Mediterranean Diet Influences Treatment Outcomes for Patients Treated with Immune Checkpoint Blockade for Advanced Melanoma

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Key Points

Question: Does habitual diet affect tumor response to immune checkpoint blockade (ICB) in advanced melanoma?

Findings: Higher adherence to the principles of a Mediterranean diet was associated with improved response rates, both within and across cohorts from the UK and the Netherlands. Patients consuming more whole grains and vegetables were less likely to develop immune-related adverse events.

Meaning: While further studies across different countries will be needed to confirm our findings and to offer patient-tailored advice, patients starting ICB may benefit from dietary counselling.

Abstract

Importance: Immune checkpoint blockade (ICB) has improved the survival of patients with advanced melanoma. Durable responses are observed for 40-60% of patients, depending on treatment regimens. However, there is still large variability in the response to ICB and patients experience a range of immune-related adverse events (irAEs) of differing severity. Nutrition, through its wide effects on the immune system and the gut microbiome, is a poorly explored but appealing target with potential to improve efficacy and tolerability of ICB.

Objective: To investigate the link between habitual diet and response to immune checkpoint blockade.

Design: Multi-center cohort study (the *PRIMM* study)

Setting: Cancer centers in the Netherlands and United Kingdom

Participants: 91 ICB-naïve patients with advanced melanoma

Exposure: Patients were treated with anti-PD-1 and anti-CTLA-4 monotherapy or combination therapy. Dietary intake was assessed through food frequency questionnaires before treatment.

Main Outcomes and Measures: Clinical endpoints were defined as overall response rate (ORR), progression-free survival at 12 months (PFS-12) and immune-related adverse events (irAEs) grade ≥ 2.

Results: Logistic generalized additive models revealed 25 associations between 7 unique food groups, 4 dietary patterns and 6 nutrients with response and irAEs. Patients who responded to ICB treatment were more likely to follow a Mediterranean dietary pattern enriched in whole grains, fish, nuts, fruit, and vegetables at baseline (probability 0.74 for PFS-12, P= .007, edf=1.54; probability 0.77 for ORR, P= .021,

edf=0.83). Plant-derived foods including whole grain foods and legumes were associated with improved response parameters as well as with a lower probability of irAEs (all *P< .05)*.

Conclusions and Relevance: In this study we report a potential benefit of Mediterranean diet, a widely recommended model of healthy eating, for improving ICB treatment outcomes. These findings suggest a role for dietary counselling prior to commencing ICB. Large prospective studies from different geographies are needed to further elucidate the role of diet in the context of ICB.

Trial Registration: PRIMM-UK (NCT03643289) and PRIMM-NL (made up of POINTING [NCT04193956] and OncoLifeS [METc number 2010/109]).

Introduction

While immune checkpoint blockade (ICB) has revolutionized the treatment of advanced melanoma, a significant number of patients do not tolerate and/or respond to this treatment (1). Recent evidence suggests that variability in the efficacy of ICB is partly explained by the gut microbiome (2). Interestingly, the abundance of many of the gut bacteria predictive of response to ICB are associated with diet (3). For example, dietary fiber is degraded to short-chain fatty acids (SCFAs) by microbiota such as Bifidobacteria and high fiber intake and high fecal SCFA concentrations have been associated with improved progression-free survival in both mice and ICB-treated patients (4-6). While evidence on immunomodulatory and anti-tumor activities of specific nutrients is increasing (7), studies comprehensively assessing the impact of overall diet composition on ICB response are still lacking. In this study, we aim to investigate how different diets associate with ICB response and toxicity, using a multinational prospective cohort of patients with advanced melanoma.

Methods

We prospectively collected dietary and clinical data from 91 patients receiving ICB between 2018 and 2021 for advanced melanoma in the UK (PRIMM-UK) and the Netherlands (PRIMM-NL**, eFigure 1** in the Supplement). Clinical endpoints were defined as overall response rate (ORR), progression-free survival at 12 months (PFS-12) and immune-related adverse events (irAEs). Patients were classified as responders [complete response (CR), partial response (PR) and stable disease (SD)], or non-responders [progressive disease (PD)], using the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria. IrAEs were assessed using the common

terminology criteria for adverse events (CTCAE) v5. As an outcome variable we focused on the development of irAEs ≥ grade 2 in order to avoid the subjectivity and inter-individual variability associated with the mildest of adverse events.

