Methodological considerations in nutritional epidemiology

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”I want to study diet as a risk factor for this disease”

Diet is a complex exposure

Where to start?

What should be prioritized?

How to do it?

Methodological considerations in nutritional epidemiology

1. Diet is a complex exposure. Nutrients, foods, dietary patterns... Which exposure is relevant?
2. Large within-person day-to-day variation vs small between-person variation
3. We are all exposed to some extent. Which level is associated with health effects?
4. A larger person with high physical activity needs more energy. How can we separate the effect of energy intake from intake of nutrients/foods?
5. How can we measure diet with high precision? Can we use objective markers?
6. How can we deal with change in food habits over time?
7. How to deal with misreporting?
8. Dietary habits is part of a whole lifestyle pattern. How can we seperate the effect of diet from other lifestyle factors?

frontiers in Nutrition

ORIGINAL RESEARCH
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Check for updates

Dairy Consumption, Lactase Persistence, and Mortality Risk in a Cohort From Southern Sweden

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Background to the paper

- Whether high consumption of dairy products is related to longevity is still unclear.
- Because dairy products differ in their composition and processing (e.g. fermentation) it is important to examine them separately.
- Substantial heterogeneity driven by sex, country and study quality has been shown when examining the association between non-fermented milk consumption and mortality.
- Additional studies of prospective cohorts with high-quality dietary data from populations with wide consumption ranges of diverse dairy products are required.
- For example, studies examining the risk with very high intake levels (i.e., more than 1 liter of milk per day) are lacking.

Diet can be studied on different levels

Vitamins
Minerals

Nutrients

Fat
Carbohydrates
Protein

Nutrients can be directly related to biology. Rely on food composition database.

Foods

Foods is not fully represented by nutrient composition. How to group them?

Food pattern

Try to take the synergy of nutrients/foods into account. More than the pieces.

Dough
Tomato sauce
Cheese
Peperoni
Olives
Basil

Energy
Carbohydrate
Protein
Fat
Saturated fat
Mono-unsaturated fat
Vitamin C
Lycopene
Gluten
Calcium
Sodium
Iron
etc..

1 meal out of
1 day out of
1 week out of
1 year out of
1 lifetime.

Nutrient databases are used to calculate nutrient intakes

Room for errors…

OUT

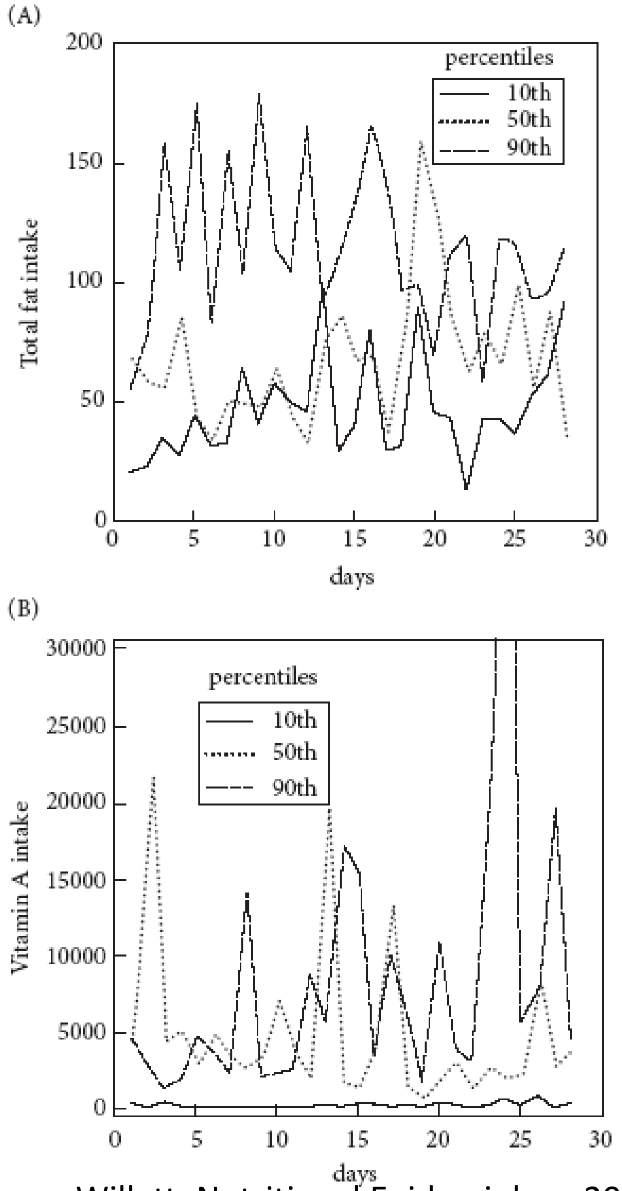
Nutrient content

Nutrient (unit)	Pizza Capricciosa (100g)
Energy (kcal)	267
Carbohydrates (g)	24,97
Fatt (g)	13,4
Protein (g)	11
Fibers (g)	1,37
Alcohol (g)	0
Monosaccharides (g)	1
Disaccharides (g)	1,3
Sucrose (g)	0,2
Total sugars (g)	2,3
Saturated fatty acids (g)	6,34
Thiamine (mg)	0,16
Riboflavin (mg)	0,16
Vitamin C (mg)	3,2
Niacin (mg)	1,95
Vitamin B6 (mg)	0,13
Vitamin B12 (µg)	0,26
Folate (µg)	24,6
Retinol (µg)	73,6
Vitamin A (µg)	85,4
β-Carotene (µg)	142
Vitamin D (µg)	0,13
Vitamin E (mg)	1,17
Vitamin K (µg)	not analyzed
Phosphate (mg)	177,2
Iodine (µg)	7,5
Iron (mg)	0,52
Calcium (mg)	119
Potassium (mg)	174
Copper (mg)	not analyzed
Magnesium (mg)	18,4
Sodium (mg)	625
Salt (g)	1,56
Selenium (µg)	4,68
Zinc (mg)	2

Long-term diet is the relevant exposure

- Large day-to-day variation, but underlying consistent pattern
- Degree of random variation differs according to nutrient
  - Energy and macronutrients have least degree of day-to-day variation
  - Micronutrients tends to be concentrated in certain foods and have larger day-to-day variation
- A single day provides poor estimate of a person´s true long-term nutrient intake, but average of multiple days will improve the estimate

Daily intakes for three women at the 10th, 50th, and 90th percentiles of distribution for fat intake (A) and vitamin A intake (B).



