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Dear Dr. Brennan:

The more than 700 medical doctors, researchers, and health professionals signing below request your intervention in what appears to be the persistent and egregious failure on the part of the journal *Nutrients* to adhere to MEDLINE's ethics policies.

As health professionals, we depend on the integrity of journals that report research on nutrition and health. *Nutrients* is one of them. Some of us have also been contributors to or reviewers for *Nutrients* over the years. However, the journal's repeated policy violations have caused us to lose confidence in its integrity, and its refusal to act on repeated complaints has led us to view these problems as systemic and requiring a more fundamental solution.

We ask that you limit or suspend *Nutrients'* participation in MEDLINE until these problems are rectified, noncomplying articles have been retracted, and sound editorial practices are in place. This request is aligned with MEDLINE's policy for reevaluation.¹

We do not bring this request lightly. We recognize that Congress authorizes the National Library of Medicine to "aid the dissemination and exchange of scientific...information important to the progress of medicine and to the public health." The decision to publish a journal on MEDLINE according to its ethics standards is an agency action that must not be "arbitrary or capricious, an abuse of discretion, or otherwise not in accordance with the law." The National Library of Medicine must apply its standards in a consistent and rational manner. It is with this in mind that we respectfully ask for your intervention.

In recent years, there has been a proliferation of online medical publications, and we regret to observe that scientific integrity does not appear to be a core value for all of them. Between January and June 2022, *Nutrients* published 2,545 articles in 12 issues. Its article-processing charge is 2,600 Swiss Francs

(about 2,645 US dollars) per article, which suggests the journal has received approximately \$6.7 million in author fees over this period, the equivalent of more than \$13 million per year.

In 2018, ten senior editors at *Nutrients* resigned due to what was reported to be growing pressures from the publisher MDPI to accept "manuscripts of mediocre quality and importance." In an interview, one of the former editors stated "Since it now costs so much for articles to be published, we were encouraged to publish more to make to make MDPI's profits greater."

An important aspect of the integrity of a journal is its willingness to uphold ethical principles for the conduct and reporting of research, whether the subjects are humans or animals. About 20% of *Nutrients'* publications report animal use.

The standards for inclusion in MEDLINE¹ include, among other provisions, evidence supporting affirmative responses to the following questions:

- Are the journal's ethical policies findable, clearly stated, and consistent with current best practices?
- Are ethical policies adhered to in individual articles?

For *Nutrients*, with regard to policies related to articles reporting animal research, the answer to question 1, we believe, is a qualified yes. The answer to question 2 is no. There is an additional relevant policy:

• Is the rationale/justification for conducting the study clear?

Again, the answer to this question is often no, as well.

We have found that the journal routinely publishes research articles that violate its own ethical standards as well as generally accepted standards for research ethics and fails to correct transgressions that are brought to its attention. The net effects are (1) readers are misled regarding the ethical approvals received, (2) readers have no confidence in the quality of research published, (3) the journal effectively communicates to potential authors—including students and early-career investigators—that ethical standards are meaningless and that ethical violations pose no barrier to publication, (4) the journal communicates to readers, including young scientists, that unnecessary, cruel, and even sadistic experiments are routine parts of the scientific enterprise, (5) scientific investigation is degraded by the publication of studies that do not meet appropriate standards, and (6) animals are harmed and killed in the process, without justification.

Nutrients' Instructions for Authors include the following provisions related to articles reporting the use of animals in research:

Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed

are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs [1]':

Replacement of animals by alternatives wherever possible, Reduction in number of animals used, and Refinement of experimental conditions and procedures to minimize the harm to animals.⁵

We note for particular attention the first of the "3Rs," above, related to the replacement of animals by alternatives wherever possible. This central tenet of animal research ethics is explicitly incorporated into *Nutrients'* requirements. In addition, section 2143 of the Animal Welfare Act and C.F.R. Title 9, Section 2.31(d)(ii, iii) of the Animal Welfare Act's implementing regulations require that the principal investigator consider alternatives to any animal used for research or educational purposes and that the principal investigator does not duplicate previous experiments. While federal regulations call on investigators to consider alternatives, *Nutrients'* guidelines explicitly call for alternatives to be used whenever possible.

In virtually every issue, *Nutrients* publishes articles that violate the journal's ethical guidelines. In some cases, the study methods could be considered by objective observers to constitute sadistic behavior inflicted on small animals in the guise of science. As one example, *Nutrients* recently published a submission from the manufacturer of saffron-based nutrition supplements. The supplements were purported to improve mood. Rather than administering saffron to volunteers and tracking depressive symptoms with validated instruments as other researchers have done, the manufacturer force-fed its supplement to mice via gavage, dropped them in water tanks, and then timed the duration of the animals' panicked struggles as a crude index of mood. The journal's ethics guidelines should have barred this publication for violating the principle of "replacement of animals by alternatives wherever possible." However, the article was indeed published. Complaints to the journal about this ethical violation were rebuffed.

