第７回「栄養とエイジング」国際会議
“健康寿命の延伸を目指して”

The 7th International Conference on “Nutrition and Aging”
: To Stretch Our Healthy Life Expectancy

特定非営利活動法人
国際生命科学研究機構　編

The 7th International Conference on “Nutrition and Aging”
: To Stretch Our Healthy Life Expectancy

Edited by

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目 次

＜特集：国際会議 スライド・データ集＞
第７回「栄養とエイジング」国際会議
“健康寿命の延伸を目指して”

基調講演：健康寿命の延伸にむけて

美味しさの科学：高齢者の食嗜好について ……3
伏木　亨

セッション１：和食（世界無形文化遺産）

現代の日本食は「和食」か？ …… 21
大谷　敏郎
日本の特徴的な食事と疾病：多目的コホート研究からのエビデンス…… 31
津金　昌一郎
疫学研究から見た日本食と循環器疾患 …… 49
三浦　克之

セッション２：先制医療としての栄養の最適化

糖脂質代謝のエピゲノム制御と先制医療 …… 61
小川　佳宏
肥満・メタボリックシンドローム予防のための人生早期の栄養状態 …… 68
岡田　知雄
食事や味刺激がもたらす大脳皮質の変化 …… 79
川上　晋平
機能性食品とエピジェネティクス …… 86
阿部　啓子
ポリフェノールがエネルギー代謝に与える影響 …… 94
日比　壮信

セッション３：食事と脳・神経機能

老齢脳における栄養：最適な食事に関する科学的根拠を得るために
—ILSI Europe の活動概況— ……107
Diána Bánáti
食事パターンと認知症の関係：久山町研究 ……118
二宮　利治
食による体内時計の制御を目指した時間栄養学研究 ……123
大石　勝隆

セッション４：腸内細菌の研究展望

ヒト腸内細菌叢のメタゲノミクス—日本人の特徴— ……139
服部　正平
共生体としての腸内細菌 ……146
Philip M. Sherman
セグメント細菌の腸免疫システムにおける重要性と腸炎モデルへの応用 ……157
梅﨑　良則
加齢に伴う腸内細菌叢の変化—０歳から 100 歳以上まで— ……165
小田巻　俊孝
ヨーグルトが腸内環境および生体機能に及ぼす影響 ……169
木村　勝紀
Lactobacillus gasseri SBT2055 の経口投与による生体防御機能の強化 ……175
酒井　史彦

セッション５：“不活動”の生理学（身体活動と栄養学）

筋萎縮を予防・治療できる新規機能性食材の開発 ……187
二川　健
老化筋肉における身体活動と栄養センシングならびにシグナルの制御 ……196
Blake B. Rasmussen
サルコペニアに対するアミノ酸栄養の重要性 ……206
小林　久峰
健康のために 10 分多く体を動かそう
：用量反応分析に基づいた日本の新しい身体活動ガイドライン ……213
宮地　元彦

ポスタープレゼンテーション要旨

ポスタープレゼンテーション・リスト ……225
ポスタープレゼンテーション要旨 ……227

2015 No. 124
イルシー ILSI JAPAN

CONTENTS

< Special Issue: ILSI Japan Conference Abstracts and Slides >
The 7th International Conference on "Nutrition and Aging"
: To Stretch Our Healthy Life Expectancy

Keynote Lecture: To Stretch Our Healthy Life Expectancy

Science of Palatability: A Study of Elderly Eating Behavior ····· 3
Tohru Fushiki

Session 1: Traditional Japanese Cuisine (Intangible Cultural Heritage)

Can We Regard Modern Japanese Food as "Washoku"? ····· 21
Toshio Ohtani
Typical Japanese Diet and Chronic Diseases: Evidence from the JPHC Study ····· 31
Shoichiro Tsugane
Epidemiologic Findings on Japanese Diet and Cardiovascular Diseases ····· 49
Katsuyuki Miura

Session 2: Optimization of Nutritional Status as Preemptive Medical Care

Epigenetic Regulation of Glucose and Lipid Metabolism and Preemptive Medicine ····· 61
Yoshihiro Ogawa
Nutritional State of Early in Life for Prevention of Obesity and Metabolic Syndrome ····· 68
Tomoo Okada
Changes in the Cerebral Cortex in Response to Food and Chemical Stimulation ····· 79
Shinpei Kawakami
Functional Foods and Epigenetics ····· 86
Keiko Abe
Enhanced Body Fat Utilization as Energy by Dietary Polyphenols ····· 94
Masanobu Hibi

Session 3: Diet and Cranial Nerve Function

Nutrition for the Ageing Brain: Towards Evidence of an Optimal Diet –An Overview of ILSI Europe's Activities– ····· 107
Diána Bánáti

The Relationship between Dietary Pattern and Dementia: The Hisayama Study ····· 118
Toshiharu Ninomiya

Chrono-nutrition Research Aimed at Biological Clock Regulation ····· 123
Katsutaka Oishi

Session 4: Research Perspective of Intestinal Microorganism

Metagenomics of Human Gut Microbiome –Profile of Japanese Gut Microbiome– ····· 139
Masahira Hattori

Intestinal Bacteria as a Symbiont ····· 146
Philip M. Sherman

Importance of Segmented Filamentous Bacteria in Intestinal Immune System and Its Application to Gut Inflammation Model ····· 157
Yoshinori Umesaki

Age-related Change of the Gut Microbiota –People Aged 0 to Over 100 Years Old– ····· 165
Toshitaka Odamaki

Effects of Yogurt on Intestinal Environment and Body Functions ····· 169
Katsunori Kimura

Augmentation of Host Defense Mechanism by Oral Administration of *Lactobacillus gasseri* SBT2055 ····· 175
Fumihiko Sakai

Session 5: Physiology of "Physical Inactivity" (Physical Activity and Nutrition)

A Novel Nutritional Approach against Unloading-mediated Muscle Atrophy ····· 187
Takeshi Nikawa

Physical Activity and the Regulation of Nutrient Sensing and Signaling in Aging Muscle ····· 196
Blake B. Rasmussen

The Importance of Amino Acid Nutrition in Sarcopenia Prevention ····· 206
Hisamine Kobayashi

"Add 10 Min. for Your Health": The New Japanese Recommendation for Physical Activity Based on Dose-response Analysis ····· 213
Motohiko Miyachi

Poster Presentation Abstracts

Poster Presentation List ····· 225
Poster Presentation Abstracts ····· 227

基調講演

健康寿命の延伸にむけて

基調講演：健康寿命の延伸にむけて

美味しさの科学：高齢者の食嗜好について

龍谷大学　農学部　食品栄養学科
伏木　亨

生活の質（QOL）を高めるためには、食嗜好にかなう食の提供が重要である。特に、食物選択や咀嚼などに制限の多い高齢者に対しては、食嗜好にあう食事の確保は非常に大きな問題である。

高齢者は一般に咀嚼や嚥下の問題があるため、離乳食に似た柔らかさや食べやすさが重要視されるが、幼児と高齢者とは大きく異なる部分がある。食体験が浅く食物に対して新奇恐怖の顕著な幼児に比べ、高齢者は食経験が豊富である。しかも、嗜好性がはっきりしている場合が多い。いわば、好みのはっきりしている健康な成人の延長として美味しさを考えねばならない。

一般に高齢者は疾患を持つ人が多く、一律ではなく個別の対応が必要な場合がある。また、栄養素の消化吸収機能低下がある場合があるので、これらの制限のなかで、食べる楽しみをどのように感じてもらえるかが我々の責務であると思う。

いくつかの制限がある中でも、やはり、できれば若い頃と同じものを食べたいという欲求がある。味の素㈱が 2000 年に実施した 5000 人嗜好調査でも、高齢者は若年者と同様に食に対する期待があることが確認されており、高齢者にとっての幸福感に繋がる重要な問題であると思われる。高齢者の食嗜好の特徴として以下のようなことが考えられる。

1．食体験が豊富であり、食の情報が豊かである。記憶維持には個人差が大きい。食嗜好は経験によるところが大きく、高齢者は豊かな食経験を持っている。認知や記憶の減退による影響は免れないが、部分的にははっきりとして記憶があることも考えられる。個人差が大きいため、個別の対応が必要な部分である。

2．味覚の受容体の数は減少しているといわれるが、それでも美味しさに対する感覚は維持されていることが多い。美味しさの判断の脳機序には大きな違いはない。味覚や嗅覚の受容体は加齢とともに減少するが、これらの受容体の全てが常時動員されているのではなく、大幅な減少が生じても感覚には大きな影響がないことも多い。

3．匂いの経験はかなり後期まで強固に残っており、過去の記憶とも結びついている。一般に美味しさの記憶は、味覚よりも嗅覚に依存するところが大きい、嗅覚の受容機構は味覚に比べて受容体の数も多く、しかも記憶と直結している。私たちが懐かしい食や食べ慣れた食に対して美味しさを感じるのは、ほとんど嗅覚の寄与であると言って過言ではない。「マドレーヌ効果」と呼ばれる現象の研究によると、匂いの記憶は何十年たってもぶれることがなく、しかも当時の記憶と強固に結びつき、食によって過去の体験などの様々な記憶が蘇ることがある。高齢者の認知機能に対する訓練という意味でも、注目すべき現象である。

これらの特徴を考慮した老人にやさしい食の提供が重要であると思われる。

Keynote Lecture: To Stretch Our Healthy Life Expectancy

Science of Palatability: A Study of Elderly Eating Behavior

Tohru Fushiki, Ph.D.
Faculty of Agriculture, Ryukoku University

The development of palatable foods can contribute to improvements in quality of life (QOL). In particular, the provision of tasty foods is important for elderly with restrictions on food choice and chewing ability. In this context, foods with a softness and ease of chewing, i.e., a texture similar to baby food, have been proposed in order to overcome chewing and swallowing problems. However, this does not consider the fact that elderly eating behavior differs substantially from that of an infant. While an elderly individual has accumulated substantial experience with food over the course of their lives, infants experience "neophobia" to new foods. When developing new foods, an important consideration is tailoring them to each individual, given differences between individuals in the types of issues they experience, such as problems with digestion and absorption, metabolic diseases, and, occasionally, malnourishment.

Despite the several restrictions they are bound by, many elderly individuals still desire to eat as they did when they were young, as reported by the "5000 person taste investigation" conducted by Ajinomoto Co., Inc., in 2000. We believe it our obligation to investigate ways in which to provide gustatory pleasure to elderly individuals, despite their restrictions. To this end, the following points should be considered:

1. The elderly have extensive experience and their knowledge of food is vast, although, of course, there are likely variations across individuals due to memory issues.
2. Taste buds decrease in number with age, but the sense of palatability remains. Indeed, there is no major difference in the cerebral mechanism underlying palatability in young and elderly individuals.
3. The olfactory receptor, which plays a dominant role in palatability, should also be considered. Olfaction is directly connected with memories in the brain, and such memories can remain stable for decades. For instance, one might recall past scenery and landscapes upon food intake. This phenomenon, which was first documented in a famous novel by Marcel Proust, is known as "the Madeleine effect." It may be useful for treatment of cognitive impairment.

With the above in mind, we believe that suitable cuisine could be prepared which the elderly would find satisfying.

Science of palatability: a study of elderly eating behavior

Preparing a cuisine the elderly find satisfying.

Faculty of Agriculture, Ryukoku University

Tohru Fushiki

1

概要

１．おいしさとは何か What is palatability?
おいしさの構造 Its components

２．高齢者の食嗜好と疾病 Palatability for aged people
糖尿病と食嗜好 Diabetes and taste
カロリー制限、減塩と食の嗜好性
Suppression of energy and salt contents in a diet

３．認知障害における食の記憶の重要性
The importance of memory of eating via olfaction

2

What is palatability?

おいしいという用語は経験的に使われているが、
その定義には確固たるものはない。

In research on human food intake and acceptance, the term " palatability" has been used its colloquial sense that reflects a positive hedonic evaluation under a given set of conditions, but its usage has not always been clear and consistent.

3

おいしさは食品の中にはない？
Where is the palatability of foods?

おいしさは食品と人間の関係のなかだけにある

Palatability can exist only in the relationship between a food and person eating it.

4

Analysis & Evaluation by Specialists:

huge number of words about palatability

あらい、まるい、なめらか、あと味、さばけ、きれ、だれ、
はば、ふくらみ、こし、こくみ、ごくみ、にく、せん、押し味、
きめ、はね、もたつき、かなけ、土臭、袋香、木臭、ムレ香、
オリ香、生酵母、びん香、老香、生老香、白米臭、甘臭、
若い、重い、端麗、濃醇、　、、、、、、、、、、、、、

宇都宮ら、清酒の官能評価分析における香味に関する品質評価用語および標準見本より抜粋

5

瞬時にざっくりと評価
Rapid and rough evaluation

あまりたくさんのことを考えてはいない

6

瞬時の判断ならば要素は多くない
それらを抽出・分解できる

Although no individual has the same experiences with specific foods, some common factors affecting palatability seem present.
If so,
palatability may be dissected into componential subdomains.

7

おいしさの要素
Four hypothetical subdomains of palatability

生理　Physiological
文化 Cultural
情報 Informational
報酬 Addictive

8

生理的なおいしさ

Physiological factors

9

生きるために体が要求しているものはおいしいと感じる.

Foods we need to live are delicious!

10

生理的おいしさに関わる論文

- 人間や動物が甘味を好み苦味を忌避するのは有用な成分・有害な成分を認識する生命維持のための先天的機能（Steiner 2001）
- 動物は必須アミノ酸を欠いたえさに対する食欲を減じ、欠乏しているアミノ酸を選択的に摂取する（Mori et al. 1991）
- 長時間の運動の後では、人や動物は甘味欲求を高める（Horio、１９９７）
- 十分なエネルギーが得られている場合、甘味嗜好が抑制される（Kawai２０００）
- 高血圧症患者は電解質の代謝異常の結果として塩味嗜好性を持つ（Rogers 1970）

11

2. 食文化のおいしさ

Cultural factors

12

子供のころからの食習慣に合わないものはマズイ

Food habits are highly influenced by social factors established as part of the acquisition of culture

「タマゴ焼きは甘いのが好きですか」

Is your ideal omelet Sweet?

食べなれたものの安心感

13

食文化に合わないものには違和感

“Uneasiness occurs when one encounters foods from a different culture”.

くさや

豆腐のスイーツ

出典　recipie.rakuten.co.jp
http://recipe.rakuten.co.jp/recipe/1710011483/

14

おいしさへの食文化の影響

- 食の伝統は健康感や便利、楽しさと同じく食品選択の主要な因子である（Rappooport et al. 1992)
- 幼児期に和食の朝食を摂取していたヒトは成長してからも和食への関心が高い（Kimura et al.2010)
- 高齢者の好みを観察すると、料理の選択には若年時の食習慣が関係している（Laurati et al. 2006)
- 大人の野菜の摂取状態は家庭での昔からの食習慣を反映している(Uglem et al. 2007)
- 地域や民族の伝統的な食は、反復摂取によって予測が可能となったおいしさ（Zeller 1999)
- 親子の類似点はそれほど大きくない（Rozin 1991)

15

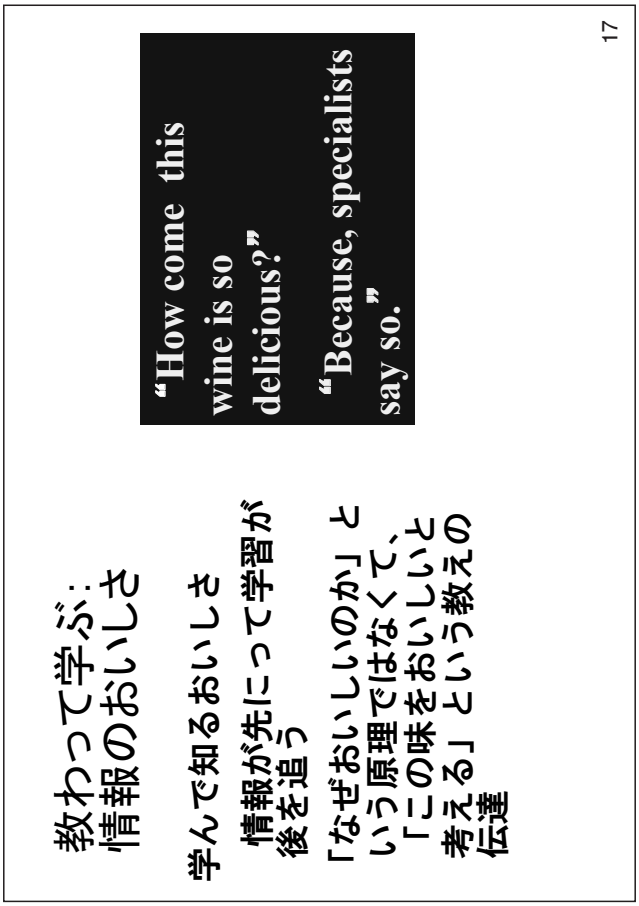
情報のおいしさ

Informational factors

16

Eat one!
Warning: one of them is hot

18


教わって学ぶ：
情報のおいしさ
学んで知るおいしさ
情報が先にって学習が
後を追う
「なぜおいしいのか」と
いう原理ではなくて、
「この味をおいしいと
考える」という教えの
伝達
"How come this wine is so delicious?"
"Because, specialists say so."

17

Worried

20

19

味覚や嗅覚の判断よりも文字情報を現代人は選んだ

We select foods in a way that highly depends on information.

Because it is safer than tasting.

食べる前に安全が判断できる

21

情報の影響に関わる論文

- おいしさの予想や期待はおいしさの評価に影響する（Cardello et al. 1985, Rozin et al. 1998)
- おいしさの予想や期待によって、予想を確認するためおいしさの外観に注目が向けられる（Wansink, 2002,2004)
- ワインボトルのラベルのような環境的因子が同時に供される食物の消費量に影響を与える（Wansink et al. 2007)
- 環境的因子として値段、表示、見た目、ネーミングなど多くの形態がある（Wansink et al. 2005)
- 色が嗜好に影響を及ぼす (Zeller 2003, Mega 1974, Johnson&Clydesdale 1982)
- 食品ラベルの内容が嗜好性に影響（Okamoto et al. 2008)
- 食品カテゴリーの重要性（Zeller 2007)

22

4.やみつき
Hedonic, Addictive factors

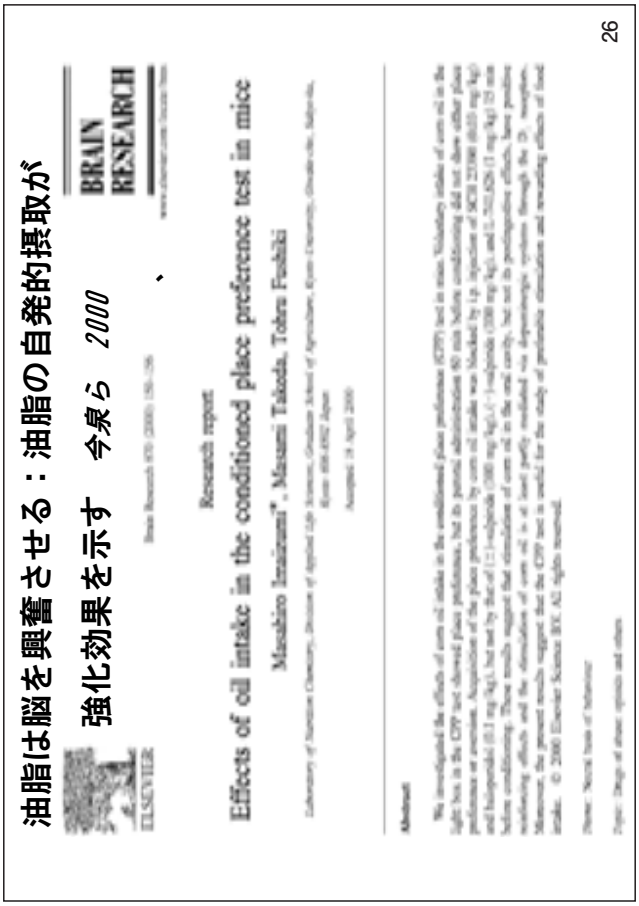
23

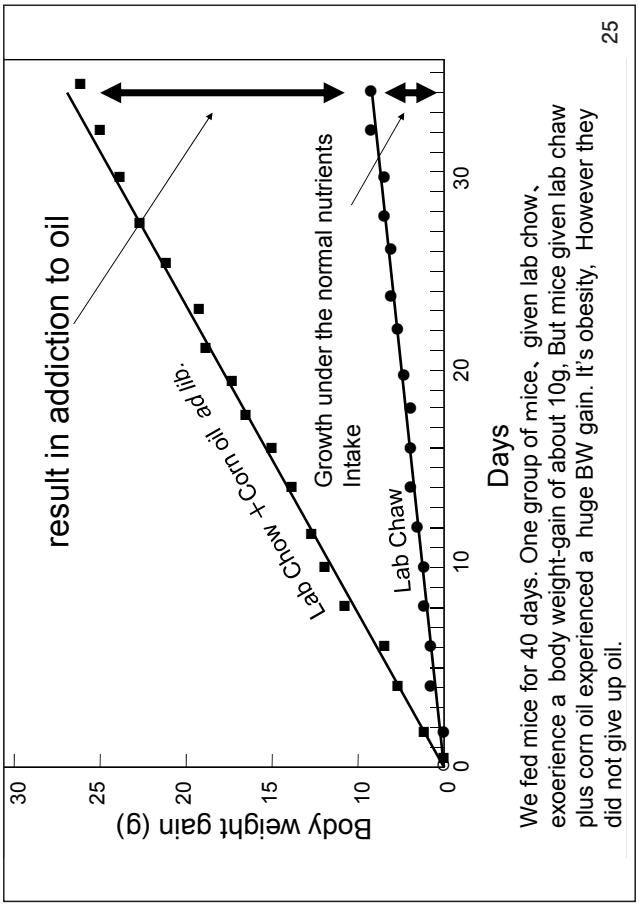
Delicious foods always contain 「Fat、Sugar、or Umami of Dashi

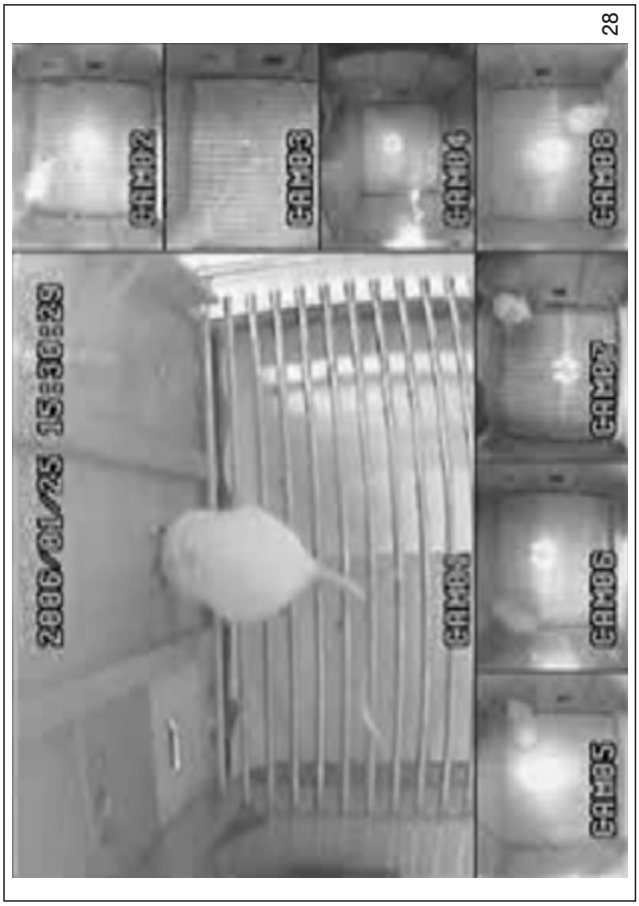
チョコレート、ケーキ、牛丼、すき焼き、ステーキ、ラーメン、ギョーザ、天ぷら、フライ、ソフトクリーム、カレー、おいしいお吸い物、おでん、
Chocolate, cake, sukiyaki, steak, tempura and Chinese noodles……. etc.

やめられない・高度の満足・幸福感
Hedonic, Addictive

24


油脂は脳を興奮させる：油脂の自発的摂取が
強化効果を示す　今泉ら　2000
BRAIN RESEARCH
Research report
Effects of oil intake in the conditioned place preference test in mice
Masakiro Imaizumi*, Masami Takeda, Tohru Fushiki
26

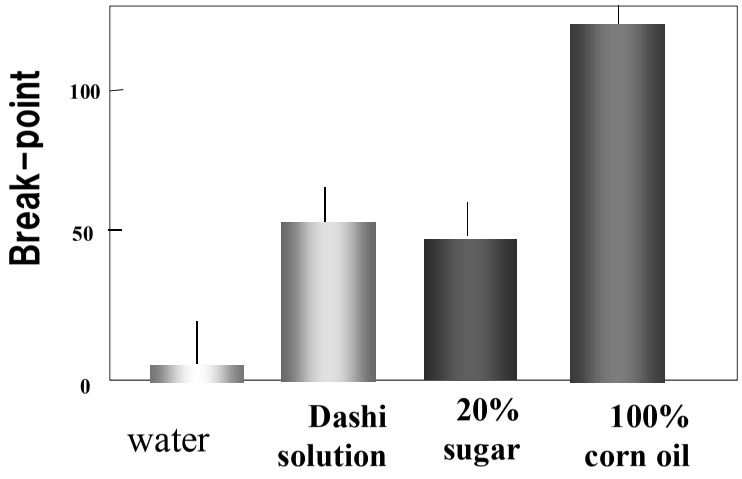

result in addiction to oil
Lab Chow +Corn oil ad lib.
Growth under the normal nutrients
Intake
Lab Chaw
Body weight gain (g)
Days
0
10
20
30
We fed mice for 40 days. One group of mice、given lab chow、exoerience a body weight-gain of about 10g, But mice given lab chaw plus corn oil experienced a huge BW gain. It's obesity, However they did not give up oil.
25


2006/01/25 15:30:29
CAM02
CAM03
CAM05
CAM06
CAM07
CAM08
28

欲求の定量的評価
Operant
lever press response
Preliminary training in lever press was performed until mice acquired a skill of 500 presses within 30 min.
Operant responding training using 10% sucrose solution under Fixed ratio schedule (FR) for 3 sessions
Then trained under Progressive ratio (PR) schedule with 100% corn oil for 3 sessions
27

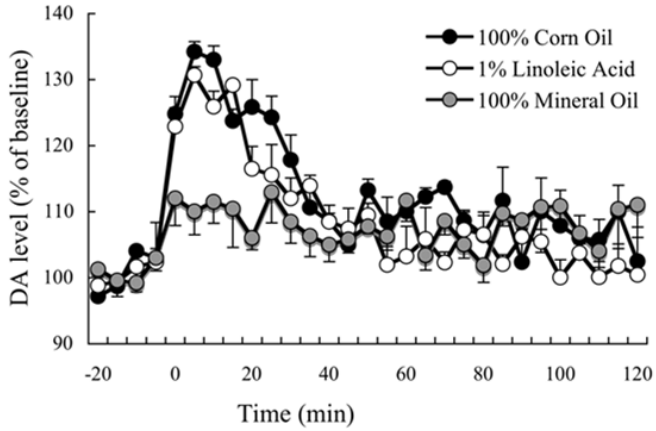
Number of times touching a lever

（at the break-point）



29

Micro-dialysis of Nucleus Accumbens (側坐核）



Adachi et al. Biosci. Biotechnol. Biochem., 77, 2175, 2013

30

報酬系の影響

- ヒトの食べたい強い欲求の構成因子として、高脂肪、甘味、炭水化物　（White et al. 2002)
- 禁断、我慢不能、やみつきになる傾向は食品成分によって異なる（Rozin et al. 1993)
- 油脂や砂糖などへの極端な嗜好は高栄養への欲求であり、やみつきと表現できる（Takede et al 2001)
- 脂質の摂取は、報酬系に影響を及ぼし、動物の強い満足感と摂食意欲をもたらす（Imaizumi et al, 2001,Sawano et al. 2000, Yoneda et bal. 2007)
- ナロキソンを投与した過食症の女性は甘味の強い高脂肪食品の消費が抑制された（Drewnowski et al. 1995)
- 報酬イコールかならずしも快楽ではない（Hironaka 1997, Shultz 2000, Geldman et al. 2003)

31

Conclusion

Palatability consists of four elements

Physiological

Cultural

Informational

Addictive

32

Fifteen questionnaire items for the three componential factors of food palatability

A: Items putatively related to reward

A-1: Is the taste likely to be addictive to you?

A-2: Does the taste make you feel compelled to pick up the food?

A-3: Does the taste make you take another bite if you take a bite?

A-4: Are you satisfied with the taste?

A-5: Do you think the food tastes good because of rich fat sweetness or umami?

B: Items putatively related to culture

B-1: Are you used to the taste?

B-2: Have you had a food that has the same or a similar taste to the food?

B-3: Have you eaten food like this many times?

B-4: Do you think your family (your parents, siblings, spouse, etc.) would like the taste of the food?

B-5: Have you liked the taste of the food since your childhood?

C: Items putatively related to information

C-1: Does the food appear tasty?

C-2: Have you ever seen this food in advertisements or heard of it by word-of-mouth?

C-3: Have you ever heard anything good about the healthfulness of the food?

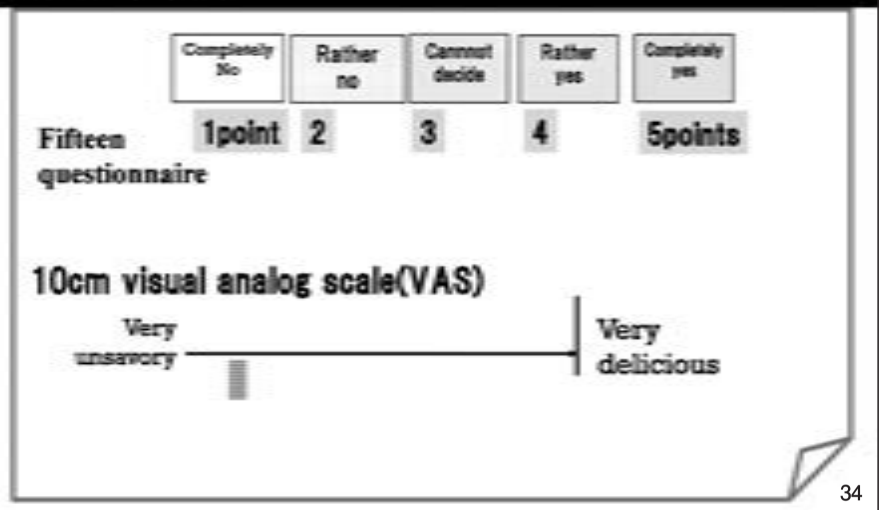
C-4: Do you feel secure about the ingredients of the food?

C-5: Do you think that the food seems expensive?

Nakano et al. 2013

33

評価方法 Estimation of Constitution of palatability



34

標準偏回帰係数

The standard regression coefficient

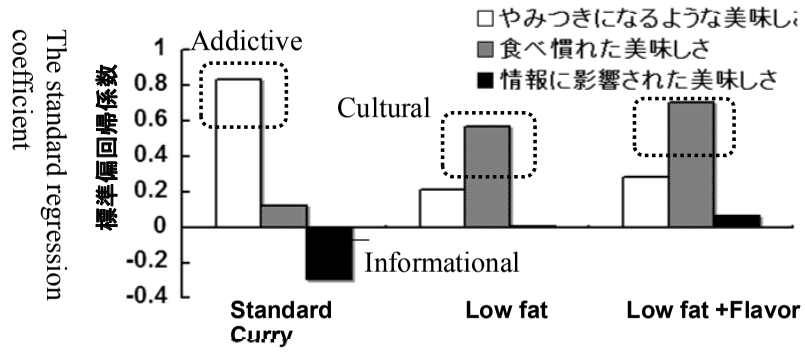
$$Y = b_1X_1 + b_2X_2 + b_3X_3$$

b1, b2, b3を 標準化 =標準偏回帰係数

・標準偏回帰係数とは、各独立変数Xのデータをその標準偏差で除した後に重回帰分析を行った場合の係数であり、各独立変数が従属変数に与える影響を比較することができる

35

The standard regression coefficient



	a	b	c
やみつきになるような美味しさ	0.84	0.12	0.29
食べ慣れた美味しさ	0.12	0.57	0.70
情報に影響された美味しさ	-0.29	0.00	0.07

36

２、**Palatability for senior citizens with diseases: Analysis of their palatability experiences by dissection into componential subdomains**

疾病のある高齢者の食嗜好性：
嗜好性要素解析の応用

37

Diabetes and physiological craving for sweetness
糖尿病と生理的甘味嗜好

糖は血液中に過剰量存在するが臓器に吸収されない

Craving for sugar in the brain
糖飢餓情報が脳を刺激する
生理的な糖欲求が生じる

38

生理的糖嗜好の緩和
食べなれ感 Cultural
情報 Cephalic stimuli by foods

頭相信号（Cephalic phase signal: 視覚、味覚・嗅覚・食感）を強化することで低カロリーでも満足感
Satisfaction by enhancement of the cephalic stimuli by food

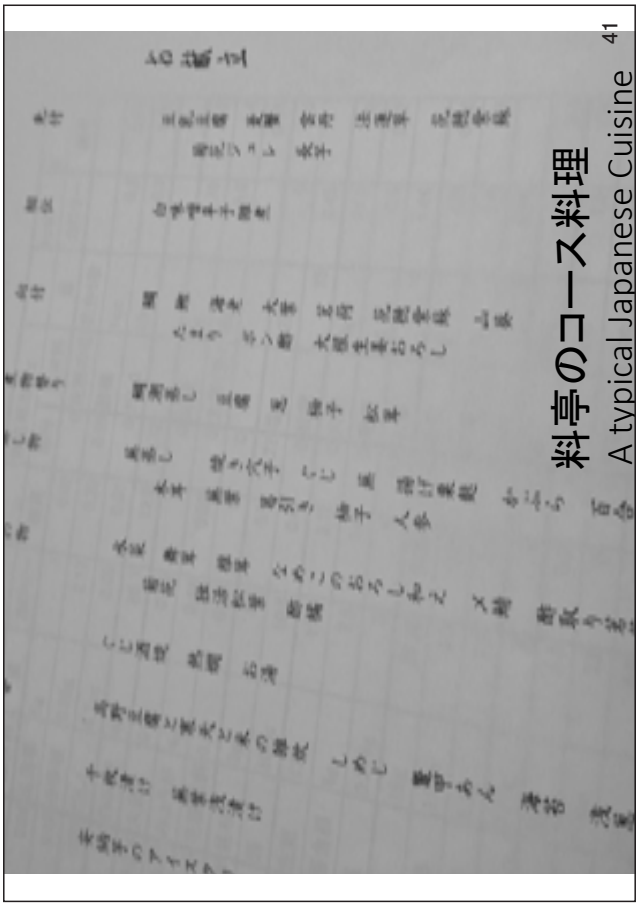
39

低カロリーの日本食
Japanese cuisine as a low-calorie diet

食材数が多い Huge number of ingredients
油脂含量が低い Low fat
視覚的な魅力 Visual satiety

味覚・嗅覚・食感（Cephalicな情報）を最大限に活用Abundant cephalic stimuli
うま味による満足強化
Satiety by UMAMI

40



精進料理
Shoujin Cuisine: Vegetarian food. The cooking technology remarkably developed in monasteries ~13thC AD

獣肉食の禁止
禅寺による料理技術の創意と調理技術の洗練

低カロリー、低脂肪、高い満足感として、特に注目を浴びつつある

43

Low-sodium food
減塩食と食の文化

食文化（食べなれた味）の嗜好性変換

塩分抑制と満足感
食べなれたおいしさを変えることは容易ではない

44

吸いものの塩加減にも生理的背景

血液中の塩分濃度に近いものがおいしい

塩化ナトリウム濃度0.9%前後
（154mEq, 0.9% NaCl)

薄いと物足りない・濃いと塩辛い

からだが求める適当な塩分は体液と同じ

45

食文化（食べなれた味）の嗜好改変の可能性

嗅覚による塩味の増強
Enhancement of salty taste by flagrance

うま味増強による塩味欲求の緩和
Tempering the craving for salt by Umami taste

46

47

料亭の一番だし

塩分濃度　0.64%
7 Kcal/100ml

48

高齢者の認知の減退と嗜好性
Memory loss (dementia) and preference for senior citizens
食べなれたおいしさの刺激

懐かしさの記憶
ある程度までの記憶減退には、懐かしい食の風味の記憶を刺激することが有効

49

嗅覚の記憶は様々な関連する記憶のタグとなって長期間 脳に保存される

An olfactory memory will be a tag of various related memories, and is preserved for a long period.

マドレーヌ効果

「物語は、ふと口にした紅茶に浸したマドレーヌの味から、幼少期に家族そろって夏の休暇を過ごしたコンブレーの町全体が自らのうちに蘇ってくる、という記憶を契機に・・・」

（プルースト「失われた時を求めて」）より

50

食べなれたおいしさの刺激

Stimulation of senior citizen's memory through the olfaction of foods he or she is used to eating

高齢者の、ある程度までの記憶減退には、懐かしい食の風味の記憶を刺激することが有効

51

The flavour of the Showa period?

昭和の懐かしい食べ物は？

クジラベーコン、クジラ竜田揚げ、すいとん、麦こがし、脱脂粉乳、ラムネ、
タケダのプラッシー、ハムカツ、魚肉ソーセージ、お子様ランチ、カルミン、りんごアメ、
マルシンハンバーグ、渡辺の粉末ジュース
ニッケ味のお菓子、はったい粉、カルメ焼き
ノースキャロライナ、肝油、コッペパン、磯自慢、
スパゲッティナポリタン、鱈昆布汁、グリコ、お茶ずけ、みつ豆缶、たくあんの煮付け、いも子のおづけ、なご炒り、かいもち、あけび、ふかしパン、ねぎぬた、イワシの煮つけ、たにし、、、

52

セッション1

和食（世界無形文化遺産）

セッション 1-1

現代の日本食は「和食」か？

国立研究開発法人　農業・食品産業技術総合研究機構　理事
食品総合研究所　所長
大谷　敏郎

おせち料理など日本の伝統的な食文化としての「和食」が、2013 年 12 月にユネスコの無形文化遺産に登録された。和食は、自然の素材に敬意を払った調理法や食材の知識、伝統文化などを包括する概念として定義されている。例えばお正月には、それぞれに意味のある新鮮な食材で美しく飾り付けた特別な食事が用意され、家族やそれぞれの地域で祝われる。また、和食に関する知識や調理法は日々の食事を通じて家庭の中で受け継がれるとされている。

一方、農林水産省は、和食が、栄養バランスに優れ、低脂質であることから、日本人の健康や長寿、肥満を防ぐのに役立っていると考えている。事実、2011 年の OECD のデータでは、最長寿国の一つにもかかわらず、肥満の割合がわずか 3.9% に留まっていることが明らかになっている。

この二つの和食の定義には大きな認識のギャップがある。ユネスコが、食材や調理法、文化、特に行事食も含めて、広い範囲を和食としているのに対し、農林水産省はバランスの取れた健康な生活を和食と考えている。

ただ多くの日本人は、正直なところ、和食は鮨や天ぷらなど伝統的で特別な食事か、両親や祖父母から受け継がれた古くさい食事くらいにしか思っていないのではないかと思われる。

日本人の平均寿命は、第二次大戦以降、大幅に改善された。1950 年の男女の平均寿命は、それぞれ 58 歳と 61.5 歳であったのに対し、2014 年では、80.2 歳と 86.6 歳に順調に伸びている。この平均寿命の伸びは、栄養状態の大幅な改善とそれに続く健康管理システムの改善が要因と分析されている。1950 年代の一日あたりの熱量供給量は 2300 キロカロリーであったが、1960 年代以降、様々な食品が一日あたり 2400 から 2600 キロカロリー供給されるようになった。この時期、米の消費量は減少し、肉や乳製品の消費量が増加したことで、PFC 比（タンパク質、脂質、炭水化物の比）が大幅に改善された。1977 年の米国の報告では、ヒトの健康を維持するのに最適な PFC 比として、15：25-30：60-55% が推奨されているが、これは、その時の日本人の PFC 比であり、理想的な栄養バランスとされている。1980 年代以降、食事の急速な西洋化に対して、日本の消費者は栄養バランスについて大きな関心を払うようになった。

日本の各年齢層のこの 30 年間の PFC 比は、タンパク質 15-16%、脂質 23-26%、炭水化物 60-62% で、実質上、大きく変化しておらず、平均寿命は順調に伸びている。一方、この間、成人病が大幅に増加したことで、消費者の食への大きな関心事項が、「健康維持のために、栄養に付加すべき機能性」に移ってきたことが報告されている。

和食が、第二次大戦以降の日本人の平均寿命の延伸に大きく貢献したことについては疑いの余地はない。日本人の食生活、特にこの 50 年間の栄養状態は大きく改善した。「和食」という言葉は、非常に広い意味と概念を含むため、「和食」という用語を使用する場合は、現代の日本食とは区別して使うべきではないかと考えている。

Session 1－1

Can We Regard Modern Japanese Food as “Washoku” ?

Toshio Ohtani, Ph.D.
Director General, National Food Research Institute, NFRI
Vice President, National Agriculture and Food Research Organization, NARO

Washoku, traditional dietary cultures of the Japanese, notably for the celebration of New Year was listed as an UNESCO’s Representative List of the Intangible Cultural Heritage of Humanity in December 4, 2013. Washoku is a comprehensive set of skills, knowledge and traditions relating to the preparation and consumption of food, and respect for natural resources. Typically seen during Japanese New Year celebrations, it takes the form of special meals and beautifully decorated dishes using fresh ingredients, each of which has a symbolic meaning. These are shared by family members or collectively among communities. The basic knowledge and skills related to Washoku are passed down in the home at shared mealtimes.

On the other hand, the Ministry of Agriculture, Forestry and Fisheries of Japan（MAFF）explain that Washoku contributes to a healthy life, long life expectancy and prevention of obesity among the Japanese since it requires well-balanced and low-fat diets. In fact, Japanese people have the highest life expectancy and the lowest obesity rate, 3.9%, in OECD Health Data, 2011.

It is a wide perception gap between both two organizations, because the definition of the Washoku of UNESCO is a wide range of all things including materials, cooking and culture, especially special meals for the celebration, but MAFF explains the well-balanced healthy life.

Honestly, most Japanese recognize Washoku as traditional special meal, such as sushi and tempura, or only old Japanese diet in their parents’ or grand parents’ generation.

Japanese life expectancy improved drastically after World War II. Average life expectancy of female and male was only 61.5 and 58.0 years’ old, respectively, in 1950 and was gradually increased to 86.6 and 80.2 years’ old in 2014. It is expected that the major reasons for this increased life expectancy were due to the improvement of nutritional food quality and subsequent improvement in the health-care system. Daily calorie supply of foods was below 2300 kcal in 1950’s, and a wide variety of foods with 2400-2600 kcal/day have been provided after 1960’s. During this period, rice consumption was reduced by half, and the consumption of meats and dairy products increased, consequently, PFC（Protein, Fat, and Carbohydrate）ratio was improved. In 1977, McGovern recommended PFC ratio of 15:25-30:60-55% as the optimal nutritional balance for maintaining human health. This ratio referred to the Japanese PFC ratio at that time, which was recognized as the ideal ratio for nutritional balance. Since 1980s, Japanese consumer expressed concern on nutrient balance due to the rapid westernization of daily meal.

The average PFC ratio for the people of all ages in Japan virtually remains unchanged for the last 30 years, i.e, P = 15-16%, F = 23-26%, C = 60-62% and the life expectancy has increased gradually. On the contrary, adult diseases have been increasing in recent years; therefore, the greatest concern of consumers for food is “the functionality for health maintenance in addition to the nutrition “as reported in different surveys.

There is no doubt that Washoku contributes the extension of the life expectancy after World War II. Japanese diet was drastically changed, especially, nutrient condition was improved in these 50 years. I believe that Washoku in a broad sense should be distinguished from modern Japanese diet, because a word of Washoku has too much meaning and concept.

ILSI Japan The 7th International Conference on Nutrition and Aging, Tokyo, Sep. 29, 2015

Can we regard modern Japanese food as "Washoku" ?

Toshio Ohtani, Ph.D

Vice-president
National Agriculture and Food Research Organization, NARO
Director General
National Food Research Institute, NFRI

1

December 4 2013

Washoku was listed as an UNESCO's Representative List of the Intangible Cultural Heritage of Humanity.

Washoku, traditional dietary cultures of the Japanese, notably for the celebration of New Year - Japan

Washoku is a comprehensive set of skills, knowledge and traditions relating to the preparation and consumption of food, and respect for natural resources. Typically seen during Japanese New Year celebrations, it takes the form of special meals and beautifully decorated dishes using fresh ingredients, each of which has a symbolic meaning. These are shared by family members or collectively among communities. The basic knowledge and skills related to Washoku are passed down in the home at shared mealtimes.

Washoku contributes to a healthy life, long life expectancy and prevention of obesity among the Japanese since it requires well-balanced and low-fat diets. - MAFF -

2

Japanese Traditional Holiday -Sekku-

In Edo-era, Japanese government specified 5 holidays called Sekku.

January 7; Nanakusa	Festival of Seven Herbs	
March 3; Momo	Doll Festival	Girls' Festival Day
May 5; Tango	Boys' Festival	Children's Day
July 7; Tanabata	Star Festival	
September 9; Kiku	Chrysanthemum Festival	

To celebrate these holidays, special dishes and/or sweets are provided.

3

What is daily WASHOKU?

Vegetarian Cuisine
Vegetarian Cuisine, Shojin-ryori, was originally brought back to Buddhist temple by priest studied in China from the 12th century onward. The major ingredients are vegetables, beans and potatoes. Ganmodoki, upper right, is made of fried tofu and vegetables that mimics goose meat. "Gan" = goose, "modoki" = mimic.

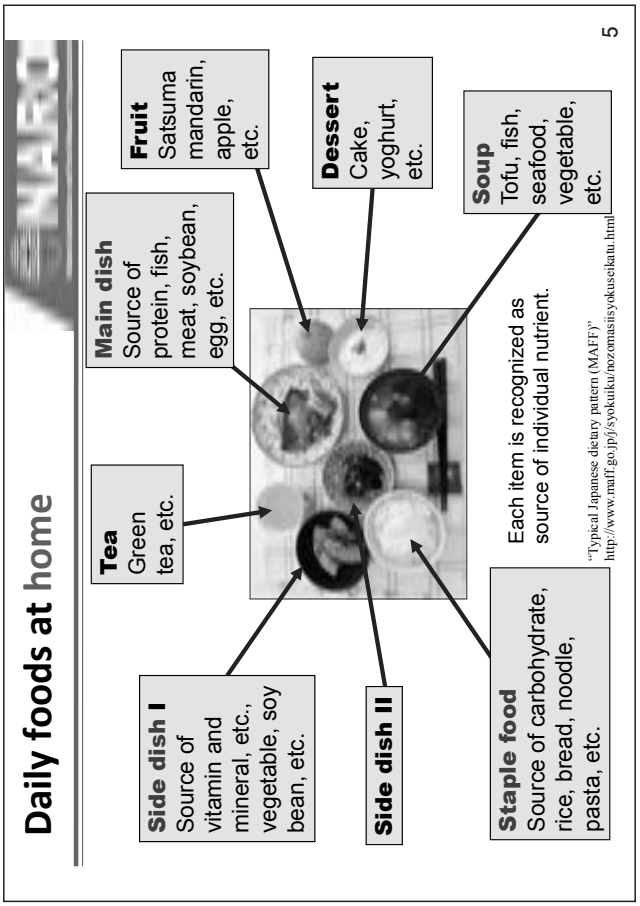
Miso Soup and Clear Soup
DASHI soup stock contains glutamic acid from seaweed and inosinic acid from bonito flakes.

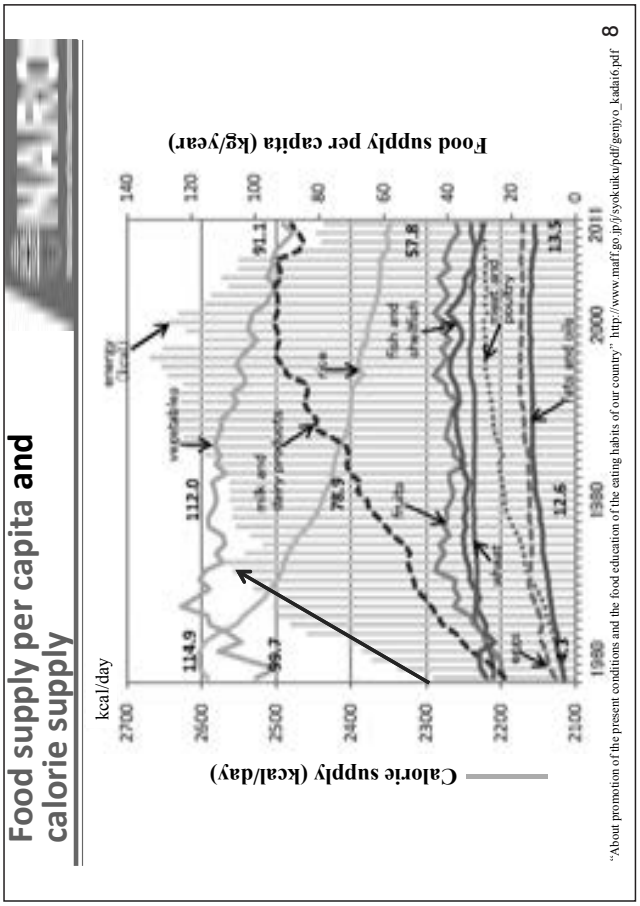
Rice

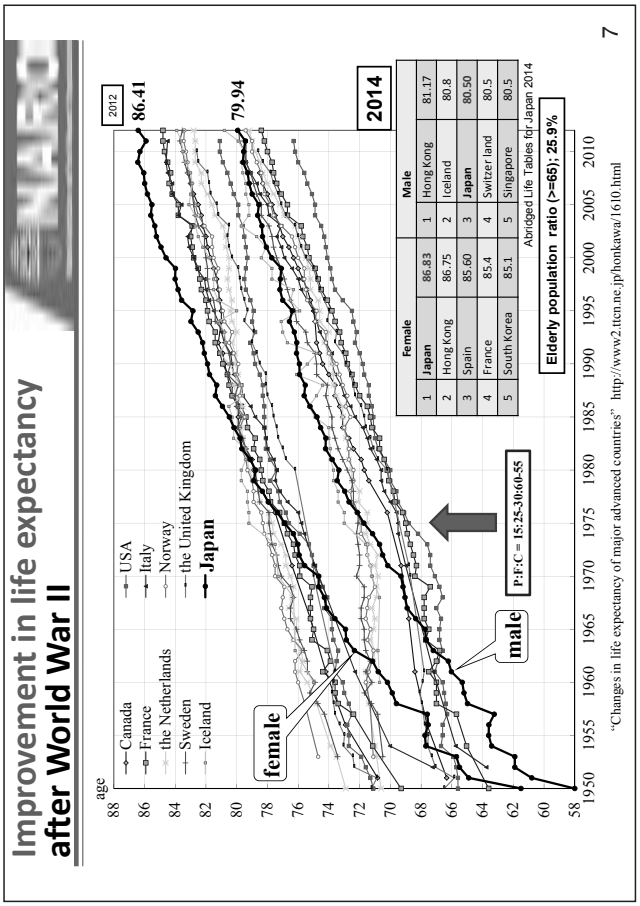
"10 Great Tastes of Japan (MAFF)"
http://www.maff.go.jp/j/shokusan/export/e_info/pdf/all.pdf

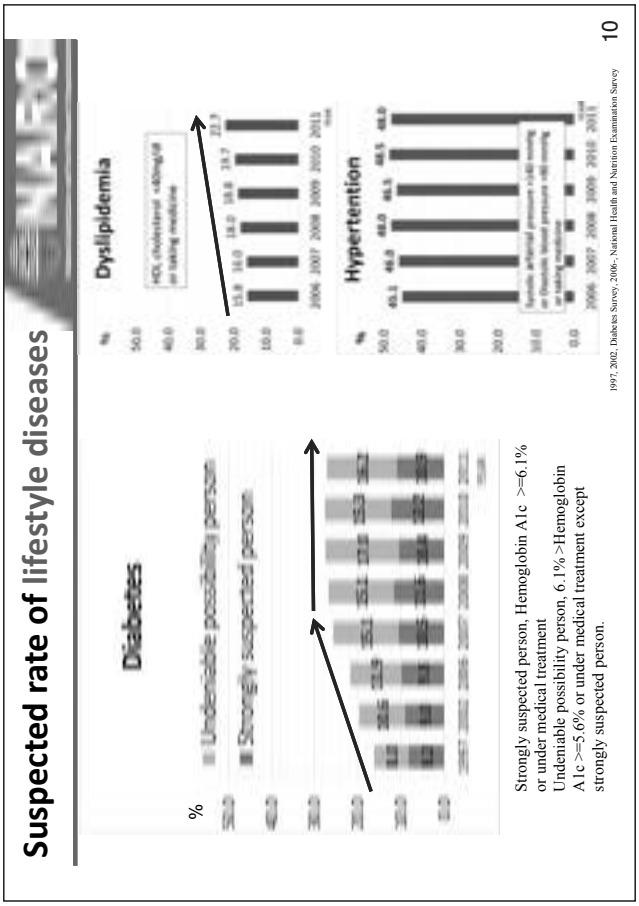
4

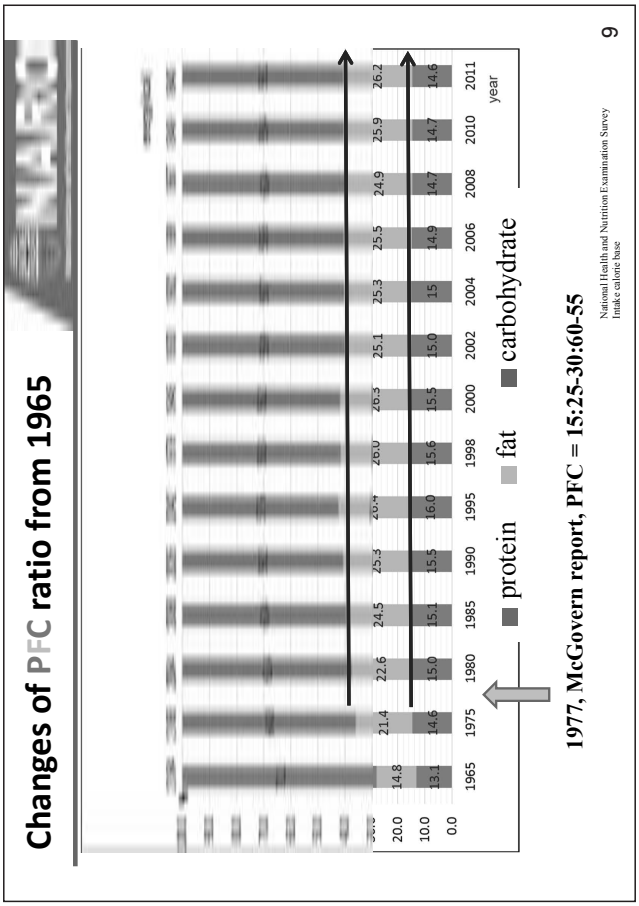
Daily foods at NARO cafeteria
Variations to simplify
"Rice balls (Onigiri)"
ja.wikipedia.org
6

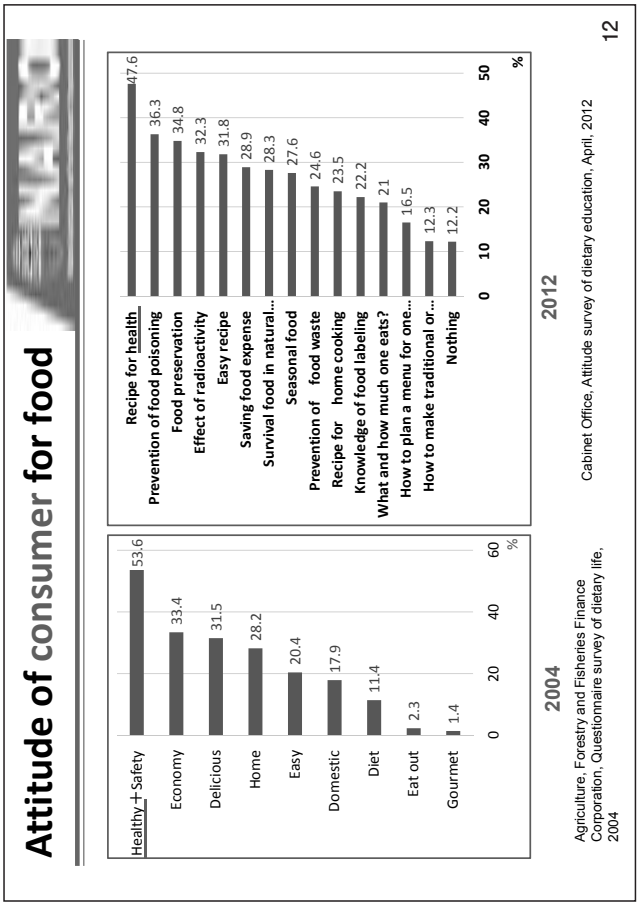

Daily foods at home
Tea
Green tea, etc.
Side dish I
Source of vitamin and mineral, etc., vegetable, soy bean, etc.
Side dish II
Staple food
Source of carbohydrate, rice, bread, noodle, pasta, etc.
Main dish
Source of protein, fish, meat, soybean, egg, etc.
Fruit
Satsuma mandarin, apple, etc.
Dessert
Cake, yoghurt, etc.
Soup
Tofu, fish, seafood, vegetable, etc.
Each item is recognized as source of individual nutrient.
"Typical Japanese dietary pattern (MAFF)"
http://www.maff.go.jp/j/syokuiku/nozomasisyokuseikatu.html
5

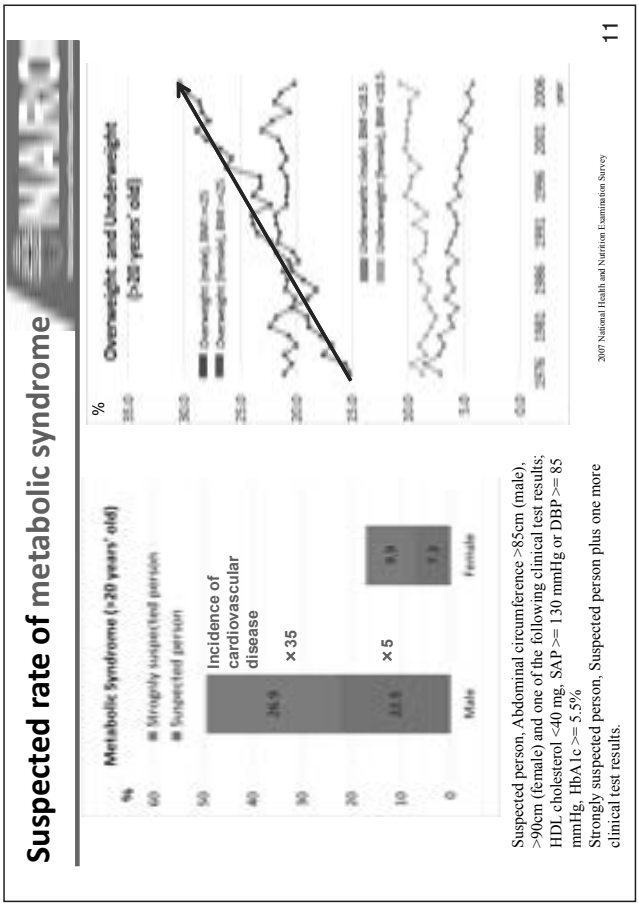

Food supply per capita and calorie supply
kcal/day
Calorie supply (kcal/day)
Food supply per capita (kg/year)
"About promotion of the present conditions and the food education of the eating habits of our country" http://www.maff.go.jp/j/syokuiku/pdf/geniyo_kadai6.pdf
8


Improvement in life expectancy after World War II
age
Canada
France
the Netherlands
Sweden
Iceland
USA
Italy
Norway
the United Kingdom
Japan
female
male
2012
86.41
79.94
2014
P:F:C = 15:25.30:60.55
Female
Male
Elderly population ratio (>=65); 25.9%
Abridged Life Tables for Japan 2014
"Changes in life expectancy of major advanced countries" http://www2.ttcn.ne.jp/honkawa/1610.html
7


Suspected rate of lifestyle diseases
Diabetes
Undeniable possibility person
Strongly suspected person
Dyslipidemia
Hypertention
Strongly suspected person, Hemoglobin A1c >=6.1% or under medical treatment
Undeniable possibility person, 6.1% >Hemoglobin A1c >=5.6% or under medical treatment except strongly suspected person.
1997, 2002, Diabetes Survey, 2006-, National Health and Nutrition Examination Survey
10


Changes of PFC ratio from 1965
protein
fat
carbohydrate
1977, McGovern report, PFC = 15:25-30:60-55
National Health and Nutrition Examination Survey
Intake calorie base
9


Attitude of consumer for food
Healthy+Safety
Economy
Delicious
Home
Easy
Domestic
Diet
Eat out
Gourmet
2004
Recipe for health
Prevention of food poisoning
Food preservation
Effect of radioactivity
Easy recipe
Saving food expense
Survival food in natural...
Seasonal food
Prevention of food waste
Recipe for home cooking
Knowledge of food labeling
What and how much one eats?
How to plan a menu for one...
How to make traditional or...
Nothing
2012
Agriculture, Forestry and Fisheries Finance Corporation, Questionnaire survey of dietary education, April, 2012
12

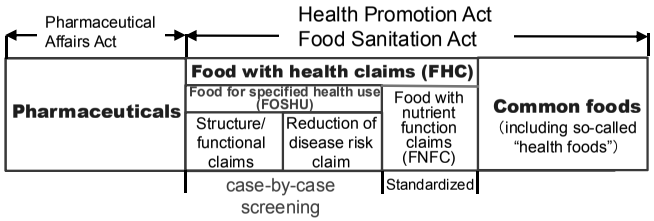

Suspected rate of metabolic syndrome
Metabolic Syndrome (≥20 years' old)
Strongly suspected person
Suspected person
Incidence of cardiovascular disease
×35
×5
Male
Female
Overweight and Underweight (>20 years' old)
Suspected person, Abdominal circumference >85cm (male), >90cm (female) and one of the following clinical test results; HDL cholesterol <40 mg, SAP >= 130 mmHg or DBP >= 85 mmHg, HbA1c >= 5.5%
Strongly suspected person, Suspected person plus one more clinical test results.
2007 National Health and Nutrition Examination Survey
11

Functional Foods in Japan

- **Concept of functional food is proposed in 1984, when the first research project of functional food started in Japan.**
- **The food functionality is generally categorized into three functions as nutritional function (the first function), sensory function (second function), and health-promoting function (third function).**

13

System and Labeling of Functional Foods in Japan



Food with Health Claims (FHC):
FHC refer to foods that comply with the specifications and standards established by the Minister of Health, Labor and Welfare (MHLW) and are labeled with certain nutritional or health functions , currently approved by the Consumer Affairs Agency.

a) Food with Nutrient Function Claims (FNFC)
Foods that are labeled with the functions of nutritional ingredients (vitamins, minerals).

b) Foods for Specified Health Uses (FOSHU) 1991-
Foods officially approved to claim their physiological effects

The world's first legal and labeling system

14

Foods for specified health uses (FOSHU)

Requirements for FOSHU Approval

- **Effectiveness on the human body is clearly proven**
- **Absence of any safety issues**
  (animal toxicity tests, confirmation of effects in cases of excess intake, etc.)
- **Use of nutritionally appropriate ingredients**
  (e.g. no excessive use of salt, etc.)
- **Guarantee of compatibility with product specifications at the time of consumption**
- **Established quality control methods,** such as specifications of products and ingredients, processes, and methods of analysis

15

FOSHU, 983 items（2011）

Approved FOSHU Products	No. of items
1. Food to modify gastrointestinal conditions	355
（contain oligosaccharides）	(82)
（contain lactic acid bacteria）	(81)
（contain dietary fibers）	(192)
2. Food related to blood cholesterol level	139
3. Food related to blood sugar levels	148
4. Food related to blood pressure	119
5. Food related to dental health	75
6. Food related to serum triglyceride levels	90
7. Food related to osteogenesis & mineral absorption	57
	983

(1072 items July, 2013)

16

FOSHU, unsuitable for entire agricultural product

High Barrier of FOSHU Approval for Entire Food

- **Because of the case-by-case screening, it takes huge amount of money and very long time to approve only one item.**
- **Effectiveness is biased toward gastrointestinal conditions, blood sugar levels, etc. excluding new effectiveness, such as immunity, stress, anti-tiredness, etc.**
- **Food functions should be approved entirely because of multi-functions in the future.**

17

Review of novel labeling system in the Regulatory Reform Council

Current status

Only two labeling systems for health claims are allowed.

1. Food with Nutrient Function Claims (FNFC) (Nutritive function claims)
2. Foods for Specified Health Uses (FOSHU) (Structure/ Functional Claims and Reduction of disease risk Claims)

Allowed functional claims

Food with Nutrient Function Claims (FNFC)	Food for Specified Health Uses (FOSHU)	Common foods

Main point of discussion

【FNFC】
Limited to nutritional information.
Only 12 vitamins, and five minerals are allowed.

【FOSHU】
Because of the case-by-case screening, it takes huge amount of money and very long time to approve only one item

➡ It is high barrier for small companies.

2013.6

After the review

To satisfy the public need for the prevention of care and illness, maintain their health and long life time. To realize the healthy society ahead of other counties.

1. Manufacturers should take a responsibility for health claim and safety on their products based on a scientific evidence in novel labeling system.
2. Supplement, processed food and agriculture-product including forestry and marine product (fresh food)should be covered in novel labeling system .
3. Novel labeling system have to refer to U.S. "The Dietary Supplement Health and Education Act (DSHEA)" .
4. Novel system should be establish before April 2015.

Japan Revitalization Strategy and the Regulatory Reform Work Plan approved by the Cabinet 2013.6.14

Recommended functional claims

Food with Nutrient Function Claims (FNFC)	Food for Specified Health Uses (FOSHU)	Common foods	

Novel labeling system

18

Schedule

1. December, 2013
   Scientific panel meeting started discussions.
2. July, 2014
   Scientific panel meeting submitted a draft policy report to Consumer Affairs Agency (CAA).
3. September, 2014
   CAA finished the public comment process and started to prepare the relevant bills.
4. December, 2014
   CAA will introduce the bill to the Diet.
5. April, 2015
   The Act will enter into force if it goes well.
6. June 15, 2015
   The first labeled product (processed food) was marketed.

CAA = Consumer Affairs Agency

19

Categories of Foods in Japan

- Food
  - Food with health claims
    - Foods for patients, pregnant women, baby, people having difficulty swallowing — The examination is required by CAA
    - FOSHU Food for specified health uses (Case-by case screening) — The examination is required by CAA
    - FNFC Food with nutrient function claims（Standardized） — No examination is necessary by CAA
    - Food with function claims（Notification） — No examination is necessary by CAA
  - Common food — Labeling of health claim is not admitted.

CAA = Consumer Affairs Agency

In 'Food with function claims', the manufacturers will be responsible for the health claim and safety of their products based on concrete scientific evidences.

20

Fundamental view point of the novel labeling system

Ensure Food Safety

The novel labeling system should be established to promote self-motivating and reasonable choice of the health foods, and prevent misunderstanding by the consumer.

Scientific Evidence

Correct labeling without misunderstanding

21

Requirements of this labeling system

- A manufacturer is responsible for scientific evidence and safety of the health food (registration system).
- The functional ingredient has been identified using validated method.
- The consideration of action mechanism by *in vitro/in vivo* test and human clinical trial are required.
- The acquisition of scientific evidence by human clinical trial of end product or research review of end product or functional ingredient is indispensable.
- The labeling functionality is limited to contribution of the maintenance and improvement of health.
- Enough eating experience and enough ensuring safety are required.
- Register to Consumer Affairs Agency within 60 days before sale and display contents for container.
- Over intake of the functional ingredient of the registered product is not permitted.

22

Examples of the supposed health claim of processed food

Product [functional ingredient] (cultivar)	Image of health claim
Green tea [O-methylated EGCG] (Benifuuki)	This food contains *O*-methylated catechin and conditions eyes and the nose of person who is worried about pollen
Soy milk [β-conglycinin] (Nanahomare)	This food helps the maintenance of serum triglyceride levels to reduce free fatty acid by β-conglycinin.
Tartary buckwheat [rutin] (Manten kirari)	This food contains rutin and helps the maintenance of the normal serum cholesterol level

(From a handout of the fifth panel meeting on the novel functional labeling system)

23

Functional labeling food（Green tea）

Functional labeling food
（Notified Number A67）

Name of product:
Benifuuki green tea bag

Health claim to be notified：
This food contains *O*-methylated catechin. ***O*-methylated catechin is reported to relieve discomfort in the eyes and/or the nose by house dust, etc.**

Attention: Japanese government does not evaluate the functionality and safety of this product. This product is not intended to diagnose, treat, cure, or prevent any disease.

24

Functional labeling food (reverse side)

Nutrition information Per container (Three tea bags)
Energy 35 kcal Protein 2.3 g Total fat 0.2 g
Carbohydrate 5.9 g Sodium 0g

Recommended consumption per day : Three tea bags (9 g)
Content of the functional ingredient per day : *O*-methylated catechin (34mg/ 3 tea bags (after infusion))

Way to intake: Pour boiling water more than 150 ml per one tea bag and extract well stirring it up and down more than two minutes, and please have it after cooling it enough.

Caution : This food is not aimed at the illness, the treatment, and the prevention. This food is not for a patient, person under age, pregnant, and lactating woman. The person who takes medicines should take this food after consultation with a doctor or a pharmacist. When you felt abnormality in physical condition, you should cancel an intake immediately, and, please talk to a doctor. The balance of the meal is important to the eating habits on the basis of a staple food, a main dishes, and some dishes.

manufacturer : JA Kagoshima chagyo
Contact information : ○○ ○○ ○○
Detailed information about the functionality and the safety : refer to web site

25

Examples of the supposed health claim of fresh food

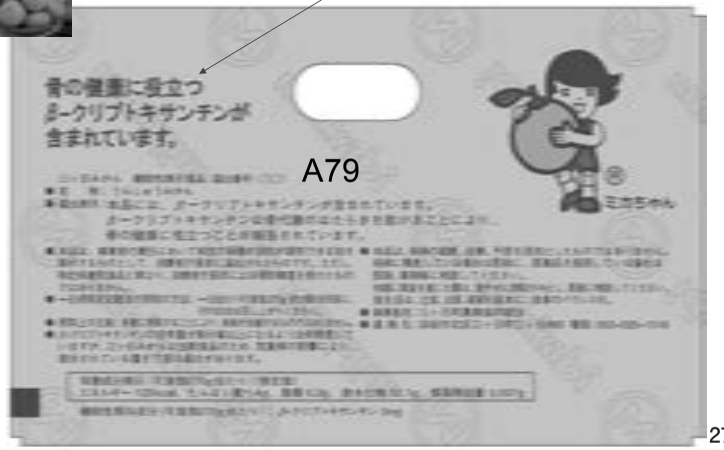
Product 【functional ingredient】 (cultivar, production area)	Image of health claim
Satsuma mandarin Unshu 【β-cryptoxanthin】 (Mikkabi Production Area)	This food contains β-cryptoxanthin and may keep your bone health. This food is suitable for a woman after the menopause
Barley 【β-glucan】 (Kirakimochi, name of cultivar)	This food contains β-glucan and helps maintenance of normal blood sugar level by suppressing the absorption of the sugar
Spinach 【lutein】 (Different cultivation method which expose to cold climate)	This food supplements lutein and helps the health maintenance of eyes

(From a handout of the fifth panel meeting on the novel functional labeling system)

26

Functional labeling food (Satsuma mandarin orange)

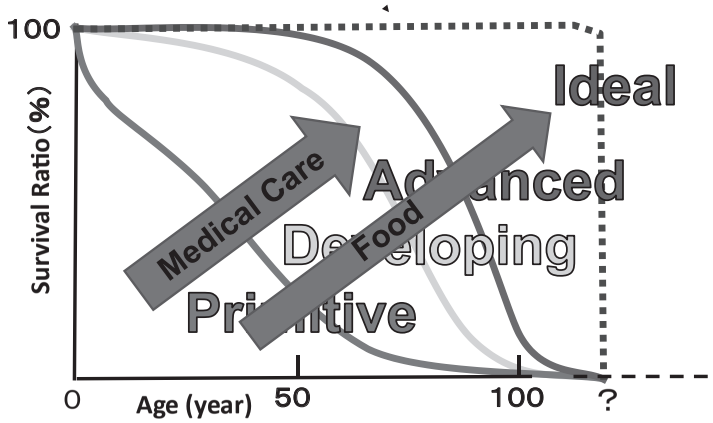
"The Satsuma mandarin orange contains beta-cryptoxanthin which helps in bone metabolism."



27

Age Distribution & Society

Science on food & health is essential for the development of society.



28

Differences between life expectancy and healthy life expectancy (Male)

Country	Life expectancy (years)	Healthy life expectancy (years)	Differences (years)
Japan	80.05	70.4	8.94
USA	76.33	65.84	10.49
UK	79.09	68.48	10.61
Germany	78.18	67.27	10.91
France	78.38	68.43	9.95
China	73.53	65.89	7.64
Italy	79.45	69.11	10.34
Spain	78.97	68.40	10.57
Norway	79.09	68.56	10.53
Cuba	76.29	67.31	8.98
Australia	79.71	68.43	11.28
Canada	79.44	69.11	10.33

Average 10.15 years

The Lancet, Published online August 27,2015

29

Differences between life expectancy and healthy life expectancy (Female)

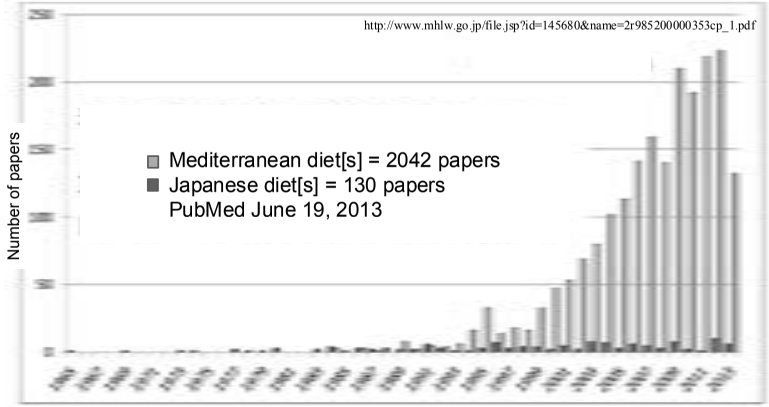
Country	Life expectancy (years)	Healthy life expectancy (years)	Differences (years)
Japan	86.39	75.56	10.83
USA	81.42	68.61	12.81
UK	82.84	70.56	12.28
Germany	83.14	70.31	12.83
France	84.91	72.32	12.59
China	79.99	70.28	9.71
Italy	84.60	71.36	13.24
Spain	84.43	71.83	12.6
Norway	83.71	71.66	12.05
Cuba	80.43	69.06	11.37
Australia	83.99	70.63	13.36
Canada	83.43	71.04	12.39

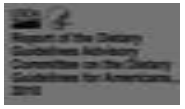
Average 12.29 years

The Lancet, Published online August 27,2015

30

More evidences are necessary





Both traditional Japanese and Okinawan dietary patterns have been associated with a low risk of coronary heart disease. Nonetheless, compared to the evidence supporting DASH and Mediterranean diets, detailed information on diet composition as well as epidemiologic and clinical trial evidence on health benefits, similar to that available for the other types of diets, is sparse. Also, over time, dietary intakes in these countries have changed and may no longer reflect the healthiest choices.

31

Thank you for your attention!

Healthy food is indispensable for the improvement of our QOL.

ILSI Japan The 7th International Conference on Nutrition and Aging, Tokyo, Sep. 29, 2015

32

セッション 1-2

日本の特徴的な食事と疾病：多目的コホート研究からのエビデンス

国立研究開発法人　国立がん研究センター
がん予防・検診研究センター長
津金　昌一郎

多目的コホート研究（JPHC Study）は、著者を主任研究者とするコホート研究で、研究開始時に 11 保健所管内の特定市町村に住民登録していた 40-69 歳住民 14 万人を対象とした。ベースライン、5 年後、10 年後と 5 年毎に計 3 回の要因把握のための包括的アンケート調査（妥当性が検証された食物摂取頻度調査（FFQ）を含む）を実施したが、約 13 万人がいずれかに回答し、約 7 万 8 千人が全てに回答している住民ベースのコホート研究である。また、約 6 万人が健診結果や血液を提供しており、ゲノム解析を含めた様々なバイオマーカーが、コホート内症例対照研究やサブコホート研究という手法を用いて検討されている。2012 年の追跡までに、25,000 人の死亡、18,000 のがん罹患、6,200 の脳卒中発症、1,200 の心筋梗塞発症を登録している。そして、様々な要因と死亡、がん・脳卒中・心筋梗塞・糖尿病・白内障・骨折・歯の喪失などとの関連について、250 編を超える英文原著論文として出版している。研究方法や成果概要をホーム・ページ（http://epi.ncc.go.jp/jphc/）で参照可能である。

日本人は、ダイドゼインやゲニステインなどのイソフラボンを多く摂取する傾向にあり、それらには発がん抑制効果やエストロゲン様作用があるこが試験管内で、また、いくつかの部位のがんの抑制効果が動物実験で確かめられている。それゆえ、イソフラボンには、ホルモンに関連する乳がんや前立腺がんを予防することが期待される。多目的コホート研究においては、イソフラボンが閉経後乳がんや局所の前立腺がんリスクを低下させるという知見が得られた。更に、保存血液を用いたコホート内症例対照研究においても、血中イソフラボン濃度が高いと、これらのがんのリスクが低下することが示された。他にも、非喫煙における肺がんや循環器疾患の予防効果が示された。一方、肝がんについては、女性においてリスクを増加させることが示された。

その他の日本の特徴的な食事として、魚、塩分／塩蔵食品、緑茶の摂取が多いことと、赤肉／加工肉、脂肪／脂肪酸、ソフトドリンクの摂取が少ないという傾向がある。本会議においては、いくつかの日本の特徴的な食事とがん、循環器疾患、糖尿病などとの関連について紹介する。

Session 1−2

Typical Japanese Diet and Chronic Diseases: Evidence from the JPHC Study

Shoichiro Tsugane, M.D., DMS.
Director, Research Center for Cancer Prevention and Screening, National Cancer Center

The Japan Public Health Center-based prospective Study (JPHC Study) conducted a baseline survey for 140 000 registered residents aged 40–69 years within 11 public health center areas nationwide in 1990–94. Five- and 10-year follow-up surveys were conducted to update information on detailed dietary habits by a comprehensive and validated food frequency questionnaire (FFQ) and health conditions. In total, 130,000 participants responded to at least one of the three questionnaire surveys, and 78,000 to all three. In addition, 60,000 participants provided blood samples on at least one of the two sampling times, and 23,000 on both. The subjects have been followed for vital status and the occurrence of cancer and other diseases. Up to 2012, 25,000 deaths have been documented, as well as 18,000 cases of cancer, 6,200 cases of stroke and 1,200 cases of myocardial infarction. We have tested the association between potential causal factors and the incidence of or mortality from cancer and other diseases, and published over 250 papers to date.

Japanese populations consume large quantities of soy foods which contains isoflavones such as genistein and daidzein. Genistein and daidzein exhibited anti-carcinogenic properties and estrogenic activities in vitro, and demonstrated a protective effect against some cancers in animal studies. On these bases, isoflavones have been recognized as key substances that may decrease the incidence of breast and prostate cancers in Japan. In the JPHC study, we found an inverse association between dietary isoflavones and post-menopausal breast cancer and localized prostate cancer. We also found that high blood levels of isoflavones were associated with a decreased risk of these cancers in nested case-control studies. In addition, isoflavones were suggested to have a protective effect against lung cancer among nonsmokers and against cardiovascular infarction in women. In contrast, isoflavones increased the risk of liver cancer in women.

Other typical dietary habits in Japan are high consumption of fish, salt/salted foods and green tea, and low consumption of red/processed meats, fat/fatty foods and soft drinks. In this conference, I will present some of the findings on some Japanese typical diet and risk of cancer, cardiovascular disease and diabetes,which may be unique for Japanese cohort studies.

Typical Japanese Diet and Chronic Diseases: evidence from the JPHC Study

日本の特徴的な食事と疾病：
多目的コホート研究からのエビデンス

Shoichiro Tsugane
Research Center for Cancer Prevention and Screening
National Cancer Center

The 7th International Conference on Nutrition and Aging
By ILSI Japan
September 29 (Tue), 2015 10:50-11:30
University Tokyo

1

JPHC STUDY

Japan Public Health Center-based prospective Study

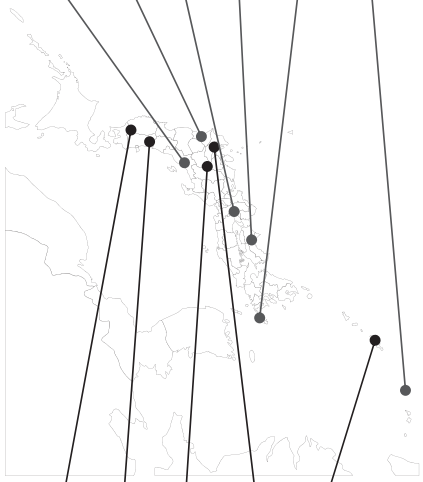
2

Map of the JPHC Study area

(Total number of subjects =140,420)

Cohort I
since 1990
(age: 40-59 yrs)
(n=61,595)

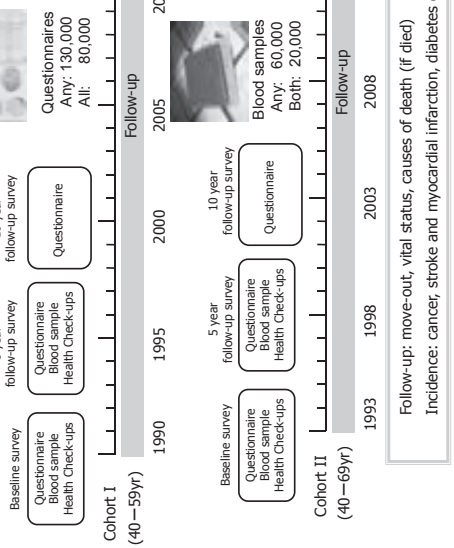
Cohort II
since 1993
(age: 40-69 yrs)
(n=78,825)



*Study participants only (otherwise number of registered residents)

3

SCHEDULE



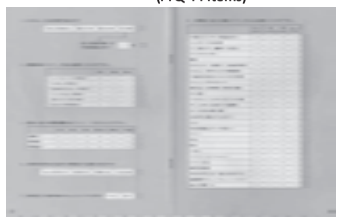
4

Food frequency questionnaire (FFQ)
- JPHC Study, baseline survey -

➢44 or 46 food item FFQ
developed based on experience of nutritional researchers.

✓4 or 5 frequency categories
✓without portion size
except limited food items in Cohort II

Cohort I (FFQ 44 items)



Cohort II (FFQ 46 items)

5

Validity of 44 item FFQ with 28-d DR for nutrients
- JPHC FFQ Validation Study , Cohort I -

Dietary intake	Men (n= 94)		Women (n=107)	
	Crude	Energy adjusted	Crude	Energy adjusted
Alcohol (g)	0.79	0.81	0.44	0.34
Carbohydrate (g)	0.61	0.51	0.48	0.33
Calcium (mg)	0.56	0.56	0.46	0.37
Phosphorus (mg)	0.58	0.56	0.41	0.44
Energy (kJ)	0.52	---	0.38	---
Sodium (mg)	0.49	0.33	0.54	0.49
Vitamin B_2 (mg)	0.45	0.43	0.36	0.39
Protein (g)	0.43	0.28	0.33	0.34
Potassium (mg)	0.37	0.38	0.35	0.37
Iron (mg)	0.36	0.31	0.38	0.30
Vitamin B_1 (mg)	0.35	0.36	0.32	0.22
Retinol (μg)	0.35	0.36	0.38	0.34
Vitamin C (mg)	0.33	0.38	0.35	0.29
β–Carotene (μg)	0.29	0.26	0.41	0.38
Niacin (mg)	0.25	0.14	0.27	0.11
Total fat (g)	0.18	0.30	0.10	0.41
Median	0.40	0.36	0.38	0.36

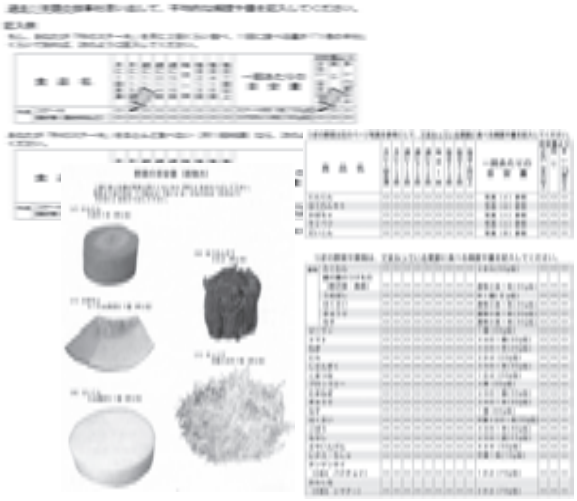
Tsubono Y, et al. J Epidemiol 2003;13:S125-S133.

6

Food frequency questionnaire (FFQ)
- JPHC Study, 5- and 10-year follow-up surveys -

➢138 food item FFQ
developed based on 3-day dietary record for 335 random sampled subjects in 5 JPHC study areas

✓9 frequency categories
✓with portion size



7

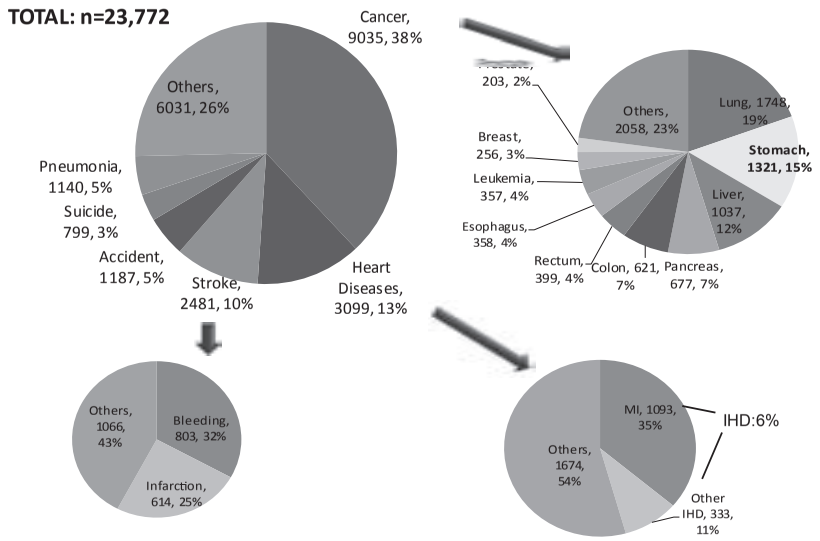
Validity of 138 item FFQ with 28-d DR for nutrients
- JPHC FFQ Validation Study, Cohort I -

Dietary intake	Men (n=102)		Women (n=113)	
	Crude	Energy adjusted	Crude	Energy adjusted
Alcohol (g)	0.81	0.82	0.51	0.42
Carbohydrate (g)	0.71	0.56	0.56	0.37
Calcium (mg)	0.65	0.43	0.53	0.47
Phosphorus (mg)	0.61	0.37	0.49	0.42
Sodium (mg)	0.59	0.41	0.55	0.48
Energy (kJ)	0.55	---	0.44	---
Vitamin B_2 (mg)	0.54	0.34	0.43	0.45
Iron (mg)	0.52	0.49	0.41	0.33
Potassium (mg)	0.52	0.39	0.40	0.31
Protein (g)	0.50	0.30	0.41	0.27
Vitamin B_1 (mg)	0.49	0.40	0.31	0.41
Vitamin C (mg)	0.42	0.37	0.32	0.18
Niacin (mg)	0.42	0.35	0.27	0.15
Retinol (μg)	0.40	0.22	0.35	0.43
Carotene (μg)	0.39	0.35	0.33	0.34
Total fat (g)	0.31	0.52	0.22	0.46
Median	0.52	0.39	0.41	0.41

Tsugane S, et al. J Epidemiol 2003;13:S51-S56.

8

Number of deaths by cause: 1990-2011



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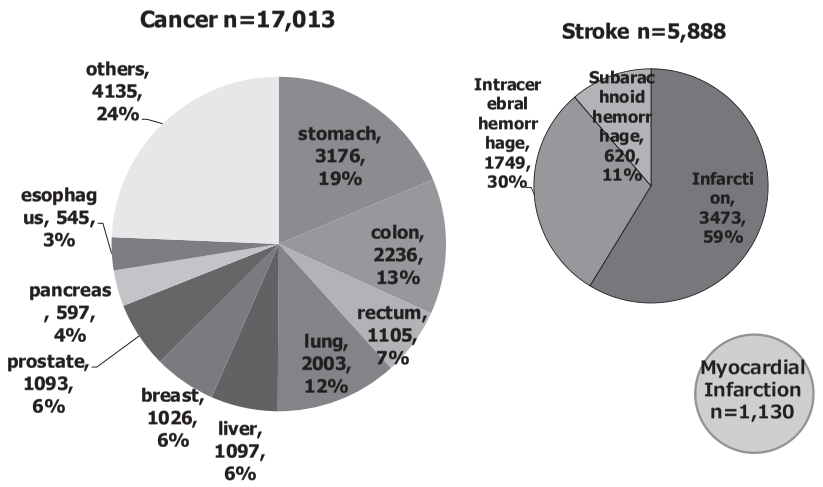
Number of registered diseases (1990-2011)



10

Strength

- Large-scale, cover areas throughout Japan
- Population-based with over 80% response rate
- Validated questionnaires (3 times) e.g. FFQ, PAQ
- Blood samples (2 times) e.g. Plasma, DNA
- Health check-up data (2 times)
- Follow-up for vital status and cause of death (if died) among all subjects (over 98% during 20- yrs)
- Follow-up for incidence of cancer, stroke and myocardial infarction among subjects in study area

11

Weakness

- Cover mainly rural areas
- Restricted age range: 40-69 yrs
- Exposure assessment: repeated only 2-3 times and no information after 10 yrs
- Blood samples only a half of subjects
- No follow-up for incidence of cancer, stroke and myocardial infarction among subjects outside study area: approximately 20% after 20- yrs

12

13

SOME FINDINGS on TYPICAL JAPANESE DIET and CHRONIC DISEASESS

14

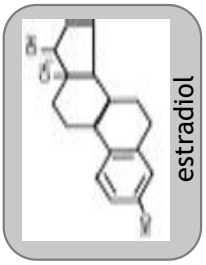
SOY FOODS AND ISOFLAVONE

15

Soy foods and Isoflavone

genistein

daidzein



estradiol

Tofu (bean curd)

Miso, Miso soup (fermented soybean paste)

Natto (fermented soybeans)

Yushi tofu

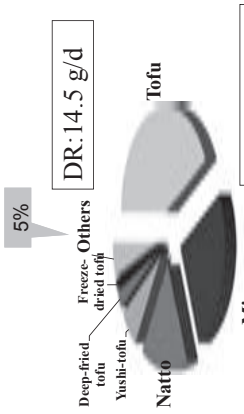
Freeze-dried tofu

Deep-fried tofu

Soymilk

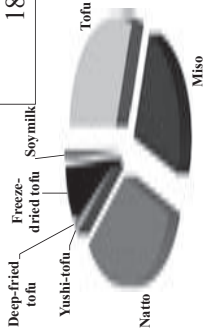
16

Validity of 138 item FFQ to assess daidzein intake
- JPHC FFQ Validation Study, Cohort I -



Spearman's correlation coefficient

	DR	Serum	Urine
FFQ	**0.57**	**0.31**	**0.29**
DR		0.39	0.43
Serum			0.23

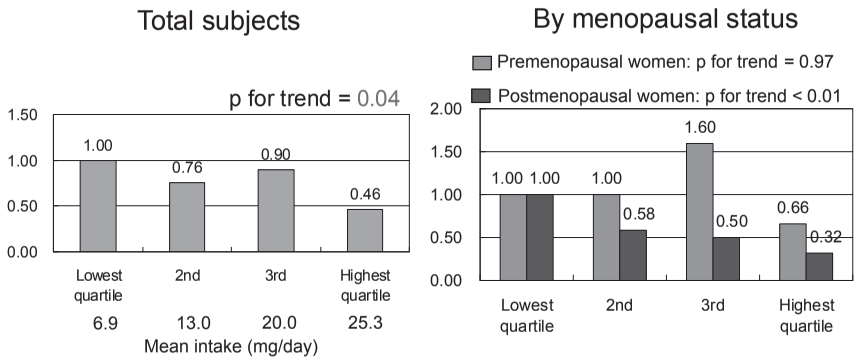


- The finding for genistein was similar.

Yamamoto S, et al. J Nutr 2001;131:2741-7

Isoflavone intake and breast cancer

21,852 women ages 40 to 59 years in Cohort I and 179 cases



*Genistein intake was reported as isoflavone intake because of high correlation among isoflavones.

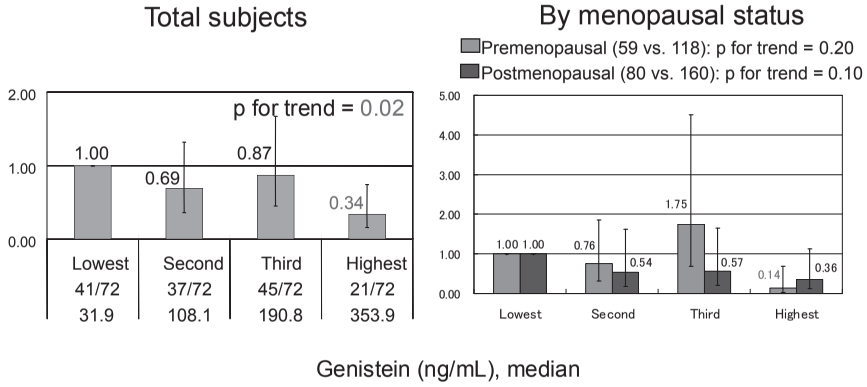
Adjusted for area, age, age at menarche, number of pregnancies, menopausal status, age at first pregnancy, smoking history, alcohol consumption, physical activity, educational level, total energy, meat, fish, vegetable, and fruits intake.

Yamamoto S, et al. J Natl Cancer Inst 2003;95:906-913.

17

Plasma isoflavone level and breast cancer

Nested case-control study: 144 cases and 288 matched controls



*Adjusting for number of birth and age at first birth.

Iwasaki M, et al. J Clin Oncol. 2008;26:1677-83.

18

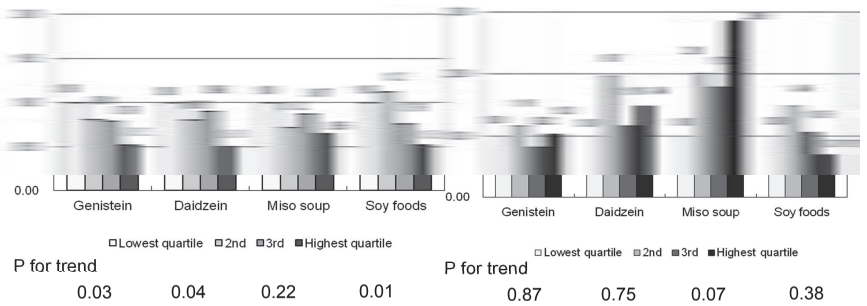
Isoflavone intake and breast cancer risk: meta-analysis

	No. of studies	Odds ratio	95% confidence interval
Studies conducted in Asia and in Asian Americans			
Highest (> 20 mg/day) vs. Lowest (< 5 mg/day)			
All studies	8	**0.71**	0.61-0.85
Case-control studies	7	**0.75**	0.62-0.89
Premenopausal women	6	**0.65**	0.50-0.85
Postmenopausal women	6	**0.63**	0.46-0.85
Moderate (median 10 mg/day) vs. Lowest (< 5 mg/day)			
All studies	8	**0.88**	0.78-0.98
Studies conducted in Western populations			
Highest (> 0.8 mg/day) vs. Lowest (< 0.15 mg/day)			
All studies	11	**1.04**	0.97-1.11
Cohort / nested case-control	4	**1.08**	0.95-1.24
Case-control studies	7	**1.02**	0.95-1.11

Wu AH, et al. Br J Cancer 2008;98:9-14.

19

Isoflavone intake and prostate cancer in older men (> 60 years old)

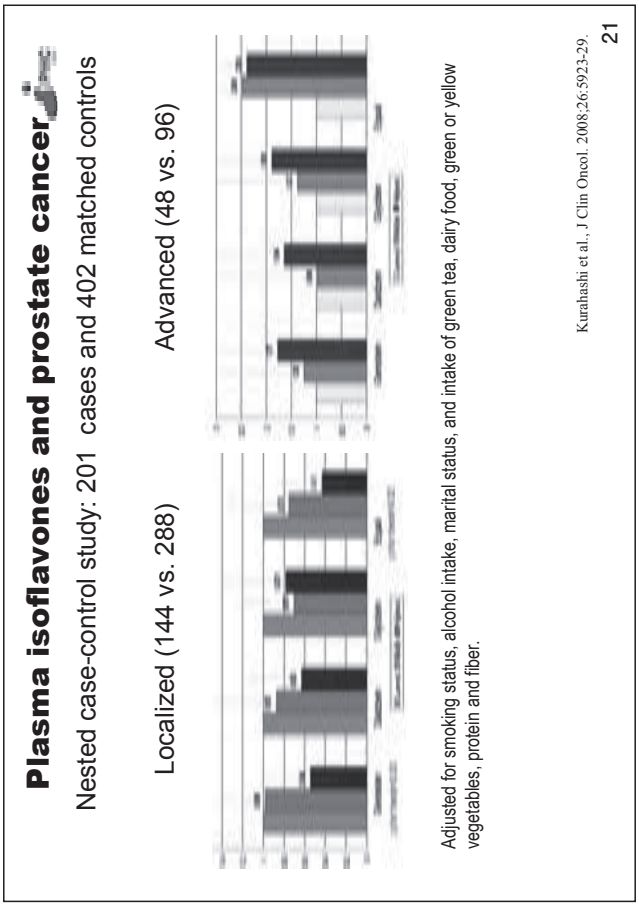


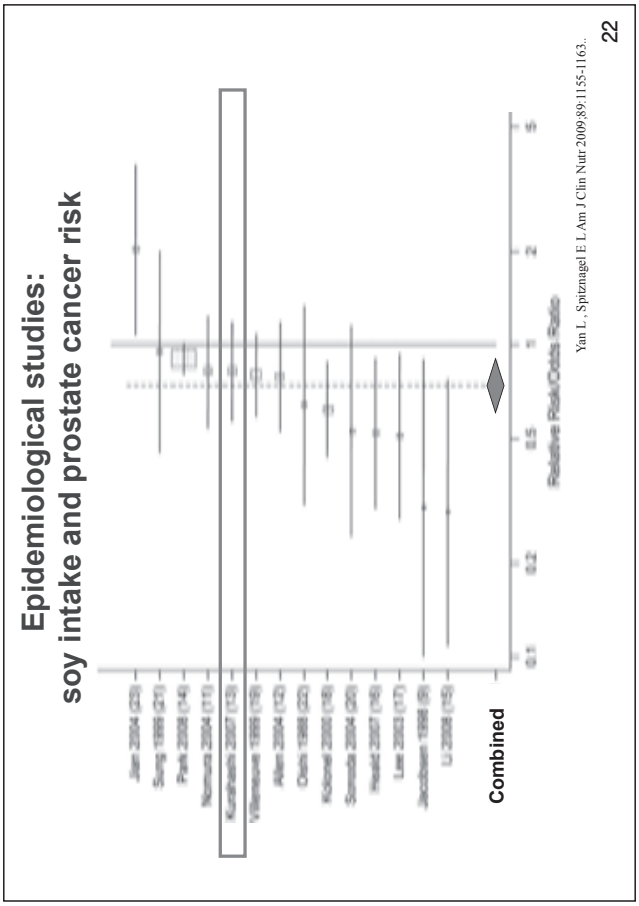
Advanced : stage C,D or Gleason Score8-10 or poor differentiation

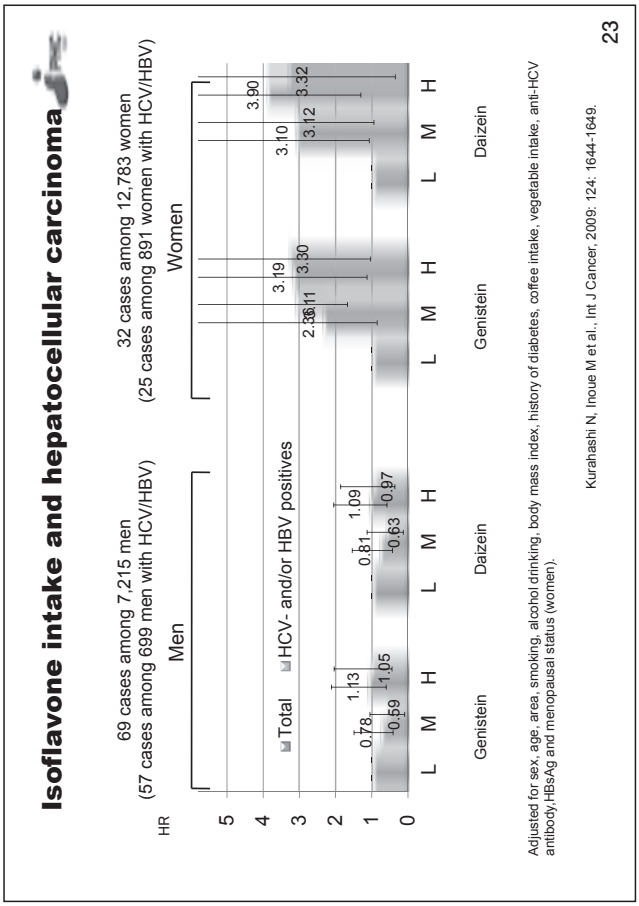
Adjusted for age, area, smoking status, drinking frequency, marital status, BMI, and intake of total fatty acids, dairy, vegetables and fruits.

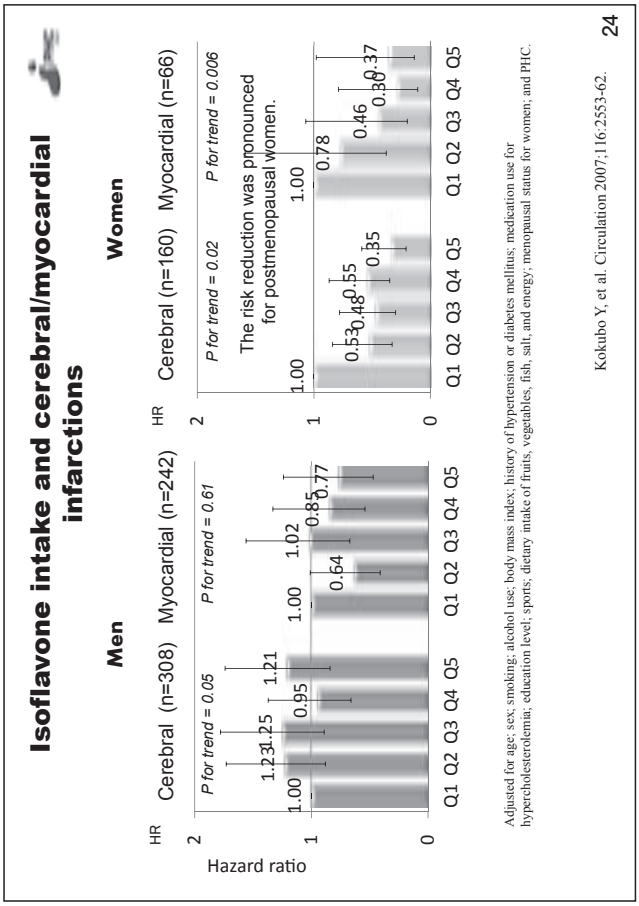
Kurahashi N, et al., Cancer Epidemiol Biomarkers Prev. 2007;16:538-45.

20


Plasma isoflavones and prostate cancer
Nested case-control study: 201 cases and 402 matched controls
Localized (144 vs. 288)
Advanced (48 vs. 96)
Adjusted for smoking status, alcohol intake, marital status, and intake of green tea, dairy food, green or yellow vegetables, protein and fiber.
Kurahashi et al., J Clin Oncol. 2008;26:5923-29.
21


Epidemiological studies:
soy intake and prostate cancer risk
Combined
Relative Risk/Odds Ratio
Yan L, Spitznagel E L Am J Clin Nutr 2009;89:1155-1163.
22


Isoflavone intake and hepatocellular carcinoma
69 cases among 7,215 men
(57 cases among 699 men with HCV/HBV)
Men
32 cases among 12,783 women
(25 cases among 891 women with HCV/HBV)
Women
HR
Total
HCV- and/or HBV positives
Genistein
Daizein
Adjusted for sex, age, area, smoking, alcohol drinking, body mass index, history of diabetes, coffee intake, vegetable intake, anti-HCV antibody,HBsAg and menopausal status (women).
Kurahashi N, Inoue M et al., Int J Cancer, 2009; 124: 1644-1649.
23


Isoflavone intake and cerebral/myocardial infarctions
Men
Women
Cerebral (n=308)
Myocardial (n=242)
Cerebral (n=160)
Myocardial (n=66)
P for trend = 0.05
P for trend = 0.61
P for trend = 0.02
P for trend = 0.006
The risk reduction was pronounced for postmenopausal women.
Hazard ratio
HR
Q1 Q2 Q3 Q4 Q5
Adjusted for age; sex; smoking; alcohol use; body mass index; history of hypertension or diabetes mellitus; medication use for hypercholesterolemia; education level; sports; dietary intake of fruits, vegetables, fish, salt, and energy; menopausal status for women; and PHC.
Kokubo Y, et al. Circulation 2007;116:2553-62.
24

Isoflavone intake and risk of cancer, cardiovascular disease and diabetes: evidence from the JPHC study

	Breast	Prostate	HCC	Other Cancers	CVD	DM
Soys, Isoflavones	↓ esp.post	↓ esp. local.	↑: f	↓: Lung, never smks, esp. m ↓: Colon, m esp.proximal null: Stomach, Endometrial	↓: MI, f ↓: Stroke, f esp. infarction	↓: f esp. post, obese

25

GREEN TEA AND COFFEE

26

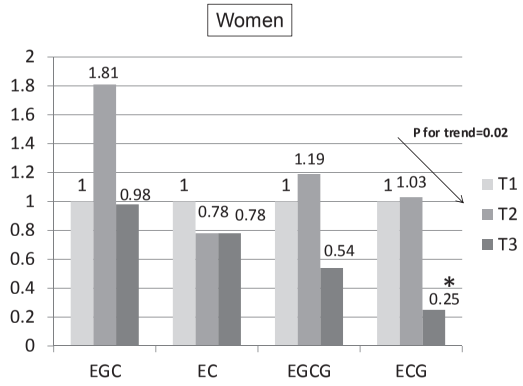
Green tea and risk of cancer

(Sasazuki S, Cancer Causes Control 2004; Lee KJ, Int J Cancer 2007; Luo J, Eur J Cancer Prev 2007; Kurahashi N, Cancer Sci 2009; Kurahashi N, Am J Epidemiol 2008; Shimazu T, Int J Cancer 2009)

27

Plasma tea polyphenol levels and gastric cancer

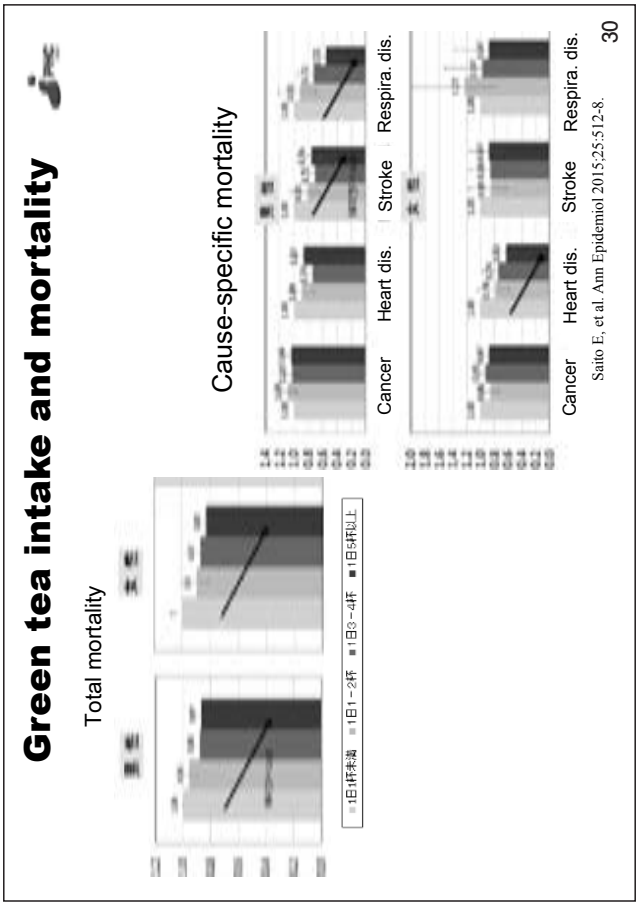
Nested case-control study in JPHC Study
(1990-2004, 163 cases:163 controls)

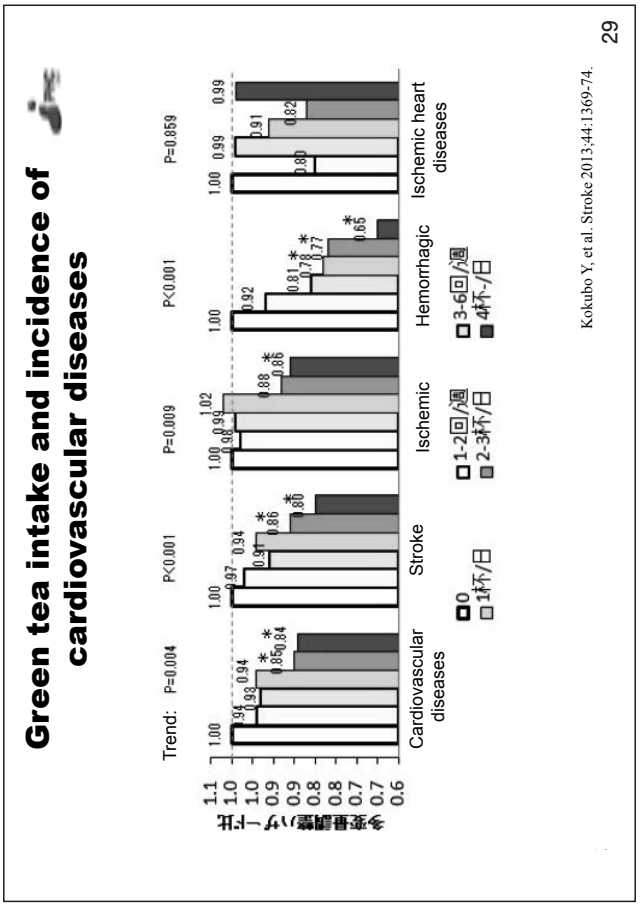


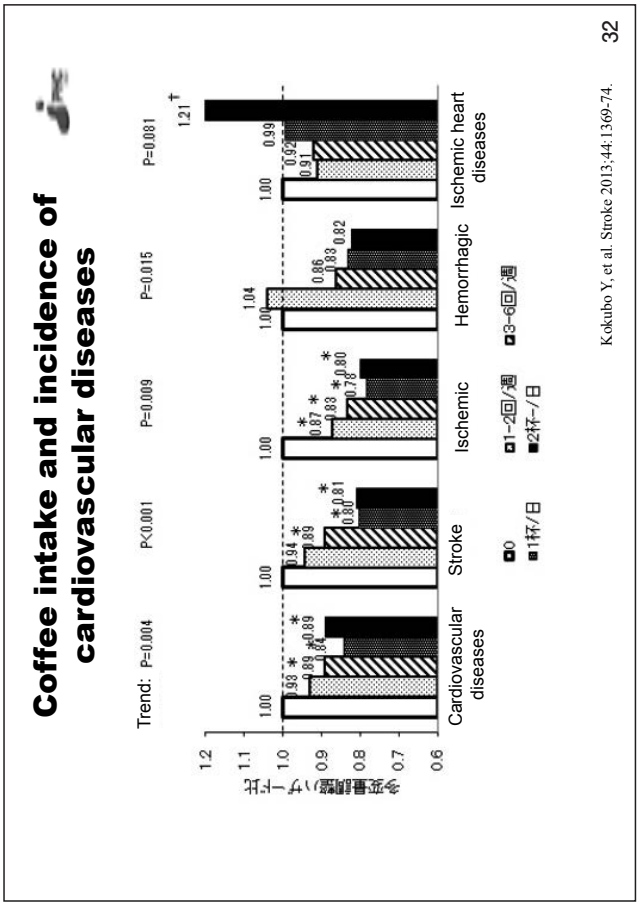
Matched for age, study area, blood donation date, and fasting time.
Adjusted for smoking, salted fish preserves, salt, fruit, green-yellow vegetables, other vegetables, BMI, family history of gastric cancer, and H.pylori infection.

