Table 4 HR for mortality from CVD according to green tea consumption: JACC Study, Japan, 1988–2003 (n=82 655)

	Green tea consumption					
	<1 cup/week	1–6 cups/week	1–2 cups/day	3–5 cups/day	≥6 cups/day	p for trend
Men						
Person-years	39 393	44 976	57 653	154 175	103 167	
Total CVD						
N	173	165	203	623	525	
Age and BMI-adjusted HR	1	0.95 (0.77–1.20)	0.89 (0.73–1.11)	0.92 (0.77–1.10)	1.02 (0.86–1.23)	0.635
Multivariable HR	1	1.09 (0.61–1.96)	1.31 (0.80–2.14)	1.31 (0.86–1.99)	1.42 (0.90–2.24)	0.327
CHD						
N	47	46	39	139	101	
Age and BMI-adjusted HR	1	1.01 (0.66–1.54)	0.59 (0.37–0.94)	0.75 (0.54–1.07)	0.75 (0.52–1.08)	0.074
Multivariable HR	1	1.28 (0.43–3.86)	0.87 (0.35–2.15)	0.88 (0.41–1.87)	0.74 (0.30–1.78)	0.273
Stroke						
N	72	67	87	272	244	
Age and BMI-adjusted HR	1	0.97 (0.68–1.38)	0.96 (0.69–1.34)	1.06 (0.76–1.32)	1.19 (0.90–1.58)	0.127
Multivariable HR	1	1.22 (0.48–3.11)	1.23 (0.56–2.70)	1.36 (0.68–2.68)	1.45 (0.69–3.06)	0.779
Women						
Person-years	68 286	68 111	71 987	233 032	136 608	
Total CVD						
N	200	139	154	598	356	
Age and BMI-adjusted HR	1	0.77 (0.61–0.98)	0.71 (0.56–0.89)	0.75 (0.63–0.89)	0.74 (0.62–0.90)	0.007
Multivariable HR	1	1.13 (0.66–1.93)	0.77 (0.48–1.26)	0.81 (0.56–1.18)	0.62 (0.40–0.98)	0.031
CHD						
N	41	24	23	108	71	
Age and BMI-adjusted HR	1	0.69 (0.40–1.18)	0.54 (0.31–0.94)	0.65 (0.44–0.96)	0.73 (0.48–1.11)	0.275
Multivariable HR	1	0.34 (0.06–1.75)	0.28 (0.07–1.11)	0.39 (0.18–0.85)	0.42 (0.15–0.92)	0.038
Stroke						
N	83	56	84	259	154	
Age and BMI-adjusted HR	1	0.74 (0.52–1.07)	0.90 (0.64–1.25)	0.76 (0.58–0.99)	0.57 (0.56–1.01)	0.061
Multivariable HR	1	1.55 (0.67–3.58)	1.10 (0.53–2.26)	1.07 (0.59–1.94)	0.72 (0.34–1.52)	0.206

Multivariable HR was adjusted for body mass index (BMI), history of hypertension, history of diabetes, smoking status, alcohol intake, education, walking hours, hours of sports participation, perceived mental stress, multivitamin use, vitamin E supplement use, consumption of total fruits, total vegetable, total beans, total meat, total fish and seaweeds, and total daily energy intake. CHD, coronary heart disease; CVD, cardiovascular disease.

Tea catechins are a major candidate compound responsible for the inverse association of green tea with CHD among women, as tea catechins were previously shown to be associated with a reduced risk of CHD rather than stroke,[23] and the antioxidant activities of catechins were proved to be higher in females than males in animal studies.[31 32] Catechins have direct effects on cardiomyocytes,[33 34] as well as vascular benefits that influence both CVD and cerebrovascular diseases. Direct cardioprotection by catechins might partly explain the more potent effects of green tea on CHD rather than stroke. A sex-specific association of green tea and CVD was also reported by Kuriyama *et al*,[9] and they speculate that confounding by smoking might account for the sex specificity. However, in the present study, no effect modification by smoking habits was observed in the stratified analysis (p=0.396 for interaction). On the other hand, the consumption of green tea was associated with healthy behaviours, including increased physical activity or the increased consumption of fruits, vegetables and beans. Fruits, vegetables and beans are enriched with flavonoids or vitamins and have beneficial effects on CVD.[35 36] Although we statistically controlled these variables and no effect modification by these healthy behaviours was observed, we could not exclude the possibility of residual confounding. Women consume a larger amount of healthy foods such as fruits, vegetables and beans than men, as was shown in the baseline characteristics of the present study. So, there remains the possibilities that healthy food intake might enhance the effect of catechins in green tea and might be associated with the sex difference of green tea benefits.