In another recent publication, experimenters ran mice and rats to exhaustion to determine whether force-feeding probiotic bacteria would reduce the animals' anxiety in the process. Probiotics are commonly sold for human consumption and their effects are readily studied in human volunteers. In another study, experimenters purported to investigate relationships between gestational diabetes and postpartum depression by injecting rats with streptozotocin to induce diabetes, then impregnating them and, after parturition, forcing the animals into behavioral tests, including the forced-swim test described above. In none of these areas does the pursuit of the research objectives require animal use.

Another recent *Nutrients* article reported an experiment in which western- and Mediterranean-like diets were fed to cynomolgus macaques with the aim of producing information about the diets' effects on human behavior, despite the fact that such diets are commonly used in human research studies and both physical and psychological endpoints can be readily assessed without the use of animals.

When investigators use animals instead of human research methods that could ethically address their research goals, they drive medical investigation away from the species of interest and toward other species whose biological responses may differ and often use scientifically unsatisfactory methods.

In Appendix I to this letter, we have described many such articles from several *Nutrients* volumes and issues. There are many more.

Over the past year, the Physicians Committee has brought to the attention of *Nutrients'* editors numerous examples of articles in its publication that ignore its explicit ethical guidelines. Despite repeated communications on our part, we have seen no evidence of an appropriate response.

In short, there is a serious ethical problem at *Nutrients* that the journal has refused to address, and its continued inclusion on MEDLINE maintains an impression that its practices are acceptable to the National Library of Medicine and are routine in the scientific world.

We anticipate the question as to whether approval by an institutional animal care and use committee is sufficient for concluding that a study has complied with a journal's ethical guidelines. It is not, nor do *Nutrients'* guidelines suggest that it would be. Such committees are often perfunctory and may not adequately review animal use protocols. Rather, the journal's review process and editorial decision-making must take the journal's guidelines and basic ethical research guidelines into account and apply them consistently. To be clear: MEDLINE's requirements that a journal's ethical policies be findable, clearly stated, and consistent with current best practices, and that ethical policies be adhered to in individual articles are not satisfied by approval by an institutional animal care and use committee.

We, therefore, request that the National Library of Medicine reevaluate *Nutrients'* participation in MEDLINE. We suggest that you consider removing *Nutrients* articles reporting animal use from consideration for listing in MEDLINE because the ethical nature of these studies is gravely in doubt. It would also be a reasonable decision to remove the journal from MEDLINE altogether. During this reevaluation process, we request that *Nutrients* participation in MEDLINE be suspended. The journal's continued presence on MEDLINE erodes confidence in MEDLINE and the National Library of Medicine, which promulgates and enforces standards for inclusion.

We are grateful to the National Library of Medicine for its attention to this matter. We look forward to your reply and would be pleased to assist you further. For the signatories below, affiliations are listed for identification only.

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Appendix I. Examples of research papers published in *Nutrients* that violate the journal's Ethical Guidelines for the Use of Animals in Research

Nutrients' Ethical Guidelines for the Use of Animals in Research state "The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs':

- Replacement of animals by alternatives wherever possible,
- · Reduction in number of animals used, and
- Refinement of experimental conditions and procedures to minimize the harm to animals."5

The examples below demonstrate violations of these Guidelines.

Volume 12, Issue 2

Short-Term Caloric Restriction Attenuates Obesity-Induced Pro-Inflammatory Response in Male Rhesus Macaques

Wright H, Handu M, Jankeel A, Messaoudi I, Varlamov O. Short-term caloric restriction attenuates obesity-induced pro-inflammatory response in male rhesus macaques. *Nutrients*. 2020; 12(2):511. https://doi.org/10.3390/nu12020511

This study aimed to demonstrate that short-term caloric restriction is an efficient approach for mitigating obesity-induced pro-inflammatory response. The researchers intended to apply their findings to humans. To do this, researchers isolated rhesus macaques to mimic a sedentary lifestyle, then fed them a high-fat "western-style diet" for six months to induce obesity. Afterward, the animals were placed on an energy-restricted diet for four months. Throughout the study, researchers collected biopsies of the animal's adipose tissue.

Animals were not required in order to address the research aims. Tests of caloric restriction have been conducted in human volunteers in clinical trials of varying duration. Inflammatory markers are readily measured in blood or tissue samples that can be ethically obtained. Many such human studies have been done, ^{8,9,10} and the retreat to another species presents no unique advantage. This study violates the principle regarding replacement of animals by alternatives wherever possible.

