B 統計学的解析

心血管危険因子/アディポカインを従属変数，アンケート項目値を独立変数として単回帰分析を行い，有意であったものについて重回帰分析を行った．変数のうち対数正規分布を示す変数については，対数変換後統計学的解析を行った．

C 結果

研究参加者の特徴を表1に示した．運動系部活動参加率および運動時間は男子に比し女子は有意に低かった．一方，総エネルギー摂取量は女子が有意に低く，食物繊維摂取量は女子が有意に高かった．

表1 研究参加者の特徴（文献3より改変）

	男子 (n = 337)	女子 (n = 442)	p value
年齢（歳）	16.5 (0.8)	16.7 (0.9)	0.01
身長（cm）	170.5 (6.1)	158.3 (5.3)	< 0.0001
体重（kg）	60.8 (10.3)	51.1 (6.5)	< 0.0001
Body mass index	20.9 (3.1)	20.4 (2.3)	0.01
腹囲（cm）	72.3 (8.2)	71.2 (5.9)	0.03
収縮期血圧（mmHg）	116 (10)	106 (10)	< 0.0001
拡張期血圧（mmHg）	63 (9)	62 (9)	0.02
総コレステロール（mg/dL）	161 (28)	174 (27)	< 0.0001
LDL コレステロール（mg/dL）	89 (24)	96 (23)	< 0.0001
HDL コレステロール（mg/dL）	59 (12)	65 (13)	< 0.0001
LDL/HDL 比	1.55 (0.54)	1.53 (0.50)	0.65
中性脂肪（mg/dL）*	55 (52, 58)	53 (50, 55)	0.23
空腹時血糖（mg/dL）	88 (7)	86 (6)	< 0.0001
インスリン（μU/mL）*	6.1 (5.7, 6.5)	6.7 (6.2, 7.0)	0.07
HOMA-IR*	1.33 (1.23, 1.41)	1.40 (1.31, 1.47)	0.26
心血管危険因子数	0.7 (0.9)	0.6 (0.8)	0.11
アディポネクチン（μg/mL）	10.5 (4.0)	12.0 (4.6)	< 0.0001
レプチン（ng/mL）	1.46 (1.35, 1.58)	5.94 (5.61, 6.28)	< 0.0001
高感度 CRP（ng/mL）	146 (129, 164)	96 (88, 106)	< 0.0001
出生時体重（g）	3154 (425)	3053 (407)	0.0009
母乳期間（month）	7.3 (5.5)	7.8 (5.2)	0.29
生活習慣			
運動系部活動参加率（%）	62	35	< 0.0001
運動時間（1日平均，min）*	53 (5)	21 (7)	< 0.0001
TV 視聴時間（1日平均，min）*	96 (2)	91 (3)	0.21
朝食の毎日摂取率（%）	96	92	0.055
食事習慣			
総エネルギー摂取量（kcal/day）	2229 (524)	1827 (455)	< 0.0001
食物繊維摂取量（g）	9.1 (3.0)	8.5 (2.9)	0.004
1000kcal 当たりの食物繊維量	4.2 (1.2)	4.7 (1.4)	< 0.0001

データは平均値（標準偏差）で表してある．

*：対数正規分布を示す変数（中性脂肪，インスリン，HOMA-IR，レプチン，高感度 CRP，運動時間，TV 視聴時間）については対数変換後，有意差検定を行った．これらの変数は平均値（95%信頼限界）で表した．

HOMA-IR: homeostasis model assessment of insulin resistance

表2　高校生および両親の生活習慣が高校生の心血管危険因子値に与える影響（文献3より改変）

	高校生の心血管危険因子値												
	BMI	Waist	SBP	HDL	LDL	ln(TG)#	FPG	ln(Insulin)#	ln(HOMA)#	No of risks	AN	ln(Leptin)#	ln(CRP)#
男子（n＝337）													
運動系部活動$	−‼	−	−2.40*	3.64***	−	−2.67**	−	−4.58***	−2.95**	−4.67***	2.36*	−3.75***	−
Ln（運動時間）#	−	−	−	−	−	−	−	−	−	−	−	−	−
ln（TV視聴時間）#	−	−	2.58*	−2.02*	−	−	−	−	−		−	−	−
毎日の朝食摂取¶	−2.70**	−3.06**	−	−	−	−	−	−	−	−	−	2.03*	−
総エネルギー摂取量	3.08**	2.96**	2.35*	−	−	−	−	−	−	−	−	−	−
Fiber/1000kcal	−	−	−	−	−	−	−2.47*	−	−	−	−	−	−
父のBMI	5.01***	4.58***	−	−	−	−	−	2.57*	2.14*	−	−	3.10**	2.47*
Ln（父の運動時間）#	−	−	−	−	−	−	−	−	−	−	−	−	−
母のBMI	3.58***	3.08**	−	−	−	−	−	−	−	−	−	−	−
Ln（母の運動時間）#	−	−	2.44*	−	−	−	−	−	−	−	−	−	−
女子（n＝442）													
運動系部活参加	−	−	−	4.66***	−	−2.55**	−	−2.27*	−2.20*	−	2.60**	−3.53***	−
ln（運動時間）#	−	−	−	−	−	−	−	−	−	−	−	−2.06*	−
ln（TV視聴時間）#	−	1.98*	−	−2.59*	−	2.09*	−	−	−	−	−2.52*	3.14**	−
総エネルギー摂取量	−	−	−	−	−2.93**	−	−	−	−	−	−	−	−
Fiber/1000kcal	−	−	−	−	−	−	−2.23*	−4.07***	−4.14***	−	−	−	2.21*
父のBMI	−	−	−	−	−	−	−	−	−	−	−	−	−
母のBMI	3.95***	2.83**	−	−	−	−	−	−	−	−	−	−	−

表内の数値は重回帰分析でのt値を示している．
‼：単回帰もしくは重回帰分析において有意でなかった変量については(−)で示してある．
AN: アディポネクチン（μg/mL），BMI: body mass index（kg/m²），CRP: 高感度CRP（ng/mL），FPG: fasting plasma glucose（mg/dL），Fiber/1000kcal: 1000kcal当たりの食物繊維摂取量（g），HDL: HDLコレステロール（mg/dL），HOMA: homeostasis model assessment of insulin resistance, LDL: LDLコレステロール（mg/dL），SBP: systolic blood pressure（mmHg）
*P < 0.05，**P < 0.01，***P < 0.001
#：対数正規分布を示す変数（中性脂肪，インスリン，HOMA＝IR，レプチン，高感度CRP，運動時間・TV視聴時間）については対数変換後統計学的解析を行っている．これらの変数はln（変数）として表した．
$：アンケートは運動系部活動に（1: 参加している，0: 参加していない）という設問にした．
¶：朝食摂取については（1: ほとんど食べない，2: 時々食べる，3: 毎日食べる）という設問にした．

高校生および保護者の生活・食習慣が心血管危険因子/アディポカイン値に及ぼす影響について重回帰分析の結果を表2に示した．高校生の運動系部活動への参加は男女ともに，特に男子においては心血管危険因子値に大きな影響を与えていた．男子では朝食の習慣をつけることがBMIや腹囲と有意な関係があり，女子では高い食物繊維摂取が血糖値，インスリン値，HOMA-IR値の低値と強い関係を認めた．

表3　高校生の肥満の有無に与える保護者の影響（文献3より改変）

	男子		女子	
	オッズ比（95% CI）	p値	オッズ比（95% CI）	p値
父が肥満	2.47（1.28, 4.77）	0.007	1.09（0.44, 2.70）	0.86
母が肥満	1.74（0.71, 4.26）	0.23	3.00（1.13, 8.00）	0.03
両親とも肥満	6.36（1.86, 21.8）	0.003	2.72（0.56, 13.2）	0.21

保護者の肥満は日本の基準によりBMI ≧ 25kg/m² としてある[6]．したがって，高校生の肥満の定義はInternational Obesity Task Force standardのBMI 25kg/m² に相当する値以上としている[7]．CI: 信頼限界

