Table 1. HLA-DRB1, DQB1, and DPB1 allele frequencies among Japanese children with Type 1A diabetes

		Type 1A		Control		Type 1A vs. Control		
HLA	Allele	n = 860	%	n	%	Pc	OR	(95% CI)
DRB1	*04:05	244	28.37	322	13.26	$<10^{-20}$	2.60	(2.15–3.14)
	*08:02	103	11.98	102	4.18	$<10^{-12}$	3.11	(2.34–4.14)
	*09:01	283	32.91	342	14.08	$<10^{-29}$	3.00	(2.50–3.60)
	*04:06	6	0.70	73	3.00	$<10^{-3}$	0.23	(0.10–0.52)
	*08:03	11	1.28	202	8.29	$<10^{-14}$	0.14	(0.08–0.26)
	*15:01	4	0.47	173	7.11	$<10^{-16}$	0.06	(0.02–0.16)
	*15:02	9	1.05	246	10.13	$<10^{-21}$	0.09	(0.05–0.18)
	*13:02	39	4.53	166	6.83	NS		
	Others	161	18.72	806	33.14			
DQB1	*03:02	167	19.42	227	9.32	$<10^{-12}$	2.34	(1.88–2.91)
	*03:03	282	32.79	361	14.86	$<10^{-26}$	2.80	(2.34–3.35)
	*04:01	222	25.81	317	13.03	$<10^{-15}$	2.32	(1.91–2.82)
	*03:01	31	3.60	282	11.61	$<10^{-11}$	0.29	(0.20–0.42)
	*06:01	21	2.44	440	18.11	$<10^{-36}$	0.11	(0.07–0.18)
	*06:02	0	0.00	151	6.22	$<10^{-19}$	0.00	
	*06:04	37	4.30	167	6.88	NS		
	Others	100	11.63	486	19.98			
DPB1	*02:01	244	28.37	273	21.02	$<10^{-2}$	1.49	(1.22–1.82)
	*03:01	59	6.86	48	3.68	<0.05	1.92	(1.30–2.84)
	*04:02	53	6.16	135	10.40	$<10^{-2}$	0.57	(0.41–0.79)
	*09:01	21	2.44	118	9.12	$<10^{-8}$	0.25	(0.16–0.40)
	*04:01	42	4.88	49	3.80	NS		
	Others	433	50.35	647	26.60			

CI, confidence interval; n, total number of alleles; Pc, corrected p values; OR, odds ratio; NS, not significant.
The total number of alleles in the control data for DRB1 and DQB1 was 2432, while the total number of alleles in the control data for DPB1 was 1298 (21).
Others for DRB1: *01:01, *03:01, *04:01, *04:03, *04:04, *04:07, *04:10, *07:01, *10:01, *11:01, *11:05, *11:06, *12:01, *12:02, *14:01, *14:03, *14:06, *16:02.
Others for DQB1: *02:01, *04:02, *05:01, *05:02, *06:09.
Others for DPB1: *01:01, *02:02, *05:01, *06:01, *13:01, *14:01, *17:01, *19:01, *25:01, *26:01, *29:01, *38:01, *41:01, *48:01.
Corrected p values (Pc), or the p values multiplied by the number of comparisons at each locus, are shown. A Pc value < 0.05 was considered significant.

DRB1*15:01-DQBI*06:02 ($Pc < 10^{-31}$; OR, 0.0), DRB1*15:02-DQBI*06:01 ($Pc < 10^{-14}$; OR, 0.11), and DRB1*08:03-DQBI*06:01 ($Pc < 10^{-6}$; OR, 0.18) (Table 2).

In the transmission disequilibrium test (TDT), the susceptible alleles associated with Type 1A diabetes in Japanese children were DRBI*04:05 ($Pc < 10^{-5}$; OR, 2.83), DRBI*09:01 ($Pc < 10^{-5}$; OR, 2.58), DRBI*08:02 ($Pc < 10^{-3}$; OR, 5.33), DQBI*04:01 ($Pc < 10^{-5}$; OR, 2.76), DQBI*03:03 ($Pc < 10^{-5}$; OR, 2.69), and DQBI*03:02 ($Pc < 10^{-3}$; OR, 2.88) (Table 3). DPB1*02:01 and DPB1*03:01 were not significant when examined using the TDT. The protective alleles were DRBI*15:02 ($Pc < 10^{-6}$; OR, 0.08), DRBI*15:01 ($Pc < 10^{-5}$; OR, 0.00), DRBI*08:03 ($Pc < 0.05$; OR, 0.26), DQBI*06:01 ($Pc < 10^{-9}$; OR, 0.13), DQBI*06:02 ($Pc < 10^{-5}$; OR, 0.00), DQBI*03:01 ($Pc < 10^{-4}$; OR, 0.18), and DPBI*09:01 ($Pc < 10^{-4}$; OR, 0.20); DRB1*04:06 and DPB1*04:02 were not significant when examined using the TDT (Table 3).

Association of HLA-A, C, and B with Type 1A diabetes

In the case-control study, the susceptible alleles associated with Type 1A diabetes in Japanese children were A*24:02 ($Pc < 10^{-2}$; OR, 1.44), C*01:02 ($Pc < 10^{-2}$; OR, 1.56), C*08:01 ($Pc < 0.05$; OR, 1.60), B*07:02 ($Pc < 10^{-3}$; OR, 2.39), B*40:06 ($Pc < 10^{-3}$; OR, 2.21), and B*54:01 ($Pc < 10^{-10}$; OR, 2.82). The protective alleles were A*26:01 ($Pc < 10^{-4}$; OR, 0.43), A*33:03 ($Pc < 10^{-2}$; OR, 0.47), A*11:01 ($Pc < 0.05$; OR, 0.60), C*12:02 ($Pc < 10^{-8}$; OR, 0.28), C*14:03 ($Pc < 10^{-3}$; OR, 0.41), C*15:02 ($Pc < 10^{-3}$; OR, 0.28), B*15:01 ($Pc < 10^{-6}$; OR, 0.30), B*52:01 ($Pc < 10^{-9}$; OR, 0.26), and B*44:03 ($Pc < 0.05$; OR, 0.47) (Table 4).

In the TDT, the susceptible alleles associated with Type 1A diabetes in Japanese children were C*01:02 ($Pc < 10^{-2}$; OR, 1.92), C*08:01 ($Pc < 0.05$; OR, 2.15), and B*54:01 ($Pc < 10^{-5}$; OR, 4.13) (Table 3). The protective alleles were A*33:03 ($Pc < 10^{-2}$; OR, 0.32),

Table 2. Haplotype frequencies of HLA-DRB1-DQB1 among Japanese children with Type 1A diabetes

HLA haplotype	Type 1A		Control			Type 1A vs. Control		
DRB1-DQB1	n = 860	%	n = 1032	%	p	Pc	OR	(95% CI)
*09:01-*03:03	275	31.98	138	13.37	2.19E-22	$<10^{-20}$	3.05	(2.42–3.83)
*04:05-*04:01	222	25.81	134	12.98	1.43E-12	$<10^{-10}$	2.33	(1.84–2.96)
*08:02-*03:02	83	9.65	20	1.94	1.01E-13	$<10^{-11}$	5.41	(3.29–8.89)
*04:05-*03:02	35	4.07	0	0.00	7.05E-13	$<10^{-11}$		
*08:03-*06:01	10	1.16	62	6.01	9.35E-09	$<10^{-6}$	0.18	(0.09–0.36)
*15:02-*06:01	9	1.05	92	8.91	4.24E-16	$<10^{-14}$	0.11	(0.05–0.22)
*15:01-*06:02	0	0.00	118	11.43	3.75E-33	$<10^{-31}$	0.00	
*04:07-*03:02	14	1.63	4	0.39	7.45E-03	NS		
*01:01-*05:01	25	2.91	40	3.88	NS			
*13:02-*06:04	37	4.30	56	5.43	NS			
*15:01-*03:01	4	0.47	2	0.19	NS			
Others	146	16.98	366	35.47				

CI, confidence interval; n, total number of alleles; OR, odds ratio.
The control data were obtained from Ref. (22).

