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Increasing plasma glucose before the development of type 1 diabetes—the TRIGR study

Johnny Ludvigsson[1] | David Cuthbertson[2] | Dorothy J. Becker[3] | Olga Kordonouri[4] | Bärbel Aschemeier[4] | Daniele Pacaud[5] | Cheril Clarson[6,7] | Jeffrey P. Krischer[2] | Mikael Knip[8,9,10,11] | TRIGR Investigators

[1]Crown Princess Victoria Children's Hospital and Div of Pediatrics, Dept of Biomedical and Clinical Sciences, Linköping university, Linköping, Sweden

[2]Health Informatics Institute, University of South Florida, Tampa, Florida, USA

[3]Department of Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, Pennsylvania, USA

[4]Diabetes Centre for Children and Adolescents, Kinder- und Jugendkrankenhaus Auf der Bult, Hannover, Germany

[5]Department of Pediatrics, Alberta Children's Hospital, Calgary, Alberta, USA

[6]Department of Paediatrics, University of Western Ontario, London, ON, Canada

[7]Lawson Health Research Institute, London, ON, Canada

[8]Pediatric Research Center, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[9]Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland

[10]Tampere Center for Child Health Research, Tampere University Hospital, Tampere, Finland

[11]Folkhälsan Research Center, Helsinki, Finland

Correspondence
Johnny Ludvigsson, Div of Pediatrics, Dept of Biomedical and Clinical Sciences, Linköping university, SE-58185 Linköping, Sweden.
Email: johnny.ludvigsson@liu.se

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Abstract

Objective: The β-cell stress hypothesis suggests that increased insulin demand contributes to the development of type 1 diabetes. In the TRIGR trial we set out to assess the profile of plasma glucose and HbA1c before the diagnosis of clinical diabetes compared to nondiabetic children.

Research Design and Methods: A cohort of children ($N = 2159$) with an affected first-degree relative and increased HLA risk were recruited 2002–2007 and followed until 2017. To study the relationship between plasma glucose/HbA1c and the development of autoantibodies or clinical disease Kaplan-Meir curves were developed. Mixed models were constructed for plasma glucose and HbA1c separately.

Results: A family history of type 2 diabetes was related to an increase in plasma glucose ($p < 0.001$). An increase in glucose from the previous sample predicted clinical diabetes ($p < 0.001$) but not autoantibodies. An increase of HbA1c of 20% or 30% from the previous sample predicted the development of any autoantibody ($p < 0.003$ resp <0.001) and the development of diabetes ($p < 0.002$ resp <0.001. Participants without autoantibodies had lower HbA1c (mean 5.18%, STD 0.24; mean 33.08 mmol/mol, STD 2.85) than those who progressed to clinical disease (5.31%, 0.42; 34.46 mmol/mol, 4.68; $p < 0.001$) but higher than those who developed any

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autoantibody (5.10%, 0.30; 32.21 mmol/mol, 3.49; $p < 0.001$), or multiple autoantibodies (5.11%, 0.35; 32.26 mmol/mol, 3.92; $p < 0.003$).

Conclusions: A pronounced increase in plasma glucose and HbA1c precedes development of clinical diabetes, while the association between plasma glucose or HbA1c and development of autoantibodies is complex. Increased insulin demand may contribute to development of type 1 diabetes.

KEYWORDS
accelerator hypothesis, autoantibodies, HbA1c, plasma glucose, type 1 diabetes, β-cell stress

1 | INTRODUCTION

The development of type 1 diabetes is usually preceded by a long preclinical autoimmune process. Several environmental and developmental components, such as viral infections, rapid growth and psychological stress, alone or together, may contribute to development of the the autoimmune process and/or the clinical manifestation of type 1 diabetes, especially in genetically predisposed individuals.[1] Viral infection could be the trigger of the autoimmune process,[2,3] but recent infections before diagnosis may also increase the β-cell decline and precipitate the manifest disease.[4] Incidence of clinically manifest type 1 diabetes usually peaks in adolescence,[5] or in some areas a bit earlier.[6] This age of children is not characterized by increasing attacks by viruses or other infections, nor by a more active immune system, but it is characterized by increased insulin demand in response to increased insulin resistance[7] associated with rapid growth and hormonal changes. The accelerator hypothesis suggests that the increasing insulin requirement associated with rapid growth may be an important cause of type 1 diabetes.[8] This hypothesis is based on the incidence peak in early puberty, but observations that rapid growth early in life may increase the disease risk may suggest effects on the early disease process.[9] Other factors may also increase the insulin demand.[10] Both physical and psychological stress, which increases cortisol levels and induces insulin resistance, may be related both to to development of β-cell related autoimmunity early in life[11,12] and to the clinical manifestation of overt type 1 diabetes.[13,14] Insulin resistance, increasing glucose levels and the resulting demands on increasing insulin secretion leads to β-cell stress through two potential mechanisms: (1) increasing cellular oxidative stress resulting in β-cell apoptosis[15] and (2) co-release of antigens[16] and abnormal peptides which can initiate an autoimmune response.[17,18]

