PATHWAY-27 Workshop

Guidance Paper for the Scientific Community

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SUBSTANTIATION OF HEALTH CLAIMS

Increasing number of foods labelled and advertised in the European Union (EU) bearing nutrition and *health claims* → **a regulation (EC No 1924/2006)** adopted in December 2006 (European Commission, 2007).

Key Issues Related to Health Claims

1. DEFINITION OF HEALTH CLAIMS: any claims that state, suggest or imply that a relationship exists between a food category, a food or one of its constituents and health.

2. Health claims can be authorised for use in the EU **only after a scientific assessment** has been carried out by the EUROPEAN FOOD SAFETY AUTHORITY (EFSA).
   The specific relationship between food/food constituent and the claimed effect has to be established, based on all robust and scientifically valid data existing.

3. EFSA GUIDANCE DOCUMENTS help in facing legal, scientific, administrative and financial troubles and clarifies scientific criteria for substantiation oh health claims.

AIM OF THE SCIENTIFIC GUIDELINES

Despite EFSA indications, many health claims dossiers have been refused, because '*the evidence provided is insufficient to substantiate this claimed effect for this food' (cause-effect relationship is inconsistent).*

PATHWAY-27 PROJECT aims to:

- Provide an application of EFSA guidance documents
- Enrich EFSA indications and produce Scientific and Industrial Guidelines for health claims related to bioactive compounds and BEFs.

SCIENTIFIC GUIDELINES aim to help scientists in:

1. Designing robust studies, thus contributing to the evidence base
2. Recognising well-designed studies in the literature as a reference for future studies