Compared with western populations, the metabolic rate of caffeine is much slower in Japanese individuals,[37 38] which might have reduced the beneficial effects of caffeine. On the other hand, Japanese people consume fish, vegetables and beans rather than western-style food including red meat. Despite such genetic and environmental differences in risk profiles of CVD, a moderate consumption of coffee and teas was consistently associated with a reduced risk of CVD in both western and Japanese populations.

There are several limitations in the present study. First, we did not have data on other dietary sources of caffeine, such as soda, which is another potential source of caffeine. Soda consumption may confound the association between caffeine intake and the risk of CVD mortality. However, soda is consumed at a rate of approximately one tenth of coffee and teas in Japan, and it is consumed much less by middle-aged than younger people.[39] Therefore, it is less likely that the consumption of soda negatively affected the results. Second, as the consumption of beverages was assessed on the basis of self-administered questionnaires, some misclassification of consumption status could arise. However, as we mentioned before, this misclassification may be non-differential and would tend to result in an underestimation of the impact of coffee, green tea and oolong tea consumption on CVD mortality. Finally, the observed inverse associations may be confounded by age because the consumption of coffee and oolong tea and caffeine intake was significantly inversely associated with age. To examine whether the association was confounded by age, we conducted both age-adjusted and age-stratified analyses. We could not observe any significant interaction with age. In addition,

Table 5 HR for mortality from CVD according to caffeine intake: JACC Study, Japan, 1988–2003 (n=82 655)

	Caffeine intake					
	Quantile 1	Quantile 2	Quantile 3	Quantile 4	Quantile 5	p for trend
Men						
Person-years	44 684	51 536	49 102	53 738	52 649	
Total CVDease						
N	225	210	176	138	125	
Age and BMI-adjusted HR	1	0.82 (0.67–1.00)	0.77 (0.62–0.94)	0.69 (0.55–0.86)	0.97 (0.91–1.02)	0.027
Multivariable HR	1	0.83 (0.61–1.13)	0.70 (0.50–0.98)	0.62 (0.43–0.92)	0.95 (0.86–1.05)	0.083*
CHD						
N	53	47	35	38	39	
Age and BMI-adjusted HR	1	0.78 (0.52–1.18)	0.65 (0.42–1.02)	0.81 (0.52–1.25)	1.03 (0.92–1.15)	0.763
Multivariable HR	1	0.96 (0.49–1.86)	0.68 (0.33–1.40)	0.46 (0.19–1.08)	0.92 (0.74–1.13)	0.158
Stroke						
N	102	85	77	59	31	
Age and BMI-adjusted HR	1	0.73 (0.54–0.98)	0.73 (0.54–1.00)	0.61 (0.43–0.86)	0.89 (0.74–0.92)	<0.001
Multivariable HR	1	0.74 (0.46–1.17)	0.63 (0.38–1.04)	0.65 (0.37–1.13)	0.80 (0.67–0.96)	0.02
Women						
Person-years	64 696	68 839	82 363	77 640	77 415	
Total CVD						
N	177	172	143	115	81	
Age and BMI-adjusted HR	1	0.85 (0.81–1.28)	0.80 (0.63–1.00)	0.81 (0.63–1.05)	0.95 (0.88–1.02)	0.025
Multivariable HR	1	0.95 (0.66–1.38)	0.95 (0.66–1.38)	0.78 (0.51–1.19)	0.96 (0.85–1.10)	0.348
CHD						
N	20	43	30	16	15	
Age and BMI-adjusted HR	1	2.86 (1.55–5.28)	1.84 (0.96–3.50)	1.32 (0.63–2.73)	1.09 (0.90–1.33)	0.866
Multivariable HR	1	2.10 (0.78–5.67)	1.18 (0.37–3.70)	0.80 (0.21–3.01)	0.98 (0.68–1.40)	0.783
Stroke						
N	83	73	52	54	46	
Age and BMI-adjusted HR	1	0.83 (0.59–1.16)	0.54 (0.37–0.78)	0.84 (0.72–1.05)	0.99 (0.90–1.09)	0.202
Multivariable HR	1	0.85 (0.51–1.40)	0.65 (0.37–1.12)	0.50 (0.26–0.94)	1.02 (0.85–1.21)	0.228

Multivariable HR was adjusted for body mass index (BMI), history of hypertension, history of diabetes, smoking status, alcohol intake, education, walking hours, hours of sports participation, perceived mental stress, multivitamin use, vitamin E supplement use, consumption of total fruits, total vegetable, total beans, total meat, total fish and seaweeds and total daily energy intake.
*Non-linear p (cubic spline) <0.001.
CHD, coronary heart disease; CVD, cardiovascular disease.

although green tea was positively associated with age, an inverse association was observed between green tea consumption and mortality from CVD. Therefore, it is less likely that the observed inverse associations between green tea and mortality were confounded by age.

In conclusion, the moderate consumption of coffee, green tea and oolong tea and caffeine intake was associated with a lower risk of mortality from CVD. Confirmation of the results of the present study by other non-western populations or randomised clinical trials will be worthwhile.

