1 Higher Susceptibility to Sunburn is Associated with Decreased Plasma Glutamine and
2 Increased Plasma Glutamate Levels among U.S. Women: An Analysis of the Nurses' Health
3 Study I and II

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47 The metabolism of glutamine and glutamate, two important amino acids synthesized in

48 the human body, may have an etiologic role in melanoma, an aggressive skin malignancy.[1, 2] Pre-

49 clinical experiments and clinical trials have demonstrated that metabotropic glutamate receptor 1

50 blocker and glutamate-release inhibitor (*e.g.,* Riluzole) can suppress melanoma cell migration,

51 invasion, and proliferation.[2] Additionally, inhibiting glutaminase, the enzyme that converts

52 glutamine to glutamate, further reduced glutamate bioavailability and suppressed tumor

53 progression.[1] Susceptibility to sunburn, a pigmentary trait, is a well-known risk factor for

54 melanoma.[3] However, it is unclear whether plasma glutamate and glutamine are affected by this

55 host factor even before cancer onset.

56 We hypothesized plasma glutamate/glutamine levels may differ by individuals' sunburn

57 susceptibility. To test this hypothesis, we examined the association among 9,129 women from

58 previous case-control studies nested within the Nurses' Health Study (NHS) I (n=5,981) and II

59 (n=3,148).[4] Non-whites and participants with a history of skin cancer or any other malignancy

60 prior to blood collection were excluded. Information on skin reaction after two or more hours'

61 sun exposure as a child/adolescent was collected and grouped into four categories (*i.e.*,

62 none/some redness only, burn, painful burn, painful burn with blisters). Plasma glutamate and

63 glutamine concentrations were obtained using liquid chromatography-tandem mass spectrometry

64 (LC-MS) at the Broad Institute of MIT and Harvard University. Details of metabolite profiling

65 methods have been published previously.[5] Age and multivariable-adjusted linear regressions

66 were performed and percentage differences in levels of glutamate, glutamine, and

67 glutamate/glutamine ratio between sunburn susceptibility groups were calculated using the

68 following equation: [exp (β-coefficient) − 1] × 100%. All analyses were performed using SAS

69 (Unix 9.4)

70   Characteristics of participants by quintiles of glutamate/glutamine ratio are presented in

71 **Table 1**. We found a significant decreasing trend of glutamine [painful burn with blisters vs.

72 reference: -9.2% (-16.9%, -0.7%), *P*-trend=0.005], and significant increasing trends of glutamate

73 [painful burn with blisters vs. reference: 19.9% (10.0%, 30.7%), *P*-trend<0.0001] as well as

74 glutamate/glutamine ratio [painful burn with blisters vs. reference: 22.8% (12.6%, 33.8%), *P*-

75 trend<0.0001] across sunburn susceptibility groups **(Table 2).** The association patterns were

76 consistent and significant in the sensitivity analysis of controls-only samples [n=4,672; painful

77 burn with blisters vs. reference: 13.4% (0.6%, 27.8%) for glutamate, -11.8% (-22.1%, -0.2%) for

78 glutamine, 15.6% (2.6%, 30.2%) for glutamate/glutamine ratio]. We did not find significant

79 interactions of sunburn susceptibility and selected covariates (*i.e.,* age, smoking, body mass

80 index, physical activity, alcohol consumption, Alternate Healthy Eating Index and personal

81 history of diabetes, cardiovascular diseases or hypercholesterolemia in relation to plasma

82 glutamate/glutamine ratio (all *P*-interaction > 0.05/7) **(Table S1).**

83   Overall, our study found that being susceptible to sunburn, especially painful burn with

84 blisters, was associated with higher glutamate and lower glutamine in plasma among cancer-free

85 participants. Although several limitations are acknowledged, such as the women-only and cross-

86 sectional study design, this is the first study demonstrating a potential association between

87 sunburn susceptibility (a highly heritable trait and melanoma risk factor) and plasma

88 glutamate/glutamine (a target of anti-melanoma therapy). Additionally, our epidemiologic

89 findings provide evidence for future investigations in terms of whether glutamatergic signaling is

90 a potential underlying pathway by which sunburn susceptibility alters melanoma risk. Further

91 studies are needed to replicate our findings.

