Published in final edited form as:

Cancer Causes Control. 2011 September ; 22(9): 1267–1275. doi:10.1007/s10552-011-9800-1.

Serum insulin-like growth factor-I and insulin-like growth factor binding-protein-3 levels with risk of malignant melanoma

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Abstract

We examined the relationship of insulin-like growth factor-I (IGF-I) and its primary growth factor, IGF binding protein-3 (IGFBP-3) with malignant melanoma using interview data and sera from cases (n=286) and controls (n=289) in a population-based case-control study conducted in 1986–1992 on Oahu, Hawaii. Serum IGF-I and IGFBP-3 concentrations were measured by ELISA. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by unconditional logistic regression and adjusted for age, sex, education, number of blistering sunburns, ability to tan, hair color, energy intake, BMI, height, smoking status, and drinking status. An inverse relationship was found between IGF-I concentration and melanoma (OR for upper vs. lower tertile: 0.44, 95% CI: 0.25–0.79), but clear associations were not observed between malignant melanoma and upper tertiles of IGFBP-3 and the IGF-1:IGFBP-3 molar ratio. The inverse association with IGF-I was strongest among subjects who did not report a history of non-melanoma skin cancer (NMSC) (OR for vs. < median: 0.39, 95% CI: 0.24–0.65), and a positive association was found among those with such a history (OR: 3.6, 95% CI: 1.0–13; $p_{\text{interaction}}=0.0035$). Our findings observed here between serum IGF-I and malignant melanoma warrants replication in studies with a larger sample size and a prospective design.

Keywords

Malignant melanoma; insulin-like growth factor-I (IGF-I); insulin-like growth factor binding protein-3 (IGFBP-3)

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Conflict of Interest

The authors state no conflict of interest.

Introduction

Insulin-like growth factor-I (IGF-I) is known for its antiapoptotic and mitogenic effects and has been implicated in the development of many cancers (1–3). A meta-analysis by Chen, et al. of 63 observational studies for 10 cancer sites found an overall positive association between cancer risk and circulating IGF-I levels (OR=1.15, 95% CI: 1.03–1.29) (2), which was predominantly observed for prostate, pre-menopausal breast and colorectal cancers. The main circulating binding protein for IGF-I, IGFBP-3, is known to possess both growth-inhibitory and potentiating effects on cells independent of IGF-I (4). A meta-analysis found an overall null effect between IGFBP-3 levels and cancer at four sites (breast, prostate, colorectal and lung), although a site-specific analysis found a positive association with pre-menopausal breast cancer (3).

Experimental studies have demonstrated that malignant melanoma relies on IGF-I for proliferation and vertical growth (5–7). Cells from early stage melanoma do not appear to independently produce IGF-I, as opposed to metastatic melanoma (8). Because malignant melanoma appears to rely on circulating IGF-I for cell growth and survival (6–7, 9), IGF-1R-kinase inhibitors have been considered for therapeutic application (10). However, unlike for these previously mentioned cancer sites, epidemiological studies have not thoroughly investigated the role of this molecular family in malignant melanoma, one of the few cancers for which incidence is increasing worldwide (11–12).

To our knowledge, only one case-control study has investigated the role of IGF-I in malignant melanoma. The authors found that IGF-I serum concentrations were lower in cases than controls ($p=0.01$); and although the distribution of the 19-CA polymorphic repeat in the *IGF-I* gene did not differ by case-control status, it was associated with melanoma thickness (Breslow index) (13). The evidence for a role of IGFBP-3 in malignant melanoma is even less clear. Two out of three studies found an increase in IGFBP-3 expression in melanoma tissue (14–16). However, it has also been suggested that IGFBP-3 may also facilitate apoptosis in melanoma cells (14). A case-only study found that levels of IGFBP-3 measured in plasma and tissue were slightly higher in patients with metastatic compared to non-metastatic disease ($p=0.26$ and $p=0.07$, respectively) (16).