Dietary intake was assessed through the EPIC-Norfolk FFQ (8) and the Dutch Healthy Diet-FFQ (DHD-FFQ) (9). FFQ processing is further described in the **Supplementary methods**. Food items were collapsed into standardized food groups, using the national food composition databases (**eTable 1**). To account for differences in nutritional profiling or diets, we performed both country-specific and joint analyses.

Four food-based scores were calculated to address dietary quality across cohorts (**eTable 2**):

- alternate Mediterranean diet score (aMED) (10)
- original plant-based diet index (oPDI) (11); further distinguished into:
    - healthy plant-based diet index (h-PDI), and
    - unhealthy plant-based diet index (u-PDI)

PCA was performed per cohort to identify data-driven dietary patterns. The first 5 principal components (PCs), collectively explaining 56.7% and 55.4% of total dietary variation in PRIMM-NL and PRIMM-UK, respectively, were retained for subsequent analyses (**eFigures 2-3**).

To determine whether a higher adherence to a particular diet is associated with a higher probability of response or irAEs, we used logistic generalized additive models (GAMs) (12). First, using the joint dataset, we modelled each outcome variable (ORR,

PFS-12, irAEs) and all four diet scores as independent variables adjusting for age, sex, BMI and cohort. Next, we modeled each outcome variable and the first 5 PCs per cohort. To test which dietary pattern had the largest influence on response and irAEs, we removed each diet score or PC from each model one at a time, keeping all other variables intact (**eTable 3**). Lastly, we analyzed specific food groups and nutrients in relation to treatment response and irAEs **(Supplementary methods).**

Results

Cohort characteristics and differences between PRIMM-NL and PRIMM-UK are summarized in **Table 1** and **eTable 4**.

The Mediterranean diet score (aMED) was the diet score with the largest influence on PFS-12, ORR, and irAEs (explained deviance by 54%, 51% and 24%, respectively, **eTable 3**). Both PFS-12 and ORR showed a positive relationship with the aMED score, where the maximum score was associated with the highest probability of response (probability 0.74 for PFS-12, *P*= .007, edf=1.54; probability 0.77 for ORR, *P*= .021, edf=0.83). With every increase in the score, we observed a consistent increase of 1.43 in the odds of being a responder. An analysis per-cohort revealed the same relationships (**eTable 5**).

PCA per cohort (**eTable 6**) revealed a positive relationship between ORR and PC1 in PRIMM-NL, where a high intake of wholemeal bread, vegetables, and potatoes and a lower intake of foods high in sugar and savoury snacks was associated with the highest probability of response (*P*= .074; edf=2.7) (**eFigures 2-4**). PC2, characterized by a high fruit intake, showed a parabolic relationship with PFS-12 (*P*= .007; edf=2.14)

and ORR (*P*= .012; edf=2.7). No significant associations were found for PRIMM-UK (**eTable 3, eTable 7**).

We identified positive linear relationships between polyunsaturated and monounsaturated fatty acids with PFS-12 (*P*= .008, edf=0.88; *P*= .024, edf=0.74) (**Figure 2, eTable 8**). While nutrient analysis was only possible for the UK dataset, this finding further supports the role of the Mediterranean diet. Other nutrients associated with ORR and PFS-12 included beta-carotene (*P*= .033, edf=0.79), vitamin C (*P*= .022, edf=1.73) and E (*P*= .054, edf=1.31).

Food groups associated with response included vegetables (*P*= .039; edf=1.4) and legumes (*P*= .057; edf=0.75) in PRIMM-UK and wholemeal bread (*P*= .046; edf=1.2) and potatoes (*P*= .014; edf=0.87) in PRIMM-NL, common sources of fiber in these populations (**eTable 9**).