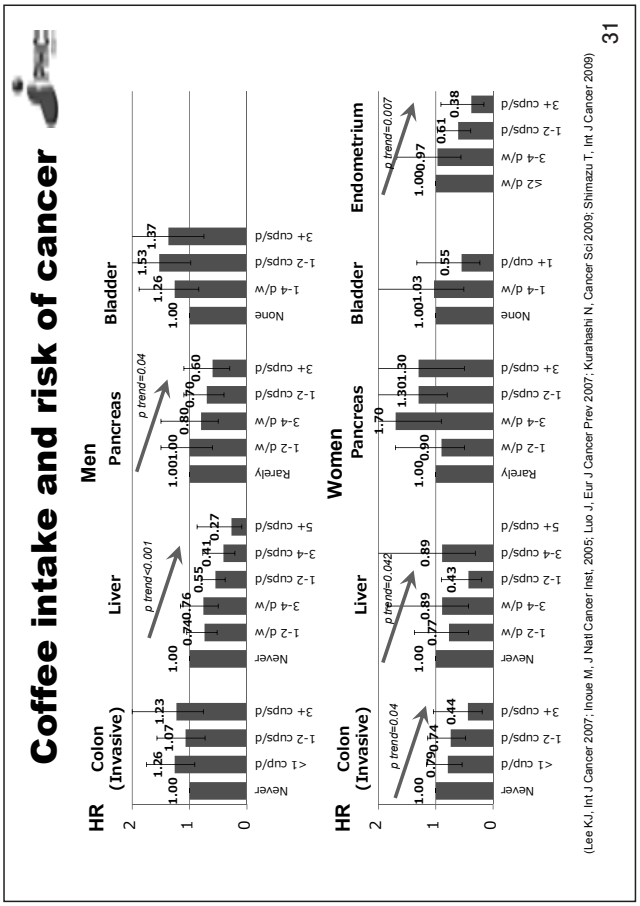
Sasazuki et al. Cancer Epidemiol Biomarkers Prev 2008;17:343-51.

28

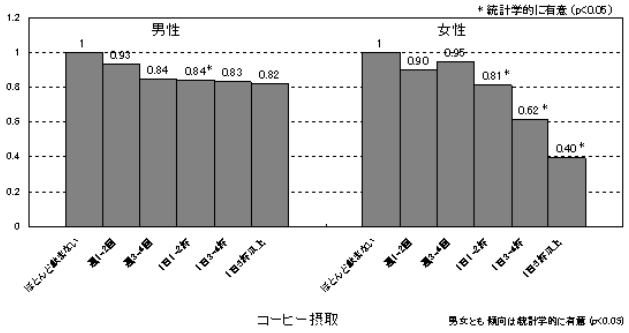

Green tea intake and mortality
Total mortality
Cause-specific mortality
Cancer
Heart dis.
Stroke
Respira. dis.
Saito E, et al. Ann Epidemiol 2015;25:512-8.
30


Green tea intake and incidence of cardiovascular diseases
Trend: P=0.004
P<0.001
P=0.009
P<0.001
P=0.859
Cardiovascular diseases
Stroke
Ischemic
Hemorrhagic
Ischemic heart diseases
Kokubo Y, et al. Stroke 2013;44:1369-74.
29


Coffee intake and incidence of cardiovascular diseases
Trend: P=0.004
P<0.001
P=0.009
P=0.015
P=0.081
Cardiovascular diseases
Stroke
Ischemic
Hemorrhagic
Ischemic heart diseases
Kokubo Y, et al. Stroke 2013;44:1369-74.
32


Coffee intake and risk of cancer
Men
Women
HR
Colon (Invasive)
Liver
Pancreas
Bladder
Endometrium
p trend=0.04
p trend=0.001
p trend=0.042
p trend=0.007
(Lee KJ, Int J Cancer 2007; Inoue M, J Natl Cancer Inst. 2005; Luo J, Eur J Cancer Prev 2007; Kurahashi N, Cancer Sci 2009; Shimazu T, Int J Cancer 2009)
31

Coffee intake and incidence of diabetes

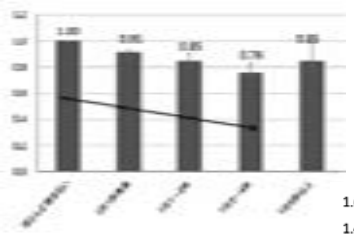


Kato M, et al. Endocr J 2009;56:459-68.

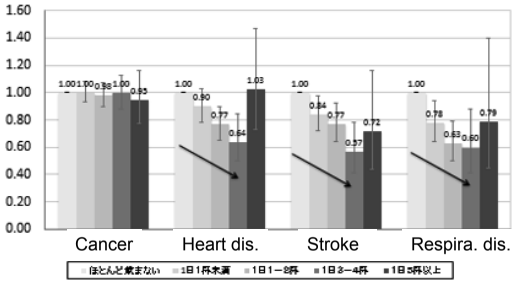
33

Coffee intake and mortality

Total mortality



Cause specific mortality



Saito E, et al. Am J Clin Nur 2015;101:1029-37.

34

Green tea and coffee intake and risk of cancer, cardiovascular disease and diabetes: evidence from the JPHC study

	Colon	Prostate	HCC	Other Cancers	CVD	DM
Green tea	null	↓ esp. adv.	null	↓:Stomach, f, esp. distal null: Breast, Pancreas	null: MI ↓: Stroke	
Coffee	↓:f esp. inv.		↓	↓: Endometrial ↓: Pancreas, m ↑: Bladder, m non smk null: Breast	null: MI ↓: Stroke	↓

35

FISH AND N-3 FATTY ACIDS
RED MEAT AND SATURATED FATTY ACIDS

36

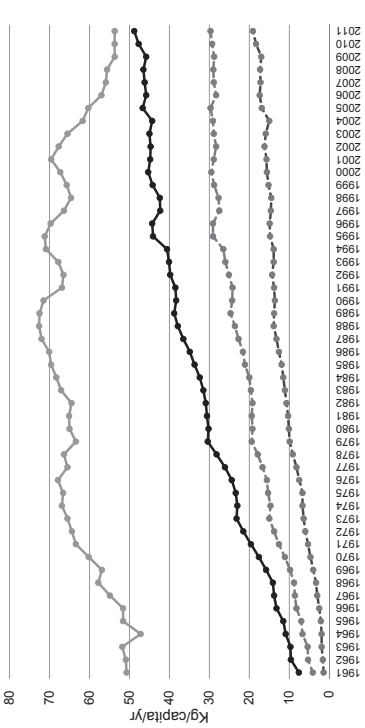
Validity of 138 item FFQ and 44 item FFQ for n-3 PUFA intake (% of TFA)

FFQ		44 item FFQ		138 item FFQ	
REFERENCE		Dietary records	Serum phospholipids	Dietary records	Serum phospholipids
Male	n	94	83	102	88
	EPA	0.51	0.44	0.49	0.59
	DHA	0.51	0.42	0.48	0.49
Female	N	107		113	
	EPA	0.50		0.48	
	DHA	0.50		0.47	

EPA: Eicosapentaenoic acid; DHA: Doxosahexaenoic acid

38

Trend of fish and meat consumption in Japan

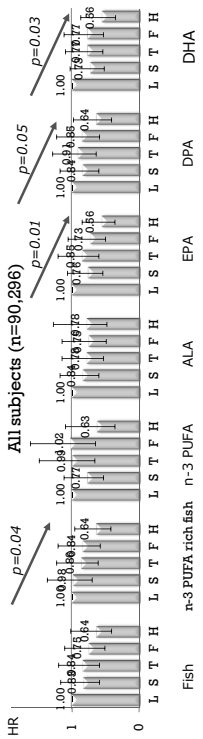


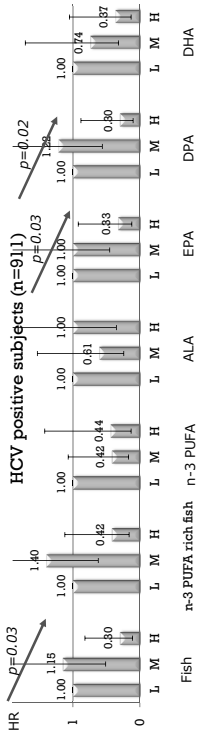
http://faostat3.fao.org/faostat-gateway/go/to/browse/FB/CL/E

37

Fish, n-3 fatty acids intake and incidence of hepatocellular carcinoma

- 398 cases among 90,296 men and women during 11 yrs-

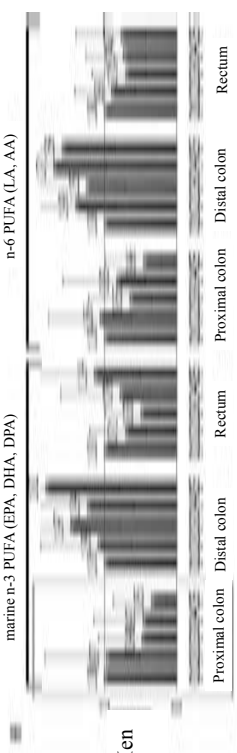


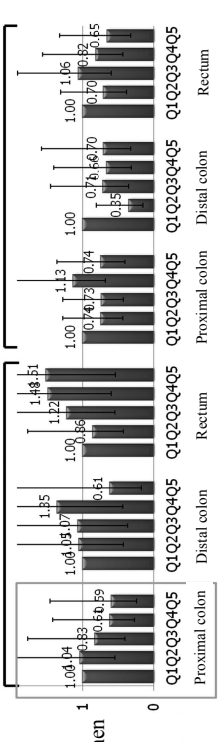


Sawada N et al. Gastroenterology 2012;142:1468-75.

40

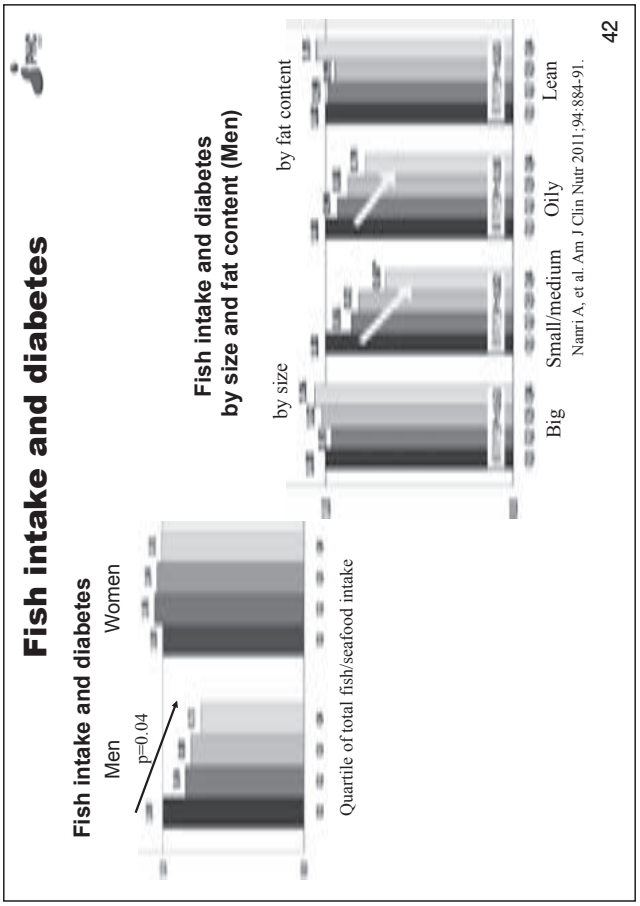
n-3 and n-6 polyunsaturated fatty acids and incidence of colorectal cancer

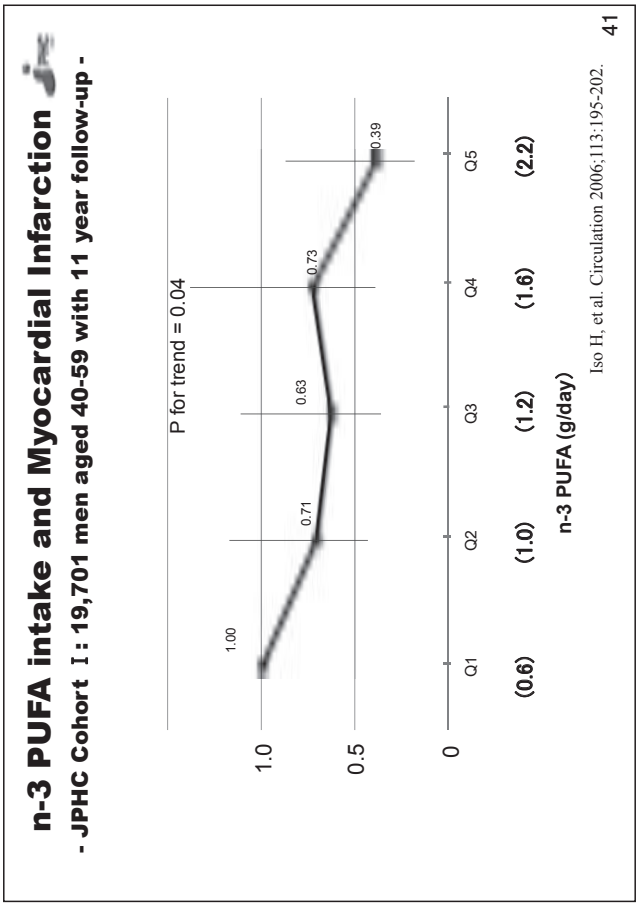


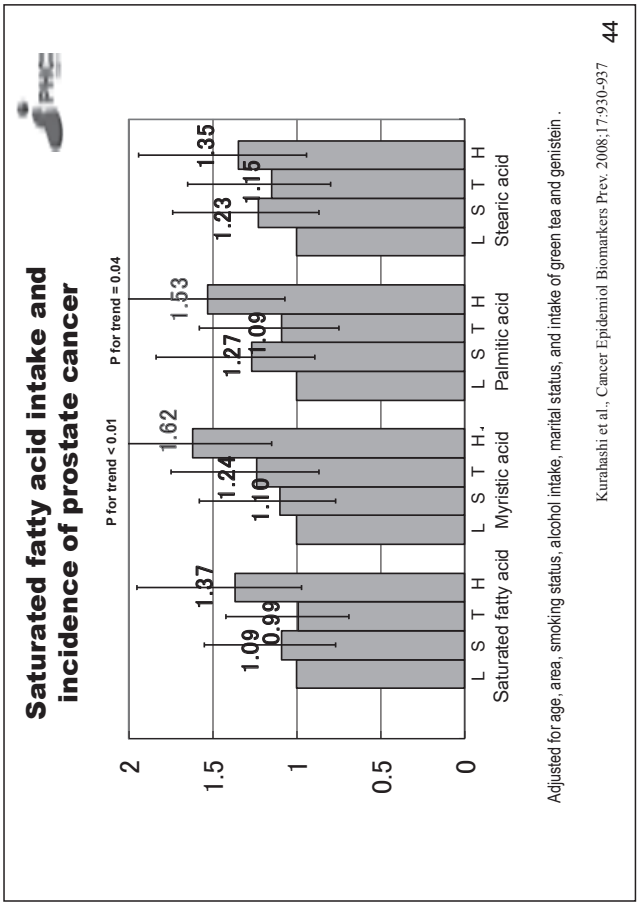


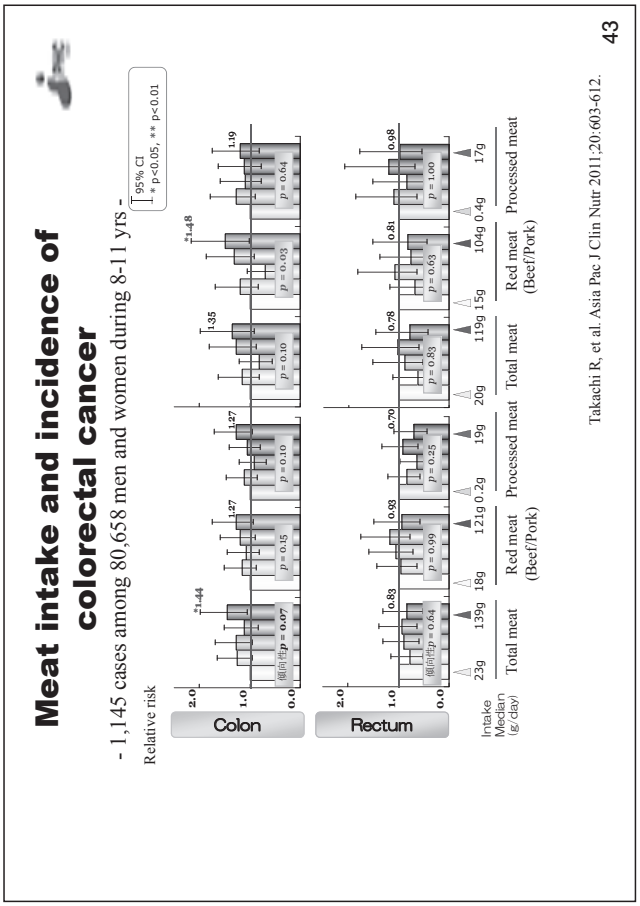
Sasazuki S, et al. Int J Cancer 2011;129:1718-29.

39


Fish intake and diabetes
Fish intake and diabetes
Men
Women
p=0.04
Quartile of total fish/seafood intake
Fish intake and diabetes
by size and fat content (Men)
by size
by fat content
Big
Small/medium
Oily
Lean
Nanri A, et al. Am J Clin Nutr 2011;94:884-91.
42


n-3 PUFA intake and Myocardial Infarction
- JPHC Cohort Ⅰ: 19,701 men aged 40-59 with 11 year follow-up -
P for trend = 0.04
1.0
0.5
0
1.00
0.71
0.63
0.73
0.39
Q1
Q2
Q3
Q4
Q5
(0.6)
(1.0)
(1.2)
(1.6)
(2.2)
n-3 PUFA (g/day)
Iso H, et al. Circulation 2006;113:195-202.
41


Saturated fatty acid intake and
incidence of prostate cancer
P for trend < 0.01
P for trend = 0.04
2
1.5
1
0.5
0
1.09
0.99
1.37
1.10
1.24
1.62
1.09
1.27
1.53
1.15
1.23
1.35
L S T H
L S T H
L S T H
L S T H
Saturated fatty acid
Myristic acid
Palmitic acid
Stearic acid
Adjusted for age, area, smoking status, alcohol intake, marital status, and intake of green tea and genistein .
Kurahashi et al., Cancer Epidemiol Biomarkers Prev. 2008;17:930-937
44


Meat intake and incidence of
colorectal cancer
- 1,145 cases among 80,658 men and women during 8-11 yrs -
95% CI
* p<0.05, ** p<0.01
Relative risk
Colon
Rectum
Intake
Median
(g/day)
23g
139g
18g
121g
0.2g
19g
20g
119g
15g
104g
0.4g
17g
Total meat
Red meat
(Beef/Pork)
Processed meat
Total meat
Red meat
(Beef/Pork)
Processed meat
Takachi R, et al. Asia Pac J Clin Nutr 2011;20:603-612.
43

Saturated fat intake and incidence of cardiovascular disease

- 82,000 men and women, 11 yr follow-up -

Incidence rate per 100,000

Ischemic stroke 1,939 / Hemorrhagic stroke 894 / Coronary Heart Disease 610

350 / 300 / 250 / 200 / 150 / 100 / 50 / 0

0.8-11.7 / 11.8-14.8 / 14.9-17.7 / 17.8-21.5 / 21.6-96.7 g/day

20g (% energy ≒ 10%)

Yamagishi K, et al. Eur Heart J 2013;34:1225-32.

46

Red meat intake and diabetes

- 1,178 newly diagnosed diabetes among 27,425 men and 36,424 women during 5 years -

Men / Women

p=0.007

P=0.27 / P=0.38 / P=0.10 / P=0.98 / P=0.56

1.8 / 1.6 / 1.4 / 1.2 / 1.0 / 0.8 / 0.6 / 0.4 / 0.2 / 0.0

Median (g/day): 15g, 83g, 0g, 15g, 0g, 20g, 12g, 72g, 0g, 14g, 0g, 18g

Red meat (Beef/Pork) / Processed meat / Chicken meat / Red meat (Beef/Pork) / Processed meat / Chicken meat

Kurotani K, et al. Br J Nutr 2013;110:1910-8.

45

Fish/n-3 PUFA and red meat/saturated fat intake and risk of cancer, cardiovascular disease and diabetes: evidence from the JPHC study

	Colon	Prostate	HCC	Other Cancers	CVD	DM
Fish, n-3 fatty acids	↓ esp.prox (n-3)		↓ (fish, n-3)	Null: Breast	↓:MI (fish, n-3)	↓:m (fish, oily)
Red meat, Saturated fat	↑ (total meat, red meat)	↑ (saturated fat)			↑:MI (saturated fat) ↓:Stroke (saturated fat)	↑:m (red meat)

48

Red meat intake and mortality

European cohort study <EPIC Study>

- 450,000 subjects, 13 yr follow-up -

0-9.9 / 10-19.9 / 20-39.9 / 40-79.9 / 80-159.9 / 160-

1.4 / 1.2 / 1.0 / 0.8 / 0.6 / 0.4 / 0.2 / 0.0

1.07 / 1.00 / 1.01 / 0.99 / 1.03 / 1.14 / 1.04 / 1.00 / 1.04 / 0.99 / 1.03 / 1.21 / 1.03 / 1.00 / 1.01 / 1.02 / 1.04 / 1.07

Total mortality / Cancer mortality / CVD mortality

Rohrmann S, et al. BMC Med 2013;11:63.

Asian cohort study <Asia Cohort Consortium>

- 300,000, 7-16 yr follow-up -

Men: Q1 / Q2 / Q3 / Q4

Total mortality / Cancer mortality / CVD mortality

Women

Total mortality / Cancer mortality / CVD mortality

Lee EJ, et al. Am J Clin Nutr 2013;98:1032-41.

47

SALT AND SALTY FOODS

49

Salt and salted food in Japanese foods

Miso: 9 – 18 %
Miso soup: 0.5 – 1.2 %

Dried fish: 1 - 10 %

Seasonings

Pickled vegetables: 1- 10 %

Salted fish gut: 11 %

Salted salmon roe: 10 %

Average Salt Intake (aged 20 years and over)
Men: **11.1g**/day, Women: **9.4g**/day
<National Health and Nutrition Survey 2013>

50

Validity of FFQ with 28-d DR and two 24-h urinary excretions for Sodium
- JPHC FFQ Validation Study, Cohort I -

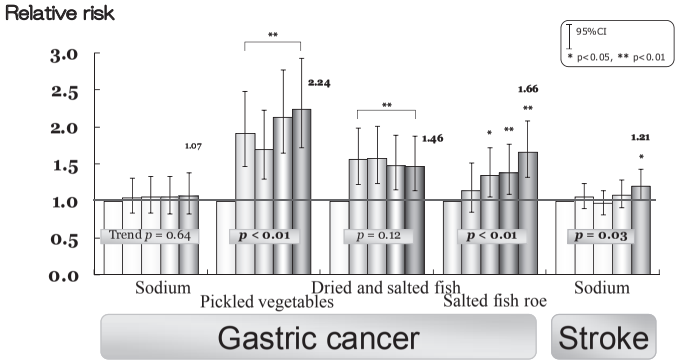
			Spearman orrelation	
			Crude	Adjusted [1]
138 item FFQ with				
DR	Men	(n=102)	0.59	0.41
	Women	(n=113)	0.55	0.48
Urine	Men	(n= 32)	0.24	0.06
	Women	(n= 57)	-0.10	0.02
44 item FFQ with				
DR	Men	(n= 94)	0.49	0.33
	Women	(n=107)	0.54	0.49
Urine	Men	(n= 32)	0.38	0.30
	Women	(n= 57)	0.12	0.16

[1] Energy intake was adjusted by residual method

Sasaki S, et al. J Epidemiol 2003;13:S102-S105.
Tsubono Y, et al. J Epidemiol 2003;13:S125-S133.

51

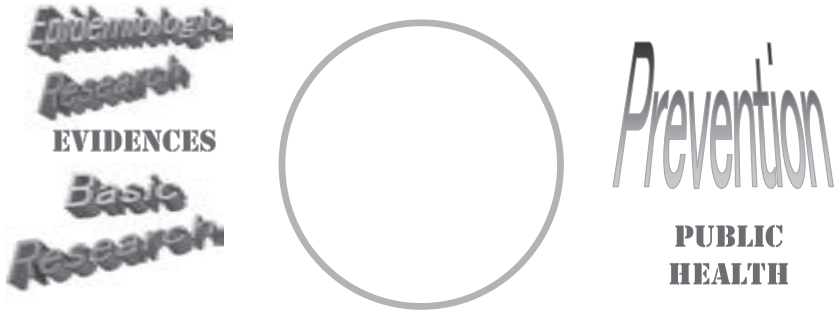
Salt, salted food intake and risk of gastric cancer and stroke



Takachi R, et al. Am J Clin Nutr 2010;91:456-464.

52

From “Cancer Research” to “Cancer Prevention”



To bridge basic results of the investigation to practical application -translational research-, through a collaborative effort of the front line medical professionals searching for causes of cancer and prevention methods

53

Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan Since 2003

- Main activities
  - Evidence assessment
    - Systematic review
    - Meta-analysis
    - Pooled analysis of Japanese large-scale population-based cohort studies
  - Development and implementation of “Current Evidence-based Cancer Prevention Recommendations for Japanese”

http://epi.ncc.go.jp/en/can_prev/index.html

54

Cause of cancer among Japanese: based on evidence assessment

Lifestyle, Infection, Others

http://epi.ncc.go.jp/can_prev/index.html

55

Cause of cancer among Japanese: based on evidence assessment

Food

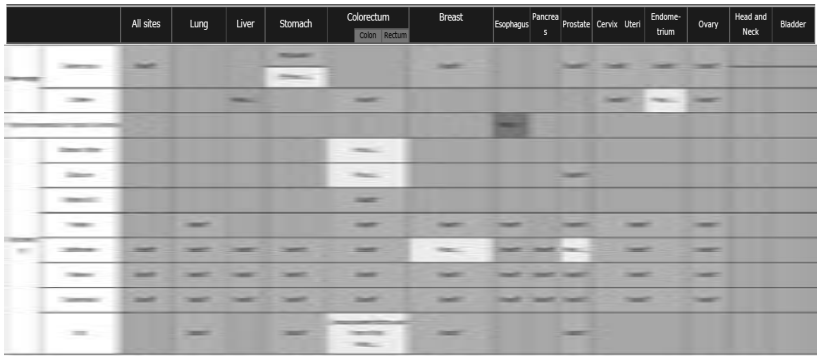
		All sites	Lung	Liver	Stomach	Colorectum (Colon / Rectum)	Breast	Esophagus	Pancreas	Prostate	Cervix Uteri	Endometrium	Ovary	Head and Neck	Bladder
Food	Vegetable	Insuff.	Insuff.	Insuff.	Poss. ↓	Insuff.	Insuff.	Prob. ↓	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.		
	Fruit	Insuff.	Poss. ↓	Insuff.	Poss. ↓	Insuff.	Insuff.	Prob. ↓	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.		
	Soy		Insuff.	Insuff.			Poss. ↓	Insuff.		Poss. ↓					
	Meat	Insuff.	Insuff.	Insuff.	Insuff.	Insuff. (Processed Meat/ Red Meat) Poss. ↑	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.		
	Fish	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Poss. ↓	Insuff.	Insuff.		
	Grain		Insuff.	Insuff.	Poss. ↑	Insuff.	Insuff.	Insuff.		Insuff.	Insuff.		Insuff.		
	salt & salted food				Prob. ↑										
	Dairy product	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.	Insuff.		
	Dietary patterns				Insuff.	Insuff.	Insuff.				Insuff.	Insuff.	Insuff.		

http://epi.ncc.go.jp/can_prev/index.html

56

Cause of cancer among Japanese: based on evidence assessment

Beverage, Nutrient

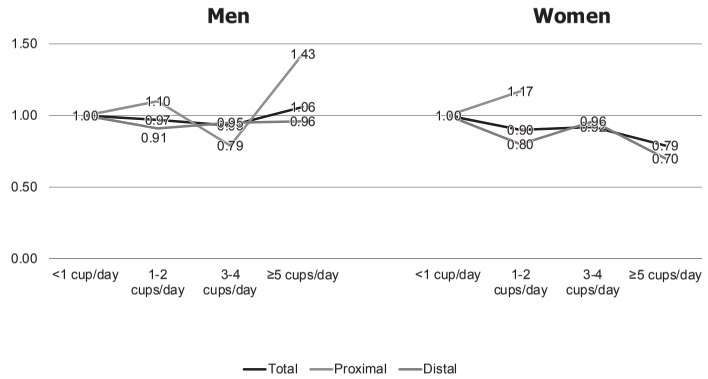


http://epi.ncc.go.jp/can_prev/index.html

57

Green tea intake and Gastric cancer: pooled analysis of six cohorts

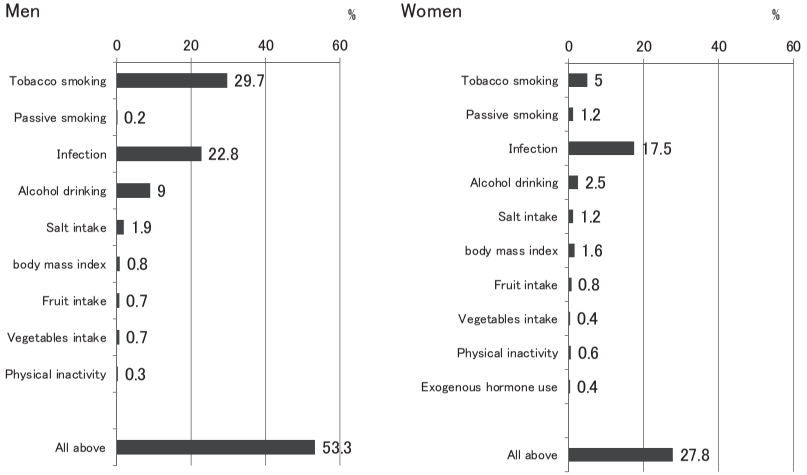
- 219,080 subjects, 3,577 cases -



Six large-scale cohort studies in Japan: JPHC-I, JPHC-II, JACC, MIYAGI, 3-pref MIYAGI, 3-pref AICHI.
Inoue M et al. GUT 2009;58:1323-1332.

58

Attributable cause of cancer in Japan, 2005 Incidence



(Inoue M et al. Ann Oncol 2011, in press)

59

Causes of Cancer in the US

Risk factor	AF%
Tobacco	30
Adult diet/obesity	**30**
Sedentary lifestyle	5
Occupational factors	5
Family history of cancer	5
Viruses/other biologic agents	**5**
Perinatal factors/growth	5
Reproductive factors	3
Alcohol	3
Socioeconomic status	3
Environmental pollution	2
Ionizing/ultraviolet radiation	2
Prescription drug/medical procedures	1
Salt/other food additives/contaminants	1

Harvard Center for Cancer Prevention: Harvard Report on Cancer Prevention, Volume 1: Causes of Human Cancer, Cancer Causes Control 1996 ;7:S3-S59.

60

Current Evidence-based Cancer Prevention Recommendations for Japanese

Risk factor	Recommendation
Smoking	Do not smoke cigarettes. Avoid passive smoking.
Drinking	Drink in moderation.
Food	Eat a balanced diet. ＊ Keep the consumption of salt-preserved foods and added salt to a minimum. ＊ Eat sufficient vegetables and fruits. ＊ Do not consume too much hot (thermally) food and drinks.
Physical Activity	Be active in daily life.
Body Weight	Maintain an appropriate weight during adulthood (do not gain or lose too much weight).
Infections	Check for the presence/absence of hepatitis virus infection, and if detected, receive appropriate treatment.

http://epi.ncc.go.jp/en/can_prev/index.html

61

Thank you for your attention!

62

セッション 1-3

疫学研究から見た日本食と循環器疾患

滋賀医科大学　社会医学講座　公衆衛生学部門／アジア疫学研究センター
三浦　克之

1960 年前後のわが国の脳卒中死亡率は世界でも有数の高さであり、平均寿命も欧米各国よりも短かった。その最大の原因が高い高血圧有病率であったが、日本人の高い食塩摂取量が大きく影響していたと考えられる。高い脳卒中死亡率を示した 1950 年代の東北地方では、食塩摂取量が 20g/ 日以上であった。国際共同疫学研究 INTERSALT では世界 32 カ国で食塩摂取量と血圧が測定され、食塩摂取量が極めて少ない未開民族では加齢による血圧上昇がほとんどないことが明らかになった。

一方、かつての日本は冠動脈疾患死亡率が低い世界有数の国であった。これに注目して 1950 年代から開始されたのが Seven Counties Study（7 カ国研究）であり、当時の日本の低い冠動脈疾患死亡率は、日本食における低い飽和脂肪酸摂取量と、これによる低い血清総コレステロール値によることが明らかになった。

国際共同疫学研究 INTERMAP では、1990 年代後半に日本、中国、英国、米国の 4 カ国で詳細な食事調査を実施し、多くの栄養素と血圧との関連を明らかにした。INTERMAP では日本食が欧米食に比べて、魚介由来 n-3 系脂肪酸（EPA, DHA）の摂取量が多く、飽和脂肪酸摂取が少ないなどの良い面、食塩摂取と男性のアルコール摂取が多いなどの悪い面を明らかにした。

我々が実施している NIPPON DATA 研究は、国による循環器疾患基礎調査および国民栄養調査の対象者を長期追跡するコホート研究であり、全国 300 地区からの国民代表集団のコホート研究に位置づけられる。NIPPON DATA80 の 24 年追跡結果から、魚介由来 n-3 系脂肪酸摂取量が高いほど将来の循環器疾患死亡リスクが低いことが明らかになった。また、野菜および果物の摂取量が多いほど循環器疾患死亡リスクが低いことも明らかになった。また、国際共同研究 ERA JUMP では、40 歳代男性の潜在性動脈硬化の日米比較を行い、日本人での高い血清 n-3 系脂肪酸が動脈硬化進展を抑制していることが明らかになった。

米国で行われた血圧低下のための摂食試験 DASH では、野菜・果物・低脂肪乳製品を増やし、総脂肪・飽和脂肪を減らした DASH 食事パターンの強い降圧効果が確認された。DASH 食と減塩の組み合わせはさらに大きな降圧を示した。DASH 食事パターンは欧米における循環器疾患予防のための食事として確立しているが、食事パターンの大きく異なるわが国でそのまま適用できるわけではない。

米飯中心の日本食パターンは、高 GI 値などの問題があるものの、低脂肪食となるなどのメリットもある。魚介中心の主菜、豊富な野菜による副菜という新しい健康的日本食パターンは、高植物性蛋白、高 n-3 系脂肪酸、低飽和脂肪酸、低エネルギーに繋がり、減塩さえしっかりと行えば循環器疾患予防に有効な食事になると考えられる。男性では適正飲酒量を守ることも重要である。

Session 1−3

Epidemiologic Findings on Japanese Diet and Cardiovascular Diseases

Katsuyuki Miura, M.D., Ph.D.
Department of Public Health/Center for Epidemiologic Research in Asia,
Shiga University of Medical Science

Japan was one of the countries with highest stroke mortality in the world around 1960, and its life expectancy was shorter than that in Western countries. A major cause of this high stroke mortality in Japan was high prevalence of hypertension, which was due to high salt (sodium) intake in Japanese. People in northeast area of Japan where stroke mortality was very high took more than 20 gram/day of salt in 1950's. An international cooperative epidemiologic study, the INTERSALT, measured salt intake and blood pressure in 32 countries, and revealed that blood pressure in people with very low salt intake does not increase by aging.

On the other hand, Japan was a country with very low mortality from coronary heart disease. The Seven Counties Study conducted from 1950' s clarified that very low mortality from coronary heart disease in Japan was due to low intake of saturated fatty acids and, therefore, low level of serum total cholesterol in Japanese.

An international cooperative study, the INTERMAP, was held in late 1990's in 4 countries (Japan, China, UK, and USA), where detailed dietary survey was performed. The INTERMAP showed that Japanese diet was better compared with Western diet for higher intake in marine-derived n-3 fatty acids (EPA and DHA) and lower saturated fat intake, and was worse for higher salt intake and higher alcohol intake in men.

The NIPPON DATA, cohort studies of the National Cardiovascular Survey and the National Nutrition Survey of Japan, showed that long-term cardiovascular mortality risk was lower in people with higher n-3 fatty acids intake. It also showed that higher intake in vegetable and fruit intake was related to lower cardiovascular mortality. An international comparison study on subclinical atherosclerosis between Japan and the USA, the ERA JUMP, showed that less progressed atherosclerosis in Japanese men compared with US men was explained largely by higher serum n-3 fatty acids in Japanese men.

A feeding trial to reduce blood pressure in the US, the DASH trial, showed that the DASH dietary pattern, which is rich in vegetables, fruits, and low fat dairy products and low in total fat and saturated fat, strongly reduced blood pressure. A combination of DASH diet and low salt reduced blood pressure further. The DASH dietary pattern has been established as a dietary pattern to prevent cardiovascular diseases in Western countries, but whether it is effective in Japanese is not clear because the Japanese dietary pattern is very different from Western one.

The present Japanese dietary pattern in which rice is the main food has a demerit for its high GI value as well as a merit for low fat intake. We need to establish a new healthy Japanese dietary pattern with high fish and vegetable and low salt; it will accomplish high vegetable protein, high n-3 fatty acids, low saturated fat, and low energy and will be effective for cardiovascular disease prevention. To avoid heavy alcohol drinking in men is also important in Japan.

7th International Conference on Nutrition and Aging
2015.9.29. University of Tokyo

疫学研究から見た日本食と循環器疾患

Epidemiologic Findings on Japanese Diet and Cardiovascular Diseases

滋賀医科大学
社会医学講座　公衆衛生学部門
アジア疫学研究センター

三浦克之

Katsuyuki Miura, MD, PhD
Department of Public Health
/Center for Epidemiologic Research in Asia,
Shiga University of Medical Science

1

伝統的日本食パターン
Traditional Japanese dietary pattern

低脂肪 low fat　　高食塩 high salt

低エネルギー low energy

野菜少ない？ low vegetable?

魚が多い rich in fish

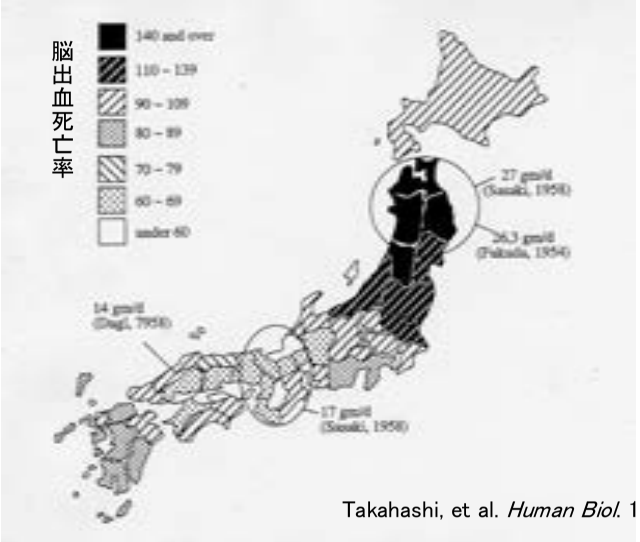
低カルシウム low calcium

大豆製品 rich in soy products

肉が少ない less meat

2

1950年代の都道府県別脳出血死亡率と食塩摂取量調査結果
Mortality from cerebral hemorrhage and salt intake in 1950's



Takahashi, et al. *Human Biol.* 1957

3

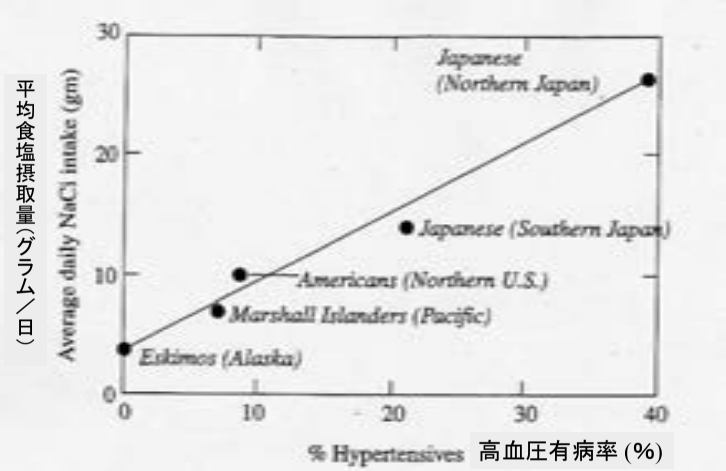
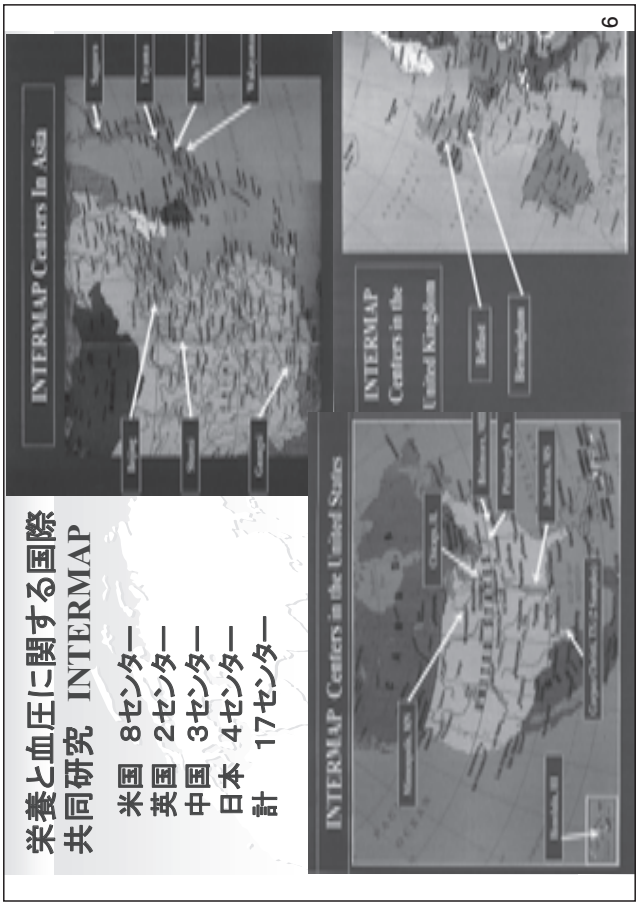
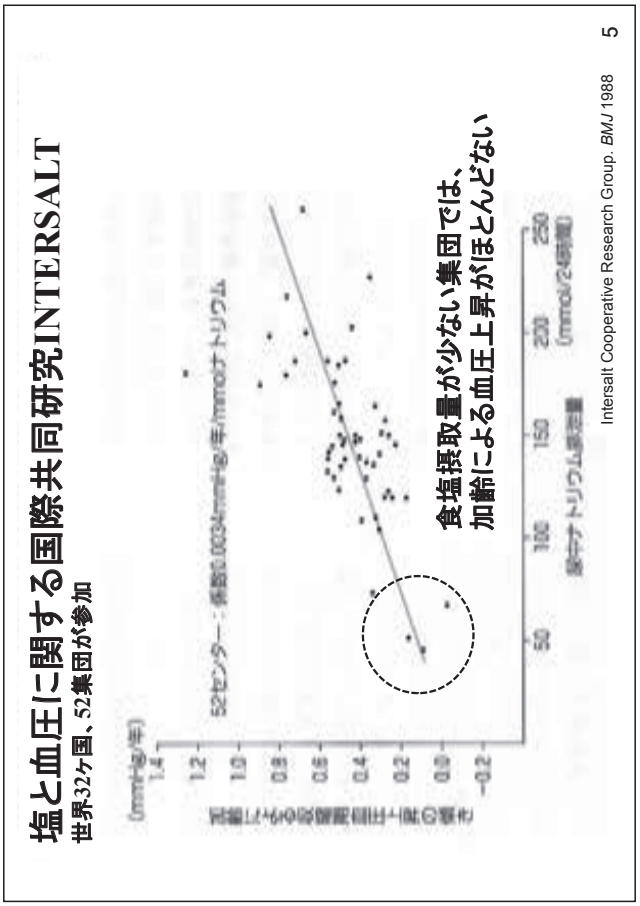


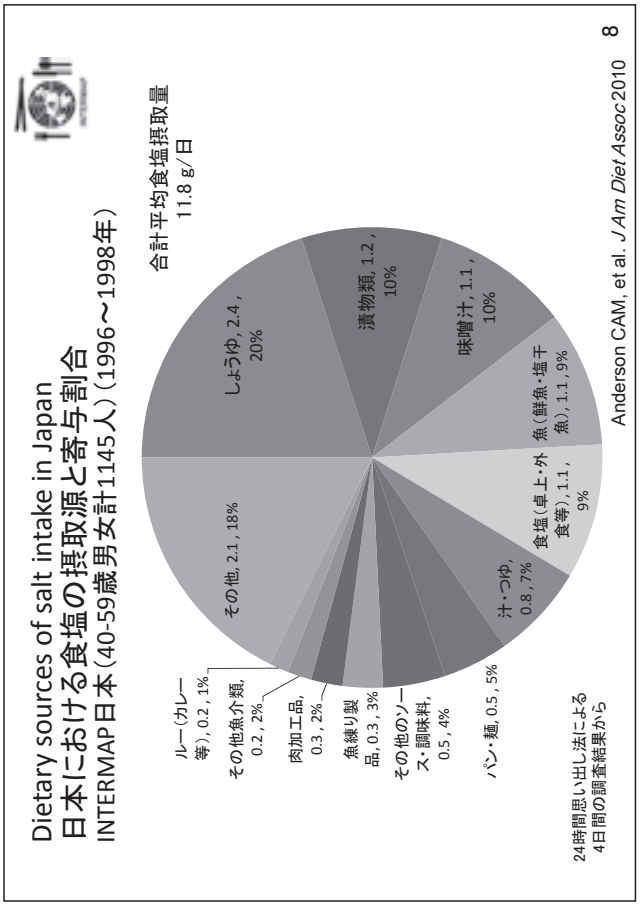
Figure 1 Correlation of average daily salt (NaCl) intakes with prevalence of hypertension in different geographic areas and among different races

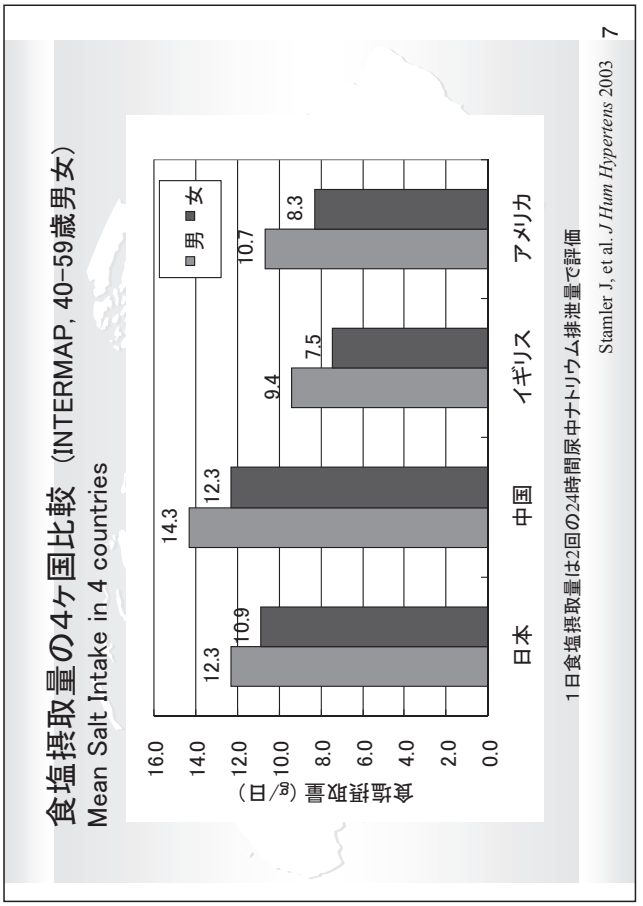
Meneely GR, Dahl LK. *Med Clin N Amer* 1961

4


栄養と血圧に関する国際
共同研究　INTERMAP
米国　8センター
英国　2センター
中国　3センター
日本　4センター
計　17センター
INTERMAP Centers in Asia
INTERMAP Centers in the United States
INTERMAP Centers in the United Kingdom
6

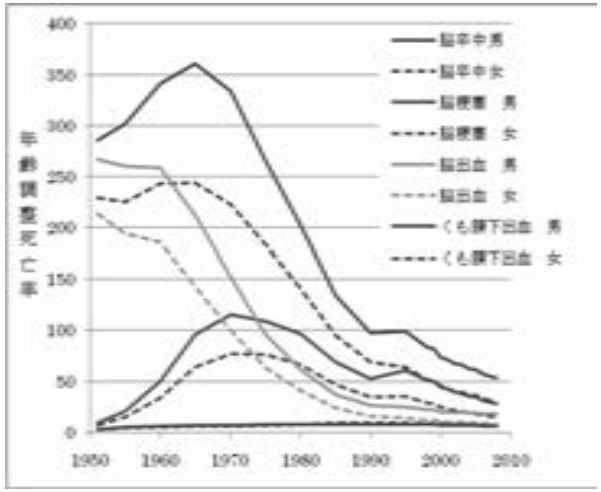

塩と血圧に関する国際共同研究INTERSALT
世界32ヶ国、52集団が参加
52センター：係数0.0034mmHg/年/mmol/ナトリウム
食塩摂取量が少ない集団では、
加齢による血圧上昇がほとんどない
Intersalt Cooperative Research Group. BMJ 1988
5


Dietary sources of salt intake in Japan
日本における食塩の摂取源と寄与割合
INTERMAP日本（40-59歳男女計1145人）（1996～1998年）
合計平均食塩摂取量
11.8 g/日
しょうゆ, 2.4, 20%
漬物類, 1.2, 10%
味噌汁, 1.1, 10%
魚（鮮魚・塩干魚）, 1.1, 9%
食塩（卓上・外食等）, 1.1, 9%
汁・つゆ, 0.8, 7%
パン・麺, 0.5, 5%
その他のソース・調味料, 0.5, 4%
魚練り製品, 0.3, 3%
肉加工品, 0.3, 2%
その他魚介類, 0.2, 2%
ルー（カレー等）, 0.2, 1%
その他, 2.1, 18%
24時間思い出し法による4日間の調査結果から
Anderson CAM, et al. J Am Diet Assoc 2010
8


食塩摂取量の4ヶ国比較（INTERMAP, 40–59歳男女）
Mean Salt Intake in 4 countries
食塩摂取量（g/日）
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
男
女
12.3
10.9
14.3
12.3
9.4
7.5
10.7
8.3
日本
中国
イギリス
アメリカ
1日食塩摂取量は2回の24時間尿中ナトリウム排泄量で評価
Stamler J, et al. J Hum Hypertens 2003
7

Trends in age-adjusted stroke mortality in Japan (1950-2008)
わが国の脳卒中の年齢調整死亡率の推移（男女別）

(人口10万対)

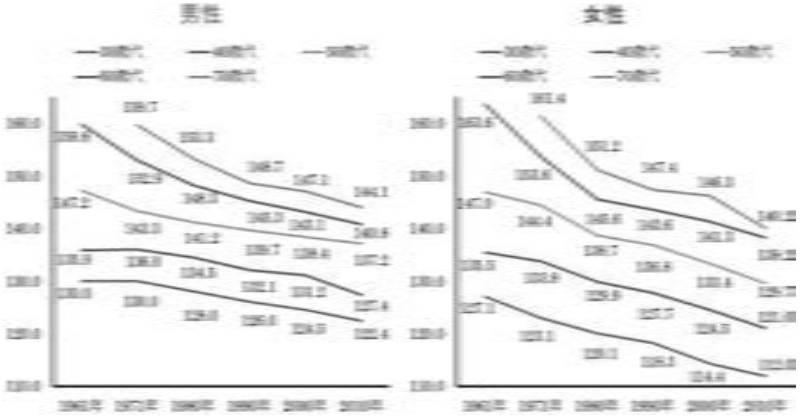


厚生労働省：人口動態統計

9

Trends in mean systolic blood pressure in Japan
収縮期血圧平均値の年次推移（1961年～2010年）

（第1次成人病基礎調査、第2次成人病基礎調査、第3次循環器疾患基礎調査(NIPPON DATA80)、第4次循環器疾患基礎調査(NIPPON DATA90)、第5次循環器疾患基礎調査、NIPPON DATA2010）



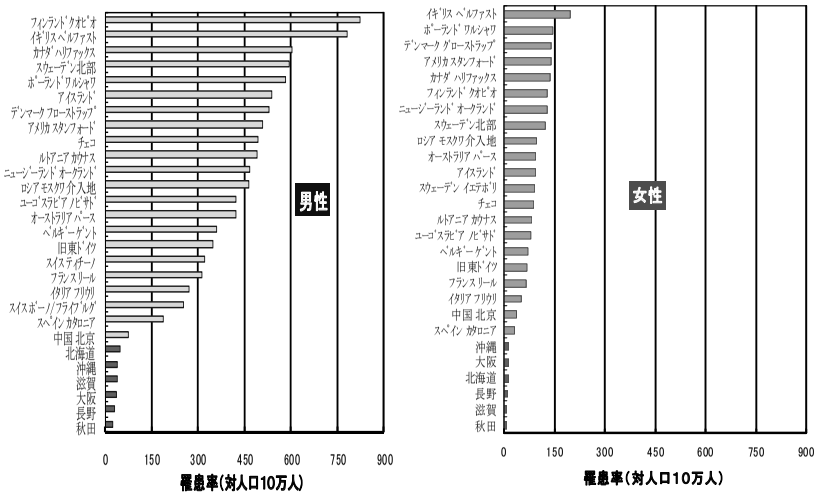
Miura K, et al. *Circ J* 2013

10

心筋梗塞罹患率の国際比較 (男女別、35-64歳)

International comparison of the incidence of myocardial infarction

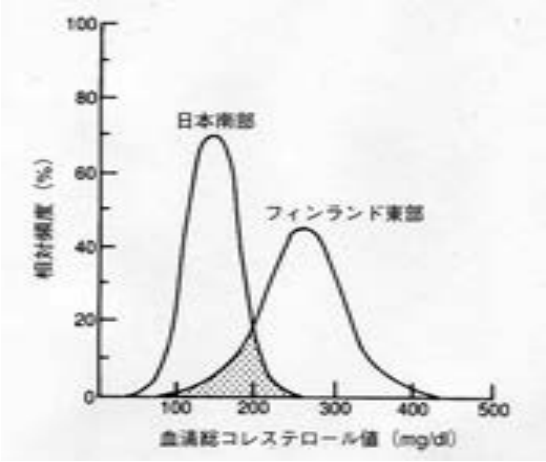
MONICA,1985-87
日本、1989-92



Ueshima H. *J Atheroscler Thromb*. 2007

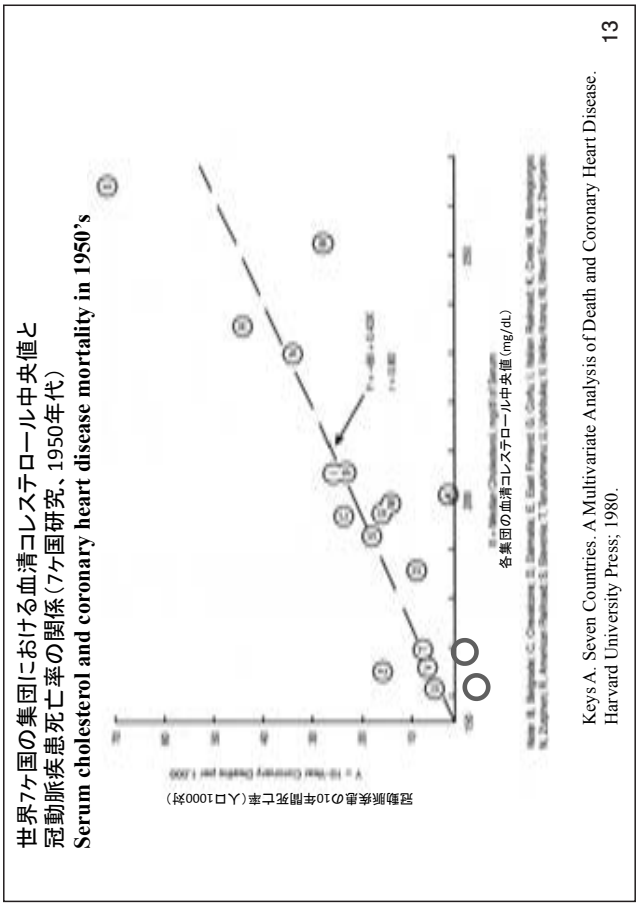
11

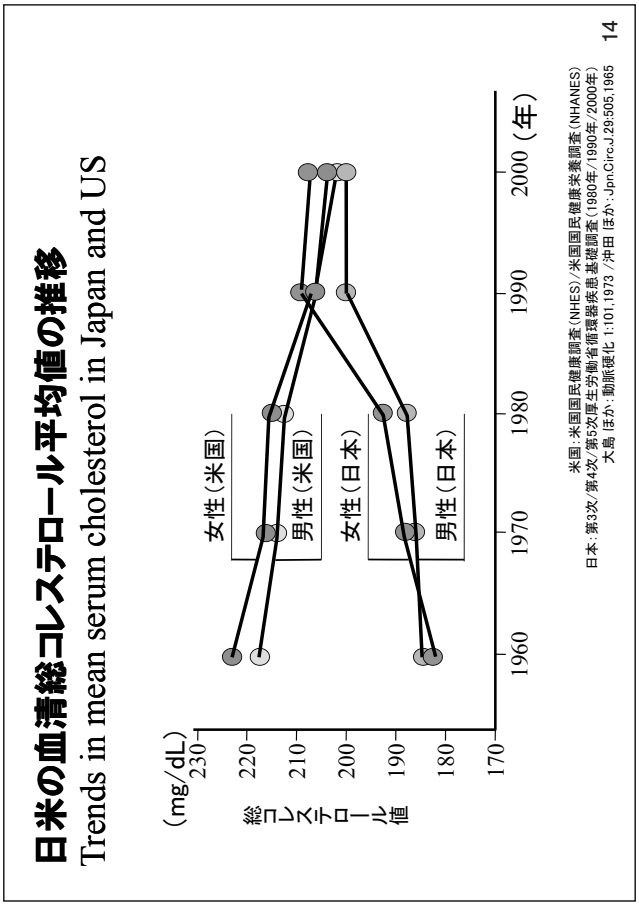
1950年代の日本とフィンランドにおける血清総コレステロールの分布の比較
Comparison of the distribution of serum total cholesterol in Japan and Finland



Keys A (ed.) Coronary heart disease in seven countries. American Heart Association Monograph 29. New York. 1970.

12


世界7ヶ国の集団における血清コレステロール中央値と
冠動脈疾患死亡率の関係（7ヶ国研究、1950年代）
Serum cholesterol and coronary heart disease mortality in 1950's
冠動脈疾患の10年間死亡率（人口1000対）
各集団の血清コレステロール中央値（mg/dL）
Keys A. Seven Countries. A Multivariate Analysis of Death and Coronary Heart Disease.
Harvard University Press; 1980.
13

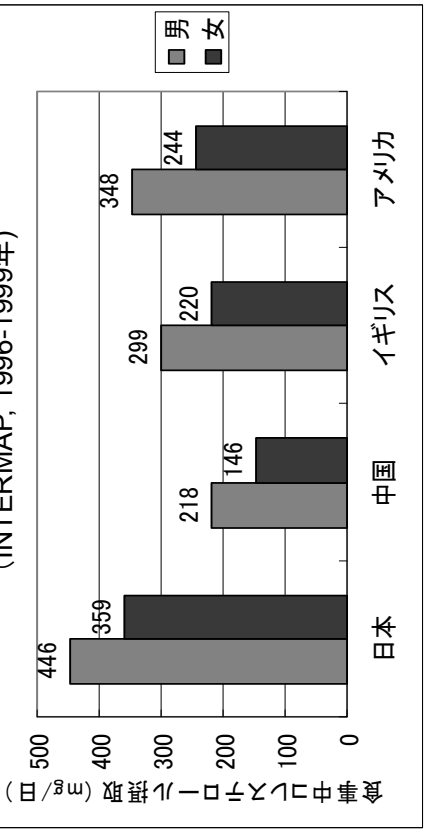

日米の血清総コレステロール平均値の推移
Trends in mean serum cholesterol in Japan and US
（mg/dL）
総コレステロール値
230
220
210
200
190
180
170
1960
1970
1980
1990
2000（年）
女性（米国）
男性（米国）
女性（日本）
男性（日本）
米国：米国国民健康調査（NHES）/米国国民健康栄養調査（NHANES）
日本：第3次/第4次/第5次厚生労働省循環器疾患基礎調査（1980年/1990年/2000年）
大島 ほか：動脈硬化 1:101,1973 /沖田 ほか：Jpn.Circ.J.29:505,1965
14

Total fat intake (% of energy) in 4 countries
脂肪エネルギー比の4ヶ国の比較
（INTERMAP, 1996-1999年）
脂肪エネルギー比（%）
35
30
25
20
15
10
23.7
26.1
20.5
19.5
33.0
32.5
33.3
32.6
男
女
日本
中国
イギリス
アメリカ
Stamler J, et al. J Hum Hypertens 2003
15

Saturated fat intake (g/day) in 4 countries
飽和脂肪酸摂取量（g/日）の4ヶ国比較
(INTERMAP研究, 40-59歳男女, 1996-1999年)
飽和脂肪酸摂取量（g/日）
40
35
30
25
20
15
10
5
0
15.6
14.4
13.6
9.4
33.8
25.6
32.8
23.3
男
女
日本
中国
イギリス
アメリカ
Stamler J, et al. J Hum Hypertens 2003
16

Dietary cholesterol intake (mg/day) in 4 countries

食事中コレステロール摂取量の4ヶ国の比較
（INTERMAP, 1996-1999年）

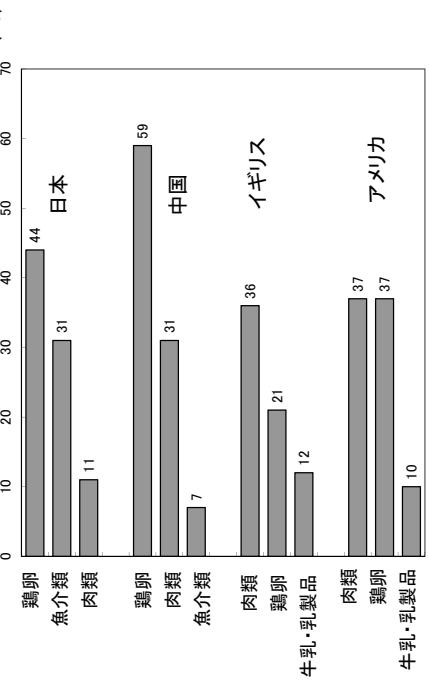


Stamler J, et al. *J Hum Hypertens* 2003

17

Dietary sources of dietary cholesterol intake in 4 countries

4カ国における食事性コレステロールの摂取源の比較
(INTERMAP, 1996-1999年)



Sakurai M, et al. *J Hypertens* 2010

18

Keysの式

Keys' formula on serum cholesterol and dietary lipids

血清総コレステロールの変化（mg/dL）

＝ 1.35 ×（2 × 飽和脂肪酸摂取量の変化（%kcal）

－ 多価不飽和脂肪酸摂取量の変化（%kcal））

＋ 1.5 × √食品中コレステロール摂取量の変化（mg/1000kcal）

Keys A. *Am J Clin Nutr* 1984

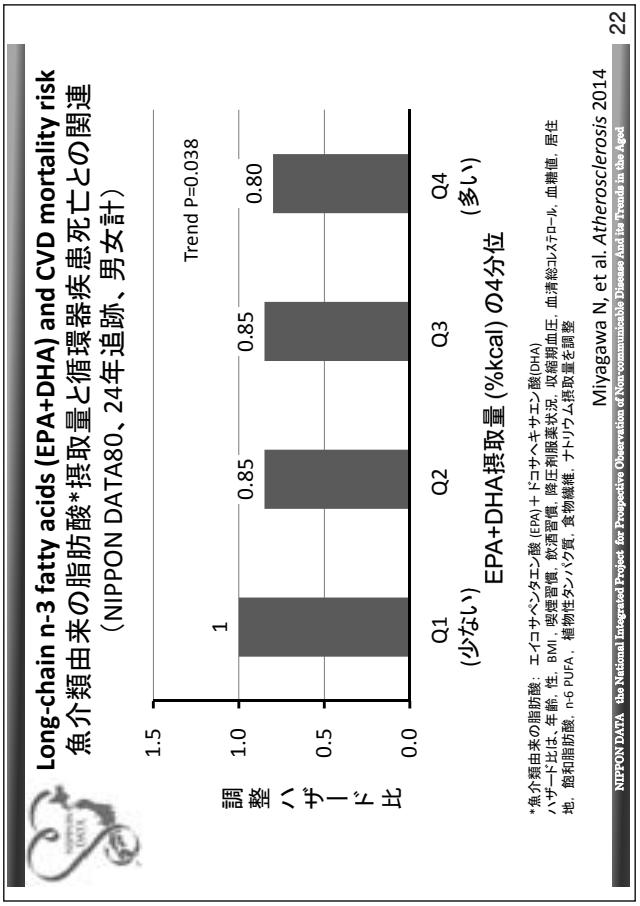
19

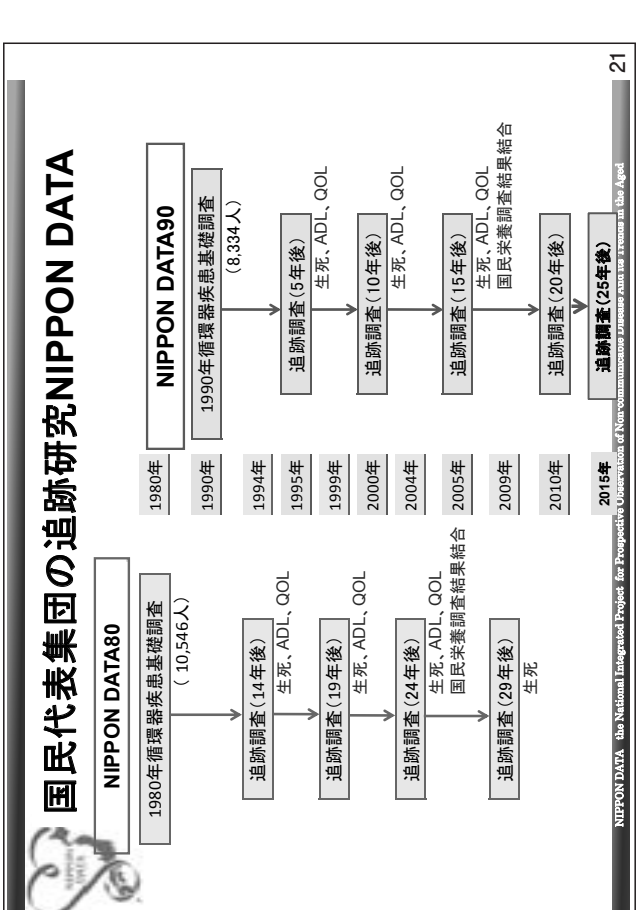
n-3 polyunsaturated fatty acids intake in 4 countries

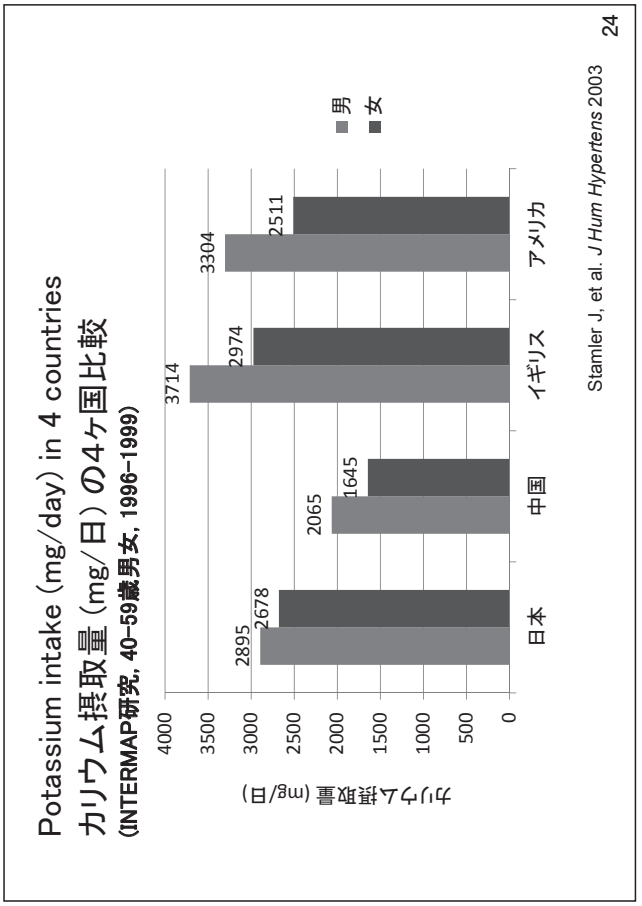
n-3多価不飽和脂肪酸摂取量（全体、α-リノレン酸、DHA+EPA）の4カ国比較　(INTERMAP, 1996-1999年)

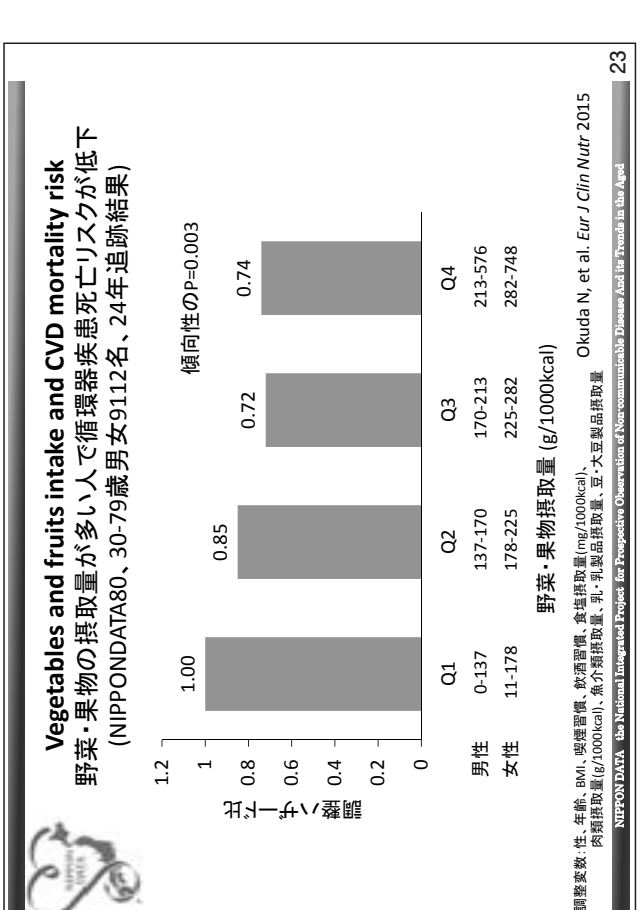
Ueshima H, et al. *Hypertension* 2007

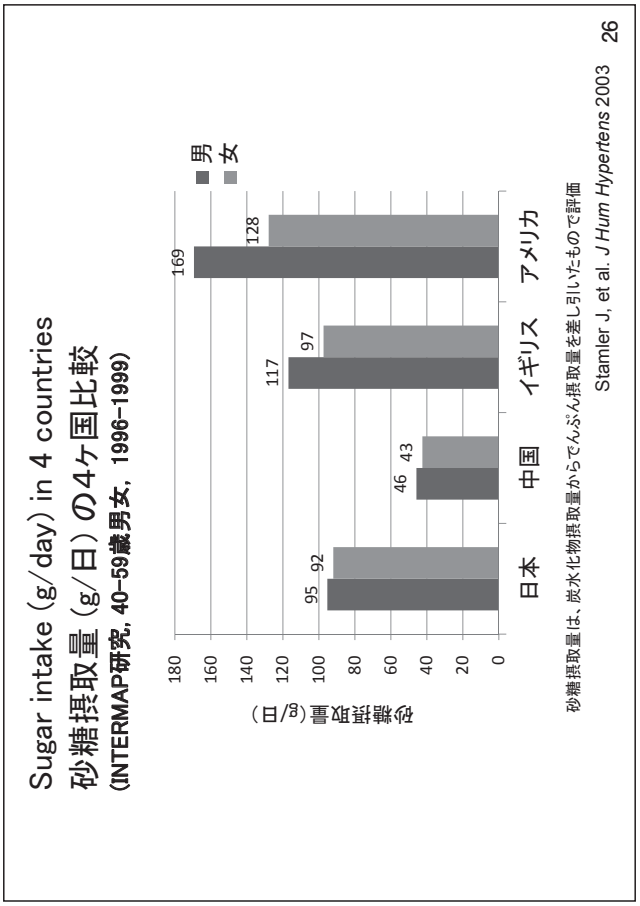
20

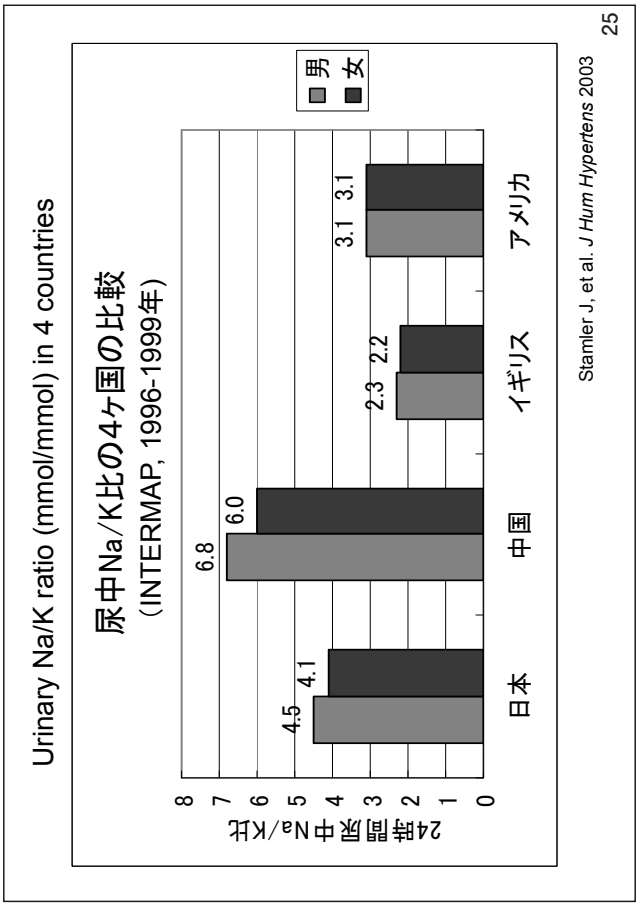

国民代表集団の追跡研究NIPPON DATA
NIPPON DATA80
1980年循環器疾患基礎調査（10,546人）
追跡調査（14年後）生死、ADL、QOL
追跡調査（19年後）生死、ADL、QOL
追跡調査（24年後）生死、ADL、QOL 国民栄養調査結果結合
追跡調査（29年後）生死
NIPPON DATA90
1990年循環器疾患基礎調査（8,334人）
追跡調査（5年後）生死、ADL、QOL
追跡調査（10年後）生死、ADL、QOL
追跡調査（15年後）生死、ADL、QOL 国民栄養調査結果結合
追跡調査（20年後）
追跡調査（25年後）
1980年
1990年
1994年
1995年
1999年
2000年
2004年
2005年
2009年
2010年
2015年
NIPPON DATA the National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged
21

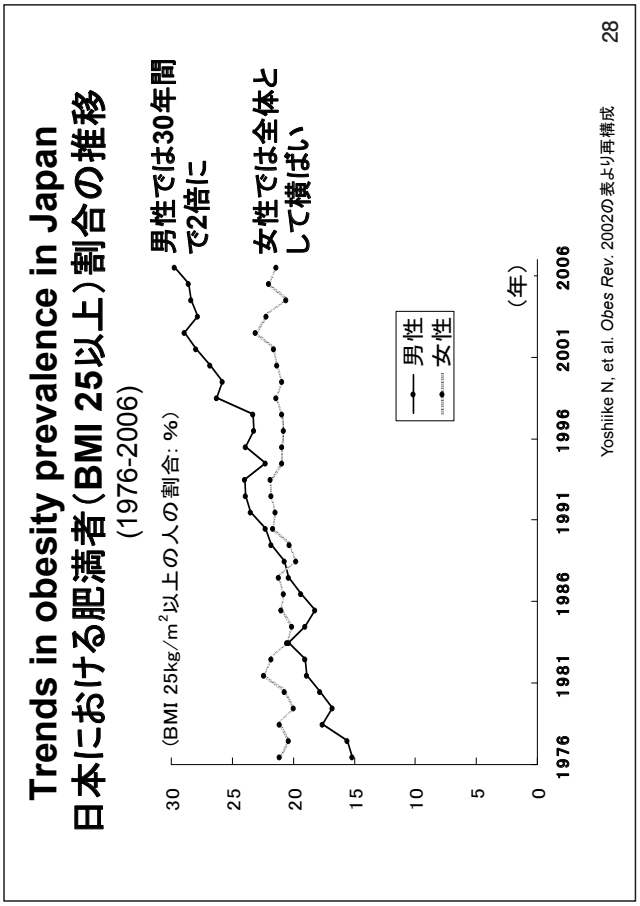

Long-chain n-3 fatty acids (EPA+DHA) and CVD mortality risk
魚介類由来の脂肪酸*摂取量と循環器疾患死亡との関連
（NIPPON DATA80、24年追跡、男女計）
調整ハザード比
1.5
1.0
0.5
0.0
1
0.85
0.85
0.80
Trend P=0.038
Q1（少ない）
Q2
Q3
Q4（多い）
EPA+DHA摂取量 (%kcal) の4分位
*魚介類由来の脂肪酸：エイコサペンタエン酸 (EPA) + ドコサヘキサエン酸(DHA)
ハザード比は、年齢、性、BMI、喫煙習慣、飲酒習慣、降圧剤服薬状況、収縮期血圧、血清総コレステロール、血糖値、居住地、飽和脂肪酸、n-6 PUFA、植物性タンパク質、食物繊維、ナトリウム摂取量を調整
Miyagawa N, et al. Atherosclerosis 2014
NIPPON DATA the National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged
22

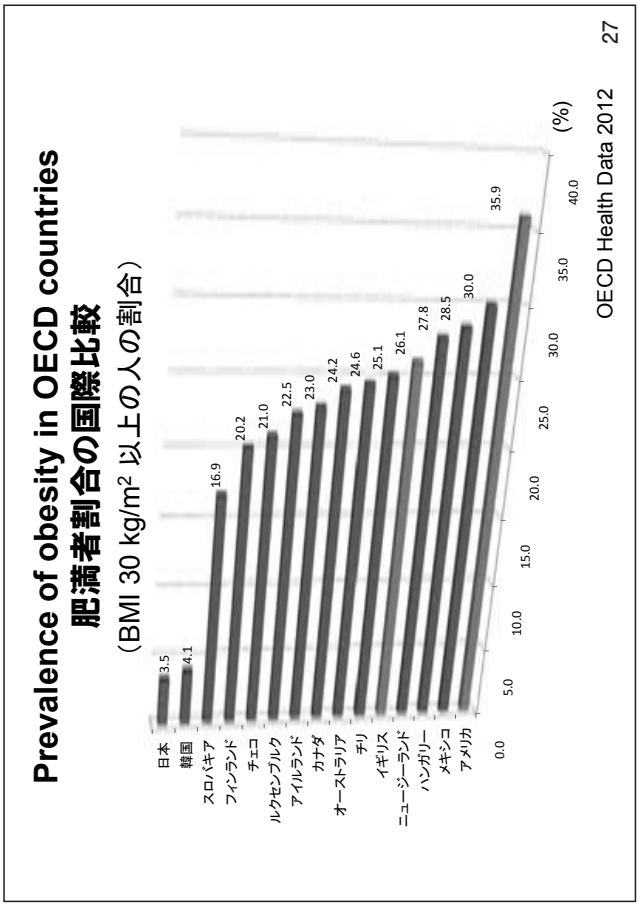

Potassium intake (mg/day) in 4 countries
カリウム摂取量（mg/日）の4ヶ国比較
(INTERMAP研究, 40–59歳男女, 1996–1999)
カリウム摂取量 (mg/日)
4000
3500
3000
2500
2000
1500
1000
500
0
2895
2678
2065
1645
3714
2974
3304
2511
日本
中国
イギリス
アメリカ
男
女
Stamler J, et al. J Hum Hypertens 2003
24


Vegetables and fruits intake and CVD mortality risk
野菜・果物の摂取量が多い人で循環器疾患死亡リスクが低下
(NIPPONDATA80、30-79歳男女9112名、24年追跡結果)
傾向性のP=0.003
循環器疾患死亡リスク
1.2
1
0.8
0.6
0.4
0.2
0
1.00
0.85
0.72
0.74
Q1
Q2
Q3
Q4
男性 0-137 137-170 170-213 213-576
女性 11-178 178-225 225-282 282-748
野菜・果物摂取量 (g/1000kcal)
調整変数：性、年齢、BMI、喫煙習慣、飲酒習慣、食塩摂取量(mg/1000kcal)、肉類摂取量(g/1000kcal)、魚介類摂取量、乳・乳製品摂取量、豆・大豆製品摂取量
Okuda N, et al. Eur J Clin Nutr 2015
NIPPON DATA the National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged
23

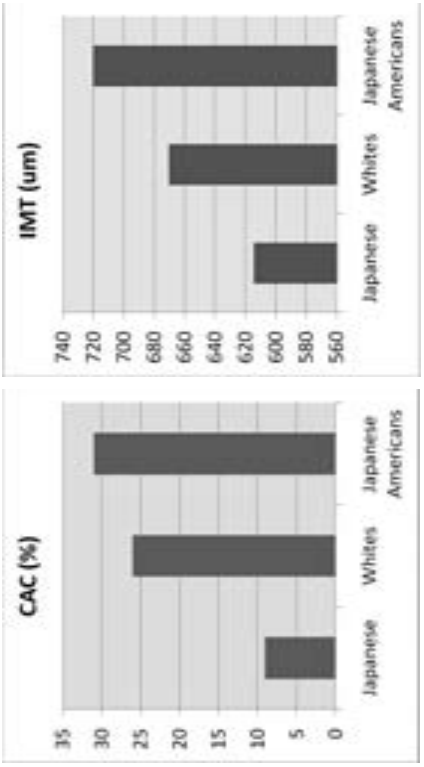

Sugar intake (g/day) in 4 countries
砂糖摂取量（g/日）の4ヶ国比較
(INTERMAP研究, 40–59歳男女, 1996–1999)
砂糖摂取量(g/日)
180
160
140
120
100
80
60
40
20
0
95
92
46
43
117
97
169
128
日本
中国
イギリス
アメリカ
男
女
砂糖摂取量は、炭水化物摂取量からでんぷん摂取量を差し引いたもので評価
Stamler J, et al. J Hum Hypertens 2003
26


Urinary Na/K ratio (mmol/mmol) in 4 countries
尿中Na/K比の4ヶ国の比較
(INTERMAP, 1996-1999年)
24時間尿中Na/K比
8
7
6
5
4
3
2
1
0
4.5
4.1
6.8
6.0
2.3
2.2
3.1
3.1
日本
中国
イギリス
アメリカ
男
女
Stamler J, et al. J Hum Hypertens 2003
25


Trends in obesity prevalence in Japan
日本における肥満者（BMI 25以上）割合の推移
(1976-2006)
(BMI 25kg/m2以上の人の割合：%)
30
25
20
15
10
5
0
男性では30年間で2倍に
女性では全体として横ばい
男性
女性
1976
1981
1986
1991
1996
2001
2006
(年)
Yoshiike N, et al. Obes Rev. 2002の表より再構成
28


Prevalence of obesity in OECD countries
肥満者割合の国際比較
(BMI 30 kg/m2 以上の人の割合)
日本 3.5
韓国 4.1
スロバキア 16.9
フィンランド 20.2
チェコ 21.0
ルクセンブルク 22.5
アイルランド 23.0
カナダ 24.2
オーストラリア 24.6
チリ 25.1
イギリス 26.1
ニュージーランド 27.8
ハンガリー 28.5
メキシコ 30.0
アメリカ 35.9
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
(%)
OECD Health Data 2012
27

3集団における冠動脈石灰化 (CAC) および頸動脈内膜中膜厚 (IMT) (ERA-JUMP)

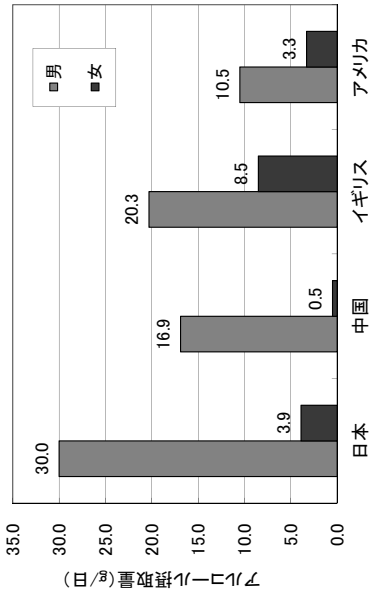


冠動脈石灰化はAgatstonスコア10以上の割合

Sekikawa et al. *J Am Coll Cardiol* 2008

29

Alcohol intake (g/day) in 4 countries
アルコール摂取量(g/日)の4ヶ国比較
(INTERMAP研究, 40–59歳男女, 1996–1999年)



Stamler J, et al. *J Hum Hypertens* 2003

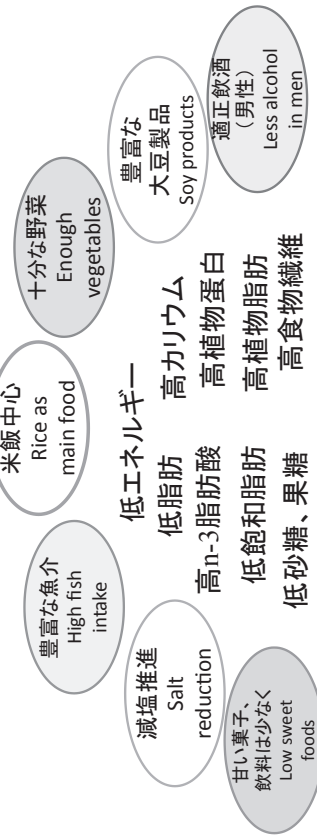
30

Good and bad sides of traditional Japanese diet
伝統的日本食パターンの光と影

- 好ましい面 **Good sides**
  - 低脂肪、低飽和脂肪、低エネルギー
  - 豊富な魚介類 → 高 n-3 脂肪酸
  - 豊富な大豆製品 → 植物蛋白、植物脂肪
  - → 低い血清コレステロール、低い肥満度
  - → 低い心筋梗塞リスク、低い糖尿病リスク
- 好ましくない面 **Bad sides**
  - 高食塩、不十分な野菜（低カリウム、低食物繊維）
  - 男性の多量飲酒
  - → 高血圧、高い脳卒中リスク

31

Establishment of "healthy Japanese dietary pattern"
「健康的日本食パターン」の確立



32

セッション２

先制医療としての栄養の最適化

セッション 2-1

糖脂質代謝のエピゲノム制御と先制医療

東京医科歯科大学大学院　医歯学総合研究科　分子内分泌代謝学分野
CREST, AMED
小川　佳宏

器官形成期に相当する胎児期は可塑性が最も高い時期であり、胎児期における急激な栄養環境の変化がエピゲノム記憶され、成人期における生活習慣病の発症に関与する可能性がある。特に、胎生期から乳児期にかけて肝臓の糖脂質代謝調節機能はダイナミックに変化するが、その分子機構には不明点が多い。我々は既に、離乳後のマウス肝臓において新規脂肪合成の律速酵素である glycerol-3-phosphate acyltransferase 1 (GPAT1) 遺伝子プロモーター領域の DNA 脱メチル化により遺伝子発現が亢進することを見出した (Diabetes 61:2442-2450, 2012)。最近では、出生後の新生仔マウスの肝臓では核内受容体 PPARα 依存的に DNA 脱メチル化に伴って脂肪酸β酸化経路を構成する酵素群の遺伝子発現が協調的に増加することを見出した。従来、新生仔マウスの肝臓では母乳中の脂肪酸がリガンドとして PPARα に結合して標的遺伝子の転写を活性化すると考えられている。授乳期にミルクに由来する脂肪酸が栄養シグナルとして PPARα を活性化し、DNA 脱メチル化により脂肪酸自体の代謝を活性化して効率良くエネルギーを得ることができる可能性が示唆された (Diabetes 64:775-784, 2015)。

本研究は、活性化された PPARα を介する DNA 脱メチル化により新生児期の肝臓の脂質代謝が制御されることを初めて証明し、代謝経路特異的あるいはライフステージ特異的な DNA 脱メチル化における脂質センサー核内受容体の重要性を示唆するものである。以上の成績は、人工乳や機能性食品により DNA メチル化の変化によりエピゲノム記憶される胎児期や新生児期の栄養環境に介入することにより、生活習慣病を含む成人期に発症する慢性疾患の発症前あるいは早期発症予防を目指す先制医療の概念に合致するものである。

(参考文献)

1. T. Ehara et al. Role of DNA methylation in the regulation of lipogenic gene expression in the neonatal mouse liver. Diabetes 61: 2442-2450, 2012.
2. T. Ehara et al. Ligand-activated PPARα-dependent DNA demethylation regulates the fatty acid β-oxidation genes in the postnatal liver. Diabetes 64: 775-784, 2015.

Session 2−1

Epigenetic Regulation of Glucose and Lipid Metabolism and Preemptive Medicine

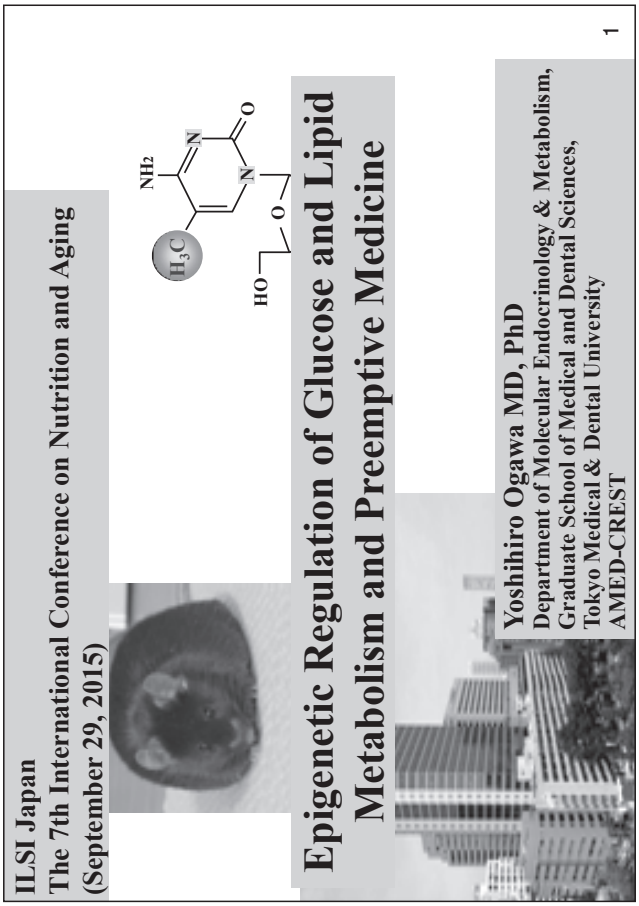
Yoshihiro Ogawa, M.D., Ph.D.
Department of Molecular Endocrinology and Metabolism
Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, CREST, AMED

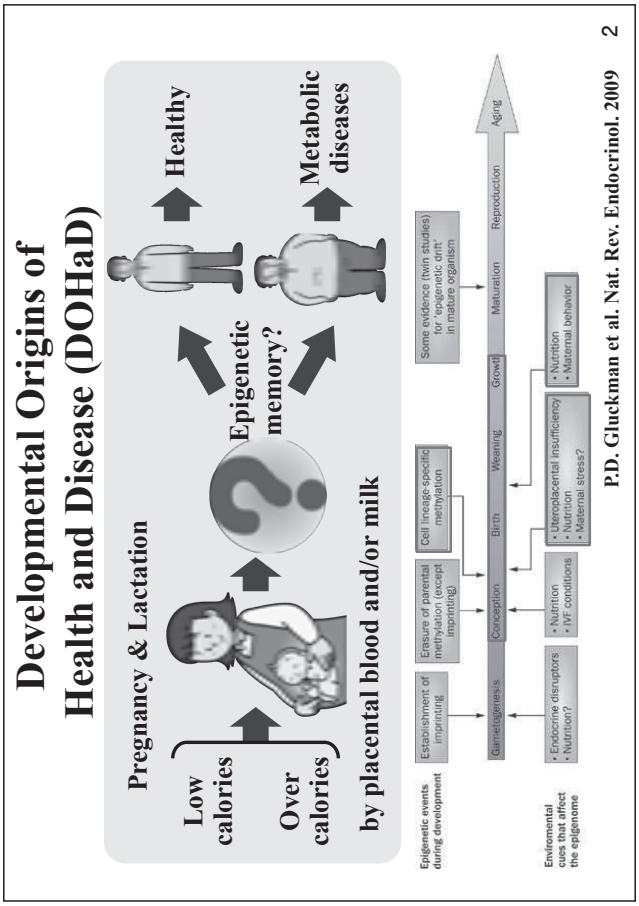
The liver is a major organ of lipid metabolism, which is markedly changed in response to physiological nutritional demand; however, the regulation of hepatic *de novo* lipogenic gene expression in early life is largely unknown. We previously demonstrated that expression of glycerol-3-phosphate acyltransferase 1 (GPAT1; *Gpam*), a rate-limiting enzyme of *de novo* lipogenesis, is regulated in the mouse liver via a mechanism involving DNA methylation, an epigenetic modification involved in the regulation of a diverse range of biological processes in mammals (**Diabetes** 61: 2442-2450, 2012). Recently, we have observed DNA demethylation with increased mRNA expression of the fatty acid β-oxidation genes in the postnatal mouse liver (**Diabetes** 64: 775-784, 2015). The DNA demethylation does not occur in the fetal mouse liver under the physiologic condition, suggesting that it is specific to the neonatal period. Analysis of mice deficient in the nuclear receptor PPARα and maternal administration of its synthetic ligand Wy14643 during the gestation and lactation periods reveal that the DNA demethylation is PPARα-dependent. Given that PPARα is known to be activated in the liver in response to milk-derived lipid ligands during the suckling period, it is tempting to speculate that milk lipids serve as a nutrient signal as well as nutrients during the neonatal period, so that they can be oxidized efficiently as an energy source.

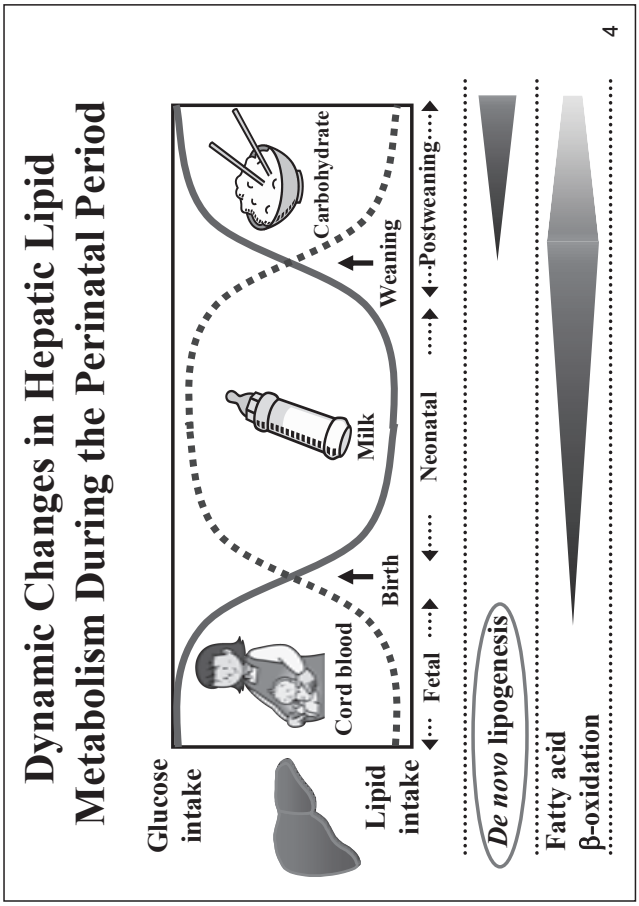
This study represents the first demonstration that the ligand-activated PPARα-dependent DNA demethylation regulates hepatic lipid metabolism during the neonatal period, thereby highlighting the role of a lipid-sensing nuclear receptor in the gene- and life stage-specific DNA demethylation of a particular metabolic pathway. Our data are consistent with the notion that the nutritional status in early life affects hepatic lipid metabolism in later life and thus provide clues to the "preemptive medicine" for adult-onset metabolic diseases in early life in the form of formula milk and functional food for both babies and mothers.

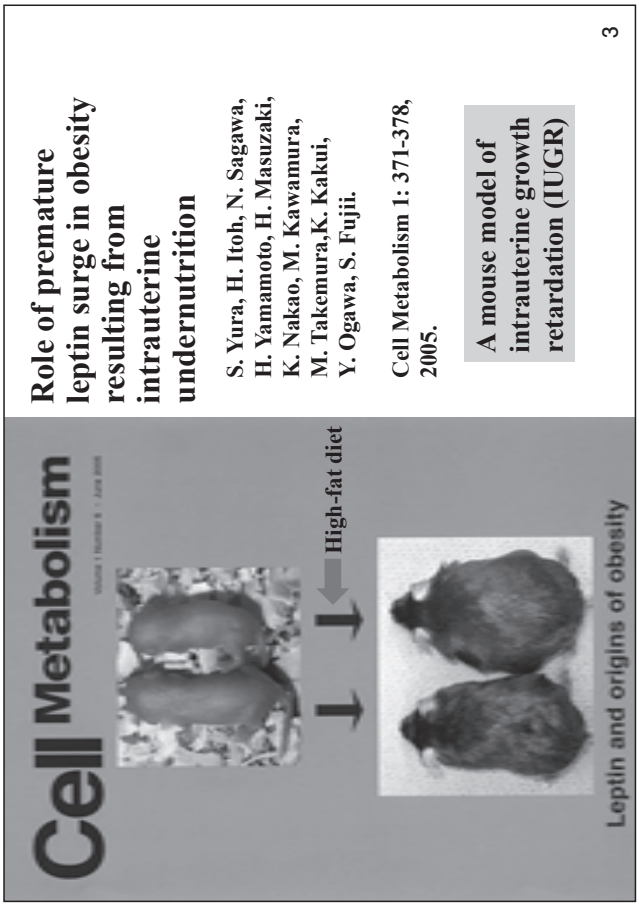
(References)

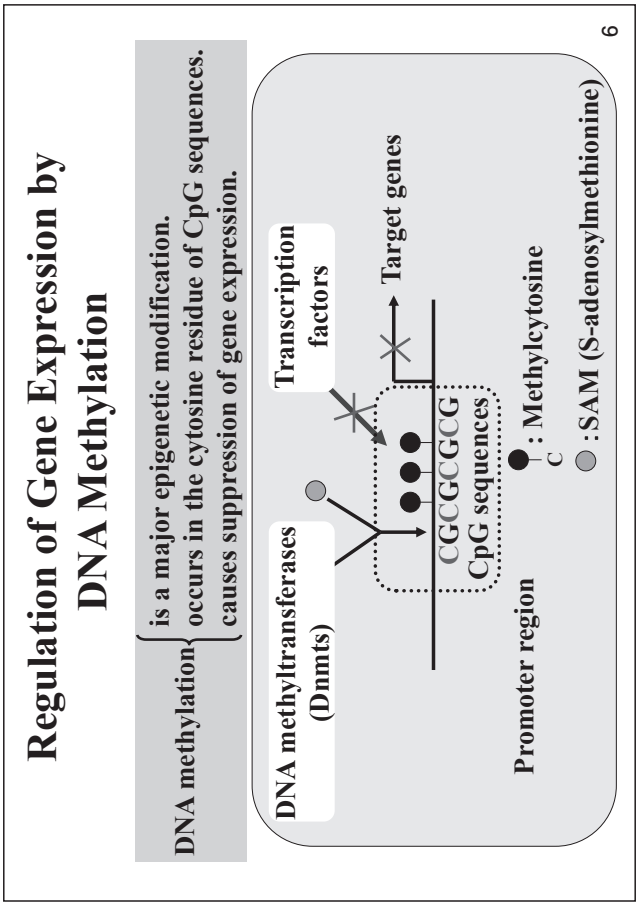
1. T. Ehara et al. Role of DNA methylation in the regulation of lipogenic gene expression in the neonatal mouse liver. **Diabetes** 61: 2442-2450, 2012.
2. T. Ehara et al. Ligand-activated PPARα-dependent DNA demethylation regulates the fatty acid β-oxidation genes in the postnatal liver. **Diabetes** 64: 775-784, 2015.

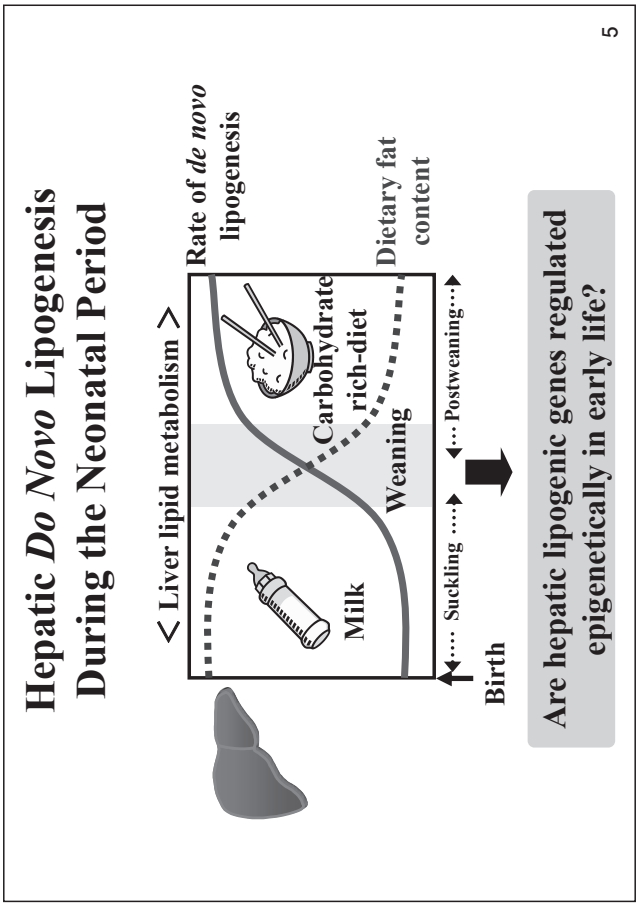

ILSI Japan
The 7th International Conference on Nutrition and Aging
(September 29, 2015)
Epigenetic Regulation of Glucose and Lipid Metabolism and Preemptive Medicine
Yoshihiro Ogawa MD, PhD
Department of Molecular Endocrinology & Metabolism,
Graduate School of Medical and Dental Sciences,
Tokyo Medical & Dental University
AMED-CREST
1

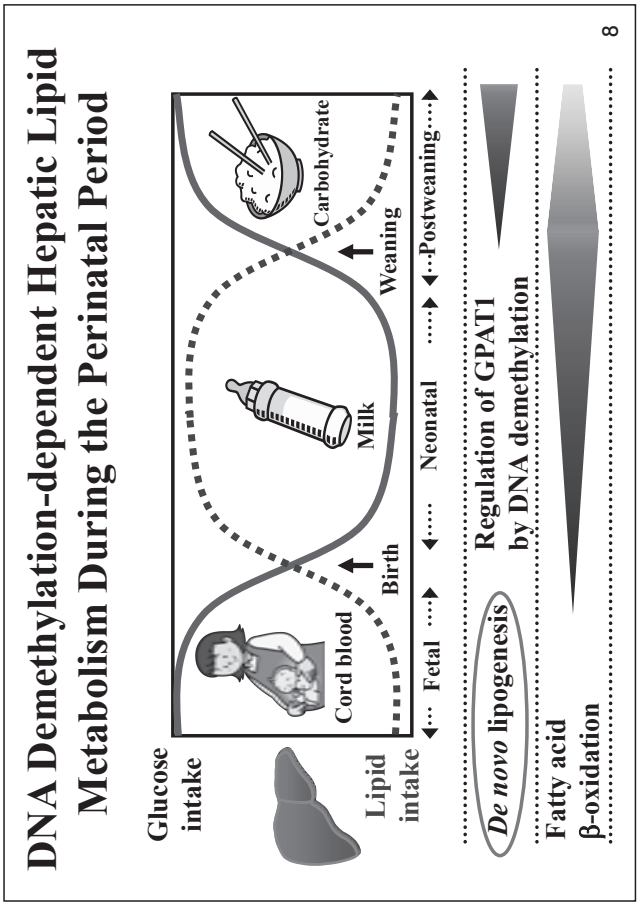

Developmental Origins of Health and Disease (DOHaD)
Pregnancy & Lactation
Low calories
Over calories
by placental blood and/or milk
Epigenetic memory?
Healthy
Metabolic diseases
Epigenetic events during development
Environmental cues that affect the epigenome
Establishment of imprinting
Erasure of parental methylation (except imprinting)
Cell lineage-specific methylation
Some evidence (twin studies) for "epigenetic drift" in mature organism
Gametogenesis
Conception
Birth
Weaning
Growth
Maturation
Reproduction
Aging
• Endocrine disruptors
• Nutrition?
• Nutrition
• IVF conditions
• Uteroplacental insufficiency
• Nutrition
• Maternal stress?
• Nutrition
• Maternal behavior
P.D. Gluckman et al. Nat. Rev. Endocrinol. 2009
2

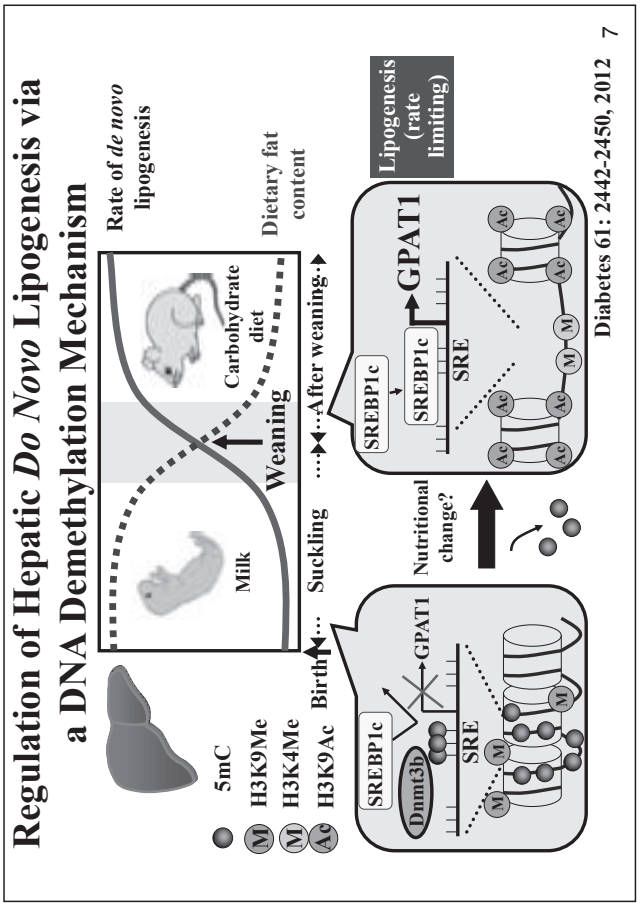

Cell Metabolism
Role of premature leptin surge in obesity resulting from intrauterine undernutrition
S. Yura, H. Itoh, N. Sagawa, H. Yamamoto, H. Masuzaki, K. Nakao, M. Kawamura, M. Takemura, K. Kakui, Y. Ogawa, S. Fujii.
Cell Metabolism 1: 371-378, 2005.
A mouse model of intrauterine growth retardation (IUGR)
High-fat diet
Leptin and origins of obesity
3


Dynamic Changes in Hepatic Lipid Metabolism During the Perinatal Period
Glucose intake
Lipid intake
Cord blood
Milk
Carbohydrate
Birth
Weaning
Fetal
Neonatal
Postweaning
De novo lipogenesis
Fatty acid β-oxidation
4


Regulation of Gene Expression by DNA Methylation
DNA methylation
is a major epigenetic modification.
occurs in the cytosine residue of CpG sequences.
causes suppression of gene expression.
DNA methyltransferases (Dnmts)
Transcription factors
Target genes
CGCGCGCG
CpG sequences
Promoter region
: Methylcytosine
C
:SAM (S-adenosylmethionine)
6

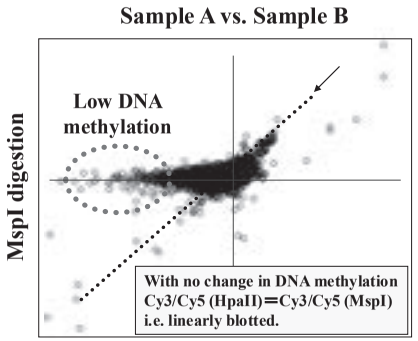

Hepatic Do Novo Lipogenesis During the Neonatal Period
< Liver lipid metabolism >
Rate of de novo lipogenesis
Milk
Carbohydrate rich-diet
Weaning
Dietary fat content
Suckling
Postweaning
Birth
Are hepatic lipogenic genes regulated epigenetically in early life?
5


DNA Demethylation-dependent Hepatic Lipid Metabolism During the Perinatal Period
Glucose intake
Lipid intake
Cord blood
Milk
Carbohydrate
Birth
Weaning
Fetal
Neonatal
Postweaning
De novo lipogenesis
Regulation of GPAT1 by DNA demethylation
Fatty acid β-oxidation
8


Regulation of Hepatic Do Novo Lipogenesis via a DNA Demethylation Mechanism
5mC
H3K9Me
H3K4Me
H3K9Ac
Rate of de novo lipogenesis
Milk
Carbohydrate diet
Weaning
Dietary fat content
Birth
Suckling
After weaning
SREBP1c
Dnmt3b
GPAT1
SRE
Nutritional change?
Lipogenesis (rate limiting)
Diabetes 61: 2442-2450, 2012
7

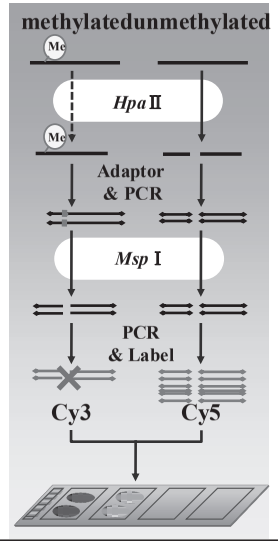
Genome-wide Analysis of DNA Methylation

(MIAMI: Microarray-based Integrated Analysis of Methylation by Isoschizomers)



Oncogene 25: 3059-3064, 2006.

