OPEN ACCESS

Clinical science

Obesity paradox in uveal melanoma: high body mass index is associated with low metastatic risk

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bjo-2022-322877).

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Received 13 November 2022
Accepted 20 March 2023
Published Online First 7 April 2023

To cite: Sabazade S, Opalko A, Herrspiegel C, *et al*. *Br J Ophthalmol* 2024;**108**:578–587.

ABSTRACT

Background Metabolic factors and obesity may influence the development and progression of cancer. In this study, we examine their association with the risk of developing metastases of uveal melanoma.

Methods Data on metabolic factors, medications, serum leptin levels, tumour leptin receptor RNA expression and clinical outcomes were examined in three cohorts. HRs for metastasis and cumulative incidences of melanoma-related mortality were calculated, and the levels of tumour leptin receptor expression were compared with prognostic factors including *BAP1* mutation, and tumour cell morphology.

Results Of 581 patients in the main cohort, 116 (20%) were obese and 7 (1 %) had metastatic disease at presentation. In univariate Cox regressions, tumour diameter, diabetes type II and use of insulin were associated with metastases, but patients with obesity had a lower risk. The beneficial prognostic implication of obesity was retained in multivariate regressions. In competing risk analyses, the incidence of melanoma-related mortality was significantly lower for patients with obesity. Serum leptin levels≥median were associated with a reduced risk for metastasis, independent of patient sex and cancer stage in a separate cohort (n=80). Similarly, in a third cohort (n=80), tumours with *BAP1* mutation and epithelioid cells had higher leptin receptor RNA expression levels, which have a negative correlation with serum leptin levels.

Conclusion Obesity and elevated serum leptin levels are associated with a lower risk for developing metastases and dying from uveal melanoma.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Obesity and metabolic factors have been associated with the prognosis in several cancers. Generally, obesity is a poor prognostic factor. It is unknown if such a relationship exists for patients with uveal melanoma.

WHAT THIS STUDY ADDS

⇒ Obesity and high serum leptin levels are associated with a lower risk for developing metastases and dying from uveal melanoma.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These results could aid prognostication of uveal melanoma, and further studies may reveal targetable metabolic factors.

INTRODUCTION

Uveal melanoma (UM) is the most common intraocular malignancy in adults. At the time of diagnosis, about 2% of patients have clinical metastases.[1] At 20 years however, the estimated relative survival rate of patients with UM is 60%.[2] This has been attributed to early seeding of micrometastases from the eye to distant organs, where they can remain dormant for years.[3] Once these tumour cells grow into larger radiologically detectable macrometastases, median patient survival is about 1 year.[4] Thus, finding factors that affect the conversion of micrometastases to macrometastases is an important step towards deepened understanding of this disease; of what affects mortality and of what targets can be modified or pharmacologically addressed to improve survival.

While many studies have focused on the interactions between UM tumour cells and the immune system, relatively few have examined the effects of metabolic factors.[5–10] Considering previous observations of obesity causing systemic inflammation that contribute to the development and progression of breast cancer; that dietary changes may modulate the immune system; and that nutrient deprivation may activate autophagy in dormant cancer cells, patients' metabolic status might indeed have an important role in the balance between dormancy and proliferation of UM micrometastases.[11–13]

In this study, we aim to examine the association between the development of UM metastases, obesity and other metabolic factors. Therefore, we collected data on several surrogate markers for patients' metabolic status including body mass index (BMI), cardiovascular diseases, diabetes, use of anticoagulants, antihypertensives, diuretics, statins and hypothyroidism replacement therapy and correlated these markers with retrospective survival data in a large cohort with nationwide coverage in Sweden. In smaller separate samples, we have also analysed levels of the major adipokine leptin in serum collected at the time of primary UM diagnosis and the primary tumour expression of leptin receptor RNA. Serum leptin levels are closely correlated with increased body fat mass and have been investigated as a contributor to angiogenesis and vasculogenic mimicry in cancer, and as a promotor of cancer stem cell proliferation, and therapeutic resistance.[14 15] Adding this analysis

allows us to assess the correlative importance of leptin and body mass for the risk for subsequent development of clinical metastases.

MATERIALS AND METHODS

Patient selection

All patients who had been diagnosed with primary UM at St Erik Eye Hospital, Stockholm, Sweden, from January 2009 through December 2017 and had clinicopathological and follow-up data available in the digitalised treatment registry at St. Erik Eye Hospital were considered for the study (n=742). The treatment registry was synchronised with data from the national cause of death registry, which is based on medical death certificates that must be submitted to the National Board of Health and Welfare within 3 weeks of death. Death certificates were usually completed by a family physician, the physician last seeing the patient before death, or a forensic pathologist.[16] To reduce the rate of misclassifications of the cause of death, typically between death from cutaneous melanoma and UM, the data had been crosschecked with digitalised medical records. St Erik Eye Hospital has the national responsibility for the care of patients with UM, and our baseline and follow-up data have been estimated to capture at least 95% of the patients with UM in the country.[17] Patients were excluded if no data on concurrent diseases and medications at time of diagnosis were available in their medical journals (n=59). For the remaining 683 patients, we had data on the presence of cardiovascular diseases, diabetes, use of anticoagulants, antihypertensives, metformin, insulin and hypothyroidism replacement therapy at the time of UM diagnosis, as well as data on patient's sex, age, presenting symptoms, tumour thickness, tumour diameter, tumour distance to the optic disc, American Joint Committee on Cancer (AJCC) T-category, AJCC stage, date of diagnosis and last follow-up. For 581 of the 683 patients (85%), data on weight and height at the time of diagnosis was available which allowed for calculation of BMI. For 200 of the 683 patients (29%), we also had data on the use of statins and diuretics. The information on patient weight, height, concurrent diseases, medications and primary tumour characteristics were collected at the time of primary tumour diagnosis when the patients' visited the hospital in person. Calculated BMI was used to stratify patients into four categories: class I, underweight (BMI<18.5); class 2, normal weight (BMI≥18.5 to 24.9); class 3, overweight (≥25 to 29.9); and class 4, obese (BMI≥30.0), according to a classification used by the National Institutes of Health (NIH).[18]

Presence of cardiovascular disease was defined as a diagnosis of coronary artery disease, cerebrovascular disease, peripheral artery disease, aortic atherosclerosis, rheumatic heart disease, congenital heart disease, pulmonary embolism or deep vein thrombosis in the past or present, as defined by the American Heart Association.[19] Within 1–4 weeks from primary tumour diagnosis, all patients underwent radiological examination with CT of the thorax and abdomen to detect subclinical metastases. Follow-up was scheduled at 1, 3 (patients treated with plaque brachytherapy only), 6 and 12 months after primary tumour treatment, and then annually or semi-annually for the remainder of the patient's life. Semi-annual screening for liver metastases by ultrasonography or CT was performed for at least 5 years after diagnosis, and then when prompted by symptoms, palpable masses, deteriorating health, jaundice, weight loss, abnormal blood tests or other features suggestive of metastasis. If ultrasound examinations were impeded by any patient factor including obesity and liver steatosis, radiologists typically suggested that the patient should be screened with CT or MRI instead.

