A population-based cross-sectional study of the association between facial morphology and cardiometabolic risk factors in adolescence

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A POPULATION-BASED CROSS-SECTIONAL STUDY OF THE ASSOCIATION BETWEEN FACIAL MORPHOLOGY AND CARDIOMETABOLIC RISK FACTORS IN ADOLESCENCE

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ABSTRACT

Objective: To determine whether facial morphology is associated with fasting insulin, glucose and lipids independently of body mass index in adolescents.

Design: Population-based cross-sectional study.

Setting: Avon Longitudinal Study of Parents and Children (ALSPAC), South West of England.

Participants: From the ALSPAC database of 4747 3D facial laser scans, collected during a follow-up clinic at the age of 15, 2348 white British adolescents (1127 males, 1221 females) were selected on the basis of complete data on cardiometabolic parameters, body mass index (BMI), and Tanner's pubertal stage.

Main outcome measures: Fasting insulin, glucose, and lipids (triglycerides, high (HDLc) and low density (LDLc) lipoprotein cholesterols).

Results: Based on the collection of 63 x, y, and z co-ordinates of 21 anthropometric landmarks, 14 facial principal components (PCs) were identified. These components explained 82 per cent of the variation in facial morphology and were used as exposure variables. With adjustment for age, gender and pubertal stage, seven PCs were associated with fasting insulin, none with glucose, three with triglycerides, three with HDLc, and four with LDLc. After additional adjustment for BMI, four PCs remained associated with fasting insulin, one PC with triglycerides, and two PCs with LDLc. None of these associations withstood adjustment for multiple comparisons.

Conclusion: These initial hypothesis generating analyses provide no evidence that facial morphology is importantly related to cardiometabolic outcomes. Further examination might be warranted. Facial morphology assessment may have value in identifying other areas of disease risk.

ARTICLE SUMMARY

Article focus

- Three-dimensional imaging opens up a new chapter in investigations of facial morphology. Previous research revealed associations of facial morphology with obesity in adolescents, but whether facial morphology can be used to identify those at future risk of adverse cardiometabolic outcomes is unknown.

Key messages

- Our results suggest that facial morphology is not strongly or consistently associated with fasting insulin, glucose, or lipids, particularly after adjustment for body mass index, in white British adolescents. Facial morphology is therefore unlikely to be useful in identifying white British adolescents at future risk of adverse cardiometabolic outcomes.

- Suggested methodology can be used in future studies to explore the associations between facial parameters and other health outcomes. It might provide valuable insights into how facial morphology can be indicative of health.

Strengths and limitations of this study

- The strengths of this study are a large sample size and the homogeneity of the sample: all participants were of white origin, born and brought up in the same region of the UK. Non-invasive, accurate, and reliable method was used for capturing details of facial soft tissue morphology. A comprehensive statistical analysis was undertaken to extract principal components of facial morphology.

- The study has some limitations. A face could not be easily represented as a single exposure due to the complexity of its morphology and the vast amount of data captured by the laser scanning system. Therefore, some data reduction was necessary prior to the analysis. Furthermore, it was not possible to control all the confounding factors in a cross-sectional study design. Since faces of adolescents are still developing, changing their shape and size, future studies might have to investigate the relationship between these changes and cardiometabolic characteristics through time.

INTRODUCTION

Recent technological advancements in imaging methods marked a transition from two-dimensional to three-dimensional (3D) approach in craniofacial research, thus opening a new era. A special emphasis has been placed on the development and application of non-invasive methods to capture human face accurately and reliably.[1, 2] Among these, laser surface scanning and stereophotogrammetry have gained wide acceptance of research community.[3] So far, a large spectrum of medical disciplines have utilised these methods in the investigations of facial growth, facial dysmorphology, craniofacial identification, as well as the influence of different medical conditions on facial phenotype.[4-12] Therefore, an exciting opportunity has occurred to explore whether facial characteristics can serve as new diagnostic measures of illnesses.

Childhood obesity is becoming an epidemic health problem.[13] It is evident from many studies that it is associated with increased risk of type 2 diabetes and cardiovascular disease later in life.[14-16] Despite this fact, the connection between obesity and craniofacial development has been rarely investigated. Bimaxillary prognathism (overdeveloped jaws in sagittal direction) and increased transverse facial dimensions seem to indicate the difference between obese adolescents and their normal-weighted peers.[17-19] However, the association between metabolic phenotype and facial form has not been addressed previously.

In order to investigate this problem, large sample and a comprehensive 3D approach to facial measurements are needed. In this cross-sectional study, which can be considered hypothesis generating, we examined the associations of facial soft tissue morphology with metabolic phenotype (fasting insulin, glucose, triglycerides, high density lipoprotein

cholesterol (HDLc), and low density lipoprotein cholesterol (LDLc)) in a large general population cohort of adolescents using an existing database of 3D facial laser scans.

MATERIAL AND METHODS

Sample

We used the data from the Avon Longitudinal Study of Parents and Children (ALSPAC), a UK-based longitudinal birth cohort study designed to explore genetic and environmental influences on health and wellbeing.[20, 21] All pregnant women were eligible to participate in ALSPAC if their estimated delivery date fell between 1st April 1991 and 31st December 1992 inclusive. 14541 pregnant women were recruited and from these women there were 14676 live born infants. Since age 7 surviving offspring have been invited to regular follow-up clinics.

In the current study, we examined the data obtained during an annual follow-up clinic at the age of 15, which was attended by 5253 adolescents. On that occasion, facial laser scanning was performed, and after a drop-out of 506 individuals due to the low quality of the scans, or some sort of facial dysmorphology, a database of 4747 individuals (2233 males and 2514 females) was formed. [22] Out of these, we selected 2348 white adolescents (1127 males and 1221 females), with complete data related to the outcome and confounding variables (see below), as facial laser scans were used to derive exposure variables. The flow-chart diagram (Fig. 1) shows gradual selection of individuals who comprised the final sample. The study was approved by the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee and informed consent was obtained from children and their parents or guardians.

Measures

Exposure variables

Facial laser scans were used to derive principal components of facial morphology, which served as the exposure variables. This is explained in detail in the section on statistical analysis. Prior to this, it was necessary to perform three steps, which will be described here. First of all, facial scans were processed. Validity and reliability of laser scanning procedure, as well as the processing stages of the scans, have been previously investigated.[23-26] Secondly, twenty-one anthropometric landmarks were manually identified on facial scans by one experienced examiner (Fig. 2), according to their respective definitions by Farkas,[27] and their *x*, *y* and *z* co-ordinates were saved for the subsequent analysis. Previous research showed that these landmarks are clinically reliable.[28,29] Finally, facial scans were initially normalized according to the natural head position, with the origin of the co-ordinate system set at the point half-way between the inner corners of the eyes (mid-endocanthion). The *x*-axis was pointing left, from right to left eye, the *y*-axis was pointing vertically upwards from chin to forehead, and the *z*-axis was pointing outwards, in the nose direction. The coronal, sagittal, and transverse planes were taken as the *xy*, *yz*, and *xz* planes, respectively.[1, 2, 8, 22, 28]

Outcome variables

Fasting insulin, fasting glucose, triglycerides, high density lipoprotein cholesterol (HDLc), and low density lipoprotein cholesterol (LDLc) were taken as the outcome variables. Full details of their assessment have been previously reported.[30]

Confounding variables

Since this study is exploratory (being the first to examine these associations) and our main motivation was to understand whether facial morphology might be able to predict those at risk of cardiometabolic disease over and above simple measurement of adiposity, we did not

adjust for a wide range of confounding variables. However, we adjusted for age, pubertal stage, and body mass index (BMI), as these are potentially important predictors of cardiometabolic risk and we would want to be clear that facial morphology predicted outcome over and above these. The age of the participants was recorded in months as they arrived at the clinic. Pubertal status was assessed on participants' self report with Tanner's questionnaires.

Statistical analyses

Participant characteristics were summarised with means (SD) for continuous approximately normally distributed variables, median (IQR) for continuous right skewed variables, and number (%) for categorical variables.

Generalized Procrustes Analysis (GPA) was performed on landmark configurations (each consisting of 63 *x*, *y*, and *z* co-ordinates of 21 facial landmarks) in order to remove differences in landmarks' position attributable to translation and rotation.[31-33] Scaling was not performed in order to preserve facial size. Principal Component Analysis (PCA) was used to reduce the set of 63 co-ordinates into a smaller number of independent components of facial morphology. According to the 'Kaiser–Guttman criterion', PCs with eigenvalues greater than the average eigenvalue value were retained [34-36] and saved as new exposure variables. The rotation method used for PCA was varimax with Kaiser normalisation.[37] GPA was performed in the open source software R project and PCA in SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

Whilst this is a cross-sectional study, in all our analyses we examined the association of principal components of facial morphology (as exposures) with fasting insulin, glucose, triglycerides, HDLc, and LDLc (as outcomes) using multivariable linear regression models.

No evidence was found for any gender interactions (all p-values ≥ 0.1) and therefore analyses are presented with both genders combined. In the first model we adjusted for age, gender, and pubertal stage. In the second model we adjusted for age, gender, pubertal stage, and BMI and examined how much this reduced any associations of facial principal components with the outcomes. Fasting insulin and triglycerides levels were right (positively) skewed and their logged values where used in the linear regression models, which ensured the model residuals were approximately normally distributed. The resultant regression coefficients with 95 per cent confidence intervals are presented.

In these multivariable analyses 140 comparisons were made (14 exposures with five outcomes and two models). In initial analyses we considered the conventional 0.05 level of statistical significance. We then adjusted for multiple comparisons using a Bonferroni correction by dividing 0.05 by 140, thus for these corrected analyses a p-value of 0.0004 would be considered statistically significant at the 0.05 level. All statistical analyses were performed in SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Table 1 shows the characteristics of the study participants. The Principal Component Analysis (PCA) identified 14 principal components (PCs) of facial morphology (Table 2). Each PC consisted of a number of co-ordinates of anthropometric landmarks. For example, the first principal component (PC 1) comprised 17 *y* co-ordinates of landmarks located in the upper and lower thirds of the face. These co-ordinates represented facial height (size). In order to facilitate understanding and interpretation of individual PCs, they are presented graphically on Figure 3. The first three PCs (facial size, inter-eye distance, and prominence of the nose and lower lip) accounted for almost half of the total variation (45.7%). The other 11 PCs

contributed to facial variation to a much lesser extent (between 1.6 and 5%), but marked those subtle features which make the faces unique.

Table 1 Characteristics of the study sample.

	Categories/units	Males N = 1127	Females N = 1221	All N = 2348
Age	Mean (months)	184.8 (3.0)	184.9 (3.2)	184.9 (3.1)
Tanner's pubertal stages	Stage I n (%)	0	0	0
	Stage II n (%)	8 (0.7%)	6 (0.5%)	14 (0.6%)
	Stage III n (%)	64 (5.7%)	118 (9.7%)	182 (7.8%)
	Stage IV n (%)	552 (49.0%)	632 (51.8%)	1184 (50.4%)
	Stage V n (%)	503 (44.6%)	465 (38.1%)	968 (41.2%)
Fasting insulin	Median (IU/l)	8.2 (5.9, 10.9)	9.7 (7.4, 13.0)	9.0 (6.6, 12.0)
Fasting glucose	Mean (mmol/l)	5.3 (0.4)	5.1 (0.3)	5.2 (0.4)
Total cholesterol	Mean (mmol/l)	3.6 (0.6)	3.9 (0.63)	3.8 (0.6)
Triglycerides	Median (mmol/l)	0.7 (0.6, 1.0)	0.8 (0.6, 1.0)	0.7 (0.6, 1.0)
HDLc	Mean (mmol/l)	1.2 (0.3)	1.4 (0.3)	1.3 (0.3)
LDLc	Mean (mmol/l)	2.0 (0.5)	2.2 (0.6)	2.1 (0.6)

Number (%) for categorical variables, mean (SD) or median (IQR) for continuously distributed variables are presented. SD, standard deviation; IQR, interquartile range; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol.

The multivariable associations of the 14 PCs with cardiometabolic outcomes are shown in Tables 3 to 7. With adjustment for age, gender and pubertal stage (model 1), seven PCs were associated with fasting insulin, none with fasting glucose, three PCs with triglycerides and HDLc, and four PCs with LDLc. After additional adjustment for BMI (model 2), four principal components remained associated with fasting insulin, none with glucose, one PC with triglycerides, none with HDLc, and two PCs with LDLc. However, none of these associations withstood adjustment for multiple comparisons.

Table 2 The results of the principal component analysis showing partial correlation coefficients between co-ordinates of anthropometric landmarks and facial principal components.

	Facial principal components													
Co-ordinates	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**	**PC14**
Ls (y)	-0.84													
Enl (y)	0.84													
Cphr (y)	-0.84													
Cphl (y)	-0.83													
Enr (y)	0.83													
Pg (y)	-0.82													
Chr (y)	-0.82													
Pil (y)	0.81													
Chl (y)	-0.81													
Pir (y)	0.81													
Psl (y)	0.79													
Li (y)	-0.78													
Psr (y)	0.78													
Exr (y)	0.75													
Exl (y)	0.74													
G (y)	0.64													
N (y)	0.62													
Psl (x)		0.94												
Psr (x)		-0.93												
Pil (x)		0.93												
Pir (x)		-0.92												
Enr (x)		-0.83												
Enl (x)		0.83												
Exr (x)		-0.79												
Exl (x)		0.75												
All (z)			-0.80											
Alr (z)			-0.79											
Sn (z)			-0.79											
Prn (z)			-0.68											
Li (z)			0.56											
Ls (z)				0.87										
Cphl (z)				0.86										
Cphr (z)				0.86										
Pg (z)				-0.78										
G (z)					-0.86									
N (z)					-0.82									
Pir (z)					0.65									
Pil (z)					0.64									
Prn (y)						0.82								
All (y)						0.79								
Alr (y)						0.77								
Sn (y)						0.72								
Chr (x)							0.82							
Chl (x)							-0.82							
Chl (z)							0.80							
Chr (z)							0.80							
Sn (x)								0.94						
Prn (x)								0.90						
G (x)									0.97					
N (x)									0.97					
Exl (z)										-0.62				
Exr (z)										-0.60				
Psl (z)											0.80			
Psr (z)											0.79			
Ls (x)												0.92		
Cphr (x)													0.82	
Cphl (x)													-0.78	
Pg (x)														0.91
Li (x)														0.76

Only major landmarks contributing to each principal component (PC) are shown (coefficients with absolute values above 0.5). Anthropometric landmarks are explained on Figure 2 and the principal components on Figure 3.

Table 3 Multivariable association of fourteen facial principal components (exposures) with fasting insulin as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	0.004	(-0.006, 0.014)	0.397	**-0.011**	**(-0.021, -0.001)**	**0.026**
PC2	**0.011**	**(0.002, 0.019)**	**0.011**	-0.003	(-0.011, 0.005)	0.419
PC3	**0.010**	**(0.002, 0.019)**	**0.015**	0.002	(-0.006, 0.010)	0.681
PC4	-0.001	(-0.009, 0.007)	0.803	0.002	(-0.005, 0.010)	0.538
PC5	**-0.011**	**(-0.020, -0.002)**	**0.019**	0.001	(-0.008, 0.010)	0.802
PC6	**0.010**	**(0.002, 0.018)**	**0.013**	0.001	(-0.007, 0.009)	0.787
PC7	0.000	(-0.008, 0.008)	0.935	0.005	(-0.003, 0.013)	0.190
PC8	**-0.017**	**(-0.028, -0.006)**	**0.003**	**-0.014**	**(-0.024, -0.004)**	**0.009**
PC9	**0.012**	**(0.003, 0.020)**	**0.005**	**0.012**	**(0.004, 0.020)**	**0.002**
PC10	0.005	(-0.003, 0.014)	0.190	0.002	(-0.006, 0.010)	0.601
PC11	**0.026**	**(0.018, 0.034)**	**<0.0001**	**0.009**	**(0.001, 0.017)**	**0.029**
PC12	0.006	(-0.002, 0.014)	0.151	0.005	(-0.002, 0.013)	0.172
PC13	-0.005	(-0.014, 0.003)	0.220	0.001	(-0.007, 0.009)	0.867
PC14	-0.003	(-0.011, 0.005)	0.485	-0.005	(-0.012, 0.003)	0.236

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.07$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.17$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 4 Multivariable association of fourteen facial principal components (exposures) with fasting glucose as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	-0.010	(-0.028, 0.0008)	0.286	-0.017	(-0.035, 0.001)	0.065
PC2	0.003	(-0.011, 0.018)	0.674	-0.003	(-0.018, 0.012)	0.724
PC3	0.010	(-0.005, 0.025)	0.197	0.006	(-0.009, 0.021)	0.458
PC4	-0.008	(-0.023, 0.007)	0.310	-0.006	(-0.021, 0.009)	0.427
PC5	0.002	(-0.014, 0.019)	0.769	0.007	(-0.009, 0.024)	0.381
PC6	0.012	(-0.003, 0.026)	0.115	0.009	(-0.006, 0.023)	0.243
PC7	0.009	(-0.006, 0.023)	0.226	0.011	(-0.004, 0.025)	0.147
PC8	-0.002	(-0.016, 0.013)	0.808	-0.001	(-0.015, 0.014)	0.924
PC9	-0.004	(-0.018, 0.011)	0.613	-0.003	(-0.018, 0.011)	0.657
PC10	-0.011	(-0.026, 0.003)	0.124	-0.013	(-0.027, 0.002)	0.083
PC11	0.008	(-0.007, 0.023)	0.301	0.002	(-0.014, 0.017)	0.838
PC12	0.005	(-0.010, 0.019)	0.535	0.005	(-0.010, 0.019)	0.521
PC13	0.003	(-0.013, 0.018)	0.730	0.004	(-0.011, 0.020)	0.563
PC14	0.001	(-0.013, 0.016)	0.866	0.002	(-0.013, 0.016)	0.812

