Self-perceived Psychological Health and Vascular Changes in Childhood

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"The greatest glory in living lies not in never falling, but rising every time we fall."

Nelson Mandela

A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarises the accompanying papers. These papers have already been published or are in manuscript at various stages (in press, submitted or in manuscript)

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Abstract

There is strong evidence that cardiovascular disease (CVD) has its origin in childhood, and that childhood cardiovascular risk factors and various other forms of adversity track into adulthood. Poorer psychological health and psychosocial factors are associated with CVD in adults, and this association is also likely to have its origins in childhood. There are no well-established validated questionnaires specifically designed to measure stress in childhood and adolescence. Currently available methods to study early atherosclerotic changes in vascular wall layers are restricted to relatively crude measurements because of low resolution. Measuring the various vessel wall layers separately will facilitate the study of early atherosclerotic changes.

The overall aim of this thesis was to develop non-invasive techniques which make it possible to detect early changes in vascular wall structures in a healthy, young population and to study associations of vascular wall structure and function with self-perceived psychological health. Our hypothesis was that psychological health, particularly "stress", is associated with endocrine measures of stress system activation and with endothelial function, and that sex differences in vascular wall function and structure already exist among the young.

A self-assessment questionnaire designed to measure perceived stress in children (SiC) was constructed. Cronbach's α for the entire SiC questionnaire was 0.86, and higher stress scores were associated with higher morning saliva cortisol levels in girls. The recently introduced Beck Youth Inventories (BYI) of Emotional and Social Impairment were also associated with saliva cortisol levels in girls.

The new very high resolution ultrasound system (55 MHz, Visualsonics) was validated *in vitro* and in humans. The resolution of ~25 μm made it possible to study the intima separately from the media. Greater intima thickness (IT) in the radial artery was seen in boys compared with girls (0.057 ±0.010 vs. 0.054 ±0.008, p=0.007). IT also increased with age (10-17 vs 60-90 years, 0.049±0.008 to 0.081±0.019 mm), and was thicker in peripheral artery disease patients compared with healthy controls (0.089±0.017 vs. 0.074±0.011 mm; P=0.05) and in the dorsal pedal artery (0.074±0.030 mm) compared with the radial artery IT (0.064±0.019 mm; P=0.007).

A total of 248 children (age 14.5±1.0 years, 136 girls, 112 boys) underwent reactive hyperemia peripheral arterial tonometry (RH-PAT) testing, a measure of endothelial function predictive of cardiovascular disease in adults. Information on self-assessed psychological health was also collected for these subjects. No sex differences were observed for the RH-PAT score (1.82±0.55). Girls had higher scores for depression, anger and anxiety, and they showed statistically significant associations between lower RH-PAT values and higher scores for anger, depression and anxiety. Among boys, disruptive behaviour was associated with higher RH-PAT scores indicating better endothelial function.

In conclusion, identifying early changes in both vascular function and psychological health in childhood demands high quality sensitive methods. Self–perceived psychological health was associated with endothelial function; and there were sex differences in structural vascular changes. These findings in childhood underline the importance of adopting a broad perspective on childhood and adolescence health to reduce adult CVD risk.

Key words: CVD, intima thickness, endothelial function, RH-PAT, stress, childhood, cortisol.

Self-perceived Psychological Health and Vascular Changes in Childhood

Overall aim:

To study the relation between self-perceived psychological health measured by self assessment questionnaires and early changes in vascular function, structure and biochemical measures.

The thesis will be based on the following papers, which will be referred to in the text by their roman numerals. In addition, some previously unpublished data will be included and discussed.

I. Osika W, Friberg P, Währborg P. A new short self-rating questionnaire to assess stress in children. *International Journal of Behavioral Medicine*. In press.

II. Osika W, Dangardt F, Grönros J, Lundstam U, Myredal A, Johansson M, Volkmann R, Gustavsson T, Gan LM, Friberg P. Increasing peripheral artery intima thickness from childhood to seniority. *Arterioscler Thromb Vasc Biol*. 2007 Mar;27(3):671-6.

III. Osika W, Dangardt F, Montgomery SM, Volkmann R, Gan LM, Friberg P. Gender differences in peripheral artery intima, media and intima media thickness in childhood and adolescence. Manuscript.

IV. Osika W, Montgomery SM, Dangardt F, Währborg P, Volkmann R, Tideman E, Friberg P. Anger, Depression and Anxiety is associated with endothelial function in childhood and adolescence. Manuscript.

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Acknowledgements

References

Papers I –IV

List of abbreviations

CVD	Cardiovascular disease	CHD	Coronary Heart Disease
DBP	Diastolic blood pressure	PWV	Pulse wave velocity
IMT	Intima media thickness	FMD	Flow mediated dilation
IT	Intima thickness	PE	Parental education
MT	Media thickness	SBP	Systolic blood pressure
RH-PAT	Reactive hyperemia – peripheral artery tonometry	SC	Saliva cortisol
BMI-z	Body mass index (kg/m^2) standardised score		

1. Introductory remarks

The WHO study of Global Burden of Disease showed that ischemic heart disease is the leading cause of death in low, middle and high income countries, while unipolar depression occupies third place in high income countries and seventh place in low income countries as the cause of disease burden (disability adjusted life years). This underlines the growing importance for public health of non-communicable diseases such as cardiovascular disease (CVD) in most low-and-middle-income countries (Lopez 2006). By the year 2020, it is estimated that the main causes of disability throughout the world will largely be depression and heart disease (Murray 1997).

Risk factors for CVD can be identified as early as in childhood, and they are predictive of future cardiovascular risk. A large number of children and adolescents regularly display a variety of symptoms such as headache, stomach ache and irritability. These symptoms, as well as self-harm, are more prevalent in girls from early adolescence and could, perhaps, be described as a form of "stress". There is however no validated test to measure stress in school children. In adults, there is an association between psychological factors such as mental stress, depression, anger and anxiety, with endothelial dysfunction and CVD. The psychosocial influence can be extremely strong; Wittstein (2005) showed that sudden emotional stress caused severe cardiac dysfunction due to very high levels of adrenaline and noradrenalin, as a consequence of high sympathetic activity.

Whether psychosocial/emotional factors are associated with cardiovascular and metabolic perturbations already in a mild form in childhood is not known. Given that present techniques only allow more crude estimations of vascular wall thickening, development of methods for detection of earlier changes is crucial.

The present study attempts to measure stress and psychological health in a population of schoolchildren, to explore and to measure very early structural and functional vascular changes using new techniques, and to identify possible associations between psychological health and vascular characteristics in the young (Fig 1).

One of the more robust factors in explaining differences in morbidity and mortality is gender. In contrast to the term "sex," "gender" is a multidimensional construct including biological/genetic, psychological, and social differences between men and women. Although gender is based on biology, and biological factors in men and women may affect behavior and vulnerability differently, these factors do not influence the entire scope of gender-related