Immunohistochemistry

BMI was correlated to BAP-1 expression in tumours from patients that underwent primary enucleation (n=88). The tumours had been immunohistochemically stained during clinical routine diagnostics, in 4 µm sections pre-treated in EDTA buffer at pH 9.0 for 20 min, and then incubated with mouse monoclonal antibodies against BAP-1 at dilution 1:40 (clone C-4; Santa Cruz Biotechnology; Cat# sc-28383, RRID:AB_626723). The dilution had been gradually titrated until optimal staining was achieved, according to manual control. The level of nuclear BAP-1 expression (nBAP-1) had then been assessed by GS. For a tumour to be classified as BAP-1 positive, at least 33% of tumour cell nuclei had to be positively stained, and accumulation of chromogen in nucleoli or similar did not suffice.

Serum leptin levels

Eighty additional patients that had been diagnosed with choroidal melanoma between 1 January 1996 and 31 December 1998 were included for a correlative analysis of the association between serum leptin levels and metastasis. These patients were part of a previously described cohort in which a panel of serum proteins were used to devise a prognostic test, but no results presented herein have been published before.[20] At baseline, none of the 80 patients had radiologically detectable metastases. Within 1 week from diagnosis, a 10 mL peripheral blood sample had been obtained from each patient. After collection, the blood was allowed to clot by leaving it undisturbed at room temperature for 30 min. The clot was then removed by centrifugation at 1500×g for 10 min. The resulting supernatant was transferred into clean polypropylene cryotubes and immediately stored and carefully preserved at −80°C until analysis. The serum concentration of leptin was determined with human leptin ELISA kits (ab179884, Abcam B.V., Amsterdam, Netherlands).

Leptin receptor RNA expression

We also collected data from 80 additional patients from The Cancer Genome Atlas (TCGA), including *BAP1* mutation status, tumour cell type, patient survival and results from RNA sequencing (transcripts per million (TPM)) of leptin receptors (LEPR), leptin receptor overlapping transcript (LEPROT) and leptin receptor overlapping transcript like 1 (LEPROTL1). LEPR is a single transmembrane domain cytokine receptor that is involved in fat metabolism. It regulates adipose tissue mass through hypothalamic effects on satiety and energy expenditure. Peripheral activation increases basal metabolism, influences reproductive function, regulates pancreatic beta-cell function and insulin secretion, is pro-angiogenic and affects innate and adaptive immunity.[21–23] Patients with obesity may suffer from leptin resistance: in non-obese subjects, a minor increase in serum leptin concentration reduces the appetite and leads to a decrease in body weight, but in obesity, the anorexic effect of leptin is decreased.[24] Leptin resistance is mediated by reduced LEPR expression or by disturbed LEPR signalling. Thus, obesity may be associated with reduced levels of LEPR.[25] High levels of LEPR have previously been identified in various cancers, including squamous cell carcinoma of the skin, breast cancer and endometroid endometrial cancer.[26–28] LEPROT and LEPROTL1 are mainly located in the Golgi apparatus, plasma membranes and endosomes and have identical binding properties as LEPR. They are involved in negative regulation of the growth hormone receptor (GHR)

signalling pathway and regulate GHR expression in the liver.[29] For sequencing, 1 μg of total RNA from each tumour had been converted to mRNA libraries using the lllumina mRNA TruSeq kit (RS-122-2001 or RS-122-2002) following the manufacturer's directions. Libraries were sequenced 48×7×48 bp on the Illumina HiSeq 2000 as described previously.[5] RNA with a mean TPM of <1 were considered non-expressed and eliminated from further analyses.

Statistical analysis

P values below 0.05 were considered statistically significant, all p values being two-sided. For tests of continuous variables that did not deviate significantly from normal distribution (Shapiro-Wilk test p>0.05), Student's t-tests were used. For non-parametrical data, Mann-Whitney U tests (2 groups) or Kruskal-Wallis tests (>2 groups) were used. In comparisons of categorical variables, we used two-by-two contingency tables and Pearson χ^2 tests (if all fields had a sample of >5) or Fisher's exact tests (if any field had a sample of <5). For comparisons of association with metastasis, univariate and multivariate Cox regressionHRs were calculated. The cumulative incidence of UM-related mortality was plotted in cumulative incidence function estimates from competing risks data with the cmprsk package for R, and the equality of survival distributions was tested with Gray's test for equality.[30] The association between serum leptin levels at diagnosis, primary tumour leptin receptor RNA expression and the development of metastases was analysed with receiver operating characteristics (ROC) and Kaplan-Meier metastasis-free survival curves. All statistical analyses except the cumulative function estimate were performed using IBM SPSS statistics V.27 (Armonk, New York, USA) or GraphPad Prism V.9.3.0 (San Diego, California, USA).

RESULTS

Descriptive statistics

Of 683 included patients in the main cohort, 352 (52%) were men and 331 (48%) were women. Their mean age at diagnosis was 64.2 years (SD 13.7) and their primary tumours had a mean apical thickness of 5.5 mm (SD 2.7) and a mean largest basal diameter of 10.9 mm (SD 3.6). Seven patients (1 %) had liver metastases that were detectable with the CT scans that were performed within a few weeks from primary tumour diagnosis. None had metastases in other thoracic or abdominal organs. Two of these seven patients (29 %) were obese. One hundred and forty-one patients developed metastases before the end of follow-up. One hundred and twenty-six patients deceased from metastatic UM and 114 from other causes. The median follow-up for the 443 survivors was 6.3 years (IQR, 4.1, table 1). The data met the proportional hazards assumption (p=0.38).

Table 1 Patient characteristics

Total n	683
Sex, n (%)	
Male	352 (52)
Female	331 (48)
Median age at diagnosis, years (IQR)	67 (17)
Mean tumour size, mm (SD)	
Thickness	5.5 (2.7)
Diameter	10.9 (3.6)
AJCC T-category, n (%)	
T1a	215 (32)
T1b	6 (1)
T1c-d	0 (0)
T2a	324 (47)
T2b	6 (<1)
T2c-d	0 (0)
T3a	115 (16)
T3b	5 (1)
T3c-d	0 (0)
T4a	11 (2)
T4b	1 (<1)
T4c-d	0 (0)
AJCC stage, n (%)	
I	215 (31)
IIA	328 (48)
IIB	118 (18)
IIIA	15 (2)
IIIB	0 (0)
IIIC	0 (0)
IV	7 (1)
Median follow-up, years (IQR)*	6.3 (4.1)

*For survivors.
AJCC, American Joint Committee on Cancer; IQR, Interquartile range; SD, Standard deviation.

