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The future of meat: health impact assessment with randomized evidence

Running head: The future of meat

Authors:

João Pedro Ferreira, MD, PhD¹; Abhinav Sharma, MD, PhD²; Faiez Zannad, MD, PhD¹

Affiliations:

¹ Université de Lorraine, INSERM, Centre d'Investigation Clinique et Plurithématique 1433, INSERM U1116, CHRU de Nancy, F-CRIN INI-CRCT (Cardiovascular and Renal Clinical Trialists), Nancy, France.

² Division of Cardiology, McGill University Health Centre, Montreal, QC, Canada.

Contact to:

Dr. João Pedro Ferreira

Centre d'Investigation Clinique 1433 module Plurithématique, CHRU Nancy - Hopitaux de Brabois, Institut Lorrain du Coeur et des Vaisseaux Louis Mathieu

4 rue du Morvan, 54500 Vandoeuvre les Nancy

Tel : +33 (0) 3 83 15 73 15

Fax : +33 (0) 3 83 15 73 24

Mail: j.ferreira@chru-nancy.fr

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Abstract

Massive animal farming for meat production poses major problems in terms of resource use, environmental impact, and biodiversity. Furthermore, excessive meat consumption has been associated with multiple deleterious health consequences. However, more and better designed randomized trials are needed to increase the level of evidence on the health impacts of meat. Novel meat alternatives, such as plant- and cell-based meat are much less impactful to the environment and might replace traditional animal meat in the future, but, despite promising early data, the health consequences of these novel products need further study. This manuscript focuses on the health impacts of meat over three main sections: 1) overview of the evidence highlighting the association of meat consumption with health; 2) novel alternatives to meat, including plant-based and cell-based alternatives; and 3) examine the rationale for randomized studies to evaluate the effects of the novel meat alternatives compared with the standard animal meat.

Key-words: animal meat; plant-based meat; cell-based meat; environmental impact; health impact; randomized controlled trials.

Introduction

The incorporation of meat (muscle obtained from an animal carcass) and marrow from large animals in the human diet occurred about 2.6 million years ago. The introduction of animal meat in the diet of early hominids expanded on a previously mainly vegetarian diet, as suggested by the tooth morphology of these hominids¹. The reason(s) why these early hominids started eating meat remains debatable. A possible explanation was the scarcity of grassland across African habitats that placed selective pressure and forced our predecessors to find new alternatives to their diet². The consumption of meat also increased the amount of proteins in the diet, which may have helped to increase the body size without loss of mobility, agility, or sociality. Throughout time, meat consumption might have also helped to select those more capable to eat and digest these sources of nutrients³. Ever since these early hominids, meat consumption has not stopped, and evidence supporting the association between large-animal extinction and human expansion exists⁴. Along with the agricultural “revolution”, around 9000 to 11000 years ago, the paradigm shifted from hunter-gathering to animal domestication for human consumption⁵. Fast-forward to the last few hundred years, technological advances enabled major increments in meat production and transportation supplying a fast-growing world population. However, it was in the last 50 years that the total meat production quadrupled. During the 60s and 70s Europe and North America were the dominant meat producers, but currently Asia is the main meat producer accounting for more than 100 million tons of meat produced every year for a world total of nearly 300 million tons, with poultry and pig dominating the current meat production⁶. The per capita meat consumption has also greatly increased, with the average person consuming around 43 kilograms of meat in 2014 compared with 20 kilograms in 1961, globally⁷. Meat production uses nearly half of the habitable land and accounts for over a

quarter of global greenhouse gas emissions, while it produces less than 20% of the total calories and less than 40% of the total protein; therefore, it is highly inefficient^{8,9}.

This massive production of animals for human consumption poses major problems in terms of resource use, environmental impact, and biodiversity. Moreover, excessive meat consumption can also have deleterious effects on human health. Innovation in novel food and meat alternatives may provide an opportunity to explore the impact of health and dietary interventions on health outcomes.

This manuscript focus on the health impacts of meat in three main sections: 1) overview of the available evidence highlighting the association of meat consumption with human health; 2) novel alternatives to meat, including plant-based and cell-based alternatives; and 3) examine whether there is a rationale for randomized studies to evaluate the effects of the novel meat alternatives compared with the standard animal meat.

Health impacts of animal meat consumption

The evidence linking animal meat consumption - mostly red (e.g., beef, pork, and lamb) and processed (i.e., transform through processes that enhance taste or improve preservation) meats - with human health is extensive, yet generally classified as “low grade” evidence, simply due to the fact that most diet studies are observational; thus, the straightforward application of the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) criteria generally classifies observational studies as “low grade” evidence¹⁰.

Notwithstanding, alternatives for evaluating and grading evidence on nutritional and lifestyle interventions have been developed^{11, 12}. The study of the impact of diet on health has specific particularities that cannot be overlooked. For example, diet patterns may change with time, diet questionnaires are self-reported, prone to measurement errors and

often not consistent across studies, and factors such as income and area of residence play a major role on the quality of the diet (and health overall)¹³. It should be also noted that the feasibility of randomized controlled trials (RCTs) assessing morbidity and mortality is also challenging with dietary interventions, because such studies would require a large sample size, with a long follow-up period, and more importantly, blinding the “treatment” allocation, keeping “treatment” adherence, and avoiding “treatment” group “cross-overs” would be practically impossible. Furthermore, the socio-cultural context of food consumption makes conducting RCTs challenging; for example, does the entire family unit have to be randomized or can only one person in a family be a part of a dietary intervention. Where there are cultures where certain foods are integrated as cultural norms, is replacement of these foods acceptable or ethical? That is why many RCTs evaluate short-term dietary interventions on surrogate markers of disease (e.g., LDL cholesterol); such studies provide important information and knowledge regarding the health effects of certain diets, but ultimately have not provided robust evidence on clinical outcomes such as cancer, major adverse cardiovascular events, or death¹⁴. Notwithstanding, the totality of evidence with extensive replication of findings in multiple settings points towards a detrimental health effect of excessive red meat and processed meat consumption¹⁵.