behavior, emotions, and attitudes. Beyond genetic and biological differences, gender refers to the socially constructed roles for men and women, implicating different social norms and expectations. These define which emotions, behaviours, and attitudes are typical and desirable for males and females (Möller-Leimkühler 2007). For consistency I have chosen to use the term sex.
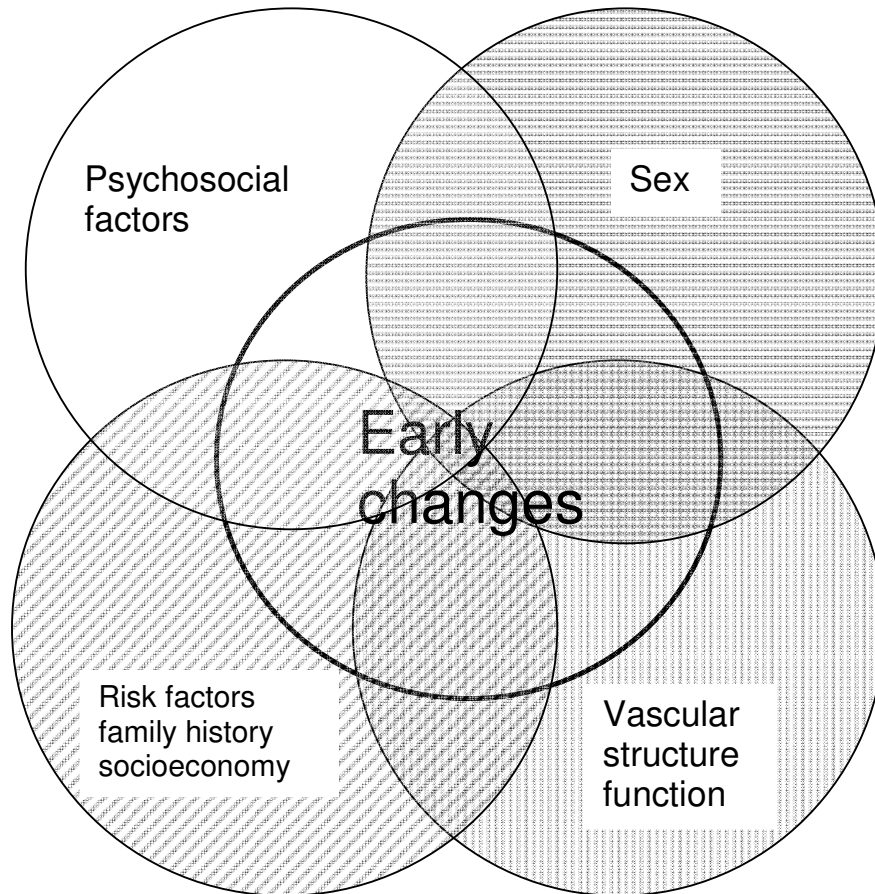

Figure 1. *Important factors for early arterial wall changes and their inherent relationships.*

2. Background

Historical context

Recognition of the importance of the link between psyche and soma can be traced to the ancient Far East. Huang Ti, the "Yellow Emperor" (2697-2597 B.C.), observed, "When the minds of people are closed and wisdom is locked out they remain tied to disease. At the beginning of the classical era, Heracleitus suggested that a static, unchanging state was not the natural condition, but rather that the capacity to undergo constant change was intrinsic to all things: "panta rei", literally everything flows, meaning that everything is constantly changing, from the smallest grain of sand to the stars in the sky. Thus, every object ultimately is a figment of one's imagination. Only change itself is real; a constant and eternal flux, like the continuous flow of a river which always renews itself.

Hippocrates considered health to be equated with a harmonious balance of the elements and qualities of life, but disease with a disharmony of these elements. He suggested that the disturbing forces that produced this disharmony derived from natural rather than supernatural sources and that counterbalancing or adaptive forces were of natural origin as well. In the years of the Renaissance, Thomas Sydenham extended this Hippocratic concept of disease as a disharmony brought about by disturbing forces, when he suggested that an individual's adaptive response to such forces could itself be capable of producing pathological changes.
In 1628 William Harvey wrote "a mental disturbance provoking pain, excessive joy, hope or anxiety extends to the heart, where it affects temper and rate".
Sir William Osler, often called the father of modern internal medicine, was perhaps the first physician to link atherosclerosis directly with behavioural excesses, which he described as "the Nemesis through which Nature exacts retributive justice for the transgression of her laws" (Allen 1996).

Claude Bernard (1878) extended the concept of harmony or the steady state in the 19th century, when he introduced the principle of a dynamic internal physiological equilibrium, *milieu interieur*.

Walter Cannon used the term homeostasis and he extended the concept of internal balance to emotional as well as physical characteristics. He proposed the "emergency reaction" hypothesis, describing the activation of the adrenal medulla and release of noradrenalin provoked by acute stress stimuli (Cannon 1929), and argued that aggression and fear are reactions to external threat and danger: he named this the "fight-flight" reaction.

In the 1930s, Hans Seley hypothesised that psychological and physiological events occurring in seriously ill patients were the consequences of severe prolonged adaptative responses. He suggested that a "stressor" induced a "stress" response in animals, which led to the definition of the "General Alarm Syndrome" (Selye 1936, 1956). According to Selye, distress (i.e. grief, resignation and despair) activates physiological mechanisms and increases the production of cortisol from the adrenal cortex.

Henry et al. performed studies in small animals and showed that if there was a disturbance in social control or support, the animals responded with defence reactions, aggression, defeat reactions and withdrawal (Henry 1977, 1986). The different neurohormonal profiles of these response patterns are illustrated in Fig. 2, showing that sympathetic components dominate in defence reactions, while hypothalamo–pituitary–adrenal (HPA) axis components dominate in defeat reactions, together with suppression of sex and growth hormones, and disturbance of immune functions (Jonsdottir 1997).
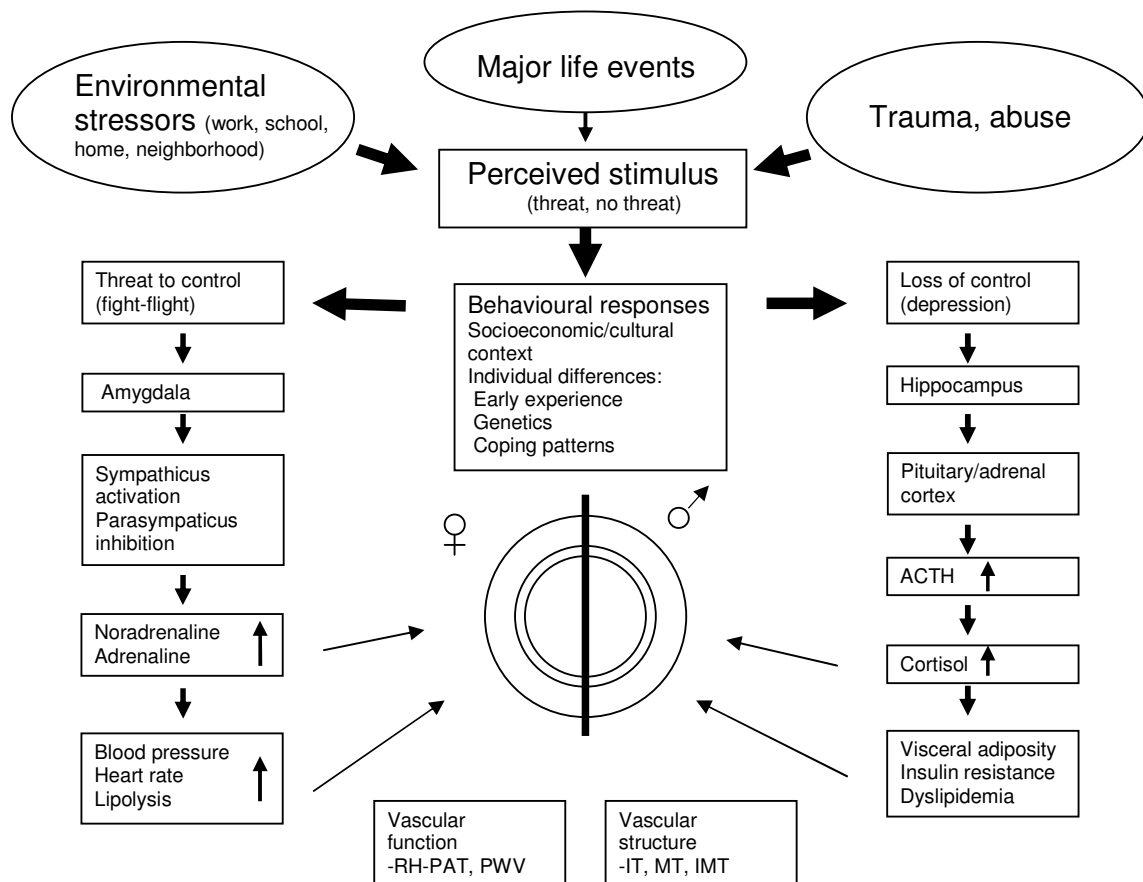
Figure 2. *Acute and chronic stress responses and their effects on vascular function and structure, in boys and girls (ACTH=Adrenocorticotrophoic hormone, RH-PAT=reactive hyperemia peripheral artery tonometry, PWV=pulse wave velocity, IT=intima thickness, MT =media thickness, IMT=intima media thickness, modified after Henry & Stephens 1977).*

Similar experiments have been performed in primates, with essentially the same results. In these studies additional somatic consequences were identified. The animals with a defeat type of reaction accumulated excess depot fat in visceral adipose tissue and developed insulin resistance, dyslipidaemia, hypertension, impaired glucose tolerance and early signs of coronary atherosclerosis (Shively 1994, 1997). In socially disrupted primates, paradoxical constriction of coronary arteries in response to acetylcholine in comparison to with socially stable controls was shown. In addition, quantitative angiography revealed larger plaques in socially stressed primates. In addition to an atherogenic effect, chronic social stress appears to impair endothelium-dependent vascular responses of the coronary arteries (Williams 1991).