C*12:02 (Pc < 10^{-5}; OR, 0.18), C*14:03 (Pc < 0.05; OR, 0.33), B*15:01 (Pc < 0.05; OR, 0.34), and B*52:01 (Pc < 10^{-5}; OR, 0.17) (Table 3).

Linkage disequilibrium (LD) between DRB1-DQB1 haplotypes and DPB1, A, C, or B alleles

DPB1*02:01 and *03:01 were assessed as susceptible alleles in the case-control study but were not specifically associated with any susceptible DRB1-DQB1 haplotype. The RD values for DPB1*02:01 to DRB1*04:05-DQB1*04:01, DRB1*09:01-DQB1*03:03, and DRB1*08:02-DQB1*03:02 were 0.246, 0.312, and 0.112, respectively. The RD values for DPB1*03:01 to DRB*04:05-DQB1*04:01, DRB1*09:01-DQB1*03:03, and DRB1*08:02-DQB1*03:02 were 0.175, 0.081, and 0.148, respectively. A*24:02 was assessed as a susceptible allele in the case-control study but was not specifically associated with any susceptible DRB1-DQB1 haplotype. The RD values for A*24:02 to DRB1*04:05-DQB1*04:01, DRB1*09:01-DQB1*03:03, and DRB1*08:02-DQB1*03:02 were 0.405, 0.310, and 0.286, respectively. However, the susceptible alleles C*01:02 and B*54:01 appeared to be associated with the DRB1*04:05-DQB1*04:01 haplotype (RD, 0.697). Meanwhile, the susceptible C*08:01 and B*40:06 alleles appeared to be associated with the DRB1*09:01-DQB1*03:03 haplotype (RD, 0.597).

DPB1*09:01, C*12:02, and B*52:01 were assessed as protective alleles in both the case-control study and the TDT and appeared to be associated with the protective DRB1*15:02-DQB1*06:01 haplotype. The RD for the C*12:02-B*52:01-DRB1*15:02-DQB1*06:01-DPB1*09:01haplotype was 0.861 among the Japanese children with Type 1A diabetes in this study. The protective alleles A*33:03, C*14:03, and B*44:03 were associated with a high LD (RD, 0.842).

Transmission of susceptible and protective alleles from maternal and paternal parents

In the TDT, the transmission of DRB1*08:02 from the father occurred more frequently than from the mother, but the difference was not significant. The transmission of DRB1*09:01 from the mother occurred more frequently than from the father, but again the difference was not significant. The DRB1, DQB1, and DPB1 alleles were not transmitted preferentially from the mother or father to the children with Type 1A diabetes (Table 3), and the same was true for the A, C, and B alleles (Table 3).

Comparison of combinations of susceptible haplotypes and protective alleles between children with Type 1A diabetes and their parents

When genetic combinations of HLA-DRB1-DQB1 haplotypes were compared between children with Type 1A diabetes and their parents (149 parent–child trios), 54.4% of the children with Type 1A diabetes and 21.3% of their parents had two susceptible haplotypes. The frequencies of DR9/9 (homozygotes for DRB1*09:01-DQB1*03:03) (Pc < 10^{-2}; OR, 3.77) in group I (homozygotes for two susceptible haplotypes) and DR4/8 (heterozygotes for DRB1*04:05-DQB1*04:01 and DRB1*08:02-DQB1*03:02) (Pc < 10^{-2}; OR, 4.38) in group II (heterozygotes for two susceptible haplotypes) were significantly higher among the children with Type IA diabetes. The frequencies of group IV (one susceptible haplotype and a protective allele) (Pc < 10^{-10}; OR, 0.16) and group VI (no susceptible haplotypes and a

Table 3. Transmission disequilibrium test (TDT) for HLA-DRB1, DQB, DPB1, A, C, and B alleles in 149 parent–child trios

HLA		Transmitted			Non-transmitted			TDT				Transmission from Maternal vs. Paternal
		Combined	Parent of origin		Combined	Parent of origin		P	Pc	OR	(95% CI)	p
			Maternal	Paternal		Maternal	Paternal					
DRB1	*04:05	85	41	44	30	12	18	2.92E-07	$<10^{-5}$	2.83	(1.87–4.30)	NS
	*08:02	32	13	19	6	5	1	2.47E-05	$<10^{-3}$	5.33	(2.23–12.76)	NS
	*09:01	85	47	38	33	13	20	1.69E-06	$<10^{-5}$	2.58	(1.72–3.85)	NS
	*08:03	6	1	5	23	13	10	1.59E-03	<0.05	0.26	(0.11–0.64)	NS
	*15:01	0	0	0	26	15	11	3.41E-07	$<10^{-5}$	0.00		NS
	*15:02	3	1	2	39	21	18	2.78E-08	$<10^{-6}$	0.08	(0.02–0.25)	NS
	*04:06	2	2	0	10	4	6	2.09E-02	NS			NS
DQB1	*04:01	80	38	42	29	12	17	1.03E-06	$<10^{-5}$	2.76	(1.80–4.22)	NS
	*03:02	49	28	21	17	9	8	8.18E-05	$<10^{-3}$	2.88	(1.66–5.00)	NS
	*03:03	86	46	40	32	15	17	6.66E-07	$<10^{-5}$	2.69	(1.79–4.03)	NS
	*03:01	7	4	3	40	17	23	1.48E-06	$<10^{-4}$	0.18	(0.08–0.39)	NS
	*06:01	8	2	6	62	34	28	1.09E-10	$<10^{-9}$	0.13	(0.06–0.27)	NS
	*06:02	0	0	0	25	15	10	5.73E-07	$<10^{-5}$	0.00		NS
DPB1	*02:01	63	30	33	43	17	26	NS				NS
	*03:01	23	12	11	14	8	6	NS				NS
	*09:01	7	3	4	35	18	17	1.56E-05	$<10^{-4}$	0.20	(0.09–0.45)	NS
	*04:02	21	10	11	24	14	10	NS				NS
A	*24:02	100	48	52	70	36	34	2.14E-02	NS			NS
	*33:03	9	4	5	28	17	11	1.79E-03	$<10^{-2}$	0.32	(0.15–0.68)	NS
	*11:01	19	11	8	26	15	11	NS				NS
	*26:01	14	9	5	20	10	10	NS				NS
C	*01:02	69	30	39	36	18	18	1.28E-03	$<10^{-2}$	1.92	(1.28–2.87)	NS
	*08:01	43	22	21	20	11	9	3.76E-03	<0.05	2.15	(1.26–3.65)	NS
	*12:02	7	4	3	40	21	19	1.48E-06	$<10^{-5}$	0.18	(0.08–0.39)	NS
	*14:03	8	4	4	24	14	10	4.68E-03	<0.05	0.33	(0.15–0.74)	NS
	*15:02	7	4	3	13	8	5	NS				NS
B	*54:01	62	27	35	15	7	8	8.50E-08	$<10^{-5}$	4.13	(2.35–7.26)	NS
	*40:06	28	14	14	12	3	9	1.14E-02	NS			NS
	*07:02	17	7	10	12	5	7	NS				NS
	*15:01	10	6	4	29	14	15	2.35E-03	<0.05	0.34	(0.17–0.71)	NS
	*52:01	7	4	3	42	23	19	5.73E-07	$<10^{-5}$	0.17	(0.07–0.37)	NS
	*44:03	9	5	4	24	14	10	9.02E-03	NS			NS

CI, confidence interval; OR, odds ratio; TDT, transmission disequilibrium test.