One of the major risk factor for malignant melanoma, UV-radiation, primarily from sun exposure, is well-established (17). However, sun exposure is not responsible for all melanoma cases (18). Melanoma is also one of the few malignancies that remains resistant to available chemotherapies. Therefore, understanding the role of these growth factors in melanoma risk and prognosis in this neoplasm may assist in identifying mechanisms of melanoma pathogenesis and may benefit disease prevention and treatment. Using previously collected data and biological specimens from a population-based case-control study of malignant melanoma conducted in Hawaii, we investigated the potential relationships between serum IGF-I and IGFBP-3 and malignant melanoma, and explored the effects of potential modifiers.

Material and methods

Details regarding the study design have been previously reported (19). In brief, this study was conducted during 1986–1992 on the island of Oahu, Hawaii. It was designed to evaluate the associations of sunlight exposure and diet with the risk of cutaneous malignant melanoma.

Cases ($n=309$) were Oahu residents identified through the rapid-reporting system of the Hawaii Tumor Registry (a member of the NCI Surveillance, Epidemiology and End Results (SEER) Program), pathology labs, and dermatologist offices. Eligible case subjects had a

prevalent (diagnosed in 1986–1987) or newly incident (diagnosed in 1988–1992) histologically confirmed diagnosis of first primary invasive malignant melanoma or melanoma *in situ*. Exclusion criteria included a history of melanoma. To reduce the poorer quality of self-reported data, the study was restricted to adult participants, 79 years. Interviews were completed for 61.8% of eligible cases (184 males and 125 females). A blood sample was successfully collected from 93.5% of interviewed cases.

Controls were randomly selected from a list of Oahu residents who participated in an ongoing annual health survey conducted by the Hawaii State Department of Health and were matched on age (± 2.5 years), sex, and race/ethnicity to cases in a 1:1 ratio. The participation rate was 57.5%. A blood sample was successfully obtained from 94.1% of interviewed controls. All participants signed an informed consent approved by the Institutional Review Board of the University of Hawaii. The present study was approved by the Institutional Review Boards of both the University of Hawaii and University of California, Los Angeles.

Data collection

Data were collected through in-person interviews, using a standardized questionnaire. Interviews were conducted in the subjects' homes during 1988–1992. Collected data included detailed demographic information, including race and ethnicity of each grandparent, information regarding propensity to sunburn after acute sun exposure, tanning ability after chronic sun exposure, extensive history of sun exposure and previous sunburns, freckling patterns, total number of moles, personal and family medical history, histories of tobacco smoking and alcohol consumption..

A food frequency questionnaire was used to obtain dietary intake. Participants reported their average frequency of consumption and portion size for >200 food items that were consumed at least 12 times during the past year. Food composition data was based, primarily, on the nutrient database from the U.S. Department of Agriculture (20); as well as other research and commercially available publications. Average daily energy intake was computed from the summation of energy from nutrients, which was calculated by multiplying the number of grams/day and the energy provided per gram of nutrients consumed.

Laboratory Methods and Quality Control

Fasting blood samples were processed within two hours of collection, and serum samples were stored at -80°C at the University Hawaii Cancer Center until shipment to UCLA on dry ice for analysis of IGF molecular family. Samples arrived at UCLA intact and frozen and were stored at -80°C until analysis. Total serum IGF-I and IGFBP-3 concentrations were measured with commercially available enzyme linked immunosorbent assay (ELISA) kits (DSL-10-5600 and DSL-10-6600) from Diagnostic Systems Laboratories (DSL) (Webster, TX), using the kit-specified protocol on 286 cases and 289 controls.

Each batch included an approximately similar number of controls and cases (± 5) and the technician was blinded to the case status of each well. Sex and age distribution did not vary between batches ($p > 0.05$). Approximately 5% of randomly selected samples were duplicated. The average intra-batch coefficient of variation (CV) was 9.8% for IGF-I and 4.6% for IGFBP-3, whereas the inter-batch CV was 16.8% and 10.3%, respectively.