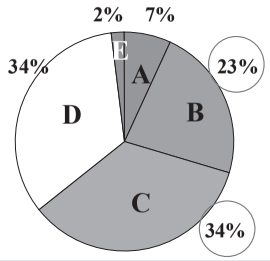
Hpa II ・・・methylation-sensitive
Msp I ・・・methylation-resistant



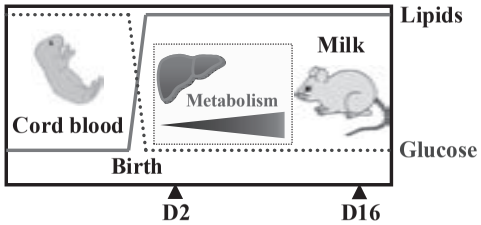
9

Genes with DNA Demethylation and Increased mRNA Expression (D2~D16)

GO analysis



A: Hematocyte function
B: Hematocyte function & Metabolism
C: Metabolism
D: Others
E: No GO annotation



Pathway analysis

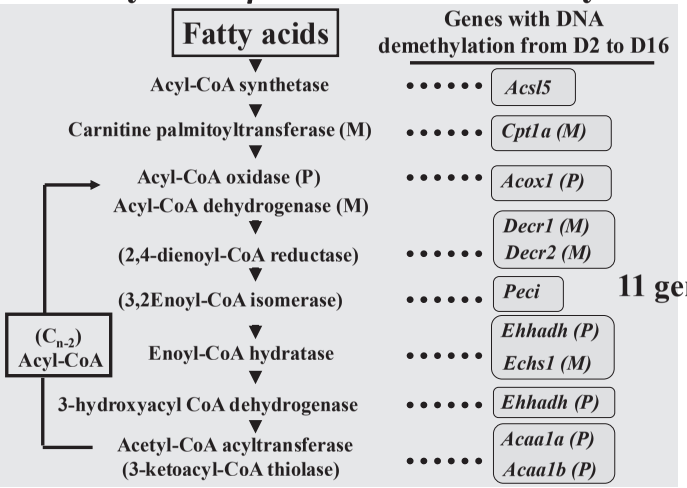
Pathways	*p* -value
PPAR signaling pathway	2.30E-06
Fatty acid metabolism	0.00020

Transcription factor binding motifs

Motifs	TFs	*p* -value
PPARG	PPAR (lipid metabolism)	8.20E-06
PPAR_DR1	PPAR	0.0015

10

Genes with DNA Demethylation in Fatty Acid β-oxidation Pathway

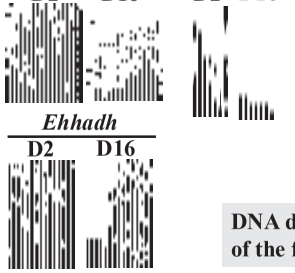


Red : known as PPARα target genes; Blue : induced by PPARα activation

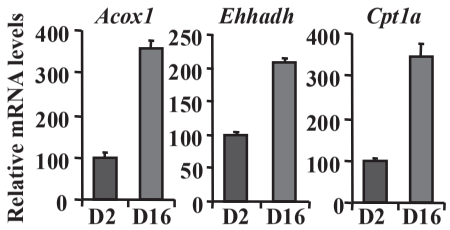
11

DNA Methylation and mRNA Expression of Fatty Acid β-oxidation Genes (D2~D16)

Bisulfide analysis



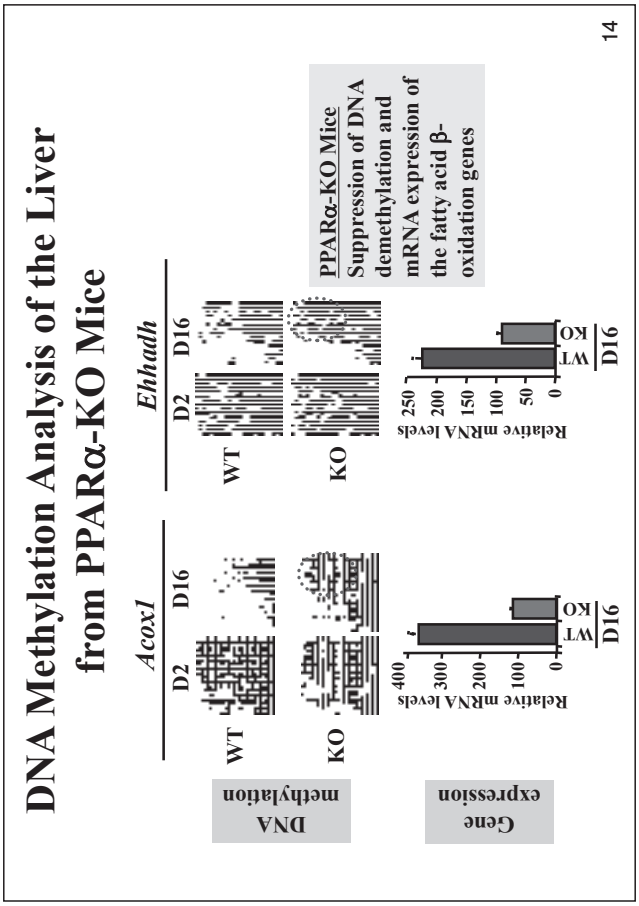
q-PCR analysis

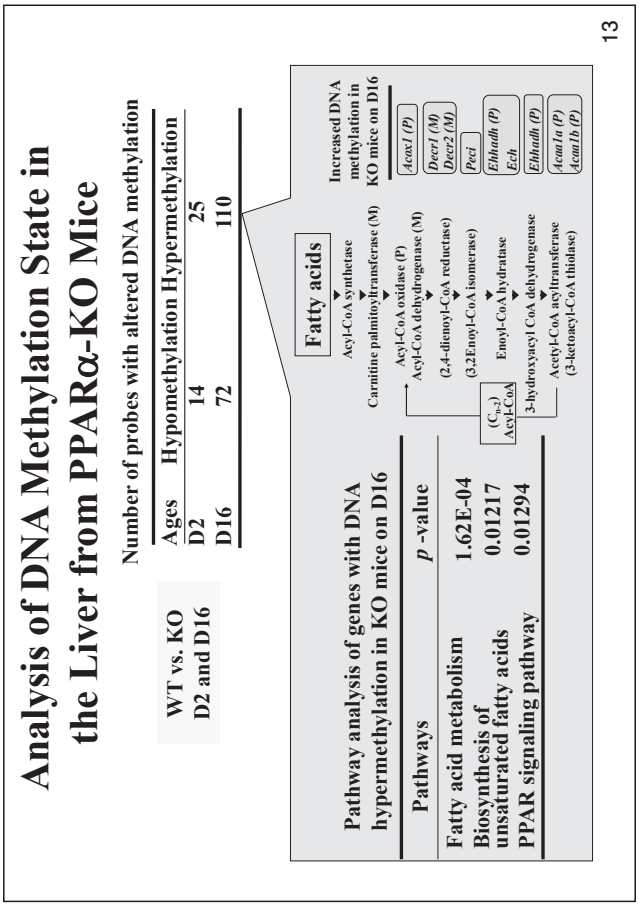


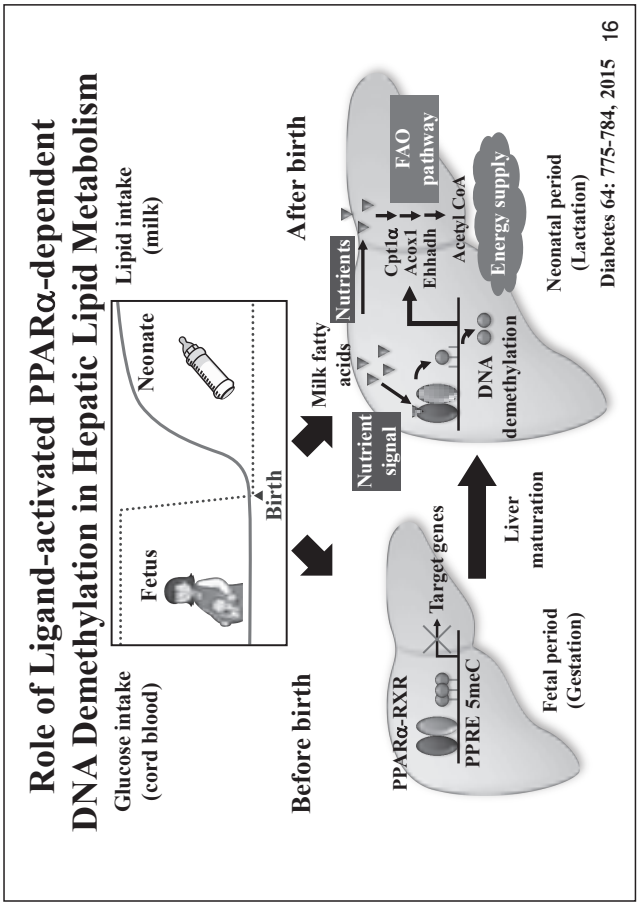
DNA demethylation and increased mRNA expression of the fatty acid β-oxidation genes from D2 to D16

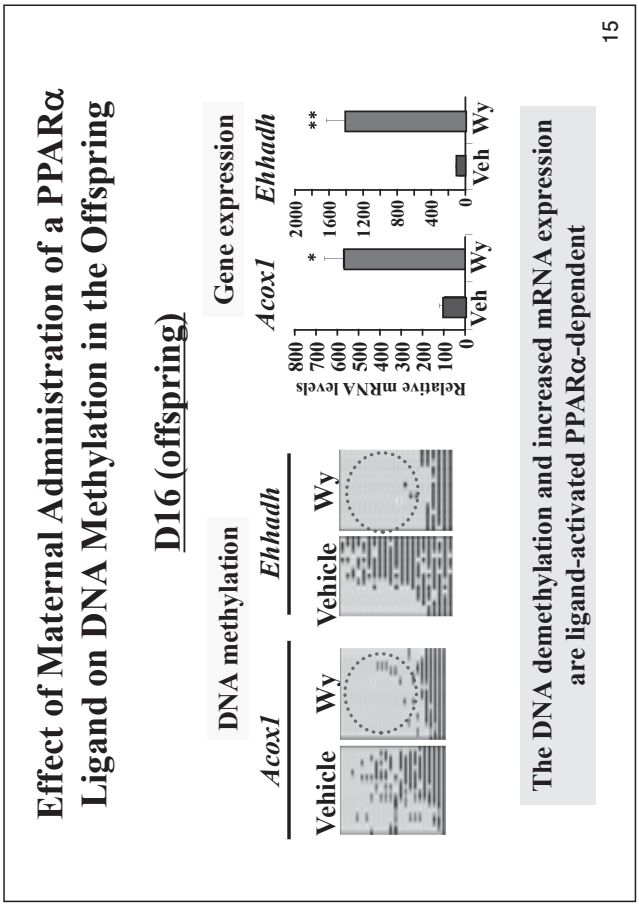
Is the DNA demethylation PPARα-dependent ?

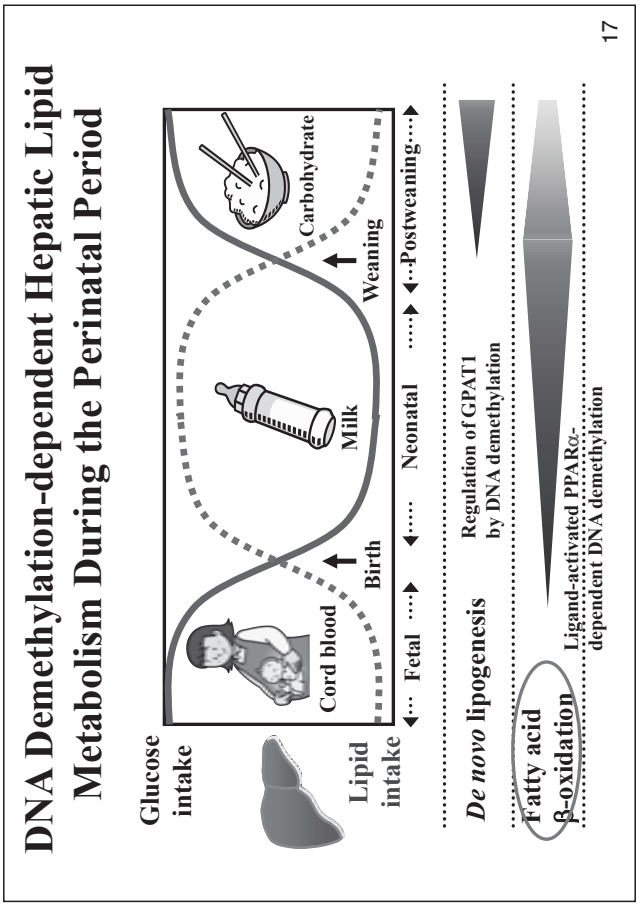
12

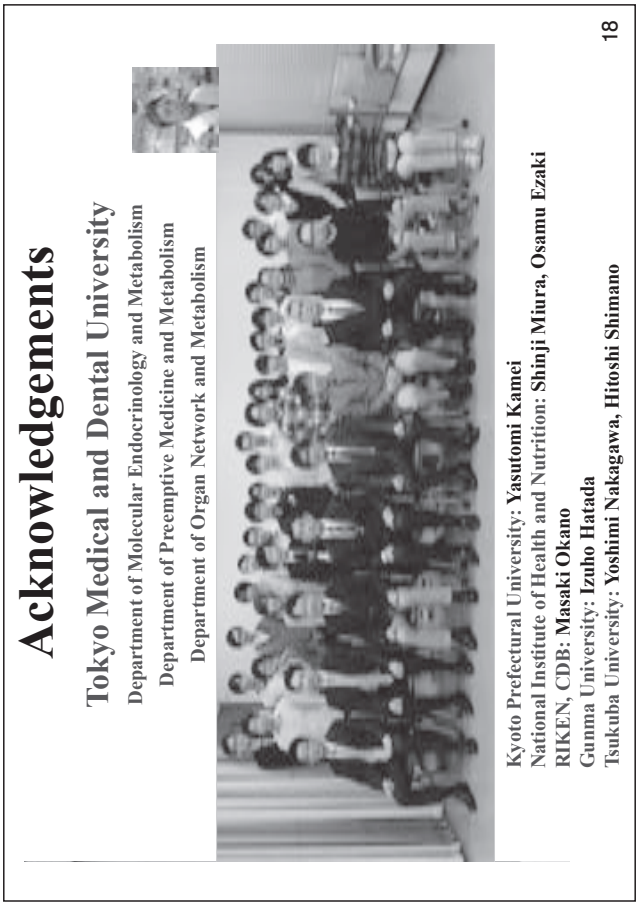

DNA Methylation Analysis of the Liver from PPARα-KO Mice
DNA methylation
Gene expression
Acox1
Ehhadh
D2
D16
WT
KO
Relative mRNA levels
PPARα-KO Mice
Suppression of DNA demethylation and mRNA expression of the fatty acid β-oxidation genes
14


Analysis of DNA Methylation State in the Liver from PPARα-KO Mice
Number of probes with altered DNA methylation
WT vs. KO
D2 and D16
Ages Hypomethylation Hypermethylation
D2 14 25
D16 72 110
Pathway analysis of genes with DNA hypermethylation in KO mice on D16
Pathways p -value
Fatty acid metabolism 1.62E-04
Biosynthesis of unsaturated fatty acids 0.01217
PPAR signaling pathway 0.01294
Fatty acids
Acyl-CoA synthetase
Carnitine palmitoyltransferase (M)
Acyl-CoA oxidase (P)
Acyl-CoA dehydrogenase (M)
(2,4-dienoyl-CoA reductase)
(3,2Enoyl-CoA isomerase)
Enoyl-CoA hydratase
3-hydroxyacyl CoA dehydrogenase
Acetyl-CoA acyltransferase
(3-ketoacyl-CoA thiolase)
Increased DNA methylation in KO mice on D16
Acox1 (P)
Decr1 (M)
Decr2 (M)
Peci
Ehhadh (P)
Ech
Ehhadh (P)
Acaa1a (P)
Acaa1b (P)
13


Role of Ligand-activated PPARα-dependent DNA Demethylation in Hepatic Lipid Metabolism
Glucose intake (cord blood)
Lipid intake (milk)
Fetus
Neonate
Birth
Before birth
After birth
Milk fatty acids
Nutrients
Nutrient signal
Cpt1α
Acox1
Ehhadh
FAO pathway
Acetyl CoA
Energy supply
DNA demethylation
PPARα-RXR
PPRE 5meC
Target genes
Liver maturation
Fetal period (Gestation)
Neonatal period (Lactation)
Diabetes 64: 775-784, 2015
16


Effect of Maternal Administration of a PPARα Ligand on DNA Methylation in the Offspring
D16 (offspring)
DNA methylation
Gene expression
Acox1
Ehhadh
Vehicle
Wy
Relative mRNA levels
Veh
The DNA demethylation and increased mRNA expression are ligand-activated PPARα-dependent
15


DNA Demethylation-dependent Hepatic Lipid Metabolism During the Perinatal Period
Glucose intake
Lipid intake
Cord blood
Milk
Carbohydrate
Birth
Weaning
Fetal
Neonatal
Postweaning
De novo lipogenesis
Regulation of GPAT1 by DNA demethylation
Fatty acid β-oxidation
Ligand-activated PPARα-dependent DNA demethylation
17


Acknowledgements
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Department of Preemptive Medicine and Metabolism
Department of Organ Network and Metabolism
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National Institute of Health and Nutrition: Shinji Miura, Osamu Ezaki
RIKEN, CDB: Masaki Okano
Gunma University: Izuho Hatada
Tsukuba University: Yoshimi Nakagawa, Hitoshi Shimano
18

セッション 2-2

肥満・メタボリックシンドローム予防のための人生早期の栄養状態

日本大学医学部小児科　客員教授
神奈川工科大学栄養生命科学科　教授
岡田　知雄

高度経済成長期以降、日本におけるこどもの肥満頻度は急速に上昇してきたが、その後、2005 年頃をピークに徐々に減少傾向に転じている。しかし、日本においては、肥満頻度と比較して、メタボリックシンドローム（MS）の頻度が高いことが指摘され、さらに、多くのこどもたちが既に高度肥満となり、治療に難渋しているのが現状である。こどもの肥満・MS 増加の背景には、生活環境の変化や、食の変貌など、社会的な要因が大きく関わっており、地域保健を含めて社会全体での対応が必要になる。

臨床の現場では、個々のこどもの肥満合併症を評価し、重症度を判定することが重要となる。積極的に医学的介入が必要なこどもの肥満状態の１つとして、2007 年小児期 MS が定義された。成人の場合と同様、内臓脂肪蓄積に高血圧、脂質異常、高血糖のうち２つ以上を有する状態で、将来、糖尿病、動脈硬化性疾患へと進展する可能性が高い。小児期 MS の診断基準の特徴は、内臓脂肪蓄積の評価に、腹囲のみでなく、腹囲身長比を用いることである。これは、個々の成長のばらつきが大きい学童では、腹囲よりも腹囲身長比の方が心血管・代謝危険因子をより正確に予測できるからである。実際に、腹囲身長比が大きい学童や MS では、頸部エコー検査など生理学的検査によって、すでに早期動脈硬化の兆候が確認されている。また、黒色表皮症は、インスリン抵抗性を合併していることが多く、臨床の現場で重要な所見である。さらに、小児期 MS では、耐糖能異常や動脈硬化の進展に関わる、組織内あるいは血液中の脂肪酸組成変化がすでに認められている。内臓脂肪蓄積を伴うこどもでは Stearoyl-CoA desaturase（SCD）を介する de nove lipogenesis が亢進してパルミトレイン酸が増加し、また、n-3 系多価不飽和脂肪酸の欠乏は SCD の活性化に関与している。一方、Delta-6 & 5 desarurase（D6D, D5D）を介する n-6 系多価不飽和脂肪酸代謝系に生じた変化は、アラキドン酸代謝系にも影響を与え炎症反応の亢進に関わっている。

また、近年、胎児発育環境が MS の起源の１つであることが示され、わが国においても出生体重と糖尿病発症には U 字型の関連性が認められている。高出生体重児だけでなく、低出生体重児も将来肥満・生活習慣病発症のリスクが高い。そして、低出生体重児は乳児期早期より特徴的な身体発育を示す。早産や子宮内低栄養による低出生体重児は、適正体重の満期産児と比較すると出生時の皮下脂肪蓄積が少ない。しかし、出生後急速に皮下脂肪を蓄積し、体重がキャッチアップする前に皮下脂肪はキャッチアップし、高体脂肪率を呈する。この皮下脂肪蓄積に、出生後のリポ蛋白リパーゼやインスリン様成長因子の著明な上昇が関与することが明らかになった。

胎児期の栄養状態は、出生時の身体所見や臍帯血所見から想像することができる。胎児の発育には多くの脂質が必要であり、特に、n-3 系多価不飽和脂肪酸は中枢神経系の発達に関与する。臍帯血では母体血よりも D6D 活性が 4 倍ほど高いという特徴があり、胎児自身の長鎖多価不飽和脂肪酸産生が亢進し、高需要に適応している。しかし、低出生体重児では D6D 活性亢進が減弱することが明らかになった。

小児期からの生活習慣病予防は、3 つのステージに分けて考えることができる。1 つ目が、胎児期のエピジェネティックな変化が生じるステージ、2 つ目は新生児期早期から幼児期の脂肪蓄積が進行するステージ、そして 3 つ目が肥満から様々な合併症へと進展するステージである。個々のこどもの背景因子は極めて多様である。将来の肥満、糖尿病、動脈硬化性疾患の発症予防のために、適正な栄養管理が確立されることを期待する。

Session 2−2

Nutritional State of Early in Life for Prevention of Obesity and Metabolic Syndrome

Tomoo Okada
Proffesser, Department of Pediatrics and Child Health, Nihon University School of Medicine

In Japan, cross-sectional analysis showed that the prevalence of obesity has gradually decreased since the early 2000s. However the prevalence of the metabolic syndrome (MS) is not lower in preteen Japanese overweight children in comparison with overweight adolescents in United States, and now many children have developed severe morbid obesity.

In 2007, the diagnostic criteria of MS in Japanese children was established. In this criteria, as an indispensable component, two markers of visceral fat accumulation, waist circumference and the waist to height ratio (WHR), were employed. Because the WHR is a better predictor of cardiovascular risks in children with height gain. Children with MS have higher risks to develop type 2 diabetes and atherosclerotic cardiovascular diseases, and early atherosclerotic changes are already detected in school-aged children with abdominal obesity by ultrasonographic examinations. In addition, acanthosis nigricans is a useful clinical marker for insulin resistance in obese children. Furthermore, children with MS exhibit the alteration of organ and plasma phospholipid composition, which affects a number of important physiological functions relating to the development of MS. The activity of stearoyl-CoA desaturase (SCD) and palmitoleic acid content, which are the biomarkers for de nove lipogenesis, are higher in children with abdominal obesity. And obese children with MS have lower DHA, which may suppress SCD and then protect against the development of obesity. Moreover, the n-6 polyunsaturated fatty acid metabolic pathway via delta-6 & 5 desaturases is demonstrated to be associated with cardiometabolic risks, insulin resistance and increased inflammation in children with abdominal obesity.

The concept of the developmental origins of health and disease (DOHaD) is based on studies by Barker and colleagues. They proposed a hypothesis that undernutrition in utero permanently changes the body's structure, function and metabolism in ways that lead to atherosclerosis and insulin resistance in later life. In addition, profound effects on the extent of body fatness and insulin sensitivity are demonstrated if there is a "mismatch" between prenatal and postnatal environments. Recently, we investigated subcutaneous fat accumulation in small for gestational age infants and found that a rapid catch-up in skinfold thickness developed prior to the body weight catch-up. Furthermore, insulin-like growth factor-I and lipoprotein lipase mass concentrations also demonstrate rapid increase during the neonatal period with fat accumulation. Investigating the precise mechanisms of DOHaD including mediating metabolic and hormonal factors may provide a new approach to prevent atherosclerosis and insulin resistance.

Better managements of undernutrition during gestation and neonatal growth during the early postnatal period, as well as lifestyle interventions aimed at changing diet, physical activity and daily life pattern during childhood, are important theme for future health.

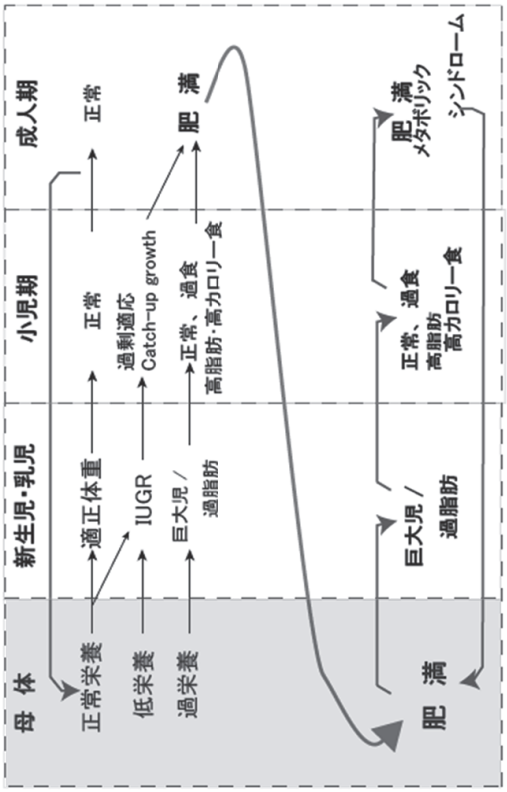
第７回「栄養とエイジング」国際会議

肥満・メタボリックシンドローム予防のための人生早期の栄養状態

Nutritional state during early life for the prevention of obesity and metabolic syndrome

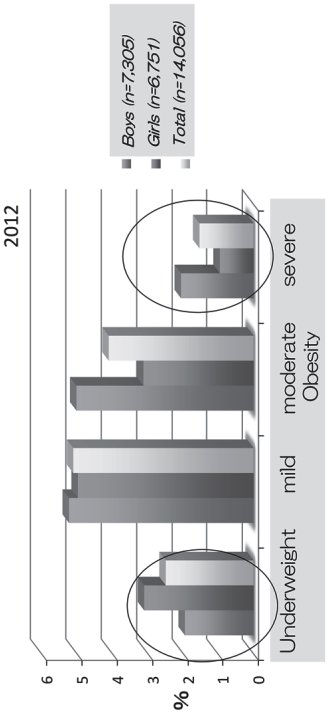
日本大学医学部小児科客員教授
神奈川工科大学栄養生命科学科特任教授
岡田知雄
Tomoo Okada

1



2

Prevalence of Obesity in Junior High School Children
足立区中学生(37校)におけるやせ・肥満の出現率



	n	prevalence
Underweight girls	351	3.11%
Severe obesity (total)	285	1.55%
Obesity (total)	1,514	10.77%

3

Metabolic Syndrome in Children

Diagnostic criteria of Metabolic Syndrome in Japan

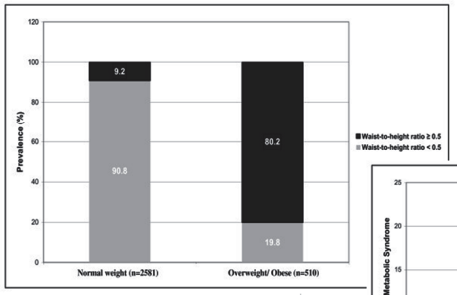
	Adults	Children
1) Abdominal circumference	≧ 85 cm (Male) ≧ 90 cm (Female)	≧ 80 cm (Boys and Girls) *: waist/height ratio ≧ 0.5
2) Serum lipids	TG≧ 150 mg/dl And/or HDLC < 40 mg/dl	TG≧ 120 mg/dl And/or HDLC < 40 mg/dl
3) Blood pressure	SBP ≧ 130 mmHg And/or DBP ≧ 85 mmHg	SBP ≧ 125 mmHg And/or DBP≧ 70 mmHg
4) Fasting glucose	≧ 110 mg/dl	≧ 100 mg/dl

4

Utility of waist-to-height ratio in assessing the status of central obesity and related cardiometabolic risk profile among normal weight and overweight/obese children: The Bogalusa Heart Study

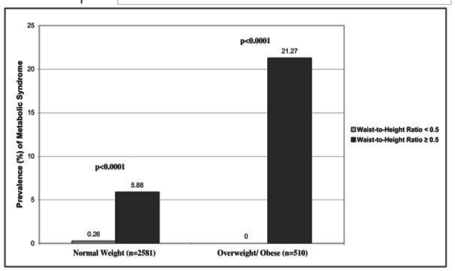
Mokha JS et al. BMC Pediatr. 2010; 10: 73.

WHtR not only detects central obesity and related adverse cardiometabolic risk among normal weight children, but also identifies those without such conditions among the overweight/obese children, which has implications for pediatric primary care practice.



Prevalence of Status of Central Obesity (WHtR <0.5 vs. ≥ 0.5) among Normal Weight and Overweight/Obese Children.

Prevalence of Metabolic Syndrome in Normal Weight and Overweight/Obese Children According to WHtR

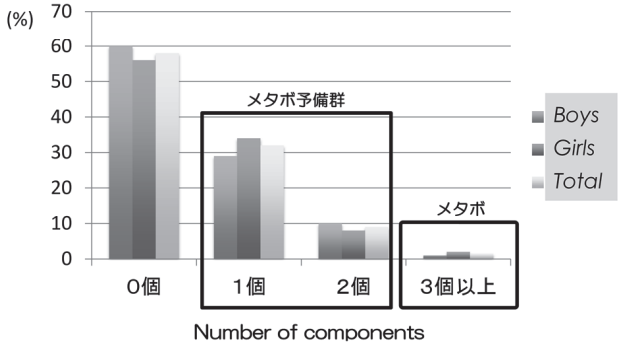


5

Prevalence of MetS in School Children

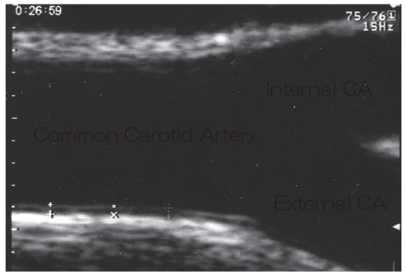
原光彦　「子供のメタボが危ない！」

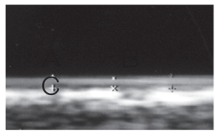
Subjects：School children (n=217)
Components of MetS（厚生労働省研究班2006）
Abdominal circ. ＞80cm (junior high), ＞75cm (elementary) or waist/height ＞0.5
TG ＞120mg/dL and/or HDLC ＜40mg/dL
SBP ＞125mmHg and/or DBP ＞70mmHg
FBS ＞100mg/dL

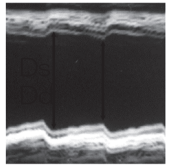


6

Ultrasonographic Examinations of Carotid Artery







Intima-media thickness (IMT)

血管の歪み度 (Strain)
= (Ds − Dd) / Dd

血管の硬さ (Elastic Property)
= (SBP − DBP) / Strain

Stiffness β
= ln (SBP / DBP) / Strain

＊SBP = 上腕で測定した収縮期血圧
DBP = 上腕で測定した拡張期血圧

＊1mmHg = 1333 dyne / cm^2 = 133.3 Pa

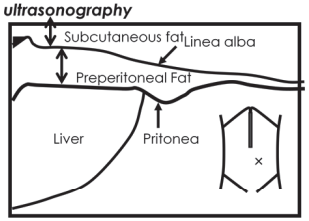
7

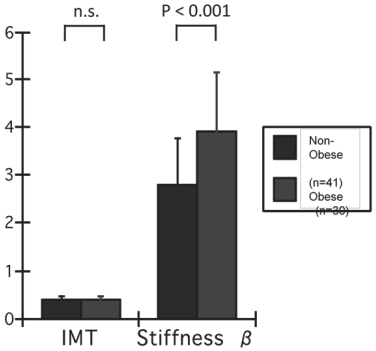
Subclinical Atherosclerosis in Obese Children

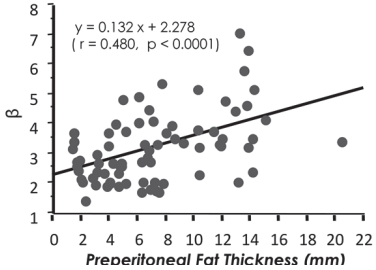
原　光彦　他：肥満研究　Vol.12(1), 2006.

Subjects:
30 obese children
Mean age; 10.7 years
Mean percentage of overweight; 52.6%

Abdominal ultrasonography







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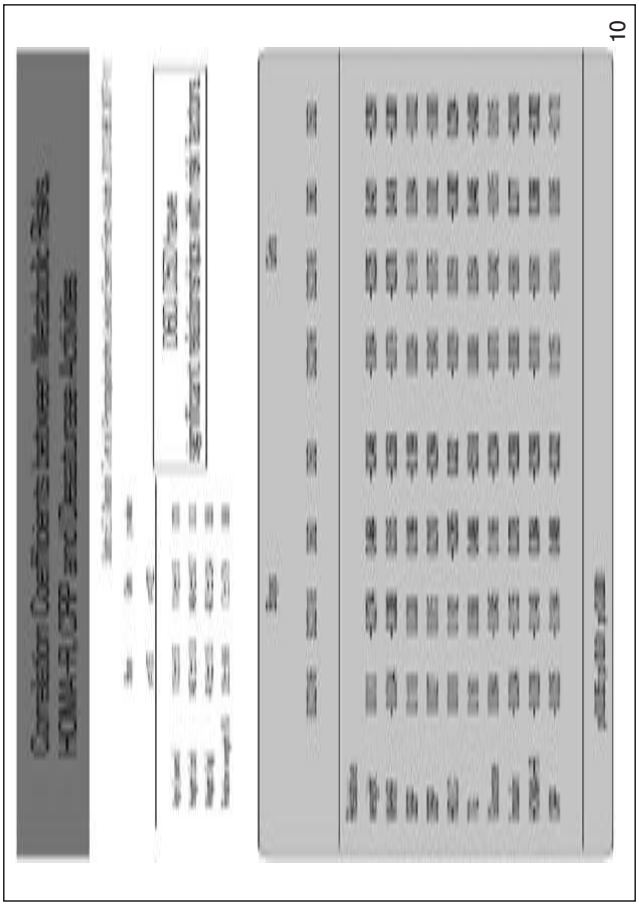
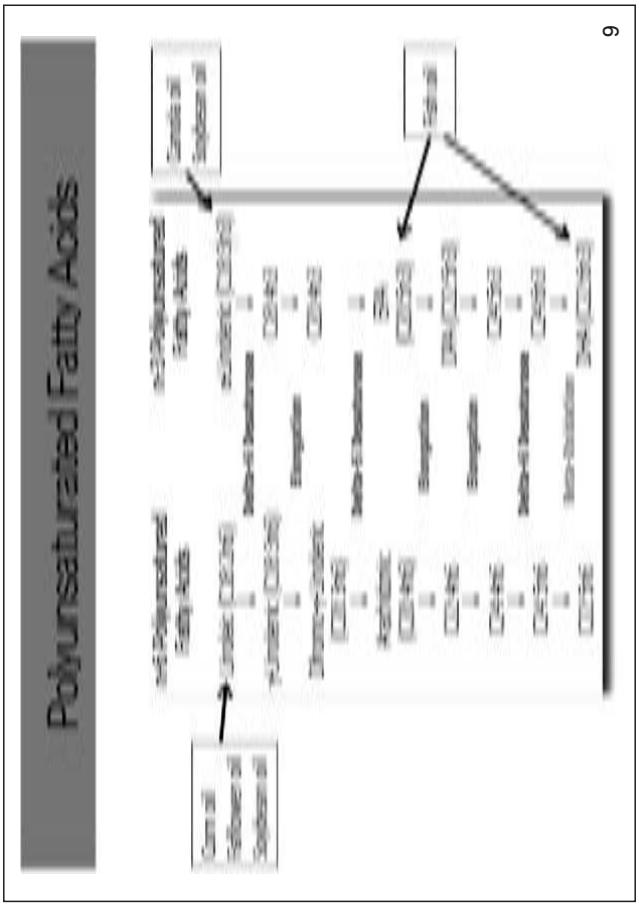
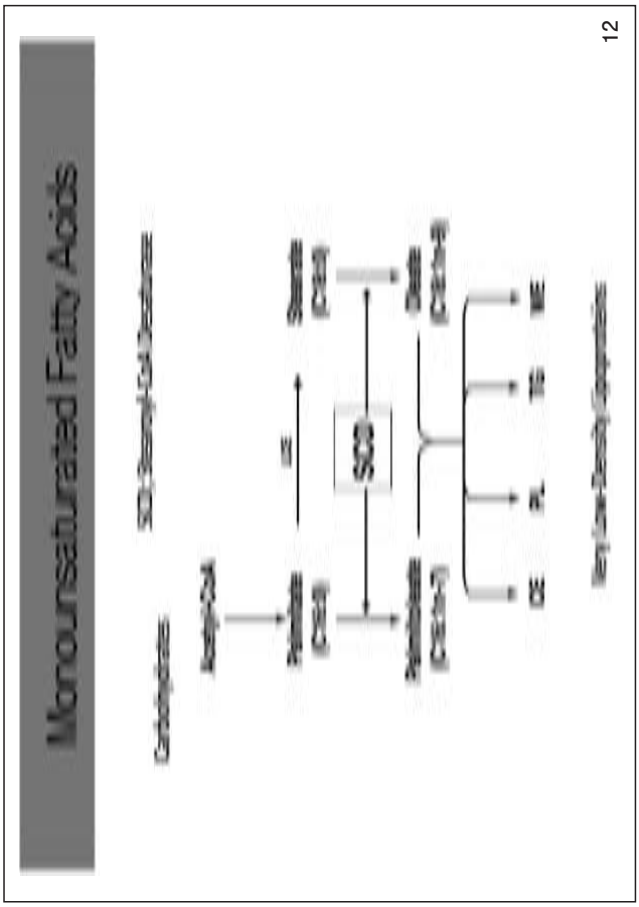
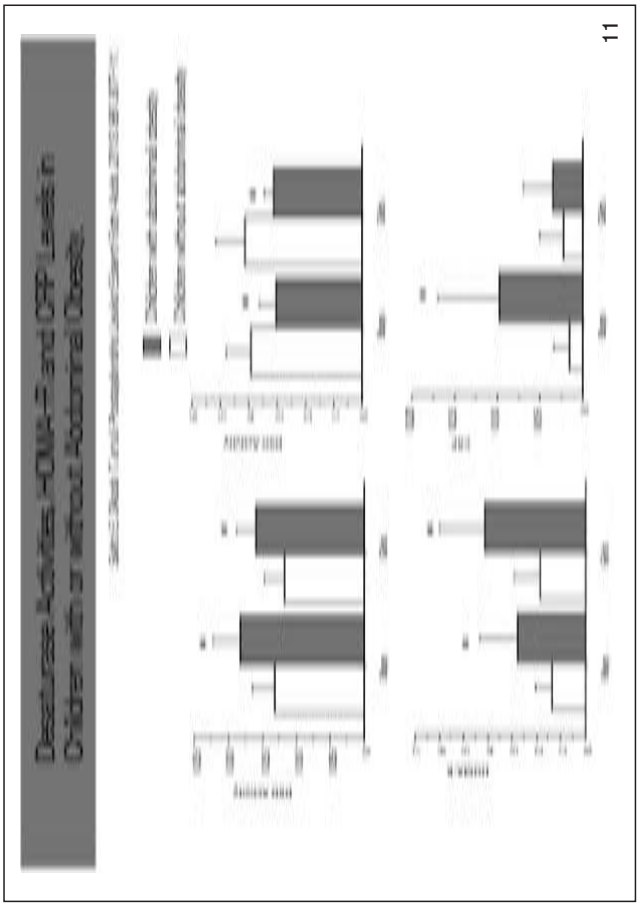
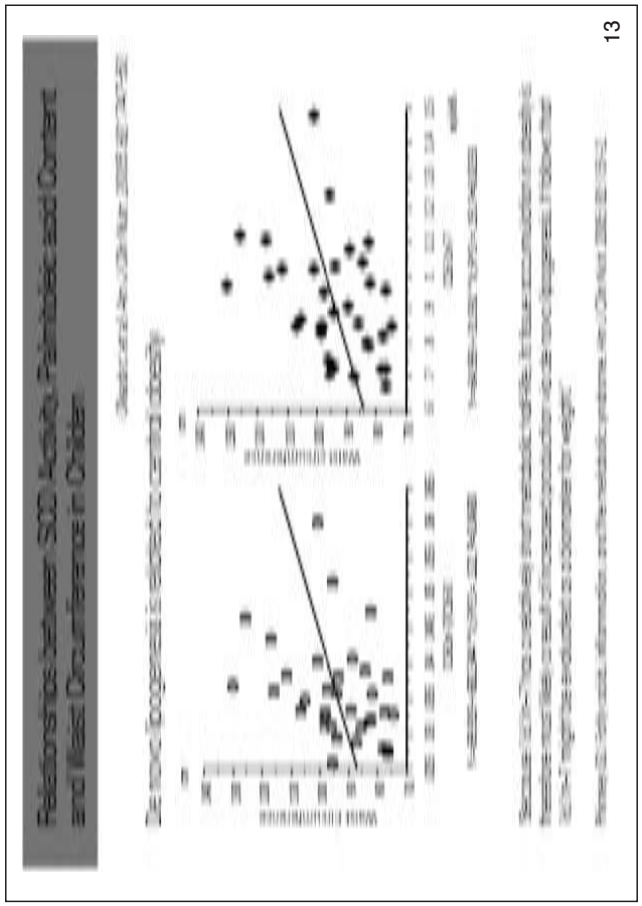
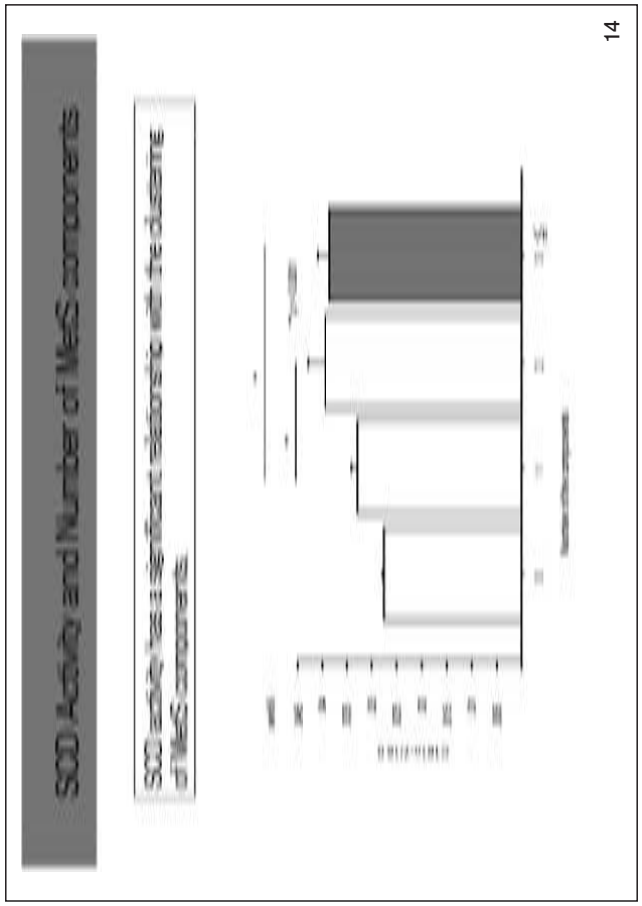

Correlation Coefficients between Metabolic Risks, HOMA-R, CRP and Desaturase Activities
10

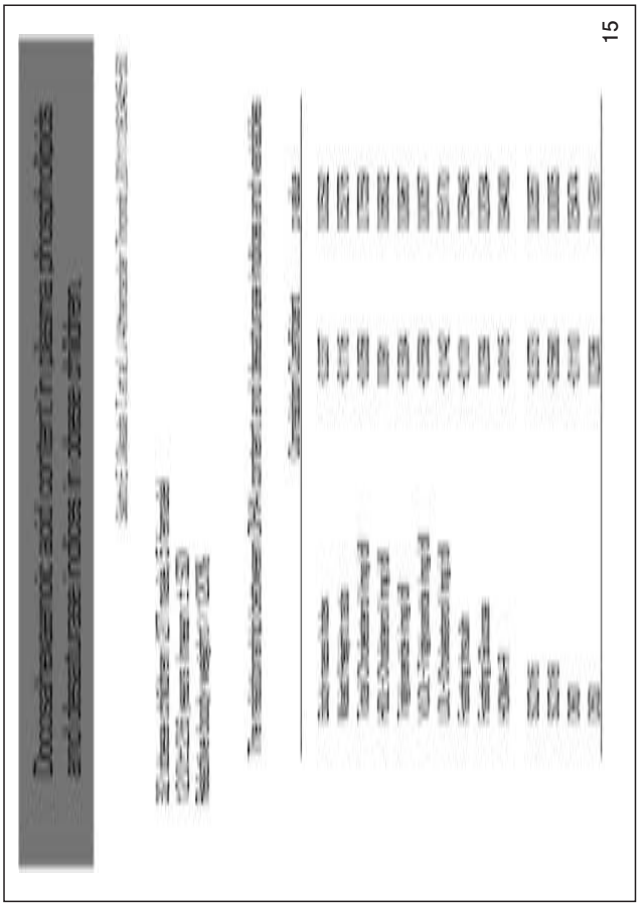

Polyunsaturated Fatty Acids
9

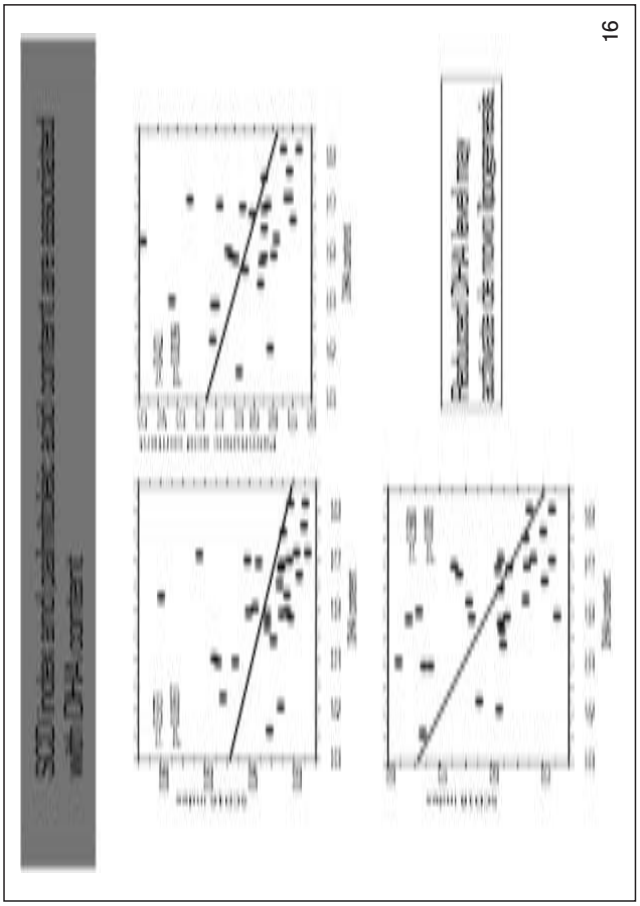

Monounsaturated Fatty Acids
12

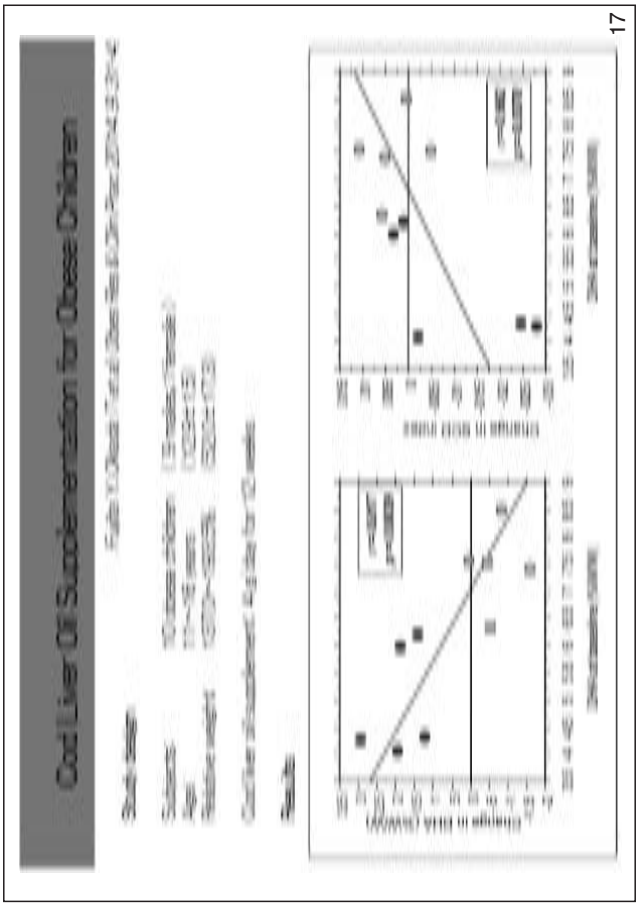

Desaturase Activities, HOMA-R and CRP Levels in Children with or without Abdominal Obesity
11

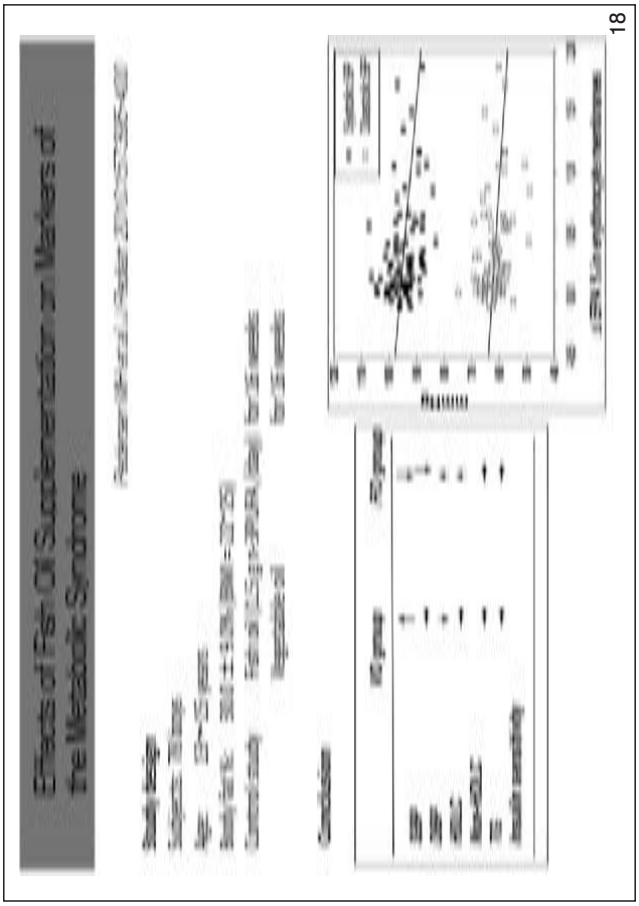

Relationships between SOD Activity, Palmitoleic acid Content and Waist Circumference in Children
13


SOD Activity and Number of MetS components
14

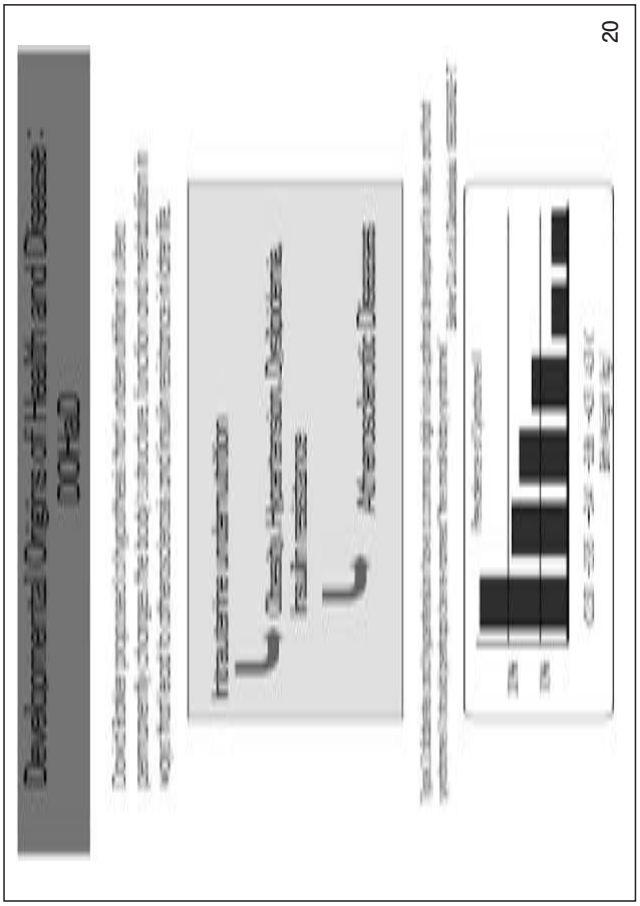

Docosahexaenoic acid content in plasma phospholipids and blood lipid indices in obese children
15

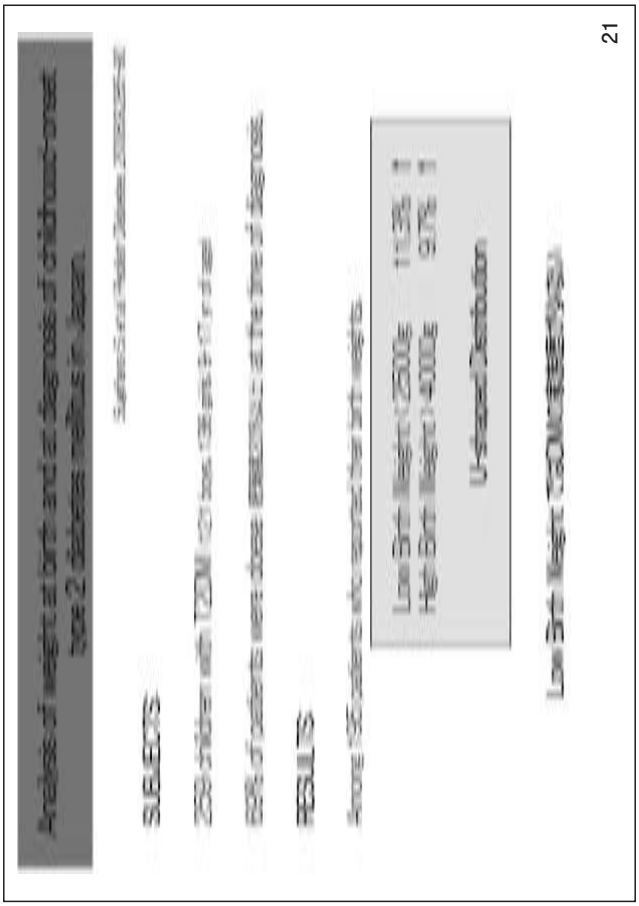

SOD Index and palmitoleic acid content are associated with DHA content
16

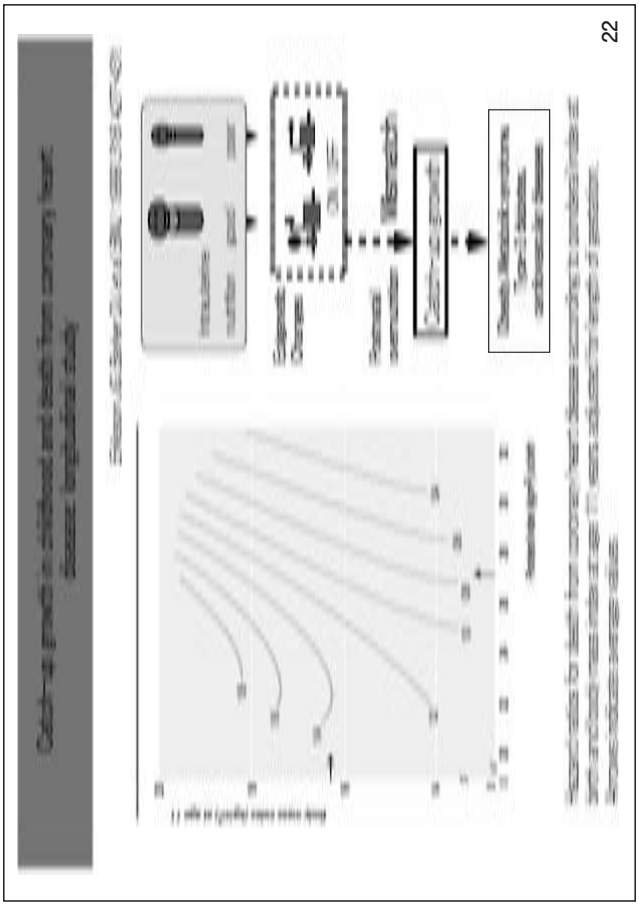

Cod Liver Oil Supplementation for Obese Children
17


Effects of Fish Oil Supplementation on Markers of the Metabolic Syndrome
18

Mechanisms for the anti-obesity effect of DHA n-3 PUFAs
19


Developmental Origins of Health and Disease : DOHaD
20


Analysis of weight at birth and at diagnosis of childhood-onset type 2 diabetes mellitus in Japan
SUBJECTS
RESULTS
Underweight Distribution
Low Birth Weight (<2500g)
Heavy Birth Weight (>4000g)
21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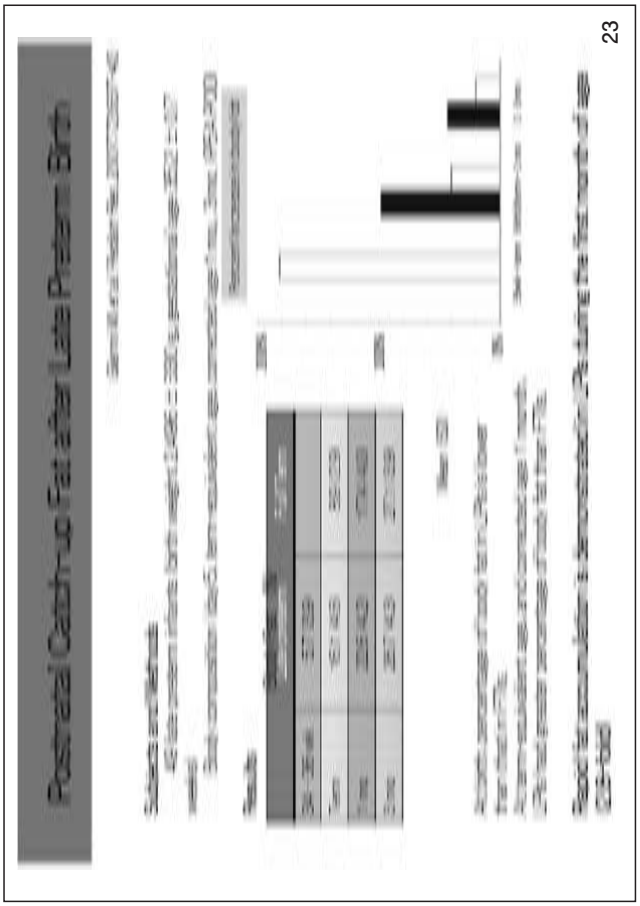
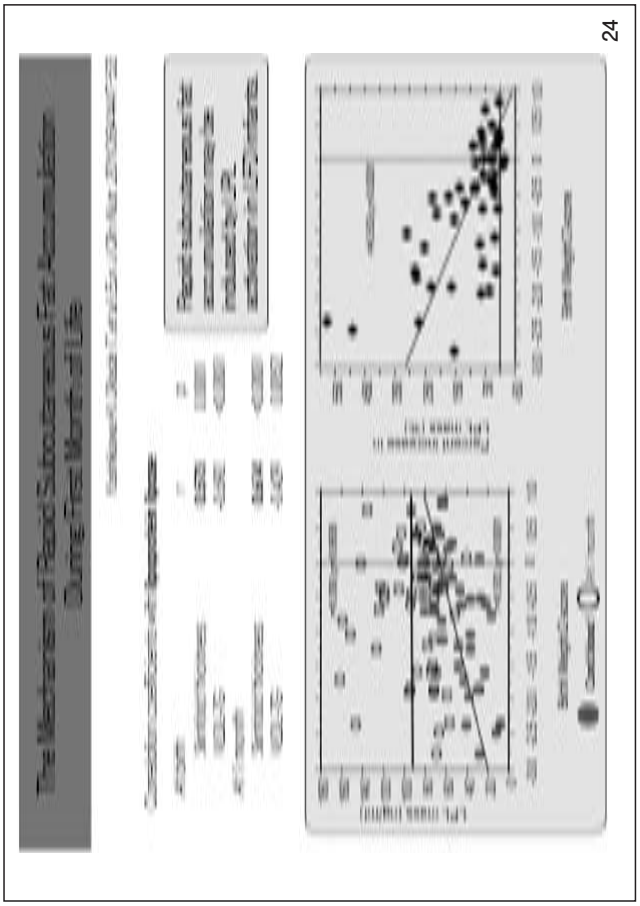
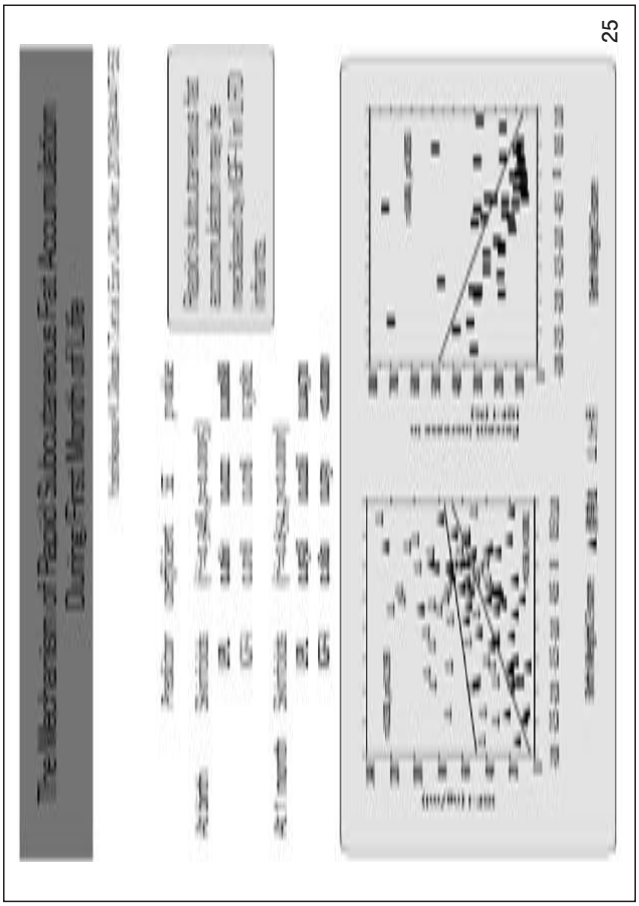
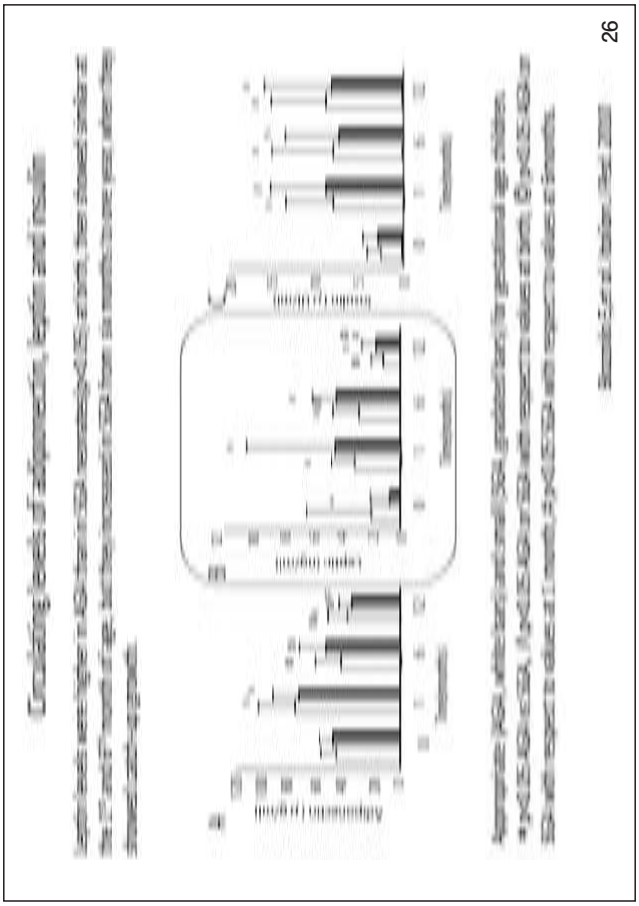
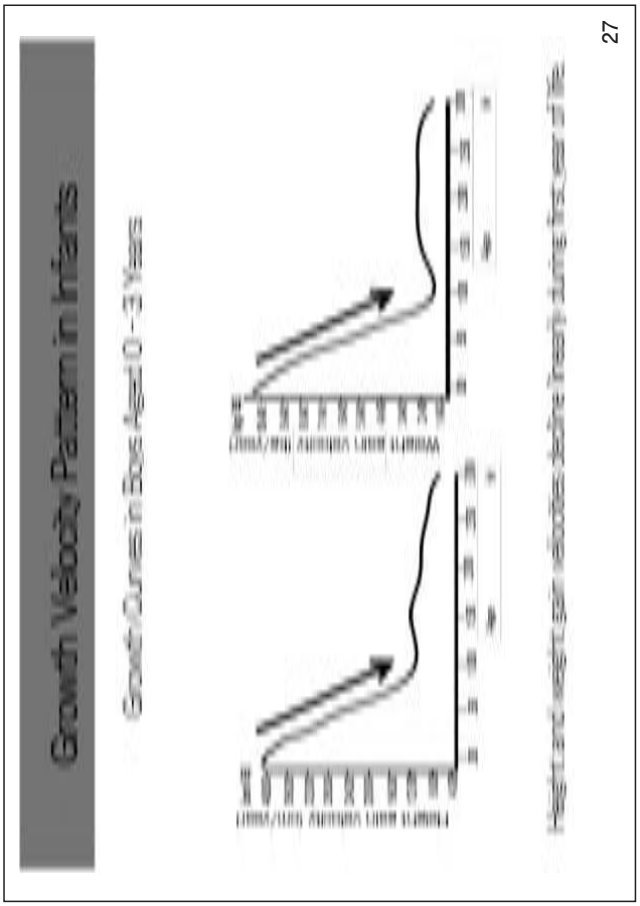
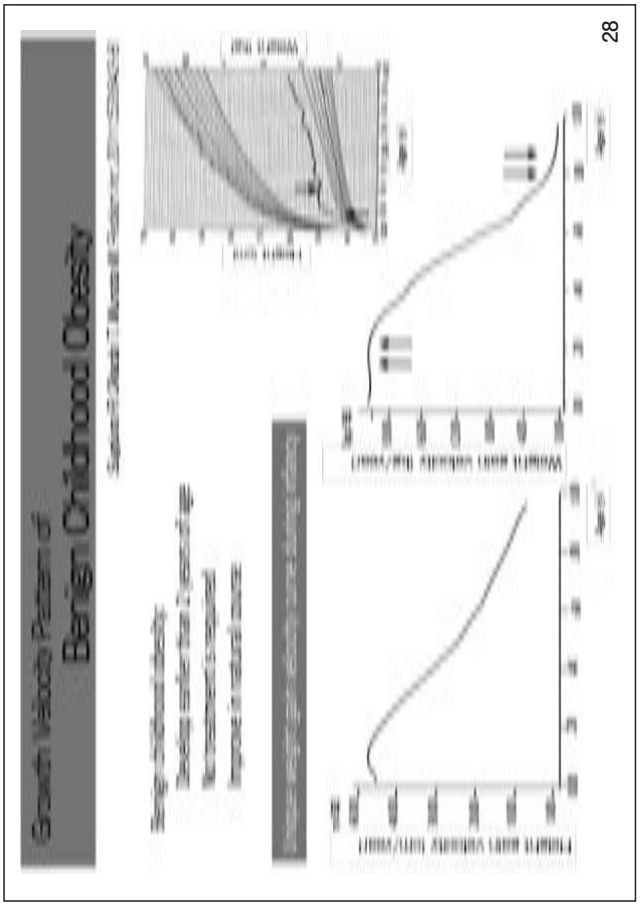

Postnatal Catch-up Fat after Late Preterm Birth
23


The Mechanism of Rapid Shortening of Fat Accumulation During First Month of Life
24


The Mechanism of Rapid Subcutaneous Fat Accumulation During First Month of Life
25


Crossing Effect of Adiposity Index and Height
26


Growth Velocity Pattern in Infants
Growth Curves in Boys Aged 0 – 3 Years
27


Growth Velocity Pattern of
Benign Childhood Obesity
28

Neonatal Intrahepatocellular Lipid

METHODS

RESULTS

CONCLUSIONS
Preterm infants have an altered body composition with significantly greater HCL

29

Aberrant Adiposity and Ectopic Lipid Deposition Characterize the Adult Phenotype of the Preterm Infant

METHODS

RESULTS

30

Childhood Obesity accompanied with LBW

低出生体重児の肥満

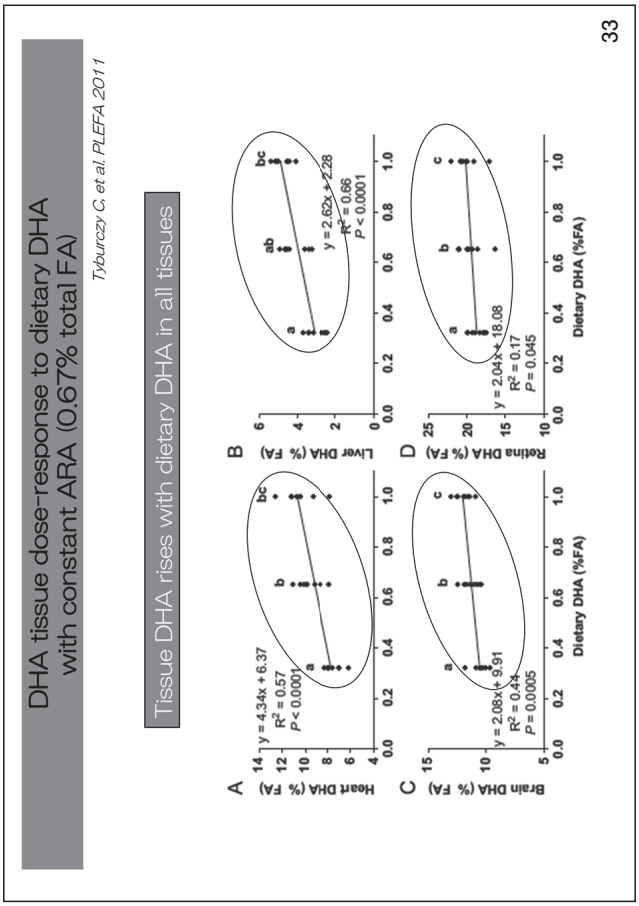
氏名	性	年齢 Y	身長m、体重kg、肥満度%	腹囲 cm	出生体重 g、在胎 (週数)	母体、妊娠合併症	肥満発症時期	その他
T.K	F	9	132, 51.4, 74.5%	102 cm	2330g (41w)	妊娠高血圧症候群、胎盤機能不全	5y	生活習慣・良、治療抵抗性、SFD
T.N	M	12	148、44.8, 10.7%	103 cm	1024g (25w)	膠原病、妊娠高血圧症候群、抗リン脂質抗体	小学生 5年11y	腹部肥満
K.S	M	12	151.5, 54.6, 27%	101 cm	2335g (41w)	特になし	小学4年10y	生活習慣・良、腹部肥満,LFD

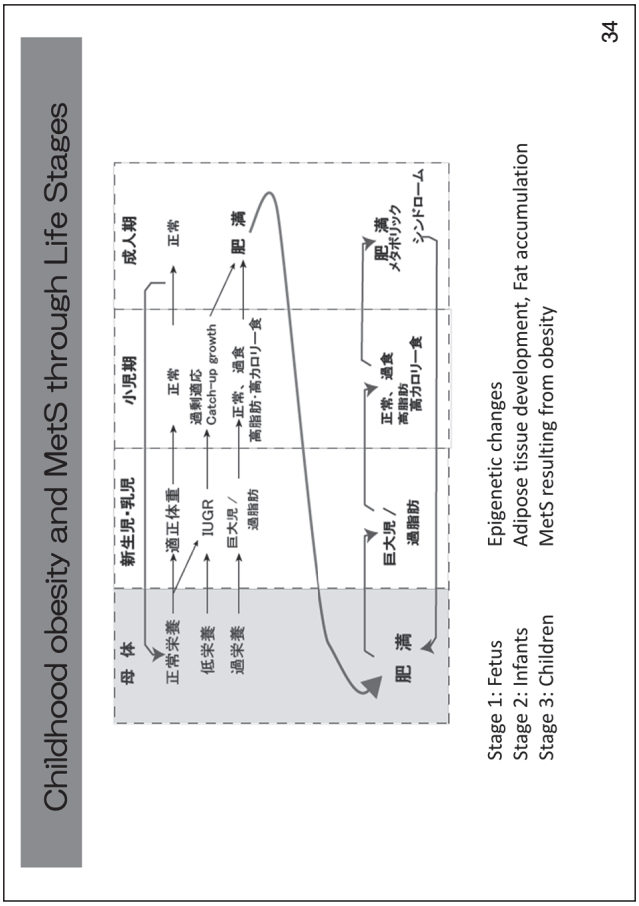
31

Cognitive Behavioral Therapy Including Self-monitoring

Daily-life Checking Sheet

32


DHA tissue dose-response to dietary DHA
with constant ARA (0.67% total FA)
Tyburczy C, et al. PLEFA 2011
Tissue DHA rises with dietary DHA in all tissues
A
Heart DHA (% FA)
y = 4.34x + 6.37
R2 = 0.57
P < 0.0001
B
Liver DHA (% FA)
y = 2.62x + 2.28
R2 = 0.66
P < 0.0001
C
Brain DHA (% FA)
y = 2.08x + 9.91
R2 = 0.44
P = 0.0005
D
Retina DHA (% FA)
y = 2.04x + 18.08
R2 = 0.17
P = 0.045
Dietary DHA (%FA)
33


Childhood obesity and MetS through Life Stages
母 体
新生児・乳児
小児期
成人期
正常栄養
適正体重
正常
低栄養
IUGR
過剰適応
Catch-up growth
過栄養
巨大児 /
過脂肪
正常、過食
高脂肪・高カロリー食
肥 満
正常
肥 満
巨大児 /
過脂肪
正常、過食
高脂肪
高カロリー食
肥 満
メタボリック
シンドローム
Stage 1: Fetus
Stage 2: Infants
Stage 3: Children
Epigenetic changes
Adipose tissue development, Fat accumulation
MetS resulting from obesity
34

セッション 2-3

食事や味刺激がもたらす大脳皮質の変化

森永製菓株式会社　研究所
川上　晋平

食事によって我々が感じる味覚情報や、硬い・柔らかいといった食感に関する情報は、口腔内の感覚器で受容され、神経伝導路に沿って大脳皮質の一次味覚野や一次体性感覚野に投射されることが知られている。このような食刺激に関する情報が伝達・処理される脳領域についてはある程度明らかにされている一方、食情報・食経験により、脳神経回路に変化が生じるのかについてはこれまでほとんど解析されてこなかった。脳には可塑性が備わっており、例えば視覚系においては、幼少期での光刺激により大脳皮質の不可逆的な可塑性が生じることが知られており、特定の時期に片方の目に光が入らないように目を覆ってしまうと、覆われた目は元に戻しても弱視になってしまう。一方、味覚に関しても、家庭の味を大人になっても好きでいるように、幼少期の食経験はその後の嗜好性に影響を及ぼすと考えられるが、幼少期の味覚刺激や食経験が脳にどのような影響を与えているのか、という点に関してはエビデンスがほとんどなく、食経験に応答した大脳皮質における分子の変動が生じているのか、といった知見は少ない。そこで我々は、食経験による脳内分子の変化を検証するにあたり、食環境が大きく変化する離乳期に注目した。離乳期は、母乳には含まれない様々な味物質や固形の食事を初めて経験する時期である。この離乳期において食経験や味刺激に応答する大脳皮質での分子変動を、東京大学と共同で解析した。

まず離乳期前後のマウス大脳皮質における DNA マイクロアレイ解析を行った結果、ほとんどの遺伝子発現プロファイルには有意な発現量変動が認められなかった一方で、生育の時間経過に伴って、発現が上昇する遺伝子や逆に低下する遺伝子の存在が明らかになった。特に、このとき発現が上昇する遺伝子群には、神経の可塑的変化への関与が報告されている immediate early gene（IEG）が複数含まれており、食刺激入力が可塑性をもたらす一つの因子として機能することが示唆された。

一方、該当する大脳皮質領域におけるタンパク質発現解析も実施した。その結果、離乳期マウスが固形餌を摂取することで、数日以内にシナプス関連タンパク質の一種である Synaptosomal-associated protein 25 (SNAP25) の蓄積量が大きく増加する事を見出した。さらに、離乳期マウスに生後味わったことのないサッカリンやカプサイシンの単一化学感覚刺激を与えた場合でも、大脳皮質において SNAP25 蓄積が見られることを確認し、離乳期における固形餌摂取や化学感覚刺激によって、大脳皮質で変動する因子を見出すことができた。SNAP25 はシナプス終末における神経伝達物質の放出に関与しており、この因子が変動しているという結果は、その局所における神経伝達に変化が生じている可能性を示唆している。離乳期という新たな味や食感を経験する時期においては、食経験により脳内分子変動が生じており、これらの知見は将来、乳幼児期の食事が味覚認知や食嗜好性などに影響をもたらすのか、といった疑問を解決する上で大きな手がかりになると考えられる。

Session 2−3

Changes in the Cerebral Cortex in Response to Food and Chemical Stimulation

Shinpei Kawakami
Research Institute, Morinaga & Co., Ltd.

Sensory signals arising from food intake, including taste, somatosensory, and tactile stimuli, are received by receptors in the oral cavity; this information is then transferred to the cerebral cortex, including the insular and somatosensory cortex. Numerous studies have evaluated cortical responses to food stimulation; however, there is little evidence regarding food-signal dependent changes in the nervous system. For example, the visual cortex has the capacity for experience-dependent cortical plasticity. In the development of the visual system, it is well established that infant optical experience plays a crucial role in the developing visual nervous system. In the gustatory cortex, it is also well known that dietary food experience, especially at the infant stage, evokes personal food preferences. However, little is known about the specific changes undergone by the gustatory nervous system or the expression of molecules in the gustatory cortex in response to feeding experiences. Mammals begin to eat various types of foods in their weaning period. Therefore, in this study, we investigated the expression patterns of molecules in mouse gustatory and somatosensory cortices during the weaning period, in cooperation with the University of Tokyo.

First, we investigated differences in whole-gene expression profiles in the mouse cerebral cortex using DNA microarray analysis before and after weaning. Among 45,037 murine genes, almost none displayed significant changes in expression during weaning, whereas 35 genes were up-regulated, and 31 down-regulated, in response to weaning. In particular, some immediate early genes (IEGs), which are known to play a role in neural plasticity by neuronal excitation, were up-regulated during weaning.

The expression of proteins in response to food signal stimulation in cortical areas was also investigated. We found that the expression of synaptosomal-associated protein 25 (SNAP25), a component of the SNARE complex, increased in the cerebral cortex after mice began to intake solid food. Chemical stimulation by saccharin or capsaicin at the weaning stage also increased SNAP25 immunoreactivity in the cortex. It is well known that SNAP25 is associated with neuronal maturation and synaptogenesis during development. Therefore, the SNAP25 accumulation observed in this study may be involved in synaptic plasticity in various cortical areas, where it may affect the synaptic transmission of gustatory and somatosensory information.

Our study reveals that the expression of IEGs and SNAP25 increases in gustatory and somatosensory cortical areas in response to food stimulation during the weaning period. These results will be vital in elucidating the effect of food intake in childhood on both synaptic plasticity, and taste palatability, in adults.

The 7th International Conference on "Nutrition and Aging"

Changes in the cerebral cortex in response to food and chemical stimulation

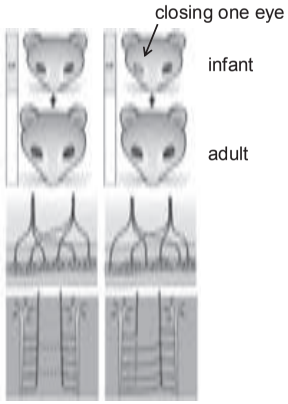
Shinpei Kawakami

Research Institute
Morinaga & Co.,Ltd.

1

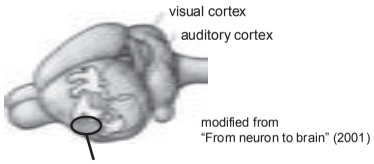
Experience-dependent changes in the brain

Visual cortex



modified from Castrén *et al.*
Trends Neurosci. 36:259-67(2013)

Gustatory cortex



Somatosensory cortex
Insular cortex

There is little evidence regarding food signal-dependent changes in the cerebral cortex

2

Novel food experience in weaning period

Mammals begin to eat various types of foods in their **weaning period**

Before weaning		**After weaning**
Milk	→	New taste, smell, texture of foods

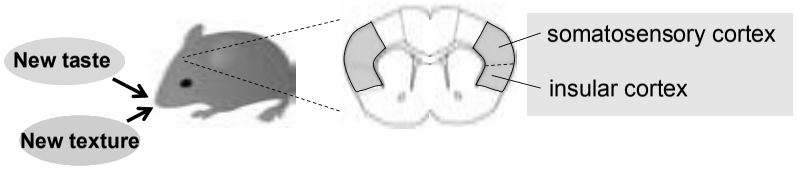
These food stimulation in this period affect the expression pattern of molecules in insular and somatosensory cortices?

3

Aim of this study

We investigated the expression patterns of molecules in mouse insular and somatosensory cortices during the weaning period

【Mouse sagittal brain section】



(1) DNA microarray analysis, *in situ* hybridization

(2) Western blotting, immunohistochemistry

4

DNA microarray analysis before and after weaning

mice

P0 (birth) → P15 → P20 → P23 → P30

Milk → Solid Food

P19 : start to eat solid food

RNA extraction
from insular and somatosensory cortices

→ DNA microarray

Animal: C57BL/6J mice

- before weaning (P15),
- after weaning (P20, P23, and P30)

Array: GeneChip Mouse Genome 430A 2.0 Array (Affimetrix)

Subject: Cerebral insular and somatosensory cortices

5

DNA microarray analysis before and after weaning

Of the total genes, 98.3% did not display significant changes in expression during weaning

31 down-regulated genes:

5 cytoskeletal protein genes, 4 oncogenes, etc

35 up-regulated genes:

5 immediate early genes (IEGs) : *egr-2/Krox-20, Ier-3, arc, homer*

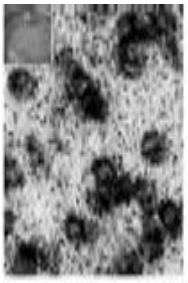
⇒IEGs is known to play a role in neural plasticity by neuronal excitation

Maeda *et al.* ***Biochem. Biophys. Res. Commun.*** 431:437-43(2013)

6

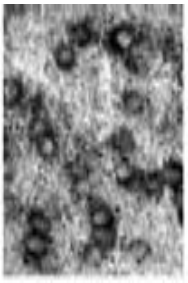
Distribution of *egr-2* mRNA

P15（without solid food）



P22（solid food）

P22（without solid food）



P22（solid food）

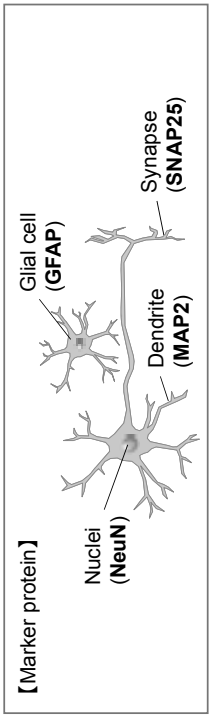
The expression pattern of IEGs changes with solid food intake

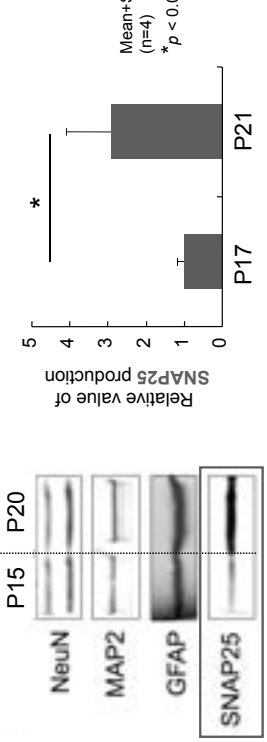
Maeda *et al.* ***Biochem. Biophys. Res. Commun.*** 431:437-43(2013)

7

Changes in the expression of neural marker proteins

【Marker protein】

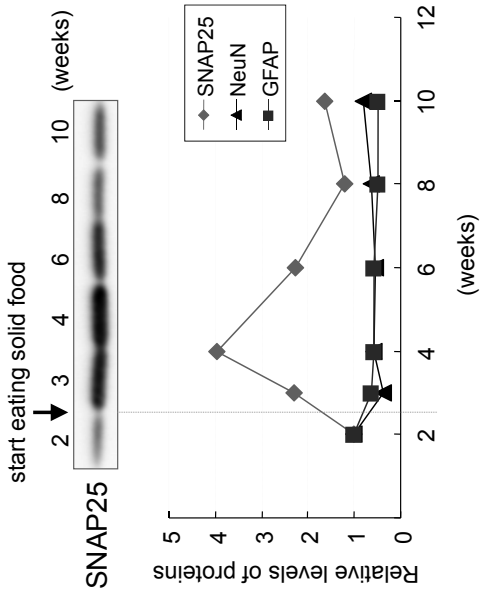




Kawakami *et al.* ***Neuroscience*** 218:326-34 (2012)

8

Age-related change of SNAP25 expression



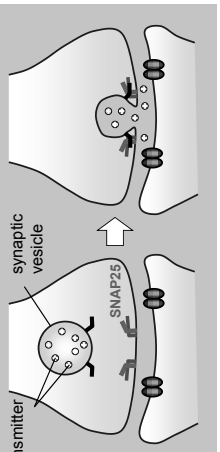
9

Accumulation of SNAP25 in mouse cerebral cortex

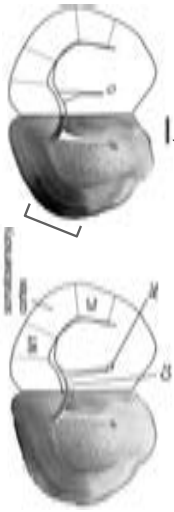
SNAP25

(Synaptosomal-associated protein 25)

- exist in the presynaptic terminal
- crucial for exocytosis and neurotransmitter release



Immunohistochemical analysis of SNAP25 protein

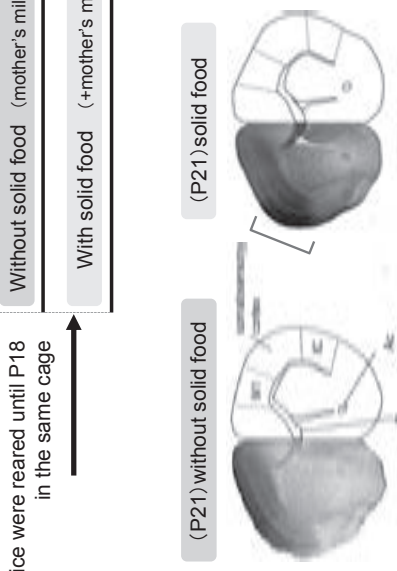


Ac: anterior commissure
Cc: corpus callosum
IC: insular cortex
M1: primary motor cortex

Kawakami *et al.* ***Neuroscience*** 218:326-34 (2012)

10

Accumulation of SNAP25 after eating solid food



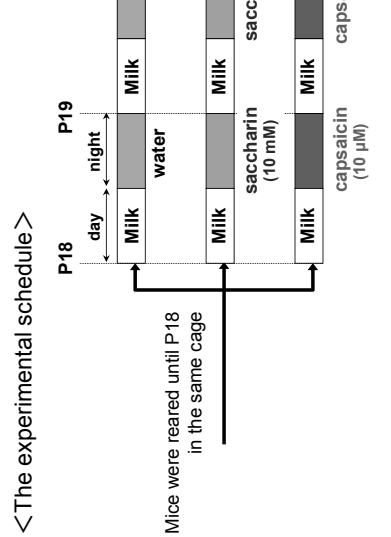
Ac: anterior commissure
Cc: corpus callosum
IC: insular cortex
M1: primary motor cortex

Kawakami *et al.* ***Neuroscience*** 218:326-34 (2012)

11

SNAP25 expression following saccharin or capsaicin intake

Saccharin (sweet) | Capsaicin (hot, pungent)



- solid food was not provided to the infant mice

12

Summary

In response to food stimulation during the weaning,
the expression of

- immediate early genes (IEGs: *egr-2/Krox-20* etc)
- SNAP25

increases in insular and somatosensory cortices

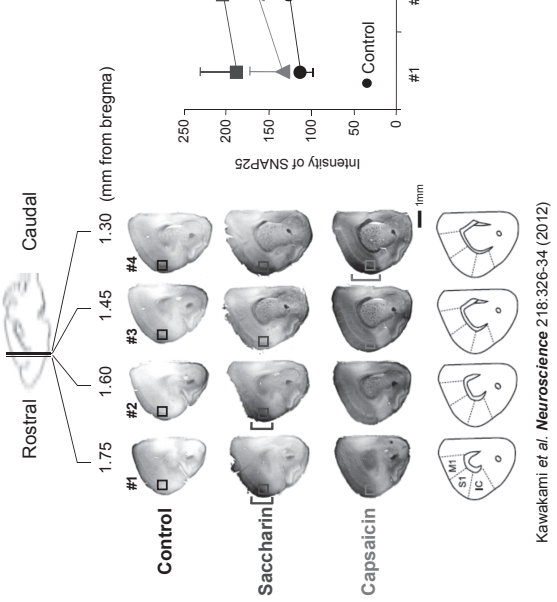
→ affect the synaptic transmission

→ affect the neural network

→ These molecular change in the cerebral cortex affect the taste preference ?

14

SNAP25 expression following saccharin or capsaicin intake



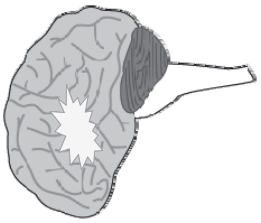
Kawakami *et al.* ***Neuroscience*** 218:326-34 (2012)

13

In the future

Dietary experience

- When?
- What kind of foods?



- synaptic transmission
- development of taste palatability

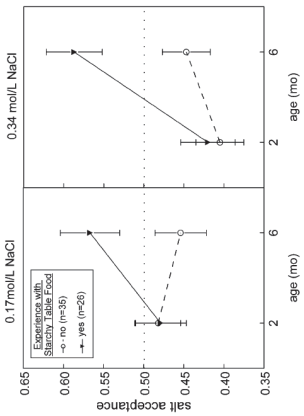
Improve the dietary life in childhood

16

Infant dietary experience affects taste preference

The development of salty taste acceptance is related to dietary experience in human infants: a prospective study[1–3]

Leslie J Stein, Beverly J Cowart, and Gary K Beauchamp (Monell Chemical Senses Center, PA, USA)

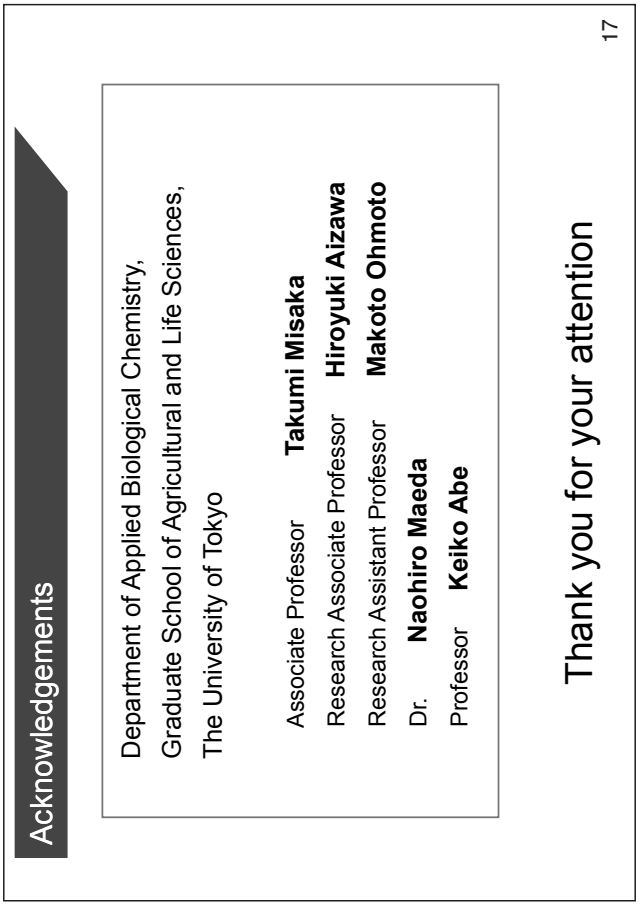


starchy table food
→ significant source of sodium

Stein L.J., *et al.*,
Am. J. Clin. Nutr., **94**, 123-129 (2012)

High-salt food experience → **Preference for salty solutions**

15


Acknowledgements
Department of Applied Biological Chemistry,
Graduate School of Agricultural and Life Sciences,
The University of Tokyo
Associate Professor Takumi Misaka
Research Associate Professor Hiroyuki Aizawa
Research Assistant Professor Makoto Ohmoto
Dr. Naohiro Maeda
Professor Keiko Abe
Thank you for your attention
17

セッション 2-4

機能性食品とエピジェネティクス

[1] 東京大学大学院 農学生命科学研究科、
[2] 公益財団法人　神奈川科学技術アカデミー
阿部　啓子[1,2]、安岡　顕人[2]、近藤　隆[2]、三坂　巧[1]、岡田　晋治[1]

我々はポリフェノール類の機能性についてゲノミクスとくにトランスクリプトームを中心とした解析を行ってきた。杜仲葉、桑葉、メープルシロップ抽出物などの不均質（複合）成分や、エラグ酸やトランスレスベラトロール（RSV）などの均質（単一）成分を用い、高脂肪食やアルコール食摂取のような代謝ストレス誘発源への効果を肝臓の DNA マイクロアレイ解析により検討した。その結果、ポリフェノール類はよく知られている抗酸化作用以外に、極めて広範囲かつ協調的な遺伝子発現調節を行っていることが明らかになった。例えば、通常食に対して高脂肪食で発現量が変化し、ポリフェノール類添加でその変化が相殺される遺伝子は、多くの場合数百の桁で検出されてくる。その中には、解糖系、糖新生、TCA 回路、脂肪酸酸化系、胆汁酸合成系、メチル基代謝系などに関与する酵素遺伝子が含まれ、予想される代謝制御は概ね異化や排出の方向である。最近我々は、エラグ酸や RSV によるアルコール性脂肪肝の抑制に、核内レセプターの一つである CAR が関与していることを見出した。他にもポリフェノール類による核内レセプターPXR、PPARs、FXR の活性化が報告されており、ポリフェノール類に対する生体応答には、これらの核内レセプターが重要な役割を担っているといえる。興味深いことは、核内レセプターによる遺伝子制御がヒストンデアセチラーゼ複合体（HDAC）やヒストンアセチルトランスフェラーゼ複合体（HAT）を通じて行われ、遺伝子のエピジェネティック修飾と深く関係している点である。

機能性食品の摂取で緩和され得る代謝ストレスも、エピジェネティック修飾に直接的に関与している。過剰な栄養素の代謝は、活性酸素分子種の発生、膜脂質や代謝系酵素の損傷、ひいては内在抗酸化系への負担を増大させる。内在抗酸化系はグルタチオンの還元性に依存しており、その合成には S-アデノシルメチオニン（SAM）や葉酸が必要である。また、過剰な糖質や脂質が代謝されることにより、酸化型ニコチンアミド（NAD）の不足、アセチル CoA の過多、AMP/ATP 比の低下が起こる。これら代謝物のうち、SAM は DNA メチルトランスフェラーゼへのメチル基供与体、NAD は HDAC 活性を持つサーチュインの補因子、アセチル CoA は HAT へのアセチル基供与体としてエピジェネティックな修飾に関連している。

代謝ストレスがエピジェネティック修飾に影響を及ぼす一方で、食品ポリフェノールは抗酸化性や転写制御を介してエピジェネティック修飾に拮抗的に働く可能性がある。さらに重要なことは、エピジェネティック修飾は卵子や精子を介して次世代の健康に影響を及ぼしうることである。例えば、父親への高脂肪食や低葉酸食の投与は仔のインスリン分泌や生殖能力に影響し、関連する遺伝子のエピジェネティックな修飾を変化させることが知られている。我々はアルコール性脂肪肝モデルマウスにおけるエピジェネティック修飾について、2 世代実験による解析を始めている。このアルコール性脂肪肝の雄マウスの仔は、エタノール摂取群で体重と血中中性脂肪値が対照群に比べて高く、RSV 共摂取群は対象群と同程度であった。親精子ゲノム DNA のメチル化パターンを解析し、仔の表現型に関与するエピジェネティック修飾の抽出を行っている。これは遺伝子発現に先行して起こる制御メカニズムであるとともに、次世代に継承される塩基配列以外の遺伝情報でもある。次世代機能性食品の評価法として注目される所以である。

Session 2−4

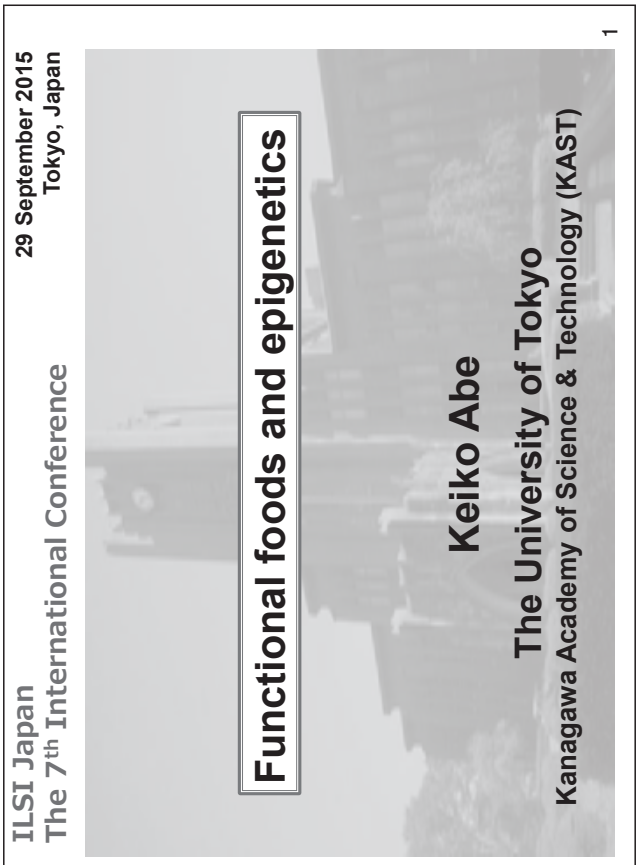
Functional Foods and Epigenetics

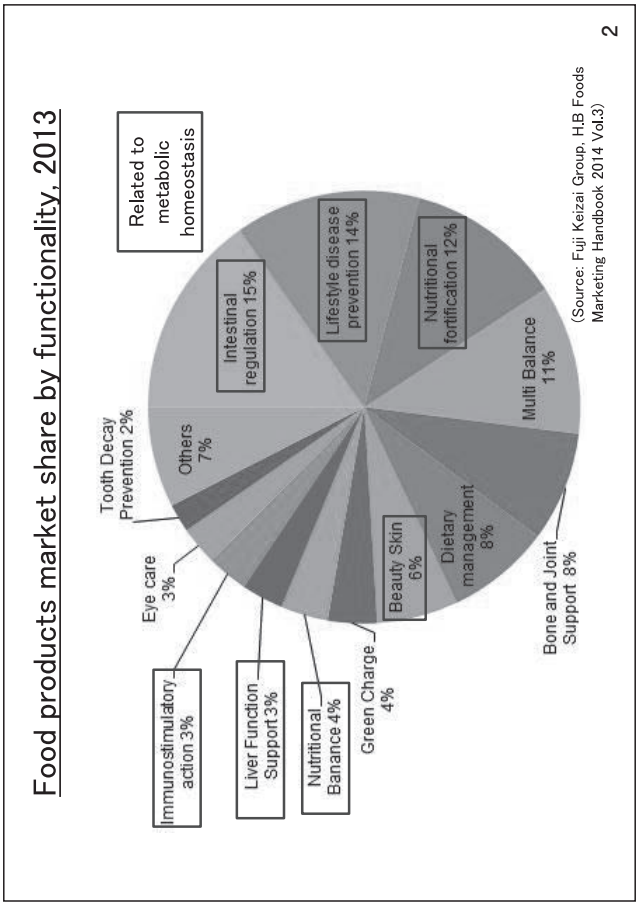
Keiko Abe[1,2], Akihito Yasuoka[2], Takashi Kondo[2], Takumi Misaka[1], Shinji Okada[1]
[1]The University of Tokyo,
[2]Kanagawa Academy of Science and Technology

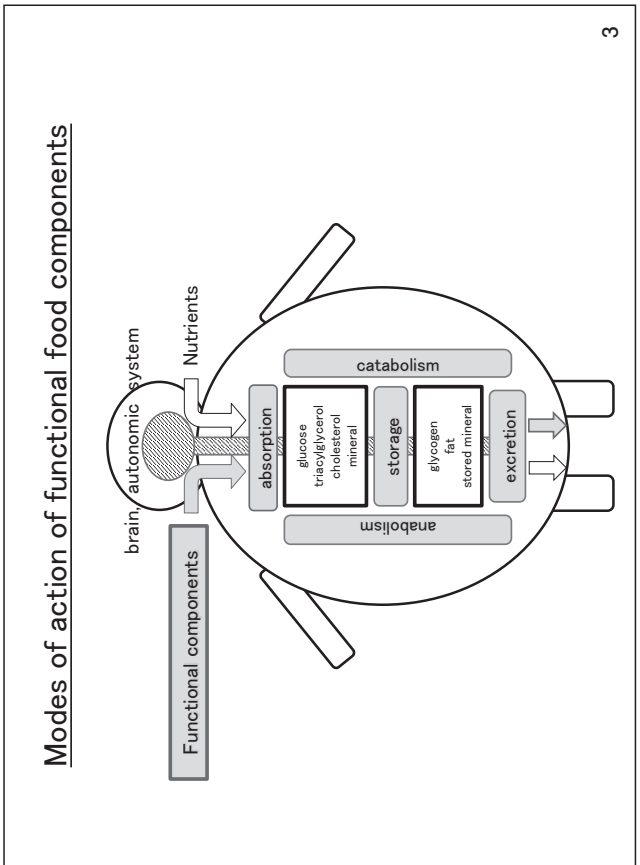
Functionality of food polyphenols influences transcriptome. The use of hepatic DNA microarray analysis on crude extracts of *Eucommia ulmoides* leaf, *Morus alba* leaf and maple syrup as well as ellagic acid and trans-resveratrol (RSV) revealed their effects on metabolic stress due to high-fat diet or alcohol-diet consumption. The results obtained indicated that these polyphenols, besides their well-known anti-oxidative activity, regulate gene expression in highly broadend and coordinated manner. The number of the genes, whose expression levels were once affected by high-fat diet and then normalized by added polyphenol reaches the order of hundreds. These genes include genes for enzymes related to glycolysis, gluconeogenesis, TCA cycle, fatty acid oxidation, bile acid synthesis, CH_3 metabolism etc., and they were predicted as up-regulated catabolism and excretion in general. We have found a nuclear receptor, CAR, as a player for amelioration of alcoholic fatty liver by ellagic acid or RSV. There are several papers reporting the activation of other nuclear receptors, PXR, PPARs and FXR by polyphenols, suggesting that these nuclear receptors play a pivotal role in the biological response to polyphenols. Of importance may be transactivation process of these nuclear receptors is deeply involved in epigenetic modification through the interaction with histone deacetylase complex (HDAC) and histone acetyltransferase (HAT).

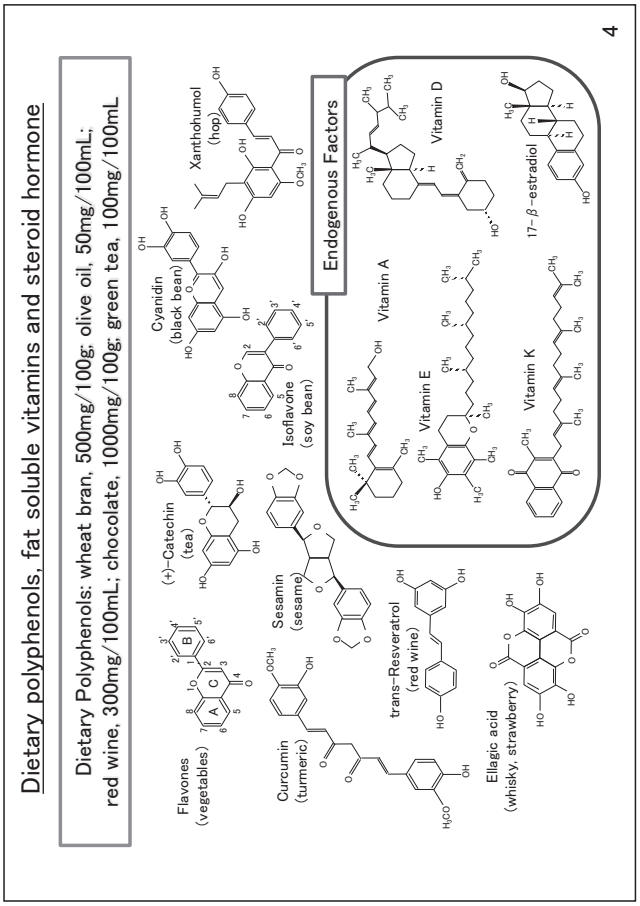
Metabolic stresses, alleviated by functional foods, are also involved directly in epigenetic modification. Metabolically excess amounts of nutrients cause generation of reactive oxygen species, damage in membrane lipids and metabolic enzymes, with arises of stress on endogenous anti-oxidative system. Endogenous anti-oxidative system depends on reductive activity of glutathion whose synthesis requires S-adenosyl methionine (SAM) and folate. Also, large amounts of sugar and lipids are coupled with shortage of reduced nicotin amide (NAD), overproduction of acetyl-CoA and lowering of AMP / ATP ratio. Among these metabolites, SAM serving as methyl donor for DNA methyltransferase, NAD as co-factor for Sirtuins with HDAC activity and acetyl-CoA as acetyl donor for HAT intimately related to epigenetic modification.

Possibly the epigenetic modification caused by metabolic stress can be antagonized by polyphenols'anti-oxidative activity and transcriptional regulation. Of more importance is that this epigenetic modifications can be transferred *via* egg and sperm to affect the health of next generation. Feeding of father with high-fat diet or low-foliate diet affects insulin secretion or reproductive potency of the progeny, respectively, with the accompanying perturbation of epigenetic modification in responsible genes. We are conducting over-two-generation experiment of epigenetic modifications in alcoholic fatty liver model mouse. The progeny of the male mice exhibited higher serum triacylglycerol level and body weight when the ethanol group were compared with control group, but equally when those co-administrated RSV were compared with control group. The sperm methylation patterns of the father mice were analyzed to extract the epigenetic modification responsible for the phenotypes of progeny. Epigenetic modifications are the regulatory mechanism that precede gene expression, and also the genetic information that can be inherited to the next generation independent of nucleotide sequences. Epigenetics is recognized indispensable as a new method to evaluate functional foods.


