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Do body mass index and waist-to-height ratio over the preceding decade predict retinal microvasculature in 11-12 year-olds and mid-life adults?

Running title: Body mass and waist pathways and microvasculature

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Abstract

Background/Objectives Microvascular changes may contribute to obesity-associated cardiovascular disease. We examined whether body mass index (BMI) and waist-to-height ratio (WHtR) (i) at multiple earlier time points and (ii) decade-long trajectories predicted retinal microvascular parameters in mid-childhood/adulthood.

Methods *Participants/design:* 1288 11-12 year-olds (51% girls) and 1264 parents (87% mothers) in the population-based CheckPoint module within the Longitudinal Study of Australian Children (LSAC). *LSAC exposure measures:* Biennial BMI z-score and waist-height ratio (WHtR) for children at 5 times points from age 2-3 to 10-11 years and self-reported parent BMI at 6 time points from child age 0-1 years to 10-11 years. *CheckPoint outcome measures*: Retinal arteriolar and venular caliber. *Analyses:* BMI/WHtR trajectories were identified by group-based trajectory modeling; linear regression models estimated associations between BMI/waist at each time point/trajectories and later retinal vascular caliber, adjusted for age, sex and family socioeconomic status.

Results In time point analyses, higher child BMI/WHtR from age 4-5 years were associated with narrower arteriolar caliber at age 11-12 years, but not venular caliber. For example, each standard deviation (SD) higher in BMI z-score at 4-5 years was associated with narrower arteriolar caliber at 11-12 years (standardized mean difference (SMD) -0.05, 95% CI -0.10 to 0.01); by 10-11 years, associations had doubled to -0.10 (95% CI -0.16 to -0.05). In adults, these finding were similar, except the magnitude of BMI and arteriolar associations were similar across all time points

(SMD -0.11 to -0.13). In child and adult BMI trajectory analyses, less favorable trajectories predicted narrower arteriolar (p-trend <0.05), but not venular (p-trend >0.1), caliber. Compared to those in the average BMI trajectory, SMDs in arterial caliber for children and adults in the highest trajectory were -0.25 (95% CI -0.44 to -0.07) and -0.42 (95% CI -0.73 to -0.10) respectively. Venular caliber showed late associations with child WHtR, but not with BMI in children or adults.

Conclusions

Associations of decade-long high BMI trajectories with narrowed retinal arteriolar caliber emerge in children, and are clearly evident by mid-life. Adiposity appears to exert its early adverse life course impacts on the microcirculation more via arteriolar than venular mechanisms.

INTRODUCTION

Early childhood obesity is associated with adverse cardiovascular outcomes later in life.[1, 2] However, how early obesity relates to a crucial component of the circulation system – the microcirculation has been largely overlooked. The microcirculation is implicated in obesity-associated cardiovascular disease (CVD) such as coronary artery disease.[3, 4] For example, in people with obesity, global microvascular dysfunction is a common pathway which predisposes to the development of coronary microvascular angina.[3] The microcirculation can be assessed by non-invasive retinal imaging and quantification of microvascular parameters, most frequently retinal arteriolar and venular caliber.[5] Understanding the relationship between obesity and the retinal microvasculature across the life course could be informative, as variations in retinal vascular caliber are thought to mirror pathologic processes occurring in the systemic and coronary microcirculation.[5, 6]

Most studies examining the association of body mass index (BMI) with the retinal microvasculature have used cross-sectional designs and mainly focused on adults.[7] In children, the only longitudinal study (the Singapore Cohort Study of Risk Factors for Myopia, n= 421) showed that one standard deviation (SD) higher BMI (3.03 kg/m^2) at age 7-9 years predicted 0.12SD decreased arteriolar caliber ($p = 0.01$) and 0.13SD increased venular caliber ($p < 0.01$) five years later. Inversely, arteriolar and venular caliber at baseline weakly predicted BMI at follow-up.[8] These results suggest that BMI is likely to be on the causal pathway, predicting changes to the retinal microvascular parameters, rather than the other way around.

The link between early or mid-life BMI with future retinal microvascular pathology has not been clearly elucidated. Adverse BMI trajectories from adolescence to young adulthood are associated with an unfavourable cardiovascular profile (eg, high blood pressure, insulin resistance),[9] but whether these findings extend to retinal microvascular parameters is unclear. An understanding of these relationships may be enlightening because distinct BMI trajectories impact differentially on the risk of cardio-metabolic disease later in life.[9, 10] For example, a 23-year longitudinal study found that compared to a normal BMI trajectory, a high-increasing childhood BMI trajectory was associated with poorer indicators of adult subclinical CVD.[11] In addition, most studies have focused on BMI, neglecting the possible impact of fat distributions on retinal microvasculature.[12-14] One of the few studies to examine fat mass and distribution is the Generation R study. Using dual-energy X-ray absorptiometry (DEXA), higher total body and abdominal fat mass in 4145 6-year-olds were associated with worse arteriolar but not venular caliber.[13] However, the cumulative effects of fat distribution patterns remain unclear since evidence is limited to cross-sectional studies. Waist girth (rather than direct body composition measurement) remains a common proxy for central adiposity, with waist-height ratio (WHtR) considered more predictive of CVD than BMI in adults.[15] Furthermore, if retinal vascular caliber changes reflect cumulative life course responses to systemic risk factors,[16] and if adiposity tracks strongly through life, then associations should be larger in adults than in children. However, this is yet to be investigated. If there is a gradient in the risk with age, then this adds further weight to the importance of early obesity prevention.