The cardiovascular impact of the defence reaction does not only occur in moments of manifest threat to the individual: it appears to occur whenever alertness is raised, thus not only due to potential harmful influences, but also through "stimulating" or even amusing challenges for the individual. Therefore, the term "defence reaction" may be somewhat misleading, as it suggests an involvement only in troubled situations. It appears, in fact, that this response pattern, in its milder or moderate forms, regularly influences the cardiovascular system in normal life through neuro-hormonal mechanisms. The defence reaction does not only imply an acute rise in the blood pressure, caused by an increased cardiac output, but the cardiovascular system is also affected by concomitant hormonal activation involving the release of ACTH, as well as glucocorticoids, adrenaline and aldosterone.

Chrousos (1992, 1998) proposed that stressful events trigger cognitive and affective responses which, in turn, induce sympathetic nervous system and endocrine changes. Living organisms survive by maintaining an immensely complex and harmonious equilibrium, or homeostasis, that is constantly challenged or threatened by intrinsic or extrinsic forces or stressors.

In humans, there is great individual variability in the adaptive response, because of differences in earlier experiences and differences in the choice of coping strategies (Eriksen 2005). It is not just the dramatic stressful events exacting their toll, but rather the many life events that elevate and sustain the activities of physiological systems and cause sleep deprivation, overeating, and other health damaging behaviour. Over time this results in wear and tear on the body, "allostatic load", which reflects not only the impact of life experiences but also of genetic load, and individual lifestyle habits reflecting factors such as diet, exercise, and substance abuse, as well as developmental experiences that set life-long patterns of behaviour and reactivity (McEwen 2006).

Barker has shown that foetal programming i.e. under-nutrition in utero is associated with persisting changes in blood pressure, cholesterol metabolism, insulin response to glucose, which can be considered in terms of 'early stress' (Barker 1998). Longitudinal studies have shown that birth weight is predictive of diabetes mellitus, coronary heart disease CHD) and stroke risk (Curhan 1996, Rich-Edwards 2005).

There is evidence that childhood exposure to stress can influence the stress response throughout life (Bauer 2004), via mechanisms such as a reduction in the number of glucocorticoid receptors in the hippocampus and other tissues, reducing negative feedback sensitivity (Liu 1997, Sapolsky, 1986, 1997).

One example of persistently augmented stress system activation has been suggested by Björntorp (1991, 2001): visceral fat and metabolic disturbances with dyslipidemia, insulin resistance and hypertension (metabolic syndrome) could be the missing link between psychosocial factors and CVD, and that the HPA axis mediates this association (Björntorp 1991, Rosmond 1998). Acutely (within hours), glucocorticoids directly inhibit further activity in the HPA axis, but the chronic actions (within days) of these steroids on brain are directly excitatory (Young 1995). The time-limited nature of this process renders its accompanying catabolic and immunosuppressive effects temporarily beneficial and of little adverse consequences (McEwen 1998). Persistent stress system activation on the other hand, would lead to the state or syndrome described by Selye.

It may be that people eat 'comfort food' in an attempt to reduce the activity of the chronic stress-response network with its attendant anxiety (Dallman, 2003). This can result in obesity, in particular visceral adiposity, with resultant raised inflammatory activity.

Autonomic Nervous system ANS, Sympathetic and parasympathetic function

ANS dysfunction through subsequent hemodynamic (i.e., hypertension) alterations increases future CVD risk (Montani 2002). Increased sympathetic nervous system activity in children has been shown to be associated with an increased prevalence of hypertension in adulthood and thus may indicate the role of the sympathetic nervous system in altering vascular function and tone (Yakinci 2000).

In recent years it has become convincingly clear that impaired parasympathetic/vagal function confers an increased risk for cardiovascular disease. Impaired heart rate recovery after physical exercise is marker of decreased vagal function, prognostic for morbidity and mortality (Cole 1999). There are interactions between the ANS and endothelial function (Harris 2004) Lower socioeconomic status is linked to impaired heart rate variability (Hemingway 2005), as is impaired heart rate recovery (Shishehbor 2006). Most interestingly,

this study showed that by regular physical exercise and prevailing low socioeconomic status, it is possible to influence the ten year survival. Decreased heart rate variability was associated with higher levels of fibrinogen in depressed patients with CHD (Carney 2007), and altered sympathovagal balance has been shown to be predictive of acute coronary syndromes and sudden cardiac death in certain populations (Kleiger 1987, Tsuji 1994, Astrup 2006). In policemen, increased hostility and depression scores were associated with higher DBP and recent life change scores. (Ely 1986), and in a recently published study, certain emergency fire fighting duties were associated with a risk of death from coronary heart disease that was markedly higher than the risk associated with non-emergency duties. Fire suppression was associated with the highest risk, which was approximately 10 to 100 times as high as that for non-emergency duties (Kales 2007).

The HPA axis and the ANS have been examined extensively as mediators between psychosocial factors and health (Hemingway 2005) and cortisol reactivity and regulation have long been recognised as related to helplessness and depression (Anisman 1982) and to long-lasting effects of trauma, such as in "post traumatic stress disorder" (Yehuda, 2002). In the Whitehall II study social inequality was associated with coronary disease risk and central obesity; and the Metabolic Syndrome was identified as a likely mediating factor (Brunner 1997). Psychosocial stress and greater susceptibility to stress are plausible risks for CHD (Hemingway 1999): a recent case-control study found that presence of psychosocial stress, including due to financial stressors, was associated with increased risk of acute myocardial infarction (Rosengren 2004).

Vascular biology

The arterial blood vessel wall consists of three concentric layers: intima, media and adventitia. The intima, adjacent to the blood vessel lumen, is composed of a monolayer of endothelial cells with underlying connective tissue. The atherosclerotic process begins morphologically in the intima, and the media thickness is more closely related to blood pressure level and becomes thicker as blood pressure increases (Folkow 1982, Hansson 2005).

Endothelial cells have pivotal roles, including: providing a non-thrombotic surface, maintaining vascular tone by releasing nitric oxide, prostacyklin and endothelin, which modulate vasodilation or vasoconstriction, and providing a non-adherent surface for leukocytes, thus preventing the atherosclerotic process.

Conduit arteries dilate in response to an increase in blood flow (Andersson 1989, Sinoway 1989, Nabel 1990). This physiological response is dependent on the presence of an intact

endothelium, (Smiesko 1985). There is a close relationship between endothelial function in the coronary and peripheral components of human circulation (Andersson 1995).

Injury to the endothelium is one of the earliest manifestations of subclinical atherosclerosis. Endothelial function can be evaluated by measuring changes in blood vessel diameter in response to specific stimuli. Flow-mediated dilation (FMD) of the brachial artery involves local release of nitric oxide from endothelial cells in response to shear forces following a hyperaemic stimulus (Davies 1995, Traub 1998). Measurement of FMD in vivo has been widely adopted as an assessment of endothelial function (Andersson 1995). The changes in diameter measured by this technique can mostly be blocked by selective inhibitors of nitric oxide synthase, indicating that these changes provide an indirect assessment of nitric oxide production.

In the Framingham study FMD was associated with many CVD risk factors, suggesting that FMD may signal several of the effects of CVD risk factors on the arterial wall (Mitchell 2004). Moreover, several studies have suggested that brachial FMD has prognostic value in identifying subjects at risk of developing CVD (Coretti 2002, Chan 2004) and impaired FMD was seen in the offspring of patients with premature myocardial infarction (Gaeta 2000). Celermajer (1992) showed altered endothelial function in both children and adults at risk of atherosclerosis, and recent studies have shown that attenuated endothelial function in children is associated with type 1 diabetes mellitus (Järvisalo 2004), with obesity (Woo 2004) and childhood infection (Charakida 2005).