Even though β-cells in most individuals have the capacity to increase their insulin secretion enough to keep blood glucose within normal range, it is still possible that even a slight increase of blood glucose could stimulate or exacerbate the autoimmune reaction against the β-cells and to β-cell failure and clinical manifestation of diabetes. In the Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study,[19] HbA1c and plasma glucose of a large number of children with genetic predisposition for type 1 diabetes have been followed from the age of 1 year until development of single or multiple auto-antibodies, and subsequently until the diagnosis of type 1 diabetes.[20] We, therefore asked in this selected population whether plasma glucose increases more in children who later develop diabetes-related autoantibodies and/or clinical type 1 diabetes than in those who do not.

2 | MATERIAL AND METHODS

The TRIGR study is an international double-blind multi-center trial designed to determine whether weaning to a hydrolyzed infant formula compared to a cow's milk based formula reduces the incidence of diabetes-related autoantibodies and the incidence of type 1 diabetes in children with an affected first-degree relative and HLA-conferred disease susceptibility.[19] Children ($N = 2159$) were recruited between May 2002 and January 2007 and followed until February 2017 when all children were at least 10 years of age.[15] HbA1c and random plasma glucose were measured at each participating center at 12, 18, and 24 months of age and annually thereafter during the total follow-up or until the diagnosis of type 1 diabetes. None of the participant developed type 2 diabetes, and none who developed T1D were autoantibody-negative. Swedish and Czech HbA1c values were adjusted due to the use of different IFCC reference ranges.[21] Diabetes-related autoantibodies were analyzed in a central laboratory (Scientific Laboratory, Children's Hospital, University of Helsinki, Helsinki, Finland) in samples taken at the same time-points and in addition at birth and at 3, 6, as well as 9 months. Autoantibodies to glutamic acid decarboxylase (GADA), to the tyrosine phosphatase-related insulinoma-associated 2 molecule (IA-2A), to insulin (IAA), and to zinc transporter 8 (ZnT8A) were analyzed with specific radiobinding immunoassays.[22] The reported disease specific sensitivity and specificity for each autoantibody were GADA sensitivity 70%–92%, specificity 90% to 98%, IA-2A sensitivity 62%–80%, specificity 93% to 100%, IAA sensitivity 42%–62%, and specificity 93% to 99%.[23] Type 1 diabetes was diagnosed according to the World Health Organization criteria.[24]

The TRIGR study was approved by the Research Ethics Boards/Committees in all participating countries, and the parents had given their informed consent to the follow-up of their children.

2.1 | Statistical analysis

Data were analyzed using the Statistical Analysis System software (Version 9.4, SAS Institute, Cary, NC). Characteristics of the study population were summarized and compared using Pearson χ^2 or Fisher exact tests by autoantibody and type 1 diabetes status. To study the relationship between plasma glucose/HbA1c and the development of autoantibodies (single or multiple) or to manifestation of type 1 diabetes, Kaplan-Meir curves were developed. Duration of time was calculated from the start where the blood glucose/HbA1c increased by a set percentage or greater from the previous sample to the development of each outcome. The set percentages used in these analyses were >5, >10, >20, and >30. These groups were not mutually exclusive. Thus, for example, those who achieved a 30% increase or greater would also be included in those who achieved a 5% increase or greater. Participants who did not achieve the outcome were censored as of their last visit or date of loss to follow-up or end of participation. The log-rank statistic was used to compare those who achieved these set percentages versus those who did not achieve an increase of 5%. Based on the nature of these analyses, participants were limited to those who a) had 2 or more blood glucose or Hba1c measurements (depending on the analysis), and b) did not experience the outcome of interest prior to the start of increasing blood glucose/increasing HbA1c. Additionally, the average plasma glucose/HbA1c and annual change in plasma glucose/HbA1c during the period prior to the development of single or multiple autoantibodies or manifest type 1 diabetes were examined. The mean plasma glucose/HbA1c values for each participant up until (but not including) the outcome of interest were calculated. To compare the average mean plasma glucose/HbA1c values for those participants who never developed any autoantibodies versus those who developed single or multiple autoantibodies or manifest type 1 diabetes, the *t* test was applied. Additionally, for each participant a linear regression was fit using again the plasma glucose/HbA1c values up until (but not including) the outcome of interest. All participants with Hba!c and glucose, respectively, were included in the multivariate analysis. For those participants who did develop diabetes, all measurements post diagnosis were excluded. From these models both the intercept (interpreted as the predicted 12 month value) and slope (interpreted as the change in plasma glucose/HbA1c per year) were calculated. These values were compared using t-tests between those participants who never developed any autoantibodies versus those who developed single or multiple autoantibodies or clinical type 1 diabetes. Lastly, mixed models were constructed for plasma glucose and HbA1c separately to examine predictors for each. Body mass index (BMI) z-score and family history of type 2 diabetes were considered as co-variates, as well as sex, age, HLA risk status and type 1 diabetes proband status, to determine whether these factors influenced the relationship between HbA1c, and plasma glucose. Due to multiple testing, to maintain a family-wise error rate of 0.05, *p*-values <0.0022 are considered significant. No imputation of missing data was performed.