Volume 13, Issue 1

Beneficial Effects of a Mixture of Algae and Extra Virgin Olive Oils on the Age-Induced Alterations of Rodent Skeletal Muscle: Role of HDAC-4

González-Hedström D, Priego T, López-Calderón A, Amor S, de la Fuente-Fernández M, Inarejos-García AM, et al. Beneficial effects of a mixture of algae and extra virgin olive oils on the age-induced alterations of rodent skeletal muscle: Role of HDAC-4. Nutrients. 2021;13(1):44. doi: 10.3390/nu13010044

In this study, rats were force-fed algae oil and/or olive oil via gavage to investigate whether a mixture of oils can reduce age-induced impairments in skeletal muscle physiology. This study is not consistent with the 3Rs because both algae oil supplements and extra virgin olive oil are safe for human consumption, and abundant prior human studies have examined the effects of oil intake on skeletal muscle in aging adults. Several human-based in vitro models have also been developed to study the molecular aspects of skeletal muscle cells and understand the mechanisms of sarcopenia using primary muscle cells from humans. This study violates the principle regarding replacement of animals by alternatives wherever possible.

Volume 13, Issue 3

Saffron Extract-Induced Improvement of Depressive-like Behavior in Mice is Associated with Modulation of Monoaminergic Neurotransmission

Monchaux De Oliveira C, Pourtau L, Vancassel S, Pouchieu C, Capuron L, Gaudout D, et al. Saffron extract-induced improvement of depressive-like behavior in mice Is associated with modulation of monoaminergic neurotransmission. *Nutrients*. 2021; 13(3):904. doi: 10.3390/nu13030904

This article reports an attempt to gauge saffron's anti-depressant effects by force-feeding saffron to mice, then putting the mice in water in the "forced swim test" as a crude gauge of mood. The study is not consistent with the 3Rs or Nutrients' ethical policies because the research goals can readily be addressed without the use of animals. Abundant prior research in humans has already established that saffron is safe for therapeutic use and has already provided data regarding potential efficacy for depression. A systematic review analyzed 23 studies and demonstrated that saffron had a large positive effect size when compared with placebo for depressive symptoms. Another systematic review included eight randomized controlled trials and concluded that saffron was as effective in treating depression as fluoxetine. Nutrients' reviewers and editors should have recognized this article as a violation of its ethical policies related to the use of animals in research.

Volume 13, Issue 5

Gut Microbiota Induced by Pterostilbene and Resveratrol in High-Fat-High-Fructose Fed Rats: Putative Role in Steatohepatitis Onset

Milton-Laskibar I, Marcos-Zambrano LJ, Gómez-Zorita S, Fernández-Quintela A, Carrillo de Santa Pau E, Martínez JA, et al. Gut microbiota induced by pterostilbene and resveratrol in high-fat-high-fructose fed rats: Putative role in steatohepatitis onset. Nutrients 2021;13(5). doi: 10.3390/nu13051738

The investigators aimed to study the metabolic effects of pterostilbene and resveratrol—two natural compounds found in plants and sold as nutritional supplements—on gut microbiota in rats displaying non-alcoholic fatty liver disease (NAFLD). To do this, they fed rats a diet high in saturated fat and fructose over an eight-week period in order to induce NAFLD. Some rats also received pterostilbene or resveratrol.

The rationale for the study given by the study's investigators was that lifestyle factors such as diet can influence intestinal microbiota composition, with possible influence on body weight homeostasis and subsequently liver diseases. This study is not consistent with the 3Rs because the research objectives can be readily studied in humans. Prior studies in humans have already investigated the use of that resveratrol for the management of NAFLD. A meta-analysis reviewed seven randomized clinical trials with a total of 302 patients with NAFLD.¹³

Volume 13, Issue 6

Probiotic Supplementation and High-Intensity Interval Training Modify Anxiety-like Behaviors and Corticosterone in High-Fat Diet-Induced Obesity Mice

Foroozan P, Koushkie Jahromi M, Nemati J, Sepehri H, Safari MA, Brand S. Probiotic supplementation and high-intensity interval training modify anxiety-like behaviors and corticosterone in high-fat diet-induced obesity mice. Nutrients. 2021;13(6):1762-1778. https://doi.org/10.3390/nu13061762

The researchers aimed to study the effect of probiotic supplementation and high-intensity interval training on anxiety-like behaviors in obese mice. To do so, mice and rats were given probiotic supplements by gavage and forced to do high-intensity interval training via running on a treadmill for 30-minute intervals. The animals were also put through open field tests and elevated plus maze tests.