Table 4. HLA-A, C, and B allele frequencies among Japanese children with Type 1A diabetes

		Type 1A		Control		Type 1A vs. Control		
HLA		n = 860	%	n = 1046	%	Pc	OR	(95% CI)
A	*24:02	390	45.35	382	36.52	$<10^{-2}$	1.44	(1.20–1.73)
	*26:01	45	5.23	118	11.28	$<10^{-4}$	0.43	(0.30–0.62)
	*33:03	33	3.84	82	7.84	$<10^{-2}$	0.47	(0.31–0.71)
	*11:01	58	6.74	112	10.71	<0.05	0.60	(0.43–0.84)
	Others	334	38.84	352	33.65			
C	*01:02	204	23.72	174	16.63	$<10^{-2}$	1.56	(1.24–1.95)
	*08:01	127	14.77	102	9.75	<0.05	1.60	(1.21–2.12)
	*12:02	29	3.37	116	11.09	$<10^{-8}$	0.28	(0.18–0.42)
	*14:03	26	3.02	74	7.07	$<10^{-3}$	0.41	(0.26–0.65)
	*15:02	11	1.28	46	4.40	$<10^{-3}$	0.28	(0.14–0.55)
	Others	450	52.33	534	51.05			
B	*07:02	71	8.26	38	3.63	$<10^{-3}$	2.39	(1.59–3.58)
	*40:06	86	10.00	50	4.78	$<10^{-3}$	2.21	(1.54–3.18)
	*54:01	152	17.67	74	7.07	$<10^{-10}$	2.82	(2.10–3.78)
	*15:01	26	3.02	98	9.37	$<10^{-6}$	0.30	(0.19–0.47)
	*52:01	27	3.14	114	10.9	$<10^{-9}$	0.26	(0.17–0.41)
	*44:03	28	3.26	70	6.69	<0.05	0.47	(0.30–0.73)
	Others	470	54.65	602	57.55			

CI, confidence interval; n, total number of alleles; OR, odds ratio.
The control data was obtained from Ref. (22).
Others for A: *01:01, *02:01. *02:06, *02:07, *02:10, *11:02, *24:02, *24:08, *26:02, *26:03, *26:05, *31:01, *32:01.
Others for C: *03:02, *03:03, *03:04, *04:01, *05:01, *06:02, *07:02, *07:04, *08:03, *14:02.
Others for B: *08:01, *13:01, *13:02, *15:02, *15:07, *15:11, *15:18, *27:04, *35:01, *37:01, *38:01, *39:01, *39:02, *39:04, *40:01, *40:02, *40:03, *46:01, *48:01, *51:01, *54:12, *55:02, *55:04, *56:01, *58:01, *59:01, *67:01.

protective allele) (Pc < 0.05; OR, 0.20) were significantly lower among the children with Type 1A diabetes than among their parents (Table 5). Of note, the frequency of group III (one susceptible haplotype and no protective allele) was similar between the children with Type 1A diabetes and their parents (Table 5).

GADAb and/or IA-2Ab were positive in 21 (7.1%) of the 296 parents: one in group I, five in group II, six in group III, four in group IV, three in group V, and two in group VI. Three parents (1.0%) had type 1 diabetes mellitus: two in group II and one in group III.

Comparison between children with Type 1A diabetes and their siblings

When the frequencies of the HLA-DRB1, DQB1, and DPB1 alleles were compared between 66 children with Type 1A diabetes and their 83 healthy siblings, the prevalences of all the alleles except for DQB1*06:01 were not significantly different. The frequency of the DQB1*06:01 protective allele was lower (Pc < 10^{-2}; OR, 0.13) among the patients than among their siblings.

When genetic combinations of HLA-DRB1-DQB1 haplotypes were compared between children with Type 1A diabetes and their siblings, the frequency of group VI (no susceptible haplotypes and a protective allele) was lower (Pc < 10^{-2}; OR, 0.09) among the children with Type 1A diabetes (3.03%) than among the siblings (25.3%) (Table 6). Of note, 44.6% of the siblings had protective alleles (groups IV + VI), compared with 10.6% of the children with Type 1A diabetes.

GADAb and/or IA-2Ab were positive in 7 (8.4%) of the 83 siblings: three in group II, three in group III, and one in group V. Groups II, III, and V can be characterized as having no protective alleles.

Onset age and HLA genotype

The DRB1 allele frequencies in four age groups, determined according to the patient's age at the time of Type 1A diabetes onset (0–1, 2–5, 6–9, and 10–16 years), are shown in Fig. 1. The frequency of DRB1*09:01 was higher (Pc < 0.01) in the 2–5-year onset group than in the other age groups, while the frequency of DRB1*08:02 tended to be higher in the 6–16-year onset group, although the difference was not significant (Fig. 1). The distribution of the DRB1*04:05 allele was not different among the four age groups. The distributions of other alleles, including DPB1*02:01, DPB1*03:01, A*24:02, C*01:02, C*08:01, and B*54:01, were not different among the four age groups (data not shown).

Discussion

This study is the first nationwide multicenter collaborative study examining genetic factors associated with

Table 5. Genetic combinations of HLA-DRB1-DQB1 haplotypes in Japanese children with Type 1A diabetes and their parents

Genetic combination of	Type 1A all		Type 1A in trio		Parents in trio		Type 1A in trio vs. Parents			
HLA-DRB1-DQB1 haplotype	n = 430	%	n = 149	%	n = 296	%	p	Pc	OR	(95% CI)
I. Two susceptible haplotypes in homozygote	82	19.07	37	24.83	21	7.09	4.33E-07	$<10^{-5}$	4.33	(2.43–7.72)
DR4/4 (*04:05-*04:01)	8	1.86	7	4.70	5	1.69	NS			
DR4/4 (*04:05-*03:02)	10	2.33	6	4.03	2	0.68	1.92E-02	NS		
DR9/9 (*09:01-*03:03)	58	13.49	22	14.77	13	4.39	2.68E-04	$<10^{-2}$	3.77	(1.84–7.72)
DR8/8 (*08:02-*03:02)	6	1.40	2	1.34	1	0.34	NS			
II. Two susceptible haplotypes in heterozygote	143	33.26	44	29.53	42	14.19	1.96E-04	$<10^{-2}$	2.53	(1.57–4.10)
DR4/9	65	15.12	19	12.75	26	8.78	NS			
DR4/8	61	14.19	18	12.08	9	3.04	4.59E-04	$<10^{-2}$	4.38	(1.92–10.01)
DR9/8	17	3.95	7	4.70	7	2.36	NS			
III. One susceptible haplotype and no protective allele	135	31.40	44	29.53	66	22.30	NS			
DR4/X	62	14.42	23	15.44	23	7.77	1.99E-02	NS		
DR9/X	64	14.88	18	12.08	32	10.81	NS			
DR8/X	9	2.09	3	2.01	10	3.38	NS			
IV. One susceptible haplotype and a protective allele	43	10.00	15	10.07	121	40.88	2.62E-12	$<10^{-10}$	0.16	(0.09–0.29)
V. No susceptible haplotype and no protective allele	13	3.02	6	4.03	19	6.42	NS			
VI. No susceptible haplotype and a protective allele	14	3.26	3	2.01	28	9.46	2.66E-03	<0.05	0.20	(0.06–0.66)