Willett, Nutritional Epidemiology 2013

Number of days depends on degree of accuracy needed and the variability of the nutrient

Table 3–7. Number of repeated days needed per person for 95% of observed values to lie within specified percent of true mean

Nutrient	Within-person coefficient of variation	Number of days needed to lie within specified % of true means			
		10%	20%	30%	40%
Total fat	38.4	57	14	6	4
Calorie-adjusted[a]	19.8	15	4	2	1
Cholesterol	62.2	149	37	17	9
Calorie-adjusted[a]	61.5	145	36	16	9
Sucrose	60.3	140	35	16	9
Calorie-adjusted[a]	50.1	96	24	11	6
Vitamin A	105.0	424	106	47	26
Calorie-adjusted[a]	104.7	424	106	47	26

([a]) Adjusted for total caloric intake using regression analysis.

- We have to have a variation in the population, and we need to have an instrument that can measure diet and can discriminate among subjects
- We have to mainly rely on self-report instruments, which introduce measurement errors
- It is difficult to measure diet, but it is not impossible
- Many aspects of diet can be measured with sufficient accuracy to provide useful information

Dietary assessment methods

	Type of method	Gives information about
Food frequency questionnaire (FFQ)	Retrospective	Usual diet (long-term)
24-hour recalls	Retrospective	Current diet (short-term)
Dietary records	Prospective	Current diet (short-term)

How often did you eat certain foods during the last year?
+ Captures irregular consumption
-Difficult to remember what you ate
-Many foods are not included in the questionnaire/ lack of details

Describe your food intake during the last 24 hours
+ Very detailed
+ Less cognitively challenging (relies on short-term recall)
- Need more than one recall to capture usual intake

Write down everything you eat during several days
+ Very detailed information
+ Gives better estimation of absolute intakes
+ Cognitive aspects is not a problem (does not rely on memory)
- Registration may influence food habits

The ”best” dietary assessment method?

It depends on what we want to measure

→Do we want to describe mean intakes in a population?

→or intake in each of the individual?

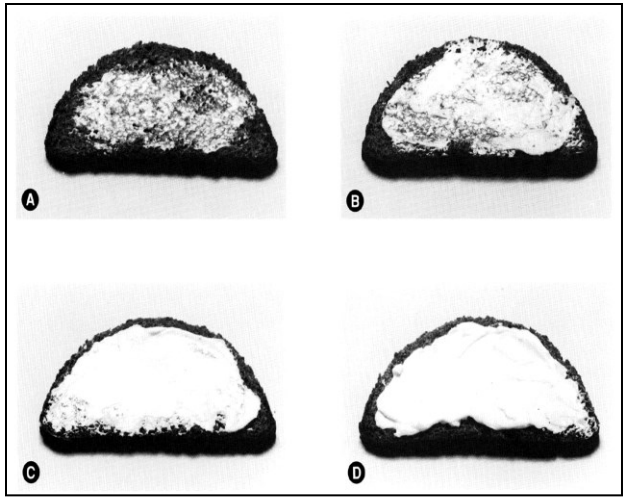
→or study associations with disease?

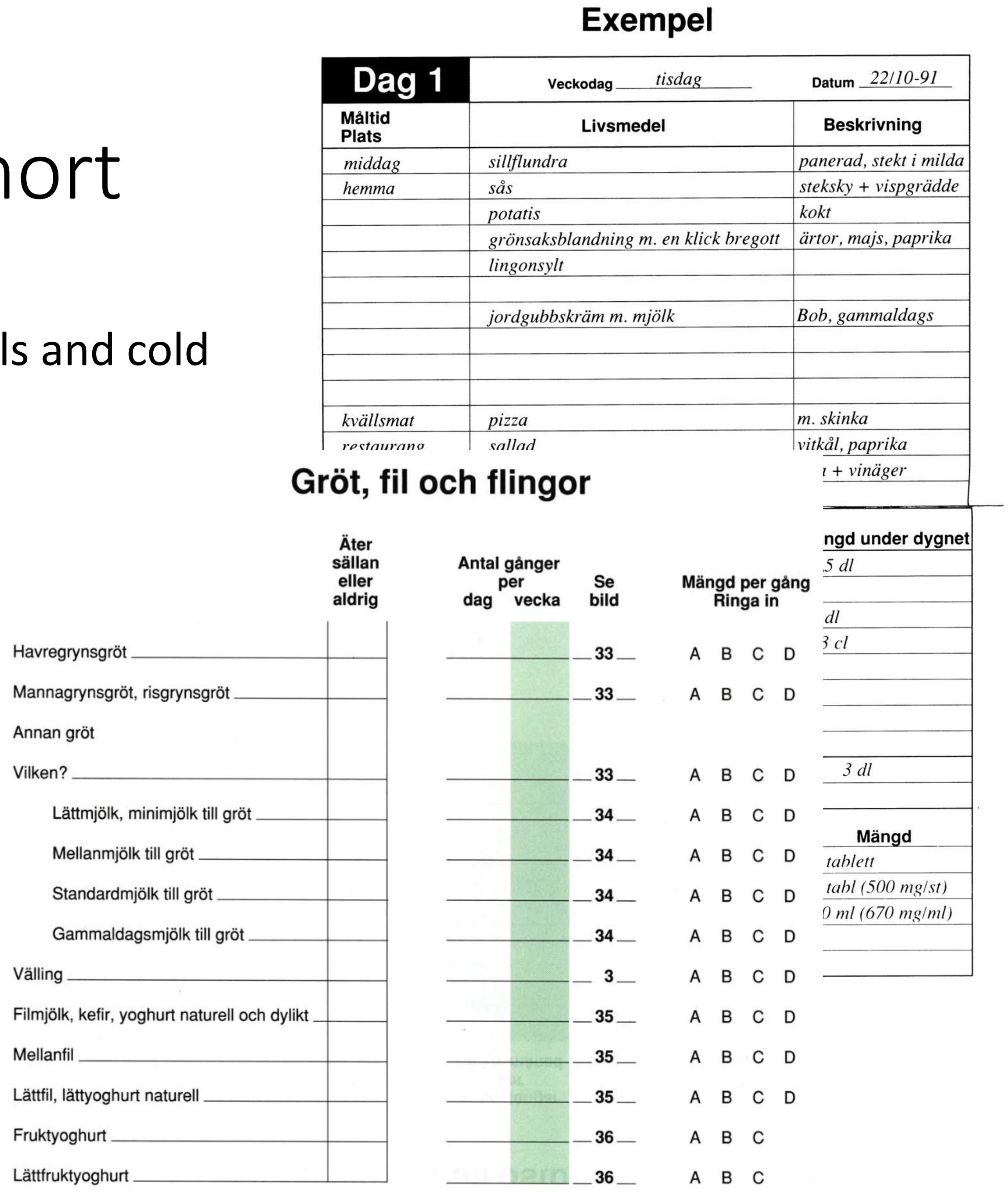
7-21 days needed to rank individuals (depends on nutrient and population group examined)

