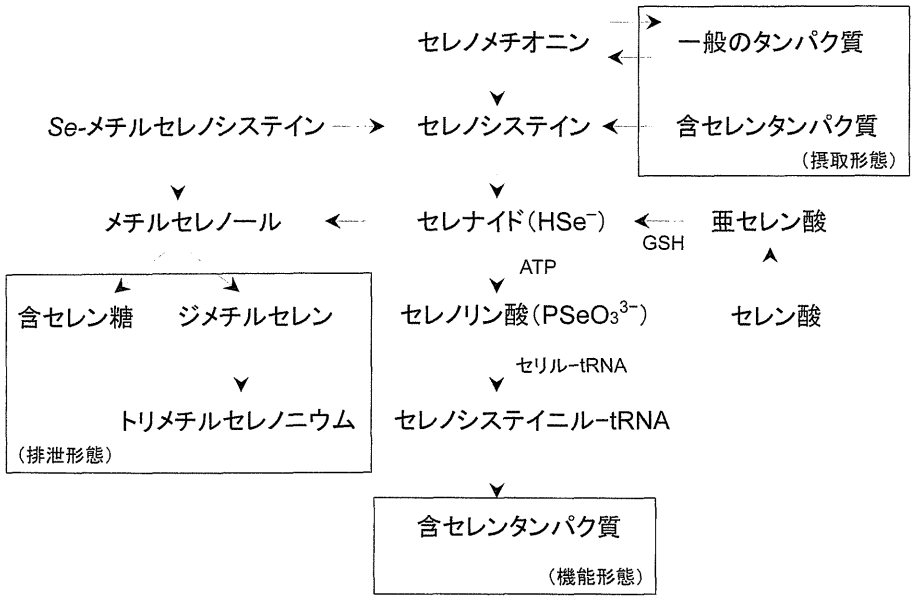

図 1. ヒトにおけるセレン化合物の代謝

一般の食物中のセレンはタンパク質のアミノ酸配列中にメチオニンと誤って取り込まれたセレノメチオニン残基，または含セレンタンパク質中のセレノシステイン残基として存在している．Se-メチルセレノシステインは意図的にセレンを蓄積させた野菜類の主形態である．食物を介して無機セレンを摂取することはほとんどない．いずれのセレン化合物も，効率に差はあるが，セレナイドに変換後，セレノリン酸，セレノシステイニル tRNA を経て，含セレンタンパク質のセレノシステイン残基として機能を発現する．セレンの主排泄経路は尿である．セレン摂取量が日常レベルであれば，含セレン糖，やや多くなればトリメチルセレノニウムとして排泄される．中毒レベルのセレン摂取の場合は呼気中にジメチルセレンとして排泄される．

分表 2010 から抜き出した．100 g あたり 10 μg 以上のセレンを含むのは，魚介類，畜肉・卵，一部の小麦および大豆製品であり，これらが主要なセレン供給源である．植物性食品のセレン濃度が生育土壌のセレン濃度の影響を受けるため，小麦および大豆製品では，原料原産地が高セレン土壌地域の北米大陸中央部であるパン，パスタ，納豆が高セレン濃度となる[22)23)]．わが国は家畜飼料も米国に依存するため，畜肉や卵のセレン濃度も高い．ラム肉は低セレン土壌地域であるニュージーランドからの輸入品なのでセレン濃度が低い．

意図的にセレンを蓄積させた場合を除き，食品中セレンの多くはタンパク質に結合している．動物は含セレンタンパク質を生成するので，動物の筋肉や内臓を摂取した場合，含有セレンの大半は含セレンタンパク質中のセレノシステイン残基，もしくはメチオニンと誤って一般のタンパク質に取り込まれたセレノメチオニン残基と考えられる．ただし，セレノシステインは不安定なので，調理加工中に別の形態に変化しているかもしれない．

植物には含セレンタンパク質が存在しない．植物は無機セレンからセレノシステインを生成するが，これを積極的に利用せずにセレノメチオニンに変換し，タンパク質中のセレノメチオニン残基として含有する．事実，通常のセレン濃度の大豆タンパク質の分解物からセレノメチオニンが検出されており[24)]，穀物や大豆を摂取した場合，含有セレンの大半はタンパク質中のセレノメチオニン残基と考えられる．また，セレンを意図的に蓄積させた高セレン酵母中のセレンも大半がタンパク質中のセレノメチオニン残基である[25)26)]．

セレノシステインは反応性が高く，生体内に高濃度に存在すれば有害である．動物はセレノシステイン-γ-リアーゼによって遊離のセレノシステインを分解するが[27)]，植物にこの酵素は存在しない．このため，高セレン環境下で植物を栽培すると，セレノシステインからセレノメチオニンへの代謝系が飽和し，セレノシステインは特殊な含セレンアミノ酸に変換される．含セレンアミノ酸の分子種としては，Se-メチルセレノシステインが大半を占めるが[28)-30)]，植物の種類によってはセレノホモランチオニン[31)32)]，γ-グルタミル

表1. ヒトに存在する含セレンタンパク質[17]

略号	名称
GPX1	古典的(Classical)グルタチオンペルオキシダーゼ
GPX2	胃腸グルタチオンペルオキシダーゼ
GPX3	血漿グルタチオンペルオキシダーゼ
GPX4	リン脂質ヒドロペルオキシドグルタチオンペルオキシダーゼ
GPX6	グルタチオンペルオキシダーゼ6
DI 1	ヨードチロニン5'-脱ヨウ素酵素1(Type I DI)
DI 2	ヨードチロニン5'-脱ヨウ素酵素2(Type II DI)
DI 3	ヨードチロニン5'-脱ヨウ素酵素3(Type III DI)
TRR1	チオレドキシン還元酵素1
TRR2	チオレドキシン還元酵素2
TRR3	チオレドキシン還元酵素3
SPS 2	セレノリン酸合成酵素2
SELP	血漿セレノプロテインP
SELW	筋肉セレノプロテインW
SELV	セレノプロテインV
SEP15	15 kDのセレノプロテイン
SELR	メチオニン-R-スルホキシド還元酵素
SELT	セレノプロテインT
SELM	セレノプロテインM
SELN	セレノプロテインN
SELH	セレノプロテインH
SELI	セレノプロテインI
SELK	セレノプロテインK
SELO	セレノプロテインO
SELS	セレノプロテインS

表2. 主な食品のセレン，およびモリブデン含有量(μg/100 g)

食品	セレン	モリブデン	食品	セレン	モリブデン
精白米(水稲)	2	69	まいわし	54	Tr
薄力粉(1等)	4	12	かつお	100	Tr
中力粉(1等)	7	9	まさば	64	0
強力粉(1等)	39	26	まだい	38	0
じゃがいも	0	4	くろまぐろ	110	0
小豆(乾)	1	210	あさり	38	9
国産大豆(乾)	5	260	くるまえび	35	1
米国産大豆(乾)	28	300	するめいか	42	1
ごま(乾)	10	92	牛肉(和牛, リブロース, 赤肉)	11	1
なす	0	10	牛肝臓	50	94
にんにく	1	15	豚肉(ロース, 赤肉)	25	1
ほうれんそう	3	5	ラム肉	4	Tr
温州みかん	0	0	鶏肉(もも, 皮なし)	16	2
りんご	0	0	卵黄	56	14
しいたけ	4	3	卵白	21	1
真昆布(素干し)	2	12	牛乳(ホルスタイン)	3	4

いずれも日本食品標準成分表2010より転載した.