Several long-term prospective cohort studies have studied the association between red and processed meat consumption with cardiovascular disease (including stroke and myocardial infarction), type 2 diabetes, cancer (colorectal cancer in particular), and mortality. Overall, these studies consistently show that the consumption of red and processed meat are associated with a higher risk of cardiovascular disease, type 2 diabetes, cancer, and mortality¹⁶⁻²⁰. For example, a meta-analysis studying the health effects of diets with lower content of red and processed meat compared with diets with higher content of

red and processed meat, found that lower red and processed meat consumption was associated with a 13% relative decrease in all-cause death (HR 0.87, 95%CI 0.82-0.92), 14% decrease in cardiovascular death (HR 0.86, 95%CI 0.79-0.94), and a 24% decrease in new-onset type 2 diabetes (HR 0.76, 95%CI 0.68-0.86)²⁰. Another meta-analysis which included over 6 million people found that a reduction of red and processed meat intake was associated with a 7% decrease in overall cancer mortality (HR 0.93, 95%CI 0.91-0.94)^{19, 21-23}. Moreover, higher plant protein intake and replacement of red meat or processed meat with plant protein has been consistently associated with lower all-cause, cancer-related and cardiovascular mortality^{21, 24,25}. A recent meta-analysis of RCTs on red meat and cardiovascular risk factors, comparing diets with red meat with diets that replaced red meat with a variety of foods, showed that replacing red meat by high-quality plant protein sources leads to more favorable lipid profile¹⁴, and increasing the consumption of whole nuts (or extra virgin oil) decrease the rate of major adverse cardiovascular events²⁶.

Consistent with the totality of the evidence, current guidelines recommend dietary patterns low in red and processed meats and high in minimally processed plant-based foods²⁷⁻³¹. Also consistently, the International Agency for Research on Cancer of the World Health Organization (WHO) classified processed meats (e.g., bacon and sausages) as carcinogenic to humans, and unprocessed red meats, such as beef and pork, as “probably carcinogenic”³⁰. Knowing that most cardiovascular diseases and deaths attributable to cardiovascular risk factors can be modified, including by dietary change, there is strong rationale to explore dietary changes as a strategy to reduce future cardiovascular disease risk³².

Beyond the health impact of red meat consumption, its environmental impact should also be taken under consideration. Climate-related issues including air and water

pollution, greenhouse gas emissions, loss of forests and other important carbon sinks, and extreme weather events pose a serious threat to human health, and animal farming is one of the major contributors to these environment-related problems^{33, 34}. These factors should also be taken into account in dietary recommendations and guidelines³⁵.

Alternatives to meat

The development of innovative companies that are aiming to compete with traditional meat-based industries has the potential to impact large scale changes in health and environment^{33,36,37}. Meat alternatives may have a plant- or cell-based origin, and usually provide a meat-like sensorial experience to the consumer. Technological advances allowed the creation of food products that taste and smell exactly like meat, but do not result from the killing of animals and have much less environmental impact. For example, a study funded by Beyond Meat® based on a comparative assessment of a plant-based burger production system with the beef life cycle assessment, identified that the plant-based burger generates 90% less greenhouse gas emissions, uses 46% less energy, and has almost no impact on water resources and land use³⁸. Some estimates find cell-based meat even less detrimental to the environment³⁹. Despite these potential advantages, in a survey study conducted in the USA, most consumers (>70%) still prefer traditional farm raised animal beef, and even if plant- and cell-based beef price would decrease by half, most people still would prefer traditional meat. Features that predicted preferences to red meat alternatives included being vegetarians, males, younger, and higher levels of education. Furthermore, more participants did not support taxing conventional beef for environmental and animal welfare objectives. Whether individuals were more open to substitute limited quantities of red meat with plant-based meats remains to be explored. These results might be related to

the relative novelty of these products and the lack of evidence on their impact on human health⁴⁰. A thorough comparison of plant- and cell-based meat with traditional farm meat based on randomized evidence, to demonstrate the safety and efficacy of meat alternatives, could be important to change the dietary guidelines and the view of the consumer.

Plant-based

Plant-based meat alternatives are designed to look, smell, and taste like animal meat to attract traditional meat consumers, beyond vegetarians and vegans. However, plant-based meat is produced using purified plant extracts, rather than whole plant protein. Important differences also exist between manufacturers. For example, the Beyond Meat® burger uses proteins of multiple vegetables, fats from vegetable oils, colour and taste from beet and apple juice extracts, plus sodium, potassium, iron and calcium as minerals. The Impossible Foods® burger uses protein from genetically modified soy and potatoes, and taste from iron-containing heme from genetically modified soy yeast (soy leghemoglobin), to look, smell and taste like animal blood (hemoglobin). The comparison of the ingredients and nutritional facts of these two products is depicted in *Table 1*.

These details are relevant, because food processing may lead to the loss of nutrients and fibers naturally present in plants, and processed foods may be higher in saturated fats that are linked to metabolic derangements (even if coming from vegetable sources)^{41,42}. The Beyond Meat® and Impossible Foods® burger have no cholesterol, less total fat, similar protein and calory content, and more salt than a grass-fed beef burger (*Table 1*). The heme content is exclusive to the Impossible Foods® burger, which is slightly higher than that of a grass-feed beef burger.