Malmö diet and cancer cohort

Modified diet history method

- 7-day food dairy: information of the hot meals and cold beverages
- 168-item food frequency questionnaire
  - intake frequencies and portion sizes
  - Photo-aid for portion size estimation
- Interview





Exempel

Dag 1	Veckodag *tisdag*	Datum *22/10-91*
Måltid Plats	Livsmedel	Beskrivning
middag	*sillflundra*	*panerad, stekt i milda*
hemma	*sås*	*steksky + vispgrädde*
	potatis	*kokt*
	grönsaksblandning m. en klick bregott	*ärtor, majs, paprika*
	lingonsylt	
	jordgubbskräm m. mjölk	*Bob, gammaldags*
kvällsmat	*pizza*	*m. skinka*
restaurang	*sallad*	*vitkål, paprika*
		+ vinäger

ngd under dygnet: *5 dl*, *dl*, *3 cl*, *3 dl*

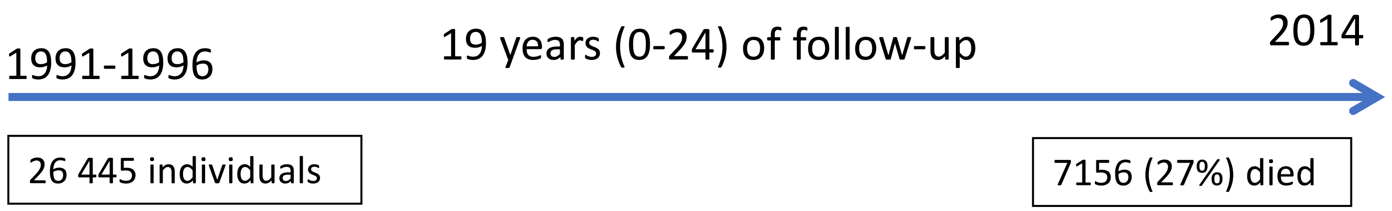
Mängd: *tablett*, *tabl (500 mg/st)*, *0 ml (670 mg/ml)*

Gröt, fil och flingor

	Äter sällan eller aldrig	Antal gånger per dag	Antal gånger per vecka	Se bild	Mängd per gång Ringa in
Havregrynsgröt				33	A B C D
Mannagrynsgröt, risgrynsgröt				33	A B C D
Annan gröt					
Vilken?				33	A B C D
Lättmjölk, minimjölk till gröt				34	A B C D
Mellanmjölk till gröt				34	A B C D
Standardmjölk till gröt				34	A B C D
Gammaldagsmjölk till gröt				34	A B C D
Välling				3	A B C D
Filmjölk, kefir, yoghurt naturell och dylikt				35	A B C D
Mellanfil				35	A B C D
Lättfil, lättyoghurt naturell				35	A B C D
Fruktyoghurt				36	A B C
Lättfruktyoghurt				36	A B C

Dairy products

- Non-fermented milk (regular milk)
- Fermented milk (yoghurt and filmjölk)
- Cream
- Cheese
- Butter



How was intakes of dairy products estimated?

7-day food record: dairy products in cooked meals, glasses of milk (as a drink) with a list of four types of milk with various fat content
FFQ: milk/cream in coffee, tea, chocolate milk, milk on cereals, porridge, fruit compote

Much more detailed questions compared to other studies!

	Äter sällan eller aldrig	Antal gånger per dag	Antal gånger per vecka	Se bild	Mängd per gång Ringa in
Havregrynsgröt				33	A B C D
Mannagrynsgröt, risgrynsgröt				33	A B C D
Annan gröt					
Vilken?				33	A B C D
Lättmjölk, minimjölk till gröt				34	A B C D
Mellanmjölk till gröt				34	A B C D
Standardmjölk till gröt				34	A B C D
Gammaldagsmjölk till gröt				34	A B C D

	Dricker sällan eller aldrig	Antal koppar per dag	Antal koppar per vecka	Se bild	Mängd per kopp Ringa in
Kaffe					
Kaffe, svart				1	A B C D E F
Kaffe med mjölk eller grädde				1	A B C D E F
Lättmjölk, minimjölk i kaffe				2	A B C
Mellanmjölk i kaffe				2	A B C
Standardmjölk i kaffe				2	A B C
Gammaldagsmjölk i kaffe				2	A B C
Kaffegrädde i kaffe				2	A B C
Vispgrädde i kaffe				2	A B C
Socker i kaffe					___ bitar eller ___ tsk per kopp
Sötningsmedel i kaffe					___ bitar eller ___ tsk per kopp

How do we know that we measure what we want to measure?