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.05$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.06$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 5 Multivariable association of fourteen facial principal components (exposures) with triglycerides as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	0.010	(-0.001, 0.020)	0.073	0.000	(-0.011, 0.010)	0.975
PC2	0.004	(-0.003, 0.010)	0.291	-0.003	(-0.010, 0.003)	0.351
PC3	**0.008**	**(0.001, 0.015)**	**0.019**	0.004	(-0.003, 0.010)	0.287
PC4	-0.005	(-0.001, 0.002)	0.177	-0.003	(-0.009, 0.004)	0.397
PC5	-0.001	(-0.008, 0.007)	0.818	0.005	(-0.002, 0.012)	0.193
PC6	**0.011**	**(0.004, 0.017)**	**0.001**	**0.007**	**(0.000, 0.013)**	**0.045**
PC7	0.000	(-0.007, 0.006)	0.945	0.002	(-0.005, 0.008)	0.563
PC8	-0.005	(-0.012, 0.001)	0.118	-0.004	(-0.011, 0.002)	0.206
PC9	0.005	(-0.001, 0.012)	0.113	0.006	(-0.001, 0.012)	0.086
PC10	0.005	(-0.001, 0.012)	0.113	0.004	(-0.003, 0.010)	0.263
PC11	**0.008**	**(0.001, 0.014)**	**0.024**	<0.001	(-0.007, 0.007)	0.988
PC12	-0.003	(-0.010, 0.003)	0.341	-0.003	(-0.010, 0.003)	0.317
PC13	-0.004	(-0.011, 0.003)	0.213	-0.002	(-0.009, 0.005)	0.601
PC14	-0.005	(-0.011, 0.002)	0.143	-0.005	(-0.012, 0.001)	0.118

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.03$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.06$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 6 Multivariable association of fourteen facial principal components (exposures) with high density lipoprotein cholesterol (HDLc) as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	**-0.028**	**(-0.042, -0.014)**	**<0.001**	-0.013	(-0.027, 0.001)	0.073
PC2	-0.005	(-0.017, 0.006)	0.351	0.008	(-0.003, 0.020)	0.154
PC3	-0.006	(-0.017, 0.006)	0.339	0.003	(-0.008, 0.014)	0.611
PC4	0.007	(-0.004, 0.019)	0.228	0.004	(-0.008, 0.015)	0.538
PC5	0.007	(-0.006, 0.020)	0.279	-0.005	(-0.018, 0.008)	0.443
PC6	**-0.014**	**(-0.025, -0.003)**	**0.016**	-0.005	(-0.016, 0.006)	0.408
PC7	0.003	(-0.008, 0.015)	0.570	-0.001	(-0.012, 0.010)	0.797
PC8	0.009	(-0.003, 0.020)	0.129	0.007	(-0.004, 0.018)	0.205
PC9	0.001	(-0.010, 0.012)	0.883	0.000	(-0.011, 0.011)	0.940
PC10	-0.010	(-0.022, 0.001)	0.078	-0.007	(-0.018, 0.004)	0.229
PC11	**-0.022**	**(-0.034, -0.011)**	**<0.001**	-0.006	(-0.017, 0.006)	0.340
PC12	0.005	(-0.007, 0.016)	0.413	0.005	(-0.006, 0.016)	0.348
PC13	0.005	(-0.007, 0.017)	0.387	-0.001	(-0.012, 0.011)	0.905
PC14	0.000	(-0.012, 0.011)	0.959	0.001	(-0.010, 0.012)	0.798

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.08$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.13$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 7 Multivariable association of fourteen facial principal components (exposures) with low density lipoprotein cholesterol (LDLc) as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	0.004	(-0.023, 0.030)	0.793	-0.014	(-0.041, 0.013)	0.307
PC2	-0.004	(-0.026, 0.018)	0.708	-0.020	(-0.042, 0.002)	0.074
PC3	**0.038**	**(0.016, 0.061)**	**0.001**	**0.028**	**(0.006, 0.050)**	**0.013**
PC4	-0.002	(-0.025, 0.020)	0.855	0.002	(-0.020, 0.024)	0.851
PC5	0.006	(-0.019, 0.031)	0.621	0.021	(-0.004, 0.046)	0.108
PC6	**0.027**	**(0.005, 0.049)**	**0.014**	0.016	(-0.005, 0.038)	0.140
PC7	0.012	(-0.009, 0.034)	0.263	0.018	(-0.004, 0.040)	0.103
PC8	**-0.024**	**(-0.045, -0.002)**	**0.033**	**-0.022**	**(-0.043, 0.000)**	**0.048**
PC9	-0.014	(-0.035, 0.008)	0.222	-0.013	(-0.034, 0.008)	0.235
PC10	0.006	(-0.015, 0.028)	0.561	0.002	(-0.019, 0.024)	0.824
PC11	**0.031**	**(0.009, 0.053)**	**0.006**	0.011	(-0.011, 0.034)	0.322
PC12	0.021	(-0.001, 0.043)	0.056	0.020	(-0.001, 0.042)	0.061
PC13	-0.006	(-0.029, 0.016)	0.582	0.001	(-0.022, 0.023)	0.961
PC14	0.013	(-0.009, 0.035)	0.238	0.011	(-0.010, 0.033)	0.315

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.05$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.06$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

DISCUSSION

Laser surface scanning is a non-invasive technology, which enables accurate and precise analysis of facial morphology.[1, 2, 23-25] Due to its portability, easy application, and relatively low cost, this technique is very suitable for epidemiological field studies. The vast amount of data captured by the system (more than 40,000 points, each consisting of *x*, *y*, and *z* co-ordinates) is a testimony of the complexity of facial surface. For this reason, face cannot be easily represented as a single exposure.

Therefore, it was necessary to make some facial data-reduction prior to its meaningful use. First of all, Generalised Procrustes Analysis (GPA; a widely established method in statistical shape analysis) was used to place landmark co-ordinates in the same space

reducing confounding errors (rotation and translation). Secondly, Principal Component Analysis (PCA) was applied on the set of co-ordinates and 14 facial principal components (PCs) were identified, which accounted for almost 82 per cent of the total variation in normal facial form, consisting of size and shape. Normal facial variation was recently analysed on a complete sample of 4747 faces from the ALSPAC database and the same number of PCs was extracted, with almost identical order of individual PCs and very similar percentages of variation.[22]

The application of this statistical technique is not new. Previously, PCA was performed on two-dimensional data sets, obtained from either lateral skull radiographs or photographs of both children and adults.[38-40] The resultant number of principal components in these studies was between 6 and 8, and these explained up to 90 per cent of the total variance in facial profile, based on linear measurements between anthropometric landmarks, or their co-ordinates. However, with the introduction of sophisticated 3D imaging techniques, the amount of data entering PCA significantly increased. Therefore, the number of PCs which represent facial variation also increased: between 14 and 16 PCs have been reported to account for between 86 and 92 per cent of the total variation.[10, 11, 41, 42]

Although the first three components in the current study explain almost half of the total variation, other components are also important, since they represent subtle changes that make the face unique. Therefore, a decision was made to keep all of them in the subsequent multivariable analyses. Following adjustment for BMI and taking account of multiple statistical testing, we did not find that any of these PCs were associated with fasting insulin or associated cardiometabolic risk factors, suggesting that facial morphology is unlikely to be a reliable way of predicting young people at future risk of type 2 diabetes or cardiovascular disease. Consistent with other large epidemiological studies conducted in healthy general

population samples, we were not able to directly measure insulin resistance using the gold standard euglycaemic hyperinsulinemic clamp. Fasting insulin has been shown to have modest to strong correlations with clamp assessed insulin resistance (correlation coefficients 0.5 to 0.9) in children and adolescents.[43, 44] Any measurement error is likely to be non-differential and therefore would be expected to bias results towards the null. Since strong associations of these outcomes with BMI have been shown in ALSPAC,[30] any associations with a better measure of insulin resistance are unlikely to be stronger than those of BMI.

Facial variation can be affected by many different factors. Whilst it is possible to control the age, gender, and ethnicity of the sample, environmental factors present a greater challenge, even with a good research strategy, as many of them can be unknown at the time of the study. The face changes throughout life, increasing in size and changing shape.[1, 2, 7] This holds true for the present sample consisted of 15-year old adolescents. The cross-sectional design of the study did not allow us to track these changes and analyse their relationship with metabolic phenotype through time. That may be more important than the assessment of variation among individuals and thus should be considered in future studies.

CONCLUSION

Our results do not provide strong evidence that facial morphology is robustly and importantly associated with cardiometabolic risk factors. The associations identified were not consistent across outcomes, were weak in magnitude, attenuated with adjustment for BMI, and did not withstand correction for multiple statistical testing. Further study of facial parameters with cardiometabolic and/or other health outcomes might provide valuable insights into how facial morphology can be indicative of health.

Acknowledgements We are extremely grateful to all the mothers, their parents and the children who are taking part and to the midwives for their co-operation and help in recruitment. The whole ALSPAC study team comprises interviewers, computer technicians, laboratory technicians, clerical workers, research scientists, volunteers, and managers who continue to make the study possible. This study could not have been undertaken without the financial support of the Medical Research Council, the Wellcome Trust, the UK Department of Health, the Department of the Environment, and DfEE, the National Institutes of Health, a variety of medical research charities and commercial companies. The ALSPAC study is part of the WHO initiated European Longitudinal Study of Pregnancy & Childhood.

Contributors JDj, DAL and SR were responsible for the conception and the design of the study. JDj and SR initiated the study. JDj and AMT collected the data. JDj, DAL, and RP were responsible for statistical analyses. JDj, AMT, AIZ and SR analysed and interpreted data on facial parameters. JDj, DAL and RP analysed and interpreted data on metabolic parameters. JDj and DAL wrote the first draft of the paper. All authors contributed to and approved the final version of the paper.

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Competing interests None.

Ethics approval The study was approved by the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee.

Data sharing statement There is no additional data available.

REFERENCES

1 Kau CH, Richmond S. Three-dimensional imaging for orthodontics and maxillofacial surgery. Iowa: Wiley Blackwell 2010.

2 Huang GJ, Richmond S, Vig KWL. Evidence-Based Orthodontics. Ames, IA: Wiley Blackwell 2011.

3 Hammond P, Suttie M. Large-scale objective phenotyping of 3D facial morphology. *Hum Mutat* 2012;33:817–25.

4 Brons S, van Beusichem ME, Bronkhorst EM, et al. Methods to quantify soft-tissue based facial growth and treatment outcomes in children: a systematic review. *PloS One* 2012;7:e41898.

5 Hammond P. The use of 3D face shape modelling in dysmorphology. *Arch Dis Child* 2007;92:1120–6.

6 Richmond S, Zhurov A, Toma A. Three-dimensional facial imaging. In: Wilkinson C, Rynn C, eds. Craniofacial Identification. Cambridge: Cambridge University Press 2012: 154–65.

7 Richmond S, Al Ali AM, Beldie L, et al. Detailing patient specific modeling to aid clinical decision-making. In: Calvo B, Peña E, eds. Patient-Specific Computational Modeling: 5 (Lecture Notes in Computational Vision and Biomechanics). Dordrecht: Springer 2012:105–31.

8 Ali AA, Richmond S, Popat H, et al. The influence of asthma on face shape: a three-dimensional study. *Eur J Orthod* Published Online First: 4 October 2012. doi: 10.1093/ejo/cjs067

9 Lee RW, Sutherland K, Chan AS, *et al*. Relationship between surface facial dimensions and upper airway structures in obstructive sleep apnea. *Sleep* 2010;33:1249–54.

10 Hennessy RJ, Baldwin PA, Browne DJ, *et al*. Three-dimensional laser surface imaging and geometric morphometrics resolve frontonasal dysmorphology in schizophrenia. *Biol Psychiatry* 2007;61:1187–94.

11 Hennessy RJ, Baldwin PA, Browne DJ, *et al*. Frontonasal dysmorphology in bipolar disorder by 3D laser surface imaging and geometric morphometrics: comparisons with schizophrenia. *Schizophr Res* 2010;122:63–71.

12 Ferrario VF, Sforza C, Dellavia C. Abnormal variations in the facial soft tissues of adults uremic patients on chronic dialysis. *Angle Orthod* 2005;75:320–5.

13 Herouvi D, Karanasios E, Karayianni C, *et al*. Cardiovascular disease in childhood: the role of obesity. *Eur J Paediatr* Published Online First: 23 January 2013. doi: 10.1007/s00431-013-1932-8

14 Raitakari OT, Juonala M, Kahonen M, *et al*. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the cardiovascular risk in young Finns study. *JAMA* 2003;290:2277–83.

15 Owen CG, Whincup PH, Orfei L, *et al*. Is body mass index before middle age related to coronary heart disease risk in later life? Evidence from observational studies. *Int J Obes* (Lond) 2009;33:866–77.

16 Han JC, Lawlor DA, Kimm SYS. Childhood obesity. (Peer reviewed commissioned seminar). *Lancet* 2010;375:1737–48.

17 Ohrn K, Al-Kahlili B, Huggare J, et al. Craniofacial morphology in obese adolescents. *Acta Odontol Scand* 2002;60:193–7.

18 Ferrario VF, Dellavia C, Tartaglia GM, et al. Soft tissue facial morphology in obese adolescents: a three-dimensional non-invasive assessment. *Angle Orthod* 2004;74:37–42.

19 Sadeghianrizi A, Forsberg CM, Marcus C, et al. Craniofacial development in obese adolescents. *Eur J Orthod* 2005;27:550–5.

20 Boyd A, Golding J, Macleod J, *et al*. Cohort Profile: The 'Children of the 90s': the index offspring of The Avon Longitudinal Study of Parents and Children (ALSPAC). *Int J Epidemiol* Published Online First: 16 April 2012. doi:10.1093/ije/dys064

21 Fraser A, Macdonald-Wallis C, Tilling K, *et al*. Cohort Profile: The Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *Int J Epidemiol* Published Online First: 16 April 2012. doi:10.1093/ije/dys066

22 Toma AM, Zhurov AI, Playle R, *et al*. The assessment of facial variation in 4747 British schoolchildren. *Eur J Orthod* 2012;34:655–64.

23 Kau CH, Zhurov AI, Scheer R, *et al*. The feasibility of measuring three-dimensional facial morphology in children. *Orthod Craniofac Res* 2004;7:198–204.

24 Kau CH, Richmond S, Zhurov AI, *et al*. Reliability of measuring facial morphology with a 3-dimensional laser scanning system. *Am J Orthod Dentofacial Orthop* 2005;128:424–30.

25 Kovacs L, Zimmermann A, Brockmann G, et al. Accuracy and precision of the three-dimensional assessment of the facial surface using a 3-D laser scanner. *IEEE Trans Med Imaging* 2006;25:742–54.

26 Zhurov AI, Kau CH, Richmond S. Computer methods for measuring 3D facial morphology. In: Middleton J, Shrive MG, Jones ML, eds. Proceedings of the 6th International Symposium on Computer Methods in Bomechanics & Biomedical Engineering. Cardiff: First Numerics Ltd 2005 (CD ROM, paper 151D).

27 Farkas LG. Anthropometry of the head and face. New York: Raven Press 1994:21–5.

28 Toma AM, Zhurov A, Playle R, *et al.* Reproducibility of facial soft tissue landmarks on 3D laser-scanned facial images. *Orthod Craniofac Res* 2009;12:33–42.

29 Djordjevic J, Pirttiniemi P, Harila V, *et al*. Three-dimensional longitudinal assessment of facial symmetry in adolescents. *Eur J Orthod* Published Online First: 7 February 2011. doi: 10.1093/ejo/cjr006

30 Lawlor DA, Benfield L, Logue J, *et al*. Association between general and central adiposity in childhood, and change in these, with cardiovascular risk factors in adolescence: prospective cohort study. *BMJ* 2010;341:c6224.

31Bookstein FL. Morphometric tools for landmark data. Cambridge: Cambridge University Press 1991:256–8.

32 Dryden IL, Mardia KV. Statistical shape analysis. Chichester, UK: Wiley 1998.

33 Zelditch ML, Swiderski DL, Sheets HD, et al. Geometric morphometrics for biologists: A Primer. New York: Elsevier academic press 2004.

34 Guttman L. Some necessary conditions for common factor analysis. *Psychometrika* 1954;19:149–61.

35 Cliff N. The eigenvalues-greater-than-one rule and the reliability of components. *Psychol Bull* 1988;103:276–9.

36 Jackson DA. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* 1993;74:2204–14.

37 Kaiser HF. The varimax criterion for analytic rotation in factor analysis. *Psychometrika* 1958;23:187–200.

38 Cleall JF, BeGole EA, Chebib FS. Craniofacial morphology: a principal component analysis. *Am J Orthod* 1979;75:650–66.

39 Halazonetis DJ. Morphometric evaluation of soft tissue profile shape. *Am J Orthod Dentofacial Orthop* 2007;131:481–9.

40 Krey KF, Dannhauer KH. Morphometric analysis of facial profile in adults. *J Orofac Orthop* 2008;69:424–36.

41 Moss JP. The use of three-dimensional imaging in orthodontics. *Eur J Orthod* 2006;28:416–25.

42 Hennessy RJ, McLearie S, Kinsella A, et al. Facial surface analysis by 3D laser scanning and geometric morphometrics in relation to sexual dimorphism in cerebral-craniofacial morphogenesis and cognitive function. *J Anat* 2005;207:283–95.

43 Gungor N, Saad R, Janosky J, *et al*. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. *J Pediatr* 2004;144:47–55.