The natural history of adult CVD begins in childhood and progresses throughout life; the speed and extent of development depending on environmental influences and genetic factors. The atherosclerotic process develops silently for decades during childhood, adolescence and early adulthood before cardiovascular complications such as myocardial infarction and stroke occur in later life. Autopsy studies have demonstrated that the earliest atherosclerotic lesions, fatty streaks, are already present in foetal aortas, (Napoli 1997) and that the atherosclerotic process thereafter progresses (Enos 1953, Holman 1958, McNamara 1971, Berenson 1987). By puberty, fatty streaks appear to be gradually transformed into more advanced atherosclerotic lesions (Stary 1989).

Structural changes of the vascular vessel wall can be determined non-invasively by ultrasound (Pignoli 1986), and the composite measure of intima media thickness (IMT) has been shown to correlate well with traditional risk factors; blood pressure, lipids and smoking by several large studies and has been shown to convey important information about risk for

future cardiovascular disease (Berenson 1998, Davis 2001, Bhuiyan 2006, Raitakari 2003). Thus, IMT measurement of the carotid arteries is considered a valuable measure of atherosclerosis (Burke 1995).

Increased carotid IMT has been identified in children with known cardiovascular risk factors such as hypercholesterolemia, Type 1 diabetes and hypertension (Tonstad 1996, Järvisalo 2002, Litwin 2004). There is evidence of continuity of risk, as childhood CVD risk factors are associated with carotid IMT in adulthood (Berenson 2002, Li 2003).

Arterial stiffness is determined by the arterial wall structure and is, in adults, associated with numerous well-established risk factors for CVD (Arnett 1994). Ultrasonographically assessed arterial stiffness is a useful way to study early pathophysiological changes in the arteries relevant to the development of atherosclerosis (Oliver 2003) and cardiovascular risk factors identified in childhood predict increased arterial stiffness in adulthood (Jounala 2005). Only a few studies have measured arterial stiffness in children, showing a positive association between serum cholesterol and arterial stiffness (Leeson 2000, Aggoun 2000).

Another measure of arterial stiffness is pulse wave velocity (PWV). PWV reflects the time needed for a pulse wave to travel a given distance along the blood vessel; and the stiffer the arteries, the higher the velocity. In adults there is a strong relationship between aortic stiffness and degree of CHD assessed by coronary angiography (Hirai 1989) and PWV is associated with cardiovascular morbidity and mortality (van Popele 2001, Laurent 2001, Blacher 2003).

So what is stress, and how can it be measured? In the previous paragraphs I have described several components of rather complex mechanisms relevant to the concept of stress (Fig 3). In addition to the interplay between stressors, neurohormonal responses (HPA, ANS), metabolic (metabolic syndrome) and cardiovascular consequences (vascular structure and function), concepts relevant to mental aspects of stress and how such stress may be measured will be discussed.
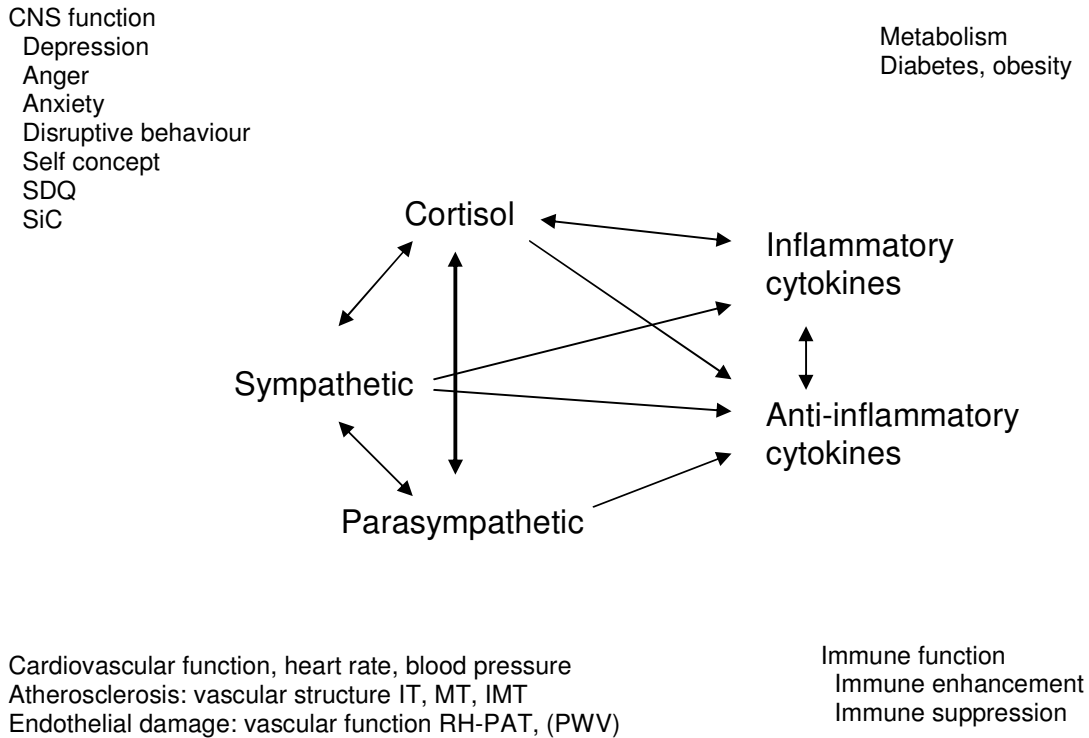
CNS function
  Depression
  Anger
  Anxiety
  Disruptive behaviour
  Self concept
  SDQ
  SiC

Metabolism
Diabetes, obesity

Cortisol

Inflammatory cytokines

Sympathetic

Anti-inflammatory cytokines

Parasympathetic

Cardiovascular function, heart rate, blood pressure
Atherosclerosis: vascular structure IT, MT, IMT
Endothelial damage: vascular function RH-PAT, (PWV)

Immune function
  Immune enhancement
  Immune suppression

Figure 3. *Relationship between CNS (central nervous system), psychological factors, metabolism, stress response systems and their impact on developing cardiovascular disease (modified after McEwen 2006).*

Psychosocial factors and their relationship with cardiovascular features

Anger The word anger derives from the Old Norse *angr*, signifying grief, circa 1000 AD. Its earliest meanings in English were trouble, affliction, irritation and sorrow (Allen 1996). The putative association between anger and CHD was described initially by investigators from the Framingham Heart Study, who reported that suppressed anger independently predicted the 8-year incidence of CHD among both men and women (Haynes 1980). Trait anger predicted IMT level progression among middle-aged women over three years (Räikkönen 2004), and anger-proneness in adults predicts CHD (Williams 2000, Chang 2002). Acute coronary syndromes can be triggered by anger, and in a recent study anger was more common in younger, socioeconomically deprived patients who presented with a myocardial infarction (Strike 2006). The biological mechanisms and processes by which anger influences CVD are yet to be unravelled. Heightened sympathetic arousal and excessive levels of circulating catecholamines are known to cause direct damage to the endothelium (Haft 1974), and anger provocation impairs endothelial function (Shimbo 2007). Possibly as a result of increased sympathetic activity, raised blood pressure tracks from childhood into adulthood, and may augment CVD risk (Bao 1995).

Depression Episodes of serious depression are characterized by the presence of depressed mood and markedly decreased interest in all activities, persisting for at least two weeks and accompanied by at least four of the following additional symptoms: change in appetite, sleep disturbance, fatigue, psychomotor retardation or agitation, feelings of guilt or worthlessness, problems concentrating and suicidal thoughts (DSM IV APA 1994). The one-month prevalence of serious depression was approximately 5% in a general population based study from the US (Blazer 1994).