Patient BMI, concurrent diseases and medications

The mean weight at diagnosis for the 581 patients with available data was 79.4 kg (SD 16.6). Most patients had a BMI between 18.5 and 29.9, (n=460, 79 %). One hundred and sixteen patients (20 %) were obese. Most patients had American Society of Anesthesiologists (ASA) physical status class 1 or 2 (n=582 of 683 patients with available data, 88 %). Twenty-eight and four patients had type II and I diabetes, respectively. Eleven of the patients with diabetes used metformin, and eight used insulin. One hundred and forty-four patients (21 %) used any kind of anti-coagulants including platelet inhibitors (n=102, 15 %), warfarin (n=34, 5 %), non-vitamin K antagonist oral anticoagulants (NOAC, n=7, 1 %) and factor Xa inhibitors (n=1, <1 %). One hundred and twenty-four patients (18 %) had a diagnosis of cardiovascular disease. Forty-one patients (6 %) used levothyroxine and 286 patients (42 %) used anti-hypertensive medication. In a smaller sample with available data on other factors (n=200), 39 patients (20 %) used statins and 30 (15 %) used diuretics (table 2).

Regression analyses

Clinical factors (patient's sex, age, BMI class, ASA class), primary tumour diameter, concurrent diseases (cardiovascular, diabetes) and medications (anticoagulants, antihypertensive, levothyroxine, statin, diuretics, metformin, insulin) were entered into univariate Cox regressions. Primary tumour diameter (HR 1.2 per increasing mm, 95% CI 1.1 to 1.2, p<0.001) was associated with development of metastases, whereas obesity was negatively correlated (HR 0.6, 95 % CI 0.5 to 1.0, p=0.04). The 28 patients with diabetes type II and 8 patients with insulin medication had greater risk for metastasis. In multivariate Cox regressions, tumour diameter, obesity and insulin medication retained their significance (table 3).

Patients with and without obesity

As obesity was an independent predictor of metastasis in regression analysis, we next examined the distribution of clinicopathological characteristics across patients with obesity vs without obesity. Patients with obesity were significantly younger

Table 2 Characteristics of patients and specific data collected

BMI (n=581)	
Weight, mean kg (SD)	79.4 (16.6)
BMI, mean (SD)	26.8 (4.7)
BMI<18.5, n (%)	5 (1)
BMI 18.5–24.9, n (%)	216 (37)
BMI 25–29.9, n (%)	244 (42)
BMI 30–34.9, n (%)	84 (15)
BMI 35–39.9, n (%)	21 (4)
BMI>40, n (%)	11 (2)
BMI<18.5–29.9, n (%)	465 (80)
BMI≥30, n (%)	116 (20)
ASA classification (n=683)	
ASA class 1, n (%)	253 (37)
ASA class 2, n (%)	348 (51)
ASA class 3, n (%)	76 (11)
ASA class 4, n (%)	6 (<1)
Anti-coagulants (n=683)	
Anti-coagulants, n (%)	144 (21)
Platelet inhibitors, n (%)	102 (15)
Warfarin, n (%)	34 (5)
NOAC, n (%)	7 (1)
Anti-Xa (fragmin, LMWH), n (%)	1 (<1)
No anti-coagulant treatment, n (%)	539 (79)
Medication for cardiovascular disease (n=683)	
Cardiovascular disease, n (%)	124 (18)
No cardiovascular disease, n (%)	559 (82)
Medication for hypothyroidism (n=683)	
Levothyroxine, n (%)	41 (6)
No levothyroxine treatment, n (%)	642 (93)
Medication for hypertension (n=683)	
Anti-hypertensives, n (%)	286 (42)
No anti-hypertensive treatment, n (%)	397 (59)
Diabetes (n=683)	
Type I, n (%)	4 (<1)
Type II, n (%)	28 (4)
Metformin treatment, n (%)	11 (2)
Insulin treatment, n (%)	8 (1)
Other medications (n=200)	
Statins, n (%)	39 (20)
Diuretics, n (%)	30 (15)

Anti-Xa, factor Xa inhibitors; ASA, American Society of Anesthesiologists physical status classified from 1 to 4; BMI, body mass index; LMWH, low-molecular-weight heparin; NOAC, non-vitamin K antagonist oral anticoagulants; SD, standard deviation.

Table 3 Cox regression, hazard for metastasis

	B	SE	Wald	P value	Exp(B)	95 % CI lower	95 % CI upper
Univariate							
Male sex	0.08	0.08	0.88	0.35	1.08	0.92	1.26
Age at diagnosis*	0.04	0.03	2.05	0.15	1.04	0.99	1.10
Tumour diameter, mm†	0.16	0.01	205.60	**<0.001**	1.17	1.15	1.20
BMI class 1–4‡	−0.25	0.13	3.66	0.07	0.78	0.60	1.02
BMI≥30	−0.58	0.29	4.08	**0.04**	0.56	0.32	0.98
ASA class 1–4§	−0.09	0.14	0.40	0.53	0.92	0.70	1.21
Cardiovascular disease	−0.03	0.23	0.01	0.91	0.98	0.62	1.53
Diabetes type I	0.43	1.00	0.18	0.67	1.53	0.21	10.97
Diabetes type II	0.66	0.33	4.01	**0.04**	1.94	1.01	3.69
Antiocoagulant medication	−0.07	0.22	0.11	0.74	0.93	0.60	1.43
Antihypertensive medication	−0.03	0.18	0.02	0.89	0.98	0.69	1.39
Levothyroxine medication	0.21	0.32	0.46	0.50	1.24	0.67	2.29
Metformin medication	0.69	0.47	2.17	0.14	1.99	0.80	4.98
Insulin medication	1.05	0.47	5.04	**0.03**	2.86	1.14	7.17
Statin medication¶	0.19	0.31	0.37	0.55	1.21	0.65	2.24
Diuretic medication¶	−0.26	0.40	0.43	0.51	0.77	0.35	1.69
Multivariate							
Tumour diameter, mm†	0.16	0.05	11.33	**<0.001**	1.18	1.07	1.29
BMI≥30	−1.30	0.61	4.50	**0.03**	0.27	0.08	0.91
Diabetes type II	0.19	0.56	0.12	0.73	1.21	0.41	3.61
Insulin medication	0.95	0.73	1.68	0.19	2.58	0.62	10.85
Multivariate							
Tumour diameter, mm†	0.16	0.05	11.33	**<0.001**	1.17	1.07	1.29
BMI≥30	−1.29	0.61	4.43	**0.03**	0.28	0.08	0.91
Insulin medication	1.11	0.55	4.06	**0.04**	3.04	1.03	8.99
Multivariate							
Tumour diameter, mm†	0.18	0.05	15.46	**<0.001**	1.20	1.09	1.31
BMI≥30	−1.22	0.61	4.06	**0.04**	0.29	0.09	0.97
Diabetes type II	0.59	0.42	1.93	0.17	1.80	0.79	4.10

Boldface indicates significant p values.
*Per increasing decade.
†Per increasing mm.
‡For each increased step in BMI category 1 through 4.
§American Society of Anesthesiologists physical status classification, for each increased step in class 1 through 4.
¶Based on a smaller patient sample (n=200) with available data.
ASA, American Society of Anesthesiologists; BMI, body mass index.

at diagnosis (median age 64 vs 67 years, Mann-Whitney U p=0.046), had higher ASA class and were more often treated with anti-hypertensive medication, but the number of patients with radiologically detectable metastases at the time of primary tumour diagnosis, patient sex, primary tumour diameter, apical thickness, distance to the optic disc, CBI, cardiovascular disease, diabetes type II, treatment with anti-coagulants or levothyroxine, as well as observation periods were all similarly distributed between the groups (table 4). When examining these factors across all four BMI classes, patient age, sex, ASA class, anti-hypertensive medication and diabetes type II were dissimilarly distributed. However, other than type II diabetes, which was more common in BMI class 3 and 4, the distributions followed no general trend: Patients were younger in BMI class 1 and 4; women were over-represented in BMI class 3, and under-represented in BMI class 1; and ASA class 3 was over-represented in BMI class 4 and 1. Tumour diameter, apical thickness, distance to the optic disc, CBI, cardiovascular disease and treatment with anti-coagulants and levothyroxine were all similarly distributed between the groups. These findings should be interpreted with caution, as the number of patients in BMI class 1 was very low (n=5, online supplemental table 1).