We **validate** the diet assessment instrument: compare the diet method with the ”golden standard” method (e.g. double labeled water for energy intake, or extensive diet records)

MDC validation study

- The diet assessment method was compared against a reference method of 18-day weighted food records collected over 1 year among 206 individuals living in Malmö in 1984–85 (Elmståhl 1996)
- The diet method generally over-reported milk intake by 50% in women and 32% in men. Cream was over-reported by 22% in women and under-reported by 11% in men. Cheese was over-reported by 9% in women and 12% in men.
- The energy-adjusted correlation coefficients were as follows:
  - milk 0.83 (men) and 0.84 (women)
  - cream 0.47 and 0.52
  - cheese 0.47 and 0.59

Ranking of individuals is the most important!

Confounders

High intake of non-fermented milk: higher BMI, lower number of women, lower educational level, and lower alcohol consumption
High intakes of fermented milk: lower age and higher educational level.

TABLE 1 | Participant characteristics according to intake groups of dairy products.

Non-fermented milk						
Intake groups (g/day)	0–200	200–400	400–600	600–800	800–1000	>1000
N	11,655	8,011	4,155	1,482	495	392
Age, y	57.2 (7.5)	58.4 (7.7)	58.6 (7.7)	58.3 (7.6)	57.6 (7.6)	57.1 (6.8)
BMI, kg/m^2	25.3 (3.8)	25.7 (3.9)	25.9 (4.0)	26.1 (4.2)	26.4 (4.3)	26.5 (4.2)
Smokers (%)	26.4	27.7	29.8	34.0	35.2	51.8
Women (%)	64.5	64.6	60.0	50.6	43.8	24.5
University degree (%)	16.3	13.9	12.3	11.7	14.3	11.0
Zero-consumers of alcohol (%)	4.2	6.0	8.9	9.0	11.9	12.5
Low leisure-time physical activity (%)	9.3	8.8	9.7	10.1	11.5	13.8
Fermented milk						
Intake groups (g/day)	0	0–100	100–200	200–300	>300	
N	9,102	7,940	5,728	2,364	1,056	
Age, y	58.4 (7.5)	57.3 (7.7)	57.9 (7.6)	57.9 (7.5)	57.3 (7.5)	
BMI, kg/m^2	25.8 (4.0)	25.6 (4.0)	25.6 (3.9)	25.2 (3.5)	25.2 (3.6)	
Women (%)	51.5	70.1	68.1	63.2	56.3	
Smokers (%)	33.4	27.4	24.1	24.1	24.4	
University degree (%)	10.3	14.7	16.9	20.3	24.3	
Zero-consumers of alcohol (%)	7.3	5.5	5.0	5.3	7.0	
Low leisure-time physical activity (%)	12.2	8.5	7.3	7.1	7.9	

		Intake categories					
		1	**2**	**3**	**4**	**5**	**6**
Non-fermented milk	Intake	0–200	200–400	400–600	600–800	800-–1,000	>1,000
	N/deaths	11,655/2,853	8,011/2221	4,155/1,277	1,482/484	495/161	392/160
	PY/deaths per 1,000 PY	22,0034/13.0	149,717/14.8	76,601/16.7	26,864/18.0	9,057/17.8	6,716/23.8
	HR (basic model)	1.00	1.03 (0.97–1.09)	1.11 (1.04–1.19)	1.22 (1.10–1.34)	1.26 (1.07–1.48)	1.78 (1.52–2.09)
	HR (full model)	1.00	1.00 (0.94–1.05)	1.05 (0.98–1.12)	1.08 (0.98–1.20)	1.09 (0.93–1.29)	1.34 (1.14–1.59)
	HR (energy-adjusted values)	1.00	0.99 (0.94–1.05)	1.07 (1.00–1.15)	1.12 (1.00–1.26)	1.18 (0.99–1.40)	1.34 (1.09–1.66)
Fermented milk	Intake	0	0–100	100–200	200–300	>300	
	N/deaths	9,102/2,896	7,940/1,960	5,728/1,446	2,364/601	1,056/253	
	PY/deaths per 1,000 PY	166,162/17.4	149,226/13.1	108,745/13.3	44,894/13.4	19,962/12.7	
	HR (basic model)	1.00	0.88 (0.83–0.93)	0.82 (0.77–0.88)	0.82 (0.75–0.90)	0.79 (0.69–0.89)	
	HR (full model)	1.00	0.95 (0.89–1.00)	0.93 (0.87–0.99)	0.93 (0.85–1.02)	0.90 (0.79–1.03)	
	HR (energy-adjusted values)	1.00	0.93 (0.88–0.99)	0.94 (0.88–1.00)	0.95 (0.87–1.04)	0.90 (0.79–1.03)	

Basic model was adjusted for age, sex; Full model was adjusted for age, sex, method, season, energy, BMI, education, physical activity, smoking, alcohol habits, and diet (fruit and vegetables, meat, fiber, sugar-sweetened beverages).

Why is it important to adjust for energy?