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93 **Supplementary File:**

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95 Supplementary Table 1. Percentage differences (95% CIs) in plasma glutamate/glutamine ratio
96 according to sunburn susceptibility, stratified by selected covariates in all 9129 participants

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Table 1. Characteristics of 9,129 cancer-free participants from NHS I and II by quintiles of plasma glutamate/glutamine ratio[1]

Characteristics	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Number of participants	1825	1826	1826	1826	1826
Age at blood collection, years	51.5 (8.0)	52.0 (8.3)	52.7 (8.7)	53.5 (8.6)	53.1 (8.5)
Plasma glutamate level, z-score	-1.1 (0.5)	-0.5 (0.5)	-0.1 (0.5)	0.4 (0.5)	1.3 (0.8)
Plasma glutamine level, z-score	0.5 (0.9)	0.2 (0.9)	0.1 (0.9)	-0.2 (0.9)	-0.6 (1.0)
Plasma glutamate/glutamine ratio, z-score	-1.3 (0.3)	-0.6 (0.1)	-0.1 (0.1)	0.5 (0.2)	1.5 (0.6)
Painful burn or blistering after ≥2h sun exposure, %	13.5	15.1	18.2	20.2	21.3
Red or blonde hair, %[2]	14.8	16.1	15.6	17.8	17.7
≥6 moles on the extremity, %[3]	8.8	11.6	12.8	13.0	10.8
Average July noon-time erythemal UV, mW/m2	188.7 (29.4)	190.1 (29.2)	189.8 (28.9)	192.6 (30.5)	191.4 (30.2)
Body mass index, kg/m2	23.7 (3.5)	24.6 (4.2)	25.8 (4.7)	26.8 (5.3)	27.8 (6.1)
Physical activity, metabolic equivalents, hours/wk	17.1 (18.8)	17.5 (25.7)	15.7 (19.1)	17.0 (25.8)	15.2 (18)
Cigarette smoking					
Never smoker, %	58.1	55.6	52.5	51.0	46.7
Past smoker, %	31.5	33.4	35.3	35.9	40.3
Current smoker, %	10.4	10.9	12.2	13.1	13.0
Alcohol intake, g/d	4.6 (7.8)	5 (9.0)	5.2 (9.3)	5.6 (10.2)	5.2 (9.4)
Alternate healthy eating index	46.6 (10.0)	46.4 (10.0)	46.4 (9.6)	46.3 (9.9)	46.5 (9.7)
History of cardiovascular diseases	0.8	0.9	1.1	0.9	1.6
History of diabetes	0.2	0.4	1.0	2.2	5.5
History of hypercholesterolemia	30.0	34.5	32.8	37.7	37.8

Note: [1] Information presented was collected at the time of blood collection or at the questionnaire cycle closest to blood collection (NHS I: 1989-1990, NHS II: 1996-1999) except for otherwise noted. Values are means (SD) for continuous variables, percentages for categorical variables, and are standardized to the age distribution of the study population except for age.

[2] Natural color of hair at 21 and 18 was asked in NHS I and II, respectively.

[3] Total number of moles > 3mm diameter was asked for the left arm in NHS I, for both lower legs in NHS II.

Table 2. Percentage differences (95% CIs) in plasma levels of glutamate, glutamine, and glutamate/glutamine ratio according to sunburn susceptibility among 9,129 cancer-free participants from NHS I and II

	None or some redness only	Burn	Painful burn	Painful burn with blisters	P for trend
Number of participants	5445	2042	1106	536	
Glutamate					
Age-adjusted model	0 (ref)	4.1% (-1.1%, 9.5%)	15.2% (8.0%, 22.9%)	34.7% (23.3%, 47.1%)	<0.0001
Multivariable-adjusted model	0 (ref)	1.7% (-3.1%, 6.8%)	7.0% (0.5%, 13.9%)	19.9% (10.0%, 30.7%)	<0.0001
Glutamine					
Age-adjusted model	0 (ref)	-0.3% (-5.2%, 4.8%)	-8.4% (-14.1%, -2.4%)	-10.1% (-17.7%, -1.9%)	0.002
Multivariable-adjusted model	0 (ref)	-0.4% (-5.4%, 4.7%)	-8.0% (-13.8%, -1.8%)	-9.2% (-16.9%, -0.7%)	0.005
Glutamate/glutamine ratio					
Age-adjusted model	0 (ref)	4.3% (-0.8%, 9.7%)	17.7% (10.4%, 25.6%)	37.6% (26.0%, 50.3%)	<0.0001
Multivariable-adjusted model	0 (ref)	2.0% (-2.9%, 7.1%)	9.6% (2.9%, 16.7%)	22.8% (12.6%, 33.8%)	<0.0001

Notes: Covariates adjusted in multivariable-adjusted model include continuous age, body mass index, physical activity, alcohol consumption, alternate healthy eating index, smoking status (never, past, current), fasting status, case-control status in previous nested case-control studies, history of diabetes, cardiovascular diseases or hypercholesterolemia, hair color (black/dark brown, light brown, blonde, red), mole counts (none, 1-2, 3-5, 6+), and cohort.

160   **Supplementary Table 1. Percentage differences (95% CIs) in plasma glutamate/glutamine ratio according to sunburn susceptibility,**
161   **stratified by selected covariates among all 9,129 participants**
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