ILSI Japan
The 7th International Conference
29 September 2015
Tokyo, Japan
Functional foods and epigenetics
Keiko Abe
The University of Tokyo
Kanagawa Academy of Science & Technology (KAST)
1


Food products market share by functionality, 2013
Related to metabolic homeostasis
Immunostimulatory action 3%
Liver Function Support 3%
Nutritional Banance 4%
Green Charge 4%
Eye care 3%
Tooth Decay Prevention 2%
Others 7%
Intestinal regulation 15%
Lifestyle disease prevention 14%
Nutritional fortification 12%
Multi Balance 11%
Bone and Joint Support 8%
Dietary management 8%
Beauty Skin 6%
(Source: Fuji Keizai Group, H.B Foods Marketing Handbook 2014 Vol.3)
2


Modes of action of functional food components
Functional components
brain, autonomic system
Nutrients
absorption
glucose
triacylglycerol
cholesterol
mineral
storage
glycogen
fat
stored mineral
excretion
anabolism
catabolism
3

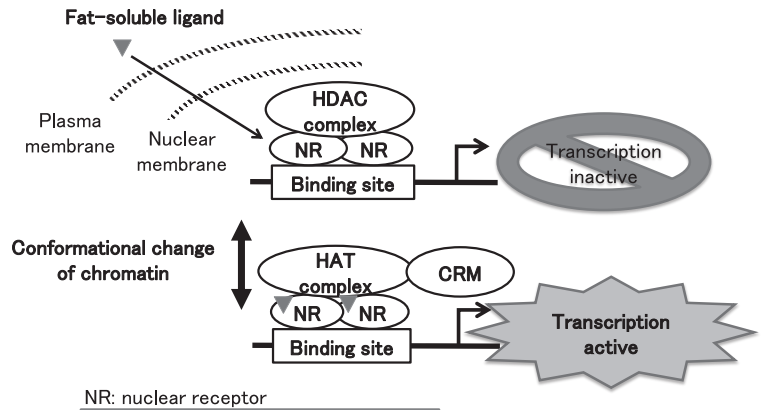

Dietary polyphenols, fat soluble vitamins and steroid hormone
Dietary Polyphenols: wheat bran, 500mg/100g; olive oil, 50mg/100mL; red wine, 300mg/100mL; chocolate, 1000mg/100g; green tea, 100mg/100mL
Flavones (vegetables)
(+)-Catechin (tea)
Cyanidin (black bean)
Xanthohumol (hop)
Curcumin (turmeric)
Sesamin (sesame)
Isoflavone (soy bean)
trans-Resveratrol (red wine)
Ellagic acid (whisky, strawberry)
Endogenous Factors
Vitamin A
Vitamin E
Vitamin K
Vitamin D
17-β-estradiol
4

Nuclear receptors activated by dietary polyphenols

Nomenclature		Name	Abbrev.	subtype	Endogenous activator	Exogeneous activator
NR1	A1,2	Thyroid receptor	TR	α, β	Thyroid hormone	
	B1,2,3	Retinoic acid receptor	RAR	α, β, γ	Retinoic acid	Carotenoid (pro-vitamin A)
	C1,2,3	Peroxisome proliferator-activated receptor	PPAR	α, β, γ	Fatty acid	Vitamin E,Vitamin K, Flavonoid, trans-Resveratrol
	D1,2	Reverse erbA	Rev-erb	α, β	reduced heme	
	F1,2,3	RAR-related orphan receptor	ROR	α, β, γ	Cholesterol	Cholesterol
	H2,3	Liver X receptor	LXR	α, β	Oxysterol	
	H4,5	Farnesoid receptor	FXR	α	Bile acid	Epicatechin
	I1	Vitamin D receptor	VDR		Vidamin D	Vidamin D
	I2	Pregnene X receptor	PXR		Bile acid, Billiirubin	Vitamin K, Flavonoid, trans-Resveratrol
	I3	Constitutive androstane receptor	CAR			
NR2	A1,2	Hepetocyte nuclear factor	HNF4	α, β	acyl-CoA	
	B1,2,3	Retinoid X receptor	RXR	α, β, γ	9-cis Retinoic acid	Carotenoid (pro-vitamin A)
	C,E,F	Testis receptor, others		7	unknown	
NR3	A1,2	Estrogen receptor	ER	α, β	17 beta-estradiol	Isoflavone, Lignan
	B1,2,3	Estrogen receptor-related receptor	ERR	α, β, γ	unknown	Isoflavone
	C1	Glucocorticoid receptor	GR		Cortisol	
	C2	Minearlcorticoid receptor	MR		Alsosterone	
	C3	Progesteron receptor	PR		Progesterone	
	C4	Androgen receptor	AR		Testosteron	
NR0,4,5,6		NOR1, others		8	almost unknown	
total				48		

5

Transcriptional machinery of nuclear receptor



NR: nuclear receptor
HDAC: histone deacetylase
HAT: histone acetyltransferase
CRM: chromatin remodeling complex

Epigenetic modification factor

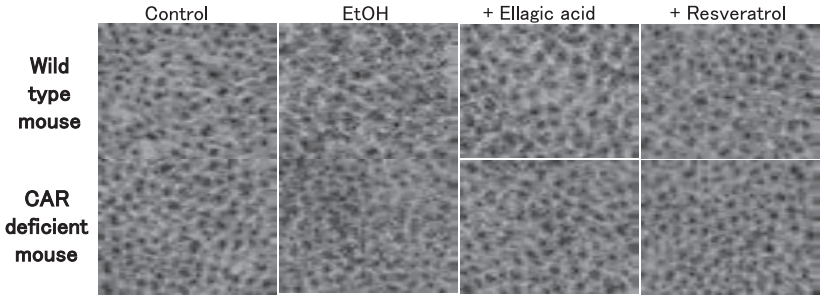
6

CAR-dependent alleviation of alcoholic fatty liver and the effect of polyphenols

0 1 2 6 (weeks)

Liquid diet	Liquid diet	Liquid diet 500 g / kg weight・day
Liquid diet	EtOH	EtOH 25 g / kg weight・day
Liquid diet	EtOH	EtOH + Polyphenol 40 mg / kg weight・day

Free feeding — Calorie restriction

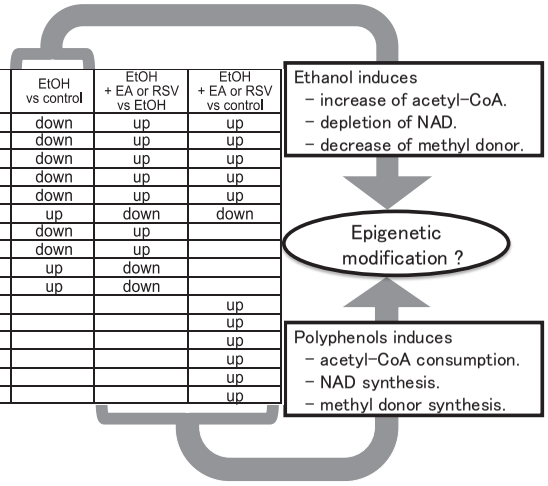


Yao R, Yasuoka A, Kamei A, Ushiama S, Kitagawa Y, Rogi T, Shibata H, Abe K, Misaka T. PLoS One. 2014

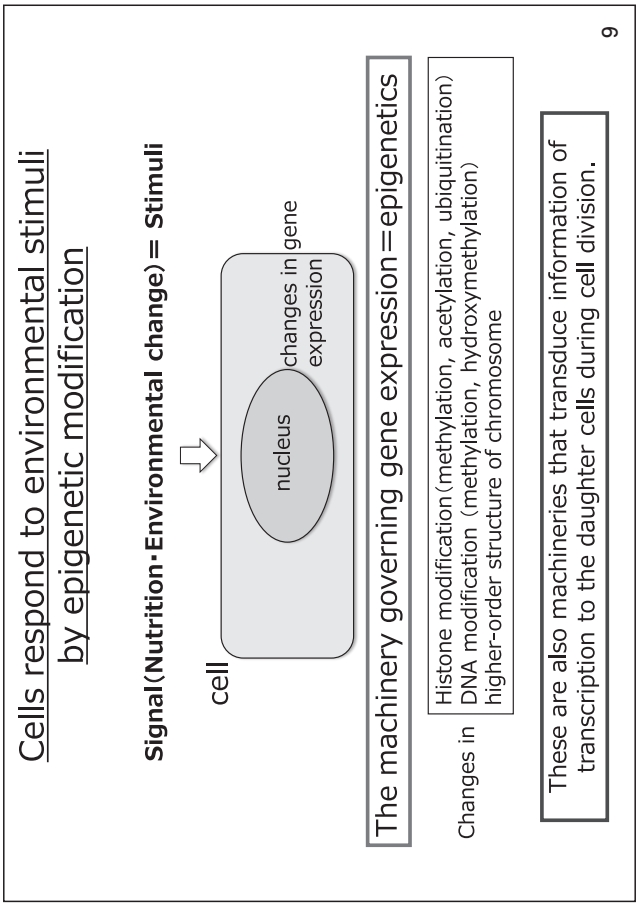
7

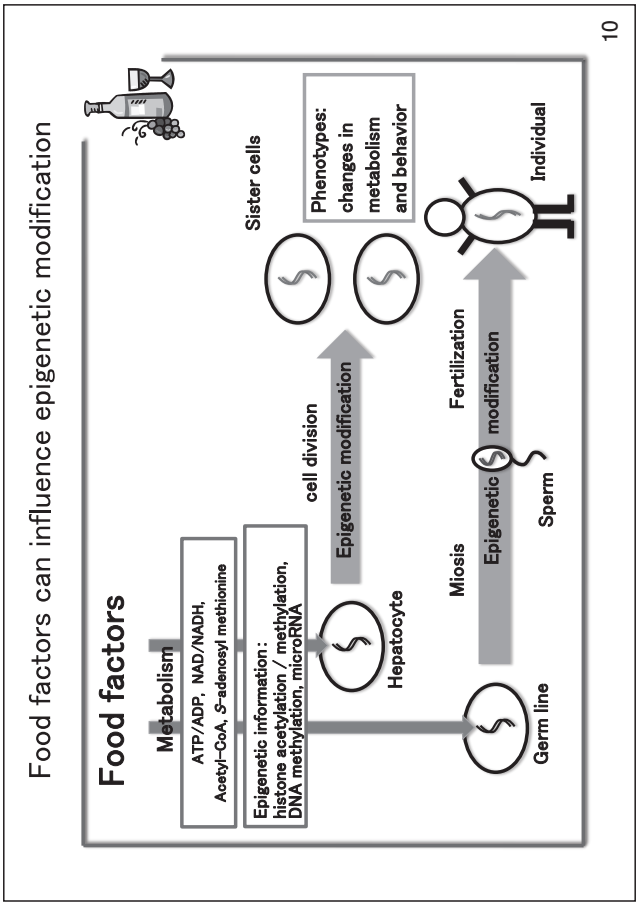
Metabolic regulation by polyphenol is possibly related to epigenetic modification in liver

Metabolic pathways	EtOH vs control	EtOH + EA or RSV vs EtOH	EtOH + EA or RSV vs control
TCA cycle	down	up	up
Steoid and Bile acid synthesis	down	up	up
β-oxidation	down	up	up
α-oxidation	down	up	up
Tetrahydrofolate metabolism	down	up	up
Core clock (day)	up	down	down
Glycogen synthesis	down	up	
Long chain unsaturated fatty acid synthesis	down	up	
Glycolysis	up	down	
Pentose phosphate pathway	up	down	
Gluconegenesis			up
NAD synthesis			up
Methionine metabolism			up
Urea cycle			up
Core clock (night)			up
Clock output regulators			up



8

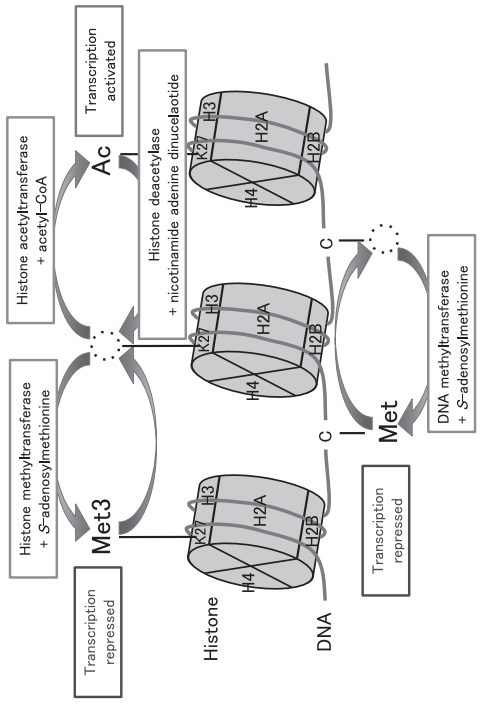

Cells respond to environmental stimuli
by epigenetic modification
Signal（Nutrition・Environmental change）= Stimuli
cell
nucleus
changes in gene expression
The machinery governing gene expression＝epigenetics
Changes in
Histone modification（methylation, acetylation, ubiquitination）
DNA modification（methylation, hydroxymethylation）
higher-order structure of chromosome
These are also machineries that transduce information of transcription to the daughter cells during cell division.
9


Food factors can influence epigenetic modification
Food factors
Metabolism
ATP/ADP, NAD/NADH,
Acetyl-CoA, S-adenosyl methionine
Epigenetic information：
histone acetylation / methylation,
DNA methylation, microRNA
Germ line
Hepatocyte
Epigenetic modification
cell division
Sister cells
Phenotypes:
changes in
metabolism
and behavior
Miosis
Epigenetic
modification
Sperm
Fertilization
Individual
10

Study 1 Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring (Ng et al. Nature 467, 2010)
Male rats fed a control diet or a High fat diet (HFD) for 13 weeks
♂
x ♀
Fed a control diet prior to sacrifice at 13-week-old age.
Subjected to serum and islet transcriptome analysis.
▼ The offspring of HFD-administered father showed decreases in insulin secretion and glucose tolerance disorder.
▼ 642 genes were differentially expressed in the islet of the offspring of such a father.
Study 2 Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals (Carone et al. Cell 143, 2010)
Male mice fed a control diet or low protein diet (LPD) for 12 weeks
♂
x ♀
Sacrificed 3 weeks after birth.
Subjected to serum and liver transcriptome analysis.
▼ The offspring of LPD-administered father showed increased expression of genes involved in lipogenesis and cholesterol synthesis.
▼ Lower level of methylation occurred in the PPARα enhancer region of offspring of LPD-administered father.
11

Transgenerational study of polyphenol functionality
Feed
Control diet
Ethanol diet
Ethanol + Resveratrol diet
male mice for 5 weeks
♂
Epigenetics of sperm
Mate with female mice fed normal diet
♀
Offspring fed a normal diet
Epigenetics of brain and peripheral tissues
12

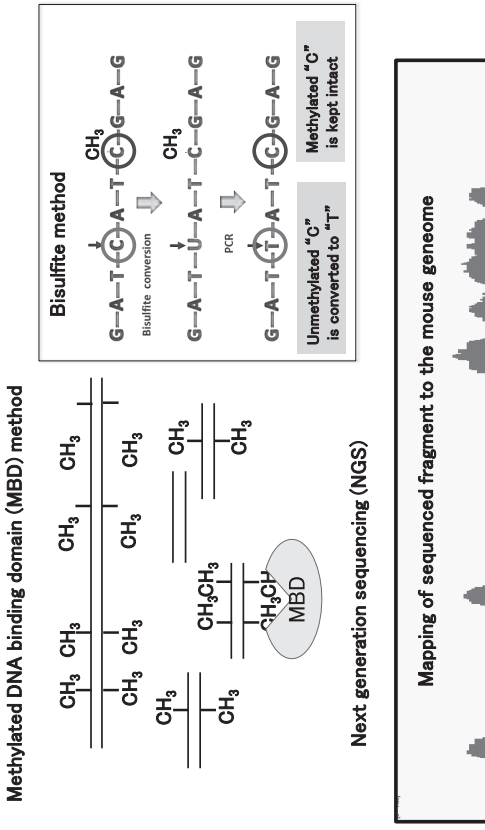
Epigenetic modification of chromatin components



Met: methylated, Met3: tri-methylated, Ac: acetylated

13

Genome-wide detection of DNA methylation



14

Two questions asked about epigenetic inheritance

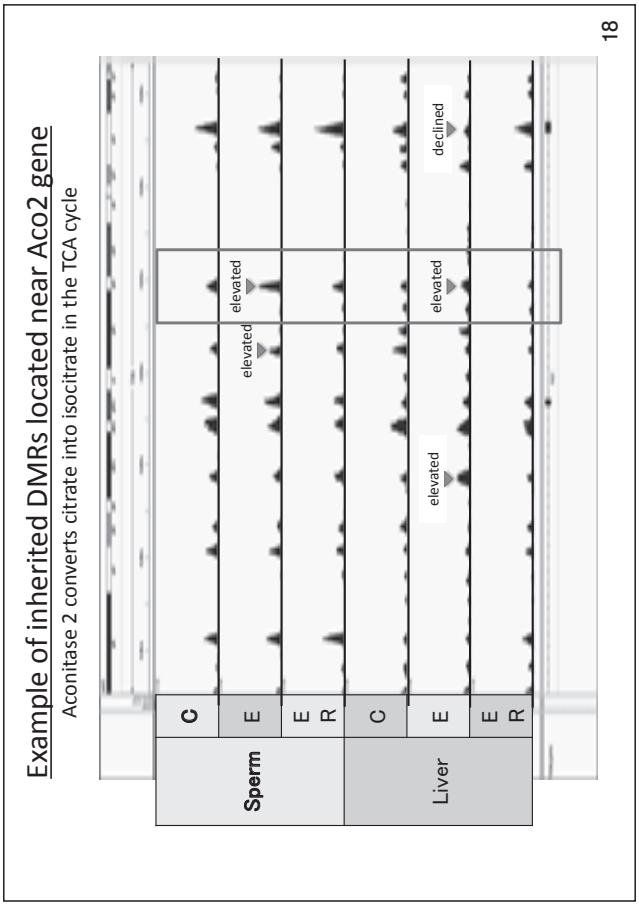
15

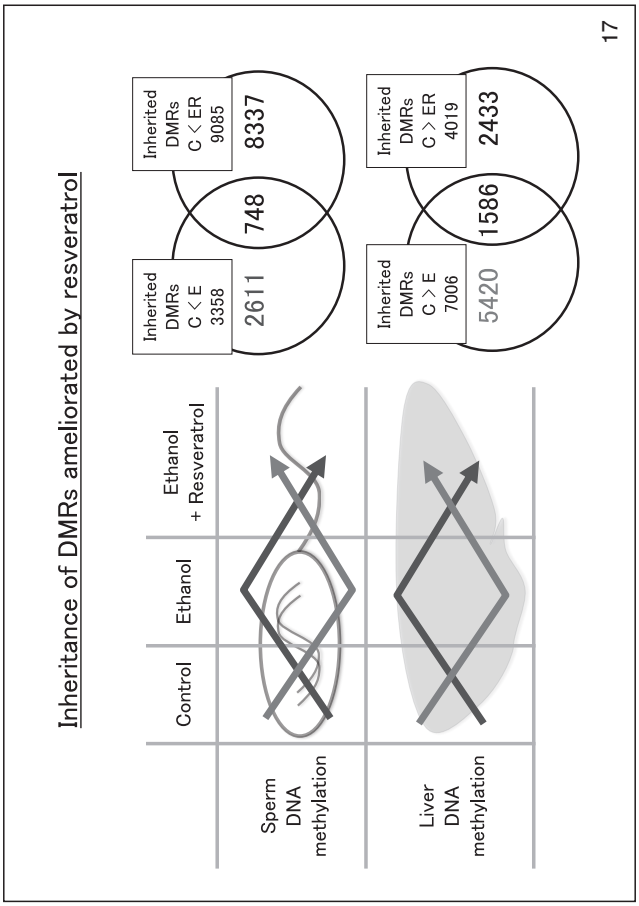
Comparison of total methylation level

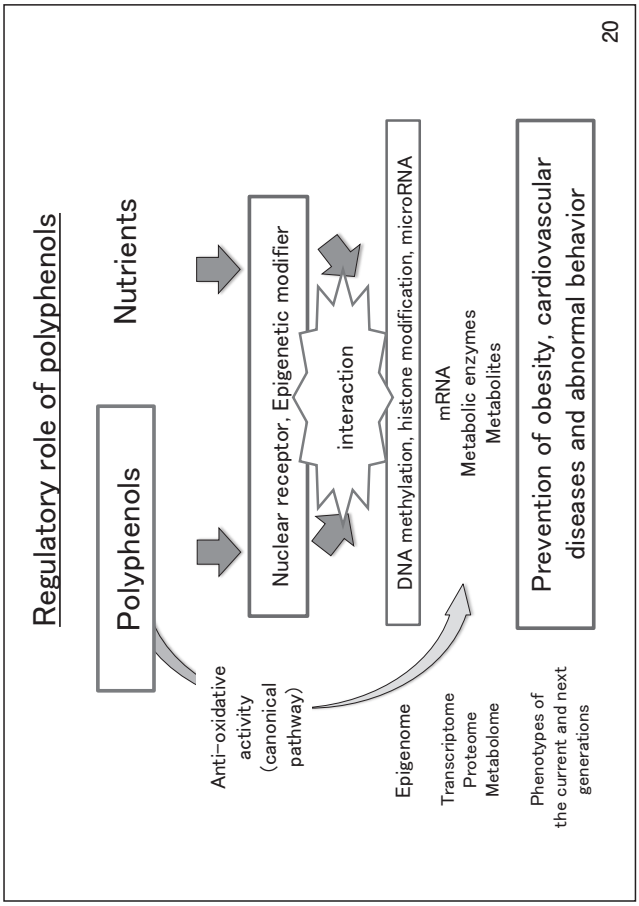
	Father			Offspring		
	control	ethanol	ethanol + resveratrol	control	ethanol	ethanol + resveratrol
Total read	41,923,440	35,938,111	36,883,755	35,098,977	34,792,315	37,615,604
MBD peak number	341,298	330,827	339,096	138,243	108,116	202,061

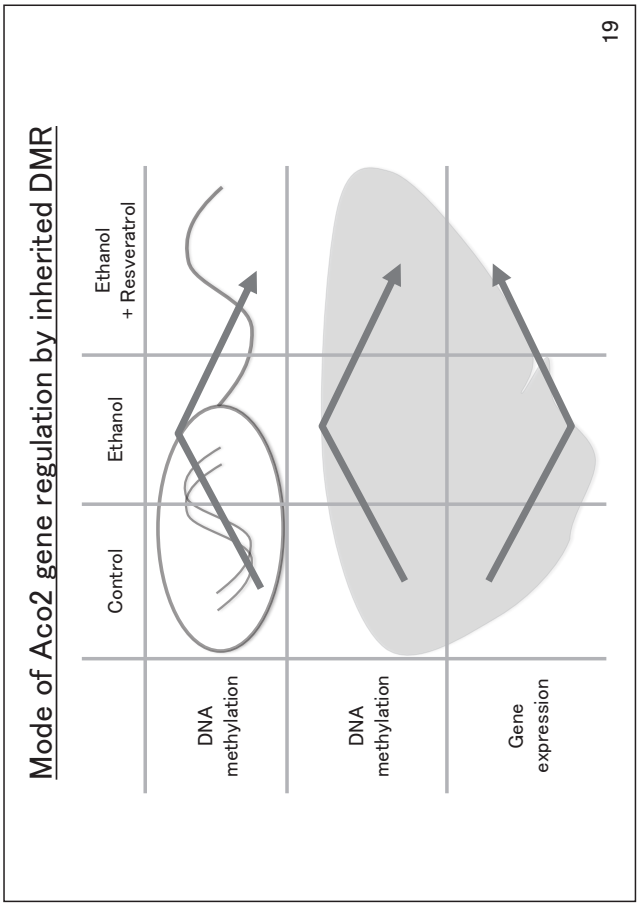
- Higher levels in sperm than in liver
- Sperm: no between-group difference
- Liver: decreasing in E group and increasing in E + R group

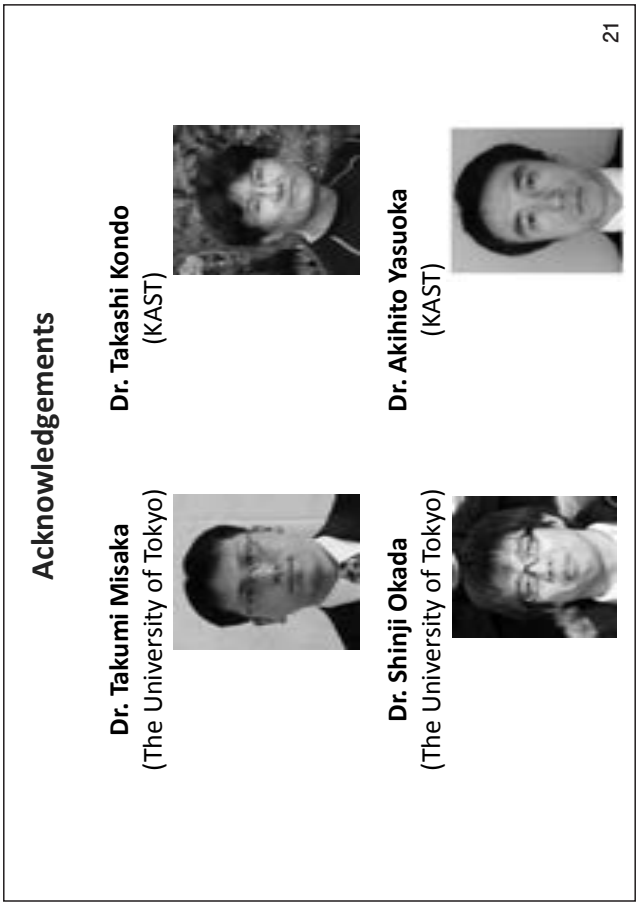
16


Example of inherited DMRs located near Aco2 gene
Aconitase 2 converts citrate into isocitrate in the TCA cycle
Sperm
C
E
E R
Liver
C
E
E R
elevated
elevated
elevated
elevated
elevated
declined
18


Inheritance of DMRs ameliorated by resveratrol
Control
Ethanol
Ethanol + Resveratrol
Sperm DNA methylation
Liver DNA methylation
Inherited DMRs C < E 3358
Inherited DMRs C < ER 9085
2611
748
8337
Inherited DMRs C > E 7006
Inherited DMRs C > ER 4019
5420
1586
2433
17


Regulatory role of polyphenols
Polyphenols
Nutrients
Anti-oxidative activity (canonical pathway)
Nuclear receptor, Epigenetic modifier
interaction
DNA methylation, histone modification, microRNA
mRNA
Metabolic enzymes
Metabolites
Epigenome
Transcriptome
Proteome
Metabolome
Phenotypes of the current and next generations
Prevention of obesity, cardiovascular diseases and abnormal behavior
20


Mode of Aco2 gene regulation by inherited DMR
Control
Ethanol
Ethanol + Resveratrol
DNA methylation
DNA methylation
Gene expression
19


Acknowledgements
Dr. Takumi Misaka
(The University of Tokyo)
Dr. Takashi Kondo
(KAST)
Dr. Shinji Okada
(The University of Tokyo)
Dr. Akihito Yasuoka
(KAST)
21

セッション 2−6

ポリフェノールがエネルギー代謝に与える影響

花王株式会社　開発研究第２セクター　ヘルスケア食品研究所
健康機能評価室　主任研究員
日比　壮信

緑茶及びコーヒーは世界で最も多くの国で飲用されている嗜好飲料であり、これまでの疫学研究において、メタボリックシンドロームを改善する可能性のある様々な生理機能が報告されている。緑茶及びコーヒーに豊富に含まれるポリフェノールの一種である、茶カテキン類とコーヒークロロゲン酸類が生理機能の発現に関与していると考えられているが、その効果は完全には理解されていない。我々は茶カテキン類及びコーヒークロロゲン酸類がエネルギー及び脂質代謝に及ぼす影響について検証することを目的として、ヒト試験及び動物試験を中心に検討を行った。本講演では茶カテキン類及びコーヒークロロゲン酸類の効果について、1）ヒト試験における脂質代謝の亢進、2）ヒト試験における体脂肪の減少、3）動物試験における抗肥満効果の作用機序解析、4）様々な対象者における健康効果、の４つの成果について紹介する。茶カテキン類またはコーヒークロロゲン酸類の継続的な摂取は、エネルギーとして脂質の利用を亢進させる効果を通じて、体脂肪、特に内臓脂肪を減少させ、メタボリックシンドロームの予防に寄与すると考えられる。

Session 2-6

Enhanced Body Fat Utilization as Energy by Dietary Polyphenols

Masanobu Hibi, Ph.D.
R&D - Development Research - Health Care Food Research,
Kao Corporation

Green tea and coffee, the most popular beverages consumed all over the world, have been reported to possess various favorable physiological effects associated with metabolic syndrome primarily based on the epidemiological studies. They are main source of dietary polyphenols known as catechins and chlorogenic acids, those are supposed as active components for their health benefits. Since health benefits of those polyphenols are not fully clarified by the clinical trials, we examined the effects of tea catechins or coffee chlorogenic acids on the energy metabolism and body fat metabolism in human clinical studies. With pre-clinical studies, we found favorable effects on body fat metabolism after repeated ingestions of green tea catechins or coffee chlorogenic acids. We further conducted a series of studies in animals and human to clarify the enhanced body fat metabolism induced by green tea catechins or coffee chlorogenic acids. In this presentation, we will summarize the results from these studies into following four sections: 1) enhanced body fat metabolism in human studies 2) body weight reduction in human studies 3) animal studies supporting the anti-obesity effects 4) studies examining other health benefits in various populations. These studies suggested that ingestion of green tea catechins or coffee chlorogenic acids may reduce body fat, especially abdominal fat, through enhanced body fat utilization as energy, which may contribute to the prevention of metabolic syndrome.

Enhanced Body Fat Utilization As Energy by Dietary Polyphenols

- Green Tea Catechins
- Coffee Chlorogenic Acids

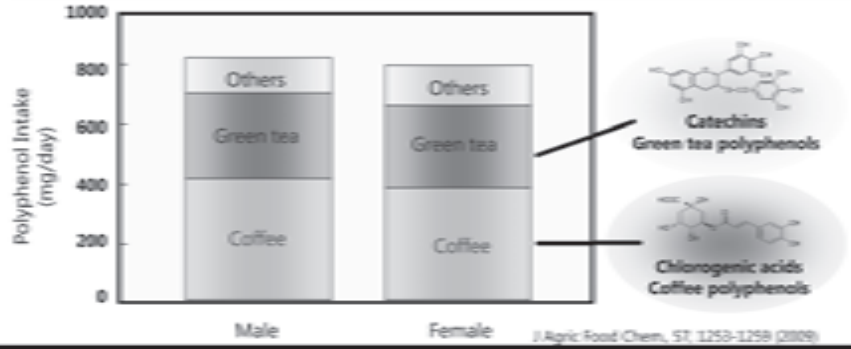
Masanobu HIBI
R&D Product Development
-Health Care Food Research
Kao Corporation

1

Polyphenols Consumption from Beverages in Japan

- Japanese from teens to fifties, Male; n=3896 , Female; n=4871
- Total polyphenol consumption from beverages except alcohol drink was surveyed.

Coffee　Green Tea
Others: Black tea, Oolong tea, other teas, fruit juice, tomato juice/vegetable juice, cocoa/chocolate drink



J Agric Food Chem, 57, 1253-1259 (2009)

Green tea and Coffee are two predominant sources of dietary polyphenols from beverages in Japan

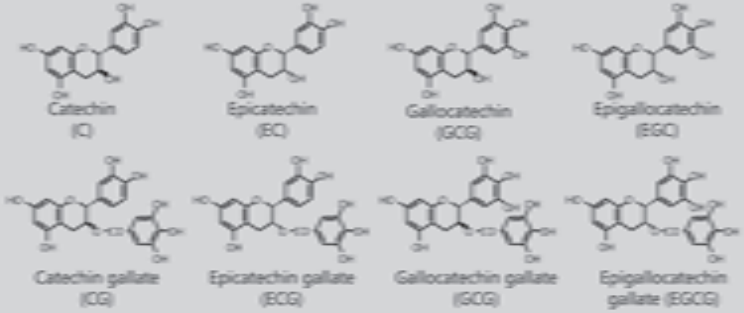
2

Chemical Identity of Green Tea Catechins

● Identity

Green Tea Catechin is a complex mixture of Catechin monomers which is extracted from crude Green Tea extract. It is a brown aqueous solution with a bitter and astringent taste. Green Tea Catechin is defined as a complex mixture of 8 catechin monomers in this presentation

● Structural Formula

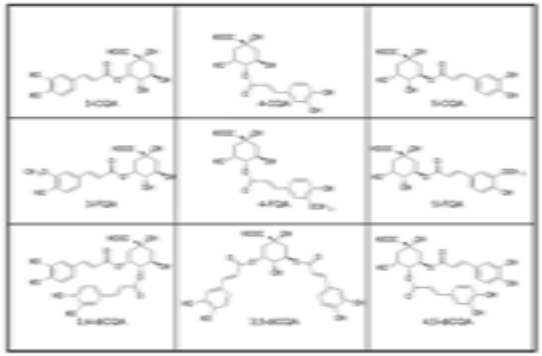


3

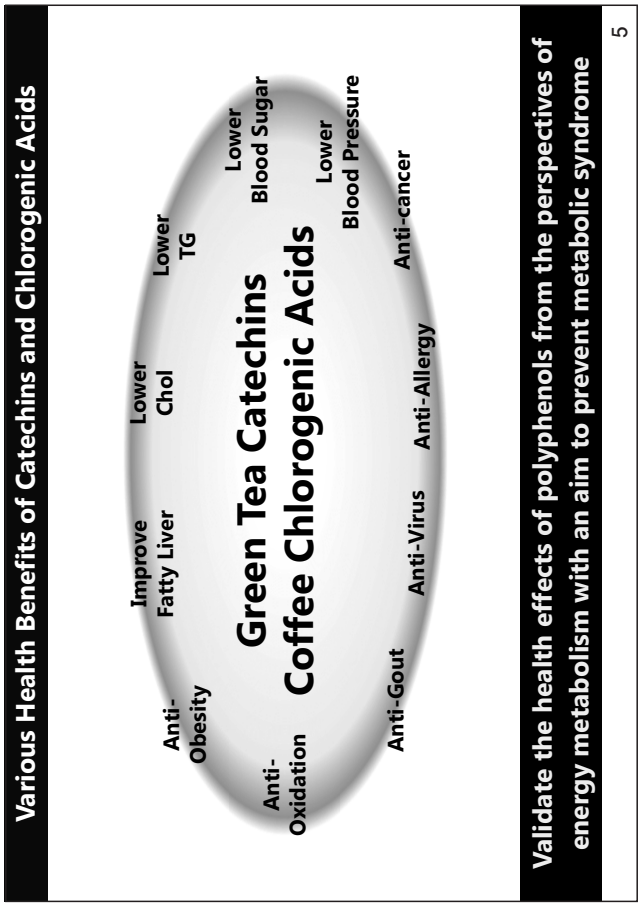
Chemical Identity of Coffee Chlorogenic Acids

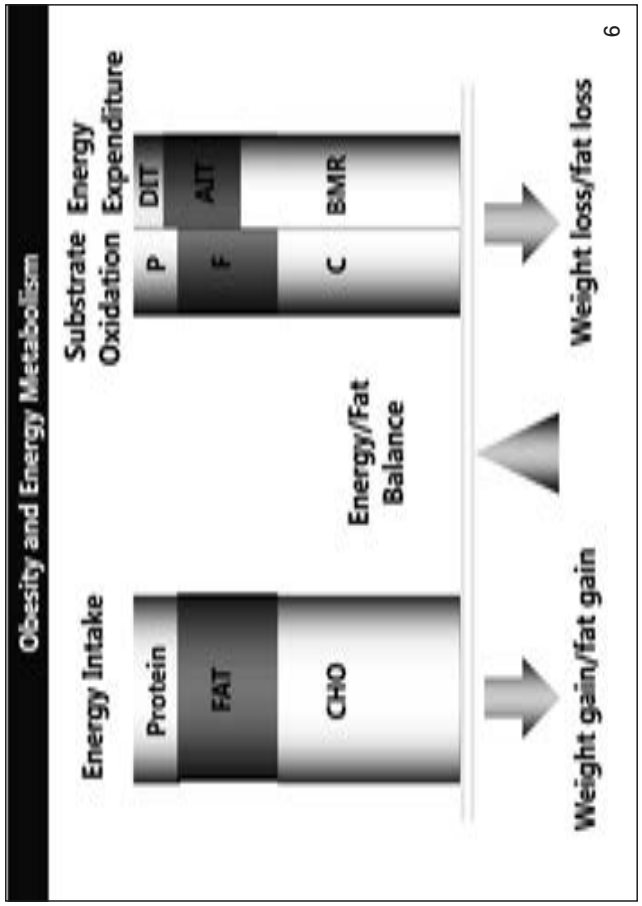
●Identity

Chlorogenic acids are found naturally in fruits and leaves of many plants as esters of cinnamic acid (e.g., caffeic, ferulic acids) with quinic acid. Chlorogenic acids are defined as a complex mixture of 9 chlorogenic acids monomers in this presentation.



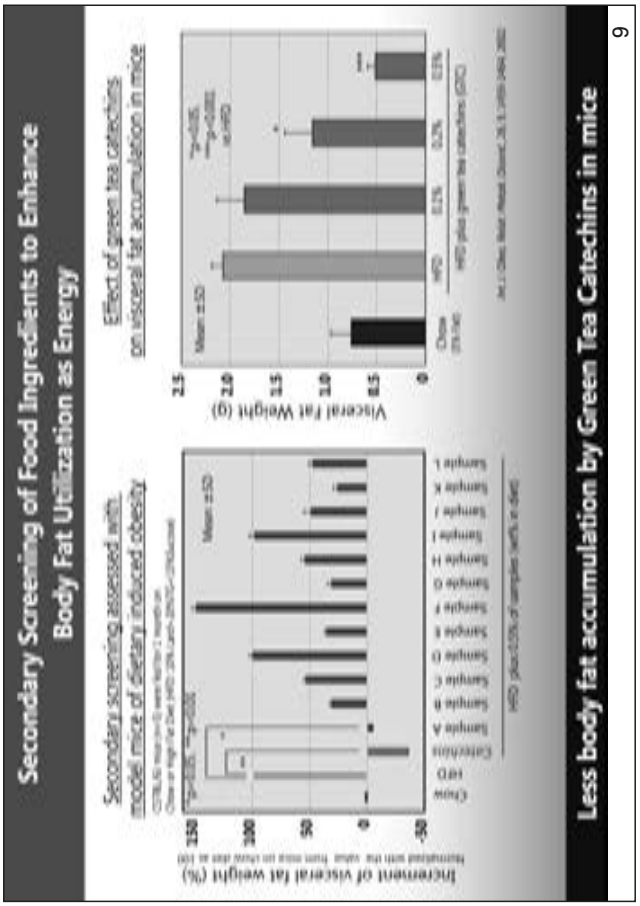
4

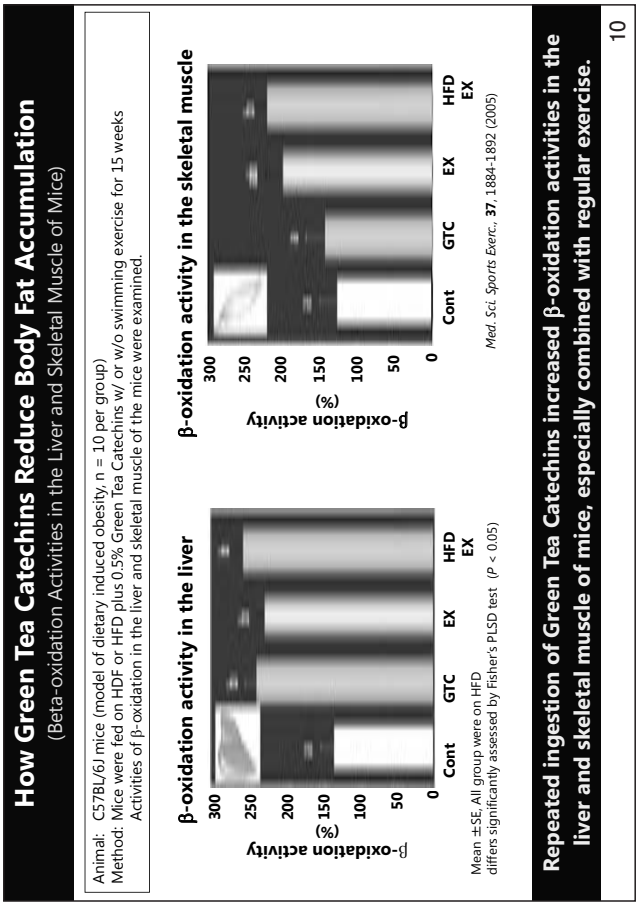

Various Health Benefits of Catechins and Chlorogenic Acids
Anti-Obesity
Improve Fatty Liver
Lower Chol
Lower TG
Lower Blood Sugar
Green Tea Catechins
Coffee Chlorogenic Acids
Lower Blood Pressure
Anti-cancer
Anti-Allergy
Anti-Virus
Anti-Gout
Anti-Oxidation
Validate the health effects of polyphenols from the perspectives of energy metabolism with an aim to prevent metabolic syndrome
5


Obesity and Energy Metabolism
Energy Intake
Protein
FAT
CHO
Substrate Oxidation
P
F
C
Energy Expenditure
DIT
AIT
BMR
Energy/Fat Balance
Weight gain/fat gain
Weight loss/fat loss
6

Enhanced Body Fat Utilization
As Energy by Dietary Polyphenols
• Green Tea Catechins
• Coffee Chlorogenic Acids
7

Primary Screening of Food Ingredients to Enhance Body Fat utilization as Energy
c.a. 8000 kinds of ingredients mainly comprised of polyphenols
Selected c.a. 100 kinds of ingredients
Primary screening with cultured cell
Assessed by the induction of m-RNA expressions of enzyme associated with fat oxidation
13 ingredients containing polyphenols were found effective
Secondary screening
with model animals of diet induced obesity
8


Secondary Screening of Food Ingredients to Enhance Body Fat Utilization as Energy
Secondary screening assessed with model mice of dietary induced obesity
Effect of green tea catechins on visceral fat accumulation in mice
Increment of visceral fat weight (%)
Visceral Fat Weight (g)
Less body fat accumulation by Green Tea Catechins in mice
9


How Green Tea Catechins Reduce Body Fat Accumulation
(Beta-oxidation Activities in the Liver and Skeletal Muscle of Mice)
Animal: C57BL/6J mice (model of dietary induced obesity, n = 10 per group)
Method: Mice were fed on HDF or HFD plus 0.5% Green Tea Catechins w/ or w/o swimming exercise for 15 weeks
Activities of β-oxidation in the liver and skeletal muscle of the mice were examined.
β-oxidation activity in the liver
β-oxidation activity in the skeletal muscle
β-oxidation activity (%)
Cont GTC EX HFD EX
Mean ±SE. All group were on HFD
differs significantly assessed by Fisher's PLSD test (P < 0.05)
Med. Sci. Sports Exerc., 37, 1884-1892 (2005)
Repeated ingestion of Green Tea Catechins increased β-oxidation activities in the liver and skeletal muscle of mice, especially combined with regular exercise.
10

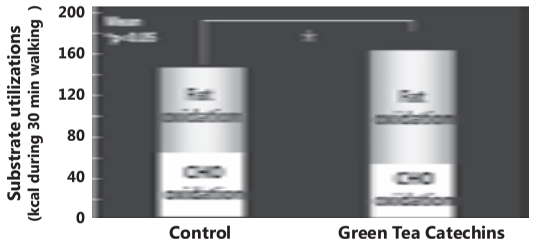
How Green Tea Catechins Reduce Body Fat Accumulation
(Increased m-RNA Expressions of Enzyme Associated with Lipid Metabolism in the Live of Mice)
Animal: C57BL/6J mice (model of dietary induced obesity, n = 5 per group)
Method: Mice were fed on either chow, HDF or HFD plus 0.5% Green Tea Catechins for 4 weeks
m-RNA expressions of enzyme associated with lipid metabolism in the liver of mice were examined.
m-RNA expressions of enzyme for lipid metabolism in liver
ACO; Acyl-CoA oxidase
MCAD; medium-chain acyl-CoA dehydrogenase: enzyme responsible for the rate-limiting step of β-oxidation
ACO
MCAD
Relative m-RNA level (%)
Green Tea Catechins
Chow HFD
Int. J. Obes. Relat. Metab. Disord., 26, 1459-1464 (2002)
Repeated ingestion of Green Tea Catechins increased m-RNA expressions of enzyme associated with lipid metabolism in the liver of mice.
11

Diet-induced Thermogenesis and Postprandial Dietary Fat Oxidation After Repeated Ingestion of Green Tea Catechins
Screening Period (2 wks)
Treatment Period (12 wks)
4wk
Meal Test for DIT
Active (n=6) (catechin 600mg / 350 ml)
Placebo (n=6) (catechin 80mg/ 350 ml)
Subjects: 12 male, Age = 27-48 yrs, BMI=24.7±0.8 kg/m2
Postprandial respiratory analysis: week 0, 4, 8, 12. Test meal: 800 kcal spiked with 13C tripalmitate
Diet-induced thermogenesis
Dietary fat oxidation
Postprandial increase in oxygen consumption (L)
Postprandial increase in 13CO2 excretion (%)
Time after meal ingestion (hrs)
J. Health Sci., 51, 248-252, 2005
Repeated ingestion of Green Tea Catechins for 12 weeks increased diet induced thermogenesis and dietary fat oxidation.
12

Fat Oxidation During Walking After Repeated Ingestion of Green Tea Catechins Combined with Regular Exercise in Human Study

Active (n=7)
(catechin 570mg / 500 mL)
Placebo (n=7)
(catechin 0mg / 500 mL)

Subjects: 14 male, Test article: Green Tea Catechins in canned beverage (500 ml sized)
Regular exercise: treadmill walking at 5 km/h for 30 min 3 times a week



J. Health Sci., **51**, 233-236, 2005

Repeated ingestion of Green Tea Catechins combined with regular walking exercise for 8 weeks increased fat oxidation at walking exercise.

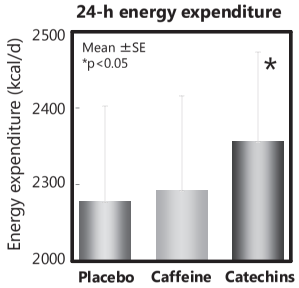
13

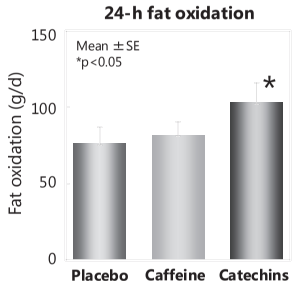
Enhanced Energy Metabolism by Consumption of Green Tea Catechins

(Assessed by Human Metabolic Chamber)

24-h measurement — X-over — 24-h measurement — X-over — 24-h measurement

Subjects: 10 male, Age=25±1 (y), BMI=25.1±1.2 (kg/m^2)
Test article: Tea extract (150mg caffeine and 375mg catechin)/Caffeine (150mg caffeine)/Placebo (cellulose)
Measurement of energy expenditure and fat oxidation for 24-h using by metabolic chamber





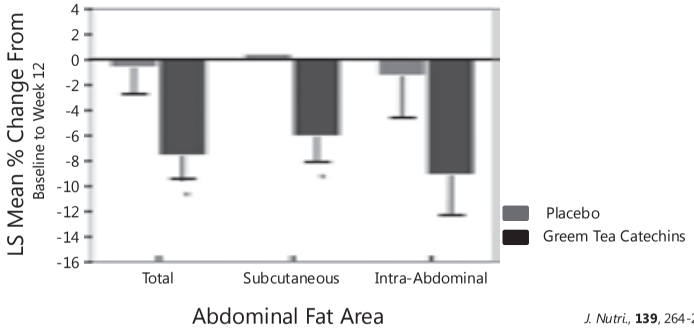
A.G. Dulloo et.al., *AJCN* (1999) 70,1040

Ingestion of Green Tea Catechins increased 24-hr total energy expenditure and fat oxidation significantly assessed by human metabolic chamber

14

Green Tea Catechins Consumption Enhances Exercise-Induced Abdominal Fat Loss in Overweight and Obese US Adults

Study design: RCT with a 2-wk screening period followed by 12wk of treatment
Subjects: n=128 (active: 65, placebo: 63), avg age 48yrs, avg BMI: 32.2 (kg/m^2),
Test article: 500 mL/d of a beverage providing ;625 mg Green Tea Catechins or a control beverage
Exercise intervention: 180 min/wk of moderate-intensity physical activity, at least 3 times per week



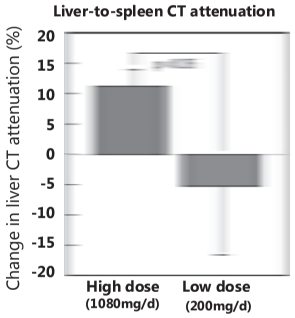
J. Nutri., **139**, 264-270, 2009

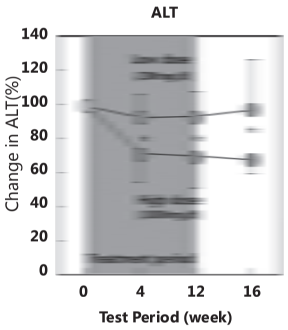
Consumption of a beverage containing Green Tea Catechins enhanced exercise-induced loss of abdominal fat and improve circulating TG levels

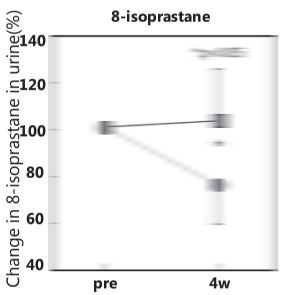
15

Liver Functions and Fat Infiltration in NAFLD Patients

Study design: Randomized double-blind controlled trial with 12wk of treatment
Subjects: n=17 Non-alcoholic fatty liver disease (NAFLD) patients, age 20-70yrs,
Test article: Two cans 340 mL sized beverage providing: high dose;1080mg, low dose; 200mg of Catechins







Int. J. Mol. Med., **32**, 989-994 (2013)

Consumption of beverage containing Green Tea Catechins improved liver fat content and liver function and inflammation marker in NAFLD patients

16

Summary-1; Green Tea Catechins

From *in vitro* and *in vivo* studies

- **Based on our screening experiments among polyphenol food ingredients, Green Tea Catechins showed highest potential in enhancing fat oxidation and least accumulation of body fat.**
- **Green Tea Catechins increased energy expenditure and fat oxidation in mice partly due to increased β-oxidation in the liver and skeletal muscle**

From human studies

- **Repeated consumption of beverage rich in Green Tea Catechins: increased energy expenditure, dietary fat oxidation and fat oxidation during exercise enhanced exercise-induced loss of abdominal fat improved fatty liver and liver functions in NAFLD patients**

17

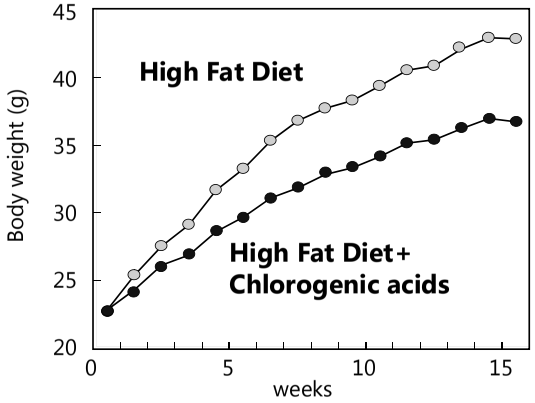
Enhanced Body Fat Utilization As Energy by Dietary Polyphenols

- **Green Tea Catechins**
- **Coffee Chlorogenic Acids**

18

Weight Changes After Repeated Ingestion of Chlorogenic Acids

Animal: C57BL/6J mice (model of dietary induced obesity, n = 10 per group)
Method: Mice were fed on HDF or HFD plus 1% Chlorogenic Acids for 15 weeks.
A body weight was measured every week.

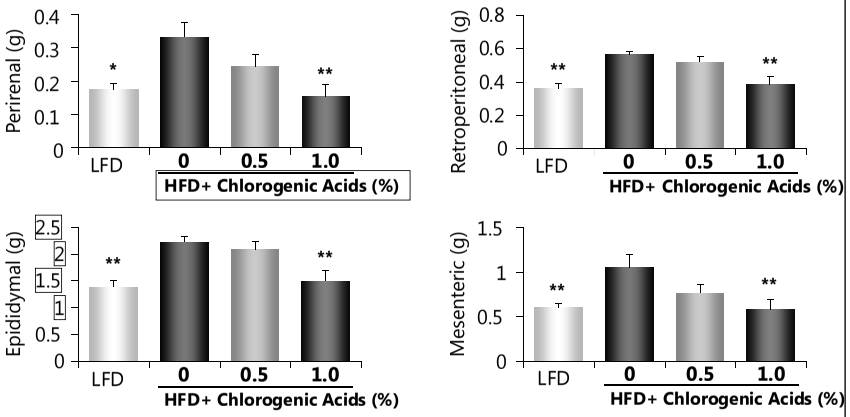


Murase T et al. Am J Physiol Endocrinol Metab 2011 300: E122–E133

19

Effect of Chlorogenic Acids on Visceral Fat Accumulation

Each fat depot was extracted and weighed after 15 week treatment



LFD: Low Fat Diet
HFD: High Fat Diet

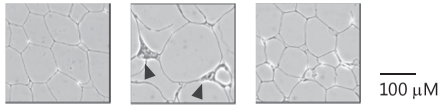
*$P<0.05$, **$P<0.01$

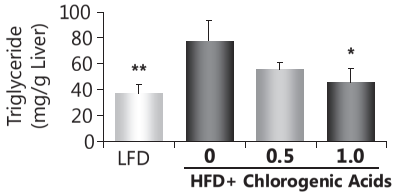
Murase T et al. Am J Physiol Endocrinol Metab 2011 300: E122–E133

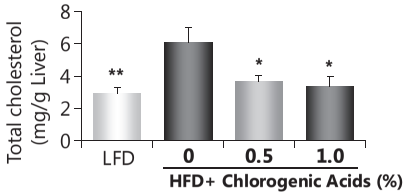
20

Effect of Chlorogenic Acids on Liver Fat Accumulation

F4/80 immunostaining







LFD: Low Fat Diet
HFD: High Fat Diet

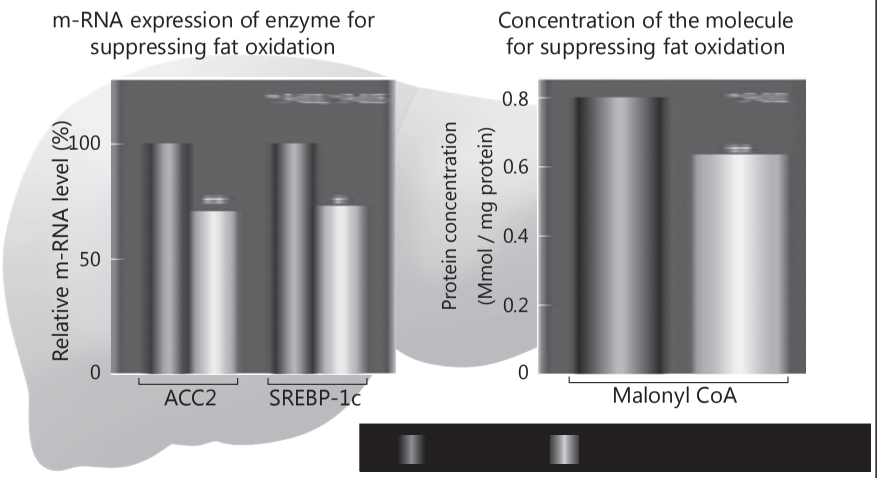
*$P<0.05$, **$P<0.01$

Upper Figures: Liver section was stained with anti-F4/80 antibody for Macrophages.
Lower Figures: Liver lipids were extracted and TG and Cholesterol were quantified.

Murase T et al. Am J Physiol Endocrinol Metab 2011 300: E122–E133

21

Mechanism of Chlorogenic Acids for Increased Fat Utilization in the Liver



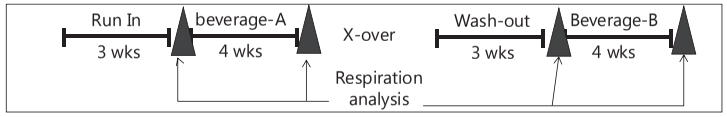
ACC2: acetyl-CoA carboxylase2 SREBP-1c: sterol regulatory element-binding protein-1c

Murase T et al. Am J Physiol Endocrinol Metab 2011 300: E122–E133

22

Energy Metabolism After Repeated Ingestion of Chlorogenic Acids Beverage in Human

Design: Randomized double-blind placebo controlled cross-over trial



Subjects: 27-47 yrs healthily male n=18

Test beverage

	Active	Placebo
Chlorogenic Acids (mg/can)	329	0
Caffeine (mg/can)		51
Calorie (kcal/can)	20	22

Intervention

All through the test period: Coffee and green tea prohibited
2 days prior to respiratory analysis: Controlled diet, alcohol and intense exercise prohibited
1 night before respiratory analysis: take designated supper before 9pm, no food and drink except water

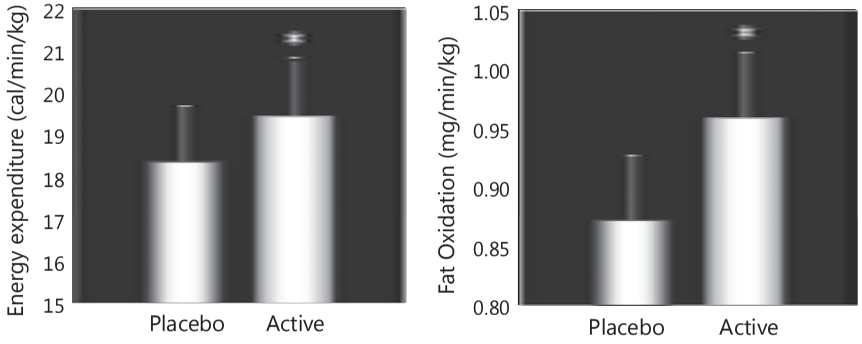
Soga S et al., Biosci. Biotechnol.. Biochem 77: 1633-1636, 2013

23

Energy metabolism After Repeated Ingestion of Chlorogenic Acids Beverage in Human

After 4 week ingestion of Chlorogenic Acids beverage

MEAN±SE, n=18
*: p<0.05 paired t-test

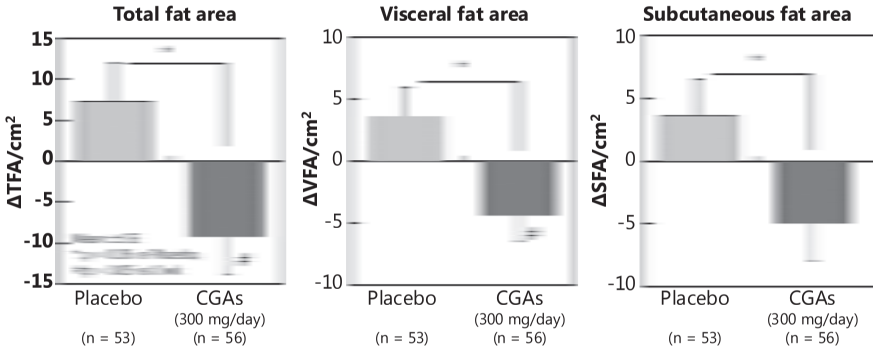


Repeated ingestion of Chlorogenic Acids beverage for 4 week increased dietary induced thermogenesis and fat oxidation after meal

24

Chlorogenic Acids Consumption Decreased Intra-abdominal Fat in Obese Adults

The effect of Chlorogenic Acids was examined in obese subjects (N=109, 25≦BMI<30) via a placebo-controlled RCT for 12 wk. Abdominal fat area was determined by CT.

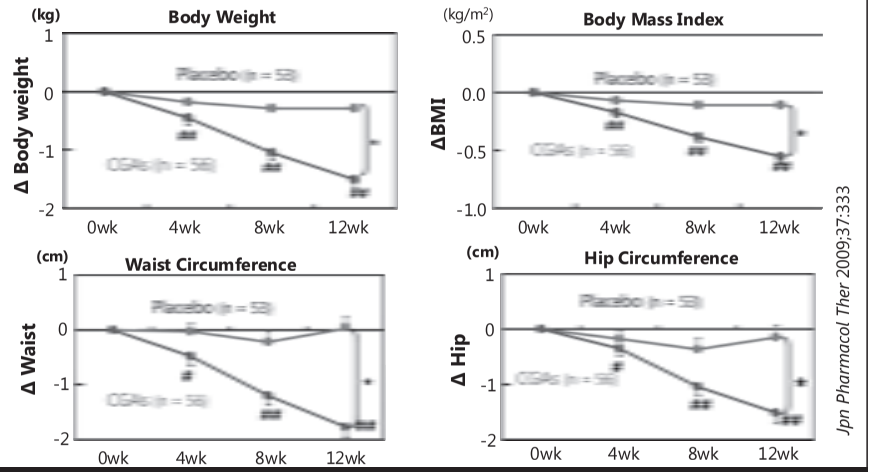


Jpn Pharmacol Ther 2009;37:333

Daily intake of Chlorogenic Acids beverage decreased abdominal fat in obese subjects

25

Chlorogenic Acids Consumption Decreased Body Weight and Waist in Obese Adults



Jpn Pharmacol Ther 2009;37:333

Daily intake of Chlorogenic Acids beverage decreased weight and waist

26

Summary-2: CGAs

From animal studies

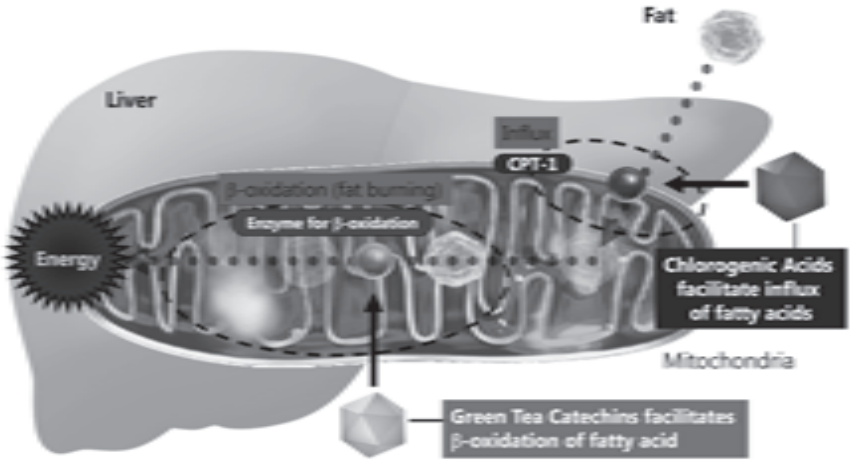
- **Coffee Chlorogenic Acids increased fat oxidation in mice partly due to suppress fat synthesizes in the liver**

From human studies

- **Repeated consumption of beverage rich in Coffee Chlorogenic Acids : increased DIT and fat oxidation after consuming diet enhanced loss of abdominal fat**

27

Chlorogenic Acids Increased Influx of Fatty Acids and Green Tea Catechins Increased β-oxidation Activities in Mitochondria



Murase T et al. *Am J Physiol Endocrinol Metab* 2011 300: E122–E133

28

Summary

Obesity
Blood Cholesterol
Fatty liver
Body fat utilization by Green Tea Catechins Coffee Chlorogenic Acids
Blood TG
Anti-Oxidation
Anti-inflammation
Blood Glucose
Blood Pressure

Consumption of Green Tea Catechins or Coffee Chlorogenic acids enhances body fat utilization, potentially leading to improve visceral fat obesity and metabolic syndrome

29

Acknowledgements

Kao Corporation
Dr. Ichiro Tokimitsu
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Mr. Tomonori Nagao
Mr. Ushio Harada
Dr. Kazuya Kozuma
Mr. Masao Takeshita
Dr. Hideto Takase
Mr. Takuya Watanabe
Dr. Yoshihisa Katsuragi

Kurume University
Dr. Sakata Ryuichiro
Dr. Nakamura Toru
Dr. Torimura Takuji
Dr. Ueno Takato
Dr. Sata Michio

Provident Clinical Research Lab
Dr. Kevin Maki
Dr. Mathew Reeves

Meridien Research
Dr. Mildred Farmer

30

セッション3

食事と脳・神経機能

セッション 3-1

老齢脳における栄養：最適な食事に関する科学的根拠を得るために
ー ILSI Europe の活動概況ー

ILSI Europe
Prof. Diána Bánáti

世界中のほとんど全ての国において、高齢者（例えば 60 歳以上）の人口比率はこれからの数十年間で急上昇すると見込まれている。しかし現在、どの主要な保健機関からも脳の健康や認知能力を維持するための予防的食事勧告は示されていない。また、そのような効果が科学的に立証されたヘルスクレームの表示を認められた食品や栄養素というものも、市場には存在しない。

近年、栄養が知的能力に及ぼす役割についての科学的証拠がかなり強化されてきている。ヒトは本質的に、食品や栄養素が脳機能や認知能力、知的能力に及ぼす影響には関心があるため、非常に多くの研究が成され、中にはマスメディアの高い関心を呼んでいる研究も存在する。神経発達を最大限にかなえ、認知能力低下のリスク低減を目指すにあたり、食品にはイノベーションの多くの可能性が秘められている。この発展途上の領域にあって、ILSI Europe の「栄養と知的能力」タスクフォースは、食事や食品成分が知的能力に及ぼす影響についての科学的知見を進展させ普及させるとともに、ヒトの生涯にわたる脳機能にとっての栄養の重要性をさらに広く知らしめることを目的として活動している。

ILSI Europe では、この分野の研究のために基本的なガイダンスを作成した。信頼のおける最新の学術論文では、方法論と評価に焦点が当てられ、子供や高齢者、あるいは一般の健常者といったさまざまなグループを対象として知的能力を測定している。 ILSI Europe はまた、食品や栄養素が脳に及ぼす影響について関心の高い研究者のための基礎作りや、幼少期の脳の発達に栄養が及ぼす影響をみることのできる脳イメージングといった新しい技術がもたらす可能性の世界的な普及に努めている。さらに、2014 年 7 月には「老齢脳における栄養」というワークショップを開催した。食事介入による認知機能維持の可能性を議論するために多くの著名な専門家が参加した。このワークショップの成功を受けて、2016 年に第 2 回のワークショップを開催し、栄養が認知能力に及ぼす効果についての最新の研究成果を取り扱う予定である。

ILSI Europe は栄養、食品安全、毒性学、リスクアセスメント、および環境分野での科学的根拠に基づいたコンセンサスを得るために、優秀な科学者どうしのコラボレーションを推進している。このコラボレーションを促進することにより、ILSI Europe は、公的私的にかかわらずさまざまなセクターに属する科学者たちが、彼らの優れた知識や視点を共有することで、複雑な科学的問題や健康問題にベストな取り組みができるよう支援している。

Session 3－1

Nutrition for the Ageing Brain: Towards Evidence of an Optimal Diet – An Overview of ILSI Europe's Activities –

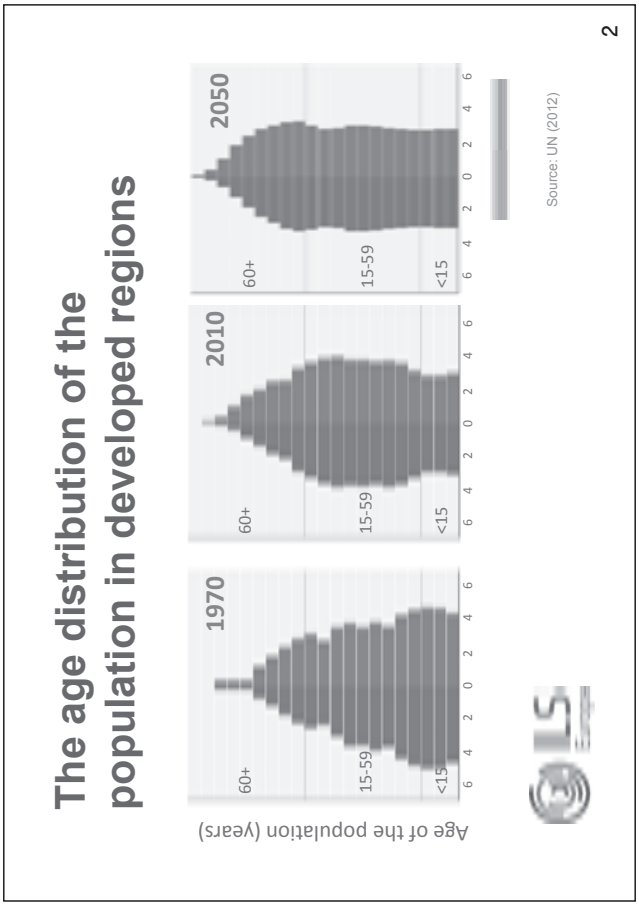
Prof. Diána Bánáti
ILSI Europe

The percentage of the aged population (e.g. 60 years and older) in almost every country will skyrocket in the next few decades. Currently, there are no preventive dietary recommendations for preserving brain health and cognition by any major health organisations. Nor are there foodstuffs or nutrients with scientifically substantiated health claims on the market.

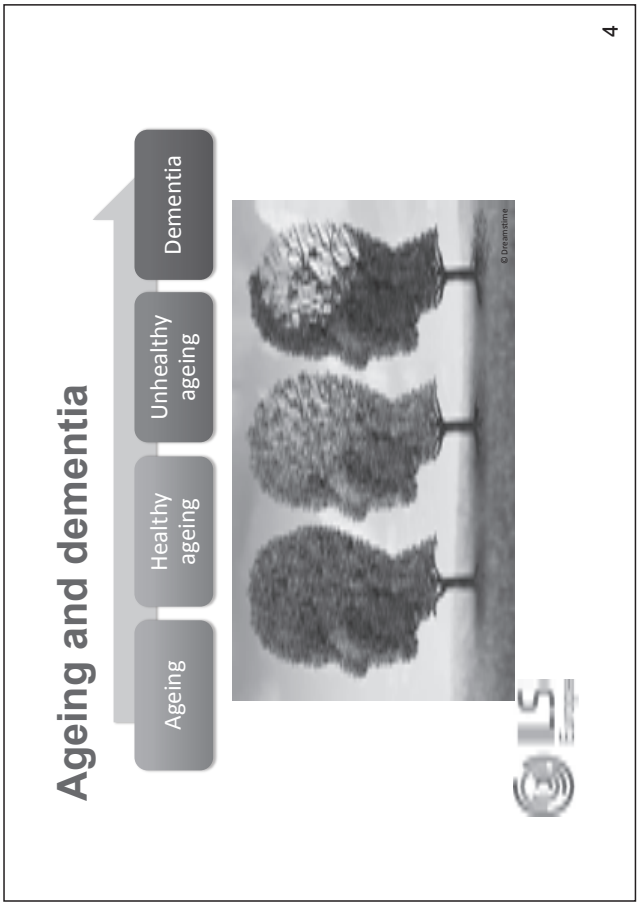
The evidence of a role for nutrition in mental performance has strengthened substantially in recent years. By their very nature people are interested in the impact of food or nutrients on brain function, cognition and mental performance, leading to numerous research studies alongside the accompanying media fascination. Great opportunities remain for food innovation to optimise neurodevelopment and to reduce risk of cognitive decline. In this developing field, the ILSI Europe Nutrition and Mental Performance Task Force aims to advance and disseminate scientific knowledge on the effects of diet and food components on mental performance, as well as to increase awareness of the importance of nutrition for brain functions across ones lifespan.

ILSI Europe has produced elemental guidance for research in this field. The latest peer-reviewed papers are focusing on methodologies and assessment, and covering measurement of mental performance in different population groups: children, the elderly, and the general healthy population. ILSI Europe has laid the groundwork for researchers interested in the brain effects of food and nutrients, and has disseminated globally the possibilities offered by emerging technologies, such as brain imaging, to indicate early effects of nutrition. In July 2014, we organised a workshop on 'Nutrition for the Ageing Brain' where numerous renowned experts were convened in order to discuss the potential for maintaining cognitive function via dietary interventions. Building on this successful event, ILSI Europe will organise the second workshop in July 2016 to address the latest breakthrough related to the effects of nutrition on cognitive health.

ILSI Europe fosters collaboration among the best scientists to provide evidence-based scientific consensus on the areas of nutrition, food safety, toxicology, risk assessment, and the environment. By facilitating their collaboration, ILSI Europe helps scientists from many sectors of society – public and private – to best address complex science and health issues by sharing their unique knowledge and perspectives.


The age distribution of the population in developed regions
1970
2010
2050
60+
15-59
<15
6 4 2 0 2 4 6
Age of the population (years)
Source: UN (2012)
ILSI Europe
2

Nutrition for the Ageing Brain:
Towards Evidence of an Optimal Diet
An overview of ILSI Europe's activities
Prof. Diána Bánáti
Executive and Scientific Director, ILSI Europe
ILSI Europe
International Life Sciences Institute
1


Ageing and dementia
Ageing
Healthy ageing
Unhealthy ageing
Dementia
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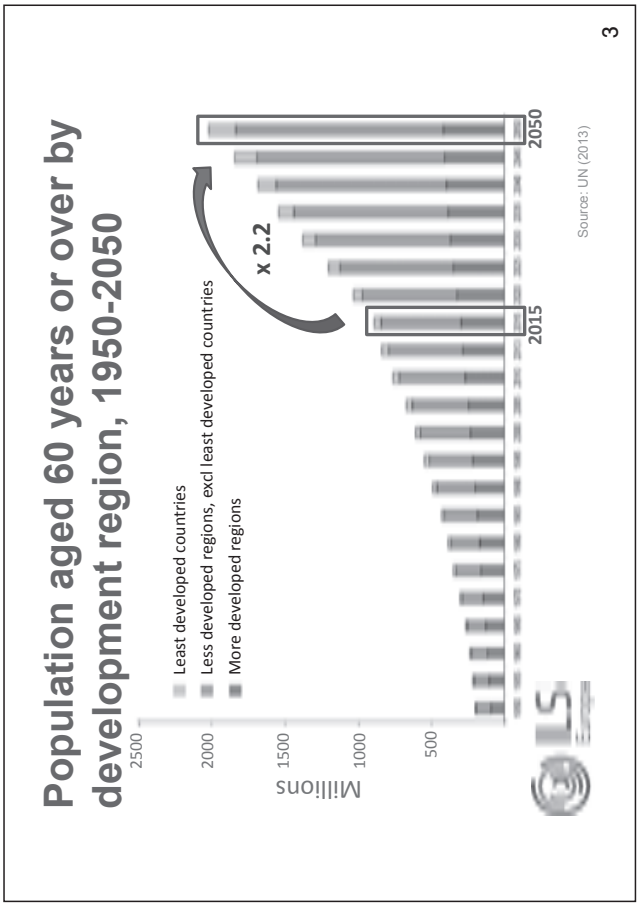
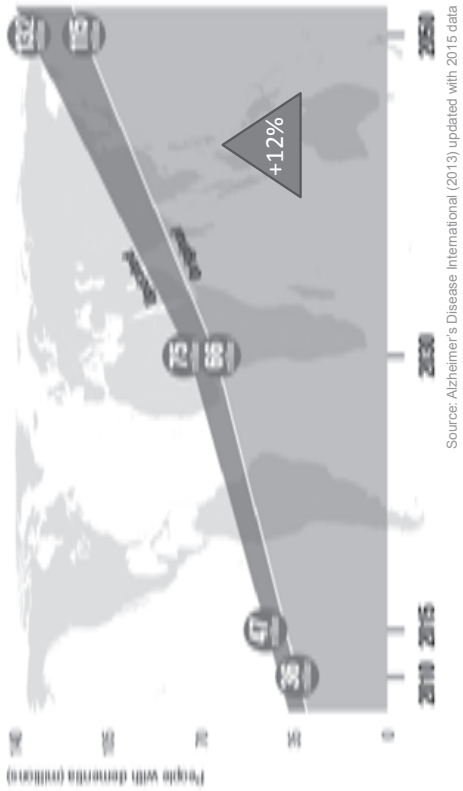
ILSI Europe
4


Population aged 60 years or over by development region, 1950-2050
Least developed countries
Less developed regions, excl least developed countries
More developed regions
x 2.2
2015
2050
Millions
2500
2000
1500
1000
500
Source: UN (2013)
ILSI Europe
3

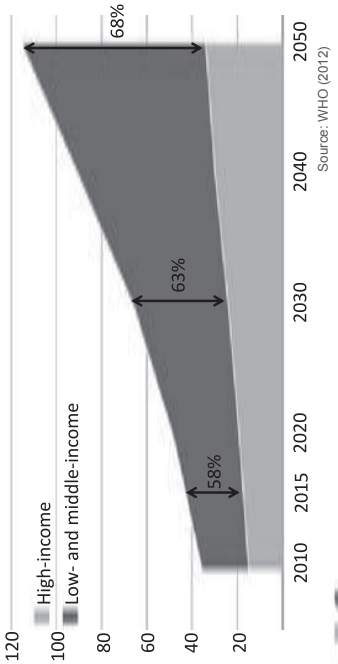
Growing number of people with dementia worldwide (2010-2050)



Source: Alzheimer's Disease International (2013) updated with 2015 data

5

Growing number of people with dementia



Source: WHO (2012)

6

Nutrition and mental performance

- Alzheimer's disease is the most commonly recognised cause of dementia in the ageing population.
- The cause of Alzheimer's disease is unknown but much is known about the changes in the brain.
- As currently no effective treatment exists, the focus has recently been shifting to modifiable risk factors of dementia.

7

Risk factors of healthy ageing

Non-modifyable risk factors	Intermediate risk factors	Modifyable risk factors
• Age • Family history • Genetics	• Cardiovascular disease • Obesity • Diabetes • Pulmonary disease • Mental illness	• **Unhealthy diet** • Physical inactivity • Tabacco/alcohol/drugs • Stress

8

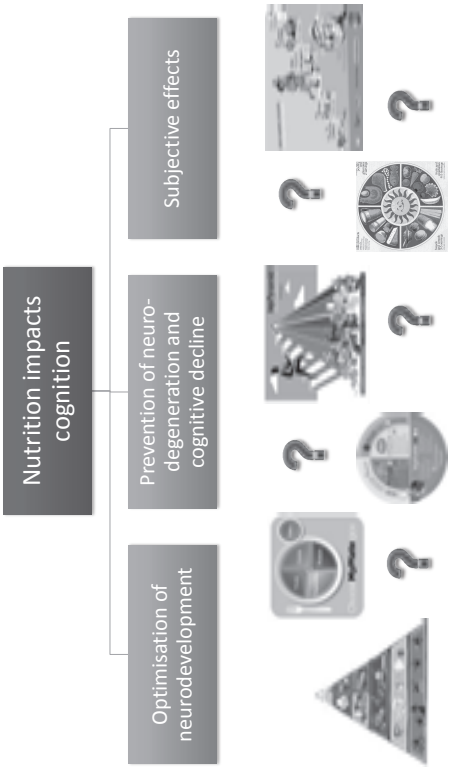
Nutrition and mental performance

- The interest in nutrition and mental performance has grown substantially in the recent years
- The evidence of a role for nutrition in mental performance has strengthened substantially in the recent years

But we still know very little

9

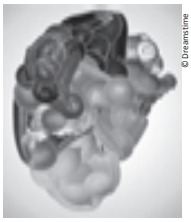
Nutrition and mental performance



10

Nutrition and Mental Performance Task Force

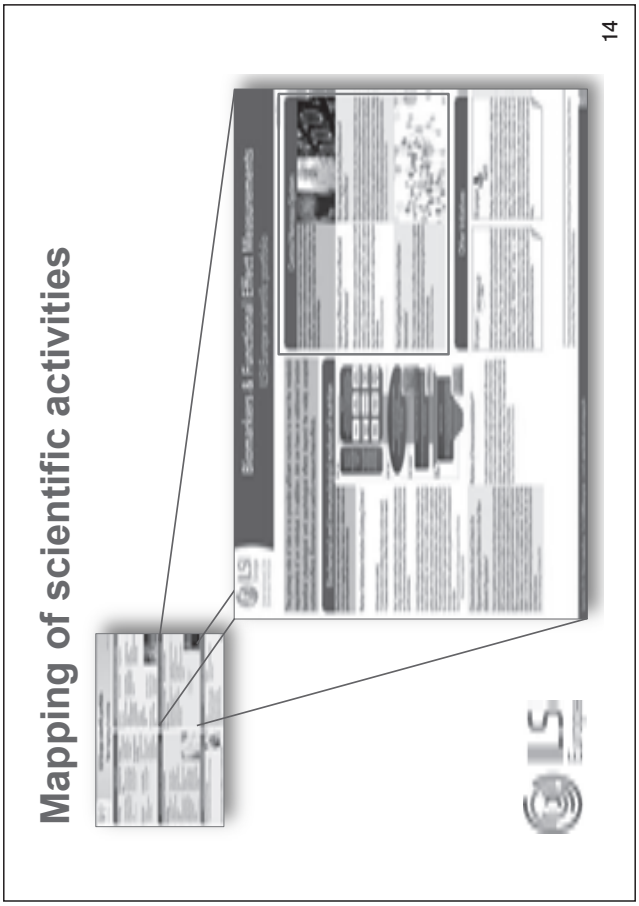
- The Nutrition and Mental Performance Task Force aims:
  - To advance and disseminate scientific knowledge on the effects of diet and food components on mental performance
  - To increase awareness of the importance of nutrition for brain function across the lifespan

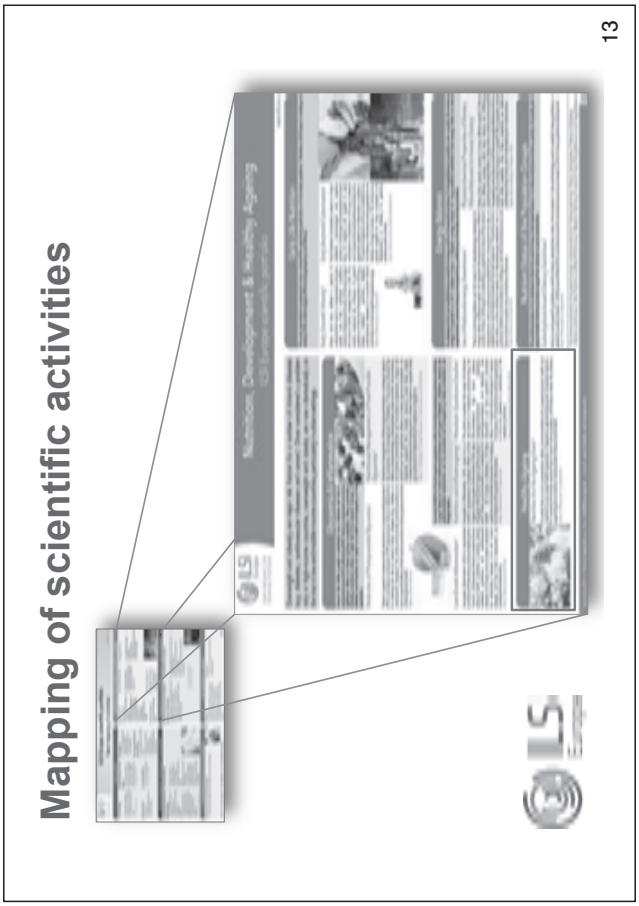


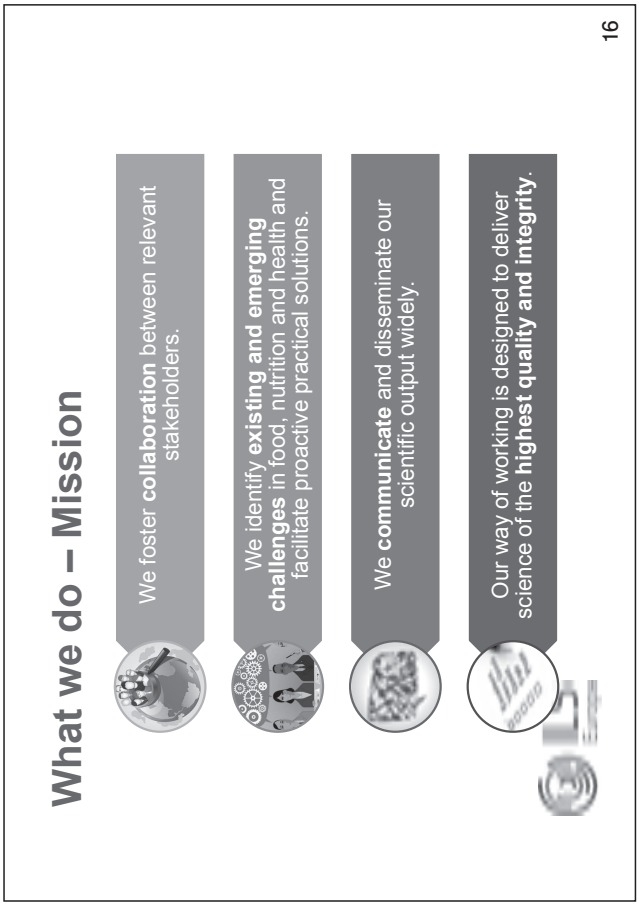
11

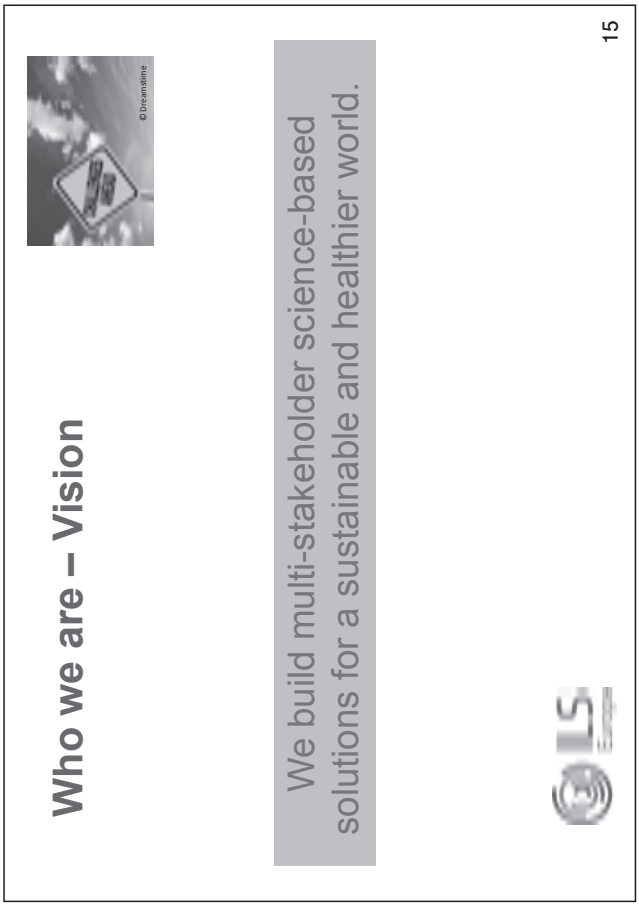
Mapping of scientific activities

12


Mapping of scientific activities
14


Mapping of scientific activities
13


What we do – Mission
We foster collaboration between relevant stakeholders.
We identify existing and emerging challenges in food, nutrition and health and facilitate proactive practical solutions.
We communicate and disseminate our scientific output widely.
Our way of working is designed to deliver science of the highest quality and integrity.
16


Who we are – Vision
© Dreamstime
We build multi-stakeholder science-based solutions for a sustainable and healthier world.
15

Driven by Science

- Science-driven approach
  - Stringent scientific review of our portfolio and new activities by SAC, BOD and external academic reviewers
- Scientific outputs
  - Peer-reviewed articles in high impact factor journals
  - Workshops and symposia with best scientists in the fields
  - ILSI Europe sessions at key conferences
- At least 50% academics in EGs, SAC and BOD
  - Close collaboration with key organisations (e.g. WHO, FAO, EC, national authorities, etc)
  - Wide network

- More than 400 publications
- More than 18,900 citations
- *h*-index 71

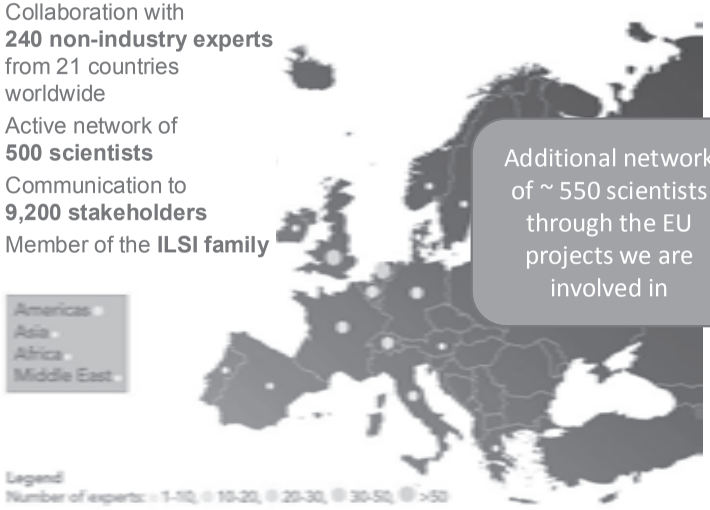
17

ILSI Key principles

18

A network of highly renowned experts

- Collaboration with **240 non-industry experts** from 21 countries worldwide
- Active network of **500 scientists**
- Communication to **9,200 stakeholders**
- Member of the **ILSI family**



Additional network of ~ 550 scientists through the EU projects we are involved in

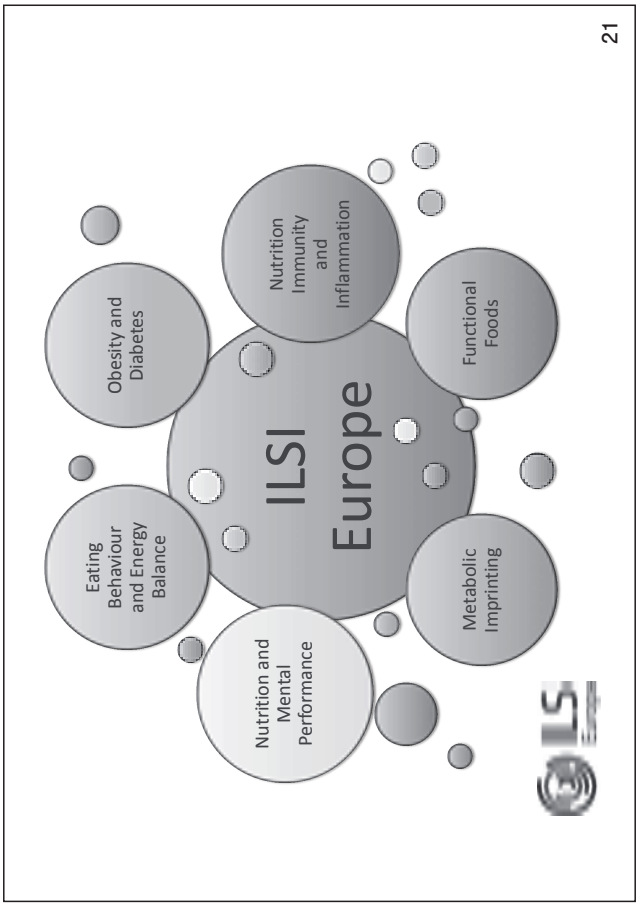
19

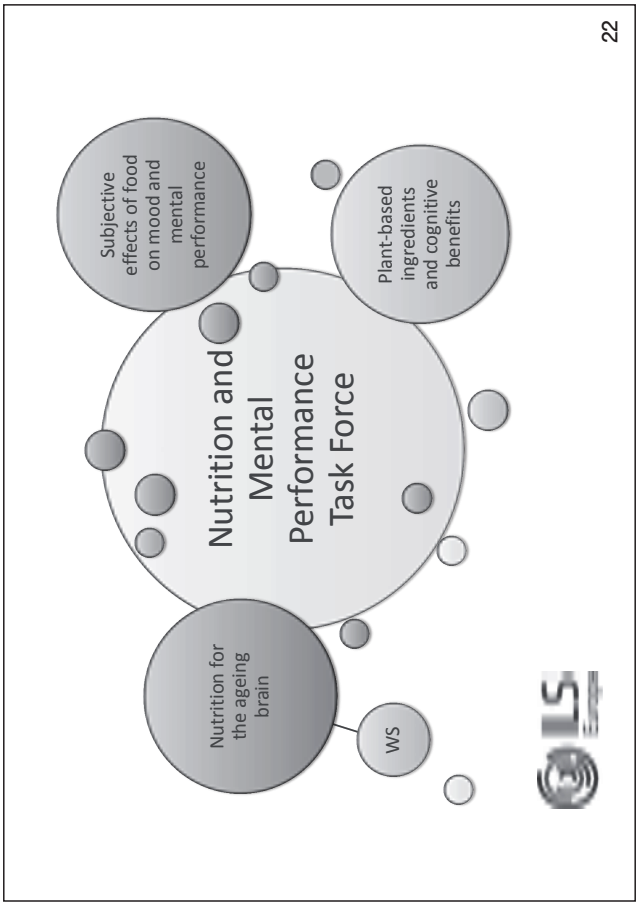
ILSI Europe Membership in 2015

55 Members

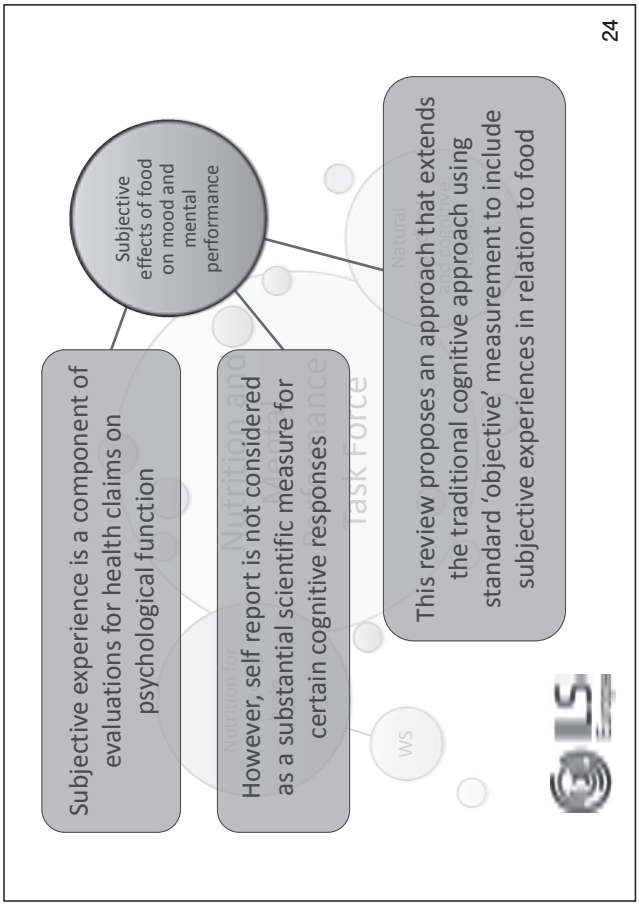
Abbott Nutrition
ADM – Rudolf Wild
Ajinomoto Europe
Arla Foods
Barilla G&R Fratelli
BASF SE
Bunge Europe
Cargill
Chr. Hansen
Coca-Cola Europe
Cosucra Groupe Warcoing
Danone
Dow Europe
DSM
DuPont de Nemours
Firmenich
General Mills
Givaudan International
Indoor Biotechnologies
Institut Mérieux
Johnson & Johnson EAME
Kao Corporation
Kikkoman Foods Europe
Lonza
Luigi Lavazza
Mars
McDonald's Europe
Mead Johnson Nutrition
Merck Consumer Healthcare
Mondelēz International
Monsanto Europe
Nestlé
Nexira
PepsiCo International
Pfizer Consumer Healthcare
Pierre Fabre Dermo Cosmétique
Premier Foods
Procter & Gamble
Puratos
Red Bull
Roquette Group
Royal FrieslandCampina
Sanofi-Aventis Group
Schwabegroup
Sealed Air
Sensus
Südzucker/BENEO Group
Swiss Quality Testing Services
Tate & Lyle Ingredients
Tereos
Tetra Pak Research
Ülker Bisküvi
Unilever
WALA Heilmittel
Yakult Europe

20


ILSI Europe
Obesity and Diabetes
Nutrition Immunity and Inflammation
Eating Behaviour and Energy Balance
Functional Foods
Nutrition and Mental Performance
Metabolic Imprinting
21

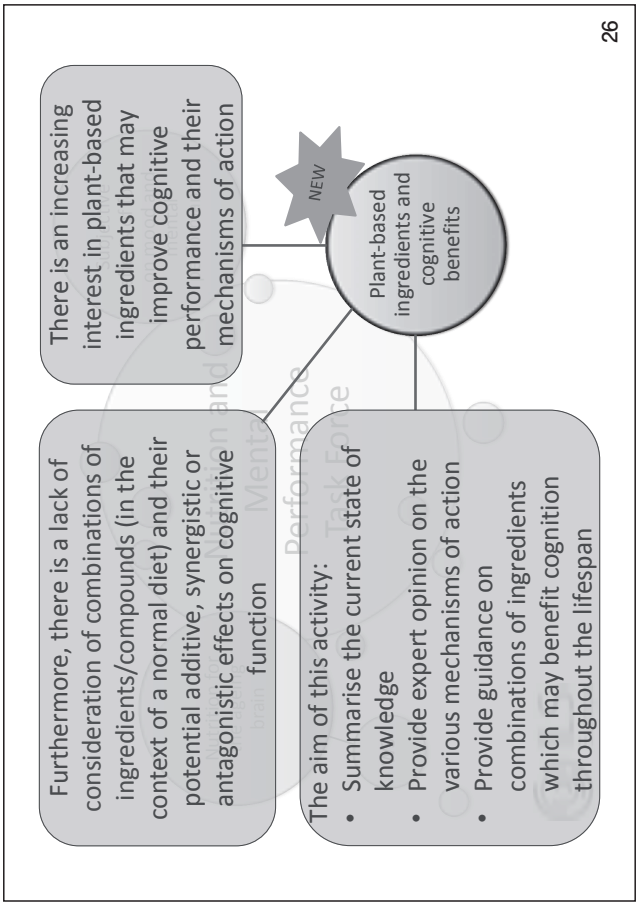

Subjective effects of food on mood and mental performance
Nutrition and Mental Performance Task Force
Plant-based ingredients and cognitive benefits
Nutrition for the ageing brain
WS
22

Subjective effects of food on mood and mental performance
Nutrition and Mental Performance Task Force
Pland-based ingredients and cognitive benefits
Nutrition for the ageing brain
WS
23


Subjective effects of food on mood and mental performance
Subjective experience is a component of evaluations for health claims on psychological function
However, self report is not considered as a substantial scientific measure for certain cognitive responses
This review proposes an approach that extends the traditional cognitive approach using standard 'objective' measurement to include subjective experiences in relation to food
WS
24

Subjective effects of food on mood and mental performance
Nutrition and Mental Performance Task Force
Nutrition for the ageing brain
WS
NEW
Plant-based ingredients and cognitive benefits
ILSI

25


There is an increasing interest in plant-based ingredients that may improve cognitive performance and their mechanisms of action
Furthermore, there is a lack of consideration of combinations of ingredients/compounds (in the context of a normal diet) and their potential additive, synergistic or antagonistic effects on cognitive function
NEW
Plant-based ingredients and cognitive benefits
The aim of this activity:
• Summarise the current state of knowledge
• Provide expert opinion on the various mechanisms of action
• Provide guidance on combinations of ingredients which may benefit cognition throughout the lifespan

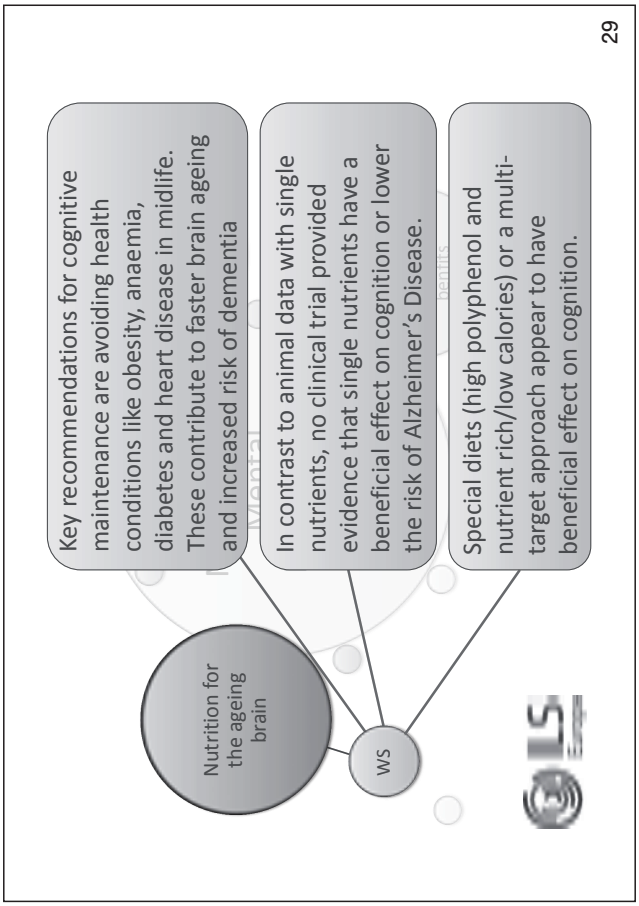
26

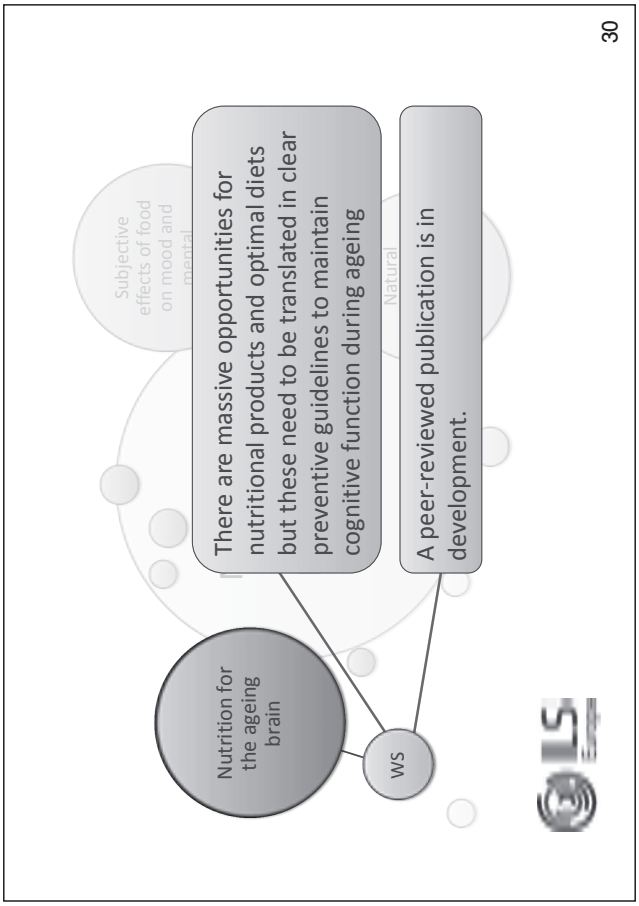
Subjective effects of food on mood and mental performance
Nutrition and Mental Performance Task Force
Plant-based ingredients and cognitive benefits
Nutrition for the ageing brain
WS
ILSI

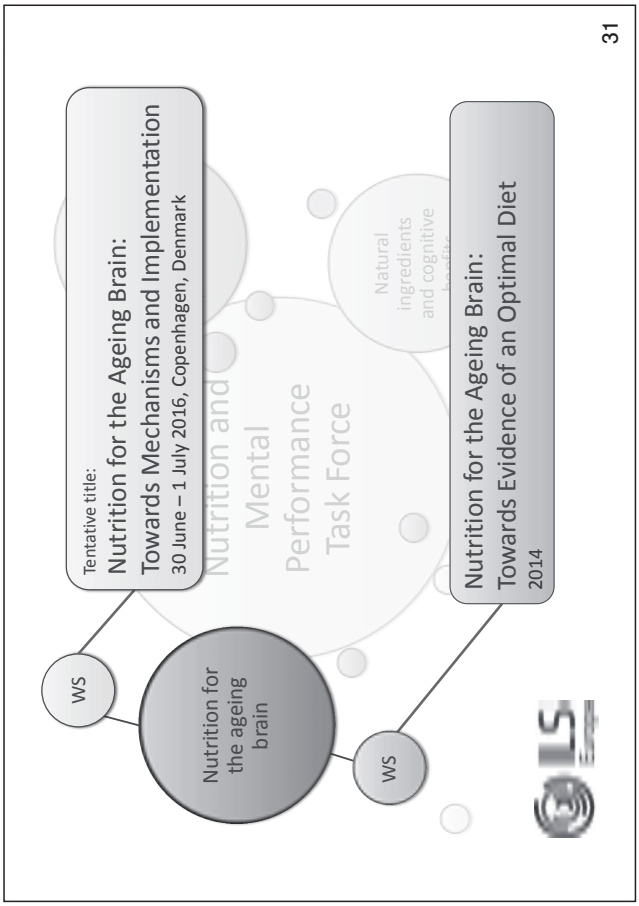
27

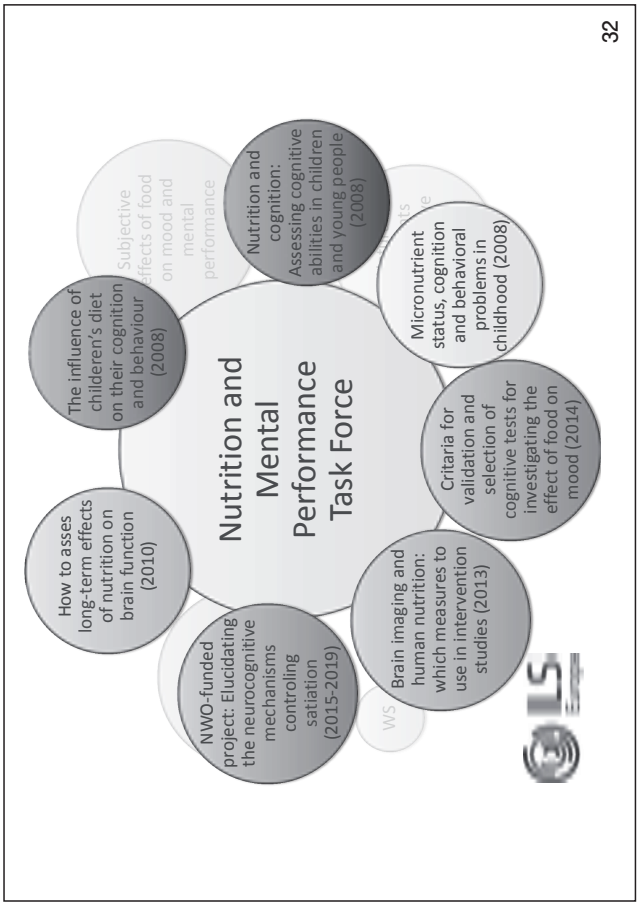
Workshop on 'Nutrition for the Ageing Brain: Towards Evidence for an Optimal Diet', July 2014, Milan, IT
Objectives of the workshop:
• Convene experts to debate the potential for maintaining cognitive function via dietary interventions
• Define mechanisms and timeframes of brain ageing and identify when neuroprotection via nutrition can begin
Nutrition for the ageing brain
WS
ILSI

28

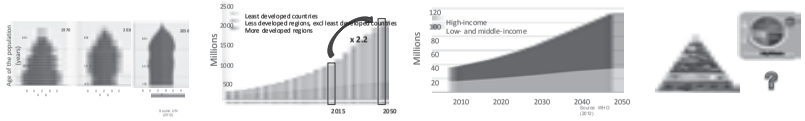

29
Key recommendations for cognitive maintenance are avoiding health conditions like obesity, anaemia, diabetes and heart disease in midlife. These contribute to faster brain ageing and increased risk of dementia
In contrast to animal data with single nutrients, no clinical trial provided evidence that single nutrients have a beneficial effect on cognition or lower the risk of Alzheimer's Disease.
Special diets (high polyphenol and nutrient rich/low calories) or a multi-target approach appear to have beneficial effect on cognition.
Nutrition for the ageing brain
WS


30
There are massive opportunities for nutritional products and optimal diets but these need to be translated in clear preventive guidelines to maintain cognitive function during ageing
A peer-reviewed publication is in development.
Nutrition for the ageing brain
WS


31
Tentative title:
Nutrition for the Ageing Brain:
Towards Mechanisms and Implementation
30 June – 1 July 2016, Copenhagen, Denmark
Nutrition and Mental Performance Task Force
Natural ingredients and cognitive benefits
Nutrition for the Ageing Brain:
Towards Evidence of an Optimal Diet
2014
Nutrition for the ageing brain
WS
WS


32
Nutrition and Mental Performance Task Force
Subjective effects of food on mood and mental performance
Nutrition and cognition: Assessing cognitive abilities in children and young people (2008)
Micronutrient status, cognition and behavioral problems in childhood (2008)
Critaria for validation and selection of cognitive tests for investigating the effect of food on mood (2014)
Brain imaging and human nutrition: which measures to use in intervention studies (2013)
NWO-funded project: Elucidating the neurocognitive mechanisms controling satiation (2015-2019)
How to asses long-term effects of nutrition on brain function (2010)
The influence of childeren's diet on their cognition and behaviour (2008)
WS

Future steps



- WHO recommendations (2012):
  - Making dementia a national public health and social care priority worldwide
  - Improving public and professional attitudes to, and understanding of, dementia
  - Improving access to education
- Neurodevelopment is a little understood area with significant long-term implications
  - More research needed to identify modifiable risk factors

33

Potential new activities of the Nutrition and Mental Performance Task Force

- New activities:
  - Follow-up workshop on 30 June-1 July 2016, Copenhagen, Denmark
  - A review of plant-based ingredients and their cognitive benefits with a focus on their likely mechanisms of action
- Potential new activities:
  - Nutrient interactions: implications for effects on brain function and research directions
  - Simple sugar consumption & mental performance

34

Nutrition and Mental Performance Task Force Members 2015

Dr Siobhan Mitchell – Chair	Nestlé	CH
Dr Caroline Saunders – Vice-Chair	PepsiCo International	UK
Prof. Keith Wesnes – Co-Chair	Northumbria University	UK
Ms Anja Holz	Südzucker/BENEO Group	DE
Dr Sophie Kergoat	Mars	US
Dr Tami Mackle	Pfizer Consumer Healthcare	US
Dr Hasan Mohajeri	DSM	CH
Dr María Ramírez	Abbott Nutrition	ES
Dr John Sijben	Danone	NL
Dr Berenike Stracke	Schwabegroup	DE
Dr David Vauzour	University of East Anglia	UK
Mr Jeroen Schuermans	ILSI Europe	BE

35

www.ilsi.eu

36

セッション 3-2

食事パターンと認知症の関係：久山町研究

九州大学大学院　医学研究院附属総合コホートセンター
二宮　利治

わが国は、4 人に 1 人が高齢者という超高齢社会を迎え、急増する認知症が大きな医療・社会問題となっている。厚生労働省が実施した認知症の全国調査では、2012 年時点での 65 歳以上における認知症の有病率は 15 ％で、全国の患者数は約 462 万人と推計された。その数は今後さらに増加し、2025 年には約 700 万人に達すると見込まれている。認知症の病型別にみると、アルツハイマー病（AD）の有病率が急速に上昇しているが、それを反映して医療機関を受診する AD 患者数も増加傾向にある。わが国の高齢人口は 2025 年以降も増加し続けると予測されているため、認知症の予防、治療、介護を含めた総合的な対策を講じることは喫緊の国民的課題となっている。この問題の抜本的な対策を講じる上で、疫学研究によって地域住民の認知症の実態を把握し、その危険因子・防御因子を明らかにすることが不可欠である。なかでも、食習慣の改善による認知症の予防が期待されている。海外の臨床・疫学研究の成績では、地中海式食事パターン（オリーブオイル、穀物、野菜、果物、ナッツ、豆、魚、鶏肉を中心とし、乳製品および赤肉を控える食事に少量のワイン）は AD の発症リスクを減少させるという報告が散見される。しかし、わが国には固有の食文化があり、海外の食習慣をそのまま国内に持ち込むことは容易ではない。そのため、認知症予防に有効なわが国固有の食事パターンを同定することは極めて重要である。

福岡県久山町では、地域住民を対象に 30 年以上にわたり継続している精度の高い認知症の前向きコホート研究を行っている。1988 年の久山町循環器病健診に参加した 60-79 歳の認知症のない久山町住民 1006 名を 17 年間前向きに追跡し、食事パターンが認知症発症に与える影響を検討した。食事パターン解析には reduced rank regression（RRR）解析を用いた。RRR 解析を用いて 7 つの食事パターンが導き出されたが、そのうちの第 1 食事パターン（DP1）には、大豆製品と豆腐、緑黄色野菜、淡色野菜、藻類、牛乳・乳製品、芋類、果実類、魚の摂取量が多く、米やアルコールの摂取が低いという特徴がみられた。この DP1 のスコアの 4 分位別にみた認知症発症のハザード比（性年齢調整）は、スコアの最も低い第 1 分位群に比べて第 4 分位群で 0.66（95 ％信頼区間［CI］0.47-0.94）と有意に低かった。認知症の病型別にみると、第 4 分位群の第 1 分位群に対するハザード比は、アルツハイマー病では 0.62（95 % CI 0.39-0.99）、脳血管性認知症では 0.48（95 %CI 0.24-0.93）であった。多変量調整後も同様の傾向を認めた。

将来の認知症を予防するためには、高血圧や糖尿病、喫煙などの危険因子の予防・管理に加え、食習慣にも留意することが重要である。特に、認知機能障害が顕性化していない健康な時から適切な食習慣と生活習慣を心がけ、脳保護を早期に開始することが、認知症予防対策において有効であると考えられる。食事性因子と認知症との因果関係を明らかにするために、質の高い前向きコホート研究や介入研究による更なる研究が必要である。

Session 3-2

The Relationship between Dietary Pattern and Dementia: The Hisayama Study

Toshiharu Ninomiya, M.D., Ph.D.
Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu University

Dementia is a syndrome that affects memory, thinking, behavior and ability to perform everyday activities. The prevalence of dementia has increased rapidly over the past two decades in Japan, with approximately 15% of people aged ≥ 65 years in 2012. The shift from a traditional Japanese diet toward a Western diet may be related to the rapid rise in the burden of dementia in Japan. The effect of diet on the onset of dementia is of scientific and public interest. In Western countries, several epidemiological studies have reported that a higher adherence to a Mediterranean dietary pattern is associated with a reduced risk of the incidence of dementia. On the other hand, a Mediterranean diet is very different from a traditional Japanese diet. Therefore, it is important to determine some dietary patterns that could help to reduce the burden of dementia specifically in the population of Japan. However, there are few epidemiological studies investigating this issue in a Japanese population.

The Hisayama study, which is a prospective longitudinal study exploring the risk factors for cardiovascular disease and dementia, evaluated the relation between dietary patterns and the risk of dementia in 1,006 community-dwelling Japanese individuals without dementia aged 60-79 years during a 17-year follow-up. Dietary patterns associated with the risk of dementia were determined by using a reduced rank regression analysis, resulting 7 dietary patterns were extracted. Among them, a higher score of dietary pattern 1 (DP1) was characterized by a high intake of soybeans and soybean products, green vegetables, other vegetables, algae and milk and dairy products, potatoes, fruits and fruit juices and fish and a low intake of rice and alcohol. Individuals with higher adherence to DP1 had 34% (95% confidence interval [CI] 6-53%) lower risk of dementia after adjusting for age and sex. With regard to subtypes of dementia, individuals with the highest quartile of scores for DP1 had a significantly lower risk of either Alzheimer's disease (hazard ratio 0.62, 95%CI 0.39-0.99) or vascular disease (hazard ratio 0.48, 95%CI 0.24-0.93). These associations were not substantially altered after adjusting for potentially confounding factors.