The Child Health CheckPoint study nested within the Longitudinal Study of Australian Children (LSAC) provided an opportunity to examine these issues. In two generations – 11-12 year-olds and mid-life adults (their parents) – we examined whether retinal vascular caliber is predicted by 1) BMI and (in children only) WHtR at multiple time points and 2) BMI and WHtR trajectories, all spanning the preceding decade.

MATERIALS AND METHODS

1. Study design and participants

Details of the LSAC design and recruitment are outlined elsewhere.[17, 18] Briefly, in 2004, LSAC recruited a nationally-representative birth cohort of 5107 infants (aged 0-1 year) and their parents using a two-stage clustered design and has since followed the children and their families biennially. The response rate to the initial invitation in 2004 was 57.2%, of which 73.7% (n=3764) were retained to wave six in 2014 (ie, when children were aged 10–11 years).

The Child Health CheckPoint (CheckPoint) study, LSAC's physical health and biomarkers module, was conducted between LSAC wave six (2014) and seven (2016).[19] At the wave six visit, interviewers invited all contactable families (n=3513) to provide consent for their contact details to be shared with the CheckPoint team. In total, 1874 children (53.3%) aged 11-12 years took part in CheckPoint's cross-sectional biophysical assessment with one attending parent (detailed methods[20] and procedures[21] are published elsewhere).

Data collection was approved by the Australian Institute of Family Studies Ethics Committee (14-26) and the Royal Children's Hospital Melbourne Human Research Ethics Committee (33225D). Parents provided written informed consent for themselves and their children at each LSAC wave and the CheckPoint.

2. Procedures

Trained LSAC interviewers visited each family at home every two years from waves one to six, during which they collected the anthropometric markers. Information from all waves was used.

The CheckPoint team booked an appointment for interested families from the same cohort between February 2015 and March 2016. The CheckPoint was a special one-off physical health assessment offered to the 11-12 year-olds children and one of their parents. Most families attended a 3.5-hour appointment comprising multiple measurement stations at CheckPoint's main assessment centers, which took place in the seven largest cities (mainly state capitals) around Australia. A small number of families (n = 518) who attended mini-assessment centers in smaller regional cities (2.5-hour appointments) or received a home visit (1.5 hours) were not included in this study, because the large and delicate equipment for retinal photography could not be readily transported to these centers.

3. Measures

3.1 Exposures from LSAC

In children, height (to the nearest 0.1 cm) and weight (to the nearest 0.1 kg) were measured in light clothing and without shoes or socks. Two height measurements were taken, and if these measurements differed by 0.5 cm or more, a third measurement was taken; the average of the three measures was used. BMI was calculated as weight (kg)/height (m^2), and then converted to age- and gender-specific z-scores using the US Centers for Disease Control growth reference charts.[22] Waist circumference (cm) was measured horizontally around the navel by lifting the shirt or jumper and lowering the belt or waistband in children. Mean of two waist measurements were used; if there was more than 0.5 cm difference on the first two, mean of three was used. WHtR was calculated as waist (cm)/height (cm). In all waves, parents' height and weight were self-reported and BMI, but not WHtR, was calculated.

3.2 Outcomes from CheckPoint

During CheckPoint's 3.5-hour visit, each child and parent took part separately in a 15 min retinal photograph assessment. Two optic disc centered digital photographs from each eye were taken by a fundus camera (EOS 60D SLR).

Right eye images were selected as the first choice for scoring. When right eye images were deemed ungradable, left-eye images were used given the high correlation that has previously been reported between the two.[23] Details of retinal image grading are described elsewhere.[24] Briefly, four experienced graders scored each of the images using the Integrative Vessel Analysis (IVAN, University of Wisconsin, Wisconsin, USA) software program by masking the participant characteristics. **Figure 1** shows the grading platform of IVAN. Retinal vessels were identified by the software as arterioles or venules from a specific area (one-half to one disc diameter from the margin of the optic disc). A segment of each vessel within this area was selected by the grader for measurement. Diameters of all the selected segments were measured by the IVAN software. For each participant, summary estimates of the average retinal vascular caliber were calculated by the software according to the Big-Six (revised Knudston-Parr) formula,[25] which combines measurements of the six largest arterioles or venules. Inter-grader reliability correlation coefficients were (r) = 0.79 for retinal arteriolar and r = 0.92 for venular caliber. Intra-grader reliability ranged from r = 0.90 to 0.99 for retinal arteriolar and r = 0.92 to 0.98 for venular caliber.

3.3 Covariates

Age, sex and family socioeconomic position (SEP) were selected as *a priori* potential confounders as they have been shown to associate with both BMI and retinal vascular caliber.[26] Age at CheckPoint was calculated to nearest week using date of birth, either imported from Medicare Australia's database at the time of LSAC enrolment (child) or self-reported (parent), and date of assessment. Children's sex was from LSAC record which was originally exported from the Medicare Australia database. Parents self-reported their sex in the CheckPoint questionnaire. SEP components were measured by questionnaires at LSAC wave six, which summarized parent-reported combined household income, current or most recent occupation of each parent and highest achieved educational qualification of each parent.[27] Each component of the score was scaled and an unweighted average was calculated over three values in a single-parent household, or over five values in a dual-parent household. The unweighted average variable at LSAC was then standardized within the wave to have a mean 0, and SD of 1, with higher scores indiating better SEP.