One neurobiological factor which has been shown to be of importance for depression and suicide attempts and suicide is the serotonin metabolite 5-hydroxyindolacetic acid (5- HIAA) (Åsberg 1976, 1997). Serotonin is a neurotransmitter thought to play a crucial role in depression, and patients who make serious or violent suicide attempts have lower levels of 5-HIAA than other patients and healthy control subjects (Träskman 1981). A low 5-HIAA concentration in the cerebrospinal fluid is predictive of subsequent successful suicide among those who have made previous attempts (Nordström 1994). The most likely explanation for theses findings is the influence of serotonin on aggressive impulses. This interpretation is supported by the extent of the inverse association between the frequencies of aggressive actions over the life-course with 5-HIAA concentration in the cerebrospinal fluid (Åsberg 1994). Increased HPA-axis activity and a diminished response to dexamethasone has also been shown in depression (Carroll 1981). However, the interplay between the HPA-axis and serotonin is complex.

Increases in the incidence of cardiovascular morbidity and mortality may be important sequelae of the central nervous system changes in depression (Anda 1993, Barefoot 1996, Rozanski 2005, Hemingway 1999, Ferketich 2000, Rosengren 2004). It is conceivable that alterations in the neurotransmitter and hormonal system due to depression in young people, also influences cardiovascular regulation and creates the conditions necessary for later atherosclerosis.

Depression seems to be under-recognized among children and adolescents, and adverse childhood experiences have strong associations with depressive symptoms, antisocial behaviour and drug use during the transition into early adulthood (Schilling 2007). Depressive symptoms among youths are often attributed to the normal stress of adolescence, misdiagnosed as primarily conduct, attention or substance abuse disorders, or seen as a stage the youth is going through. Studies of high school students have shown that, similar to depression in adults, the incidence of depression among adolescents is greater among females than males (Lewinsohn 1998). These sex differences have been reported as emerging between the ages of 13 and 15 years, and prior to this, rates of depression appear

to be similar for young boys and girls (Hankin 1999, Sweeting 2003). In a study by Saluja (2004) 18% of youths in the US (on average aged 15 years or less) reported symptoms of depression, and a higher proportion of females (25%) reported depressive symptoms than males (10%). The increase in prevalence of depressive symptoms with age was far more pronounced for females than males. Youths who reported experiencing somatic symptoms also reported significantly more depressive symptoms than other youths. In studies from the UK, Hawton and colleagues have shown that girls report more symptoms and exhibit more deliberate self harm than boys (Hawton 2002, 2003).

Studies from Sweden have also shown that girls have a higher prevalence of depression than boys (ages 16-17 years). The one-year prevalence of major depression was 5.8% and the lifetime prevalence was 11.4%, with a male to female ratio of four to one (Olsson 1999). In a study from Finland (Haarasilta 2001) the one-year prevalence of depression in adolescents (aged 15-19 years) was 5.3%, (females 6.0%, males 4.6%). During the last 20 years it seems that internalised problems such as depression have increased among adolescents, at least in high-income countries (Rutter 1995, Prosser 1996, Fombonne 1998, Collishaw 2004). Thus, depression in the young represents a substantial clinical problem, and there is a need to develop knowledge in identifying early signs expressed both mentally and physically.

Self-assessment of psychological characteristics

Self-assessment questionnaires have been shown to be of great value when studying psychological health in children and adolescents. Self-assessments convey unique information about the psychological state of the child or adolescent, and are especially valuable when internalised symptoms and thoughts are the topic of interest, as in anxiety, depression and anger. There is a high correlation between children's self-assessed depression, feelings of hopelessness and low self-perception, with suicidal thoughts and attempted suicide (Kazdin 1990). Self-assessment of disruptive behaviour often provides specific information that is otherwise impossible to obtain (Gothelf 1997, Reitman 1998). Kazdin (1987) stated that self-assessment scales are particular valuable, because they can be used to identify social adjustment problems among children and adolescents, which could be hidden from their parents.

The discrepancy between self-assessments of psychological state made by children or adolescents compared with assessments made by their parents or teachers could be because children and adolescents tend to under-report problems to adults or have difficulties in verbalising them (Birmaher 1996). An explanation could be that some children, especially

boys, do not express their feelings or communicate their thoughts in a way that is understandable to others (Jolly 1994). It may also be easier to report lack of wellbeing in a questionnaire, which leads to higher scores for symptoms, than in a conversation.

It is a common observation that parents can be less reliable when giving information about psychological states in their own children, than the children themselves, who often deliver more precise information about various forms of internalised symptoms (Broberg 2003). According to Rutter (1980) it is possible for parents to miss serious manifestations of depression in their child.

Nowadays, the diagnosis of depression and estimation of treatment effects are often facilitated by validated questionnaires (Beck 1961, Montgomery 1979).

When Aaron Beck began studying depression in the 1950s, the prevailing psychoanalytic theory attributed the syndrome to inverted hostility against the self. In contrast with this explanation, he observed that depressed patients might be described as having negative views of themselves, their present experiences, and their futures. Beck later proposed this negative triad as a framework for understanding the phenomenology of depression (Beck, 1967).

He developed the Beck Depression Inventory in a way that was novel at that time; by collating patients' verbatim descriptions of their symptoms and using these to structure a scale which could reflect the intensity or severity of a given symptom (Beck 1961).

To study psychiatric disease and psychological ill-health in children and adolescents, the Beck Youth Inventories of Emotional and Social Impairment (BYI) were developed and recently introduced as five self-completion scales that can be used to assess and screen for depression, anxiety, anger, disruptive behaviour, and self-perception in children between the ages of 7 and 14 years (Beck 2001).

Another questionnaire developed for screening mental health in children and adolescents, the Strengths and Difficulties Questionnaire (SDQ) has been shown to have the ability to identify child psychiatric morbidity, and provides screening on empathy and pro-social behaviour, which are aspects of child development emphasized in current child psychiatry (Goodman 1997, 1999, 2001). The design of the SDQ makes the questionnaire especially suitable for studies in the general population where the majority of children are healthy, and the questionnaire has been translated into Swedish (Smedje 1999). However, no well-established questionnaires exist that measure self perceived stress in childhood.

There are other important factors affecting health, both in the young and in adults. Socioeconomic circumstances such as parental education (PE) which partly indicates family cultural and material factors have been reported to be associated with CVD risk factors in childhood (Leino 2000, Kocaoglu 2005, Goodman 2005, Kivimäki 2006) and later mortality (Strand 2007). Health and the risk of premature death are influenced by socioeconomic factors acting throughout life and socioeconomic influences on particular causes of death may have different critical times. The risk of premature death from CVD is particularly sensitive to socioeconomic influences acting in early life (Smith 1997).

Psychosocial factors may already be related to early physiological changes in childhood; and these changes are relevant to future CVD risk. Thus, it vital to study whether, and to what extent, psychological and psychosocial factors measured by validated tests, are associated with physiological characteristics in the young.

In conclusion, early influences on our stress response systems; psychological, metabolic and cardiovascular systems, and the interaction between them, have a bearing on future health. As psychological factors in adulthood are connected with CVD, it would be of great value to unravel whether this association is already evident in childhood. If so, this could identify an important factor in the development of CVD, providing a basis for preventive interventions.

In order to identify small deviations of both psychological and physiological measures among a healthy population of children and adolescents, the methods used have to be highly sensitive. Clearly, it is a challenging task to identify such small changes. Nevertheless, we need to improve our knowledge in this field for the further advance of possible prevention and treatment strategies.

Present methods to study vascular wall layers are restricted to crude measurements because of low resolution. The present way to assess FMD is highly operator dependent and therefore complicated to use in large-scale field studies. Measuring the different vessel wall layers separately, as well as using a new operator independent method for assessing endothelial function, will facilitate research. This will help to study relationships among a variety of risk factors such as psychosocial exposures, and specific pathophysiological processes, for example structural and functional vascular wall changes. Therefore there is a need to develop improved techniques, which will facilitate such studies.

3. Current status and unresolved issues

What was known before the study?

I.   Psychological health is relevant to CVD in adults

II.   Imaging of early vascular changes is possible, but limitations in resolution does not allow more specific imaging of different wall layers

III.   Males have a higher CHD lifetime risk both in middle and old age and an increased magnitude of atherosclerosis at other arterial sites, compared to women

IV.   HPA axis activity is related to stress and cardiovascular and metabolic perturbations in adults

4. Aims of the study

General

To develop non-invasive techniques which make it possible to detect early changes in vascular wall structures in a healthy, young population and study associations between vascular wall structure and function and self perceived psychological health.