Growing evidence suggests that it is important to pay attention to dietary habits as well as the prevention and amelioration of risk factors such as hypertension, diabetes and smoking for reducing the societal burden of dementia in the future. Especially, it is crucial to begin protecting the brain before any cognitive impairment becomes manifest. Primary prevention of dementia with favorable dietary habits and lifestyles may be appropriate strategy to apply for healthy people without any cognitive symptom. Further researches, especially well-planned prospective cohort studies or intervention trials, are warranted to establish a causative role of specific nutrients, foods, and dietary patterns for the prevention of dementia.

The 7th international Conference on Nutrition and Aging

The relationship between dietary pattern and dementia: the Hisayama Study

Toshiharu Ninomiya

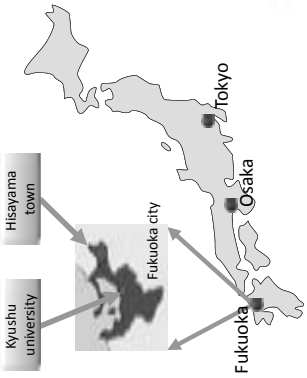
Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu University

Sep 30 (Wed), 2015
Ichijo-Hall,
Yayoi auditorium
University of Tokyo

1

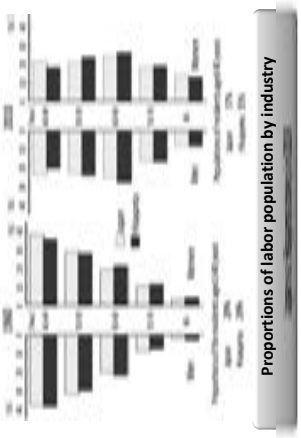
Population of Hisayama town

Location of Hisayama town

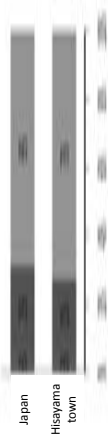


Year	1960	2010
Hisayama town	6,500	8,400
Fukuoka city	650,000	1,430,000
Japan	93 million	128 million

Age-distribution
Japan vs. Hisayama town, in 1960 and 2010



Proportions of labor population by industry



2

Subjects and Methods

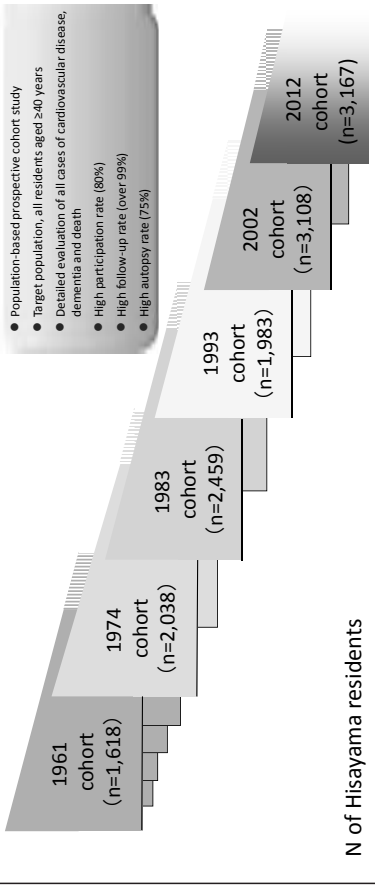
Participants	:	1,006 residents in Hisayama town, aged 60-79 years or older in 1988. (Excluded : subjects with dementia, no blood sample and dietary information)
Study design	:	Prospective cohort study for 17 years (December 1988 to November 2005)
Assessment of food intakes	:	70-item semi-quantitative food frequency questionnaire (Intakes of each food were adjusted for energy intake by using the nutrient density method)
Outcomes	:	Total dementia: 271 subjects Alzheimer's disease: 144 subjects Vascular dementia : 88 subjects
Confounders	:	Age, sex, education level, hypertension, diabetes, serum total cholesterol, body mass index, history of stroke, smoking habits, exercise, and regular energy intake
Statistical analyses	:	Dietary patterns: Reduced rank regression analysis Hazard ratio: Cox proportional hazards model

Ozawa M, et. al. Am J Clin Nutr 97:1076, 2013

4

The Hisayama Study

- Population-based prospective cohort study
- Target population, all residents aged ≥40 years
- Detailed evaluation of all cases of cardiovascular disease, dementia and death
- High participation rate (80%)
- High follow-up rate (over 99%)
- High autopsy rate (75%)



3

Pearson's correlation coefficients between nutrients and extracted dietary patterns

Hisayama 1,006 residents aged 60-79 years, 1988

Nutrients	Dietary patterns						
	1	2	3	4	5	6	7
Saturated fatty acid	0.50*	0.49*	0.51*	0.02	0.06	0.11*	0.04
Monounsaturated fatty acid	0.57*	0.58*	0.08*	0.34*	-0.06	-0.13*	-0.07
Polyunsaturated fatty acid	0.59*	0.45*	-0.50*	0.16*	0.08	0.07	0.05
Vitamin C	0.51*	-0.70*	0.10	0.35*	-0.02	-0.06	0.10
Potassium	0.84*	-0.44*	-0.04*	0.07	-0.10	0.14*	-0.09
Calcium	0.84*	0.16*	-0.05*	-0.42*	-0.15*	-0.06	0.06
Magnesium	0.84*	-0.30*	0.03	-0.21*	0.21*	-0.07	-0.05

Ozawa M, et. al. Am J Clin Nutr 97:1076, 2013

5

Factor loadings of food groups associated with a dietary pattern favorable for the prevention of dementia

Hisayama 1,006 residents aged 60-79 years, 1988

Food groups	Factor loadings (Dietary pattern 1)
Rice	**-0.45**
Breads	0.10
Noodles and other cereals	0.01
Potatoes	0.16
Soybeans and soybean products	**0.37**
Miso	0.01
Pickles	0.04
Green vegetables	**0.40**
Other vegetables	**0.36**
Fruits and fruit juices	0.19
Algae	**0.24**
Fish	0.17
Meat	0
Egg	0.15
Milk and dairy products	**0.37**
Fats and oils	0.12
Sugar and confection	-0.10
Alcoholic drinks	-0.17
Salt	-0.008

- Factor loadings represent the magnitude and direction of the contribution of each food group to a dietary pattern 1 score.
- A positive value of factor loading indicates an increased intake of the food group. A negative value of factor loading indicates a reduced intake of the food group.

Ozawa M, et. al. Am J Clin Nutr 97:1076, 2013

6

Effect of diet on risk of total dementia

Hisayama 1,006 residents, aged 60-79 years,1988-2005, multivariable-adjusted

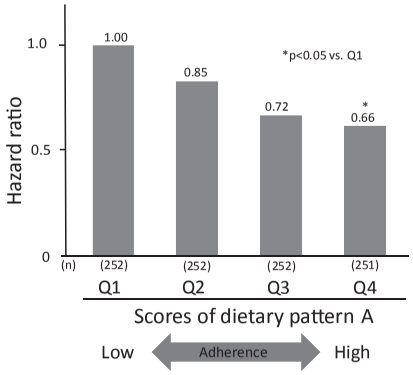
People adhere to dietary pattern A had a significantly lower risk of dementia

Dietary pattern A

Higher intake	Less intake
Soybean products	**Rice**
Vegetable	**Alcohol**
Algae	
Milk and dairy products	
Fruits	
Potato	
Fish	
Eggs	

Amounts of each food intake was corrected by total energy intake using a nutritional density method.

Balance of food groups are more important rather than amounts.



Adjusted for age, sex, education level, hypertension, diabetes, serum total cholesterol, body mass index, history of stroke, smoking habits, exercise, and energy intake

Ozawa M, et. al. Am J Clin Nutr 97:1076, 2013

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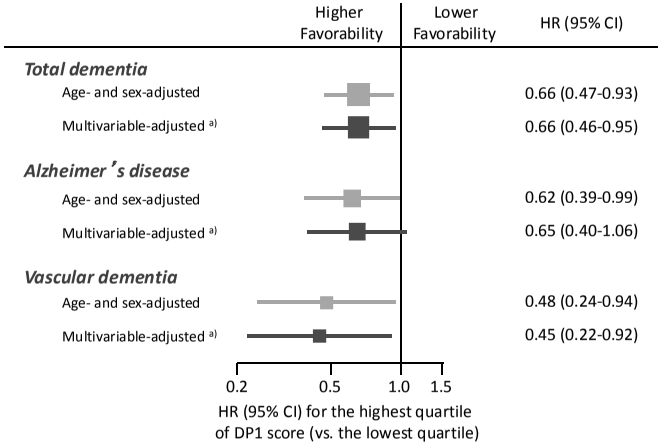
Effect of dietary pattern 1 on risk of dementia subtypes

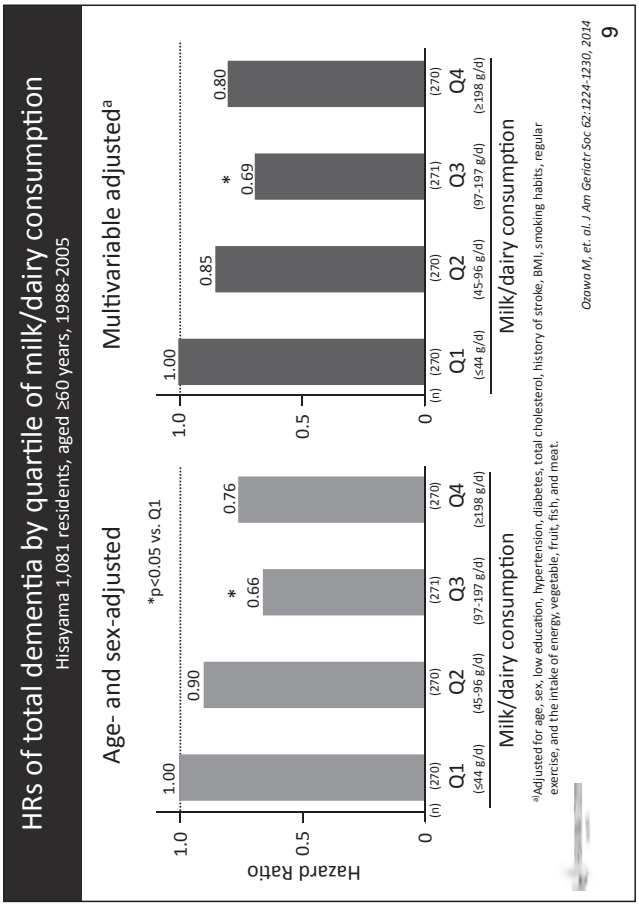
Hisayama 1,006 residents, aged 60-79 years, 1988-2005, multivariable-adjusted

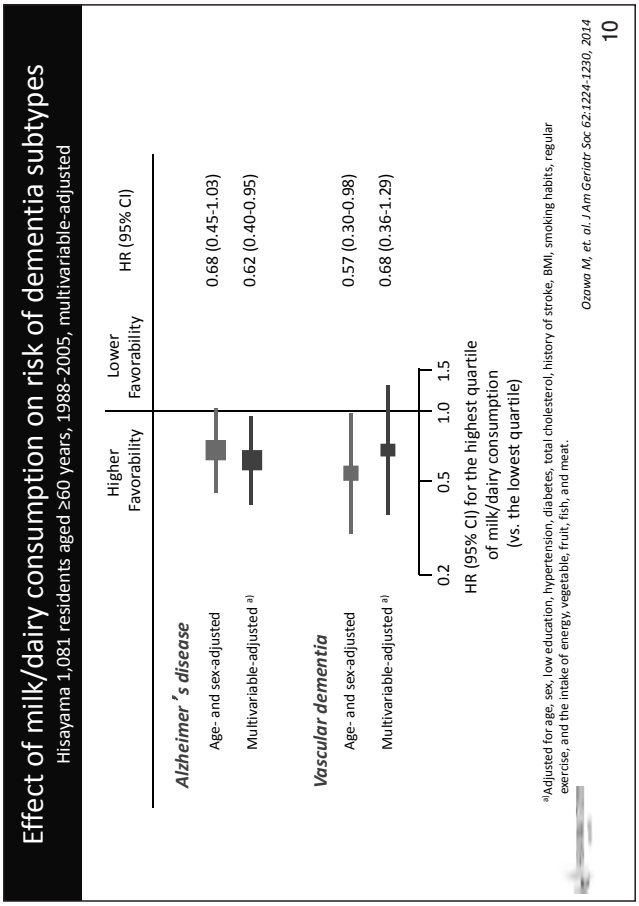


a) Adjusted for age, sex, education level, hypertension, diabetes, serum total cholesterol, body mass index, history of stroke, smoking habits, exercise, and energy intake

Ozawa M, et. al. Am J Clin Nutr 97:1076, 2013

8


HRs of total dementia by quartile of milk/dairy consumption
Hisayama 1,081 residents, aged ≥60 years, 1988-2005
Age- and sex-adjusted
Multivariable adjusted[a]
*p<0.05 vs. Q1
Hazard Ratio
1.0
0.5
0
1.00
0.90
0.66
0.76
1.00
0.85
0.69
0.80
(n) (270) (270) (271) (270)
Q1 Q2 Q3 Q4
(≤44 g/d) (45-96 g/d) (97-197 g/d) (≥198 g/d)
Milk/dairy consumption
[a]Adjusted for age, sex, low education, hypertension, diabetes, total cholesterol, history of stroke, BMI, smoking habits, regular exercise, and the intake of energy, vegetable, fruit, fish, and meat.
Ozawa M, et. al. J Am Geriatr Soc 62:1224-1230, 2014
9


Effect of milk/dairy consumption on risk of dementia subtypes
Hisayama 1,081 residents aged ≥60 years, 1988-2005, multivariable-adjusted
Higher Favorability
Lower Favorability
HR (95% CI)
Alzheimer's disease
Age- and sex-adjusted
0.68 (0.45-1.03)
Multivariable-adjusted [a]
0.62 (0.40-0.95)
Vascular dementia
Age- and sex-adjusted
0.57 (0.30-0.98)
Multivariable-adjusted [a]
0.68 (0.36-1.29)
0.2
0.5
1.0
1.5
HR (95% CI) for the highest quartile of milk/dairy consumption (vs. the lowest quartile)
[a]Adjusted for age, sex, low education, hypertension, diabetes, total cholesterol, history of stroke, BMI, smoking habits, regular exercise, and the intake of energy, vegetable, fruit, fish, and meat.
Ozawa M, et. al. J Am Geriatr Soc 62:1224-1230, 2014
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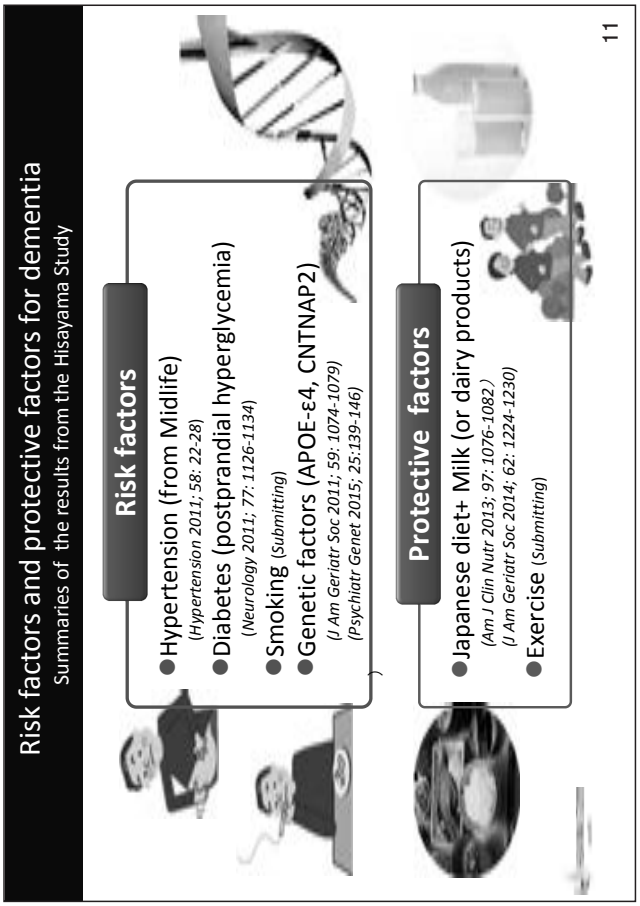

Risk factors and protective factors for dementia
Summaries of the results from the Hisayama Study
Risk factors
●Hypertension (from Midlife)
(Hypertension 2011; 58: 22-28)
●Diabetes (postprandial hyperglycemia)
(Neurology 2011; 77: 1126-1134)
●Smoking (Submitting)
●Genetic factors (APOE-ε4, CNTNAP2)
(J Am Geriatr Soc 2011; 59: 1074-1079)
(Psychiatr Genet 2015; 25:139-146)
Protective factors
●Japanese diet+ Milk (or dairy products)
(Am J Clin Nutr 2013; 97: 1076-1082)
(J Am Geriatr Soc 2014; 62: 1224-1230)
●Exercise (Submitting)
11

セッション 3-3

食による体内時計の制御を目指した時間栄養学研究

国立研究開発法人　産業技術総合研究所
バイオメディカル研究部門　生物時計研究グループ　研究グループ長
大石　勝隆

サーカディアンリズムの乱れは、うつ病や睡眠障害などの精神疾患や、肥満・糖尿病、高血圧、メタボリックシンドロームなどの生活習慣病の原因となっている。我々は、睡眠・覚醒リズムの乱れや睡眠の断片化、行動リズムの乱れなどを特徴とするストレス性の睡眠障害モデルマウスの開発に成功した。本モデルマウスにおいては、血中の遊離アミノ酸プロファイルや腸内フローラが大きく影響を受けている一方で、体内時計の制御に必須な役割を担っている時計遺伝子の発現リズムは正常であった。また、本モデルマウスにおいて睡眠障害を長期間継続することにより、非肥満性の耐糖能異常が誘発されることも判明した。本モデルマウスは、心理的ストレスによる睡眠障害や関連疾患の発症メカニズムの解明や、睡眠障害のバイオマーカーの開発、睡眠障害や生体リズムの乱れを改善するための食品成分の探索を行っている。

一方我々は、生体リズムを積極的に制御するための天然化合物の探索を目指し、PER2::LUC マウス（時計遺伝子 *Per2* の下流にホタルルシフェラーゼ遺伝子を導入し、PER2::LUCIFERASE 融合タンパク質を産生するマウス）胎仔脳より作製した神経細胞を用いて、リアルタイム体内時計レポーターシステムを構築した。本システムを用いて、体内時計や睡眠に作用する食品由来成分や乳酸菌、生薬由来化合物等のスクリーニングを行っており、本講演においては、その成果について紹介する。

Session 3−3

Chrono-nutrition Research Aimed at Biological Clock Regulation

Katsutaka Oishi, Ph.D.
Group Leader, Biological Clock Research Group, Biomedical Research Institute,
National Institute of Advanced Industrial Science and Technology（AIST）

Circadian rhythm disruption causes various psychological and physiological disorders such as depression, obesity, diabetes and sleep disorders. We established a mouse model of a psychophysiological stress-induced chronic sleep disorder（CSD）characterized by hyperphagia, sleep fragmentation, disrupted circadian sleep-wake cycles, and unusual wheel-running activity. Plasma free amino-acid profiles and the intestinal microbiota were extremely disrupted in the mice with CSD, whereas the circadian expression of clock genes was not altered. We found that long term CSD caused glucose intolerance without obesity. Using this mouse model, we are trying to elucidate the underlying mechanisms of stress-induced sleep disorders and related diseases, to identify biomarkers for sleep disorders, and to screen dietary supplements that could improve sleep disorders.

To find natural compounds that could modulate the biological clock, we also developed a real-time molecular clock reporter system using neuronal cells derived from PER2::LUC mouse embryos. I will discuss the expectations of the regulation of sleep and circadian rhythms by dietary nutrients, *Lactobacillus* strains, and herbal medicine-derived natural compounds.

AIST

ILSI Japan 2015
September 30, 2015

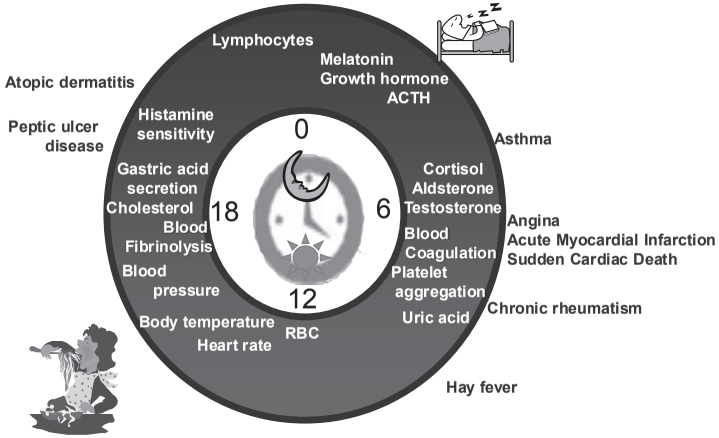
Chrono-nutrition Research Aimed at Biological Clock Regulation

食による体内時計の制御を目指した時間栄養学研究

国立研究開発法人　産業技術総合研究所（AIST）
大石 勝隆
Katsutaka OISHI
E-mail: k-ooishi@aist.go.jp

AIST

24 h rhythms in physiological functions and diseases

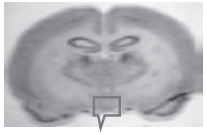


AIST

SCN is the central clock in mammals

Circadian rhythms in physiology

- ✧ Sleep-Wake cycle
- ✧ Blood pressure
- ✧ Heart rate
- ✧ Hormone secretion
- ✧ Neural activity
- ✧ Lipid metabolism
- ✧ Glucose metabolism
- ✧ Cell cycle
- ✧ Immune functions



SCN
8000 X 2 = 16000 neurons

AIST

Circadian clock genes

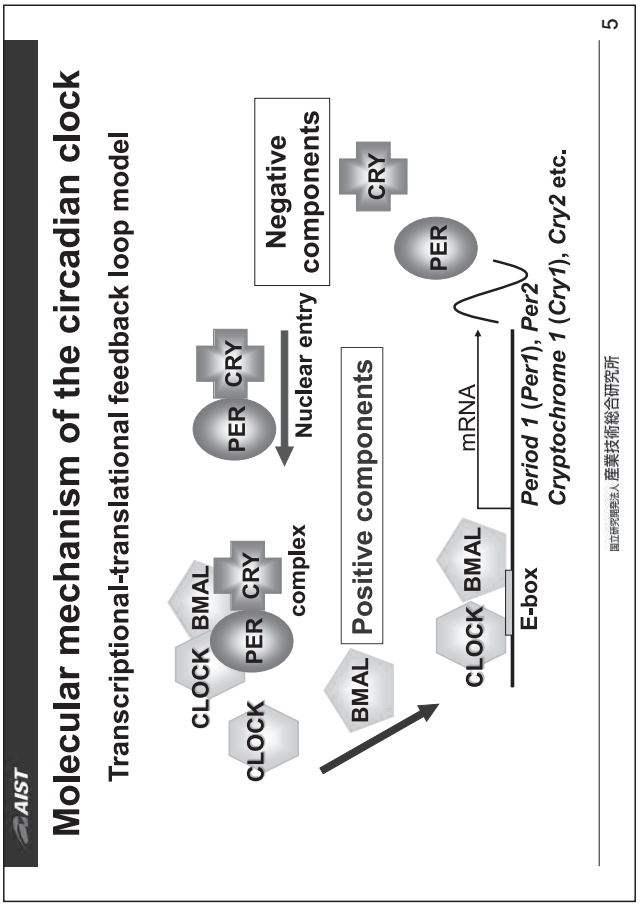
Drosophila

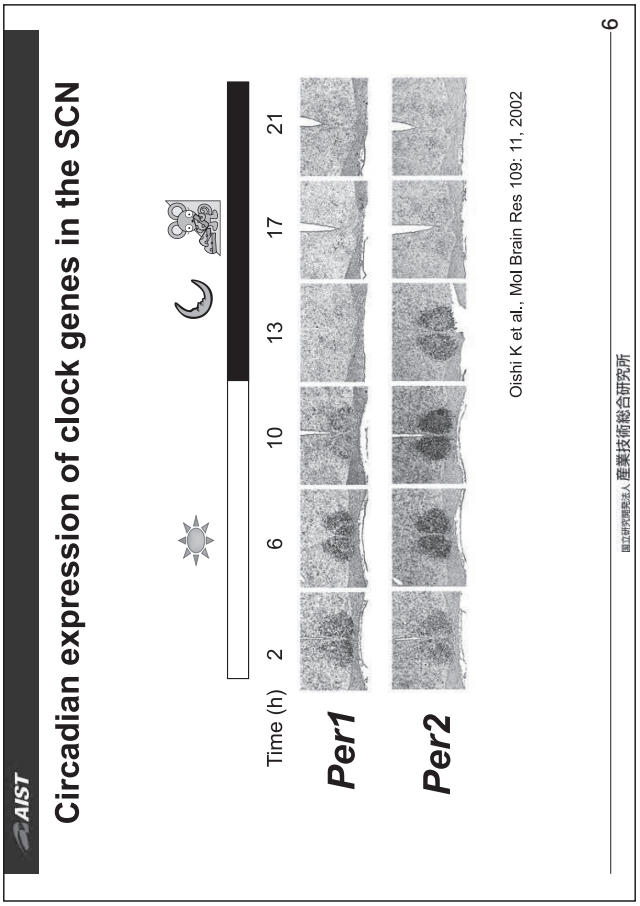
Period
Timeless
Clock
BMAL
Cryptochrome

Highly conserved

Period1
Period2
Clock
BMAL1
Cryptochrome1
Cryptochrome2

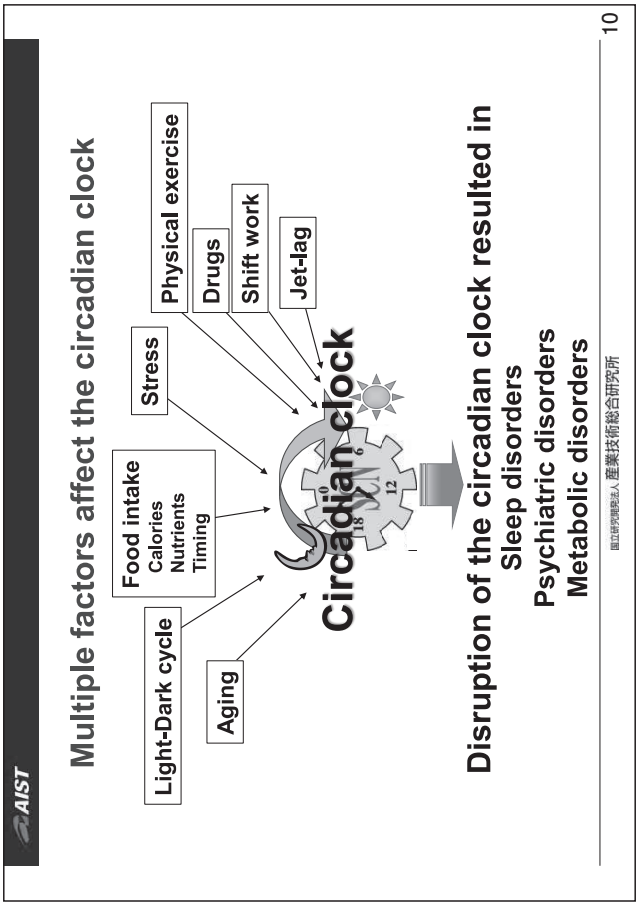
Mouse
Human

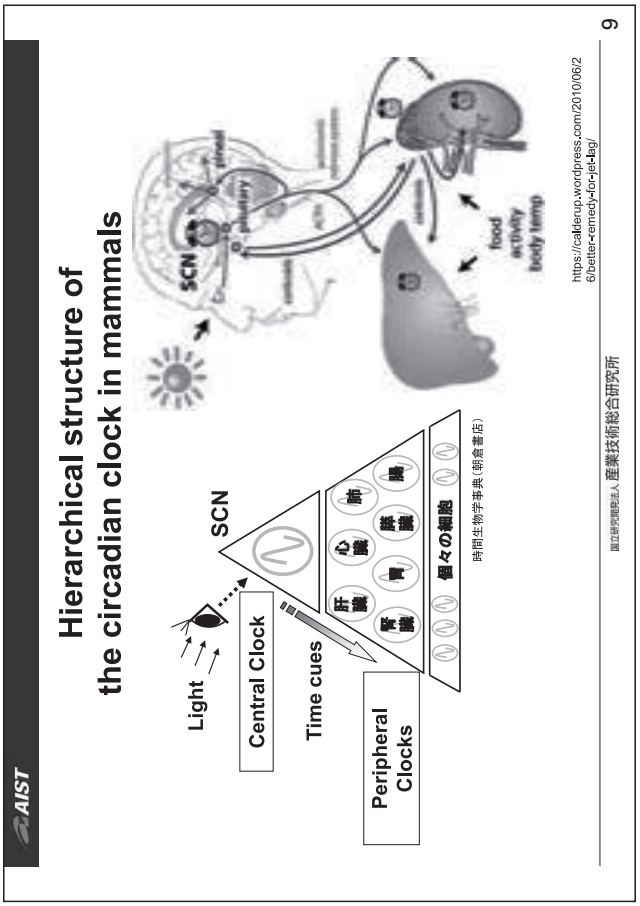

AIST
Molecular mechanism of the circadian clock
Transcriptional-translational feedback loop model
CLOCK
BMAL
PER
CRY
complex
Nuclear entry
Negative components
Positive components
mRNA
E-box
Period 1 (Per1), Per2
Cryptochrome 1 (Cry1), Cry2 etc.
5

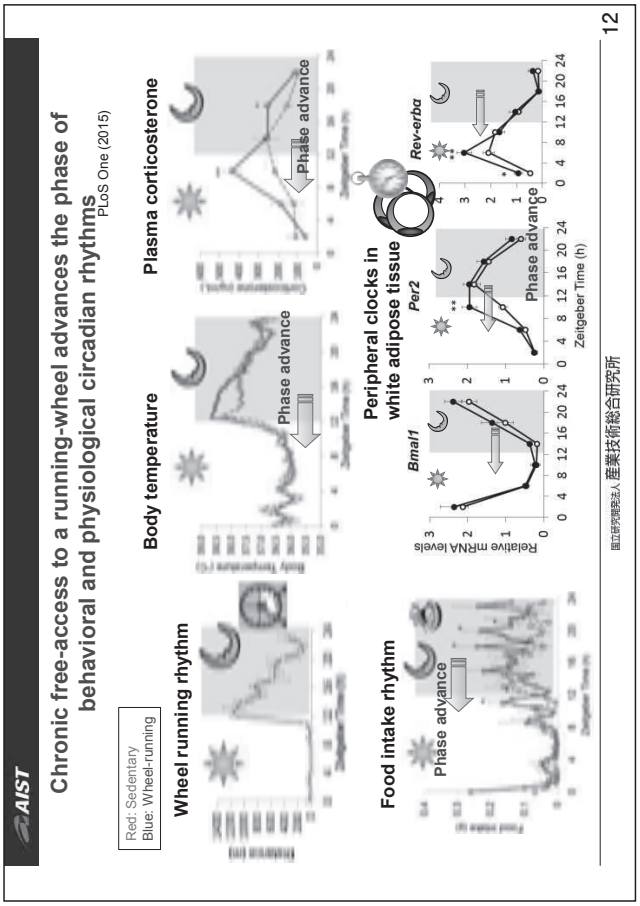

AIST
Circadian expression of clock genes in the SCN
Time (h)
2
6
10
13
17
21
Per1
Per2
Oishi K et al., Mol Brain Res 109: 11, 2002
6

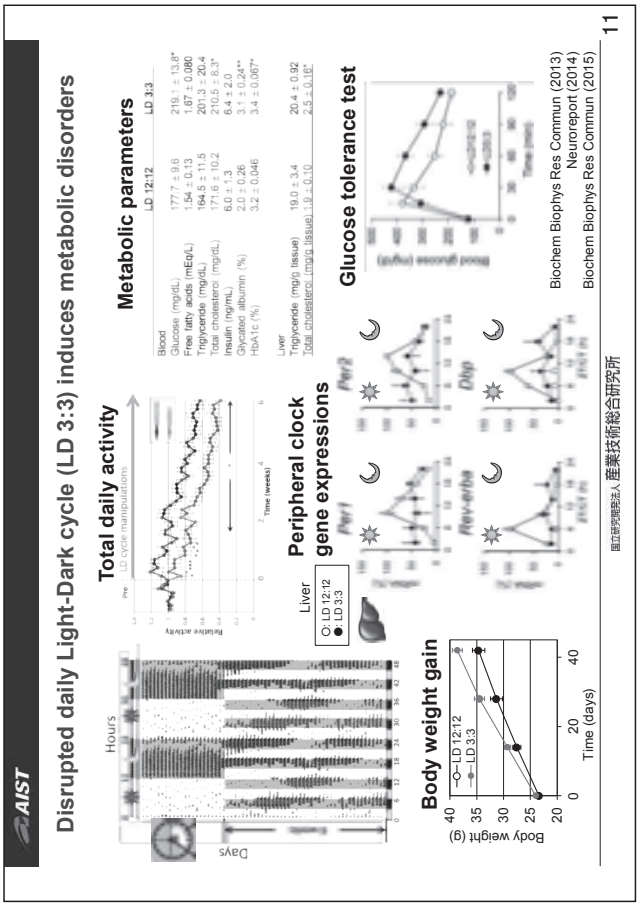
AIST
Circadian expression of clock genes in peripheral tissues
Circadian clock exists in peripheral tissues
Peripheral clocks
Time (h)
2 5 8 11 14 17 20 23
Lung
Heart
Kidney
Per2
Bmal1
Oishi K et al., BBRC 253: 199, 1998
7

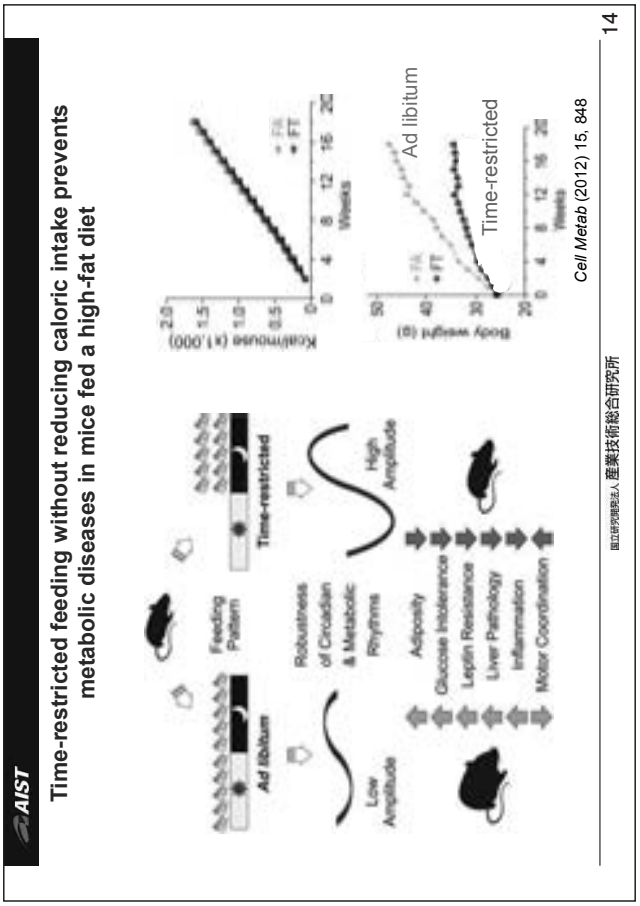
AIST
A number of circadian genes in peripheral tissues
Microarray analysis
In the mouse liver
High
Low
mRNA level
Nocturnal circadian genes
Diurnal circadian genes
Oishi et al., DNA Res 2005
Pck1
Gck
Slc2a2
Gys2
Hmgcr
Cyp7a1
mRNA (%)
Time (h)
8

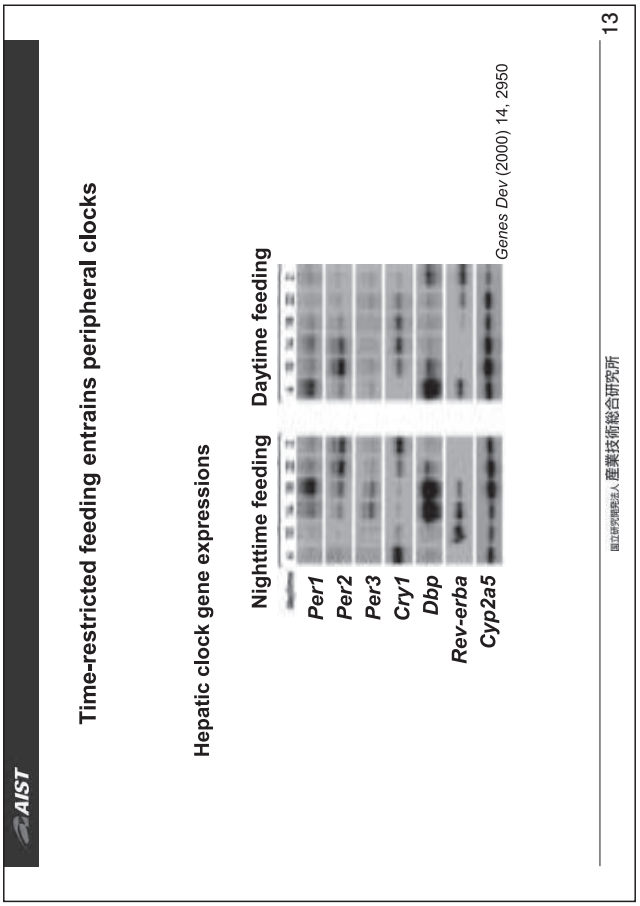

AIST
Multiple factors affect the circadian clock
Light-Dark cycle
Aging
Food intake
Calories
Nutrients
Timing
Stress
Physical exercise
Drugs
Shift work
Jet-lag
Circadian clock
Disruption of the circadian clock resulted in
Sleep disorders
Psychiatric disorders
Metabolic disorders
10

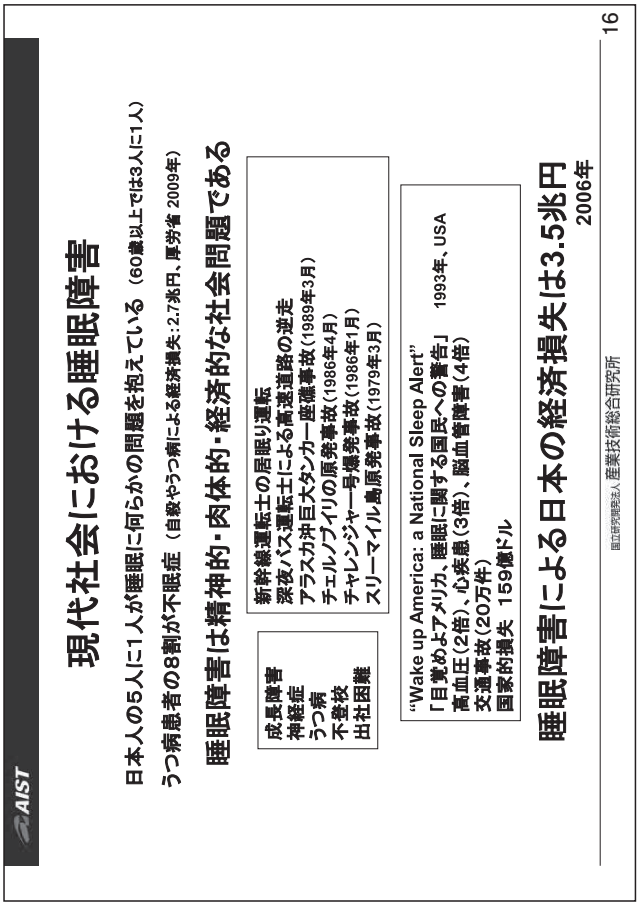

AIST
Hierarchical structure of
the circadian clock in mammals
Light
Central Clock
SCN
Time cues
Peripheral
Clocks
肝臓
心臓
脂肪
腎臓
胃
機能
腸
個々の細胞
時間生物学事典（朝倉書店）
https://calderup.wordpress.com/2010/06/26/better-remedy-for-jet-lag/
9

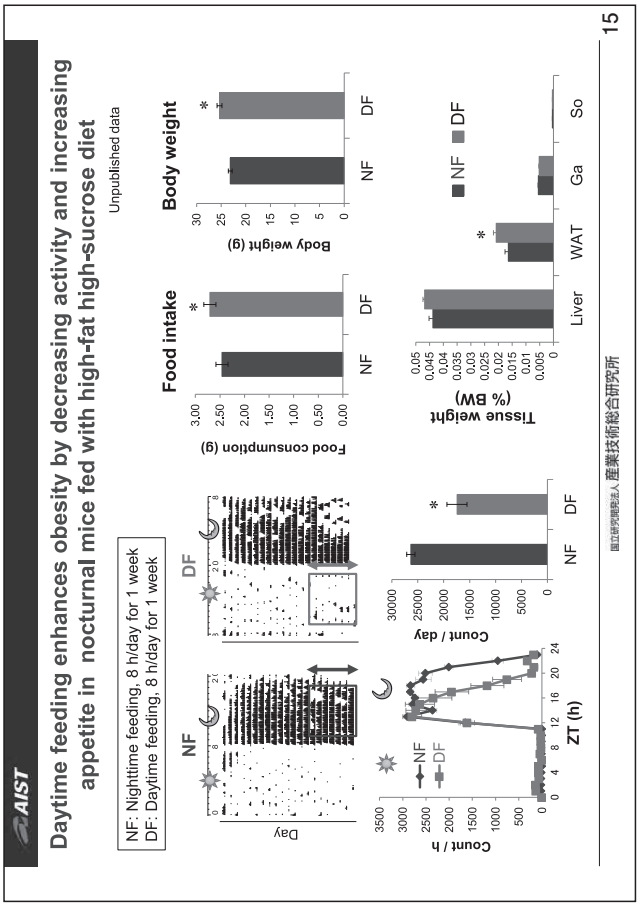

AIST
Chronic free-access to a running-wheel advances the phase of
behavioral and physiological circadian rhythms
PLoS One (2015)
Red: Sedentary
Blue: Wheel-running
Wheel running rhythm
Body temperature
Plasma corticosterone
Phase advance
Food intake rhythm
Peripheral clocks in
white adipose tissue
Bmal1
Per2
Rev-erbα
Relative mRNA levels
Zeitgeber Time (h)
12

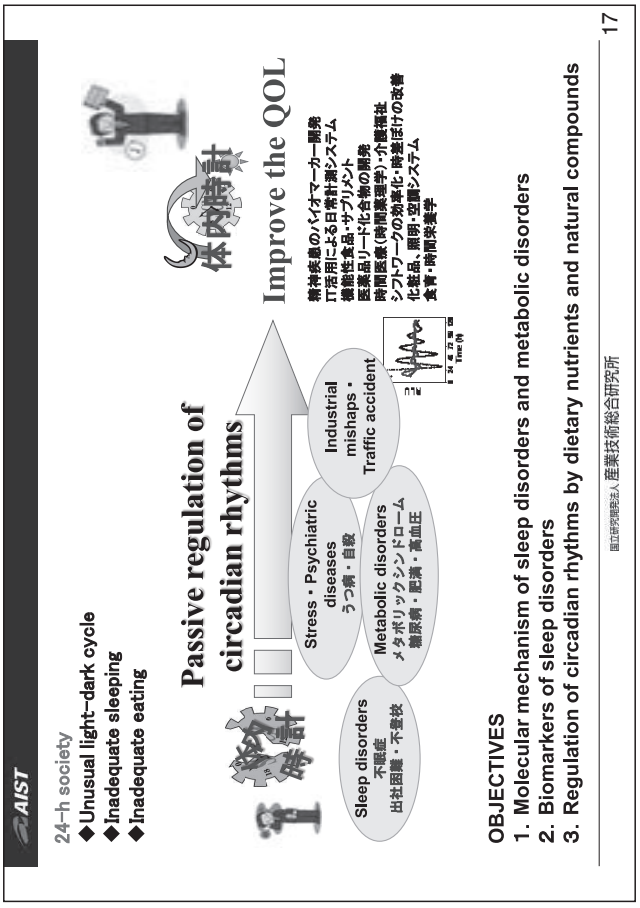

AIST
Disrupted daily Light-Dark cycle (LD 3:3) induces metabolic disorders
Total daily activity
Metabolic parameters
Glucose tolerance test
Peripheral clock
gene expressions
Liver
Body weight gain
Time (days)
Biochem Biophys Res Commun (2013)
Neuroreport (2014)
Biochem Biophys Res Commun (2015)
11

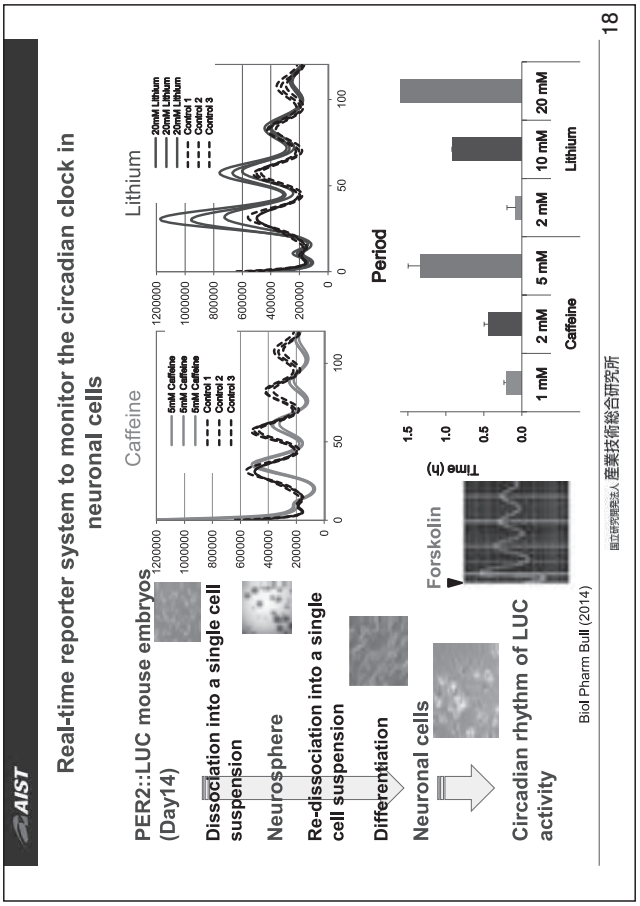

AIST
Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet
Ad libitum
Feeding Pattern
Time-restricted
Robustness of Circadian & Metabolic Rhythms
Low Amplitude
High Amplitude
Adiposity
Glucose Intolerance
Leptin Resistance
Liver Pathology
Inflammation
Motor Coordination
Kcal/mouse (x 1,000)
Weeks
FA
FT
Body weight (g)
Ad libitum
Time-restricted
Cell Metab (2012) 15, 848
14
独立行政法人 産業技術総合研究所


AIST
Time-restricted feeding entrains peripheral clocks
Hepatic clock gene expressions
Nighttime feeding
Daytime feeding
Per1
Per2
Per3
Cry1
Dbp
Rev-erba
Cyp2a5
Genes Dev (2000) 14, 2950
13
独立行政法人 産業技術総合研究所


AIST
現代社会における睡眠障害
日本人の5人に1人が睡眠に何らかの問題を抱えている（60歳以上では3人に1人）
うつ病患者の8割が不眠症（自殺やうつ病による経済損失：2.7兆円、厚労省 2009年）
睡眠障害は精神的・肉体的・経済的な社会問題である
成長障害
神経症
うつ病
不登校
出社困難
新幹線運転士の居眠り運転
深夜バス運転士による高速道路の逆走
アラスカ沖巨大タンカー座礁事故（1989年3月）
チェルノブイリの原発事故（1986年4月）
チャレンジャー号爆発事故（1986年1月）
スリーマイル島原発事故（1979年3月）
"Wake up America: a National Sleep Alert"
「目覚めよアメリカ、睡眠に関する国民への警告」 1993年、USA
高血圧（2倍）、心疾患（3倍）、脳血管障害（4倍）
交通事故（20万件）
国家的損失 159億ドル
睡眠障害による日本の経済損失は3.5兆円
2006年
16
独立行政法人 産業技術総合研究所

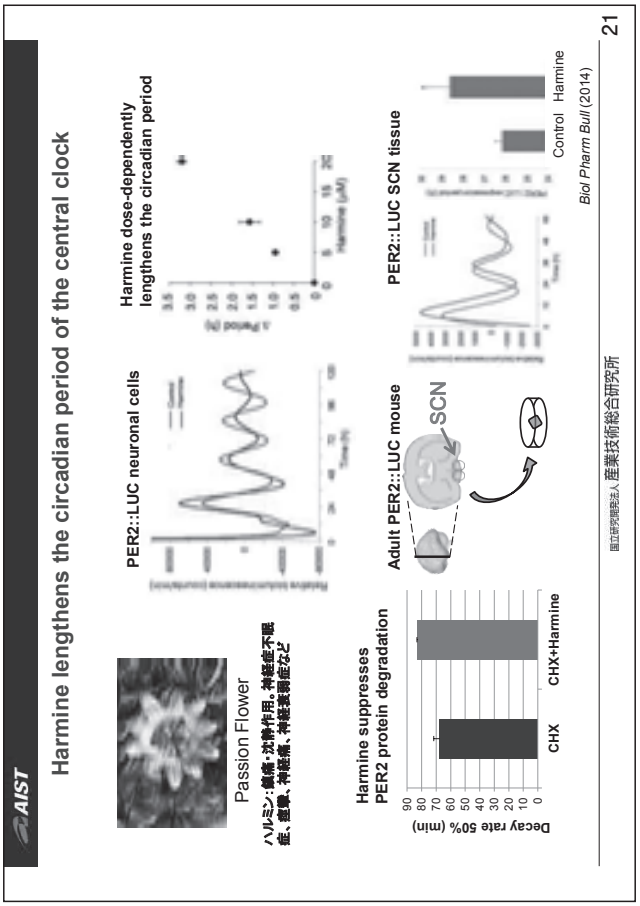

AIST
Daytime feeding enhances obesity by decreasing activity and increasing appetite in nocturnal mice fed with high-fat high-sucrose diet
Unpublished data
NF: Nighttime feeding, 8 h/day for 1 week
DF: Daytime feeding, 8 h/day for 1 week
NF
DF
Day
Count / h
ZT (h)
Count / day
Food intake
Food consumption (g)
Body weight
Body weight (g)
Tissue weight
(% BW)
Liver
WAT
Ga
So
15
独立行政法人 産業技術総合研究所

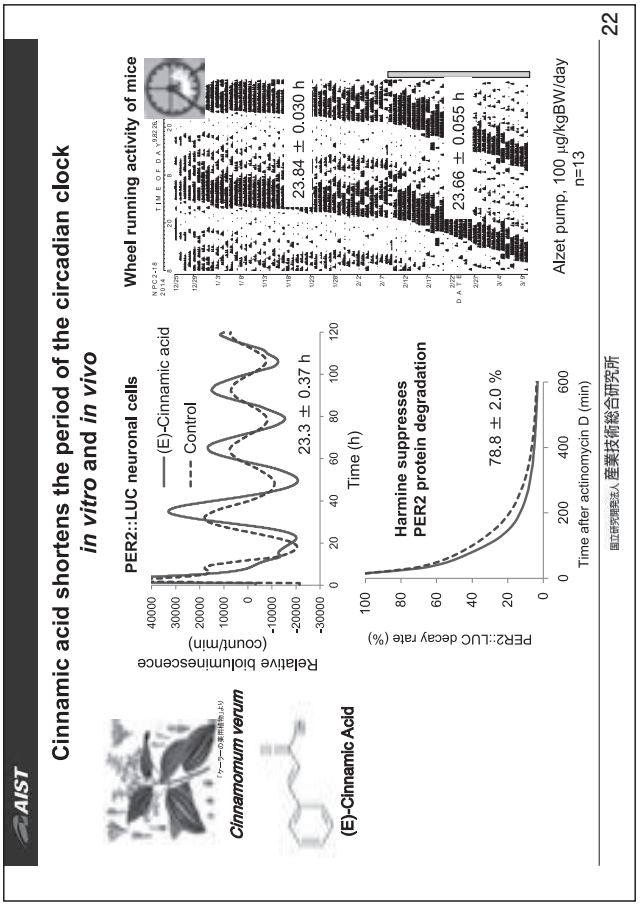

AIST
24-h society
◆Unusual light-dark cycle
◆Inadequate sleeping
◆Inadequate eating
Passive regulation of circadian rhythms
Stress・Psychiatric diseases
うつ病・自殺
Industrial mishaps・Traffic accident
Metabolic disorders
メタボリックシンドローム
糖尿病・肥満・高血圧
Sleep disorders
不眠症
出社困難・不登校
体内時計
Improve the QOL
精神疾患のバイオマーカー開発
IT活用による日常計測システム
機能性食品・サプリメント
医薬品リード化合物の開発
時間医療(時間薬理学)・介護福祉
シフトワークの効率化・時差ぼけの改善
化粧品、照明・空調システム
食育・時間栄養学
OBJECTIVES
1. Molecular mechanism of sleep disorders and metabolic disorders
2. Biomarkers of sleep disorders
3. Regulation of circadian rhythms by dietary nutrients and natural compounds
17


AIST
Real-time reporter system to monitor the circadian clock in neuronal cells
PER2::LUC mouse embryos (Day14)
Dissociation into a single cell suspension
Neurosphere
Re-dissociation into a single cell suspension
Differentiation
Neuronal cells
Circadian rhythm of LUC activity
Caffeine
Lithium
Forskolin
Period
Time (h)
1 mM
2 mM
5 mM
2 mM
10 mM
20 mM
Caffeine
Lithium
Biol Pharm Bull (2014)
18

AIST
Functional screening of natural compounds in herbal medicine
Period
Amplitude
Phase shift
19

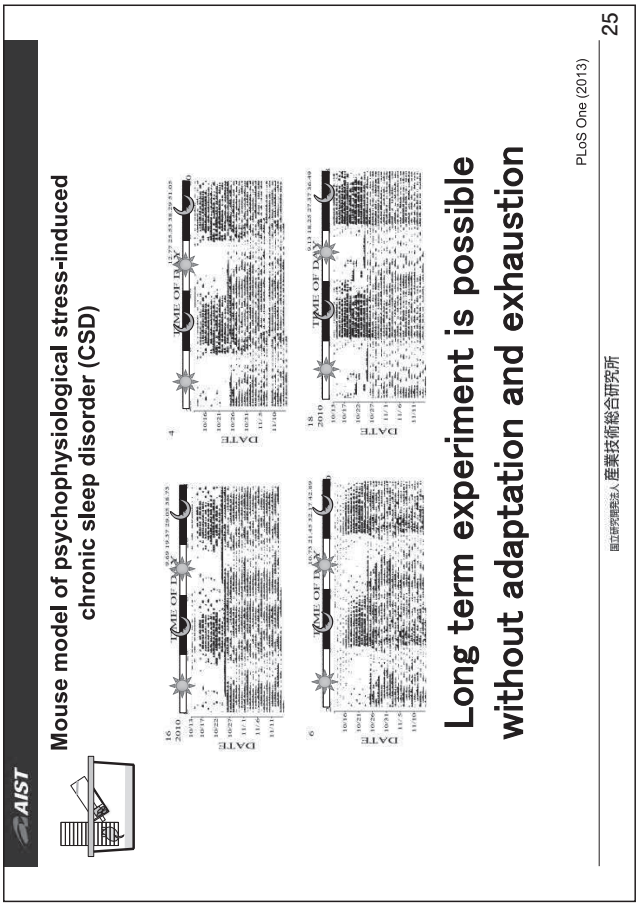
AIST
Functional screening of natural compounds in herbal medicine
Aconitine
Alisol A
Paeonol
[6]-shogaol
Period
Per1 / GAPDH
Per2 / GAPDH
Bmal1 / GAPDH
Time (h)
20

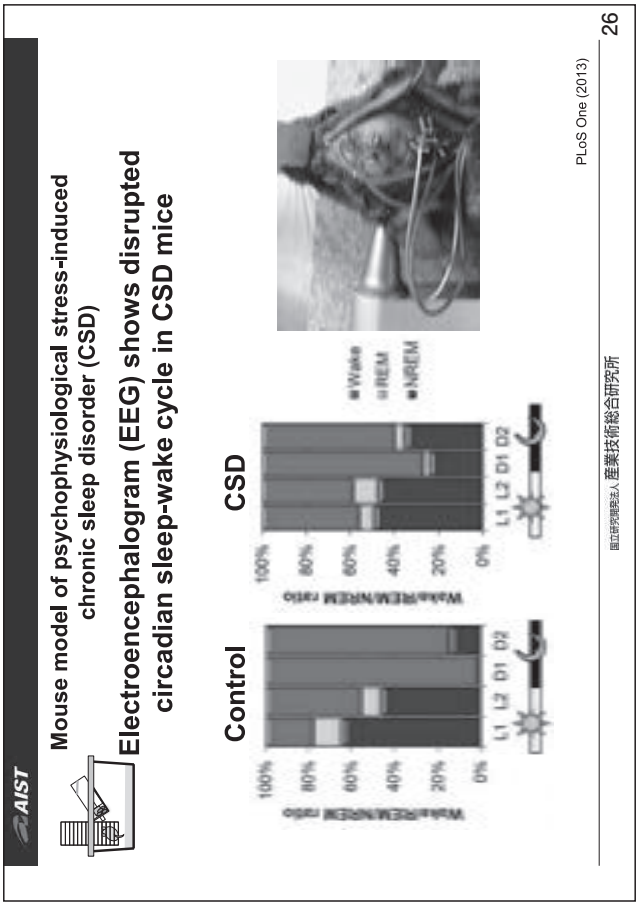

AIST
Harmine lengthens the circadian period of the central clock
Passion Flower
ハルモン：鎮痛・沈静作用。神経症不眠症、躁鬱、神経痛、神経表現症など
PER2::LUC neuronal cells
Harmine dose-dependently lengthens the circadian period
Harmine suppresses PER2 protein degradation
Decay rate 50% (min)
CHX
CHX+Harmine
Adult PER2::LUC mouse
SCN
PER2::LUC SCN tissue
Control
Harmine
Biol Pharm Bull (2014)
21


AIST
Cinnamic acid shortens the period of the circadian clock
in vitro and in vivo
Cinnamomum verum
(E)-Cinnamic Acid
PER2::LUC neuronal cells
(E)-Cinnamic acid
Control
23.3 ± 0.37 h
Relative bioluminescence (count/min)
Time (h)
Harmine suppresses PER2 protein degradation
78.8 ± 2.0 %
PER2::LUC decay rate (%)
Time after actinomycin D (min)
Wheel running activity of mice
23.84 ± 0.030 h
23.66 ± 0.055 h
Alzet pump, 100 μg/kgBW/day n=13
22

AIST
Development of a mouse model of chronic sleep disorder (CSD)
Genetic factors
◆Clock gene mutation
Environmental factors
◆Shift work
◆Stress
◆Irregular feeding
◆Jet-lag
Disruption of the circadian clock
Stress・Psychiatric diseases
うつ病・自殺
Sleep disorders
不眠症
出社困難・不登校
Industrial mishaps・Traffic accident
Metabolic disorders
メタボリックシンドローム
糖尿病・肥満・高血圧
23

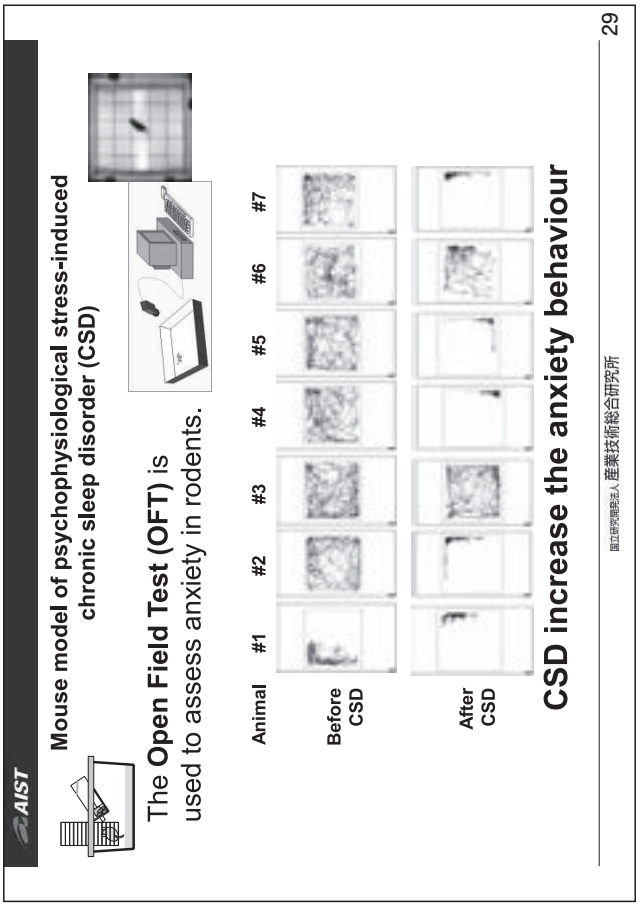
AIST
Mouse model of psychophysiological stress-induced chronic sleep disorder (CSD)
Continuous stress imposed by the water avoidance on a wheel
DATE
➢ Increase in daytime activity
➢ Reduction in nighttime activity
PLoS One (2013)
24

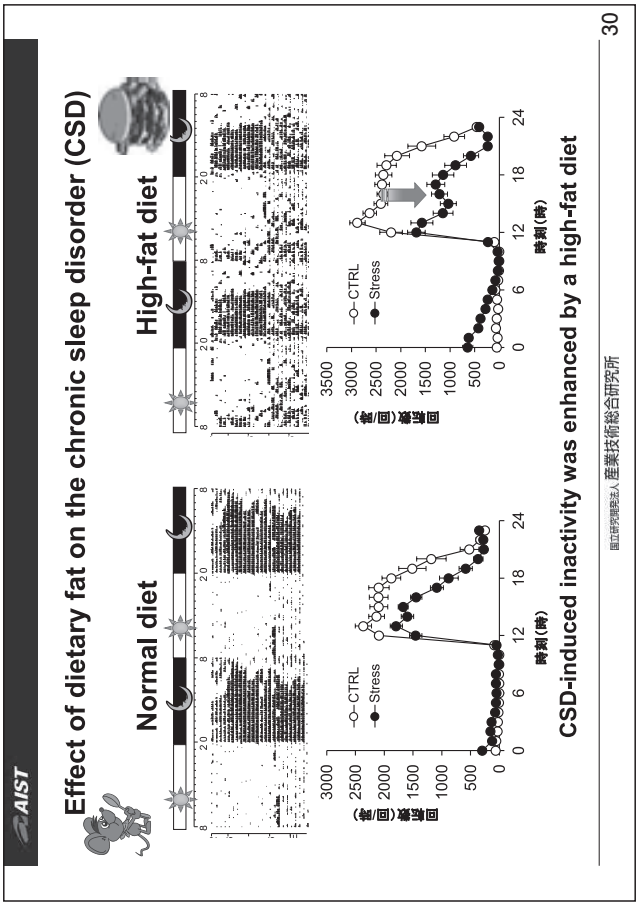

AIST
Mouse model of psychophysiological stress-induced chronic sleep disorder (CSD)
Long term experiment is possible without adaptation and exhaustion
TIME OF DAY
DATE
PLoS One (2013)
25


AIST
Mouse model of psychophysiological stress-induced chronic sleep disorder (CSD)
Electroencephalogram (EEG) shows disrupted circadian sleep-wake cycle in CSD mice
Control
CSD
Wake
REM
NREM
L1 L2 D1 D2
PLoS One (2013)
26

AIST
Mouse model of psychophysiological stress-induced chronic sleep disorder (CSD)
CSD has little effect on peripheral clock gene expressions
Liver
CTRL
CSD
Per1
Per2
Bmal1
Dbp
mRNA (%)
Zeitgeber Time (h)
Biochem Biophys Res Commun (2014)
27

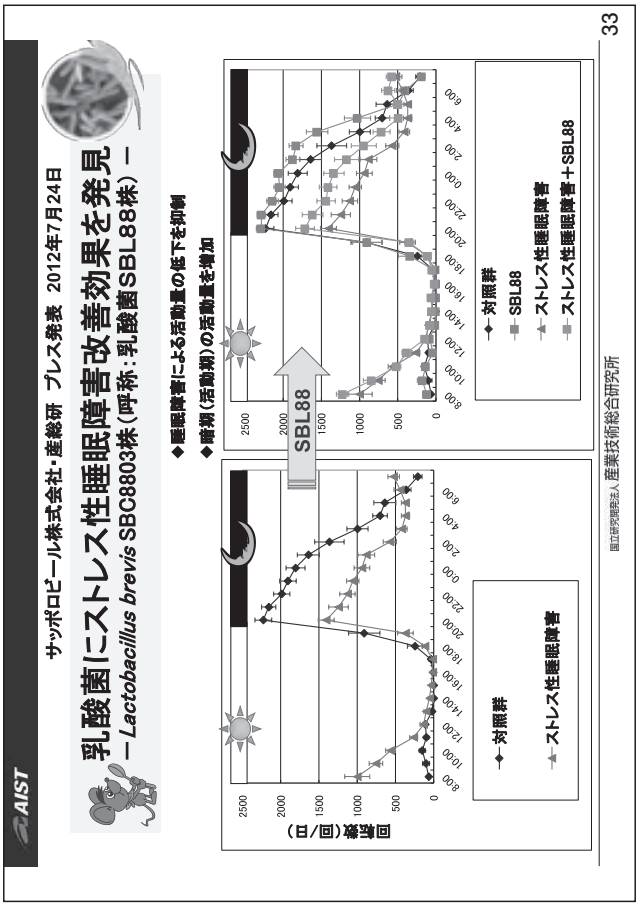
AIST
Mouse model of psychophysiological stress-induced chronic sleep disorder (CSD)
Plasma amino acid profile might be a putative biomarker for circadian rhythm disorders
ZT6 (Inactive phase)
ZT18 (Active phase)
Biochem Biophys Res Commun (2014)
28

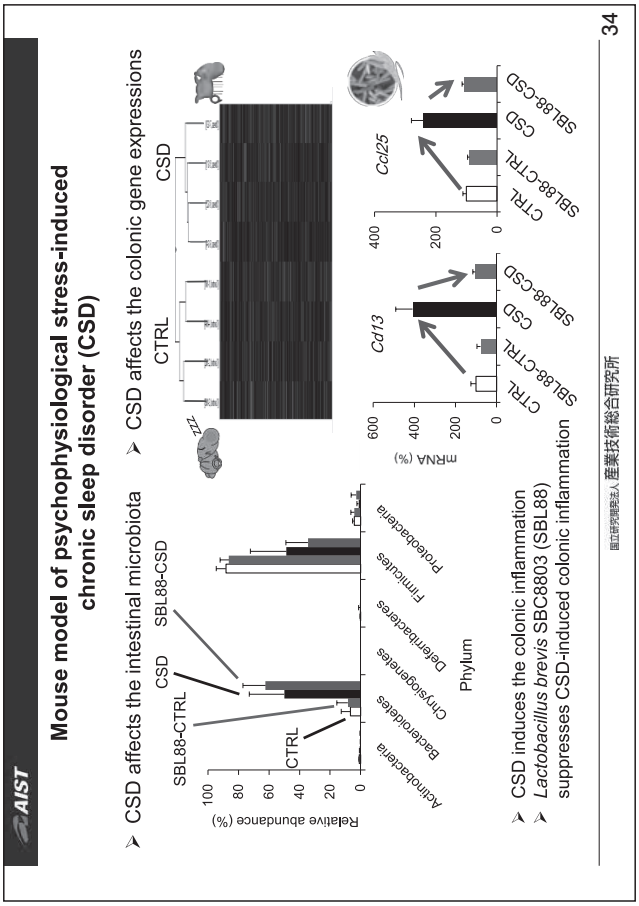

AIST
Mouse model of psychophysiological stress-induced chronic sleep disorder (CSD)
The Open Field Test (OFT) is used to assess anxiety in rodents.
Animal #1 #2 #3 #4 #5 #6 #7
Before CSD
After CSD
CSD increase the anxiety behaviour
29


AIST
Effect of dietary fat on the chronic sleep disorder (CSD)
Normal diet
High-fat diet
CTRL
Stress
回転数（回/時）
時刻（時）
CSD-induced inactivity was enhanced by a high-fat diet
30

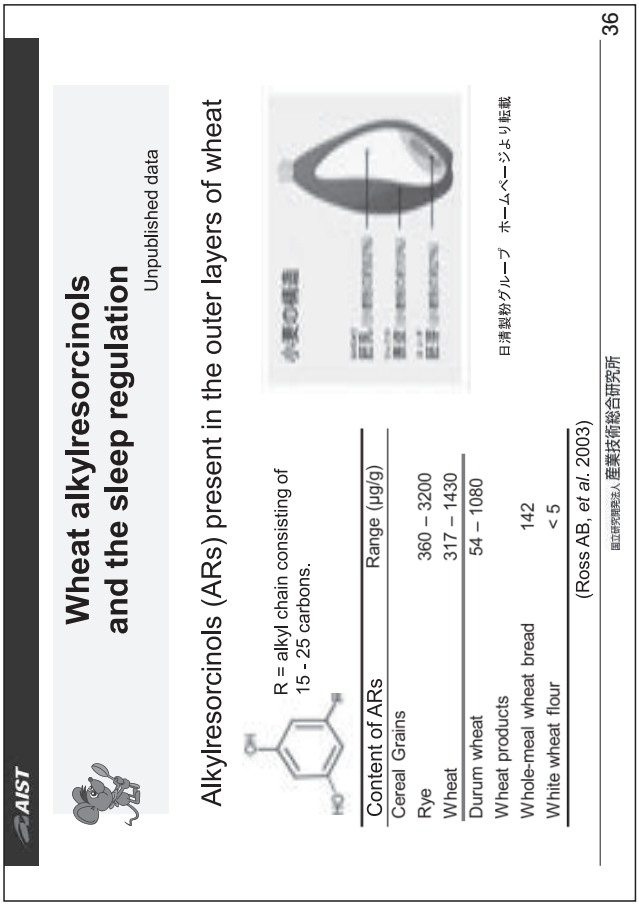
AIST
CSD enhances high-fat diet-induced metabolic disorders
HFD feeding with or without CSD for 6 weeks
CSD induces hyperphagia and gluconeogenesis
Body weight
Food intake
Plasma leptin
Leptin mRNA
Plasma glucose
GTT
Pepck
Glucokinase
Control
Stress
31

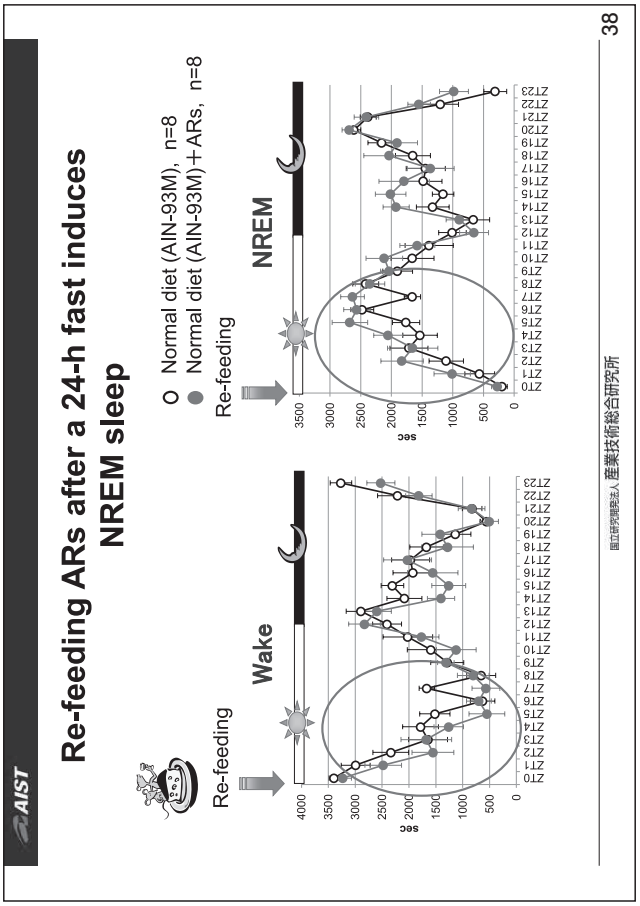
AIST
サッポロビール株式会社・産総研 プレス発表 2012年7月24日
乳酸菌にストレス性睡眠障害改善効果を発見
－ Lactobacillus brevis SBC8803株（呼称：乳酸菌SBL88株）－
SBL88 decreases NREM sleep during the active phase
CTRL, n=9
SBL88, n=10
Wake
NREM
REM
Duration of wakefulness (min/h)
Duration of NREM sleep (min/h)
Duration of REM sleep (min/h)
Time (h)
Life Sci (2014)
32

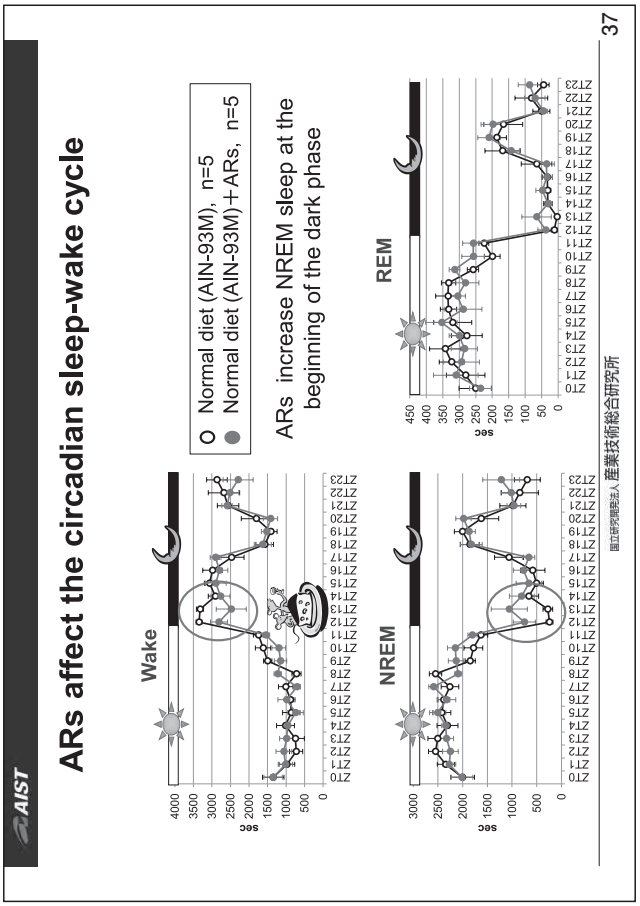

AIST
サッポロビール株式会社・産総研 プレス発表 2012年7月24日
乳酸菌にストレス性睡眠障害改善効果を発見
－Lactobacillus brevis SBC8803株（呼称：乳酸菌SBL88株）－
◆睡眠障害による活動量の低下を抑制
◆暗期（活動期）の活動量を増加
SBL88
回転数（回/日）
対照群
ストレス性睡眠障害
ストレス性睡眠障害＋SBL88
33


AIST
Mouse model of psychophysiological stress-induced chronic sleep disorder (CSD)
CSD affects the intestinal microbiota
CSD affects the colonic gene expressions
SBL88-CTRL
CSD
SBL88-CSD
CTRL
Relative abundance (%)
Actinobacteria
Bacteroidetes
Chrysiogenetes
Deferribacteres
Firmicutes
Proteobacteria
Phylum
CSD induces the colonic inflammation
Lactobacillus brevis SBC8803 (SBL88) suppresses CSD-induced colonic inflammation
Cd13
Ccl25
mRNA (%)
34

AIST
腸脳相関を介したLactobacillus brevis SBC8803（SBL88乳酸菌）の作用
脳
自律神経系（求心性）
腸から脳への伝達
腸内分泌細胞への伝達
（腸：蠕動運動）
膵器（胃：食欲）
（肌：保湿）
腸
5-HT3
SBL88
SBL88のEC細胞刺激による セロトニン分泌と受容
Beneficial Microbes, In press
Neuroscience Letters, 539, 32-37 (2013)
J. Appl. Microbiol. 116 (5), 1274 – 1281 (2014)
Food and Nutrition Sciences, 4 (8), 767-771 (2013)
サッポロビール（株）中北様よりご提供
35


AIST
Wheat alkylresorcinols and the sleep regulation
Unpublished data
Alkylresorcinols (ARs) present in the outer layers of wheat
R = alkyl chain consisting of 15 - 25 carbons.
Content of ARs
Range (µg/g)
Cereal Grains
Rye 360 – 3200
Wheat 317 – 1430
Durum wheat 54 – 1080
Wheat products
Whole-meal wheat bread 142
White wheat flour < 5
(Ross AB, et al. 2003)
日清製粉グループ ホームページより転載
36


AIST
Re-feeding ARs after a 24-h fast induces NREM sleep
Re-feeding
Wake
Re-feeding
NREM
Normal diet (AIN-93M), n=8
Normal diet (AIN-93M)＋ARs, n=8
sec
ZT
38
独立行政法人 産業技術総合研究所


AIST
ARs affect the circadian sleep-wake cycle
Wake
NREM
REM
Normal diet (AIN-93M), n=5
Normal diet (AIN-93M)＋ARs, n=5
ARs increase NREM sleep at the beginning of the dark phase
sec
ZT
37
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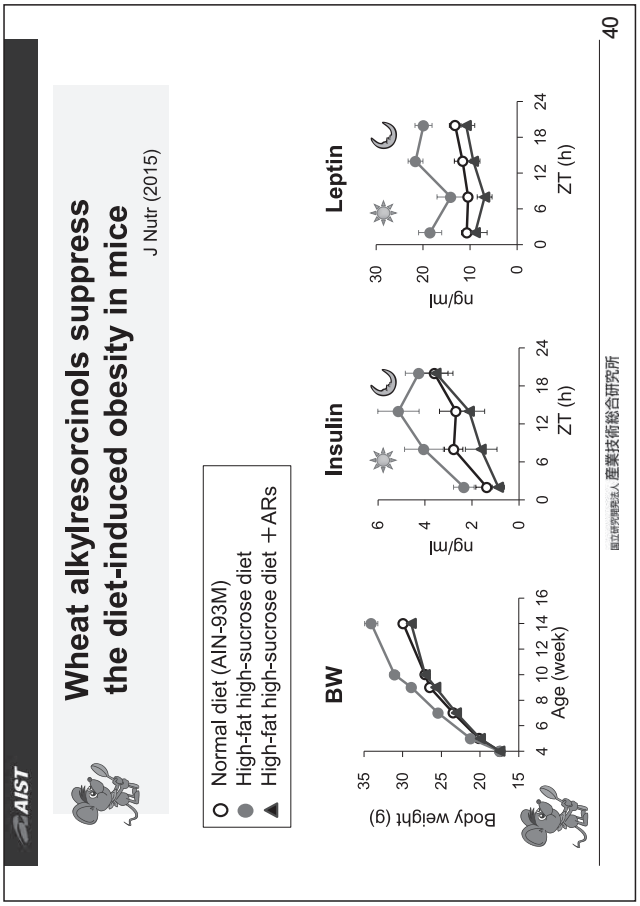
AIST
Wheat alkylresorcinols suppress the diet-induced obesity in mice
J Nutr (2015)
Normal diet (AIN-93M)
High-fat high-sucrose diet
High-fat high-sucrose diet ＋ARs
BW
Insulin
Leptin
Body weight (g)
ng/ml
Age (week)
ZT (h)
40
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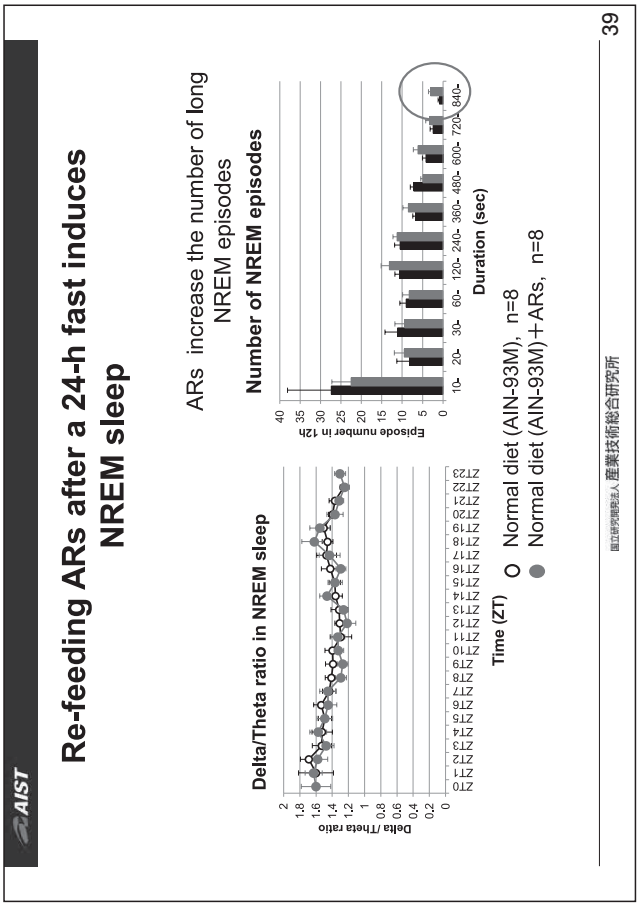
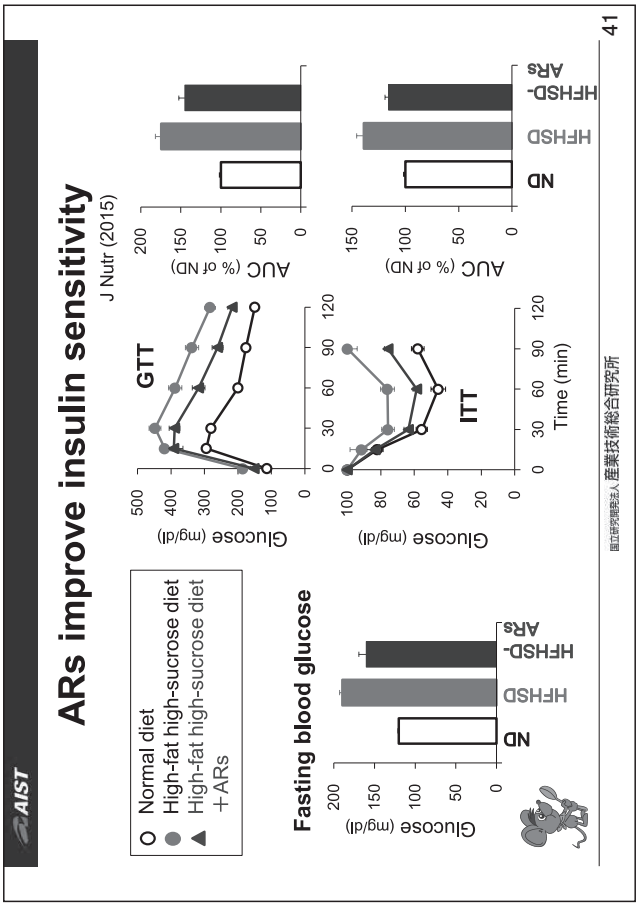
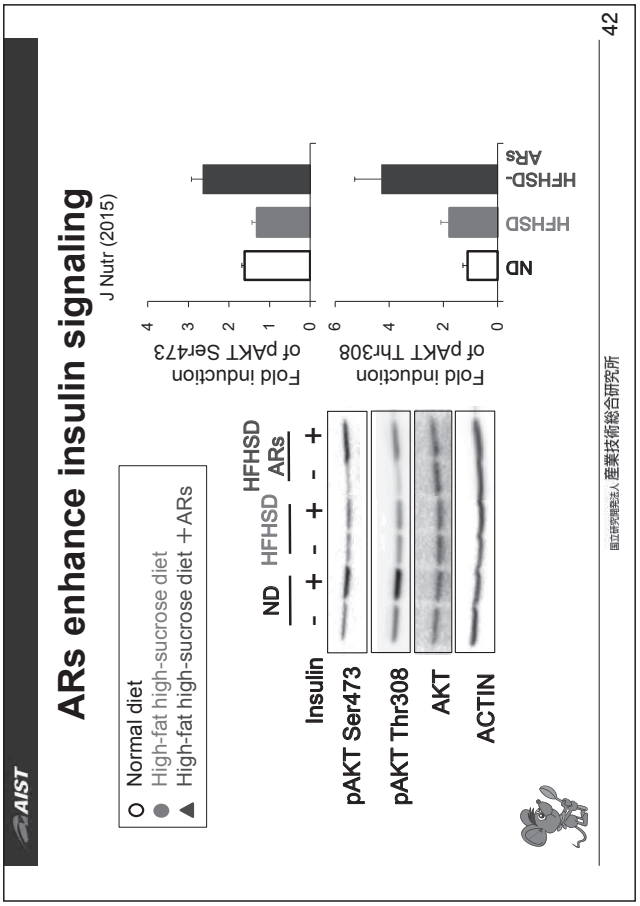
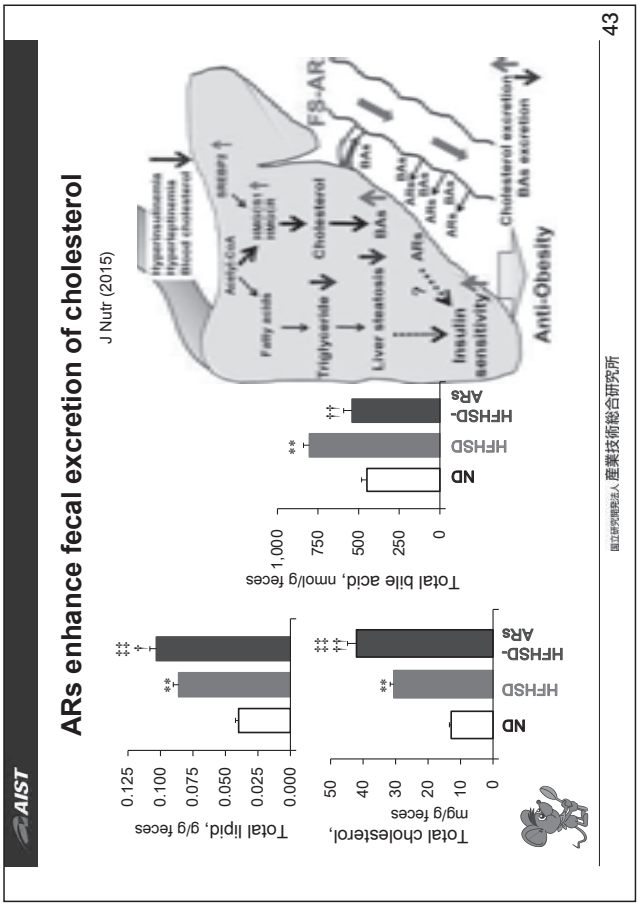
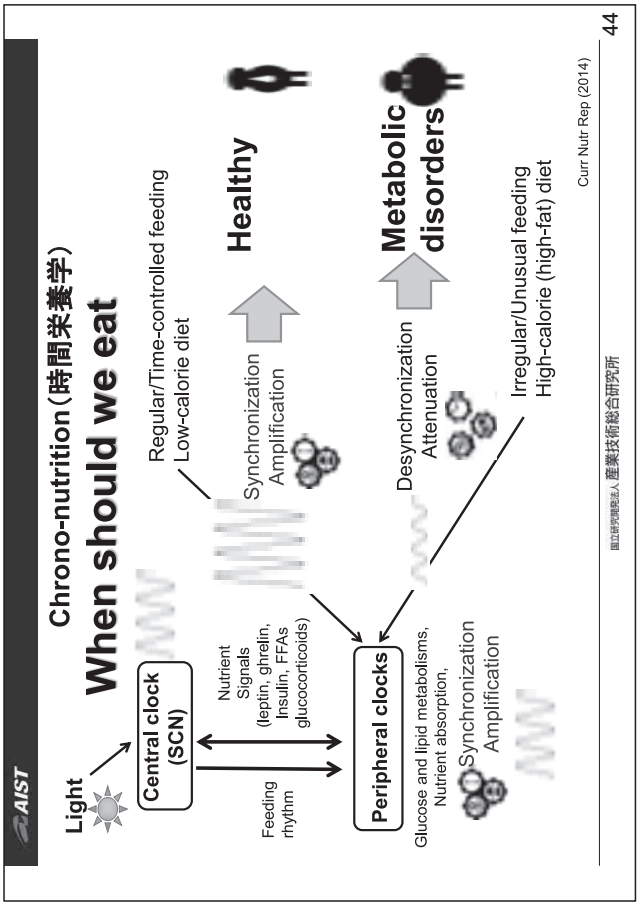
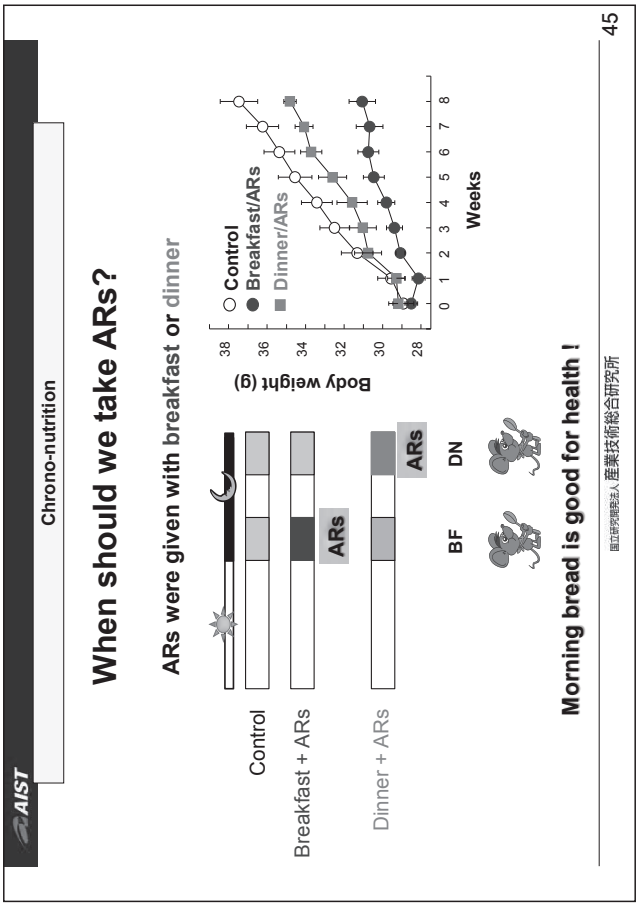

AIST
Re-feeding ARs after a 24-h fast induces NREM sleep
ARs increase the number of long NREM episodes
Number of NREM episodes
Episode number in 12h
Duration (sec)
Delta/Theta ratio in NREM sleep
Delta /Theta ratio
Time (ZT)
Normal diet (AIN-93M), n=8
Normal diet (AIN-93M)+ARs, n=8
39
独立行政法人 産業技術総合研究所

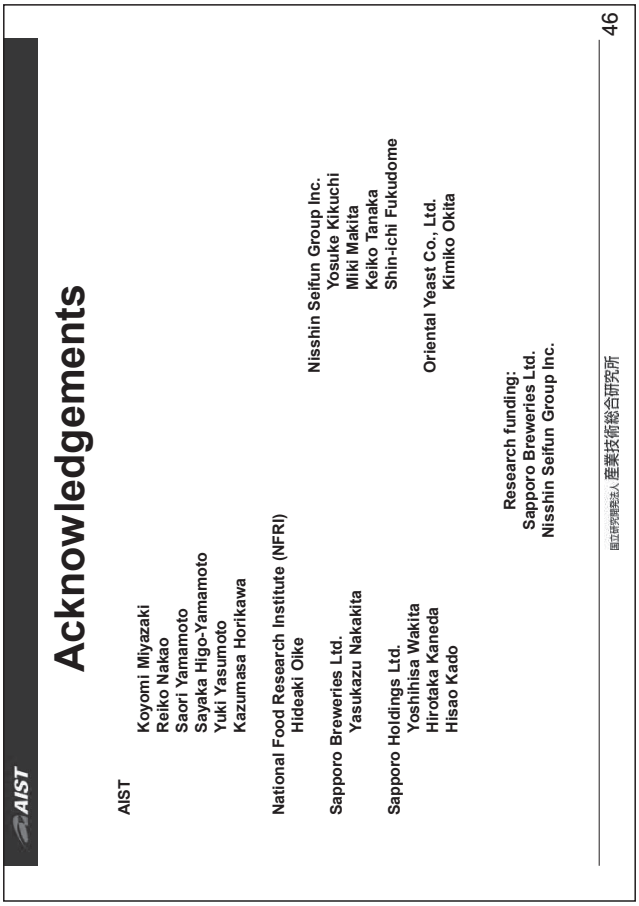

AIST
ARs improve insulin sensitivity
J Nutr (2015)
Normal diet
High-fat high-sucrose diet
High-fat high-sucrose diet +ARs
Fasting blood glucose
Glucose (mg/dl)
ND
HFHSD
HFHSD-ARs
GTT
ITT
AUC (% of ND)
Time (min)
独立行政法人 産業技術総合研究所
41


AIST
ARs enhance insulin signaling
J Nutr (2015)
Normal diet
High-fat high-sucrose diet
High-fat high-sucrose diet +ARs
ND
HFHSD
HFHSD-ARs
Insulin
pAKT Ser473
pAKT Thr308
AKT
ACTIN
Fold induction of pAKT Ser473
Fold induction of pAKT Thr308
独立行政法人 産業技術総合研究所
42


AIST
ARs enhance fecal excretion of cholesterol
J Nutr (2015)
Total lipid, g/g feces
Total cholesterol, mg/g feces
Total bile acid, nmol/g feces
ND
HFHSD
HFHSD-ARs
FS-AR
Anti-Obesity
独立行政法人 産業技術総合研究所
43


AIST
Chrono-nutrition（時間栄養学）
When should we eat
Light
Central clock (SCN)
Feeding rhythm
Nutrient Signals (leptin, ghrelin, Insulin, FFAs glucocorticoids)
Peripheral clocks
Glucose and lipid metabolisms, Nutrient absorption,
Synchronization Amplification
Regular/Time-controlled feeding
Low-calorie diet
Synchronization Amplification
Healthy
Desynchronization Attenuation
Metabolic disorders
Irregular/Unusual feeding
High-calorie (high-fat) diet
Curr Nutr Rep (2014)
独立行政法人 産業技術総合研究所
44


AIST
Chrono-nutrition
When should we take ARs?
ARs were given with breakfast or dinner
Control
Breakfast + ARs
Dinner + ARs
ARs
ARs
BF
DN
Control
Breakfast/ARs
Dinner/ARs
Body weight (g)
38
36
34
32
30
28
0 1 2 3 4 5 6 7 8
Weeks
Morning bread is good for health !
国立研究開発法人 産業技術総合研究所
45


AIST
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AIST
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Saori Yamamoto
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Sapporo Holdings Ltd.
Yoshihisa Wakita
Hirotaka Kaneda
Hisao Kado
Nisshin Seifun Group Inc.
Yosuke Kikuchi
Miki Makita
Keiko Tanaka
Shin-ichi Fukudome
Oriental Yeast Co., Ltd.
Kimiko Okita
Research funding:
Sapporo Breweries Ltd.
Nisshin Seifun Group Inc.
国立研究開発法人 産業技術総合研究所
46

セッション4

腸内細菌の研究展望

セッション4−1

ヒト腸内細菌叢のメタゲノミクス―日本人の特徴―

早稲田大学　理工学術院　先進理工学研究科
服部　正平

人体には数百兆個の細菌が生息しており、その多数は腸内において複雑な細菌集団（腸内細菌叢）を形成している。腸内細菌叢の集合ゲノムである腸内マイクロバイオームはヒトゲノムの約1000倍数の遺伝子をコードしており、病気を含む様々なヒト生理に密接に関係している。このヒト腸内マイクロバイオームの解析は近年における次世代シークエンス（NGS）技術の著しい進歩によりかなり現実的となった。

最近、私たちは106人の日本人の腸内細菌叢からNGSを用いてメタゲノムシークエンスデータを収集し、そこに約500万の遺伝子を同定した。この日本人データを中国やアメリカ等の数カ国の合計1000人以上のメタゲノムデータと比較解析を行った。その結果、これらの国間で、腸内マイクロバイオームの全体構造が大きく多様化していることが分かった。本ミーティングでは、日本人腸内マイクロバイオームの生態学的及び機能的特徴を紹介する。

Session 4－1

Metagenomics of Human Gut Microbiome
－Profile of Japanese Gut Microbiome－

Masahira Hattori, Ph.D.
Graduate School of Advanced Science and Engineering, Waseda University

Our human bodies are colonized with over 100 trillion cells of microbes, the majority of which reside in the intestinal tract and form a complex bacterial community (gut microbiota). The gut microbiome (collective genomes of the gut microbiota) is estimated to encode approximately 1000-fold more genes than that of human genome, and has profound influences on various human physiologies including disease. Analysis of human gut microbiome has recently become fairly practical due to remarkable advances in next-generation sequencing technologies (NGS).

We have currently identified five million unique genes in NGS-based metagenomic sequence data obtained from fecal DNA samples of 106 Japanese healthy individuals. We then compared the Japanese data with metagenomic data of more than 1,000 individuals publicly available from several countries including China and the USA. The analysis found significantly high variances in the overall structure of the gut microbiomes across these countries. In this meeting I will present ecological and functional features of the Japanese gut microbiome as compared with those of other populations.

Metagenomics of Human Gut Microbiome

-Profiling of the Japanese Gut Microbiome-

Masahira HATTORI

Graduate School of Advanced Science and Engineering,
Waseda University, Tokyo, JAPAN
&
Graduate School of Frontier Sciences,
The University of Tokyo, Chiba, JAPAN

Sept. 30, 2015 @ The 7th International Conference on "Nutrition and Aging", *Tokyo, Japan*

1

Human microbiota & microbiome

Body site	Bacteria/ g, ml, or cm^2	#Species
Nasal	10^3-10^4	
Oral (total)	10^{10}	>700
Saliva	10^8-10^{10}	>600
Gingival crevice	10^{12}	
Tooth surface	10^{11}	
Gastrointestinal (total)	10^{14}	>1000
Stomach	10^0-10^4	
Small intestine	10^4-10^7	
Colon (feces)	10^{11}-10^{12}	>1000
Skin (total)	10^{12}	
Skin surface	10^5	>150
Urogenital (total)	10^{12}	
Vagina	10^9	

A huge numbers of bacteria reside various human body sites to form site-specific bacterial communities called **microbiota**. Among them, the gut microbiota is the largest and most complex, and is composed of more than 1,000 different microbial species. **Microbiome** is the collective genomes of microbes constituting the microbiota.

2

Methods for bacterial community analysis

Microbiota

Microbiome
(the collective genomes of bacteria constituting the microbiota)

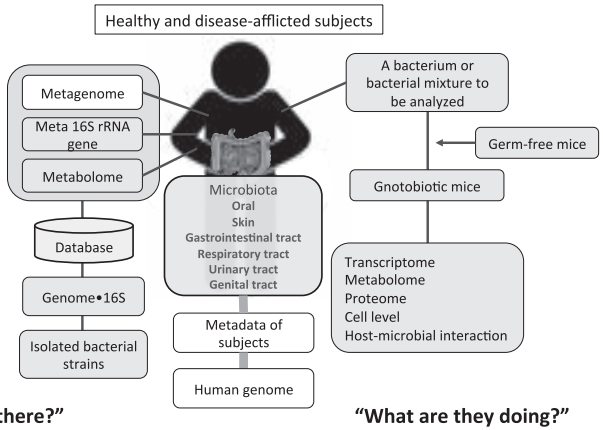
Bacterial culture method (1960's ~)
- Presence of unculturable bacteria that cannot be detected.

Bacterial 16S rRNA gene analysis (1980's ~)
- Can detect the unculturable bacteria
- But no functional information (⇒Ecological study)

Metagenomics (2004 ~)
- Shotgun sequencing of microbiome
- Can get both ecological and functional information
- **Large-scale analysis by NGS (2008~)** (NGS: Next-generation sequencing)

3

Outline of human microbiota research



"Who's there?"
Data-driven study
(Technology development, Database construction)

"What are they doing?"
Functional study
Targeted metagenomics

4

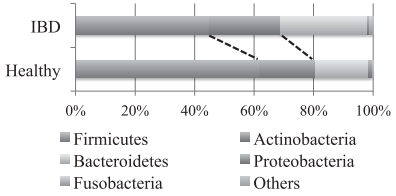
Dysbiosis of gut microbiota and disease

Related diseases

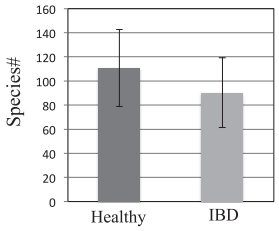
- Obesity
- Metabolic syndrome
- Inflammatory bowl disease (IBD)
- Irritable bowel syndrome
- Atherosclerosis
- Rheumatism
- Type 2 Diabetes
- Allergy
- Asthma
- Celiac disease
- Colon cancer
- Liver cancer
- Autism
- Multiple sclerosis

Metabolism system
Immune system
Nervous system

Change in bacterial composition



Decrease in microbial diversity



5

However, much basic knowledge on the human gut microbiome are still insufficient…….

Our recent research focuses are:

- To elucidate the population-level diversity in the human gut microbiome by metagenomics.
- To explore environmental factors which profoundly contribute to the diversity in gut microbiomes across countries.
- To characterize Japanese gut microbiome as compared with those of other countries.

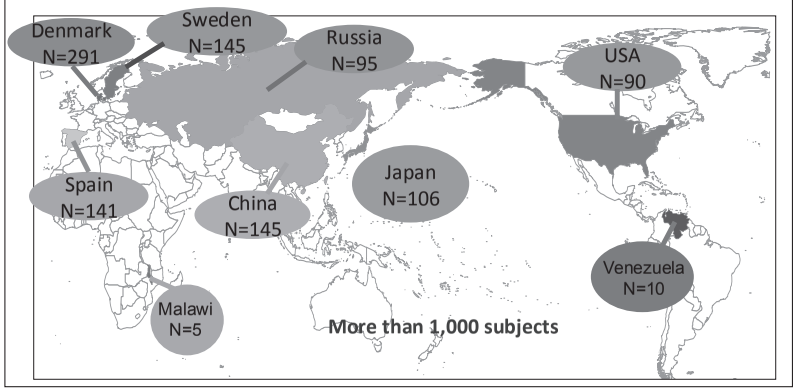
- We compared the metagenomic data of the bacterial composition in gut microbiomes of healthy individuals in 9 countries including the Japanese data newly collected.
- The 9 countries included the United States (**US**), China (**CN**), Russia (**RU**), Sweden (**SE**), Denmark (**DK**), Spain (**ES**), Japan (**JP**), and Venezuela / Malawi natives (**VM**).