3 | RESULTS

There were 2159 participants in TRIGR. Table 1 provides characteristics of the study population by autoantibody and type 1 diabetes status. Participants who developed at least a single autoantibody differed from those who did not by HLA risk status ($p < 0.001$). Participants who developed multiple autoantibodies differed from those who did not develop any autoantibodies by HLA status ($p < 0.001$) and relative proband status ($p < 0.001$). Likewise, those who progressed to type 1 diabetes differed from those who did not develop any autoantibodies by HLA status (<0.001) and relative proband status ($p < 0.001$). Region, mode of delivery, BMI z-score and sex did not differ between those who remained autoantibody-negative and those who developed single or multiple autoantibodies or manifest diabetes.

Figure 1 shows the Kaplan-Meir curves for the time from the percent increase in plasma glucose from the previous sample to the development of any autoantibody, multiple autoantibodies and type 1 diabetes. An increase in HbA1c of 20% or 30% (but not 5% or 10%) from the previous sample was predictive of the development of any autoantibody (p 0.003 and <0.001, respectively). Any increase in HbA1c was, however, not predictive of the development of multiple autoantibodies. An increase of HbA1c of 20% or 30% (but not 5% or 10%) from the previous sample was predictive of the development of clinical disease (p 0.002 and <0.001, respectively).

The Kaplan-Meir curves for the time from the percent increase in plasma glucose from the previous sample to the development of any autoantibody, multiple autoantibodies and type 1 diabetes are presented in Figure 2. In contrast to HbA1c, there were no effect of any measure of increases in plasma glucose on the development of any autoantibody or multiple autoantibodies. An increase in plasma glucose of 5% or 10% was predictive of the development of type 1 diabetes ($p < 0.001$ and 0.002, respectively).

Among those children, who progressed to type 1 diabetes HbA1c started to increase approximately 12 months before the diabetes diagnosis (Figure S1), where after the values continued to rise up to the diagnosis, The first increase in the plasma glucose concentrations could be observed around 24 months before the diagnosis with the steepest increase seen during the last 12 months preceding the clinical diagnosis (Figure S2). For HbA1c, the three groups were significantly different (at *p*-values ≤0.0102) starting at a year prior to diagnosis (or last HbA1C), and for glucose, starting at 1.5 years prior to diagnosis (or last glucose).

The participant mean HbA1c and plasma glucose by autoantibody and type 1 diabetesT1D status during the period prior to the development of single or multiple autoantibodies or manifest diabetesT1D are shown in Table S1. Participants who never developed any autoantibodies had a statistically, but clinically insignificantly, higher mean HbA1c (mean 5.18%, STD 0.24; mean 33.08 mmol/mol, STD 2.85) than those who later developed IA-2A (mean 5.08%, STD 0.31; 31.98 mmol/mol, STD 3.50; $p < 0.001$), IAA (mean 5.10%, STD 0.30; mean 32.19 mmol/mol, STD 3.28; $p < 0.001$), GADA (mean 5.12%, STD 0.36; 32.34 mmol/mol, STD 4.15; $p < 0.001$), ZnT8A (mean 5.09%, STD 0.31; 32.05 mmol/mol, STD 3.48; $p < 0.001$), any