CI, confidence interval; OR, odds ratio.
Susceptible haplotype: *04:05-*04:01, *09:01-*03:03, *08:02-*03:02, *04:05-*03:02.
Protective allele in DRB1: *08:03, *15:01, *15:02, *04:06.
Protective allele in DQB1: *06:01, *06:02, *03:01.
X in DRB1: *01:01, *03:01, *04:01, *04:03,*04:04,*04:07, *04:10, *07:01,*10:01, *11:01, *11:06, *12:01, *12:02, *13:02, *16:02.
X in DQB1: *02:01, *04:02, *05:01, *05:02, *06:04, *06:09.

Table 6. Genetic combinations of HLA-DRB1-DQB1 haplotypes in Japanese children with Type 1A diabetes and their siblings

Genetic combination of HLA-DRB1-DQB1 haplotype	Type 1A n = 66	%	Siblings n = 83	%	Type 1A vs. Siblings p	Pc	OR	(95% CI)
I. Two susceptible haplotypes in homozygote	16	24.24	11	13.25	NS			
DR4/4 (*04:05-*04:01)	4	6.06	3	3.61	NS			
DR4/4 (*04:05-*03:02)	3	4.55	2	2.41	NS			
DR9/9 (*09:01-*03:03)	9	13.64	6	7.23	NS			
DR8/8 (*08:02-*03:02)	0	0.00	0	0.00	NS			
II. Two susceptible haplotypes in heterozygote	19	28.79	17	20.48	NS			
DR4/9	9	13.64	13	15.66	NS			
DR4/8	8	12.12	2	2.41	2.31E-02	NS		
DR9/8	2	3.03	2	2.41	NS			
III. One susceptible haplotype and no protective allele	20	30.30	15	18.07	NS			
DR4/X	9	13.64	5	6.02	NS			
DR9/X	10	15.15	7	8.43	NS			
DR8/X	1	1.52	3	3.61	NS			
IV. One susceptible haplotype and a protective allele	5	7.58	16	19.28	NS			
V. No susceptible haplotype and no protective allele	4	6.06	3	3.61	NS			
VI. No susceptible haplotype and a protective allele	2	3.03	21	25.30	1.50E-04	$<10^{-2}$	0.09	(0.02–0.41)

CI, confidence interval; OR, odds ratio.
Susceptible haplotype: *04:05-*04:01, *09:01-*03:03, *08:02-*03:02, *04:05-*03:02.
Protective allele in DRB1: *08:03, *15:01, *15:02, *04:06.
Protective allele in DQB1: *06:01, *06:02, *03:01.
X in DRB1: *01:01, *03:01, *04:01, *04:03,*04:04,*04:07, *04:10, *07:01,*10:01, *11:01, *11:06, *12:01, *12:02, *13:02, *16:02.
X in DQB1: *02:01, *04:02, *05:01, *05:02, *06:04, *06:09.

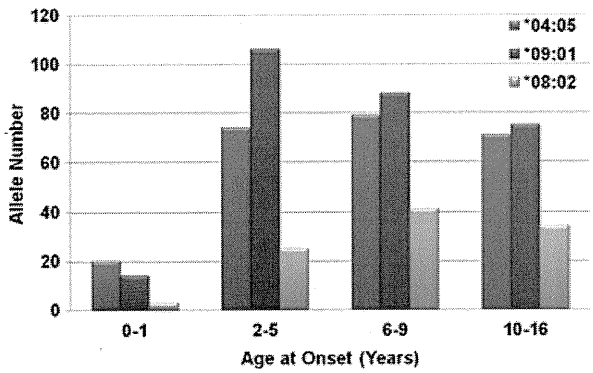

Fig. 1. DRB1 allele frequencies in four age groups of Japanese children with Type 1A diabetes according to the age at onset. The frequency of DRB1*09:01 was higher (Pc < 0.01) in the 2–5-year onset group, while the frequency of DRB1*08:02 tended to be higher in the 6–16-year onset group, although the difference was not significant. The distribution of the DRB1*04:05 allele frequency was not different among the four age groups.

childhood-onset type 1 diabetes mellitus in Japan. In the study, a TDT was performed for the first time in a population of Japanese children with Type 1A diabetes; the results confirmed the identities of susceptible and protective DRB1, DQB1, DPB1, A, C, and B, alleles.

We confirmed previously reported HLA-DRB1-DQB1 susceptible and protective haplotypes and obtained new findings regarding the DPB1 allele. DPB1*02:01 and DPB1*03:01 were identified as susceptible alleles among Japanese children with Type 1A diabetes (Table 1). DPB1*02:01 is unique to the Japanese population, while DPB1*03:01 is observed in multiple ethnic groups (4, 5, 16–19). This finding is noteworthy because neither the susceptible DRB1 allele nor the DQB1 allele is common to both Japanese and Caucasian populations. Moreover, the DPB1*02:01 and DPB1*03:01 alleles were not specifically associated with any susceptible DRB1-DQB1 haplotype. DPB1*04:02 was identified as a protective allele among Japanese children with Type 1A diabetes, similar to cases in multiple ethnic groups (4, 5, 16–19). Moreover, the DPB1*04:02 allele was not associated with any protective DRB1-DQB1 haplotypes. Of note, however, the association of DPB1*02:01, DPB1*03:01, and DPB1*04:02 with Type 1A diabetes was relatively weak in Japanese children, as the association was significant in the case-control study but not in the TDT. However, DPB1*09:01 was identified as a protective allele in both the case-control study and the TDT,

and DPB1*09:01 appeared to be associated with the protective DRB1*15:02-DQB1*06:01 haplotype, which is a major protective haplotype in the Japanese population but is rare in Caucasian populations.

The independent effects of HLA-A and B have been demonstrated in Caucasian populations (2, 4, 5). Following adjustment for LD to haplotypes at the DR-DQ region, both susceptible and protective alleles were found at HLA-A (e.g., A*24:02, susceptible allele; A*11:01, protective allele) and HLA-B (e.g., B*39:06, susceptible allele; B*57:01, protective allele) (4, 5). A*24:02 was a susceptible allele independent of the susceptible DRB1-DQB1 haplotypes among Japanese children with Type 1A diabetes. A*11:01 was also a protective allele among Japanese children with Type 1A diabetes. However, the association of A*24:02 and A*11:01 with Type 1A diabetes was relatively weak in the Japanese children, as the association was significant in the case-control study but not in the TDT (Tables 3 and 4). Of note, the B*39:06 and B*57:01 alleles were not observed in this study.

The analysis of LD between DRB1-DQB1 haplotypes and DPB1, A, C, or B alleles demonstrated both susceptible (C*08:01-B*40:06-DRB1*09:01-DQB1*03:03 and C*01:02-B*54:01-DRB1*04:05-DQB1*04:01) as well as protective (C*12:02-B*52:01-DRB1*15:02-DQB1*06:01-DPB1*09:01 and A*33:03-C*14:03-B*44:03) haplotypes among Japanese children with Type 1A diabetes.

