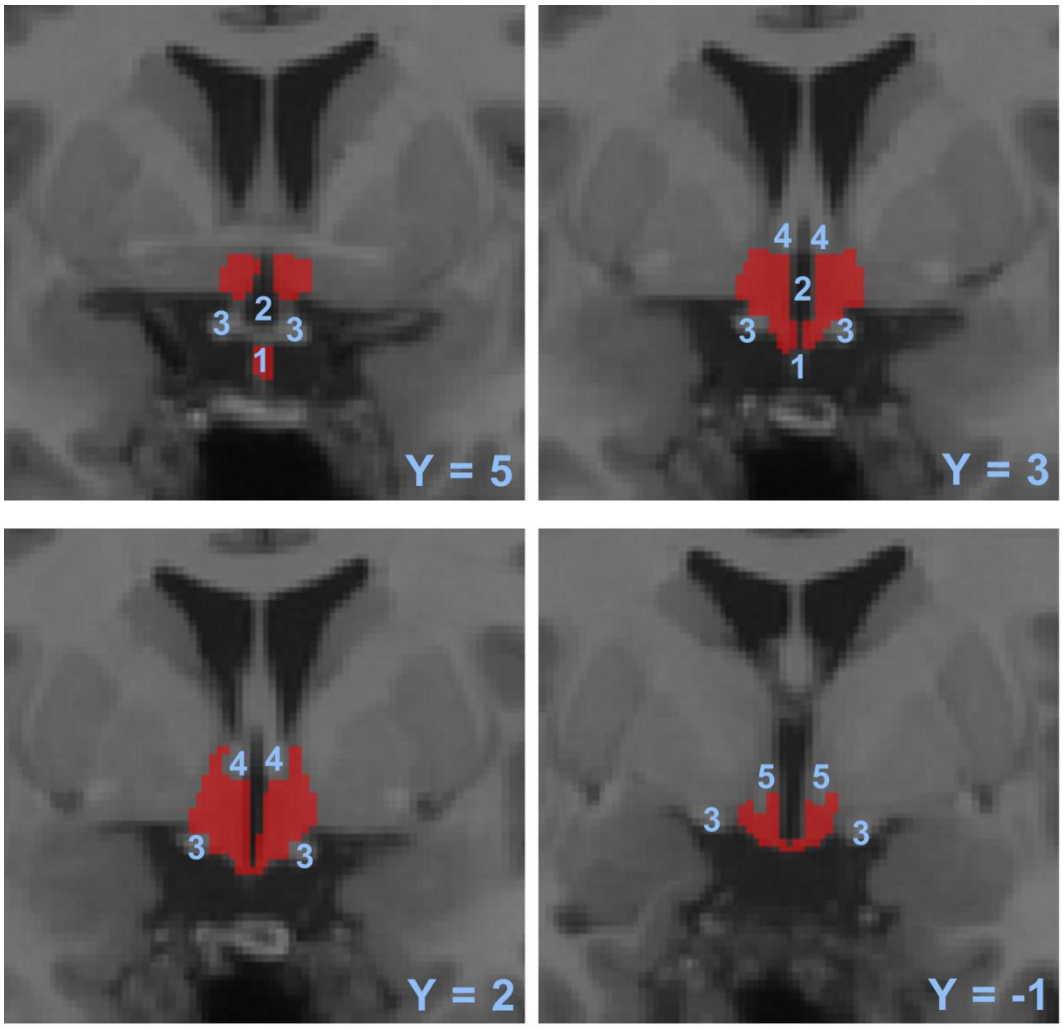
Supplementary Material

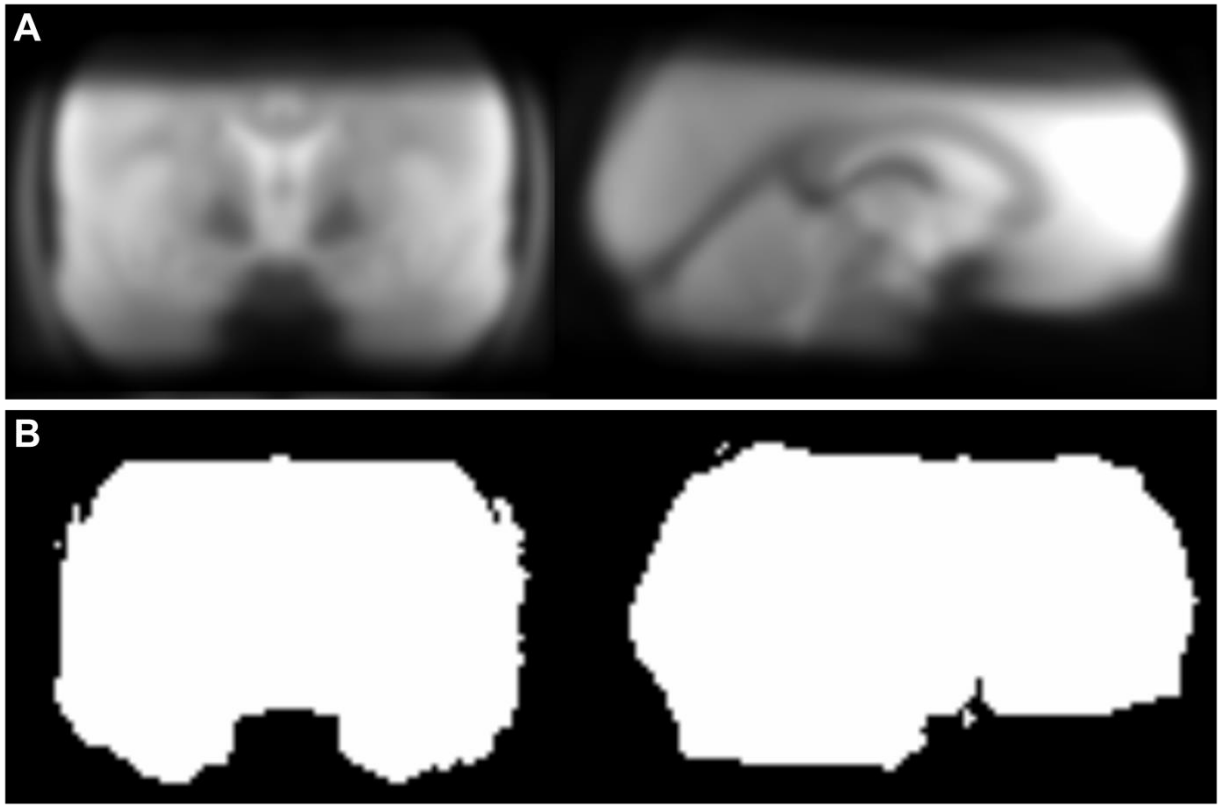
Methods

Supplementary Figure1: Manual segmentation of the hypothalamus.



Manual segmentation of the hypothalamus shown on a T1-weighted template image with annotations depicting anatomical landmarks used to identify the hypothalamic area, 1=Infundibular Stalk, 2=Third Ventricle, 3=Optic Tract, 4=Fornix, 5=Hypothalamic Sulcus.