This study is not consistent with the 3Rs because the objectives of the study can be readily addressed without animal use. Abundant prior research in humans has examined the effects of high-intensity interval training on stress, anxiety, and depression, the effects of the gut microbiome on depression and anxiety, and the influences of diet alterations on mood. Hundreds of clinical trials have studied the effect of physical activity on anxiety, and numerous clinical trials have explored

the association between probiotic supplementation and anxiety. To the extent further research is of interest in this area, animal use is not ethically justifiable given the ready availability of other methods.

Volume 13, Issue 11

Milk Formula Diet Alters Bacterial and Host Protein Profile in Comparison to Human Milk Diet in Neonatal Piglet Model

Rosa F, Zybailov BL, Glazko GV, Rahmatallah Y, Byrum S, Mackintosh SG, et al. Milk formula diet alters bacterial and host protein profile in comparison to human milk diet in neonatal piglet model. Nutrients. 2021;13(11):3718. https://doi.org/10.3390/nu13113718

This article reports an attempt to investigate the interaction between neonatal diets and gut microbial activity. The investigators specifically intended to apply their findings to humans. To do this, the investigators fed neonatal pigs pasteurized human milk or Similac dairy-based infant formula. After 21 days, all the pigs were killed for analysis. This study is not consistent with the 3Rs or Nutrients' ethical principles because differences in gut microbiota in breast-fed and formula-fed infants have been studied extensively in clinical studies. For instance, a publication from the CHILD cohort study, including a subset of 1249 mother-infant pairs, used a multi-analytic approach to associate breastfeeding practices and milk microorganisms with infant gut microbiota. ¹⁴ Similarly, a meta-analysis examined seven microbiome studies with a total of 1825 stool samples of 684 infants from five countries and found consistent differences between breast-fed and non-breast-fed infants in gut microbial diversity, microbiota age, microbial composition, and microbial predicted functional pathways. ¹⁵ Furthermore, several in vitro gut models have been developed to study the interactions between bacteria and the cecal mucus commensal microbiota. ¹⁶

Volume 13, Issue 12

Fruit and Vegetable Supplemented Diet Modulates the Pig Transcriptome and Microbiome After a Two-Week Feeding Intervention

Solano-Aguilar GI, Lakshman S, Shao J, Chen C, Beshah E, Dawson HD, et al. Fruit and vegetable supplemented diet modulates the pig transcriptome and microbiome after a two-week feeding intervention. Nutrients. 2021; 13(12):4350. https://doi.org/10.3390/nu13124350

In this study, experimenters used pigs to evaluate the effect of a two-week intervention diet of fruit and vegetable consumption on the pig's whole blood cell transcriptome and fecal microbiome. The research was specifically intended to be relevant to human nutrition. To do this, pigs were fed either a standard diet or a standard diet supplemented with fruits and vegetables. Investigators collected blood and fecal samples to carry out their analysis. This study is not consistent with the 3Rs because the aims of the study could have been readily achieved with human participants. It is well established that diet changes, including the consumption of vegetables and fruits, influence the gut microbiome. A meta-analysis analyzed 27 human-based studies, reporting that increased polyphenol intake increased the abundance of human gut microbiota, contributing to benefits in human health.¹⁷ Furthermore, a complex human intestine-on-a-chip has been developed and is being used to study the development of microbiome-related therapeutics, probiotics, and nutraceuticals.¹⁸

High-Fructose, High-Fat Diet Alters Muscle Composition and Fuel Utilization in a Juvenile Iberian Pig Model of Non-Alcoholic Fatty Liver Disease

Spooner HC, Derrick SA, Maj M, Manjarín R, Hernandez GV, Tailor DS, et al. High-fructose, high-fat diet alters muscle composition and fuel utilization in a juvenile iberian pig model of non-alcoholic fatty liver disease. Nutrients. 2021; 13(12):4195. https://doi.org/10.3390/nu13124195

The aim of this study was to determine the effect of a "Western-style diet" on pediatric skeletal muscle composition and metabolism. To do this, the researchers fed juvenile pigs either a control diet or a high-fructose, high-fat diet for 10 weeks. Then the pigs were killed to examine the connection between "Western diet"-induced pediatric NAFLD and altered skeletal muscle fuel utilization and lipid storage. This study is not consistent with the 3Rs because human studies have already

considered these endpoints and demonstrated the capacity for nonanimal methods to advance this area of research. It has already been determined that a high-fat and high-sugar diet promotes atrophy of skeletal muscle and induces protein degradation and peripheral inflammation.¹⁹ A prolonged high-fructose and high-fat diet accelerates skeletal muscle atrophy, and function, and impairs peripheral glucose transport. Additionally, the Korean Sarcopenic Obesity Study is a large prospective observational cohort study that is studying the association between non-alcoholic fatty liver disease and sarcopenia.²⁰