Statistical Analyses

All statistical analyses were conducted using SAS v.9.1 (SAS Institute, Cary, NC). Chi-square tests were conducted for differences between cases and controls for categorical variables. To test for differences in the distributions of continuous variables, one-way ANOVA was employed.

IGF-I and IGFBP-3 were assayed using non-glycosylated standards (ng/ml). To calculate the IGF-I:IGFBP-3 molar ratio, which may represent bioavailable IGF-I, molecular measures in ng/ml were converted to nM using the following conversions 1 ng/ml IGF-I=0.13 nM and 1 ng/ml IGFBP-3= 0.035 (as provided by the DSL protocol). Therefore, IGF-I:IGFBP-3 molar ratio=IGF-I*0.13/IGFBP-3*0.035. The associations of IGF-I, IGFBP-3, and their molar ratio with malignant melanoma were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) using unconditional logistic regression. Parameter estimates were similar when using conditional logistic regression on the subset of complete matched case-control pairs. Exposures of interest were categorized into tertiles, and a linear trend was tested by treating tertiles as a continuous variable. We used three adjustment models. For the minimally adjusted model, we adjusted for the matching factors of age (continuous), sex, and race/ethnicity (White, Japanese Americans, Chinese Americans, Hawaiians). In the fully-adjusted model, we additionally adjusted for the melanoma risk factors identified in an earlier study (19) and factors known to influence IGF levels, including education (high school, >high school), lifetime number of blistering sunburns (continuous), propensity to burn (severe sunburn with blistering, painful sunburn followed by peeling, mild burn with some degree of tanning, brown without sunburn), hair color (black/dark brown, medium brown, light brown, blonde/red), height (continuous), BMI (<25 kg/m², 25 to <30 kg/m², 30 kg/m²), daily energy intake (continuous), smoking status (never, former, current), and drinking status (never, former, current). Current smokers and drinkers were those who reported to presently smoke or drink alcoholic beverages on a daily or weekly basis at interview, respectively. Former smokers or drinkers were those who reported to have smoked any tobacco product daily, or drunk alcoholic beverages weekly, for 6 months or more, in the past. The mutually-adjusted model was additionally adjusted for IGF-I concentrations (continuous) for the IGFBP-3 models or for IGFBP-3 concentrations for the IGF-I models. The Pearson's correlation between the two hormone measures was r=0.66 among controls.

To maximize our power in stratified analyses, we collapsed continuous hormone concentrations for IGF-I and IGFBP-3 into <median and median. Heterogeneity by subgroups defined by potential modifiers, such as sex, race and sun sensitivity, was examined using the likelihood ratio test, comparing the fully adjusted model including cross-product terms of subgroup membership and IGF hormone levels divided at the median and the fully adjusted model including only main effect terms. We adjusted our significance threshold to $\alpha=0.0042$ (0.05/12) using a Bonferroni correction to account for multiple testing of 13 interactions (10 from Table 4, plus sex and race).

Results

Table 1 presents the characteristics of the study participants who provided serum samples by case-control status. The distribution of these characteristics did not differ between those who provided serum and those who did not, with the exception of education level in cases (data not shown). Cases who did not provide serum all had a high school degree or greater (p=0.019). The distribution of age, sex, race, smoking and drinking status, and BMI did not differ between cases and controls (Table 1). A higher proportion of cases reported having >high school education level than controls (p=0.006). The median distributions of IGF-I, IGFBP-3, their molar ratio, or energy intake did not vary between cases and controls. When stratified by sex, females controls had higher serum IGFBP-3 levels than males (p<0.001). Associations of established risk factors and malignant melanoma among Caucasians in this study population were previously published (19).