Specific

To construct a questionnaire with reliable psychometric properties that measures different aspects of the stress concept in children.

To develop a new ultrasound technique for use in humans to structurally define the intima thickness (IT) and media thickness (MT) separately from each other, that can be used from childhood to old age and among CVD patients.

To study whether childhood sex differences exist in vascular wall structures and vascular wall function, given the greater CVD risk in adult males.

To investigate whether vascular function is impaired in children with self perceived psychological ill-health.

5. Methodological considerations

5.1 Ethics

The local ethics committees approved the study protocols (Örebro University Hospital Dnr 304/03, Sahlgrenska Academy at Göteborg University, Dnr 203-05), and the studies were carried out in accordance with the Helsinki Declaration (World Medical Association) and national guidelines (Swedish Research Council). All study participants received verbal and written information about the purpose and content of the study and written consent was obtained from all study participants. In the studies involving children, written consent was obtained from the children and at least one of the parents, if possible both.

5.2 Study populations

Study I. *Development and validation of a new short self-rating questionnaire to assess stress in children*

The study was performed in three stages:

Stage 1. A total of 24 children from the 4th grade (ages 10-11 years) of a primary school in a suburban and predominantly middle-class area of Göteborg were invited to participate. Some 23 children, 11 girls and 12 boys agreed to participate. They answered the first version of the SiC questionnaire with ten items.

Stage 2. Some 74 children, 42 girls and 32 boys in 4th and 5th grade (ages 10 to 12 years) from a small town in Sweden participated in the second pilot study. They were presented with an extended version of the SiC questionnaire with additional questions about social support, comprising 21 items in total.

Stage 3. A total of 222 children from eleven 3rd and 4th grade classes in four primary schools in small rural towns (Karlskoga and Granbergsdal, Örebro County), were asked to participate during an ordinary lesson. Some 84 children, 50 girls and 34 boys (aged 9.9±0.5 years) participated and completed the sample collection for urinary catecholamines and among these, 55 children provided all five saliva samples for the measurement of cortisol.

Study II. *Assessing vascular wall layers with new very high resolution ultrasound*

Some 90 normotensive healthy volunteers, aged 10 to 90 years were recruited. They comprised twenty-five schoolchildren (17 girls) aged 10 to 17 years, fourteen younger adults (7 females) aged 21 to 29 years, 23 adults (7 females) aged 30 to 57 years and 28 (15 females) elderly subjects aged 60 to 90 years. The children were recruited from a school in the city of Göteborg and the adults through advertisements at primary health care facilities in the Göteborg and Varberg areas. To participate in the study, the subjects had to be apparently healthy, non-smokers, and without any known chronic disease such as hypertension, dyslipidemia, or diabetes mellitus, and were not taking any medication.

A total of 12 patients aged 64 to 83 years (73.3±7.6 years; 5 female, 7 male) with peripheral artery disease (PAD) in the lower extremities and with angiographic verified stenosis were recruited from the outpatient clinic at the department of vascular surgery at Sahlgrenska University Hospital, Gothenburg. Of these, six had undergone operations with femoral bypass or percutaneous transluminal angioplasty, and the other six were awaiting an operation. They were compared with 12 healthy age- and sex-matched controls (73.5±8.4 years; 5 female, 7 male).

Study III and IV *Vascular wall structure and function in relation to psychological factors*

Some 534 healthy school children (age 14.2 ±0.9 years female/male 279/255) in the 7th 8th and 9th grade from two schools in the Gothenburg region were invited to participate in the study (Fig. 4).

A total of 252 children (139 girls, 113 boys) participated in the vascular studies and underwent RH-PAT testing and their peripheral arteries were investigated using very high resolution ultrasound. Four subjects had missing values of RH-PAT due to technical difficulties in obtaining signals, and there was also missing data for some ultrasound measures, see table 2. Weight was measured by an electronic scale and height by a stadiometer allowing calculations of BMI (kg/m^2) and BMI-z scores were calculated according to Cole (2000).

> 534 children in 30 7th 8th and 9th grade classes from two schools were invited to participate in the study (age 14.2 ±0.9 years) f/m 279/255

> From 252 children f/m 139/113 ultrasound images were obtained
> In 248 children f/m 136/112 RH-PAT signals were recorded. The participation rate was 46% for the whole group, 48% in girls and 43% in boys.

Figure 4. *Flowchart, study III and IV.*

5.3 Self assessment questionnaires

Beck Youth Inventories

The Beck Youth Inventories of Emotional and Social Impairment (BYI) were recently introduced as five self-completion scales that can be used to assess and screen for depression, anxiety, anger, disruptive behaviour, and self-perception in children between the ages of 7 and 14 years (Beck 2001). Each subscale of 20 items can be completed in less than 10 minutes. The items are written at a 2nd-grade reading level. The subject rates each statement on a four-point Likert scale ranging from never (0) to always (3). The resulting scores are calculated for each inventory by summing their 20 responses and these scores can range from 0 to 60.

The psychometric properties of the BYI were studied in a nationally drawn, standardised sample of 800 children in the USA. The internal consistency in each of the five inventories was high (coefficients > 0.84) and the magnitude for each of the median 7-day, test-retest reliability correlations for the five inventories was large (rs> 0.73). Evidence supporting the construct validity of the BYI was reported in a study of paediatric psychiatric outpatients (Steer 2001). The inventories have been translated into Swedish and this version was studied extensively among standardised samples drawn from different Swedish regions and from different clinical and school settings (Tideman, 2004). These normative data are presented separately for girls and boys, as the authors noticed a slight sex difference. The 95% standard error of the mean (SEM) for the whole study population of 2358 children and adolescents from 9 to 18 years of age was calculated to 4 points for the subscales for anxiety, depression, anger and disruptive behaviour and 5 points for the self concept scale as described by Tideman.

Strengths and difficulties (SDQ)

The Strengths and Difficulties Questionnaire (SDQ) comprises 25 items, divided into five subscales (hyperactivity, emotional symptoms, conduct problems, peer problems and pro-social behaviour). It was developed in the UK, but has been translated into a large number of languages, and in Europe it has replaced or complemented the American Youth Self Report, which is designed to measure both internalized and externalized psychological difficulties (Goodman 1999). A validation study has shown that the instrument identifies child psychiatric morbidity, and provides screening on empathy and pro-social behaviour, which are aspects of child development emphasized in current child psychiatry. The design of the SDQ makes the questionnaire especially suitable for studies in the general population where the majority of children are healthy (Goodman 1997, 2001, Smedje 1999).

Stress in Children (SiC)

A new questionnaire was constructed to assess stress in schoolchildren. The selection of the final items was based on the following criteria: they should identify different aspects of stress as defined here; they should be easy to understand; and physical, emotional and symptomatic aspects should be represented. A final pool of 21 items was chosen for this purpose and a first test version of the questionnaire was constructed, using Likert-scale response categories with four alternatives. The children were asked to indicate the category that best applied to them. These categories were: never, sometimes, often and very often. The SiC is a self-rating questionnaire and can be completed in a few minutes. When analyzing data from the SiC questionnaire each participant's total score was calculated and divided by the number of questions to provide a mean score, i.e. SiC Global Mean Score (GMS) for each participant. When the participants missed or had chosen not to answer questions, the mean score was calculated by dividing the total score by the numbers of questions answered. A maximum of two missing questions was accepted.

<u>Rasch analysis</u> When developing psychometric questionnaires, it is of importance to study how the questionnaire and its items work in more detail. The Rasch analysis is a member of the group of models based on latent trait analysis, focusing on the operating characteristics across the whole range of the latent trait and the properties of those characteristics. The Rasch model offers opportunities to examine latent trait constructs in a rigorous way, e.g. composite measures of subjective health (Hagquist 2001). It requires uni-dimensionality of the latent trait, and an underlying continuum from low to high for values of the trait is assumed. The new SiC questionnaire was analysed by using the RUMM 2020 software package (Sheridan 1997).