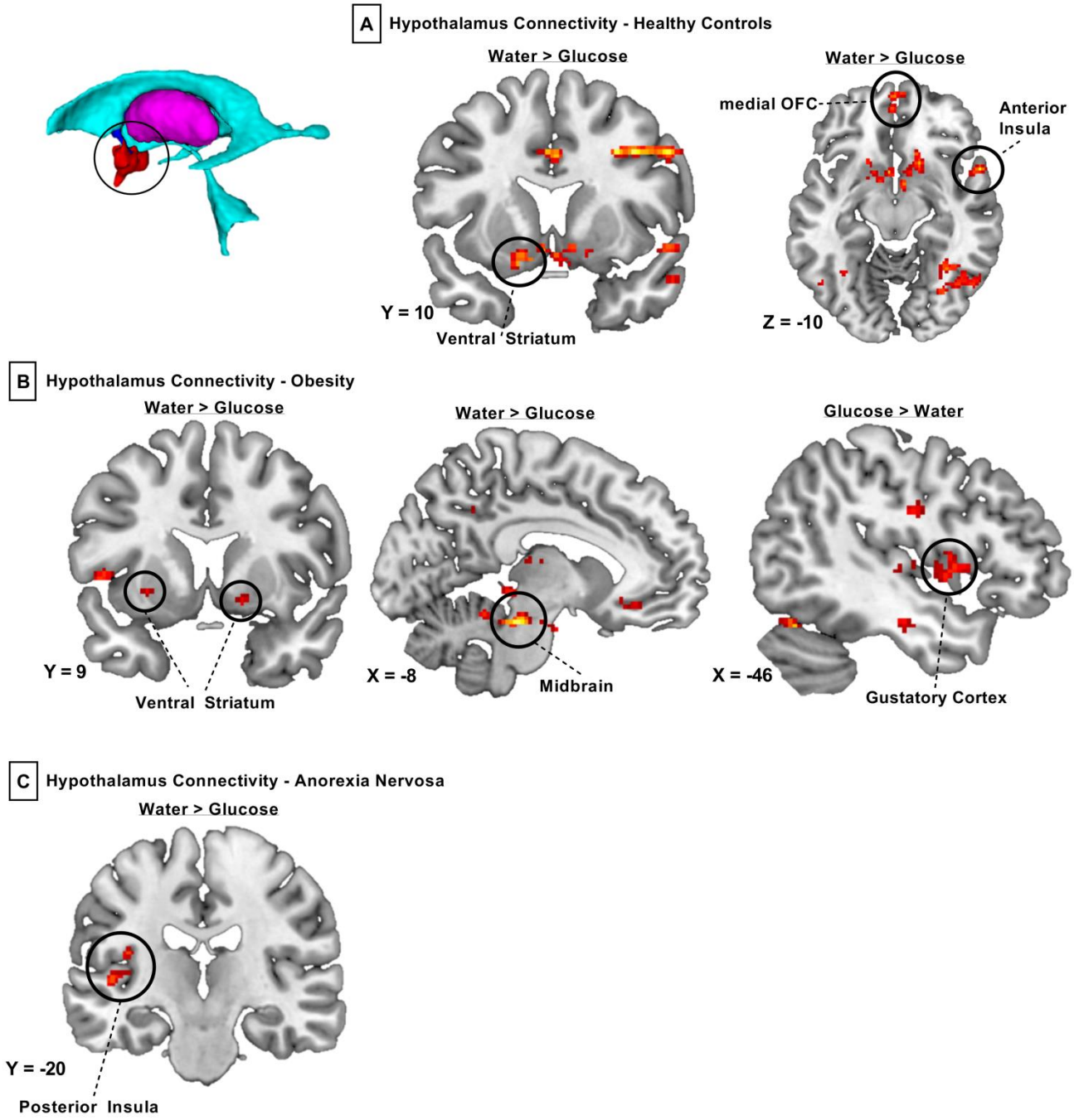
Supplementary Figure 2: Brain coverage of EPI-sequence.



A. Group-averaged functional MRI data (EPI-images) of all participants included in the study. B. 2nd-level mask used in group comparisons.

Results

Supplementary Figure 3: Satiety-state-dependent functional connectivity of the hypothalamus.



A, Satiety-state-dependent functional connectivity in healthy controls between the hypothalamus and left ventral striatum (water>glucose, mean difference: 0.136, t_{27} =

4.31, $P < 0.001$), anterior insula (water>glucose, mean difference: 0.149, $t_{27} = 4.18$, $P < 0.001$) and medial orbitofrontal cortex (water>glucose, mean difference: 0.13, $t_{27} = 4.06$, $P < 0.001$). **B**, Satiety-state-dependent functional connectivity in controls with obesity between the hypothalamus and right and left ventral striatum (right: water>glucose, mean difference: 0.144, $t_{23} = 3.57$, $P = 0.002$; left: water>glucose, mean difference: 0.138, $t_{23} = 3.73$, $P = 0.001$), midbrain (water>glucose, mean difference: 0.159, $t_{23} = 4.03$, $P = 0.001$) and gustatory cortex (glucose>water, mean difference: 0.155, $t_{23} = 4.11$, $P < 0.001$). **C**, Satiety-state-dependent functional connectivity in patients with AN between the hypothalamus and posterior insula (water>glucose, mean difference: 0.15, $t_{23} = 4.18$, $P < 0.001$).