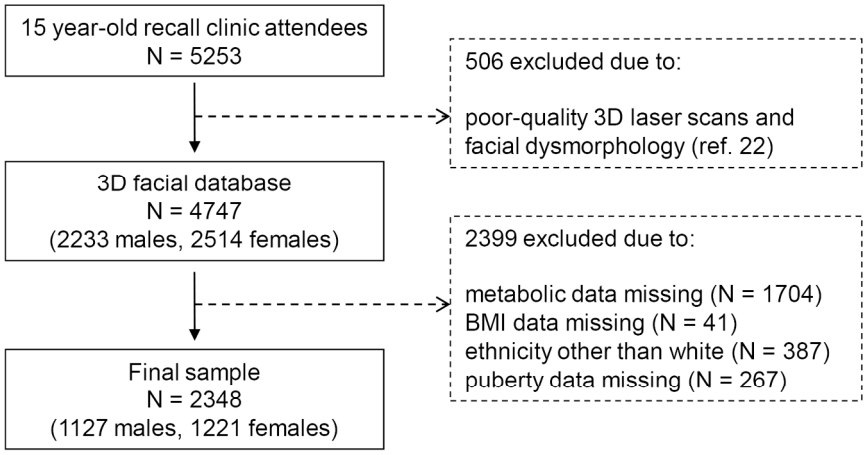
44 Schwartz B, Jacobs DR, Jr., Moran A, *et al*. Measurement of insulin sensitivity in children: comparison between the euglycemic-hyperinsulinemic clamp and surrogate measures. *Diabetes Care* 2008;31:783–8.

FIGURE LEGENDS:

Fig. 1 Flowchart showing the selection of study sample from 15+ year follow-up clinic of the Avon Longitudinal Study of Parents and Children (ALSPAC). All analyses presented in this paper are based on 2348 participants with complete data on facial soft tissue morphology (exposure), blood-based indicators of insulin resistance and associated cardiometabolic risk factors (outcomes), and body mass index and pubertal stage (covariables).

Fig. 2 Twenty-one anthropometric landmarks which were identified on facial laser scans of participants. (1) Glabella (g); (2) Nasion (n); (3) Endocanthion left (enl); (4) Endocanthion right (enr); (5) Exocanthion left (exl); (6) Exocanthion right (exr); (7) Palpebrale superius left (psl); (8) Palpebrale superius right (psr); (9) Palpebrale inferius left (pil); (10) Palpebrale inferius right (pir); (11) Pronasale (prn); (12) Subnasale (sn); (13) Alare left (all); (14) Alare right (alr); (15) Labiale superius (ls); (16) Crista philtri left (cphl); (17) Crista philtri right (cphr); (18) Labiale inferius (li); (19) Cheilion left (chl); (20) Cheilion right (chr); (21) Pogonion (pg). Definitions by Farkas [27] were used. Reprinted with permission from 'John Wiley and Sons'.

Fig. 3 Facial principal components (PCs). Numbers indicate percentages of normal facial variation explained by the given principal component. Co-ordinates which constitute each principal component are marked on the face (refer to Table 2), and arrows indicate *x*, *y*, and *z* directions.



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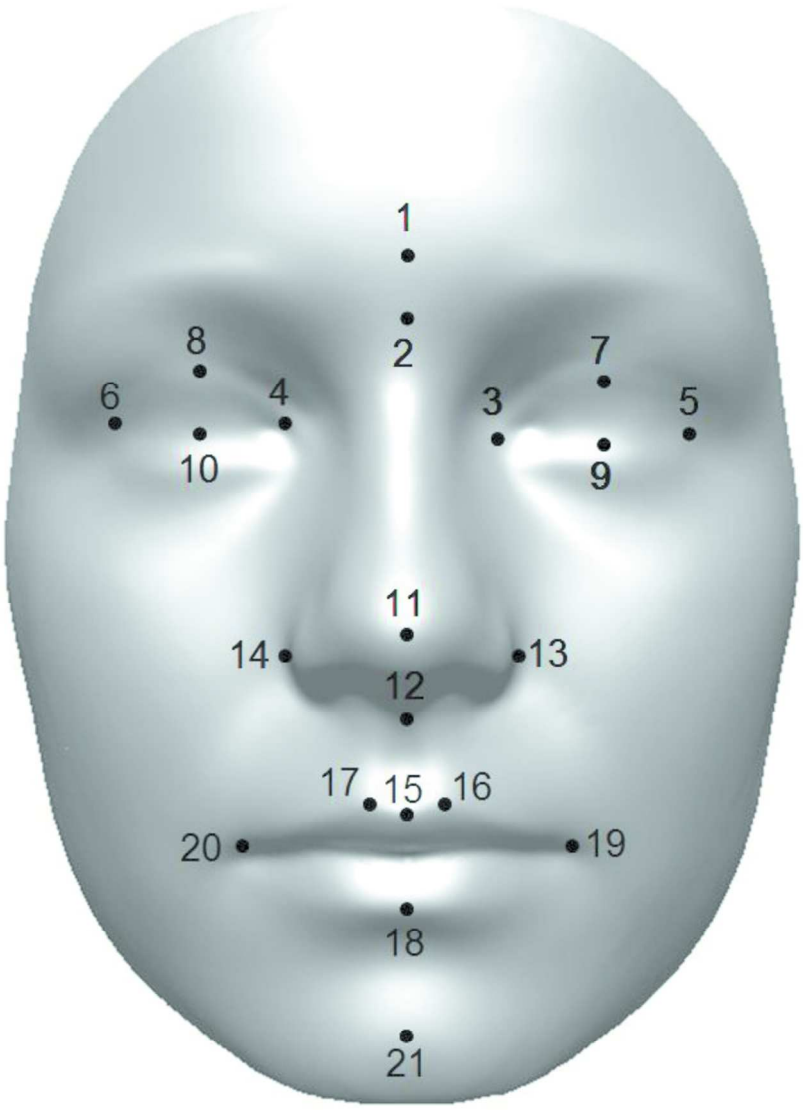
180x94mm (300 x 300 DPI)



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113x145mm (300 x 300 DPI)

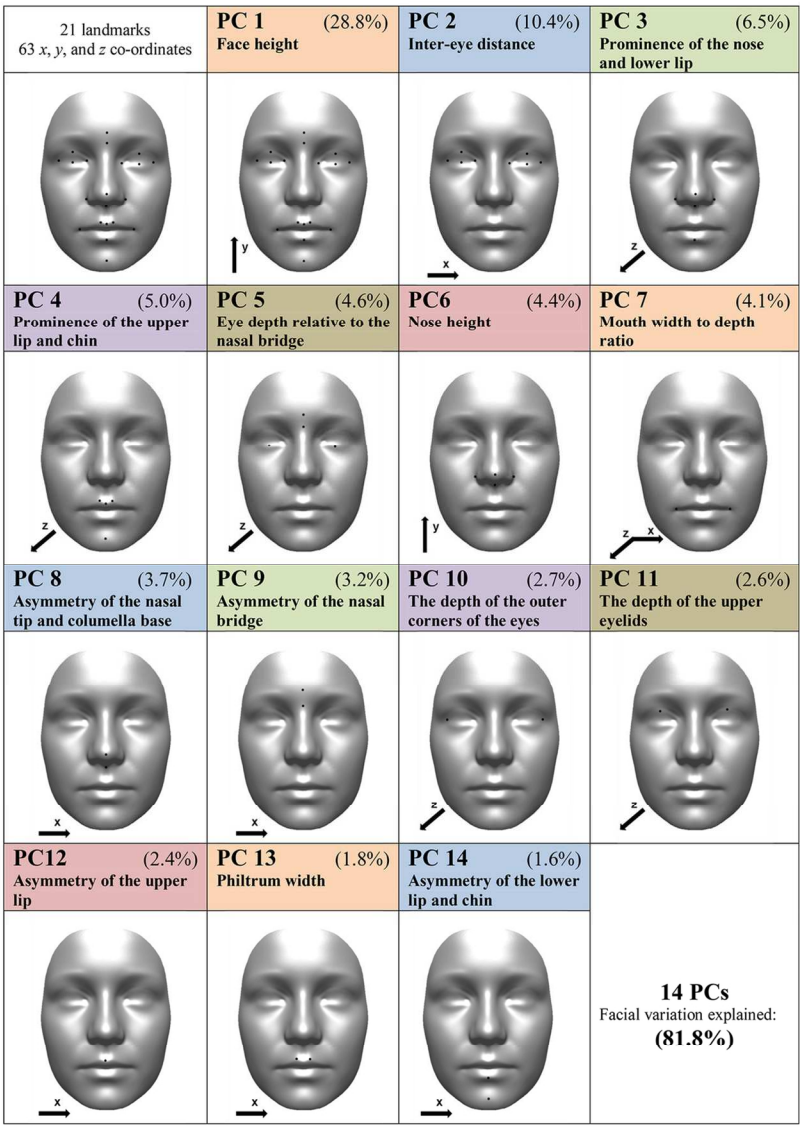

Fig. 3 Facial principal components (PCs). Numbers indicate percentages of normal facial variation explained by the given principal component. Co-ordinates which constitute each principal component are marked on the face (refer to Table 2), and arrows indicate x, y, and z directions.
99x149mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**1, 2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**2**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**4**
Objectives	3	State specific objectives, including any prespecified hypotheses	**4, 5**
Methods			
Study design	4	Present key elements of study design early in the paper	**2, 5**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**5-7**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	**5**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	**6, 7**
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	**6, 7**
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	**Page 5 and Figure 1**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	**7**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	**7, 8**
		(*b*) Describe any methods used to examine subgroups and interactions	**7, 8**
		(*c*) Explain how missing data were addressed	**NA**
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	**NA**
		(*e*) Describe any sensitivity analyses	**NA**
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	**Page 5, Figure 1, Table 1**
		(b) Give reasons for non-participation at each stage	**Figure 1**
		(c) Consider use of a flow diagram	**Figure 1**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**Table 1, Page 6**
		(b) Indicate number of participants with missing data for each variable of interest	**Figure 1**
Outcome data	15*	Report numbers of outcome events or summary measures	**6**
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	**Page 8, Tables 3-7**
		(*b*) Report category boundaries when continuous variables were categorized	**NA**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**NA**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**8, 10, Table 2**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**14**
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	**15**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**13-15**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**15**
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	**16**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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 www.strobe-statement.org.

To all the reviewers:

We would like to thank the reviewers for their valuable comments on our manuscript. You all seem to agree that the manuscript is well written and explained and that the proposed methodology is interesting and novel. We thank you for these encouraging thoughts. Since one reviewer and an associate editor raised a question about our motivation to do the study, we would like to explain this issue further before we proceed with answering specific questions of each reviewer.

First of all, the topic presented in the paper is of multidisciplinary nature and required a collaboration of several researchers with different areas of expertise. We understand that the journal probably does not have many submissions of this kind (excluding a review paper by Hammond (2007) on the use of 3D face shape modelling in dysmorphology). In that sense, this paper probably represents an 'unknown territory' for its readers, as well as the reviewers, and we are fully aware of this fact. Although the paper is not a classical example of what a paediatric specialist probably reads most often, we believe it deserves to be published in the journal. The reason is the following: three-dimensional (3D) imaging methods provide a possibility to analyse relationship between phenotypic measures and faces non-invasively, accurately, and relatively quickly, if valid methodology is accepted. It can be assumed that the interest in facial research related to different illnesses and/or metabolic phenotypes will be increased in the near future.

For us, the motivation to undertake the study was very clear: on one hand, the importance of cardiometabolic disorders in the modern world, increasingly witnessed even in the early stages of life, and on the other hand diagnostic possibilities utilizing modern 3D imaging technology. Initial idea to investigate the relationship between metabolic phenotype and face came mainly from three studies (mentioned in the introduction in the revised version) which investigated the effects of obesity on facial morphology. In these studies, the samples were small and the methods two-dimensional, and we wanted to test the hypothesis using more robust methods and larger sample. As collaborators, we were directly involved in generating large database of 3D facial scans within the ALSPAC, and we were also in charge for the processing and analysing of this data. Since ALSPAC study is very well planned and conducted, it provided a unique opportunity to investigate our new hypothesis.

We did our best to revise the manuscript according to your suggestions. Changes in the manuscript are marked in red and detailed explanations are provided below.

To Reviewer 1:

1. Reviewer 1: The authors proposed / tested a novel and interesting hypothesis that variation in facial morphology may be associated with differences in metabolic phenotype in adolescence. They have used the large and very well characterised ALPSAC study to undertake a cross sectional analysis of 15 year olds already studied for their metabolic phenotype. The problem with the methodology (as acknowledged by the authors) is that in order to carry out the facial morphology analysis multiple parameters need to be measured. When appropriate corrections are made for multiple analysis no particular associations are found.

The manuscript is very well written and explained and the statistical methods are valid for the kind of facial measurements undertaken but due to the very detailed measurement information that needs to be presented and the fact there seems no way around ending up with multiple measurements, all individually tested against the hypothesis, it then seems unlikely that any single or grouping of measurements will be associated with a feature of metabolic status independent of BMI. In view of this I don't think this manuscript will be of sufficient interest to merit full publication. In summary, I think this may be better written as a letter just stating that the hypothesis has been examined but no clear association has been found.

1. Authors: As the reviewer noticed, and we explained in more details in completely revised discussion, multiple measurements are the necessity, because facial morphology is a very complex issue to investigate. At present, we are unaware of any more advanced or more comprehensive method to analyse variation in facial morphology than the one we used in this study. Of course, the fact that we got negative results cannot be changed (after adjustments for multiple testing no association between cardiometabolic risks and facial features was found). However, we believe that suggested methodology can be a valuable source for future studies. In order to fulfill this role, methodology has to be thoroughly explained and that is why a letter or short communication would probably not be sufficient to convey the information of the study.

2. Reviewer 1: The data presented in table 2 and 4 are difficult to take any useful clinical message from since it has by necessity comprised of a lot of data on the measurements made.

2. Authors: We agree with this observation. Therefore, we decided to make a compromise here. Since it was necessary to present how principal components were identified from the groups of facial landmarks, we decided to keep Table 2 in the revised version of the manuscript. However, in order to facilitate understanding of the results, we added a new figure (Figure 4) combining graphical presentation of principal components with their respective descriptions and percentages of variation (the latter was taken from table 3 in the original submission, which is omitted from the revised version). In the title of the table 2 and the text, the reader is encouraged to refer to this figure for easier understanding.

Table 4 in the original manuscript presented the results of 140 analyses, which could also be overwhelming for the readers. Therefore, another decision was made to turn it into 5 separate tables (Tables 3 to 7), one for each cardiometabolic outcome considered. Besides regression coefficients and their respective 95% confidence intervals, we provided p values. Thus, it can be more obvious than in previous version which principal components were significantly associated with the outcome before and after adjustment for BMI and at which level of significance. This is also important to indicate that none of the components withstood the adjustment for multiple comparisons.

3. Reviewer 1: There is no real need to repeat the methods used for metabolic phenotyping described and published in previous ALPSAC studies.

3. Authors: Associate Editor also suggested to shorten the manuscript and according to his suggestion and your observation we decided to delete detailed explanations about metabolic data collection and calculation. The reference is provided.

Finally, we would like to thank you for all your advice and careful consideration of the manuscript.

To Reviewer 2:

1. Reviewer 2: I enjoyed reading this article. Though there is not strong correlation, it represents a good step moving forward with facial morphometrics.

1. Authors: Reviewer 2 supported the idea of the study and we are grateful for his/her support.

To Reviewer 3:

1. Reviewer 3: This is an interesting cross sectional study, which aims to investigate the relationship between facial morphology with fasting insulin, glucose and lipids in the ALSPAC cohort at age 15+ years. Whilst there is some information in the introduction, referring to other studies that have looked at facial morphology, in association with sleep apnoea and mental health, the authors have provided no reasons to suggest why they would expect any relationship in their data. This is a major weakness of the study in my opinion.

1. Authors: According to this comment, introduction has been completely revised and new references provided. Our motivation to undertake the study has been explained above, in our addressing to all reviewers.

2. Reviewer 3: The sample, methods and technical details are explained well. However, I have some other specific comments. There is no mention of dropouts in the sample – original sample was over 14000 and this study looks at just over 2000 adolescents.

2. Authors: ALSPAC study is a longitudinal study which was initiated in the early 90's. The initial sample consisted of approximately 14,000 children. At the age of 15, 5235 individuals attended the recall clinic. On that occasion, adolescents were laser scanned and 4,747 3D facial images were retained in the 3D facial database. The reason why we looked at 2348 adolescents was that at the age of 15 these individuals had complete data records on investigated parameters (cardiometabolic outcomes) and confounding variables (age, ethnicity, pubertal

stage, and BMI). A step-by-step selection of the participants can be found on the updated flowchart (Figure 1).

3. Reviewer 3: In the statistical analysis section, the authors mention generalized Procrustes analysis; this is not a standard technique in the general literature and should be explained and/or referenced. The remainder of this section is very clear, although I am not sure a Bonferroni correction is suitable when you perform such a large number of tests (140).

3. Authors: Generalised Procrustes Analysis is a widely established method in statistical shape analysis. This is essentially mathematical technique which places landmark co-ordinates in the same space reducing confounding errors (rotation and translation). As the readership of this journal probably lacks adequate knowledge on this topic, your suggestion to clarify the issue is reasonable. However, we are afraid that detailed explanations of this technique would be too complicated and unnecessary for medical practitioners. For those who would like to investigate this issue in more detail, three references have been provided.

You expressed a doubt related to appropriateness of Bonferroni correction. In this study, 140 tests were performed on a data set. Let us investigate the chance (*P*) of identifying at least one significant result:

***P* (at least one significant result) = 1 – *P* (no significant results).**

***P* (at least one significant result) = $1 - (1-0.05)^{140}$**

***P* (at least one significant result) = 1 – 0.00075 = 0.99925**

It means that the chance to discover at least one statistically significant association in this study (even if the test is actually not significant), i.e. to find a false-positive, was approximately 99.9%. Therefore, the chance of type I error was extremely high and p value of 0.05 had to be reduced to 0.0004 (0.05/140). The simple way to do this was to apply a Bonferroni correction.

However, we are aware that although the Bonferroni correction controls for false positives, it can become very conservative with this large number of tests. This, in turn, increases the risk of generating false-negative (type II error). In order to make it clear that this was not the case in this study, we decided to enter additional data on p values into new Tables 3 to 7. From these results, it is obvious that p values in all the analyses were far above the set limit.

4. Reviewer 3: The principal components analysis does not seem to have worked too well if you need 14 PC to account for 82% of variation. Are PCs which each explain less than 5% of variability in outcome really useful? Also why did you cut-off at 14?