IrAEs exhibited negative associations with whole grain foods (*P*= .018; edf=0.84), legumes (*P*= .052; edf=0.75) and magnesium (*P*= .016, edf=1.45) in PRIMM-UK and were positively associated with a group of processed meats (*P*= .020; edf=0.85). Due to a lower number of cases (n=21), these relationships did not reach statistical significance in PRIMM-NL where a non-linear association between irAEs and fruit intake was identified (*P*= .037, edf=0.79).

Discussion

To our knowledge, this study represents the first examining detailed dietary data in relation to patient outcome when receiving ICB. We show that a Mediterranean-style diet is associated with a higher probability of response in ICB-treated patients with advanced melanoma. The traditional principles of Mediterranean diet remain the most widely used dietary recommendations of public health institutions globally. Interestingly, Mediterranean diet, rich in unsaturated fatty acids, fiber, and polyphenols, is associated with an increased abundance of microbiota producing SCFA (3) that have been linked to immunotherapy response in several studies (2,4-6).

We observe positive associations between ORR and PFS-12 with a number of fiber-rich foods, including vegetables, whole grains, potatoes and legumes which aligns with a recent observational study reporting an association between high fibre intake and improved PFS (5). Separately, Vitamin E and C have recently gained interest related to their immunomodulatory and anti-tumor activities (7,13-15, **eTable 10**) and are associated with response to ICB in our study.

Lastly, we show that plant-derived foods are associated with a lower probability of irAEs with the opposite association observed for processed meat. The current challenge in the treatment with ICB lies in maintaining or improving treatment efficacy, while minimizing severe and long-term irAEs. Plant-dominated diets have the potential to assist in this goal due to their diverse effects on the immune system. For example, fiber-derived SCFA affect both, regulatory and effector T cells and have been implicated in immunotolerance as well as anti-tumor response (2-6).

Strenghts & Limitations

Collecting extensive dietary data from patients with advanced cancer is challenging, and the primary strength of this study lies in the prospective dietary assessment, and the depth of data collected from a real-world population of patients across two European countries. Limitations include sample size and the difference between the UK and Dutch FFQs. However, these differences have been accounted for in the statistical models used. We move a step past the studies that have linked specific dietary components to favorable outcomes and chose to complement the analysis of nutrients by whole foods and dietary pattern analyses.

Conclusions

We found that a Mediterranean dietary pattern is linked to higher likelihood of PFS and ORR in a cohort of patients due to receive ICB for advanced melanoma. Plant-derived foods high in fiber have a potential to reduce irAEs. These findings underline the importance of dietary assessment in patients starting ICB and support a role for dietary strategies to improve treatment outcomes.

References

(1) Wolchok JD, Chiarion-Sileni V, Gonzalez R. Long-term outcomes with nivolumab plus ipilimumab or nivolumab alone versus ipilimumab in patients with advanced melanoma*. J Clin Oncol*. 2022;40(2):127-137. doi: 10.1200/JCO.21.02229.

(2) Lee KA, Thomas AM, Bolte LA, Björk JR, de Ruijter LK, Armanini F et al. Cross-cohort gut microbiome associations with immune checkpoint inhibitor response in advanced melanoma. *Nat Med.* 2022;28(3):535-544. doi: 10.1038/s41591-022-01695-5.

(3) Bolte LA, Vich Vila A, Imhann F, Collij V, Gacesa R, Peters V et al. Long-term dietary patterns are associated with pro-inflammatory and anti-inflammatory features of the gut microbiome. *Gut* 2021;70(7):1287-1298. doi: 10.1136/gutjnl-2020-322670.

(4) Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science.* 2015;350(6264):1084-9. doi: 10.1126/science.aac4255.

(5) Spencer CN, McQuade JL, Gopalakrishnan V, McCulloch JA, Vetizou M, Cogdill AP et al. Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. *Science.* 2021;374(6575):1632-1640. doi: 10.1126/science.aaz7015.