Structural brain differences

We observed no significant differences in hypothalamic volume between normal-weight control participants and patients with AN (mean percentage of hypothalamic volume for control participants: 0.0676%, SD = 0.0052%, mean volume for patients with AN: 0.0663%, SD = 0.0069%, mean difference between groups: 0.0013%, t_{50} = 0.75, P = 0.455), but increased volume when compared to controls with obesity (mean percentage of hypothalamic volume for obese controls: 0.0639%, mean difference between groups: 0.0036%, t_{50} = 2.15, P = 0.036). Although gray and white matter volume was higher in healthy controls than in patients with AN (mean gray matter volume for control participants: 738.957 cm^3, mean gray matter for patients with AN: 664.093 cm^3, mean difference between groups: 74.863 cm^3, t_{50} = 4.676, P < 0.001, mean white matter volume for control participants: 446.929 cm^3, mean white matter for patients with AN: 412.667 cm^3, mean difference between groups: 34.259 cm^3, t_{50} = 3.765, P < 0.001), there was no significant difference in white and gray matter between healthy controls and controls with obesity (mean gray matter for participants with obesity: 727.796 cm^3, mean difference between groups: 11.16 cm^3, t_{50} = 0.647, P = 0.52, mean white matter for participants with obesity: 459.574 cm^3, mean difference between groups: -12.648 cm^3, t_{50} = -1.401, P = 0.167).

Furthermore, there was no significant difference in hypothalamic volume between patients with AN and controls with obesity (mean difference between groups: -0.0024%, t_{46} = 1.18, P = 0.242), although white and gray matter volume was significantly higher in controls with obesity than in patients with AN (mean difference in gray matter between groups: -63.702 cm^3, t_{46} = -3.104, P = 0.003, mean difference in white matter between groups: -46.907 cm^3, t_{46} = -3.941, P < 0.001).

Glucose and water induced BOLD activation in additional reward-related brain regions

Normal-weight control participants showed glucose-induced attenuation of activity in the nucleus caudatus (t_{27}=2.46, *P*=0.021), putamen (t_{27}=3.01, *P*=0.006), insular cortex (t_{27}=2.67, *P*=0.013), medial orbitofrontal cortex (t_{27}=2.93, *P*=0.007) and inferior operculum (t_{27}=2.29, *P*=0.03). Patients with AN and controls with obesity did not show a significant glucose induced deactivation in the nucleus caudatus (*P*=0.347 and *P*=0.722, respectively), putamen (*P*=0.677 and *P*=0.929), insular cortex (*P*=0.58 and *P*=0.438), medial orbitofrontal cortex (*P*=0.912 and *P*=0.587) and inferior operculum (*P*=0.827 and *P*=0.462). A group comparison revealed significant differences between all three groups in BOLD signal response in the nucleus caudatus ($F_{2,73}$=3.89, *P*=0.025): no significant differences between normal-weight control participants and patients with AN (*P*=0.065) and no significant difference between controls with obesity and patients with AN (*P*=0.345) but a stronger decrease in activation in normal-weight control participants when compared tocontrols with obesity (t_{50}=-2.65, *P*=0.011). Signal response in the putamen also proved to be different between groups ($F_{2,73}$=3.99, *P*=0.023), however, there were no significant difference between normal-weight control participants and patients with AN (*P*=0.084) but a stronger signal decrease in patients with AN as well as normal-weight control participants when compared to controls with obesity (t_{46}=-2.29, *P*=0.026 and t_{50}=-3.52, *P*=0.001, respectively). Furthermore, we observed significant differences between groups in the insular cortex ($F_{2,73}$=3.79, *P*=0.027). There were no significant differences between normal-weight control participants and patients with AN (*P*=0.132), but a stronger decrease in patients with AN and normal weight controls when compared to controls with obesity (t_{46}=-2.18, *P*=0.034 and t_{50}=-3.28, *P*=0.002, respectively). Finally, there were no groups differences in activation in the

medial orbitofrontal cortex ($F_{2,73}$=2.11, *P*=0.128) and inferior operculum ($F_{2,73}$=2.55, *P*=0.085).

Supplementary Table 1: Within group results - influence of metabolic state on hypothalamus connectivity