Regardless of the nutritional comparisons, more important is to compare these products directly on health effects. For this purpose, the use of surrogate markers of

cardiovascular disease may be particularly useful for use in early phase RCTs by reducing sample size and follow-up time while providing insight on how the metabolism is changed with product exposure. In this regard, and beyond the more traditional risk factors like LDL cholesterol and blood pressure, trimethylamine N-oxide (TMAO) is a gut microbiota-generated metabolite that has been associated with atherosclerosis, stroke, myocardial infarction and cardiovascular death^{43, 44}. The association of high plasma TMAO with cardiovascular disease is positive and dose-dependent⁴⁵. As consequence, decreasing the TMAO levels has become a potential therapeutic target for reducing cardiovascular risk^{46,47}. TMAO is generated via gut microbiota-dependent formation of trimethylamine (TMA) from phosphatidylcholine, the major dietary source of choline⁴⁸. Carnitine is enriched in red meat, and, consequently, higher total choline content is found in beef (and other meats), liver, and egg yolks^{48,49,50}. In some observational studies, plasma levels of TMAO were increased in omnivores compared to vegans/vegetarians, and in recent clinical challenge studies, TMA and TMAO generation from oral carnitine was observed to be substantially reduced with a vegetarian diet⁵¹⁻⁵³.

Recently, a randomized crossover trial on the effect of plant-based compared with animal-based meat on TMAO and cardiovascular disease risk factors in generally healthy adults: Study With Appetizing Plantfood - Meat Eating Alternative Trial (SWAP-MEAT) found that the plant-based burger (Beyond Meat®) reduced the TMAO levels compared with animal meat⁵⁴. The SWAP-MEAT was a single-centre, randomized cross-over trial with no washout period comparing two or more serving a day of plant-based burger with animal burger for eight weeks, while keeping the rest of the diet as similar as possible between the study phases, in 36 health participants (67% women and 69% white), with a mean age of 50±14 years and a mean body mass index of 28±5 Kg/m². The mean TMAO concentrations

were overall significantly lower for plant-based burger than for animal (2.7 ± 0.3 vs. 4.7 ± 0.9 ; $P = 0.012$), particularly among those who received animal meat first. Furthermore, there was a clinically meaningful LDL cholesterol decrease during the plant-based meat period with a mean reduction of around 10 mg/dL, and a statistically significant weight reduction. Importantly, the mean daily sodium intake was similar between periods and the saturated fat intake lower in the plant-based period. Exploratory analyses of the microbiome failed to reveal possible responder compared with non-responder factors. These findings support the health benefit of plant-based burgers compared with animal meat burgers. Another ongoing trial with a cross-over design and TMAO change as the primary outcome, the Plant-Based Meat vs Animal "Red" Meat (FOOD-1) trial (ClinicalTrials.gov Identifier: NCT04510324), randomly assigns 30 adults to either beef burger or plant-based burger, and may provide further independent replication and insight into the effects of plant-based vs. animal burgers.

Cell-based

Another line of products in the pipeline is cell-based (or cultured) meat that produces animal meat without the need to raise or kill the animal. These products look, smell and taste exactly like animal meat, because they are made with animal cells. Several companies are currently producing cell-based meat but as these companies are navigating uncharted territory, the regulatory procedures involving these products has been challenging.

Notwithstanding, the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) recently created a formal agreement to jointly oversee the production of cell-based meat in order to establish regulations and product labelling⁵⁵. While barriers to commercialization remain, cell-based meat may soon be widely available for the consumer. Moreover, the price of these products has sharply decreased over the last years, and they

may become affordable to most consumers along with companies' scalability and increasing demand.

To the best of our knowledge, currently there are no large-scale randomized trials powered for clinical outcomes, on the horizon involving these products. However, these may be important to assess the health impacts of cell-based meat and reassure the consumers about their safety and potential health benefits. Cell-based meat is produced in a sterile environment without antibiotics and is not handled in slaughterhouses or butcheries, thus has a low risk of contamination by either bacteria, viruses or prions (e.g., salmonella, Escherichia coli, "mad cow" disease, swine flu). Furthermore, the "fat" content of the meat can be controlled in the laboratory. These features are encouraging, but do not eliminate the need for formal testing in experimental models and RCTs to evaluate their potential health impacts compared with traditional and plant-based meat.

Need for randomized evidence

RCTs are the gold standard test of whether or not a treatment is beneficial⁵⁶⁻⁵⁸. The numerous biases and limitations of non-randomised, observational studies have been thoroughly documented, and also apply to food based observational studies⁵⁹. However, with dietary interventions it is challenging to conduct large RCTs with long-term follow-up⁶⁰. Albeit challenging, these large studies are not impossible if they are designed as pragmatic trials with adequate power to accommodate the inherent variations in people's diet. Furthermore, blinding of the allocation should be attempted whenever possible – for instance plant- or cell-based patties are similar to traditional animal meat patties in taste and appearance, and these could be given to participants in a double-blind fashion. Using

digital health technologies to facilitate the acquisition of outcomes can be leveraged to facilitate pragmatic acquisition of outcomes relating to such dietary RCTs^{61,62}.

Moreover, smaller, short-term trials, assessing surrogate markers of disease (e.g., TMAO, LDL cholesterol, blood pressure, body weight, insulin resistance, salt intake, gut microbiome or circulating “multi-omics” markers) can be conducted to compare the effects of the plant- and cell-based meat with traditional animal meat on cardiometabolic risk.

Relevant considerations to take into account when designing dietary RCTs are described in the *Table 2* and the summary of this manuscript’s content is depicted in the *Central Illustration*.