-Se-メチルセレノシステイン[32)33)]なども存在する．

魚類も含セレンタンパク質を生成するので，魚肉摂取はセレノシステイン残基の摂取につながる．しかし，魚肉中セレンは高濃度なので，すべて含セレンタンパク質由来とは考えにくい．魚の筋肉や内臓中セレンに関しては様々な形態が提唱・報告されている．マグロなどの大型回遊魚がセレンとともに水銀を蓄積することから，セレンと水銀の複合体の存在が示唆されている[34)]．しかし，クロマトグラフィーにおいてセレンと水銀が同じ位置に溶出されるといった報告にとどまっている[35)]．マグロなどの血合肉，血液，内臓には，低分子セレン化合物の存在が示唆されていたが[36)]，最近，その一部が図2に示すセレノネインであると同定された[37)]．しかし，この化合物は筋肉には検出されないので，魚肉中セレンの主形態とはみなせない．

4. 摂取量

(1) 食品標準成分表2010を用いたセレン摂取量の算定

筆者らは，食品標準成分表2010記載のセレン含有量をもとに献立からのセレン摂取量を算定し，実測値と比較した[38)]．すなわち，病院または介護施設の一般食または介護食を8日分収集し，成分表記載の数値からセレン摂取量を算定して実測値と比較した．なお，献立に使用された食品の約半数は成分表にセレン含有量の記載がなかったため，近縁食品の数値の転用，または属する食品群のセレン含有量の平均値を代用などの方法で数値を当てはめた．表3に示すように，病院一般食からのセレン摂取量の実測値(90～150 μg/日)は，これまでの日本人のセレン摂取量の推定値(約100 μg/日)[39)]に近似していた．実測値が計算値の2倍近い場合もあるが，この程度の乖離は微量栄養素においてしばしば認められる．すなわち，セレン含有量表示のない食品に対して数値を当てはめるという問題はあるが，食品標準成分表2010を用いてセレン摂取量を推定することはおおむね可能といえる．

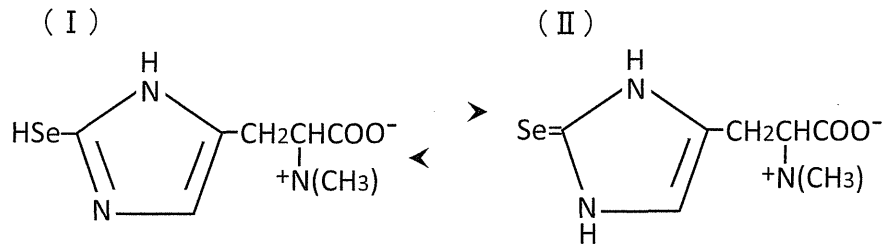

図2．セレノネインの構造[37)]

ジクロルメタンなど非極性溶媒中において，−20℃であればⅠの構造であるが，室温下ではⅠが酸化された二量体(ジセレニド(-Se-Se-))となる．水，メタノール，アセトニトリルなどの極性溶媒中ではⅡの構造をとる．

表3．セレン，およびモリブデン摂取量の計算値と実測値の比較[39)]

食事	セレン(μg/日)		モリブデン(μg/日)	
	計算値	実測値	計算値	実測値
病院普通食1	108	101	242	302
病院普通食2	146	114	269	289
病院普通食3	73	90	253	247
病院普通食4	120	125	223	177
病院普通食5	82	151	218	333
病院普通食6	86	146	267	480
介護施設普通食1	58	59	157	230
介護施設介護食1	17	24	65	106

介護施設の食事での摂取量が低いのは食事量そのものが少ないためである．

(2)適正な摂取範囲

食事摂取基準2010年版における成人のセレン摂取の推奨量(25～30 μg/日)[40]は，体格差を考慮しても欧米(55～75 μg/日)より低い．この違いは，欧米の推奨量が血清GPX活性の飽和に必要なセレン摂取量にもとづくのに対して[41]，日本の推奨量の策定根拠が血清GPX活性の飽和値の3分の2値を維持するのに必要なセレン摂取量であること[40]に起因する．日本の策定根拠は血清GPX活性が飽和値の3分の2であれば克山病は発症しないというWHOの報告[42]に従っている．推奨量は欠乏症予防のための指標なので日本の推奨量に大きな問題はない．欧米が血清GPX活性の飽和にこだわるのは，平均セレン摂取量が50～60 μg/日を下回る集団では低セレン摂取ががんの発症リスクを高めることが疫学的に証明されているからである[9)-11)]．50～60 μg/日は血清GPX活性の飽和に必要なセレン摂取量にほぼ一致するだけでなく，血清GPX以外の含セレンタンパク質の生合成量をも飽和する摂取量である．低セレン摂取ががん発症リスクを高める機構は不明だが，25種類の含セレンタンパク質のどれかが関連すると推定できることから，がん予防を念頭におく場合，50～60 μg/日のセレンを摂取してすべての含セレンタンパク質の生合成量を飽和水準まで高めるのは妥当かもしれない．摂取基準では生活習慣病予防を念頭においた指標を目標量と定義しているので，50～60 μg/日は目標量の下限値とすべきである．

一方，日本の成人のセレン摂取の耐容上限量(210～300 μg/日)[40]は欧米(約400 μg/日)に比較して厳しい値である．この差は，欧米が爪の変形を主症状とする慢性セレン中毒予防を念頭におくのに対して[41]，日本は継続的な300 μg/日程度のセレン摂取が2型糖尿病発症リスクを増加させるという報告[43]を上限量策定の根拠としたためである．しかし，耐容上限量は慢性中毒予防のための指標なので，次回の改訂では欧米の策定根拠を採用すべきであろう．すなわち現在の日本のセレンの耐容上限量は，糖尿病という生活習慣病の発症リスクを念頭においているので，目標量の上限値といえる．

以上より，成人のセレン摂取に関する食事摂取基準としては，推奨量を現行どおり25～30 μg/日，耐容上限量を食事摂取基準2005年版が採用していた350～450 μg/日とし，新たに50から250 μg/日の範囲を目標量とするのが適切と判断できる．図3に，世界各国のセレン摂取量をこれらの摂取の指標と比較して示した[44)-56)]．なお，フィンランドやニュージーランドは低セレン摂取を解消するための対策を講じているが，

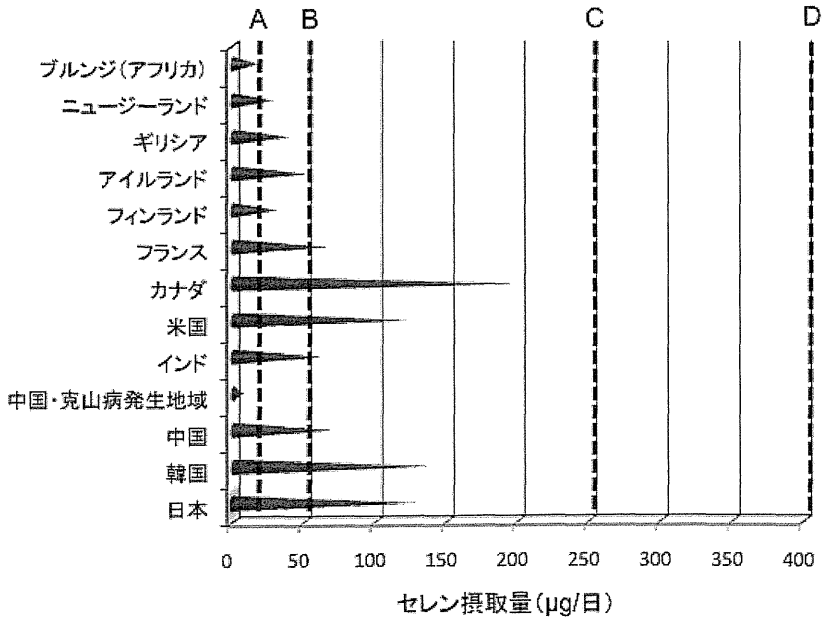

図3．世界各国の平均的なセレン摂取量[44)-56)]

A：セレン欠乏を予防するための推奨摂取量

B：がん発生リスクを高めないための目標摂取量の下限

C：糖尿病発生リスクを高めないための目標摂取量の上限

D：慢性セレン中毒を避けるための耐容上限量

図にはあえて対策以前の数値を用いた．セレン摂取が適正範囲に収まるのは米国，カナダ，日本，適正範囲より少ないのは欧州，オセアニア，アフリカである．すなわち，欧州とオセアニアの一部ではがん予防のための目標量を下回り，アフリカの一部では現在でも欠乏症の生じる危険性がある．すなわち，食品へのセレン強化やセレンサプリメントに意味があるのは欧州，オセアニア，アフリカであり，日本では普通に食事を摂取するかぎりセレンを意図的に摂取する必要はないことを強調したい．