The distributions of serum IGF-I and IGFBP-3 levels in relation to tumor site and stage are shown in Table 2. We found that median IGF-I levels were on lower when tumors were

located on the face as opposed to on the trunk ($p=0.07$, data not shown); whereas IGFBP-3 levels were lower when the tumor was located on the face or upper limbs compared to the trunk or lower limbs ($p=0.061$). IGF-I levels were higher for patients with localized compared to *in situ* tumors ($p=0.036$). IGF-I:IGFBP-3 molar ratios increased with more advanced stage of disease at diagnosis ($p=0.028$). In females the median IGFBP-3 levels was higher with advancing stage (*in situ*=4530 ng/mL, localized=5079 ng/mL, and regional=5543 ng/mL; $p=0.043$).

The ORs for serum IGF-I, IGFBP-3, and their molar ratios can be found in Table 3. The minimally adjusted model suggests an inverse relationship between the highest IGF-I tertile and malignant melanoma. After further adjusting for covariates, this inverse association was strengthened and the confidence interval no longer included 1.0 (OR=0.59, 95% CI: 0.37–0.95). This inverse relationship was strengthened further by additionally adjusting for IGFBP-3 concentrations. In contrast, we did not detect an association between IGFBP-3 and malignant melanoma (Table 3). The associations for the molar ratio were similar to those of IGF-I whereby, after adjusting for covariates, the OR for the highest tertile was 0.69 (95% CI: 0.43–1.11).

Similar results were obtained when restricting the analysis to Caucasians. The precision of the ORs decreased when we stratified by sex and used sex-specific tertiles; however, the directions of the associations were the same and the p -values for heterogeneity between sex were all >0.05 . The inverse association with the upper tertile of the IGF-I:IGFBP-3 molar ratio appeared to be somewhat stronger in females (OR=0.43, 95% CI: 0.18–0.98) than in males (OR=0.82, 95% CI: 0.45–1.52); however, the test for heterogeneity was not quite noteworthy ($p=0.10$).

The associations between serum IGF-I and malignant melanoma stratified by tumor characteristics and various risk factors are presented in Table 4. The association for IGF-I was similar across disease stage (*in situ* and localized) and tumor site (face, upper limbs, trunk, and lower limbs). In contrast, a personal history of non-melanoma skin cancer (NMSC) modified the association of IGF-I and malignant melanoma. Among study participants who did not report a history of NMSC, we observed an inverse relationship between IGF-I and malignant melanoma (OR for vs. < median=0.39, 95% CI: 0.24, 0.65), while among participants reporting a prior history, the association was positive (OR=3.6, 95% CI: 1.0–13). This difference remained noteworthy ($p_{\text{interaction}}=0.0035$) even after correction for multiple comparisons. No clear effect modification was detected for IGFBP-3 with malignant melanoma, and the results for the molar ratio were similar to those found for IGF-I.

Discussion

In this study, we found an inverse association between serum levels of IGF-I and malignant melanoma and no clear association with serum IGFBP-3. The association with IGF-I did not considerably vary across sexes and disease stages, but was stronger among subjects with no personal history of NMSC.

The relationship between IGF-I and malignant melanoma has not been well described. Our finding of a positive association between circulating IGF-I levels and malignant melanoma contrasts with the positive associations found for other cancer sites, as well as with results from experimental studies of melanoma cell lines suggesting that IGF-I promotes melanoma cell growth, survival and metastasis (6, 21–22). Our results may be due to chance, although we believe this is unlikely. We replicated within our control population the expected inverse relationship between circulating IGF-I and age (23). Moreover, our findings are consistent

with the only other study of IGF hormones and melanoma (13), which found lower mean serum IGF-I concentrations for cases than controls ($p=0.01$) and a difference in circulating IGF-I concentration in relation to tumor location ($p<0.001$). They reported average IGF-I concentrations that were lower in subjects with tumors on the upper limbs and face compared to those with melanoma on the lower limbs and trunk (13). Similarly, we found lower IGF-I levels for patients with melanoma on the face compared to those with melanoma on the trunk.