Future directions

The landscape of the meat substitutes is rapidly evolving, this it is important to ensure that these new products are beneficial to human health as well as the health of the planet. Long-term behavioural change should also be assessed, to identify the factors that lead to a person transition (or not) toward plant-based or cell-based products and stick to these products in a long-term basis. A “multi-omics” approach (instead of a single marker approach) could find novel biomarkers reflecting pathophysiologic changes directly linked to meat consumption.

Conclusions

Novel meat alternatives to meat (plant- or cell-based) may provide a similar sensorial experience to traditional meat, but with a potentially smaller environmental impact. Furthermore, plant-based alternatives showed promising short-term results on surrogate markers of cardiovascular disease. Cell-based alternatives are, theoretically, also very

promising, but studies are lacking. Well-designed randomized trials are key to a better understanding of the health impact of these products that might be an alternative to traditional meat in a near future.

Disclosures

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Bibliography

1. Aiello, L.; Wheeler, P., The expensive-tissue hypothesis: The brain and digestive system in human and primate evolution. . In *Current Anthropology*, 1995.
2. Blumenshine, R., Carcass consumption sequences and the archaeological distinction of hunting and scavenging. *Journal of Human Evolution*, 1986.
3. Babbitt, C., Genomic signatures of diet-related shifts during human origins. *Proceedings of the Royal Society B. Biological Sciences* 2011.
4. Werdelin, L.; Lewis, M., Plio-Pleistocene Carnivora of eastern Africa: species richness and turnover patterns. *Zoological Journal of the Linnean Society*, 2005.
5. Schoeninger, M., The Agricultural "Revolution": Its Effect on Human Diet in Prehistoric Iran and Israel. Paléorient, 1981.
6. Food and Agriculture Organization of the United Nations (FAO). <http://www.fao.org/faostat/en/?#data/>, 2020 (online access on the 19/09/2020).
7. Rammohan, A.; Awofeso, N.; Robitaille, M., Addressing Female Iron-Deficiency Anaemia in India: Is Vegetarianism the Major Obstacle? ISRN Public Health, 2011.
8. Ellis, E.; Klein Goldewijk, K.; Siebert, S.; Lightman, D.; Ramankutty, N., Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography*, 2010.
9. Poore, J.; Nemecek, T., Reducing food's environmental impacts through producers and consumers. *Science* **2018**, *360* (6392), 987-992.

