermore, the plasma K concentration still decreased due to the IV glucose load during treatment with dexamethasone.

Plasma K concentration is known to influence insulin secretion, and a glucose-K cycle has been proposed.[50] The present study might add to this theory that the decrease in muscle K content during glucose infusion, seen before treatment with dexamethasone, could aid in increasing insulin secretion from the pancreatic β cells. The largest effect should then be found in relatives compared with patients with type 2 diabetes. A reflection of this might be the positive coefficient of correlation between basal muscle K content and first phase insulin secre-

tion and the inverse relationship between the decrease in muscle K content and first phase insulin secretion, both found only in relatives.

Na excess has been implied as a possible factor in the etiology of essential hypertension, but any firm establishment of this has not been achieved.[51-53] In this study, the intramuscular accumulation of Na was found after both glucose injection and after the induction of insulin resistance by treatment with dexamethasone. As the tendency towards intramuscular accumulation of Na was larger in relatives to patients with type 2 diabetes mellitus compared with controls, one could speculate whether an inherited tendency towards insulin resistance might be a prerequisite for an association between Na homeostasis and blood pressure. This would explain why it has not been possible to obtain concensus regarding the importance of Na balance upon the regulation of blood pressure.

[^{3}H]Ouabain Binding Capacity

This study confirms the finding of an increase in muscle [^{3}H]ouabain binding capacity after treatment with glucocorticoids.[54,55] An increase in muscle [^{3}H]ouabain binding capacity has previously been found when the Na,K-ATPase has been stimulated by a β_2-agonist[56] or by physical activity.[57] If the Na,K-pumps are stimulated by treatment with dexamethasone, this increase in [^{3}H]ouabain binding capacity could be yet another reflection of a general tendency towards an increase in the number of Na,K-ATPase when stimulated. Furthermore, the associations between muscle [^{3}H]ouabain binding capacity and insulin concentrations and secretions could possibly point towards an increase in the number of Na,K-pumps when stimulated by insulin, as found also in other studies.[29,31] However, these associations were only found in relatives, and the infusion of glucose did not affect muscle [^{3}H]ouabain binding capacity; therefore, this issue needs further studies. These results also point towards differences in electrolyte homeostasis between relatives and controls.

Despite the increase in muscle [^{3}H]ouabain binding capacity, muscle K/Na ratio decreased in both groups, so one could speculate whether dexamethasone induced a decreased activity of the Na,K-ATPase, a decrease that the relatives could not totally compensate. Interestingly, long-term glucocorticoid treatment is known to induce myopathy and muscle weakness, whereas muscle endurance capacity during physical exercise is probably partly dependent on the capacity of the Na,K-ATPase.[58] This might also argue in favor of a decreased functional capacity of the Na,K-ATPase during treatment with dexamethasone. Persons who are not predisposed to the development of type 2 diabetes seem to be able to compensate for the reduced function by increasing the Na,K-ATPase number, whereas relatives do increase the Na,K-ATPase number, but functionally, they do not seem to compensate as their muscle Na content increases. However, we did not measure Na,K-ATPase activity, and of course, an increased influx of Na and efflux of K during treatment with dexamethasone would yield the same result. Thus, the relatives should have higher transmembrane fluxes than controls.

Possible Pathophysiologic Significance of the [^{3}H]Ouabain Binding Capacity Results of the Present Study

In a recent study, we have found a decreased [^{3}H]ouabain binding capacity in patients with type 2 diabetes mellitus associated with an increase in waist/hip ratio.[28] As no difference was found between groups in muscle [^{3}H]ouabain binding capacity in the present study, this study might confirm that the reduction in muscle [^{3}H]ouabain binding capacity found in patients with type 2 diabetes probably was associated with environmental factors.

Mg

Muscle Mg content did not differ between groups, and it decreased to the same extent during both glucose infusion and treatment with dexamethasone, which induces a reduction in insulin sensitivity. We have previously shown that Mg supplementation decreases insulin sensitivity,[16] and that the infusion of insulin and glucose decreases muscle Mg content.[16] As both hyperinsulinemia[17] and hyperglycemia[18] is known to increase renal Mg excretion, a feed-back mechanism has been proposed, partly regulating both Mg and glucose homeostasis.[16] The present study contributes to this by showing that muscle Mg content decreases with the hyperglycemia and hyperinsulinemia induced by a short, IV infusion of glucose. Furthermore, dexamethasone reduces muscle Mg content, and as it also deteriorates glucose homeostasis, it is suggested that the decrease in muscle Mg content is a consequence of the increased insulin and glucose concentrations. Plasma Mg concentration followed the same pattern as muscle Mg content.

Possible Pathophysiologic Significance of the Mg Results of the Present Study

The study did not support Mg deficit as a factor in the development of type 2 diabetes, as suggested by earlier studies,[5] but rather, that the Mg deficit is a consequence of hyperinsulinemia and hyperglycemia when type 2 diabetes develops, in accordance with the previously suggested hypothesis.[16] This decrease in whole body Mg content might be a risk factor in the development of atherosclerosis.[16]

Conclusions

In conclusion, IV glucose injection increases muscle Na content and decreases muscle K content. Muscle K content decreased more in persons predisposed to the development of type 2 diabetes, and they showed associations between variables related to glucose homeostasis and electrolyte content and changes in electrolyte contents, which were not found in controls. Even though muscle [^{3}H]ouabain binding capacity increased after treatment with dexamethasone, muscle Na content increased and muscle K/Na ratio decreased, and the response to dexamethasone differed between groups. The differences in electrolyte handling between groups might possibly relate to the development of type 2 diabetes mellitus.

IV glucose injection decreases muscle Mg content, as does an increase in insulin resistance induced by treatment with dexamethasone, without any differences between groups, probably confirming that muscle Mg content decreases in hyperinsulinemic states, not due to, but as a consequence of the hyperinsulinemia itself.

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