- Energy intake varies between individuals
  - Variation is due to body size (affect energy needed for resting metabolic rate), metabolic efficiency and physical activity (+ weight change if not in energy balance)
- Intake of most nutrients tends to be positively correlated with total energy intake
- Nutritional factors may be examined in terms of absolute amounts or in relation to energy intake
- Absolute amount will have less of an effect for a larger (thus higher energy-consuming) person than for a smaller person

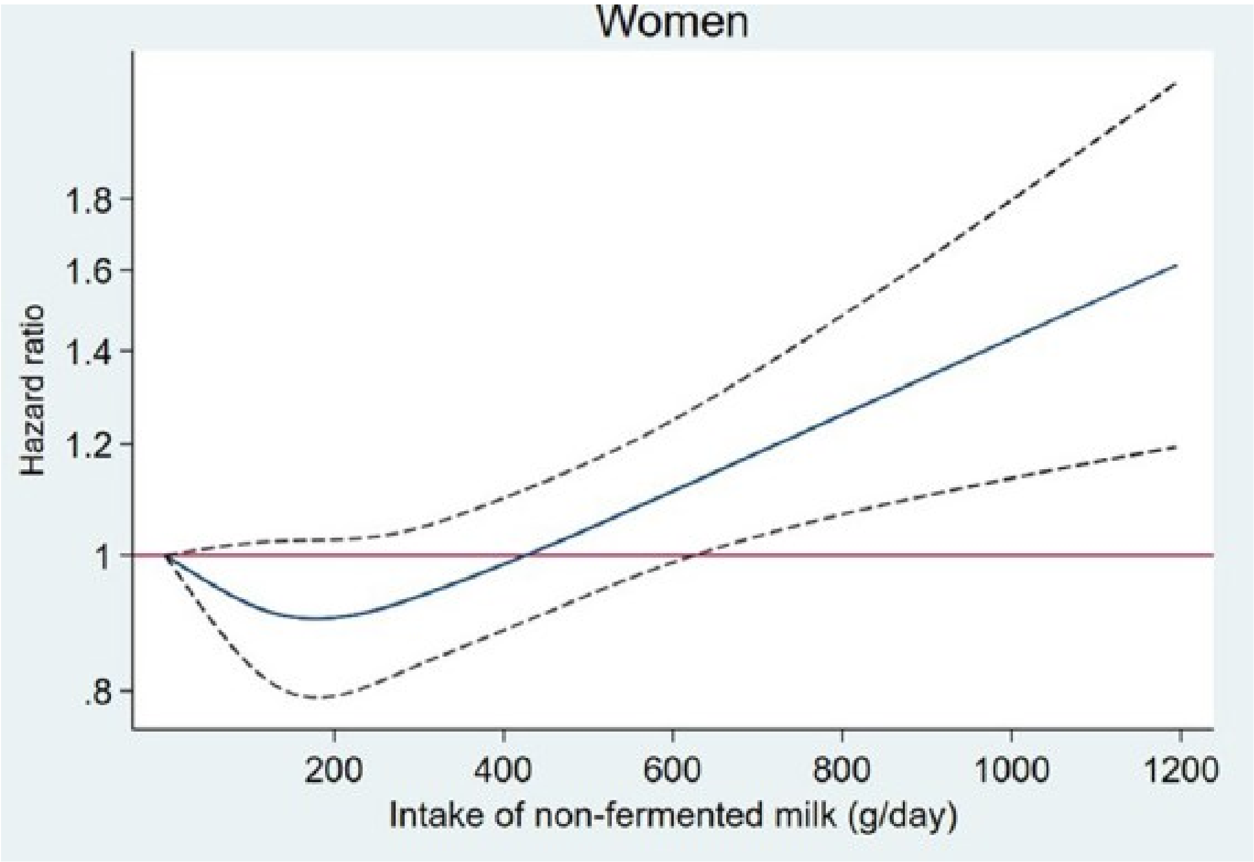
		Intake categories					
		1	2	3	4	5	6
Non-fermented milk	Intake	0–200	200–400	400–600	600–800	800-–1,000	>1,000
	N/deaths	11,655/2,853	8,011/2221	4,155/1,277	1,482/484	495/161	392/160
	PY/deaths per 1,000 PY	22,0034/13.0	149,717/14.8	76,601/16.7	26,864/18.0	9,057/17.8	6,716/23.8
	HR (basic model)	1.00	1.03 (0.97–1.09)	1.11 (1.04–1.19)	1.22 (1.10–1.34)	1.26 (1.07–1.48)	1.78 (1.52–2.09)
	HR (full model)	1.00	1.00 (0.94–1.05)	1.05 (0.98–1.12)	1.08 (0.98–1.20)	1.09 (0.93–1.29)	1.34 (1.14–1.59)
	HR (energy-adjusted values)	1.00	0.99 (0.94–1.05)	1.07 (1.00–1.15)	1.12 (1.00–1.26)	1.18 (0.99–1.40)	1.34 (1.09–1.66)
Fermented milk	Intake	0	0–100	100–200	200–300	>300	
	N/deaths	9,102/2,896	7,940/1,960	5,728/1,446	2,364/601	1,056/253	
	PY/deaths per 1,000 PY	166,162/17.4	149,226/13.1	108,745/13.3	44,894/13.4	19,962/12.7	
	HR (basic model)	1.00	0.88 (0.83–0.93)	0.82 (0.77–0.88)	0.82 (0.75–0.90)	0.79 (0.69–0.89)	
	HR (full model)	1.00	0.95 (0.89–1.00)	0.93 (0.87–0.99)	0.93 (0.85–1.02)	0.90 (0.79–1.03)	
	HR (energy-adjusted values)	1.00	0.93 (0.88–0.99)	0.94 (0.88–1.00)	0.95 (0.87–1.04)	0.90 (0.79–1.03)	

Basic model was adjusted for age, sex; Full model was adjusted for age, sex, method, season, energy, BMI, education, physical activity, smoking, alcohol habits, and diet (fruit and vegetables, meat, fiber, sugar-sweetened beverages).