4. Statistical analysis

To visualize our findings, we internally constructed standardized scores ([observed value - mean]/SD) for retinal vascular caliber, BMI for adults and WHtR for children, and used the existing BMI z-scores for children. Thus, regression coefficients represent the standardized mean difference (SMD) for a one SD higher score in the exposure (or 1 unit higher BMI z-score). Multivariable linear regression models were performed for both aims with estimates adjusted for age, sex and SEP. We did not adjust for glucose, lipids or blood pressure since these would most likely reflect effect modification rather than confounding of any relationship between BMI/WHtR and retinal vascular caliber. All analyses were performed in Stata 14.0 (StataCorp LP, TX, USA), with children and adults considered separately.

Aim 1: Linear regression models were performed to assess whether BMI/WHtR at each of the preceding time points predicted retinal arteriolar and/or venular caliber at the CheckPoint assessment.

Aim 2: We identified BMI z-score/WHtR trajectories in children based on measures taken during LSAC waves two to six and the BMI trajectories for adults based on self-reported data gathered in LSAC waves one to six. The ‘traj’ plug-in from Stata 14.0 was used for the group-based trajectory modeling.[28] Methods of how we generated the trajectories have been published by our research team.[29] Briefly, BMI or WHtR scores were modeled with censored normal distribution, which is designed for the repeatedly measured continuous variables. For trajectory modeling, we included participants who had a BMI or WHtR value for at least four of the six waves. In order to extract the most meaningful and distinct trajectories, Bayesian information criterion values, average posterior probabilities and the proportion of the sample in each trajectory were taken into account (eTable 1 and 2).[30]

Based on these criteria, trajectories were selected and named from visual inspection. A five-trajectory solution was selected for child BMI z-score, with 6.2% in the 'low', 31.3% 'average', 45.6% 'always high', 12.1% 'always very high' and 4.8% 'low to high' trajectories (**Figure 2a**). For adults, we selected a four-trajectory solution (51.0% 'normal', 32.8% 'overweight', 12.8% 'obese', and 3.4% 'severely obese'; **Figure 2b**). Adult BMI trajectories were quite flat, but one child trajectory ('low to high') was characterized by a steeply rising BMI z-score over time, while the 'average' and 'high' trajectories appeared to fall slightly. For children's WHtR trajectories, a three-trajectory solution was selected and, in line with the clinical cut-point of 0.5,[12] named as 'normal' (72.3%), 'high normal' (23.9%) and 'always very high' (3.8%); **Figure 2c)**.

Multivariable linear regression models were performed to examine whether longitudinal BMI/WHtR trajectories predicted retinal vascular caliber in children and adults.

Sensitivity analysis: Previous studies reported that lower birth weight predicted poor retinal vascular caliber,[31, 32] so we conducted a sensitivity analysis further adjusting birth weight (kg) for Aim 1 in children.

RESULTS

Sample characteristics

Figure 3 shows the study flow from wave one of LSAC onward. Of the 1874 CheckPoint families, 1288 11-12 year-olds and 1264 adults (mean age 44 years (SD 5.1)) had retinal vascular caliber data available (**Table 1**). Around half of children (50.9%) were girls, while most adults (86.6%) were mothers. Families included in our analysis were slightly more advantaged (mean SEP 0.3, SD 1.0) than all families in LSAC wave six (mean 0.0, SD 1.0).

Aim 1: BMI and (children only) WHtR across the preceding multiple time points predict retinal vascular caliber

In children, higher BMI and WHtR from 4-5 years modestly predicted adverse retinal arteriolar, but not venular, caliber at age 11-12 years, and the associations strengthened with age (**Table 2**). At 4-5 years of age, per unit higher BMI z-score was associated with slight narrowing of arteriolar caliber (SMD -0.05, 95% CI -0.10 to 0.01). By age 10-11 years, the effect size of BMI on arteriolar caliber had doubled (SMD -0.10, 95% CI -0.16 to -0.05). In adults, the magnitude of associations was similar across the six-time points (SMD -0.11 to -0.13). In children, the association of WHtR with arteriolar caliber changed little with age (at 10-11 years SMD -0.08, 95% CI -0.14 to -0.01).

In comparison, the association between BMI and venular caliber was weak and did not vary with age in children or adults. However, an association between WHtR and venular caliber in children emerged from 8-9 years onward. Overall across each wave, the explanatory power of adult BMI for both arteriolar and venular caliber was larger than in children (Partial R^2 children from 2-3 to 10-11 years 0.9-1.8%, adults from mean age 33 to 44 years 2.0-2.6%).