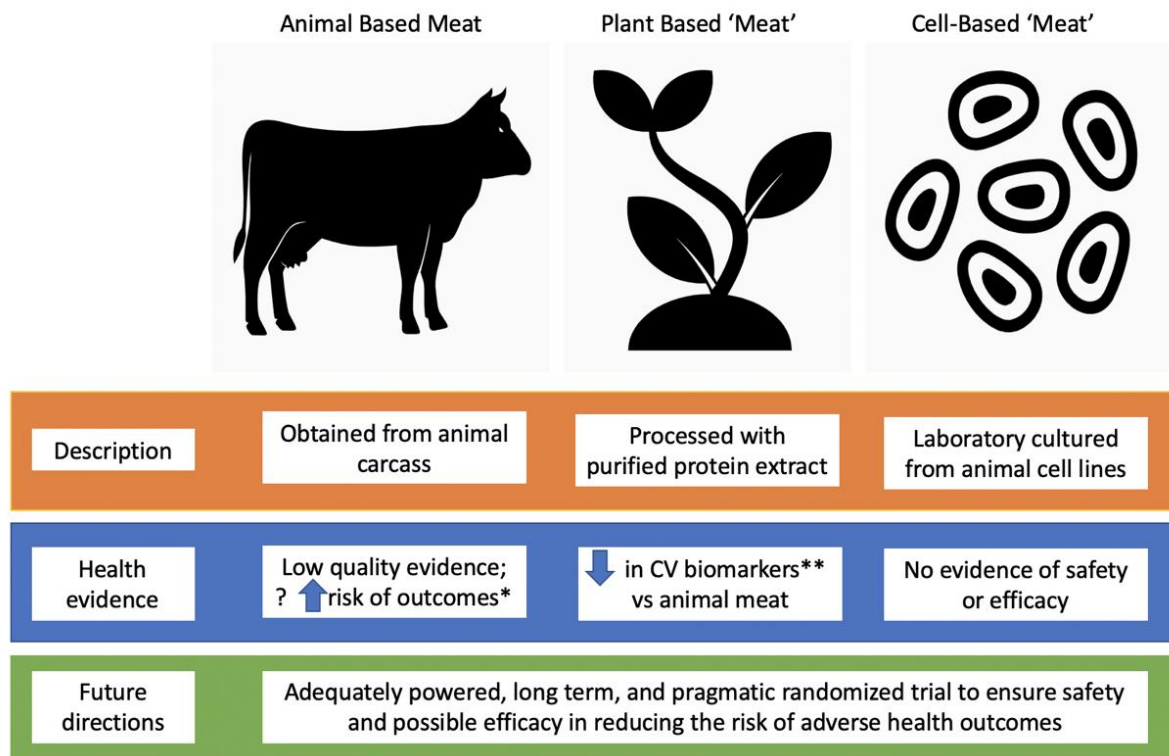
10. Guyatt, G. H.; Oxman, A. D.; Vist, G. E.; Kunz, R.; Falck-Ytter, Y.; Alonso-Coello, P.; Schünemann, H. J., GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *Bmj* **2008**, *336* (7650), 924-6.
11. Katz, D. L.; Karlsen, M. C.; Chung, M.; Shams-White, M. M.; Green, L. W.; Fielding, J.; Saito, A.; Willett, W., Hierarchies of evidence applied to lifestyle Medicine (HEALM): introduction of a strength-of-evidence approach based on a methodological systematic review. *BMC Med Res Methodol* **2019**, *19* (1), 178.
12. Schwingshackl, L.; Knüppel, S.; Schwedhelm, C.; Hoffmann, G.; Missbach, B.; Stelmach-Mardas, M.; Dietrich, S.; Eichelmann, F.; Kontopantelis, E.; Iqbal, K.; Aleksandrova, K.; Lorkowski, S.; Leitzmann, M. F.; Kroke, A.; Boeing, H., Perspective: NutriGrade: A Scoring System to Assess and Judge the Meta-Evidence of Randomized Controlled Trials and Cohort Studies in Nutrition Research. *Adv Nutr* **2016**, *7* (6), 994-1004.
13. Mente, A.; Dehghan, M.; Yusuf, S., Diet and health: the need for new and reliable approaches. *Eur Heart J* **2020**, *41* (28), 2641-2644.
14. Guasch-Ferré, M.; Satija, A.; Blondin, S. A.; Janiszewski, M.; Emlen, E.; O'Connor, L. E.; Campbell, W. W.; Hu, F. B.; Willett, W. C.; Stampfer, M. J., Meta-Analysis of Randomized Controlled Trials of Red Meat Consumption in Comparison With Various Comparison Diets on Cardiovascular Risk Factors. *Circulation* **2019**, *139* (15), 1828-1845.
15. Qian, F.; Riddle, M. C.; Wylie-Rosett, J.; Hu, F. B., Red and Processed Meats and Health Risks: How Strong Is the Evidence? *Diabetes Care* **2020**, *43* (2), 265-271.
16. Abete, I.; Romaguera, D.; Vieira, A. R.; Lopez de Munain, A.; Norat, T., Association between total, processed, red and white meat consumption and all-cause, CVD and IHD mortality: a meta-analysis of cohort studies. *Br J Nutr* **2014**, *112* (5), 762-75.
17. Zeraatkar, D.; Guyatt, G. H.; Alonso-Coello, P.; Bala, M. M.; Rabassa, M.; Han, M. A.; Vernooij, R. W. M.; Valli, C.; El Dib, R.; Johnston, B. C., Red and Processed Meat Consumption and Risk for All-Cause Mortality and Cardiometabolic Outcomes. *Ann Intern Med* **2020**, *172* (7), 511-512.
18. Han, M. A.; Zeraatkar, D.; Guyatt, G. H.; Vernooij, R. W. M.; El Dib, R.; Zhang, Y.; Algarni, A.; Leung, G.; Storman, D.; Valli, C.; Rabassa, M.; Rehman, N.; Parvizian, M. K.; Zworth, M.; Bartoszko, J. J.; Lopes, L. C.; Sit, D.; Bala, M. M.; Alonso-Coello, P.; Johnston, B. C., Reduction of Red and Processed Meat Intake and Cancer Mortality and Incidence: A Systematic Review and Meta-analysis of Cohort Studies. *Ann Intern Med* **2019**, *171* (10), 711-720.
19. Zeraatkar, D.; Johnston, B. C.; Bartoszko, J.; Cheung, K.; Bala, M. M.; Valli, C.; Rabassa, M.; Sit, D.; Milio, K.; Sadeghirad, B.; Agarwal, A.; Zea, A. M.; Lee, Y.; Han, M. A.; Vernooij, R. W. M.; Alonso-Coello, P.; Guyatt, G. H.; El Dib, R., Effect of Lower Versus Higher Red Meat Intake on Cardiometabolic and Cancer Outcomes: A Systematic Review of Randomized Trials. *Ann Intern Med* **2019**, *171* (10), 721-731.
20. Vernooij, R. W. M.; Zeraatkar, D.; Han, M. A.; El Dib, R.; Zworth, M.; Milio, K.; Sit, D.; Lee, Y.; Gomaa, H.; Valli, C.; Swierz, M. J.; Chang, Y.; Hanna, S. E.; Brauer, P. M.; Sievenpiper, J.; de Souza, R.; Alonso-Coello, P.; Bala, M. M.; Guyatt, G. H.; Johnston, B. C., Patterns of Red and Processed Meat Consumption and Risk for Cardiometabolic and Cancer Outcomes: A Systematic Review and Meta-analysis of Cohort Studies. *Ann Intern Med* **2019**, *171* (10), 732-741.
21. Song, M.; Fung, T. T.; Hu, F. B.; Willett, W. C.; Longo, V. D.; Chan, A. T.; Giovannucci, E. L., Association of Animal and Plant Protein Intake With All-Cause and Cause-Specific Mortality. *JAMA Intern Med* **2016**, *176* (10), 1453-1463.
22. Chen, Z.; Glisic, M.; Song, M.; Aliahmad, H. A.; Zhang, X.; Moumdjian, A. C.; Gonzalez-Jaramillo, V.; van der Schaft, N.; Bramer, W. M.; Ikram, M. A.; Voortman, T., Dietary protein intake and all-cause and cause-specific mortality: results from the Rotterdam Study and a meta-analysis of prospective cohort studies. *Eur J Epidemiol* **2020**, *35* (5), 411-429.
23. Zheng, Y.; Li, Y.; Satija, A.; Pan, A.; Sotos-Prieto, M.; Rimm, E.; Willett, W. C.; Hu, F. B., Association of changes in red meat consumption with total and cause specific mortality among US women and men: two prospective cohort studies. *Bmj* **2019**, *365*, l2110.