In multivariate Cox regression with tumour diameter and patient age (per decade) as covariates, obesity was associated with decreased HR for metastasis (HR 0.4, 95% CI 0.2 to 0.8, p=0.008, figure 1A). Similarly, patients had decreasing HR for metastasis with increasing BMI class 1 to 4 (HR per step, 0.7, 95%

Table 4 Clinicopathological characteristics of patients without vs with obesity

	BMI<30 (n=465)	BMI≥30 (n=116)	P value
Age at diagnosis, median (IQR)	67 (17)	64 (20)	0.046
Radiologically detectable metastases at the time of primary tumour diagnosis	5 (1)	2 (2)	0.33
Sex, n (%)			0.92
Male	246 (53)	62 (53)	
Female	219 (47)	54 (47)	
Tumour diameter, mean mm (SD)	10.9 (2)	11.1 (10)	0.64
Tumour thickness, mean mm (SD)	5.9 (1)	5.6 (5)	0.70
Distance to optic disc, mean mm (SD)	3.9 (1)	4.2 (4)	0.50
CBI, n (%)	15 (3)	5 (4)	0.52
ASA classification, n (%)			<0.001
1	194 (42)	17 (15)	
2	230 (50)	64 (57)	
3	32 (7)	32 (28)	
4	1 (<1)	0 (0)	
Anti-coagulants, n (%)			0.23
No	371 (80)	86 (75)	
Yes	90 (20)	28 (25)	
Cardiovascular disease, n (%)			0.56
No	382 (82)	92 (80)	
Yes	82 (18)	23 (20)	
Levothyroxine, n (%)			0.17
No	433 (93)	103 (90)	
Yes	31 (7)	12 (10)	
Anti-hypertensives, n (%)			<0.001
No	277 (61)	46 (40)	
Yes	180 (39)	69 (60)	
Diabetes type II, n (%)			0.32
No	449 (97)	110 (95)	
Yes	16 (3)	6 (5)	
Median follow-up, years (IQR)			
Dead from metastatic UM	2.0 (1.6)	1.8 (2.2)	
Dead from other causes	4.4 (2.6)	4.7 (2.9)	
Survivors	5.9 (3.7)	6.1 (4.5)	

Diabetes type I not shown, as BMI was available for one patient only.
ASA, American Society of Anesthesiologists physical status, classified from 1 to 4; CBI, ciliary body involvement; IQR, Interquartile range; SD, Standard deviation; UM, uveal melanoma.

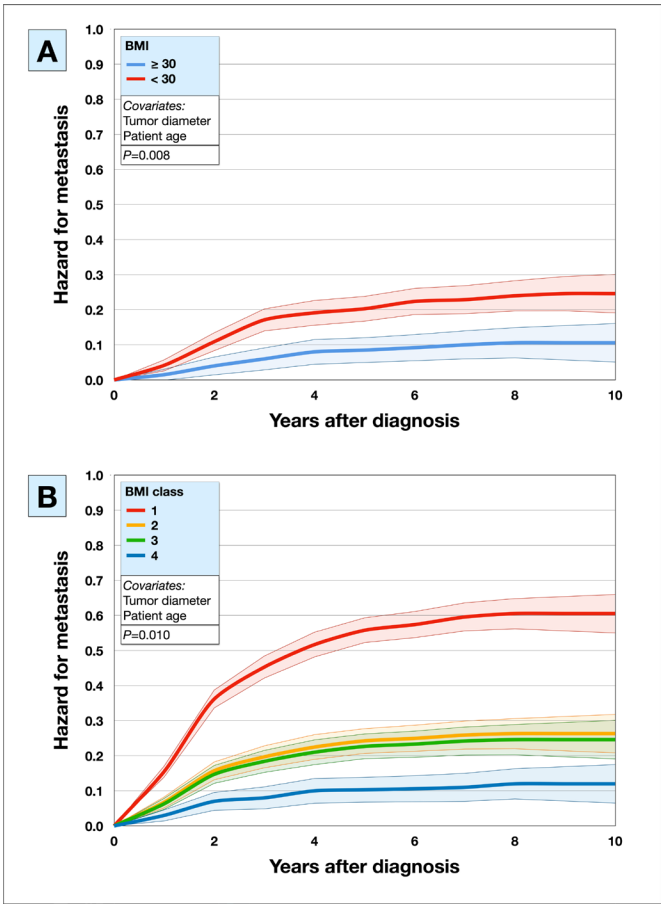

Figure 1 Hazard for metastasis in relation to body mass index (BMI). (A) Multivariate Cox regression HR for metastasis for patients with and without obesity (HR 0.4, 95% CI 0.2 to 0.8, p=0.008), adjusted for patient age and tumour diameter. (B) HR for metastasis for BMI class 1 to 4, also adjusted for patient age and tumour diameter (HR per step, 0.7, 95% CI 0.5 to 0.9). Coloured fields represent 95% CIs. HR, hazard ratio.

CI 0.5 to 0.9, p=0.010, figure 1B). In linear regression, there was no correlation between primary tumour nBAP-1 expression and patients' BMI, or in the distribution of BMI between tumours with high and low nBAP-1 (online supplemental figure).

The cumulative incidence function estimate of UM-related mortality from competing risks data five and ten years after diagnosis was 9.9 and 11.3% for patients with obesity and 20.3 and 24.6% for patients without obesity. The incidence of UM-related mortality was significantly higher for patients without obesity (Grey's test for equality p=0.01). There was no significant difference in death from other causes (p=0.08, figure 2A). Similarly, there was no difference in all-cause mortality (ie, the sum of UM-related and other mortality) between patients with and without obesity (p=0.85, figure 2B).

Serum leptin at diagnosis

Next, we examined the correlation between levels of serum leptin at diagnosis and development of metastases in the separate cohort of 80 patients with long-term follow-up (online supplemental table 2). Patients that developed metastases had significantly lower leptin concentrations (typically corresponding to lower volumes of adipose tissue, 8.9 vs 12.9 ng/mL, Mann-Whitney U p<0.01, figure 3A). Patients with obesity had significantly higher serum leptin levels (p=0.03, figure 3B). When dividing the sample into four groups (patients with obesity that developed metastases vs patients with obesity that did not develop metastases vs patients without obesity that developed metastases vs patients without obesity that did not develop metastases), there was no significant difference in serum leptin levels between the groups (Kruskal-Wallis p=0.63, figure 3C). Women had significantly higher serum leptin levels (p<0.001), but in multivariate Cox regression with patient sex and AJCC stage as covariates, serum leptin levels≥median retained its association with a decreased risk for metastases (HR 0.3, CI 0.1 to 0.9, p=0.03).