6

Current status of metagenomics of the human gut microbiome

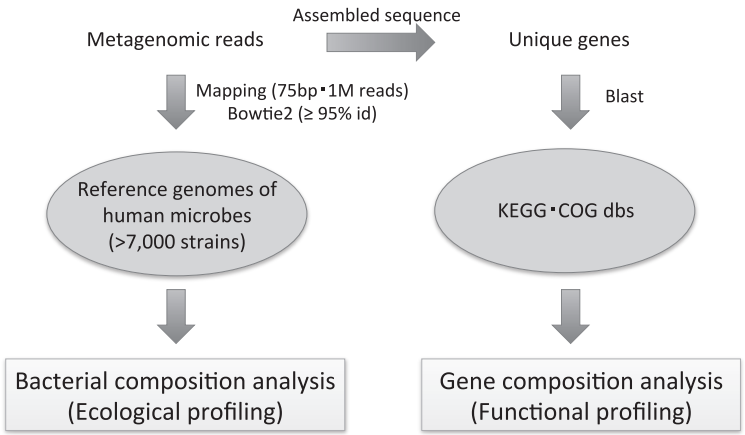
Country	Subject#	Sequencer	Unique gene#	Published year
USA	2	Sanger	0.05M	2006
Japan (HMGJ)	13	Sanger	0.7M	2007
IHMC launched in 2008				

Most researches have been done by metagenomic analysis using NGS after 2010



7

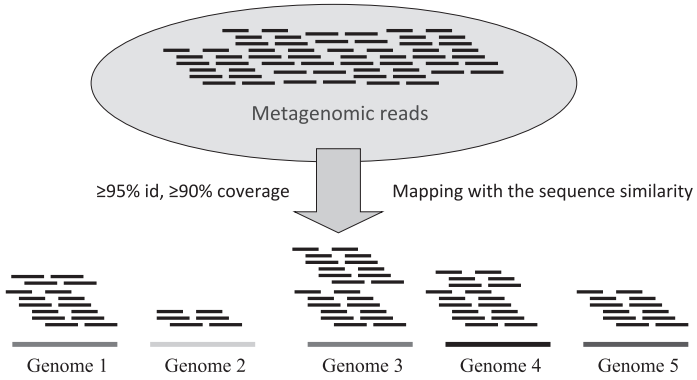
Analysis work flow of the metagenomic data



8

Mapping of metagenomic reads onto reference genomes

Taxonomic assignment of metagenomc reads to the species/genome
Quantification of the bacterial composition



Reference genomes (>7,000 human microbes)

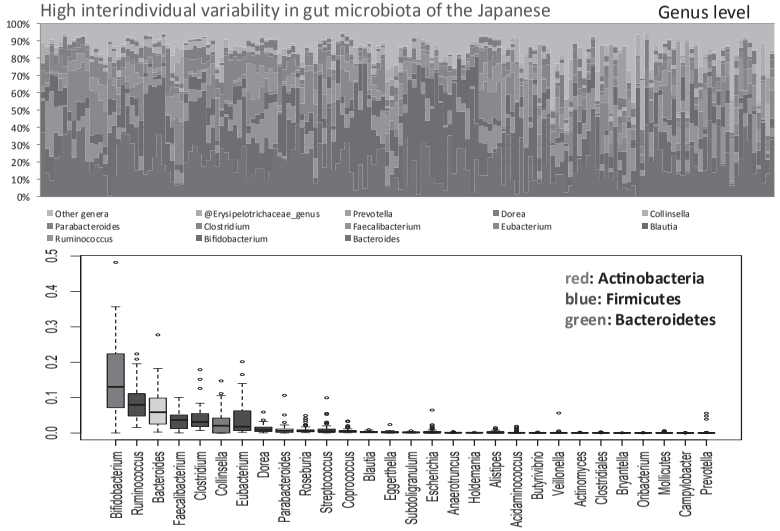
About 80% of the total reads can be mapped

9

Bacterial composition in gut microbiota of 106 Japanese individuals

High interindividual variability in gut microbiota of the Japanese

Genus level



10

Search of factors which profoundly contribute to the population-level diversity in human gut microbiota across the countries

Association study of the microbial composition with:

・Epidemiological data of dietary intake of 119 food items (g/capita/day) from:
FAOSTAT (Food and Agriculture Organization of the United Nations) database

・Epidemiological data of Antibiotic use from:
IMS Health MIDAS database (In human)
A recently published paper (In farm)

11

Meal information (FAOSTAT)

(g/capita/day)

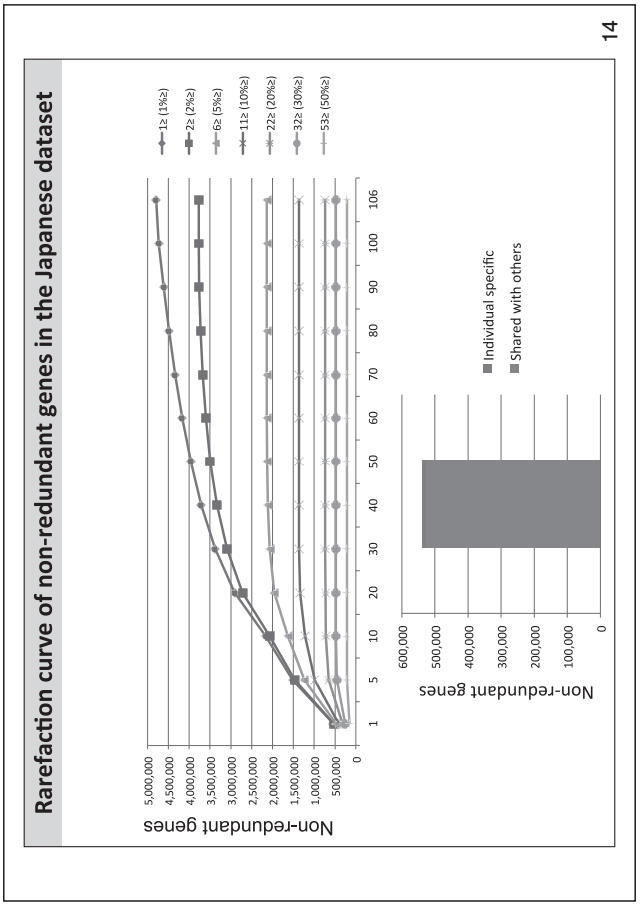
Food and Agriculture Organization of the United Nations

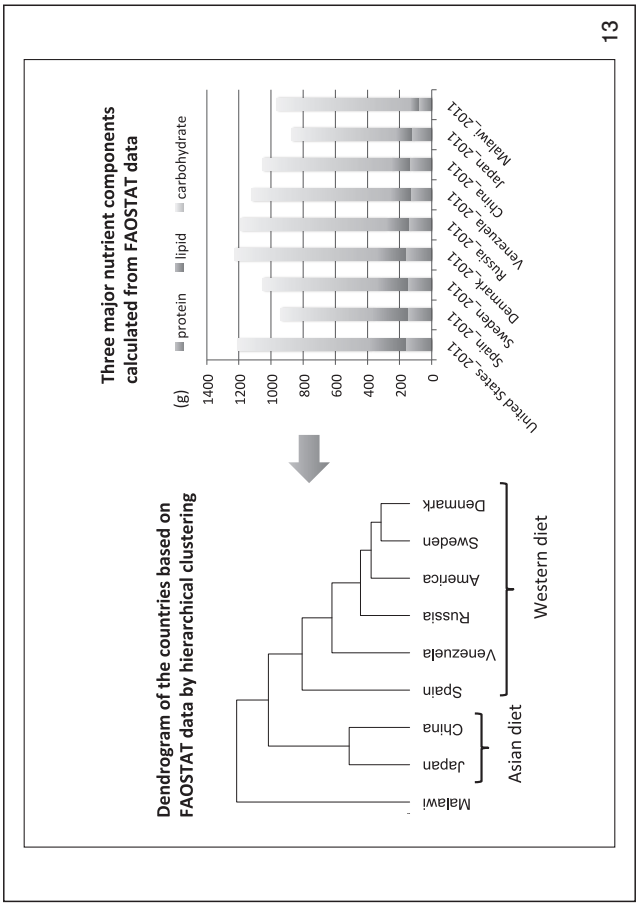
http://faostat.fao.org

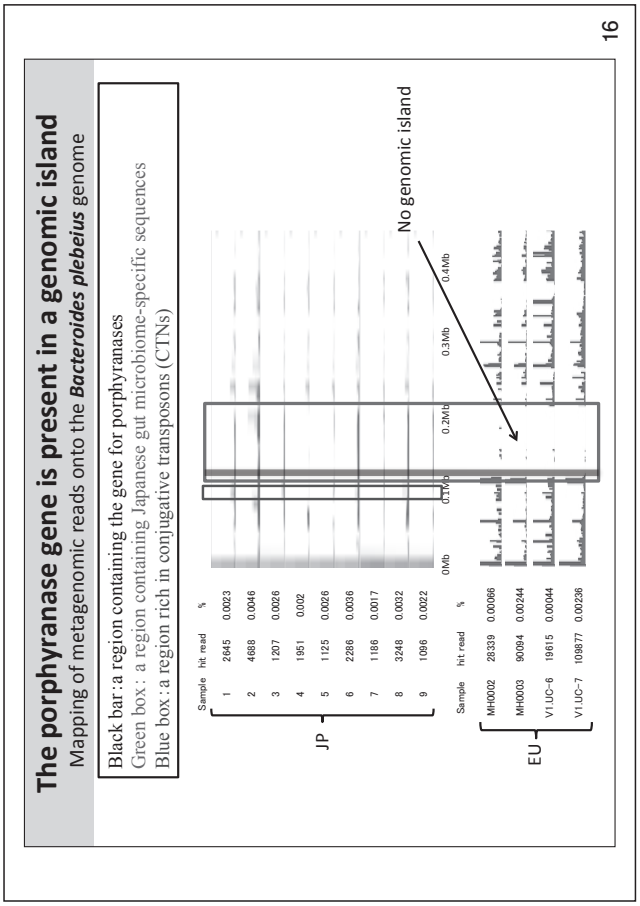
Data from 245 countries and 35 regional areas from 1961 through the most recent year

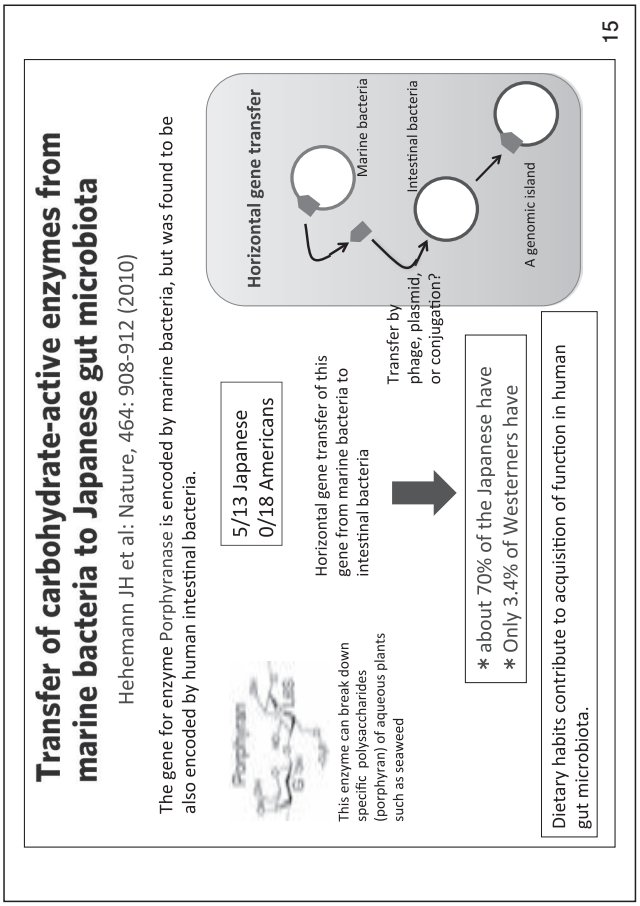
Food (119 items)	Japan	Russia	Sweden		America	China	Denmark		Venezuela	Malawi
Milk - Excluding Butter	203	481	979		700	82	710		403	14
Vegetables, Other	228	240	170		186	766	234		84	40
Wheat	131	358	218		222	182	275		134	18
Milk, Whole	127	350	193		310	75	98		259	11
Potatoes	58	312	159		147	101	208		49	297
Beer	73	211	113		229	71	195		222	6
Rice (Paddy Equivalent)	222	21	23		34	314	20		132	24
Sugar, Raw Equivalent	75	97	109		175	17	142		114	35
Maize	31	2	3		35	19	13		176	365
Sugar (Raw Equivalent)	47	87	101		91	16	114		108	35
Pig meat	54	54	99		82	101	132		21	9
Sugar, Refined Equiv	43	80	93		84	15	105		99	32
Rice (Milled Equivalent)	148	14	16		23	209	14		88	16
Poultry Meat	46	62	42		134	35	52		99	4
Oranges, Mandarines	28	22	139		83	27	120		39	0
Fruits, Other	28	60	62		62	79	64		44	48
Tomatoes	23	64	61		121	78	56		33	7
Bovine Meat	24	48	68		109	13	71		67	6
Cassava	0				0	5			42	194

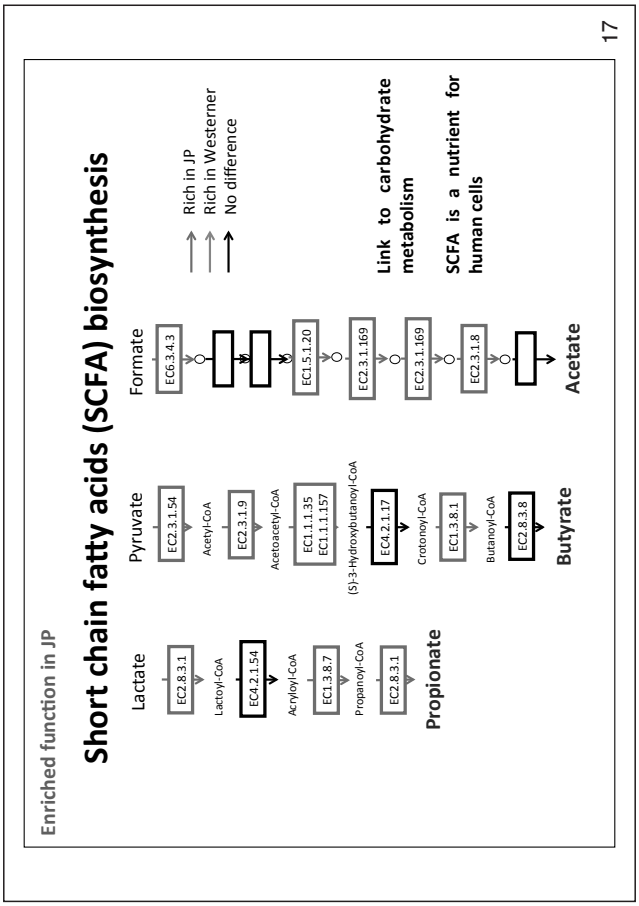
12

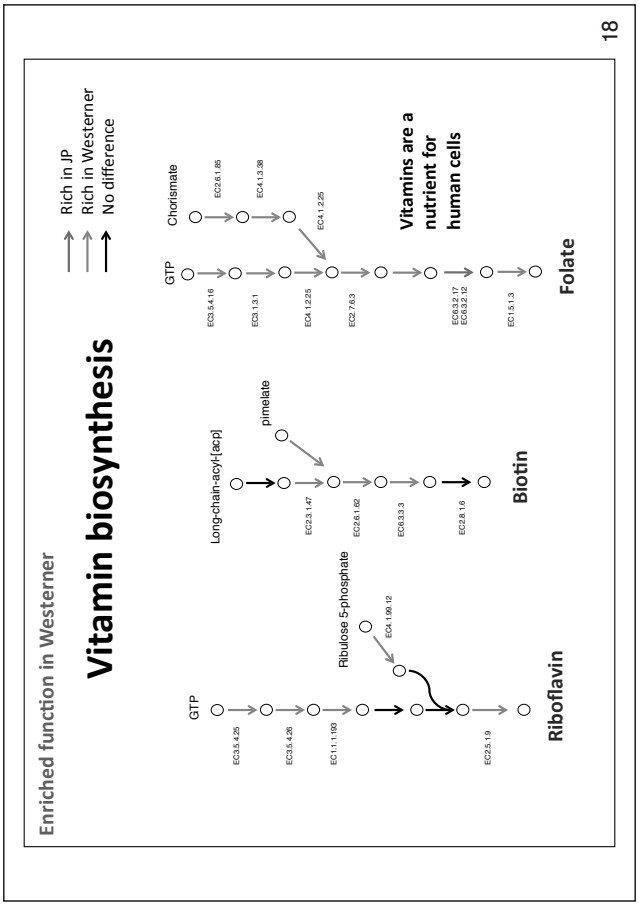

Rarefaction curve of non-redundant genes in the Japanese dataset
Non-redundant genes
1≥ (1%≥)
2≥ (2%≥)
6≥ (5%≥)
11≥ (10%≥)
22≥ (20%≥)
32≥ (30%≥)
53≥ (50%≥)
Non-redundant genes
Individual specific
Shared with others
14


Three major nutrient components calculated from FAOSTAT data
(g)
protein
lipid
carbohydrate
Dendrogram of the countries based on FAOSTAT data by hierarchical clustering
Malawi
Japan
China
Spain
Venezuela
Russia
America
Sweden
Denmark
Asian diet
Western diet
United States_2011
Spain_2011
Sweden_2011
Denmark_2011
Russia_2011
Venezuela_2011
China_2011
Japan_2011
Malawi_2011
13


The porphyranase gene is present in a genomic island
Mapping of metagenomic reads onto the Bacteroides plebeius genome
Black bar : a region containing the gene for porphyranases
Green box : a region containing Japanese gut microbiome-specific sequences
Blue box : a region rich in conjugative transposons (CTNs)
No genomic island
JP
EU
16


Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota
Hehemann JH et al: Nature, 464: 908-912 (2010)
The gene for enzyme Porphyranase is encoded by marine bacteria, but was found to be also encoded by human intestinal bacteria.
5/13 Japanese
0/18 Americans
This enzyme can break down specific polysaccharides (porphyran) of aqueous plants such as seaweed
Horizontal gene transfer of this gene from marine bacteria to intestinal bacteria
Horizontal gene transfer
Marine bacteria
Intestinal bacteria
Transfer by phage, plasmid, or conjugation?
A genomic island
* about 70% of the Japanese have
* Only 3.4% of Westerners have
Dietary habits contribute to acquisition of function in human gut microbiota.
15


Enriched function in JP
Short chain fatty acids (SCFA) biosynthesis
Lactate
Pyruvate
Formate
EC2.8.3.1
Lactoyl-CoA
EC4.2.1.54
Acryloyl-CoA
EC1.3.8.7
Propanoyl-CoA
EC2.8.3.1
Propionate
EC2.3.1.54
Acetyl-CoA
EC2.3.1.9
Acetoacetyl-CoA
EC1.1.1.35
EC1.1.1.157
(S)-3-Hydroxybutanoyl-CoA
EC4.2.1.17
Crotonoyl-CoA
EC1.3.8.1
Butanoyl-CoA
EC2.8.3.8
Butyrate
EC6.3.4.3
Acetate
Rich in JP
Rich in Westerner
No difference
Link to carbohydrate metabolism
SCFA is a nutrient for human cells
17


Enriched function in Westerner
Vitamin biosynthesis
GTP
EC3.5.4.25
EC3.5.4.26
EC1.1.1.193
Ribulose 5-phosphate
EC4.1.99.12
EC2.5.1.9
Riboflavin
Long-chain-acyl-[acp]
pimelate
EC2.3.1.47
EC2.6.1.62
EC6.3.3.3
EC2.8.1.6
Biotin
GTP
Chorismate
EC3.5.4.16
EC2.6.1.85
EC3.1.3.1
EC4.1.3.38
EC4.1.2.25
EC4.1.2.25
EC2.7.6.3
EC6.3.2.17
EC6.3.2.12
EC1.5.1.3
Folate
Vitamins are a nutrient for human cells
Rich in JP
Rich in Westerner
No difference
18

セッション 4−2

共生体としての腸内細菌

Professor of Paediatrics, Microbiology, and Dentistry
Hospital for Sick Children, University of Tronto
Philip. M. Sherman, M.D., FRCPC

現在では、複雑で多様な腸内細菌叢が人の健康増進に重要であると理解されている。dysbiosis（腸内菌の共生バランス失調）を通じて、人の健康状態は生涯に亘り、腸の構成や機能の変化と関連しているまた、dysbiosis が種々の疾病の病因に関わっているとの認識も高まってきている。プロバイオティクスは、「十分な量を摂取することにより腸内細菌叢の恒常性を回復し、宿主の健康に役立つ生きた微生物」と定義されている。過敏性腸症候群による腹痛、急性腸炎の持続期間、抗生物質誘発性あるいは *Clostridium difficile* 誘発性の下痢など、様々な腸内環境において特定のプロバイオティクスに改善効果が認められ、プラセボより有効であることが複数の無作為化臨床試験で示されている。*Helicobacter pylori* 除菌治療の第一選択肢である三剤併用あるいは連続療法において、補助剤としてプロバイオティクスを用いると副作用の発生率が低下し、除菌の成功率が上昇する。しかし、これらの知見が現行の治療法を変えるに至るほど十分なものであるかは、激しく議論されているところである。

我々は、選ばれた乳酸菌が腸管障害の予防に有効であることを示す一連の研究に取り組んでいる。これらの試験では、組織培養系における *Escherichia coli* O157:H7 感染上皮細胞の細胞骨格の再構築、*Citrobacter rodentium* 誘導マウス大腸炎、ストレス（水回避ストレスや母子分離ストレス）によるげっ歯類大腸の変化など、種々の実験的アプローチによって腸上皮傷害が誘導された。プロバイオティクスは、腸内細菌叢の多様性を回復し、病原菌の定着・繁殖を抑制するが、有用な効果を示すためには介入のタイミングが重要である。プロバイオティクスがもたらす効果成分の正確な構成を明らかにすることが現在の研究課題となっている。

腸の dysbiosis のコントロールにプロバイオティクスを用いた場合の試験結果が多様であることは、Colonization resistance（外来菌の定着抑制機構）、上皮バリアーの完全性、シグナル伝達の調節、自然免疫および獲得免疫に関する効果が菌株特異的であることによって説明できると考えられる。将来、プロバイオティクスは、効果の根底にあるメカニズムに基づき、特定の病態に対する治療法として選択されるようになるであろう。さらに、特定の疾病を標的にデザインされたプロバイオティクスが開発される可能性もある。未解決の課題は、最適な用量、摂取のタイミング、単一と複数の菌株の投与のどちらが好ましいか、保存中の生菌数維持、プロバイオティクス由来製品を利用するメリットなどに関連するものである。

Session 4−2

Intestinal Bacteria as a Symbiont

Philip M. Sherman, M.D., FRCPC
Professor of Paediatrics, Microbiology, and Dentistry
Hospital for Sick Children, University of Tronto

The complex and diverse intestinal bacterial microflora is now recognized as important in promoting human health. An altered gut microflora, referred to as dysbiosis, correlates the life continuum with changes in gut composition and function at the beginning and at the end of life span. Dysbiosis is also increasing recognized to have an etiologic role in a variety of diseases. Probiotics are defined as live microorganisms that, when ingested in sufficient amounts, restore microbial homeostasis and have a benefit on health. Randomized controlled trials indicate that certain probiotics are more effective than placebo in a variety of intestinal conditions including, for example, abdominal pain in irritable bowel syndrome, the duration of acute enteritis, antibiotic- and *Clostridium difficile*-induced diarrhea. When used as an adjunct with either first-line triple or sequential therapies, probiotics also reduce the frequency of adverse effects and increase rates of successful *Helicobacter pylori* eradication. However, whether the findings are sufficient to change current practice remains the subject of intense debate.

We have undertaken a series of studies demonstrating the efficacy of selected lactic acid-producing bacteria in preventing the consequences of intestinal injury. In these models, damage to the gut epithelium was induced by employing a variety of experimental approaches including *Escherichia coli* O157:H7-induced rearrangement of the cytoskeleton in infected epithelial cells grown in tissue culture, *Citrobacter rodentium*-induced colitis in mice, and stress-induced (using either water avoidance or maternal separation) changes in the large bowel of rodents. Probiotics restore gut microbial diversity and reduce pathogen colonization, but timing of the intervention is critical for demonstrating a beneficial effect. The precise component of the probiotic agent mediating the observed effects is the subject of current research.

Strain-specific effects on colonization resistance, epithelial barrier integrity, modulation of signal transduction events, as well as innate and adaptive immune responses likely serve to explain the observed variability in findings when employing probiotics to manage gut dysbiosis. In the future, probiotics are likely to be chosen for use in a defined clinical setting based on their underlying mechanisms of action. In addition, there is the possibility of developing specific, disease-targeted designer probiotics. Unresolved issues relate to optimal dosages, timing of ingestion, single versus combination formulations, maintenance of viability in storage, and the merits of employing probiotic-derived products.

Selected References:

Vong L, Pinnell LJ, Maattanen, Yeung CW, Lurz, **P. Sherman PM**: Selective enrichment of commensal gut bacteria protects against Citrobacter rodentium-induced colitis. Am J Physiol, 2015; In Press.

Smith CJ, Emge JR, Berzins K, Lung L, Khamishon R, Shah P, Rodrigues DM, Sousa AJ, Reardon C, **Sherman PM**, Barrett KE, Gareau MG: Probiotics normalize the gut-brain-microbiota axis in immunodeficient mice. Am J Physiol 2014;307:G793−G802.

Sanders M-E, Guarner F, Guerrant R, Holt PR, Quigley EM, Sartor RB, **Sherman PM**, Mayer EA. An update on the use and investigation of probiotics in health and disease. Gut 2013;62:787−796.

Backhed F, Fraser C, Ringel Y, Sanders M-E, Sartor RB, **Sherman PM**, Versalovic JE, Young V, Finlay BB: Defining a healthy human gut microbiome:current concepts, future directions, and clinical applications. Cell Host and Microbe 2012;15:611−622.

Defining a healthy microbiome

ILSI-Japan 7th International Conference on Nutrition and Aging

Wednesday September 30, 2015

Tokyo, Japan

Philip M. Sherman, MD, FRCPC
Professor of Paediatrics, Microbiology, and Dentistry
Hospital for Sick Children, University of Toronto
Scientific Director, CIHR Institute of Nutrition, Metabolism & Diabetes
Canada Research Chair in Gastrointestinal Disease

SickKids CIHR IRSC

1

Learning objectives:

1. **Update what is known about the gut microbiome in health & over the life span.**
2. **Consider the role of prebiotics and probiotics in management of dysbiosis.**
3. **Report on the Canadian Microbiome Initiative (CIHR & Genome Canada).**

2

Disclosures

I have the following financial relationships to disclose:

***Lallemand Human Nutrition**

(research contract)

***Abbott Nutrition (honorarium)**

Jamieson Laboratories (honorarium)

***Mead Johnson Nutrition (honorarium)**

***Nestlé Nutrition (honorarium)**

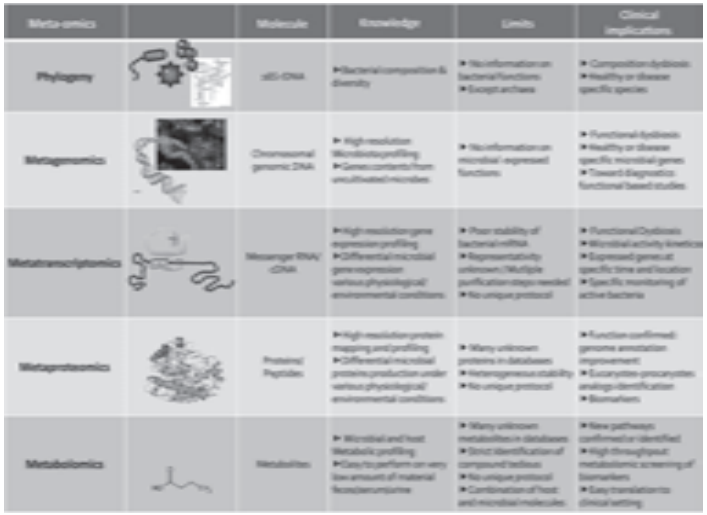
***Procter & Gamble (honorarium)**

Antibe Therapeutics (stockholder)

** Products or services produced by this company are relevant to my presentation.*

3

Differing levels of analysis of the gut microbiome



P Lepage et al. Gut 2013;62:146-158

4

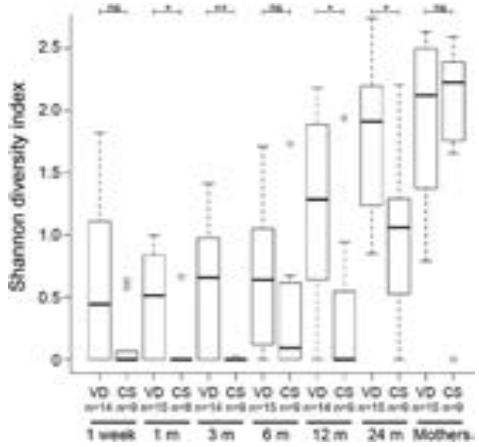
Development of the gut microbiota

- **Fetal intestine: "sterile"**
- **Initial colonization determined by:**
  - **Delivery mode (caesarian section vs. vaginal)**
  - **Diet (breast feeding vs. formula feedings)**
  - **Hygiene (exposure to pathogens)**
  - **Medication (antibiotics*)**
- **Temporal changes over the life span: premature infants & the elderly**

S Rautava, et al. Nat Rev Gastroenterol 2012;9:565-576
M-E Sanders, et al. Gut 2013;62:787-796
* F Raymond et al. ISME J 2015; In Press
ES Lim et al. Nat Med 2015; In Press

5

Gut diversity in 15 vaginally delivered and 9 caesarean section infants

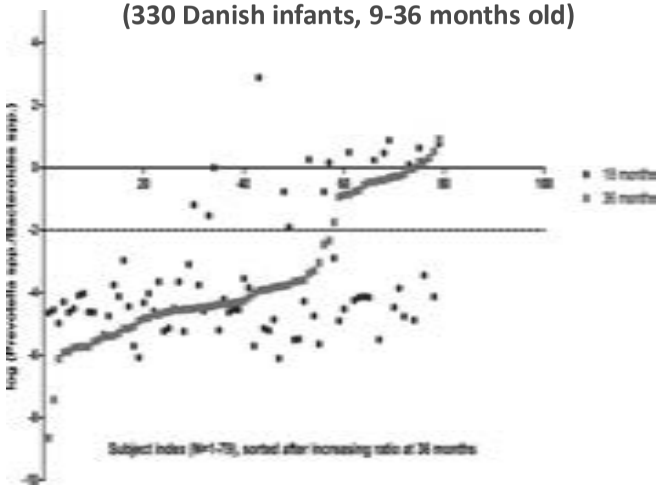


HE Jakobsson, TR Abrahamsson, et al. Gut 2014;63:559-566
F Backhed et al. Cell Host Microbe 2015;17:690-703

6

Changing gut microbiota diversity

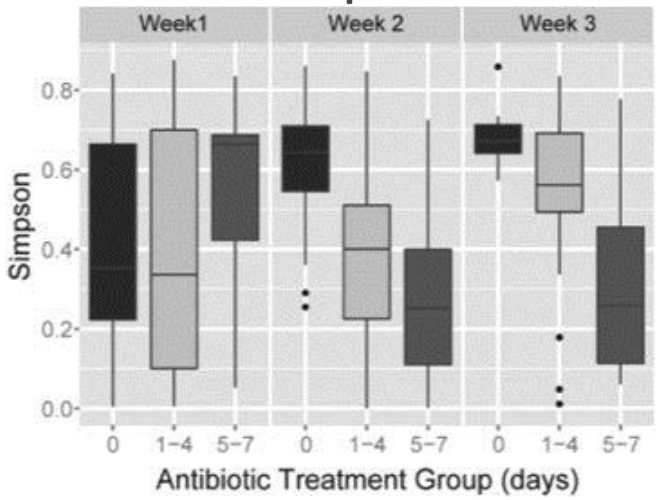
(330 Danish infants, 9-36 months old)



A. Bergstrom et al. Appl Environ Microbiol 2014;80:2889-2900

7

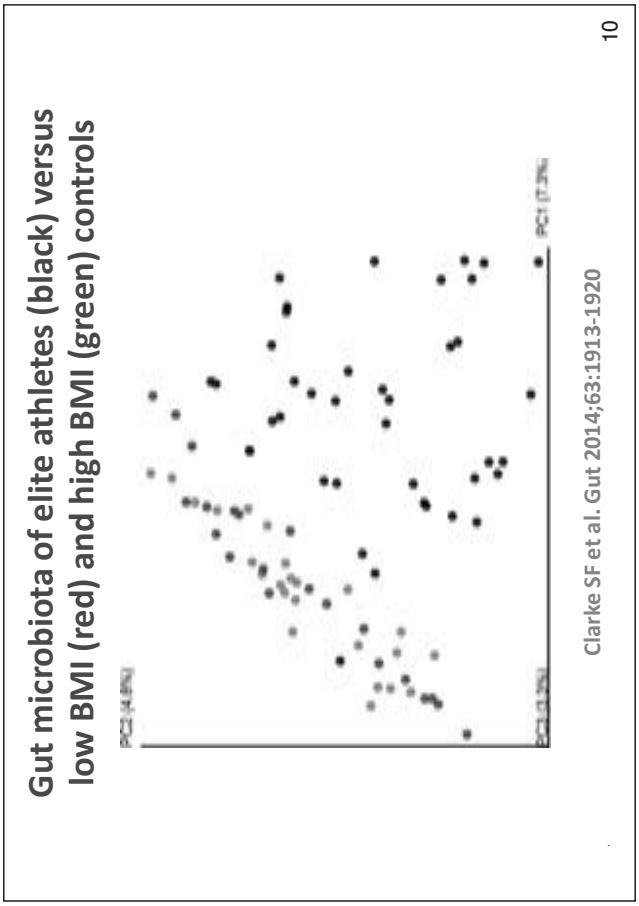
Antibiotic use in preterm infants

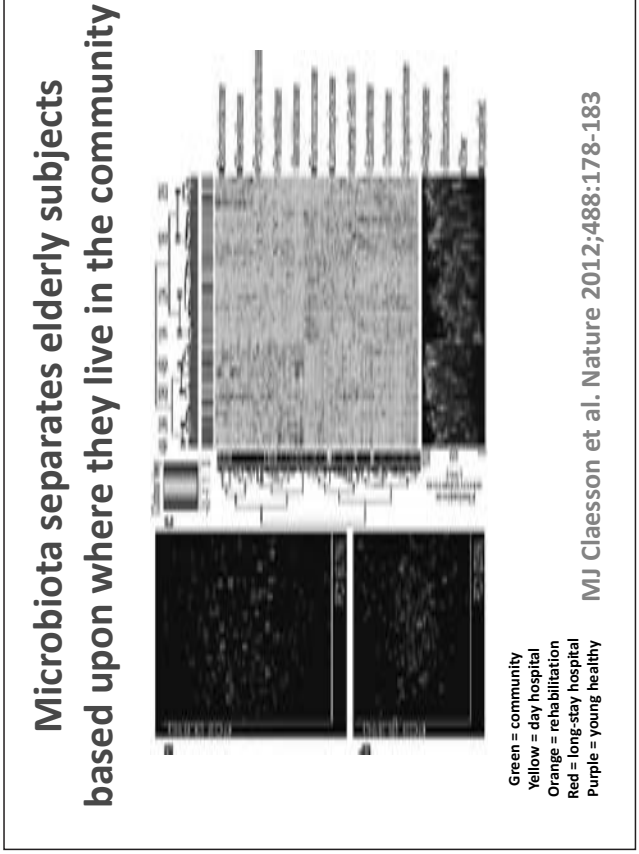


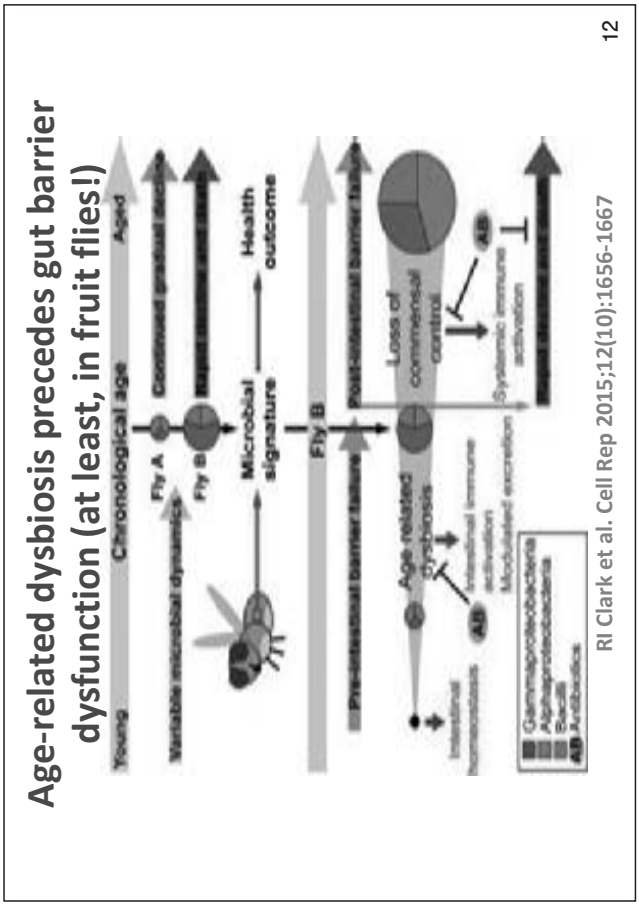
C Greenwood et al. J Pediatr 2014;165:23-29
S Arboleya et al. J Pediatr 2015;166:538-544

8

Gut Microbiome Clusters by Diet:
Herbivore
Carnivore
Omnivore
Ley et al, Science 2008;320:1647-1651
9


Gut microbiota of elite athletes (black) versus low BMI (red) and high BMI (green) controls
Clarke SF et al. Gut 2014;63:1913-1920
10


Microbiota separates elderly subjects based upon where they live in the community
Green = community
Yellow = day hospital
Orange = rehabilitation
Red = long-stay hospital
Purple = young healthy
MJ Claesson et al. Nature 2012;488:178-183
11


Age-related dysbiosis precedes gut barrier dysfunction (at least, in fruit flies!)
Young
Chronological age
Aged
Fly A
Fly B
Microbial signature
Health outcome
Fly B
Age-related dysbiosis
Loss of commensal control
Gammaproteobacteria
Alphaproteobacteria
Bacilli
Antibiotics
RI Clark et al. Cell Rep 2015;12(10):1656-1667
12

Reduced bacterial diversity (dysbiosis): an emerging theme across diseases

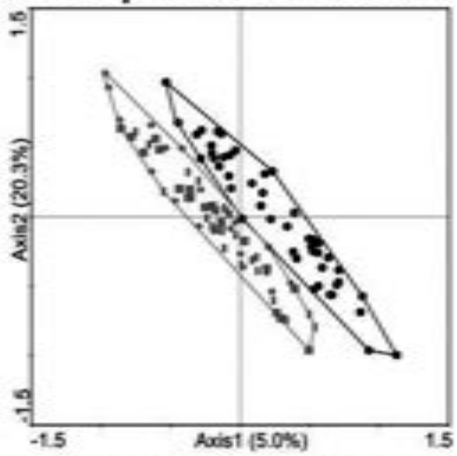
- Microbiota affected by:
  - Infections
  - Antibiotics
  - Xenobiotics
- Diabetes mellitus & Obesity
- Cancers: gastric, colonic
- Inflammatory bowel diseases
- Necrotizing enterocolitis
- Irritable bowel syndrome, colic
- Celiac disease

Scientific American
June 2012

C Peterson & J Round
Cellular Microbiology 2014;16:1024-1033

13

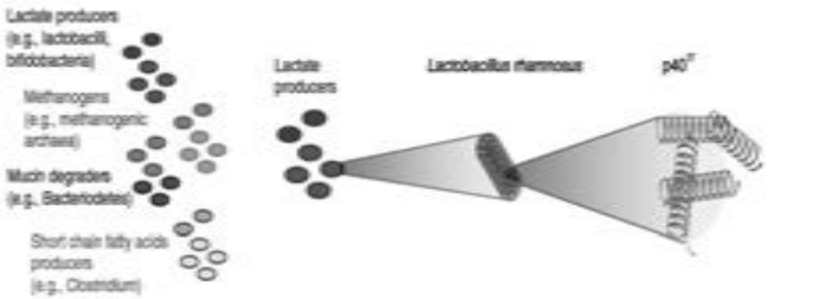
Microbial composition of IBS vs. controls



Adults: M. Rajlic-Stojanovic et al. Gastroenterology 2011;141:1792-1801
Children: D. Saulnier et al. Gastroenterology 2011;141:1782-1791
Colicky Infants: C deWeerth, et al. Pediatrics 2013;131:e550-e558

14

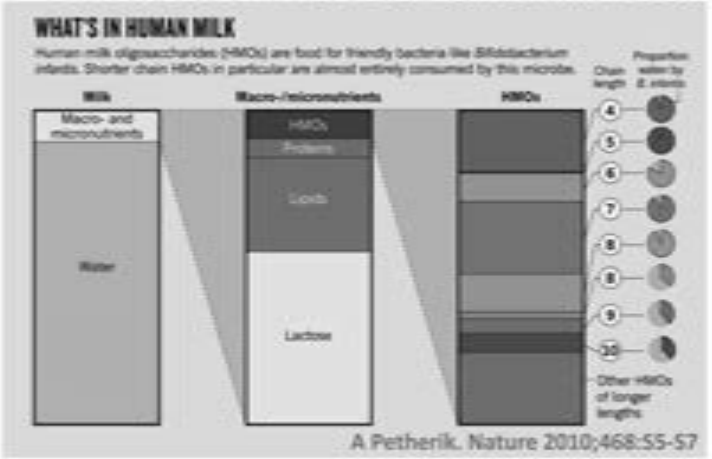
How does one increase diversity?



B. Olle. Nat Biotechnol 2013;31:309-315
"Precision microbiome reconstitution":
C. Buffie et al. Nature 2015;517:205-208

15

Nature's First Functional Food

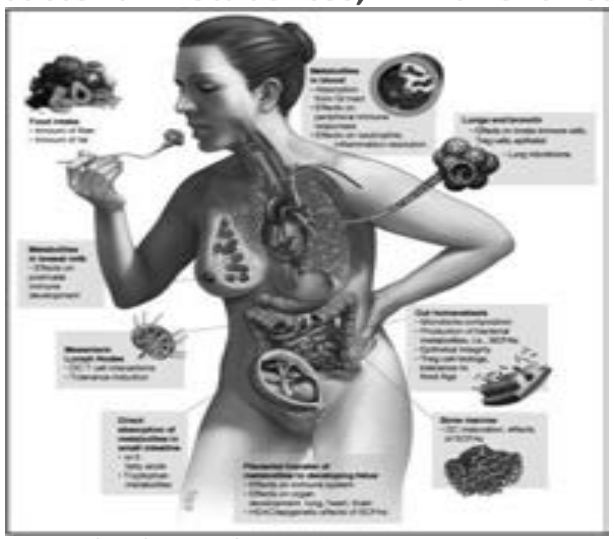


A Petherik. Nature 2010;468:S5-S7

S Musilova, et al. Beneficial Microbes 2014;5:273-283
TR Abrahamsson & PM Sherman. J Infect Dis 2014;209:323-324
T. Gura. Science 2014;345:747-749

16

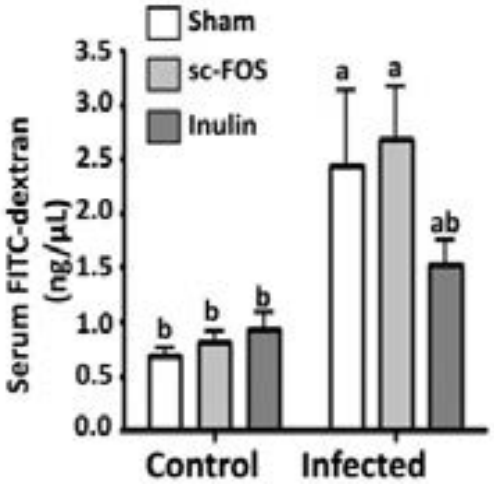
Diet, bacterial metabolites, immune function



AN Thornburn et al. Immunity 2014;40:833-42

17

Gut epithelial barrier integrity impacted by diet



K. Johnson-Henry et al. J Nutr 2014;144:1725-1733

18

Benefits of prebiotics for infants

systematic review of 11 RCT's (n=1,459)

- Results in softer, more frequent stools
- Increases #'s of Bifidobacter & Lactobacilli
- Reduces stool pH
- No effect on weight accretion
- Clinical impact not assessed as an outcome
  - might protect against harmful bacteria
  - may reduce infection rates

S Rao, et al. Arch Pediatr Adolesc Med 2009;163:755-764
R Oozer, et al. Am J Clin Nutr 2013;98:561S-571S

19

Definition & examples of a probiotic

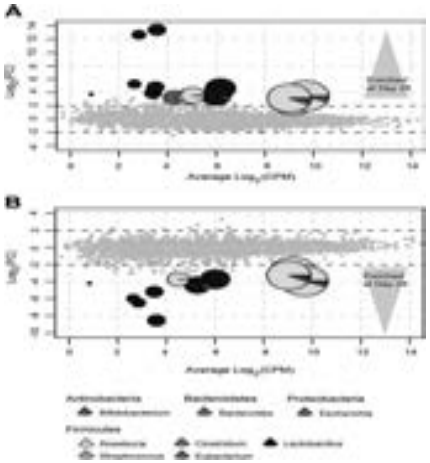
Is:	Is not:	Examples:
Microbe	Synonomous with "commensal"	*Bifidobacterium (longum, bifidum)*
Alive	Synonomous with "live, active culture"	*Streptococcus thermophilus*
Defined and properly named	Live vaccine	*Lactobacillus (GG, acidophilus, rhamnosus, casei, plantarium)*
Safe	Fecal enema	*Lactococcus (lactis, cremoris)*
Regulatory categories -Food -Dietary supplement -Drug -Designer/genetically modified -Direct fed (animal uses)		*Escherichia coli Nissle 1917*
		Saccharomyces (boulardii, cerevisiae)

C. Hill et al. Nat Rev Gastroenterol Hepatol 2014;11(8):506-514

20

Functional dynamics of the gut microbiome in elderly: differential bacterial gene expression following probiotic

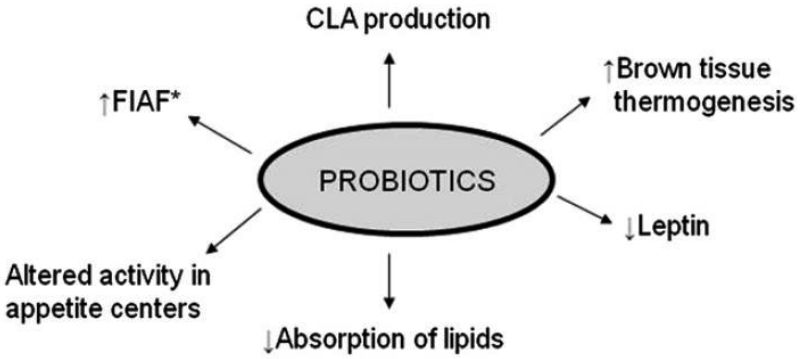
N=12, age 65-80 yrs
L. rhamnosus GG
for 4 wks (panel A)
and 8 wks (panel B)



EA Eloe-Fadrosh et al. mBio 2015;6(2):e00231-15
J Vulevic et al. Br J Nutr 2015;114:586-595

21

Anti-obesity effects of probiotics:



CLA : linoleic acid
FIAF: fasting-induced adipose factor

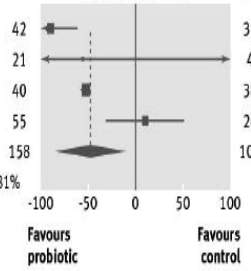
T Arora et al., Nutrition 2013;29:591-596

22

Infant colic trials with *L reuteri* DSM 17938

Study or subgroup	Probiotic Mean	Probiotic SD	Probiotic Total	Control Mean	Control SD	Control Total	Mean difference IV, random (95% CI)	Weight (%)	Mean difference IV, random (95% CI)
Savino 2007[32]	81.70	38.08	41	171.60	84.24	42		31.4	-89.90 (-117.92 to -61.88)
Savino 2010[33]	111.20	335.01	25	167.00	261.18	21		4.1	-55.80 (-228.21 to 116.61)
Szajewska 2013[36]	75.60	9.91	40	128.40	10.64	40		38.2	-52.80 (-57.31 to -48.29)
Sung 2014	217.20	129.50	63	207.10	92.80	55		26.3	10.10 (-30.20 to 50.40)
Total			169			158		100.0	-48.05 (-84.76 to -11.34)

Test for heterogeneity: τ^2=912.25, χ^2=16.09, df=3, P=0.001, I^2=81%
Test for overall effect: z=2.57, P=0.01



Double-blinded placebo controlled, except Savino et al 2007
Exclusive breast milk, except Sung et al 2014
Different gut microbiota: Italy, Poland, Australia

V Sung et al. BMJ Open 2014;Dec 4 4(12)e006475
Toronto Study: K Chau et al. J Pediatr 2015;166(1):74-78

23

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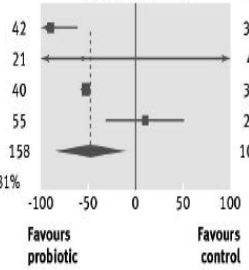
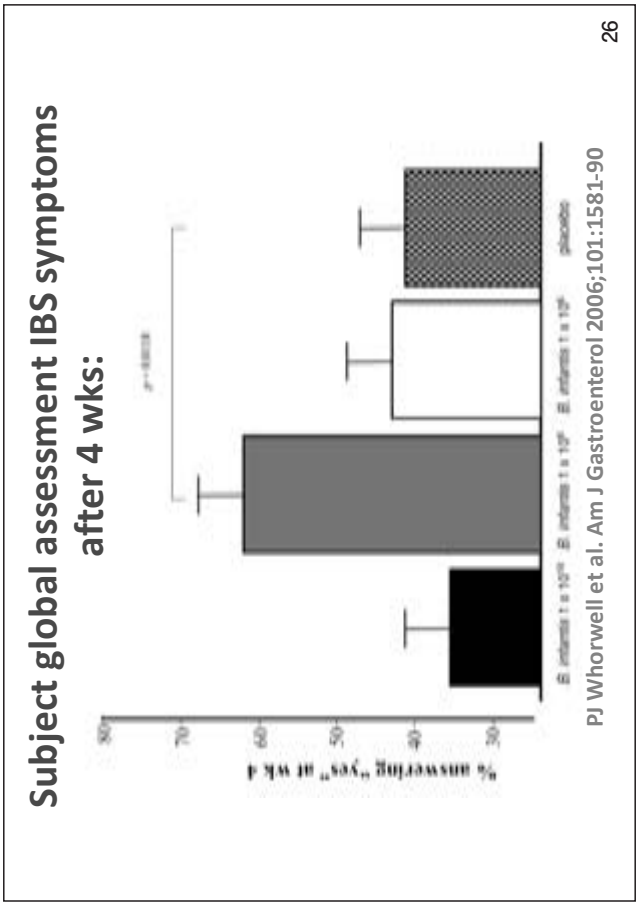
Test for heterogeneity: τ^2=912.25, χ^2=16.09, df=3, P=0.001, I^2=81%
Test for overall effect: z=2.57, P=0.01

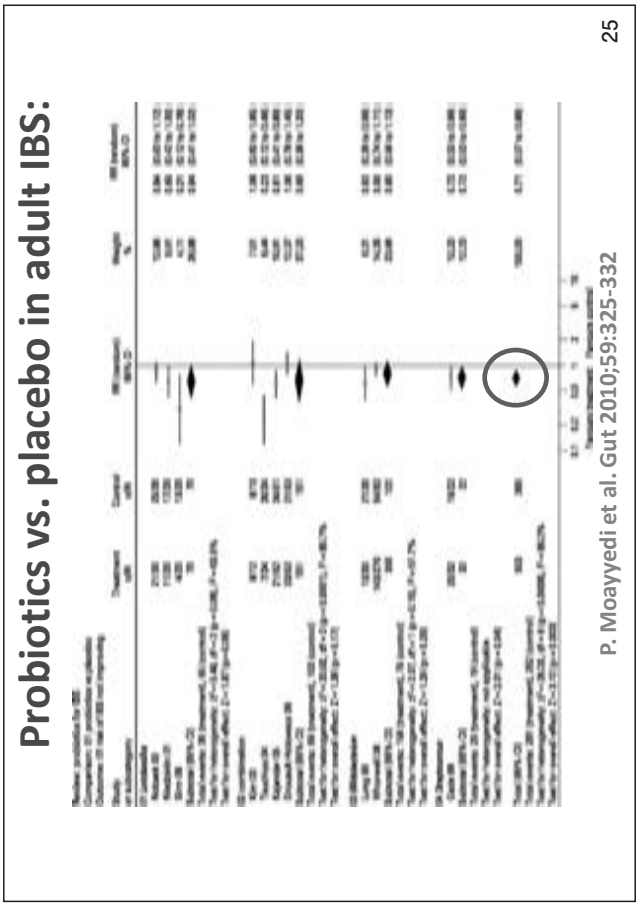


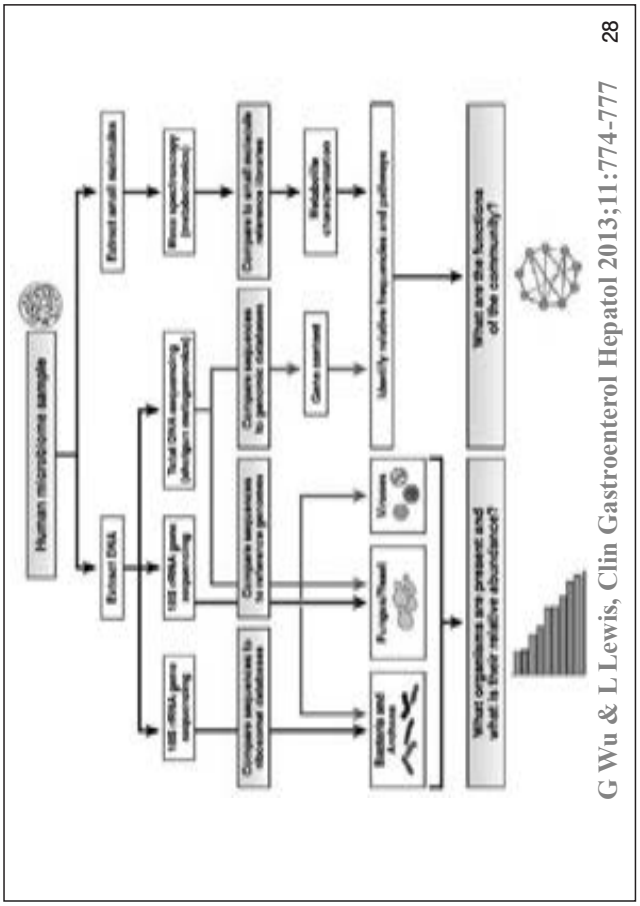
Double-blinded placebo controlled, except Savino et al 2007
Exclusive breast milk, except Sung et al 2014
Different gut microbiota: Italy, Poland, Australia

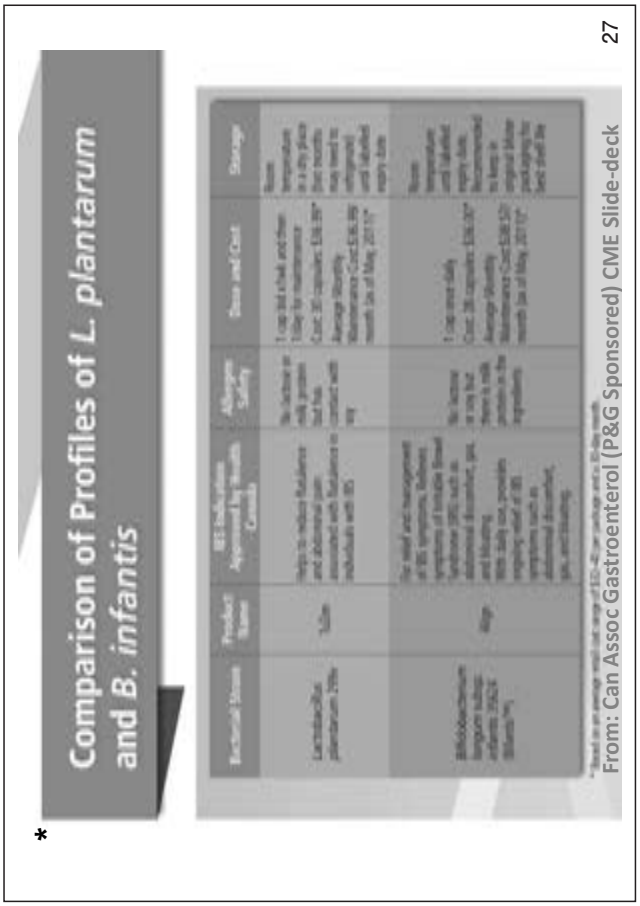
V Sung et al. BMJ Open 2014;Dec 4 4(12)e006475
Toronto Study: K Chau et al. J Pediatr 2015;166(1):74-78

24


Subject global assessment IBS symptoms after 4 wks:
% answering "yes" at wk 4
B. infantis 1 x 10^10
B. infantis 1 x 10^9
B. infantis 1 x 10^8
placebo
PJ Whorwell et al. Am J Gastroenterol 2006;101:1581-90
26


Probiotics vs. placebo in adult IBS:
P. Moayyedi et al. Gut 2010;59:325-332
25


Human microbiome sample
Extract DNA
Extract small molecules
16S rRNA gene sequencing
Total DNA sequencing (shotgun metagenomic)
Mass spectrometry (metabolomic)
Compare sequences to reference genomes
Compare sequences to genomic databases
Compare to small molecule reference libraries
Gene assembly
Metabolite characterization
Bacteria and Archaea
Fungi/Yeast
Viruses
Identify relative frequencies and pathways
What organisms are present and what is their relative abundance?
What are the functions of the community?
G Wu & L Lewis, Clin Gastroenterol Hepatol 2013;11:774-777
28


Comparison of Profiles of L. plantarum and B. infantis
*
From: Can Assoc Gastroenterol (P&G Sponsored) CME Slide-deck
27

CIHR: 13 virtual institutes (funding of CDN $ 1B/year)

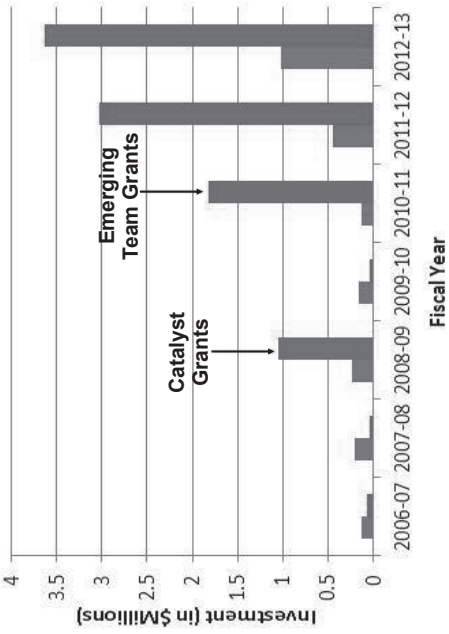
29

Canadian Microbiome Initiative (CMI)

- Creation of the CMI in 2007
- Workshop with Genome Canada, June 2008
- Strategic Funding
  - Catalyst Grants (1.2 M for 12 NPI's)
  - Emerging Team Grants (15.5M for 7 NPI's)
  - Roadmap Signature Initiatives
- Networking/outreach activities
  - Workshop for Teams
  - International connections
  - Journalism workshop (Nat Med)
  - Café scientifique for public dissemination
  - Workshop – discovery to applications

30

CIHR funding of microbiota research



31

Emerging Team Grants: Canadian Microbiome Initiative

Principal Investigator	Institution	Title
Michael G SURETTE	McMaster Univ	Respiratory tract microbiome dynamics and the interplay of commensal bacteria with resident pathogens
Deborah M MONEY	Univ British Columbia	Vaginal microbiome project
Anita L KOZYRSKYJ	Univ Alberta	Synergy in microbiota research (infant and children)
David S GUTTMAN	Univ Toronto	Assessing the breadth and depth of cystic fibrosis-associated polymicrobial respiratory tract infections
Barton B FINLAY	Univ British Columbia	Impact of the microbiota on immune development and disease
W Ford DOOLITTLE	Dalhousie Univ	Modeling and mapping microbial diversity and function with marker genes, genomes and metagenomes
Kenneth CROITORU	Univ Toronto	Influences of Host Genome on the Human Gut Microbiome: Healthy Cohort Carrying Crohn's Disease Risk Alleles - Crohns & Colitis Canada /Helmsley Foundation GEM Project)

32

Joint Programming Initiative: A Healthy Diet for a Healthy Life (JPI-HDHL)

- 25 member states and associated countries of the European Union
- Strategic research agenda developed in 3 areas:
  1. Determinants of Diet and Physical Activity
  2. Diet and Food Production
  3. Diet-Related Chronic Diseases
- Joint Action: **Biomarkers in Nutrition** and Health - aimed at validation of biomarkers and the investigation of intake/exposure and nutritional status of biomarkers in the area of nutrition and health
  - Funded: FOODBALL consortium, which includes 20 research organizations from 9 EU countries, **Canada,** and New Zealand
- Future Priority Topic: **Intestinal Microbiomics**

For more information: http://www.healthydietforhealthylife.eu

33

Would you like a copy of the pdf?: philip.sherman@sickkids.ca

34

Take home messages in 2015

Gut microbiota is increasingly recognized to play a role in promoting health.

Intestinal dysbiosis appears to play a role in a variety of disease states.

Prebiotics & probiotics: comparative efficacy and relative safety profiles are needed.

Advocate for further research to define the FUNCTIONS of the microbiota, and which strain(s), dose of probiotics should be used in specific conditions.

35

36

セッション4-3

セグメント細菌の腸免疫システムにおける重要性と腸炎モデルへの応用

株式会社ヤクルト本社　中央研究所
梅﨑　良則

健常者の腸内（糞便）フローラを再発性の *Clostridium difficile* 腸炎の患者に移植することにより病態の改善が認められるなど、近年、健康・疾病における腸内フローラの役割が大きく注目されている。これまで腸内フローラと健康・疾患との関連性に関する研究はヒトフローラの細菌学的解析と動物モデルでのフローラに対する宿主応答の解析を中心に進められてきた。特に無菌動物への糞便フローラの移植による通常化実験はフローラに対する腸管の生理応答や免疫応答のプロセスを明らかにし、一方ノートバイオート実験はその構成菌の役割を明らかにした。その中で我々は腸上皮細胞の細胞膜成分の合成および腸粘膜の免疫系を指標に腸内フローラの解析を行った結果、マウスの小腸腸上皮細胞の主要糖脂質アシアロ GM1（GA1）のフコシル化反応、ならびに腸粘膜免疫システムを構成する腸上皮間リンパ球（IELs）のリクルートの亢進、Th17 細胞の誘導、IgA 分泌促進のいずれもがマウス回腸を主要なニッチとする難培養性の Segmented filamentous bacteria（SFB）によってもたらされることを明らかにした。IELs やフコシル化 GA1（FGA1）の生理的な役割についてはまだ不明な点が残っているが、腸内フローラあるいは SFB がこれらの諸形質を誘導する機構については近年著しい進展が認められた。

現在、腸内細菌の刺激で顕著に増幅される IELs は、その前駆細胞（TCR α β+、CD8 α β+）が絨毛あるいはパイエル板などの抹消組織において腸内細菌由来の何らかの抗原で活性化され、その後上皮細胞間にリクルートされること[1]、SFB による IELs のリクルートの亢進は IELs の TCR 鎖と CD8 分子サブセットの解析から腸内フローラ全体の効果とは異なり、末梢の抗原に応答した CD8 α β（+）TCR α β T 細胞のみがリクルートされたものであると推定された。また SFB による IgA の産生促進を解析した結果、小腸パイエル板で腸内細菌によって B 細胞のプライミングが促進された後、小腸、大腸の粘膜固有層に移動し、IgA 産生が増強されることが推定された。さらに SFB は近年発見された Th17 細胞を単独で誘導することができる小腸常在菌であることが明らかとなり、これは腸管免疫の一つのトピックとなっている。Th17 細胞は消化管の病態生理に重要な役割を担っており、自己免疫疾患や腸炎などの疾患と腸内細菌の密接な関係が明らかにされてきた。SFB による免疫形質の誘導には明確な宿主特異性が認められ、たとえばラット由来の SFB はマウスではこれらの諸免疫形質を誘導できない。すでに我々は両 SFB の全ゲノム構造を決定したが、宿主特異性を決定する遺伝子候補を見いだせなかった。しかし現時点ではそれに関与する複数の遺伝子候補が Th17 細胞の誘導活性より推測される[2]。いずれにしても、宿主動物種に依存した SFB の小腸上皮への強い接着がこれらの免疫誘導メカニズムの起点になっていると思われる。

一方、SFB の免疫誘導活性が明らかになると腸炎発症における SFB の役割が追求された。その解析には SFB と他の常在菌を組み合わせてノートバイオート化した腸炎自然発症のミュータントマウスやデキストラン硫酸誘導腸炎モデルが用いられた。いずれの成績も SFB の単独定着、あるいは炎症に関わることが推定される主要な常在菌の混合定着のみでは炎症は発症しないが、両者を組み合わせることによって腸炎を発症する[3]。したがって、腸炎の発症や抑制に関わる腸内細菌の探索およびそのメカニズムの解析には SFB を含む腸内菌をノートバイオート化した腸炎モデルマウスはきわめて有用なツールとなると考えられる。

参考文献　1）Cheroutre et al., Nat Rev Immunol, 2011 、2）Yang et al., Nature, 2014 、3）Stepankova et al., Inflamm Bowel Dis, 2007

Session 4−3

Importance of Segmented Filamentous Bacteria in Intestinal Immune System and Its Application to Gut Inflammation Model

Yoshinori Umesaki
Yakult Central Institute

Now, fecal microbiota transplantation has been shown to be safe and effective for the refractory Clostridium difficile infection (CDI) or inflammatory bowel disease (IBD). This may pave the way to the individual treatment for the patients suffering some types of gut disorders. The analysis of the role of the microbiota in the development of the physiological and immunological functions of the intestine using gnotobiotic animals as well as comprehensive characterization of the human microbiota has contributed to the understanding of the importance of the microbiota. Today I would like to introduce the importance of segmented filamentous bacteria (SFB), a key indigenous microbe, in the development of the gut immune system using gnotobiotic mouse. Finally I would like to discuss on the application of SFB to IBD model mouse to elucidate the bacterial species including probiotics concerning the disease development and control and their mechanisms.

In the first step of our study, we have determined suitable biochemical and immunological markers for monitoring microbial effects in host animals. Comparing between the germ-free mice and conventional or conventionalized ones, we have found clear differences in epithelial cell glycolipid biosynthesis and intraepithelial lymphocyte dynamics. Furthermore, using these markers we have been able to identify a key microbe that activates the mucosal immune system in the small intestine. This is a kind of indigenous bacteria colonized the ileum, called segmented filamentous bacteria (SFB). SFB monocolonization of the germ-free mice enhances the recruitment for CD8$\alpha\beta$+TCR$\alpha\beta$-bearing intraepithelial lymphocytes (IELs). They also induce IgA secretion not only in the small intestine but also in the colon, probably through effective priming of B cell response by SFB in Peyer's patches. Moreover, it is a notable discovery that they can induce Th17 cells, a new helper T cell subset involving the defense against infections and inflammatory diseases, in the lamina propria. This immune stimulatory effect by SFB as well as the tight adhesion to the intestinal epithelial cell surface is host-species specific. Mouse-derived SFB efficiently bind to the epithelial cells and induce the immune system only in the mouse but not in the rat, and vice versa. Although we have determined the whole genome sequences of both mouse- and rat derived SFB, the candidate genes for the host specificity have not known clearly.

Judging from the immunostimulatory activities of SFB as mentioned above, it is easy to speculate that SFB shall contribute to the progression of the IBD in some way. The experiments with gnotobiotic IBD model mice suggest that SFB augment the disease development in cooperation with another kind of indigenous intestinal microbe, such as Bacteroides sp., despite no sign of the disease in the case of their monocolonization. Accordingly, gnotobiotic IBD model mouse based on SFB colonization is expected to be a useful tool for the analyses of the occurrence or repression of the disease and their mechanisms.

ILSI-Nutrition & Aging, 2015

Importance of Segmented Filamentous Bacteria in Intestinal Immune System and Its Application to Gut Inflammation Model

Yoshinori Umesaki
Yakult Central Institute

1

1. Background and Aims

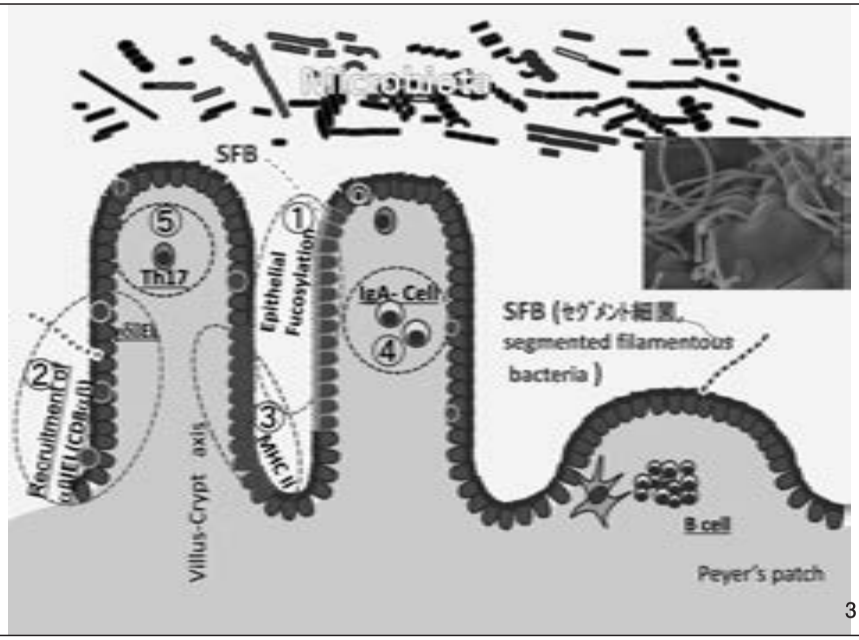
- Development of DNA Sequencing Technology with NG Sequencer
- Establishment of Animal Disease Models with Defined Bacterial Species

- Importance of Composition and Diversity of Microbiota
- Close Association of Microbiota with Health and Disease

(1) Analysis of the Physiological Responses to Microbiota
"What and How Key Bacteria Activate Gut Immune System? "

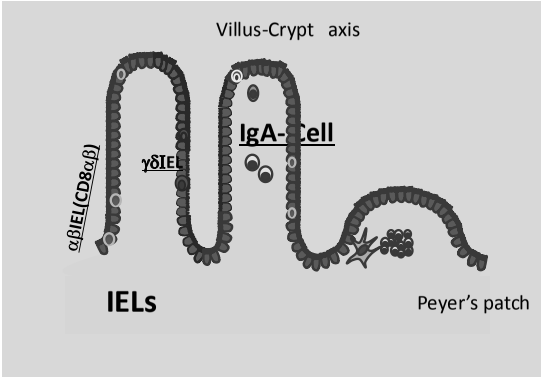
(2) Its Application
"Use of Probiotics for Improvement of Colitis"

2



3

2. IELs and IgA Responses to the Intestinal Microbes



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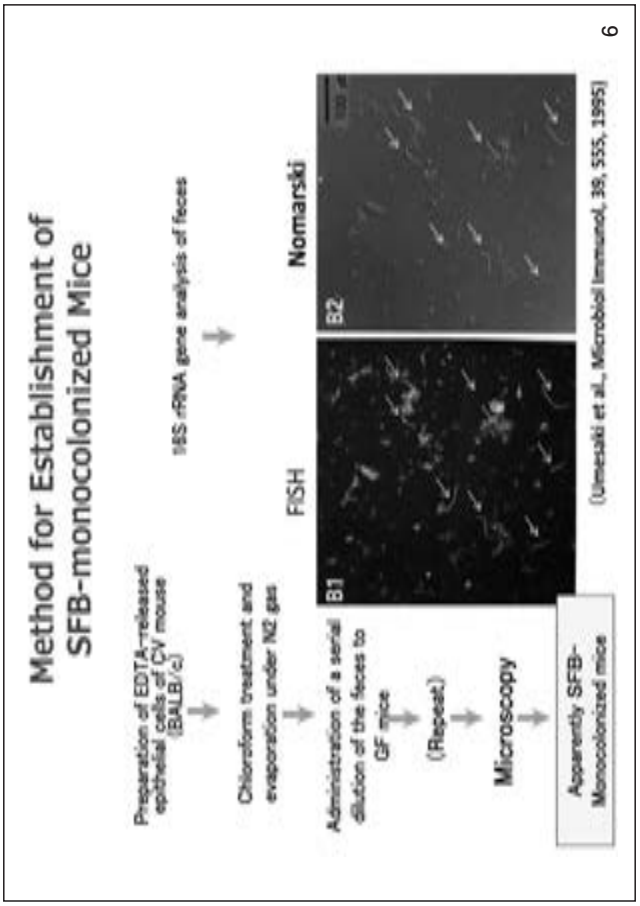
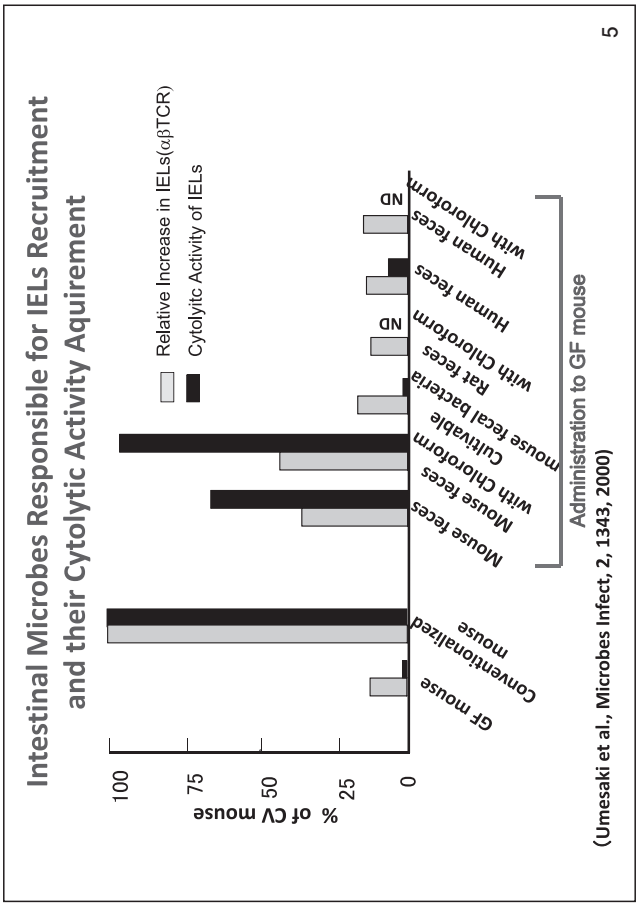
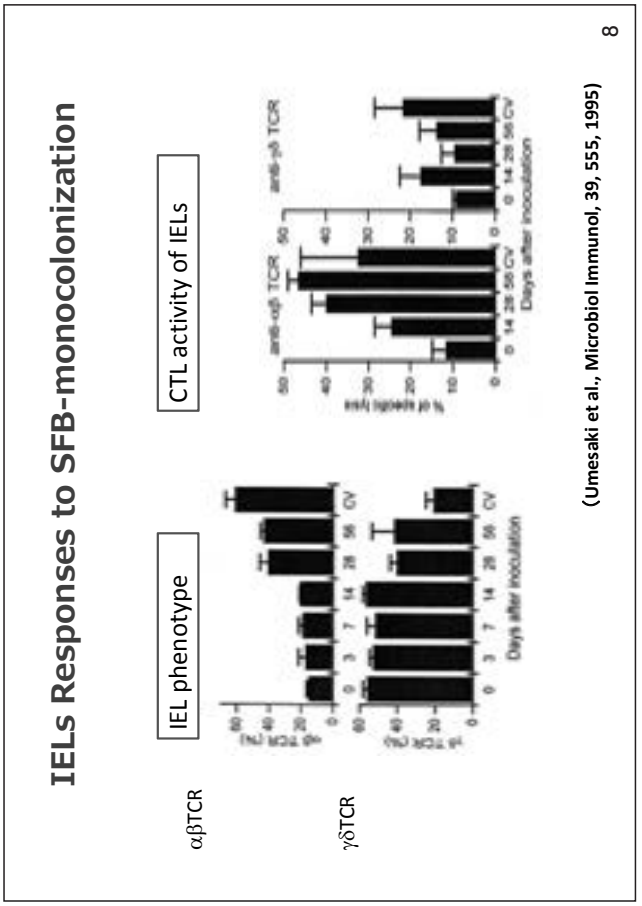
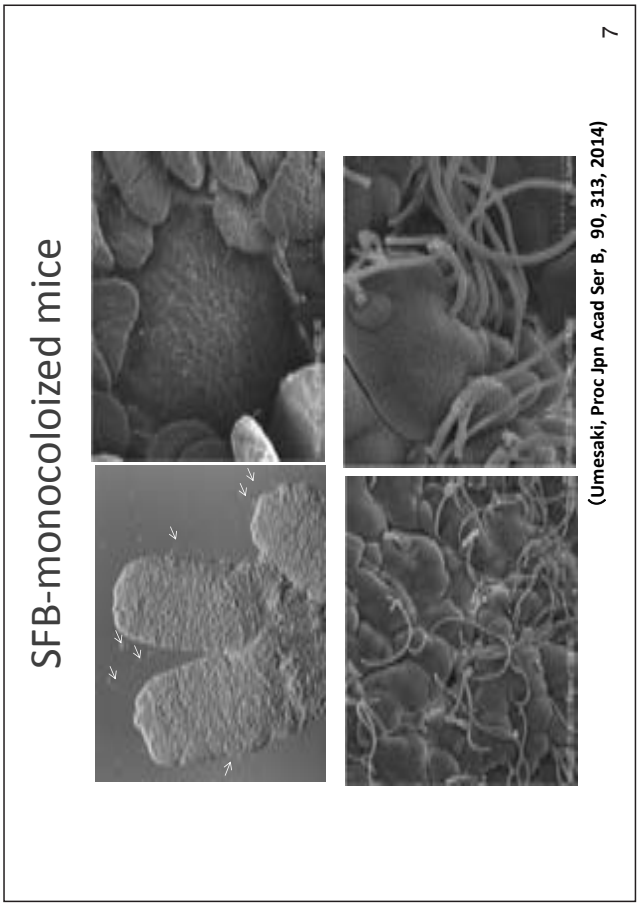

Method for Establishment of SFB-monocolonized Mice
Preparation of EDTA-released epithelial cells of CV mouse (BALB/c)
Chloroform treatment and evaporation under N2 gas
Administration of a serial dilution of the feces to GF mice
(Repeat)
Microscopy
Apparently SFB-Monocolonized mice
16S rRNA gene analysis of feces
FISH
Nomarski
B1
B2
(Umesaki et al., Microbiol Immunol, 39, 555, 1995)
6


Intestinal Microbes Responsible for IELs Recruitment and their Cytolytic Activity Aquirement
Relative Increase in IELs(αβTCR)
Cytolytic Activity of IELs
% of CV mouse
100
75
50
25
0
GF mouse
Conventionalized mouse
Mouse feces
Mouse feces with Chloroform
Cultivable mouse fecal bacteria
Rat feces with Chloroform
Human feces
Human feces with Chloroform
ND
ND
Administration to GF mouse
(Umesaki et al., Microbes Infect, 2, 1343, 2000)
5

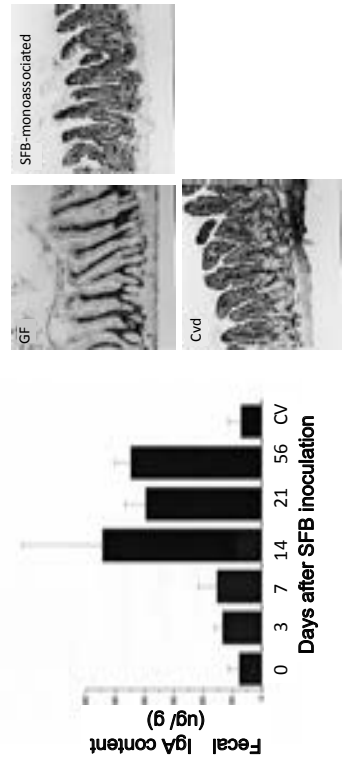

IELs Responses to SFB-monocolonization
αβTCR
γδTCR
IEL phenotype
CTL activity of IELs
anti-αβ TCR
anti-γδ TCR
Days after inoculation
(Umesaki et al., Microbiol Immunol, 39, 555, 1995)
8


SFB-monocoloized mice
(Umesaki, Proc Jpn Acad Ser B, 90, 313, 2014)
7

IgA Produciton and Epithelial MHC class II Response to SFB-monocolonization

IgA production

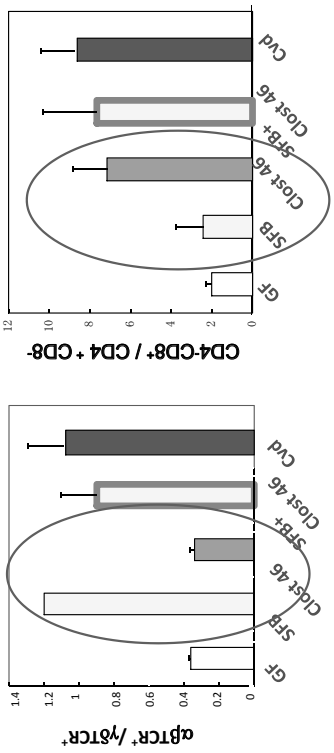
Epithelial MHC class II



(Umesaki et al., Microbiol Immunol, 39, 555, 1995)

9

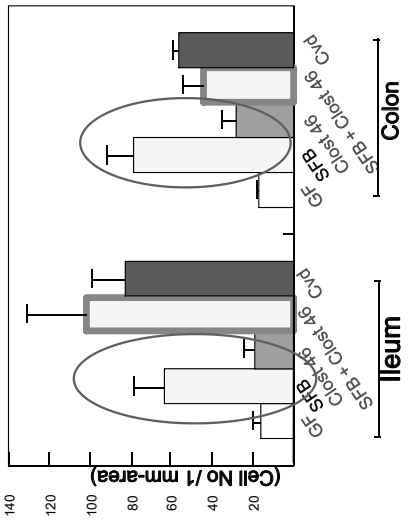
IELs Recruitment in Mono- or Dicolonized Mice with SFB and/or Clostridia in the Ileum and Colon



(Umesaki et al., Infect Immun, 67, 3504, 1999)

10

IgA Responses in Mono- or Dicolonized Mice with SFB and/or Clostridia in the Ileum and Colon



(Umesaki et al., Infect Immun, 67, 3504, 1999)

11

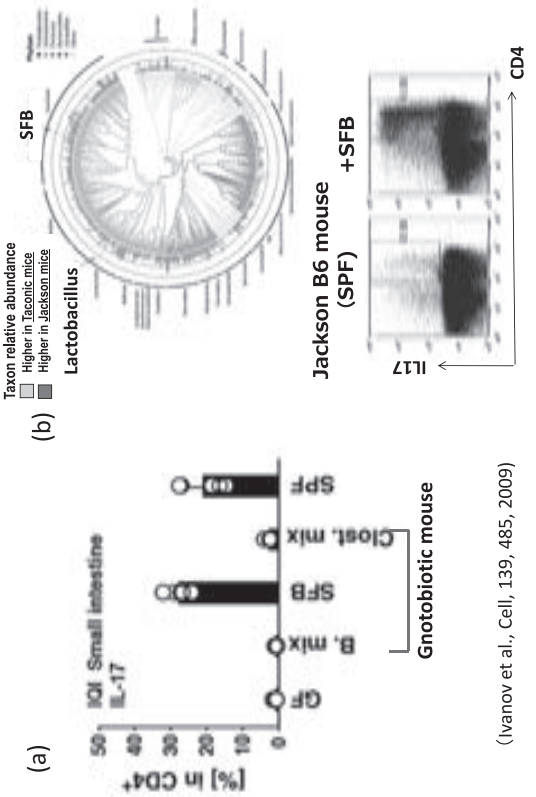
3. Th17 Cell Induction by SFB

12

4. Host-Specificity for Development of Gut Immune System by SFB

14

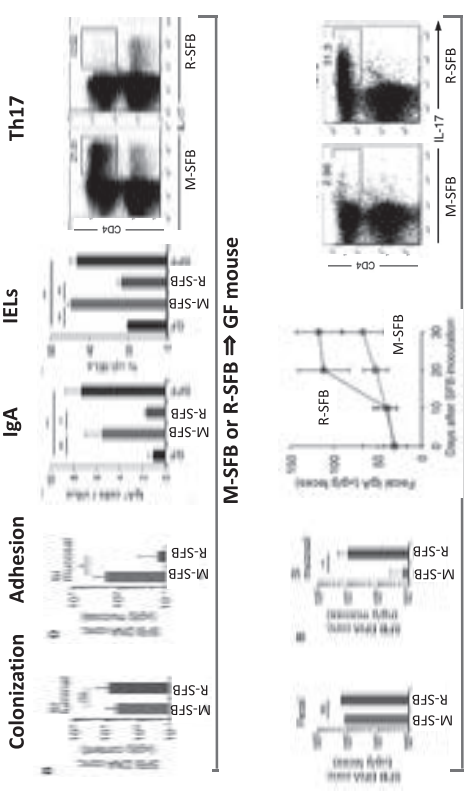
Th17 Cell Induction by Association of SFB with GF mice or Jax SPF Mice



(Ivanov et al., Cell, 139, 485, 2009)

13

Induction of Gut Immune System by Host-matched SFB



(Atarashi et al., Cell, Sept. 24, 2015)

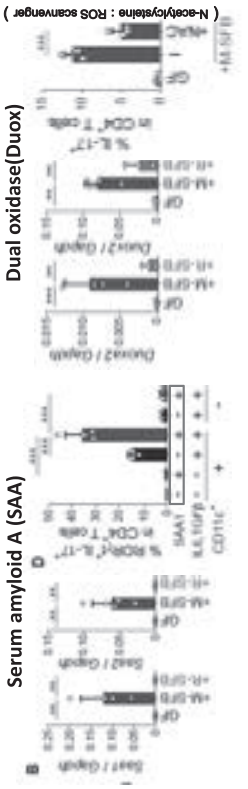
15

Mechanism for Th17 Cell Induction by SFB

Differences in Whole Genome Structure

	Genome size (kbp)	G+C content (%)	Number of CDSs	rRNA	tRNA	Flagellin genes	CRISPRS	Prophages
M-SFB	1,586*	28	1420	5	38	FliC1 to FliC3	3	2
R-SFB	1,515	28	1346	5	39	FliC1 to FliC4	1	0

(Prakash et al., Cell Host & Microbe, 10, 273, 2011)



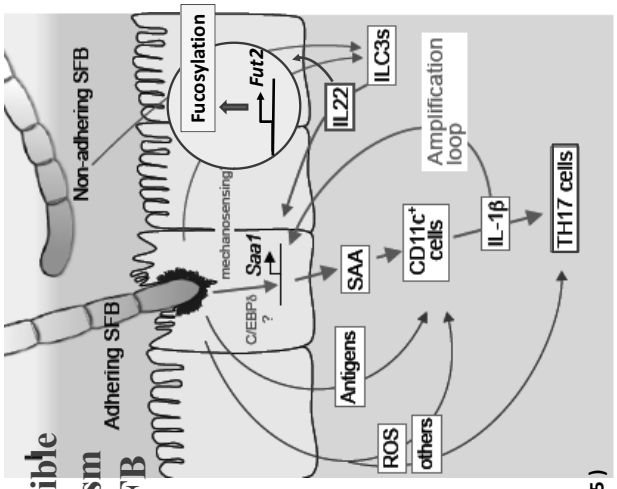
(Atarashi et al., Cell, Sept. 24, 2015)

16

5. Colitis Model Based on SFB Colonization and Evaluation of Repression of Colitis by Probiotics or Fermented Milk

18

Illustration of Possible Induction Mechanism of Th17 Cells by SFB

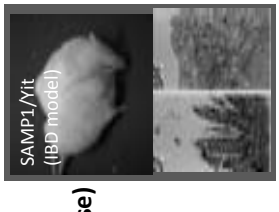


(Atarashi et al., Cell, Sept. 24, 2015)

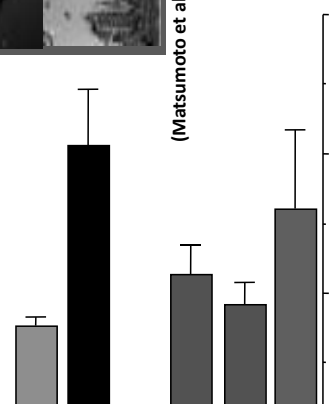
17

Role of SFB in Gnotobiotic IBD Model

(SAMP1/Yit-derived MLN CD4+cells →*scid* GB mouse)



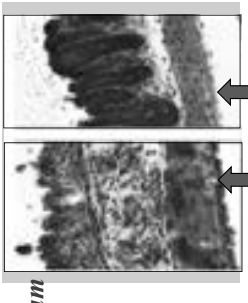
(Matsumoto et al., Gut, 43, 71, 1998)

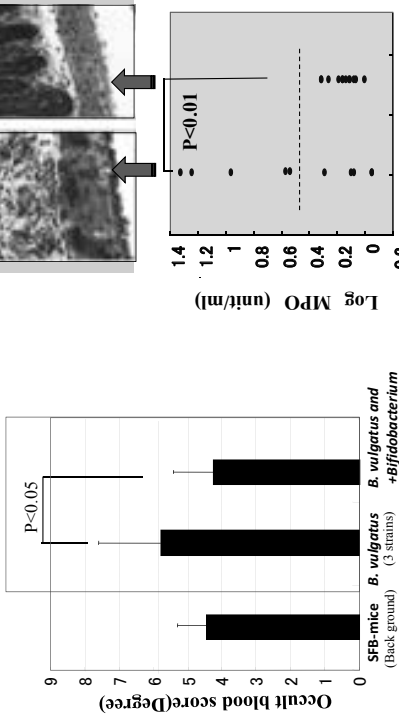


(Matsumoto et al., unpublished)

19

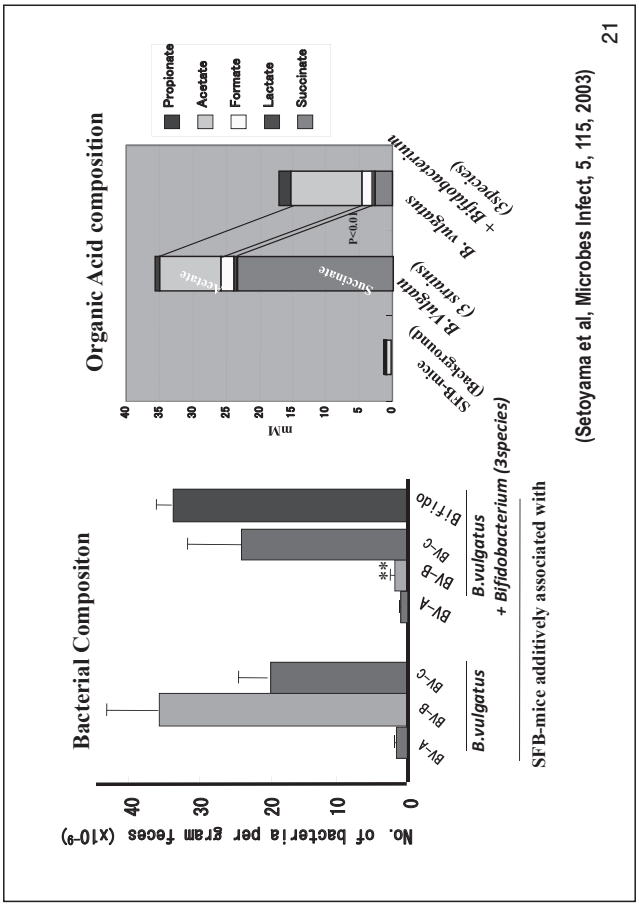
Repression of Colitis by *Bifidobacterium* in Gnotobiotic Mouse





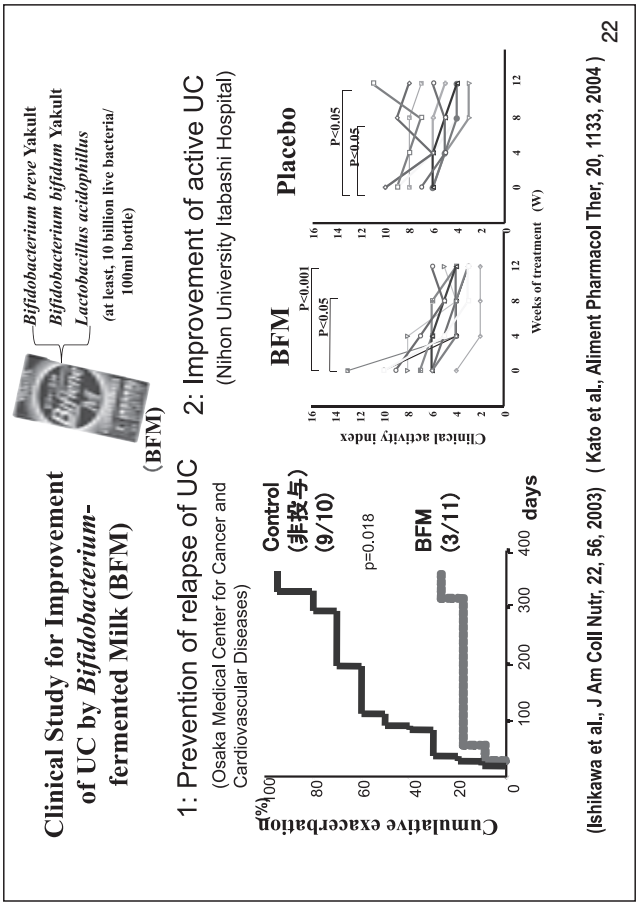
(Setoyama et al, Microbes Infect, 5, 115, 2003)

20



(Setoyama et al, Microbes Infect, 5, 115, 2003)

21



(Ishikawa et al., J Am Coll Nutr, 22, 56, 2003) (Kato et al., Aliment Pharmacol Ther, 20, 1133, 2004)

22

セッション 4-4

加齢に伴う腸内細菌叢の変化―0 歳から 100 歳以上まで―

森永乳業株式会社　基礎研究所
小田巻　俊孝

我々のライフステージは、健康と密接な関わりをもつ腸内菌叢の構成に大きな影響を及ぼす。1970 年代前半には、培養法による解析結果から加齢に伴う腸内細菌叢の変動が示唆されており、近年の分子生物学的手法を用いた解析によっても乳幼児や成人、高齢者との間には明確な差が存在していることが明らかとなっている。しかし、それら研究の多くは被験者を成人や高齢者といった年齢に基づいたグループ分けの後に比較しているため、加齢に伴う腸内菌叢の連続的な変動についてはほとんど明らかにされていない。本課題解決に向けて、我々は 0～104 歳の健常な日本人 400 人以上から糞便を収集し、16S rRNA 遺伝子の V3-V4 領域を対象としたメタゲノム解析を実施した。

培養法から示唆されていた結果と一致し、Actinobacteria 門の割合は離乳後急激に低下し、その後も徐々に減少していくことが示された。Firmicutes 門は離乳後最優勢となり、Bacteroidetes 門および Proteobacteria 門が増加する 70 歳代以降までその割合は安定していた。更に属レベルでの階層的凝集型クラスター解析の結果、特定の年代で割合が高い、つまり加齢に伴い変化が認められる菌群と成人の間は割合が保たれる 2 つのグループに分けられた。これらの結果から、加齢に伴う腸内菌叢の変化は単一方向に増加あるいは減少する属ばかりではなく、特定の年代に特異的な変化を示す菌群の存在が示唆された。更に JS ダイバージェンスを用いたクラスタリングの結果、主に乳幼児や児童、成人、高齢者から形成される 3 つのエンテロタイプが示された。離乳後 9 歳までと 70 歳代の被験者はそれぞれ約半数ずつ乳幼児と大人の、大人と高齢者のエンテロタイプに分類されたことから、これら年代は腸内菌叢構成が特に変化する年齢層に該当するのではないかと考えられた。

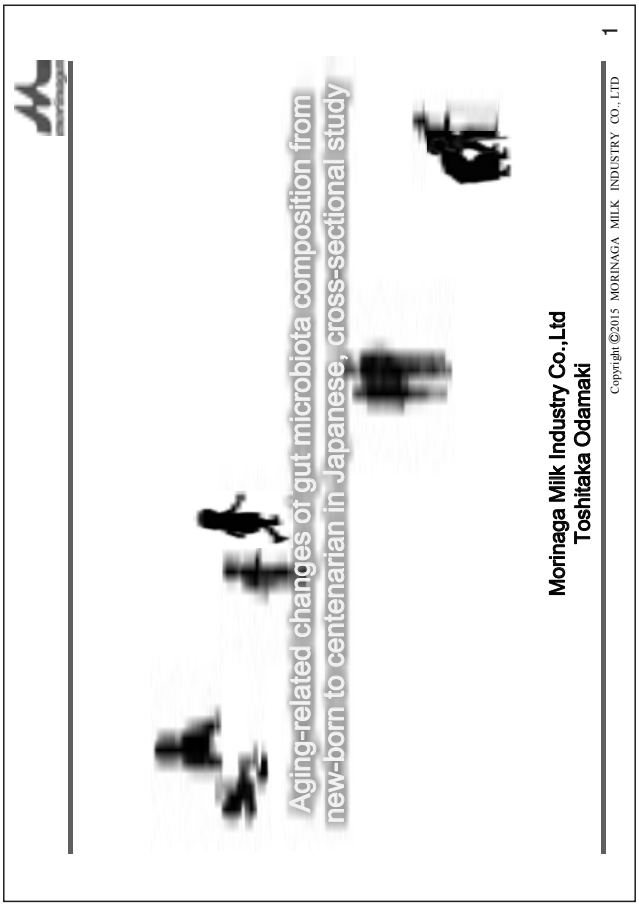
Session 4-4

Age-related Change of the Gut Microbiota -People Aged 0 to over 100 Years Old-

Toshitaka Odamaki Ph.D.
Next Generation Science Institute, Morinaga Milk Industry Co., Ltd.

Our life stage impacts the gut microbiota composition, which is known to be closely associated with our healthy conditions. In the early 1970s, Dr. Mitsuoka outlined the composition changes of gut microbiota during the life span of Japanese people based on the results using culture-based method. Recent studies using molecular methods have also indicated clear differences in the composition of gut microbiota between infants, adults and elderly. However, most of these studies were performed on subjects with segmented ages, therefore it is unclear when and how the microbiota composition shift from infants to adults and elderly. To address this issue, fecal samples of over 400 healthy Japanese between the ages of 0 and 104 years were analyzed by high-throughput sequencing of amplicons derived from the V3-V4 region of the 16S rRNA gene.

In accordance with previous result by culture-based method, we found that the relative abundance of Actinobacteria substantially decreased after weaning and the decrease was continued through the life stage. Instead, those of Firmicutes turned to be the most predominant phylum after weaning and kept the composition until the increase of Bacteroidetes and Proteobacteria over 70 years old. Agglomerative hierarchical clustering based on the abundance at genus level indicated the existence of two categories of the members of gut microbiota: those whose abundances change with age and those whose abundance maintain at relative stable during adulthood. In addition, enterotype clustering by Jensen-Shannon divergence indicated the existence of three clusters, each mainly corresponding to segments of infant/children, adult and elderly. Both subject groups at ages of post-weaning to 9 years old and from 70 to 79 years old were found to be almost equally distributed into two different clusters, respectively, suggesting that there is significant reshaping of the composition of gut microbiota at these age stages.


Aging-related changes of gut microbiota composition from new-born to centenarian in Japanese, cross-sectional study
Morinaga Milk Industry Co.,Ltd
Toshitaka Odamaki
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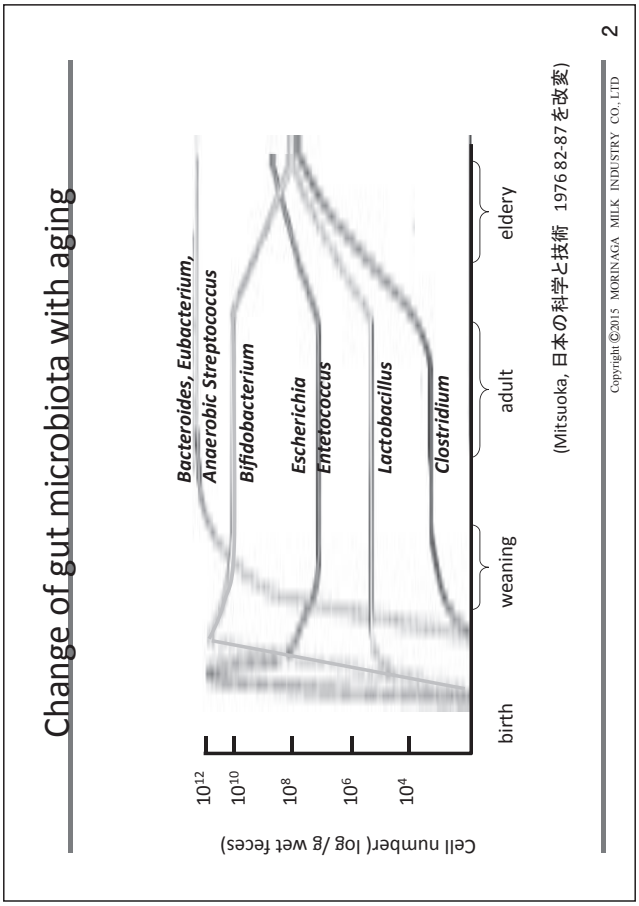
Change of gut microbiota with aging
Cell number(log /g wet feces)
10^{12}
10^{10}
10^{8}
10^{6}
10^{4}
Bacteroides, Eubacterium, Anaerobic Streptococcus
Bifidobacterium
Escherichia
Entetococcus
Lactobacillus
Clostridium
birth
weaning
adult
eldery
(Mitsuoka, 日本の科学と技術 1976 82-87 を改変)
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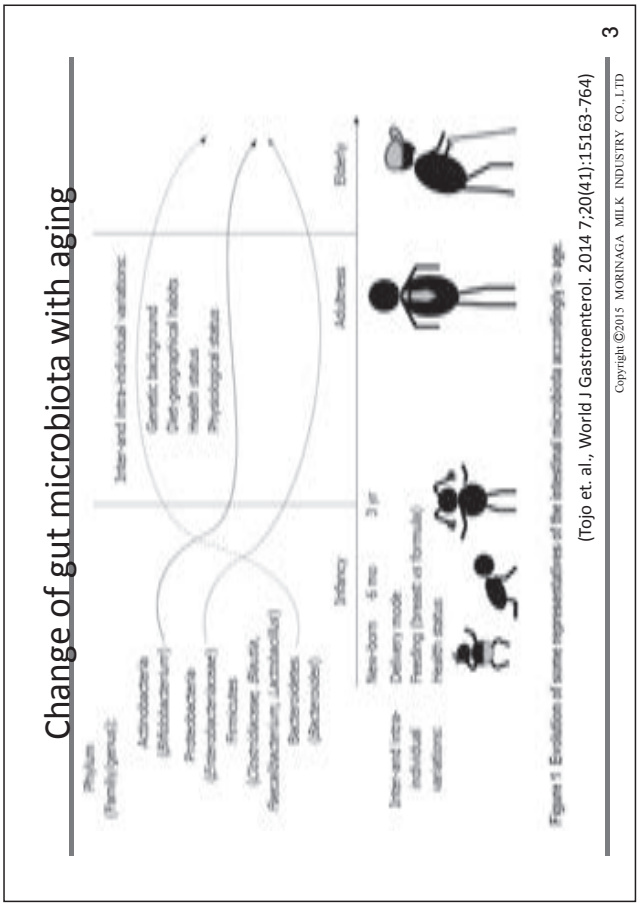
Change of gut microbiota with aging
(Tojo et. al., World J Gastroenterol. 2014 7;20(41):15163-764)
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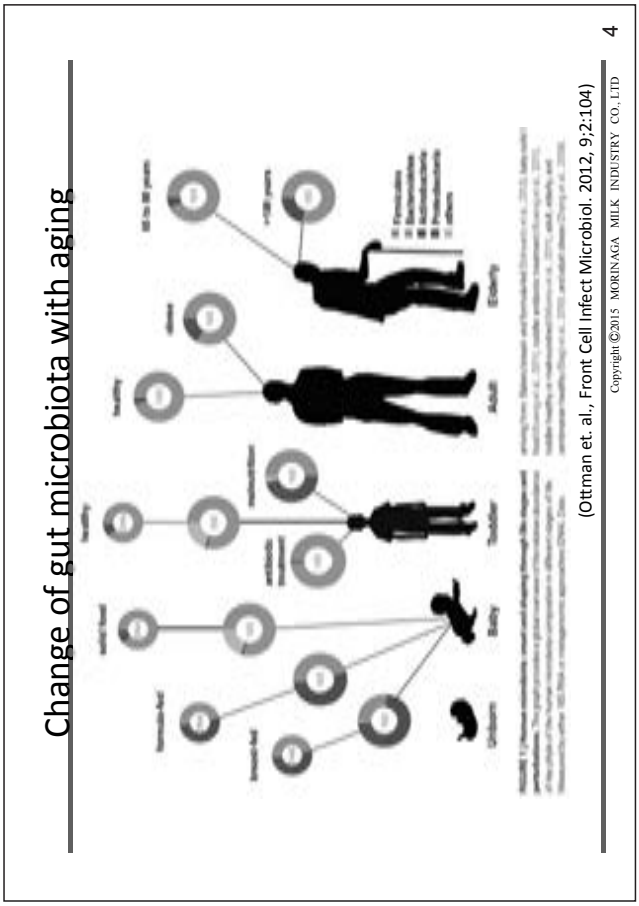
Change of gut microbiota with aging
(Ottman et. al., Front Cell Infect Microbiol. 2012, 9;2:104)
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4

Study design

<sample>
・371 samples
(367 healthy Japanese subjects aging 0 to 104 years old of community-dwelling)

<Method>
・ DNA extracted from fecal samples by beads beating method.
・ V3-V4 region of the 16S rRNA gene were sequenced by Miseq

Study protocols were approved by the ethical committee of a medical institution and written informed consent was obtained from all subjects or their guardian.

<Sample distribution>

age	number of sample	(M/F)
preweaning	14	(7/7)
weaning	12	(6/6)
weaned-9	32	(16/16)
10-19	10	(7/3)
20-29	40	(16/24)
30-39	88	(45/43)
40-49	34	(13/21)
50-59	25	(12/13)
60-69	28	(11/17)
70-79	15	(5/10)
80-89	48	(16/32)
90-99	19	(4/15)
100-	6	(0/6)
sum	371	(158/213)

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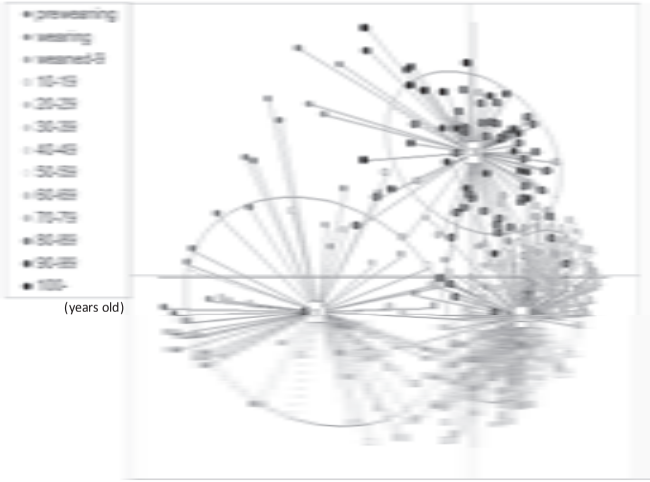
Three enterotype clustering related with age



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Take home messages

・It seemed to have some patterns and turning points in the composition change of gut microbiota with aging

・Higher positive correlations were observed in the elderly-associated CAGs, implying that these genera can grow well in the same environmental condition, or they develop a mutualistic relationship.

・The composition of bifidobacterial species was also changed with aging, especially at weaning and centenarian.

・Factors such as nutrients and drugs existing in the gut might play an important role in shaping the composition of gut microbiota with aging.

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セッション4-5

ヨーグルトが腸内環境および生体機能に及ぼす影響

株式会社明治　研究本部　食機能科学研究所
木村　勝紀

1907年、ロシアのノーベル賞受賞者であるイリヤ・メチニコフは、著書「The Prolongation of Life」において、腸内の腐敗菌が産生する毒性物質が老化に関与しており、ヨーグルトを摂取することによって腸内菌叢のバランスを正常に保つことが長寿の秘訣であると説いた。これは現在のプロバイオティクスの概念に通じる考え方であり、以降ヨーグルトは世界中に広まった。我々は、ヨーグルトの整腸作用をはじめとする様々な健康機能について科学的に検証を続けている。今回、ヨーグルトが腸内環境および生体機能に及ぼす影響を中心に紹介する。

ヒトの大腸内には500種、100兆個以上もの微生物が生息しており、いわゆる腸内菌叢を形成している。腸内菌叢を構成する微生物には、宿主に有害な作用を示すもの、あるいは健康維持に役立つものなどがあり、構成菌群の相互作用により、一定のバランスを維持している。この腸内菌叢のバランスの乱れが、下痢症、便秘症および胃腸障害などの原因になると考えられている。

便秘状態が腸内環境および生体機能に及ぼす影響について検討したところ、便秘群では非便秘群に比べて、便中および血中腐敗産物が有意に高く、便秘群では腐敗菌の一種である *Clostridium perfringens* の検出率が有意に高いことが認められた。また、便秘群では非便秘群に比べて、皮膚の角層水分量が低い傾向が認められた。すなわち、便秘による腸内環境の悪化が皮膚機能の低下に影響することが示唆された。*Lactobacillus delbrueckii* subsp. *bulgaricus* 2038および *Streptococcus thermophilus* 1131で発酵させたヨーグルトの摂取により、腸内の有用菌であるビフィズス菌数および排便回数の増加、腸内腐敗産物の減少などがヒト試験により確認されている。そこで、本ヨーグルトを慢性的便秘で乾燥皮膚を有する女性に摂取させたところ、便秘の症状が有意に改善されるとともに、皮膚機能の改善効果が認められた。

次に、加齢による腸管バリア機能および免疫恒常性の低下に対するヨーグルトの効果について検討した。*L. delbrueckii subsp. bulgaricus* 2038および *S. thermophilus* 1131で発酵させたヨーグルトをマウスに長期投与し、各組織を採取して加齢による変化とヨーグルトの効果に関する解析に供した。さらにDSS投与により大腸炎を誘発した場合についても同様に検討した。結腸のDNAマイクロアレイデータを用いてパスウェイ解析した結果、加齢によりT細胞、B細胞、Toll様受容体やNOD様受容体などに関わるシグナル伝達系に強い影響が認められ、免疫機構の恒常性の低下が推定された。実際にマウス血液中の炎症性サイトカイン濃度は加齢により上昇し、慢性的な炎症状態が誘導されていた。同時に多くの抗菌ペプチド遺伝子の発現低下も認められ、腸管バリア機能や腸内菌叢への影響が予想された。ヨーグルトの投与はこれらの加齢による遺伝子発現変動に影響を与え、抗菌ペプチドであるReg3β およびReg3γ 遺伝子の発現低下を抑制するとともに、血液中のIL-1βの上昇を抑制した。またDSS大腸炎を誘発した際、老齢マウスにおいてより強い炎症反応が観察されたが、ヨーグルトの投与によりMPO活性の上昇抑制など重症化の抑制効果が認められた。

以上の結果より、ヨーグルトは腸内環境や免疫機能の改善を通じて、生体機能を高めることが示唆された。

Session 4−5

Effects of Yogurt on Intestinal Environment and Body Functions

Katsunori Kimura
Food Science Research Laboratories, R&D Division, Meiji Co., Ltd.

In 1907, Nobel Prize winner Elie Metchnikoff of Russia suggested that aging process was the result of the toxic products of putrefactive microbes in the intestine and intake of yogurt which influenced the intestinal microbiota to maintain an optimal balance was the secret to longevity in his book titled "The Prolongation of Life". This is a way of thinking related to a current probiotic concept, and after that the yogurt spread out in the world. We are researching on a variety of health benefits of yogurt. At this time, I would like to introduce some of the results of investigation into the effects of yogurt on intestinal environment and body functions.

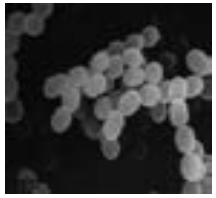
The human gut harbors a diverse microbial community (intestinal microbiota) that consists of more than 10^{14} bacterial cells comprising 500 species. Some species of bacteria are harmful and others are helpful to health of host. An imbalanced composition of intestinal microbiota causes constipation, diarrhea, and gastrointestinal disorders.

We examined the influence of constipation on intestinal environment and body functions. The putrefactive products in feces and blood of constipation group were significantly higher compared with those of non-constipation group. Detection rate of *Clostridium perfringens* which was kind of putrefactive bacteria in constipation group was significantly higher. In addition, the tendency that the horny layer moisture of skin in the constipation group was lower than that of non-constipation group was recognized. It was suggested that aggravation of the intestinal environment due to the constipation influenced the reduction of skin functions. It was shown that intake of yogurt fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* 2038 and *Streptococcus thermophilus* 1131 increased the number of bifidobacteria and stool frequency and decreased the putrefactive products in feces. This yogurt also improved a symptom of the constipation and skin functions of women with chronic constipation who had dry skin.

Then, we examined the effects of the yogurt on reduction in intestinal tract barrier function and immunological homeostasis by aging. The yogurt fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* 2038 and *Streptococcus thermophilus* 1131 was administered to mice for a long period of time and each organ was taken for immunological analysis. In addition, the effects of this yogurt on intestinal tract barrier function and immunological homeostasis of mice with DSS-induced colitis were examined. As a result of pathway analysis using DNA microarray data of the colon, signal transduction system related to T cell, B cells, Toll-like receptors and NOD-like receptors were strongly affected by aging, and the reduction in immunological homeostasis was indicated. In practice, the inflammatory cytokine density in mouse blood was increased and a chronic inflammatory state was derived by aging. The expression of many antibacterial peptide genes was decreased at the same time, and influence on intestinal tract barrier function and intestinal microbiota was expected. Administration of yogurt affected the gene expression change by aging and inhibited the decrease of expression of antibacterial peptides, Reg3β and Reg3γ, and the increase of IL-1β in blood. In case of aged mice with DSS-induced colitis, stronger inflammatory responses were observed. Administration of yogurt inhibited the severe inflammatory reaction including inhibition of MPO activity.

Therefore, intake of yogurt enhanced several body functions by improving intestinal environment and immunological functions.

Effects of yogurt on intestinal environment and body functions



Food Science Research Laboratories,
R&D Division, Meiji Co., Ltd.
Katsunori Kimura

1

Yogurt

Symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.
(CODEX STANDARD FOR FERMENTED MILKS)

Elie Metchnikoff theorized that health could be enhanced and senility delayed by manipulating the intestinal microbiome with host-friendly bacteria found in yogurt.

2

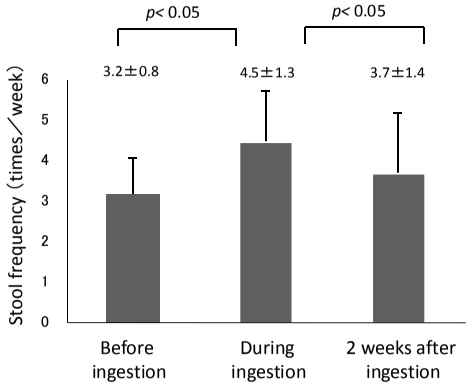
Meiji Bulgaria Yogurt LB81

Meiji Bulgaria Yogurt LB81
fermented by LB81 lactic acid bacteria
(*L. delbrueckii* subsp. *bulgaricus* 2038
and *S. thermophilus* 1131)
was approved for a FOSHU claim.

3

Effect of Meiji Bulgaria Yogurt LB81 on stool frequency

Thirty six female students with constipation ingested 100g of LB81 yogurt once a day for 2 weeks.



4

Effects of LB81 yogurt on intestinal microflora and putrefactive metabolites (130g × 2, 2 weeks)

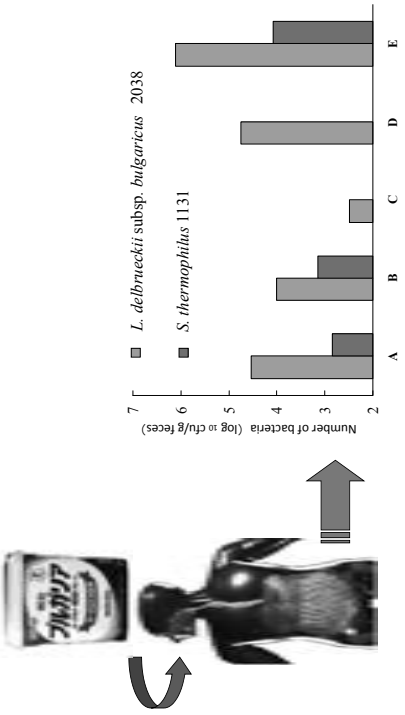
	Before	After
	Log 10 (cfu/g feces)	
Total bacteria	10.9±0.2	10.9±0.2
Bacteroidaceae	10.6±0.2	10.6±0.2
Bifidobacterium	**9.7±0.4**	**10.1±0.2***
Bifidobacterium (%)	6.3	15.9
Eubacterium	9.9±0.5	9.3±0.9
Clostridium (lecithinase +)	5.5±2.1	4.7±1.9
Clostridium (lecithinase −)	9.1±0.2	9.4±0.1
Lactobacillus	9.0±0.6	9.1±0.9
Enterobacteriaceae	9.3±0.8	9.1±0.7
Enterococcus	9.2±0.7	8.7±1.0
	ppm	
Ammonia	**78.4±45.4**	**17.1±11.7***
p-Cresol	63.6±51.0	38.5±36.8
Indole	42.8±36.5	31.8±26.5
Skatole	54.2±47.0	20.9±19.3
pH	7.0±0.9	6.7±0.9

paired t test: * $p<0.05$

5

Survival of LB81 lactic acid bacteria in the gastrointestinal tract of human

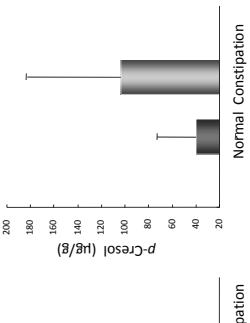
Five people ingested 100g of LB81 yogurt once a day for 6 days.