表4 思春期（高校生）の生活習慣病予防に関する提言―ガイドライン策定に向けて―

1. 運動習慣を身につけよう
   - 可能なら運動系部活に参加しよう
   - 運動系部活に参加していない場合は，休日に60分以上運動しよう
   平日は学校で結構運動しています．春休み，夏休み，冬休み，あるいは休日に肥満になりやすいものです．休日の運動量を増やしましょう．
2. テレビやテレビゲームから離れよう
   - 平日は1日合計50分以内，休日は1日合計100分以内に，テレビ（テレビゲームも含みます）から離れよう，テレビを消そう
3. よい食習慣を身につけよう
   - 朝食を毎日とろう
   - 食物繊維を積極的に摂取しよう
4. 腹囲が80cmを超えたら，医療機関に相談しよう
   肥満（内臓肥満）は生活習慣病の源流にあります．肥満治療や生活習慣病指導が行える医療機関を本人あるいは保護者に紹介してください．日本肥満学会「認定肥満症専門病院リスト」http://wwwsoc.nii.ac.jp/jasso/data/pdf/hplist.pdf も参考になると思います．

保護者の肥満が高校生の肥満に与える影響には性差を認め，父が肥満であると高校生が肥満であるオッズ比は男子にのみ有意に高く，母が肥満であると，高校生が肥満であるオッズ比は女子にのみ有意に高かった（表3）．

むすび

このような結果を踏まえ，表4のような提言を行っている．今後prospective studyを行い，これらにより心血管危険因子値の改善がみられるか検討を続けていきたい．

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〈吉永正夫〉

脂肪肝・非アルコール性脂肪性肝障害

従来から，脂肪肝（fatty liver: FL）は肥満の合併症としてよく知られている．小児期のFLの多くは無症状で，血液検査や腹部超音波検査，腹部CT検査を契機として偶然発見される場合が多い．以前は，肥満に合併するFLは減量すれば速やかに改善し予後良好と考えられてきたが，病理学的検討から，小児期でも脂肪性肝炎から肝硬変に進行する例もあることが明らかとなり，適切な介入と慎重な経過観察が必要である．FLは，メタボリックシンドロームの病態と関係が強く，日本人を含むアジア人に高頻度で発生し，近年増加傾向にあることから，特に注目されている．

A 脂肪肝・非アルコール性脂肪性肝障害・非アルコール性脂肪性肝炎の定義

近年，世界的な肥満や2型糖尿病（type 2 diabetes mellitus: T2DM）の増加に伴って，過栄養性のFLが増加している．FLとは，肝細胞内に中性脂肪が過剰に蓄積した状態を指し，組織学的には肝小葉の1/3以上に脂肪滴が沈着している場合をいう．

過栄養性FLの中には，飲酒歴がないにも関わらず，アルコール性肝障害に類似した組織像を呈す例があり，非アルコール性脂肪性肝障害（non-alcoholic fatty liver disease: NAFLD）と呼ばれている[1]．NAFLDと診断するためには，種々のウイルス性肝炎や自己免疫性肝炎，代謝疾患に伴う肝障害などを除外する必要がある．NAFLDは，単純性脂肪肝から，脂肪性肝炎，肝線維症，肝硬変までを含む幅広い概念で，FLに炎症所見や肝細胞の変成，線維化が加わった場合を非アルコール性肝炎（non-alcoholic steatohepatitis: NASH）という[2]．NASHはNAFLDの重症型であり，診断には肝生検が必要である．

B NAFLD・NASHの疫学

我が国の一般成人におけるNAFLDの頻度は約10%程度と推定されており，肥満者やT2DM者におけるNAFLDの頻度はより高率である．

小児FLについてTominagaらは，4～12歳の810名を対象として腹部超音波検査を行い，男児の3.4%，女児の1.8%にFL所見が認められ，肥満が高度な学童にFLが多かったと報告している[3]．また，Tazawaらは6～11歳の肥満小児310名を対象として，FLを反映する高ALT血症の頻度を検討し，肥満小児の約25%に高ALT血症が認められ，肥満の持続期間が長いほど高ALT血症の頻度が増加していたと報告している[4]．

NASHの診断には肝生検が必要であり，小児期NASHの頻度に関する大規模疫学調査報告はない．Kinugasaらは，肝機能障害のある肥満小児11例に肝生検を行い，単純性FLはわずか3例のみで2例は脂肪性肝炎，5例は脂肪性肝線維症，1例は肝硬変であったと報告している[5]．乾らは，30名のNAFLD小児に肝生検を施行し，うち9例（30%）はNASHであったと報告している[6]．このように，小児のNASHは想像以上に高頻度であり，肝硬変まで進行している例も散見される

ため，決して軽視できない肥満合併症である．

C NAFLD・NASHの病因・病態

NAFLDやNASHの病因として，はじめに脂肪肝が生じ，その後に脂肪性肝炎に移行するという，Two hit hypothesisが，以前から最も支持されている[7]（図1）．肥満，特に過剰な内臓脂肪蓄積は，インスリン抵抗性を惹起させ肝細胞に過剰な脂肪蓄積を招く（1st hit），その後，過酸化脂質や酸化ストレス，エンドトキシンなどの肝細胞障害因子が2nd hitとなってNASHに進展するという考え方である．

しかし近年，幼児期からすでに肥満にNAFLDを合併した例も報告されており，遺伝的素因や子宮内環境と出生後の環境のミスマッチ（いわゆるDOHaDの概念）もNAFLDやNASHの発症に関与している可能性もある[8]．

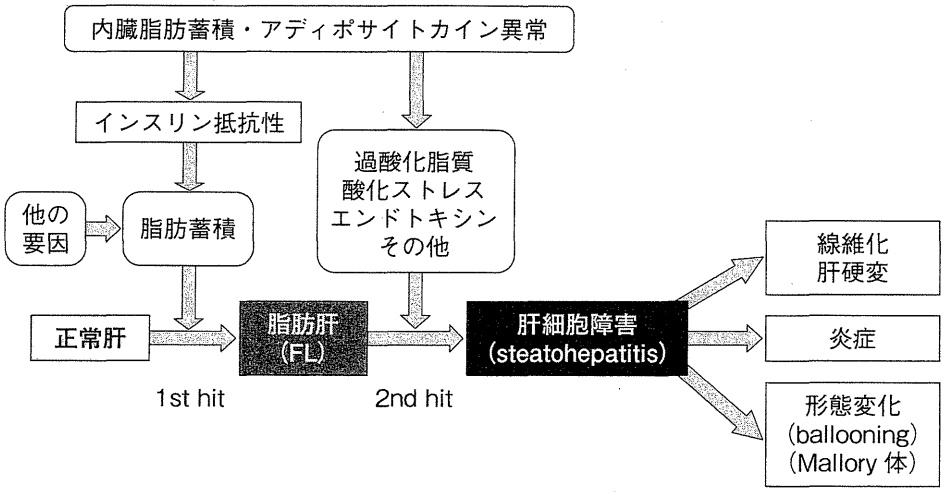

図1　NASHの病因論（Two hit hypothesis）

D NAFLDやNASHの診断

NAFLDやNASHは無症状の場合も多く，たとえ症状があっても，倦怠感や，上腹部不快感，軽度の腹痛などの非特異的な症状の場合が多い．NAFLDやNASHの合併が疑われる診察所見としては，肝臓の腫大や肝臓部分の叩打痛，頸部の黒色表皮症（achanthosis nigricans: AN）があげられる．肥満小児に認められるANは内臓脂肪蓄積に伴う高インスリン血症やインスリン抵抗性を反映しており，ANが認められる肥満小児はメタボリックシンドロームやNAFLD・NASHの合併が多い[9]．

NAFLDやNASHの診断には，アルコール摂取歴がないことと，FLをきたしうる他の肝疾患の除外が必要である．近年，思春期小児のアルコールアビュースも問題となっており，思春期以降の若年者の診察の際には20歳前でもアルコールを摂取している可能性もありうるという立場で診察に当たるべきである．また，FLの鑑別診断として，栄養障害，ウイルス感染症，代謝性疾患，自己免疫性疾患，薬剤性などに起因する肝障害を否定しなければならない[10]．表1に小児期からFLを生じうる疾患群を示す．

表1 小児期に脂肪肝を生じうる疾患群（文献 10 より改変）

全身性疾患/栄養障害
- 肥満，メタボリックシンドローム
- 急性疾患（脱水症，重症感染症）
- 飢餓
- クワシオルコル
- celiac 病
- 完全静脈栄養法に関連したもの
- その他