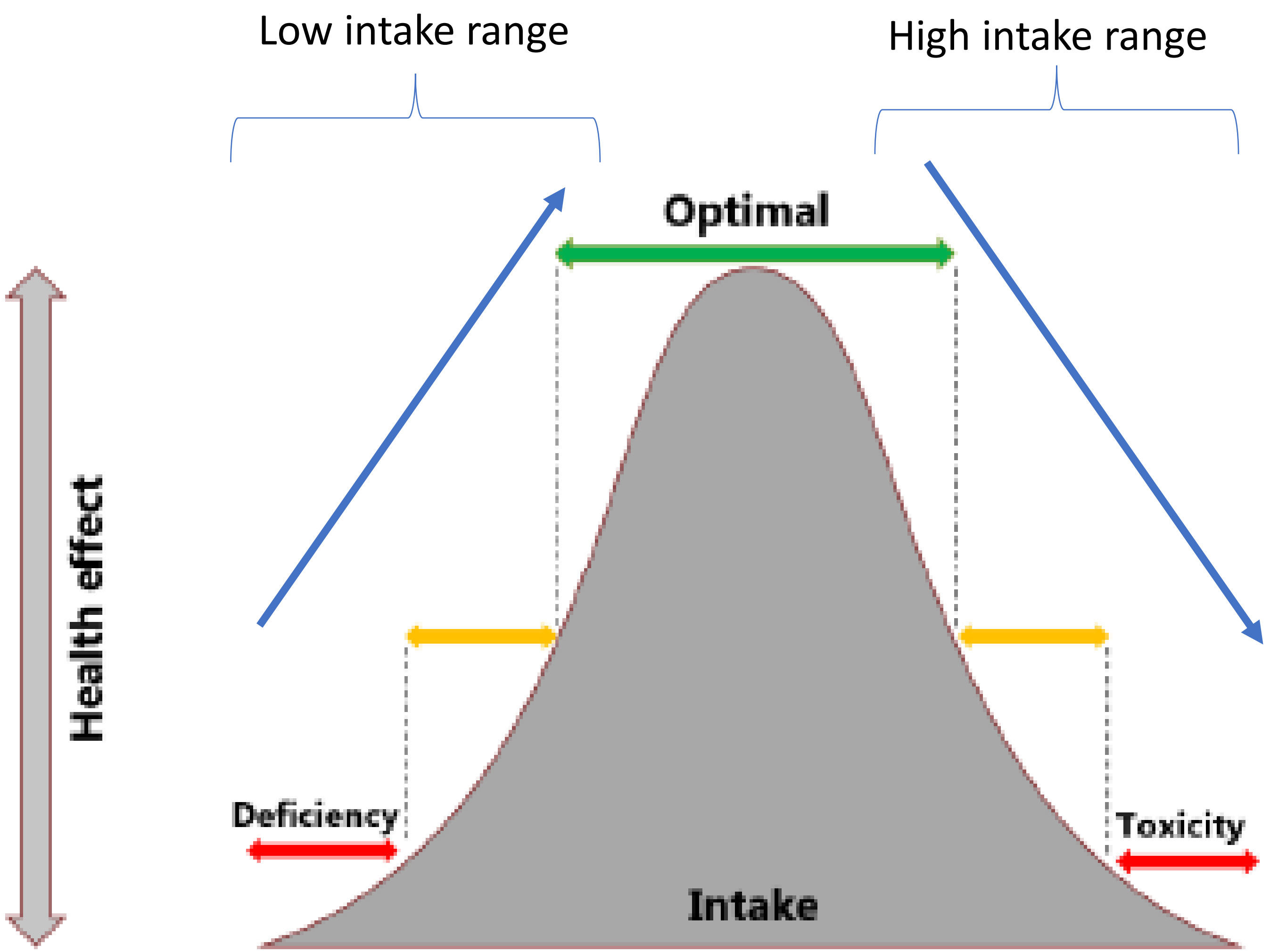
How to deal with misreporting?

- Excluding individuals who potentially misreport their energy intakes
- Excluding individuals reporting a substantial change in food habits before baseline
- After these exclusions (35% of the population): no major influence on HR

	Intake categories					
	1	2	3	4	5	6
Non-fermented milk	1.00	1.03 (0.96-1.11)	1.07 (0.98-1.16)	1.06 (0.94-1.21)	1.13 (0.92-1.38)	1.38 (1.12-1.71)
Fermented milk	1.00	0.94 (0.87-1.01)	0.93 (0.86-1.01)	0.95 (0.85-1.06)	0.90 (0.76-1.06)	


Women
Hazard ratio
1.8
1.6
1.4
1.2
1
.8
200
400
600
800
1000
1200
Intake of non-fermented milk (g/day)

