tended to the *DQ-LTR13* integration, where we also find a contribution of *DQ-LTR13* to RA susceptibility in *DQ8*-positive individuals. Furthermore we recently published that patients with Addison's disease—another *HLA DQ8*-associated autoimmune disease of the adrenals which may occur in combination with Type 1 diabetes as part of the autoimmune pluriglandular syndrome Type 2—have more often the *DQ8-DQ-LTR13* combination in contrast to the *DQ-LTR3* insertion. Although both *DQ-LTR3* and *DQ-LTR13* are linked to *DQ8*, *DQ-LTR13* enhances the risk for Addison's disease [7].

Whether *DQ-LTR13* has a functional significance or not is currently under investigation. Preliminary data indicate that *DQ-LTR13* harbours regulatory capacity and shows functional activity of an upstream element as revealed by analyses of nuclear initiation of mRNA transcription and steady state cytoplasmic mRNA levels. The further elucidation of this retroviral insertion in the most important IDDM susceptibility haplotype is an important issue.

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Observations

Compensatory hypochloraemic alkalosis in diabetic ketoacidosis

Keywords Diabetic ketoacidosis, acid-base imbalance, hypochloraemia

Diabetic ketoacidosis (DKA) is a life-threatening complication of diabetes mellitus. Metabolic acidosis caused by ketoacids is an essential component of DKA and can have detrimental effects on cardiac, respiratory and metabolic function [1]. The only known compensatory response to metabolic acidosis in DKA is hyperventilation with consecutive respiratory alkalosis [1].

The effect of chloride on acid-base state has been known for many years. Hyperchloraemia and hypochloraemia cause metabolic acidosis and metabolic alkalosis, respectively [2, 3]. Recent research indicates that changes in chloride play an important role in the compensation of lactic acidosis [4]. Although chloride concentrations are frequently decreased in DKA, it is not known, whether these changes play a role in the acid-base state in this entity. The aim of this study was to investigate the effect of hypochloraemic alkalosis on acid-base state of patients with DKA.

A total of 21 patients with DKA (11 women, 10 men, 44±16 years) admitted to the emergency department of a primary care hospital were studied. Fluid, insulin or bicarbonate had not been administered before the investigation. Of these patients, four had new onset diabetes and 17 patients had known insulin-dependent diabetes. DKA was triggered by inadequate insulin dose in three patients, by infection in nine patients and by unknown cause in nine patients. Arterial blood samples were collected from an indwelling arterial catheter and laboratory parameters were measured immediately after drawing. The pH was ≤7.3 or serum bicarbonate ≤15 mmol/l and ketonuria and glucosuria were present in all patients. The ten healthy control subjects consisted of five women and five men with a mean age of 39±12 years.

Acid-base state was assessed using a physical-chemical analysis, which is based on the electroneutrality of plasma.

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Abbreviations: DKA, Diabetic ketoacidosis; [$HCO3^-$], concentration of bicarbonate in arterial plasma; [Na^+], concentration of sodium in arterial plasma; [Cl^-], concentration of chloride in arterial plasma; [Na^+]/[Cl^-] ratio, sodium/chloride ratio in arterial plasma; [XA^-], concentration of unmeasured anions in arterial plasma; [A^-], sum of negative charged albumin and phosphate in arterial plasma; [K^+], concentration of potassium in arterial plasma

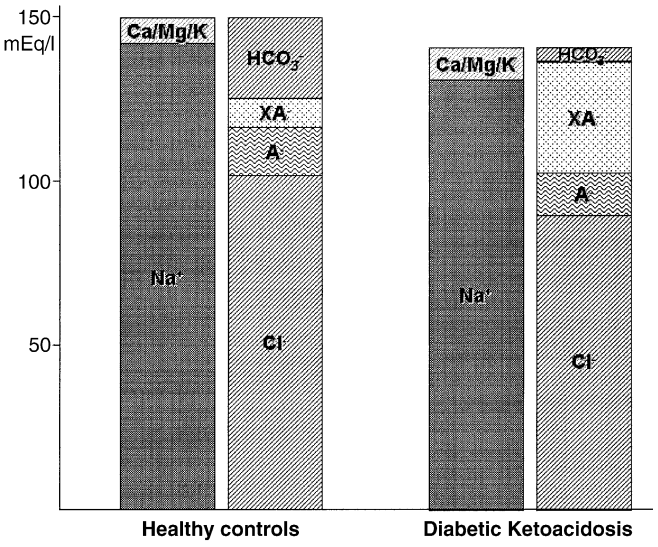

Fig. 1. Plasma ion gamblegram in healthy control subjects and diabetic ketoacidosis. Hypochloraemic alkalosis might be a metabolic compensation for metabolic acidosis due to unmeasured anions (XA^-)

The principles of this acid-base model are described elsewhere in detail [2].