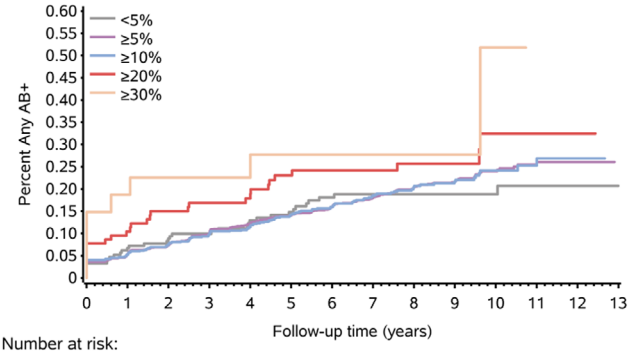
TABLE 1 Participant characteristics by antibody and diabetes status

		AB-	Any AB+		Multi-AB+		Type 1 diabetes progressors	
		N = 1593	*N* = 566	*p*	*N* = 255	*p*	*N* = 173	*p*-value
HLA risk category, % (*N*)	HLA-DQB1*0302/DQB1*02	21.3 (340)	31.1 (176)	<0.001	38.8 (99)	<0.001	42.2 (73)	<0.001
	HLA-DQB1*0302/x	44.0 (701)	44.5 (252)		41.6 (106)		37.0 (64)	
	HLA-DQA1*05-DQB1*02/y	33.5 (534)	23.7 (134)		18.8 (48)		19.7 (34)	
	HLA-DQA1*03-DQB1*02/y	1.1 (18)	0.7 (4)		0.8 (2)		1.2 (2)	
Region, % (*N*)	Canada	23.8 (379)	26.3 (149)	0.364	26.7 (68)	0.376	26.6 (46)	0.826
	USA	18.7 (298)	17.1 (97)		16.9 (43)		18.5 (32)	
	Northern Europe	25.8 (411)	25.4 (144)		26.7 (68)		28.3 (49)	
	Central Europe II	8.2 (131)	9.5 (54)		11.0 (28)		6.9 (12)	
	Southern Europe	5.7 (91)	4.1 (23)		3.1 (8)		3.5 (6)	
	Central Europe I	12.7 (203)	13.8 (78)		11.4 (29)		11.6 (20)	
	Australia	5.0 (80)	3.7 (21)		4.3 (11)		4.6 (8)	
Relative with type 1 diabetes, % (*N*)	Mother only	50.5 (805)	43.6 (247)	0.003	33.7 (86)	<0.001	34.1 (59)	<0.001
	Father only	33.3 (530)	33.9 (192)		37.6 (96)		34.7 (60)	
	Sibling(s) only	13.1 (209)	17.5 (99)		20.0 (51)		22.0 (38)	
	More than family member	3.1 (49)	4.9 (28)		8.6 (22)		9.2 (16)	
Mode of delivery, % (*N*)	Caesarean section	45.2 (720)	39.4 (223)	0.017	39.2 (100)	0.074	39.3 (68)	0.139
	Vaginal delivery	54.8 (873)	60.6 (343)		60.8 (155)		60.7 (105)	
Gender, % (*N*)	Male	52.3 (833)	53.9 (305)	0.514	60.0 (153)	0.022	52.6 (91)	0.938
	Female	47.7 (760)	46.1 (261)		40.0 (102)		47.4 (82)	
BMI (z-score), mean (STD, *N*)		0.2 (1.9, 1593)	0.1 (1.7, 566)	0.148	0.0 (2.0, 255)	0.079	0.0 (1.9, 173)	0.311
IAA+, % (*N*)		0.0 (0)	60.2 (341)	<0.001	82.4 (210)	<0.001	85.0 (147)	<0.001
GADA+, % (N)		0.0 (0)	65.2 (369)	<0.001	85.9 (219)	<0.001	80.9 (140)	<0.001
IA-2A+, % (N)		0.0 (0)	37.6 (213)	<0.001	75.7 (193)	<0.001	79.2 (137)	<0.001
ZnT8A+, % (N)		0.0 (0)	32.5 (184)	<0.001	63.9 (163)	<0.001	64.2 (111)	<0.001

Note: *p*-value based on comparison versus Ab- participants.

autoantibody (mean 5.10%, STD 0.30: mean 32.21 mmo/mol, STD 3.49; $p < 0.001$), and multiple autoantibodies (mean 5.11%, STD 0.35; mean 32.26 mmol/mol, STD 3.92; $p = 0.003$). Participants who never developed any autoantibodies had a lower mean HbA1c (mean 5.18%, STD 0.24; mean 33.08 mmol/mol, STD 2.85) than those who later developed clinical diabetes (mean 5.31%, STD 0.42; mean