Aim 2: Decade-long BMI and (children only) WHtR trajectories predict retinal vascular caliber

In children, less favorable BMI trajectories were associated with narrower arteriolar caliber, with similar effects seen for higher WHtR trajectories (*p* for trend <0.05, **Figure 4**). Compared to children following the 'average' BMI trajectory, those following the 'always very high' trajectory had arteriolar caliber that was -0.25 SMD (95% CI -0.44 to -0.07) narrower. Compared to children following 'normal' WHtR trajectory, those following a 'high normal' and 'always very high' trajectory had narrower arteriolar calibers of 0.14 (95% CI -0.27 to -0.01) and 0.25 (95% CI -0.54 to 0.03) SMD respectively. Similarly, adults following an 'overweight', 'obese' or 'severely obese' trajectory had narrower arteriolar caliber compared to those following the 'normal' BMI trajectory, with the strongest effect seen for those who followed the 'severely obese' trajectory (SMD -0.42, 95% CI -0.73 to -0.10).

In contrast, we found little evidence for a gradient of higher BMI/WHtR trajectories with wider (ie poorer) venular caliber in either children or adults (*p* for trend >0.05). Nonetheless, point estimates for children were in the expected direction (**Figure 4**), with trajectories characterized by 'low to high', 'always very high' BMI and 'always very high' WHtR showing venular caliber that was wider by 0.13 (95% CI -0.13 to 0.40), 0.19 (95% CI 0.00 to 0.37) and 0.26 (95% CI -0.03 to 0.55) SMD respectively compared to 'normal' trajectories.

Sensitivity analysis

When Aim 1 analyses were repeated including birth weight as a confounder, associations were largely unchanged (eTable 3).

DISCUSSION

1. Principal findings

We found that higher BMI and WHtR from 4-5 years of age onwards predicted adverse retinal arteriolar caliber by age 11-12 years. Similar associations were seen in mid-life adults' BMI but with higher explanatory power. There was less convincing evidence that BMI over the preceding time points was associated with venular caliber in either children or adults, but there was evidence that WHtR from 8-9 years was associated with venular caliber in children. We observed a gradient of suboptimal decade-long higher BMI and (child only) WHtR trajectories predicting poorer retinal arteriolar, but not venular, caliber in children; again, we saw larger effects in adults. Only the least favorable BMI and WHtR trajectories were associated with adverse venular caliber in children.

2. Strengths and limitations

Strengths of our study include the large, population-based, cross-generational cohort with BMI and (children only) WHtR measured biennially across the preceding decade. The outcome measurements for children and adults were taken at the same time, with the same equipment, using the same protocols. Furthermore, the average posterior probability value for each trajectory was 0.82-0.97 for each group (see eTable 2), well above the recommended minimum value of 0.70,[33] indicating the models had good assignment accuracy.

Some limitations also warrant consideration. First, parent height and weight were self-reported and limited data were available from adult males (n=169), as mothers typically accompanied their children to the CheckPoint assessment center. Nevertheless, evidence suggests self-reported BMI in longitudinal studies is acceptable for epidemiologic research and the value correlates very highly with actual measurements in adults.[34] However, our estimates may lack precision in men given that our adult sample comprised 87% mothers. Second, retinal microvascular parameters were only collected at one-time point, limiting our ability to precisely pinpoint when the association first emerges. Future studies with repeated measures of both BMI and retinal vascular caliber are needed to establish exactly when these associations emerge. We recognize that both selection bias and attrition limit the population representativeness of our cohort. However, the sample covered a wide social and geographic range which means that the risk factor associations are likely to be generalizable.[35] Lastly, WHtR is a proxy measure for central body fat. Replications are warranted in studies with longitudinal fat mass measures.

3. Interpretation in light of other studies

We showed that the adverse microvascular variation at 11-12 years of age could be predicted from BMI as early as 4-5 years of age. This finding is consistent with the literature suggesting that the association between BMI and adverse retinal vascular variations may commence early in life. The youngest population-based sample among which this relationship has previously been examined were children aged 55.5 months (SD 10.3) taking part in the cross-sectional Sydney Pediatric Eye Disease Study.[36] In this small community sample (n = 379), each unit higher BMI was cross-sectionally associated with 1.06 µm narrower arteriolar caliber ($p = 0.01$) and 1.12 µm wider venular caliber ($p = 0.02$).[36] Taken together with our findings, early BMI from 4-5 years may not only associate with cross-sectional, but also predict future retinal microvascular parameters. Our findings are also consistent with the Singapore Cohort Study of Risk Factors for Myopia, which included children of the same age with similar size of associations for arteriolar caliber, but not venular caliber.[8]

In addition, by using two generations of participants with identical outcome measures, we can speculate that increasing adiposity may have cumulative effects on retinal microvascular parameters from childhood to mid-adulthood. Although the effects were similar in children and adults, the explanatory power (ie R^2) was higher in adults than in children. Furthermore, we found that consistently suboptimal decade-long BMI and/or WHtR trajectories were associated with adverse retinal vascular caliber. This supports our hypothesis that high BMI/WHtR has cumulative effects on vascular caliber. The only other study that has examined the effect of BMI trajectories on retinal caliber was from our research team.[37] In a small cohort (n=187), we did not see the association of children's BMI trajectories (10 time points from 2 weeks to 14 years) and retinal vascular caliber.[37] However, the small size of the study and the fact that 90% of children were of normal-weight BMI may have limited the power to detect small associations.[37]