$[XA^-]$ describes the amount of unmeasured anions in blood, e.g. ketoacids or lactate: $[XA^-]=([Na^+]+[K^+]+[Ca^{2+}]+[Mg^{2+}])-([Cl^-]+[HCO_3^-]+[A^-])$. $[A^-]$ is the sum of negative charged albumin {[Alb] mmol/l×(0.123×pH–0.631)} and negative charged phosphate {[Pi] mmol/l×(0.309×pH–0.469)}.

Significant lactic acidosis was excluded. Creatinine was increased in some patients; however, falsely high creatinine can be observed as a result of acetoactetate interference and renal function does not influence acid-base state, unless plasma creatinine exceeds 264 μmol/l [1].

Laboratory and acid-base parameters of patients with DKA and of healthy control subjects were compared by a Mann-Whitney U test. Correlation was calculated using Spearman's correlation coefficient. A p value of less than 0.05 was considered significant. Severe metabolic acidosis was found (pH 6.940±0.178 vs 7.403±0.012) with reduced $[HCO_3^-]$ (4.1±3.3 vs 24.2±1.7 mmol/l) due to increased $[XA^-]$ (34±5 vs 9±3 mmol/l). $[Na^+]$ and $[Cl^-]$ were decreased (131±8 vs 142±2; 88±9 vs 102±2 mmol/l, respectively). The $[Na^+]/[Cl^-]$ ratio was increased (1.49±0.09 vs 1.39±0.03) All were compared to healthy control subjects (all $p<0.05$, Fig. 1).

An inverse correlation between $[XA^-]$ and $[Cl^-]$ was found in partial correlation, adjusting for $[Na^+]$ ($r_s=-0.58$, $p<0.007$).

We did not measure ketoacids in blood; however, ketonuria is an established diagnostic tool for DKA. A 2+ reaction on the ketostix reagent was used for qualitative diagnosis of ketonaemia. We estimated indirectly the plasma concentration of ketoacids by calculating unmeasured anions $[XA^-]$. Lactic and renal acidosis played a minor role. Thus, we could assign the major part of the increase of $[XA^-]$ to the ketoacids.

$[Cl^-]$ was markedly decreased in patients with DKA. Hypochloraemia in DKA is generally explained by plasma dilution. However, the $[Na^+]/[Cl^-]$ ratio was increased compared to the healthy control subjects, indicating a lack of $[Cl^-]$, relative to $[Na^+]$. Thus, hypochloraemia in DKA cannot be explained by plasma dilution alone, since under this condition, all electrolytes should be diluted equally, leaving the $[Na^+]/[Cl^-]$ ratio unchanged.

It has been suggested that hypochloraemia, caused by chloride moving into the intracellular compartment, could be a response to accumulating tissue anions in blood [4]. Our data suggest that this concept could be applicable to DKA, also. Hypochloraemia implies hypochloraemic alkalosis, which could be a metabolic compensation for metabolic acidosis in DKA. The inverse correlation of $[Cl^-]$ and $[XA^-]$ supports this assumption: The more ketoacids, the less chloride (Fig. 1). Adjusting $[Cl^-]$ and $[XA^-]$ for $[Na^+]$ is an established tool to appreciate and quantitate an excess or deficit of a certain plasma ion, when plasma dilution is present [2]. Chloride channels, activated by acidic extracellular pH, and enhanced renal chloride excretion could be possible mediators of acidosis-induced hypochloraemia [4, 5].