Volume 14, Issue 1

Jasmine Tea Attenuates Chronic Unpredictable Mild Stress-Induced Depressive-like Behavior in Rats via the Gut-Brain Axis

Zhang Y, Huang J, Xiong Y, Zhang X, Lin Y, Liu Z. Jasmine Tea Attenuates Chronic Unpredictable Mild Stress-Induced Depressive-like Behavior in Rats via the Gut-Brain Axis. *Nutrients*. 2021 Dec 27;14(1):99. doi: 10.3390/nu14010099.

The authors used rats to evaluate the effect of jasmine tea on chronic unpredictable mild stress-induced depression using behavioral tests and analyzing neurotransmitters and gut microorganisms. Among these tests was the forced swim test, a controversial and inaccurate method to identify depressive-like behavior in animals.

It is readily apparent that animals were not required to address the study aims.

Volume 14, Issue 2

Centenarian-Sourced Lactobacillus casei Combined with Dietary Fiber Complex Ameliorates Brain and Gut Function in Aged Mice

Ren M, Li H, Fu Z, Li Q. Centenarian-Sourced Lactobacillus casei Combined with Dietary Fiber Complex Ameliorates Brain and Gut Function in Aged Mice. *Nutrients*. 2022; 14(2):324. https://doi.org/10.3390/nu14020324

The authors used older mice to study the potential roles of a probiotic and the dietary fiber complex on brain and gut function. Over the 12-week study, the mice were gavaged daily and forced to complete the Morris Water Maze Test numerous times. It is clear that animals were not required to address the study aims.

Volume 14, Issue 3

Sargassum plagiophyllum Extract Enhances Colonic Functions and Modulates Gut Microbiota in Constipated Mice

Khuituan P, Huipao N, Jeanmard N, Thantongsakul S, Promjun W, Chuthong S, Tipbunjong C, Peerakietkhajorn S. Sargassum plagiophyllum Extract Enhances Colonic Functions and Modulates Gut Microbiota in Constipated Mice. *Nutrients*. 2022; 14(3):496. https://doi.org/10.3390/nu14030496

In this study, the authors used mice to investigate the effects of Sargassum plagiophyllum extract on functions of the gastrointestinal tract and gut microbiota. Sargassum plagiophyllum is an alga that is safe for human consumption therefore this study could have easily been conducted on human volunteers.

Volume 14, Issue 4

Time-of-Day Circadian Modulation of Grape-Seed Procyanidin Extract (GSPE) in Hepatic Mitochondrial Dynamics in Cafeteria-Diet-Induced Obese Rats

Rodríguez RM, Cortés-Espinar AJ, Soliz-Rueda JR, Feillet-Coudray C, Casas F, Colom-Pellicer M, Aragonès G, Avila-Román J, Muguerza B, Mulero M, Salvadó MJ. Time-of-Day Circadian Modulation of Grape-Seed Procyanidin Extract (GSPE) in Hepatic

Mitochondrial Dynamics in Cafeteria-Diet-Induced Obese Rats. *Nutrients*. 2022; 14(4):774. https://doi.org/10.3390/nu14040774

The authors state their study purpose as "we wondered whether some of the beneficial effects of grape-seed procyanidin extract (GSPE) on metabolic syndrome could be mediated by a circadian modulation of mitochondrial homeostasis." They used 96 rats to evaluate changes in mitochondrial function. The study aims could have been addressed in clinical studies without the use of animals.

Volume 14, Issue 5

Soy Formula Is Not Estrogenic and Does Not Result in Reproductive Toxicity in Male Piglets: Results from a Controlled Feeding Study

Ronis MJJ, Gomez-Acevedo H, Shankar K, Hennings L, Sharma N, Blackburn ML, et al. Soy Formula Is Not Estrogenic and Does Not Result in Reproductive Toxicity in Male Piglets: Results from a Controlled Feeding Study. *Nutrients*. 2022; 14(5):1126. https://doi.org/10.3390/nu14051126

This study aimed to determine whether soy formula is estrogenic and if it can significantly alter male reproductive development. The experimenters intended to apply the results to humans. To do this, the investigators fed male piglets cow's milk or soy formula, then killed them to carry out their analysis.

Numerous human studies have investigated the effects of soy formula in human infants. Hormone-related endpoints have been part of such studies. As one of many examples, a clinical trial that included 50 boys and 51 girls concluded that no early infant feeding effects were found on reproductive organ volumes and structural characteristics in children by age five. Another longitudinal study followed infants for up to nine months and found no significant differences between boys fed cow-milk and those fed soy formula and that estradiol was not detectable. As these human studies suggest, all relevant endpoints can be addressed in clinical studies, and animals are not required.