感染症
- C 型肝炎
- EB ウイルス感染症

自己免疫性疾患
- 自己免疫性肝炎

代謝性疾患
- シトリン欠損症
- Wilson 病
- αアンチトリプシン欠損症
- ガラクトース血症
- 遺伝性果糖不耐症
- 糖原病
- オルニチントランスカルバミラーゼ欠損症
- リポジストロフィー
- βリポ蛋白欠損症
- 糖尿病，他

薬剤性
- アミオダロン
- メトトレキサート，L-アスパラギナーゼ
- ステロイド，ビタミン A，エタノール，他

上述したように，NAFLD や NASH の合併があっても特異的な症状に乏しいため，血液検査や画像診断で NAFLD や NASH の存在に気付かれる場合が多い．一般に，血液検査では ALT 優位の肝逸脱酵素の上昇が認められる．ただし，肝硬変まで進行すると AST 優位になることに留意する必要がある．腹部超音波検査では，肝臓の肥大と，肝実質の点状高エコー（bright liver），肝臓深部のエコー減衰（deep attenuation），肝内脈管の不鮮明化，肝腎コントラストの増強などの所見が，腹部 CT 検査では，肝実質の CT 値の低下（肝臓の CT値/脾臓の CT 値＜ 0.9）が認められる．

NAFLD の診療で一番問題となるのは，NASH に進行しているか否かの判断である．現時点では，NASH を診断する有益な血液検査指標はなく，NASH への進行の有無を判断するには肝生検が必要である．肝生検は侵襲的な検査であるため，成人における肝生検を考慮すべき指標[11]などを参考にして個別に対応する（表 2）．

NASH の診断は組織学的所見でなされる．NASH は進行性であるため活動性と進行度の診断に Brunt の分類が汎用されている．最近，小児期 NASH の中には，成人 NASH と組織学的に異なるパターンを呈する者が多いことが明らかとなった[12]（表 3）．“Pediatric type” の NASH は，肥満が高度な有色人種の男児に多いと報告されている．

表2 肝生検を考慮すべき症例（文献 11 より改変）

① ALT が高値（100IU/L 以上）で増悪傾向にある
② NASH の危険因子を有する
（内臓脂肪蓄積，糖代謝異常，脂質異常症，高血圧，メタボリックシンドローム，高カロリー輸液，小腸手術後，急激な体重減少・飢餓など）
③ 肝硬変への進行が疑われる
（肝機能低下，AST ＞ ALT，血小板減少，線維化マーカーの上昇）
④ 食事運動療法で改善が認められない
⑤ 脂肪肝を生じる他疾患（特に代謝性疾患との鑑別）との鑑別が困難

表3 小児期 NASH の組織像の相違点

	Pediatric-type NASH	Adult-type NASH
小児における頻度	多い	少ない
脂肪変性	強い	弱い
炎症性細胞浸潤	門脈域が主体	小葉内が主体
肝細胞の ballooning	認めない	認める
線維化	認めないかあっても門脈域	類洞周辺か小葉中心周囲
肝硬変	あり	あり

E NAFLD や NASH の予後

成人の NASH は進行性で，肝線維化から肝硬変へ，さらには肝細胞癌が発症する場合があることが報告されている．小児期 NASH の予後はまだ明らかになっていない点も多いが，肝硬変を呈する例が報告されており，小児期 NASH も進行性であると考えられる．むしろ，小児期に NASH を発症した者は，肥満の治療成績が芳しくないことや NASH の病態が成人発症例よりも長期的に継続する可能性が高いことを考慮すると，成人の NASH より予後が悪い可能性も否定できない．乾らは，小児の NAFLD 例は，治療途中で脱落する者や再燃する者が 93% を占めており，社会的予後はきわめて悪いと報告している[6]．

F NAFLD や NASH の治療

肥満に伴う NAFLD や NASH の治療の第一段階は，肥満の是正である．小児肥満治療の原則は，正常な成長発達を妨げることなく，肥満に伴う合併症や肥満の程度を改善させることである．したがって，強いエネルギー制限は行わず，性別年齢別の推定エネルギー必要量の 90% 程度のエネルギー制限を行う．三大栄養素のバランスは，糖尿病食に準じ，単純糖質や高果糖コーンシロップに代表される異性化糖を制限し，グリセミックインデックスの小さい複合糖質の摂取を勧める．NAFLD や NASH を合併する肥満小児の多くは身体活動が不十分である．適度な運動は，ストレス解消や，インスリン感受性の改善効果があるため，運動系部活動や地域のスポーツ活動に参加するようにアドバイスする．さらに肥満改善には，生活リズムの適正化がきわめて重要であり，夜更かしをしないようにして，起床したら朝日を浴びて必ず朝食を摂取するように指導する．指導が奏効して肥満が改善すると，血液検査における肝逸脱酵素の上昇や画像診断における FL 所見は改善する場合が多いが，高度肥満例や，肥満の経過が長期にわたる思春期の肥満例は，肥満治療の成績が悪いため NAFLD や NASH も改善しにくい．

NAFLD や NASH に対する薬物用法としては，ビタミンＥやビタミンＣなどの抗酸化薬や，ウルソデオキシコール酸，ポリエンホスファチジルコリンなどの肝庇護剤が用いられる場合もある[13]．成人 NASH に対してはメトホルミンやチアゾリジン誘導体などのインスリン抵抗性改善薬が使用されている．表4に小児期の NASH や NAFLD に対する薬剤を示す．FL に保険適用があるのはポリエンホスファチジルコリンのみである点に注意を要する．小児ではビタミンＥやメトホルミンが使用される場合が多く，我々も T2DM に合併した NAFLD にメトホルミンが著効した

表4 小児期 NAFLD・NASH の薬物療法

	薬剤名	商品名	用量
インスリン抵抗性改善薬	メトホルミン	メルビン錠（1 錠 200mg）	2〜3 錠　分 2〜3
	チオゾラジン誘導体	アクトス錠（1 錠 15mg）	1〜2 錠　分 1
抗酸化薬	ビタミン E	ユベラ錠（1 錠 50mg）	3〜6 錠　分 2〜3
	ビタミン C	シナール配合顆粒（1 包 200mg）	3〜9 包　分 1〜3
肝庇護剤	ウルソデオキシコール酸	ウルソ錠（1 錠 100mg）	3〜6 錠　分 3
	ポリエンホスファチジルコリン	EPL カプセル（1Cap 250mg）	3〜6 錠　分 3
漢方薬	大柴胡湯	ツムラ大柴胡湯エキス顆粒	7.5g　分 2〜3　食前

例を経験しているが，メトホルミンの有効性に関する大規模臨床試験は現在施行中である[14]．

むすび

FL は，小児肥満によくみられる合併症である．NAFLD は，メタボリックシンドロームの病態を反映している点が重要で，一部は小児期から NASH や肝硬変に移行するため予後は楽観できない．

現在の NAFND・NASH 診療の問題点は，NASH 診断のために有益な検査法が肝生検以外にないことや，大規模疫学調査結果に裏付けされた有効な治療法が明らかになっていないことである．現状では，NASH・NAFLD の温床となる肥満やメタボリックシンドロームそのものの予防対策がきわめて重要なことは論を待たないが，メタボリックシンドロームの病態を有し肥満治療に抵抗する肝障害が持続する例では，NASH の有無や進行度を評価するために肝生検を考慮すべきである．

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〈原　光彦〉

門脈圧亢進症

portal hypertension

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病態

小児の門脈圧亢進症の原因として，肝内性では，胆道閉鎖症，肝内胆汁うっ滞症，代謝性疾患，先天性肝線維症がある．肝前性には肝外門脈閉塞症があり，食道静脈瘤からの吐下血，脾腫で発見されることが多い．肝後性では慢性うっ血性心不全，Budd-Chiari(バッド-キアリ)症候群などがある．

治療方針

胃食道静脈瘤からの出血，脾機能亢進に伴う汎血球減少や腹水に対する治療，予防が重要である．腹部超音波，腹部造影CT，胃食道内視鏡検査などを定期的に行い，管理法を変更する．

A 静脈瘤出血の治療

1. 出血性ショック　酸素吸入を行いながら乳酸リンゲル20 mL/kgを急速静注する．さらに輸血，胃チューブ挿入による胃洗浄を行う．

2. 静脈瘤出血の止血　Sengstaken-Blakemore tubeを挿入する．食道バルーン圧は30～40 mmHgとし，圧迫による壊死を防ぐため，留置は18～24時間以内にとどめる．出血部位の内視鏡的静脈瘤結紮術も可能であれば行う．制酸薬としてH_2受容体拮抗薬を用い，凝固異常があれば血小板，新鮮凍結血漿を輸血する．