In ROC analysis, the leptin level achieved an area under the curve (AUC) of 0.69 (95% CI 0.56 to 0.82, figure 3D). Patients with leptin levels below the median had significantly shorter metastasis-free survival (log-rank p=0.049, figure 3E).

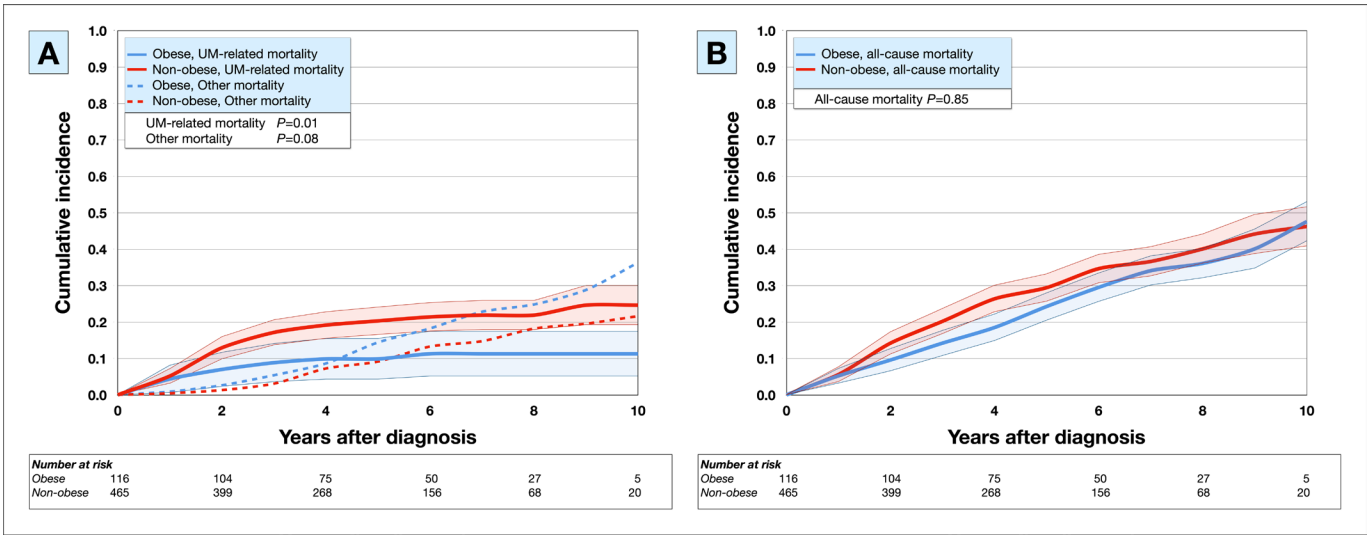

Figure 2 Cumulative incidence of mortality in competing risk analysis. (A) Patients without obesity (BMI<30) had significantly greater incidence of UM-related mortality than patients with obesity (BMI≥30, Gray's test for equality p=0.01). There was no significant difference in death from other causes (dashed lines, p=0.08). (B) Similarly, there was no difference in all-cause mortality (ie, the sum of UM-related and other mortality) between patients with and without obesity (p=0.85). Coloured fields represent 95% CIs. UM, uveal melanoma.

Leptin receptor RNA expression

Lastly, we examined the relation between RNA expression levels of LEPR, LEPROT and LEPROTL1, and *BAP1* mutation, histological cell types (spindle-shaped, epithelioid or mixed) and patient survival in tumours from 80 patients in the TCGA cohort. LEPR expression was very low (mean 0.3 TPM, range <0.1 to 2.1) and it was eliminated from further analyses. LEPROT and LEPROTL1 expression was higher (mean TPM 32.1, range 2.1–77.6; and 29.1, range 8.0–77.4, respectively). LEPROT expression was higher in tumours with *BAP1* mutation and epithelioid vs spindle-cell histology, but patients with LEPROT>median did not have worse melanoma-specific survival (figure 4A–4G). The expression of LEPROTL1 was higher in tumours from patients who died from metastatic UM, but not in tumours with *BAP1* mutation or epithelioid histology (p=0.06 and >0.99, respectively, figure 4H–4M). Patients with LEPROTL1>median did not have worse melanoma-specific survival (figure 4N).

DISCUSSION

In this study, we have examined the association between BMI, metabolic factors, concurrent cardiovascular diseases, medications, the risk of UM metastases and the incidence of UM-related mortality. We find that patients with obesity have a reduced risk for metastasis, regardless of patient age, diabetes and tumour diameter. Further, high serum leptin levels were associated with better patient survival and low tumour leptin receptor RNA expression with markers for less aggressive tumours in separate cohorts.

We believe that the lower expression of leptin receptors in tumours with favourable prognostic factors such as *BAP1* wild type and spindle-shaped morphology could be a marker for leptin resistance and obesity, thus corroborating and nuancing the results on the serum and patient levels. In turn, higher expression of leptin receptors in aggressive tumours may be a marker for a leptin starvation and possibly metabolic changes. Leptin can activate several pathways such as JAK-STAT3, MAPK/ERK, PKC, JNK, p38 and PI3K-Akt that induce the expression of angiogenic factors and vasculogenic mimicry.[14] The latter is a strong prognostic factor in UM.[31 32] Leptin has also been described as having a role in promoting the aerobic glycolytic pathway and in enhancing epithelial to mesenchymal transition.[33]

Thus, one may propose that the prognostic implication of obesity in UM may be related to elevated serum leptin levels, which suppresses tumour leptin receptor expression, which in turn reduces the activity of pathways that are associated with vasculogenic mimicry, extracellular matrix interactions and invasive behaviour. A similar beneficial effect of high serum leptin levels has been found in several other cancers, including pancreatic and colorectal carcinoma (CRC), as well as in cutaneous melanoma.[34–36] In hepatocellular carcinoma, leptin has been observed to activate the p38-MAPK-dependent signalling pathway and inhibit tumour growth.[37] Moreover, increasing efficacy of immunotherapy has been observed in patients with obesity with CRC, cutaneous melanoma, lung carcinoma and ovarian carcinoma, which was attributed to leptin signalling and T cell exhaustion.[38] However, it should be stressed that the role of high serum leptin levels is dual, that they have also been associated with immune ageing and tumour progression, and that the leptin hormonal axis and its targets are not necessarily direct causal factors in neither a favourable nor unfavourable direction. Leptin may be a mere surrogate marker for a range of other patient and tumour metabolic and inflammatory changes, of which one or several may be the actual culprit.[38 39]