We considered the possibility that, due to the retrospective design of our study, cancer status may have influenced our findings (i.e., reverse causation) and stratified our analysis by cancer stage. If reverse causation was an issue, we would expect to find no or a weaker inverse relationship for melanoma *in situ*. In contrast, we found that the inverse relationship between IGF-I levels and malignant melanoma was similar but, if anything, stronger for *in situ* than localized disease, arguing against reverse causality. It may also be of concern that some of the *in situ* cases were misclassified and actually were dysplastic or compounded nevi cases. However, the fact that the inverse association was slightly stronger in these cases suggests that these cases were more similar to malignant melanoma cases than controls. In addition, these results would suggest that our findings may be more relevant to early stage disease and/or presence of dysplastic or compounded nevi.

There is a possibility that the total serum IGF-I concentrations did not reflect levels of either the local tissue or the relevant exposure period (24). Flemings et al. (24) found that advanced primary and metastatic melanomas presented with lower IGF-I mRNA compared to early primary lesions or dysplastic nevi. Also, it may be that the potential mitogenic effects of IGF-I that are important for early melanoma cells occur prior to detection of disease and/or during the dysplastic nevus stage. In addition, although we do not expect disease recovery or progression to influence the concentrations of this hormone, if they did, measures at time of blood draw in this study (median interval since diagnosis: 13.2 months) would not reflect those prior to diagnoses.

The observed inverse association of serum IGF-I with melanoma risk in our data mirrors trends previously reported for cancers of the endometrium (2, 25–26) and colorectum (27). The investigators of a colorectal cancer study hypothesized that the role of IGF-I may be analogous to the dual effects of TNF-beta (27), where the usual activity for TNF-beta is growth inhibitory, but is stimulatory in the presence of tumor cells (28). It is unclear whether IGF-I signaling is altered when melanoma is established. Similarly, additional measures for proteins within the IGF family, such as ALS, a glycoprotein forming the IGF ternary complex (29), or proteins found to interact with IGF-I and melanoma cell progression--such as, Bcl-2, an apoptosis regulator (21); IL-8, an inflammatory marker (22); or IGF-IR, a marker of melanoma progression (30)--might shed light on possible mechanisms for the inverse association of IGF-I with malignant melanoma. Experimental studies are needed to test these hypotheses.

Findings from our stratified analyses suggests that history of NMSC may modify the association between IGF-I and malignant melanoma and that those with a personal history of NMSC and elevated IGF-I levels would be at a substantial increased risk of malignant melanoma. However, due to the small sample size, precision of this risk estimates was limited. When evaluating sun exposure patterns (chronic and acute) in relation to IGF-I or IGFBP-3 levels, no clear association was found. Nevertheless, this potential interaction and the relationship between these hormones and patterns sun exposure deserves further investigation in larger studies.

Similar to studies of other cancer sites, we found that the association with serum IGF-I was strengthened when adjusting for IGFBP-3 levels (31), suggesting that the relevant biomarker may be free (unbound) IGF-I, as opposed to total IGF-I. Alternatively, this could imply that the role of IGFBP-3 in malignant melanoma development may be in the opposite direction to that of IGF-I. We failed to detect a clear relationship between tertiles of IGFBP-3 and malignant melanoma, possibly due to insufficient power. However, when we categorized IGFBP-3 using quartiles, subjects in the highest quartile of IGFBP-3 had a suggestive increased risk of malignant melanoma ($p_{\text{trend}}=0.076$). Consistent with this possible direct relationship, two of three studies using immunohistochemical analysis found an increased IGFBP-3 expression in metastatic melanoma tumor tissues compared to those with primary disease (14–15). In the study that found no difference in hormone expression, the authors did find that the median serum IGFBP-3 levels were higher for patients with metastatic disease ($p=0.09$)(16).

On the other hand, the lack of a clear association with IGFBP-3 levels could be the consequence of the potential dual apoptotic and proliferative function of this protein (14). Alternatively, it may be due to the limitations of the commercially available assay used in our study, which measured total IGFBP-3, i.e., both the intact and proteolytically-cleaved forms of IGFBP-3. Cancer status may result in increased IGFBP-3 proteolysis; therefore measures of the cleaved hormones may be a more appropriate biomarker for disease (32).