What is already known on this subject

- Many studies have showed the inverse association between coffee consumption and CVD and have focused on CHD in which the association of coffee consumption with stroke remains unclear. Few studies have examined the relationship between tea consumption and CVD. These data are lacking in Asian countries.

What this study adds

- This study showed a U-shaped relationship of coffee consumption and caffeine intake with the risk of mortality from CVD, especially stroke among Japanese men and women.
- A higher amount of green tea consumption consistently decreased the risk of mortality from CVD, especially CHD among Japanese women.

Author affiliations:
[1]Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan
[2]Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan
[3]Department of Medical Informatics, Kansai Rosai Hospital, Osaka, Japan
[4]Department of Social and Environmental Health, Osaka University Graduate School of Medicine, Osaka, Japan
[5]Department of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan
[6]Department of Food Science and Nutrition, Faculty of Human Life and Environment, Nara Women's University, Nara, Japan
[7]Infectious Disease Surveillance Center; Infectious Disease Research Division, Hyogo Prefectural Institute of Public Health and Environmental Sciences, Kobe, Japan
[8]Department of Public Health, Aichi Medical University, Aichi, Japan
[9]Department of Epidemiology and Environmental Health, Jissen Women's University, Tokyo, Japan
[10]Department of Public Health/Health Information Dynamics, Aichi Medical University, Aichi, Japan

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APPENDIX

The present members of the JACC Study and their affiliations are as follows: Dr Akiko Tamakoshi (Nagoya University Graduate School of Medicine, present chairman of the study group), Dr Mitsuru Mori (Sapporo Medical University School of Medicine), Dr Yutaka Motohashi (Akita University School of Medicine), Dr Ichiro Tsuji (Tohoku University Graduate School of Medicine), Dr Yosikazu Nakamura (Jichi Medical School), Dr Hiroyasu Iso (Osaka University Graduate School of Medicine), Dr Haruo Mikami (Chiba Cancer Center), Dr Yutaka Inaba (Juntendo University School of Medicine), Dr Yoshiharu Hoshiyama (University of Human Arts and Sciences), Dr Hiroshi Suzuki (Niigata University School of Medicine), Dr Hiroyuki Shimizu (Gifu University School of Medicine), Dr Hideaki Toyoshima (Nagoya University Graduate School of Medicine), Dr Kenji Wakai (Aichi Cancer Center Research Institute), Dr Shinkan Tokudome (Nagoya City UniversityMedical School), Dr Shuji Hashimoto and Dr Yoshinori Ito (Fujita Health University School of Health Sciences), Dr Shogo Kikuchi (Aichi Medical University School of Medicine), Dr Akio Koizumi (Kyoto University Graduate School of Medicine), Dr Takashi Kawamura (Kyoto University Center for Student Health), Dr Yoshiyuki Watanabe and Dr Tsuneharu Miki (Kyoto Prefectural University of Medicine), Dr Chigusa Date (Faculty of Human Life and Environmental Sciences, Nara Women's University), Dr Kiyomi Sakata (Wakayama Medical University), Dr Takayuki Nose (Tottori University Faculty of Medicine), Dr Norihiko Hayakawa (Research Institute for Radiation Biology and Medicine, Hiroshima University), Dr Takesumi Yoshimura (Fukuoka Institute of Health and Environmental Sciences), Dr Akira Shibata and Dr Katsuhiro Fukuda (Kurume University School of Medicine), Dr Tomoyuki Kitagawa (Cancer Institute of the Japanese Foundation for Cancer Research), Dr Toshio Kuroki (Gifu University), Dr Yoshiyuki Ohno (Asahi Rosai Hospital), Dr Naoyuki Okamoto (Kanagawa Cancer Center), Dr Hideo Shio (Moriyama Municipal Hospital), Dr Kazuo Tajima (Aichi Cancer Center Research Institute), Dr Takashi Shimamoto (Osaka Medical Center for Health Science and Promotion) and Dr Heizo Tanaka (Medical Research Institute, Tokyo Medical and Dental University).

遺伝疫学

POINTS

- 遺伝性出血性末梢血管拡張症（HHT）は常染色体優性遺伝形式をとり，責任遺伝子として *ENG*（HHT1 型），*ACVRLK1*（別名 *ALK-1*）（HHT2 型），*SMAD4*（HHT と若年性ポリポーシス合併型）の 3 つが同定されている．いずれも血管新生に関与する TGF-β/BMP シグナリングカスケード関連の遺伝子である．
- 3 遺伝子の検査により突然変異を検出できるのは，臨床的症例のうち約 80～85%にとどまる（うち *ENG* と *ALK-1* が大半で，*SMAD4* は 1～2%）．
- 他に 2 つの遺伝子座が特定されている（HHT3 型と HHT4 型）．しかし遺伝子は不明．
- 登録された *ENG*，*ALK-1* の突然変異はそれぞれ約 400，330 種類（うち遺伝子多型が約 1 割）である．現在でも報告される変異の約 3 割は新規で，増え続けている．国内では 7 種の *ENG*，1 種の *ALK-1* の変異が報告されている．
- 人口集団に特有の変異はほとんどない．
- 世界の有病率は少なく見積もっても 10,000 人に 1 人．日本は欧米より低いとは限らず，遺伝疫学調査が行われた地域では 5,000～8,000 人に 1 人であった．