HOW TO DESIGN
A HIGH QUALITY INTERVENTION STUDY

Key Elements

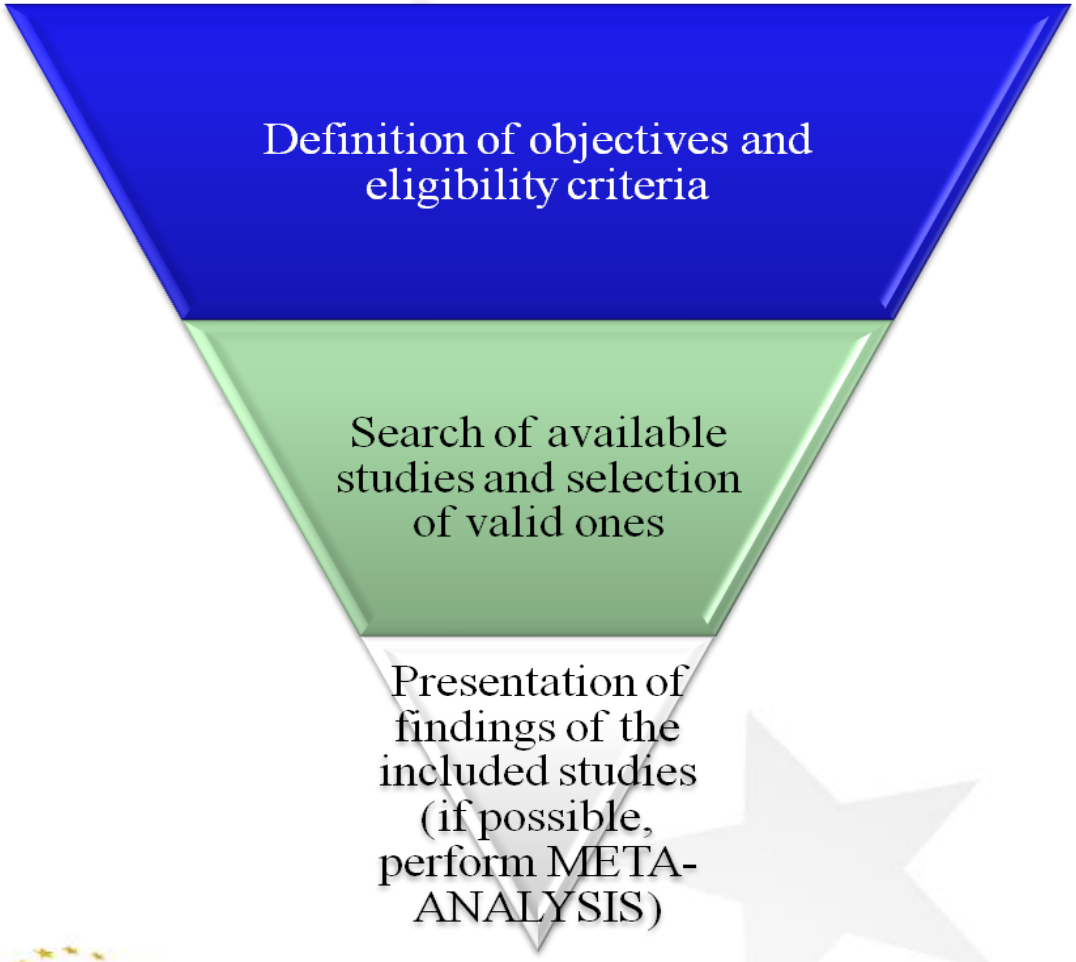
1. Review of the evidence

2. Definition of the research question and of the study hypothesis

3. Study design

Review of the evidence

SYSTEMATIC REVIEWS



- Are the gold standard for synthesizing the existing evidence because of their methodological rigor
- Provide reliable findings from which conclusions can be drawn

Review of the evidence

PATHWAY-27

Metabolic effect of bioactives

- PUFA-3 improve the blood lipid profile, reduce hypertension, inflammation and may also interact with other dietary factors to modulate the risk factors of MS;
- EFSA has approved an Article 13 Health Claim related to DHA effectiveness on blood triglycerides in adults, but other claims (obesity, oxidative stress) had a negative opinion.

Molecular mechanisms involved in the metabolic effect

Examples

- Cyanidin-3-O-β-Glucoside (C3G) improves obesity and triglyceride metabolism in mice: the underlying mechanism is partly related to the activation of lipoprotein lipases in plasma and skeletal muscle, and to their inhibition in adipose tissue following the activation of pAMPK
- Extensive data from epidemiological studies and clinical trials on the effects n-3 PUFA on multiple biological processes → the interpretation of experimental data for DHA-enriched foods is complicated by two main issues: (a) its interaction with the food matrix and consequent bioavailability/bioactivity of DHA (the absorption rate varied depending on the food matrix), and (b) its effective dose.
- From bioactive to functional foods (BEF) (very few have addressed n-3 LC-PUFA-enriched foods);

DHA is commercially available and, according to EU legislation, may be used in foods

The effectiveness of DHA for prevention of MS requires evidence-based scientific support

DEFINING THE RESEARCH QUESTION AND STUDY HYPOTHESIS

Research question should consider the ‘PICO’ elements:

Patient problem or population

Intervention

Comparison

primary Outcome(s)

Study hypothesis: theory that has to be tested.

It should be clearly stated as it will directly influence all other aspects of the study, such as the study design (Welch et al.,2011)

DEFINING THE RESEARCH QUESTION AND STUDY HYPOTHESIS
Pathway-27

Research question: In men and women aged 18 to 80 years at risk of metabolic syndrome (P), studied in a 12-week multi-centre, randomised, double-blind, placebo-controlled, parallel-arm dietary intervention (I), what would be the effects of bioactive enrichment of food and the food matrix used (DHA+beta-glucans or DHA+anthocyanins vs. placebo, in dairy, egg and bakery foods (C)) on markers of the metabolic syndrome, primarily fasting blood triglycerides (O).

Hypothesis: It was hypothesised that markers of metabolic syndrome would be improved (i.e. lower fasting blood triglycerides) in subjects consuming a BEF compared with placebo and that the food matrix would impact the changes in these markers.

CHOICE OF THE STUDY TYPE AND STUDY DESIGN

- In the field of dietary human intervention studies, there is no rule on **which type** of study is the best → randomised controlled trials (RCTs) are the most rigorous way of determining whether a cause-effect relation exists between a food/constituent and a certain health outcome and for assessing the cost-effectiveness of an intervention.

- Running **exploratory or pilot studies** beforehand can be a cost-effective fact-finding way to see what effects may be expected from a larger and well-controlled intervention and whether such an intervention would be feasible.

- The minimum **sample size** required to detect a given effect can be calculated based on the effect size, estimates of standard deviation, on the level of statistical significance (alpha), and on the statistical power (Zhong, 2009). Power/sample size calculations will greatly depend on the primary outcome; the impact on secondary outcomes must be considered too.

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CHOICE OF THE STUDY TYPE AND STUDY DESIGN (2)

- The **minimum study duration** required should be used, for ethical as well as budget reasons, and to minimise non-compliance and/or dropout. Study duration will vary widely depending on the outcome targeted by the health claim, but also volunteers' compliance should be taken into account.
- **Randomisation** determines each participant's treatment independently with equal allocation to two treatment groups. **Block randomisation** can be used to keep numbers in each group very close at all times.
- '**Blinding**' refers to keeping either study participants or the research team involved in the study ('***single blinding***'), or both participants and the research team ('***double blinding***'), unaware of the assigned treatment. This avoids influences on participants/research team behaviour.
    - Double-blinding with food products is more difficult and often not fully feasible: both test and placebo foods should ideally be manufactured to be identical in taste, texture, and appearance, differing only in the target component (Yao et al., 2013), which can be very challenging when dealing with bioactives.

