1   **Title :** Arterial stiffening, insulin resistance and acanthosis nigricans in a

2   community sample of adolescents with obesity.

3   **Running head :** Arterial stiffening and acanthosis nigricans

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10 **Word count:** 1619 (not including abstract (165words), tables and references)

13 **Conflict of interest statement :** No conflict of interests to declare by any author.

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1 **Abstract**

2 Acanthosis Nigricans (AN) is a common finding in adolescents with obesity. Little

3 is known about its relevance for cardiovascular (CVS) risk, in particular arterial

4 stiffening. We investigated associations between AN, conventional markers of

5 CVS risk and carotid-radial pulse wave velocity (PWV) in a community sample of

6 adolescents with obesity aged 12-19 recruited to an obesity trial. AN was present

7 in 63% of subjects and 43% had severe grading. Presence of AN and severe AN

8 were associated with BMIz. Presence of AN (but not severity) was associated

9 with abnormal or fasting hyperinsulinaemia but not after adjustment for BMIz.

10 PWV data were available for 147 (84% of participants) Severe grade AN was

11 associated with PWV (co-efficient 0.51, 95% CI 0.13 to 0.89, p = 0.01) but not

12 when adjusted for BMIz, ethnic grouping and age. In our study presence and

13 severity of AN offered little additional information on CVS risk beyond the

14 degree of obesity itself. The relevance of AN for CVS risk should be interpreted

15 with caution.

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Introduction

Acanthosis nigricans (AN) is a dark, velvety skin pigmentation associated with obesity(1) frequently encountered in clinical practice in young people with obesity. Correctly interpreting its relevance and communicating this to patients and families is important. Studies have identified AN in adolescence as a marker for type 2 Diabetes Mellitus (DM)(2, 3) and insulin resistance (IR)(4-7) yet it is unclear whether AN in adolescent obesity is associated with increased cardiovascular risk separately to risk for diabetes. Whilst attempts to identify cardiovascular risk by presence of AN in adolescents have been made using adult-based components of the metabolic syndrome,(6) (7) evidence for associations between AN and contemporary arterial pathological processes, such as arterial stiffening, has not been investigated. Pulse Wave Velocity (PWV), is a non-invasive proxy for arterial stiffness, demonstrated to be a reliable predictor of future cardiovascular mortality and morbidity in adults,(8, 9) and shown to correlate with degree of atheroma in adults.(10) Meta-analyses have demonstrated greater PWV in obese children and adolescents. (11, 12)

We investigated for associations between AN at the neck and 1) measures of insulin resistance and 2) PWV using baseline data from a community sample of obese adolescents (>95th centile Body Mass Index (BMI)) from greater London recruited into a randomized controlled trial for an obesity intervention.(13)

Methods

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1 Subjects were aged 12 to 19 with obesity (>95th centile BMI), recruited from

2 community sources (GPs, schools, youth groups, self referral) between January

3 2011 and July 2013 for the HELP trial.(13) Genetic or endocrine causes of

4 obesity, and chronic illness (including DM types 1 and 2), were excluded. The

5 Central London ethics committee provided ethics permission.

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7 Ethnicity was self-reported, grouped as white, black, South Asian or

8 mixed/other. Pubertal status was self reported using standardized diagrams(14)

9 and grouped into pre/early (tanner 1 and 2), mid (tanner 3 and 4), late/complete

10 (5). Family history of type 2 DM (1st degree relative) was recorded. BMI and

11 waist circumference z-scores (BMIz and waistz) were derived using LMSgrowth

12 program version 2.69 (Harlow Healthcare, UK) using UK 1990 population

13 growth data.(15) Fasting insulin and glucose were measured and Homeostatic

14 Model Assessment Insulin resistance (HOMA-IR) was derived (insulin x

15 glucose/2).(16) Definitions of metabolic abnormalities were taken from a UK

16 consensus statement(16) (abnormal HOMA $\geq$ 4.4, fasting hyperinsulinaemia

17 using pubertal stage specific cut-offs : >10 mU/L pre/early puberty, >30 mU/L

18 mid puberty, mU/L>20 late and complete puberty). Information on smoking was

19 also collected by self-report. Participants with HbA1c or fasting glucose

20 indicative of diabetes mellitus were excluded.

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22 AN was measured by appearance and texture at the neck by a single

23 paediatrician (LH) using a previously reported grading system(17) : absent,

24 present/mild (limited to base of skull not reaching lateral margins of the neck),

25 moderate (extending to lateral margins of neck but not visible from front) and

severe (visible from the front). These were dichotomized into two variables for analysis : 1) AN present or not and 2) severe grade or not (i.e milder grade or not present).