24. Budhathoki, S.; Sawada, N.; Iwasaki, M.; Yamaji, T.; Goto, A.; Kotemori, A.; Ishihara, J.; Takachi, R.; Charvat, H.; Mizoue, T.; Iso, H.; Tsugane, S., Association of Animal and Plant Protein Intake With All-Cause and Cause-Specific Mortality in a Japanese Cohort. *JAMA Intern Med* **2019**, *179* (11), 1509-1518.
25. Baden, M. Y.; Liu, G.; Satija, A.; Li, Y.; Sun, Q.; Fung, T. T.; Rimm, E. B.; Willett, W. C.; Hu, F. B.; Bhupathiraju, S. N., Changes in Plant-Based Diet Quality and Total and Cause-Specific Mortality. *Circulation* **2019**, *140* (12), 979-991.
26. Estruch, R.; Ros, E.; Salas-Salvadó, J.; Covas, M. I.; Corella, D.; Arós, F.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; Fiol, M.; Lapetra, J.; Lamuela-Raventos, R. M.; Serra-Majem, L.; Pintó, X.; Basora, J.; Muñoz, M. A.; Sorlí, J. V.; Martínez, J. A.; Fitó, M.; Gea, A.; Hernán, M. A.; Martínez-González, M. A., Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. *N Engl J Med* **2018**, *378* (25), e34.
27. Evert, A. B.; Dennison, M.; Gardner, C. D.; Garvey, W. T.; Lau, K. H. K.; MacLeod, J.; Mitri, J.; Pereira, R. F.; Rawlings, K.; Robinson, S.; Saslow, L.; Uelmen, S.; Urbanski, P. B.; Yancy, W. S., Jr., Nutrition Therapy for Adults With Diabetes or Prediabetes: A Consensus Report. *Diabetes Care* **2019**, *42* (5), 731-754.
28. Kahleova, H.; Levin, S.; Barnard, N., Cardio-Metabolic Benefits of Plant-Based Diets. *Nutrients* **2017**, *9* (8).
29. Arnett, D. K.; Blumenthal, R. S.; Albert, M. A.; Buroker, A. B.; Goldberger, Z. D.; Hahn, E. J.; Himmelfarb, C. D.; Khera, A.; Lloyd-Jones, D.; McEvoy, J. W.; Michos, E. D.; Miedema, M. D.; Muñoz, D.; Smith, S. C., Jr.; Virani, S. S.; Williams, K. A., Sr.; Yeboah, J.; Ziaeian, B., 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* **2019**, *140* (11), e596-e646.
30. Bouvard, V.; Loomis, D.; Guyton, K. Z.; Grosse, Y.; Ghissassi, F. E.; Benbrahim-Tallaa, L.; Guha, N.; Mattock, H.; Straif, K., Carcinogenicity of consumption of red and processed meat. *Lancet Oncol* **2015**, *16* (16), 1599-600.
31. Mozaffarian, D., Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity: A Comprehensive Review. *Circulation* **2016**, *133* (2), 187-225.
32. Yusuf, S.; Joseph, P.; Rangarajan, S.; Islam, S.; Mente, A.; Hystad, P.; Brauer, M.; Kutty, V. R.; Gupta, R.; Wielgosz, A.; AlHabib, K. F.; Dans, A.; Lopez-Jaramillo, P.; Avezum, A.; Lanas, F.; Oguz, A.; Kruger, I. M.; Diaz, R.; Yusoff, K.; Mony, P.; Chifamba, J.; Yeates, K.; Kelishadi, R.; Yusufali, A.; Khatib, R.; Rahman, O.; Zatonska, K.; Iqbal, R.; Wei, L.; Bo, H.; Rosengren, A.; Kaur, M.; Mohan, V.; Lear, S. A.; Teo, K. K.; Leong, D.; O'Donnell, M.; McKee, M.; Dagenais, G., Modifiable risk factors, cardiovascular disease, and mortality in 155 722 individuals from 21 high-income, middle-income, and low-income countries (PURE): a prospective cohort study. *Lancet* **2020**, *395* (10226), 795-808.
33. Willett, W.; Rockström, J.; Loken, B.; Springmann, M.; Lang, T.; Vermeulen, S.; Garnett, T.; Tilman, D.; DeClerck, F.; Wood, A.; Jonell, M.; Clark, M.; Gordon, L. J.; Fanzo, J.; Hawkes, C.; Zurayk, R.; Rivera, J. A.; De Vries, W.; Majele Sibanda, L.; Afshin, A.; Chaudhary, A.; Herrero, M.; Agustina, R.; Branca, F.; Lartey, A.; Fan, S.; Crona, B.; Fox, E.; Bignet, V.; Troell, M.; Lindahl, T.; Singh, S.; Cornell, S. E.; Srinath Reddy, K.; Narain, S.; Nishtar, S.; Murray, C. J. L., Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet* **2019**, *393* (10170), 447-492.
34. Godfray, H. C. J.; Aveyard, P.; Garnett, T.; Hall, J. W.; Key, T. J.; Lorimer, J.; Pierrehumbert, R. T.; Scarborough, P.; Springmann, M.; Jebb, S. A., Meat consumption, health, and the environment. *Science* **2018**, *361* (6399).
35. Dunk, J. H.; Jones, D. S.; Capon, A.; Anderson, W. H., Human Health on an Ailing Planet - Historical Perspectives on Our Future. *N Engl J Med* **2019**, *381* (8), 778-782.