In terms of genomic imprinting of the HLA-class II gene, several studies have been reported (26–29). In a Caucasian population, a striking feature of the data was that HLA-DR3/DR4 patients inherit their DR3 allele from their mother and the DR4 allele from their father more often than vice versa. Margaritte-Jeannin et al. (27) proposed that parental imprinting for a specific allelic combination may explain the HLA genotypes observed in the patients and their relatives. Sadauskaite-Kuehne et al. (28) also studied diabetes-associated allelic transmission rates from mothers and fathers to children with diabetes in 125 families in Lithuania, an area with a low incidence of type 1 diabetes. They reported that the DR4-DQB1*03:02-DQA1*03:01 haplotype was transmitted significantly more frequently from both parents, but that the DR3-DQB1*02:01-DQA1*05:01 haplotype was transmitted more frequently from only mothers. In Japan, Sasaki et al. (29) reported that maternal alleles in a susceptible DQA1*03:01-DQB1*03:02 haplotype showed a strong transmission disequilibrium with GADAb-positive type 1 diabetes, while paternal alleles in the same haplotype did not in 28 nuclear families, supporting the hypothesis that an epigenetic mechanism including genomic imprinting at the HLA-DQ region is involved in the pathogenesis and the genetic complexity of Japanese type 1 diabetes. However, none of the DRB1, DQB1, DPB1, A, C, or B alleles were preferentially transmitted from the mother or the father to the children with Type 1A diabetes in this study (Table 3). Our study suggests that the genomic imprinting of HLA-class II and class I genes is not involved in the pathogenesis of Type 1A diabetes in Japanese patients.

The frequency of subjects with two susceptible DRB1-DQB1 haplotypes was significantly higher among the children with Type 1A diabetes than among their parents. Of note, the frequencies of homozygosity for DRB1*09:01-DQB1*03:03 and of heterozygosity for DRB1*04:05-DQB1*04:01 and DRB1*08:02-DQB1*03:02 were significantly higher among children with Type IA diabetes, while the frequency of subjects with one susceptible haplotype and without a protective allele (group III) was not different between children with Type 1A diabetes and their parents. The frequencies of subjects with one susceptible haplotype and a protective allele (group IV) and with no susceptible haplotype and a protective allele (group VI) were lower among the children with Type 1A diabetes than among their parents (Table 5). These results suggest a dose effect of susceptible DRB1-DQB1 haplotypes and the effect of protective alleles.

The siblings of children with Type 1A diabetes may also represent a high-risk group for type 1 diabetes in the Japanese population, as the high prevalence (about 4%) of diabetes among Japanese siblings is comparable with that among Caucasian siblings (about 6%) (7, 8). The prevalences of the susceptible DRB1 and DQB1 alleles were similar between the children with Type 1A diabetes and their siblings. However, the prevalence of the protective DQB1*06:01 allele was higher among non-diabetic siblings. The frequency of group IV (no susceptible haplotype and a protective allele) was higher among the siblings than among the children with Type 1A diabetes. These results suggest the role of the protective allele among the siblings.

Only the allele frequency of DRB1*09:01 was significantly different among four age groups of Japanese children with Type IA diabetes determined according to the age at the time of onset (0–1, 2–5, 6–9, and 10–16 years). DRB1*09:01 may be strongly associated with an early onset in preschool children, whereas DRB1*08:02 may be weakly associated with a later onset in school-age children. Murao et al. (15) focused on the differences in the contributions of HLA-DR and -DQ haplotypes to the susceptibility to Type 1 diabetes during adulthood (later than 20 years of age) and childhood (1.0–18 years of age) in Japanese patients. They reported that the DRB1*09:01-DQB1*03:03 (DR9) frequency/DRB1*04:05-DQB1*04:01 (DR4) frequency increased with an increasing age of onset, and that another susceptible haplotype, DRB1*08:02-DQB1*03:02 (DR8), was involved only in the childhood-onset group. They did not mention any

difference among childhood-onset type 1 diabetes, and our results complement the data reported by Murao et al. The present results are also compatible with and complementary to our previous report, in which the frequency of the DR9 genotype was found to be significantly higher among a younger age group (0–10 years) than among an older age group (11–16 years) at the time of onset, and the frequency of DR4-DQ4 was higher in the older age group (11–16 years) (13).

Kawabata et al. (30) reported the age-related association of the MHC class I chain-related gene A and a marker in the class I C region with Japanese type 1 diabetes. However, this study did not show an association of susceptible class I A*24:02, C*01:02, C*08:01, or B*54:01 alleles with age at the time of onset in children with Type 1A diabetes (data not shown).

The amino acid residue at position 57 of the DQβ chain has been shown to play a key role in genetic susceptibility to type 1 diabetes. The lack of aspartic acid at this position at both DQ alleles is strongly associated with type 1 diabetes in Caucasian populations (31, 32). However, this Asp57 hypothesis is not tenable for Japanese type 1 diabetic patients (33). The influence of the HLA-DR and HLA-DQ molecules on the risk of type 1 diabetes is probably related to their central role in antigen presentation and the activation of a helper T cell-mediated immune response (2, 32). The HLA-class II and class I pocket structure is critical to the etiology of autoimmunity, as different pocket variants may have different affinities to the antigenic peptides of specific proteins from pancreatic β cells, including insulin and GAD; therefore, certain variants are more likely to present autoantigenic peptides to T cells than others (32, 34). In a future study, an analysis of how variations in amino acids, especially those found within the peptide-binding domains, are correlated with changes in disease risk would be valuable, providing a possible link between genetic association studies and the causal mechanism(s) of Type 1A diabetes.

In conclusion, this study demonstrated the characteristic association, which was mostly different but partly the same as that in Caucasian populations, of HLA-DRB1, DQB1, DPB1, and A, C, B, genes with Type 1A diabetes among Japanese children. A TDT did not reveal the genomic imprinting of HLA-class II and class I genes in Type 1A diabetes in the present population. A comparison of children with Type 1A diabetes and their parents and siblings suggested a dose effect of susceptible DRB1-DQB1 haplotypes and the effect of protective alleles on the immunological pathogenesis of Type 1A diabetes. These results may provide fundamental data for further genetic studies examining other immune-related and insulin resistance or beta cell function-related genes in Japanese patients with type 1 diabetes.

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Conflict of interest

No potential conflicts of interest relevant to this article were present.

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Insulin Resistance in Children: Consensus, Perspective, and Future Directions

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Insulin Resistance in Children: Consensus, Perspective, and Future Directions

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Objective: Emerging data indicate that insulin resistance is common among children and adolescents and is related to cardiometabolic risk, therefore requiring consideration early in life. However, there is still confusion on how to define insulin resistance, how to measure it, what its risk factors are, and whether there are effective strategies to prevent and treat it. A consensus conference was organized in order to clarify these points.

Participants: The consensus was internationally supported by all the major scientific societies in pediatric endocrinology and 37 participants.

Evidence: An independent and systematic search of the literature was conducted to identify key articles relating to insulin resistance in children.

Consensus Process: The conference was divided into five themes and working groups: background and definition; methods of measurement and screening; risk factors and consequences; prevention; and treatment. Each group selected key issues, searched the literature, and developed a draft document. During a 3-d meeting, these papers were debated and finalized by each group before presenting them to the full forum for further discussion and agreement.