We were unable to stratify our analysis by histological types or to evaluate the association of plasma IGFs by level of Breslow index. This is regrettable because it is possible that the association of IGF-I and melanoma risk may vary by histological type or stage. Santonocito C, et al. reported that IGF-I was positively associated with tumor thickness, which is predictive of disease stage. IGF-I may only play a role in the radial growth phase of melanoma cells (6) and length of time in, or presence of, this phase differs by histology (e.g. radial growth is not present in nodular melanoma).

Another limitation of this study included limited power to assess for effect modification, although we were powered to detect an interaction OR of 3.0. Selection bias may have occurred if those who provided a blood sample differ in important ways from those who did not. This seems unlikely since a very high proportion (94%) of subjects agreed to the blood collection, and since we found that the distributions of covariates that are likely to affect IGF-I or IGFBP-3 levels, such as sex, age, race, BMI, and energy intake, were similar in among those who provided and did not provide blood samples. Our study also did not include abdominal circumference which is important to consider in relation to IGF-I (33). Prolonged storage of serum may be of concern; however, all samples were stored together in -80°C freezers and they underwent an equal number of freeze-thaw cycles during the analysis, suggesting that if degradation occurred it was likely similar for cases and controls. We note that serum IGF-I and IGFBP-3 levels were found to remain relatively stable over 9 years of storage at -80°C (34). The inter-batch CV value for IGF-I was relatively high (16.8%) in our study. Because cases and controls were analyzed in similar numbers in each batch and the intra-batch CV was low (9.8%), the consequence of this high inter-batch CV is more likely to be a loss in power than a differential bias. Nevertheless, in a sensitivity analysis, we adjusted for batch effect, as well as removed each plate successively from our analysis and found that the inverse association between IGF-I and melanoma risk persisted. The validity of the self-report of NMSC may be of concern. However, some studies have found self-report of NMSC to be fairly accurate (35–36). However, self-report of NMSC may be subject to differential recall bias. Because cases and controls were unaware of their IGF-I levels, it is unlikely that such a bias would have created spurious associations. Finally, we note that this study had a number of important strengths, including a population-based

design, careful assessment of the known risk factors for melanoma, and the ability to adjust for a variety of confounding variables.

In conclusion, our study suggests that high circulating levels of IGF-I may be inversely associated with risk of malignant melanoma and that this association differ by history of NMSC. These results warrant confirmation in studies conducted with a larger sample size and a prospective design.

Acknowledgments

This work was supported by NCI grant P01 CA33619 and US department of Health and Human Services contract N01-PC67010. S.L.P and the lab assays were supported by NCI T32 CA09142. We would like to thank Dr. Gang Zeng and his research group at UCLA for use of their laboratory space and equipment. In addition, we are in debt to the study participants for contributing their time and specimens.

Abbreviations used

IGF-I	insulin-like growth factor-I
IGFBP-3	insulin-like growth factor binding protein-3
ELISA	Enzyme-linked immunosorbent assay
OR	Odds Ratio
CI	Confidence Interval
NMSC	non-melanoma skin cancer
BMI	body mass index.

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Table 1

Characteristics of study subjects^a

	Cases	%	Controls	%	P-value
Total	286		289		
Age (years)^a					
< 40	65	22.7	62	21.5	
40 to 49	63	22.0	73	25.3	
50 to 59	45	15.7	42	14.5	
60 to 69	63	22.0	62	21.5	
70	50	17.5	50	17.3	0.74 ^b
Sex^a					
Males	170	59.4	171	59.2	
Females	116	40.6	118	40.8	0.95
Race/ethnicity^a					
Caucasian	255	89.2	258	89.3	
Chinese	11	3.9	10	3.5	
Hawaiian	5	1.8	5	1.7	
Japanese	15	5.2	16	5.5	0.99
Education					
High school	54	18.9	83	28.7	
>High school	232	81.1	206	71.3	0.0056
Smoking status					
Never	137	47.9	120	41.5	
Former	114	39.9	113	39.1	
Current	35	12.2	56	19.4	0.051
Drinking status					
Never	71	24.8	89	30.8	
Former	61	21.3	65	22.5	
Current	154	53.9	135	46.7	0.18
BMI (kg/m²)					
< 25	149	52.1	126	43.6	