Volume 14, Issue 6

Probiotic Strains Isolated from an Olympic Woman's Weightlifting Gold Medalist Increase Weight Loss and Exercise Performance in a Mouse Model

Lin W-Y, Kuo Y-W, Lin J-H, Lin C-H, Chen J-F, Tsai S-Y, et al. Probiotic Strains Isolated from an Olympic Woman's Weightlifting Gold Medalist Increase Weight Loss and Exercise Performance in a Mouse Model. *Nutrients*. 2022; 14(6):1270. https://doi.org/10.3390/nu14061270

The authors used 144 mice to test the weight loss and exercise performance enhancement effectiveness of probiotic strains of different origins, including four isolated from an Olympic weightlifter. The mice were gavaged with the probiotic strains and then forced to perform several tests that would likely be characterized as cruel by dispassionate observers.

Effect of Gestational Diabetes on Postpartum Depression-like Behavior in Rats and its Mechanism

Zhao R, Zhou Y, Shi H, Ye W, Lyu Y, Wen Z, et al. Effect of gestational diabetes on postpartum depression-like behavior in rats and its mechanism. *Nutrients*. 2022; 14(6):1229. https://doi.org/10.3390/nu14061229

This study intended to determine the relationship between gestational diabetes mellitus and the development of postpartum depression, aiming to provide a basis for prevention and early intervention. Humans were the species of interest. However, to do this investigation, rats were fed a high-fat diet and injected with streptozotocin to induce diabetes, then rats were mated. After the females gave birth, they were forced into several behavioral tests, including the elevated maze test, the forced swim test, and the sucrose preference test, all of which are controversial in the scientific literature.

It is clear that animals were not required in order to address the study aims, as human studies can address them readily. As the editors and reviewers would be well aware, the relationship between gestational diabetes and postpartum depression has been investigated in numerous human studies. The National Institute of Child Health and Human Development Fetal Growth Studies-Singleton cohort found a modest association between depressive symptoms early in pregnancy and an increased risk of incident gestational diabetes, as well as between gestational diabetes and subsequent postpartum depression risk.²³ Another cohort study that included 1,066 women found that mothers diagnosed with gestational diabetes mellitus have an elevated risk of developing postpartum depression symptoms.²⁴

Volume 14, Issue 7

Evaluation of the Safety of a Plant-Based Infant Formula Containing Almonds and Buckwheat in a Neonatal Piqlet Model

Rosa F, Yelvington B, Terry N, Tripp P, Pittman HE III, Fay BL, Ross TJ, Sikes JD, Flowers JB, Bar-Yoseph F, Yeruva L. Evaluation of the Safety of a Plant-Based Infant Formula Containing Almonds and Buckwheat in a Neonatal Piglet Model. *Nutrients*. 2022; 14(7):1499. https://doi.org/10.3390/nu14071499

This article describes an experiment on piglets who were given either dairy-based or plant-based infant formula to determine if the plant-based formula supported similar development and growth compared with piglets fed dairy-based milk formula.

Animals are clearly not required for such studies and are, moreover, not suitable for infant growth studies if the goal is to apply the results to human infants. Human-based research methods are readily available and are routinely used. Therefore this study constitutes a violation of the journal's ethical guidelines.

Volume 14, Issue 8

Network Pharmacology Exploration Reveals Gut Microbiota Modulation as a Common Therapeutic Mechanism for Anti-Fatigue Effect Treated with Maca Compounds Prescription

Zhu H, Wang R, Hua H, Cheng Y, Guo Y, Qian H, Du P. Network pharmacology exploration reveals gut microbiota modulation as a common therapeutic mechanism for anti-fatigue effect treated with maca compounds prescription. *Nutrients*. 2022; 14(8):1533. https://doi.org/10.3390/nu14081533

The study aimed to investigate effects of maca compounds on gut bacteria and fatigue. To do this, the authors tied lead blocks to the tails of mice, dropped them into water, and watched as some of the mice swam in vain for as long as 25 minutes before sinking. The researchers reported on the bacterial populations in feces.

The study would likely be viewed as sadistic by dispassionate observers. With regard to the journal's ethics guidelines, it is noteworthy that maca compounds are available for human use, and fecal samples are readily obtained. This study should have been rejected as a violation of Nutrients' ethical policies.