6

Effects of constipation on putrefactive metabolites in feces

Forty eight female students were divided into two groups.
・Constipation group (4 or less weekly defecation days, n=15)
・Normal group (more than 4 weekly defecation days, n=33)

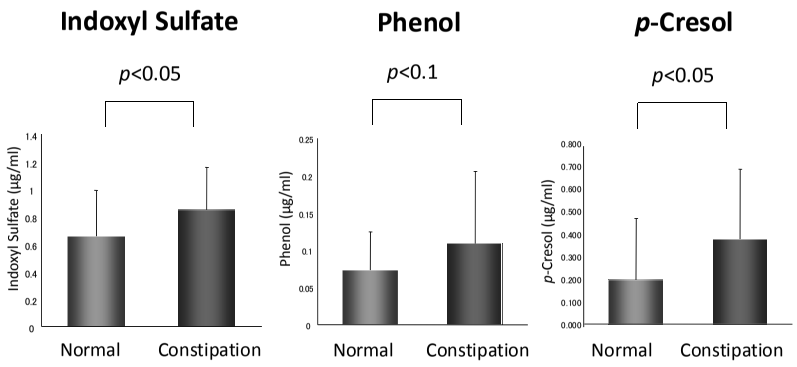


7

Effects of constipation on putrefactive metabolites in feces

8

Effects of constipation on putrefactive metabolites in blood



9

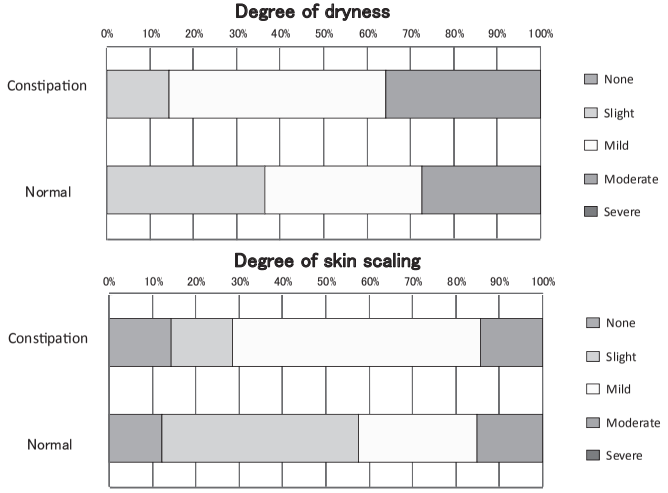
Effect of constipation on intestinal microbiota

	Normal (n=33)	Constipation (n=15)
	Number of cells Log_{10} cfu/g feces (Detection rate: %)	
Bifidobacterium	10.16±0.88 (100)	10.39±0.60 (100)
Lactobacillus	5.96±0.92 (100)	6.35±0.99 (100)
Streptococcus	8.53±0.46 (100)	8.64±0.38 (100)
Bacteroides fragilis group	10.10±0.55 (100)	10.13±0.33 (100)
Clostridium coccoides group	10.48±0.21 (100)	10.47±0.17 (100)
Clostridium leptum subgroup	10.42±0.40 (100)	10.45±0.30 (100)
Clostridium perfringens	**5.67±0.98 (33) ***	**5.42±0.71 (67)**

* χ^2 test ($p<0.05$)

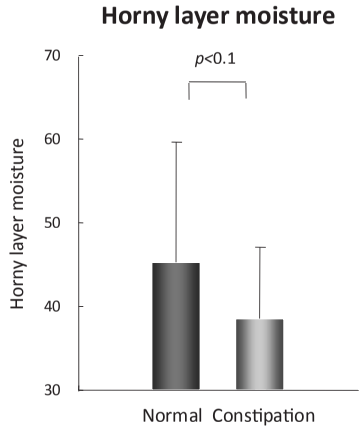
10

Effects of constipation on skin condition evaluated by dermatologists



11

Effect of constipation on dermal characteristics

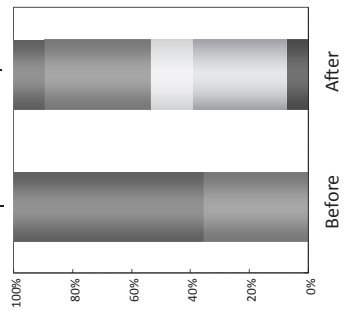


12

Effects of LB81 yogurt on constipation

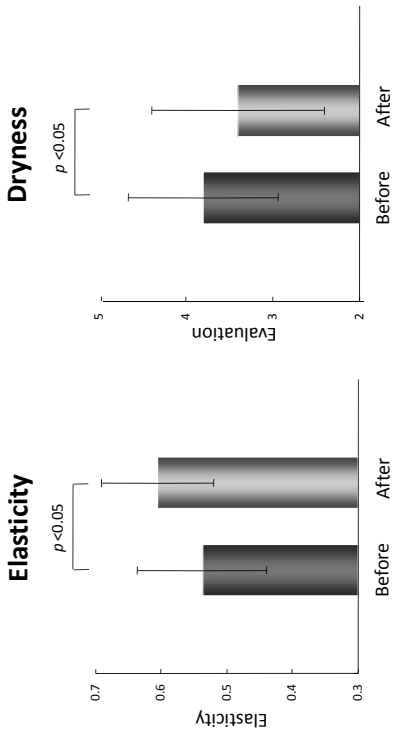
Twenty eight female volunteers with constipation ingested 120g of yogurt twice a day for 4 weeks.

Questionnaire survey regarding constipation



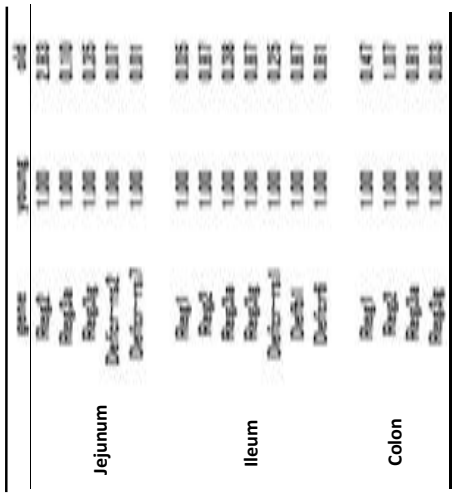
13

Effects of LB81 yogurt on dermal characteristics



14

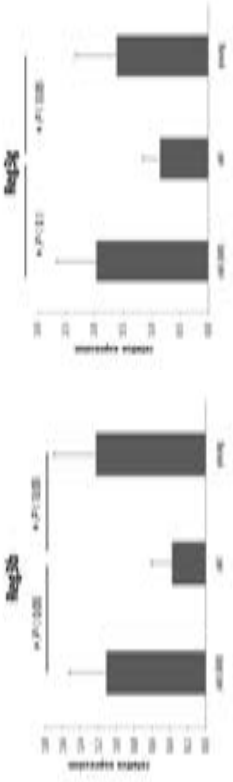
Effects of aging on intestinal barrier function of mice



15

The effect of LB81 yogurt on Reg3 gene expression in small intestine

The age of 28 months, yogurt intake for 20 months



16

セッション4−6

Lactobacillus gasseri SBT2055の経口投与による生体防御機能の強化

雪印メグミルク株式会社　ミルクサイエンス研究所
酒井　史彦

Lactobacillus gasseri はヒトの腸管に存在する常在菌であり、特に小腸で優勢に検出される菌種の一つである。近年では、ヒトに有益な作用をもたらす乳酸菌「プロバイオティクス」として発酵乳などに応用され、その多様な機能性が注目されている。我々は、ヒト由来の *L. gasseri* SBT2055（LG2055）の機能性について研究を続けており、LG2055を用いた発酵乳を摂取することで、腸内環境を整える作用のほか、血中コレステロール低下作用、内臓脂肪蓄積抑制作用、などの有益な作用がもたらされることを報告してきた。一方、様々なプロバイオティクスが腸内フローラのバランスを改善するとともに、生体防御機能を高める可能性が以前から報告されている。本発表では、LG2055をマウスに経口投与した際に認められる生体防御機能の強化作用と、これまでに明らかとなったその作用機序について報告する。

1. 腸管でのIgA産生誘導作用[1)]：　マウスにLG2055を含んだ食餌を5週間摂食させた結果、小腸組織中のIgA量及びパイエル板のIgA陽性細胞の割合が増加した。また、*in vitro* での樹状細胞とnaïve B細胞との共培養系にLG2055を添加すると、B細胞からのIgA産生が強く誘導された。一方LG2055は、樹状細胞からのBAFF、IL-6、IL-10、TGF-β、といったIgA産生の促進に関与するサイトカインの産生を誘導した。さらに、LG2055刺激で誘導されたTGF-βは、樹状細胞からのBAFF、IL-6、IL-10の産生促進に関与することが示された。これらの結果から、LG2055は樹状細胞からのサイトカイン産生刺激を介してB細胞からのIgA産生を誘導し、そこにはTGF-βによる樹状細胞へのautocrine/paracrine的な作用が関与している可能性が示唆された。
2. インフルエンザウイルス感染防御作用[2)]：　マウスにLG2055を3週間経口投与した後にインフルエンザウイルスに感染させた結果、LG2055を投与したマウスでは投与しないマウスに比べて感染後の生存率が有意に上昇した。また、肺及び肺胞洗浄液（BALF）中のウイルス量、IL-6量、およびBALFへの浸出細胞数が対照群よりも有意に低い値を示した。一方、正常マウスへのLG2055の経口投与により、I型IFNによって誘導される抗ウイルス遺伝子（Mx1、Oas1a）の発現量が肺で上昇していることが明らかとなった。さらにその作用機序を詳細に検討した結果、LG2055は腸管でのI型IFN産生誘導を介して肺のウイルス増殖を抑制している可能性が示唆された。
3. 歯周病の抑制作用：　マウスにLG2055を3週間経口投与し、その後2週間にわたり口腔内に歯周病原性細菌 *Porphylomonas gingivalis* 381を口腔感染させた。その結果、感染に伴う歯肉上皮の肥厚や歯槽骨吸収は、LG2055投与により有意に抑制された。また、LG2055投与群の口腔内では、抗菌ペプチドであるβ-Defensinの発現が上昇し、歯茎での *P. gingivalis* の菌数が減少していることが明らかとなった。さらに、歯肉粘膜組織での炎症性サイトカインの発現が抑制されていた。経口投与されたLG2055は、口腔内でのβ-Defensinの発現を刺激することで *P. gingivalis* の増殖を抑制し、歯周病を抑制する可能性が示唆された。

1）Sakai *et al.* 2014 *PloS ONE* 9(8) : e105370. DOI:10.1371/journal.pone.0105370
2）Nakayama *et al.* 2014 *Sci Rep* 4, 4638, DOI:10.1038/srep04638.

Session 4−6

Augmentation of Host Defense Mechanism by Oral Administration of *Lactobacillus gasseri* SBT2055

Fumihiko Sakai
Milk Science Research Institute, Megmilk Snow Brand Co., Ltd.

Lactobacillus gasseri is indigenous bacteria inhabiting the intestinal tract of human, and used in fermented dairy products owing to its beneficial features. It has been proven that the human intestine-originated probiotic bacterium *L. gasseri* SBT2055 (LG2055) has several beneficial effects on human health: improvement of the intestinal environment, lowering the serum cholesterol concentration, and preventing abdominal adiposity. It is also reported that orally administrated probiotic bacteria contribute to augmentation of host defense mechanism by altering the microbial balance or by interacting with the host immune system. This study shows that oral-administration of LG2055 augments the host defense mechanism.

1. Induction of IgA in the small intestine[1] : Oral-administration of LG2055 induced IgA production in the small intestine and increased the ratio of IgA+ cell population in Peyer's patch in mice. LG2055 markedly increased the IgA production from B cells co-cultured with dendritic cells. In addition, it is demonstrated that LG2055 stimulates dendritic cells to promote the production of BAFF, IL-6, IL-10, and TGF-β all of which are critical for IgA production from B cells. Furthermore, TGF-β was critical for the production of BAFF, IL-6, IL-10, and TGF-β itself from LG2055-stimulated dendritic cells. These results demonstrate that LG2055 induces IgA production from B cells by stimulating the cytokine secretion from dendritic cells, and TGF-β produced by dendritic cells stimulated by LG2055 induces the production of BAFF, IL-6, and IL10 by an autocrine/paracrine fashion.
2. Protection from influenza virus infection[2] : Oral-administration of LG2055 to mice increased the survival rate of them after a lethal infection with influenza A virus and reduced both the virus replication and inflammatory responses in the lungs. Expression of antiviral genes, such as Mx1 and Oas1a, was induced in the lung tissue by oral-administration of LG2055. The further analysis has led us to the speculation that the suppression of virus replication in the lungs of LG2055 fed mice derives from the protective effect in the intestine, where the type I IFN is suggested to be involved. Therefore, it is suggested that oral-administrated LG2055 initially stimulates the intestine, and then enhances the host defense against influenza A virus infection in the lungs which are remote organs from the intestine.
3. Prevention of periodontal disease: Mice were orally administrated with LG2055 for 3 weeks, and then subsequently orally infected with *Porphyromonas gingivalis*. LG2055 treatment significantly reduced alveolar bone loss and *P. gingivalis*-specific 16S rRNA levels in the gingival tissue. Inflammation caused by *P. gingivalis* infection was also suppressed by LG2055 treatment. Furthermore, production of β-defensin-14 was significantly enhanced in the saliva of mice 3 weeks after the oral-administration of LG2055. These results suggest that oral administration of LG2055 prevents periodontitis via up-regulation of the production of β-defensin-14 in the oral cavity.

1) Sakai*et al.* 2014*PloS ONE*9(8): e105370. DOI:10.1371/pone.0105370
2) Nakayama*et al.* 2014*Sci Rep*4,4638, DOI:10.1038/srep04638.

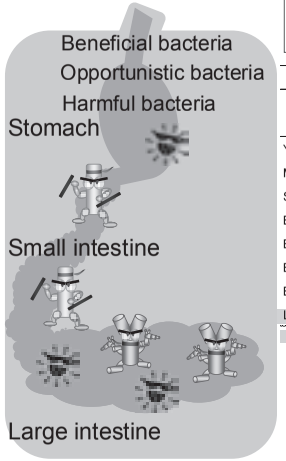
Augmentation of host defense mechanism by oral administration of *Lactobacillus gasseri* SBT2055

Fumihiko Sakai
(Megmilk Snow Brand co., Ltd.)

ILSI Japan, The 7th International Conference on Nutrition and Aging, Sep. 30. 2015

1

Intestinal Flora



500～1000 trillion cells (10^{14}),1000 bacterial species
The human gut microbiota plays a key role in the human health, gastrointestinal disease and other diseases.

Microbiota in the intestine of 9 adults

	Jejunum		Ileum		Caecum/Colon	
	n	log10 (median)	n	log10 (median)	n	log10 (median)
Yeasts and Fungi	4	4.5－7.0 (6.7)	2	4.9-7.6	2	6.8-7.4
Micrococci	3	4.7-7.2 (6.7)	1	6.7	1	6.4
Streptococci	4	6.1-8.0 (7.6)	6	5.9-8.0 (7.8)	8	6.7-8.2 (7.7)
Enterobacteria	1	7.9	2	5.9-8.0	2	5.7-7.9
E. coli	4	7.3-7.8 (7.6)	5	4.8-8.7 (7.5)	8	6.9-8.6 (7.9)
Bifidobacteria	0	-	2	7.4-8.0	5	7.6-10.2 (8.6)
Bacteroides	3	6.3-9.4 (7.8)	3	7.8-9.4 (8.2)	6	6.4-11.2 (8.8)
Lactobacilli	7	5.6-9.4 (7.7)	6	6.0-8.4 (7.6)	9	6.2-10.2 (8.0)
L. gasseri	7	5.5-9.3 (6.9)	6	5.8-7.5 (6.8)	8	6.1-9.2 (6.9)
L. reuteri	5	5.8-8.4 (7.4)	5	5.8-7.5 (6.8)	8	5.5-7.4 (6.7)
L. salivarious	3	5.6-8.6 (7.8)	4	5.5-8.2 (6.7)	4	5.8-7.2 (6.3)
L. casei	1	8.3	1	6.3	5	5.5-6.8 (5.8)
L. plantarum	1	8.7	1	6.3	3	5.5-7.0
L. buchnen	2	3.4-7.5	1	6.0	0	-

Reuter, G., *Curr. Issues Intest. Microbiol.*, (2001) 2(2): 43-53.

2

Lactobacillus gasseri SBT2055 (LG2055)

LG2055

- Originated from human feces
- Resistant to gastric and bowel fluid

Administration of LG2055

- Highly established in intestinal tract
- Lower the fecal populations of *Staphylococcus* and *p*-cresol
- Regulate the functions of the intestines

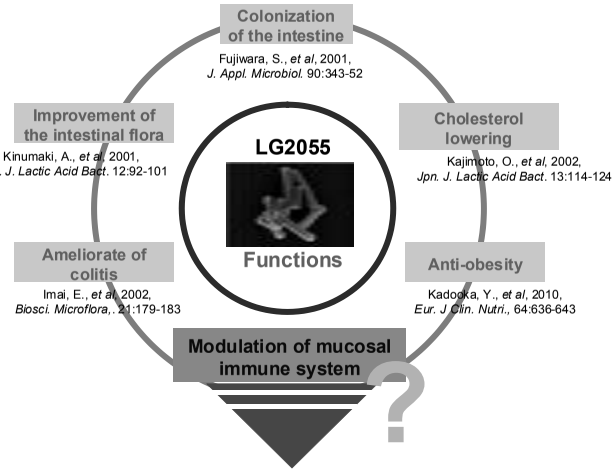
Probiotics

LG2055 is used in fermented milk products.

Fujiwara et al, J. Appl. Microbiol. 90 (3), (2001) 343-52

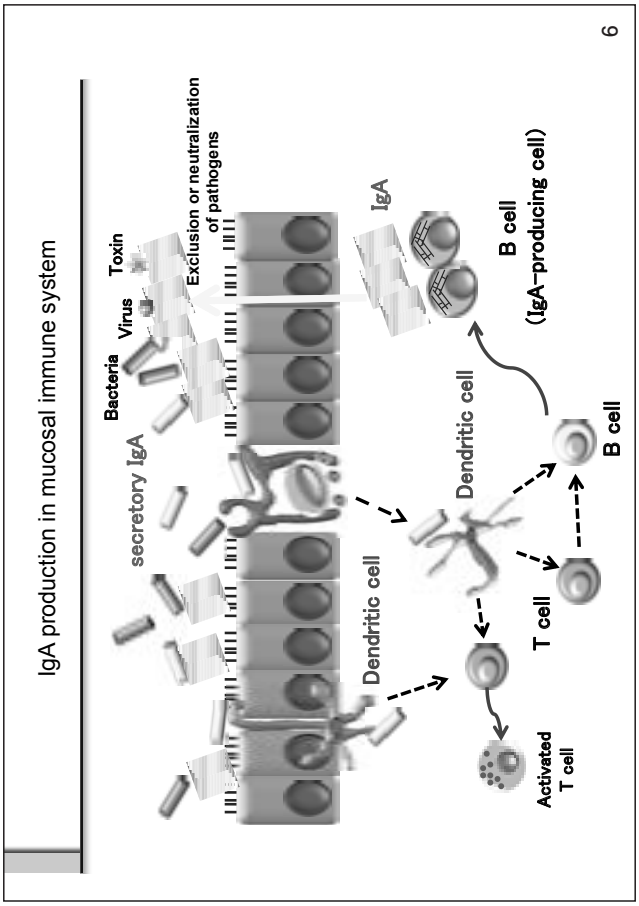
3

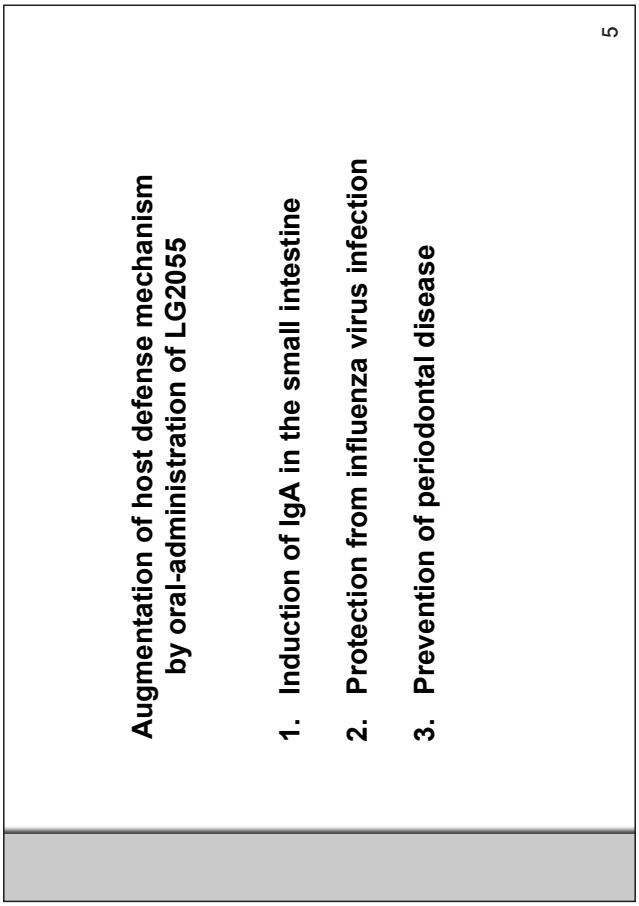
Functions of LG2055

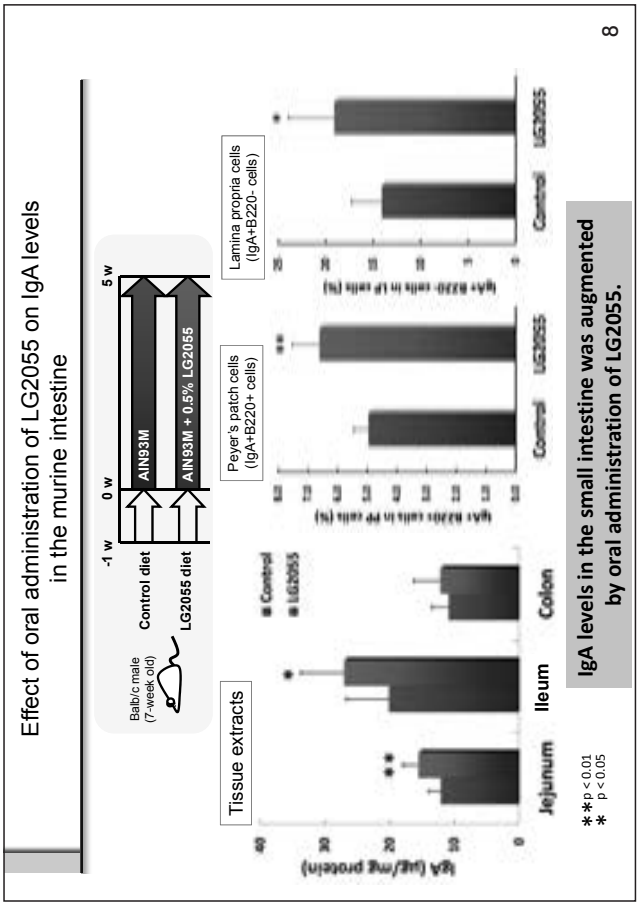


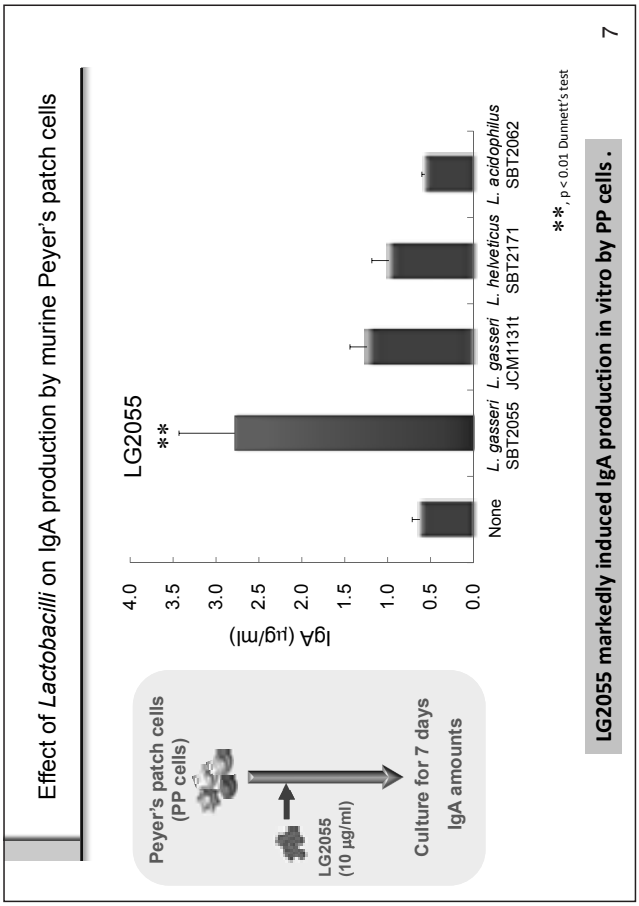
Effect of LG2055 oral administration on mucosal immune system

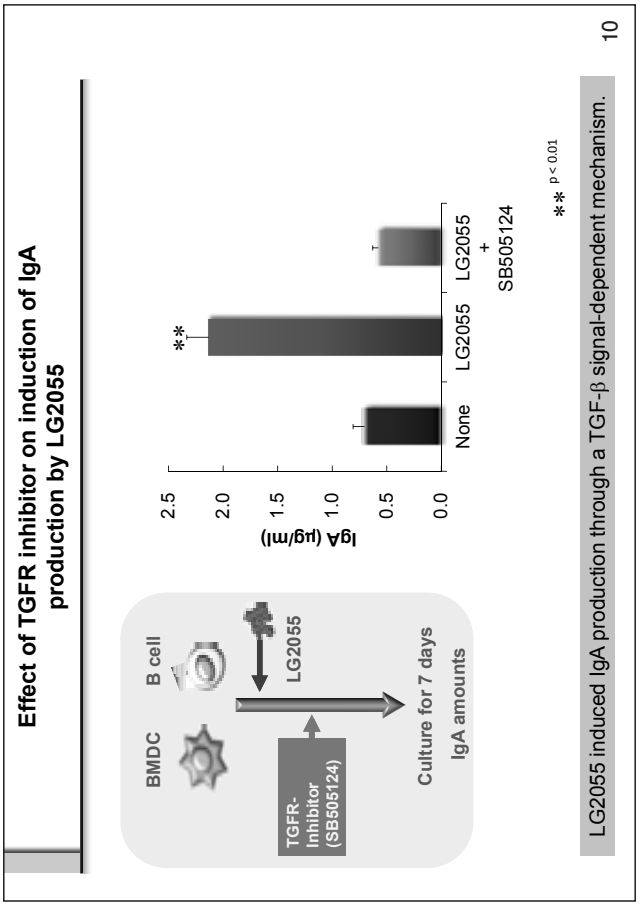
4

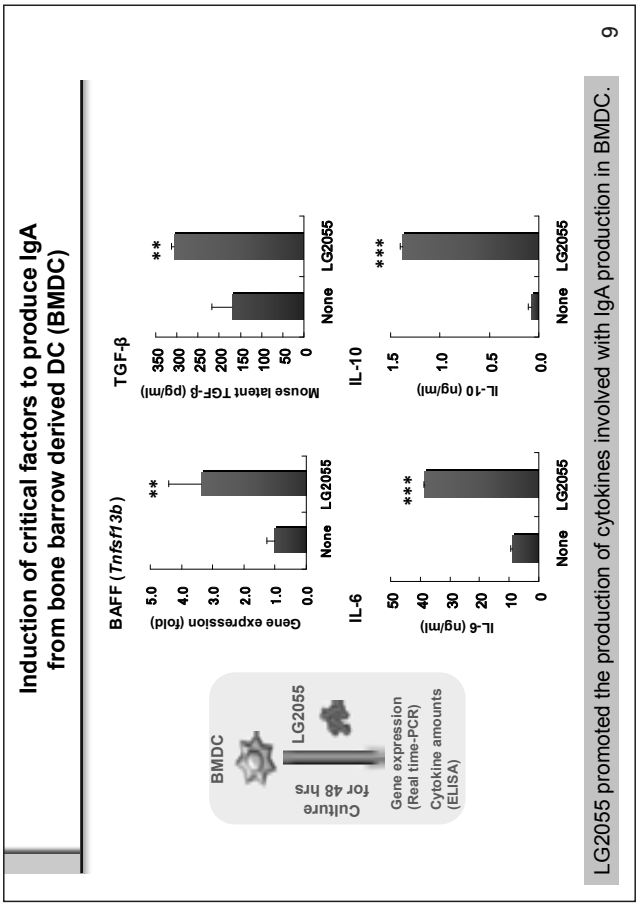

IgA production in mucosal immune system
secretory IgA
Bacteria
Virus
Toxin
Exclusion or neutralization of pathogens
IgA
Dendritic cell
Dendritic cell
B cell (IgA−producing cell)
B cell
T cell
Activated T cell
6


Augmentation of host defense mechanism by oral-administration of LG2055
1. Induction of IgA in the small intestine
2. Protection from influenza virus infection
3. Prevention of periodontal disease
5


Effect of oral administration of LG2055 on IgA levels in the murine intestine
Balb/c male (7-week old)
-1 w
0 w
5 w
Control diet
LG2055 diet
AIN93M
AIN93M + 0.5% LG2055
Tissue extracts
Control
LG2055
IgA (μg/mg protein)
Jejunum
Ileum
Colon
Peyer's patch cells (IgA+B220+ cells)
Lamina propria cells (IgA+B220− cells)
IgA+ B220+ cells in PP cells (%)
IgA+ B220− cells in LP cells (%)
** p < 0.01
* p < 0.05
IgA levels in the small intestine was augmented by oral administration of LG2055.
8


Effect of Lactobacilli on IgA production by murine Peyer's patch cells
Peyer's patch cells (PP cells)
LG2055 (10 μg/ml)
Culture for 7 days
IgA amounts
IgA (μg/ml)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
LG2055
**
None
L. gasseri SBT2055
L. gasseri JCM1131t
L. helveticus SBT2171
L. acidophilus SBT2062
**, p < 0.01 Dunnett's test
LG2055 markedly induced IgA production in vitro by PP cells .
7


Effect of TGFR inhibitor on induction of IgA production by LG2055
BMDC
B cell
LG2055
TGFR-Inhibitor (SB505124)
Culture for 7 days
IgA amounts
IgA (μg/ml)
2.5
2.0
1.5
1.0
0.5
0.0
**
None
LG2055
LG2055 + SB505124
** p < 0.01
10
LG2055 induced IgA production through a TGF-β signal-dependent mechanism.


Induction of critical factors to produce IgA from bone barrow derived DC (BMDC)
BMDC
LG2055
Culture for 48 hrs
Gene expression (Real time-PCR)
Cytokine amounts (ELISA)
BAFF (Tnfsf13b)
Gene expression (fold)
TGF-β
Mouse latent TGF-β (pg/ml)
IL-10
IL-10 (ng/ml)
IL-6
IL-6 (ng/ml)
None
LG2055
9
LG2055 promoted the production of cytokines involved with IgA production in BMDC.

Hypothetical model for enhancement of IgA production
Small intestine
LG2055
Virus
Bacteria
?
IgA ↑
BAFF
IL-6
IL-10
TGF-β
Dendritic cells
naïve B cells
IgA+ B cells
Plasma cells
Sakai, F., et al, 2014, PLoS ONE., 9:e105370
12

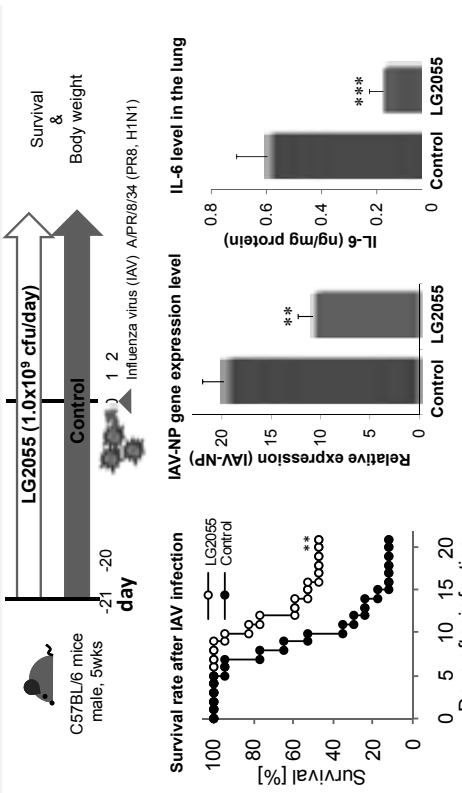
Effect of TGF-β receptor inhibitor on cytokine production in BMDC
None
inhibitor
LG2055
LG2055 + inhibitor
BAFF
BAFF (pg/ml)
TGF-β
Mouse Latent TGF-β (pg/ml)
IL-6
IL-6 (ng/ml)
IL-10
IL-10 (ng/ml)
BMDC
LG2055
TGFR-Inhibitor (SB505124)
Culture for 3 days
Cytokine amounts
BAFF
IL-6
IL10
TGF-β
11
LG2055 promoted cytokine production in BMDC through the TGF-β signal-dependent manner.

Augmentation of host defense mechanism by oral-administration of LG2055

1. Induction of IgA in the small intestine
2. Protection from influenza virus infection
3. Prevention of periodontal disease

13

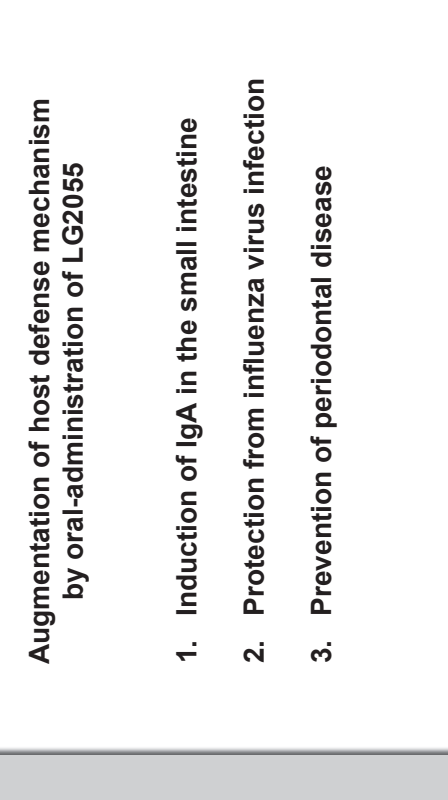
Effect of oral administrations of LG2055 on the prevention of influenza



SBT2055 administration was effective for the prevention against influenza virus A/PR8 infection in mice.

14

Expression changes in antiviral genes in the lung tissues after LG2055 administration



Oas1a : 2'-5'-oligoadenylate synthetase 1a
Mx1 : myxovirus resistance 1

Oral administration of LG2055 induces the expression of the antiviral gene, Mx1 and Oas1a mRNAs in the lung tissues.

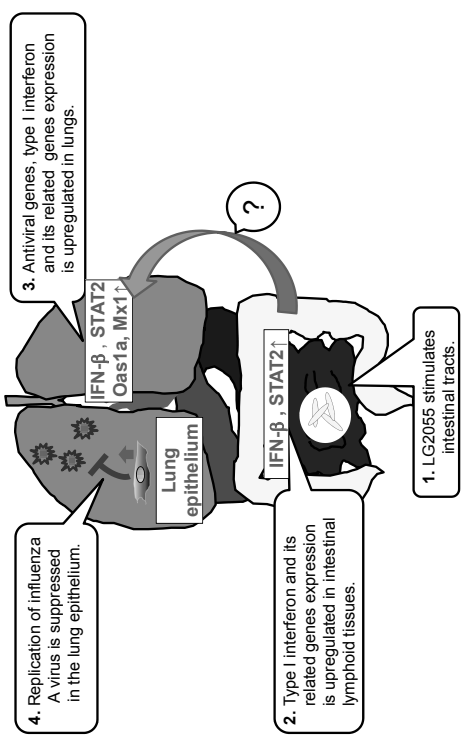
15

Expression changes in type I interferon and the signaling molecules genes in the lung and intestinal tract tissues

Significant differences were observed in the expression levels of type I interferon and its signaling molecule.

16

Proposed mechanism of preventive effect of LG2055 against influenza virus infection.



17

Augmentation of host defense mechanism by oral-administration of LG2055

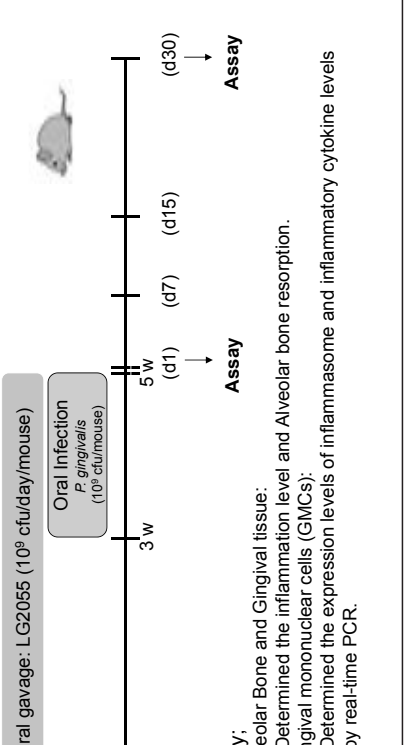
1. Induction of IgA in the small intestine
2. Protection from influenza virus infection
3. Prevention of periodontal disease

18

Experimental Protocol

Mouse: BALB/c mice (7 - 8 wks old)

Porphylomonas gingivalis ATCC 381 (10^9cfu / mouse)



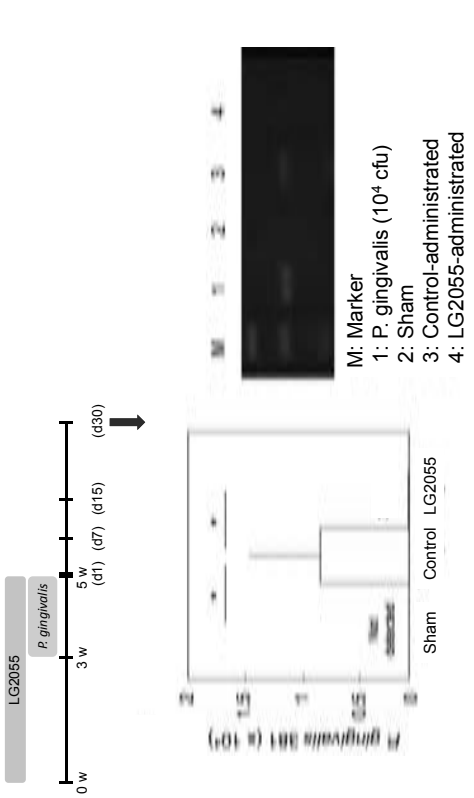
Assay:
1) Alveolar Bone and Gingival tissue:
Determined the inflammation level and Alveolar bone resorption.
2) Gingival mononuclear cells (GMCs):
Determined the expression levels of inflammasome and inflammatory cytokine levels by real-time PCR.

19

Significant reduction of alveolar bone loss were detected of mice administered *L. gasseri* SBT2055

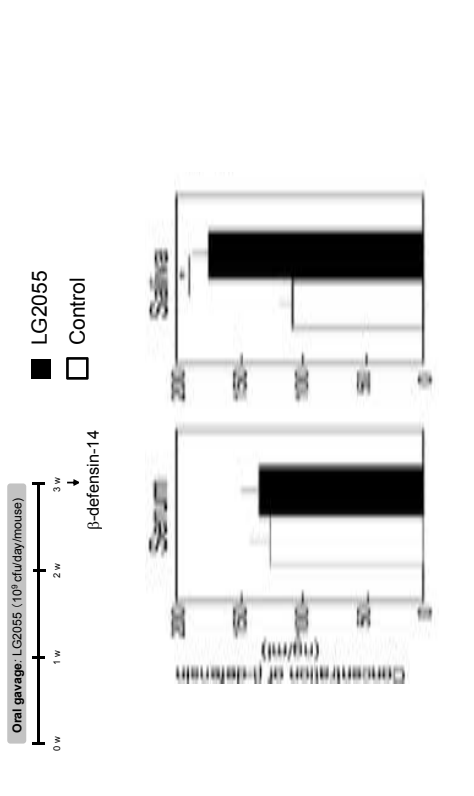
20

Detection of *P. gingivalis*-specific 16S rRNA.



21

Anti-microbial peptide responses in saliva of mice orally given LG2055



22

Summary

Oral administered Probiotic bacterium LG2055 induces;

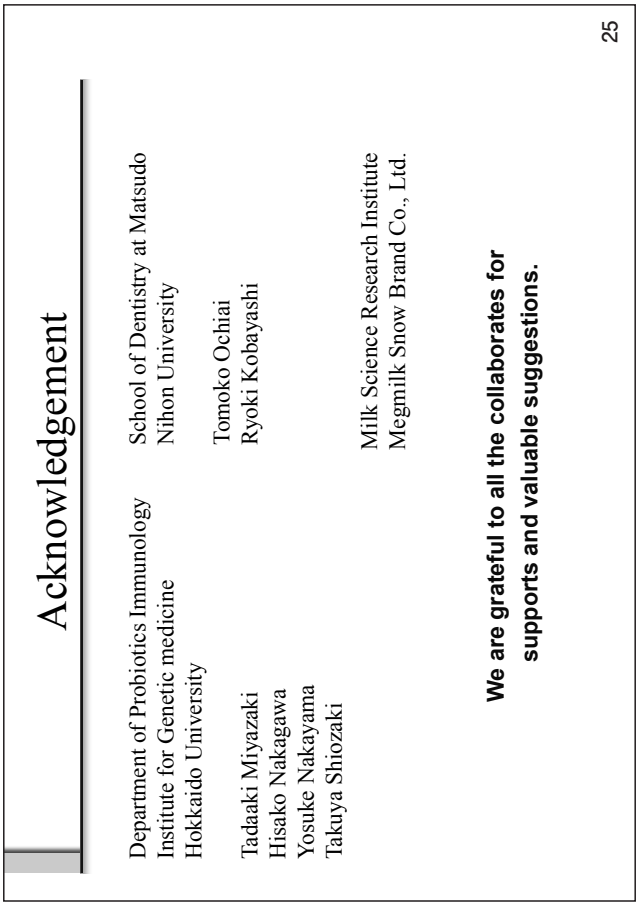
1. Significant reduction of alveolar bone loss and inflamed gingiva.
2. Low production of inflammation cytokines in gingiva.
3. Anti-microbial peptide (β-defensin) were induced in oral mucosa.

LG2055 enhances not only gut but also oral immune system for prevent periodontal disease (Inflammation)

23

Summary

24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Hokkaido University
School of Dentistry at Matsudo
Nihon University
Tadaaki Miyazaki
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Yosuke Nakayama
Takuya Shiozaki
Tomoko Ochiai
Ryoki Kobayashi
Milk Science Research Institute
Megmilk Snow Brand Co., Ltd.
We are grateful to all the collaborates for supports and valuable suggestions.
25

セッション5

“不活動”の生理学（身体活動と栄養学）

セッション 5-1

筋萎縮を予防・治療できる新規機能性食材の開発

徳島大学
医学部医科栄養学科　生体栄養学
二川　健

無重力環境や寝たきりなど、筋肉に機械的な負荷がかからない状態を Unloading という。Unloading 状態では、筋肉が萎縮し、運動能力の著しい障害が起こる。これを廃用性筋萎縮という。廃用性筋萎縮が社会問題となる一方で、未だ予防法・治療法は開発されていない。そこで我々は、栄養学的な廃用性筋萎縮の治療法の開発を目的とした。これまでに、Unloading 状況下ではユビキチンリガーゼ Casitas B-lineage Lympnoma b（Cbl-b）の発現が増大し、筋細胞の成長に重要な IGF-1 の細胞内シグナル分子である Insulin receptor substrate-1（IRS-1）をユビキチン化することで、その加水分解を促進することが筋萎縮の原因の一つであることを明らかにした。そして、Cbl-b と IRS-1 の結合を阻害し、筋萎縮を抑制するペンタペプチドである Cblin（Cbl-b inhibitor）を開発した。しかし、Cblin はペプチドであり、生体内でアミノペプチターゼに分解されるため大量の投与を必要とした。本研究では、Cblin の実用化に向け、消化酵素による分解を受け、Cblin に似た配列が派生されるイネを作成し、その筋萎縮効果を検討した。アグロバクテリウム法を用いて Cblin 様配列を組込んだイネ（以下 Cblin 米）の開発を行った。Cblin 米は、パンクレアチンによる消化を受け Cblin 様ペプチドを派生した。また、Cblin 米の経口摂取は坐骨神経切除による IRS-1 の分解を阻害し、筋萎縮を抑制した。さらに、Cblin 米を経口摂取したマウスの門脈中に Cblin 様ペプチドが存在することを確認した。以上の結果は、Cblin の機能性食材化の実現に繋がると考え、実現できればより効率的な Cblin の利用が可能となる。

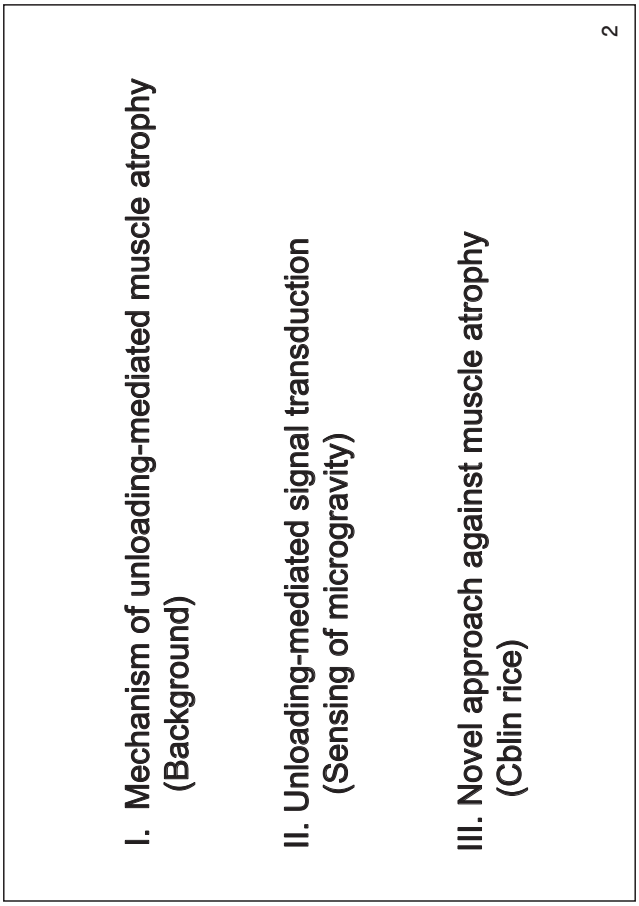
Session 5－1

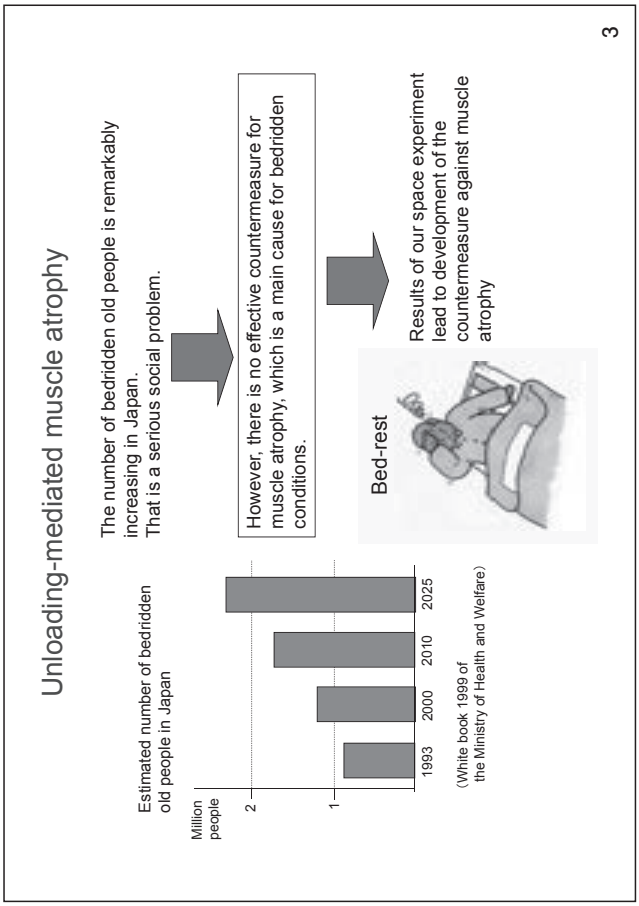
A Novel Nutritional Approach against Unloading-mediated Muscle Atrophy

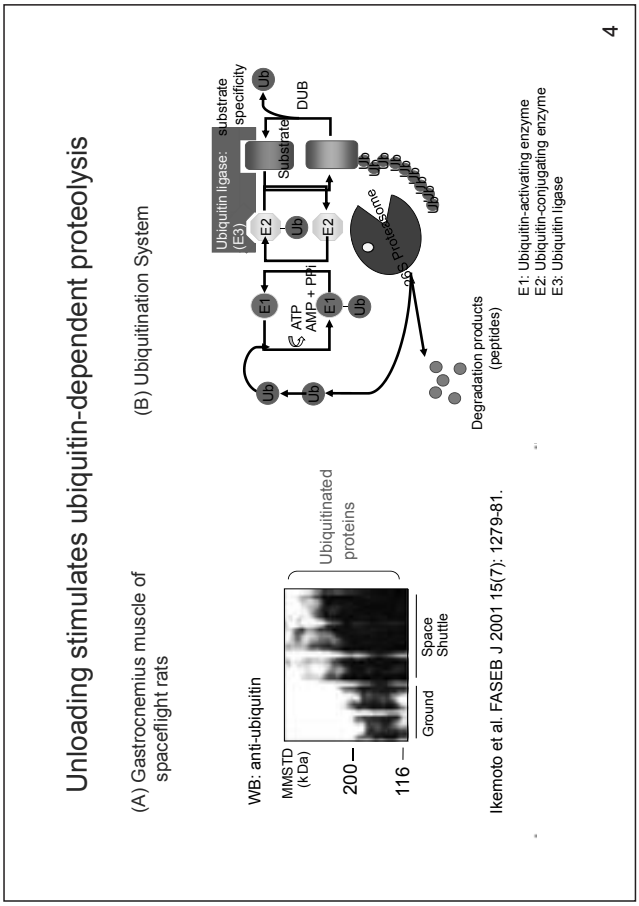
Takeshi Nikawa, M.D.
Department of Nutritional Physiology, Institute of Medical Nutrition,
Tokushima University Medical School

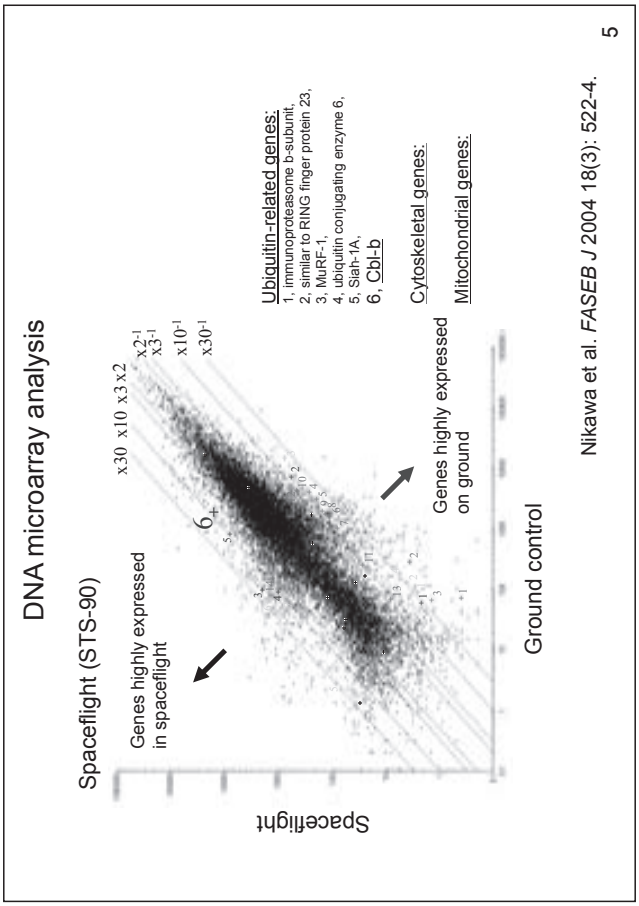
Skeletal muscle subjected to unloading conditions is vulnerable to be atrophied. We reported that ubiquitin ligase Cbl-b play a role in unloading-mediated skeletal muscle atrophy: Cbl-b ubiquitinate and lead to degrad IRS-1, an important IGF-1 signaling intermediate molecule, resulting in muscle mass loss. We also reported that intramuscular injection of a pentapeptide, DGpYMP, mimetic of phosphorylated site sequence of IRS-1, significantly inhibited denervation-induced skeletal muscle loss. In the present study, we examined effects of oral administration of Cblin-like peptide（QDGYMPW）transgenic rice on denervation-induced muscle mass loss. We first generated transgenic rice seeds in which 15-tandem repeated Cblin-like peptide were fused to storage protein glutelin for expression. We confirmed Cblin-like peptide was released from recombinant protein by digestion with chymotrypsin or pancreatin in vitro. In addition, the digestive Cblin-like products inhibited Cbl-b-mediated IRS-1 ubiquitination. Moreover, dietary Cblin-like peptide transgenic rice seeds had inhibitory effects on denervation-induced degradation of IRS-1 and skeletal muscle atrophy in vivo. Furthermore, we found Cblin-like peptide in postprandial blood in mouse. Our present results suggest that Cblin-like peptide transgenic rice may be available as a dietary therapy to prevent skeletal muscle atrophy.

Novel nutritional approach against unloading-mediated muscle atrophy
Department of Nutritional Physiology,
Institute of Medical Nutrition,
Tokushima University Graduate School
Takeshi Nikawa
1

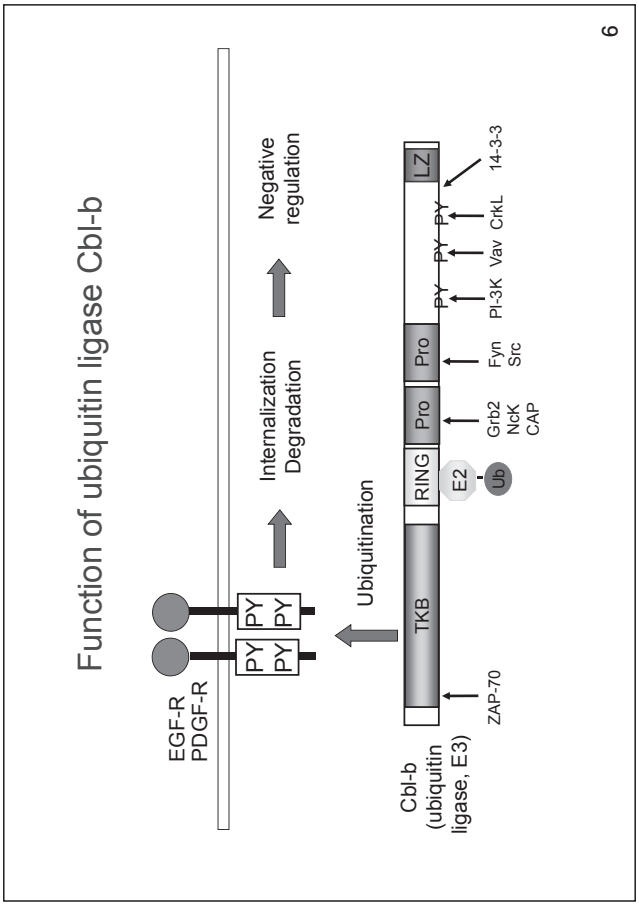

I. Mechanism of unloading-mediated muscle atrophy
(Background)
II. Unloading-mediated signal transduction
(Sensing of microgravity)
III. Novel approach against muscle atrophy
(Cblin rice)
2


Unloading-mediated muscle atrophy
Estimated number of bedridden old people in Japan
Million people
2
1
1993
2000
2010
2025
(White book 1999 of the Ministry of Health and Welfare)
The number of bedridden old people is remarkably increasing in Japan.
That is a serious social problem.
However, there is no effective countermeasure for muscle atrophy, which is a main cause for bedridden conditions.
Bed-rest
Results of our space experiment lead to development of the countermeasure against muscle atrophy
3


Unloading stimulates ubiquitin-dependent proteolysis
(A) Gastrocnemius muscle of spaceflight rats
WB: anti-ubiquitin
MMSTD (kDa)
200
116
Ubiquitinated proteins
Ground
Space Shuttle
(B) Ubiquitination System
Ubiquitin ligase: (E3)
substrate specificity
Ub
E1
E2
ATP
AMP + PPi
Substrate
DUB
26S Proteasome
Degradation products (peptides)
E1: Ubiquitin-activating enzyme
E2: Ubiquitin-conjugating enzyme
E3: Ubiquitin ligase
Ikemoto et al. FASEB J 2001 15(7): 1279-81.
4



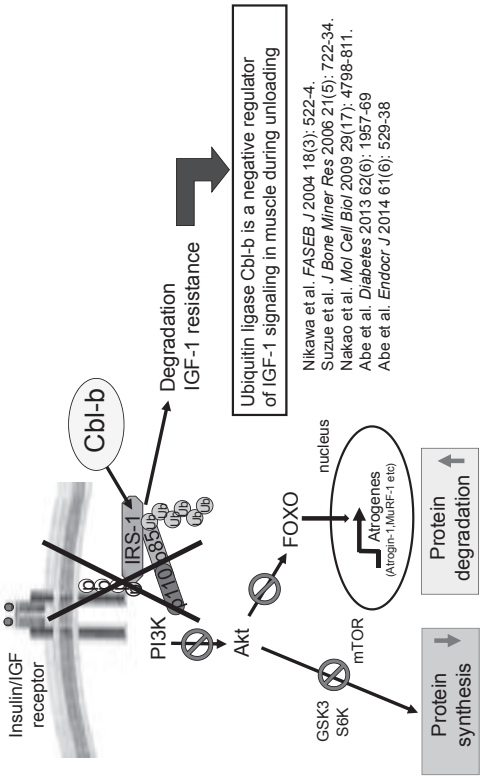
Nikawa et al. *FASEB J* 2004 18(3): 522-4.



Nakao et al. *Mol Cell Biol* 2009 29(17): 4798-811.

Nakao et al. *Mol Cell Biol* 2009 29(17): 4798-811.

Role of Cbl-b in unloading-induced muscle atrophy

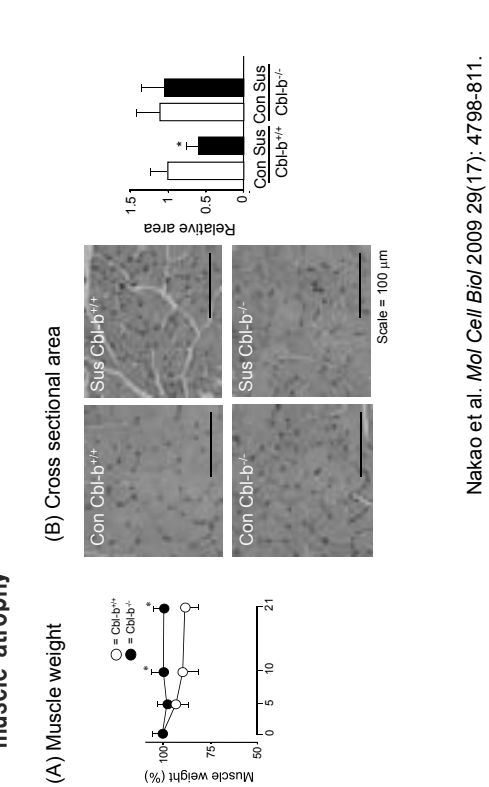


Ubiquitin ligase Cbl-b is a negative regulator of IGF-1 signaling in muscle during unloading

Nikawa et al. *FASEB J* 2004 18(3): 522-4.
Suzue et al. *J Bone Miner Res* 2006 21(5): 722-34.
Nakao et al. *Mol Cell Biol* 2009 29(17): 4798-811.
Abe et al. *Diabetes* 2013 62(6): 1957-69
Abe et al. *Endocr J* 2014 61(6): 529-38

10

Cbl-b$^{-/-}$ mice are resistant to suspension-induced muscle atrophy



Nakao et al. *Mol Cell Biol* 2009 29(17): 4798-811.

9

Sensing mechanism of unloading stress = Regulation of Cbl-b expression (hypothesis)

The regulation of Cbl-b expression is still unknown.

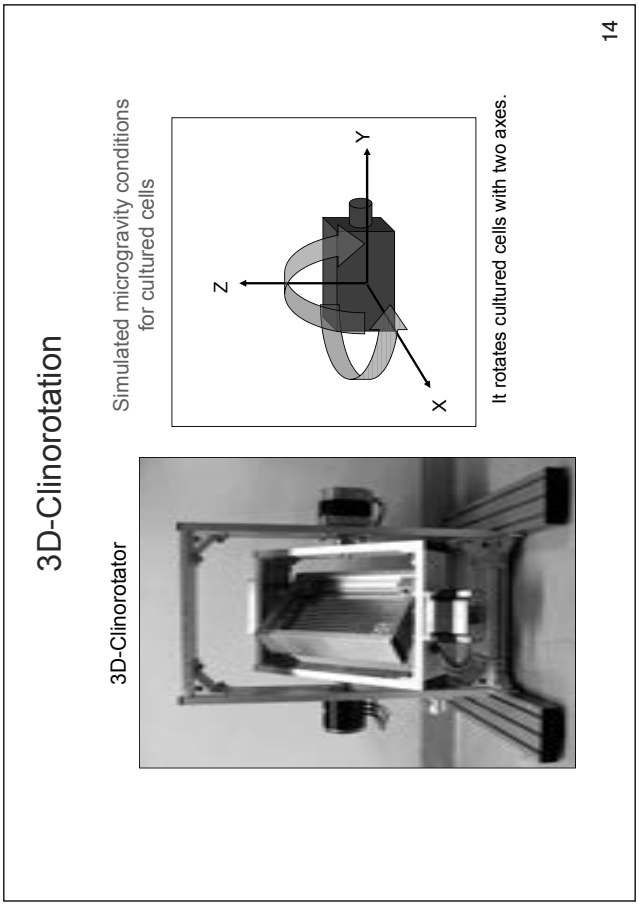
12

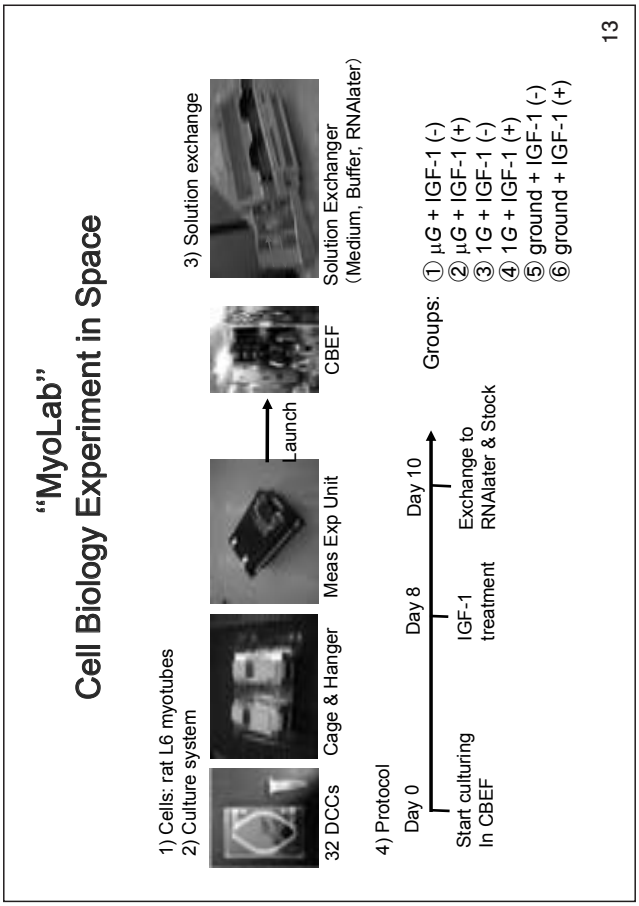
I. Mechanism of unloading-mediated muscle atrophy (Background)

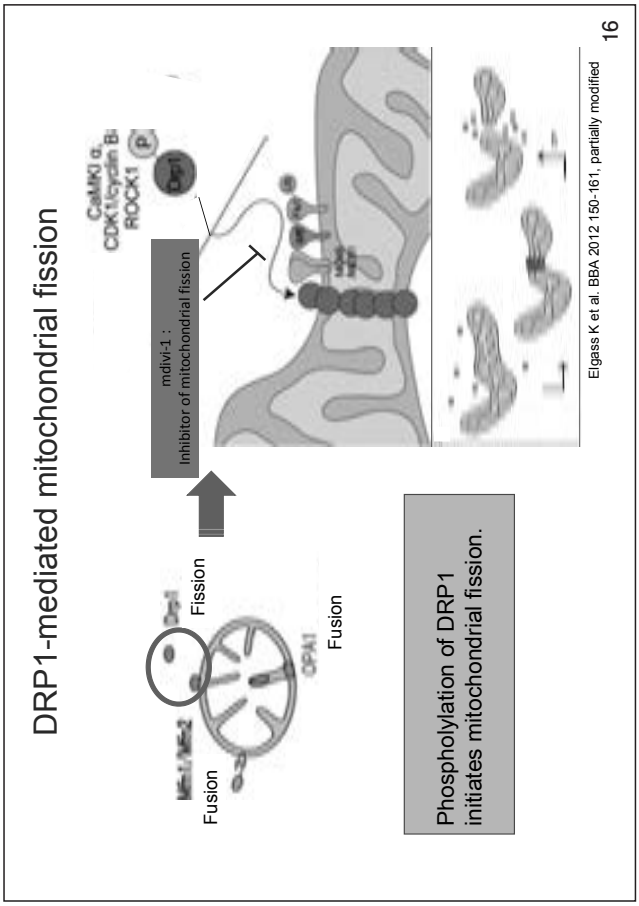
II. Unloading-mediated signal transduction (Sensing of microgravity)

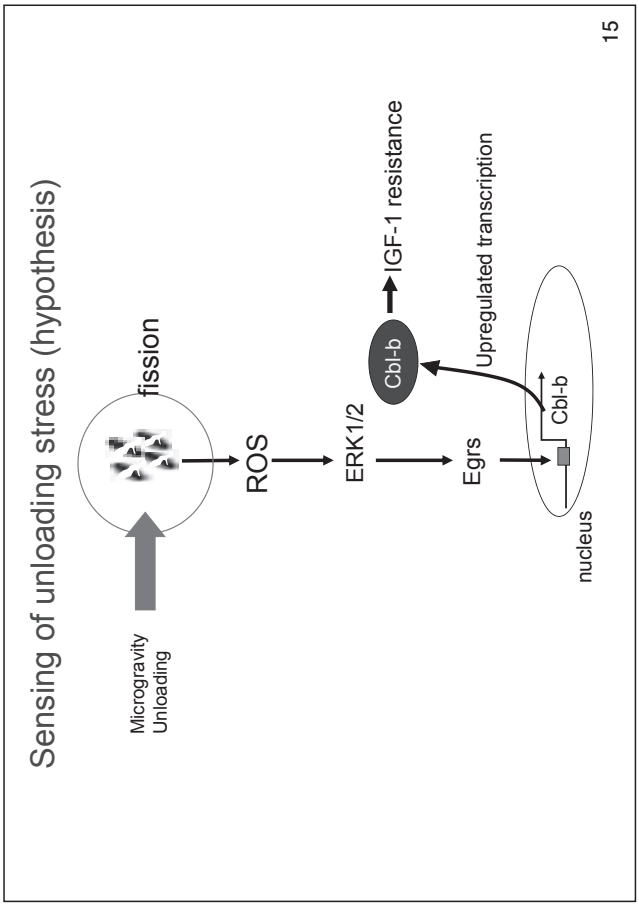
III. Novel approach against muscle atrophy (Cblin rice)

11

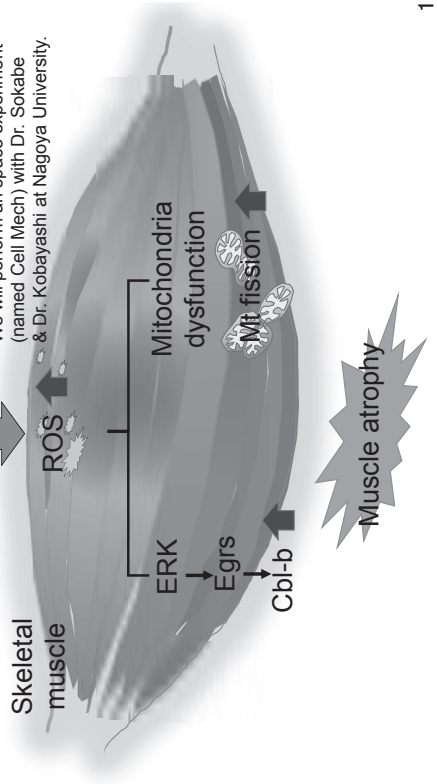

3D-Clinorotation
3D-Clinorotator
Simulated microgravity conditions
for cultured cells
Z
Y
X
It rotates cultured cells with two axes.
14


“MyoLab”
Cell Biology Experiment in Space
1) Cells: rat L6 myotubes
2) Culture system
32 DCCs
Cage & Hanger
Meas Exp Unit
Launch
CBEF
3) Solution exchange
Solution Exchanger
(Medium, Buffer, RNAlater)
4) Protocol
Day 0
Day 8
Day 10
Start culturing
In CBEF
IGF-1
treatment
Exchange to
RNAlater & Stock
Groups: ① μG + IGF-1 (-)
② μG + IGF-1 (+)
③ 1G + IGF-1 (-)
④ 1G + IGF-1 (+)
⑤ ground + IGF-1 (-)
⑥ ground + IGF-1 (+)
13


DRP1-mediated mitochondrial fission
Fission
Fusion
Fusion
OPA1
mdivi-1 :
Inhibitor of mitochondrial fission
Phospholylation of DRP1
initiates mitochondrial fission.
Eigass K et al. BBA 2012 150-161, partially modified
16


Sensing of unloading stress (hypothesis)
Microgravity
Unloading
fission
ROS
ERK1/2
Cbl-b
IGF-1 resistance
Egrs
Upregulated transcription
Cbl-b
nucleus
15

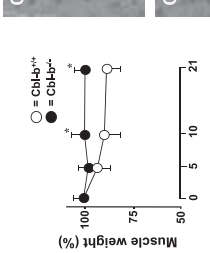
Conclusion



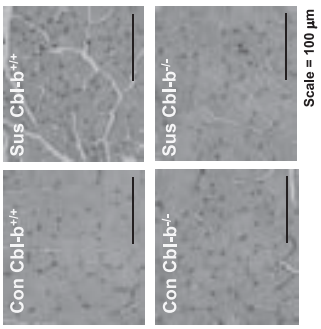
17

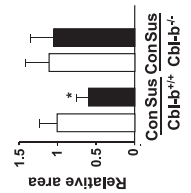
Cbl-b$^{-/-}$ mice are resistant to suspension-induced muscle atrophy

(A) Muscle weight



(B) Cross sectional area





Inhibition of Cbl-b-mediated ubiquitination led to development of countermeasure against muscle atrophy.

Nakao et al. *Mol Cell Biol* 2009 29(17): 4798-811.

19

I. Mechanism of unloading-mediated muscle atrophy (Background)

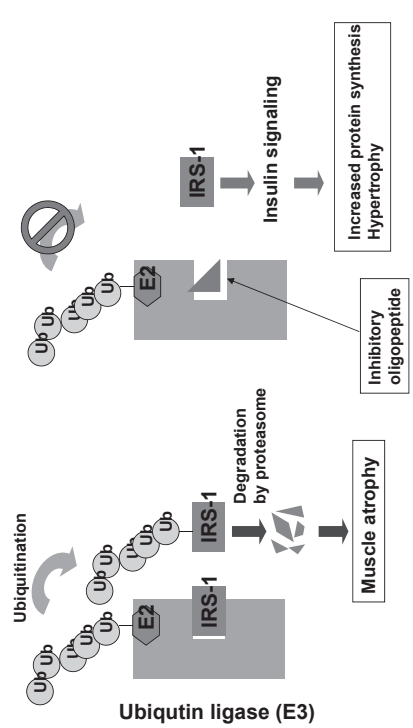
II. Unloading-mediated signal transduction (Sensing of microgravity)

III. Novel approach against muscle atrophy (Cblin rice)

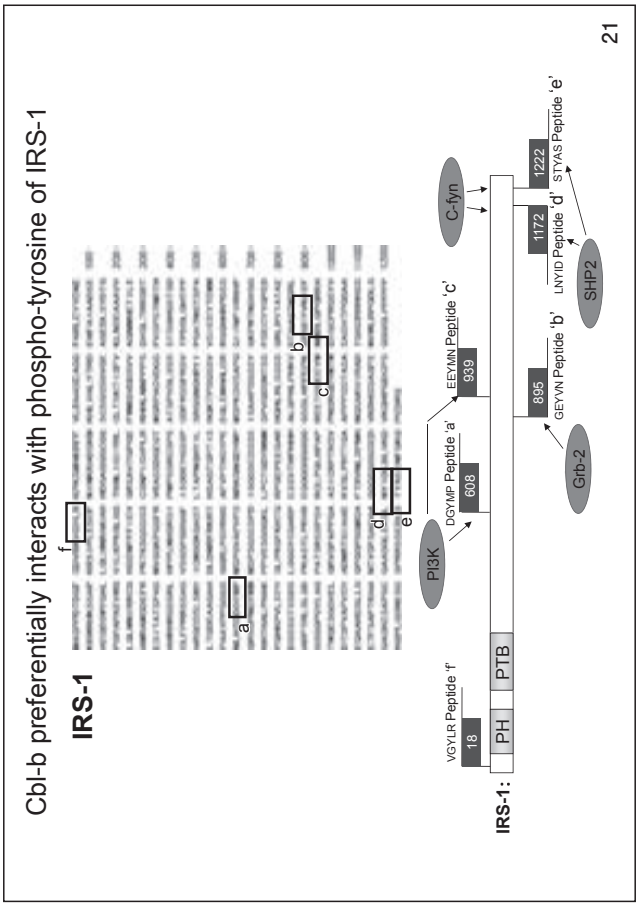
18

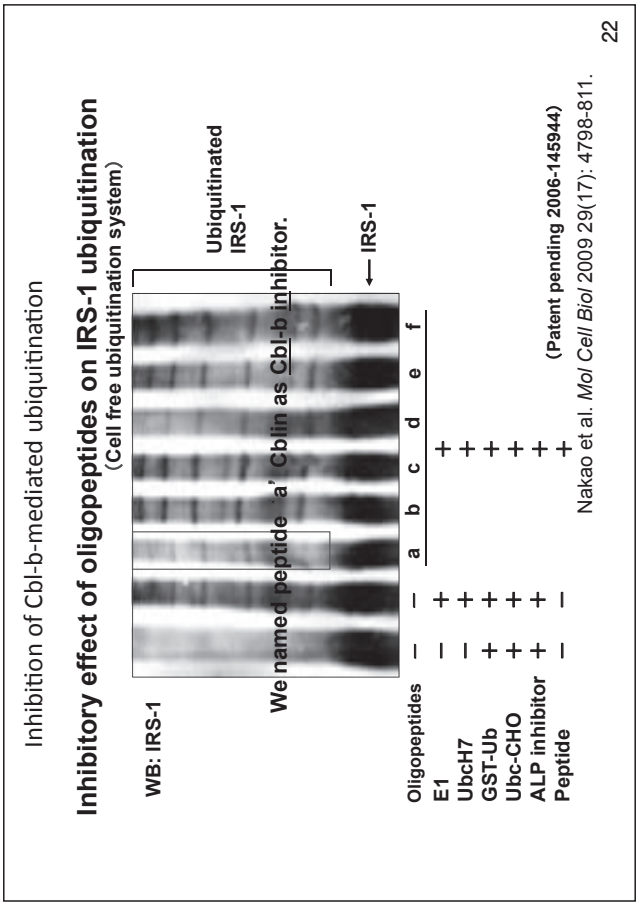
How do we inhibit ubiquitin ligase Cbl-b?

We focus on competitively inhibitory function of oligopeptides.



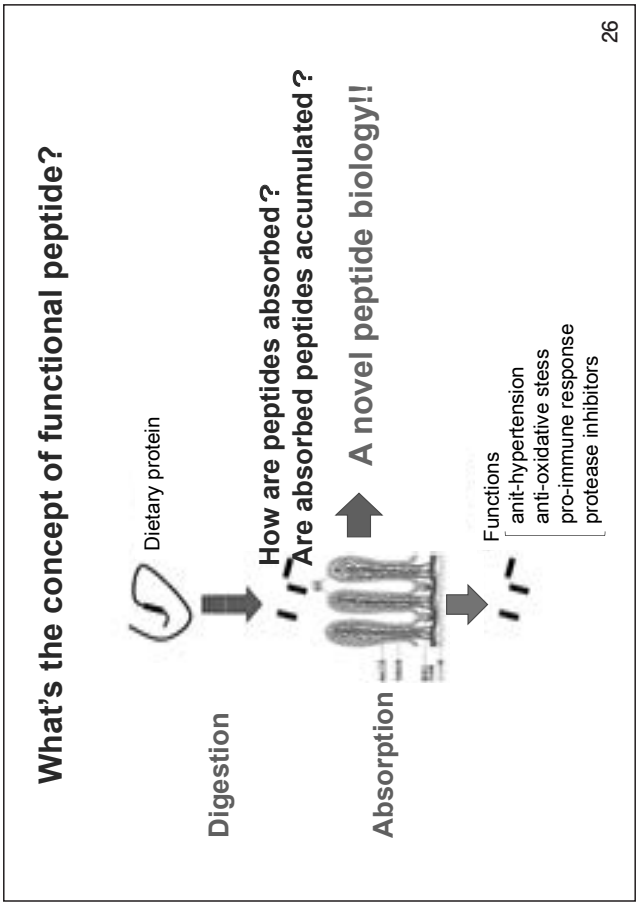
20

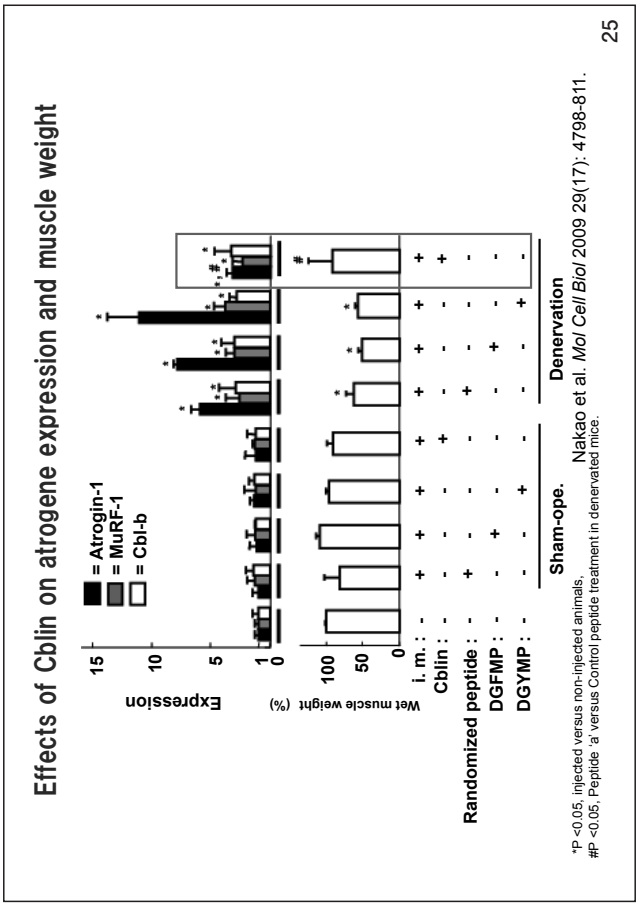

Cbl-b preferentially interacts with phospho-tyrosine of IRS-1
IRS-1
IRS-1:
VGYLR Peptide 'f'
18
PH
PTB
PI3K
DGYMP Peptide 'a'
608
EEYMN Peptide 'c'
939
C-fyn
895
GEYVN Peptide 'b'
Grb-2
1172
1222
LNYID Peptide 'd'
STYAS Peptide 'e'
SHP2
21

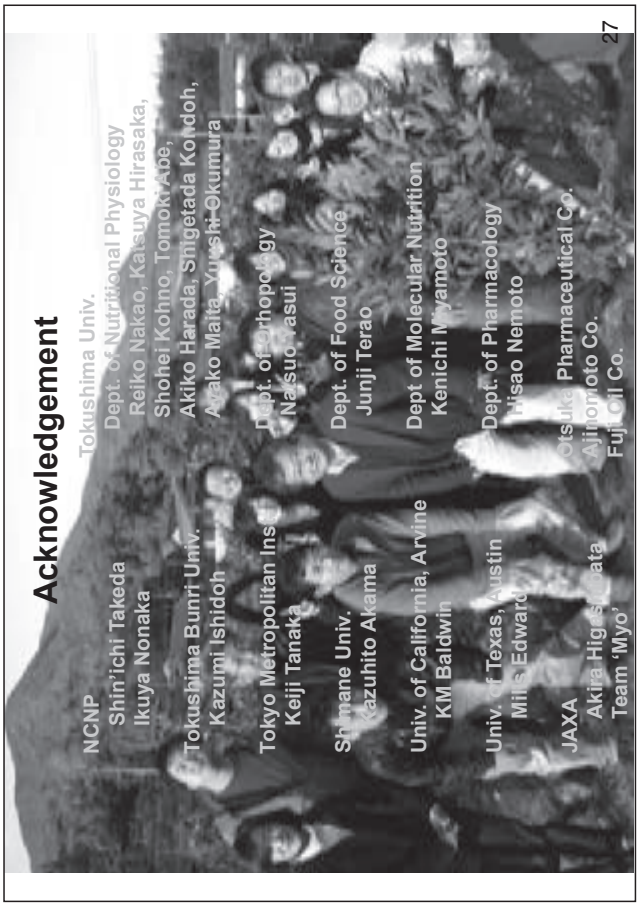

Inhibition of Cbl-b-mediated ubiquitination
Inhibitory effect of oligopeptides on IRS-1 ubiquitination
(Cell free ubiquitination system)
WB: IRS-1
Ubiquitinated IRS-1
We named peptide 'a' Cblin as Cbl-b inhibitor.
IRS-1
Oligopeptides
E1
UbcH7
GST-Ub
Ubc-CHO
ALP inhibitor
Peptide
(Patent pending 2006-145944)
Nakao et al. Mol Cell Biol 2009 29(17): 4798-811.
22

Effect of Cblin on IRS-1 ubiquitination in denervated muscle
Experimental design
C57/BL6 mice (7 weeks old, male)
Group
・Control [peptide administration (-)]
・Loading control [Peptide 'a' or control peptide]
・Denervation [Peptide 'a' or control peptide]
Denervation
= Cutting sciatic nerve
denervation
Day 0 Day 2 Day 4 Day 6 Day 8 Day 10
←1week→
breeding start
(normal diet)
administration of oligopeptides
by intramuscular injection
Dose of oligopeptides : 6 mg/mouse
Day10
Administration of oligopeptides
after 6 hr
Isolation of gastrocnemius muscles
23

Inhibitory effect of Cblin on Cbl-b-mediated ubiquitination in vivo
Ubiquitinated IRS-1
IRS-1
IP: IRS-1
IB: Ub
IB: IRS-1
IB: β-Actin
i. m. :
Cblin :
Randomized peptide :
DGFMP :
DGYMP :
Sham-ope.
Denervation
Nakao et al. Mol Cell Biol 2009 29(17): 4798-811.
24


What's the concept of functional peptide?
Digestion
Dietary protein
How are peptides absorbed?
Are absorbed peptides accumulated?
Absorption
A novel peptide biology!!
Functions
anit-hypertension
anti-oxidative stess
pro-immune response
protease inhibitors
26


Effects of Cblin on atrogene expression and muscle weight
= Atrogin-1
= MuRF-1
= Cbl-b
Expression
Wet muscle weight (%)
i. m. :
Cblin :
Randomized peptide :
DGFMP :
DGYMP :
Sham-ope.
Denervation
*P <0.05, injected versus non-injected animals,
#P <0.05, Peptide 'a' versus Control peptide treatment in denervated mice.
Nakao et al. Mol Cell Biol 2009 29(17): 4798-811.
25


Acknowledgement
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Shimane Univ.
Kazuhito Akama
Univ. of California, Arvine
KM Baldwin
Univ. of Texas, Austin
Mike Edward
JAXA
Akira Higashibata
Team 'Myo'
Tokushima Univ.
Dept. of Nutritional Physiology
Reiko Nakao, Katsuya Hirasaka,
Shohei Kohno, Tomoki Abe,
Akiko Harada, Shigetada Kondoh,
Ayako Maita, Yuushi Okumura
Dept. of Anthropology
Nasuo Yasui
Dept. of Food Science
Junji Terao
Dept of Molecular Nutrition
Kenichi Miyamoto
Dept. of Pharmacology
Hisao Nemoto
Otsuka Pharmaceutical Co.
Ajinomoto Co.
Fuji Oil Co.
27

セッション 5−2

老化筋肉における身体活動と栄養センシングならびにシグナルの制御

Professor & Chair
Department of Nutrition & Metabolism, University of Texas Medical Branch
Blake B. Rasmussen, Ph.D.

サルコペニアは加齢に伴う骨格筋量の減少と機能の低下状態を指すが、加齢に伴う筋肉のタンパク合成障害がその原因の一つではないかと考えられる。今回の講演では、加齢が骨格筋タンパク合成にどの程度影響するのか、また加齢に伴うタンパク合成障害を克服しサルコペニアの進行を遅らせるために、栄養や運動あるいは身体活動をいかに戦略的に活用できるかについて、データを基に報告する。続いて身体活動とアミノ酸がタンパク合成と筋肉増加の制御にどのように関わっているのか、その細胞内分子メカニズムについて論ずる。さらに、摂取した栄養素や身体活動に相応した骨格筋タンパク合成の増加がみられないといった、タンパク同化抵抗性が生じる原因に対して、栄養センシングと mTORC1 シグナルの果たす重要な役割に着目した、ヒトでの最近の研究内容も紹介する。

Session 5-2

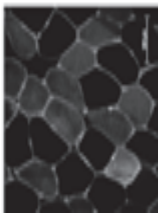
Physical Activity and the Regulation of Nutrient Sensing and Signaling in Aging Muscle

Blake B. Rasmussen, Ph.D.
Professor & Chair, Department of Nutrition & Metabolism, University of Texas Medical Branch

The loss of skeletal muscle size and function with aging, sarcopenia, may be related, in part, to an age-related muscle protein synthesis impairment. In this presentation, data will show the extent to which aging affects skeletal muscle protein synthesis and how nutrition and exercise or physical activity can be strategically employed to overcome age-related protein synthesis impairments and slow the progression of sarcopenia. The cellular and molecular mechanisms responsible for how physical activity and amino acids regulate protein synthesis and muscle growth will be discussed. In addition, the presentation will include recent data from human studies highlighting the important role of nutrient sensing and mTORC1 signaling in the etiology of anabolic resistance (i.e., the inability to increase skeletal muscle protein synthesis in response to nutrients or physical activity).

Physical Activity and the Regulation of Nutrient Sensing & Signaling in Aging Muscle

Blake Rasmussen, PhD
Professor & Chair
Department of Nutrition & Metabolism
Sealy Center on Aging
Claude Pepper Older Americans Independence Center
University of Texas Medical Branch



utmb Health

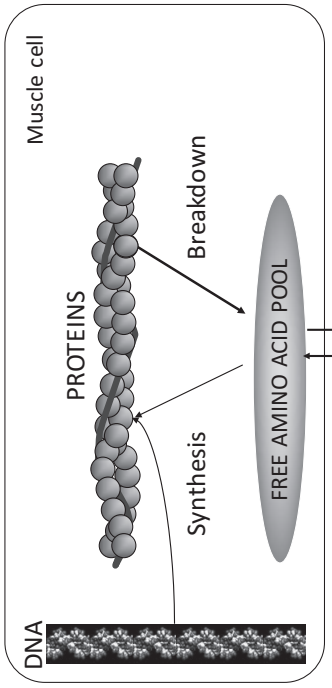
1

Presentation Outline

- Sarcopenia & Aging
- Protein Turnover & mTORC1 Signaling in Aging
- Role of Insulin in Muscle Anabolism
- Amino Acids & Insulin Sensitivity
- Anabolic Resistance
- Endothelial Function & Muscle Metabolism
- Amino Acid Sensing in Muscle
- Physical Activity/Inactivity & Muscle Sensitivity to Insulin and Amino Acids

2

Metabolic Control of Muscle Mass

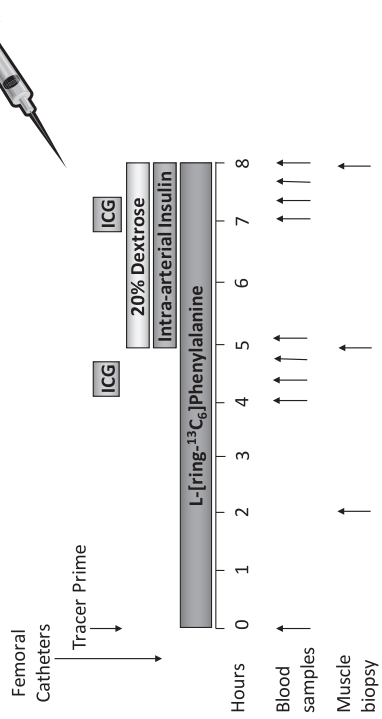


Biolo et al Am J Physiol, 1995

3

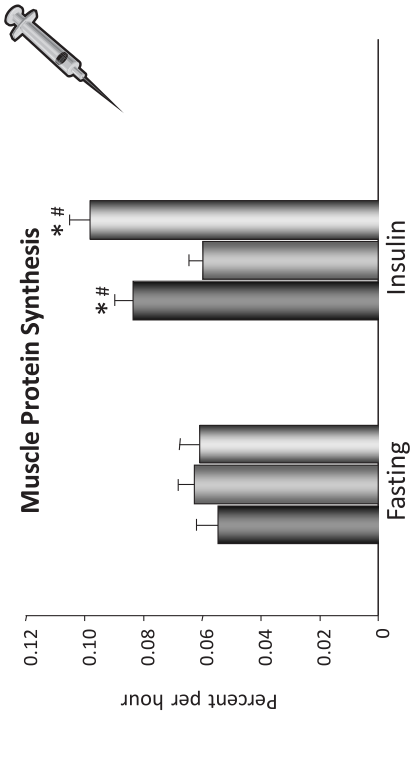
Effect of Insulin on Muscle Protein Metabolism in Aging

Study Design



4

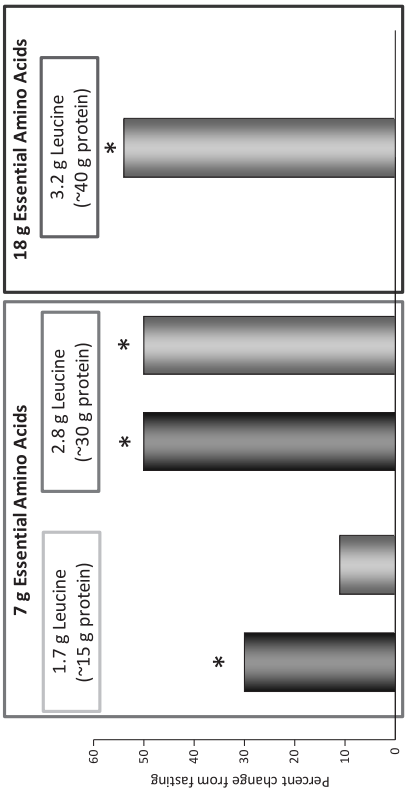
Muscle Protein Synthesis is Resistant to the Anabolic Effect of Insulin in Older Adults



Rasmussen et al., FASEB J, 2006
Fujita et al. Diabetologia, 2009

5

Response of Muscle Protein Synthesis to Low and High Amino Acids Doses



Katsanos et al, AJP, 2006
Volpi et al, AJCN, 2003

6

What is Anabolic Resistance?

An impaired ability to acutely increase muscle protein synthesis in response to nutrition (e.g., essential amino acids/protein), muscle contraction (e.g., resistance exercise), or growth factors (e.g., insulin).

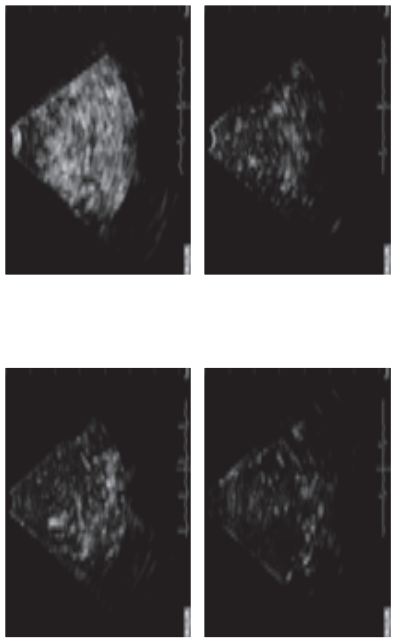
7

Conditions that Reduce Muscle Sensitivity to Amino Acids

- Injury
- Following Surgery
- Elderly (Sarcopenia)
- Forced Inactivity / Bed Rest
- Cancer, AIDS, Space Flight, Muscular Dystrophy
- Trauma, Burns, Type 1 and 2 Diabetes

8

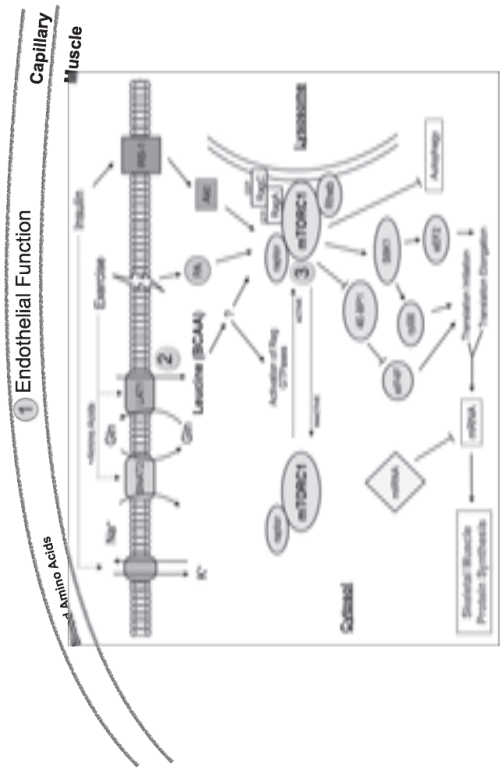
Endothelial Dysfunction Contributes to Anabolic Resistance in Older Adults



Timmerman et al, JCEM, 2010
Timmerman et al, Diabetes, 2010

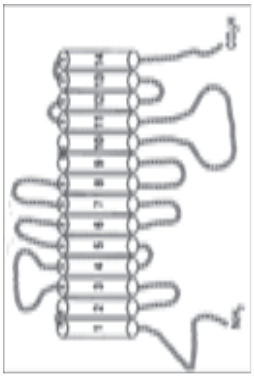
10

Potential Mechanisms of Reduced Amino Acid Sensing in Skeletal Muscle



9

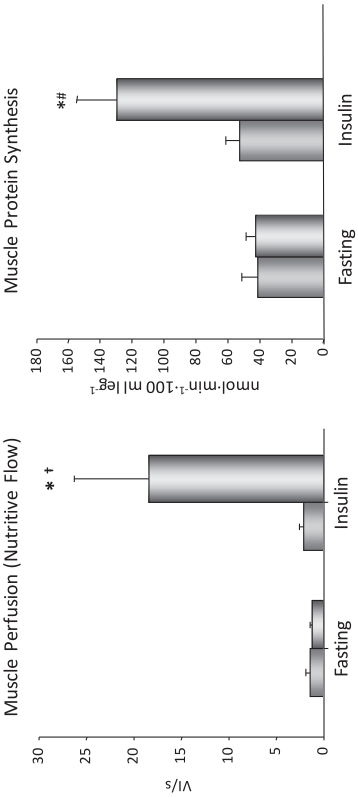
#2 Potential Mechanism for Reduced Amino Acid Sensing (Amino Acid Transport)



System	Protein	Gene	AA substrates	Notes
A	SNAT2	SLC38A2	Gly, Pro, Ala, Ser, Cys, Gln, Asn, His, Met	Sodium-Dependent
ASC	ASCT2	SLC1A5	Ala, Ser, Cys, Thr, Gln	Sodium-Dependent
L	LAT1	SLC7A5	His, Met, Leu, Ile, Val, Phe, Tyr, Trp, Gln	Sodium Independent
Imino	PAT1	SLC36A1	Pro, Gly, Ala	Sodium Independent, H+ coupled
y+	CAT1	SLC7A1	Arg, Lys, His	Sodium Independent

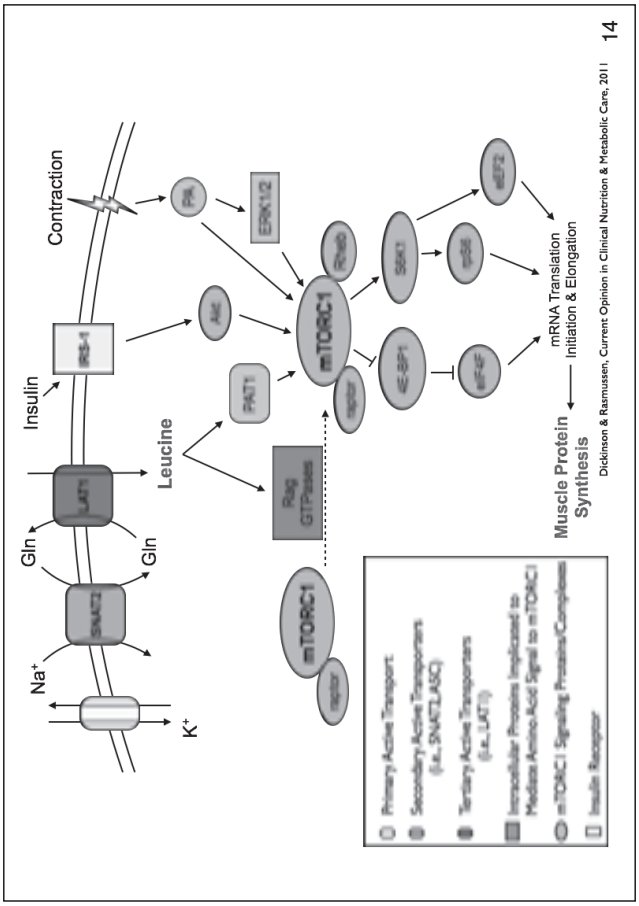
12

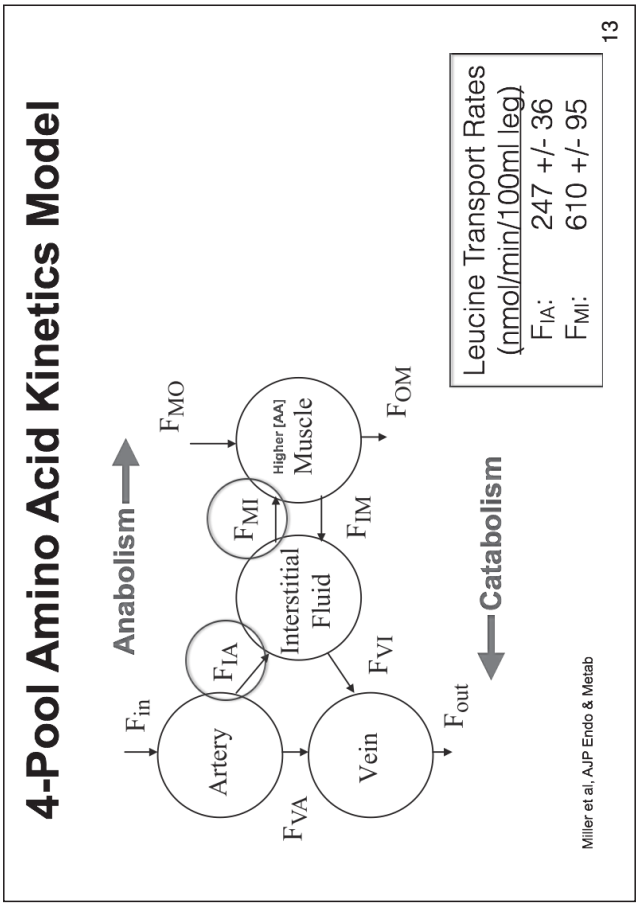
Pharmacological Vasodilation Restores the Muscle Anabolic Response to Insulin in Older Adults

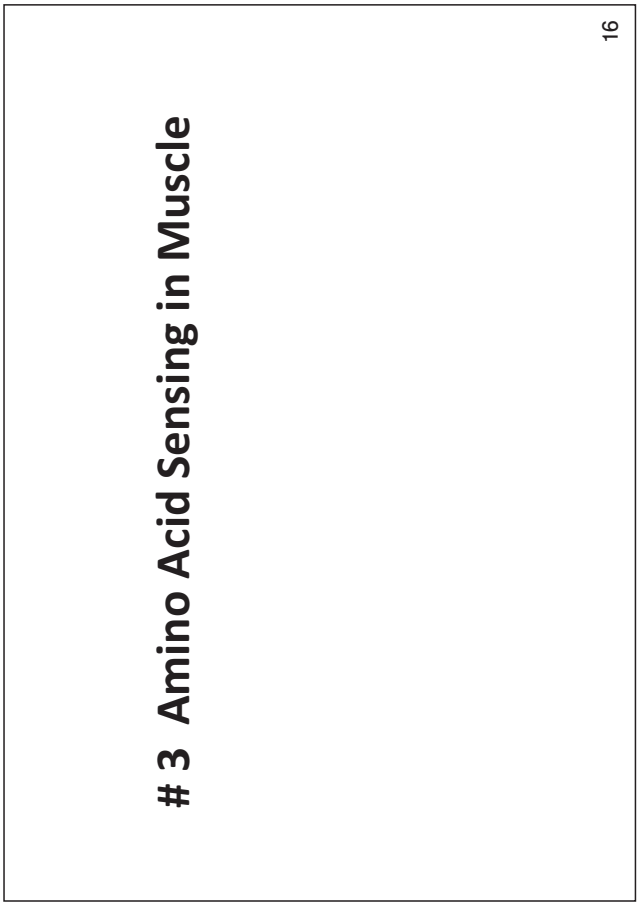


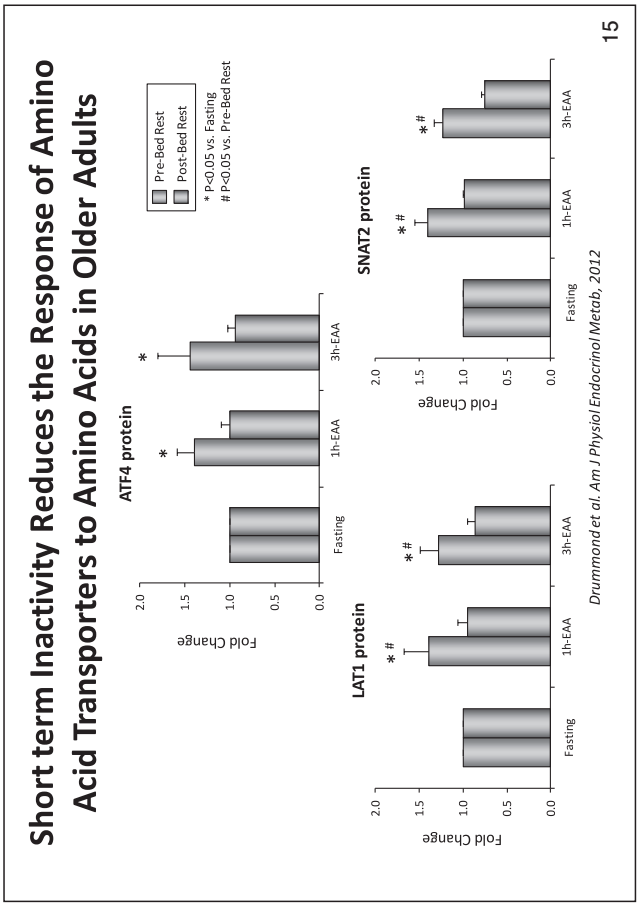
Timmerman, Lee et al., Diabetes, 2010

11


Contraction
Insulin
Gln
Gln
Leucine
Na+
K+
mTORC1
mTORC1
Muscle Protein Synthesis
mRNA Translation
Initiation & Elongation
Dickinson & Rasmussen, Current Opinion in Clinical Nutrition & Metabolic Care, 2011
14

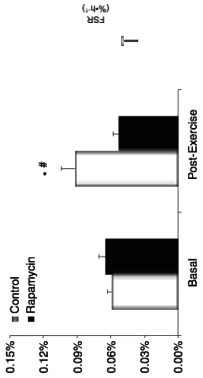

4-Pool Amino Acid Kinetics Model
Anabolism
Catabolism
Artery
Vein
Interstitial Fluid
Higher [AA] Muscle
Leucine Transport Rates
(nmol/min/100ml leg)
FIA: 247 +/- 36
FMI: 610 +/- 95
Miller et al, AJP Endo & Metab
13


3 Amino Acid Sensing in Muscle
16


Short term Inactivity Reduces the Response of Amino Acid Transporters to Amino Acids in Older Adults
ATF4 protein
LAT1 protein
SNAT2 protein
Pre-Bed Rest
Post-Bed Rest
* P<0.05 vs. Fasting
# P<0.05 vs. Pre-Bed Rest
Fold Change
Fasting
1h-EAA
3h-EAA
Drummond et al. Am J Physiol Endocrinol Metab, 2012
15

mTORC1 Activation is Necessary for the Exercise and Amino Acid - Induced Increase in Muscle Protein Synthesis

Resistance Exercise

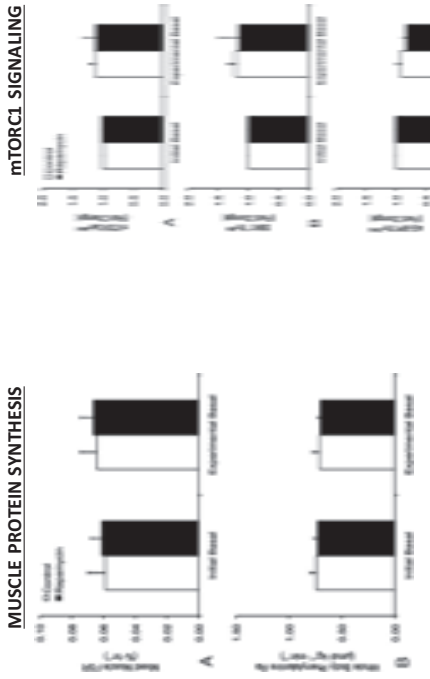


Essential Amino Acids

Drummond et al, J Physiol, 2009
Dickinson et al, J Nutrition, 2011
Dickinson et al, Clin Nutr, 2013

17

Low Dose Rapamycin in Humans Has No Effect on Basal Muscle mTORC1 Signaling & Protein Synthesis



Dickinson et al, Metabolism, 2013

18

Aging Induces Anabolic Resistance to Exercise

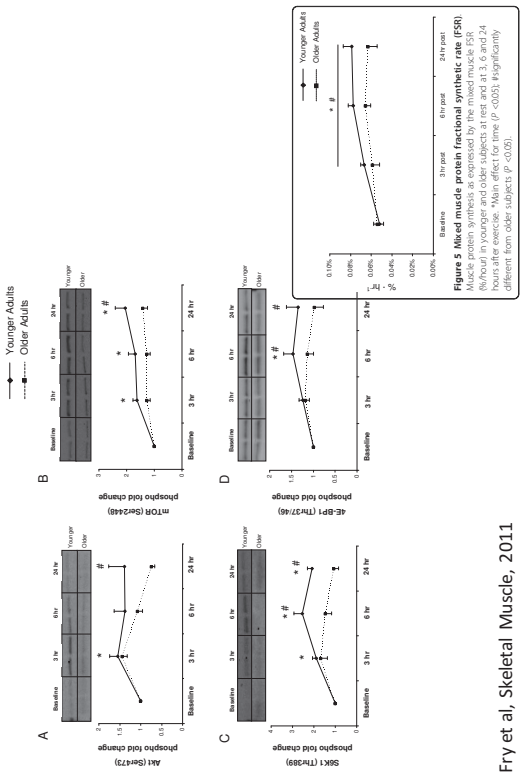


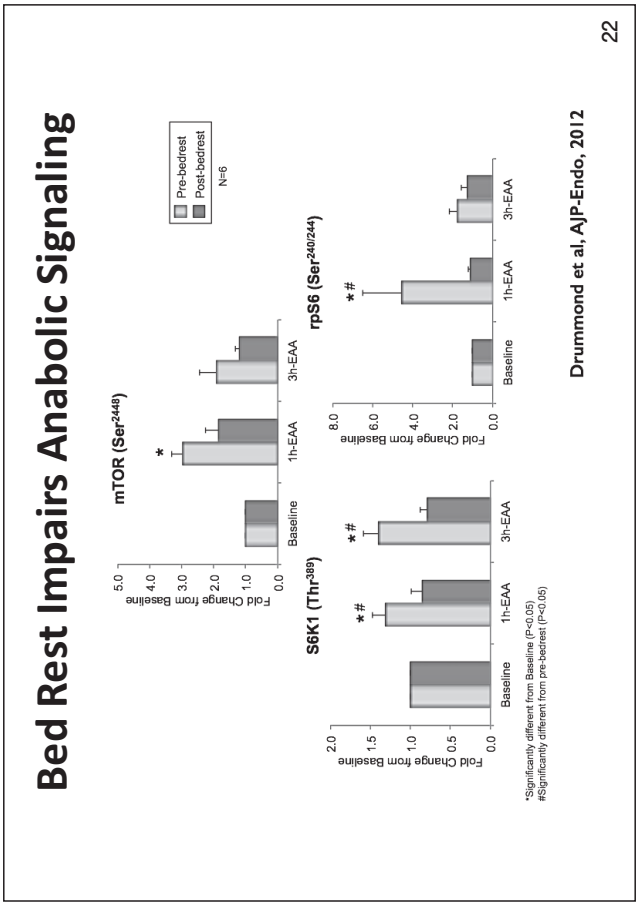
Figure 5 Mixed muscle protein fractional synthetic rate (FSR) Muscle protein synthesis as expressed by the mixed muscle FSR (%/hour) in younger and older subjects at rest and at 3, 6 and 24 hours after exercise. *Main effect for time (P <0.05); #significantly different from older subjects (P <0.05).