36. Singh, S., Meat Substitutes Market worth \$3.5 billion by 2026. <https://www.marketsandmarkets.com/PressReleases/meat-substitutes.asp>, 2020 (online access on the 19/09/2020).
37. Forum, W. E., Meat: the Future series - Alternative Proteins. http://www3.weforum.org/docs/WEF_White_Paper_Alternative_Proteins.pdf, 2019 (online access on the 19/09/2020).
38. Heller; Martin C; Gregory A; Keoleian, Beyond Meat's Beyond Burger Life Cycle Assessment: A detailed comparison between a plant-based and an animal-based protein source. CSS Report, University of Michigan: Ann Arbor 1-38., 2018.
39. Dent M, Plant-based and Cultured Meat 2020-2030. <https://www.idtechex.com/en/research-report/plant-based-and-cultured-meat-2020-2030/702>, 2020 (online access on the 19/09/2020).
40. Van Loo E.J.; Caputo V.; J.L., L., Consumer preferences for farm-raised meat, lab-grown meat, and plant-based meat alternatives: Does information or brand matter? *Food Policy*, 2020.
41. Hall, K. D.; Ayuketah, A.; Brychta, R.; Cai, H.; Cassimatis, T.; Chen, K. Y.; Chung, S. T.; Costa, E.; Courville, A.; Darcey, V.; Fletcher, L. A.; Forde, C. G.; Gharib, A. M.; Guo, J.; Howard, R.; Joseph, P. V.; McGehee, S.; Ouwkerk, R.; Raisinger, K.; Rozga, I.; Stagliano, M.; Walter, M.; Walter, P. J.; Yang, S.; Zhou, M., Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake. *Cell Metab* **2019**, *30* (1), 67-77.e3.
42. Hu, F. B.; Otis, B. O.; McCarthy, G., Can Plant-Based Meat Alternatives Be Part of a Healthy and Sustainable Diet? *Jama* **2019**, 1-3.
43. Wang, Z.; Klipfell, E.; Bennett, B. J.; Koeth, R.; Levison, B. S.; Dugar, B.; Feldstein, A. E.; Britt, E. B.; Fu, X.; Chung, Y. M.; Wu, Y.; Schauer, P.; Smith, J. D.; Allayee, H.; Tang, W. H.; DiDonato, J. A.; Lusic, A. J.; Hazen, S. L., Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **2011**, *472* (7341), 57-63.
44. Tang, W. H.; Wang, Z.; Levison, B. S.; Koeth, R. A.; Britt, E. B.; Fu, X.; Wu, Y.; Hazen, S. L., Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* **2013**, *368* (17), 1575-84.
45. Schiattarella, G. G.; Sannino, A.; Toscano, E.; Giugliano, G.; Gargiulo, G.; Franzone, A.; Trimarco, B.; Esposito, G.; Perrino, C., Gut microbe-generated metabolite trimethylamine-N-oxide as cardiovascular risk biomarker: a systematic review and dose-response meta-analysis. *Eur Heart J* **2017**, *38* (39), 2948-2956.
46. Wang, Z.; Roberts, A. B.; Buffa, J. A.; Levison, B. S.; Zhu, W.; Org, E.; Gu, X.; Huang, Y.; Zamanian-Daryoush, M.; Culley, M. K.; DiDonato, A. J.; Fu, X.; Hazen, J. E.; Krajcik, D.; DiDonato, J. A.; Lusic, A. J.; Hazen, S. L., Non-lethal Inhibition of Gut Microbial Trimethylamine Production for the Treatment of Atherosclerosis. *Cell* **2015**, *163* (7), 1585-95.
47. Roberts, A. B.; Gu, X.; Buffa, J. A.; Hurd, A. G.; Wang, Z.; Zhu, W.; Gupta, N.; Skye, S. M.; Cody, D. B.; Levison, B. S.; Barrington, W. T.; Russell, M. W.; Reed, J. M.; Duzan, A.; Lang, J. M.; Fu, X.; Li, L.; Myers, A. J.; Rachakonda, S.; DiDonato, J. A.; Brown, J. M.; Gogonea, V.; Lusic, A. J.; Garcia-Garcia, J. C.; Hazen, S. L., Development of a gut microbe-targeted nonlethal therapeutic to inhibit thrombosis potential. *Nat Med* **2018**, *24* (9), 1407-1417.
48. Koeth, R. A.; Wang, Z.; Levison, B. S.; Buffa, J. A.; Org, E.; Sheehy, B. T.; Britt, E. B.; Fu, X.; Wu, Y.; Li, L.; Smith, J. D.; DiDonato, J. A.; Chen, J.; Li, H.; Wu, G. D.; Lewis, J. D.; Warriar, M.; Brown, J. M.; Krauss, R. M.; Tang, W. H.; Bushman, F. D.; Lusic, A. J.; Hazen, S. L., Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* **2013**, *19* (5), 576-85.
49. Conlon, M. A.; Bird, A. R., The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* **2014**, *7* (1), 17-44.
50. Miller, C. A.; Corbin, K. D.; da Costa, K. A.; Zhang, S.; Zhao, X.; Galanko, J. A.; Blevins, T.; Bennett, B. J.; O'Connor, A.; Zeisel, S. H., Effect of egg ingestion on trimethylamine-N-oxide