遺伝性出血性末梢血管拡張症 hereditary hemorrhagic telangiectasia（HHT）は血管新生に重要な役割を果たしている TGF-β/BMP シグナリングカスケードの障害が関与する疾患であり，常染色体優性遺伝形式をとる．その責任遺伝子として現在までに 3 つが同定されている．すなわち，細胞表面の co-receptor である endoglin をコードする *ENG*，同じく細胞表面の receptor である serine/threonine-protein kinase receptor R3（別名 activin A receptor，type II -like kinase 1 または activin receptor-like kinase 1，または TGF-β superfamily receptor type I）をコードする *ACVRLK1*（別名 *ALK-1*），細胞内の信号伝達分子 mothers against decapentaplegic homolog 4（別名 deletion target in pancreatic carcinoma 4，または SMAD family member 4）をコードしている *SMAD4* の 3 遺伝子である．これらの遺伝子以外に，今のところ少なくとも 2 つが疾患発症に関与していると想定されているが，現在までのところそれらは同定されていない．このことと技術的問題からか，以上の 3 遺伝子の分子遺伝学的検査により突然変異を検出できるのは，臨床診断された症例のうち約 80～85%にとどまるのが現状である[1]．

1．責任遺伝子同定の歴史的経緯

患者家族のマイクロサテライトマーカーを用いた解析から 1993 年，9 番染色体長腕（9q33-q34.1）にあることが推定された．1994 年に McAllister らがその頃マッピング・クローニングされた endoglin 遺伝子 *EGN* を調べた結果，血縁関係のない HHT 患者の 64 の DNA サンプルから 3 症例で複数の突然変異が同定された[2]．その後，突然変異が endoglin の不安定な蛋白質や短縮した蛋白質を生成することから，突然変異はハプロ不全（haploinsufficiency）の機序で HHT を引き起こしていると推定された．これが HHT1 型（HHT1）である．

一方，activin A receptor，type II -like kinase 1（*ACVRLK1*，または *ALK-1*）は 1993 年にクローニングされていた．endoglin 遺伝子に異常がある HHT1 患者

の血管内皮細胞で *ALK-1* の発現が低下しているという報告が1996年にあり，1997年に9番染色体長腕に連鎖しないが12番染色体長腕（12q13）に連鎖するHHT6家系のうち全てで *ALK-1* に突然変異がみつかった．うち2症例では中途終止コドンがみつかり，突然変異のmRNAがほとんど発現していないことから突然変異 *ALK-1* は機能的にヌル対立遺伝子と考えられた[3]．これがHHT2型（HHT2）である．

SMAD4 は膵臓がんに関係する18番染色体長腕（18q21.1）上のがん抑制遺伝子として，1996年にマッピング・クローニングされていた．1998年，若年性ポリポーシス juvenile polyposis syndrome（JPS）家系の連鎖解析の結果，遺伝子座から *SMAD4* が候補遺伝子としてあげられ，実際に *SMAD4* の突然変異が同定された．2004年，JPSとHHTが併存する6症例すべてで *ENG* と *ALK-1* に突然変異を認めない代わりに *SMAD4* に変異を認めたことから，HHTの責任遺伝子として *SMAD4* が認識されることとなった[4]．

上記3つの遺伝子変異がみつからない症例の家系を用いた連鎖解析で，2006年頃に5番染色体（HHT3型：HHT3）と7番遺伝子短腕（7p14）（HHT4型：HHT4）にそれぞれ遺伝子座が特定された．しかし遺伝子は2010年現在同定されておらず，疾患発生機序の複雑さや未知の機序の存在が解明を困難にしていると考えられている[5]．

2．遺伝子検査

a．配列解析 sequencing analysis または突然変異走査検索 mutation scanning

上記3つの遺伝子の配列解析によって約75%のHHT患者を同定できると報告されている[6]．コード領域の配列解析によって検出できるのは，ミスセンス・ナンセンス変異，短い挿入や欠失，スプライス部位変異である．1本鎖高次構造多型（SSCP）検出法などに代表される突然変異走査検索は，突然変異の分布が特定遺伝子の広い範囲にわたっている場合や家族内の個々人で異なった変異をもっている場合などに用いられるが，検出率は配列解析より若干低いと推測されている[1]．

b．重複/欠失解析 duplication/deletion analysis

上記の配列解析では検出できない長い範囲の重複や欠失を検出するためのもので，定量的PCRやmultiplex ligation-dependent probe amplification（MLPA）法に代表される手法である．2003年頃からHHTの検査にも適用され出し，配列解析に加えて用いると約10%分検出率が上昇すると報告されている[6]．