In subjects with obesity, plasma levels of adiponectin are lower and levels of leptin higher.[40 41] Together with adiponectin, leptin is one of the major adipokines.[41] Low serum adiponectin, which is primarily produced in adipose tissue, has previously been found to correlate with the development of UM metastases.[42] Adiponectin is involved in regulating glucose levels and fatty acid breakdown and has cardioprotective properties, whereas leptin has the opposite effect.[41] Further, the liver synthesised growth factors, insulin-like growth factor 1 (IGF-1) and hepatocyte growth factor/scatter factor are associated with UM-related mortality and possibly with tumour cell homing to the liver.[43] In turn, IGF-1 levels are affected by levels of insulin, exercise, stress, nutrition and BMI.[44] A subset of UM with high risk for metastasis have been found to express oestrogen receptors and are

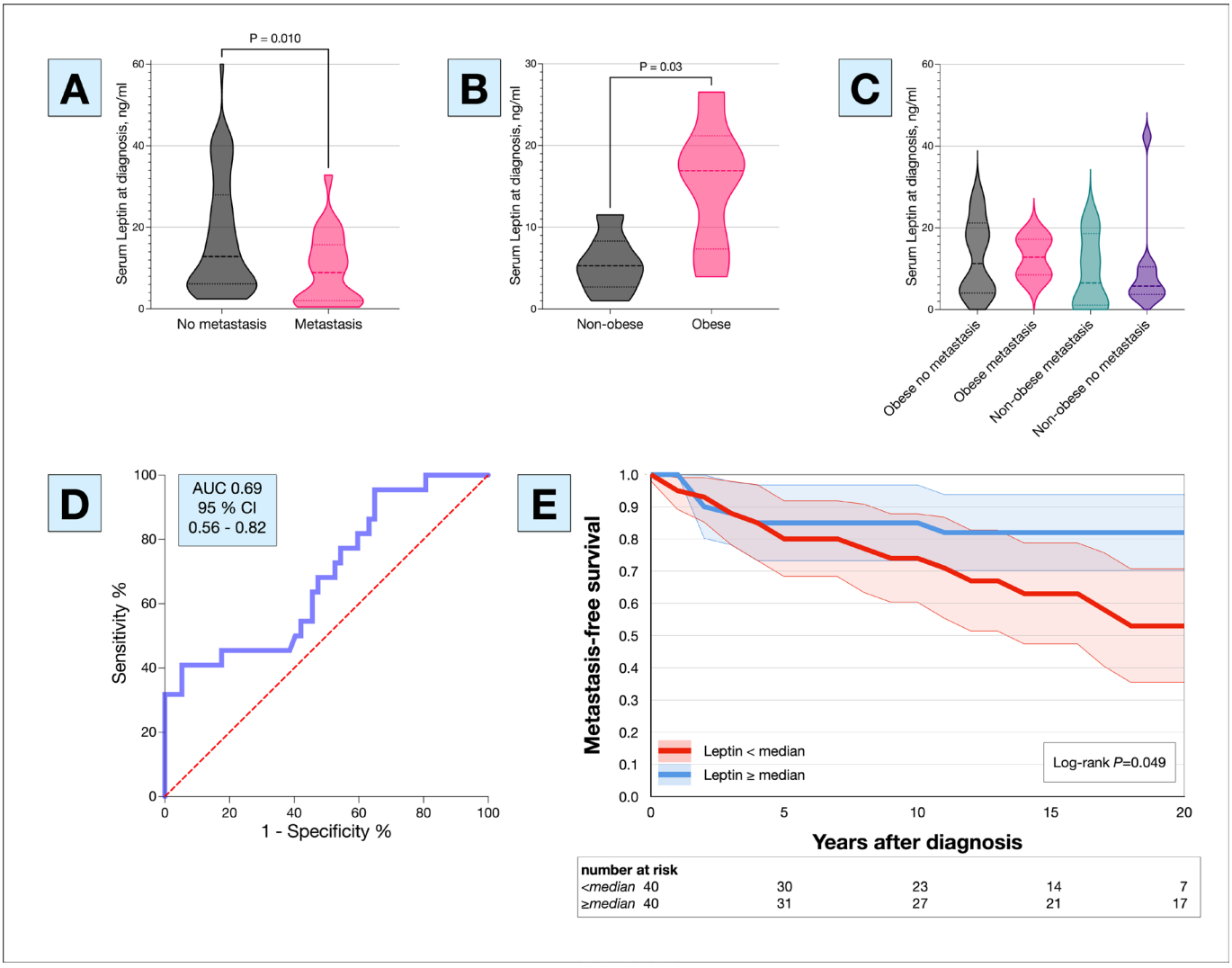

Figure 3 Development of metastases in relation to obesity and serum leptin levels at diagnosis. (A) Patients that eventually developed metastases had significantly lower leptin concentrations (Mann-Whitney U $p<0.01$). (B) Patients with obesity had higher serum leptin levels (p=0.03) than patients without obesity. (C) There was no significant difference in leptin levels between the groups when splitting the sample into four groups based on obesity and the development of metastases (Kruskal-Wallis p=0.63). (D) In ROC, serum leptin levels achieved an AUC of 0.69 (95% CI 0.56 to 0.82) for metastasis. (E) Patients with leptin levels<median had significantly shorter Kaplan-Meier metastasis-free survival (log-rank p=0.049). Coloured fields represent 95% CIs. AUC, area under the curve; ROC, receiver operating characteristics.

possibly stimulated by oestrogen levels, which in turn correlate to patient's sex and volume of subcutaneous fat.[45] Multiple other hormonal and metabolic systems may be involved, including melatonin, endogenous glucocorticoids and the gut microbiome. Deregulation of circadian clock-lipid metabolism interplay can increase the risk of obesity, which in turn may exacerbate circadian disorganisation.[46 47]

As observations of the beneficial implication of obesity and high leptin levels are seemingly contradictory to the general notion that obesity is a poor prognostic factor in cancer, the term 'the obesity paradox' has been transferred to cancer after being coined by Fleischmann *et al*[48] in a study of the beneficial influence of BMI on mortality in patients on haemodialysis.[49 50] Herein, we have shown that this concept can be transferred to UM.

Diabetes type II and the use of insulin were associated with metastases, and the latter retained its significance in multivariate analysis, independent of tumour diameter and obesity. Although based on a very limited number of patients (only eight used insulin), this is noteworthy and should be examined closer. Conversely, metformin has previously been identified as a potential anti-cancer drug with a cytostatic effect.[51] We found no beneficial prognostic effect of metformin in the present study, based on a similarly limited number of patients.