Ⅱ．モリブデン

1．生理機能と欠乏症

ヒトを含む高等動物にはモリブデンを含む酵素が3種存在する．すなわち，キサンチン酸化酵素，アルデヒド酸化酵素，亜硫酸酸化酵素であり，モリブデンはモリブドプテリン補因子として存在する．ヒトには遺伝的にモリブデン含有酵素を合成できない欠損症[57]とモリブドプテリン補因子が合成できず複数のモリブデン含有酵素が機能しない欠損症[58]が存在する．とくに亜硫酸酸化酵素の機能欠損は，亜硫酸の蓄積が中枢神経障害を引き起こすため，新生児の段階で死に至ることがほとんどである．モリブデン含有酵素である亜硫酸酸化酵素の機能欠損が死につながることは，モリブデンがヒトの生存に必須であることを意味する．しかし，ヒトの必須微量ミネラルとしてのモリブデンの地位は，モリブデン非添加の高カロリー輸液を長期間投与された患者において，上記の亜硫酸酸化酵素欠損症に類似した中枢神経症状が出現し，栄養水準のモリブデン酸投与によって回復したというわずか一例の症例報告[59]によってようやく確定した．

モリブデンは表2に示すように穀物と豆類に高濃度で存在し，ヒトでの食事性欠乏の報告例はない．動物実験では，モリブデン非添加 AIN76 飼料（モリブデン濃度 25 ng/g）を用いてモリブデン含有酵素の低下を引き起こした研究が一例存在する[60]．しかし，筆者らの測定によれば，飼料用カゼインとデンプンのモリブデン濃度は，それぞれ 120 ～ 237 ng/g，および 50 ～ 71 ng/g であり，さらに大豆タンパク質や小麦グルテンにはより高濃度のモリブデンが混入しているため，一般的には食品タンパク質とデンプンを用いてモリブデン濃度 50 ng/g 未満の飼料を調製するのは難しい．事実，筆者の研究室で作成したモリブデン非添加 AIN93G 飼料のモリブデン濃度は 80 ng/g であり，これを低モリブデン基本食とした場合，表4のように血清モリブデン濃度のみがモリブデン投与量に依存して変化するだけで，臓器中モリブデン濃度とキサンチン酸化酵素活性はモリブデン投与量とは無関係に一定だった[61]．このように食事性モリブデン欠乏を作成するのが困難であるため，モリブデンと化学的挙動が類似するタングステンを投与してモリブデン含有酵素活性を低下させることが試みられている[62]．

2．吸収と排泄

食品中モリブデンの化学形態に関する報告はきわめて少ない．モリブデンがリン酸と高い親和性を有することからモリブドリン酸として存在すると考えられるが[63]，十分な証明はない．筆者らは，出納実験によってモリブデン摂取量 150 ～ 320 μg/ 日の範囲で食事中モリブデンの吸収を 90％以上と算定した[64]．また，主排泄経路は尿であり，摂取量と尿排泄量との間に強い相関が成立する．一般人を対象とした研究でも，モリブデン摂取量と 24 時間尿中排泄量との間に有意な関連が認められる[65]．一方，米国で行われた出納実験では，約 20 ～ 1500 μg/ 日のモリブデン摂取範囲で出納値ゼロが維持されること[66]，および血清モリブデン濃度が摂取量と強く相関することが報告されている[67]．すなわち，モリブデンは広い摂取範囲において高い吸収率であるが，速やかに尿に排泄されるので必要以上の臓器蓄積は生じないといえる．

表4．飼料中モリブデン濃度が臓器モリブデン濃度と肝臓キサンチン酸化酵素に及ぼす影響[61]

飼料へのモリブデン添加量（μg/g）	モリブデン濃度（ng/g）			肝臓キサンチン酸化酵素活性
	肝臓	腎臓	血清	(unit/mg protein)
0	839 ± 24 [a]	478 ± 9 [a]	5.7 ± 0.2 [a]	0.16 ± 0.01 [a]
0.1	949 ± 32 [a]	508 ± 24 [a]	6.5 ± 1.3 [a]	0.13 ± 0.02 [a]
0.5	893 ± 44 [a]	496 ± 17 [a]	12.4 ± 2.1 [b]	0.12 ± 0.02 [a]

4 週齢の Wistar 系雄ラットを 4 週間飼育．基本飼料はモリブデン非添加 AIN93G 飼料（モリブデン濃度，80 ng/g）．
値は平均値 ± 標準誤差．共通の添字のない群間には有意差（$p<0.05$）がある．

3. 摂取量

日本人の食事摂取基準では，成人のモリブデン摂取の推奨量を，20 μg/日の摂取でもゼロ出納が維持されること[66]にもとづき，20～25 μg/日としている[40]．モリブデンは穀物と豆類に豊富に含まれ，日本人の食事からの摂取量は欧米人よりもやや多い150～350 μg/日と推定される[64)68)]．つまり日本人は，日常的に推奨量の約10倍に相当するモリブデンを摂取しており，通常の食生活で不足が起こることはない．成人のモリブデンの耐容上限量はヒトでの情報が少ないため，ラットの健康障害非発現量にもとづき450～600 μg/日とされている[40]．厳密な菜食習慣を持つ場合，穀物や豆類の消費が多くなるのでモリブデン摂取量が日常的に耐容上限量を上回ることがあるが，健康障害は認められない[69]．米国のモリブデン摂取の耐容上限量が2000 μg/日[70]であることを考慮すると，1000 μg/日程度まで耐容上限量を高めてもいいかもしれない．

表3に，セレンと同様に食品標準成分表2010に記載されたモリブデン含有量をもとに食事からのモリブデン摂取量を算定し，実測値と比較した結果を示す[39]．計算値と実測値はおおむね一致している．モリブデンの供給源は穀物，および豆製品であり，献立における使用量把握が容易であることから，食事調査においてモリブデン摂取は比較的正確に見積もることが可能と思われる．

おわりに

モリブデンは推奨量の100倍近く摂取しても健康障害は生じないと思われ，推奨量の10倍を超える日本人のモリブデン摂取量を問題視する必要はない．一方，セレンの望ましい摂取範囲は50～250 μg/日と考えられるが，ほとんどの日本人の平均的な摂取量はこの範囲に収まっている．米国において，前立腺がん発生に対する200 μg/日の付加的なセレン摂取の影響を調べる大規模な疫学研究が実施されたが，予防的な効果は認められなかった[71]．日常の食事において適切なセレン摂取を達成している一般の日本人がセレンサプリメントを使用することは，目標量の上限を超える可能性があり，健康の維持・増進にとって何のメリットもないことを重ねて強調しておきたい．

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Review

The Optimal Dietary Fat to Carbohydrate Ratio to Prevent Obesity in the Japanese Population: A Review of the Epidemiological, Physiological and Molecular Evidence

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Summary The prevention of obesity, which leads to diabetes and other diseases, is a major concern for public health. There might be an optimal dietary fat to carbohydrate ratio for prevention and treatment of obesity. According to the Japanese Dietary Reference Intakes (RDA) for 2010, the optimal fat intake is 20–30% of energy for ages 1–29 y and 20–25% for ages 30 y and over. Upper boundary values of this recommendation were the median of the percentage of energy from dietary fat in Japanese. In a systematic review to estimate the optimal dietary fat to carbohydrate ratio, it was found that obese subjects with hyperinsulinemia (or insulin resistance) lost more weight on a mild low-carbohydrate (LC) (or low-glycemic load diet; 40% carbohydrate, 30–35% fat) than on a low-fat (LF) diet (55–60% carbohydrate, 20% fat), whereas those without hyperinsulinemia showed the opposite. In non-obese primarily insulin-sensitive subjects, decreasing fat rather than carbohydrate intake is generally more effective to prevent obesity. Physiological and molecular evidence supports this conclusion. Increased carbohydrate intake, especially in high-glycemic food, leads to postprandial hyperglycemia and hyperinsulinemia, which are exaggerated in obese insulin-resistant subjects. Even in an insulin-resistant state, insulin is able to stimulate fatty acid synthesis in liver, activate lipoprotein lipase, and prevent lipolysis in adipose tissues, which all facilitate adipose tissue enlargement. Optimal dietary fat to carbohydrate ratio may differ in populations depending on their prevalence for obesity. Because the prevalence of overweight/obesity in Japanese is low, a LF diet is recommended in the general population.