Retinal arteriolar and venular caliber had different patterns of association with BMI and WHtR. The association of BMI with narrower arteriolar caliber in children and adults is in line with previous studies and a recent meta-analysis.[14, 38] We found little evidence of associations with retinal venular caliber for adults but did see some evidence in children. For instance, we found that WHtR, an index of central fat distribution, was related to venular caliber in children from 8-9 years; the 'low to high', 'always very high' BMI and WHtR trajectories among children were associated with wider venular caliber. These observations indicate venular associations may appear later and be more closely related to central adiposity. Previous research has demonstrated mixed evidence regarding the relationship between BMI and WHtR with retinal venular caliber among children and adults.[13, 39]

4. Implications

Mounting evidence suggests obesity has adverse effects on both preclinical and clinical cardiovascular health.[1, 2] Our study suggests that greater BMI and WHtR predict adverse retinal microvascular parameters, a recognized early marker of later CVD.[38] Adverse microvascular parameters are predicted by BMI from age four years onwards and strengthen across the life course. Even though effects were relatively modest, at the population level they may have clinical implications. Data from 16 community-based studies estimated that the natural change in arteriolar caliber, without considering BMI, was estimated to be -0.02 µm per decade.[24] Taken together with our current findings, high or rising BMI appears to accelerate adverse changes in microvascular parameters. For example, if a child's BMI z-score increased by two SD units (ie, moved from normal into the obese range) at age 6-7 years, we estimate that his or her arteriolar caliber would be 1.7 µm narrower than the average. The Cardiovascular Risk in Young Finns Study reported that the improved ideal cardiovascular health from childhood to adulthood was significantly associated with wider arteriolar caliber in adulthood.[40] Thus, our estimated the effect of BMI on arteriolar calibers may translate into substantial effects on future cardiovascular health. Our findings emphasize the importance of tackling obesity from early childhood, where its adverse effects are more likely to be reversible.[41, 42]

How increasing levels of adiposity may contribute to microvascular variations is still unclear. Some studies suggest that variation of arteriolar and venular caliber may be determined by different risk factors.[43-45] For example, elevated blood pressure has been found to have stronger associations with arteriolar narrowing,[26, 46] while inflammation markers have been more consistently associated with venular dilatation.[13] Further research is needed to elucidate the potential mechanisms by which adiposity adversely affects microvascular parameters. Prompt intervention in these pathways may prevent future microvascular disease.

5. Conclusion

Higher BMI and WHtR from 4-5 years, and less favorable decade-long trajectories, consistently predicted poorer retinal arteriolar caliber at 11-12 years. Similar results were observed in mid-life adults with stronger effects. There was little evidence of relationships with venular caliber, which may appear later and have closer relationships with central adiposity. Our findings suggest that greater adiposity may be a driver of poor microvascular parameters across the life course, but the underlying mechanisms of this relationship warrant further investigation to guide interventions.

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Figure legends

Figure 1. Retinal images of a child with normal weight and a child with obesity on the grading platform of IVAN software

Blue (venule) and red (arteriole) marks are identified via IVAN software. For each participant, a segment of each vessel within the specific area (one-half to one disc diameter from the margin of the optic disc) was selected by the grader for measurement; the IVAN software then estimates summary values for the average retinal vascular caliber according to the Big-Six (revised Knudston-Parr) formula, which combines measurements of the six largest arterioles or venules.

Figure 2. Trajectories of body mass index and waist-to-height ratio in children and adults

Figure 3. Flowchart of Longitudinal Study of Australian Children and Child Health CheckPoint

Figure 4. Associations of decade-long body mass index and waist-to-height ratio trajectories with retinal vascular caliber

Symbols in circles, diamonds and triangles represent adjusted standardized mean difference of outcomes according to body mass index z-score and waist-to-height ratio trajectories in children and body mass index trajectories in adults respectively. Horizontal bars indicate 95% confidence intervals of standardized mean difference; dash and solid bars represent results from children and adults respectively. Ref: reference group

Table 1. Characteristics of analytic samples (ie participants with retinal images in CheckPoint)

Characteristics	Children Means (SD [a]) or %	Adults Means (SD [a]) or %
Demographics		
Age (years)	11.4 (0.5)	43.8 (5.1)
Gender (% female)	50.9	86.6
Birth weight (kg)	3.45 (0.55)	
Family socioeconomic position (wave 6)	0.3 (0.1)	
Exposures collected in LSAC		
BMI (z-score[b] for children; kg/m^2 for adults)		
Wave 1 (child 0-1y)	-	25.2 (4.8)
Wave 2 (child 2-3ys)	0.51 (1.06)	25.0 (4.7)
Wave 3 (child 4-5ys)	0.51 (1.07)	26.0 (5.0)
Wave 4 (child 6-7ys)	0.33 (0.93)	26.0 (5.3)
Wave 5 (child 8-9ys)	0.27 (1.02)	26.5 (5.8)
Wave 6 (child 10-11ys)	0.24 (0.97)	26.9 (6.0)
Waist-to-height ratio		
Wave 1 (child 0-1y)	-	
Wave 2 (child 2-3ys)	0.53 (0.04)	
Wave 3 (child 4-5ys)	0.49 (0.03)	
Wave 4 (child 6-7ys)	0.46 (0.04)	
Wave 5 (child 8-9ys)	0.45 (0.05)	
Wave 6 (child 10-11ys)	0.45 (0.05)	
Outcomes collected in CheckPoint		
Retinal arteriolar caliber (µm)	159.1 (11.9)	151.0 (14.0)
Retinal venular caliber (µm)	230.7 (16.6)	218.9 (18.5)

a. Standard deviation; b. Body mass index was transformed to z-score with Centers for Disease Control and Prevention (US)-growth charts. CheckPoint, Longitudinal Study of Australian Children (LSAC)'s biophysical assessment module.