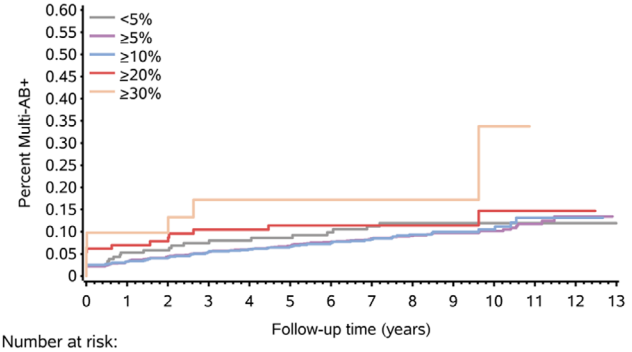
(A) Outcome: Development of any autoantibody



Number at risk:

<5%	246	182	167	156	144	131	121	112	104	82	49	24	13
≥5%	1525	1381	1321	1260	1176	1088	989	844	681	468	265	126	36
≥10%	797	713	665	627	578	523	460	384	311	198	111	47	11
≥20%	116	98	93	87	80	71	63	55	48	33	12	3	1
≥30%	27	21	20	17	15	14	13	13	12	6	2		

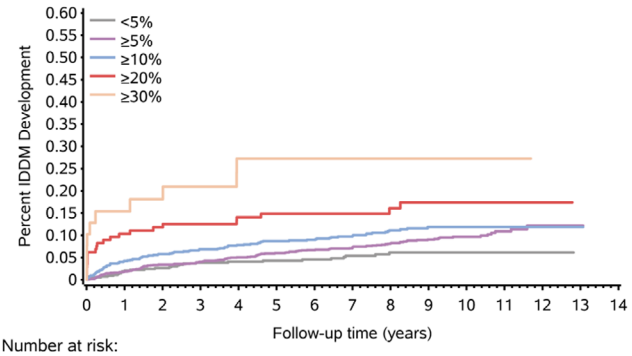
(B) Outcome: Development of multiple autoantibodies



Number at risk:

<5%	253	190	177	165	157	146	138	126	116	92	55	27	15
≥5%	1666	1533	1474	1431	1360	1277	1168	1009	811	557	326	154	42
≥10%	871	788	745	709	663	616	544	463	373	239	139	60	15
≥20%	130	110	107	100	94	89	80	70	58	41	18	6	2
≥30%	31	27	26	21	19	19	17	16	14	8	4		

(C) Outcome: Development of type 1 diabetes

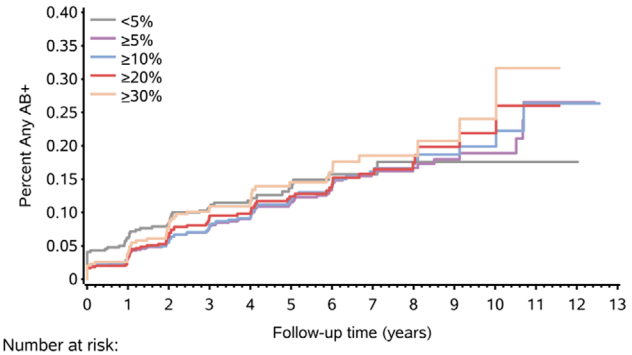


Number at risk:

<5%	542	512	491	464	437	398	363	305	236	174	100	48	19	
≥5%	1449	1410	1374	1353	1313	1260	1195	1078	941	703	434	223	75	1
≥10%	933	869	826	794	745	699	637	554	459	315	191	90	26	1
≥20%	146	126	120	117	112	107	96	85	68	52	28	12	6	
≥30%	39	32	29	26	23	21	18	17	15	11	7	2		

FIGURE 1 Time from various percent increases in successive HbA1c to various outcomes; (A). Outcome: Development of any autoantibody; (B). Outcome: Development of multiple autoantibodies; (C) Outcome: Development of type 1 diabetes

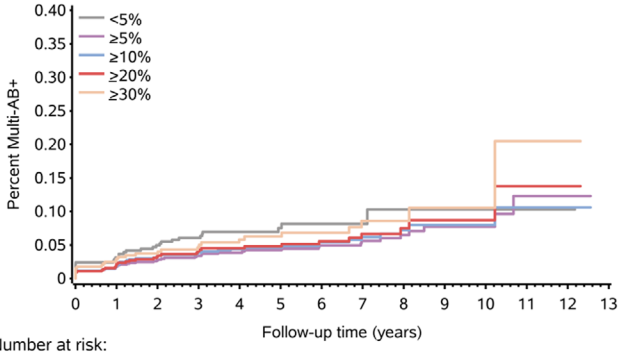
(A) Outcome: Development of any autoantibody



Number at risk:

<5%	568	390	352	308	247	145	89	57	26	17	10	2	1
≥5%	1084	779	665	558	464	377	300	229	160	104	50	22	5
≥10%	985	701	602	502	412	335	262	191	131	80	36	16	2
≥20%	725	516	437	365	304	237	174	127	80	50	21	7	
≥30%	489	334	277	226	182	142	106	76	47	29	10	4	

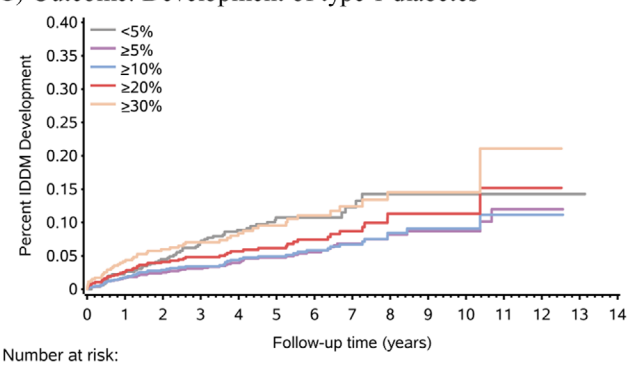
(B) Outcome: Development of multiple autoantibodies



Number at risk:

<5%	461	387	351	321	253	151	90	52	26	18	11	3	2
≥5%	1248	899	772	651	544	449	358	266	186	122	58	27	6
≥10%	1137	814	699	588	485	402	311	224	153	94	44	21	3
≥20%	847	602	517	436	361	289	210	148	95	60	27	11	1
≥30%	581	396	336	277	220	174	129	92	58	37	15	7	1

(C) Outcome: Development of type 1 diabetes



Number at risk:

<5%	504	477	455	427	374	251	167	99	51	41	30	13	7	1
≥5%	1322	1001	872	744	619	524	417	322	231	151	81	35	7	
≥10%	1206	899	784	671	555	475	366	270	187	113	58	25	4	
≥20%	913	669	580	494	412	343	248	178	116	74	36	13	2	
≥30%	636	446	379	319	256	210	150	110	69	45	21	9	2	

FIGURE 2 Time from various percent increases in successive plasma glucose to various outcomes; (A). Outcome: Development of any autoantibody; (B). Outcome: Development of multiple autoantibodies; (C) Outcome: Development of type 1 diabetes

34.46 mmol/mol, STD 4.68; $p < 0.001$). Participants who never developed any autoantibodies had a higher mean plasma glucose (mean 5.27 mmoL/L, STD 0.75) than those who later developed IAA (mean 5.00 mmoL/L, STD 0.83;p-value $p < 0.001$), and any autoantibody (mean 5.06 mmoL/L, STD 0.84; $p < 0.001$). Participants who never developed any autoantibodies had a statistically lower mean blood

TABLE 2 Multivariate mixed models of HbA1c (%) and plasma glucose (mmol/l)

		HbA1c (%)			Blood glucose (mmol/l)		
		Estimate	SE	*p*-value	Estimate	SE	*p*-value
Intercept		5.819	0.089	<0.001	7.887	0.553	<0.001
First degree with type 2 diabetes?	Yes	0.107	0.127	0.403	3.905	0.656	<0.001
BMI z-score		0.002	0.004	0.648	0.045	0.019	0.017
Sex	Female	−0.013	0.017	0.443	0.016	0.082	0.849
Age		−0.024	0.003	<0.001	−0.101	0.023	<0.001
HLA risk	HLA-DQA1*03-DQB1*02/y	0.081	0.085	0.344	−0.072	0.405	0.858
	HLA-DQA1*05-DQB1*02/y	−0.006	0.019	0.769	−0.080	0.095	0.400
	HLA-DQB1*0302/DQB1*02	0.037	0.021	0.078	0.158	0.105	0.133
Family member with type 1 diabetes	Father only	−0.047	0.026	0.073	−0.041	0.127	0.749
	More than one family member	0.033	0.049	0.497	0.322	0.246	0.190
	Mother only	−0.033	0.025	0.184	0.125	0.122	0.306

glucose (mean 5.27 mmoL/L, STD 0.75) than those who later developed overt diabetes (mean 6.16 mmoL/L, STD 1.85; $p < 0.001$).