HHT全体に占める各遺伝子変異の寄与割合と遺伝子毎の各検査検出割合を表5に示す．

臨床的にHHTと診断され，かつ検査により変異が検出された症例の大半が *END*，*ALK-1* のいずれかの変異であり，若干 *ENG* の方が多い．両者に変異を認めない症例の約10%に *SMAD4* の変異が検出される[1]．

3．検査で得られた突然変異の，家系調査などによる意味づけ

endoglinおよびALK-1遺伝子の変異の種類は，新たな変異の報告により年々その数は増加しており，HHT Mutation Database（http://www.hhtmutation.org）

表5　HHTの検出に用いられる遺伝子検査と検出割合（文献1より改変）

遺伝子	HHT全体に占める変異の割合	検査法	遺伝子毎の検出割合
ENG	39～59%	配列解析	～90%
		重複/欠失解析	～10%
ALK1	25～57%	配列解析	～95%
		重複/欠失解析	～5%
SMAD4	1～2%（*ENG*，*ALK-1* に変異認めないHHTで約10%に検出）	配列解析	不明
		重複/欠失解析	不明

において 2010 年末現在，前者が 397 種，後者が 332 種も登録されている．発端者を多数調査した最近の報告でも新規の突然変異は 30%を占めている[7]．人口流入が少なく HHT 有病率の高い地域では founder effect，すなわち同じ変異が高い確率で認められることもあるが，多くの場合同じ地域内でも複数の変異が検出され，地域での遺伝疫学調査を難しいものにしている[8]．親に変異がない de novo の変異がみつかるなど，同じ家系内ですら変異の異なる場合がある．

新たな変異がみつかった場合，その変異が疾患の原因となるかどうかを見極めることが重要となる．家系内の健常者と患者とを調査できる場合は分離比分析（segregation analysis）などにより，発症とその遺伝子変異との関連を検証する．その家系で疾患との関連がみられても，後の他の症例や家系を用いた解析によって疾患関連性を否定される場合もある[5,7]．遺伝子変異の意義を予測する以下のようなデータベースサイトがある．

SIFT（http://blocks.fhcrc.org/sift/SIFT.html）
PolyPhen（http://genetics.bwh.harvard.edu/pph/）
NetGene2（http://genome.cbs.dtu.dk/services/NetGene2/）
BDGP（http://www.fruitfly.org/seq_tools/splice.html）

これらは完璧なものではなく，当然のことながらサイトよって結果が異なることもある．最終的な判定には症例家系調査の積み重ねが重要である[5,7]．これらの蓄積により，前述の HHT Mutation Database に登録されている *ENG* の変異 397 種のうち，10%（40 個）が疾病とは関連しない単なる遺伝子多型(polymorphism)，7%（28 個）が意義不明（unknown significance）と暫定的に判定されている．同様に *ALK-1* の変異 332 種のうち，9%（31 個）が遺伝子多型，9%（29 個）が意義不明と判定されている．

4．わが国における遺伝疫学の調査

1976 年に徳島での 5 家系 15 名の症例の臨床遺伝学調査の報告がなされている[9]．その遺伝子変異の報告が 1997 年に行われ[10]，これがわが国で初めてのものと思われる．その当時，海外では endoglin 遺伝子の変異が 9 種報告されていたが，この徳島の家系の変異は *ENG* の exon4 上のコドン 479 番目とこれによるアミノ酸 160 番目の変化（c. 479C>A，p. Ala160Asp）であり，これまで報告されていないものであった．