(6) Nomura M, Nagatomo R, Doi K, Shimizu J, Baba K, Saito T et al. Association of short-chain fatty acids in the gut microbiome with clinical response to treatment with nivolumab or pembrolizumab in patients with solid cancer tumors*. JAMA Netw Open*. 2020;3(4):e202895. doi: 10.1001/jamanetworkopen.2020.2895.

(7) Holly AE, Lee KA, Daniel CR, Spector TD, McQuade JL. Patient Nutrition: An Overlooked Yet Emerging Variable in the Precision Oncology Equation. *J Immunother Precis Oncol.* 2020;3(3):108-112. doi: 10.36401/JIPO-20-7.

(8) Bingham SA, Welch AA, McTaggart A, Mulligan AA, Runswick SA, Luben R et al. Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. *Public Health Nutr.* 2001;4(3):847-58. doi: 10.1079/phn2000102.

(9) van Lee L, Feskens EJ, Meijboom S, Hooft van Huysduynen EJ, van't Veer P, de Vries JH et al. Evaluation of a screener to assess diet quality in the Netherlands. *Br J Nutr.* 2016;115(3):517-26. doi: 10.1017/S0007114515004705.

(10) Fung TT, McCullough ML, Newby PK, Manson JE, Meigs JB, Rifai N, Willett WC, Hu FB. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr.* 2005;82(1):163-73. doi: 10.1093/ajcn.82.1.163.

(11) Satija A, Bhupathiraju SN, Spiegelman D, Chiuve SE, Manson JE, Willett W et al. Healthful and Unhealthful Plant-Based Diets and the Risk of Coronary Heart Disease in U.S. Adults. *J Am Coll Cardiol.* 2017;70(4):411-422. doi: 10.1016/j.jacc.2017.05.047.

(12) Wood SN. *Generalized Additive Models: An Introduction with R* (*2nd edition)*. New York, NY: Chapman and Hall/CRC; 2017. doi: 10.1201/9781315370279.

(13) Yuan X, Duan Y, Xiao Y, Sun K, Qi Y, Zhang Y, Ahmed Z et al. Vitamin E Enhances Cancer Immunotherapy by Reinvigorating Dendritic Cells via Targeting Checkpoint SHP1. *Cancer Discov.* 2022;12(7):1742–1759. doi: 10.1158/2159-8290.CD-21-0900.

(14) Magrì A, Germano G, Lorenzato A, Lamba S, Chilà R, Montone M et al. High-dose vitamin C enhances cancer immunotherapy. *Sci Transl Med.* 2020;12(532):eaay8707. doi: 10.1126/scitranslmed.aay8707.

(15) Huang J, Liu D, Wang Y, Liu L, Li J, Yuan J et al. Ginseng polysaccharides alter the gut microbiota and kynurenine/tryptophan ratio, potentiating the antitumour effect of antiprogrammed cell death 1/programmed cell death ligand 1 (anti-PD-1/PD-L1) immunotherapy. *Gut.* 2022;71(4):734-745. doi: 10.1136/gutjnl-2020-321031.

Figures and Tables

Table 1. Cohort characteristics

	PRIMM-NL (n=44)	PRIMM-UK (n=47)	*P*-value
Age (years) at stage IV diagnosis, *mean (SD)*	59.43 (12.74)	66.21 (16.63)	**.020**
BMI (kg/m^2), *mean (SD)*	27.51 (5.55)	29.06 (5.32)	.189
Gender, *n (%)*			.123
Male	22 (50)	32 (68)	
Female	22 (50)	15 (32)	
Outcomes following ICB, *n (%)*			
PFS-12	20 (46)	23 (49)	.930
ORR	26 (59)	27 (58)	1.000
irAEs (CTCAE grade ≥ 2)	21 (48)	25 (53)	.756
Metastatic stage, *n (%)*			**.014**
Stage 3, unresectable	1 (2)	4 (9)	
M1a	6 (14)	11 (23)	
M1b	8 (18)	11 (23)	
M1c	12 (27)	17 (36)	
M1d*	17 (39)	4 (9)	
BRAF mutant, *n (%)*	23 (52)	14 (30)	.049
ECOG Performance score ≥1, *n (%)*	16 (36)	33 (70)	**.002***
ICB regimen, *n (%)*			**0.043**
Ipilimumab-nivolumab combination	11 (25)	23 (49)	
Single agent PD-1/PDL-1 inhibition	32 (73)	24 (51)	
Single agent CTLA-4 inhibition	1 (2)	0 (0)	
Previous BRAF or MEK inhibition, *n (%)*	17 (39)	9 (19)	.068
Antibiotic use at baseline, *n (%)*	10 (23)	8 (18)	.710
PPI use at baseline, *n (%)*	19 (43)	12 (26)	.120