4. Authors: The explanation is provided in completely revised discussion. Until recently, Principal Component Analysis (PCA) has been performed on data collected from two-dimensional facial records: either lateral skull radiographs or photographs (Cleall et al., 1979; Halazonetis, 2007; Krey and Danhauer, 2008).

Using relatively small number of landmarks it was possible to describe most of the variation in the face shape by just several principal components (6 to 8). However, with the introduction of 3D imaging methods, the situation changed dramatically. Imagine that facial surface captured by the laser scanning device consists of tens of thousand data points, each consisting of x, y, and z co-ordinates. That clearly illustrates the complexity of 3D facial shape. The first step towards reducing this extremely large amount of data was to focus on collection of 63 x, y, and z co-ordinates of 21 anthropometric landmarks, previously proven to be reliable for clinical research. When this data was entered into PCA analysis, 14 principal components (PCs) were extracted which explained 82% of the variation in facial form (size and shape together). This outcome reflects the complexity of facial morphology, rather than failure of the analysis itself. Other authors agree with this opinion (please see discussion).

In their recent review paper, Hammond and Suttie (2012) stated: "…as few as 50–100 modes of dense surface models are required to cover 99% of shape variation in a set of faces. Thus, a face can be represented by an ordered sequence of 50 or so numbers. This is a huge data compaction."

In our previous study, in which we investigated normal facial variation in a whole sample of 4,747 faces in the ALSPAC database, 14 PCs also explained 82% of the facial variation. This is a commentary written by Kuijpers-Jagtman (2012) in the same journal: " In their study, Toma et al (2012) made clever use of the existing technique of principal component analysis to identify key components of facial variation.....(conclusion): The data presented is not only important to quantify facial variation in a normal population but can help to analyse facial dysmorphology and understand genotype/phenotype associations. This seems to be just the beginning of a meaningful use of data derived from novel non-invasive techniques. The study methodology and statistical handling of the data provide a good basis for future studies into facial variation."

Why did we cut-off at 14?

The 'Kaiser–Guttman criterion' was used as the stopping rule to identify key principal components (Guttman, 1954; Cliff, 1988; Jackson, 1993). According to this rule, the PCs with eigenvalues greater than the average eigenvalue were retained (this is a standard approach). The rotation method used for PCA was varimax with Kaiser normalisation (Kaiser, 1958). We added this information in the statistical analysis in the revised manuscript.

Are PCs which each explain less than 5% of variability in outcome really useful?

The first three components (which can be interpreted as facial height, width, and convexity) explained almost half of the total variation (45.7%) and other 11 components (explaining between 1.6% and 5% of the variation in 3D facial shape) contributed to subtle changes that make the face unique. We did not want to discard them, since previous studies showed that these subtle differences can play an immense role in delineating different craniofacial syndromes for example. Even if a certain principal component contributes little to the overall normal facial morphology, it might be important for capturing the difference between individuals with a certain condition and normal controls. In that sense, keeping all 14 PCs seemed as a reasonable choice.

5. Reviewer 3: Each of the 14 PCs are then looked at in turn in relation to each of the 5 outcomes. The PCs are not looked at simultaneously; they are orthogonal.

5. Authors: Ten regression analyses were conducted. In each of these, 14 PCs were considered the exposures. They were entered into the analysis simultaneously.

6. Reviewer 3: Table 4 is difficult to read and some sort of shading would have been useful. Additional I believe there are several errors, as many estimated effects are not contained within the corresponding 95% CIs.

6. Authors: Please, read our answer to the comment 2 of the Reviewer 1. Instead of a very large Table 4, which is difficult to read and understand, we chose to divide the information into 5 separate tables (Tables 3 to 7), for each cardiometabolic outcome that we looked at: fasting insulin, fasting glucose, triglycerides, HDLc, and LDLc.

You have noticed correctly that many regression coefficients were outside of their corresponding 95% confidence intervals. We would like to thank you for thoroughly checking the table. Indeed, the mistakes occurred while reading the numbers from SPSS table and entering them into the manuscript table. Just in case, we run the analyses again to double check the validity of the numbers. P values have been also provided to make a clear distinction between principal components which significantly contribute to the model(s) and those which do not. Also, p values facilitate the observations that after adjustments for multiple comparisons, none of these principal components remained significantly associated with the outcomes.

7. Reviewer 3: The authors conclude that facial morphology is not strongly associated with fasting insulin, glucose and lipids in this sample. Of course this assumes that the principal components have captured and characterised facial morphology sufficiently – I would suggest this is unlikely to be the case. Your main conclusions relate to PCs 9, 11 and 3, the largest of which, PC3 explains 6.5% of variability in facial morphology.

7. Authors: Regarding the percentage of variance in facial shape explained by PCs, please read our previous comment (4). An additional explanation: increasing the number of landmarks identified on the facial scan could increase the number of PCs which would, in turn, describe higher proportion of the total variance. Prior to using additional landmarks it is necessary to prove that they are reliable. In this study, no landmarks were placed on larger area of the forehead and cheeks, and therefore these facial regions tended to be underrepresented in the analysis.

Our main conclusion states the following:

"Our results do not provide strong evidence that facial morphology is robustly and importantly associated with cardiometabolic risk factors. The associations identified were not consistent across outcomes, were weak in magnitude, attenuated with adjustment for BMI and did not withstand correction for multiple statistical testing. Further study of facial parameters with cardiometabolic and/or other health outcomes might provide valuable insights into how facial morphology can be indicative of health."

Although associations have been found for some principal components (such as number 3, 6, 9, and 11), just a few withstood adjustments for BMI, and none withstood adjustment for multiple comparisons.

8. Reviewer 3: Maybe age 15 is not the best time to perform this study, as adolescents are still developing physically. A comment on the stability of these landmarks at this age and later would be interesting.

8. Authors: It is true that adolescents still develop physically. Large sample of 3D facial images on which to test the hypothesis was available from the ALSPAC only in this age group, so there was no option to use older sample. However, if the whole idea is to detect a risk for cardiometabolic disease, then early prediction is favourable. The title of the study clearly states that this is an exploration of the possible association in the adolescent period. Of course, cross-sectional study design has its own limitations, and we mentioned this in the revised discussion. In addition, facial morphology depends on many factors: some of which can be controlled by careful study planning. However, there are some confounding factors, which cannot be known (and that is a general limitation of observational studies). Face changes both in size and shape during time, and these changes might be more important in relation to metabolic phenotype than just an exploration of variations among individuals. This is an interesting topic, which could be considered in future studies.

Once again, we would like to thank you for all the comments and careful reading of the manuscript.

A population-based cross-sectional study of the association between facial morphology and cardiometabolic risk factors in adolescence

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A POPULATION-BASED CROSS-SECTIONAL STUDY OF THE ASSOCIATION BETWEEN FACIAL MORPHOLOGY AND CARDIOMETABOLIC RISK FACTORS IN ADOLESCENCE

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ABSTRACT

Objective: To determine whether facial morphology is associated with fasting insulin, glucose and lipids independently of body mass index in adolescents.

Design: Population-based cross-sectional study.

Setting: Avon Longitudinal Study of Parents and Children (ALSPAC), South West of England.

Participants: From the ALSPAC database of 4747 3D facial laser scans, collected during a follow-up clinic at the age of 15, 2348 white British adolescents (1127 males, 1221 females) were selected on the basis of complete data on cadiometabolic parameters, body mass index (BMI), and Tanner's pubertal stage.

Main outcome measures: Fasting insulin, glucose, and lipids (triglycerides, high (HDLc) and low density (LDLc) lipoprotein cholesterols).

Results: Based on the collection of 63 x, y, and z co-ordinates of 21 anthropometric landmarks, 14 facial principal components (PCs) were identified. These components explained 82 per cent of the variation in facial morphology and were used as exposure variables. With adjustment for age, gender and pubertal stage, seven PCs were associated with fasting insulin, none with glucose, three with triglycerides, three with HDLc, and four with LDLc. After additional adjustment for BMI, four PCs remained associated with fasting insulin, one PC with triglycerides, and two PCs with LDLc. None of these associations withstood adjustment for multiple comparisons.

Conclusion: These initial hypothesis generating analyses provide no evidence that facial morphology is importantly related to cardiometabolic outcomes. Further examination might be warranted. Facial morphology assessment may have value in identifying other areas of disease risk.

ARTICLE SUMMARY

Article focus

- Three-dimensional imaging opens up a new chapter in investigations of facial morphology. Previous research revealed associations of facial morphology with obesity in adolescents, but whether facial morphology can be used to identify those at future risk of adverse cardiometabolic outcomes is unknown.

Key messages

- Our results suggest that facial morphology is not strongly or consistently associated with fasting insulin, glucose, or lipids, particularly after adjustment for body mass index, in white British adolescents. Facial morphology is therefore unlikely to be useful in identifying white British adolescents at future risk of adverse cardiometabolic outcomes.

- Suggested methodology can be used in future studies to explore the associations between facial parameters and other health outcomes. It might provide valuable insights into how facial morphology can be indicative of health.

Strengths and limitations of this study

- The strengths of this study are a large sample size and the homogeneity of the sample: all participants were of white origin, born and brought up in the same region of the UK. Non-invasive, accurate, and reliable method was used for capturing details of facial soft tissue morphology. A comprehensive statistical analysis was undertaken to extract principal components of facial morphology.

- The study has some limitations. First of all, the study is ethnic-specific. Secondly, a face could not be easily represented as a single exposure due to the complexity of its morphology and the vast amount of data captured by the laser scanning system. Therefore, some data reduction was necessary prior to the analysis. Furthermore, it was not possible to control all the confounding factors in a cross-sectional study design. Since faces of adolescents are still developing, changing their shape and size, future studies might have to investigate the relationship between these changes and cardiometabolic characteristics through time.

INTRODUCTION

Recent technological advancements in imaging methods marked a transition from two-dimensional to three-dimensional (3D) approach in craniofacial research, thus opening a new era. A special emphasis has been placed on the development and application of non-invasive methods to capture human face accurately and reliably.[1, 2] Among these, laser surface scanning and stereophotogrammetry have gained wide acceptance of research community.[3] So far, a large spectrum of medical disciplines have utilised these methods in the investigations of facial growth, facial dysmorphology, craniofacial identification, as well as the influence of different medical conditions on facial phenotype.[4-12] Therefore, an exciting opportunity has occurred to explore whether facial characteristics can serve as new diagnostic measures of illnesses.

Childhood obesity is becoming an epidemic health problem.[13] It is evident from many studies that it is associated with increased risk of type 2 diabetes and cardiovascular disease later in life.[14-16] Despite this fact, the connection between obesity and craniofacial development has been rarely investigated. Bimaxillary prognathism (overdeveloped jaws in sagittal direction) and increased transverse facial dimensions seem to indicate the difference between obese adolescents and their normal-weighted peers.[17-19] However, the association between metabolic phenotype and facial form has not been addressed previously.

In order to investigate this problem, large sample and a comprehensive 3D approach to facial measurements are needed. In this cross-sectional study, which can be considered hypothesis generating, we examined the associations of facial soft tissue morphology with metabolic phenotype (fasting insulin, glucose, triglycerides, high density lipoprotein

cholesterol (HDLc), and low density lipoprotein cholesterol (LDLc)) in a large general population cohort of adolescents using an existing database of 3D facial laser scans.

MATERIAL AND METHODS

Sample

We used the data from the Avon Longitudinal Study of Parents and Children (ALSPAC), a UK-based longitudinal birth cohort study designed to explore genetic and environmental influences on health and wellbeing.[20, 21] All pregnant women were eligible to participate in ALSPAC if their estimated delivery date fell between 1st April 1991 and 31st December 1992 inclusive. 14541 pregnant women were recruited and from these women there were 14676 live born infants. Since age 7 surviving offspring have been invited to regular follow-up clinics.

The current study was approved by the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee and informed consent was obtained from children and their parents or guardians. The data collected during an annual follow-up clinic at the age of 15, which was attended by 5235 adolescents, was examined. On that occasion, facial laser scanning was performed, and after a drop-out of 488 individuals due to the low quality of the scans, or some sort of facial dysmorphology, a database of 4747 individuals (2233 males and 2514 females) was formed.[22] Out of these, we selected 2348 white adolescents (1127 males and 1221 females), with complete data related to the outcome and confounding variables (see below), as facial laser scans were used to derive exposure variables. The flow-chart diagram (Fig. 1) shows gradual selection of individuals who comprised the final sample. In order to make sure there was no selection bias, we first compared facial principal components (i.e. exposure variables; see below) of the study sample (2348 adolescents) with

those of 4747 adolescents forming 3D facial database[22] and concluded that there was no reason to believe that selected individuals were significantly different in terms of facial morphology. Secondly, we compared observed values for outcomes and confounding variables in the study sample (2348 adolescents) with imputed variables in the eligible sample of the follow-up clinic (5235 adolescents), which were published as supplementary online material of the previous study.[23] The distributions in imputed datasets were very similar to those observed, providing some evidence that the missing data were missing at random.

Measures

Exposure variables

Facial laser scans were used to derive principal components of facial morphology, which served as the exposure variables. This is explained in detail in the section on statistical analysis. Prior to this, it was necessary to perform three steps, which will be described here. First of all, facial scans were processed. Validity and reliability of laser scanning procedure, as well as the processing stages of the scans, have been previously investigated.[24-27] Secondly, twenty-one anthropometric landmarks were manually identified on facial scans by one experienced examiner (Fig. 2), according to their respective definitions by Farkas,[28] and their *x*, *y* and *z* co-ordinates were saved for the subsequent analysis. Previous research showed that these landmarks are clinically reliable.[29,30] Finally, facial scans were initially normalized according to the natural head position, with the origin of the co-ordinate system set at the point half-way between the inner corners of the eyes (mid-endocanthion). The *x*-axis was pointing left, from right to left eye, the *y*-axis was pointing vertically upwards from chin to forehead, and the *z*-axis was pointing outwards, in the nose direction. The coronal, sagittal, and transverse planes were taken as the *xy*, *yz*, and *xz* planes, respectively.[1, 2, 8, 22, 29]

Outcome variables

Fasting insulin, fasting glucose, triglycerides, high density lipoprotein cholesterol (HDLc), and low density lipoprotein cholesterol (LDLc) were taken as the outcome variables. Full details of their assessment have been previously reported.[23]

Confounding variables

Since this study is exploratory (being the first to examine these associations) and our main motivation was to understand whether facial morphology might be able to predict those at risk of cardiometabolic disease over and above simple measurement of adiposity, we did not adjust for a wide range of confounding variables. However, we adjusted for age, pubertal stage, and body mass index (BMI), as these are potentially important predictors of cardiometabolic risk and we would want to be clear that facial morphology predicted outcome over and above these. The age of the participants was recorded in months as they arrived at the clinic. Pubertal status was assessed on participants' self report with Tanner's questionnaires.

Statistical analyses

Participant characteristics were summarised with means (SD) for continuous approximately normally distributed variables, median (IQR) for continuous right skewed variables, and number (%) for categorical variables.

Generalized Procrustes Analysis (GPA) was performed on landmark configurations (each consisting of 63 *x*, *y*, and *z* co-ordinates of 21 facial landmarks) in order to remove differences in landmarks' position attributable to translation and rotation.[31-33] Scaling was not performed in order to preserve facial size. Principal Component Analysis (PCA) was used to reduce the set of 63 co-ordinates into a smaller number of independent components of

facial morphology. According to the 'Kaiser–Guttman criterion', PCs with eigenvalues greater than the average eigenvalue value were retained [34-36] and saved as new exposure variables. The rotation method used for PCA was varimax with Kaiser normalisation.[37] GPA was performed in the open source software R project and PCA in SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

Whilst this is a cross-sectional study, in all our analyses we examined the association of principal components of facial morphology (as exposures) with fasting insulin, glucose, triglycerides, HDLc, and LDLc (as outcomes) using multivariable linear regression models. No evidence was found for any gender interactions (all p-values ≥ 0.1) and therefore analyses are presented with both genders combined. In the first model we adjusted for age, gender, and pubertal stage. In the second model we adjusted for age, gender, pubertal stage, and BMI and examined how much this reduced any associations of facial principal components with the outcomes. Fasting insulin and triglycerides levels were right (positively) skewed and their logged values where used in the linear regression models, which ensured the model residuals were approximately normally distributed. The resultant regression coefficients with 95 per cent confidence intervals are presented.

In these multivariable analyses 140 comparisons were made (14 exposures with five outcomes and two models). In initial analyses we considered the conventional 0.05 level of statistical significance. We then adjusted for multiple comparisons using a Bonferroni correction by dividing 0.05 by 140, thus for these corrected analyses a p-value of 0.0004 would be considered statistically significant at the 0.05 level. All statistical analyses were performed in SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Table 1 shows the characteristics of the study participants. The Principal Component Analysis (PCA) identified 14 principal components (PCs) of facial morphology (Table 2). Each PC consisted of a number of co-ordinates of anthropometric landmarks. For example, the first principal component (PC 1) comprised 17 *y* co-ordinates of landmarks located in the upper and lower thirds of the face. These co-ordinates represented facial height (size). In order to facilitate understanding and interpretation of individual PCs, they are presented graphically on Figure 3. The first three PCs (facial size, inter-eye distance, and prominence of the nose and lower lip) accounted for almost half of the total variation (45.7%). The other 11 PCs contributed to facial variation to a much lesser extent (between 1.6 and 5%), but marked those subtle features which make the faces unique.

The multivariable associations of the 14 PCs with cardiometabolic outcomes are shown in Tables 3 to 7. With adjustment for age, gender and pubertal stage (model 1), seven PCs were associated with fasting insulin, none with fasting glucose, three PCs with triglycerides and HDLc, and four PCs with LDLc. After additional adjustment for BMI (model 2), four principal components remained associated with fasting insulin, none with glucose, one PC with triglycerides, none with HDLc, and two PCs with LDLc. However, none of these associations withstood adjustment for multiple comparisons.

Table 1 Characteristics of the study sample.