Conclusions: Given the current childhood obesity epidemic, insulin resistance in children is an important issue confronting health care professionals. There are no clear criteria to define insulin resistance in children, and surrogate markers such as fasting insulin are poor measures of insulin sensitivity. Based on current screening criteria and methodology, there is no justification for screening children for insulin resistance. Lifestyle interventions including diet and exercise can improve insulin sensitivity, whereas drugs should be implemented only in selected cases. ***(J Clin Endocrinol Metab* 95: 5189–5198, 2010)**

Insulin resistance in adults has been recognized for decades as a cardinal feature in the development of type 2 diabetes (T2D) and has been associated with obesity, the metabolic syndrome, hypertension, and heart disease (1). It is also clear that insulin resistance is significantly related to obesity and cardiometabolic risk in children (2). However, there is a lack of clarity as to how insulin resistance in childhood is best assessed, in what clinical disorders it occurs, and whether it can be treated or prevented.

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Abbreviations: DM, Diabetes mellitus; FSIVGTT, frequently sampled iv glucose tolerance test; GDM, gestational DM; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; LOE, level of evidence; NAFLD, nonalcoholic fatty liver disease; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; SSPP, steady-state plasma glucose; T2D, type 2 diabetes.

To address the current state of the art related to insulin resistance in children, the European Society for Pediatric Endocrinology (ESPE), the Lawson Wilkins Pediatric Endocrine Society (LWPES), the International Society for Pediatric and Adolescent Diabetes (ISPAD), the Asia Pacific Pediatric Endocrine Society (APPES), the Australasia Pediatric Endocrine Society (APEG), the Sociedad Latino-Americana de Endocrinologia Pediatrica (SLEP), and the Japanese Society for Pediatric Endocrinology (JSPE) convened a panel of experts for a consensus conference on childhood insulin resistance.

Methods

The conference used an evidence-based approach. An independent and systematic search of the literature was conducted through EMBASE and PubMed based on MeSH terms. Grading of the evidence was based on previously published American Diabetes Association standards (3). See Supplemental Data, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

Definition and Background

1. Insulin resistance refers to reduced whole body glucose uptake [level of evidence (LOE) A; mostly in adults]

Insulin resistance is defined as the decreased tissue response to insulin-mediated cellular actions and is the inverse of insulin sensitivity. The term "insulin resistance," as generally applied, refers to whole-body reduced glucose uptake in response to physiological insulin levels and its consequent effects on glucose and insulin metabolism. Euglycemic hyperinsulinemic clamp studies have shown that insulin resistance is determined primarily by the response of skeletal muscle, with over 75% of infused glucose taken up by muscle and only 2–3% by adipose tissue (4).

2. Insulin resistance is a continuum (LOE A in adults)

Insulin sensitivity is a continuum from very low levels in individuals with high insulin resistance to very high levels in individuals without insulin resistance.

3. Insulin resistance is commonly associated with obesity (LOE A in adults and children)

Insulin resistance is most commonly associated with obesity, although not all obese people are insulin resistant and insulin resistance may occur in nonobese children and adults (5–7). Insulin resistance can also occur during normal physiological conditions, such as pregnancy or puberty (8).

4. One of the consequences of insulin resistance is chronic compensatory hyperinsulinemia (LOE A in adults, B in children)

Although the primary interest has been in insulin resistance, the adverse effects related to insulin resistance are more likely mediated via compensatory hyperinsulinemia (9). Despite the hyperinsulinemic response to insulin resistance, the current LOE does not support development of a definition of insulin resistance based on fasting insulin.

5. Standards for insulin resistance in children, with definitions for normal and abnormal levels, are nonexistent (LOE C in children)

Standards for insulin resistance in children have not been established. This is due, in part, to the use of a variety of techniques to measure insulin sensitivity, lack of sufficient cohort sizes to establish normative distributions for insulin sensitivity, and lack of adequate longitudinal studies to relate definitions for insulin resistance to long-term outcomes. Clinical features, such as acanthosis nigricans, can point to the likelihood of insulin resistance but cannot define it. Fasting insulin is not an optimal tool for individual assessment of peripheral insulin sensitivity, but it may provide information regarding compensatory hyperinsulinemia and liver insulin metabolism. Depending on the study population, fasting insulin is not always well correlated with insulin resistance in children (10), and differences exist between the heritability of fasting insulin and insulin resistance (11). Many studies have used fasting insulin alone or in combination with fasting glucose as surrogates for insulin resistance, but these are poor substitutes for the direct measures, thus limiting their precision. Fasting insulin as an index of insulin resistance may be applicable in epidemiological studies using large populations of children and/or well-defined cohorts.

Methods of Measurement

6. The euglycemic hyperinsulinemic clamp is the "gold standard" for measuring insulin sensitivity; the frequently sampled iv glucose tolerance test (FSIVGTT) and steady-state plasma glucose (SSPG) methods are also valid measurements (LOE A in adults, C in children)

The hyperinsulinemic euglycemic clamp, the FSIVGTT with modeling, and the SSPG are generally accepted as valid and reliable for measurement of insulin sensitivity. However, each of these methods is time consuming, requires iv infusions and frequent blood sampling, is bur-

densome for participants, is costly, and requires a research setting.

Less intensive methods, such as measurement of insulin during the oral glucose tolerance test (OGTT), offer the advantage of a smaller number of blood samples. High correlations were reported in adult studies comparing the OGTT with the euglycemic hyperinsulinemic clamp (12). The OGTT has not been studied as well in children. In a group of 38 obese 8–18 yr olds, the correlation between the OGTT (whole-body insulin sensitivity index) and the euglycemic hyperinsulinemic clamp was 0.78 (13).

7. The homeostasis model assessment (HOMA) and the quantitative insulin-sensitivity check index do not offer any advantages over fasting insulin in euglycemic children (LOE A in adults, B in children)

In an attempt to further simplify the measurement of insulin sensitivity, a number of methods using single simultaneously obtained samples of fasting insulin and glucose have been developed. Each of these uses a mathematical formula that adjusts for individual variability in insulin and glucose secretion and clearance. Although the goal for these methods was to improve the accuracy of fasting insulin alone by the addition of fasting glucose, it is now agreed that they yield similar results to fasting insulin. For instance, HOMA, the most widely used of the surrogate measures in children, is highly correlated with fasting insulin ($r \geq 0.95$) in children (10) and adults. These high correlations can be attributed to the narrow range of fasting glucose even among obese children and those with abnormal glucose tolerance (14, 15), whereas there is a 53-fold variation in fasting insulin in children (10).

8. Fasting insulin is a poor measure of whole body insulin sensitivity in an individual child (LOE A)

The accuracy of fasting insulin as a measure of insulin sensitivity has been assessed through correlation analyses with the euglycemic hyperinsulinemic clamp, FSIVGTT, or SSPG and found to be disappointingly low (16). Studies of cohorts (with more than 50 participants for this consensus statement) containing both grade school-aged and high school-aged children have reported correlations from 0.42–0.91 between fasting insulin and the clamp (10, 17) and from 0.18–0.8 between fasting insulin and FSIVGTT (18–21). In the largest cohort reported to date, the correlation between fasting insulin and the clamp was 0.42 at mean age of 13 yr (n = 323) and 0.29 at mean age of 15 yr (n = 300), with slightly higher correlations in obese than thin children (10). It can be concluded from these studies that fasting insulin is a poor measure of whole body insulin sensitivity in an individual child, and it should not be used for clinical decision making in daily clinical practice.