Volume 14, Issue 9

The Administration of Panax Ginseng Berry Extract Attenuates High-Fat-Diet-Induced Sarcopenic Obesity in C57BL/6 Mice

Shin J-E, Jeon S-H, Lee S-J, Choung S-Y. The Administration of Panax Ginseng Berry Extract Attenuates High-Fat-Diet-Induced Sarcopenic Obesity in C57BL/6 Mice. *Nutrients*. 2022; 14(9):1747. https://doi.org/10.3390/nu14091747

The authors describe their study as a means to address the combination of obesity and sarcopenia and to evaluate the possible clinical value of ginseng berry extract for this condtion. To create a model for obesity, they fed mice a high-fat diet for nine weeks. They then added ginseng berry extract for an additional four weeks. To access muscle strength, mice were

lifted by the tail and placed on a grid and were then pulled horizontally until their grip was broken.

Obesity and sarcopenia can be and have been extensively studied in humans, and ginseng extracts can be and have been used in human trials. Animals were not required to address the study outcomes.

Volume 14, Issue 11

Prevention of Ulcerative Colitis in Mice by Sweet Tea (Lithocarpus litseifolius) via the Regulation of Gut Microbiota and Butyric-Acid-Mediated Anti-Inflammatory Signaling

He X-Q, Liu D, Liu H-Y, Wu D-T, Li H-B, Zhang X-S, Gan R-Y. Prevention of Ulcerative Colitis in Mice by Sweet Tea (Lithocarpus litseifolius) via the Regulation of Gut Microbiota and Butyric-Acid-Mediated Anti-Inflammatory Signaling. *Nutrients*. 2022; 14(11):2208. https://doi.org/10.3390/nu14112208

This study used mice to investigate the preventive effect and potential mechanism of sweet tea extract against ulcerative colitis. First, the mice were given dextran sulfate sodium to induce ulcerative colitis then the mice received the sweet tea extract via gavage for 14 days. This study could have easily been done with humans.

Volume 14, Issue 14

Mediterranean Diet Reduces Social Isolation and Anxiety in Adult Female Nonhuman Primates

Johnson CSC, Frye BM, Register TC, Snyder-Mackler N, Shively CA. Mediterranean Diet Reduces Social Isolation and Anxiety in Adult Female Nonhuman Primates. *Nutrients*. 2022; 14(14):2852. https://doi.org/10.3390/nu14142852

This article described the effects of a Mediterranean diet on the behavior of monkeys, aiming to draw conclusions that would be important for understanding effects on humans. Specifically, the experimenters fed 38 female cynomolgus macaques either a Mediterranean diet or a westernized diet for more than two years and conducted a series of behavioral tests. These observations followed on previous reports by the investigators regarding diet effects on body weight, body fat, insulin resistance, and hepatosteatosis, fecal bacterial populations, responses to psychosocial stress, and other factors. Animals are not necessary for such research. These diets are frequently studied in clinical trials, and physical and psychological responses to diet changes are readily studied in detail in humans.

Appendix II. Summary of Requests to Nutrients for Action on Apparent Violations

This list summarizes communications the Physicians Committee has had with *Nutrients*. The list includes the research articles in question, the date(s) contact was made, and *Nutrients'* actions. For each manuscript, the Physicians Committee sent *Nutrients* a letter describing the study, explaining the relevant violations of *Nutrients'* ethical policies, and summarizing how the research aims could have been met without the use of animals.

Monchaux De Oliveira C, Pourtau L, Vancassel S, Pouchieu C, Capuron L, Gaudout D, et al. Saffron extract-induced improvement of depressive-like behavior in mice Is associated with modulation of monoaminergic neurotransmission. *Nutrients*. 2021; 13(3):904.

Initial contact: May 2021

Follow up: June 2021, July 2021, November 2021

Nutrients' action: No response

Foroozan P, Koushkie Jahromi M, Nemati J, Sepehri H, Safari MA, Brand S. Probiotic supplementation and high-intensity interval training modify anxiety-like behaviors and corticosterone in high-fat diet-induced obesity mice. *Nutrients*. 2021;13(6):1762-1778.

Initial contact: June 2021

Follow up: July 2021, August 2021, November 2021

Nutrients' action: No response

Milton-Laskibar I, Marcos-Zambrano LJ, Gómez-Zorita S, Fernández-Quintela A, Carrillo de Santa Pau E, Martínez JA, et al. Gut microbiota induced by pterostilbene and resveratrol in high-fat-high-fructose fed rats: Putative role in steatohepatitis onset. *Nutrients* 2021;13(5).

Initial contact: July 2021

Follow up: August 2021, September 2021, November 2021

Nutrients' action: No response

López-Fernández-Sobrino R, Soliz-Rueda JR, Suárez M, Mulero M, Arola L, Bravo FI, et al. Blood pressure-lowering effect of wine lees: Dose-response study, effect of dealcoholization and possible mechanisms of action. *Nutrients*. 2021; 13(4):1142.