Diet scores, *mean (SD)*			
aMED	3.07 (1.25)	2.55 (1.28)	.083
OriginalPDI	30.52 (4.29)	34.23 (4.45)	**$1.222x10^{-4}$***
hPDI	32.84 (5.81)	35.49 (7.37)	.130
uPDI	31.70 (4.56)	34.32 (5.65)	**.021**

Characteristics of the PRIMM cohorts. Baseline characteristics are presented as mean and standard deviation (SD) for continuous variables and as counts and percentages for categorical variables. χ^2 tests for categorical variables and Mann-Whitney U test (MWU) for continuous data were performed to calculate differences between cohorts. *P*-values written in **bold** indicate nominally significant differences between PRIMM-UK and PRIMM-NL ($P < .05$). Asterisks (*****) indicate statistical significance under a false discovery rate (FDR) of 5%. BMI, body-mass index; aMED, alternate Mediterranean diet score; original PDI, original plant-based diet index, further differentiated into hPDI, healthy; and uPDI, unhealthy plant-based diet index; ICB, immune checkpoint blockade; ECOG, Eastern Cooperative Oncology Group; BRAF, v-raf murine sarcoma viral oncogene homolog B1, MEK, mitogen-activated protein kinase, PFS-12, progression-free survival at 12 months; ORR, overall response rate; CTCAE, common terminology criteria for adverse events; irAE, immune-related adverse event; SD, standard deviation.

Figure 1. Relationship between ORR and the alternate Mediterranean diet score (aMED) across both cohorts. The Y-axis shows the probability of ORR on a scale from 0 to 0.9. The X-axis shows adherence to a Mediterranean diet high in vegetables, legumes, fruit, and whole grains and low in red and processed meat, expressed by the aMED score ranging from 0 (minimum score) to 5 (maximum score). Abbreviations: ORR, overall response rate; PFS-12, progression-free survival at 12 months; aMED, alternate Mediterranean diet score.

Figure 2. Relationships between treatment outcomes and specific nutrients and food groups. Associations between response and specific dietary factors in PRIMM-UK (grams per day) and PRIMM-NL (frequencies per day). Abbreviations: PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; PFS-12, progression-free survival at 12 months; ORR, overall response rate; irAEs, immune-related adverse events; gr, grams.

Ethical approval and informed consent

PRIMM-UK (NCT03643289) is sponsored by East & North Hertfordshire NHS Trust with UK central ethical approval. PRIMM-NL, consisting of eligible patients from the POINTING (NCT04193956) and OncoLifeS studies (METc 2010/109; https://www.trialregister.nl/trial/7839), was approved by the Medical Ethical Committee of the University Medical Center Groningen in the Netherlands.

Data availability

All relevant data supporting the key findings of this study are available within the article and the supplementary files. Other data are available from the corresponding author upon reasonable requests. All statistical analysis scripts are written in R and can be found here: https://github.com/WeersmaLabIBD.