Although fasting insulin is a poor surrogate, much of the data relating to prevalence, intervention, and prevention are based on it or other surrogates, bringing into question the precision of the results from those studies.

Methods of Screening

9. Based on current screening criteria and methodology, there is no justification for screening children for insulin resistance, including obese children (LOE A)

The prevalence of insulin resistance is unknown, but it is clear that insulin-resistant obese children have significantly greater cardiovascular risk profiles, and childhood insulin resistance appears to predict future cardiovascular risk (21). Although this suggests that screening has the potential to identify at-risk children, the key issue for any screening program is availability of an accurate, reliable, reproducible, and easily applicable method of measurement. It is impractical to use any lengthy methods requiring multiple samples because of the complexity, time, and cost of individual testing. In the clinical setting, fasting insulin is an unreliable measure of insulin sensitivity, and testing of aliquots of a common sample assayed in different laboratories has shown disparate results (22). Even if a uniformly reliable insulin assay became available, separate standards would need to be developed by genders, ethnic groups, and pubertal stages (8, 23, 24). Currently, there is no recommended pharmacological treatment for isolated insulin resistance. Therefore, screening for insulin resistance is not justified in the clinical setting for children, including those with obesity. The mere presence of obesity should call for intervention to lower weight and consequently improve insulin sensitivity without a need to measure insulin levels.

Assessment of Risk Factors of Childhood Insulin Resistance

10. The two most important biological conditions associated with insulin resistance in childhood are ethnicity and puberty (LOE A)

Using a variety of methods, studies show that African-American, Hispanic, Pima Indian, and Asian children are less insulin sensitive compared with Caucasian children (25–27). The insulin resistance in minority ethnic groups is manifested as lower insulin-stimulated glucose uptake, concomitant with hyperinsulinemia, evidence of increased insulin secretion from the β-cell and decreased insulin clearance (25–27).

During puberty there is ~25–50% decline in insulin sensitivity with recovery when pubertal development is

complete (8). The compensatory increase in insulin secretion during puberty may be blunted in African-American and Hispanic youth, thus increasing their risk for T2D around the time of puberty (28, 29).

11. Obesity, particularly increased abdominal visceral adiposity, and nonalcoholic fatty liver disease (NAFLD) are associated with insulin resistance in children (LOE A)

Obesity is the most prevalent pathophysiological cause of insulin resistance. Insulin sensitivity is inversely associated with body mass index and percentage body fat, and obese youth have lower insulin sensitivity than their normal-weight peers (30, 31). Independent of the relation between total body fat and insulin resistance, increased abdominal visceral adipose tissue in obese youth is associated with lower insulin sensitivity and higher acute insulin response (23). Limited studies show that ectopic fat deposition such as intramyocellular lipid in obese adolescents is also associated with decreased peripheral insulin sensitivity (32).

Studies using the clamp methodology demonstrate that NAFLD is associated with hepatic and peripheral insulin resistance (33). The relation between insulin sensitivity and NAFLD seems to be, in part, driven by abdominal fat content (34).

The relationship between lifestyle factors, *e.g.* nutrition and physical activity, and insulin sensitivity is poorly defined in children.

Increased caloric intake leading to obesity, rather than the dietary macronutrient composition, is associated with insulin resistance and hyperinsulinemia. Limited cross-sectional data suggest that dietary saturated fat and sugar-sweetened beverages may be associated with alterations in insulin sensitivity and secretion (35).

The effect of physical activity on insulin sensitivity, independent of changes in weight and adiposity, remains controversial.

12. Polycystic ovary syndrome (PCOS), independent of weight, is characterized by insulin resistance in childhood (LOE B)

Adolescent girls with PCOS can have severe insulin resistance with increased risk for impaired glucose tolerance (IGT) and T2D, and the impairment in insulin sensitivity is more pronounced in obese than lean PCOS girls (36, 37). In some ethnic groups, girls with premature pubarche, a potential antecedent of PCOS, have increased insulin levels, and a causal relation between hyperinsulinemia and adrenal and/or ovarian androgen hypersecretion has been hypothesized (38, 39). However, population studies of normal girls have shown that rapid weight gain is associated with higher adrenal androgens and body fatness, and that insulinemia was related to early menarche (40). Thus, the association of higher insulin levels with premature pubarche and subsequent PCOS may be driven, at least in part, by obesity.

13. Genetics and heritability play a role in childhood insulin resistance (LOE B)

In studies of adult twins, approximately half of the variance in insulin sensitivity and secretion can be attributed to genetic factors (41, 42). Healthy children with a family history of T2D are more insulin resistant, with an impaired balance between insulin sensitivity and secretion (43, 44). Recently, common genetic variants have emerged that identify heritable components of insulin sensitivity (45). The T2D protective variant Pro12Ala in PPAR-γ is associated with higher insulin sensitivity in Caucasian children (46).

14. Intrauterine exposure to poorly controlled maternal diabetes increases the risk of obesity, insulin resistance, and IGT in childhood (LOE B)

Epidemiological and clinical studies have demonstrated that offspring of mothers with preexisting diabetes mellitus (DM) or gestational DM (GDM) have an increased risk of obesity and altered glucose metabolism (47). Small size at birth or being large for gestational age is independently associated with increased risk of childhood obesity (and possibly altered glucose metabolism) (48), but the risk of obesity and IGT/diabetes is also higher in normal-weight offspring of mothers with DM or GDM (49). Infants of mothers with GDM have more body fat than infants born to mothers with normal glucose tolerance (50), but less is known about whether excess adiposity in these infants is a risk factor for obesity or insulin resistance in later life.

Higher levels of maternal glucose during pregnancy, with or without meeting criteria for the diagnosis of GDM, might play a role in the future risk of childhood obesity and insulin resistance in the offspring (51).

15. Postnatal and childhood weight gain increase the risk of insulin resistance in normal-birth-weight and small-for-gestational-age children (LOE B)

Rapid postnatal weight gain has consistently been associated with risk of insulin resistance and greater adiposity in children and young adults (52–56) and predicts insulin resistance-related outcomes in adults (57, 58). However, the timing of rapid weight gain with respect to future insulin resistance remains controversial, with some

studies relating it to early infancy (0–6 months) and others between ages 2 and 11 yr (54–56).

The association between small-for-gestational-age infants and an increased risk of obesity, insulin resistance, and T2D is accentuated by weight gain during early life with increased percentage body fat (52, 59, 60).

Preterm children have reduced insulin sensitivity, which persists in adulthood and is associated with truncal obesity (61).

Consequences of Childhood Insulin Resistance

16. Insulin resistance is a risk factor for prediabetes and T2D in childhood (LOE B)

Insulin resistance and impaired β-cell function are the two key components in the pathogenesis of T2D in youth (62). Despite limited and conflicting cross-sectional data, it is well accepted that youth with IGT have impairment in insulin secretion compared with equally obese youths with normal glucose tolerance (63–65). In some studies, this has been associated with similar levels of insulin sensitivity (63, 65), whereas in others obese adolescents with IGT were more insulin resistant than adolescents with normal glucose tolerance and a similar degree of adiposity (32, 66). However, there are very limited longitudinal data on whether insulin resistance predicts the development of IGT and T2D. A recent longitudinal study has shown that obese adolescents progressing to IGT manifest primary defects in β-cell function, which are aggravated by a progressive decline in insulin sensitivity (67).

