Childhood obesity related endothelial dysfunction : an update on pathophysiological mechanisms and diagnostic advancements

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Abstract

Childhood obesity jeopardizes a healthy future for our society's children as it is associated with increased cardiovascular morbidity and mortality later on in life. Endothelial dysfunction, the first step in the development of atherosclerosis, is already present in obese children and may well represent a targetable risk factor. Technological advancements in recent years have facilitated non-invasive measurements of endothelial homeostasis in children. Thereby this topic ultimately starts to get the attention it deserves. In this paper, we aim to summarize the latest insights on endothelial dysfunction in childhood obesity We discuss methodological advancements in peripheral endothelial function measurement and newly identified diagnostic markers of vascular homeostasis. Finally, future challenges and perspectives are set forth on how to efficiently tackle the catastrophic rise in cardiovascular morbidity and mortality that will be inflicted on obese children if they are not treated optimally.

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INTRODUCTION

The onset of atherosclerosis, dysfunction of the arterial endothelium, starts early in life and is greatly accelerated in the setting of childhood obesity. Obesity therefore poses a serious health threat to children, increasing the child's risk of developing clinically overt cardiovascular disease in adult life [1]. Notwithstanding the dramatic increase in the prevalence of childhood obesity worldwide over the past three decades, rates now seem to have reached a plateau in most countries. Currently, about 20% of children and adolescents between 12 and 19 years in the USA are obese [2].

Endothelial dysfunction is the primum movens in the pathogenesis of atherosclerosis, appearing long before clinical symptoms arise, and might qualify as a surrogate endpoint for cardiovascular disease risk. The endothelium's primary role is the tight control of the blood vessel diameter. In response to stimuli for increased blood flow demand, endothelial Nitric Oxide Synthase (eNOS) in endothelial cells is activated and produces Nitric Oxide (NO), which diffuses into the vessel wall to fine-tune vasodilation [3]. Although NO is considered a key regulator of endothelial function, several other factors are involved as well [4,5]. The location of the endothelium close to the blood circulation, however, exposes the endothelial cells to many damaging factors. These factors cause harm to endothelial cells ultimately leading to endothelial dysfunction, commonly defined as "an imbalance between vasodilating and vasoconstricting substances produced by (or acting on) endothelial cells" [6]. At first, testing of the vascular endothelium required in vitro experimentation. In recent years, several approaches were introduced enabling the non-invasive assessment of endothelial function in vivo. These novel developments in the field have encouraged clinical researchers to examine alterations in vascular endothelial function not only in adults, but also in children and adolescents. Endothelial dysfunction was found to be present in the major conduit arteries of obese children and is now referred to as macrovascular endothelial dysfunction [7]. Although endothelial dysfunction is considered a 'systemic disorder', dysfunction of small resistance vessels, also called microvascular endothelial dysfunction, precedes the development of macrovascular endothelial dysfunction [8].

This growing interest in endothelial homeostasis introduced improved and novel laboratory methods for investigating the endothelium at a finer cellular level. Endothelial Micro Particles (EMP) [9] as well

as Endothelial Progenitor Cells (EPC) [10] and Circulating Angiogenic Cells (CAC) [11] emerged as markers of respectively endothelial damage and repair. All of them have been implicated in the process of childhood obesity related endothelial dysfunction [12,13]. Therefore, the combined assessment of these cell-based markers and endothelium vasodilatory function may represent a promising option to optimize the risk stratification and the primary care management of obese children.

In this review, we aim to summarize the latest insights on endothelial dysfunction in obese children. We discuss the methodological advancements in peripheral endothelial function measurement and new biomarkers of vascular homeostasis. Finally, we explore future challenges and perspectives for the treatment of childhood obesity.

NEW DETERMINANTS OF CHILDHOOD OBESITY RELATED ENDOTHELIAL DYSFUNCTION

In obese children multiple cardiovascular risk factors are present, which all negatively affect endothelial function [14]. Endothelial dysfunction summarizes the cumulative burden of these risk facts and might therefore represent an excellent surrogate marker for the diagnosis of early cardiovascular disease (figure 1). Risk factors in obese children include classical cardiovascular risk factors such as hypertension and dyslipidaemia [15] but also newly discovered cytokines and signaling molecules including micro RNA (miRNAs). The impact of each of these factors has been demonstrated in clinical and fundamental studies, and below we discuss the latest data in this field.