The concept of compensatory hypochloraemic alkalosis could have an important effect on fluid replacement therapy in DKA. The widely recommended administration of isotonic saline in DKA should be reconsidered in light of our findings, as this fluid's sodium to chloride ratio of 1:0 leads to an increase of $[Cl^-]$ relative to $[Na^+]$ and thus could counteract compensatory hypochloraemic alkalosis. Hyperchloraemic acidosis has been described as a complication during fluid replacement with isotonic saline in DKA [6]. Saline-driven acidosis in DKA could possibly be avoided by the use of solutions with chloride concentrations at a physiologic level or beneath. Actually, the use of 0.45% NaCl in DKA is recommended in the ADA guidelines for hyperglycaemic crises in diabetes mellitus [6].

NaCl 0.9% has not been compared to a fluid with a physiologic $[Na^+]/[Cl^-]$ ratio (e.g. lactated Ringer's solution) with regard to acid-base changes during fluid resuscitation in DKA.

We investigated the impact of hypochloraemia on the acid-base state of patients with DKA and found hypochloraemic alkalosis to be a possible metabolic compensation for metabolic acidosis. Hypochloraemic alkalosis could prevent the patients from a worse metabolic acidosis. In this view, administration of isotonic saline in DKA should be reconsidered.

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Multiple autoantibodies as predictors of Type 1 diabetes in a general population

To the Editor: Autoantibodies have been widely used to predict the development of Type 1 diabetes [1]. Most studies have been carried out on first-degree relatives of Type 1 diabetic patients [2, 3, 4] who are at a 10 to 15-fold higher risk of developing the disease than people in the general population. However, approximately 85% of all patients who develop Type 1 diabetes do not have an affected family member.

To evaluate the predictive value of autoantibodies in a general population, we screened 9698 Florida school children, who were between 5 and 18 years of age, for islet-cell autoantibodies (ICA). Informed consent was obtained from all subjects under a protocol approved by the institutional review board at the University of Florida. We followed 3854 of these children for 6 to 12 years for the subsequent development of Type 1 diabetes.

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At the initial screening, 55 children were ICA positive. These children were then tested for autoantibodies to insulin, GAD, IA-2 and IA-2β. Of the 55 children positive for ICA 13 also had antibodies to insulin, 18 to GAD, 13 to IA-2 and 8 to IA-2β (Fig. 1). Of the 55 ICA-positive children, 11 progressed to Type 1 diabetes. Of these 11 ICA-positive children, 6 had autoantibodies to insulin, 10 to GAD, 9 to IA-2 and 7 to IA-2β. During the course of the study, only one ICA-negative child developed Type 1 diabetes.

Table 1 shows the autoantibody profiles of the 11 ICA-positive children who developed Type 1 diabetes. All had multiple autoantibodies at the initial screening. Clinical disease developed 3 months to 10 years later.

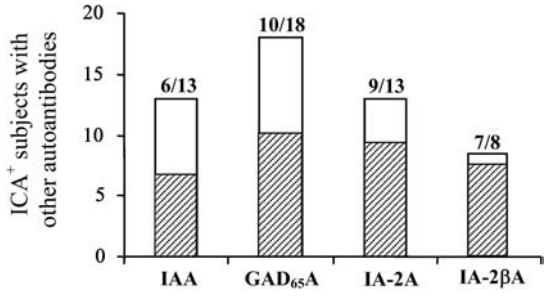

Fig. 1. Autoantibodies in 55 ICA-positive subjects. Shaded areas show number of subjects who progressed to Type 1 diabetes in the presence of each of the autoantibodies

Table 1. Autoantibody profile of children progressing to Type 1 diabetes

Subjects	Age (years/months)[a]	Sex	Autoantibodies					Time to Onset of Diabetes (years/months)
			ICA	$GAD_{65}A$	IA-2A	IA-2βA	IAA	
1	7/7	M	+	+	–	–	–	4/4
2	7/5	M	+	–	+	+	+	4/3
3	7/9	M	+	+	+	+	–	1/2
4	10	F	+	+	+	+	+	3/6
5	13/5	F	+	+	+	–	–	6/8
6	15/6	M	+	+	+	+	–	7/1
7	7/9	M	+	+	+	+	+	6/2
8	9/5	F	+	+	+	+	+	0/3
9	7	F	+	+	–	–	–	6/8
10	8/4	F	+	+	+	–	+	2/0
11[b]	9/8	F	+	+	+	+	+	10/1

[a] Age when samples were collected
[b] Initially autoantibody negative, but became autoantibody positive 3 years later