Key Words low-carbohydrate diet, low-fat diet, RDA, insulin resistance, obesity

Obesity in the United States and in much of the westernized world has increased dramatically over the past several decades: 64.5% of adults in the United States are overweight (body mass index [BMI]≥25 kg/m^2 and <30 kg/m^2) or obese (BMI≥30 kg/m^2) (*1*). Overweight/obesity (BMI≥25 kg/m^2) was the most important predictor of diabetes. In the Nurses' Health Study, during 16 y of follow-up, 3,300 new cases of type 2 diabetes were observed in the baseline population of 84,941 female nurses. The relative risk of diabetes was 38.8 for women with a BMI of 35.0 kg/m^2 or higher, 20.1 for women with BMI of 30.0 to 34.5 kg/m^2, and 7.59 for women with BMI of 25.0 to 29.9 kg/m^2, as compared with women who had a BMI of less than 23.0 kg/m^2 (*2*).

In Japan, the prevalence of overweight/obesity (BMI≥25 kg/m^2) in adults is very low compared with the United States: 30.4% in men and 20.2% in women in 2007, according to Japanese cross-sectional nationwide surveys (*3*). However, a strong positive association between baseline BMI and the incidence of diabetes in the follow-up period was observed similar to that in the United States. In a Japanese cohort of healthy men (n=16,829) and women (n=8,370) followed for 7.4 y, new cases of diabetes were documented in 869 men and 224 women (*4*). The relative risk of diabetes was 5.55 for men with a BMI of 25.2 to 26.3, compared with men who had a BMI of 15.0 to 19.7, and the relative risk of diabetes was 5.70 for women with a BMI of 24.4 to 25.9, compared with women who had a BMI of 14.9 to 19.1. Therefore, in Japan also, the prevention of overweight/obese subjects is a major public issue.

The role of dietary fat and carbohydrate in the obesity epidemic has been a hotly debated topic for decades and remains unresolved. To reduce the incidence of obesity in general populations, public statements on optimal ratios of dietary fat to carbohydrate have been issued. Health organizations have recommended diets that are low in total and saturated fat and high in carbohydrates obtained from vegetables, fruits, and whole grains or fiber-rich foods (*5–7*). Dietary guidelines for Americans published in 2005 emphasized the importance of the amount of energy consumed rather than the proportions of protein, fat, and carbohydrate in the diet, pro-

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vided that the macronutrients are within the AMDR, the acceptable macronutrient distribution range: 10–35% of energy from protein, 45–65% from carbohydrate, and 20–35% from fat (*8*). Dietary reference intakes for Japanese issued by the Ministry of Health, Labour, and Welfare in 2010 indicated that optimal fat intake is 20–30% for ages 1–29 y and 20–25% for ages 30 y and over. Upper boundary values of this recommendation were a median of the percentage of energy from dietary fat in Japanese, a recommendation that most Japanese are able to follow.

The present review was conducted to determine the optimal dietary fat to carbohydrate ratio to prevent obesity in the Japanese population. As a result, it was suggested that a mild low-carbohydrate (LC) diet was effective in reducing body weight in obese subjects with hyperinsulinemia (or insulin resistance), whereas a low-fat (LF) diet favored prevention of obesity in non-obese subjects or treatment of obese subjects without hyperinsulinemia. In addition, to elucidate the molecular mechanisms of obesity in response to a carbohydrate-rich diet, several aspects of insulin actions, namely lipogenesis in the liver, activation of lipoprotein lipase (LPL), and lipolysis under insulin-resistance state were also reviewed.

Methods of Review and Definitions

Selection of publications of epidemiological studies. For epidemiological studies, key words "(Diet, Fat-Restricted [MESH]) AND (dietary OR intake OR consumption) AND ((randomized controlled trial [PTYP] OR random [WORD]) OR (cohort studies [MESH] OR risk [MESH] OR (odds [WORD] AND ratio [WORD]) OR (relative [WORD] AND risk [WORD]) OR case control [WORD] OR case-control studies [MESH]))" with a limitation of "humans" were used in PubMed to select all publications through June 1, 2011 (n=1,004), initially to review the effects of dietary fat on mortality and mobility reported therein. From these publications, those related to changes in body weight were selected and reviewed. Other important topics, such as the effects of dietary fat subtypes, i.e., saturated, mono-unsaturated, n-6, and n-3 fatty acids, on obesity, are not discussed in this review. Because several reviews and meta-analyses have been published since the original search date, publications that appeared after this date are presented in this study with comments relating their findings to those of the previous reviews and meta-analyses. To show a visual representation of the results of the review, findings from representative publications are presented here in figures.

Current body weight is the result of the accumulated daily balance of energy intake and expenditure over previous days. Therefore, the causes of obesity are multifactorial, including such factors as physical activity level, energy intake, and food availability. It is difficult to assess these factors, and there are strong limitations to examining the effects of dietary macronutrients on obesity in cross-sectional and prospective studies (confounding factors may not be measured adequately). For this reason, carefully conducted intervention studies in which dietary fat to carbohydrate ratios were changed were mostly selected for this review.

Selection of publications of physiological and molecular studies. In a review of the mechanism of lipogenic action of insulin (covered later in this review), key words "insulin AND obesity AND ((lipogenesis AND liver) OR LPL OR (lipolysis and adipose tissues))" were used initially in PubMed to select appropriate publications, including reviews. Additional publications, which were necessary to describe the effects of insulin in an insulin-resistance state, were included from citations obtained from review articles and personal reference lists.

Definitions of LF and LC diets. The term LF diet is used relative to that of a high-fat diet in the literature; therefore, the absolute amounts of fat were diverse. In general, a high-fat diet means fat intake provides more than 30% of energy and a LF diet means less than 30%. The LC diet has been used in two different types of diet: a very LC diet (ketogenic diet) and a mild LC diet (low-glycemic load diet). Glycemic load is the mathematical product of glycemic index and carbohydrate amount. In the ketogenic diet, carbohydrate intake is less than 40 g/d (*9*), whereas in the low-glycemic load diet, the total amount of carbohydrate is decreased by 10–20% of energy, and foods containing carbohydrate with lower glycemic index were used. In Japanese, median intake of energy in adults was 1,856 kcal/d, and median intakes of carbohydrate, fat, and protein were 258 g/d (56% of energy), 51 g/d (24.8%), and 68 g/d (15%), respectively, according to The National Health and Nutrition Survey in Japan, 2007 (*3*). In this review, these two types of LC diets are reviewed separately.

Results and Discussion

A LF diet prevents obesity in general populations

In a meta-analysis of general populations under free-living conditions, weight loss was positively and independently associated with a reduction in the percentage of energy as fat (0.37 kg/%, p<0.005) (*10*). Another meta-analysis of intervention studies also supports this conclusion (*11*). For every 1% decrease in energy from fat, there was a 0.28-kg decrease in body weight.