There are several limitations to this study. First, the results were based on retrospective cohorts, and we had limited control over confounding factors. Other diseases and risk factors for metastasis than the ones collected may have influenced the outcomes. Second, the use of BMI as measurement of obesity has been criticised since it does not differentiate between fat and lean mass.[50] This means that a healthy individual with well-developed musculature can have a higher BMI than an individual that does not exercise and have a relatively higher proportion of subcutaneous fat. Third, if some of the included patients had widespread metastatic disease and cachexia at diagnosis, the association between BMI and metastasis would be influenced. However, only 1% of the patients had detectable metastases by CT at the time of diagnosis, and among these, obesity was more prevalent

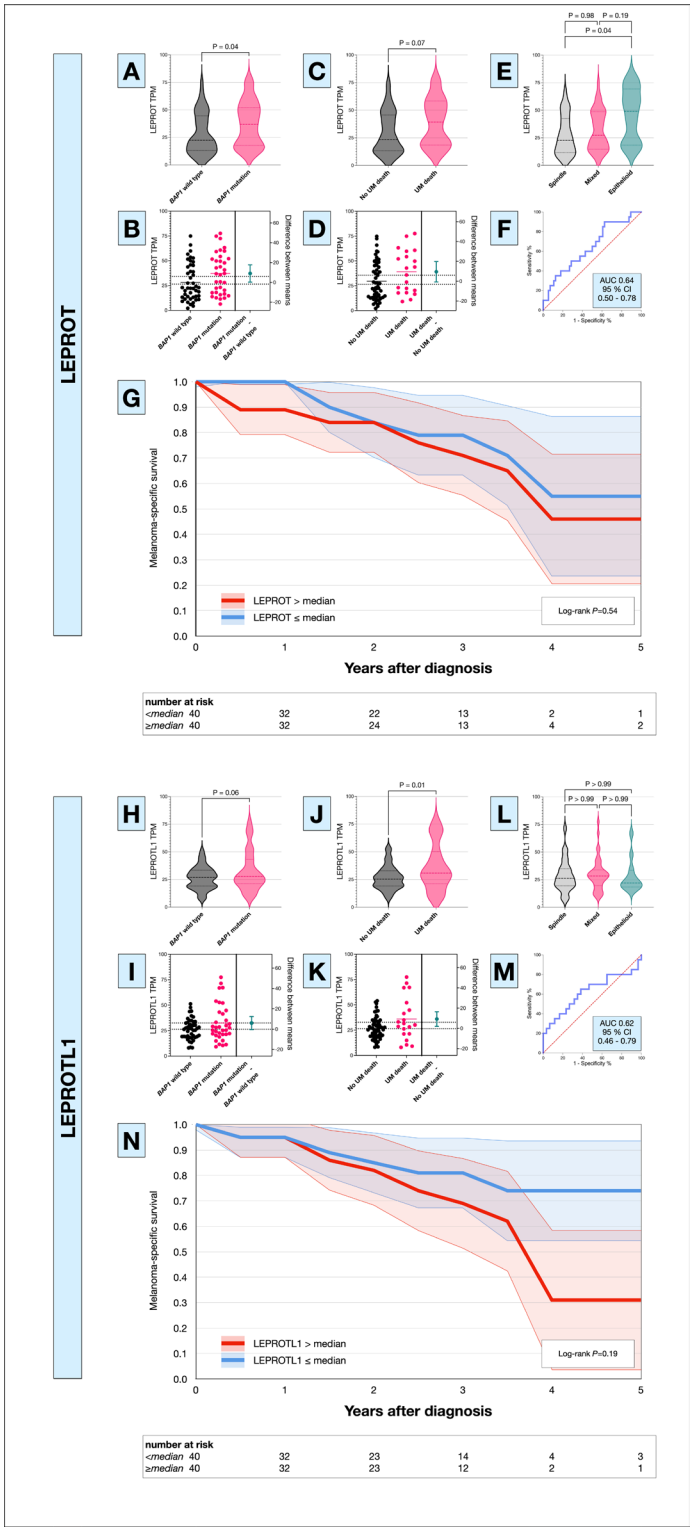

Figure 4 Genetic, histological and clinical factors in relation to tumour RNA expression levels of leptin receptor overlapping transcript (LEPROT) and leptin receptor overlapping transcript like 1 (LEPROTL1). Levels of LEPROT were significantly higher in tumours with (A and B) *BAP1* mutation, but (C and D) the difference was not significant on the 0.05 level in tumours from patients who died from metastatic uveal melanoma (UM), or in (E) tumours with a mix of spindle and epithelioid tumour cells. However, tumours with >90% epithelioid cells had greater expression of LEPROT than tumours with >90% spindle cells. (F) In ROC, LEPROT levels achieved an AUC of 0.64 (95% CI 0.50 to 0.78) for metastasis. (G) There was no difference in melanoma-specific survival between patients that had tumours with LEPROT levels above or below the median value. (H and I) The difference in LEPROTL1 levels between tumours with and without *BAP1* mutation was not significant on the 0.05 level. (J and K) Tumours from patients who died from metastatic UM had higher expression of LEPROTL1. (L) The expression of LEPROTL1 was similar across cell types. (M) In ROC, the LEPROTL1 levels achieved an AUC of 0.62 (95% CI 0.46 to 0.69). (N) There was no difference in melanoma-specific survival between patients that had tumours with LEPROTL1 levels above or below the median value. Coloured fields represent 95% CIs. AUC, area under the curve; ROC, receiver operating characteristics.

(two of seven, 29%) than in the total sample (116 of 581, 20%). If obesity or any other patient factor is impeding ultrasound examinations during the 5 years of metastatic screening, radiologists will typically suggest that the patient is examined with an alternative modality such as MRI or CT. Further, even if some metastases are missed or detected with a delay in patients with obesity, which will affect HRs for metastasis, it will not influence data on melanoma-related mortality to the same extent. The cause of death is not based on ultrasound screening, but on medical death certificates that are completed by physicians and/or forensic pathologists that have access to results from a range of examinations, typically including radiology other than ultrasound, laboratory investigations, biopsies of lesions suspected to be metastases and autopsy findings. Considering that the lower baseline BMI of patients that developed metastases and died of melanoma cannot be explained by a higher rate of metastases at presentation; that radiologists will recommend the radiological modality that has the best chances of detecting metastases; and that a similar difference was seen between patients without and with obesity in both the risk for metastasis and melanoma-related mortality which are based on data from different sources, we think that there is a very low risk that missed metastases in patients with obesity influenced our results. Fourth, BMI was only registered at the time of diagnosis. It is possible that some patients that were obese at diagnosis were no longer obese at the time of metastasis, and vice versa. Fifth, it should be mentioned that serum leptin levels have a diurnal variation that follows the circadian rhythm with peak levels at night.[52] All of the blood samples from the rather small cohort used to examine the prognostic implication of serum leptin levels were collected in daytime, but it cannot be excluded that the time of blood collection during the day may have influenced leptin levels for the individual patients.

In conclusion, our results indicate that obesity and high serum leptin levels are associated with lower risk for metastases in UM, independent of primary tumour size, diabetes, patient age and patient sex. Our findings pave the way for future studies aimed to further clarify the underlying mechanism for this association and elaborate on the implications of the obesity paradox in patients.