	Z-values	*k*	x	y	z
Healthy Controls					
Middle temporal gyrus	>8	222	56	2	-24
Pons	>8	48	16	-24	-32
Anterior insula	>8	30	56	12	-10
Ventral Striatum	7.69	229	-16	10	-16
Inferior frontal gyrus	>8	66	12	38	-24
Inferior parietal lobule	>8	71	44	-32	34
Inferior temporal gyrus	>8	97	-60	-8	-18
Temporal pole	>8	33	-36	20	-28
Hippocampus	>8	40	-36	-20	-18
Medial orbitofrontal cortex	7.39	98	0	60	-10
Patients with Anorexia Nervosa					
Cerebellum	>8	49	-10	-86	-30
Fusiform gyrus	>8	32	56	-2	-28
Middle frontal gyrus	>8	50	34	30	36
Occipital lobe	7.84	63	10	-100	8
Posterior insula	7.56	113	-38	-18	18
Middle temporal gyrus	7.52	42	68	-26	-8
Controls with obesity					
Midbrain	>8	191	-12	-26	-20
Temporal pole	>8	50	38	24	-36
Ventral striatum	>8	41	-22	-2	-2
Ventral striatum	6.95	57	24	-4	-6
Anterior Cingulate	7.61	37	2	38	8
Superior temporal gyrus	7.54	31	-56	-8	2
Gustatory cortex	7.33	85	-48	6	2
Thalamus	6.94	52	-6	-18	12
Middle temporal gyrus	6.48	33	60	-40	-8

k=Cluster size (voxels). All clusters were significant after whole-brain family-wise error correction at the cluster level $P_{FWE}<0.05$ with a minimum cluster size of $k>30$.

Supplementary Table 2: Between group results - interaction between metabolic state and group - hypothalamus connectivity

	Z-values	*k*	x	y	z
Healthy Controls vs. Patients with Anorexia Nervosa					
Middle frontal gyrus	>8	182	34	30	36
Middle temporal gyrus	>8	110	-40	8	-42
Inferior temporal gyrus	7.2	64	-58	-4	-26
Middle temporal gyrus	>8	151	56	-2	-28
Ventral Striatum	>8	151	-16	10	-14
Cuneus	>8	104	12	-100	10
Insula	>8	63	-34	-26	20
Rolandic Operculum	7.72	41	52	-24	22
Inferior parietal lobule	7.54	75	38	-36	44
Medial frontal gyrus	7.5	114	-6	48	30
Hippocampus	7.49	53	28	-34	4
Middle Occipital gyurs	7.22	31	-52	-72	4
Putamen	6.97	49	16	14	-10
Inferior orbitofrontal cortex	6.89	35	-46	40	-14
Healthy Controls vs. Controls with obesity					
Brainstem	>8	141	-6	-14	-22
Lentiform nucleus	>8	44	-18	-4	-10
Medial orbitofrontal cortex	>8	41	2	64	-2
Superior frontal gyrus	7.46	117	16	66	22
Rolandic operculum	6.89	32	56	-8	14
Superior temporal gyrus	6.69	35	-56	-8	2
Controls with obesity vs. Patients with Anorexia Nervosa					
Middle temporal gyrus	>8	178	62	-42	-10
Inferior operculum	>8	57	-48	8	4
Superior temporal gyrus	>8	165	-48	-18	2
Superior frontal gyrus	>8	108	-32	34	34
Ventral striatum	>8	48	-10	4	-6
Midbrain	>8	72	-12	-30	-6
Putamen	>8	49	-28	-8	-6
Middle frontal gyrus	7.52	71	26	50	26
Hippocampus	7.49	35	24	-32	-4
Inferior parietal lobule	7.4	49	-34	-64	40
Anterior cingulate cortex	6.66	33	-4	30	16

k=Cluster size (voxels). All clusters were significant after whole-brain family-wise error correction at the cluster level P_{FWE}<0.05 with a minimum cluster size of *k*>30.

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	✓
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	✓
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓
Objectives	3	State specific objectives, including any prespecified hypotheses	✓
Methods			
Study design	4	Present key elements of study design early in the paper	✓
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	✓
		(*b*) For matched studies, give matching criteria and the number of controls per case	✓
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓
Bias	9	Describe any efforts to address potential sources of bias	✓
Study size	10	Explain how the study size was arrived at	✓
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	✓
		(*b*) Describe any methods used to examine subgroups and interactions	✓
		(*c*) Explain how missing data were addressed	✓
		(*d*) If applicable, explain how matching of cases and controls was addressed	✓
		(*e*) Describe any sensitivity analyses	✓
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓
		(b) Give reasons for non-participation at each stage	✓
		(c) Consider use of a flow diagram	✓
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓

		(b) Indicate number of participants with missing data for each variable of interest	✓
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	✓
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓
		(*b*) Report category boundaries when continuous variables were categorized	✓
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	✓
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓
Discussion			
Key results	18	Summarise key results with reference to study objectives	✓
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.