A single operator (using tonometry) recorded pulse waveforms at the carotid and radial pulses. PWV was derived using waveforms, blood pressure and distances between pulse sites (distance between the carotid and sternal notch, then sternal notch to the radial pulse via the mid shoulder), using software and protocolled quality indices (Sphygmocor, AtCor Medical, Sydney Australia). Blood Pressure was measured at the right arm and averaged 3 times as per published guidelines.(18) Raw blood pressure was converted into standardized z-scores (diastolic z and systolic z respectively). A single measurement of PWV occurred at baseline following a standard operating procedure for all participants, with quality control adhered to as per the manufacturer's guidance (only PWV statistics on wave readings with SDs < 6% mean time and with only final derived PWV with an SD <10% were accepted). Measurement of PWV was performed in the supine position, following 30 minutes rest at room temperature.

Analysis

STATA version 13 (StataCorp, Texas, USA) was used for analyses. Associations between. Associations between AN, adiposity measures and insulin resistance (as fasting hyperinsuinaemia and abnormal HOMA-IR), were tested using logistic regression with odd's ratios (OR) and linear regression models between AN ,

1 PWV and adiposity. Multivariable models were then applied to adjust for other

2 covariables also found to be associated.

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4 **Results**

5 174 subjects were recruited, with even distribution across age groups (mean

6 15.4 years, SD 2.1 years) with predominance of later stages of puberty (12%

7 pre/early, 22% mid, 66% late/complete). 62% were female. Ethnicity

8 composition was white 38%, black 30%, South Asian 21%, mixed/other 11%.

9 Information on smoking was available for 168 participants, with 120 (70%)

10 having never smoked and 48 (30%) currently smoking or having smoked

11 previously. Mean BMIz was 2.80 (SD 0.55).

12

13 Assessment of AN was possible in 173 subjects, with AN present in 109 (63%)

14 and 75 (43%) of the sample having severe grade AN. Presence of AN was

15 associated with BMIz (OR 2.12, 95%CI 1.17 to 3.82, p =0.01); and was more

16 common in all non-white ethnic groups (black OR 29.65, 95% CI 9.38 to 93.75, p

17 <0.001; South Asian OR 8.47, 95%CI 3.27 to 21.92, p <0.001; mixed OR 5.24,

18 95%CI 1.73 to 15.85, p < 0.01). There was no association between presence or

19 severity of AN and sex, age or pubertal stage. Severe grade AN was associated

20 with BMIz (OR 2.06, 95% CI 1.15 to 3.67, p = 0.02) and was more common in all

21 non-white ethnic groups compared to white: (black – OR 21.92, 95% CI 8.31 to

22 57.78 p <0.001; South Asian OR 8.90, 95%CI 3.30 to 23.96, p <0.001; mixed OR

23 4.15, 95%CI 1.26 to 13.66, p = 0.02).

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1   Fasting hyperinsulinaemia and abnormal HOMA-IR were present in 34 (24%)

2   and 35 (20%) of individuals respectively. Both fasting hyperinsulinaemia and

3   abnormal HOMA-IR were associated with BMIz and pubertal stage (data not

4   shown). Univariable models for associations of any or severe grade AN with

5   fasting hyperinsulinaemia and abnormal HOMA-IR are shown in the table.

6   Presence of AN was associated with both fasting hyperinsulinaemia and

7   abnormal HOMA-IR; however this association was attenuated after adjusting for

8   BMIz and pubertal stage; with. BMIz remaining strongly associated with insulin

9   resistance in these adjusted models (OR 9.19, 95% CI 3.40 to 25.02, p <0.001,

10 4.91 for abnormal insulin, OR 4.56, 95% CI 1.91 to 10.87, p = 0.001)

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12 Presence of AN was positively associated with fasting cholesterol before after

13 adjustment for BMIz (OR 1.62, 95% CI 1.08 to 2.42, p = 0.01) but not fasting

14 triglycerides, diastolic z or systolic z blood pressure. Severe AN was positively

15 associated with diastolic z blood pressure after adjustment for BMIz (OR 1.36,

16 95% CI 1.03 to 1.81, p = 0.03) but no other cardio-metabolic marker.

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18 PWV measurements were available for 146 (84%) participants. PWV was

19 associated with age and ethnicity but not pubertal stage or sex (data not shown).

20 PWV was associated with BMIz when adjusted for age and ethnicity (co-efficient

21 0.54, 95% CI 0.19 to 0.88, p < 0.01), but not associated with either systolic or

22 diastolic blood pressure, nor heart rate (data not shown). Smoking status was

23 not associated with PWV. Univariable models of associations between presence

24 of any AN and severe AN and PWV is shown in table. Severe grade AN was

25 associated with PWV, however, this association was attenuated in a

1   multivariable model adjusting for BMIz, ethnic grouping and age (see table).