2002 年には秋田県内の人口約 17 万人の郡部（二次医療圏にほぼ一致）に在住する 7 家系に関する遺伝疫学調査の報告がなされた[8]（図 8）．

この調査の概要は以下の通りである．臨床診断あるいは聞き取り調査により死亡例も含めて HHT32 症例が把握され，生存症例は 23 例であった．うち，肺動静脈奇形を認めたのは半数の 16 例であった（図 8 の＊）．連鎖解析が適用しやすい比較的大きな家系の SB-1 と SB-2 では，連鎖解析により責任遺伝子が 9 番染色体上の HHT1 遺伝子座付近にあるらしいことを確認し〔SB-1 と SB-2 の最大多点 LOD（対数尤度比）スコアがそれぞれ 2.4，1.1〕，次いで 12 番染色体上の HHT2 遺伝子座付近にないことを確認した（LOD スコアがいずれも−2 未満）．続いて配列解析を行い，SB-1 では新規の変異である intron 3 スプライス供与部位の変異とこれによる exon 3 のスキップ（c. 360+1 G>C，p. Gly74_Tyr120del）を認め，SB-2 と SB-3 では新規の変異である exon 7 上の A 挿入とこれによるフレームシフト（c. 828_829insA，p. Tyr277fs）を認め，SB-4 では新規の変異である exon 8 上の 4 塩基対欠失とこれによるフレームシフト（c. 1120_1123delAAAG，p. Lys374fs）を認め，SB-7 では既知の変異である exon 11 上の A の挿入とこれによるフレームシフト（c. 1470_1471insA，p. Asp491fs）をそれぞれ認めた．SB-5 と SB-6 の家系では endoglin 遺伝子の配列解析では変異を認めなかった．当時一般的ではなかった定量的な重複/欠失解析を用いることができたら，あるいは *ALK-1* や *SMAD4* の配列解析を行えば変異を検出できた可能性がある．いずれにしても共通の突然変異は家系 SB-2 と SB-3 でしか認められなかった．しかも後のハプロタイプ分析でこの 2 家系の突然変異は共通の先祖から由来したものと判明している．すなわち，比較的隔離された狭い地域においても共通の突然変異は認められなかった．このことは突然変異が比較的近い世代で起こったことを意味している．また，このことが人口集団での HHT の遺伝疫学調査を困難なものにしている．