	Categories/units	Males N = 1127	Females N = 1221	All N = 2348
Age	Mean (months)	184.8 (3.0)	184.9 (3.2)	184.9 (3.1)
Tanner's pubertal stages	Stage I n (%)	0	0	0
	Stage II n (%)	8 (0.7%)	6 (0.5%)	14 (0.6%)
	Stage III n (%)	64 (5.7%)	118 (9.7%)	182 (7.8%)
	Stage IV n (%)	552 (49.0%)	632 (51.8%)	1184 (50.4%)
	Stage V n (%)	503 (44.6%)	465 (38.1%)	968 (41.2%)
BMI	Median (kg/m^2)	20.4 (18.9, 22.3)	21.2 (19.5, 23.4)	20.8 (19.1, 23.0)
Fasting insulin	Median (IU/l)	8.2 (5.9, 10.9)	9.7 (7.4, 13.0)	9.0 (6.6, 12.0)
Fasting glucose	Mean (mmol/l)	5.3 (0.4)	5.1 (0.3)	5.2 (0.4)
Total cholesterol	Mean (mmol/l)	3.6 (0.6)	3.9 (0.63)	3.8 (0.6)
Triglycerides	Median (mmol/l)	0.7 (0.6, 1.0)	0.8 (0.6, 1.0)	0.7 (0.6, 1.0)
HDLc	Mean (mmol/l)	1.2 (0.3)	1.4 (0.3)	1.3 (0.3)
LDLc	Mean (mmol/l)	2.0 (0.5)	2.2 (0.6)	2.1 (0.6)

Number (%) for categorical variables, mean (SD) or median (IQR) for continuously distributed variables are presented. SD, standard deviation; IQR, interquartile range; BMI, body mass index; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol.

Table 2 The results of the principal component analysis showing partial correlation coefficients between co-ordinates of anthropometric landmarks and facial principal components.

	Facial principal components													
Co-ordinates	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**	**PC 9**	**PC 10**	**PC 11**	**PC 12**	**PC 13**	**PC 14**
Ls (y)	-0.84													
Enl (y)	0.84													
Cphr (y)	-0.84													
Cphl (y)	-0.83													
Enr (y)	0.83													
Pg (y)	-0.82													
Chr (y)	-0.82													
Pil (y)	0.81													
Chl (y)	-0.81													
Pir (y)	0.81													
Psl (y)	0.79													
Li (y)	-0.78													
Psr (y)	0.78													
Exr (y)	0.75													
Exl (y)	0.74													
G (y)	0.64													
N (y)	0.62													
Psl (x)		0.94												
Psr (x)		-0.93												
Pil (x)		0.93												
Pir (x)		-0.92												
Enr (x)		-0.83												
Enl (x)		0.83												
Exr (x)		-0.79												
Exl (x)		0.75												
All (z)			-0.80											
Alr (z)			-0.79											
Sn (z)			-0.79											
Prn (z)			-0.68											
Li (z)			0.56											
Ls (z)				0.87										
Cphl (z)				0.86										
Cphr (z)				0.86										
Pg (z)				-0.78										
G (z)					-0.86									
N (z)					-0.82									
Pir (z)					0.65									
Pil (z)					0.64									
Prn (y)						0.82								
All (y)						0.79								
Alr (y)						0.77								
Sn (y)						0.72								
Chr (x)							0.82							
Chl (x)							-0.82							
Chl (z)							0.80							
Chr (z)							0.80							
Sn (x)								0.94						
Prn (x)								0.90						
G (x)									0.97					
N (x)									0.97					
Exl (z)										-0.62				
Exr (z)										-0.60				
Psl (z)											0.80			
Psr (z)											0.79			
Ls (x)												0.92		
Cphr (x)													0.82	
Cphl (x)													-0.78	
Pg (x)														0.91
Li (x)														0.76

Only major landmarks contributing to each principal component (PC) are shown (coefficients with absolute values above 0.5). Anthropometric landmarks are explained on Figure 2 and the principal components on Figure 3.

Table 3 Multivariable association of fourteen facial principal components (exposures) with fasting insulin as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	0.004	(-0.006, 0.014)	0.397	**-0.011**	**(-0.021, -0.001)**	**0.026**
PC2	**0.011**	**(0.002, 0.019)**	**0.011**	-0.003	(-0.011, 0.005)	0.419
PC3	**0.010**	**(0.002, 0.019)**	**0.015**	0.002	(-0.006, 0.010)	0.681
PC4	-0.001	(-0.009, 0.007)	0.803	0.002	(-0.005, 0.010)	0.538
PC5	**-0.011**	**(-0.020, -0.002)**	**0.019**	0.001	(-0.008, 0.010)	0.802
PC6	**0.010**	**(0.002, 0.018)**	**0.013**	0.001	(-0.007, 0.009)	0.787
PC7	0.000	(-0.008, 0.008)	0.935	0.005	(-0.003, 0.013)	0.190
PC8	**-0.017**	**(-0.028, -0.006)**	**0.003**	**-0.014**	**(-0.024, -0.004)**	**0.009**
PC9	**0.012**	**(0.003, 0.020)**	**0.005**	**0.012**	**(0.004, 0.020)**	**0.002**
PC10	0.005	(-0.003, 0.014)	0.190	0.002	(-0.006, 0.010)	0.601
PC11	**0.026**	**(0.018, 0.034)**	**<0.0001**	**0.009**	**(0.001, 0.017)**	**0.029**
PC12	0.006	(-0.002, 0.014)	0.151	0.005	(-0.002, 0.013)	0.172
PC13	-0.005	(-0.014, 0.003)	0.220	0.001	(-0.007, 0.009)	0.867
PC14	-0.003	(-0.011, 0.005)	0.485	-0.005	(-0.012, 0.003)	0.236

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.07$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.17$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 4 Multivariable association of fourteen facial principal components (exposures) with fasting glucose as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	-0.010	(-0.028, 0.0008)	0.286	-0.017	(-0.035, 0.001)	0.065
PC2	0.003	(-0.011, 0.018)	0.674	-0.003	(-0.018, 0.012)	0.724
PC3	0.010	(-0.005, 0.025)	0.197	0.006	(-0.009, 0.021)	0.458
PC4	-0.008	(-0.023, 0.007)	0.310	-0.006	(-0.021, 0.009)	0.427
PC5	0.002	(-0.014, 0.019)	0.769	0.007	(-0.009, 0.024)	0.381
PC6	0.012	(-0.003, 0.026)	0.115	0.009	(-0.006, 0.023)	0.243
PC7	0.009	(-0.006, 0.023)	0.226	0.011	(-0.004, 0.025)	0.147
PC8	-0.002	(-0.016, 0.013)	0.808	-0.001	(-0.015, 0.014)	0.924
PC9	-0.004	(-0.018, 0.011)	0.613	-0.003	(-0.018, 0.011)	0.657
PC10	-0.011	(-0.026, 0.003)	0.124	-0.013	(-0.027, 0.002)	0.083
PC11	0.008	(-0.007, 0.023)	0.301	0.002	(-0.014, 0.017)	0.838
PC12	0.005	(-0.010, 0.019)	0.535	0.005	(-0.010, 0.019)	0.521
PC13	0.003	(-0.013, 0.018)	0.730	0.004	(-0.011, 0.020)	0.563
PC14	0.001	(-0.013, 0.016)	0.866	0.002	(-0.013, 0.016)	0.812

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.05$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.06$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 5 Multivariable association of fourteen facial principal components (exposures) with triglycerides as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	0.010	(-0.001, 0.020)	0.073	0.000	(-0.011, 0.010)	0.975
PC2	0.004	(-0.003, 0.010)	0.291	-0.003	(-0.010, 0.003)	0.351
PC3	**0.008**	**(0.001, 0.015)**	**0.019**	0.004	(-0.003, 0.010)	0.287
PC4	-0.005	(-0.001, 0.002)	0.177	-0.003	(-0.009, 0.004)	0.397
PC5	-0.001	(-0.008, 0.007)	0.818	0.005	(-0.002, 0.012)	0.193
PC6	**0.011**	**(0.004, 0.017)**	**0.001**	**0.007**	**(0.000, 0.013)**	**0.045**
PC7	0.000	(-0.007, 0.006)	0.945	0.002	(-0.005, 0.008)	0.563
PC8	-0.005	(-0.012, 0.001)	0.118	-0.004	(-0.011, 0.002)	0.206
PC9	0.005	(-0.001, 0.012)	0.113	0.006	(-0.001, 0.012)	0.086
PC10	0.005	(-0.001, 0.012)	0.113	0.004	(-0.003, 0.010)	0.263
PC11	**0.008**	**(0.001, 0.014)**	**0.024**	<0.001	(-0.007, 0.007)	0.988
PC12	-0.003	(-0.010, 0.003)	0.341	-0.003	(-0.010, 0.003)	0.317
PC13	-0.004	(-0.011, 0.003)	0.213	-0.002	(-0.009, 0.005)	0.601
PC14	-0.005	(-0.011, 0.002)	0.143	-0.005	(-0.012, 0.001)	0.118

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.03$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.06$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 6 Multivariable association of fourteen facial principal components (exposures) with high density lipoprotein cholesterol (HDLc) as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	**-0.028**	**(-0.042, -0.014)**	**<0.001**	-0.013	(-0.027, 0.001)	0.073
PC2	-0.005	(-0.017, 0.006)	0.351	0.008	(-0.003, 0.020)	0.154
PC3	-0.006	(-0.017, 0.006)	0.339	0.003	(-0.008, 0.014)	0.611
PC4	0.007	(-0.004, 0.019)	0.228	0.004	(-0.008, 0.015)	0.538
PC5	0.007	(-0.006, 0.020)	0.279	-0.005	(-0.018, 0.008)	0.443
PC6	**-0.014**	**(-0.025, -0.003)**	**0.016**	-0.005	(-0.016, 0.006)	0.408
PC7	0.003	(-0.008, 0.015)	0.570	-0.001	(-0.012, 0.010)	0.797
PC8	0.009	(-0.003, 0.020)	0.129	0.007	(-0.004, 0.018)	0.205
PC9	0.001	(-0.010, 0.012)	0.883	0.000	(-0.011, 0.011)	0.940
PC10	-0.010	(-0.022, 0.001)	0.078	-0.007	(-0.018, 0.004)	0.229
PC11	**-0.022**	**(-0.034, -0.011)**	**<0.001**	-0.006	(-0.017, 0.006)	0.340
PC12	0.005	(-0.007, 0.016)	0.413	0.005	(-0.006, 0.016)	0.348
PC13	0.005	(-0.007, 0.017)	0.387	-0.001	(-0.012, 0.011)	0.905
PC14	0.000	(-0.012, 0.011)	0.959	0.001	(-0.010, 0.012)	0.798

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.08$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.13$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 7 Multivariable association of fourteen facial principal components (exposures) with low density lipoprotein cholesterol (LDLc) as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	0.004	(-0.023, 0.030)	0.793	-0.014	(-0.041, 0.013)	0.307
PC2	-0.004	(-0.026, 0.018)	0.708	-0.020	(-0.042, 0.002)	0.074
PC3	**0.038**	**(0.016, 0.061)**	**0.001**	**0.028**	**(0.006, 0.050)**	**0.013**
PC4	-0.002	(-0.025, 0.020)	0.855	0.002	(-0.020, 0.024)	0.851
PC5	0.006	(-0.019, 0.031)	0.621	0.021	(-0.004, 0.046)	0.108
PC6	**0.027**	**(0.005, 0.049)**	**0.014**	0.016	(-0.005, 0.038)	0.140
PC7	0.012	(-0.009, 0.034)	0.263	0.018	(-0.004, 0.040)	0.103
PC8	**-0.024**	**(-0.045, -0.002)**	**0.033**	**-0.022**	**(-0.043, 0.000)**	**0.048**
PC9	-0.014	(-0.035, 0.008)	0.222	-0.013	(-0.034, 0.008)	0.235
PC10	0.006	(-0.015, 0.028)	0.561	0.002	(-0.019, 0.024)	0.824
PC11	**0.031**	**(0.009, 0.053)**	**0.006**	0.011	(-0.011, 0.034)	0.322
PC12	0.021	(-0.001, 0.043)	0.056	0.020	(-0.001, 0.042)	0.061
PC13	-0.006	(-0.029, 0.016)	0.582	0.001	(-0.022, 0.023)	0.961
PC14	0.013	(-0.009, 0.035)	0.238	0.011	(-0.010, 0.033)	0.315

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.05$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.06$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

DISCUSSION

Laser surface scanning is a non-invasive technology, which enables accurate and precise analysis of facial morphology.[1, 2, 24-26] Due to its portability, easy application, and relatively low cost, this technique is very suitable for epidemiological field studies. The vast amount of data captured by the system (more than 40,000 points, each consisting of *x*, *y*, and *z* co-ordinates) is a testimony of the complexity of facial surface. For this reason, face cannot be easily represented as a single exposure.

Therefore, it was necessary to make some facial data-reduction prior to its meaningful use. First of all, Generalised Procrustes Analysis (GPA; a widely established method in statistical shape analysis) was used to place landmark co-ordinates in the same space reducing confounding errors (rotation and translation). Secondly, Principal Component

Analysis (PCA) was applied on the set of co-ordinates and 14 facial principal components (PCs) were identified, which accounted for almost 82 per cent of the total variation in normal facial form, consisting of size and shape. Normal facial variation was recently analysed on a complete sample of 4747 faces from the ALSPAC database and the same number of PCs was extracted, with almost identical order of individual PCs and very similar percentages of variation.[22]

The application of this statistical technique is not new. Previously, PCA was performed on two-dimensional data sets, obtained from either lateral skull radiographs or photographs of both children and adults.[38-41] The resultant number of principal components in these studies was between 6 and 8, and these explained up to 90 per cent of the total variance in facial profile, based on linear measurements between anthropometric landmarks, or their co-ordinates. However, with the introduction of sophisticated 3D imaging techniques, the amount of data entering PCA significantly increased. Therefore, the number of PCs which represent facial variation also increased: between 14 and 16 PCs have been reported to account for between 86 and 92 per cent of the total variation.[10, 11, 41, 42]

Although the first three components in the current study explain almost half of the total variation, other components are also important, since they represent subtle changes that make the face unique. Therefore, a decision was made to keep all of them in the subsequent multivariable analyses. Following adjustment for BMI and taking account of multiple statistical testing, we did not find that any of these PCs were associated with fasting insulin or associated cardiometabolic risk factors, suggesting that facial morphology is unlikely to be a reliable way of predicting young people at future risk of type 2 diabetes or cardiovascular disease. Consistent with other large epidemiological studies conducted in healthy general population samples, we were not able to directly measure insulin resistance using the gold

standard euglycaemic hyperinsulinemic clamp. Fasting insulin has been shown to have modest to strong correlations with clamp assessed insulin resistance (correlation coefficients 0.5 to 0.9) in children and adolescents.[43, 44] Any measurement error is likely to be non-differential and therefore would be expected to bias results towards the null. Since strong associations of these outcomes with BMI have been shown in ALSPAC,[23] any associations with a better measure of insulin resistance are unlikely to be stronger than those of BMI.

The study has some limitations. First of all, it is ethnic-specific, and therefore future studies will have to address the research question in different enthnic groups. Secondly, facial variation can be affected by many different factors. Whilst it is possible to control the age, gender, and ethnicity of the sample, environmental factors present a greater challenge, even with a good research strategy, as many of them can be unknown at the time of the study. The face changes throughout life, increasing in size and changing shape.[1, 2, 7] This holds true for the present sample consisting of 15-year old adolescents. The cross-sectional design of the study did not allow us to track these changes and analyse their relationship with metabolic phenotype through time. That may be more important than the assessment of variation among individuals and thus should be considered in future studies.

CONCLUSION

Our results do not provide strong evidence that facial morphology is robustly and importantly associated with cardiometabolic risk factors. The associations identified were not consistent across outcomes, were weak in magnitude, attenuated with adjustment for BMI, and did not withstand correction for multiple statistical testing. Further study of facial parameters with cardiometabolic and/or other health outcomes might provide valuable insights into how facial morphology can be indicative of health.

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Contributors JDj, DAL and SR were responsible for the conception and the design of the study. JDj and SR initiated the study. JDj and AMT collected the data. JDj, DAL, and RP were responsible for statistical analyses. JDj, AMT, AIZ and SR analysed and interpreted data on facial parameters. JDj, DAL and RP analysed and interpreted data on metabolic parameters. JDj and DAL wrote the first draft of the paper. All authors contributed to and approved the final version of the paper.

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Competing interests None.

Ethics approval The study was approved by the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee.

Data sharing statement no additional data available.

REFERENCES

1 Kau CH, Richmond S. Three-dimensional imaging for orthodontics and maxillofacial surgery. Iowa: Wiley Blackwell 2010.

2 Huang GJ, Richmond S, Vig KWL. Evidence-Based Orthodontics. Ames, IA: Wiley Blackwell 2011.

3 Hammond P, Suttie M. Large-scale objective phenotyping of 3D facial morphology. *Hum Mutat* 2012;33:817–25.

4 Brons S, van Beusichem ME, Bronkhorst EM, et al. Methods to quantify soft-tissue based facial growth and treatment outcomes in children: a systematic review. *PloS One* 2012;7:e41898.

5 Hammond P. The use of 3D face shape modelling in dysmorphology. *Arch Dis Child* 2007;92:1120–6.

6 Richmond S, Zhurov A, Toma A. Three-dimensional facial imaging. In: Wilkinson C, Rynn C, eds. Craniofacial Identification. Cambridge: Cambridge University Press 2012: 154–65.

7 Richmond S, Al Ali AM, Beldie L, et al. Detailing patient specific modeling to aid clinical decision-making. In: Calvo B, Peña E, eds. Patient-Specific Computational Modeling: 5 (Lecture Notes in Computational Vision and Biomechanics). Dordrecht: Springer 2012:105–31.

8 Ali AA, Richmond S, Popat H, et al. The influence of asthma on face shape: a three-dimensional study. *Eur J Orthod* Published Online First: 4 October 2012. doi: 10.1093/ejo/cjs067

9 Lee RW, Sutherland K, Chan AS, *et al*. Relationship between surface facial dimensions and upper airway structures in obstructive sleep apnea. *Sleep* 2010;33:1249–54.