Parental education The Level of parental education (PE) was assessed by asking the school children about the highest level of education their parents had achieved. A classification was constructed as used by Statistics Sweden (2004) with a score of 1 for nine-year compulsory school, for children aged 7-16; 2 for completed upper secondary education and; 3 for university or college for higher education. The scores for each parent where then added for a combined measure. We performed analysis to confirm that if single parenthood was not a confounding factor for associations with this measure of parental education.

5.4 Neuroendocrine measurements

Saliva cortisol

Subjects were instructed carefully how to provide samples of saliva for the measurement of cortisol (I). Saliva samples were collected on an ordinary school day by chewing a cotton swab for 30-45 seconds which was then placed in a test tube (Salivette, Sarstedt, AG&Co Nümbrecht, Germany). The first sample (SC1) was collected immediately upon waking in the morning, irrespective of time. The next sample (SC2) was collected 15 minutes later, before eating or brushing teeth. The quotient between these two values (SC1/SC2) provided a measure of the cortisol waking response (Pruessner 1995,1997). The third sample (SC3) was obtained at 09.00 hours at school. The fourth sample (SC4) was collected approximately 15 minutes before lunch at 11.00 hours. The fifth and final sample (SC5) was collected at bedtime, after 15 min at rest in bed, before falling asleep. The area under the curve (AUC) for the five salivary cortisol samples was calculated (S1+S2+S3+S4+S5)/5) (Pruessner 2003).

Salivary cortisol concentrations were determined using a commercial radioimmunoassay (Spectria CORTISOL [125I] Coated Tube Radioimmunoassay) designed for quantitative in vitro measurement of cortisol concentration in serum, plasma, urine or saliva. Duplicate determinations were used for salivary cortisol (Aardal 1995, Clinical Chemistry Lab, Örebro University Hospital).

Urinary catecholamines

Urine catecholamine levels were determined for each child using samples collected in plastic containers over a 24-hour period. Sampling began at 14:00 hours and continued to the same time the next day. The samples were stored in a cold room during the collection period and then transported to the laboratory at Örebro University Hospital. Catecholamine levels were determined using high performance liquid chromatography (HPLC) (Gerlo 1985).

5.5 Vascular function

Reactive hyperemia –peripheral artery testing (RH-PAT)

RH-PAT endothelial function was assessed in all children using the Endo-PAT device (Itamar Medical Ltd, Caesarea, Israel). RH-PAT testing is a non-invasive technique that combines the traditional flow mediated dilatation with pneumatic finger-tip probes to measure arterial pulse wave amplitude and provide an objective measure of endothelial function (Fig 5). Briefly, the subjects sit in a reclining chair with their hands at heart level in a comfortable position such that the fingers are hanging freely. Fingertip probes are placed on both index fingers and pulse wave amplitudes are recorded for the duration of the study. After five minutes when six baseline measurements are collected, arterial flow to the arm is occluded for five minutes using a blood pressure cuff inflated to 40 mmHg above systolic pressure. After the five minute occlusion, the cuff is rapidly deflated to allow for reactive or flow-mediated hyperemia. Pulse wave amplitudes are recorded for at least five minutes after the cuff is deflated. An integrated software program compares the ratio of arterial pressure in the two fingers before and after the occlusion to calculate the RH-PAT score in an operator-independent manner. The RH-PAT score is calculated as the ratio of the average pulse wave amplitude measured over 60 seconds starting one minute after cuff deflation divided by the average pulse wave amplitude measured at baseline. This ratio is normalized using the concurrent signal from the contra-lateral finger to correct for changes in systemic vascular tone (Bonetti 2003).

The choice to use the average one-min PAT signal starting one min after cuff deflation to describe the magnitude of reactive hyperemia was based on the observation that this time interval provided the best information for detection of coronary endothelial dysfunction as determined by receiver operating characteristic curve analysis, as well as the best correlation with the coronary blood flow response to acetylcholine (Kuvin 2003, Bonetti 2004). In a separate group of healthy subjects (n=33), we investigated the reproducibility of RH-PAT measurements. Each subject was studied twice with a 10-week interval. Coefficients of variation were 11%.
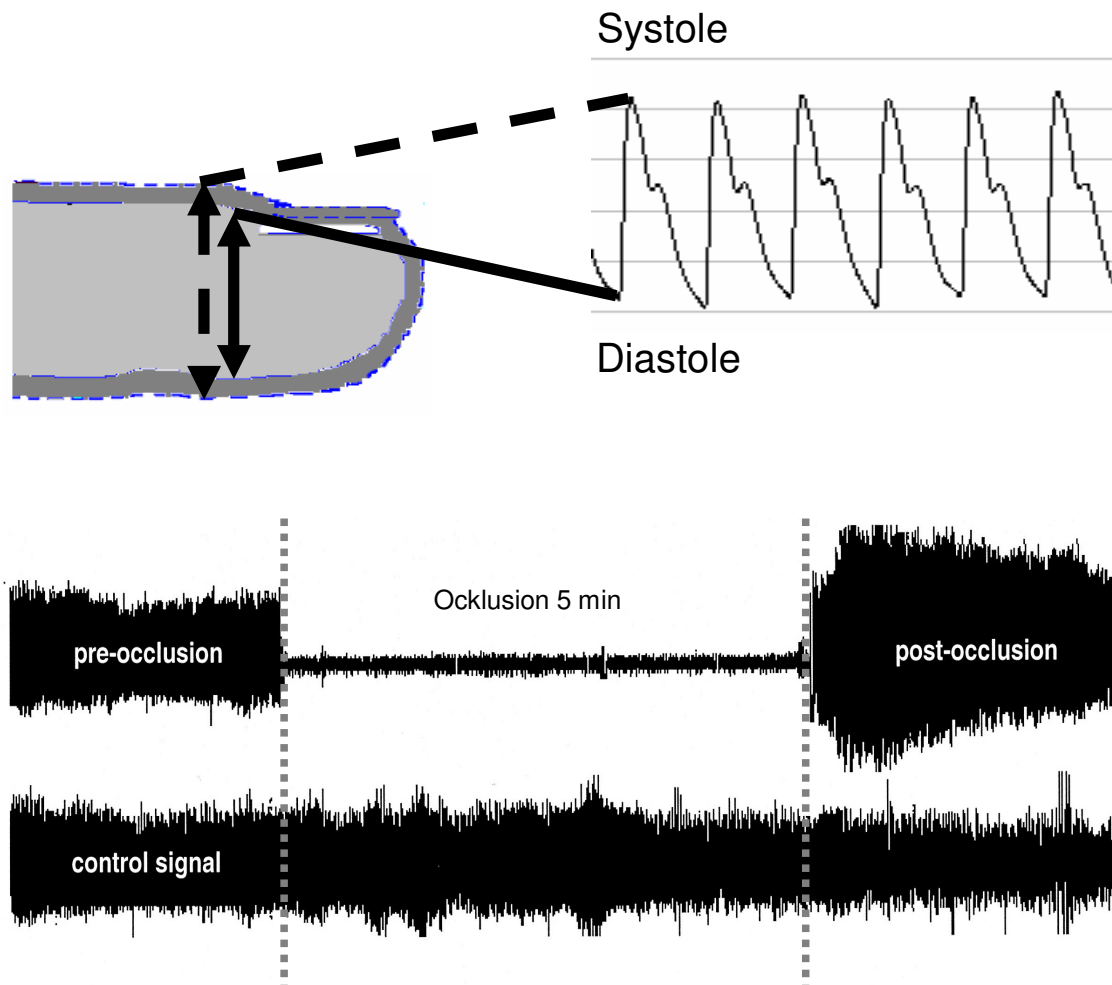
Systole

Diastole

pre-occlusion

Ocklusion 5 min

post-occlusion

control signal

Figure 5. *Reactive hyperemia–peripheral artery tonometry (RH-PAT, Itamar). Fingertip probes are placed on both index fingers and pulse wave amplitudes are recorded. Arterial flow to one arm is occluded using a blood pressure cuff. After five minute occlusion, the cuff is rapidly deflated to allow for reactive or flow-mediated hyperemia. The RH-PAT score is calculated as the ratio of the average pulse wave amplitude measured over 60 seconds starting one minute after cuff deflation divided by the average pulse wave amplitude measured at baseline. This ratio is normalized using the concurrent signal from the contra-lateral finger to correct for changes in systemic vascular tone.*

Pulse wave velocity (PWV)

A pressure tonometer was used transcutaneous recording of the pressure pulse waveform from the underlying artery (Sphygmocor system, AtCor Medical, Australia). Records were made simultaneously with an ECG signal, which provided an R-timing reference (De Angelis 2004). Pressure pulse recordings were performed consecutively at two superficial artery sites (carotid – radial segment). Integral software was used to process each set of pressure-pulse and ECG waveform data to calculate the mean time difference between R-wave and

pressure wave on a beat-to-beat basis and averaging 10 consecutive cardiac cycles. The PWV was then calculated using the mean time difference and distance between the two recording points. Quality indices included in the software were set to ensure uniformity of data. The reproducibility, calculated as coefficient of variations for the radial-carotid PWV measurements, was 9%.