℞ 処方例

1) ガスター注　1 mg/kg/日(最大40 mg/日)　20 mgを生理食塩液20 mLに溶解し，緩徐に静注あるいは輸液に混合して点滴静注

門脈血流を減少させ，門脈圧を下げるために合成バソプレシン2)を投与する．

2) ピトレシン(20単位/mL)　5%ブドウ糖液100 mLに混和して0.01 U/kg/分で持続静注

投与中は血圧低下に注意し，血圧，心電図，尿量のモニターを行う．肝血流低下による肝機能の不可逆的悪化にも注意し，減量・休止時期を判断する．

B 静脈瘤からの出血予防

内視鏡的静脈瘤硬化療法が考慮される．肝機能異常が高度でなければ，食道離断術，シャント手術なども選択されるが，肝移植の可能性が高い場合には，開腹術は避けたほうがよい．

C 脾機能亢進症

肝移植術への影響や重症感染症合併があるため，脾摘出術を避け，部分的脾動脈塞栓療法などが選択される．

D 肝内性門脈圧亢進症

吐下血を繰り返す場合あるいは重症の肝中心静脈閉塞症では肝移植の適応となる．

脂肪肝，NASH

simple steatosis, nonalcoholic steatohepatitis

原　光彦　都立広尾病院・小児科部長

病態

肝細胞に中性脂肪が過剰に蓄積した状態を脂肪肝と呼び，飲酒習慣がないのにアルコール性肝障害に類似した肝病理組織所見を示す例を非アルコール性脂肪性肝疾患(nonalcoholic fatty liver disease：NAFLD)という．NAFLDは，単純性脂肪肝(simple steatosis：SS)と炎症・線維化を伴う非アルコール性脂肪性肝炎(nonalcoholic steatohepatitis：NASH)に大別され，NASHの一部は肝硬変に進行する．小児にもNAFLDは存在し，多くの例は肥満に合併する．しかし，代謝疾患や中心静脈栄養に伴う例もある．

A 成因

NAFLDの成因としてtwo hit theoryが提唱されている．肥満や糖代謝異常によってSSが生じ(first hit)，炎症性サイトカインや酸化ストレスなどがsecond hitとなってNASHに至ると考えられている．

B 診断

画像診断で脂肪肝を示唆する所見があり，ウイルス肝炎や自己免疫性肝炎，代謝疾患，アルコール性肝炎などが除外された場合に臨床的NAFLDと診断する．SSとNASHの鑑別には肝生検が必要で，治療抵抗例や代謝疾患との鑑別困難例には生検を行う．

治療方針

肥満を伴う例は肥満治療を優先する．食事運動療法が無効な例や2型糖尿病合併例には薬物療法も考慮する．小児期NASHに対する薬物療法のエビデンスは十分ではないが，抗酸化薬は有効とする報告が多い．病態に応じて下記肝庇護薬と血糖降下薬の併用可．

℞ 処方例　肥満に伴うNAFLDには下記の薬剤を症状に応じて適宜用いる．

(抗酸化薬)

1) ユベラ錠(50 mg)　3～6錠　分3　食後

(肝庇護薬)　2)，3)のいずれかを用いる．

2) ウルソ錠(100 mg)　3～6錠　分3　食後

3) EPLカプセル(250 mg)　3～6カプセル　分3　食後

(血糖降下薬)　2型糖尿病合併例には4)を用いる．

4) メルビン錠(250 mg)　2～3錠　分2～3　食後

(漢方薬)

5) ツムラ大柴胡湯エキス顆粒　7.5 g　分2～3　食前

胆石，胆嚢炎

cholelithiasis and cholecystitis

鍵本聖一　埼玉県立小児医療センター・総合診療科部長

病態

コレステロール結石は胆汁内のコレステロールの過飽和により生じ，結晶コレステロール，蛋白，ビリルビン，炭酸塩で形成される．ビリルビン結石は溶血や静脈栄養に伴うことが多く，混合石は小児胆石の約3%を占め，胆汁うっ滞や胆道感染に続発し，胆嚢より胆管に形成されやすい．石は胆嚢粘膜を刺激し，慢性胆嚢炎や疝痛発作をきたす．胆嚢管の閉塞で急性胆嚢炎を起こし，胆嚢腫大や壊死，胆汁漏出をきたす．石が胆管に排出されれば，胆管結石，胆管炎，胆汁うっ滞，急性膵炎を惹起しうる．このほか肝硬変と慢性肝疾患，Wilson(ウィルソン)病，セフトリアキソンの長期投与，Rett(レット)症候群，心臓移植後に，コレステロール結石では肥満，回腸切除や回腸クローン，嚢胞性線維症，低βリポ蛋白血症に伴うことがある．無症候性胆石では合併症リスクは低い．超音波の胆石発見率は98%であり，感度特異度とも高い．

小児急性胆嚢炎は成人よりもまれであり，多くが11歳以上の年長児に生じる．原因は溶血性血液疾患，先天奇形，感染，中心静脈栄養，回腸切除後，短小腸症候群などである．小児無石胆嚢炎は全小児急性胆嚢炎の約2～15%であり非常にまれである．原因は，重度熱傷，大手術後，代謝性疾患，骨髄移植後などがある．

治療方針

A 症候性胆石

症状を伴う場合には胆嚢摘出を行う．繰り返す痛み発作には手術を行うが，急性胆嚢炎を合併する場合には，保存的に2～3か月待機することもある．全身状況が許さない状況では胆嚢切開，結石摘出のみ行うこともある．

腹腔鏡下手術の適応は症候性胆石と溶血性疾患をもつ胆石症で，侵襲が少ない．合併症は1%未満である．

コレステロール結石では胆汁酸製剤内服が選択肢となる．小結石では60%で溶解するが，中止すると再発が多い．長期投与の必要から，適応は限られる．

℞ 処方例　12歳女児，コレステロール結石の場合．

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Significant Associations Among Hemostatic Parameters, Adipokines, and Components of the Metabolic Syndrome in Japanese Preschool Children

Hitoshi Horigome, Yasutomi Katayama, Masao Yoshinaga, Yoshiaki Kato, Hideto Takahashi and Ryo Sumazaki

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Significant Associations Among Hemostatic Parameters, Adipokines, and Components of the Metabolic Syndrome in Japanese Preschool Children

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Abstract

Development of cardiovascular diseases could originate in early childhood. However, reference values of hemostatic parameters and adipokines in preschool children remain to be explored. We measured blood levels of adipokines and parameters of the hemostatic/fibrinolytic systems in 167 healthy children aged 4 to 6 years at 9:00 to 10:30 AM after a strictly enforced overnight fast. Participants with body mass index (BMI) values ≥90th percentile had significantly higher values of systolic blood pressure and heart rate, as well as blood levels of insulin, coagulation factor (F) VII, FX, protein S, leptin, and homeostasis model assessment of insulin resistance (HOMA-IR), and lower values of desacyl-ghrelin than children with BMI < 90th percentile. Circulating levels of fibrinogen and leptin increased with increased number of cardiovascular risk factors. Stepwise regression analysis identified many hematological variables to be associated with features of the metabolic syndrome. The results implicated the hemostatic/fibrinolytic system or adipokines in the insidious progression of cardiovascular diseases from an early age.

Keywords

cardiovascular risk factor, children, coagulation, fibrinolysis, metabolic syndrome

Cardiovascular disease is the main cause of death worldwide. The development of cardiovascular diseases is multifactorial, including a possible association with the metabolic syndrome.[1] The metabolic syndrome is defined by a constellation of clinical features including visceral or central obesity, insulin resistance, high blood pressure, high triglycerides (TGs), and low high-density lipoprotein cholesterol (HDL-C). However, the correlation among the diagnostic criteria for metabolic syndrome in the pediatric age group and future cardiovascular disease developing in adulthood remains to be investigated,[2] despite the prevailing concept that lifestyle-related diseases sometimes originate in childhood.[3] The early stage of arteriosclerosis can be detected by increased carotid intima–media thickness, and this early marker of arteriosclerosis in childhood is associated with fluctuation of some hematological variables.[4] The prevalence of obesity in children has been increasing over the last 20 years in Japan as in all Western countries.[5-8] The incidence of the metabolic syndrome in Japanese overweight children is comparable to that in US overweight children, and the critical period for the development of obesity is between 5 and 6 years of age.[5,7] Establishing hematological reference values for metabolic syndrome in preschool children would therefore be useful in the overall assessment of the potential effects of intervention.