- production in humans: a randomized, controlled, dose-response study. *Am J Clin Nutr* **2014**, *100* (3), 778-86.
51. Koeth, R. A.; Lam-Galvez, B. R.; Kirsop, J.; Wang, Z.; Levison, B. S.; Gu, X.; Copeland, M. F.; Bartlett, D.; Cody, D. B.; Dai, H. J.; Culley, M. K.; Li, X. S.; Fu, X.; Wu, Y.; Li, L.; DiDonato, J. A.; Tang, W. H. W.; Garcia-Garcia, J. C.; Hazen, S. L., l-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *J Clin Invest* **2019**, *129* (1), 373-387.
52. Wang, Z.; Bergeron, N.; Levison, B. S.; Li, X. S.; Chiu, S.; Jia, X.; Koeth, R. A.; Li, L.; Wu, Y.; Tang, W. H. W.; Krauss, R. M.; Hazen, S. L., Impact of chronic dietary red meat, white meat, or non-meat protein on trimethylamine N-oxide metabolism and renal excretion in healthy men and women. *Eur Heart J* **2019**, *40* (7), 583-594.
53. Mertens, E.; Markey, O.; Geleijnse, J. M.; Givens, D. I.; Lovegrove, J. A., Dietary Patterns in Relation to Cardiovascular Disease Incidence and Risk Markers in a Middle-Aged British Male Population: Data from the Caerphilly Prospective Study. *Nutrients* **2017**, *9* (1).
54. Crimarco, A.; Springfield, S.; Petlura, C.; Streaty, T.; Cunanan, K.; Lee, J.; Fielding-Singh, P.; Carter, M. M.; Topf, M. A.; Wastyk, H. C.; Sonnenburg, E. D.; Sonnenburg, J. L.; Gardner, C. D., A randomized crossover trial on the effect of plant-based compared with animal-based meat on trimethylamine-N-oxide and cardiovascular disease risk factors in generally healthy adults: Study With Appetizing Plantfood-Meat Eating Alternative Trial (SWAP-MEAT). *Am J Clin Nutr* **2020**.
55. **USDA and FDA Announce a Formal Agreement to Regulate Cell-Cultured Food Products from Cell Lines of Livestock and Poultry.** <https://www.fda.gov/news-events/press-announcements/usda-and-fda-announce-formal-agreement-regulate-cell-cultured-food-products-cell-lines-livestock-and>, 2019 (online access on the 19/09/2020).
56. Collins, R.; MacMahon, S., Reliable assessment of the effects of treatment on mortality and major morbidity, I: clinical trials. *Lancet* **2001**, *357* (9253), 373-80.
57. Barton, S., Which clinical studies provide the best evidence? The best RCT still trumps the best observational study. *Bmj* **2000**, *321* (7256), 255-6.
58. Rush, C. J.; Campbell, R. T.; Jhund, P. S.; Petrie, M. C.; McMurray, J. J. V., Association is not causation: treatment effects cannot be estimated from observational data in heart failure. *Eur Heart J* **2018**, *39* (37), 3417-3438.
59. Freemantle, N.; Marston, L.; Walters, K.; Wood, J.; Reynolds, M. R.; Petersen, I., Making inferences on treatment effects from real world data: propensity scores, confounding by indication, and other perils for the unwary in observational research. *Bmj* **2013**, *347*, f6409.
60. Ludwig, D. S.; Ebbeling, C. B.; Heymsfield, S. B., Improving the Quality of Dietary Research. *Jama* **2019**.
61. Marquis-Gravel, G.; Roe, M. T.; Turakhia, M. P.; Boden, W.; Temple, R.; Sharma, A.; Hirshberg, B.; Slater, P.; Craft, N.; Stockbridge, N.; McDowell, B.; Waldstreicher, J.; Bourla, A.; Bansilal, S.; Wong, J. L.; Meunier, C.; Kassahun, H.; Coran, P.; Bataille, L.; Patrick-Lake, B.; Hirsch, B.; Reites, J.; Mehta, R.; Muse, E. D.; Chandross, K. J.; Silverstein, J. C.; Silcox, C.; Overhage, J. M.; Califf, R. M.; Peterson, E. D., Technology-Enabled Clinical Trials: Transforming Medical Evidence Generation. *Circulation* **2019**, *140* (17), 1426-1436.
62. Sharma, A.; Harrington, R. A.; McClellan, M. B.; Turakhia, M. P.; Eapen, Z. J.; Steinhubl, S.; Mault, J. R.; Majmudar, M. D.; Roessig, L.; Chandross, K. J.; Green, E. M.; Patel, B.; Hamer, A.; Olgin, J.; Rumsfeld, J. S.; Roe, M. T.; Peterson, E. D., Using Digital Health Technology to Better Generate Evidence and Deliver Evidence-Based Care. *J Am Coll Cardiol* **2018**, *71* (23), 2680-2690.

Central Illustration. Graphical summary of the manuscript's content



Central figure: Meat options available or in the commercialization pipeline.

CV cardiovascular; *Outcomes refers to cardiovascular, cancer, and all cause mortality

** CV biomarkers includes trimethylamine N-oxide and low-density lipoprotein

Table 1. Comparison of the main characteristics of the plant-based burger versus a traditional beef burger.

Characteristics	Beyond Burger	Impossible Burger	Grass-fed beef burger
Serving size	1/4 pound (113 g)	1/4 pound (113 g)	1/4 pound (113 g)
Calories	250	240	240
Total fat	18 g	14 g	14 g
Saturated fat	6 g	8 g	6 g
Cholesterol	0	0	70 mg
Sodium	300 mg	370 mg	77 mg
Carbohydrate	3 g	9 g	0
Fiber	2 g	3 g	0
Protein	20 g	19 g	22 g
Iron	0	4.2 mg	2.7 mg

Sources: <https://faq.impossiblefoods.com/hc/en-us/articles/360018939274-What-are-the-nutrition-facts->; <https://www.healthline.com/nutrition/impossible-burger#nutrition>; <https://www.goodhousekeeping.com/health/diet-nutrition/a29074114/beyond-burger-nutrition-ingredients/>. (Accessed online on the 26th of August 2020).

Table 2. Considerations to take into account when designing dietary randomised controlled trials

Large outcome trials	Small “mechanistic” trials
Diet intervention cross-overs	Underpowered to study major outcome events
Change in dietary patterns with time	Easier to control dietary interventions
Self-reported diet questionnaires	Effects on surrogate markers that may not be associated with outcomes
Bias and confounding due to factors other than diet (e.g., income-inequality)	Carry-over effects in cross-over trials
Lost to follow-up	Sample not representative of the general population
Need for major adverse events that may have low incidence in the population	Not possible to assess the long-term consequences of the dietary intervention