Choice of the study type and study design PATHWAY-27

- *Multi-centre, double-blind, placebo-controlled, parallel RCT with 4 arms:*

1. Dairy BEF (DHA+beta-glucans) + egg placebo + bakery placebo;
2. Egg BEF (DHA+anthocyanins) + dairy placebo + bakery placebo;
3. Bakery BEF (DHA+anthocyanins) + dairy placebo + bakery placebo;
4. Dairy, egg and bakery placebo (control arm).

- *Small-size, double-blind pilot RCT without placebo, 3 study centres tested 5 different combinations of DHA, beta-glucans and anthocyanins in 1 food matrix each (1 centre used dairy, 1 centre used egg, and 1 centre bakery BEFs). This allowed identification in each centre of the best combination of bioactives to be used in the following larger RCT.*

In a reference article (Grimsgaard et al., 1997) the standard deviation of the decrease in triglycerides concentration was 0.34 mmol/L in the placebo group and 0.31 mmol/L in the DHA-supplemented group (difference in triglycerides concentration between groups: 0.11 mmol/L). To obtain a conservative estimation for the power, the common standard deviation was assumed to be 0.35 mmol/L. To detect a difference in the decrease of triglycerides concentration not smaller than 0.12 mmol/L, a sample size of 200 participants per group (dairy BEF, egg BEF, bakery BEF, placebo) would be required for the power of the statistical test to exceed 80%, i.e. sample size of 800 subjects.

CHOICE OF THE STUDY TYPE AND STUDY DESIGN (2) Pathway-27

- **Duration**: 12 weeks, which is more than the minimum of 8 weeks recommended when investigating changes in blood concentration triglycerides (EFSA Panel on Dietetic Products Nutrition and Allergies, 2011b).

- **Randomization**: The allocation of participants to the 4 different groups was carried out based on predefined randomisation lists created with a block size of 4 and with an allocation ratio of 1:1:1:1.

- **Blinding**: In the double-blind RCT, matching test and control products was difficult; enrichment with DHA gave the test food a recognisable fishy taste, and anthocyanins gave a purple colour. Formulation and technological processing of the enriched test food were modified in order to minimise these issues.

PITFALLS AND DIFFICULTIES IN RUNNING A LIS

There are still many studies which are not good enough but designing and running good studies is not easy, many factors need to be taken into account:

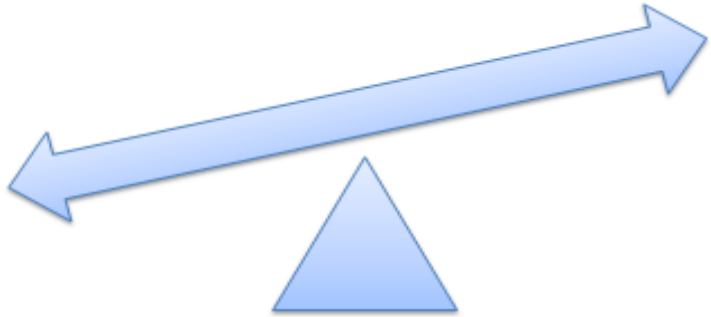
1. Strict selection of study population
2. Different ethical approval processes in a multicentric study
3. Recruitment
4. Compliance

Selection of study population: inclusion criteria

Eligibility (inclusion/exclusion) criteria: physiological or clinical characteristics, or demographic variables used to define the study population (Welch, 2011).

- INCLUSION CRITERIA: the study population should match the target population for the health claim

TOO MANY CRITERIA reduce the possibility of finding participants meeting all criteria and the application of results to the general population



TOO FEW CRITERIA decrease the homogeneity of the study group and can limit the comparisons between individuals

Selection of study population: exclusion criteria and strategies

- EXCLUSION CRITERIA are needed to avoid factors that may confound the interpretation of results (acute/chronic diseases, medication use, lifestyle…)
- **Strategies against strict criteria adopted in PATHWAY-27**:
  - screening potential subjects in several steps: phone/emails → visit and sample
  - relaxing eligibility criteria, since the recruitment has been unexpectedly difficult and slow (e.g. smokers (>5 cigarettes/day) could be included provided that their smoking habits did not change during the study; adding low HDL criteria).