The hemostatic and fibrinolytic systems as well as adipokines have been implicated in the development of metabolic syndrome,[9-12] although such variables are not included in the diagnostic criteria of the syndrome. While the developmental changes in the hemostatic/fibrinolytic systems during childhood have been studied,[13-15] the available data are mostly for Western children; this is important because the metabolic

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syndrome-related parameters such as insulin resistance show race differences.[16] A full assessment of these variables and any association with the metabolic syndrome criteria remain to be investigated, partly due to the difficulty in morning blood sampling in young children. This study was designed to establish reference values for hemostatic/fibrinolytic variables and adipokines in Japanese preschool children after strict fasting and to determine any relationships with the components of metabolic syndrome.

Methods

Participants

The study comprised 167 preschool children, aged 5 to 6 years (females, 85), who attended kindergarten in Yokohama City or Kagoshima City. Children showing illness at examination including common cold or had history of significant disease were excluded from the study. The Ethics Committee of Human Research of Tsukuba University Hospital approved the study protocol in advance. In addition, parents of the participants attended an instruction lecture about the importance of prevention of metabolic syndrome from early childhood before the commencement of the study. Written informed consent was subsequently obtained from the parents.

Anthropometric and Biometric Assessment

Height and weight were measured using standard methods (TTM-HV; TSUTSUMI Co, Kyoto, and DC-320; TANITA Co, Tokyo, Japan), and the body mass index (BMI) was calculated as weight in kilograms divided by height in meter square. Blood pressure and heart rate were measured 3 times using an automated oscillatory system (TM-2571; A&D Co, Tokyo), between 9 and 10:30 AM, after the participants had rested for at least 10 minutes in a seated position; the reported values represent the average of the second and third measurements.

Blood Sampling and Laboratory Analyses

Blood samples were collected from the antecubital vein in the morning (between 9 and 10:30 AM) after an overnight fast (except for water) and after at least a 15-minute rest immediately before sampling. Parents were required to restrict their children from taking meals or any sugar-containing liquids overnight. Children who consumed food before blood sampling were excluded from the study. The sample was drawn into 3 polypropylene tubes: 1 for serum collection to measure biochemical parameters, adipokines, and soluble thrombomodulin (sTM); 1 containing fluorescein Na, EDTA 2Na, and heparin Na to measure fasting plasma glucose; and 1 containing 1/10 volume of 3.13% sodium citrate to measure the hemostatic/fibrinolytic parameters. The parameters measured in this study are listed in Table 1. The latter 2 tubes were centrifuged, with the resultant plasma samples frozen immediately and then stored at −80°C until assayed. The homeostasis model assessment of insulin resistance (HOMA-IR) represented the product of fasting plasma glucose (mmol/L) and insulin (μIU/mL) levels divided by a constant value of 22.5.

Alanine aminotransferase (ALT), uric acid, TG, total cholesterol, HDL-C, low-density lipoprotein cholesterol (LDL-C), fibrinogen, insulin, high-sensitivity C reactive protein (hs-CRP), and fasting plasma glucose were measured by standard automated methods using the appropriate devices (JEOL, Sysmex, Mitsubishi Chemical Medience, Fujirebio, and Siemens Healthcare Diagnostics, Japan). The sTM was measured by enzyme immunoassay ([EIA]; Molecular Devices, Japan); leptin by radioimmunoassay ([RIA]; Aloka, Japan); desacylghrelin, adiponectin, and resistin by enzyme-linked immunosorbant assay ([ELISA]; Mitsubishi Chemical Medience, Otsuka Pharmaceutical Co, and BioVender Laboratory Medicine, Japan). Coagulation factor (F) VII, FVIII, and FX were measured by clotting time methods, and von Willebrand factor (vWF) was assayed by the fixed platelet agglutination method (Siemens Healthcare Diagnostics). Protein C antigen, free protein S antigen, and plasminogen activator inhibitor 1 (PAI-1) in a complex with tissue plasminogen activator 1 (tPA-PAI-1 complex) were assayed by latex photometric immunoassay (Mitsubishi Chemical Medience and JEOL).

Data Analysis

All continuous variables were expressed as mean ± standard deviation (SD), with the 5th, 10th, 50th, 90th, and 95th percentile values calculated for each parameter. Logarithms of the values were also calculated for hs-CRP. The number of data was different among parameters (maximum 167 and minimum 108; see Table 1) because the specimen volume was insufficient to allow all measurements in some children, who needed to be resampled but refused.

The study cohort was divided into 2 groups: those with <90th percentile values of BMI and those with ≥90th percentile. Each continuous variable was compared between the 2 groups using the Student *t* test. Participants were then assigned to subgroups based on the number of the following cardiovascular risk factors: (1) BMI ≥ 90th percentile, (2) blood pressure (systolic or diastolic or both) ≥90th percentile, (3) plasma glucose level ≥ 90th percentile, (4) TG ≥ 90th percentile, and (5) HDL-C ≤ 10th percentile. The hematological parameters were then compared among the subgroups using analysis of variance (ANOVA) followed by a Tukey–Kramer-type multiple comparisons.

The relationships between the hemostatic/fibrinolytic parameters or adipokines and the components of the metabolic syndrome were tested by simple linear regression model, and significant variables were then subjected to stepwise linear regression analysis to identify independent predictors of the metabolic syndrome. A P value less than .05 was considered statistically significant.

Results

Table 1 details the anthropometric, biometric, and hematological data for all participants. Analysis of differences in various

Table 1. Measured Variables[a]

	n	Mean ± SD	Minimum	Maximum	5	10	50	90	95
Age, years	165	5.9 ± 0.6	4.2	6.9	4.58	5.14	5.96	6.46	6.67
Height, cm	167	112 ± 5.9	100	125.8	101.94	104.32	112.6	120	122.84
Weight, kg	167	19.1 ± 3.1	14	32.4	15.14	16.08	18.7	23.12	26.12
BMI, kg/m^2	167	15.1 ± 1.5	12.5	21.9	12.95	13.33	14.98	16.77	17.85
Systolic BP, mm Hg	164	95.4 ± 8.0	78	117	81	86	95	107.17	109
Diastolic BP, mm Hg	164	56.1 ± 9.2	36	82	42	44	54	68	72
Insulin, μIU/mL	165	2.88 ± 1.69	0.3	9.69	1.032	1.27	2.5	4.922	6.213
FPG, mg/dL	165	86.2 ± 7.6	60	106	74	77	86	96	98
TC, mg/dL	165	171.4 ± 23.9	120	251	131.3	138	170	202	209.7
HDL-C, mg/dL	165	62.1 ± 12.6	33	92	42.3	46	61	79	84
LDL-C, mg/dL	165	102.8 ± 19.7	36	166	75	79.2	101	129.8	135
TG, mg/dL	165	44.6 ± 21.2	18	141	21	24.6	40	72.4	80.7
ALT, IU/L	165	13.2 ± 4.9	6	53	9	9	12	18	20
UA, mg/dL	165	4.16 ± 0.63	2.6	5.8	3.2	3.4	4.2	5.1	5.3
sTM, FU/mL	120	3.11 ± 0.55	2	4.9	2.3	2.5	3.1	3.89	4.29
Fbg, mg/dL	122	255.2 ± 58.0	105	455	189	204	239	332.2	383.95
FVII, %	158	90.1 ± 10.3	49	116	72	76	91.5	101	105.05
FVIII, %	115	104.2 ± 23.7	51	177	61.8	73	102	137.4	147.4
FX, %	158	95.9 ± 11.1	68	128	78.95	83	95	113	119
vWF, %	115	92.2 ± 27.7	53	187	57.8	61.6	86	133.8	151
Protein C, %	158	87.0 ± 14.7	46	144	65.95	70	86	107.2	115.1
Protein S, %	115	85.9 ± 15.8	44	125	61.8	66.6	83	108.8	117.2
PAI-1, ng/mL	158	28.0 ± 18.9	10	137	11	13	22	51.3	60.25
Ghrelin, fmol/mL	115	47.8 ± 36.6	13	182	13	13	41	102	116
Adiponectin, μg/mL	162	15.4 ± 5.1	3.1	36.1	7.95	9.19	15	22.07	24.29
Leptin, ng/mL	163	2.23 ± 1.61	0.9	16.4	1.1	1.2	1.9	4	4.96
hs-CRP, ng/mL	152	1477 ± 3478	11	23600	50	59.3	339	3253	6261.5
Ln hs-CRP	152	5.95 ± 1.52	2.40	10.07	3.91	4.08	5.83	8.09	8.74
Resistin, ng/mL	108	4.50 ± 2.54	1	12.4	1.8	2	3.7	8.3	10.22
HOMA-IR	165	0.63 ± 0.40	0.07	2.37	0.21	0.24	0.52	1.15	1.40

Abbreviations: BMI, body mass index; BP, blood pressure; Fbg, fibrinogen; FPG, fasting plasma glucose; FVII, factor VII; FVIII, factor VIII; FX, factor X; TC, total cholesterol; hs-CRP, high-sensitivity C reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PAI-1, plasminogen activator inhibitor 1; sTM, soluble thrombomodulin; TG, triglyceride; UA, uric acid; vWF, von Willebrand factor.