A large randomized intervention trial including 48,835 post-menopausal women in the United States (The Women's Health Initiative Dietary Modification Trial) also supports a LF diet for the prevention of obesity (*12*). This intervention included group and individual sessions to promote a decrease in fat intake and did not include weight loss or energy restriction goals. Energy from fat was decreased from 38.8% to 29.8% in the intervention group, whereas there was no alteration of fat intake in the control group (from 38.8% to 38.1%). Concomitantly, energy from carbohydrate was increased from 44.5% to 52.7% in the intervention group, whereas there was no alteration of carbohydrate intake in the control group (from 44.5% to 44.7%). Women in the intervention group lost weight in the first year and maintained a lower weight than the control

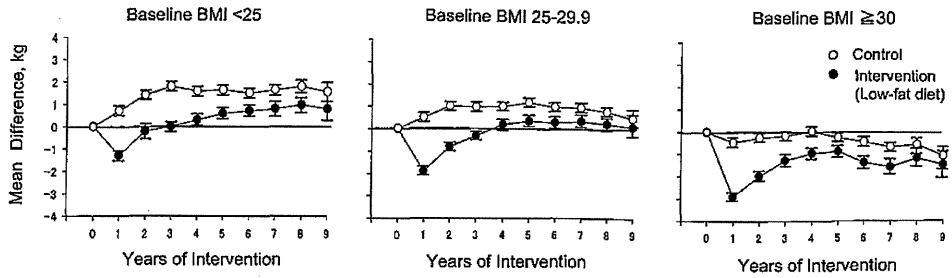

Fig. 1. Differences in body weight by body mass index (BMI) at screening in response to a low-fat (LF) diet. A large randomized intervention trial including 48,835 post-menopausal women during an average 7.5 y of follow-up supports a LF diet (energy from fat decreased from 38.8% to 29.8%) but not energy intake for the prevention of obesity. Women in the intervention groups lost weight in the first year and maintained lower weight than did women in the control groups. No tendency toward weight gain was observed in the intervention groups, whereas body weights in the control groups gradually increased. Error bars indicate 95% confidence intervals. Patient numbers at baseline for the intervention and control groups by BMI: BMI <25 kg/m^2, 5,072 and 7,585; BMI 25–29.9 kg/m^2, 6,940 and 10,446; and BMI≧30 kg/m^2, 7,442 and 11,126, respectively. Reproduced with permission (*12*).

women over an average 7.5 y of follow-up (Fig. 1). No tendency toward weight gain was observed in the intervention group, whereas body weights in the control group gradually increased. In both groups, weight loss was greatest among women who decreased their percentage of energy from fat. Weight loss in response to fat reduction was also slightly greater in subjects with a baseline BMI of <25 kg/m^2.

Several mechanisms for body fat increase in response to a high-fat intake have been proposed (*13*, *14*). Fat is the most energy-dense of the macronutrients and is palatable. Fat produces less of a thermogenic effect than does carbohydrate (*15*, *16*), and fat intake is not regulated, whereas carbohydrate intake is regulated for combustion of carbohydrate substrates (*17*). A prompt increase in glucose oxidation occurs after ingestion of carbohydrate-containing meals, whereas fat oxidation is reduced after food consumption, even when meals provide substantial amounts of fat (*18*). These findings indicate that when energy intake is not intentionally restricted, a LF diet prevents body weight increase in the general population.

A very LC diet (ketogenic diet) decreases body weight in obese subjects

Intervention studies to compare the efficacy of LF and very LC diets to reduce body weight in obese subjects have been conducted and summarized in several meta-analyses (*19–22*). All analyses revealed that a very LC diet is more effective than a LF diet in reducing body weight in obese subjects. In a recent meta-analysis performed by Hession et al., studies comparing the weight loss effects of a very LC diet (less than 60 g/d carbohydrate without intentional energy restriction) against a LF diet with energy restriction (less than 30% fat with 600 kcal/d energy restriction) of more than 6 mo were included (*21*). Among 9 studies analyzed (n=690 in total), 6 studies (*23–28*) showed greater reduction in body weight by LC diet than by LF diet, whereas 3 studies (*29–31*) reported no differences between LC and LF diets in the decrease of body weight when measured at 6 mo of intervention.

However, several adverse effects were observed in a very LC diet. A meta-analysis showed an increase in LDL cholesterol (*22*). Increased blood ketone productions showed unfavorable effects, such as hyperuricemia and orthostatic hypotension (*32*). Recently, even under energy restricted conditions, it was reported that a very LC diet (60% fat/5% carbohydrate) for 6 wk (*33*) or a very LC diet (60% fat/4% carbohydrate) for 1 y (*34*) reduced endothelium-dependent flow-mediated dilation of brachial arteries. A relatively very LC diet (60% fat/20% carbohydrate) worsened the aortic augmentation index (*35*). These adverse effects might be mediated by a large amount of dietary fat. Therefore, a very LC diet was not recommended in the general population.

Mixed evidence that a mild LC diet (low-glycemic diet) decreases body weight in obese subjects

In a Cochrane review, a low-glycemic-index or low-glycemic load diet was compared with a high-glycemic-index or high-glycemic-load diet on different indices of body fat in 6 studies (*36*). Pooled data from 4 studies (*37–40*) showed that weight loss was significantly greater in participants (n=163 in total) receiving the low-glycemic diet (−1.1 kg of difference, p<0.05). Other studies reported a favorable percent change in body mass (*41*) or a favorable change in BMI on a low-glycemic diet (*39*, *42*).

However, two recent intervention studies suggested that reduced-calorie diets resulted in meaningful weight loss, regardless of macronutrient balance. In one study, a total of 34 healthy overweight adults ate a high-glycemic load diet (20% fat, 20% protein, and 60% carbohydrate) or a low-glycemic load diet (30% fat, 30% protein, and 40% carbohydrate) under 30% energy-restricted conditions (*43*). There was no significant change in body weight between the two groups: percentage weight change at 12 mo was −8.04±4.1% in the high-glycemic load diet group and −7.81±5.0% in the low-glycemic load diet group. In the other study, a total of 811 overweight adults (BMI>25 kg/m^2) ate one of four diets for 2 y (*44*). The targeted percentages of energy derived from fat, protein, and carbohydrate in

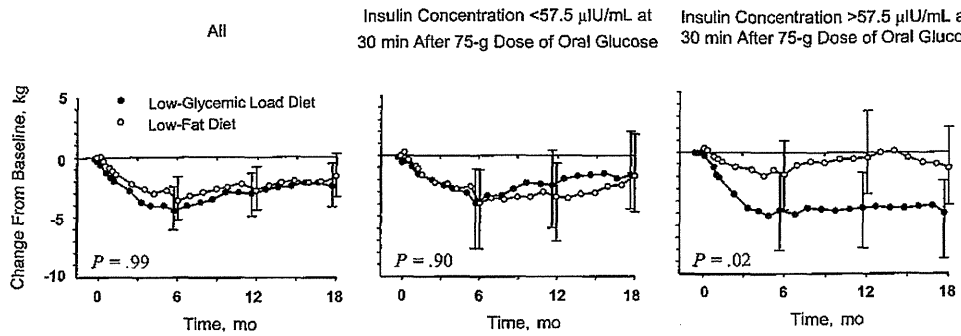

Fig. 2. Changes in body weight in insulin-sensitive and -resistant obese subjects. Obese nondiabetic insulin-sensitive (insulin concentration ≤57.5 μU/mL at 30 min after 75-g dose of oral glucose, n=28) and obese nondiabetic insulin-resistant (insulin concentration >57.5 μU/mL at 30 min after 75-g dose of oral glucose, n=28) young adults were randomized to either a low-fat diet (55% carbohydrate of energy, 20% fat, and 25% protein) or a low-glycemic load diet (or a low-carbohydrate diet; 40% carbohydrate, 35% fat, and 25% protein) for a 6-mo intervention and a 12-mo follow-up period. In the insulin-resistant groups, a low-glycemic load diet produced a greater decrease in weight than did the low-fat diet at 18 mo. Reproduced with permission (47).

the four diets were 20%, 15%, and 65% (LF/low protein [LP] diet); 20%, 25%, and 55% (LF/high protein [HP] diet); 40%, 15%, and 45% (LC/LP diet); and 40%, 25%, and 35% (LC/HP diet). At 2 y, weight loss remained similar in those who were assigned to a diet with 15% or 25% protein (3.0 and 3.6 kg, respectively), in those assigned to a diet with 20% fat or 40% fat (3.3 kg for both groups), and in those assigned to a diet with 65% carbohydrate or 35% carbohydrate (2.9 and 3.4 kg, respectively). There were no differences in reduction of body weights between groups when measured at 6, 12, and 18 mo. When considering the results of recent intervention studies, it is not conclusive that a mild LC diet is preferable for obese subjects.