17. Insulin resistance is associated with the metabolic syndrome and cardiometabolic risk factors (LOE A)

Regardless of the metabolic syndrome definition used, insulin resistance and high insulin levels are associated with the clustering of cardiometabolic risks associated with metabolic syndrome in a variety of ethnic groups (7, 68, 69).

There are no studies that directly measure *in vivo* insulin sensitivity and its relationship to atherosclerotic abnormalities in children. Very limited observations suggest a relationship between HOMA and arterial stiffness and fasting insulin levels in youth (70). However, a role for insulin resistance in the early abnormalities of vascular smooth muscles is proposed based on the observation that circulating biomarkers of endothelial dysfunction (intercellular adhesion molecule and E-selectin) are highest, whereas the antiatherogenic adipocytokine adiponectin is lowest among the most insulin-resistant youths (71).

Treatment

18. Diet and weight loss drugs improve insulin sensitivity in adolescents through weight loss and other mechanisms (LOE B)

Dietary fat intake influences insulin sensitivity, with the most consistent effect related to increased fat intake lowering insulin sensitivity rather than reduced fat intake increasing insulin sensitivity (35, 72). However, a consistent effect of fat quality on insulin sensitivity could not be found across 41 adult studies, largely because of design flaws limiting interpretation (73).

A high whole-grain or dietary fiber intake is associated with higher insulin sensitivity and weight loss, and a low intake is associated with lower insulin sensitivity, based upon a questionnaire study in adolescents and prospective crossover studies in adults (74).

Improvement in insulin sensitivity in adolescents on a low glycemic load diet is contradictory to the greater number of studies in adults in which a consistent effect of this diet is not seen on insulin sensitivity (75–77).

Although there are similarities between a low glycemic load and a low-carbohydrate diet, there are no studies evaluating the latter diet's impact on insulin sensitivity in children. In adolescents receiving either a high-fiber or low glycemic load diet, weight loss was observed with improved insulin sensitivity (74–77). It is unclear whether the improvements in insulin sensitivity were due to weight loss, the diet, or a combination of these factors.

Few studies have examined the impact of a hypocaloric diet on insulin sensitivity in children; however, adult studies have found variable weight loss and improvement in insulin sensitivity.

The weight-reducing drugs sibutramine and orlistat led to an improvement in insulin sensitivity with a reduction in weight of approximately 0.6 SD in children and adolescents (78–80).

19. Exercise and fitness improve insulin sensitivity through weight loss and also mechanisms independent of weight loss in adolescents (LOE A)

Studies specifically exploring the impact of exercise and mechanism of action on insulin resistance are few.

Lifestyle programs including supervised exercise can improve fasting insulin levels as quickly as 2 wk before measurable weight loss (81, 82). Furthermore, lifestyle intervention improved body composition without a change in body weight (83). Available studies suggest that

fitness may play a more important role than body mass index reduction on improvement in insulin sensitivity in obese adolescents (84).

Adequate studies are not available to differentiate the effect of a single session of exercise on insulin sensitivity, as opposed to the training regimen. There appears to be improvement in insulin sensitivity with prescribed aerobic exercise regimens and combinations of aerobic and resistance training (85, 86). However, there is inadequate evidence about the optimal form of exercise. Exercise intensity has not been shown to be correlated with insulin sensitivity. After the cessation of exercise, improved insulin sensitivity levels revert to preexercise levels, and there may even be a rebound phenomenon with greater insulin resistance (82).

20. Multicomponent lifestyle intervention improves insulin sensitivity more than individual lifestyle components in adolescents (LOE B)

There are limited data to show that the effects of nutrition, exercise, and behavioral modification together on insulin sensitivity are more beneficial and sustained than any one component alone (87). Short-term randomized studies of lifestyle and exercise intervention in obese adolescent girls improved insulin sensitivity when compared with no intervention (88).

21. Metformin improves insulin sensitivity in adolescence (LOE B)

Metformin has been shown to improve insulin sensitivity in adolescents with T2D and girls with PCOS, justifying consideration of metformin as a therapeutic tool in these disorders (89, 90). There are conflicting reports on the influence of metformin on insulin sensitivity in insulin-treated, insulin-resistant type 2 diabetics (91).

The safety and efficacy of metformin in the management of T2D in children were confirmed using glycemic control as a proxy for improved insulin sensitivity (92). However, other reports have emphasized that lifestyle and dietary measures can be at least as effective as metformin in these patients (91).

Metformin has been shown to be efficacious in improving insulin sensitivity in obese PCOS girls with IGT (90), but not in obese PCOS girls without IGT (96). In nonobese teenage girls with PCOS, combined flutamide-metformin therapy improved insulin sensitivity (97). Both flutamide and metformin seem to be needed to obtain maximal efficacy on parameters of insulin sensitivity and to ameliorate body composition (98).

However, it has to be stressed that metformin has not been approved for the treatment of children with insulin resistance; therefore, appropriate, well-designed, controlled trials are needed.

Prevention

22. Maternal obesity, gestational diabetes, smoking in pregnancy, and maternal undernutrition should be targeted to lessen obesity and insulin resistance in children (LOE A)

All factors affecting fetal growth are potential candidate targets for prevention purposes.

The most common and important among these risk factors are maternal obesity, gestational diabetes, maternal undernutrition, and smoking during pregnancy (49, 99–102).

23. Breast-feeding should be promoted through public health interventions as a contributing factor to reduce the prevalence of obesity and potential insulin resistance later in life. In addition, ongoing dietary advice starting from weaning has the potential to prevent insulin resistance in the long term (LOE B)

There are no specific data on a direct relationship between breast-feeding and prevention of insulin resistance, but given the association between obesity and reduced insulin sensitivity, breast-feeding should be promoted (103, 104).

Because of the strong link between obesity and insulin resistance, the impact of dietary interventions used to prevent obesity has been examined for its effect on insulin resistance (104). Increased saturated fat intake has been associated with reduced insulin sensitivity in children (35). A healthy low saturated fat and cholesterol diet, started in 7-month-old infants, showed a positive effect on insulin resistance at the age of 9 yr (105).

24. Identification of infants and preschool children at risk for obesity combined with intervention programs to prevent excessive weight gain should be developed and evaluated. Physical activity as a means of increasing insulin sensitivity is an important component of any intervention (LOE B)

Young adults born preterm have lower insulin sensitivity than controls, and weight gain velocity during childhood is associated with lower insulin sensitivity in adulthood (93). Adiposity rebound is a sensitive marker for the risk of developing obesity and its complications, and therefore it should be prevented (55, 94).

Based on available data on the beneficial effect of physical activity on surrogate measures of insulin sensitivity, such as fasting insulin and HOMA for insulin resistance (85, 95), physical activity should be promoted, although further studies using state of the art methodology for insulin sensitivity are required to validate these findings.

Conclusions

This consensus statement highlights the lack of a clear cutoff to define insulin resistance in children and shows that surrogate measures, such as fasting insulin, are poor estimates of insulin sensitivity. Based on current screening criteria and methodology, there is no justification for screening children for insulin resistance, even those who are obese. However, it appears that prevention strategies should be started early in life and, with regard to treatment, lifestyle interventions should be included, whereas metformin should be limited to selected cases. Future research should aim at assessing the following: how to best measure insulin sensitivity; standardization of insulin measurements; identification of strong surrogate biomarkers of insulin resistance; and the potential role of both lifestyle intervention and medications in the prevention and treatment of insulin resistance.

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