[a] Data are mean ± standard deviation (SD), minimum, maximum values, and 5th, 10th, 50th, 90th, 95th percentiles.

parameters between children with BMI < 90th percentile (n = 149) and those with BMI >/= 90th percentile (n = 18) showed that systolic blood pressure (92.9 ± 9.4 vs 103.8 ± 9.5 mm Hg, $P < .001$), diastolic blood pressure (55.2 ± 10.5 vs 61.7 ± 11.4 mm Hg, $P = .017$), heart rate (95.1 ± 14.5 vs 102 ± 9.5 bpm, $P = .048$), insulin (2.7 ± 1.4 vs 4.8 ± 2.6 μIU/mL, $P = .004$), FVII (89.6 ± 10.6 vs 94.1 ± 6.6, $P = .021$), FX (95 ± 10.8 vs 103.2 ± 11.6, $P = .004$), protein S (84.8 ± 15.1 vs 93.9 ± 19.8, $P = .021$), ghrelin (49.2 ± 37.7 vs 33.6 ± 19.3 fmol/mL, $P = .018$), leptin (2 ± 0.84 vs 4.5 ± 3.6 ng/mL, $P = .01$), and HOMA-IR (0.57 ± 0.32 vs 1.17 ± 0.64, $P = .003$) were significantly different. The other hematological parameters were not different between the 2 groups.

Among all participants, no, 1, and 2 cardiovascular risk factors (pertaining BMI, blood pressure, plasma glucose, TG, and HDL-C) were observed in 96 (58.5%), 44 (26.8%), and 24 (14.6%) cases, respectively. None had 3 or more risk factors. Blood levels of fibrinogen and leptin in participants with 2 or 1 risk factors were significantly higher than in those with no cardiovascular risk factors (Figure 1). The other parameters showed no significant association with the number of cardiovascular risk factors.

Stepwise regression analysis identified ALT, uric acid, fibrinogen, FVIII, FX, vWF, protein C, protein S, PAI-1, and leptin levels as significant independent risk factors for the metabolic syndrome (Table 2).

Discussion

The present study demonstrated that many hemostatic/fibrinolytic parameters and adipokines, as well as ALT and uric acid, are associated with the components of the metabolic syndrome, even in healthy preschool children. The mean BMI in this study group was 15.1 ± 1.5 kg/m^2 with a maximum value of 21.9. Based on the cutoff value of BMI for obesity in preschool children reported in our recent work,[7] only 10 children were judged overweight in this study, indicating that the study population could be considered a healthy one.[7,8] Specifically, blood levels of FVII, FX, protein S, and leptin were higher in children with BMI ≥ 90th percentile, and that of ghrelin was lower, compared to the other group. Stepwise regression analysis identified blood

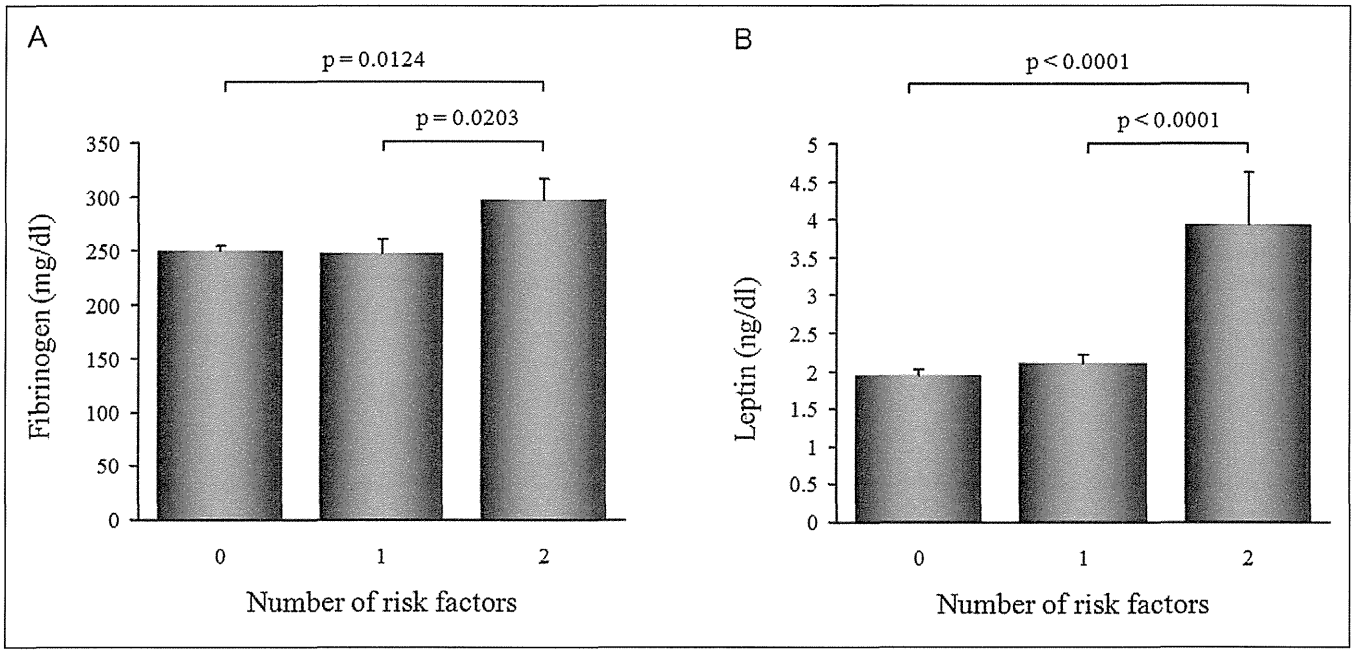

Figure 1. Comparison of various parameters among the 3 groups with no, 1, or 2 cardiovascular risk factors. Blood levels of fibrinogen (A) and leptin (B) were significantly higher in the group with 2 risk factors than in those with none or 1 risk factors. Data are expressed as mean ± standard error of the mean (SEM).

Table 2. Variables Independently Associated With Components of the Metabolic Syndrome.

Dependent Variable	Independent Variable	Parameter Estimate	Standard Error	*P* Value
ALT	FPG	−0.153	0.047	.002
	Systolic BP	0.092	0.043	.034
UA	FPG	−0.022	0.009	.025
	BMI	0.131	0.054	.033
Fibrinogen	FPG	2.01	0.936	.035
FVIII	BMI	4.558	1.707	.009
FX	TG	0.174	0.068	.013
vWF	insulin	5.955	2.264	.011
Protein C	Diastolic BP	0.28	0.157	.017
	TG	0.236	0.083	.025
Protein S	TG	0.361	0.089	.0001
PAI-1	FPG	0.689	0.244	.002
Leptin	BMI	0.371	0.086	<.0001
	insulin	0.36	0.078	<.0001

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; FVIII, factor VIII; FX, factor X; h-CRP, PAI-1, plasminogen activator inhibitor 1; TG, triglyceride; UA, uric acid; vWF, von Willebrand factor.

levels of fibrinogen (Fbg), FVIII, FX, vWF, protein C, protein S, and PAI-1 were determined by 1 or 2 components of the metabolic syndrome, although the independent components related to each dependent variable varied. Furthermore, children who had one or more known cardiovascular risk factors had significantly higher values of fibrinogen and leptin than those with no risk factors. These results lend support to the notion that hemostatic/fibrinolytic parameters or substances secreted by adipose tissues are associated with the development of metabolic syndrome in early childhood.