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Contributors SS: conceptualisation, formal analysis, investigation, writing—original draft, visualisation, data curation; AO: investigation, visualisation, data curation; CH: writing—review and editing, investigation; VTG: writing—review and editing; FP: investigation, validation; HA: investigation, writing—review and editing, resources; GS: conceptualisation, methodology, validation, formal analysis, resources, data curation, writing—original draft, writing—review and editing, visualisation, supervision, project administration, funding acquisition. GS is guarantor.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study was approved by the Swedish Ethical Review Authority (reference number 2019-04297) and adhered to the tenets of the Declaration of Helsinki and the research group's internal data security policy for sensitive data, which is based on recommendations from Karolinska Institutet, Swedish law and the General Data Protection Regulation (GDPR). Informed consent was obtained from all patients in the second cohort, before the collection of blood samples. The requirement for informed consent from the patients in the main cohort was waived because this was a retrospective registry study that did not affect treatment or follow-up of the patients, and did not require any additional examinations, tests, or interviews. Further, all patient data had been previously collected and no new clinical data collection was performed, and no tissues or other biological samples were handled or analysed.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The results published here are in part based upon data generated by the TCGA Research Network: https://www. cancer.gov/tcga.

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Supplementary Material to

The Obesity Paradox in Uveal Melanoma: High Body Mass Index is Associated with Low Metastatic Risk

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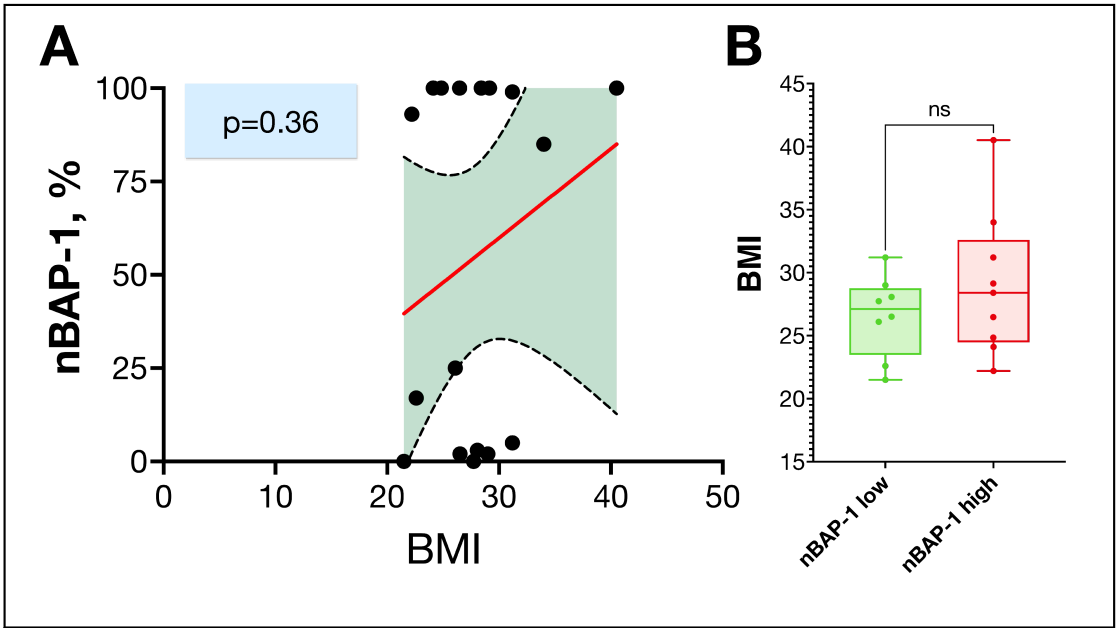
Sabazade S, *et al. Br J Ophthalmol* 2023;0:1–10. doi: 10.1136/bjo-2022-322877

Supplemental table 1. Clinicopathological characteristics across BMI class 1 through 4

BMI Class (*n*)	**1 (5)**	**2 (216)**	**3 (244)**	**4 (116)**	***P***
Age at diagnosis, mean (SD)	62 (13)	64 (16)	66 (12)	62 (13)	<0.001
Sex, n (%)					0.01*
Male	1 (20)	99 (46)	146 (60)	62 (53)	
Female	4 (80)	117 (54)	98 (40)	54 (47)	
Tumour diameter, mean mm (SD)	11.0 (3.6)	10.8 (3.5)	11.0 (3.6)	11.1 (3.9)	0.99
Tumour thickness, mean mm (SD)	7.5 (3.6)	5.7 (3.2)	5.9 (2.8)	5.6 (2.8)	0.92
Distance to OD, mean mm (SD)	3.7 (3.2)	3.9 (3.4)	3.0 (3.1)	4.2 (3.9)	0.59
CBI, n (%)	1 (20)	7 (5)	7 (4)	5 (7)	0.15*
ASA-classification, n (%)					<0.001*
1	0 (0)	103 (49)	88 (36)	17 (15)	
2	3 (60)	96 (46)	132 (54)	64 (57)	
3	2 (40)	11 (5)	21 (9)	32 (28)	
4	0 (0)	0 (0)	1 (<1)	0 (0)	
Anti-coagulants, n (%)					0.46*
No	5 (100)	174 (81)	196 (80)	88 (76)	
Yes	0 (0)	42 (19)	48 (20)	28 (24)	
Cardiovascular disease, n (%)					0.95*
No	4 (80)	178 (82)	201 (82)	93 (80)	
Yes	1 (20)	38 (18)	43 (18)	23 (20)	
Levothyroxine, n (%)					0.34*
No	0 (80)	203 (94)	227 (93)	104 (90)	
Yes	1 (20)	13 (6)	17 (7)	12 (10)	
Anti-hypertensives, n (%)					<0.001*
No	4 (80)	140 (65)	141 (58)	47 (41)	
Yes	1 (20)	76 (35)	103 (42)	69 (59)	
Diabetes type II, n (%)					0.008*
No	4 (80)	52 (95)	49 (86)	20 (77)	
Yes	1 (20)	3 (6)	8 (14)	6 (23)	

ASA, American Society of Anesthesiologists physical status, classified from 1 to 4. CBI, Ciliary body involvement. OD, optic disc. SD, standard deviation. *At least one cell has *n*<5.



Supplemental figure 1. Immunohistochemical nuclear BAP-1 expression (nBAP-1) in relation to patients' body mass index (BMI). A) In linear regression, there was no correlation between primary tumour nBAP-1 expression and BMI. B) Similarly, the distribution of BMI was similar between tumours with high and low nBAP-1 (Mann–Whitney *U P*=0.44). ns, non-significant.

Supplemental table 2. Clinicopathological characteristics of 80 patients with analysed serum leptin levels	
Age at diagnosis, median (IQR)	67 (18)
Sex, n (%)	
Male	42 (53)
Female	38 (48)
Tumour diameter, mean mm (SD)	10.7 (2.4)
Tumour thickness, mean mm (SD)	5.7 (2.6)
CBI, n (%)	3 (4)
AJCC stage, n (%)	
I	25 (31)
IIA	39 (49)
IIB	16 (20)
IIIA	0 (0)
IIIB	0 (0)
IIIC	0 (0)
IV	0 (0)
Median follow up, years (IQR)[a]	22.4 (0.7)

IQR, interquartile range. SD, standard deviation. CBI, Ciliary body involvement. AJCC, American Joint Committee on Cancer. [a]For survivors.