Initial contact: August 2021

Follow up: September 2021, November 2021, January 2022

Nutrients' action: No response

González-Hedström D, Priego T, López-Calderón A, Amor S, de la Fuente-Fernández M, Inarejos-García AM, et al. Beneficial effects of a mixture of algae and extra virgin olive oils on the age-induced alterations of rodent skeletal muscle: Role of HDAC-4. *Nutrients*. 2021;13(1):44.

Initial contact: October 2021

Follow up: November 2021, January 2022

Nutrients' action: No response

November 2021, the Physicians Committee sent a letter to Nutrients summarizing all the previous complaints.

Mosa Zhang, Managing Editor of Nutrients, responded, saying that *Nutrients* had established a committee to investigate these issues. The committee stated "animal studies are useful to understand mechanisms and we can't avoid publishing papers using animal models, because they are needed. Besides, the authors explained that the experimental procedure has been approved by Ethics Committee or Institutional Review Board." They also stated "The research we publish follows the

relevant national or international guidelines. Research procedures must be carried out in accordance with national and institutional regulations. Statements on animal welfare should confirm that the study complied with all relevant legislation."

December 2021: The Physicians Committee responded to *Nutrients*, pointing out that the points above are not responsive to the ethical violations in question and do not take into account either the letter or the spirit of the journal's ethical guidelines.

No response.

Rosa F, Zybailov BL, Glazko GV, Rahmatallah Y, Byrum S, Mackintosh SG, et al. Milk formula diet alters bacterial and host protein profile in comparison to human milk diet in neonatal piglet model. *Nutrients*. 2021;13(11):3718.

Initial contact: January 2022

Nutrients' action: *Nutrients* replied stating that the editorial processing committee and Editor-in-Chief are discussing and investigating this issue. There was no further action or response.

Solano-Aguilar GI, Lakshman S, Shao J, Chen C, Beshah E, Dawson HD, Vinyard B, Schroeder SG, Jang S, Molokin A, Urban JF Jr. Fruit and Vegetable Supplemented Diet Modulates the Pig Transcriptome and Microbiome after a Two-Week Feeding Intervention. Nutrients. 2021; 13(12):4350.

Initial contact: March 2022

Nutrients' action: *Nutrients* replied stating that the editorial processing committee and Editor-in-Chief will discuss. There was no further action or response.

Ronis MJJ, Gomez-Acevedo H, Shankar K, Hennings L, Sharma N, Blackburn ML, Miousse I, Dawson H, Chen C, Mercer KE, Badger TM. Soy Formula Is Not Estrogenic and Does Not Result in Reproductive Toxicity in Male Piglets: Results from a Controlled Feeding Study. Nutrients. 2022; 14(5):1126.

Initial contact: May 2022

Nutrients' action: Nutrients replied stating the manuscript received ethics committee approval.

Follow up: June 2021, July 2021

Rosa F, Yelvington B, Terry N, Tripp P, Pittman HE III, Fay BL, Ross TJ, Sikes JD, Flowers JB, Bar-Yoseph F, Yeruva L. Evaluation of the Safety of a Plant-Based Infant Formula Containing Almonds and Buckwheat in a Neonatal Piglet Model. Nutrients. 2022; 14(7):1499.

Initial contact: May 2022

Nutrients' action: Nutrients replied stating the manuscript received ethics committee approval.

Follow up: June 2021, July 2021

June 2022: Physicians Committee asked *Nutrients* for an update on its intended actions, if any. We note that the issues raised are straightforward, are routine parts of editorial review, and extend back more than a year.

Johnson CSC, Frye BM, Register TC, Snyder-Mackler N, Shively CA. Mediterranean Diet Reduces Social Isolation and Anxiety in Adult Female Nonhuman Primates. Nutrients. 2022; 14(14):2852.

Initial contact: August 2022

Rafało-Ulińska, A., Pochwat, B., Misztak, P., Bugno, R., Kryczyk-Poprawa, A., Opoka, W., ... & Szewczyk, B. (2022). Zinc Deficiency Blunts the Effectiveness of Antidepressants in the Olfactory Bulbectomy Model of Depression in Rats. Nutrients, 14(13), 2746.

Initial contact: August 2022

Baker JA, Breit KR, Bodnar TS, Weinberg J, Thomas JD. Choline Supplementation Modifies the Effects of Developmental Alcohol Exposure on Immune Responses in Adult Rats. Nutrients. 2022;14(14):2868.

Initial contact: August 2022

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