A mild LC diet preferentially reduces body weights in obese subjects with hyperinsulinemia (insulin resistance)

The studies described above comprised mixed populations of insulin-sensitive and insulin-resistant obese subjects. However, when only the publications that separately examine the effects of LF and mild LC diets on body weight decrease in insulin-sensitive and insulin-resistant subjects were selected, a clear picture appeared. In obese subjects with hyperinsulinemia and insulin resistance, a mild LC diet was more likely than was a LF diet to reduce body weight under energy-restricted conditions (45–47).

In the first intervention study, obese nondiabetic insulin-sensitive (fasting insulin <10 μU/mL, n=12) and obese nondiabetic insulin-resistant (fasting insulin >15 μU/mL, n=9) women were randomized to either a LF diet (60% carbohydrate, 20% fat, and 20% protein) or a mild LC diet (40% carbohydrate, 40% fat, and 20% protein) for 16 wk under a 400-kcal energy deficit/d (45). A marked difference was observed in body weight reduction. Insulin-sensitive women on the LF diet lost 13.5±1.2% (n=6) of their initial body weight, whereas those on the mild LC diet lost 6.8±1.2% (n=6). In contrast, among the insulin-resistant women, those on the mild LC diet lost 13.4±1.3% (n=5) of their initial body weight as compared with 8.5±1.4% (n=4) lost by those on the LF diet. Differences in resting metabolic rate, physical activity, or energy intake between the two dietary groups were not observed (45).

In the second intervention study, obese (BMI 25–29.9 kg/m^2) insulin-sensitive (insulin concentration ≤66 μU/mL at 30 min after 75-g dose of oral glucose, n=16) and obese nondiabetic insulin-resistant (insulin concentration >66 μU/mL at 30 min after 75-g dose of oral glucose, n=16) adults were randomized to either a LF diet (or high-glycemic diet; 60% carbohydrate, 20% fat, and 20% protein) or a mild LC diet (or low-glycemic diet; 40% carbohydrate, 30% fat, and 30% protein) for 6 mo at 30% calorie restriction compared to baseline individual energy needs (46). In the insulin-resistant groups, the mild LC diet produced a greater decrease in weight (−10.2 vs −6.2 kg) than did the LF diet at 6 mo. There were no significant differences in weight decrease between the mild LC and LF diets in the insulin-sensitive groups.

In the third intervention study, obese nondiabetic insulin-sensitive (insulin concentration ≤57.5 μU/mL at 30 min after 75-g dose of oral glucose, n=28) and obese nondiabetic insulin-resistant (insulin concentration >57.5 μU/mL at 30 min after 75-g dose of oral glucose, n=28) young adults were randomized to either a LF diet (or high-glycemic diet; 55% carbohydrate, 20% fat, and 25% protein) or a mild LC diet (or low-glycemic diet; 40% carbohydrate, 35% fat, and 25% protein) for a 6-mo intervention and 12-mo follow-up period (47). Although both the mild LF- and LC-diet groups decreased energy intake similarly by 400 kcal/d, effects of LF and LC diets on body weight reduction were markedly different between the insulin-sensitive and -resistant groups. In the insulin-resistant groups, the mild LC diet produced a greater decrease in weight (−5.8 vs −1.2 kg) and body fat percentage (−2.6 vs −0.9%) than did the LF diet at 18 mo (Fig. 2). There were no significant differences in decreases in weight and body

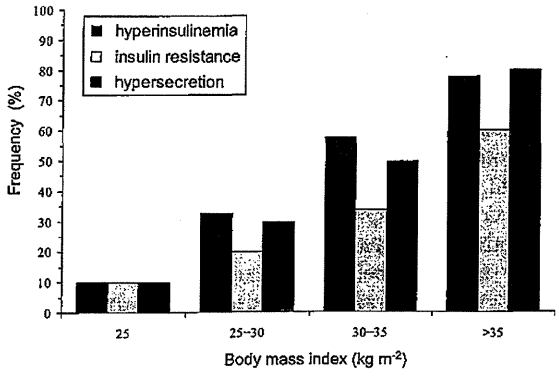

Fig. 3. Prevalence rates of insulin resistance, hyperinsulinemia, and insulin hypersecretion (all defined as the top decile of the respective distributions in lean subjects) as a function of the body mass index (BMI). Black bars, hyperinsulinemia; light gray bars, insulin resistance; dark gray bars, hypersecretion. Reproduced with permission (*51*).

fat between the mild LC and LF diets for any subjects or in the insulin-sensitive group.

Metabolic syndrome is closely associated with hyperinsulinemia (*48*). A recent study examining the effects of LF and mild LC diets in subjects with and without metabolic syndrome under 500-kcal/d energy deficit conditions indicated that a LF diet is preferable in insulin-sensitive obese subjects (*49*). In this study, 202 obese subjects were randomized to either a LF diet (55–60% carbohydrate, less than 30% fat, and 15% protein) or a mild LC diet (or low-glycemic diet; 30–35% carbohydrate, 35–40% fat, and 25–30% protein) for a 12-mo follow-up period. In the subjects with metabolic syndrome, both the mild LC and LF diets were equally effective in reducing waist circumference, whereas in subjects without metabolic syndrome, the LF diet was preferable to that of the mild LC diet: the change in waist circumference was -7.8 ± 7.1 cm in the LF diet group versus -3.8 ± 5.0 cm in the mild LC diet group.

Thus, these four studies suggest that a mild LC diet preferentially reduces body weight in obese subjects with hyperinsulinemia (insulin resistance), whereas a LF diet preferentially reduces body weight in obese subjects without hyperinsulinemia.

Physiological aspects of a mild LC diet making it preferable in obese, insulin-resistant subjects to reduce body fat

It is known that not all obese subjects show insulin resistance (*50, 51*). In a European study of insulin resistance in the obese, hyperinsulinemia, insulin resistance, and insulin hypersecretion were found to increase linearly with an increase in BMI (Fig. 3) (*51*). In this study, hyperinsulinemia was defined as the upper 10% of fasting plasma insulin concentrations in the lean groups. Insulin resistance was defined as the bottom 10% of glucose disposal estimated by euglycemic insulin clamp technique in the lean groups, and insulin hypersecretion was defined as the upper 10% of the distribution of posthepatic insulin delivery rate. According to these criteria, roughly one-half of the obese subjects (BMI>30 kg/m^2) were insulin resistant. The frequency of insulin resistance was 20% in subjects with a BMI of 25–30 kg/m^2, 34% in subjects with a BMI of 30–35 kg/m^2, and 60% in subjects with a BMI of >35 kg/m^2, relative to 10% in subjects with a BMI of 25 kg/m^2 (*51*). Similar trends were observed in regard to hyperinsulinemia and insulin hypersecretion.

Insulin resistance in liver and skeletal muscles elevates glucose concentrations, by which insulin secretion is increased. Moreover, pancreatic beta cells can acutely assess the body's sensitivity to insulin and translate this information into an insulin response that is precisely balanced to offset the severity of insulin resistance (*52*). In patients with insulin resistance, the increment of insulin secretion from β-cells in response to a fixed amount of glucose is greater than that in normal subjects (*53*). Therefore, the sensitivity of glucose to an increased blood insulin level is augmented in obese subjects. Diets with higher glycemic load resulted in higher postprandial insulin concentration in a dose-dependent manner in lean young adults (*54*). It is well known that obese subjects show hyperinsulinemia after oral glucose tolerance testing (glucose is a substance of high glycemic load) (*55, 56*). Postprandial hyperglycemia and hyperinsulinemia augmented by an increase in dietary carbohydrate intake in obese subjects may further promote fat cell enlargement (*57*).