Table S1 also provides the average estimated intercept (12 month visit) HbA1c and plasma glucose values and slope (change in HbA1c and blood glucose per year) from each participant's linear regression by autoantibody and type 1 diabetes status. The average intercept (12 month visit) HbA1c for those who never developed any autoantibodies was higher (mean 5.11%, STD 0.34; mean 32.29 mmol/mol, STD 3.98) than those who later developed IA-2A (mean 5.03%, STD 0.37; 31.41 mmol/mol, STD 4.19; $p < 0.002$), IAA (mean 5.04%, STD 0.36; 31.49 mmol/mol, STD 4.10; $p < 0.001$), ZnT8A (mean 5.03%, STD 0.39; 31.42 mmol/mol, STD 4.32; $p < 0.006$), any autoantibody (mean 5.03%, STD 0.37; 31.40 mmol/mol, STD 4.43, $p < 0.001$), and type 1 diabetes (mean 5.01%, STD 0.50; 31.20 mmol/mol, STD 5.80; p 0.017). The average change in HbA1c per year for those who never developed any autoantibodies was lower (mean 0.02%/yr, STD 0.09; mean 0.24 mmol/mol/yr, 1.03) than for those who later developed type 1 diabetes (mean 0.28%/yr, STD 0.48; 3.04 mmol/mol/yr, STD 5.32; $p < 0.001$). The average intercept (12 mos visit) plasma glucose did not differ between those who never developed any autoantibodies and those who later developed single or multiple autoantibodies or manifest diabetes. The average change in plasma glucose per year for those who never developed any autoantibodies was lower (mean 0.03 mmoL/L/yr, STD 0.22) than for those who later presented with clinical diabetes (mean 0.58 mmoL/L/yr, STD 1.72; $p < 0.001$).

Table 2 shows the results of the multivariate mixed models. For the HbA1c model, HLA risk, type 1 diabetes proband status, sex, BMI z-score and family history of type 2 diabetes were not significant, whereas age ($p < 0.001$) was a significant predictor. For the plasma glucose model, HLA risk, type 1 diabetes proband status, BMI z-score and gender were not significant, whereas age ($p < 0.001$) and family history of type 2 diabetes ($p < 0.001$) were significant predictors.

4 | DISCUSSION

In this study, we asked whether plasma glucose increases more in children who later develop diabetes-related autoantibodies, that is whether increasing plasma glucose contributes to the autoimmune process, or whether plasma glucose increases more in children who later develop clinical type 1 diabetes than in those who do not. The results show that a more rapid and pronounced increase of plasma glucose and/or HbA1c is related to the development of any but not to multiple autoantibodies. It can be speculated that the autoimmune mechanism is activated with antibodies against one autoantigen, and thereafter the diversity of autoantigens will spread, independently of blood glucose level. Therefore, only the first appearance of any autoantibody may relate to blood glucose level as shown in our study.

The onset of clinical disease is regularly preceded by a significant increase of plasma glucose and HbA1c caused by failing β-cells and insufficient insulin secretion.

The β-cell stress hypothesis suggests that any factors increasing the demand for insulin and leading to increased β-cell stress could contribute to the development of type 1 diabetes.[10] In the present study we found in a multiple regression analysis that an increasing BMI adjusted for age and gender, is associated with an increase of plasma glucose, while increasing age seems to be inversely associated with plasma glucose. That age has a negative slope in the multiple regression is probably because of collinearity with the other parameters in the model (ie BMI and sex). With higher BMI the insulin demand is increased, which explains why patients with high BMI are found to have higher C-peptide concentrations at diagnosis of type 1 diabetes,[25] while a more rapid decline of C-peptide after diagnosis is observed in such patients.[26]

As the manifestation of diabetes is often preceded by development of multiple autoantibodies by several years, one could expect that β-cell stress might first contribute to the development of an autoimmune reaction. This has been suggested to be one reason

explaining that early psychological stress has been found to be followed by the appearance of diabetes-related autoantibodies[11,12] and then later manifestation of type 1 diabetes in the same population.[14] The tendency to develop autoantibodies is influenced by the HLA-risk which we confirm in this study. However, we also observed that individuals who did not develop autoantibodies had higher plasma glucose and HbA1c than those children who did develop autoantibodies. In the former individuals, milder increase of blood glucose does not seem to trigger the development of autoantibodies suggesting that their β-cells are not attacked by the immune system. A more rapid and pronounced increase of plasma glucose and/or HbA1c is, however, related to the development of autoantibodies. These autoantibodies could either be a secondary consequence of an increased release of autoantigens[16] or development of abnormal proteins contributing to autoimmunity,[17,18] or they could be part of an immune attack causing damage to the β-cells, with decreased β-cell function and increase of plasma glucose concentrations.