Several parameters involved in blood coagulation and fibrinolysis systems are known predictors of cardiovascular diseases.[9,10,17-20] These include fibrinogen, tissue factor (TF), FVII, FVIII, FX, vWF, protein C, sTM, PAI-1, and leptin. Among them, PAI-1 and leptin have recently attracted much interest because they are secreted by adipose tissue and their blood levels correlate with obesity and the amount of visceral fat mass. Both PAI-1 and leptin levels are also related to each other independent of the fat mass,[18] and both are recognized as cardiovascular risk factors.[21-23] In the present study, PAI-1 was significantly associated with fasting plasma glucose, while leptin showed a strong relationship with BMI and fasting insulin levels, suggesting the association with insulin resistance. Increased PAI-1 levels is currently considered a true component

of the metabolic syndrome, through which the risk of development of cardiovascular disease increases.[24] In the present study, PAI-1 and leptin levels were associated with features of the metabolic syndrome, indicating that the above-mentioned association is valid even in preschool children. Desacyl-ghrelin is another adipokine considered to lower cardiovascular risk through the activation of endothelial nitric oxide synthase.[25] Participants with high BMI in the present study had low ghrelin levels. Furthermore, serum concentrations of uric acid increase in proportion with leptin[26] and uric acid is also strongly associated with several components of the metabolic syndrome,[27] tendencies that were reproduced in the present study. Increased adipose tissue, and progression of the metabolic syndrome, have also been associated with increases in other indicators of prothrombotic activity such as increased plasma levels of fibrinogen, vWF, FVII, FVIII, and FX.[19,24] These changes were also observed in the present study.

Fibrinogen is both a procoagulant factor and an activator of inflammation, thus a typical cardiovascular risk factor.[28] The present study indicated that even in children, fibrinogen levels increased with BMI and with the number of cardiovascular risk factors. Increased fibrinogen has been associated with impaired activation of protein C,[29] which is interesting because thrombin binds to both fibrinogen and TM through a common region. In addition, thrombin is a procoagulant when bound to fibrinogen but exerts potent anticoagulant activity through the activation of protein C when it binds to TM on the cell surface. It is therefore conceivable that fibrinogen levels influence serum levels of sTM and that increased sTM in the circulation is related to a decrease in cardiovascular complications.[30] The present study demonstrated a significant association between protein C/S levels and certain components of the metabolic syndrome (TG and blood pressure). Previous studies on obese adults also showed increased activated protein C levels and their decrease after weight loss.[31] Protein S also exerts anticoagulant activity usually as a cofactor of protein C but also through the stimulation of TF pathway inhibitor.[32] However, sTM did not show any significant correlations with such metabolic syndrome-related parameters in this study. This might be due to the small number of participants analyzed and/or obese children.

Our study also presented clinically significant reference values for metabolic syndrome- or overweight-related variables in healthy preschool children, including those involved in hemostasis and fibrinolysis systems. Some of these values such as developmental changes from infancy to adolescence were reported previously,[13-15] although these studies did not specify the time of blood sampling or whether fasting was applied. Some parameters, especially those related to fibrinolysis, are significantly influenced by circadian oscillation; for instance, PAI-1 activity peaks in the morning.[33] Our results obtained in the morning after strict fasting are therefore important for future research and could be useful for the establishment of diagnostic criteria or prevention strategies for the metabolic syndrome in Japanese children.

Study Limitations

The numbers of participants analyzed and that of obese children were relatively small in this study. The results should be useful to provide reference values for hemostatic/fibrinolytic parameters and adipokines in Japanese preschool children. Further studies are needed to confirm how the abnormalities observed herein in those parameters could be implicated in later-life development of cardiovascular diseases.

Conclusions

The present study demonstrated that, even in preschool children, many hemostatic/fibrinolytic or adipose tissue-related variables show significant associations with the components of the metabolic syndrome, implicating a role for these systems in the insidious progression of cardiovascular diseases from early age.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Original Article

Association of Changes in Body Fatness and Fatty Acid Composition of Plasma Phospholipids During Early Puberty in Japanese Children

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Aims: Plasma fatty acid composition can change with age, reflecting diet and levels of desaturating enzymes such as stearoyl-CoA desaturase (SCD), delta-6 desaturase (D6D) and delta-5 desaturase (D5D), which contribute to the development of insulin resistance. This study analyzed longitudinal changes in fatty acid composition in Japanese children during early puberty and the association between changes in desaturase indices and changes in body fatness and insulin resistance.

Methods: The study included 77 children (38 boys and 39 girls) aged 9.6 ± 0.5 years. Relative weight (RW) and waist-to-height ratio (WHtR) were determined. The fatty acid composition of plasma phospholipids was analyzed by gas chromatography, and the desaturase indices were calculated: SCD (16:1n-7/16:0: SCD16 and 18:1n-9/18:0: SCD18), D6D (20:3n-6/18:2n-6) and D5D (20:4n-6/20:3n-6) in 2006 and 2009.

Results: Obese children showed higher dihomo-gamma linolenic acid (DGLA; 20:3n-6), a higher D6D index and lower D5D index than non-obese children. Longitudinal changes in fatty acid composition were generally similar in both sexes. Increased D6D index and DGLA and decreased D5D index were significantly associated with increased WHtR in boys and girls. In addition, increased D6D index was associated with an increased homeostasis model of assessment ratio (HOMA-R) only in girls.

Conclusion: The change in abdominal adiposity is a determinant of longitudinal changes in D6D and D5D indices and DGLA during early puberty.

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Key words; Fatty acid profile, Desaturase, Insulin resistance, Body fatness, Longitudinal study

Introduction

Childhood obesity is a worldwide epidemic and the consequences include increased obesity-related diseases such as metabolic syndrome and type 2 diabetes[1, 2]. The increased prevalence of childhood obesity is expected to lead to the earlier onset and increased incidence of coronary artery disease. It is generally accepted that atherosclerosis originates in childhood and that diet and physical activity are key determinants of the development of obesity. Although fatty acids are important in the development of obesity, insulin resistance and cardiovascular diseases, it is difficult to determine the effect of differences in dietary fatty acid composition. Many studies have found that the fatty acid composition in blood and tissues is related to insulin resistance, type 2 diabetes, metabolic syndrome and cardiovascular diseases[3-5]. In children, higher levels of saturated fatty acids, lower levels of n-3 polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA, 22:6n-3), and linoleic acid (LA, 18:2n-6) as well as higher levels of dihomo-gamma-linolenic acid (DGLA, 20:3n-6) in phospho-

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lipids[6-10] and palmitoleic acid (16:1n-7) in plasma lipids[11] are related to obesity and metabolic syndrome; however, the fatty acid composition of human adipose tissue can change with age, independent of diet[12].

The fatty acid composition of plasma lipids reflects food intake and levels of desaturating enzymes such as stearoyl-CoA desaturase (SCD), which is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs), and delta-6 desaturase (D6D) and delta-5 desaturase (D5D), which modulate the transformation of essential fatty acids into eicosanoids. The activities of these enzymes cannot be measured easily in humans *in vivo* but can be estimated from the product/precursor ratios. Several studies have demonstrated that high SCD and D6D indices and a low D5D index, obtained from fatty acid composition in plasma phospholipids, are associated with adiposity, insulin resistance and metabolic syndrome[7, 13, 14]. A few studies have assessed longitudinal changes in serum fatty acid composition in cholesteryl esters[15] and in phospholipids[16] in children, but studies of longitudinal changes in desaturase indices and the relationship of these changes with body fat have not been reported. In this study, we analyzed the fatty acid composition and its longitudinal changes in plasma phospholipids in Japanese children during early puberty. We also investigated the association of changes in desaturase indices with changes in body fatness and insulin resistance.