Hypertension during adolescence [16] can lead to severe vascular endothelial dysfunction in adult life [17]. In pre-pubertal children, obesity is strongly associated with hypertension. Counterintuitive, however, obese pre-pubertal children demonstrate a better functional capacity of their endothelium than the normal-weight normotensive counterparts [18]. Accordingly, young obese children seem capable of developing an early vascular adaptive response to increased blood flow demands. Radtke et al recently provided evidence in support of this theory. They performed a cold pressure test to measure the change in blood pressure in response to stress in children with no known cardiovascular risk factors [19]. Children with a positive test, and thus at increased risk of hypertension, showed greater endothelial

capacity. The concept that young obese children are able to elicit an adaptive response of their endothelium to stress may also explain why 6 months of exercise training does not improve endothelium-dependent flow mediated dilation of the brachial artery in this population[20], whereas multiple previous studies have demonstrated positive effects of training on endothelial status in (post-) pubertal children [21].

Dyslipidemia is another potent factor that is involved in impaired endothelial function in obese children. Although LDL cholesterol diminishes the NO production of endothelial cells [22], a pro-atherosclerotic lipid profile is less prevalent in obese children than in obese adults. Elevated LDL cholesterol is often seen in obese adults, but is rarely observed in obese children [15]. Therefore, the focus of attention has recently shifted towards HDL cholesterol. HDL cholesterol has been called the "good cholesterol" for a long time as it strongly associates with a reduction in cardiovascular risk in the adult population [23]. Unexpectedly, pharmacological attempts to raise HDL cholesterol levels in adults did not succeed in establishing a risk reduction in major coronary events [24]. HDL, however, is a complex lipoprotein containing more than 1000 lipids and 70 proteins. [25] This multifaceted composition may underlie the diversity of HDL's actions, including its anti-oxidative and anti-inflammatory properties. Hereof, Matsuo et al showed that the function of HDL is impaired in obese children. In particular, HDL in the young obese is less capable of stimulating eNOS activity [26] and thus of endothelial function [27]. Six months of exercise training facilitated a tendency towards the improvement of HDL function, for which the authors speculated that the training was not intensive enough to achieve statistical significance [26].

Recent progresses in the field further indicate that HDL is a major carrier of **miRNAs** [28]. miRNA's are small (20-25 nucleotides) non coding RNA molecules that regulate the expression of protein coding genes and are emerging as new biomarkers and therapeutic targets. Uncovering miRNAs that specifically relate to first signs of endothelial maladaptation may allow an earlier identification of obese children who are at increased cardiovascular risk [28].Moreover, in adults exercise can thrive HDL-carried miRNA's to a more pro-angiogenic profile[29]. Whether the latter is also true for children, remains to be proven.

Both **low cardiorespiratory fitness** [30] and **physical inactivity** [31] are independent predictors of cardiovascular morbidity and mortality. While cardiorespiratory fitness can be objectively assessed by ergo spirometry, physical activity is recorded by using questionnaires and/or accelerometers. [32] In young children (5 to 10 years old), physical activity strongly correlates with endothelial function [33]. Surprisingly, no correlation of physical (in)activity or cardiorespiratory fitness with endothelial function is observed in adolescents (mean age of 14.5 years) [34]. Although adolescents demonstrate high cardiorespiratory fitness in the study of Radtke et al, only 16% of them actually adhered to the recommended 60 minutes of physical activity per day. Therefore, it could be possible that these adolescents did not perform enough physical activity to keep their endothelial function optimal, while cardiorespiratory fitness was preserved. Magnussen et al. identified 15 years and onwards as the age from which differences in physical activity will have an effect on vascular function and structure. Although small increases in physical activity already decelerate progression of intima media thickness (IMT) in young adults, larger efforts will probably be required to ascertain normal endothelial function later on in life [35]. Vice versa, in young recreationally active men (25 ± 2 years), as little as 5 days of diminished physical activity negatively impacted macrovascular endothelial function in the study by Boyle et al. [36]. Furthermore, whereas a high intensity exercise bout increases endothelial function in normal-weight young adults, this response is completely blunted in obese participants [37]. However, the differentiation between cardiorespiratory fitness and physical activity still remains complex and more research will be necessary to investigate their differential effects on childhood obesity related endothelial dysfunction.