2   BMIz remained associated with PWV in this multivariable model.

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4   **Discussion**

5

6   AN was common in our study population, with 63 % and 43% found to have AN

7   and Severe AN respectively. There was a positive association between BMIz and

8   presence of AN (with an increase of 1SD in BMI leading to a doubling in the risk

9   of AN) consistent with other published studies of AN in adolescent groups.(4-6)

10 However both presence and severity of AN were poor markers of insulin

11 resistance when adjusted for BMIz. There were no associations between PWV, as

12 a proxy for arterial stiffening, after adjusting for BMIz. Our study suggests that

13 the finding of AN (or severity) in an obese group of adolescents does not provide

14 additional information about individual cardio-metabolic risk beyond the degree

15 of obesity itself. Kobaissi et al(7) have similarly reported that presence and

16 severity of AN poorly predict insulin resistance in adolescent obesity after

17 adjusting for BMI, albeit in an exclusively Hispanic group. We believe that this is

18 the first study to examine for a relationship between the presence and severity of

19 AN, and contemporary measures of arterial stiffness as a proxy for long-term

20 cardio-metabolic risk.

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22 There were a number of strengths to our study. We used a community sample

23 with a mixture of the ethnicities seen in particular in urban areas of the UK, and

24 also we adjusted for co-variables such as BMIz in regression models. To counter

25 the challenge of assessing AN in Caucasian individuals, we also used texture as

well as appearance and used a single trained observer. Our study also had a number of limitations which must be kept in mind. The sample size may have had insufficient power to detect small effect sizes, especially in multivariable regression models, and pulse wave data in particular was not complete for all participants. Although all PWV measurements were consistent with the manufacturer's quality control indices (as described above), we measured PWV only once in each participant at baseline (so as to limit burden on participants for the bigger trial), meaning that repeated measure variablity can not be reported for our data. We measured PWV by carotid-radial methodology and recent evidence has shown that PWV in the context of obesity may vary by arterial site,(11) thus our findings may not reflect changes at other important cardio-vascular sites such as the aorta. Given exclusion and screening for DM, none of our subjects had DM, so our findings cannot be generalized to patients with established DM, obesity and AN. Our data are also limited in that it is cross-sectional and highlights the need for longitudinal data to more fully examine the relationship between AN and the development of DM and longer term cardiovascular risk within groups and individuals.

In conclusion, the cardio-metabolic relevance of AN is unclear and may offer no extra information beyond than degree of obesity. It should therefore be interpreted with caution.

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Table : Shows A) univariable models with presence of acanthosis nigricans and presence of severe acanthosis nigricans as predictors of insulin resistance; B) a multivariable, adjusted model (for BMIz and pubertal stage) as predictors of acanthosis nigricans and insulin resistance; C) univariable regression model of acanthosis nigricans and presence of severe AN as predictors of pulse wave velocity; and D) a multivariable, adjusted model (for BMIz, ethnicity and age) of presence of severe acanthosis nigricans as a predictor of pulse wave velocity.

A. Univariable models of any AN or severe grade AN as predictors of fasting hyperinsulinism and abnormal HOMA						
	Any AN present			Severe AN present		
	N	OR (95% CI)	p	N	OR (95% CI)	p
Fasting hyperinsulinism	173	2.68 (1.09 to 6.58)	0.03*	173	1.88(0.88 to 4.00)	0.10
Abnormal HOMA	173	3.44 (1.34 to 8.8)	0.01*	173	1.98 (0.94 to 4.21)	0.07
B. Multivariable model of any AN as a predictor for fasting hyperinsulinism and abnormal HOMA (adjusting for BMIz and pubertal stage)						
Fasting hyperinsulinism	173	2.40 (0.85 to 6.76)	0.10			
Abnormal HOMA	172	2.60 (0.96 to 7.01)	0.06			
C. Univariable model of Any AN or severe grade AN as predictors of PWV						
	N	Beta (95% CI)	p	N	Beta (95% CI)	p
PWV	145	0.36 (-0.03 to 0.76)	0.07	145	0.51 (0.12 to 0.89)	0.01 *
D. Mutivariable model of Severe AN as a predictor of PWV (adjusted for BMIz, ethnicity and Age)						
PWV				145	0.37 (-0.07 to 0.80)	0.10

PWV = pulse wave velocity, BMIz = z-score of Body Mass Index, AN = Acanthosis nigricans, HOMA = Homeostatic. N = total number included in each model.