10 Hennessy RJ, Baldwin PA, Browne DJ, *et al*. Three-dimensional laser surface imaging and geometric morphometrics resolve frontonasal dysmorphology in schizophrenia. *Biol Psychiatry* 2007;61:1187–94.

11 Hennessy RJ, Baldwin PA, Browne DJ, *et al*. Frontonasal dysmorphology in bipolar disorder by 3D laser surface imaging and geometric morphometrics: comparisons with schizophrenia. *Schizophr Res* 2010;122:63–71.

12 Ferrario VF, Sforza C, Dellavia C. Abnormal variations in the facial soft tissues of adults uremic patients on chronic dialysis. *Angle Orthod* 2005;75:320–5.

13 Herouvi D, Karanasios E, Karayianni C, *et al*. Cardiovascular disease in childhood: the role of obesity. *Eur J Paediatr* Published Online First: 23 January 2013. doi: 10.1007/s00431-013-1932-8

14 Raitakari OT, Juonala M, Kahonen M, *et al*. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the cardiovascular risk in young Finns study. *JAMA* 2003;290:2277–83.

15 Owen CG, Whincup PH, Orfei L, *et al*. Is body mass index before middle age related to coronary heart disease risk in later life? Evidence from observational studies. *Int J Obes* (Lond) 2009;33:866–77.

16 Han JC, Lawlor DA, Kimm SYS. Childhood obesity. (Peer reviewed commissioned seminar). *Lancet* 2010;375:1737–48.

17 Ohrn K, Al-Kahlili B, Huggare J, et al. Craniofacial morphology in obese adolescents. *Acta Odontol Scand* 2002;60:193–7.

18 Ferrario VF, Dellavia C, Tartaglia GM, et al. Soft tissue facial morphology in obese adolescents: a three-dimensional non-invasive assessment. *Angle Orthod* 2004;74:37–42.

19 Sadeghianrizi A, Forsberg CM, Marcus C, et al. Craniofacial development in obese adolescents. *Eur J Orthod* 2005;27:550–5.

20 Boyd A, Golding J, Macleod J, *et al*. Cohort Profile: The 'Children of the 90s': the index offspring of The Avon Longitudinal Study of Parents and Children (ALSPAC). *Int J Epidemiol* Published Online First: 16 April 2012. doi:10.1093/ije/dys064

21 Fraser A, Macdonald-Wallis C, Tilling K, *et al*. Cohort Profile: The Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *Int J Epidemiol* Published Online First: 16 April 2012. doi:10.1093/ije/dys066

22 Toma AM, Zhurov AI, Playle R, *et al*. The assessment of facial variation in 4747 British schoolchildren. *Eur J Orthod* 2012;34:655–64.

23 Lawlor DA, Benfield L, Logue J, *et al*. Association between general and central adiposity in childhood, and change in these, with cardiovascular risk factors in adolescence: prospective cohort study. *BMJ* 2010;341:c6224.

24 Kau CH, Zhurov AI, Scheer R, *et al*. The feasibility of measuring three-dimensional facial morphology in children. *Orthod Craniofac Res* 2004;7:198–204.

25 Kau CH, Richmond S, Zhurov AI, *et al*. Reliability of measuring facial morphology with a 3-dimensional laser scanning system. *Am J Orthod Dentofacial Orthop* 2005;128:424–30.

26 Kovacs L, Zimmermann A, Brockmann G, et al. Accuracy and precision of the three-dimensional assessment of the facial surface using a 3-D laser scanner. *IEEE Trans Med Imaging* 2006;25:742–54.

27 Zhurov AI, Kau CH, Richmond S. Computer methods for measuring 3D facial morphology. In: Middleton J, Shrive MG, Jones ML, eds. Proceedings of the 6th International Symposium on Computer Methods in Bomechanics & Biomedical Engineering. Cardiff: First Numerics Ltd 2005 (CD ROM, paper 151D).

28 Farkas LG. Anthropometry of the head and face. New York: Raven Press 1994:21–5.

29 Toma AM, Zhurov A, Playle R, *et al.* Reproducibility of facial soft tissue landmarks on 3D laser-scanned facial images. *Orthod Craniofac Res* 2009;12:33–42.

30 Djordjevic J, Pirttiniemi P, Harila V, *et al*. Three-dimensional longitudinal assessment of facial symmetry in adolescents. *Eur J Orthod* Published Online First: 7 February 2011. doi: 10.1093/ejo/cjr006

31Bookstein FL. Morphometric tools for landmark data. Cambridge: Cambridge University Press 1991:256–8.

32 Dryden IL, Mardia KV. Statistical shape analysis. Chichester, UK: Wiley 1998.

33 Zelditch ML, Swiderski DL, Sheets HD, et al. Geometric morphometrics for biologists: A Primer. New York: Elsevier academic press 2004.

34 Guttman L. Some necessary conditions for common factor analysis. *Psychometrika* 1954;19:149–61.

35 Cliff N. The eigenvalues-greater-than-one rule and the reliability of components. *Psychol Bull* 1988;103:276–9.

36 Jackson DA. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* 1993;74:2204–14.

37 Kaiser HF. The varimax criterion for analytic rotation in factor analysis. *Psychometrika* 1958;23:187–200.

38 Cleall JF, BeGole EA, Chebib FS. Craniofacial morphology: a principal component analysis. *Am J Orthod* 1979;75:650–66.

39 Halazonetis DJ. Morphometric evaluation of soft tissue profile shape. *Am J Orthod Dentofacial Orthop* 2007;131:481–9.

40 Krey KF, Dannhauer KH. Morphometric analysis of facial profile in adults. *J Orofac Orthop* 2008;69:424–36.

41 Moss JP. The use of three-dimensional imaging in orthodontics. *Eur J Orthod* 2006;28:416–25.

42 Hennessy RJ, McLearie S, Kinsella A, et al. Facial surface analysis by 3D laser scanning and geometric morphometrics in relation to sexual dimorphism in cerebral-craniofacial morphogenesis and cognitive function. *J Anat* 2005;207:283–95.

43 Gungor N, Saad R, Janosky J, *et al*. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. *J Pediatr* 2004;144:47–55.

44 Schwartz B, Jacobs DR, Jr., Moran A, *et al*. Measurement of insulin sensitivity in children: comparison between the euglycemic-hyperinsulinemic clamp and surrogate measures. *Diabetes Care* 2008;31:783–8.

FIGURE LEGENDS:

Fig. 1 Flowchart showing the selection of study sample from 15+ year follow-up clinic of the Avon Longitudinal Study of Parents and Children (ALSPAC). All analyses presented in this paper are based on 2348 participants with complete data on facial soft tissue morphology (exposure), blood-based indicators of insulin resistance and associated cardiometabolic risk factors (outcomes), and body mass index and pubertal stage (covariables).

Fig. 2 Twenty-one anthropometric landmarks which were identified on facial laser scans of participants. (1) Glabella (g); (2) Nasion (n); (3) Endocanthion left (enl); (4) Endocanthion right (enr); (5) Exocanthion left (exl); (6) Exocanthion right (exr); (7) Palpebrale superius left (psl); (8) Palpebrale superius right (psr); (9) Palpebrale inferius left (pil); (10) Palpebrale inferius right (pir); (11) Pronasale (prn); (12) Subnasale (sn); (13) Alare left (all); (14) Alare right (alr); (15) Labiale superius (ls); (16) Crista philtri left (cphl); (17) Crista philtri right (cphr); (18) Labiale inferius (li); (19) Cheilion left (chl); (20) Cheilion right (chr); (21) Pogonion (pg). Definitions by Farkas [28] were used. Reprinted from the author's previous publication with permission from 'John Wiley and Sons'.

Fig. 3 Facial principal components (PCs). Numbers indicate percentages of normal facial variation explained by the given principal component. Co-ordinates which constitute each principal component are marked on the face (refer to Table 2), and arrows indicate *x*, *y*, and *z* directions.

A POPULATION-BASED CROSS-SECTIONAL STUDY OF THE ASSOCIATION BETWEEN FACIAL MORPHOLOGY AND CARDIOMETABOLIC RISK FACTORS IN ADOLESCENCE

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ABSTRACT

Objective: To determine whether facial morphology is associated with fasting insulin, glucose and lipids independently of body mass index in adolescents.

Design: Population-based cross-sectional study.

Setting: Avon Longitudinal Study of Parents and Children (ALSPAC), South West of England.

Participants: From the ALSPAC database of 4747 3D facial laser scans, collected during a follow-up clinic at the age of 15, 2348 white British adolescents (1127 males, 1221 females) were selected on the basis of complete data on cadiometabolic parameters, body mass index (BMI), and Tanner's pubertal stage.

Main outcome measures: Fasting insulin, glucose, and lipids (triglycerides, high (HDLc) and low density (LDLc) lipoprotein cholesterols).

Results: Based on the collection of 63 x, y, and z co-ordinates of 21 anthropometric landmarks, 14 facial principal components (PCs) were identified. These components explained 82 per cent of the variation in facial morphology and were used as exposure variables. With adjustment for age, gender and pubertal stage, seven PCs were associated with fasting insulin, none with glucose, three with triglycerides, three with HDLc, and four with LDLc. After additional adjustment for BMI, four PCs remained associated with fasting insulin, one PC with triglycerides, and two PCs with LDLc. None of these associations withstood adjustment for multiple comparisons.

Conclusion: These initial hypothesis generating analyses provide no evidence that facial morphology is importantly related to cardiometabolic outcomes. Further examination might be warranted. Facial morphology assessment may have value in identifying other areas of disease risk.

ARTICLE SUMMARY

Article focus

- Three-dimensional imaging opens up a new chapter in investigations of facial morphology. Previous research revealed associations of facial morphology with obesity in adolescents, but whether facial morphology can be used to identify those at future risk of adverse cardiometabolic outcomes is unknown.

Key messages

- Our results suggest that facial morphology is not strongly or consistently associated with fasting insulin, glucose, or lipids, particularly after adjustment for body mass index, in white British adolescents. Facial morphology is therefore unlikely to be useful in identifying white British adolescents at future risk of adverse cardiometabolic outcomes.

- Suggested methodology can be used in future studies to explore the associations between facial parameters and other health outcomes. It might provide valuable insights into how facial morphology can be indicative of health.

Strengths and limitations of this study

- The strengths of this study are a large sample size and the homogeneity of the sample: all participants were of white origin, born and brought up in the same region of the UK. Non-invasive, accurate, and reliable method was used for capturing details of facial soft tissue morphology. A comprehensive statistical analysis was undertaken to extract principal components of facial morphology.

- The study has some limitations. First of all, the study is ethnic-specific. Secondly, a face could not be easily represented as a single exposure due to the complexity of its morphology and the vast amount of data captured by the laser scanning system. Therefore, some data reduction was necessary prior to the analysis. Furthermore, it was not possible to control all the confounding factors in a cross-sectional study design. Since faces of adolescents are still developing, changing their shape and size, future studies might have to investigate the relationship between these changes and cardiometabolic characteristics through time.

INTRODUCTION

Recent technological advancements in imaging methods marked a transition from two-dimensional to three-dimensional (3D) approach in craniofacial research, thus opening a new era. A special emphasis has been placed on the development and application of non-invasive methods to capture human face accurately and reliably.[1, 2] Among these, laser surface scanning and stereophotogrammetry have gained wide acceptance of research community.[3] So far, a large spectrum of medical disciplines have utilised these methods in the investigations of facial growth, facial dysmorphology, craniofacial identification, as well as the influence of different medical conditions on facial phenotype.[4-12] Therefore, an exciting opportunity has occurred to explore whether facial characteristics can serve as new diagnostic measures of illnesses.

Childhood obesity is becoming an epidemic health problem.[13] It is evident from many studies that it is associated with increased risk of type 2 diabetes and cardiovascular disease later in life.[14-16] Despite this fact, the connection between obesity and craniofacial development has been rarely investigated. Bimaxillary prognathism (overdeveloped jaws in sagittal direction) and increased transverse facial dimensions seem to indicate the difference between obese adolescents and their normal-weighted peers.[17-19] However, the association between metabolic phenotype and facial form has not been addressed previously.

In order to investigate this problem, large sample and a comprehensive 3D approach to facial measurements are needed. In this cross-sectional study, which can be considered hypothesis generating, we examined the associations of facial soft tissue morphology with metabolic phenotype (fasting insulin, glucose, triglycerides, high density lipoprotein

cholesterol (HDLc), and low density lipoprotein cholesterol (LDLc)) in a large general population cohort of adolescents using an existing database of 3D facial laser scans.

MATERIAL AND METHODS

Sample

We used the data from the Avon Longitudinal Study of Parents and Children (ALSPAC), a UK-based longitudinal birth cohort study designed to explore genetic and environmental influences on health and wellbeing.[20, 21] All pregnant women were eligible to participate in ALSPAC if their estimated delivery date fell between 1st April 1991 and 31st December 1992 inclusive. 14541 pregnant women were recruited and from these women there were 14676 live born infants. Since age 7 surviving offspring have been invited to regular follow-up clinics.

The current study was approved by the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee and informed consent was obtained from children and their parents or guardians. The data collected during an annual follow-up clinic at the age of 15, which was attended by 5235 adolescents, was examined. On that occasion, facial laser scanning was performed, and after a drop-out of 488 individuals due to the low quality of the scans, or some sort of facial dysmorphology, a database of 4747 individuals (2233 males and 2514 females) was formed.[22] Out of these, we selected 2348 white adolescents (1127 males and 1221 females), with complete data related to the outcome and confounding variables (see below), as facial laser scans were used to derive exposure variables. The flow-chart diagram (Fig. 1) shows gradual selection of individuals who comprised the final sample. In order to make sure there was no selection bias, we first compared facial principal components (i.e. exposure variables; see below) of the study sample (2348 adolescents) with

those of 4747 adolescents forming 3D facial database[22] and concluded that there was no reason to believe that selected individuals were significantly different in terms of facial morphology. Secondly, we compared observed values for outcomes and confounding variables in the study sample (2348 adolescents) with imputed variables in the eligible sample of the follow-up clinic (5235 adolescents), which were published as supplementary online material of the previous study.[23] The distributions in imputed datasets were very similar to those observed, providing some evidence that the missing data were missing at random.

Measures

Exposure variables

Facial laser scans were used to derive principal components of facial morphology, which served as the exposure variables. This is explained in detail in the section on statistical analysis. Prior to this, it was necessary to perform three steps, which will be described here. First of all, facial scans were processed. Validity and reliability of laser scanning procedure, as well as the processing stages of the scans, have been previously investigated.[24-27] Secondly, twenty-one anthropometric landmarks were manually identified on facial scans by one experienced examiner (Fig. 2), according to their respective definitions by Farkas,[28] and their *x*, *y* and *z* co-ordinates were saved for the subsequent analysis. Previous research showed that these landmarks are clinically reliable.[29,30] Finally, facial scans were initially normalized according to the natural head position, with the origin of the co-ordinate system set at the point half-way between the inner corners of the eyes (mid-endocanthion). The *x*-axis was pointing left, from right to left eye, the *y*-axis was pointing vertically upwards from chin to forehead, and the *z*-axis was pointing outwards, in the nose direction. The coronal, sagittal, and transverse planes were taken as the *xy*, *yz*, and *xz* planes, respectively.[1, 2, 8, 22, 29]

Outcome variables

Fasting insulin, fasting glucose, triglycerides, high density lipoprotein cholesterol (HDLc), and low density lipoprotein cholesterol (LDLc) were taken as the outcome variables. Full details of their assessment have been previously reported.[23]

Confounding variables

Since this study is exploratory (being the first to examine these associations) and our main motivation was to understand whether facial morphology might be able to predict those at risk of cardiometabolic disease over and above simple measurement of adiposity, we did not adjust for a wide range of confounding variables. However, we adjusted for age, pubertal stage, and body mass index (BMI), as these are potentially important predictors of cardiometabolic risk and we would want to be clear that facial morphology predicted outcome over and above these. The age of the participants was recorded in months as they arrived at the clinic. Pubertal status was assessed on participants' self report with Tanner's questionnaires.

Statistical analyses

Participant characteristics were summarised with means (SD) for continuous approximately normally distributed variables, median (IQR) for continuous right skewed variables, and number (%) for categorical variables.

Generalized Procrustes Analysis (GPA) was performed on landmark configurations (each consisting of 63 *x*, *y*, and *z* co-ordinates of 21 facial landmarks) in order to remove differences in landmarks' position attributable to translation and rotation.[31-33] Scaling was not performed in order to preserve facial size. Principal Component Analysis (PCA) was used to reduce the set of 63 co-ordinates into a smaller number of independent components of

facial morphology. According to the 'Kaiser–Guttman criterion', PCs with eigenvalues greater than the average eigenvalue value were retained [34-36] and saved as new exposure variables. The rotation method used for PCA was varimax with Kaiser normalisation.[37] GPA was performed in the open source software R project and PCA in SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

Whilst this is a cross-sectional study, in all our analyses we examined the association of principal components of facial morphology (as exposures) with fasting insulin, glucose, triglycerides, HDLc, and LDLc (as outcomes) using multivariable linear regression models. No evidence was found for any gender interactions (all p-values ≥ 0.1) and therefore analyses are presented with both genders combined. In the first model we adjusted for age, gender, and pubertal stage. In the second model we adjusted for age, gender, pubertal stage, and BMI and examined how much this reduced any associations of facial principal components with the outcomes. Fasting insulin and triglycerides levels were right (positively) skewed and their logged values where used in the linear regression models, which ensured the model residuals were approximately normally distributed. The resultant regression coefficients with 95 per cent confidence intervals are presented.