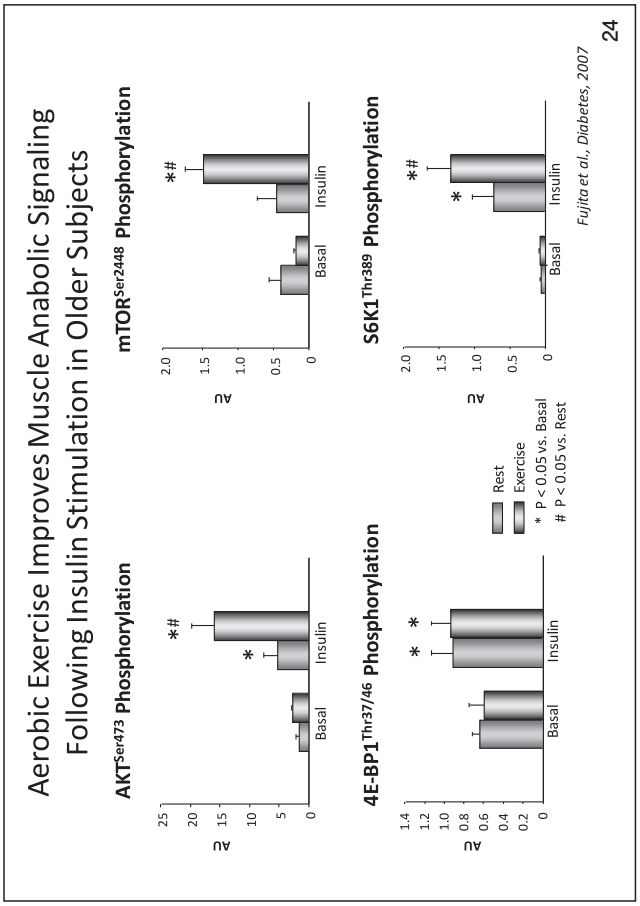
Fry et al, Skeletal Muscle, 2011

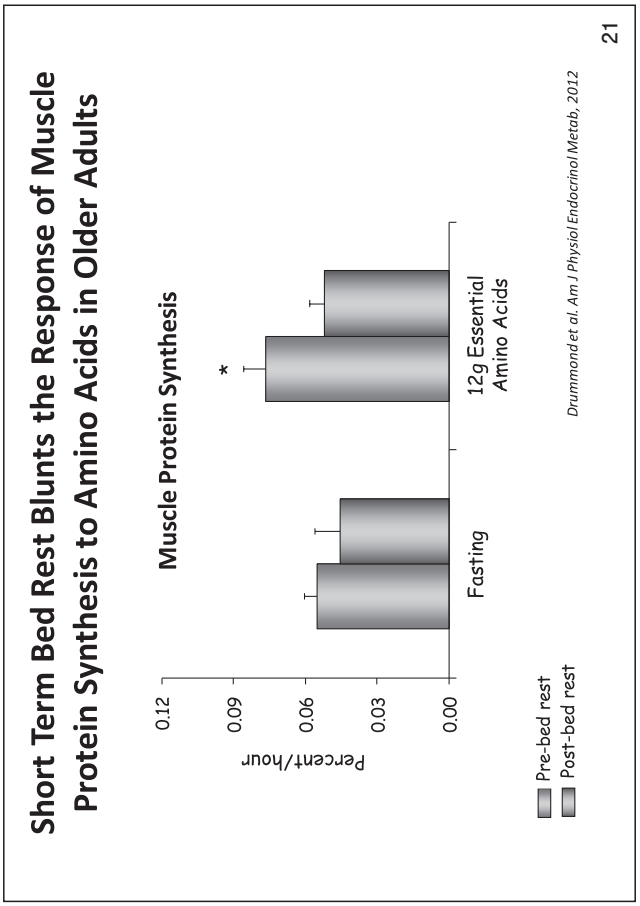
19

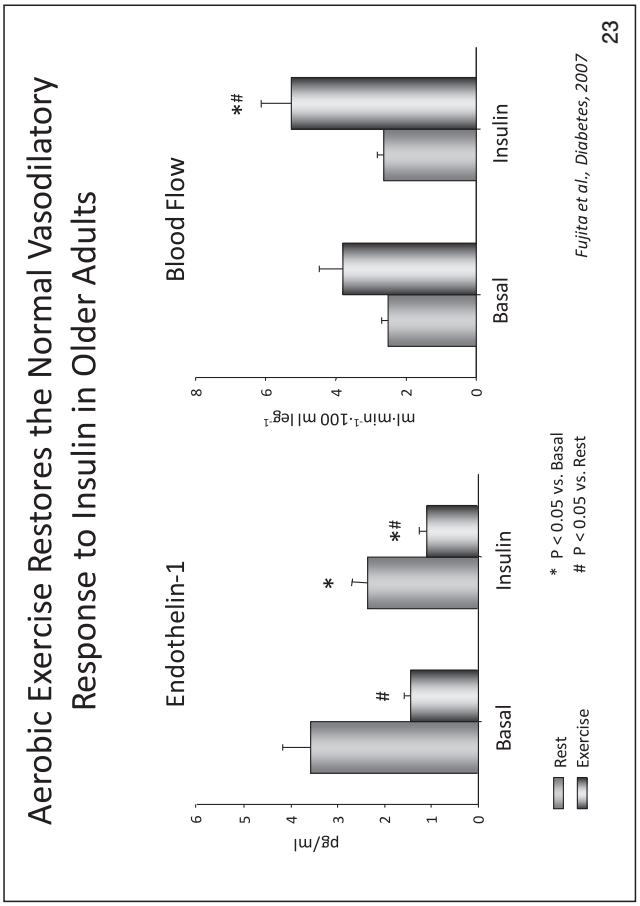
Role of Physical Activity

20

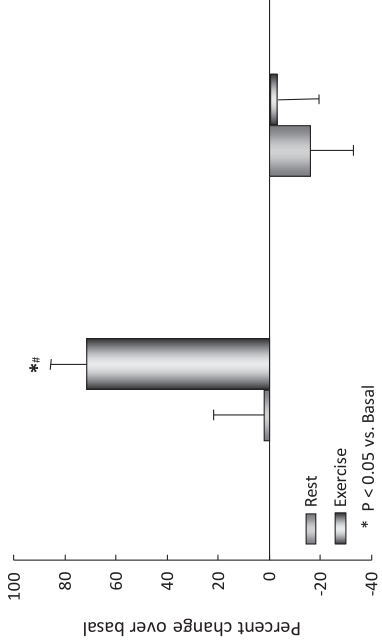

Bed Rest Impairs Anabolic Signaling
mTOR (Ser2448)
Pre-bedrest
Post-bedrest
N=6
Fold Change from Baseline
Baseline
1h-EAA
3h-EAA
S6K1 (Thr389)
rpS6 (Ser240/244)
*Significantly different from Baseline (P<0.05)
#Significantly different from pre-bedrest (P<0.05)
Drummond et al, AJP-Endo, 2012
22


Aerobic Exercise Improves Muscle Anabolic Signaling Following Insulin Stimulation in Older Subjects
AKTSer473 Phosphorylation
mTORSer2448 Phosphorylation
S6K1Thr389 Phosphorylation
4E-BP1Thr37/46 Phosphorylation
AU
Basal
Insulin
Rest
Exercise
* P < 0.05 vs. Basal
# P < 0.05 vs. Rest
Fujita et al., Diabetes, 2007
24


Short Term Bed Rest Blunts the Response of Muscle Protein Synthesis to Amino Acids in Older Adults
Muscle Protein Synthesis
Percent/hour
Fasting
12g Essential Amino Acids
Pre-bed rest
Post-bed rest
Drummond et al. Am J Physiol Endocrinol Metab, 2012
21


Aerobic Exercise Restores the Normal Vasodilatory Response to Insulin in Older Adults
Endothelin-1
pg/ml
Blood Flow
ml·min-1·100 ml leg-1
Basal
Insulin
Rest
Exercise
* P < 0.05 vs. Basal
# P < 0.05 vs. Rest
Fujita et al., Diabetes, 2007
23

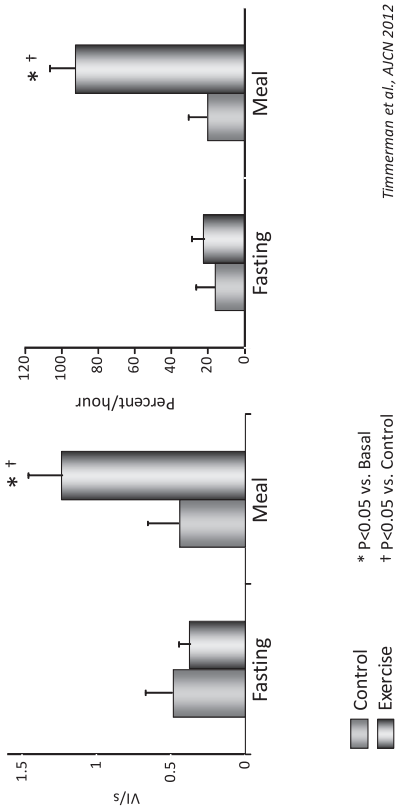
Aerobic Exercise Improves Muscle Protein Synthesis in Response to Insulin in Older Subjects



Fujita et al., Diabetes, 2007

25

Aerobic Exercise Restores Muscle Protein Anabolic Response to a Mixed Meal in Older Adults



Timmerman et al., AJCN 2012

26

Conclusions

- Insulin resistance and endothelial dysfunction decrease muscle protein anabolism and contribute to sarcopenia in older adults.
- Type 2 diabetes and inactivity induce additional alterations in muscle amino acid transport, which likely contribute to accelerated sarcopenia.
- Vasodilators, insulin sensitizers, and exercise may be important tools to enhance muscle protein anabolism and reduce muscle loss

27

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Ted Graber, PhD
Brad Lambert, PhD
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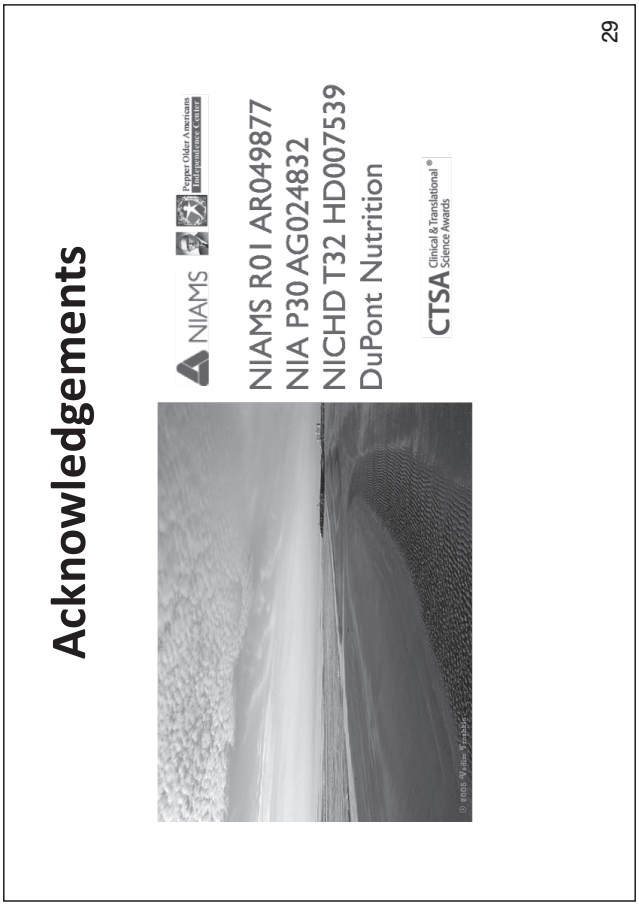
Syed Husaini
Junfang Hao
Susan Wilson
Ming Zheng

28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NIAMS
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NIA P30 AG024832
NICHD T32 HD007539
DuPont Nutrition
CTSA Clinical & Translational Science Awards
29

セッション 5-3

サルコペニアに対するアミノ酸栄養の重要性

味の素株式会社　研究開発企画部
小林　久峰

骨格筋の量は加齢とともに徐々に減少する。特に 50 歳以降毎年 1～2 ％程度筋肉量は減少すると言われている。その結果、高齢者においては、筋量と筋力・筋機能の低下を特徴とするサルコペニアという現象が生じる。サルコペニアによる筋量・筋力の低下は、歩行能力の低下に代表される運動機能障害や、転倒・骨折リスクの増大に結びついており、高齢者の生活の質の低下、フレイル（虚弱）、自立性の喪失、さらには死亡リスクの増大につながる。

40 歳代までの成人では、筋タンパク質合成と筋タンパク質分解の動的平衡により、骨格筋の量はほぼ一定に保たれている。サルコペニアは性ホルモンなど内分泌機能の変化、神経系の変化、炎症性因子の増加、運動や栄養の低下、筋サテライト細胞の減少や活性低下といった複合的な要因が関わっているが、筋タンパク質の代謝の観点からは、筋タンパク質合成反応の加齢による低下によって筋量の減少が起こる。骨格筋のタンパク質合成・分解に影響を与える生活習慣は、栄養と運動であり、栄養素の中では、特にタンパク質の摂取が重要である。タンパク質の摂取による血中アミノ酸濃度の増加と、骨格筋へのアミノ酸の供給が筋タンパク質合成を引き起こす。地域高齢者の観察研究では、タンパク質摂取量が多いほど、筋肉量の減少率が小さいことが報告されている。一方、運動は筋タンパク質合成を刺激するが、同時に筋タンパク質分解も引き起こす。しかし運動に加えてアミノ酸を与えた場合には、筋タンパク質合成がさらに刺激され、分解は抑制されるため、強い筋タンパク質の同化作用を引き起こされる。

アミノ酸の中では特に必須アミノ酸の摂取が、高齢者の骨格筋タンパク質の合成を促しタンパク質同化反応を引き起こす。さらに 9 種の必須アミノ酸の中でも、分岐鎖アミノ酸（BCAA）のひとつであるロイシンは、細胞内の mTORC1 を活性化し、タンパク質合成刺激因子として働くことが知られている。ヒト高齢者において、ロイシンの含量を約 40 ％に高めたロイシン高配合必須アミノ酸混合物（以下 Amino L40）と、通常の必須アミノ酸混合物（ロイシン含量 26 ％）の筋タンパク質合成促進作用を比較したところ、Amino L40 がより大きな筋タンパク質合成を引き起こすことを発見した。さらに最近の研究では、高齢女性において、わずか 3 g の Amino L40 の摂取が、20 g のホエイタンパク質を摂取した場合と同等の筋タンパク質合成を起こすことを確認している。このように Amino L40 は効率的に高齢者の筋タンパク質合成を促進する。

さらに、サルコペニアが顕在化している地域在住の日本人の高齢女性（75 歳以上）を対象とした、3ヶ月間の筋力・バランス・歩行機能トレーニング（1 週間に 2 回）と、Amino L40 の継続的な摂取（3 g を 1 日 2 回）、およびそれらの組合せにより、筋量、筋力、身体運動機能に与える影響を評価した無作為化比較対象試験（RCT）では、運動と Amino L40 摂取は、筋量、筋力、歩行速度の改善について相乗的に作用し、筋量、筋力、歩行速度が全て有意に改善した。また、健康な高齢者のレジスタンストレーニングに併用して Amino L40 を摂取させた場合（3 g の Amino L40 を 1 日 1 回あるいは 2 回）においても、Amino L40 の併用によって、10 m 障害物歩行の時間が短縮するなどの移動能力の改善が認められたことが報告されている。

このように Amino L40 の摂取は筋タンパク質合成を効率的に引き起こし、超高齢社会におけるサルコペニア予防対策手段として有望である。

Session 5-3

The Importance of Amino Acid Nutrition in Sarcopenia Prevention

Hisamine Kobayashi
R&D Planning Department, Ajinomoto Co., Inc.

Skeletal muscle mass gradually decreases with increasing age. In particular, after 50 years of age, about 1–2 % of the muscle mass is known to decrease every year. As a result, sarcopenia, which is characterized by reduced muscle mass, muscle strength, and muscle function, occurs in the elderly. Sarcopenia is related to impaired motor function. Elderly people with sarcopenia show a decline in walking ability and have an increased risk of falls and fractures, which lead to decreased quality of life, frailty, and loss of independence, as well as an increased risk of death.

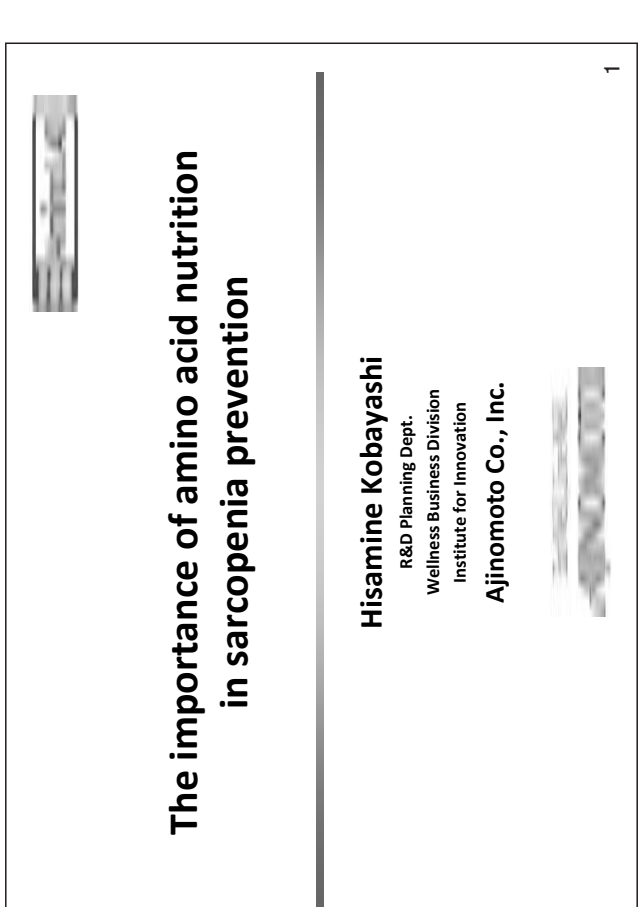
In adults aged up to 40 years, skeletal muscle mass is maintained substantially constant by the dynamic equilibrium of the synthesis and breakdown of muscle protein. Multiple factors are involved in the development of sarcopenia. However, in terms of protein metabolism, muscle mass reduction is caused by impairments in the muscle protein synthetic responses to anabolic stimuli, physical activity and dietary intake, with aging. Among nutrients, oral intake of protein is important, as it increases blood amino acid concentrations and enhances the supply of amino acids to skeletal muscles and muscle protein synthesis. Conversely, although exercise stimulates muscle protein synthesis, it also simultaneously leads to muscle protein breakdown. However, when amino acids are given in addition to exercise, muscle protein synthesis is further stimulated and breakdown is suppressed, thereby inducing muscle protein anabolism.

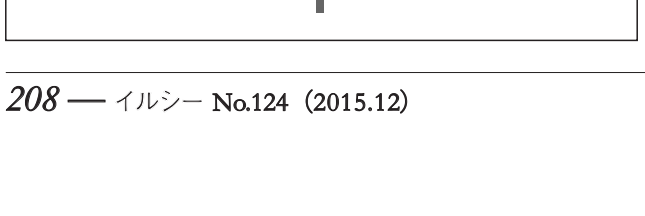
The intake of amino acids, particularly essential amino acids, promotes the synthesis of skeletal muscle protein and leads to muscle protein anabolism in the elderly. Furthermore, among the nine essential amino acids, leucine activates intracellular mTORC1 and stimulates protein synthesis. Elderly individuals who consumed an essential amino acid mixture that was rich in leucine (leucine content, about 40 %, hereafter referred to as Amino L40) showed higher muscle protein anabolism than elderly individuals who consumed a normal essential amino acid mixture (leucine content, 26 %). Furthermore, in a recent study in older women, we found that the ingestion of 3 g of Amino L40 promotes the synthesis of muscle proteins to the same extent as the ingestion of 20 g of whey protein. Thus, Amino L40 effectively promoted muscle protein synthesis in the elderly.

We then performed a randomized controlled study to evaluate the effectiveness of exercise, Amino L40, and a combination of exercise and Amino L40 in enhancing muscle mass and strength in community-dwelling elderly sarcopenic women aged over 75 years. The exercise program included a 60-minute moderate intensity comprehensive training program that was performed twice a week, and 3 g of Amino L40 was consumed twice a day for 3 months. The results showed that the combination of exercise and Amino L40 significantly increased muscle mass, muscle strength, and walking speed in sarcopenic elderly women.

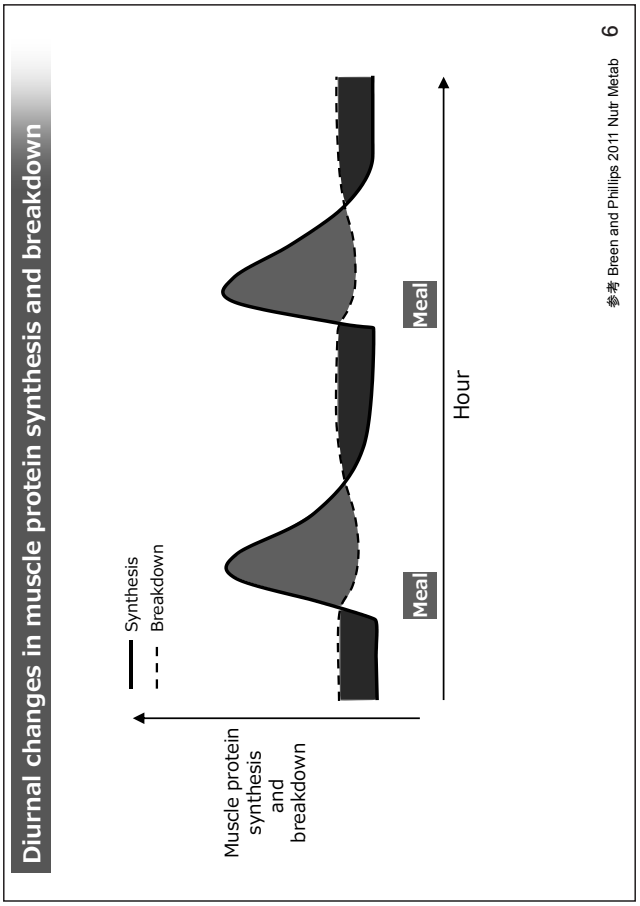
Thus, as the oral intake of Amino L40 effectively increased muscle protein synthesis, we believe that it can be effective for preventing sarcopenia in the super-aged society.

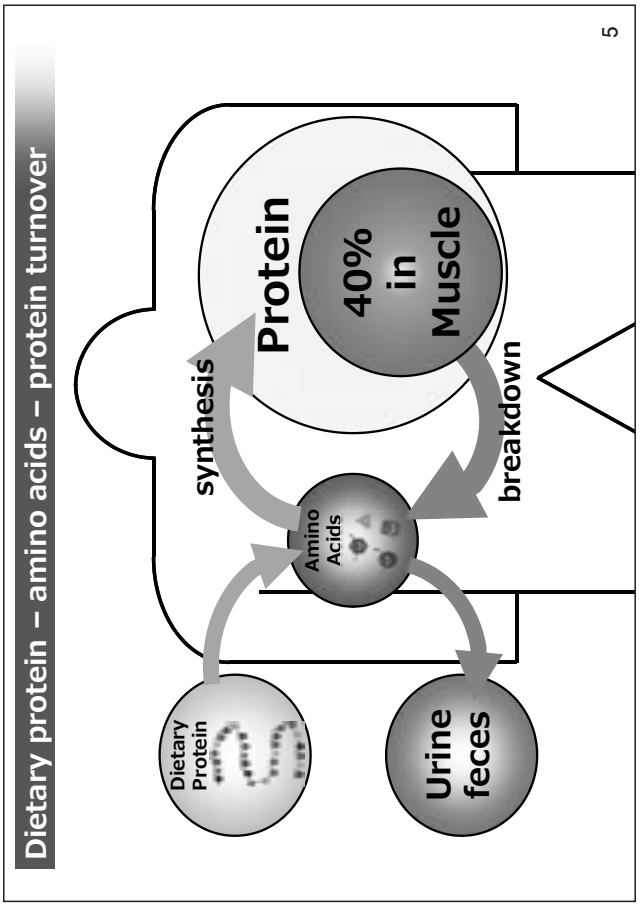
The importance of amino acid nutrition in sarcopenia prevention
Hisamine Kobayashi
R&D Planning Dept.
Wellness Business Division
Institute for Innovation
Ajinomoto Co., Inc.
1

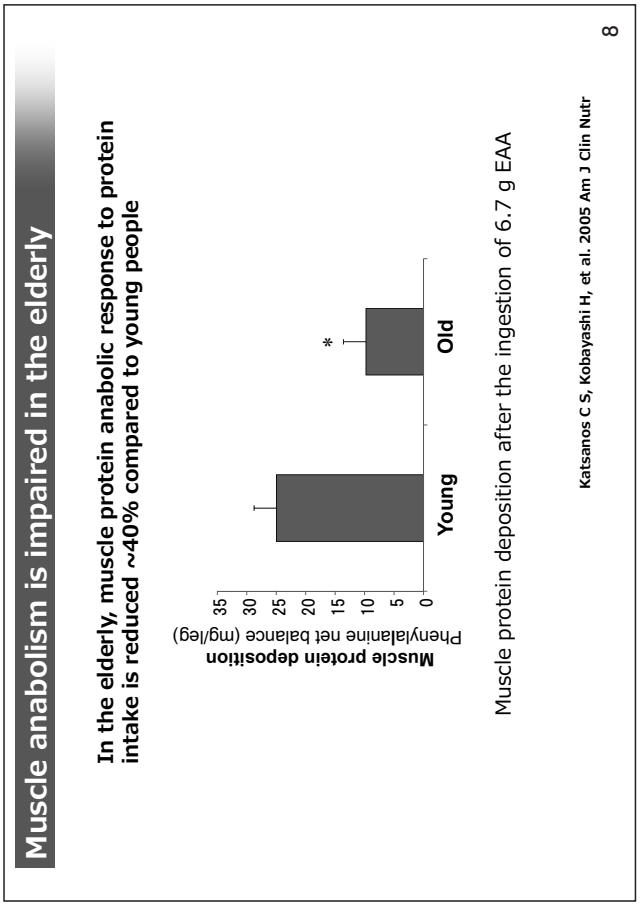

Sarcopenia
Loss of skeletal muscle mass and strength that occurs with advancing age
Impaired state of health
- Mobility disorders
- Increased risk of falls and fractures
- Impaired ability to perform activities of daily living
- Disabilities
- Loss of independence
- Increased risk of death
Provided by Prof. Fujita, Ritsumeikan University
2

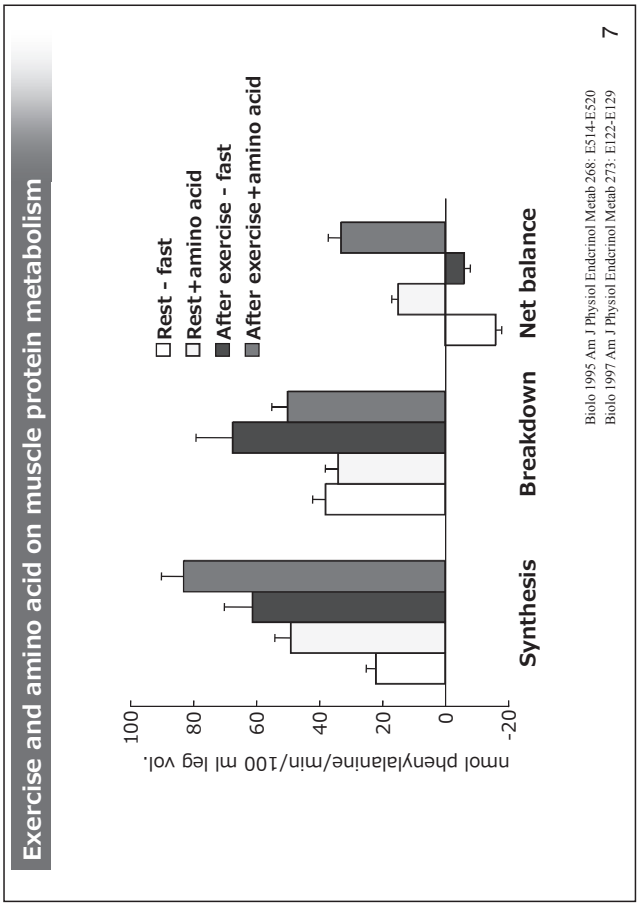

Protein intake and muscle mass
Association between protein intake and 3-year changes in lean mass in the Health ABC Cohort (70-79y, n = 2066)
Low protein intake is associated with a significant loss of muscle
Energy-adjusted total protein intake
US RDA 0.8 g/kg/d
11.2% (0.7 g/kg/d)
12.7% (0.7 g/kg/d)
14.1% (0.8 g/kg/d)
15.8% (0.9 g/kg/d)
18.2% (1.1 g/kg/d)
Changes in lean mass (kg)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Houston DK et al. Am J Clin Nutr 2008
3

Dietary protein – amino acids – protein turnover
Dietary Protein
synthesis
Amino Acids
Protein
breakdown
Urine feces
4

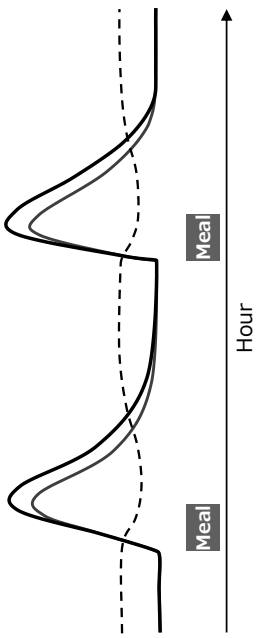

Diurnal changes in muscle protein synthesis and breakdown
Synthesis
Breakdown
Muscle protein synthesis and breakdown
Meal
Meal
Hour
参考 Breen and Phillips 2011 Nutr Metab
6


Dietary protein – amino acids – protein turnover
Dietary Protein
Urine feces
Amino Acids
synthesis
Protein
40% in Muscle
breakdown
5


Muscle anabolism is impaired in the elderly
In the elderly, muscle protein anabolic response to protein intake is reduced ~40% compared to young people
Muscle protein deposition
Phenylalanine net balance (mg/leg)
35
30
25
20
15
10
5
0
Young
Old
*
Muscle protein deposition after the ingestion of 6.7 g EAA
Katsanos C S, Kobayashi H, et al. 2005 Am J Clin Nutr
8


Exercise and amino acid on muscle protein metabolism
Rest - fast
Rest+amino acid
After exercise - fast
After exercise+amino acid
nmol phenylalanine/min/100 ml leg vol.
100
80
60
40
20
0
-20
Synthesis
Breakdown
Net balance
Biolo 1995 Am J Physiol Endcrinol Metab 268: E514-E520
Biolo 1997 Am J Physiol Endcrinol Metab 273: E122-E129
7

Diurnal changes in muscle protein synthesis and breakdown



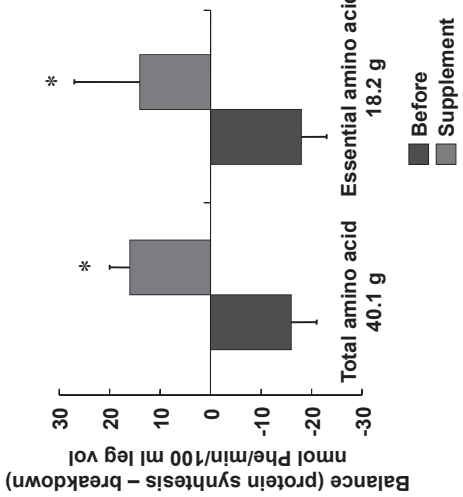
Muscle protein anabolic response to the meal is reduced in the elderly

Modified from Breen and Phillips 2011 Nutr Metab

9

Importance of essential amino acids

Essential amino acids are primarily responsible for stimulation of muscle protein anabolism in the elderly



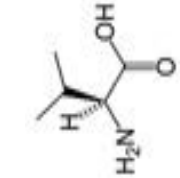
Volpi, Kobayashi, et al. Am J Clin Nutr 2003

10

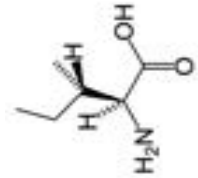
Branched-chain amino acids（BCAA）

- Aliphatic side-chains with a branch
- Essential amino acids
- BCAAs, particularly leucine, stimulate protein synthesis and reduce protein breakdown via mTOR pathway

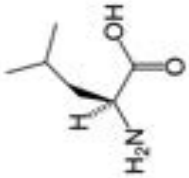
L-Leucine (Leu)



L-Isoleucine (Ile)

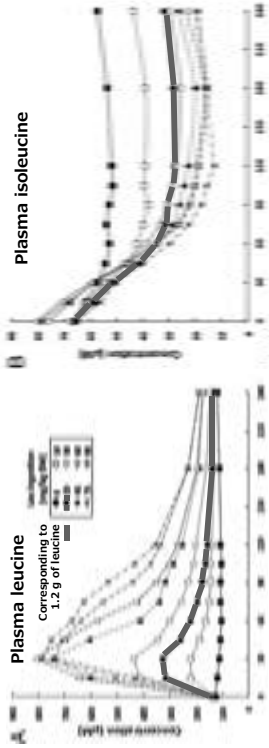


L-Valine (Val)



11

Undesiable effects of ingestion of leucine alone



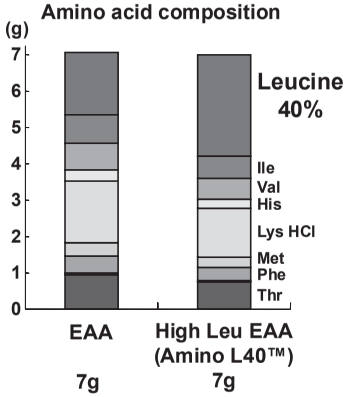
Plasma concentrations of isoleucine, valine, phenylalanine, methionine, and tyrosine decreased after leucine ingestion.

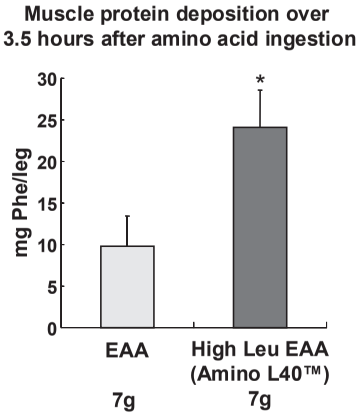
Matsumoto T et al. SpringerPlus 2014

12

Leucine-enriched essential amino acid

Leucine enriched essential amino acid mixture (Amino L40™) showed a greater skeletal muscle protein anabolism in the elderly





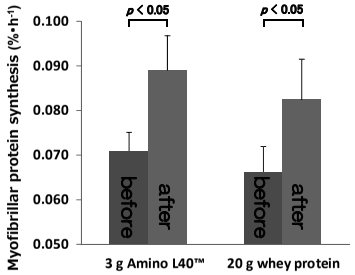
Modified from Katsanos, Kobayashi, et al. Am. J. Physiol. Endocrinol. Metab. 2006

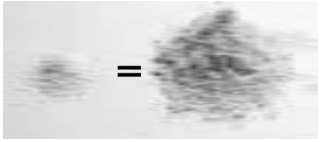
13

Low-dose Amino L40™ promotes protein synthsis

Subjects: healthy elderly women (≧ 60 y, n = 8/group)

Bolus ingestion of 3 g of Amino L40™ or 20 g of whey protein





Only 3 g of Amino L40™ stimulates muscle protein synthesis to the same extent as 20 g of whey protein.

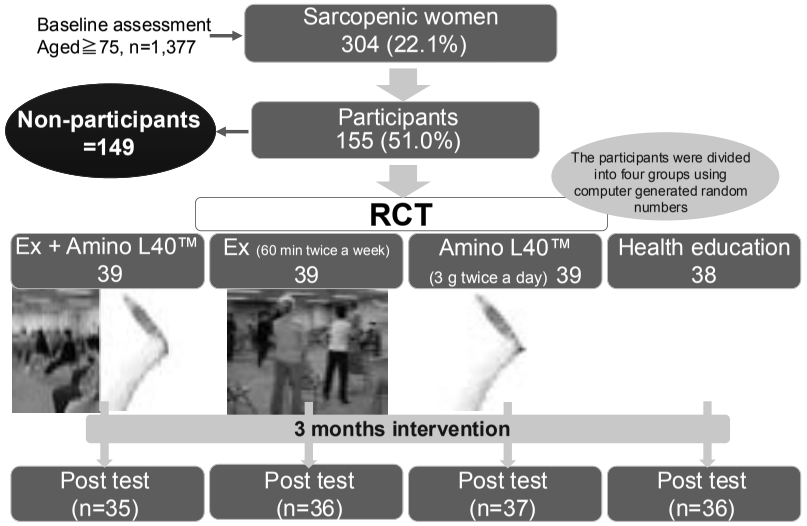
Results similar even in the exercised leg.
Also it promotes albumin synthesis.

3 g of Amino L40™ is effective enough to promote protein synthesis

Modified from Bukhari, S.S.I. et al. Am J Physiol Endcriol Metab (2015)

14

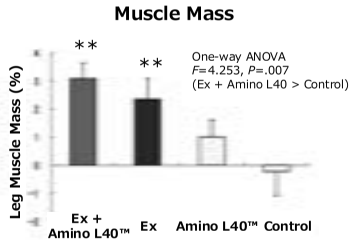
Amino L40™ randomized controlled trial

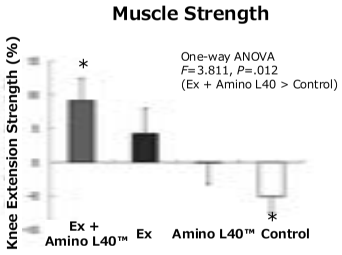


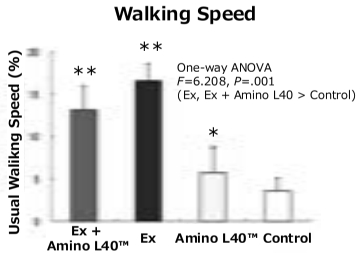
Kim et al. J Am Geriatr Soc 2012

15

Changes in Muscle Mass, Strength, Walking Speed







Exercise and Amino L40™ together are effective in enhancing muscle mass, muscle strength, and walking speed in sarcopenic women.

**: p <0.01, *: p < 0.05 in comparing the pre- and post intervention changes

Kim et al. J Am Geriatr Soc 2012

16

Summary

- Skeletal muscle mass declines with aging (sarcopenia)
- Anabolic response to dietary protein is reduced in the elderly
- Essential amino acids are primarily responsible for muscle protein anabolism
- Leucine enriched essential amino acid effectively stimulates muscle protein anabolism in the elderly and improves sarcopenia (muscle mass, muscle strength and physical performance)

17

Eat Well, Live Well.
AJINOMOTO®

18

セッション 5-4

健康のために10分多く体を動かそう：用量反応分析に基づいた日本の新しい身体活動ガイドライン

国立研究開発法人　医薬基盤・健康・栄養研究所
宮地　元彦

身体活動不足は、現在、非伝染性疾患（NCDs）による日本の死亡の3番目の主要な危険因子です。日本では、一日あたりの歩数（中高強度身体活動量（MVPA）の指標の一つ）の平均値が、1997年には1日約8000歩だったのが2009年には約7000歩に、10年間で大幅に減少しました。健康日本21（第二次）は厚生労働省が2013年に発表した健康づくり施策で、2022年まで遂行されます。その目的は、国民の健康寿命の延伸と生活の質の向であり、そのために健康日本21では、歩数を増やすなどの目標が設定されました。

国立健康・栄養研究所（NIHN）が提供する科学的証拠に基づき、厚生労働省は健康づくりのための身体活動指針「アクティブガイド」を2013年3月に策定・発表しました。アクティブガイドは日本人の成人が1日あたり60分のMVPAを実施することを推奨しています。しかし、それを日本人の多くの労働者が達成することは困難です。なぜなら、現代生活では仕事や家族との約束に当てられる時間によって、各個人が身体活動に割り当てることができる時間が短いからです。

アクティブガイドの最も重要な日本人に伝えたいメインメッセージは、「10分多く体を動かそう」を表す「+10」です。我々の知る限りでは、このような身体活動の推奨が政府の健康増進政策のための身体活動ガイドラインで示されるのは世界で初めてです。+10は、すでに1日60分の身体活動を達成している活動的な人だけでなく、座りがちな生活を送る不活動な人たちが段階的に身体活動を増やしていくことに対して最も効果的なメッセージです。

日本の「+10」の勧告は、主に次の二つの補完的なレポートの結果に基づいています：

第一に、身体活動と死亡・疾患リスクとの間の用量応答関係のメタ解析結果です。データは、身体活動量といくつかの非伝染性疾患の死亡や相対危険度（RR）との関係を調べた26のコホート研究から抽出されました。1メッツ・時／週すなわち1日あたり2-3分のMVPAの増加は、RRの0.8％の減少をもたらすことを見出しました。第二に、日本の2010年の国民健康・栄養調査（NHNS）における7876人から収集したデータは、日本の人口の61％が、一日あたり10分多く身体活動を多く行うことをいとわないことを示しました。これらの結果を総合すると、日本人の60％以上で実行可能な約10分の身体活動の増加は約3.2％のRRの減少（2～3分×4≒10分なので0.8％×4≒3.2％）を可能にし、公衆衛生の観点から有用であることを示唆しています。

ここで紹介した日本の「+10」の経験は、他国の公衆衛生当局を刺激し、各国の集団の特性やニーズに合った効率的かつ実践可能な低用量の身体活動メッセージを考慮することを奨励するでしょう。

Session 5−4

"Add 10 Min. for Your Health": The New Japanese Recommendation for Physical Activity Based on Dose-response Analysis

Motohiko Miyachi, Ph.D.
National Institutes of Biomedical Innovation, Health, and Nutrition

Physical inactivity is now identified as the third leading risk factor of Japanese mortality by non-communicable diseases (NCDs). In Japan, the mean number of steps per day (a parameter for the amount of moderate-to-vigorous PA (MVPA)) has decreased significantly over the past decade, from ～8,000 in 1997 to～7,000 steps a day in 2009. Healthy Japan 21 (2nd edition) is a new initiative launched in 2013 by the Ministry of Health, Labour and Welfare (MHWL) and will run until 2022. Its aim is to reduce the number of deaths of people in the prime of their lives, prolong healthy years of life and improve people's quality of life. To do this, Healthy Japan 21 set a goal as "increase the number of steps taken".

Based on scientific evidence provided by the National Institute of Health and Nutrition (NIHN), the MHWL established and published the Japanese official PA guidelines for health promotion "ActiveGuide" in March 2013. ActiveGuide recommends performing 60 minutes of MVPA per day for Japanese adults. However, it is difficult for the majority of the working population, because modern life and commitments to work and family indeed reduce the time that each individual can allocate to PA.

Most importantly, the main message disseminated to the Japanese population is "+10," representing "add 10 minutes of MVPA per day". To our knowledge, the implementation of such PA recommendations in a governmental health promotion policy is a world first. It allows the involvement of people who have already achieved the targeted 60 minutes per day, and to encourage the most sedentary individuals to become progressively more active, thus targeting sedentary and more active people with the same simple message.

The Japanese "+10" recommendation mainly results from the two following complementary reports: First, the completion of a PA dose–response meta-analysis. Data has been extracted from 26 cohort studies that investigated the relation between the amounts of PA and mortality / the relative risk for some non-communicable diseases. We found that an increment of 1 MET•h/week, which is equivalent to 2 − 3 minutes of MVPA per day, results in a 0.8% reduction of the average RR. Second, in the National Health and Nutrition Survey (NHNS), Japan 2010, the data collected from a sample of 7876 people indicated that 61% of the Japanese population would be willing to perform an additional 10 minutes per day of PA. Taken together, these results provide room for an approximately 3.2% reduction of the RR ($2 - 3$ min $\times$ 4 $\approx$ 10 min, so 0.8% $\times$ 4 $\approx$ 3.2%), which is promising from a public health perspective.

The Japanese "+10" experience presented here may inspire other public health agencies, encouraging them to consider an efficient but engaging low-dose PA message that fits the characteristics and needs of their populations.

"Add 10 min for your health": The new Japanese recommendation for physical activity based on dose-response analysis

National Institute of Health and Nutrition, NIBIOHN
Department of Health Promotion and Exercise
Motohiko Miyachi, Ph.D.

1

Physical activity = exercise + daily life activities

Physical activity

Exercise

Activities aiming at improving physical fitness. They are planned and completed during the leisure time.

Brisk walk, dance, gym, jogging, soccer…

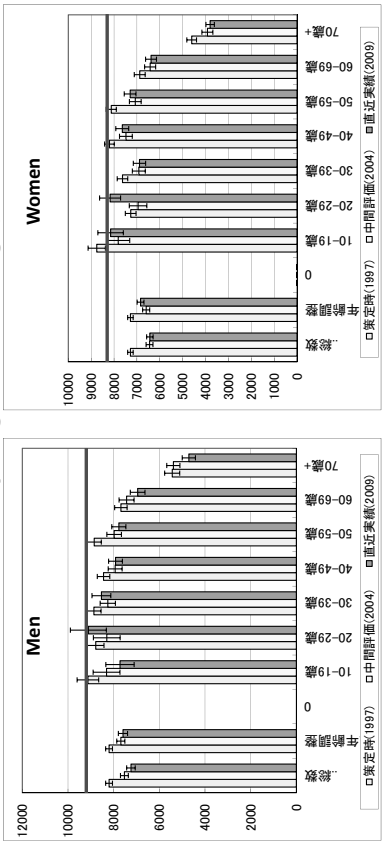
Daily life activities

Related to work and housework

Shopping, walking dog, commuting, cleaning tasks, gardening, playing with children, carrying packages, stairs, car washing…

2

Step count changes from 1997 to 2009 in Japan (relatively to gender and age)



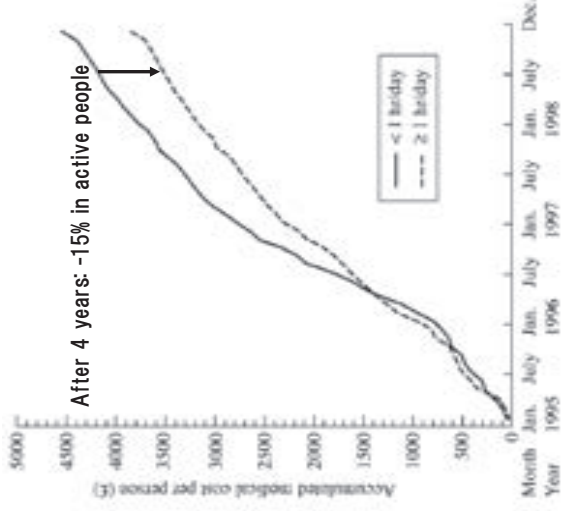
1. All generations together –> 1000 steps reduction
2. Step count = objective marker in amount of physical activity
3. The decrease in step counts is a serious health related concern.

3

Amount of physical activity *vs.* medical expenses

27,421 adults (Japanese)
40-79 years old
4-year follow-up

People who completed more than 1h of walk a day reduce their medical expenses by 15% after 4 years

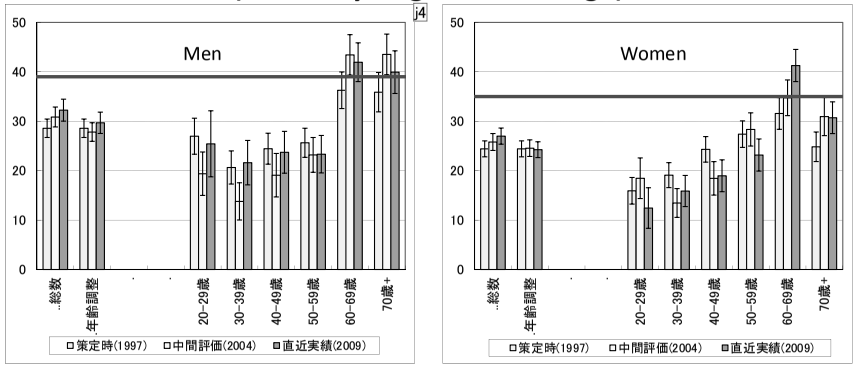


Tsuji et al. Int J Epidemiol. 32(5):809-14, 2003

4

Changes in exercise habits from 1997 to 2009 in Japan (relatively to gender and age)

j4



j3

1. No change in the the age-adjusted proportion of people who keep exercise habit over the past decade.
2. Exercise habit decreases in young and middle-aged women.
3. More exercise habits in the seniors.

5

What are the target values in physical activity? Health Japan 21 national program (2ndchapter)

- 1,000 to 1,500 steps/day increase in physical activity
  20-64 yr-old：♀ 8,500 steps/day, ♂ 9,000 steps/day
  65 yr-old or more：♀ 6,000 steps/day, ♂ 7,000 steps/day
- 10% increase in proportion of people who keep exercise habit
  20-64 yr-old (34%)：♀ 33%, ♂ 36%
  from 65 yr-old (52%)：♀ 48%, ♂ 58%
- Increase of local governments to address community and environmental developments that residents become active
  from 17 prefectures to 47 (all) prefectures

j5

6

To design new recommendations:

1. Need for evidence-based recommendations
2. What about people over 70 years old ?
3. Inclusion of the healthy life expectancy concept (prevention for NCDs, musculo-skeletal impairments, dementia, reduction of ADL)
4. Establishing of recommendations for everyone
5. Easy expression

j10

j11

7

j12

Process of the establishing of reference values

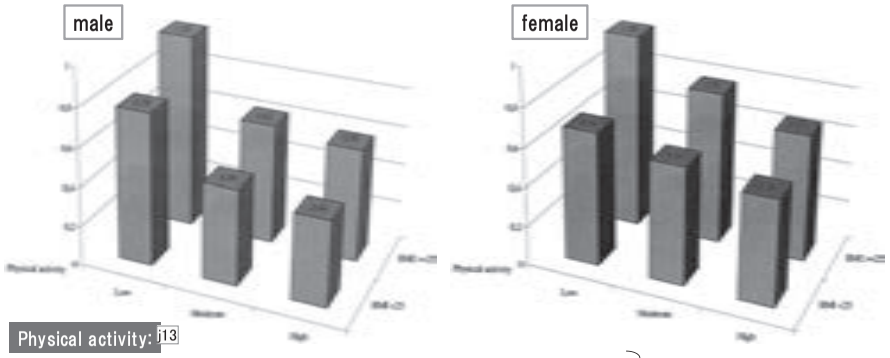
1st review board meeting (RBM) to determine the direction of review	2011/Feb/10
⇓⇓⇓	
Litereture search via PubMed and other databases	2011/Mar/25
⇓⇓⇓	
Reading the title and abstract and selection (Primary review)	2011/Mar/20～Apr/15
⇓⇓⇓	
2nd RBM to determine extraction items	2011/May/11
⇓⇓⇓	
Intensive reading of full papers and final selection (2nd review)	2011/May/15～Aug/25
⇓⇓⇓	
3rd RBM to comfirm the validity of selection and extraction	2011/Sep/1
⇓⇓⇓	
Interim report and discussion at the 66th annual meeting of JSPFSM	2011/Sep/18
⇓⇓⇓	
4th RBM to determine the methods of meta-analysis	2011/Nov/15
⇓⇓⇓	
Data reduction and statistical analysis	2011/Nov/16～2012/Mar/14
⇓⇓⇓	
5th RBM to discuss about the reference values	2012/Mar/15
⇓⇓⇓	
Writing the draft of the reference	2012/Apr～

8

Relationship of physical activity and body mass index to the risk of hypertension: a prospective study in Finland

Gang Hu, Noél C. Barengo, Jaakko Tuomilehto, Timo A. Lakka, Aulikki Nissinen, Pekka Jousilahti
(Hypertension. 2004; 43; 25-30)

♀ : N=9139, ♂: N=8302, 11-year follow-up



Physical activity: j13

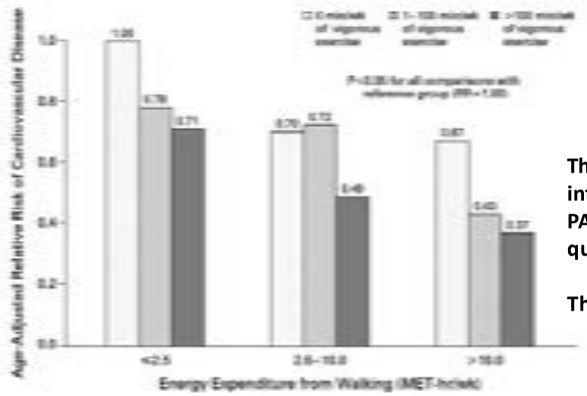
Working PA: light , moderate or active
Commuting PA: walking or bicycling < 30 min, walking or cycling ≧30 min
Leisure-time PA: low, moderate or high

After the sum, and divided into three categories

9

Walking compared with vigorous exercise for the prevention of cardiovascular events in women
Joann E. Manson et al.
(New Engl. J. Med. 2002; 347; 716-725)

N=73743 (50-79 year-old, women only)
average follow-up time 5.9 years



j14

The duration, frequency, and intensity of walking and other PA was measured by questionnaire
↓
The calculated METs・h/week

10

Systematic review for the establishment of references values

Investigated periods	Outcomes	Exposure factors
2005/4 – 2011/3	Deaths, non-communicable diseases, need for care (reduction of ADL), musculo-skeletal impairments, dementia	Physical activity, exercise, fitness (cardiorespiratory fitness, muscular strength, or others)
Before 2005/4	Need for care (reduction of ADL), musculo-skeletal impairments, dementia	

j15

For deaths and CVD results from the 2006 project were also used

j16

	Number of paper	Inclusion rate
Initial literature search	6533	—
1st review	844	12.9%
2nd review	341	40.4%
3rd review and data extraction	205	60.1%

2011: 205 papers included in the systematic review
2006 + 2011: 267 papers finally included in the systematic review

11

Japanese research for establishing physical activity references 2013

j17

Physical activity: 11 articles reviewed (meta-analysis: 3)
Exercises: 5 articles reviewed (meta-analysis: 2)
Cardiorespiratory fitness: 5 articles reviewed (meta-analysis: 4)
Total: 21 articles

- **Noda H et al. JACC study. J Am Coll Cardiol. 2005; 46(9):1761-7.**
- **Lee KJ et al. JPHC study. Cancer Causes Control. 2007; 18(2):199-209.**
- **Suzuki S. et al. JACC study. Cancer Epidemiol Biomarkers Prev. 2008;17(12):3396-401.** j18
- **Takahashi H. et al. Miyagi Cohort (Ohsaki) study. Eur J Cancer Prev. 2007 Oct;16(5):403-8.**
- **Sawada S. et al. Tokyo Gus Study. Diabetes Care. 2010 Jun;33(6):1353-7**

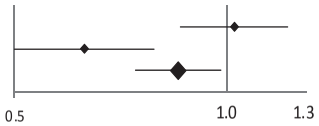
12

Meta-analysis between amount of PA and RR for death or diseases (by Japanese studies only)

3 papers→6 outcomes→11 quantile

G1 (inactive): n=6, 6.2 METs•h/wk

Group	n	PA (METs•h/d)	RR	95%CI	
G2	5	18.9 (16.6-21.2)	1.026	0.861	1.221
G3	6	27.2 (23.6-30.9)	0.629	0.501	0.788
Total	11	24.6 (21.1-28.1)	0.854	0.744	0.981



21-24METs·h/wk was boundary between G2 (moderate active) and G3 (vigorous active)

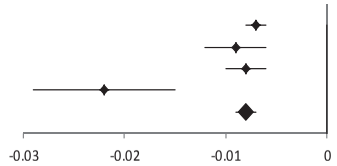
Based on studies of Japanese, the amount of physical activity of 23 METs•h/wk or more was required for health promotion.
↓
23 METs•h/wk is corresponds to 60 min/d, if intensity of physical activity is 3-4 METs.

13

Meta-analysis for associations between PA of 1METs•h/wk and RR of death or diseases

There is dose-response relationship between PA and RR of death and diseases.

Outcomes	n	Risk reduction	SD
Death	11	-0.007	0.001
CVD	5	-0.009	0.003
Cancer	15	-0.008	0.002
JMI, Dementia	5	-0.022	0.007
	36	-0.008	0.001



Increase in 1METs•h/wk of physical activity corresponds to a decrease in RR of 0.8%.

+

The proportion of population who can move the body additional 10 min/day was 61%.
(National Health and Nutrition Survey)

⇩

Let's move more than 10 minutes every day now (+10).

14

Flow of review meetings for establishment of a new PA references and guidelines

Study group report writing for the reference revision, published in the Japanese journal	2012/Apr～May
⇓⇓⇓	
Pre-award meeting for the official meeting with the Health Promotion Division of MHLW	2012/Oct/16
⇓⇓⇓	
1st official meeting on revision of PA references and guidelines (DIscussion about the direction of the revision)	2012/Nov/7
⇓⇓⇓	
2nd official meeting on revision of PA references and guidelines (DIscussion about the reference values)	2012/Nov/27
⇓⇓⇓	
3rd official meeting on revision of PA references and guidelines (Approval of drafts of PA references and guidelines)	2012/Dec/26
⇓⇓⇓	
Announce the final version of revised PA references and guidelines	2013/Mar/18

15

Physical Activity References for Japanese 2013 (PARJ2013)

Young and middle-aged: 18-year-old to 64 years old

- 23 METs•h/wk of moderate to vigorous physical activity at 3 METs or more
  => Be active 60 min every day! The more vigorous, the better.
- 4 METs•h/wk of moderate to vigorous exercise at 3 METs or more
  => 60 min/wk of exercise such as lightly sweat

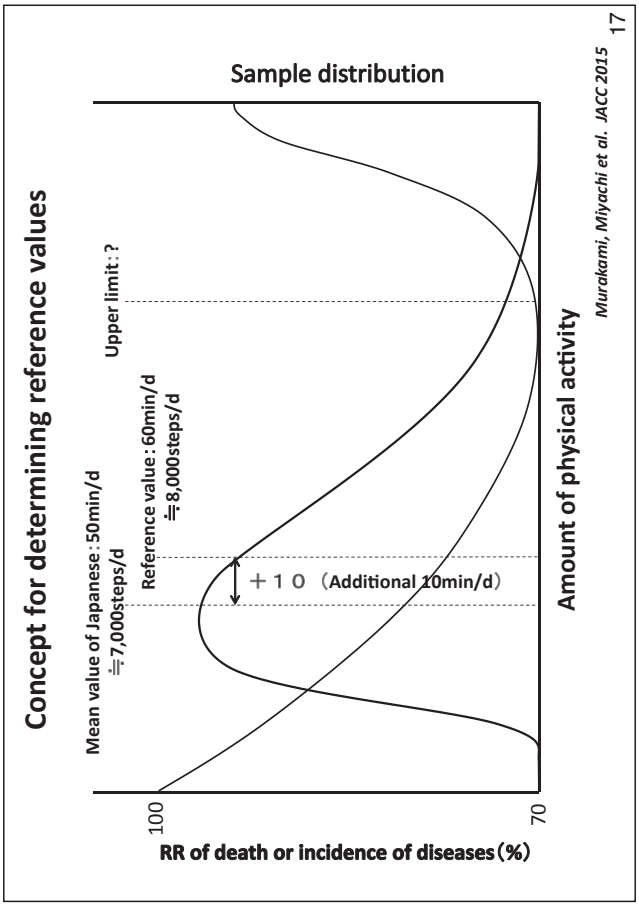
Elderly people over the age of 65

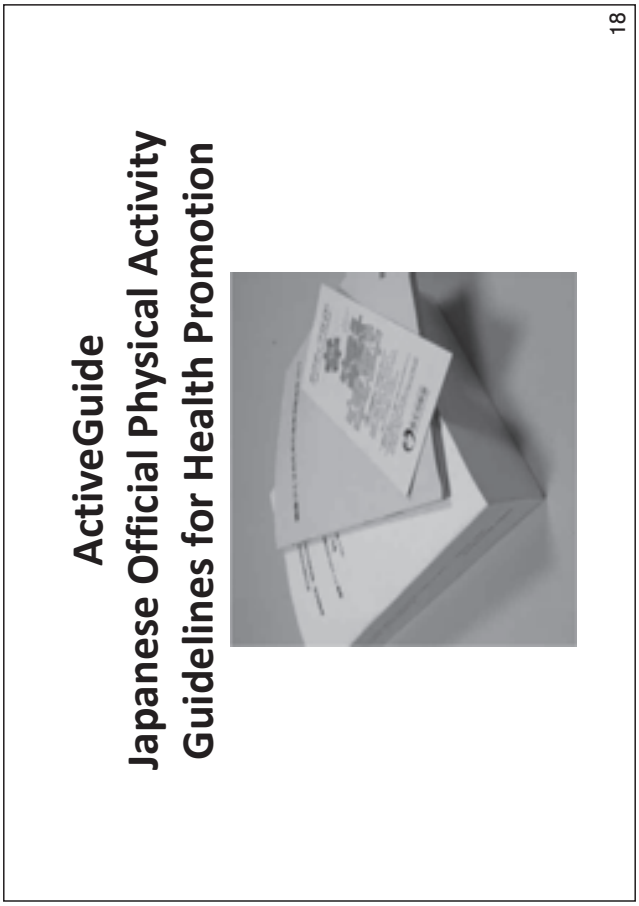
- 10 METs•h/wk of physical activity regardless of intensity
  => Move 40 min every day! Do not keep your body inactive.

All generations

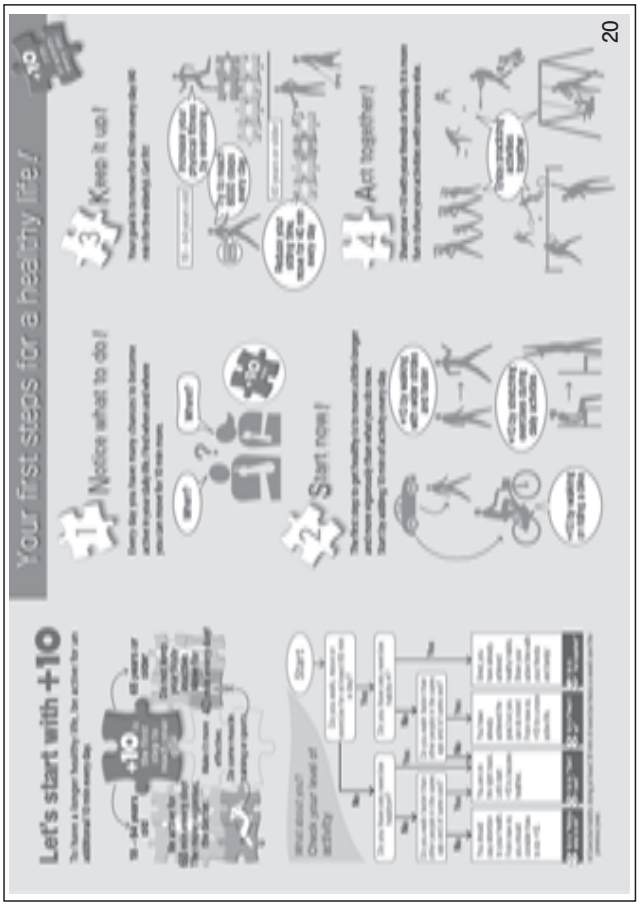
- Based on the dose-response relationship, to increase even a little amount of physical activity
  => Be active for an additional 10 min every day.

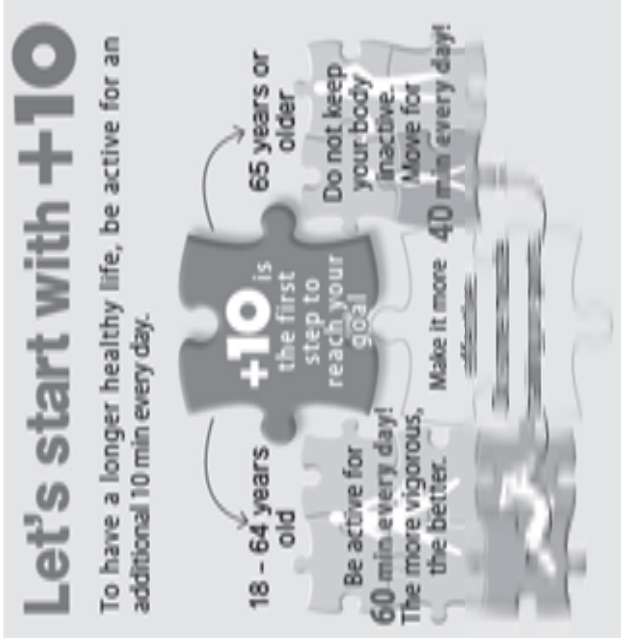
16


Concept for determining reference values
Mean value of Japanese : 50min/d
≒7,000steps/d
Upper limit : ?
Reference value : 60min/d
≒8,000steps/d
＋１０（Additional 10min/d）
Sample distribution
100
70
RR of death or incidence of diseases（%）
Amount of physical activity
Murakami, Miyachi et al. JACC 2015
17

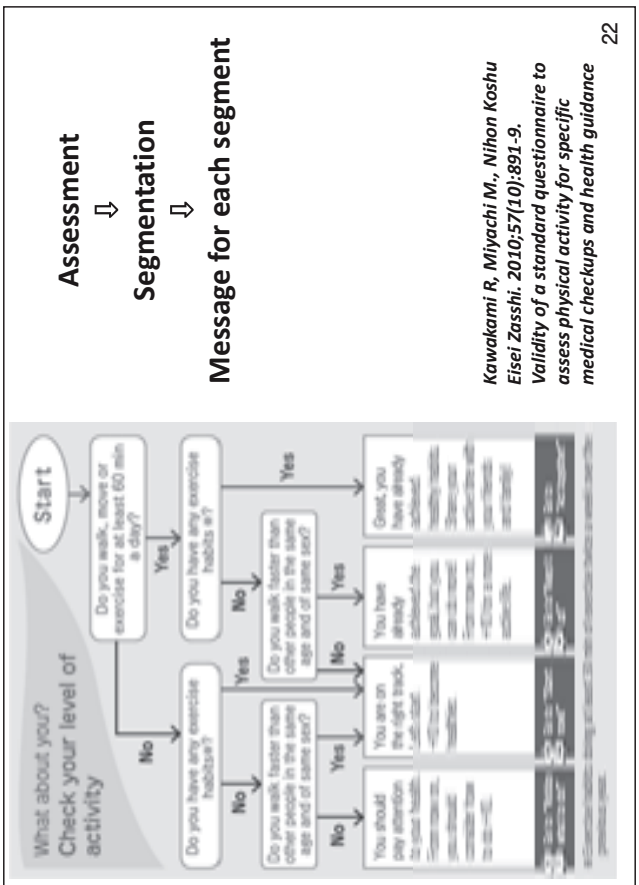

ActiveGuide
Japanese Official Physical Activity
Guidelines for Health Promotion
18

ActiveGuide
19


Let's start with +10
Your first steps for a healthy life!
1 Notice what to do!
2 Start now!
3 Keep it up!
4 Act together!
20



21



Assessment
⇩
Segmentation
⇩
Message for each segment

Kawakami R, Miyachi M., Nihon Koshu Eisei Zasshi. 2010;57(10):891-9. Validity of a standard questionnaire to assess physical activity for specific medical checkups and health guidance

22

1. Notice what to do!
⇩
2. Start now!
⇩
3. Keep it up!
⇩
4. Act together!

Use of the concept of behavior trans-theoretical model and social capital

23

Environment and connection with others affect physical activity and exercise habits.
Inoue S et al. J Epidemiol. 2011;21(6):481-90.
⇓
Increasing awareness about the environment and connection with others are more important.
Harada K et al. J Aging Phys Act. 2011;19(3):262-72.
⇓
Let's think about area of residence, workplace, and the connection with colleagues, family and friends.

24

Internationalization of Activeguide

Japanese

English

Malay

25

謝辞 Acknowledgment

厚生労働省「健康づくりのための運動基準・運動指針の改定ならびに普及・啓発に関する研究班」

基準策定WG
田畑泉（立命館大学）、宮武伸行（香川大学）、小熊祐子（慶應義塾大学）、澤田亨（東京ガス）、種田行男（中京大学）

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栄研メンバー
田中茂穂、高田和子、川上諒子、田中憲子、村上晴香

厚生労働省「運動基準・運動指針改定に関する検討会」

戸山芳昭※、鎌形喜代実、下光輝一、鈴木志保子、鈴木隆雄、須藤美智子、田中喜代次、田畑泉、内藤義彦、福永哲夫、藤川眞理子、道長麻里、宮地元彦

26

ポスタープレゼンテーション

要旨

POSTER PRESENTATION LIST

	名前	表題
P-1	Mami Takahashi, Takashi Matsumoto	Effectiveness of the Steaming Cake Containing *Monascus* koji in the Stroke-prone Spontaneously Hypertensive Rats (SHRSP/Izm)
P-2	Saiko Shirakawa, Hatsuho Zeniya, Takako Ikeda, Naoya Hayashi, Kyoko Kodani, Kazuhiko Kawabata, Yukiko Nakanishi	Relationship between Drive for Thinness and Physical Fitness of Female University Students Using the Structural Analysis
P-3	Takako Ikeda, Yukiko Nakanishi, Mikiko Kurose, Hiroko Nakamura, Nori Karasawa, Asako Kojima, Hatsuho Zeniya, Saiko Shirakawa, Shuichi Kimura	The Surface Analysis by Flow Cytometry on the Balance of Calcium and Magnesium Intakes
P-4	Te-Hua Liu Tsung-Yu Tsai,	Effects of *Latcobacillus plantarum* Strain TWK10-Fermented Soymilk on the Deoxycorticosterone Acetate-salt Induced Hypertension and Associated Dementia in Rats
P-5	Shizuko Satoh-Kuriwada, Misako Kawai, Noriaki Shoji, Hisayuki Uneyama, Takashi Sasano	Risk of Malnutrition in the Elderly with Umami Taste Sensitivity Loss Revealed by the Newly Developed Umami Taste Sensitivity Test
P-6	Tamami Iwamoto, Andrea Wakita, Hideki Matsumoto, Hisayuki Uneyama, Shigeru Yamamoto	Health Benefit of Umami Taste Substance, Monosodium Glutamate (MSG) for Sodium Reduction
P-7	Yuki Yamada, Tomohiro Yano,, Masako Ota	Consideration of Vitamin C Effects in Prevention and Treatment of Cancers
P-8	Michiko Saito, Mami Matsuoka, Masako Ota, Anna Oue	Relationship between Life Habit and Venous Function in Young Humans
P-9	Nobuya Shiozawa, Chiaki Sato, Masako Ota, Tomohiro Yano	A Role of Tocotrienol-Rich Fraction as a Potential Anticancer Agent in Prostate Cancer
P-10	Kakeru Kono, Wakana Yamada, Masako Ota, Tomohiro Yano	Investigating the Possibility of Tocotrienol-Rich Fraction in the Prevention of Deterioration of Bone Quality – A Preliminary Study
P-11	Asuka Uchida, Masako Ota, Tomohiro Yano	Potential for Boysenberry as Functional Food Ingredient

	名前	表題
P-12	Koh Okuno, Takako Ikeda, Yukiko Nakanishi	Effects of Maternal Magnesium Deficiency on the Blood Profiles of Weaning Rats
P-13	Tomoyuki Okamoto, Yasuyuki Sakata, Kazutaka Oshio, Hirohiko Nakamura, Hiroshi Iwamoto, Kazuyoshi Namba, Yasuhiro Takeda, Fumiaki Yoshizawa	The Effects of Long-term Shiikuwasha Extract Supplementation on Age-related Loss of Skeletal Muscle Mass
P-14	Yasuaki Wada, Bo Lönnerdal	Effects of Industrial Heating Processes of Milk-based Enteral Formulas on Site-specific Protein Modifications and Their Relationship to in vitro and in vivo Protein Digestibility
P-15	Hsiao-Yin Lei, Yuko Ishida, Aiko Ono-Ohmachi, Yoshikazu Morita, Ken Kato	Milk Basic Protein（MBP）Increases Bone Mineral Density and Improves Bone Metabolism in Human Study
P-16	Tatsuya Arai, Satoshi Higurashi, Yuko Haruta, Hiroshi Urazono, Toshiya Kobayashi, Yukio Kadooka	Improvement in Skin Conditions by Oral Supplementation of a Sphingomyelin-containing Milk Phospholipids Concentrate in a Double-blind, Placebo-controlled, Randomized Trial
P-17	Keishi Kameyama, Takayoshi Fujii, Sachiko Nishikawa, Takayuki Kajiura, Eiji Nakamura, Yoshihiro Usuda	Intestinal Colonization by a *Lachnospiraceae* Bacterium Contributes to the Development of Diabetes in Obese Mice
P-18	Kyoko Miura, Hiroyuki Kato, Sayako Nakano, Katsuya Suzuki	Leucine Enriched Amino Acid Mixture Protects against Muscle Damage Induced by Resistance Training
P-19	Takayuki Tanaka, Yoko Kageyama, Akira Imaizumi, Hiroko Jinzu, Maiko Mori	Plasma Free Amino Acid Profile for Evaluating Nutritional Status and Various Risks Associated with Lifestyle
P-20	Toshi Kinouchi, Megumu Igawa, Yumi Kato	Later Taste Preferences Modified by Flavor Experience in Infancy in Rats
P-21	M. Takeshita, M. Hibi, N. Osaki, H. Hashimoto, T. Yoneshiro, M. Saito, Y. Katsuragi	Daily Consumption of Catechin-rich Beverage Affects Energy Metabolism and Metabolic Syndrome in Humans
P-22	Hideto Takase, Mitsuhiro Katashima	Dietary Habits and Nutrition Balance Relating to Visceral Fat Accumulation in Japanese. A Cross-sectional Population Study.

P-1

Effectiveness of the Steaming Cake Containing *Monascus* koji in the Stroke-prone Spontaneously Hypertensive Rats (SHRSP/Izm)

Mami Takahashi[1], Takashi Matsumoto[2]
[1] Department of Food Science and Nutrition, Showa Women's University, Tokyo, Japan
[2] Graduate School of Life Sciences, Showa Women's University, Tokyo, Japan

Background and Objective

Monascus koji was gradually regarded as the functional food because the monacolin K and γ-aminobutyric acid were found. In the EU, *Monascus* koji is offered as a food supplement for lowing the blood cholesterol level. It is well recognized that *Monascus* koji would prevent metabolic diseases. Monacolin K, an inhibitor for cholesterol synthesis, is the secondary metabolites of the *Monascus* species. However, the effective utilization of steaming cake containing *Monascus* koji *in vivo* was unknown in terms of its application to food. The stroke-prone spontaneously hypertensive rats (SHRSP/Izm) is a genetic model for hypertension and hypertension-related disorders such as cerebral stroke and renal failure. The purpose of this study is to evaluate the application of steaming cake containing *Monascus* koji, and their respective effectiveness.

Methods

SHRSP/Izm were provided by Japan SLC, Inc. (Sizuoka, Japan). Male rats 6 weeks of age were used in all experiments. The breeding room was kept at a controlled temperature (23±3 ℃) and a relative humidity of 55±10%, 12 h light/ dark cycle. Free access to food and water and enough space to exercise were provided for all animals. The steaming cake containing *Monascus* koji were prepared by steaming for 100 ℃ and 30 minutes.

Results

The body weights of SHRSP/Izm continued to increased gradually to 5weeks. The increased body weights by feed intake in the steaming cake containing *Monascus* koji groups was lower than that in the control groups. However, the intake did not have the significant difference between the steaming cake containing *Monascus* koji and the control groups. This studies demonstrated that the steaming cake containing *Monascus* koji may have great benefit for development of foods with functionality.

P-2

Relationship between Drive for Thinness and Physical Fitness of Female University Students Using the Structural Analysis

Saiko Shirakawa[1], Hatsuho Zeniya[1], Takako Ikeda[1], Naoya Hayashi[2], Kyoko Kodani[3], Kazuhiko Kawabata[2], Yukiko Nakanishi[4]

[1] Department of Live Sciences, Showa Women's University
[2] Faculty of Mastery for service, Kwansei Gakuin Univeristy
[3] Faculty of Literature, Tezukayama Gakuin University
[4] Department of Health and Nutrition, University of Human Arts and Sciences

【Objective】 "Health" is one of the most serious concern in Japan. Especially, extension of healthy life expectancy has become a major national challenge. In the previous studies, we have examined the association among Physical fitness, Body mass index, and Body images in female university students. Recently, we have focused on the Body images and Thinness. The present study aimed to find the factors which associated to Drive of Weight and Height (Actual value –Ideal value) using the structural analysis of Physical Fitness, Body Mass Index, and Desired Body Image in female university students.

【Methods】 Female Students in the classes of Practices of Sports Sciences, Health Sciences, and Physical Education Methods examined the Physical Fitness Test of Japanese Ministry of Education and Science and Anthropometric measurements in K University. Also, Questionnaire on Ideal body images was applied. Data were collected twice at December, 2013 and April, 2014 and obtained from 359 participants. SPSS ver21, Amos ver21 was used for the structural analysis.

【Results and Discussion】 Factor analysis measured items were analyzed to find the structure models. There is no association between Physical Fitness and Drive for Thinness. On the other hands, there is a significant association between Ideal Weight and Actual Height, Ideal Weight and Actual Weight, and Ideal Weight and Ideal Height. To analyze the relationship between Physical Fitness and Body, covariance structure analysis was conducted. Body fat percentages was suggested as a factor on the relationships among the measured items.

P-3

The Surface Analysis by Flow Cytometry on the Balance of Calcium and Magnesium Intakes

Takako Ikeda[1)] , Yukiko Nakanishi[2)] , Mikiko Kurose[3)] , Hiroko Nakamura[3)] ,
Nori Karasawa[4)] , Asako Kojima[2)] , Hatsuho Zeniya[1)] , Saiko Shirakawa[1)] , Shuichi Kimura[3)]
[1)] Department of Live Sciences, Showa Women's University
[2)] Department of Health and Nutrition, University of Human Arts and Sciences
[3)] Research Institute for Nutrition and Aging,
[4)] Research and Development Center, Nippon Meat Packers, Inc.

【Objective】 In the United States in 2010, it has been also reported that inadequate intake of magnesium is approximately 60% among adult population, associating with increasing of obesity, arteriosclerosis, hypertension, osteoporosis, diabetes, and cancer. In the present study, the effects of balance of calcium (Ca) and Mg intakes on the immune function among in mice were tested by the surface analysis of immunocompetent cells using flow cytometry.

【Methods】 Four-week-old male ICR mice were divided into 4 groups (Control group, Mg deficient group (MgD), high Ca group (HCa), MgD+HCa group (MgD+HCa) with 6 animals in each group fed the basal diet (AIN-93G, using a mixture of minerals, including magnesium oxide), or the Mg deficient diet or the high Ca diet including twice Ca compared to AIN-93G for 3 weeks. Food intake and body weight were measured daily and after the autopsy, blood, thymus and spleen were collected and weighed. Blood glucose and Mg concentrations were measured. In addition, the blood, spleen and thymus were applied to a surface analysis by flow cytometry.

【Results】 Serum Mg concentration which is an indicator of Mg status of the body, showed significantly lower and was apparently confirmed to be Mg deficient state among MgD, and MgD+HCa groups compared to the control group. The thymus and spleen weight in MgD group were significantly higher than in control group. NK cells in thymus of MgD+HCa mice showed significantly higher than in MgD mice. T cells in serum of MgD+HCa mice were significantly higher than control. B cells in serum of MgD and MgD+HCa mice were significantly lower than control. Also, T cells and helper-T cells in serum of HCa mice were significantly lower than control. B cells in serum of HCa mice was tend to be higher than control, MgD, groups, respectively.

【Discussion】 It was reported that the Mg deficiency lowered the resistance to infection increasing lymphocytes and lymphoid tissues. There is no contradiction among our results showed that NK cells, which are lymphocytes for biological defense has increased by Mg deficiency or diabetes. Also, when is Mg deficiency, by increasing the percentage basis T cells than B cells, and is a high Ca state, by increasing the percentage basis B cells than T cells, that forms the body's defense system by limited immunocompetent cells was suggested.

P-4

Effects of *Latcobacillus plantarum* Strain TWK10-Fermented Soymilk on the Deoxycorticosterone Acetate-salt Induced Hypertension and Associated Dementia in Rats

Te-Hua Liu and Tsung-Yu Tsai*
Department of Food Science, Fu Jen Catholic University, Taipei, Taiwan

Oxidative stress due to the excessive production of reactive oxygen species is the major reason for neuronal cell degeneration observed in neurodegenerative diseases, such as Alzheimer's disease (AD) and vascular dementia (VaD). Hypertension has been found to increase the incidence of VaD. The objective of this study was to investigate the effect of *Lactobacillus plantarum* TWK10 (TWK10)-fermented soy milk on VaD induced by deoxycorticosterone acetate (DOCA)-salt hypertension in rats. The results revealed that water and ethanol extracts from TWK10-fermented soy milk were orally administrated and significantly decreased the blood pressure of DOCA-salt hypertension rats by 11.24% and 14.51%, respectively ($p < 0.05$). In addition, oral administration of the mixture of uracil and glycerol which were identified as bioactive ingredients in TWK10-fermented soy milk also can significantly decreased the blood pressure of DOCA-salt hypertension rats by 14.81-19.43%. Extracts and combination of uracil and glycerol decreased escape latency and total swimming distance in reference and working memory task and significantly increased target crossing in the probe test to 2.83 ± 1.33, 3.33 ± 1.03 and 2.67±1.37-3.17±1.17 times, respectively ($p < 0.05$). In conclusion, TWK10-fermented soy milk extracts and a mixture of uracil and glycerol, can act as neuroprotective agents and improve the learning and memory ability of rats with VaD induced by DOCA-salt hypertension.

Keywords: learning and memory ability, deoxycorticosterone acetate (DOCA)-salt hypertension, glycerol, *Latcobacillus plantarum* TWK10-fermented soy milk, uracil and glycerol

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P-5

Risk of Malnutrition in the Elderly with Umami Taste Sensitivity Loss Revealed by the Newly Developed Umami Taste Sensitivity Test

Shizuko SATOH-KURIWADA [1], Misako KAWAI [2], Noriaki SHOJI [1],
Hisayuki UNEYAMA [2], Takashi SASANO [1]
[1] Div. Oral Diagnosis, Dept. Oral Med. Surgery, Tohoku Univ. Grad. Sch. Dentistry
[2] Institute for Innovation, Ajinomoto Co., Inc.

Objectives: Patients with taste disorder often complain of persistent impairment of palatability related to umami-taste, although the other four basic taste sensations such as sweet, salty, sour, and bitter, are normal. Since there was no clinical test for umami-taste sensitivity, it could not be confirmed the decline of umami-taste sensation in the patient. Hence, we have developed a clinical umami-taste sensitivity test to assess the patient with umami taste disorder.

Methods: As for a new umami-taste test, we employed the filter paper disc method (FPD), in order to possibly compare other four basic taste disorder that became evident for the existing taste tests in Japan (Taste Discs™, Sanwa Chemical Laboratory Inc.). Monosodium glutamate (MSG) was used as an umami solution with six levels of concentration.

Results & Findings: We clinically applied the FPD methods to the patients who visited our clinic complaining of taste disorder. Recognition threshold (RT) of the umami sensation was compared to healthy volunteers with normal RT. In 16 % of patients, RT for only umami-taste was higher than that in healthy volunteers, whereas the other four basic tastes were all within normal levels. Those patients with loss of umami-taste sensitivity were all over 65 years old, and they all complained of appetite loss and weight loss, resulting in poor overall health. After treatment, the RT for umami-taste of the patients returned to normal levels, and their subjective umami-taste sensation were recovered. Concomitantly, they also remarkably regained their appetite and weight. The patients were pleased with regaining of taste sensation and health.

Conclusion: Umami-taste test is useful for the detection of umami-taste disorder, because the decline of umami-taste sensitivity trigger malnutrition particularly in the elderly.

References: S.Satoh-Kuriwada, N.Shoji, T. Sasano, et al., "Development of an umami taste sensitivity test and its clinical use" *Pros One*, www.plosone org, vol. 9: e95177, 2014.
T. Sasano, S.Satoh-Kuriwada, N.Shoji, et al., "Important role of umami taste sensitivity in oral and overall health," *Current Pharmaceutical Design*, vol. 20, pp. 2750-2754, 2014.

P-6

Health Benefit of Umami Taste Substance, Monosodium Glutamate (MSG) for Sodium Reduction

Tamami Iwamoto[1], Andrea Wakita[2], Hideki Matsumoto[2], Hisayuki Uneyama[2], and Shigeru Yamamoto[3]

[1] Department of Food and Nutritional Sciences Jumonji University, Saitama, Japan
[2] Frontier Research Labs, Institute for Innovation, Ajinomoto Co., Inc. Kanagawa, Japan
[3] Asian Nutrition and Food Culture Research Center, Jumonji University, Saitama, Japan

In 2013, Japanese food culture, WASHOKU was approved as the fifth entree UNESCO food cultural heritage and humanity, followed by French, the Mediterranean, Mexican and Turkish cuisines. The perceived essence of Japanese cuisine could be the well sophisticated soup stock (Dashi) enriched in umami taste substances. Many sensory analysis studies have reported that the dried bonito soup stock or the pure umami taste seasoning monosodium glutamate (MSG) is helpful for sodium reduction to increase palatability in low sodium diet. Psychological stress increase is one of major factors to make it difficult in early-phase low-sodium food acceptance.

In this study, we observed effect of MSG supplementation to low-sodium diet on the mood and psychological stress conditions in healthy volunteers. A cross-over randomized, single-blind, placebo-controlled trial study was carried out on 31 Japanese female from Jumonji University, aged 20 to 35 years old. The study had 5 days for baseline (control) and 10 days for interventions (reduced-sodium diet with or without MSG). Profile of mood states (POMS) questionnaire, body mass index, saliva (Chromogranin-A/protein rate) and urine samples were taken. As a result, the stress marker showed that in the control group, the stress increased significantly during the intervention phase but not in the MSG-treated group. When the stress marker was calculated as increased percentage from baseline, the control group was significantly higher than the MSG group during intervention period. However, POMS did not show changes between both groups. These results may indicate that MSG fortification to the low-sodium diet could ameliorate the psychological stress in the early phase of low-sodium diet replacement from the normal diet. This means that the conserved essence of WASHOKU, umami taste might have contributed to the healthy eating related to salty taste food satisfaction. We propose the positive usage of the pure umami taste substance; MSG might be very helpful to improve the acceptance for the low-sodium food with pleasantness and deliciousness.

P-7

Consideration of Vitamin C Effects in Prevention and Treatment of Cancers

Yamada, Yuki[1], Yano, Tomohiro[2], Ota, Masako[2]
[1] Graduate school of Life Sciences, Toyo University,
[2] Faculty of Food Life Science, Toyo University

Aims: This study aimed to consider effects of protective and treatment for cancers by intake and administration of vitamin C.

Methods: Literature database of PubMed was utilized to find relevant randomized controlled trials protective efficacy of vitamin C on cancers in August 2013. Results of collected literatures were used systematic review. Case reports were investigated to treatment effect for cancers by intake of vitamin C.

Results: We evaluated the quality of 347 literatures by Jadad Scale and ABC grade method. Two literatures were conducted systematic review on incidence of total cancer, colorectal cancer and lung cancer. Subjects of two literature consumed 500mg/day vitamin C for averaged to 10 years. Lung cancer incidence significantly increased by intake of vitamin C in one literature (RR:1.84, 95%CI:1.14-2.97).Total cancer incidence increased compared to smoker than non-smoker by intake of vitamin C in two literatures (one literature：smoker [RR:1.08, 95%CI:0.84-1.39] non-smoker [RR:1.04, 95%CI:0.91-1.17] the other literature：smoker [RR:1.19, 95%CI:0.82-1.74] non-smoker [RR:1.23, 95%CI:0.80-1.88]).

It has reported that concomitant administration intravenous vitamin C (60mg vitamin C twice a week) and anticancer drug were useful in treatment of cancers (J. Am. Coll. Nutr, 2003 22:118-123).These treatments decreased hepatic metastasis in Patient with breast cancer and hepatic metastasis. Lung cancer tumor also decreased in lung cancer patients by these treatments.

Conclusions: History of smoker was suggested that served as a confounding factor between intake of vitamin C and lung cancer. Intravenous vitamin C after the onset of cancers was suggested that useful in treatment of cancers by confirmation of decreasing cancer tumor.

P-8

Relationship between Life Habit and Venous Function in Young Humans

Michiko Saito [1], Mami Matsuoka [2], Masako Ota [2], Anna Oue [2]
[1] Graduate School of Life Sciences, Toyo University,
[2] Faculty of Food and Nutritional Sciences, Toyo University

The venous system plays an important role in hemodynamic control because venous vessels have high distensibility and contain 60-70 % of whole blood at resting condition. Ageing and/or the lower physical activity associate with the increased stiffness of veins (Hernandez et al. 2004, Monahan et al. 2001, Young et al. 2006), causing the cardiovascular disease (e.g. hypertension) (Olsen and Lanne 1998, Safar and London 1987). To reduce the risk for cardiovascular disease, it is significant to improve venous function in young, although the factors which effect venous function are not understood well. Thus, based on previous studies (Kawano et al. 2010, Monahan et al. 2001, Yoshioka et al. 1991), the purpose of this study was to reveal our hypothesis that life habit (e.g. fitness levels and dietary habit) might be related to the venous function.

In 58 health young subjects, fitness levels, diet survey and venous function were investigated. As fitness levels, muscle strength (grip strength and back strength), high power (standing broad jump), agility (jumping side to side), muscular endurance (sit-up), flexibility (long seat body anteflexion and bending the upper part of the body), and whole body endurance (multi-stage fitness test) were measured. BDHQ (brief-type self-administered diet history questionnaire) was used to assess diet survey. To investigate the venous function, venous compliance was determined using the first derivation of the cuff pressure-venous volume relation obtained during cuff deflation protocol (Hallwill et al. 1999).

Fitness levels except for flexibility have significant positive interrelation with venous compliance ($P < 0.05$). And, there was significant positive relationship between the retinol and venous compliance ($P < 0.05$). On the other hand, α- and β- carotene and δ- tocopherol have significant negative correlation with venous compliance ($P < 0.05$). These results suggest that various fitness levels and the intake of vitamins might be concerned in venous function in healthy young subject in our study.

P-9

A Role of Tocotrienol-Rich Fraction as a Potential Anticancer Agent in Prostate Cancer

Nobuya Shiozawa[1], Chiaki Sato[2], Masako Ota[1) 2)], Tomohiro Yano[1) 2)]
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【Objective】

In Japan of super-aged society, the number of deaths of cancer is increasing. Especially, the prostate cancer patients increased rapidly in Japanese men. Therefore, we focused on prostate cancer prevention. Prostate cancer categorized as androgen-dependent and androgen-independent cells with clinical diagnosis. They initially respond to androgen ablation therapy by undergoing programmed cell death. However, patients with advanced prostate cancer develop hormone refractory disease that results in a fatal effect due to the growth of androgen-independent prostate cancer cell. Therefore, an alternative methodology to enhance the apoptotic response is necessary to develop new therapeutic drugs for the treatment of prostate cancer. We investigated Tocotrienol-Rich Fraction (TRF) extracted from annatto could act as a potential anti-cancer agent in prostate cancer cells.

【Material & Method】

We used androgen-dependent prostate cancer cell (LNCaP cell) and androgen-independent prostate cancer cell (PC3 cell). LNCaP and PC3 cells were treated with TRF for 12h, and subsequently extracted mRNA from prostate cancer cells. The expression of PSA mRNA was determined by RT-real time-PCR. Also, we checked PSA mRNA expression in prostate cancer cells by a Src inhibitor (PP2) and/or a Stat3 inhibitor (AG490).

【Result】

TRF significantly suppressed the growth of LNCaP cell as well as PC3 cell. TRF induced the simultaneous inactivation of Src and Stat3 in both types of prostate cancer cells. Furthermore, co-treatment of a PP2 and AG490 synergistically suppressed growth of the prostate cancer cells.

【Conclusion】

TRF suppresses LNCaP and PC3 cells growth via simultaneous inactivation of Src and Stat3. These results suggest that TRF is effective in prostate cancer prevention.

P-10

Investigating the Possibility of Tocotrienol-Rich Fraction in the Prevention of Deterioration of Bone Quality – A Preliminary Study

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Background/Aims

In Japan of a super-aged society, the prevention of osteoporotic fracture is important for extend of health expectancy. Not only low bone mineral density (BMD), but low bone quality is also an important risk factor for osteoporotic fracture. Bone quality is determined by collagen cross-links which are regulated by enzyme lysyl oxidase (LOX) in osteoblastic cells. LOX expression is known to be inhibited by activation of Janus kinase (JAK) signaling located in the upstream of LOX. But, JAK signaling is reported to be inhibited by Tocotrienol-Rich Fraction (TRF), a member of the vitamin E family. However, the effect of TRF on the LOX expression in osteoblastic cells has not been understood. Here, we have investigated the relation between TRF and LOX expression.

Methods

A human osteosarcoma cell line (MG-63) was cultured in medium containing 5 μg/ml or 10 μg/ml TRF. After 12 h and 24 h of treatment TRF, we analyzed LOX mRNA expression by RT-real time PCR. mRNA expression levels were compared TRF-treated group and untreated control group, and statistical analysis were performed by using Dunnett's test.

Results

For TRF of 10 μg/ml, LOX mRNA levels increased for both the treatment duration of 12 h and 24 h with the respective statistical significance being $p < 0.05$ and $p < 0.01$. However, there were no significant changes after treatments of 5 μg/ ml TRF for both 12 h and 24 h durations.

Conclusions

Our results show that TRF may be effective to prevent the deterioration of collagen cross-linking formation. We are conducting more experiments to elucidate the signal mechanisms of TRF.

P-11

Potential for Boysenberry as Functional Food Ingredient

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Objectives

According to the statistics of Ministry of Health, heart disease is the second leading cause of death in Japan, and antioxidants are known to reduce such risk. The purpose of this study was to select functional food ingredient for prevention of arteriosclerosis by means of antioxidant activity. From the viewpoint of local production for local consumption, I studied the berries especially boysenberry from Gunma with the campus where I belonged to. Boysenberry is said to contain more polyphenols than any other common berries and to be a potential antioxidant with the main polyphenol being ellagic acid.

Materials and methods

In this study, we used boysenberry produced in Gunma and raspberry as a control. Each berry was frozen, dried, triturated and were dissolved in water. The ellagic acid solution, which had the equal level with that involved in the boysenberry, was prepared.We assay the total polyphenol content using the Folin-Denis method and the antioxidant activity using DPPH free radical and ABTS assay by the difference in color with the reagent.

Results

Boysenberry had twice as much polyphenol content and antioxidant activity of catechin equivalence as raspberry. Although the ellagic acid is one of polyphenols and is said to be contained a lot in boysenberry, it accounts for only 15% -20% of antioxidant activity.

Conclusion

Boysenberry contains polyphenols more than other berries and has potent antioxidant activity as a functional food ingredient. Overall, it seems that the ellagic acid does not greatly influence antioxidation.

P-12

Effects of Maternal Magnesium Deficiency on the Blood Profiles of Weaning Rats

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National Health and Nutrition Examination Survey (2013) showed the average value of magnesium intake was 239 mg in Japan. The result was lower than the estimated average amount of the Dietary Reference Intakes for Japanese (4. 5 mg / kg body weight / day) particularly intakes were reported as not enough among reproductive aged women. The present study aimed to examine the effects of maternal magnesium deficiency on the weaning rats.

Female SD rats were divided into 4 groups. 1) Control Group (AIN-93G), 2) MgD Group (1/3 Magnesium against AIN93G), 3) HF Group (35% energy from fat), 4) MgD+HF Group. After 2weeks, serum magnesium level in MgD was significantly lower than it in Control. Then those rats were applied to the mating process. Mother rats were fed with the each diets based on the group for three weeks. One mother rats fed 10 pups during 3 weeks of lactation periods. Mother rats and the weaning rats were collected blood and dissected. There is no significance of serum magnesium level among the meaning rats of 4 groups. Triglyceride concentration in the weaning rats of MgD was significantly lower than other 3 groups. Serum glucose level in the weaning rats of MgD were significantly lower than it in the HF rats, while there was no significance compared to the Control Group. There was no synergistic or additive effect between maternal magnesium deficiency and High Fat on the blood profiles of the weaning rats.

P-13

The Effects of Long-term Shiikuwasha Extract Supplementation on Age-related Loss of Skeletal Muscle Mass

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Objectives: Age-related loss of skeletal muscle mass, referred to as sarcopenia, is a significant problem that has serious implications for quality of life of the elderly in Japan, a super-aged society. It has been reported that the etiology of skeletal muscle atrophy is multifaceted, involving a number of intrinsic and extrinsic factors. Recently, we reported that Shiikuwasha extract (SE) has an inhibitory effect on dexamethasone-induced skeletal muscle atrophy. The aim of this study is to examine whether long-term supplementation with SE has an inhibitory effect on loss of skeletal muscle mass with aging.

Methods: Fischer 344 × Brown Norway F1 rats (28 months old) were divided into 4 groups: REF group (animals sacrificed at the start point), CTL group (animals fed a normal diet), SE 0.2% group (animals fed a diet containing 0.2% SE), and SE 1% group (animals fed a diet containing 1% SE), The animals were fed *ad libitum* for 15 weeks, during which age-related skeletal muscle atrophy progressed. Following this, animals were sacrificed and the skeletal muscles were dissected and weighed. Further, signaling components involved in protein metabolism and the degrees of oxidative stress were examined in the muscles.

Results: In the CTL group, mass of the gastrocnemius (GAST) and soleus muscles decreased significantly when compared to those in the REF group, indicating that age-related skeletal muscle atrophy was induced. The masses of GAST and extensor digitorum longus per body weight were significantly higher in the SE1% group than in the CTL group. Moreover, CuZn SOD activity was significantly higher in the SE1% group than in the CTL group. However, there were no significant differences in thiobarbituric acid reactive substances (TBARS) level and catalase activity in all groups. Furthermore, there was no significant effect on the signaling components involved in protein metabolism.

Discussion: Our data suggest that long-term supplementation with SE attenuates loss of skeletal muscle mass with aging in the elderly rat model, although the mechanism by which SE acts on is unclear. Given that supplementation with SE significantly increased CuZn SOD activity but did not alter signaling components involved in protein metabolism, further studies are needed to determine the mechanism. In conclusion, SE may not only have an inhibitory effect on dexamethasone-induced skeletal muscle atrophy but also age-related skeletal muscle atrophy.

P-14

Effects of Industrial Heating Processes of Milk-based Enteral Formulas on Site-specific Protein Modifications and Their Relationship to in vitro and in vivo Protein Digestibility

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Heat treatments are applied to milk and dairy products to ensure their microbiological safety and shelf lives. Types of heating processes may have different effects on protein modifications, leading to different protein digestibility. In this study, milk-based liquid nutritional formulas (simulating enteral formulas) were subjected to steam injection ultra-high-temperature treatment or in-can sterilization, and the formulas were investigated by proteomic methods and in vitro and in vivo digestion assays. Proteomic analyses revealed that in-can sterilization resulted in higher signals for N^{ϵ}-carboxymethyllysine and dephosphorylation of Ser residues in major milk proteins than in steam-injected formula, reflecting the more severe thermal process of in-can sterilization. In vitro and in vivo digestion assays indicated that steam injection improved protein digestibility, supposedly by denaturation, while the improvement seemed to be overwhelmed by formation of aggregates that showed resistance to digestion in in-can sterilized formula. Adverse effects of heat treatment on protein digestibility are more likely to be manifested in milk-based formulas than in cow's milk. Although the differences might be of limited significance in terms of amino acid bioavailability, these results emphasize the importance of protein quality of raw materials and selection of heating processes. [J Agric Food Chem. 2015; 63(30):6787-98.]

P-15

Milk Basic Protein (MBP) Increases Bone Mineral Density and Improves Bone Metabolism in Human Study

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Introduction

It is well known that cow's milk is useful to keep bones healthy, because it contains high levels of calcium, one of nutrient essentials for bone formation. However, we hypothesized that milk may contain some functional components other than calcium, that affect bone metabolism. In the previous *in vitro* and *in vivo* studies, we have demonstrated that milk basic protein (MBP) promoted bone formation and suppressed bone resorption. In addition, we confirmed the bone-strengthening effect of MBP in human study.

Aim

In this presentation, we explain how MBP affects the bone mineral density and the biochemical markers of bone metabolism in healthy adult volunteers.

Methods

We conducted five human studies, in which MBP was supplemented for adult women, adult men, adult young women, menopausal women, or old women and bone parameter were analyzed. In the adult women study, 33 healthy women were randomly assigned to treatment with either placebo or MBP (40 mg per day) for six months. The bone mineral density of each volunteer was measured at the beginning of the study and after six months of treatment. Serum and urine indices of bone metabolism were measured at 0, 3, and 6 months.

Results

The adult women study showed that an intake of MBP significantly decreased urinary excretion of N-end telopeptides (NTx) of collagen, a marker indicating bone resorption. In addition, the rate of increase in bone mineral density at 6 months was significantly higher in the MBP group as compared to the placebo group. Other studies also showed similar results.

Conclusion

These results suggest that MBP supplementation is effective in bone mineral density, and that this increase of bone mineral density may be mediated by the improvement of bone metabolism. We consider that MBP is a superior component for maintenance of human bone metabolism.

P-16

Improvement in Skin Conditions by Oral Supplementation of a Sphingomyelin-containing Milk Phospholipids Concentrate in a Double-blind, Placebo-controlled, Randomized Trial

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BACKGROUND: Milk fat is known as a convenient source of dietary sphingomyelin (SPM), a physiological effect of which includes elevating ceramide levels in the body, leading to improvement in skin conditions. A more concentrated source of SPM than unprocessed milk fat is the milk fat globule membrane-enriched fractions, which are produced during the manufacture of dairy products and abundant in phospholipids; such a SPM-containing milk phospholipids concentrate (SPM-MPC) is also useful for investigating the benefits of dietary SPM. Here we examined the effect of consuming SPM-MPC on skin conditions in a double-blind, placebo-controlled, randomized trial.

METHODS: A total of 96 healthy subjects aged 20-39 with lower skin hydration levels (less than 55 arbitrary units below the eye region) were randomly assigned to three groups: the high-SPM group supplemented with SPM-MPC at a dose equivalent to 10 mg/day of SPM, the low-SPM group supplemented with SPM-MPC equivalent to 5 mg/day of SPM, and the placebo group fed a vehicle comprised of olive oil and beeswax. During the supplementation for 12 weeks, skin conditions were evaluated at baseline and every 3 weeks: skin hydration, transepidermal water loss, sebum production, skin elasticity and several subjective perceptions of skin such as moisture and smoothness.

RESULTS: Skin hydration at the heel was significantly increased at weeks 9 and 12 in the low-SPM group compared with the placebo group. Skin elasticity (parameter R2) at the region below the eye was significantly increased at week 9 in the high-SPM group versus placebo. Questionnaire-based subjective perceptions of skin conditions were significantly improved in the facial skin moisture at weeks 3 and 12, and in the wrinkle around the eyes at weeks 9 and 12, in the high-SPM group versus placebo.

CONCLUSION: It is suggested that a constant and long-term supplementation of SPM-MPC is capable of improving skin conditions.

P-17

Intestinal Colonization by a *Lachnospiraceae* Bacterium Contributes to the Development of Diabetes in Obese Mice

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Recent findings suggest that gut microbiota is a causative factor in the development of metabolic syndrome such as obesity and type 2 diabetes (T2D). We investigated gut microbiota of fecal samples from two mouse models of obesity and T2D using terminal restriction fragment length polymorphism (T-RFLP) analysis. The aim of the present study was to identify bacteria that may contribute to the development of metabolic dysfunctions. Comparison analysis of the homozygous *db/db* (diabetic) mice with the heterozygous *db/+* (non-diabetic) mice revealed that a specific bacterium (282 bp peak by T-RFLP analysis) was present at significantly higher population in *db/db* mice than *db/+* mice. On the other hand, a few of *ob/ob* (obese) mice show severe hyperglycemia similar to *db/db* mice, whereas most of them generally show normal fasting blood glucose levels. Interestingly, the 282 bp-specific bacterium was also dominantly detected in the hyperglycemic *ob/ob* mice, and the 16S rDNA sequence was completely consistent with the specific bacterium found in *db/db* mice. Therefore, we hypothesized that the specific bacterium influences the development of T2D. We successfully isolated and identified the bacterium belonging to *Lachnospiraceae* (strain ID: AJ110941). The colonization of germ-free *ob/ob* mice by AJ110941 induced significant increases in fasting blood glucose levels as well as liver and mesenteric adipose tissue weights, and decreases in plasma insulin levels and HOMA-β values. In addition, quantitative PCR analysis targeted for the bacterium in fecal samples from human volunteer with or without T2D revealed that the bacterium was detected in 71% of T2D subjects (n=34), and in 38% of healthy subjects (n=34).

These results indicate that AJ110941 should be one of the important causative gut commensal bacteria for the induction of obesity and insulin resistance in mice, and possibly in human. These findings would explore new directions for the diagnosis, treatment and prevention of metabolic syndrome by regulating the bacteria in the gut.

P-18

Leucine Enriched Amino Acid Mixture Protects against Muscle Damage Induced by Resistance Training

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Resistance training (RT) such as eccentric training (ET) improves muscle insulin sensitivity and sarcopenia, and is thought to be beneficial as a basal treatment for patients with T2DM. However, RT often results in muscle damage and muscle pain, leading to difficulty in continuing exercise. Leucine (LEU) is known to promote muscle protein synthesis, and is expected to suppress muscle damage after RT and improve sarcopenia. We established a high-intensity ET model using normal rats, and evaluated the effect of a L-enriched amino acid mixture (LEAA) containing 40% LEU on muscle damage in this model. Male SD rats (8 wks old) were subjected to ET. Dorsiflexion of the distal hind limb was induced by electrical stimulation of the tibialis anterior (TA) muscle (5 mA, 1100 msec pulse at 100 Hz). Lengthening contraction (LC) of TA was induced at an angular velocity of 100 deg/sec from 45° to 135° (10 contractions every 10 sec). Five sets of LC were induced at 60 sec intervals. Transient muscle weakness was observed 1 day after RT, along with an increase of plasma CPK to 3-fold above the level in sedentary rats. Inflammatory cell infiltration into TA was detected from 1 to 3 days after RT. Furthermore, expression of IL-6 and IL-1β was markedly increased in TA. These changes returned to the levels in sedentary rats by 15 days after RT. Oral administration of LEAA (1g/kg) once daily suppressed elevation of CPK and muscle strength recovered to the pre-RT level within 6 days. In addition, the increase of IL-6 and IL-1β expression and inflammatory cell infiltration into TA were suppressed by LEAA. Furthermore, the wet weight of TA and its glycogen content at 7 days after RT were increased by LEAA. These findings indicate that LEAA provides protection against muscle damage due to RT by suppressing the inflammatory response and improving glucose metabolism in skeletal muscle, and also enhances the recovery of muscle strength. Therefore, LEAA supplementation may be beneficial for continuing exercise training in patients with T2DM.

P-19

Plasma Free Amino Acid Profile for Evaluating Nutritional Status and Various Risks Associated with Lifestyle

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Keywords: plasma free amino acid, nutritional status, lifestyle-related diseases, multivariate analysis

Abstract

Background: Recently with the advance of 'omics' technology is being generated with an increasing speed. In clinical settings, biomarkers developed from metabolomics are becoming one of the most important criteria that can be quantitatively measured and evaluated as indicators of normal or pathological states. Although many biomarkers exist and are being developed, currently there are only a few screening methods that have both high sensitivity and the ability for early detection. In addition, some studies show the potential of treating plasma free amino acid (PFAA) profile as a metabolomics subset. However, to date, few studies of PFAA profile have been performed in a large population to evaluate the risk of lifestyle. In this study, we aimed to find a quantitative model for evaluating nutritional status and various risks associated with lifestyle, based on PFAA profiles.

Methods: We quantified PFAA levels in 1,890 subjects to evaluate a condition where levels of essential amino acids were found to be lower than minus two standard deviations from the average value of a healthy person, in 865 subjects who had undergone evaluations of their visceral fat area (VFA) by CT scan, in 1,160 subjects who had each been subjected to an oral glucose tolerance test to determine their 2-h post-challenge insulin levels (Ins120min), and in 2,000 subjects who had undergone abdominal ultrasound scan in a comprehensive health screening for discriminating the fatty liver disease (FLD). For the above subjects whose plasma amino acid concentrations were measured by high-performance liquid chromatography–electrospray ionization mass spectrometry followed by precolumn derivatization, we performed the multivariate analysis to model the relationships between the PFAA profiles with the VFA or Ins120 min values or fatty liver occurrence data (PFAA model).

Results and Discussion: Lower PFAAs showed significant association with malfunction of serological tests for anemia (e.g. hematocrit, hemoglobin), protein intake (e.g. albumin, total protein), and immunity (e.g. lymphocyte). The correlation coefficients of the obtained PFAA models against VFA or Ins120 min were higher than single PFAA level. The area under the receiver operating characteristic curve for the PFAA model of FLD was 0.83, which was higher than those of other existing liver function-associated markers (e.g. AST, ALT, γ-GTP). PFAA profiles confer independent and differing contributions to increasing the nutritional and lifestyle-related disease risks in addition to the currently known factors in a general Japanese population, suggesting the usefulness as versatile markers for health monitoring.

P-20

Later Taste Preferences Modified by Flavor Experience in Infancy in Rats

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Some kinds of milk formulas such as protein hydrolysate formulas have particular flavor of amino acids. We examined the impact of feeding of such formulas in infancy on the taste preferences after weaning using our rat artificial rearing systems.

Rats were fed commercial standard infant formula, whey hydrolysate formula, or casein hydrolysate formula with a formula-feeding bottle for rats during the daytime and fed milk formula for rats through a intragastric catheter during the night-time from 9 to 20 days of age. Other rats were raised on the casein hydrolysate formula by means of a formula-feeding device for rats from10 to 20 days of age. All rats were fed a standard solid diet after 20 days of age. Taste preferences of each rat to leucine and glutamate were evaluated by two-bottle preference tests at 7 and 10 weeks of age.

At 7 weeks of age, dam-fed rats, standard formula-fed rats, and whey hydrolysate formula-fed rats prefered leucine or glutamate solution against water, whereas the preferences of two casein hydrolysate formula-fed groups were significantly low compared with the dam-fed rats, and the preference rates were close to 50%. At 10 weeks of age, threre was no significant difference in preference of amino acid solutions among the groups.

Our results suggest that experience of strong flavor of amino acids in infancy may modify taste preferences and the modification could persist in the later life.

P-21

Daily Consumption of Catechin-rich Beverage Affects Energy Metabolism and Metabolic Syndrome in Humans

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Introduction: We have investigated effects of daily consumption of catechin-rich beverage on energy metabolism including dietary induced thermogenesis (DIT) and brown fat-dependent thermogenesis, and metabolic syndrome (MetS).

Methods: Study I: Twelve healthy men consumed a beverage containing 593 mg or 78 mg (control) of catechins for 12 weeks. Postprandial energy expenditure (EE) and excretion of $^{13}CO_2$ were monitored after ingestion of a test meal containing ^{13}C-labelled triacylglycerol. Study II: Ten healthy men with low brown adipose tissue activities assessed by PET/CT participated. They consumed a beverage containing 1,080 mg or 0 mg (placebo) of catechins daily for 5 weeks in a crossover manner. EE at 27 ℃ and after 2-h cold exposure at 19 ℃ was measured. Study III: Japanese overweight and obese adults (n=166) consumed a beverage containing 554 mg or 87 mg (control) of catechins daily for 12 weeks.

Results: StudyI: DIT was significantly higher in the catechin group than the control group. Also, the excretion of $^{13}CO_2$ in the catechin group was significantly increased (90.3 kcal to 51.4 kcal) than control group. Study II: Resting EE at 27 ℃ did not change, whereas EE at 19 ℃ increased significantly, particularly after the treatment with catechins. Cold-induced thermogenesis increased after the catechin treatment from 92 to 198 kcal/d ($p<0.001$) while did not change after the placebo treatment. The catechin treatment also increased fat oxidation to the 2-h cold exposure ($p<0.05$). Study III: Body weight, waist circumference, blood pressure, and visceral fat were significantly lower in the catechin group than the control group. Postprandial glucose response after a 75-g oral glucose tolerance test was significantly lower in subjects with impaired glucose tolerance in the catechin group than the control group.

Discussion: Daily consumption of a catechin-rich beverage may be useful for the management of energy metabolism and MetS, linked to type-2 diabetes mellitus and CVD.

Key words: green tea, catechins, energy metabolism, metabolic syndrome

P-22

Dietary Habits and Nutrition Balance Relating to Visceral Fat Accumulation in Japanese. A Cross-sectional Population Study.

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Background and objective: Visceral obesity is thought to be a large risk factor for metabolic syndrome (MetS) and associated lifestyle-related diseases. To prevent visceral obesity, it is important to clarify the dietary habits and nutrition balance involved in visceral fat accumulation.

Methods: Visceral fat area (VFA), anthropometric measurements, and blood parameters were surveyed in two large populations (7224 adults aged 20 to 65 in a workplace base and 5005 elderly adults aged over 65 in a community base). VFA was measured using bioelectrical impedance analysis (BIA). The incidence of MetS was diagnosed based on the Japanese criteria. The workplace group was also administered a questionnaire containing 35 items with 5 ranking scales to survey diet and lifestyle. Six factors of diet and lifestyle relating to obesity (i.e., overeating, irregular meal time, night eating, eating fast, food choice, and sedentary behavior) were scored by factor analysis of the responses to the questionnaire. A dietary survey was performed based on a 3-day photographic diet record in 606 Japanese adults in a metropolitan area. Food choice and nutrition balance relating to visceral fat accumulation were investigated.

Results: More than 30% of men had visceral obesity with a VFA greater than 100 cm^2. In women, visceral obesity was rare (3%) in the younger population but increased to 12% in the older population. Visceral obesity had the highest odds ratio against MetS compared to body mass index or waist circumference in both populations. The dietary factor score represents food choice was significantly related to VFA. The subgroup with a dietary pattern rich in vegetables, fruit, fish, and less in meat and edible oils was estimated to be lower in VFA. Three nutrition balance factors, protein/fat ratio, dietary fiber/carbohydrate ratio, and n3 fatty acids/fat ratio were significantly different among subgroups stratified by dietary factor score, which represents food choice.

Conclusion: Visceral obesity was the greatest risk factor for MetS in Japanese adults and an elderly population. The results suggested that balanced food choice and nutrition balance are key factors to preventing visceral obesity.

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