Increased blood insulin stimulates the synthesis of fatty acid in liver and the preferential uptake of fatty acids in adipose tissues to store fat and prevents lipolysis in adipose tissues, all of which facilitate adipose tissue enlargement. Furthermore, these lipogenic effects of insulin are not impaired in obese subjects, whereas the glucose-lowering effects of insulin (inhibition of gluconeogenesis/glycolysis in the liver and stimulation of glucose uptake in skeletal muscles) is severely impaired. Recently, it was shown that hyperinsulinemia is associated with increased production of intestinal apoprotein B-48, which is one of the causes of postprandial hypertriglycemia (*58*). This effect of insulin also indirectly promotes obesity. In the following sections, the mechanisms of insulin-mediated increases in lipid synthesis and fat accumulation in the insulin-resistant state are reviewed.

Insulin-induced lipogenesis in liver is not impaired in insulin-resistant animals or humans

The insulin signaling pathway is thought to proceed through receptor-mediated tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and/or IRS-2. This leads to activation of phosphoinositide 3-kinase (PI3K) and activated Akt (also known as protein kinase B). In activating hepatic lipogenesis, insulin increases transcription of genes encoding acetyl-CoA carboxylase, fatty acid synthase, and others. These actions are caused by an insulin-induced increase in sterol regulatory element-binding protein-1c (SREBP-1c) mRNA (*59*).

To examine the insulin signaling pathway and lipogenesis in the insulin-resistant state, two different ani-

mal models of insulin resistance and hyperinsulinemia, those of lipodystrophy induced by overexpression of the aP2-SREBP1c transgene in adipocytes and obesity induced by mutational disruption of the leptin gene (ob/ob mice) were investigated (*60*). Both animal models showed a reduction of IRS-2 mRNA and protein and increased gluconeogenesis in livers, whereas they showed an increase in SREBP-1c mRNA and lipogenesis. IRS-1 mRNA in the liver was not altered in these animal models. In addition, prolonged insulin treatment in isolated rat hepatocytes led to a fall in IRS-2 mRNA and protein and an increase in SREBP-1c transcript, suggesting that chronic hyperinsulinemia promotes gluconeogenesis in the liver and hyperglycemia, whereas it stimulates fatty acid synthesis in the liver and hypertriglycemia (*60*). It was shown with IRS-1 and IRS-2 liver knockout mice that IRS-1 could convey signals to increase SREBP-1c mRNA and lipogenesis (*61*, *62*). The complete blockage of insulin signaling observed in liver insulin receptor knockout mice showed a decrease in the expression of SREBP-1c (*63*), suggesting that selective insulin resistance may occur in animal models of insulin resistance (*64*). Recently, a branch point in the insulin signaling pathway that may account for selective insulin resistance (in which insulin loses its ability to block glucose production but retains its ability to stimulate lipogenesis) was identified (*65*). In rat hepatocytes, subnanomolar concentrations of rapamycin, an inhibitor of the mammalian target of rapamycin complex 1 (mTORC1), blocked insulin induction of SREBP-1c but had no effect on insulin suppression of phosphoenolpyruvate carboxylase (PEPCK), suggesting that the kinase complex designated mTORC1 was a branch point in the insulin signaling pathway. Therefore, the IRS-1/Akt/mTORC1 pathways are thought to mediate the increase of lipogenesis in the insulin-resistant state.

The finding that insulin-induced lipogenesis in the liver was not impaired in the insulin-resistant state in animal studies could apply to humans. The pattern of stored energy distribution derived from a high-carbohydrate meal is different in young, lean, insulin-resistant individuals (fasting insulin concentration of 12.1±1.2 μU/mL) compared with young, lean, insulin-sensitive individuals (fasting insulin concentration of 7.6±0.6 μU/mL) (*66*). In contrast to the insulin-sensitive subjects, who stored most of their ingested energy in the liver as glycogen, the insulin-resistant subjects had a marked defect in muscle glycogen synthesis and diverted much more of their ingested energy into hepatic de novo lipogenesis, as assessed by incorporation of deuterated water into plasma triglyceride, resulting in increased liver and plasma triglycerides (TGs). Increasing very-low-density lipoprotein-TG secretion from the liver may lead to increased fat accumulation in adipose tissue (*67*). Therefore, insulin activation of the liver IRS-1/Akt/mTORC1 pathway in the insulin-resistant state may lead to obesity.

An increase in lipoprotein lipase (LPL) activity in adipose tissue in response to insulin is not impaired in obese subjects

LPL, located on the capillary endothelium of tissues, catalyses the rate-limiting step in the hydrolysis of TGs from circulating chylomicrons and very-low-density lipoproteins. Most LPL is found in adipose tissues and skeletal muscles, where some of the liberated free fatty acids are taken up and are either stored or oxidized, respectively (*68*). In healthy humans, a combination of stable isotope labeling and arteriovenous difference measurements in adipose tissues showed that in postprandial periods, there is preferential uptake of fatty acids released from chylomicrons by LPL in adipose tissues and also a release of LPL-derived fatty acids into plasma (*69*). Therefore, an increase in LPL activity in adipose tissues may promote fat cell enlargement via increased uptake of fatty acids into adipocytes, in addition to an increased supply of fatty acids to muscle and liver.

Regulation of LPL activity is complex and is controlled by several modulators, such as apoproteins and angiopoietin-like proteins ANGPTL3 and ANGPTL4 (*70*). LPL is active as a dimer, whereas its monomer is inactive. ANGPTL4 inhibits LPL activity by promoting the conversion of active LPL dimers into inactive LPL monomers. Insulin not only increases the level of LPL mRNA but may also regulate LPL activity through both posttranscriptional and posttranslational mechanisms (*71*). The fact that feeding increases active dimeric LPL from inactive monomeric LPL in adipose tissues suggests that insulin may stimulate dimer formation of LPL by an unknown mechanism (*72*). Glucose also increases adipose tissue LPL activity and enhances the stimulatory effects of insulin, possibly by the glycosylation of LPL (*73*).

In humans, feeding or insulin/glucose infusion stimulates LPL activity in adipose tissues, whereas its activity decreases in skeletal muscles (*74*). This divergent response would serve to direct lipoprotein TG-derived fatty acids away from muscle to adipose tissue for storage. A high-carbohydrate diet for 16 d in normal-weight subjects increased postprandial LPL activity in adipose tissue, with elevation of blood glucose and insulin concentrations after meals, relative to a high-fat diet (*75*). Therefore, increased insulin and glucose from a high-carbohydrate diet may promote obesity via activation of LPL in adipose tissues.

The LPL activity in adipose tissues in response to insulin during maintenance of euglycemia was examined in 22 obese and 8 normal-weight subjects (*76*). Basal levels of LPL activity per g of fat tissue in the obese and control groups were 18.7±2.0 and 9.6±2.7 nEq/g/min, respectively. When the responses of LPL in absolute change from basal values were compared between the obese and control groups, no significant differences were found. However, because of the higher baseline LPL activity in the obese subjects, the percent increase in LPL from the basal value was significantly blunted in obese subjects. Basal LPL activity expressed per 10^6 cells correlated positively with cell size, and both the

obese and normal-weight subjects were found to respond similarly to insulin. These data suggest that insulin activates LPL in adipose tissues in obese subjects, irrespective of insulin resistance.

Inhibition of lipolysis in adipocytes in response to insulin is not impaired in insulin-resistant subjects

The concentration of blood free fatty acids (FFA) is determined primarily by their rate of appearance from adipose tissues (lipolysis) and also by their rate of disappearance from plasma. Blood FFA concentrations are elevated during fasting and decreased after feeding. Lipolysis is stimulated by catecholamines during fasting and inhibited by insulin after feeding. If the antilipolytic effect of insulin in obese subjects were impaired due to insulin resistance, fat mass would be smaller in obese subjects. However, most of the studies suggested that insulin resistance is not observed at this step in obese subjects (see following paragraph), although the resistance of insulin to increased glucose oxidation in enlarged adipocytes was clearly shown and is due to a marked decrease in GLUT4 in adipocytes (*77*, *78*).