Adipocytes secrete a vast array of cytokines called **adipokines** and, as such, adipose tissue meets the criteria of an endocrine organ [38]. In childhood obesity, hypertrophic adipose tissue is invaded by macrophages, resulting in the up-regulation of adipocyte adhesion molecules. This process leads to the diapedesis of monocytes and initiates a vicious circle of adipogenesis and **inflammation** [39]. Several adipokines have a direct effect on endothelial function, including leptin and adiponectin [40,41]. Chemerin, a novel adipokine at the crossroad between inflammation and obesity, may possibly influence the vascular endothelium as well. In obese children, increased circulating levels of chemerin

have been observed, [42] closely correlating with the degree of endothelial dysfunction. Also, addition of chemerin to cultured endothelial cells up regulated the expression of adhesion molecules for white blood cells.

As in adults, **sleep apnea** in obese children is highly prevalent [43] and impairs endothelial function [44]. Kim et al. have recently investigated the link between sleep apnea**,** childhood obesity and inflammation by measuring circulating levels of Pentraxin-3 [45]. Plasma pentraxin-3 levels correlate positively with BMI and with the severity of obstructive sleep apnea syndrome in obese children. In addition, pentraxin-3 correlates with the number of circulating EMP in obese children [46]. More research on Pentraxin-3 as a new biomarker in the setting of childhood obesity could be of interest.

Psychological psychosocial distress is highly prevalent in obese children [47]. In adults, anger and hostility have been associated with cardiovascular morbidity and mortality [48]. In addition, scores for anger, depression and anxiety are negatively correlated with endothelial function in healthy children [49]. Mechanisms, however, are most likely of postnatal origin, since a recent large population study found no correlation between maternal stress during pregnancy and endothelial function in children at the age of 10 to 12 years [50]. It remains to be examined, however, whether psychological traits impose an increase in the cardiovascular risk of obese children.

To conclude, the rapidly increasing prevalence of childhood obesity has clearly awoken the scientific community. Still, to prevent a catastrophic increase in the prevalence of cardiovascular disease we need to step up even more and improve our understanding of the mechanisms underlying childhood obesity related endothelial dysfunction. Novel non-invasive methods for the evaluation of endothelial function can now be easily applied in children and are devoid of the ethical concerns and difficulties associated with invasive imaging modalities, thus setting the scene for thorough clinical and translational studies. In the following paragraphs we discuss these technological advancements in methodology.

TECHNOLOGICAL ADVANCEMENTS IN OBESITY ASSOCIATED ENDOTHELIAL FUNCTION MEASUREMENT

More than 20 years ago, a first non-invasive method, called Flow Mediated Dilation (FMD), was introduced to measure endothelial function [51]. More recently, Peripheral Arterial Tonometry (Endo-PAT) was developed to overcome the user dependent disadvantage of FMD [52]. For Endo-PAT, a pneumatic probe is placed on both index fingers. Then, similar to the FMD method, a sphygmomanometer is insufflated to supra-systolic pressures forcing a transient occlusion of the brachial artery. The shear stress induced dilation of the small resistance vessels will cause pressure differences, which are registered and expressed as pulse wave amplitudes. The software provided with the Endo-PAT system will then automatically calculate the Reactive Hyperaemia Index (RHI) as the ratio of the pulse wave amplitude (PWA) starting 90 seconds after occlusion, for 60 seconds, divided by the baseline PWA.

In recent past, Chen et al. and Radtke et al. demonstrated that the time needed to reach maximal dilation (i.e.; peak response) with the Endo-PAT device is more variable in children and adolescents than in adults [19,53]. The Endo-PAT algorithm might not correctly account for physiological adaptations in childhood and puberty [54,55]. Therefore, if RHI is used in children, the true peak dilation could be missed. To overcome this hurdle, we recently proposed to use peak response instead of the automatically calculated RHI in children [56,57]. Although Endo-PAT was initially set forth as an alternative technique for FMD, a large population based study recently pointed out that micro- (measured with Endo-PAT) and macrovascular (detected with FMD) endothelial dysfunctions can develop independently of each other [58]. Microvascular endothelial dysfunction would precede endothelial dysfunction at the macrovascular level in obese children [59]. To conclude, the analysis of Endo-PAT testing in children requires caution, and it seems more correct to use peak response instead of RHI. Further, the uncoupling of macrovascular from microvascular endothelial dysfunction urges the assessment of both Endo-PAT and FMD measures in obese children. Cellular markers of endothelial damage and repair may add further to our knowledge of the vascular endothelium in childhood obesity. These will be discussed in the following paragraphs.