In these multivariable analyses 140 comparisons were made (14 exposures with five outcomes and two models). In initial analyses we considered the conventional 0.05 level of statistical significance. We then adjusted for multiple comparisons using a Bonferroni correction by dividing 0.05 by 140, thus for these corrected analyses a p-value of 0.0004 would be considered statistically significant at the 0.05 level. All statistical analyses were performed in SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Table 1 shows the characteristics of the study participants. The Principal Component Analysis (PCA) identified 14 principal components (PCs) of facial morphology (Table 2). Each PC consisted of a number of co-ordinates of anthropometric landmarks. For example, the first principal component (PC 1) comprised 17 *y* co-ordinates of landmarks located in the upper and lower thirds of the face. These co-ordinates represented facial height (size). In order to facilitate understanding and interpretation of individual PCs, they are presented graphically on Figure 3. The first three PCs (facial size, inter-eye distance, and prominence of the nose and lower lip) accounted for almost half of the total variation (45.7%). The other 11 PCs contributed to facial variation to a much lesser extent (between 1.6 and 5%), but marked those subtle features which make the faces unique.

The multivariable associations of the 14 PCs with cardiometabolic outcomes are shown in Tables 3 to 7. With adjustment for age, gender and pubertal stage (model 1), seven PCs were associated with fasting insulin, none with fasting glucose, three PCs with triglycerides and HDLc, and four PCs with LDLc. After additional adjustment for BMI (model 2), four principal components remained associated with fasting insulin, none with glucose, one PC with triglycerides, none with HDLc, and two PCs with LDLc. However, none of these associations withstood adjustment for multiple comparisons.

Table 1 Characteristics of the study sample.

	Categories/units	Males N = 1127	Females N = 1221	All N = 2348
Age	Mean (months)	184.8 (3.0)	184.9 (3.2)	184.9 (3.1)
Tanner's pubertal stages	Stage I n (%)	0	0	0
	Stage II n (%)	8 (0.7%)	6 (0.5%)	14 (0.6%)
	Stage III n (%)	64 (5.7%)	118 (9.7%)	182 (7.8%)
	Stage IV n (%)	552 (49.0%)	632 (51.8%)	1184 (50.4%)
	Stage V n (%)	503 (44.6%)	465 (38.1%)	968 (41.2%)
BMI	Median (kg/m^2)	20.4 (18.9, 22.3)	21.2 (19.5, 23.4)	20.8 (19.1, 23.0)
Fasting insulin	Median (IU/l)	8.2 (5.9, 10.9)	9.7 (7.4, 13.0)	9.0 (6.6, 12.0)
Fasting glucose	Mean (mmol/l)	5.3 (0.4)	5.1 (0.3)	5.2 (0.4)
Total cholesterol	Mean (mmol/l)	3.6 (0.6)	3.9 (0.63)	3.8 (0.6)
Triglycerides	Median (mmol/l)	0.7 (0.6, 1.0)	0.8 (0.6, 1.0)	0.7 (0.6, 1.0)
HDLc	Mean (mmol/l)	1.2 (0.3)	1.4 (0.3)	1.3 (0.3)
LDLc	Mean (mmol/l)	2.0 (0.5)	2.2 (0.6)	2.1 (0.6)

Number (%) for categorical variables, mean (SD) or median (IQR) for continuously distributed variables are presented. SD, standard deviation; IQR, interquartile range; BMI, body mass index; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol.

Table 2 The results of the principal component analysis showing partial correlation coefficients between co-ordinates of anthropometric landmarks and facial principal components.

	Facial principal components													
Co-ordinates	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**	**PC 9**	**PC 10**	**PC 11**	**PC 12**	**PC 13**	**PC 14**
Ls (y)	-0.84													
Enl (y)	0.84													
Cphr (y)	-0.84													
Cphl (y)	-0.83													
Enr (y)	0.83													
Pg (y)	-0.82													
Chr (y)	-0.82													
Pil (y)	0.81													
Chl (y)	-0.81													
Pir (y)	0.81													
Psl (y)	0.79													
Li (y)	-0.78													
Psr (y)	0.78													
Exr (y)	0.75													
Exl (y)	0.74													
G (y)	0.64													
N (y)	0.62													
Psl (x)		0.94												
Psr (x)		-0.93												
Pil (x)		0.93												
Pir (x)		-0.92												
Enr (x)		-0.83												
Enl (x)		0.83												
Exr (x)		-0.79												
Exl (x)		0.75												
All (z)			-0.80											
Alr (z)			-0.79											
Sn (z)			-0.79											
Prn (z)			-0.68											
Li (z)			0.56											
Ls (z)				0.87										
Cphl (z)				0.86										
Cphr (z)				0.86										
Pg (z)				-0.78										
G (z)					-0.86									
N (z)					-0.82									
Pir (z)					0.65									
Pil (z)					0.64									
Prn (y)						0.82								
All (y)						0.79								
Alr (y)						0.77								
Sn (y)						0.72								
Chr (x)							0.82							
Chl (x)							-0.82							
Chl (z)							0.80							
Chr (z)							0.80							
Sn (x)								0.94						
Prn (x)								0.90						
G (x)									0.97					
N (x)									0.97					
Exl (z)										-0.62				
Exr (z)										-0.60				
Psl (z)											0.80			
Psr (z)											0.79			
Ls (x)												0.92		
Cphr (x)													0.82	
Cphl (x)													-0.78	
Pg (x)														0.91
Li (x)														0.76

Only major landmarks contributing to each principal component (PC) are shown (coefficients with absolute values above 0.5). Anthropometric landmarks are explained on Figure 2 and the principal components on Figure 3.

Table 3 Multivariable association of fourteen facial principal components (exposures) with fasting insulin as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	0.004	(-0.006, 0.014)	0.397	**-0.011**	**(-0.021, -0.001)**	**0.026**
PC2	**0.011**	**(0.002, 0.019)**	**0.011**	-0.003	(-0.011, 0.005)	0.419
PC3	**0.010**	**(0.002, 0.019)**	**0.015**	0.002	(-0.006, 0.010)	0.681
PC4	-0.001	(-0.009, 0.007)	0.803	0.002	(-0.005, 0.010)	0.538
PC5	**-0.011**	**(-0.020, -0.002)**	**0.019**	0.001	(-0.008, 0.010)	0.802
PC6	**0.010**	**(0.002, 0.018)**	**0.013**	0.001	(-0.007, 0.009)	0.787
PC7	0.000	(-0.008, 0.008)	0.935	0.005	(-0.003, 0.013)	0.190
PC8	**-0.017**	**(-0.028, -0.006)**	**0.003**	**-0.014**	**(-0.024, -0.004)**	**0.009**
PC9	**0.012**	**(0.003, 0.020)**	**0.005**	**0.012**	**(0.004, 0.020)**	**0.002**
PC10	0.005	(-0.003, 0.014)	0.190	0.002	(-0.006, 0.010)	0.601
PC11	**0.026**	**(0.018, 0.034)**	**<0.0001**	**0.009**	**(0.001, 0.017)**	**0.029**
PC12	0.006	(-0.002, 0.014)	0.151	0.005	(-0.002, 0.013)	0.172
PC13	-0.005	(-0.014, 0.003)	0.220	0.001	(-0.007, 0.009)	0.867
PC14	-0.003	(-0.011, 0.005)	0.485	-0.005	(-0.012, 0.003)	0.236

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.07$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.17$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 4 Multivariable association of fourteen facial principal components (exposures) with fasting glucose as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	-0.010	(-0.028, 0.0008)	0.286	-0.017	(-0.035, 0.001)	0.065
PC2	0.003	(-0.011, 0.018)	0.674	-0.003	(-0.018, 0.012)	0.724
PC3	0.010	(-0.005, 0.025)	0.197	0.006	(-0.009, 0.021)	0.458
PC4	-0.008	(-0.023, 0.007)	0.310	-0.006	(-0.021, 0.009)	0.427
PC5	0.002	(-0.014, 0.019)	0.769	0.007	(-0.009, 0.024)	0.381
PC6	0.012	(-0.003, 0.026)	0.115	0.009	(-0.006, 0.023)	0.243
PC7	0.009	(-0.006, 0.023)	0.226	0.011	(-0.004, 0.025)	0.147
PC8	-0.002	(-0.016, 0.013)	0.808	-0.001	(-0.015, 0.014)	0.924
PC9	-0.004	(-0.018, 0.011)	0.613	-0.003	(-0.018, 0.011)	0.657
PC10	-0.011	(-0.026, 0.003)	0.124	-0.013	(-0.027, 0.002)	0.083
PC11	0.008	(-0.007, 0.023)	0.301	0.002	(-0.014, 0.017)	0.838
PC12	0.005	(-0.010, 0.019)	0.535	0.005	(-0.010, 0.019)	0.521
PC13	0.003	(-0.013, 0.018)	0.730	0.004	(-0.011, 0.020)	0.563
PC14	0.001	(-0.013, 0.016)	0.866	0.002	(-0.013, 0.016)	0.812

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.05$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.06$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 5 Multivariable association of fourteen facial principal components (exposures) with triglycerides as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	0.010	(-0.001, 0.020)	0.073	0.000	(-0.011, 0.010)	0.975
PC2	0.004	(-0.003, 0.010)	0.291	-0.003	(-0.010, 0.003)	0.351
PC3	**0.008**	**(0.001, 0.015)**	**0.019**	0.004	(-0.003, 0.010)	0.287
PC4	-0.005	(-0.001, 0.002)	0.177	-0.003	(-0.009, 0.004)	0.397
PC5	-0.001	(-0.008, 0.007)	0.818	0.005	(-0.002, 0.012)	0.193
PC6	**0.011**	**(0.004, 0.017)**	**0.001**	**0.007**	**(0.000, 0.013)**	**0.045**
PC7	0.000	(-0.007, 0.006)	0.945	0.002	(-0.005, 0.008)	0.563
PC8	-0.005	(-0.012, 0.001)	0.118	-0.004	(-0.011, 0.002)	0.206
PC9	0.005	(-0.001, 0.012)	0.113	0.006	(-0.001, 0.012)	0.086
PC10	0.005	(-0.001, 0.012)	0.113	0.004	(-0.003, 0.010)	0.263
PC11	**0.008**	**(0.001, 0.014)**	**0.024**	<0.001	(-0.007, 0.007)	0.988
PC12	-0.003	(-0.010, 0.003)	0.341	-0.003	(-0.010, 0.003)	0.317
PC13	-0.004	(-0.011, 0.003)	0.213	-0.002	(-0.009, 0.005)	0.601
PC14	-0.005	(-0.011, 0.002)	0.143	-0.005	(-0.012, 0.001)	0.118

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.03$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.06$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 6 Multivariable association of fourteen facial principal components (exposures) with high density lipoprotein cholesterol (HDLc) as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	**-0.028**	**(-0.042, -0.014)**	**<0.001**	-0.013	(-0.027, 0.001)	0.073
PC2	-0.005	(-0.017, 0.006)	0.351	0.008	(-0.003, 0.020)	0.154
PC3	-0.006	(-0.017, 0.006)	0.339	0.003	(-0.008, 0.014)	0.611
PC4	0.007	(-0.004, 0.019)	0.228	0.004	(-0.008, 0.015)	0.538
PC5	0.007	(-0.006, 0.020)	0.279	-0.005	(-0.018, 0.008)	0.443
PC6	**-0.014**	**(-0.025, -0.003)**	**0.016**	-0.005	(-0.016, 0.006)	0.408
PC7	0.003	(-0.008, 0.015)	0.570	-0.001	(-0.012, 0.010)	0.797
PC8	0.009	(-0.003, 0.020)	0.129	0.007	(-0.004, 0.018)	0.205
PC9	0.001	(-0.010, 0.012)	0.883	0.000	(-0.011, 0.011)	0.940
PC10	-0.010	(-0.022, 0.001)	0.078	-0.007	(-0.018, 0.004)	0.229
PC11	**-0.022**	**(-0.034, -0.011)**	**<0.001**	-0.006	(-0.017, 0.006)	0.340
PC12	0.005	(-0.007, 0.016)	0.413	0.005	(-0.006, 0.016)	0.348
PC13	0.005	(-0.007, 0.017)	0.387	-0.001	(-0.012, 0.011)	0.905
PC14	0.000	(-0.012, 0.011)	0.959	0.001	(-0.010, 0.012)	0.798

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.08$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.13$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

Table 7 Multivariable association of fourteen facial principal components (exposures) with low density lipoprotein cholesterol (LDLc) as an outcome.

	Model 1			Model 2		
PC	**B**	**(95% CI)**	**p Value**	**B**	**(95% CI)**	**p Value**
PC1	0.004	(-0.023, 0.030)	0.793	-0.014	(-0.041, 0.013)	0.307
PC2	-0.004	(-0.026, 0.018)	0.708	-0.020	(-0.042, 0.002)	0.074
PC3	**0.038**	**(0.016, 0.061)**	**0.001**	**0.028**	**(0.006, 0.050)**	**0.013**
PC4	-0.002	(-0.025, 0.020)	0.855	0.002	(-0.020, 0.024)	0.851
PC5	0.006	(-0.019, 0.031)	0.621	0.021	(-0.004, 0.046)	0.108
PC6	**0.027**	**(0.005, 0.049)**	**0.014**	0.016	(-0.005, 0.038)	0.140
PC7	0.012	(-0.009, 0.034)	0.263	0.018	(-0.004, 0.040)	0.103
PC8	**-0.024**	**(-0.045, -0.002)**	**0.033**	**-0.022**	**(-0.043, 0.000)**	**0.048**
PC9	-0.014	(-0.035, 0.008)	0.222	-0.013	(-0.034, 0.008)	0.235
PC10	0.006	(-0.015, 0.028)	0.561	0.002	(-0.019, 0.024)	0.824
PC11	**0.031**	**(0.009, 0.053)**	**0.006**	0.011	(-0.011, 0.034)	0.322
PC12	0.021	(-0.001, 0.043)	0.056	0.020	(-0.001, 0.042)	0.061
PC13	-0.006	(-0.029, 0.016)	0.582	0.001	(-0.022, 0.023)	0.961
PC14	0.013	(-0.009, 0.035)	0.238	0.011	(-0.010, 0.033)	0.315

Model 1 is adjusted for age, gender, and puberty (adjusted $R^2 = 0.05$); Model 2 is adjusted for age, gender, puberty, and BMI (adjusted $R^2 = 0.06$). PC, principal component of the face (refer to the text, Table 2, and Figure 3 for an explanation); B, regression coefficient, CI, confidence interval. Figures in bold indicate statistically significant associations at the level $p < 0.05$ (before Bonferroni corrections).

DISCUSSION

Laser surface scanning is a non-invasive technology, which enables accurate and precise analysis of facial morphology.[1, 2, 24-26] Due to its portability, easy application, and relatively low cost, this technique is very suitable for epidemiological field studies. The vast amount of data captured by the system (more than 40,000 points, each consisting of *x*, *y*, and *z* co-ordinates) is a testimony of the complexity of facial surface. For this reason, face cannot be easily represented as a single exposure.

Therefore, it was necessary to make some facial data-reduction prior to its meaningful use. First of all, Generalised Procrustes Analysis (GPA; a widely established method in statistical shape analysis) was used to place landmark co-ordinates in the same space reducing confounding errors (rotation and translation). Secondly, Principal Component

Analysis (PCA) was applied on the set of co-ordinates and 14 facial principal components (PCs) were identified, which accounted for almost 82 per cent of the total variation in normal facial form, consisting of size and shape. Normal facial variation was recently analysed on a complete sample of 4747 faces from the ALSPAC database and the same number of PCs was extracted, with almost identical order of individual PCs and very similar percentages of variation.[22]

The application of this statistical technique is not new. Previously, PCA was performed on two-dimensional data sets, obtained from either lateral skull radiographs or photographs of both children and adults.[38-41] The resultant number of principal components in these studies was between 6 and 8, and these explained up to 90 per cent of the total variance in facial profile, based on linear measurements between anthropometric landmarks, or their co-ordinates. However, with the introduction of sophisticated 3D imaging techniques, the amount of data entering PCA significantly increased. Therefore, the number of PCs which represent facial variation also increased: between 14 and 16 PCs have been reported to account for between 86 and 92 per cent of the total variation.[10, 11, 41, 42]

Although the first three components in the current study explain almost half of the total variation, other components are also important, since they represent subtle changes that make the face unique. Therefore, a decision was made to keep all of them in the subsequent multivariable analyses. Following adjustment for BMI and taking account of multiple statistical testing, we did not find that any of these PCs were associated with fasting insulin or associated cardiometabolic risk factors, suggesting that facial morphology is unlikely to be a reliable way of predicting young people at future risk of type 2 diabetes or cardiovascular disease. Consistent with other large epidemiological studies conducted in healthy general population samples, we were not able to directly measure insulin resistance using the gold

standard euglycaemic hyperinsulinemic clamp. Fasting insulin has been shown to have modest to strong correlations with clamp assessed insulin resistance (correlation coefficients 0.5 to 0.9) in children and adolescents.[43, 44] Any measurement error is likely to be non-differential and therefore would be expected to bias results towards the null. Since strong associations of these outcomes with BMI have been shown in ALSPAC,[23] any associations with a better measure of insulin resistance are unlikely to be stronger than those of BMI.

The study has some limitations. First of all, it is ethnic-specific, and therefore future studies will have to address the research question in different enthnic groups. Secondly, facial variation can be affected by many different factors. Whilst it is possible to control the age, gender, and ethnicity of the sample, environmental factors present a greater challenge, even with a good research strategy, as many of them can be unknown at the time of the study. The face changes throughout life, increasing in size and changing shape.[1, 2, 7] This holds true for the present sample consisting of 15-year old adolescents. The cross-sectional design of the study did not allow us to track these changes and analyse their relationship with metabolic phenotype through time. That may be more important than the assessment of variation among individuals and thus should be considered in future studies.

CONCLUSION

Our results do not provide strong evidence that facial morphology is robustly and importantly associated with cardiometabolic risk factors. The associations identified were not consistent across outcomes, were weak in magnitude, attenuated with adjustment for BMI, and did not withstand correction for multiple statistical testing. Further study of facial parameters with cardiometabolic and/or other health outcomes might provide valuable insights into how facial morphology can be indicative of health.

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Contributors JDj, DAL and SR were responsible for the conception and the design of the study. JDj and SR initiated the study. JDj and AMT collected the data. JDj, DAL, and RP were responsible for statistical analyses. JDj, AMT, AIZ and SR analysed and interpreted data on facial parameters. JDj, DAL and RP analysed and interpreted data on metabolic parameters. JDj and DAL wrote the first draft of the paper. All authors contributed to and approved the final version of the paper.