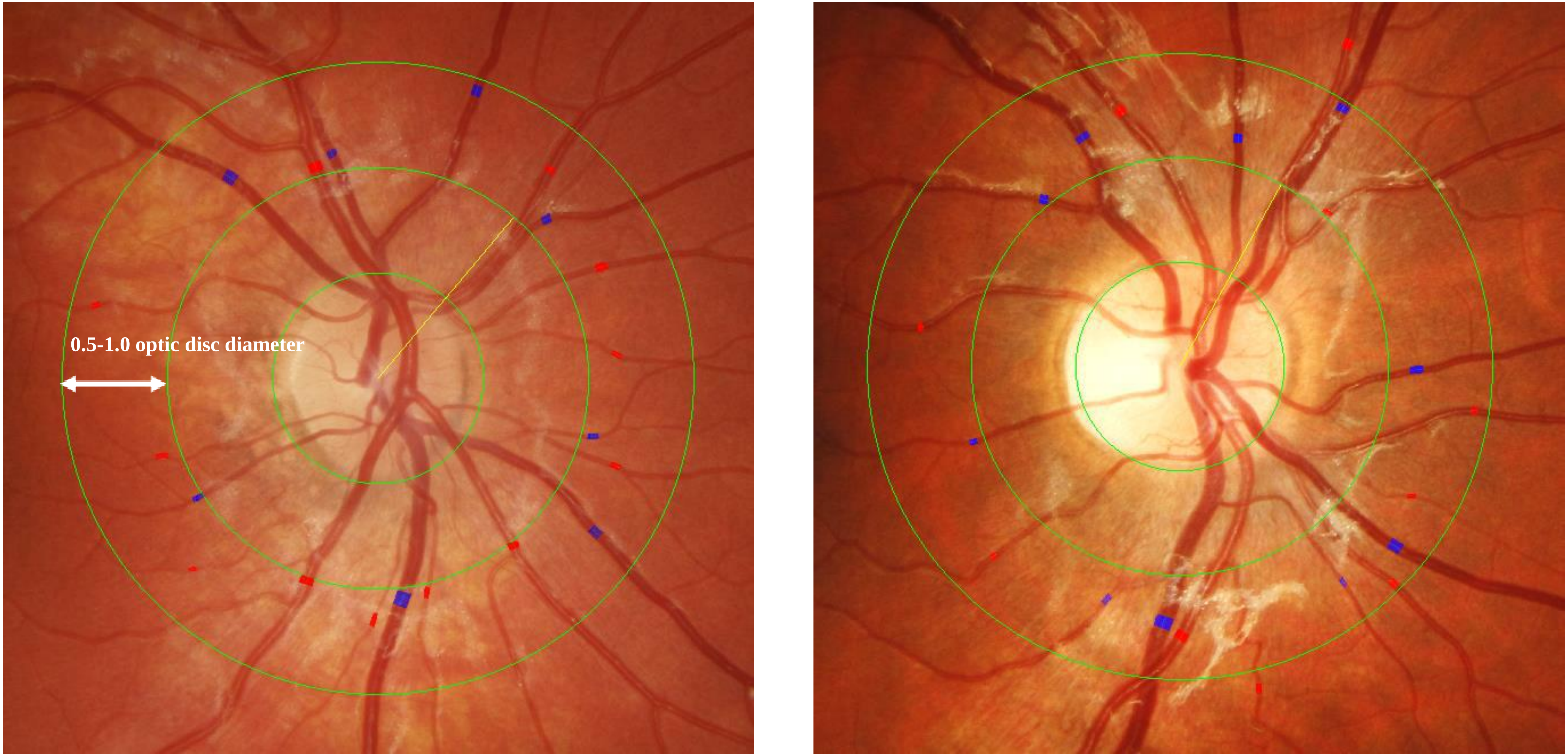
Table 2. Associations of body mass index and waist-to-height ratio at multiple time points over the past decade with retinal vascular caliber in children and adults; model estimates adjusting for age, sex and socioeconomic position