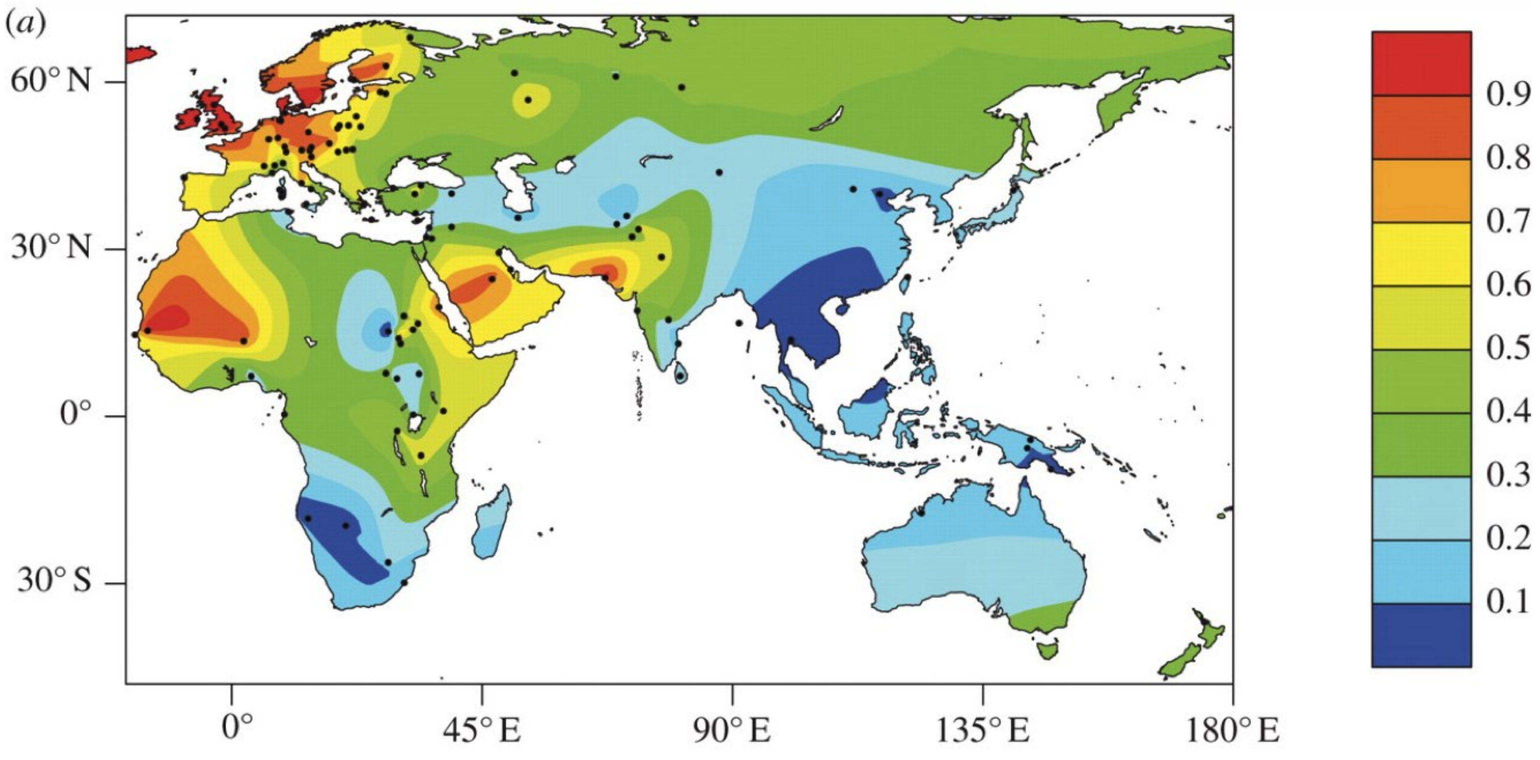
Which intake level is optimal?



Nutritional biomarkers

- Objective markers of dietary intake

Lactase persistence worldwide



https://lbc.msu.edu/evo-ed/Pages/Lactase/anthro_biogeogr.html

Lactase persistence genotype as a marker of long-term adult milk intake

	rs4988235 genotype			P-trend
	CC (lactase non-persistence)	TC (lactase persistence)	TT (lactase persistence)	
N	1,038	7,459	13,737	
Age, years	57.6 (57.1–58.1)	57.9 (57.7–58.1)	58.1 (58.0–58.3)	0.003
Females	61.6%	62.5%	62.1%	0.77
BMI, kg/m^2	25.2 (25.0–25.4)	25.4 (25.4–25.5)	25.6 (25.5–25.6)	<0.001
Energy intake, kcal/day	2,304 (2,270–2,339)	2,275 (2,262–2,288)	2,283 (2,273–2,293)	0.98
Carbohydrates, E%	45.3 (44.9–45.6)	45.1 (45.0–45.3)	44.9 (44.8–45.0)	0.005
Protein, E%	15.6 (15.4–15.7)	15.8 (15.7–15.8)	15.8 (15.7–15.8)	0.13
Fat, E%	39.1 (38.8–39.5)	39.1 (39.0–39.3)	39.3 (39.2–39.4)	0.03
Saturated fat, E%	16.9 (16.7–17.1)	16.9 (16.8–17.0)	17.0 (16.9–17.1)	0.04
Fiber, g/1,000 kcal	9.10 (8.94–9.25)	9.00 (8.94–9.06)	8.89 (8.85–8.93)	<0.001
Fruit and vegetables, g/day	378 (367–388)	371 (367–375)	366 (363–369)	0.003
Coffee, g/day	501 (478–524)	526 (518–535)	528 (521–534)	0.14
Meat, g/day	131 (128–135)	133 (131–134)	134 (133–135)	0.06
Fish, g/day	42.1 (40.2–44.0)	41.7 (41.0–42.5)	41.3 (40.8–41.9)	0.28
Non-fermented milk, g/day	222 (208–235)	279 (273–284)	283 (279–286)	<0.001
Fermented milk, g/day	83.3 (76.9–89.7)	86.6 (84.2–89.0)	88.4 (86.5–90.2)	0.08
Cheese, g/day	45.5 (43.8–47.2)	42.8 (42.1–43.4)	42.5 (42.0–42.9)	0.009
Cream, g/day	16.4 (15.4–17.3)	15.5 (15.1–15.9)	15.3 (15.0–15.6)	0.06
Butter, g/day	11.7 (10.4–12.9)	11.1 (10.6–11.5)	11.3 (11.0–11.6)	0.80

<table>
<tr><th></th><th colspan="3">rs4988235 genotype</th><th></th></tr>
<tr><th></th><th>CC
(lactase non-persistence)</th><th>TC
(lactase persistence)</th><th>TT
(lactase persistence)</th><th>P-trend</th></tr>
<tr><td>N</td><td>1,038</td><td>7,459</td><td>13,737</td><td></td></tr>
<tr><td>PY/deaths/deaths per 1,000 PY</td><td>19,658/268/13.6</td><td>139,195/2,079/14.9</td><td>257,023/3,814/14.8</td><td></td></tr>
<tr><td>HR of mortality (95% CI): additive model</td><td>1.00</td><td>1.11 (0.97–1.26)</td><td>1.07 (0.95–1.22)</td><td>0.94</td></tr>
<tr><td>HR of mortality (95% CI): dominant model</td><td>1.00</td><td colspan="2">1.08 (0.96–1.23)</td><td>0.20</td></tr>
</table>

Biomarkers for dairy intake?