	Cases	%	Controls	%	P-value
25 to 25.9	99	34.6	112	38.8	
30	38	13.3	51	17.6	0.10
IGF-I, median (25–75% percentile range) (ng/mL)	285.3 (216.9–368.1)		300.4 (216.9–376.7)		0.33 ^b
IGFBP-3, median (25–75% percentile range) (ng/mL)	4621 (3931–5366)		4536 (3923–5184)		0.43 ^b
IGF-I:IGFBP-3 Molar ratio, median (25–75% percentile range)	0.2318 (0.1886–0.2801)		0.2403 (0.1935–0.2977)		0.061 ^b
Energy intake, median 25–75% percentile range)	1913 (1426–2626)		1947 (1429–2623)		0.83 ^b

^aOriginally matched variables

^bP-values represent comparison between cases and controls using one-way ANOVA, all other p-values were calculated using χ^2 test.

Table 2

Distribution of IGF hormone levels in relation to tumor characteristics among 286 cases

	N	%	Median IGF-I (25–75% percentile range)	Median IGFBP-3 (25–75% percentile range)	Median IGF-I:IGFBP-3 Molar ratio (25–75% percentile range)
Tumor site^a					
Face	42	14.7	256.9 (190.2–309.1)	4469 (3632–5186)	0.2055 (0.1761–0.2565)
Upper limbs	76	26.6	289.7 (220.7–387.8)	4656 (3990–5248)	0.2475 (0.1888–0.2853)
Trunk	99	34.6	304.9 (235.9–398.0)	4727 (3924–5522)	0.2464 (0.1975–0.2854)
Lower limbs	62	21.7	289.9 (198.9–352.2)	4590 (4038–5574)	0.2197 (0.1760–0.2733)
P-value			0.082	0.061	0.056
Tumor stage^a					
<i>in situ</i>	101	35.3	257.4 (200.0–330.0)	4538 (3931–5091)	0.2156 (0.1801–0.2670)
Localized	167	58.4	305.0 (232.8–398.0)	4784 (3990–5482)	0.2428 (0.1928–0.2838)
Regional	6	2.1	253.9 (173.0–329.2)	3983 (2752–5488)	0.2515 (0.1167–0.4009)
P-value			0.036	0.143	0.028

^aFor tumor site, one case with tumor overlapping several sites and seven cases with missing information were excluded; for tumor stage, 12 patients with missing stage were excluded

Table 3

Associations between IGF-I and IGFBP-3 levels and malignant melanoma

	Cases	Controls	Model 1 ^a	Model 2 ^{b, c}	Model 3 ^d
IGF-I (ng/mL)					
< 252.0	113	96	1.0	1.0	1.0
252.0 to < 350.6	94	97	0.80 (0.53–1.2)	0.76 (0.49–1.2)	0.66 (0.42–1.1)
350.6	79	96	0.67 (0.43–1.0)	0.59 (0.37–0.95)	0.44 (0.25–0.79)
P-trend			0.074	0.031	0.0059
Continuous (10 ng/mL increase)			0.99 (0.98–1.0)	0.99 (0.97–1.0)	0.98 (0.96–1.0)
IGFBP-3 (ng/mL)					
<4175.8	98	97	1.0	1.0	1.0
4175.8 to < 4987.9	84	96	0.87 (0.58–1.3)	0.83 (0.53–1.3)	0.95 (0.60–1.5)
4987.9	104	96	1.1 (0.73–1.7)	1.0 (0.64–1.6)	1.3 (0.77–2.2)
P-trend			0.65	0.97	0.31
Continuous (100 ng/mL increase)			1.0 (0.99–1.0)	1.0 (0.99–1.0)	1.0 (1.0–1.0)
IGF-I:IGFBP-3 molar ratio					
< 0.2063	103	96	1.00	1.00	
0.2063 to <0.2753	102	97	0.97 (0.64–1.5)	1.1 (0.70–1.7)	
0.2753	81	96	0.77 (0.50–1.2)	0.69 (0.43–1.1)	
P-trend			0.25	0.14	
Continuous (0.010 unit increase)			0.98 (0.95–1.0)	0.97 (0.95–1.0)	