	Children age from 2-3 to 11-12 years				**Adults mean age from 33 to 44 years**			
	Retinal arteriolar caliber		**Retinal venular caliber**		**Retinal arteriolar caliber**		**Retinal venular caliber**	
Adiposity marker by study wave	Standardized mean difference (95% CI)	*p*	Standardized mean difference (95% CI)	*p*	Standardized mean difference (95% CI)	*p*	Standardized mean difference (95% CI)	*p*
Body mass index (z-score[a] for children)								
Wave 1 (child 0-1y)					-0.11 (-0.17, -0.05)	<0.001	-0.01 (-0.07, 0.05)	0.69
Wave 2 (child 2-3ys)	-0.03 (-0.09, 0.02)	0.21	0.02 (-0.03, 0.08)	0.35	-0.12 (-0.18, -0.05)	<0.001	-0.01 (-0.07, 0.05)	0.69
Wave 3 (child 4-5ys)	-0.05 (-0.10, 0.01)	0.07	0.02 (-0.03, 0.07)	0.42	-0.11 (-0.17, -0.04)	0.001	0.03 (-0.03, 0.10)	0.28
Wave 4 (child 6-7ys)	-0.07 (-0.13, -0.02)	0.01	0.03 (-0.03, 0.09)	0.31	-0.13 (-0.19, -0.07)	<0.001	-0.01 (-0.07, 0.05)	0.73
Wave 5 (child 8-9ys)	-0.06 (-0.11, -0.01)	0.03	0.05 (-0.01, 0.10)	0.09	-0.13 (-0.19, -0.07)	<0.001	0.01 (-0.05, 0.07)	0.73
Wave 6 (child 10-11ys)	-0.10 (-0.16, -0.05)	<0.001	0.04 (-0.02, 0.09)	0.22	-0.11 (-0.16, -0.05)	<0.001	0.04 (-0.01, 0.10)	0.14
Waist-to-height ratio								
Wave 1 (child 0-1y)								
Wave 2 (child 2-3ys)	-0.03 (-0.09, 0.03)	0.34	0.02 (-0.03, 0.08)	0.42				
Wave 3 (child 4-5ys)	-0.10 (-0.16, -0.04)	<0.01	0.02 (-0.04, 0.07)	0.60				
Wave 4 (child 6-7ys)	-0.07 (-0.13, -0.01)	0.02	0.03 (-0.03, 0.09)	0.39				
Wave 5 (child 8-9ys)	-0.07 (-0.13, -0.01)	0.02	0.07 (0.01, 0.13)	0.03				
Wave 6 (child 10-11ys)	-0.08 (-0.14, -0.01)	0.02	0.08 (0.02, 0.14)	0.01				

a. Body mass index was transformed to z-score with widely used Centers for Disease Control and Prevention (US)-growth charts. The SDs for retinal arteriolar and venular caliber are 11.92, 16.56 µm for children, 14.01 and 18.53 µm for adults respectively.

Figure 1. Retinal images of a child with normal weight and a child with obesity on the grading platform of IVAN software

Child with normal weight (BMI z-score <85th percentile) | Child with obesity (BMI z-score >95th percentile)



Retinal arteriolar caliber = 163.2 μm
Retinal venular caliber = 202.8 μm

Retinal arteriolar caliber = 155.5 μm
Retinal venular caliber = 232.3 μm

Figure 2. Trajectories of body mass index and waist-to-height ratio in children and adults