その後の遺伝子変異の国内での報告は，関東地方在住で親族関係ではないと思われる 3 人の肺動静脈奇形を伴う HHT 症例の 3 つの変異がある[11]．1 例目で

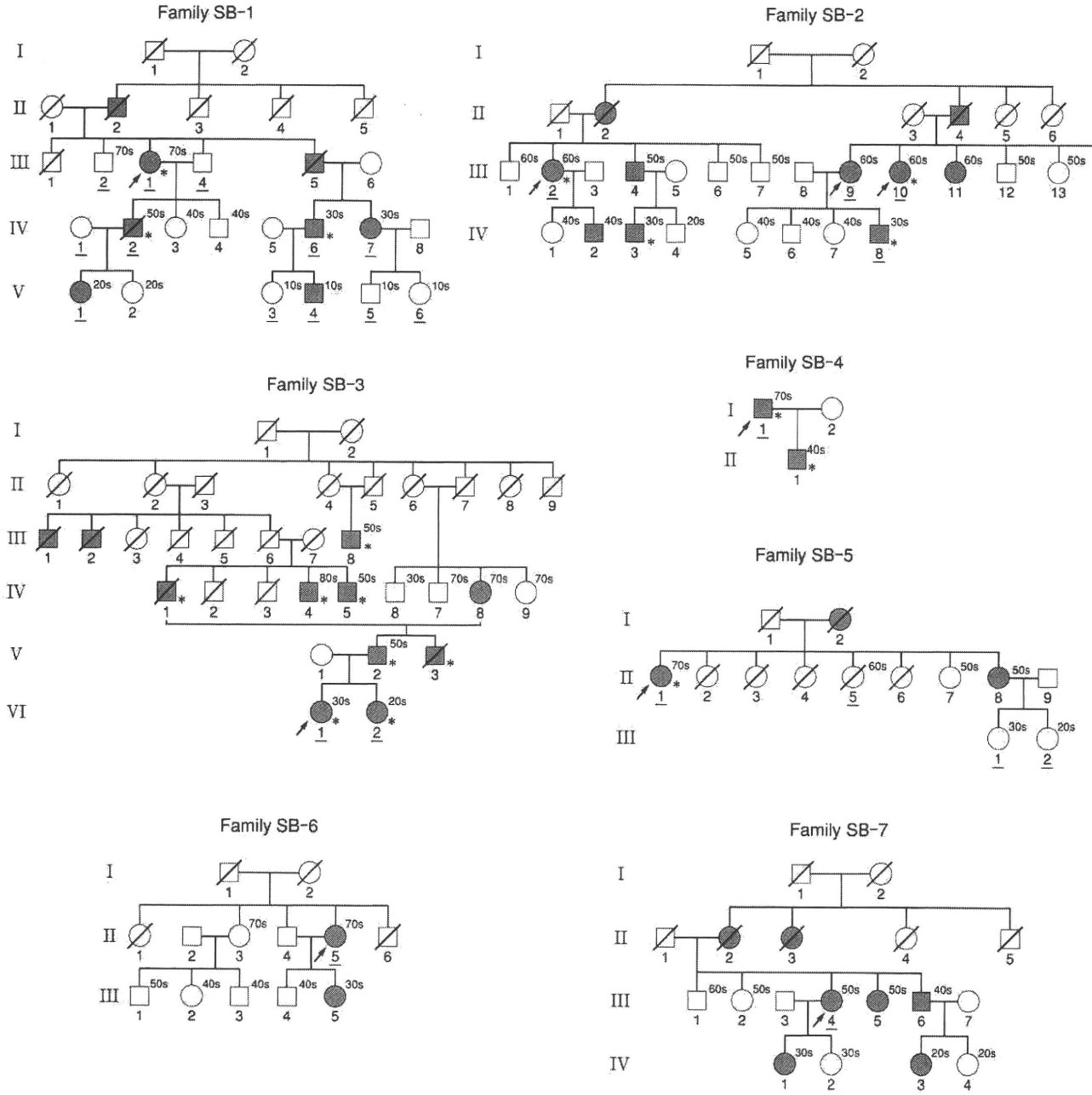

図 8　秋田県一郡内における HHT の 7 家系（文献 8 より改変）
青は HHT 罹患者，矢印は三次医療機関受診者，＊は肺動静脈奇形を有する HHT 症例，
下線は調査参加・遺伝子検査用の血液試料提供者をそれぞれ示す．

は *ENG* の exon 8 上のスプライス不全を起こす既知の変異（c. 1134 G>A, p. Ala378Ala），2 例目では *END* の exon 8 上のフレームシフトを起こす 2 塩基対の欠失（c. 1084_1085delAA, p. Lys362fs）という新規変異，3 例目では *ALK-1* の exon 5 上のミスセンス変異（c. 598C>G, p. Arg200Gly）の新規変異がそれぞれ検出されている．これらの変異に関して家系調査などによる疾患関連性の検討が行われているかは不明である．

SMAD4 の変異のわが国における報告は，複数のがん関連症例と 1 例の JPS 症例ではあるが，HHT 症例ではみあたらない．

以上のように国内での遺伝疫学調査は十分とはいえない状況である．国内で（英文により）報告されている遺伝子変異を表 6 にまとめた．

表 6 国内で（英文により）報告されている HHT 関連の遺伝子変異.

地域	遺伝子	部位	変異の型	遺伝子とアミノ酸の変異	報告
徳島	*END*	Exon 4	ミスセンス変異	c. 479C>A, p. Ala160Asp	新規, 後に欧米でも
秋田県内の郡部（7 家系のうちの 5 家系）	*END*	Intron 3	スプライス供与部位	c. 360+1G>C, p. Gly74_Tyr120del	新規
	END	Exon 7	挿入	c. 828_829insA, p. Tyr277fs	新規
	END	Exon 8	欠失	c. 1120_1123delAAAG, p. Lys374fs	新規
	END	Exon 11	挿入	c. 1470_1471InsA, p. Asp491fs	既知, 欧米で
関東地方？（独立の 3 症例？）	*END*	Exon 8	スプライス不全	c. 1134G>A, p. Ala378Ala	既知, 欧米で
	END	Exon 8	挿入	c. 1084_1085delAA, p. Lys362fs	新規
	ALK-1	Exon 5	ミスセンス変異	c. 598C>G, p. Arg200Gly	新規

5. 有病率

人口集団中の有病率の推定は時代とともにその値が上昇しているようであり，欧米の 1964 年の報告（Tuente ら）[12]では 50,000～100,000 人に 1 人であったが，1992 年報告（Porteous ら）[13]では少なくとも 40,000 人に 1 人，遺伝疫学の調査報告（1998 年 Marchuk ら）[14]ではさらに 10,000 人に 1 人となっている．しかし，重篤な症状をもたない患者は未算入になりがちで，これらの頻度は過小評価されていると考えられている．集積地域での推定は例えばデンマークのフィン島では 1,641～7,246 人に 1 人[15]，カリブ海のオランダ領アンティルでは世界最高頻度の 1,331 人に 1 人と報告されている[16]．

わが国は，古くは前述の徳島での調査において 11,111～50,000 人に 1 人の有病率と推定された[9]．その後も欧米よりも少ないと考えられていたが，前述の秋田県内の調査で必ずしも少なくないことが判明した[8]．この調査では生存症例は 23 人であったが，聞き取り調査できなかった家系内の子孫がいたため，1990 年のわが国の出生率から，30 歳以上の症例は 1 人あたり 2 人の子供をもち，うち 1 人が患者であると仮定しての推計も行った．結果，約 17 万人の二次医療圏内に HHT 症例 23～36 人が存在，すなわち郡内における HHT 患者の有病率は約 8,000～5,000 人に 1 人と推計された．

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〈和田安彦　小泉昭夫〉

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

Claire Levy-Marchal, Silva Arslanian, Wayne Cutfield, Alan Sinaiko, Celine Druet, M. Loredana Marcovecchio, Francesco Chiarelli and on behalf of ESPE-LWPES-ISPAD-APPES-APEG-SLEP-JSPE, and the Insulin Resistance in Children Consensus Conference Group

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

Claire Levy-Marchal, Silva Arslanian, Wayne Cutfield, Alan Sinaiko, Celine Druet, M. Loredana Marcovecchio, and Francesco Chiarelli, on behalf of ESPE-LWPES-ISPAD-APPES-APEG-SLEP-JSPE, and the Insulin Resistance in Children Consensus Conference Group

Institut National de la Santé et de la Recherche Médicale, Unité 690 (C.L.-M., C.D.), Hôpital Robert Debré, 75019 Paris, France; Université Paris Diderot (C.L.-M., C.D.), 75013 Paris, France; Children's Hospital of Pittsburgh (S.A.), University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15224; Liggins Institute (W.C.), University of Auckland, Auckland 1142, New Zealand; Department of Pediatrics (A.S.), University of Minnesota, Minneapolis, Minnesota 55455; and Department of Pediatrics (M.L.M., F.C.), University of Chieti, Chieti 66013, Italy

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.