CELLULAR BIOMARKERS OF ENDOTHELIAL DAMAGE AND REPAIR IN OBESE CHILDREN

The location of the endothelium close to the circulation exposes the endothelial cells to damaging factors such as lipids and inflammatory proteins. Upon this activation or following apoptosis, endothelial cells will shed small (100 nm- 1µm) particles of the cell membrane into the circulation, called **EMP** [60], which can be enumerated using flow cytometry. In the largest study to date, involving 844 adults without a history of cardiovascular disease, EMP counts strongly correlate with cardiovascular and metabolic risk factors, in particular with dyslipidemia [61]. Indeed in obese adults, numbers of EMP are increased and also negatively correlated with macrovascular endothelial function [62]. Likewise, obese children have higher blood EMP than their normal-weight counterparts [63]. Moreover, EMP urged to be important indicators of microvascular endothelial health in children [57]. The relation between EMP and endothelial function, however, might well be bidirectional [64]. Evidence from in vitro by Agouni et al already demonstrated that EMP from adults with the metabolic syndrome have reduced endothelial NO production and directly influence the endothelium dependent vasorelaxation [65]. Limits for blood sampling volumes in children, however, have so far hampered research on EMP functioning in pediatric populations.

Next to EMP as markers of endothelial damage, **EPC** emerged as markers of endothelial repair. The theory that lost endothelial cells are solely replaced by neighboring endothelial cells has now been largely refuted. After endothelial injury, EPC are mobilized from the bone marrow into the circulation under the influence of several chemotactic factors [66]. EPC migrate to and incorporate into sites of damaged endothelium [67]. Obesity is a prominent predictor of low EPC mobilization, and weight-loss is associated with increased circulating EPC and improved macrovascular endothelial function [68]. Remarkable, Jung et al reported an elevated level of circulating EPC in obese children [12]. Methodological issues, raised by the lack of assay standardization and phenotypic definition, could have accounted for this rather unexpected finding. Enumeration of EPC according to recent recommendations [69], shows that the EPC level in obese children is negatively correlated with BMI [57], which is in accordance with data obtained in adults [68]. In addition, obese children have less EPC in

their bloodstream than normal-weight controls [57]. Moreover, in analogy to high EMP, low circulating EPC turns out to be an independent predictor of reduced peak response [57]. Increased EMP and reduced EPC blood counts in obese children support the existence of an imbalance between endothelial damage and repair mechanisms in this population (figure 2).

CAC are the most novel cellular players in vascular regeneration. They contribute to endothelial repair by attracting circulating EPC in the blood and by stimulating their integration into the injured endothelium via the secretion of angiogenic growth factors. Obese adults have reduced and dysfunctional CAC [70]. In obese children, CAC are also functionally defective [71], thereby shifting the balance in these children even further towards higher endothelial damage and reduced repair capacity (figure 2).

Still, in contrast to CAC from obese adults, CAC of obese youngsters are not yet resistant to the pro-angiogenic effects of leptin. The adipokine leptin, mainly known for its role in regulating human energy homeostasis and appetite [72], has several atherogenic, thrombotic and angiogenic actions on cardiovascular homeostasis [73]. In this regard, leptin enhances the migratory activity of CAC in normal-weight adults [74]. In obese adults, leptin resistance of CAC can be defeated by weight loss [75]. Interestingly, the migratory ability of CAC in normal-weight children cannot be improved any further upon stimulation with leptin, indicating that in normal-weight children CAC function is likely at its maximum [71].

To conclude, new cellular markers of endothelial damage and repair may allow researchers to gain a greater insight into the endothelial biology of obese children. In vitro work now suggests that these markers do not merely reflect the status of the endothelium, but also effectively contribute to the progression or reversion of endothelial dysfunction in pathological conditions. Study results relating to young obese children are discussed in the next paragraphs.