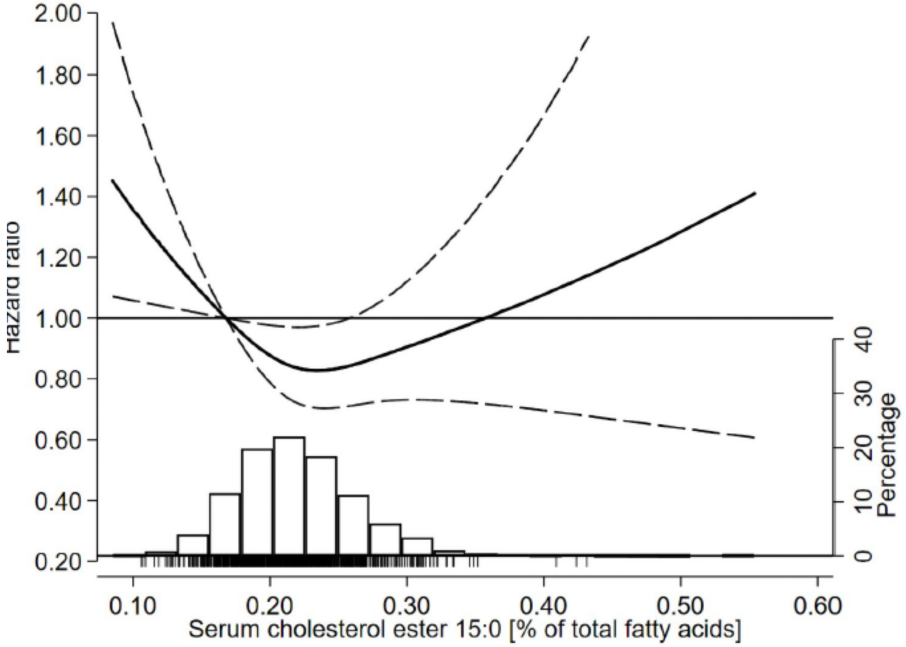
RESEARCH ARTICLE

Biomarkers of dairy fat intake, incident cardiovascular disease, and all-cause mortality: A cohort study, systematic review, and meta-analysis

Kathy Trieu[1☯], Saiuj Bhat[2☯], Zhaoli Dai[3,4], Karin Leander[5], Bruna Gigante[6], Frank Qian[7,8], Andres V. Ardisson Korat[9], Qi Sun[7,9], Xiong-Fei Pan[1,10,11], Federica Laguzzi[5], Tommy Cederholm[12], Ulf de Faire[5], Mai-Lis Hellénius[5], Jason H. Y. Wu[1‡], Ulf Risérus[12‡], Matti Marklund[1,12,13‡*]

Dairy fat intake: concentration of 15:0 and 17:0 in blood or adipose tissue

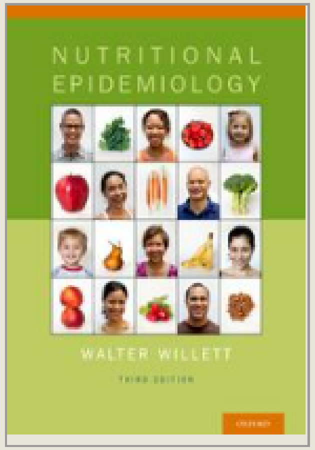
Their findings do not support that dairy fat intake (even at high intakes in Nordic countries) might contribute to higher risk of mortality



ig 2. HRs of all-cause mortality as a function of serum pentadecanoic acid (15:0) in the 60YO study. Data were

Conclusions

- Diet is a complex exposure. Studies of nutrients, foods, dietary patterns etc are all needed and relevant.
- Large day-to-day variation: to capture accurate intakes you need several days. There is a need to futher develop the diet assessment methods.
- To seperate the effect of energy from that of nutrients/foods, we should adjust for energy.
- For a few nutrients/foods there are objective markers: these can be used in combination with self-report.
- Food habits should preferable be measured more than once. We usually have same underlying dietary pattern.
- Misreporting is a challenge. Identify misreporters and exclude them.
- Important to adjust for confounding. Conduct studies in populations with various confounding structures.



Nutritional Epidemiology

Walter Willett

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Perspective: An Extension of the STROBE Statement for Observational Studies in Nutritional Epidemiology (STROBE-nut): Explanation and Elaboration

Advances in Nutrition 2017

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ABSTRACT

Nutritional epidemiology is an inherently complex and multifaceted research area. Dietary intake is a complex exposure and is challenging to describe and assess, and links between diet, health, and disease are difficult to ascertain. Consequently, adequate reporting is necessary to facilitate comprehension, interpretation, and generalizability of results and conclusions. The STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement is an international and collaborative initiative aiming to enhance the quality of reporting of observational studies. We previously presented a checklist of 24 reporting recommendations for the field of nutritional epidemiology, called "the STROBE-nut." The STROBE-nut is an extension of the general STROBE statement, intended to complement the STROBE recommendations to improve and standardize the reporting in nutritional epidemiology. The aim of the present article is to explain the rationale for, and elaborate on, the STROBE-nut recommendations to enhance the clarity and to facilitate the understanding of the guidelines. Examples from the published literature are used as illustrations, and references are provided for further reading. *Adv Nutr* 2017;8:652–78.