Study population and eligibility criteria in PATHWAY-27

- ***Study population:*** Men and women aged 18 to 80 years, at risk for Metabolic Syndrome. This study could be used for the substantiation of disease risk reduction claims targeted at people with a risk factor for the Metabolic Syndrome but otherwise healthy (subgroup of the general population).

- ***Inclusion criteria****:* Men and women aged 18 to 80 years with 2 to 4 of the Metabolic Syndrome criteria, at least one of them being fasting triglycerides ≥150 mg/dL but ≤400 mg/dL, or HDL-C ≤40 mg/dL (men) or ≤50 mg /dL

- ***Exclusion criteria:***

  - Having all 5 criteria for Metabolic Syndrome;
  - Regular drug therapy with impact on serum lipids (e.g. statins, fibrates);
  - Diabetes (fasting glucose >126 mg/dL, or anti-diabetic treatment);
  - Hypothydroidism or thyroxine treatment
  - Familial dyslipidemia;
  - Recent history of cancer or cancer treatment (less than 2 years);
  - Active or recently diagnosed intestinal malabsorption or disorders associated with malabsorption:
  - Use of medication known to cause malabsorption;
  - Illegal drug use, chronic alcoholism or active smoking;
  - Intensive physical exercise (≥5 hours per week)
  - History of allergy or intolerance to any components used in BEF, celiac disease, lactose intolerance, allergy to milk or egg proteins;
  - Consumption of nutritional supplements containing DHA, beta-glucan or anthocyanins;
  - Women who are pregnant, lactating or actively trying to conceive;
  - Institutionalised patients, those who lack autonomy to consent or are unable to meet all examinations;
  - Participation in other clinical trials that may impact on outcome;
  - Subjects deprived of their liberty by judicial or administrative decision.

Different ethical approval processes in a multicentric study

- All centres running clinical trials in Europe are expected to follow **Good Clinical Practice** (GCP) Guidelines (Directive 2001/20/EC).

- Very long time needed for the ethical approval process in order to:
  - Prepare and share the protocol and related documents + translate them in every country
  - Submit the protocol to the local/national Ethics Committees and wait for the authorization

- Necessity in Pathway-27 of starting the LIS at the same time in all centres because of risk of variability between centres if test/control products are not produced at the same time

→ **IN PATHWAY-27 DELAY IN STARTING BOTH PILOT STUDIES AND THE LIS!**

Recruitment: how to make a good recruitment plan

- Defining in advance time needed for recruitment
- Choosing large and specialized staff
- Choosing effective strategies
- Monitoring the recruitment
- Managing the production plan in parallel with the recruitment

Recruitment: strategies

Strategies in PATHWAY-27:

Contact with general practitioners;

Cooperation with clinics dedicated to treating Metabolic Syndrome;

Screening of blood donors lists;

Web announcements on the university and hospital websites;

Distribution of flyers;

Newspapers and radio advert;

Social media posts (e.g. Facebook)

Not enough → EXTENSION OF THE RECRUITMENT

Compliance

- "Compliance" = *the degree with which a volunteer adheres to the experimental protocol*
- Lack of compliance in human interventions: (Jin, 2008; Matsui, 2009)

Participant-related	Protocol-related
Misunderstanding instructions	Over-complicated regimen
Social stigma (associated with disease or status)	Inconvenient regimen (e.g. many restrictions, many tests)
Practical difficulties (affecting work, family life)	Size of dose
Forgetfulness	Frequency of dose
Boredom, apathy, frustration (e.g. long trials, no obvious benefit)	Ease of storage / preparation / consumption
Adverse effects	Product acceptability
Restrictive conditions	Product shelf-life

Compliance in PATHWAY-27

- Main compliance issues in PATHWAY-27:

FOOD PROBLEMS	LOGISTIC PROBLEMS
• taste and smell • monotony of the diet/no replacement • mild gastrointestinal side effects (diarrhoea, bloating, nausea) • daily pancakes' consumption	• excessive length • time off from work • food handling outside home (> for frozen pancakes) • holidays

Compliance in PATHWAY-27: strategies

- **Measuring compliance** through:
  - Consumption records/diaries;
  - Return of packaging/containers or unused product
- **Maximising compliance:**
  - Minimise the burden for volunteers;
  - Make palatable food products;
  - Any dietary change should fit easily in the usual diet, and test foods should be easy to prepare/cook;
  - Use of easy-to-prepare, easy-to-store food
  - Try to empower the participant in the behavioural change;
  - Friendly staff;
  - Good communication with participants.