NEW INSIGHTS INTO HOW TO REVERSE ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction is traditionally regarded as a reversible process. Weight loss by reducing caloric intake and/or increasing physical activity tackles multiple cardiovascular risk factors associated with endothelial dysfunction in obese children [76]. Besides the indirect effects, weight loss also directly influences the endothelium by increasing the expression and activity of eNOS [77]. In obese children, a combination of diet and exercise training is able to improve macrovascular endothelial function after as little as 6 to 8 weeks [78,79] However, 10-months of supervised diet and exercise is needed to enhance microvascular endothelial function [80]. Microvascular endothelial dysfunction therefore seems much harder to tackle than macrovascular endothelial dysfunction. Its longer presence may well explain the discrepancy in time needed to overcome the endothelial malfunctioning and thus reduce the cardiovascular risk.

Along with the improvement of microvascular endothelial function, a differential recruitment of EPC and EMP is observed in obese children. In our recent work, obese children have a peak in the number of blood EPC after 5 months of diet and exercise, whereas at 10 months there is a significant drop in circulating EMP. At ten months EPC levels returned to baseline. We hypothesize that the need for high EPC numbers was eliminated since endothelial damage was significantly reduced. In a large cohort of primary school children, 1 year of exercise training on every school day led to a significant increase in the number of EPC. However only 13% of these children were obese or overweight, setting limits for comparison.

To our knowledge, the effect of a diet plus exercise treatment on numbers of circulating EMP in obese children hadn't been described before. Interestingly, microparticles, including EMP, may either be viewed as beneficial or detrimental [65] and further information on EMP in obese children would therefore be of interest.

FUTURE CHALLENGES AND PERSPECTIVES

The current clinical guidelines to treat childhood obesity merely focus on weight stabilization (and thus reducing BMI by normal growth) in case of moderate obesity, and weight reduction in case of severe obesity [81]. Children are encouraged to reduce their time spent in sedentary behavior and to increase physical activity up to 60 minutes per day [82]. These recommendations are mainly based on epidemiological studies, in which reduced physical activity was clearly associated with increased incidence of obesity [37]. The major goal of obesity therapy in children, however, should be aimed at reducing the long-term risk of cardiovascular morbidity and mortality. For this purpose, longitudinal prospective clinical studies are needed with patient follow up for cardiovascular events to occur. This kind of research would be extremely costly and time-consuming. The emerging evidence that endothelial function qualifies as a clinically relevant surrogate endpoint may offer a solution to resolve this issue. We believe that thorough evaluation of the endothelial status in large-scale multicenter trials can create a setting enabling the further optimization of training protocols for obese children. One of these training modalities could be aerobic interval training. Preliminary data indicate that this type of training program is superior to other treatment options in reversing endothelial dysfunction in children [83]. Although the participation of children in clinical studies raises several ethical concerns [84], we hope that researchers will continue the fight against childhood obesity.

CONCLUSIONS

In recent years attention has shifted toward mechanisms of childhood obesity related endothelial dysfunction after an initial period where all eyes were on obese adults. Determinants of childhood obesity related endothelial dysfunction, cell based and biochemical, are now increasingly explored. In addition to extensive endothelial activation, dysfunction and damage, obese children have reduced endogenous vascular repair capacity. Endothelial dysfunction in the smaller resistance vessels also seems harder to beat than large vessel endothelial dysfunction. Obviously, much more research is necessary to fully understand childhood obesity related micro- and macrovascular endothelial

dysfunction in all its facets. We acknowledge that this requires a considerable investment, but the socioeconomic burden of obese children not being treated optimally and growing up to obese adults developing associated comorbidities, is even greater. This cost was recently estimated to be none less than $19 000 per person [85]. Eventually, studies should not merely be initiated for scientific reasons, but more importantly, to safeguard the cardiovascular future of our society's obese children.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest

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FIGURE LEGENDS

Figure 1. Determinants of obesity related endothelial dysfunction in children.

On the left, cardiovascular risk factors known to affect endothelial function in obese children are summarized. On the right, non-invasive techniques to assess macrovascular (FMD) and microvascular endothelial dysfunction (Endo-PAT) are shown.

Figure 2. Imbalance between endothelial damage and repair.

The imbalance between endothelial damage (higher counts of circulating EMP) and endothelial repair (lower numbers of EPC and impaired function of CAC) in childhood obesity related endothelial dysfunction is displayed, while the balanced situation in normal-weight children is depicted as well.