Conference Participants

1. Shin Amemiyia, Department of Pediatrics, Saitama Medical University, Saitama, Japan. 2. Silva Arslanian, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania. 3. Gerald Berenson, Tulane Center for Cardiovascular Health, New Orleans, Louisiana. 4. Sonia Caprio, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut. 5. Marie-Aline Charles, INSERM, U780, IFR69, Villejuif and University Paris–Sud, Orsay, France. 6. Francesco Chiarelli, Department of Pediatrics, University of Chieti, Chieti, Italy. 7. Stephen Cook, Department of Pediatrics, Division of General Pediatrics, and Strong Children's Research Center, University of Rochester School of Medicine and Dentistry, Rochester, New York. 8. Wayne Cutfield, Liggins Institute, University of Auckland, Auckland, New Zealand. 9. Elizabeth Davis, Department of Endocrinology and Diabetes, Princess Margaret Hospital for Children, Perth, Western Australia, Australia. 10. Larry Dolan, Department of Pediatrics, University of Cincinnati School of Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio. 11. Céline Druet, INSERM, Unité 690, Hôpital Robert Debré, Paris, France. 12. David Dunger, Department of Pediatrics, University of Cambridge, Cambridge, United Kingdom. 13. Anne Fagot-Campagna, Institut de Veille Sanitaire, Département des Maladies Chroniques et Traumatismes, Saint Maurice, France. 14. Carl-Erik Flodmark, Department of Pediatrics, Childhood Obesity Unit, University Hospital, Malmö, Sweden. 15. Earl Ford, Division of Adult and Community Health, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia. 16. Jean-François Gautier, Department of Diabetes and Endocrinology, Saint-Louis University Hospital, AP-HP, and INSERM, Paris, France. 17. Elizabeth Goodman, Center for Child and Adolescent Health Policy, MassGeneral Hospital for Children, Harvard Medical School, Boston, Massachusetts. 18. Michael Goran, Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California. 19. Morey Haymond, Department of Pediatrics, Division of Diabetes and Endocrinology, Texas Children's Hospital, Baylor College of Medicine, Houston, Texas. 20. Paul Hofman, Liggins Institute, University of Auckland, Auckland, New Zealand. 21. Anita Hokken-Koelega, Department of Pediatrics, Division of Endocrinology, Sophia Children's Hospital, Erasmus Medical Center, Rotterdam, The Netherlands. 22. Lourdes Ibanez, Endocrinology Unit, Hospital Sant Joan de Déu, University of Barcelona, 08950 Esplugues, Barcelona, Spain. 23. SoJung Lee, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania. 24. Claire Levy-Marchal, INSERM, Unité 690, Hôpital Robert Debré, Paris, France. 25. Claudio Maffeis, Regional Center for Juvenile Diabetes, University of Verona, Verona, Italy. 26. M. Loredana Marcovecchio, Department of Pediatrics, University of Chieti, Chieti, Italy. 27. Veronica Mericq, Institute of Maternal and Child Research, Faculty of Medicine, University of Chile, Santiago, Chile. 28. Boyd Metzger, Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois. 29. Svante Norgren, Department of Pediatric Medicine, Karolinska University Hospital, Stockholm, Sweden. 30. Ken Ong, Medical Research Council Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom. 31. David Pettitt, Sansum Diabetes Research Institute, Santa Barbara, California. 32. Mary Rudolf, University of Leeds and Leeds PCT, Leeds, United Kingdom. 33. Jeffrey Schwimmer, Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, University of California, San Diego School of Medicine, San Diego, California. 34. Alan Sinaiko, Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota. 35. Julia Steinberger, Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota. 36. Ram Weiss, Department of Human Metabolism and Nutrition, Braun School of Public Health, Hebrew University School of Medicine, Jerusalem, Israel. 37. Chittaranjan Yajnik, Diabetes Unit, KEM Hospital, Pune, India.

Acknowledgments

Address all correspondence and requests for reprints to: Prof. Francesco Chiarelli, M.D., Ph.D., Department of Pediatrics, University of Chieti, Via dei Vestini, 5, Chieti 66013, Italy. E-mail: chiarelli@unich.it.

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