^a Adjusted for age, race, and sex

^b Additionally adjusted for energy intake, number of lifetime blistering sunburns, height, BMI, ability to tan, education, hair color, and smoking and drinking status.

^c For Model 2 and 3, one case and one control had missing covariate information.

^d Model 2 additionally adjusted for IGF-I or IGFBP-3 concentrations (continuous).

Table 4
Associations between IGF-I levels and malignant melanoma, stratified by disease stage and various risk factors

	Cases	Controls	OR	Cases	Controls	OR (95% CI) ^a	P-value	P _{interaction}
	IGF-I < 300.4 ng/mL			IGF-I ≥ 300.4 ng/mL				
Tumor stage								
<i>In situ</i>	67	144	1.0	34	145	0.48 (0.26–0.89)	0.019	
Localized	80	144	1.0	87	145	0.74 (0.45–1.2)	0.23	0.28
Tumor site								
Face	30	144	1.0	12	145	0.43 (0.17–1.1)	0.072	
Upper limbs	44	144	1.0	32	145	0.67 (0.34–1.3)	0.24	
Trunk	48	144	1.0	51	145	0.85 (0.47–1.5)	0.59	
Lower Limbs	33	144	1.0	29	145	0.54 (0.25–1.2)	0.12	0.62
Blistering sunburn								
No	55	60	1.0	31	67	0.48 (0.22–1.1)	0.075	
Yes	103	83	1.0	96	78	0.76 (0.45–1.3)	0.31	0.073
Mole removed								
Never	52	83	1.0	43	97	0.42 (0.22–0.82)	0.011	
Ever	106	60	1.0	84	48	0.78 (0.40–1.5)	0.45	0.30
Personal history of non-melanoma skin cancer^b								
No	112	119	1.0	79	133	0.39 (0.24–0.65)	<0.001	
Yes	41	24	1.0	44	12	3.6 (1.0–13)	0.049	0.0035
Family history of skin cancer^b								
No	113	115	1.0	76	108	0.57 (0.34–0.96)	0.034	
Yes	29	20	1.0	42	27	0.73 (0.25–2.13)	0.57	0.33
Smoking status								
Never	73	53	1.0	63	67	0.43 (0.22–0.84)	0.014	
Ever	85	90	1.0	64	78	0.87 (0.48–1.6)	0.65	0.52
Alcohol intake								
Never	45	43	1.0	26	46	0.22 (0.08–0.57)	0.0018	
Ever	113	100	1.0	101	99	0.82 (0.50–1.4)	0.45	0.22
BMI (kg/m²)								

	Cases		Controls		OR	IGF-I < 300.4 ng/mL		IGF-I ≥ 300.4 ng/mL		OR (95% CI) ^a	P-value	P _{interaction}
	Cases	Controls	Cases	Controls		Cases	Controls	Cases	Controls			
<25	74	60	75	66	1.0	63 (0.32–1.25)	0.19					
25	84	83	52	79	1.0	0.59 (0.33–1.1)	0.074					0.23
Calories (kcal)												
<1921.0	82	81	63	61	1.0	0.66 (0.36–1.24)	0.20					
1921.0	76	62	64	84	1.0	0.54 (0.29–0.99)	0.047					0.23

^a Adjusted for age, race, sex, energy intake, number of lifetime blistering sunburns, height, BMI, ability to tan, education, hair color, smoking and drinking status and IGFBP-3.

^b Nine cases had missing personal history of non-melanoma skin cancer and 26 cases & 18 controls had missing family history of skin cancer