Participants and Methods

Study Population

The study population was 77 children (38 boys and 39 girls) aged 9.6 ± 0.5 years (mean ± SD), who were in the 4th grade of an elementary school located in a rural area in Shizuoka prefecture in 2006. The school participated in this study voluntarily. All children were free from diseases other than hyperlipidemia and obesity. Each child's standing height and weight were measured and relative weight (RW) was calculated according to the standard weight for sex, age and height using data from the Ministry of Education, Science, Sports and Culture[17]. Waist circumference was measured at the level of the umbilicus, and the waist-to-height ratio (WHtR) was calculated. All blood samples were obtained from the cubital vein in the morning after a 12 hour fast. Concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDLC) and triglyceride (TG) were measured by enzymatic methods. Concentrations of low-density lipoprotein cholesterol (LDLC) were calculated using the Friedewald formula[18]. Concentrations of plasma insulin and glucose were determined, and the homeostasis model of assessment ratio (HOMA-R) was obtained using Matthews' formula as an index of insulin resistance[19]. Three years later (in 2009) we approached the students about participating in the second phase of the study.

Informed consent was obtained from each child and his or her parents. The study protocol was approved by the local ethics committee, which is composed of members of the school's health education committee. This committee includes members of the local Board of Education and representatives of Nihon University Itabashi Hospital.

Fatty Acid Analysis

Total lipids were extracted as described[20]. Briefly, plasma was homogenized with an ultrasonic homogenizer in chloroform/methanol (1:1, v/v). After filtration with filter paper and a membrane filter, distilled water was added and the mixture was shaken. The lower layer (chloroform layer) was collected, the chloroform was removed by evaporation and the residue was separated into phospholipids and neutral lipids by solid-phase extraction or thin-layer chromatography. Following the addition of an internal standard (for instance, C23:0Me, tricosanoic acid methylester), phospholipids were transmethylated at 90℃ for 60 min using the (14%, w/v) boron trifluoride in methanol method. The fatty acid composition was analyzed by gas chromatography (Omegawax250 fused silica capillary column, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Supelco, Japan).

The desaturase indices were determined as: SCD (16:1n-7/16:0: SCD16 and 18:1n-9/18:0: SCD18), D6D (20:3n-6/18:2n-6) and D5D (20:4n-6/20:3n-6).

Statistical Analysis

All data are expressed as the mean ± SD. Group differences were assessed using the unpaired t-test. The correlation coefficient between two variables was determined by single regression analysis. $p \leq 0.05$ was considered significant. All statistical analyses were performed with the STATVIEW statistical package (v4.5; Abacus Concepts, Berkeley, CA, USA).

Results

Baseline Characteristics

At the start of this study, 11 of the children (4 boys and 7 girls) were obese with relative weight (RW) ≥ 120%. There was no difference between boys and girls for any of the parameters, except for fasting glucose concentration in non-obese children: according

Table 1. Subject characteristics

	Boys			Girls		
	Non-obese	Obese	*p* value	Non-obese	Obese	*p* value
1st	*n*=34	*n*=4		*n*=32	*n*=7	
Age (year)	9.6±0.5	9.8±0.5	0.4773	9.6±0.5	9.4±0.5	0.5323
Body weight (kg)	31.5±5.9	45.4±12.9	0.0005	30.3±5.5	45.7±8.3	<0.0001
Height (cm)	135.8±6.3	136.3±4.5	0.8534	134.9±6.3	139.7±6.0	0.0325
Relative weight (%)	102.8±12.4	145.4±31.8	<0.0001	101.1±11.9	139.4±13.7	<0.0001
Waist-to-height ratio	0.44±0.04	0.58±0.07	<0.0001	0.44±0.03	0.54±0.05	<0.0001
Total cholesterol (mg/dL)	176.5±26.1	178.8±20.2	0.8692	182.1±24.0	186.6±40.8	0.6999
LDL-cholesterol (mg/dL)	99.6±22.2	106.9±17.8	0.3183	106.0±21.9	114.8±32.0	0.2384
HDL-cholesterol (mg/dL)	67.2±12.0	55.3±12.5	0.0692	66.4±12.0	52.0±11.5	0.0041
Triglyceride (mg/dL)	48.6±24.1	83.0±45.6	0.0195	48.6±21.3	99.0±58.4	0.0003
Glucose (mg/dL)	93.5±5.7	93.0±5.2	0.8755	89.9±4.9$^{\#\#}$	91.4±4.7	0.4592
Insulin (IU/mL)	7.0±3.4	11.5±3.8	0.0178	7.8±3.5	20.1±12.8	<0.0001
HOMA-R	1.6±0.8	2.7±1.0	0.0220	1.7±0.8	4.5±2.6	<0.0001
2nd	*n*=32	*n*=6		*n*=31	*n*=8	
Age (year)	12.5±0.5	12.8±0.4	0.0778	12.5±0.5	12.3±0.5	0.1875
Body weight (kg)	43.4±7.6	62.6±13.3	<0.0001	43.8±5.9	61.2±7.6	<0.0001
Height (cm)	155.8±8.5	157.1±5.1	0.7084	152.7±3.5	154.0±3.4	0.3874
Relative weight (%)	93.7±8.5	131.7±22.6	<0.0001	98.0±12.1	134.1±15.3	<0.0001
Waist-to-height ratio	0.41±0.03	0.56±0.06	<0.0001	0.42±0.03$^{\#}$	0.51±0.04	<0.0001
Total cholesterol (mg/dL)	162.9±22.2**	158.3±15.0	0.6709	178.4±22.6$^{\#\#}$	166.0±24.6	0.1823
LDL-cholesterol (mg/dL)	88.4±18.3**	95.8±15.2	0.3614	102.7±20.8$^{\#\#}$	98.9±20.3	0.6468
HDL-cholesterol (mg/dL)	65.8±10.2	46.8±7.0	0.0001	64.1±11.3	50.4±10.0	0.0034
Triglyceride (mg/dL)	43.6±18.5	81.2±30.7	0.0002	58.0±25.9$^{*\#}$	83.9±38.6	0.0294
Glucose (mg/dL)	88.2±5.1**	89.3±3.5	0.6052	88.3±5.4	90.4±3.9	0.3152
Insulin (IU/mL)	6.5±2.9	15.2±4.4	<0.0001	10.5±5.5$^{**\#\#}$	18.6±7.9	0.0017
HOMA-R	1.4±0.7	3.4±1.1	<0.0001	2.3±1.3$^{*\#\#}$	4.2±1.9	0.0019

1st vs. 2nd in non-obese, $^{*}p<0.05$, $^{**}p<0.01$ Mean±SD
Male vs. Female in non-obese, $^{\#}p<0.05$, $^{\#\#}p<0.01$

to the guidelines for normal serum lipid levels for Japanese children[21], 4 girls had hypercholesterolemia (>220 mg/dL), 1 boy and 2 girls had hypertriglyceridemia (>120 mg/dL) and no child had a low concentration of HDLC (<40 mg/dL). Obese children had lower concentrations of HDLC, higher concentrations of TG and insulin and higher HOMA-R (**Table 1**). **Table 2** gives the fatty acid profiles. Obese boys had significantly higher contents of DGLA (20:3n-6) and eicosapentaenoic acid (EPA; 20:5n-3) than non-obese boys. Obese girls had higher contents of DGLA (20:3n-6) than non-obese girls; however, the difference was not statistically significant. The D6D index was higher in obese children, whereas the D5D index was lower in obese boys than in any other group. Non-obese boys had lower LA (18:2n-6) and higher DHA (22:6n-3) than non-obese girls. Obese boys had higher EPA (20:5n-3) than obese girls.

In boys, the D6D index and the content of DGLA (20:3n-6) were correlated positively with RW ($r=0.615$, $p<0.00012$; $r=0.375$, $p=0.0204$, respectively) and WHtR ($r=0.618$, $p<0.0001$; $r=0.422$, $p=0.0083$), but not with HOMA-R. The D5D index was correlated negatively with RW, WHtR and HOMA-R ($r=-0.418$, $p=0.0091$, $r=-0.464$, $p=0.0034$ and $r=-0.457$, $p=0.0039$, respectively). In girls, the D6D index and DGLA (20:3n-6) were correlated positively with RW ($r=0.547$, $p=0.0003$ and $r=0.366$, $p=0.0220$, respectively), WHtR ($r=0.575$, $p=0.0001$ and $r=0.369$, $p=0.0209$, respectively) and HOMA-R ($r=0.665$, $p<0.0001$; $r=0.391$, $r=0.0139$, respectively), whereas the D5D index was correlated negatively with HOMA-R ($r=-0.431$, $p=0.0061$) but not with D6D or D5D. The SCD16 index had no significant relationship with RW or WHtR in either sex, whereas the SCD18 index was correlated nega-