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Competing interests None.

Ethics approval The study was approved by the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee.

Data sharing statement There is no additional data available.

REFERENCES

1 Kau CH, Richmond S. Three-dimensional imaging for orthodontics and maxillofacial surgery. Iowa: Wiley Blackwell 2010.

2 Huang GJ, Richmond S, Vig KWL. Evidence-Based Orthodontics. Ames, IA: Wiley Blackwell 2011.

3 Hammond P, Suttie M. Large-scale objective phenotyping of 3D facial morphology. *Hum Mutat* 2012;33:817–25.

4 Brons S, van Beusichem ME, Bronkhorst EM, et al. Methods to quantify soft-tissue based facial growth and treatment outcomes in children: a systematic review. *PloS One* 2012;7:e41898.

5 Hammond P. The use of 3D face shape modelling in dysmorphology. *Arch Dis Child* 2007;92:1120–6.

6 Richmond S, Zhurov A, Toma A. Three-dimensional facial imaging. In: Wilkinson C, Rynn C, eds. Craniofacial Identification. Cambridge: Cambridge University Press 2012: 154–65.

7 Richmond S, Al Ali AM, Beldie L, et al. Detailing patient specific modeling to aid clinical decision-making. In: Calvo B, Peña E, eds. Patient-Specific Computational Modeling: 5 (Lecture Notes in Computational Vision and Biomechanics). Dordrecht: Springer 2012:105–31.

8 Ali AA, Richmond S, Popat H, et al. The influence of asthma on face shape: a three-dimensional study. *Eur J Orthod* Published Online First: 4 October 2012. doi: 10.1093/ejo/cjs067

9 Lee RW, Sutherland K, Chan AS, *et al*. Relationship between surface facial dimensions and upper airway structures in obstructive sleep apnea. *Sleep* 2010;33:1249–54.

10 Hennessy RJ, Baldwin PA, Browne DJ, *et al*. Three-dimensional laser surface imaging and geometric morphometrics resolve frontonasal dysmorphology in schizophrenia. *Biol Psychiatry* 2007;61:1187–94.

11 Hennessy RJ, Baldwin PA, Browne DJ, *et al*. Frontonasal dysmorphology in bipolar disorder by 3D laser surface imaging and geometric morphometrics: comparisons with schizophrenia. *Schizophr Res* 2010;122:63–71.

12 Ferrario VF, Sforza C, Dellavia C. Abnormal variations in the facial soft tissues of adults uremic patients on chronic dialysis. *Angle Orthod* 2005;75:320–5.

13 Herouvi D, Karanasios E, Karayianni C, *et al*. Cardiovascular disease in childhood: the role of obesity. *Eur J Paediatr* Published Online First: 23 January 2013. doi: 10.1007/s00431-013-1932-8

14 Raitakari OT, Juonala M, Kahonen M, *et al*. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the cardiovascular risk in young Finns study. *JAMA* 2003;290:2277–83.

15 Owen CG, Whincup PH, Orfei L, *et al*. Is body mass index before middle age related to coronary heart disease risk in later life? Evidence from observational studies. *Int J Obes* (Lond) 2009;33:866–77.

16 Han JC, Lawlor DA, Kimm SYS. Childhood obesity. (Peer reviewed commissioned seminar). *Lancet* 2010;375:1737–48.

17 Ohrn K, Al-Kahlili B, Huggare J, et al. Craniofacial morphology in obese adolescents. *Acta Odontol Scand* 2002;60:193–7.

18 Ferrario VF, Dellavia C, Tartaglia GM, et al. Soft tissue facial morphology in obese adolescents: a three-dimensional non-invasive assessment. *Angle Orthod* 2004;74:37–42.

19 Sadeghianrizi A, Forsberg CM, Marcus C, et al. Craniofacial development in obese adolescents. *Eur J Orthod* 2005;27:550–5.

20 Boyd A, Golding J, Macleod J, *et al*. Cohort Profile: The 'Children of the 90s': the index offspring of The Avon Longitudinal Study of Parents and Children (ALSPAC). *Int J Epidemiol* Published Online First: 16 April 2012. doi:10.1093/ije/dys064

21 Fraser A, Macdonald-Wallis C, Tilling K, *et al*. Cohort Profile: The Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *Int J Epidemiol* Published Online First: 16 April 2012. doi:10.1093/ije/dys066

22 Toma AM, Zhurov AI, Playle R, *et al*. The assessment of facial variation in 4747 British schoolchildren. *Eur J Orthod* 2012;34:655–64.

23 Lawlor DA, Benfield L, Logue J, *et al*. Association between general and central adiposity in childhood, and change in these, with cardiovascular risk factors in adolescence: prospective cohort study. *BMJ* 2010;341:c6224.

24 Kau CH, Zhurov AI, Scheer R, *et al*. The feasibility of measuring three-dimensional facial morphology in children. *Orthod Craniofac Res* 2004;7:198–204.

25 Kau CH, Richmond S, Zhurov AI, *et al*. Reliability of measuring facial morphology with a 3-dimensional laser scanning system. *Am J Orthod Dentofacial Orthop* 2005;128:424–30.

26 Kovacs L, Zimmermann A, Brockmann G, et al. Accuracy and precision of the three-dimensional assessment of the facial surface using a 3-D laser scanner. *IEEE Trans Med Imaging* 2006;25:742–54.

27 Zhurov AI, Kau CH, Richmond S. Computer methods for measuring 3D facial morphology. In: Middleton J, Shrive MG, Jones ML, eds. Proceedings of the 6th International Symposium on Computer Methods in Bomechanics & Biomedical Engineering. Cardiff: First Numerics Ltd 2005 (CD ROM, paper 151D).

28 Farkas LG. Anthropometry of the head and face. New York: Raven Press 1994:21–5.

29 Toma AM, Zhurov A, Playle R, *et al.* Reproducibility of facial soft tissue landmarks on 3D laser-scanned facial images. *Orthod Craniofac Res* 2009;12:33–42.

30 Djordjevic J, Pirttiniemi P, Harila V, *et al*. Three-dimensional longitudinal assessment of facial symmetry in adolescents. *Eur J Orthod* Published Online First: 7 February 2011. doi: 10.1093/ejo/cjr006

31Bookstein FL. Morphometric tools for landmark data. Cambridge: Cambridge University Press 1991:256–8.

32 Dryden IL, Mardia KV. Statistical shape analysis. Chichester, UK: Wiley 1998.

33 Zelditch ML, Swiderski DL, Sheets HD, et al. Geometric morphometrics for biologists: A Primer. New York: Elsevier academic press 2004.

34 Guttman L. Some necessary conditions for common factor analysis. *Psychometrika* 1954;19:149–61.

35 Cliff N. The eigenvalues-greater-than-one rule and the reliability of components. *Psychol Bull* 1988;103:276–9.

36 Jackson DA. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* 1993;74:2204–14.

37 Kaiser HF. The varimax criterion for analytic rotation in factor analysis. *Psychometrika* 1958;23:187–200.

38 Cleall JF, BeGole EA, Chebib FS. Craniofacial morphology: a principal component analysis. *Am J Orthod* 1979;75:650–66.

39 Halazonetis DJ. Morphometric evaluation of soft tissue profile shape. *Am J Orthod Dentofacial Orthop* 2007;131:481–9.

40 Krey KF, Dannhauer KH. Morphometric analysis of facial profile in adults. *J Orofac Orthop* 2008;69:424–36.

41 Moss JP. The use of three-dimensional imaging in orthodontics. *Eur J Orthod* 2006;28:416–25.

42 Hennessy RJ, McLearie S, Kinsella A, et al. Facial surface analysis by 3D laser scanning and geometric morphometrics in relation to sexual dimorphism in cerebral-craniofacial morphogenesis and cognitive function. *J Anat* 2005;207:283–95.

43 Gungor N, Saad R, Janosky J, *et al*. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. *J Pediatr* 2004;144:47–55.

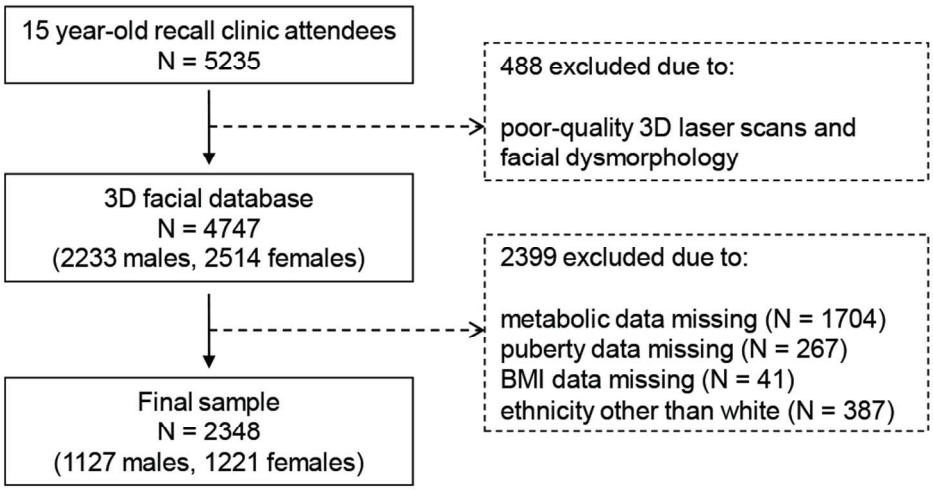
44 Schwartz B, Jacobs DR, Jr., Moran A, *et al*. Measurement of insulin sensitivity in children: comparison between the euglycemic-hyperinsulinemic clamp and surrogate measures. *Diabetes Care* 2008;31:783–8.

FIGURE LEGENDS:

Fig. 1 Flowchart showing the selection of study sample from 15+ year follow-up clinic of the Avon Longitudinal Study of Parents and Children (ALSPAC). All analyses presented in this paper are based on 2348 participants with complete data on facial soft tissue morphology (exposure), blood-based indicators of insulin resistance and associated cardiometabolic risk factors (outcomes), and body mass index and pubertal stage (covariables).

Fig. 2 Twenty-one anthropometric landmarks which were identified on facial laser scans of participants. (1) Glabella (g); (2) Nasion (n); (3) Endocanthion left (enl); (4) Endocanthion right (enr); (5) Exocanthion left (exl); (6) Exocanthion right (exr); (7) Palpebrale superius left (psl); (8) Palpebrale superius right (psr); (9) Palpebrale inferius left (pil); (10) Palpebrale inferius right (pir); (11) Pronasale (prn); (12) Subnasale (sn); (13) Alare left (all); (14) Alare right (alr); (15) Labiale superius (ls); (16) Crista philtri left (cphl); (17) Crista philtri right (cphr); (18) Labiale inferius (li); (19) Cheilion left (chl); (20) Cheilion right (chr); (21) Pogonion (pg). Definitions by Farkas [28] were used. Reprinted from the author's previous publication with permission from 'John Wiley and Sons'.

Fig. 3 Facial principal components (PCs). Numbers indicate percentages of normal facial variation explained by the given principal component. Co-ordinates which constitute each principal component are marked on the face (refer to Table 2), and arrows indicate *x*, *y*, and *z* directions.



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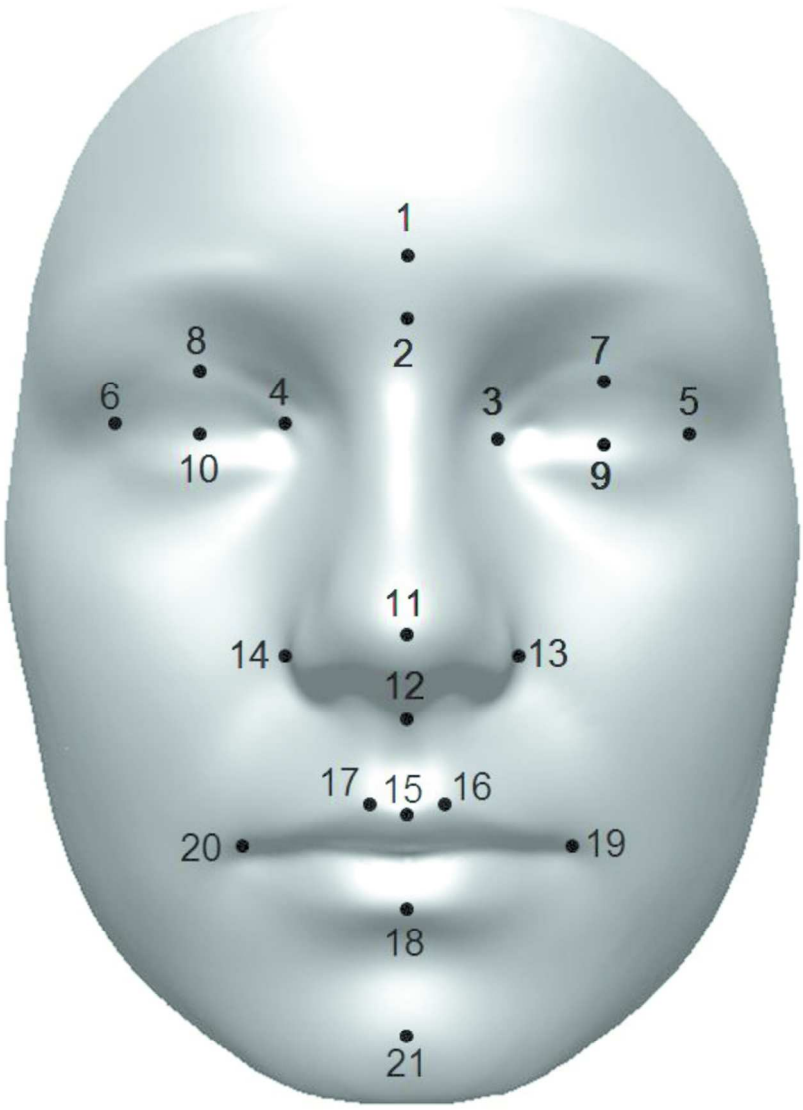

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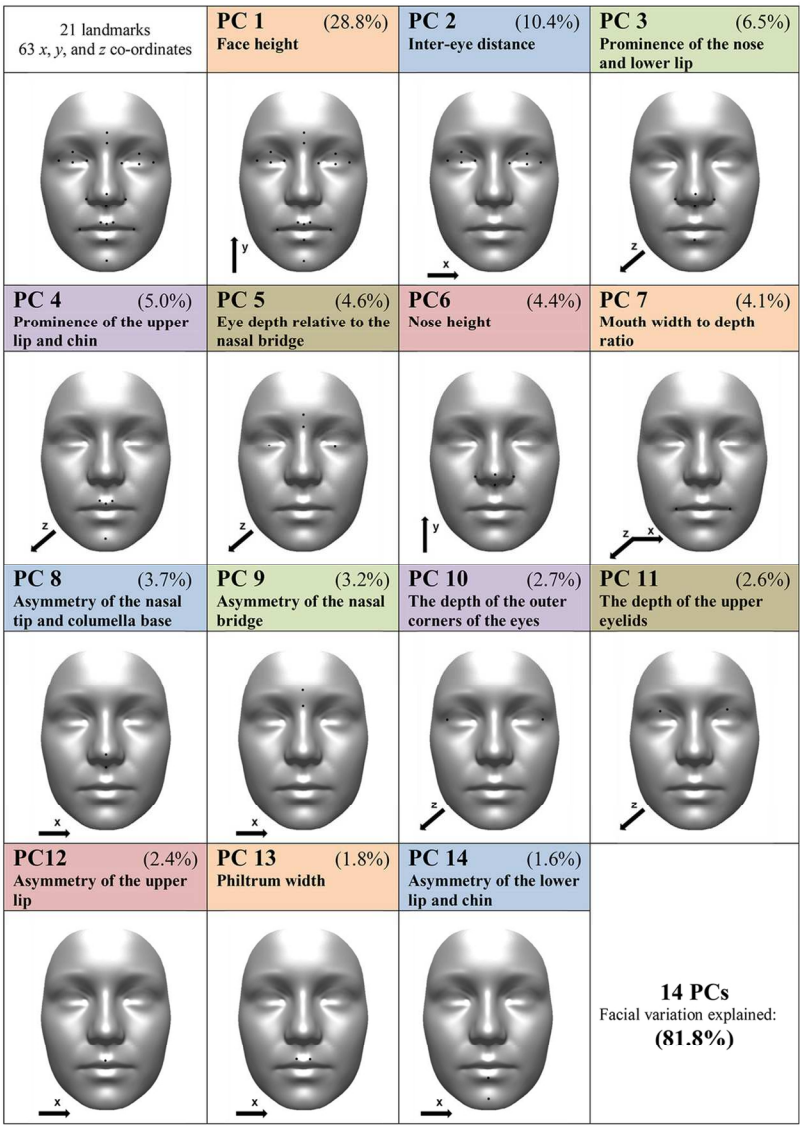
113x145mm (300 x 300 DPI)



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99x149mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**1, 2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**2**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**4**
Objectives	3	State specific objectives, including any prespecified hypotheses	**4, 5**
Methods			
Study design	4	Present key elements of study design early in the paper	**2, 5**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**5-7**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	**5**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	**6, 7**
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	**6, 7**
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	**Page 5 and Figure 1**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	**7**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	**7, 8**
		(*b*) Describe any methods used to examine subgroups and interactions	**7, 8**
		(*c*) Explain how missing data were addressed	**NA**
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	**NA**
		(*e*) Describe any sensitivity analyses	**NA**
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	**Page 5, Figure 1, Table 1**
		(b) Give reasons for non-participation at each stage	**Figure 1**
		(c) Consider use of a flow diagram	**Figure 1**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**Table 1, Page 6**
		(b) Indicate number of participants with missing data for each variable of interest	**Figure 1**
Outcome data	15*	Report numbers of outcome events or summary measures	**6**
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	**Page 8, Tables 3-7**
		(*b*) Report category boundaries when continuous variables were categorized	**NA**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**NA**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**8, 10, Table 2**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**14**
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	**15**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**13-15**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**15**
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	**16**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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 www.strobe-statement.org.