The antilipolytic effects of insulin on fat cells of different sizes were examined in the 1970s by measuring glycerol release. Basal lipolysis was larger in larger cells (*79*). The antilipolytic effects of insulin on noradrenalin-stimulated lipolysis were more pronounced in the large cells at all tested concentrations (*80*, *81*). Responsiveness and sensitivity to insulin was not altered in adipose tissues of either control or obese subjects (*82*). Rather, a marked resistance to the lipolytic effect of noradrenalin was observed in isolated adipocytes from obese subjects (*83*).

In vivo studies also show that the antilipolytic effect of insulin is not impaired in obese subjects. Both antilipolytic and antiketotic actions occurred at lower insulin concentrations (<90 μU/mL) than those required for hypoglycemic activity (>1,000 μU/mL) (*84*), suggesting that marked insulin resistance might be required to reduce antilipolytic action in adipose tissues. Decreases in blood FFA and glycerol observed during oral glucose tolerance tests were not impaired in obese subjects (*85*). Insulin and glucose infusion rapidly produced antilipolysis in obese and normal groups, as evidenced by large falls in FFA at 20 min after insulin infusion, where FFA was 47% of the basal level in the obese subjects and 31% of the basal level in the normal subjects (*76*).

Triglycerides in tissues are hydrolyzed in a sequential process involving different lipases. Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) are necessary for proper hydrolysis of tri- and diglycerides, respectively. The last step in lipolysis is performed by monoglyceride lipase (MGL), which hydrolyzes monoglycerides to form glycerol and fatty acids (*86*). The activity of ATGL and HSL is tightly regulated by catecholamines and insulin. β-Adrenergic stimulation of the G-protein-coupled receptor activates adenylate cyclase to increase cellular cAMP levels. The antilipolytic action of insulin is mediated by lowering cAMP levels via activation of phosphodiesterase 3B (*87*). The IRS-1/PI3K/PDE3IK (an insulin-stimulated protein serine kinase) signaling pathway is involved in PDE3B activation (*88*). cAMP binding to protein kinase A (PKA) induces phosphorylation of HSL and perilipin, a protein coating the lipid droplet. PKA phosphorylation of HSL causes HSL translocation from the cytosol to the lipid droplet, whereas phosphorylation of perilipin by PKA alleviates the barrier function of this protein and promotes lipolysis (*89*). ATGL is phosphorylated on two conserved serine residues (Ser 404 and 428), although PKA does not phosphorylate ATGL (*90*). However, insulin treatment downregulates ATGL mRNA levels in adipocytes (*91*, *92*). To my knowledge, it has not been shown that decreases in cAMP concentration or ATGL mRNA in adipocytes in response to insulin are blunted in adipocytes from obese subjects.

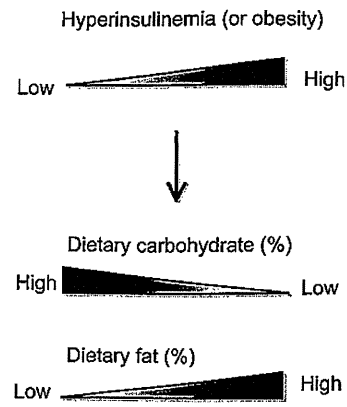

Fig. 4. A proposed model of optimal dietary fat to carbohydrate ratio according to the degree of hyperinsulinemia (or obesity). A key to macronutrient balance in the reduction of body weight is the state of hyperinsulinemia (insulin resistance or obesity); thus, optimal dietary fat to carbohydrate ratios may differ between prevention and treatment of obesity. A mild low-carbohydrate diet (40% carbohydrate) is preferable for obese, hyperinsulinemic, insulin-resistant subjects, whereas a low-fat diet (20–25% fat) is preferable for normal-weight, normoinsulinemic, insulin-sensitive subjects.

Shift from a mild LC diet to a LF diet during obesity treatment (hypothesis)

When a mild LC diet is given to obese subjects, body weights might decrease with improvement in hyperinsulinemia and insulin resistance. Data from the National Weight Control Registry of people who were successful in losing weight and maintaining reduced body weight show that despite wide variation in the methods used to lose body weight, there was remarkable similarity in how they maintained the weight loss, including a diet that was, on average, 24% fat (*93*). Therefore, fat intake might be gradually decreased with a concomitant increase in carbohydrate intake with improvement in obesity (Fig. 4).

Conclusions

In terms of epidemiological, physiological, and molecular aspects, the optimal dietary fat to carbohydrate ratio varies due to the amount of body fat present and

to hyperinsulinemia (insulin resistance). No evidence was found that the lipogenic effects of insulin were impaired in subjects with insulin resistance. In general, in non-obese subjects, most of whom are insulin sensitive, decreasing fat intake is more effective than decreasing carbohydrates to prevent obesity. However, for obese subjects with insulin resistance, a mild LC diet favors a reduction in body weight. The optimal dietary fat to carbohydrate ratio may differ depending on whether the goal is prevention or treatment of obesity, and public guidelines on macronutrients should either be based on the prevalence of obesity in the target society or individualized.

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An Evaluation of Protein Intake for Metabolic Demands and the Quality of Dietary Protein in Rats Using an Indicator Amino Acid Oxidation Method

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Summary Currently, protein requirements are generally determined based on nitrogen balance studies, but there are a variety of limitations associated with this method. The indicator amino acid oxidation (IAAO) method, with a theoretical base that differs widely from the nitrogen balance method, was developed as an alternative method for humans. The objective of the present study was to evaluate protein intakes for metabolic demands and protein quality, using protein itself, in rats employing the IAAO technique with L-[1-^{13}C]phenylalanine. Male Wistar/ST rats (5–6 wk old) received a graded casein (4.3, 8.6, 12.9, 17.2, 21.5, 25.8%), or a wheat gluten (7.2, 10.8, 14.4, 18.0, 21.6, 25.2%) diet, along with L-[1-^{13}C]phenylalanine. An isotopic plateau in breath was achieved 210 min after the start of the ^{13}C ingestion. The protein intakes for metabolic demands were calculated by applying a mixed-effect change-point regression model to breath $^{13}CO_2$ data, which identified a breakpoint at minimal breath $^{13}CO_2$ in response to graded protein intake. The protein intakes for metabolic demands determined by the IAAO method were 13.1 g/kg BW/d for casein and 18.1 g/kg BW/d for wheat gluten, showing a tendency similar to that determined by the nitrogen balance method. These results demonstrated that the IAAO method could be employed to evaluate not only the protein intakes for metabolic demands, but the dietary protein quality in freely living rats, suggesting that this method might be viable in a clinical setting.

Key Words protein metabolic demand, protein quality, indicator amino acid oxidation, rats

The nitrogen balance method is normally employed to determine protein requirements, as specified in the 2007 WHO/FAO/UNU (*1*). However, the limitations of the nitrogen balance method, which can result in considerable error in the prediction of balance (*2*, *3*), have been well described (*4–6*). In the nitrogen balance method, after the diet has been changed, a period of time is usually allowed for adaptation to be complete during the first 5–7 d (*7*). Therefore, employing the nitrogen balance method, the metabolic demand for protein cannot be assessed in patients with a widely varying metabolic demand. The indicator amino acid oxidation (IAAO) method was originally employed to study amino acid requirements in pigs (*8*), and thereafter it has been widely used for studies on pigs (*9–11*) and humans (*12–17*). Since the IAAO method does not require prior dietary adaptation (*18*) to each of the varying protein intake levels, it could be available when an assessment of the metabolic demand for protein is required for post-operative patients or patients with injuries or infections.

In 2007, Humayun et al. (*19*) applied the IAAO method and conducted a reevaluation study on the protein requirements in healthy young men by feeding the subjects graded protein intake as a crystalline amino acid mixture and measuring changes in the oxidation of orally administered L-[1-^{13}C]phenylalanine. However, no studies have previously been conducted on determining the protein requirement using protein itself in animals or humans employing the IAAO method. Therefore, sufficient evidence has not been gathered showing that the IAAO method is viable for measurements of the protein requirement, and it has not been sufficiently validated in studies employing experimental rats up to the present. We should consider that the mechanism of the assimilation of the amino acid mixture differed from that of the protein. Amino acid mix-

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