The Finnish Diabetes Prediction and Prevention (DIPP) project is a birth cohort study recruiting children from the general population carrying HLA-conferred susceptibility to type 1 diabetes.[27] Helminen et al reported based on a DIPP cohort comprising close to 500 participants testing positive for multiple (≥2) diabetes-associated autoantibodies, out of whom 43% progressed to clinical type 1 diabetes during the follow-up, that a 10% increase in the HbA1c levels in samples obtained 3–12 months apart predicted the diagnosis of overt diabetes after a median time of 1.1 years.[28] With an HbA1c level of 5.9% in two consecutive samples the median time to diagnosis was 0.9 years. The timing of the increasing HbA1c levels among progressors seems to be quite similar in the current TRIGR study (Figure S1). In another analysis based on the DIPP study Helminen et al observed that a random plasma glucose ≥7.8 mmoL/L predicted progression to clinical type 1 diabetes after a median time of 12 months[29] in participants with multiple autoantibodies. In the current TRIGR study the mean plasma glucose reached a level of 7.8 mmoL/L about 12 months before the diagnosis of clinical diabetes, a timing very similar to that seen among the progressors in the DIPP cohort.

The connection between the development of autoantibodies and increasing plasma glucose/HbA1c is complex. In our study mean plasma glucose and Hba1c values were calculated for all participants. For those who developed diabetes, all measurements post diagnosis were excluded. No matching was done between the autoantibody-negative participants and those who developed T1D (or the other outcomes noted). The number of measurements per participant and the mean age of each measurement for each group was compared, and both were significantly different between autoantibody- negative individuals and those who developed T1D. We noticed that children who never developed auto-antibodies had higher mean plasma glucose than those who did, while increasing plasma glucose was associated with development of any autoantibody. As mentioned above, these autoantibodies could either be a secondary consequence of an increased release of autoantigens[16] or development of abnormal proteins contributing to autoimmunity,[17,18] but additional mechanisms seem to be involved in the autoimmune process, as we do not find any association between increasing plasma glucose and development of multiple auto-antibodies. The picture is more clear when looking at development of type 1 diabetes. The onset of clinical disease is regularly preceded by a significant increase of plasma glucose and HbA1c caused by failing β-cells and insufficient insulin secretion leading to overt type 1 diabetes as per our existing definitions. One may speculate that a milder β-cell stress, with a slight increase of plasma glucose, is not causing any autoimmune reaction, but when the β-cell function starts to fail, the plasma glucose increases, which may cause a vicious circle by inducing further β-cell stress and several possible mechanisms exacerbating the development of autoimmunity.

5 | LIMITATIONS

The main results are based on the Kaplan–Meier analysis. There are potential limitations in these analyses, as they relate to selection of the groups and potential confounding factors (e.g. different mean ages at baseline). The TRIGR sample size is large and the determinations of autoantibodies, plasma glucose, and HbA1c were done regularly providing an extensive database for analyses with significant power. However, the sample was a selective group of individuals with certain HLA risk and history of type 1 diabetes among first-degree relatives. These individuals have an increased tendency to develop both autoantibodies and clinical diabetes, and therefore we have to be cautious before extrapolating our findings to a general population with less genetic risk.

6 | CONCLUSIONS

In conclusion, plasma glucose and HbA1c increase years before diagnosis of type 1 diabetes in a population of individuals with a family history of type 1 diabetes and a high-risk HLA genotype. A more rapid and pronounced increase in plasma glucose levels, which is apparently driven by factors known to increase insulin resistance, may support the hypothesis that increased insulin demand is one factor among others contributing to the progression to overt type 1 diabetes, while the importance of increasing blood glucose and increased insulin demand for the development of the autoimmune process is less clear.

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CONFLICT OF INTEREST

The authors have no industry links and other personal connections that could influence this study, and have no conflicts of interest to disclose

AUTHOR CONTRIBUTION

Authors of this manuscript contributed in the following manner: Johnny Ludvigsson and David Cuthbertson wrote the manuscript,

planned the analysis, researched data and contributed to discussion. David Cuthbertson made the statistical analysis, Dorothy J Becker, Jeffrey P Krischer, and Mikael Knip researched data, contributed to discussion and reviewed/edited the manuscript. Bärbel Aschemeier, Cheril Clarson, Olga Kordonouri, and Daniele Pacaud contributed to discussion and reviewed/edited the manuscript. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Johnny Ludvigsson https://orcid.org/0000-0003-1695-5234
Dorothy J. Becker https://orcid.org/0000-0001-8632-3633
Olga Kordonouri https://orcid.org/0000-0001-9563-3537
Daniele Pacaud https://orcid.org/0000-0001-6337-7241
Mikael Knip https://orcid.org/0000-0003-0474-0033

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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