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Competing interests

RKW acted as a consultant for Takeda, received unrestricted research grants from Takeda, Johnson & Johnson, Tramedico and Ferring, and received speaker fees from MSD, Abbvie and Janssen Pharmaceuticals. GAPH acted as a consultant for Amgen, Bristol-Myers Squibb, Roche, MSD, Pfizer, Novartis, Sanofi, Pierre Fabre and has received a research grant from Bristol-Myers Squibb (all payments to the institution). ERL and TDS are consultants for ZOE Global Ltd. EGEdV reports an advisory role at Daiichi Sankyo, NSABP and Sanofi (paid to University Medical Center Groningen),

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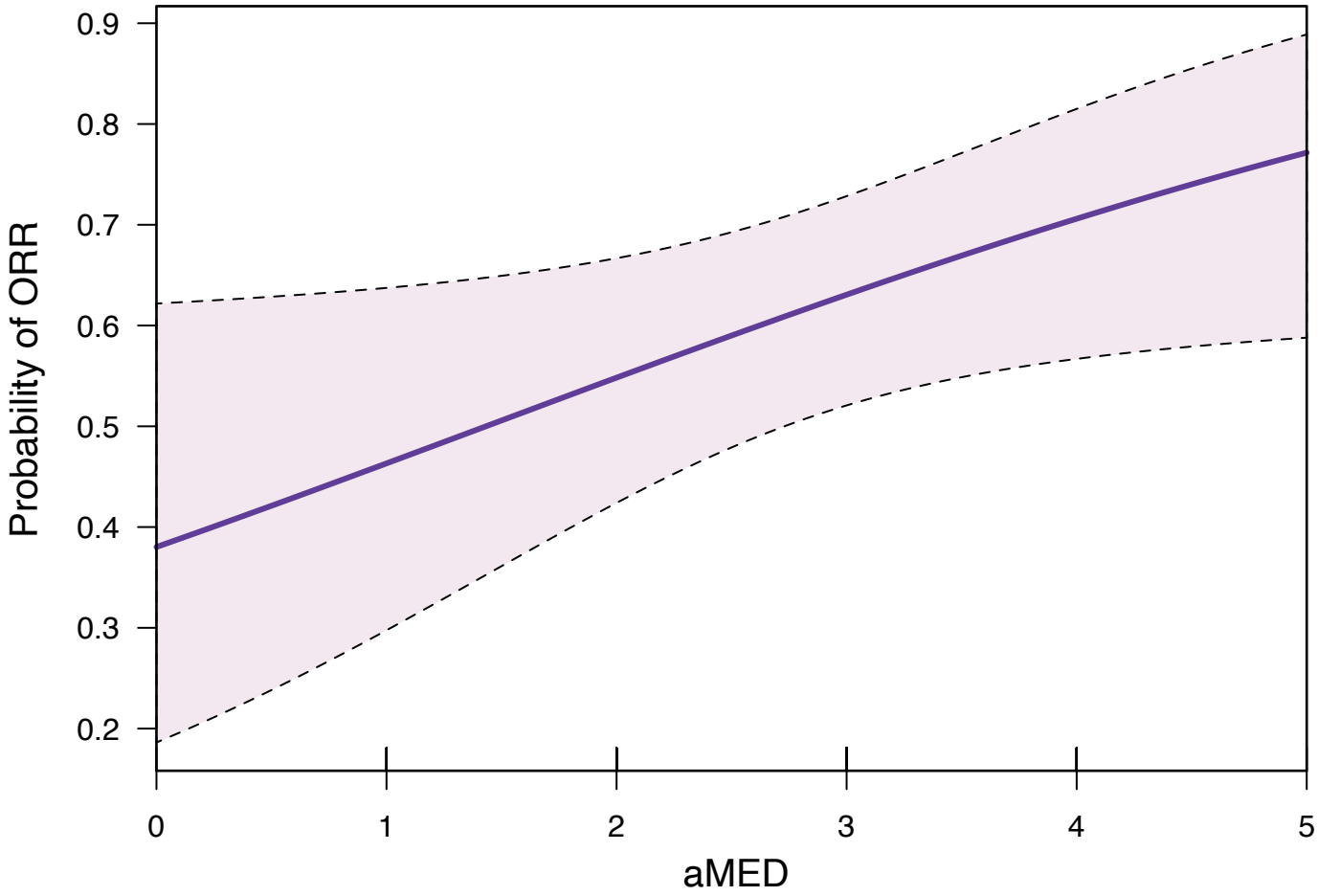
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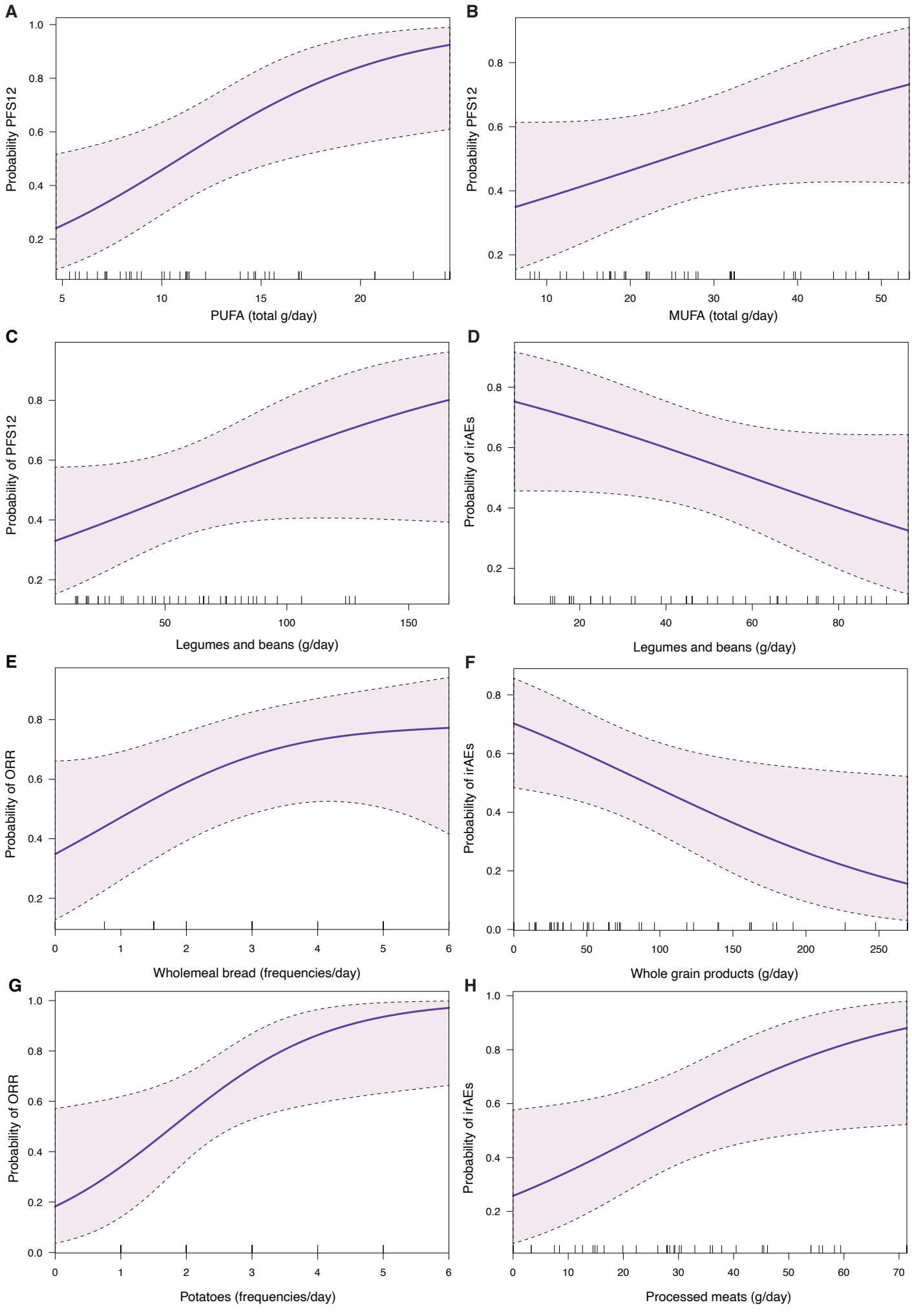
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E
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Wholemeal bread (frequencies/day)
F
Probability of irAEs
Whole grain products (g/day)
G
Probability of ORR
Potatoes (frequencies/day)
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Probability of irAEs
Processed meats (g/day)