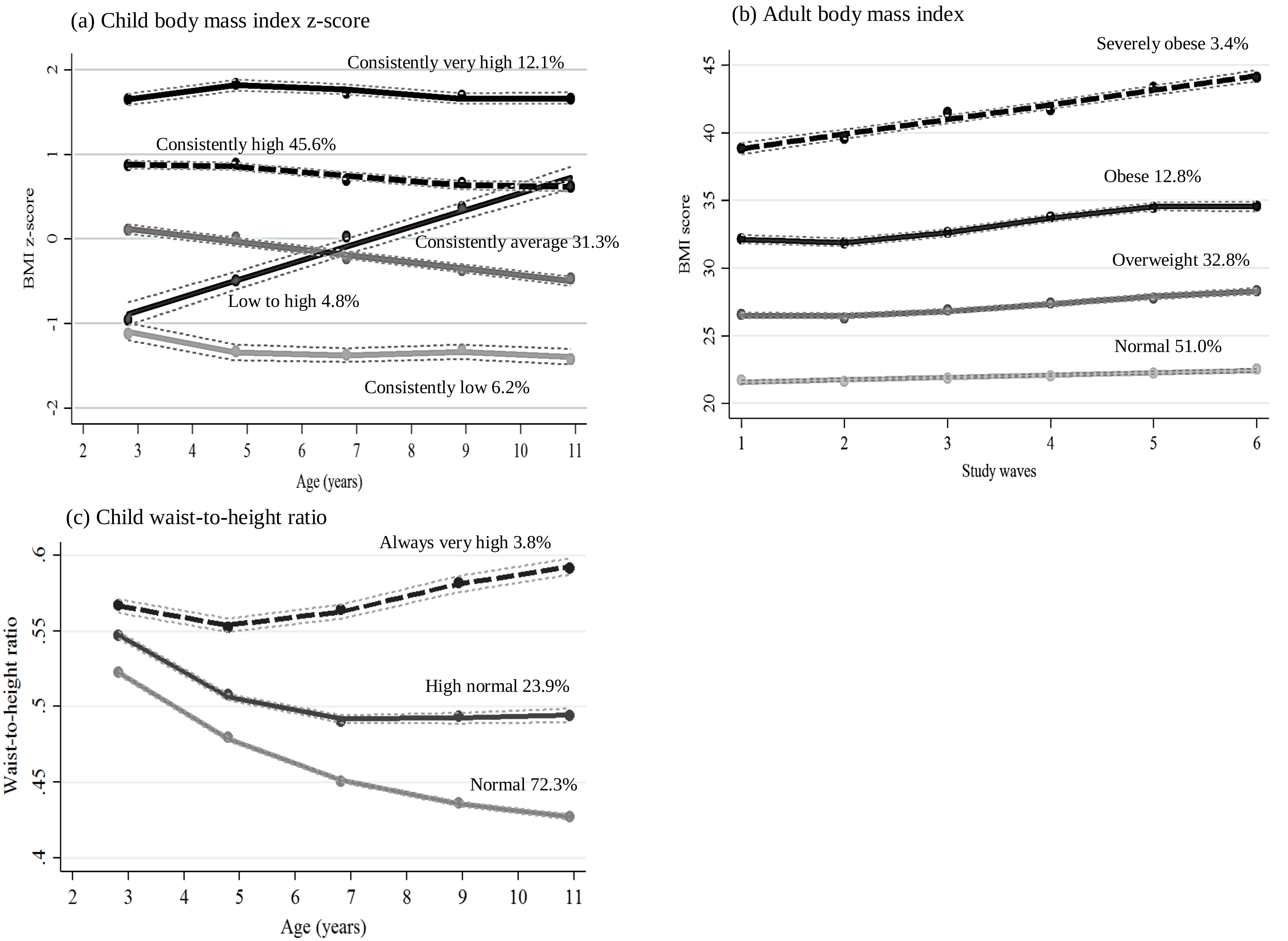
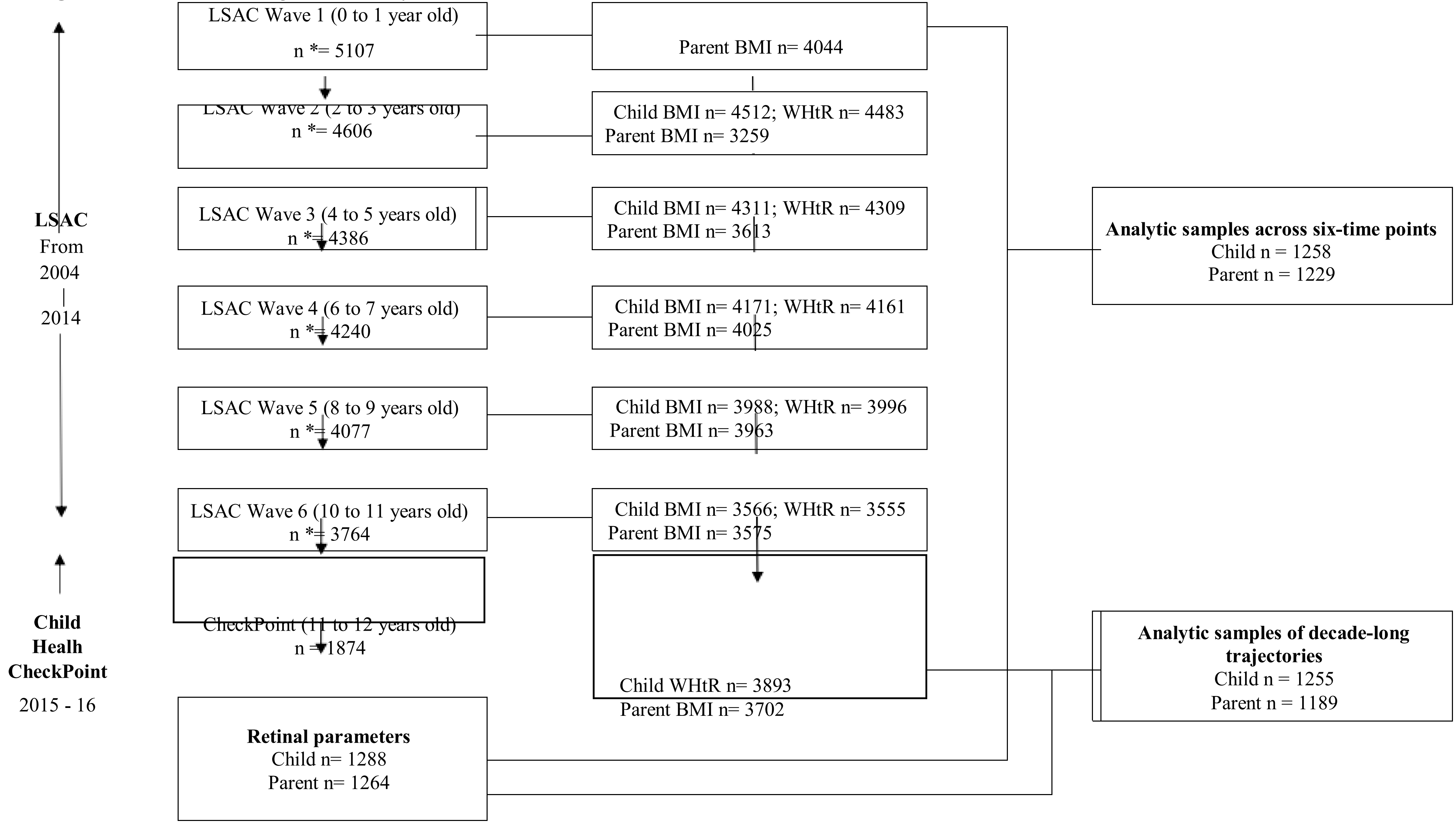


Figure 3. Flowchart of Longitudinal Study of Australian Children and Child Health CheckPoint



LSAC, Longitudinal Study of Australian Children; BMI, body mass index; WHtR, waist-to-height ratio; *number of responses

Figure 4. Associations of decade-long body mass index and waist-to-height ratio trajectories with retinal vascular caliber