5.6 Vascular structure

Carotid IMT

B-mode real-time ultrasound with a linear high-resolution transducer (8 MHz Acuson Sequoia 512; Acuson, Mountainview, Calif) was used to evaluate arterial IMT in the common carotid artery of subjects in supine position. The IMT is defined as occurring from the lumen–intimal interface to the medial–adventitial border (Wendelhag 1991). ECG triggered B-mode images from at least three consecutive cardiac cycles were stored and averaged.

Validating a new very high resolution ultrasound (55MHZ) technique for use in humans

Verifying the image system resolution

Hardware phantoms were created in order to establish a relationship between ultrasound image features and true biology when using very high resolution ultrasound. These phantoms were also intended to test the actual axial resolution of the imaging system.

The phantoms were produced by placing a small amount of silicone to a rotating disk. The thin layer of silicone that is created is proportional to rotation speed of the disk: the higher rotation speed the thinner the layer. Plastic Petri dishes provided a convenient substrate for the silicone layer. Layers from 1 to 100 microns were created using this technique. Silicone was selected because of its tissue mimicking properties.

The phantoms were submerged in water and examined using the ultrasonic image system at a transponder frequency of 55 MHz. The silicone layers were later weighed with a precision scale to verify the average thickness. Circular shapes were stamped out of the silicone layers and weighed. The identity of the layer and its weights were recorded. The area and the density of the weighed samples are known, so the thickness of the layers could be calculated. The density was obtained from data sheets provided by the manufacturer of the silicone.

Histopathological and morphometric analysis

For validation and verification purposes, specimens of the mesenteric artery were obtained from 18 patients with colon cancer undergoing total colectomy and evaluated with histopathology and in vitro very high resolution ultrasound. Apart from their cancer diagnosis,

they were healthy with no cardiovascular risk factors. The mesenteric artery was chosen because the diameter of its lumen diameter is approximately the same as for the radial artery. A one cm long segment of the vessel from the second or third mesenteric artery branch was free-dissected from the adjacent tissue and stored immediately in formalin. To validate the technical accuracy of the ultrasound, we chose to image the already fixed vessels ex vivo, to minimize artefact due to tissue shrinkage, and to perform comparisons with subsequent histological sections. The formalin-fixed arteries were placed in a Petri dish with physiological saline solution. Ultrasound imaging was then performed using a 55-MHz ultrasound probe mounted on a three-dimensional motor device connected to a holder. The vessel was scanned using the thee-dimensional software package from Visualsonics, generating a resolution of 30 x 30 x 30 um.

The fixed vessels were then embedded in paraffin and sectioned at 5 um onto slides. The exact location of the sections was noted for comparison with the thee-dimensional image data. A total of three consecutive preparations from the middle part of the vessels were stained with hematoxylin for morphological analysis. The three-dimensional image of the vessel was analyzed on an off-line workstation using the imaging analysis tool provided by Visualsonics. Three consecutive cross-sectional vessel images from the middle section of the entire three-dimensional setting were chosen for intima area measurements. The corresponding histological sites were measured on the specimen slides using Image-Pro software (Version 5.0; CyberMetrics Inc, Parameter AB, Sweden).

To further study the characteristics of the new ultrasound technique and its ability to depict biological structures, i.e. the intima separately from the media, again, a one cm vessel segment from the second or third mesenteric artery branch was free-dissected from the adjacent tissue and cut open longitudinally. An oblique cut through the intima and media layers was made and the specimen was then submerged in saline (37^OC) and studied with ultrasound (Fig. 9).

In vivo studies in humans
The ultrasound measurements in humans were performed at the hospital and a primary health care centre (II, III). Right sided radial and dorsal pedal arteries of resting subjects in a supine position were scanned to obtain two-dimensional images, which were subsequently analysed off-line. The radial artery was investigated 1-2 cm proximal to the skin fold separating the palma manus from regio antebrachii anterior, and the dorsal pedal artery was measured above the first proximal metatarsal bone in the foot. At the position of the thickest part of the far wall (visually judged), four consecutive beats were recorded in real time and

saved. In a separate group of healthy subjects (n=10) we studied the reproducibility of radial and dorsal pedal arterial IT and IMT. Each subject was studied twice with a four-week interval. Coefficients of variation for radial arterial IT and IMT were 15% and 4% respectively, and for dorsal pedal arterial IT and IMT they were 19% and 6% respectively.

The intima thickness echo and total intima thickness were assessed using callipers within a higher resolution zoom. Because the 55 MHz probe has a very short pulse length, the theoretical limit of discrimination is approximately 25 um. The first calliper was set in the intima directly after the lumen-intima interface, and the second calliper at the end of the intima-media interface echo. Since calliper thickness is approximately 10 um, we overestimated intima thickness by about 15 um using this method. We performed measurements systematically to ensure this bias was constant.

The measurements of IT were performed in systole, at the artery's largest diameter. The media thickness (MT) was then calculated as the difference between intima-media thickness (IMT) and IT (MT=IMT-IT). IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface. Lumen diameter was defined as the distance between the leading edges of the intima-lumen interface of the near wall and the lumen-intima interface of the far wall (Wendelhag 1991).

5.7 Statistical analyses

Results are expressed as mean+/-SD. The non-parametric Spearmans rho was used to measure the associations between the SIC questionnaire and BYI scores with biological measures; salivary cortisol (SC) and catecholamine levels. One-way ANOVA was used for the analysis of salivary cortisol data. Cronbach's alpha was used for the analysis of internal consistency.

The relationship between two variables was assessed using bivariate scatter plots and the Pearson correlation coefficient. Coefficient of variation is the mean SD of differences between measurements divided by the mean of these measurements multiplied by 100. The vascular parameters IT, MT, and IMT were normally distributed. Two-way ANOVA and the Student t test were used to examine differences between arterial sites among the four age groups. Intima thickness, IMT, and IT-to-lumen diameter ratio demonstrated univariate associations (P=0.05) with age, BMI, and systolic blood pressure. A multiple, forward, stepwise linear regression analysis was performed using the vascular measures as dependent variables, and age, BMI, and systolic blood pressure as independent variables.

Silicone layer thickness obtained from weighing is called W and the measure of thickness obtained from ultrasonic imaging (UBM) is called U. Bland-Altman plots were constructed according to the formula: $X=(U+W)/2$ and $y=(U-W)$.

As the distributions of the BYI subscales were skewed, we used the lnskew0 transformation procedure provided by Stata to eliminate skewness. In all of these measures, skewness was reduced to less than +/- 0.001. The SDQ was normally distributed and remained unchanged. The RH-PAT score was log-transformed before analysis because of its skewed distribution. Unpaired t-tests for age, weight, height, BMI-SD, SBP, DBP, RH-PAT and the questionnaires were performed between sexes.

A linear regression model analysed the association between the log RH-PAT score with age, sex, BMI-SD, the five log BYI subscales and SDQ; and in separate analyses also adjusted for age. Age was modelled in 5 categories and as a continuous measure. The continuous measure was chosen as it explained more of the variance in the psychometric and vascular measures. The BYI and SDQ scores were divided into quartiles and the associations of each quartile with the RH-PAT score were shown in box-plots.

The analyses are also performed for girls and boys separately, and the mean BYI and SDQ scores are presented in box-plots by sex. We also assessed whether the association of the self assessed psychological factors with endothelial function, RH-PAT, varies by sex.

Where one factor (sex) modifies the association of two others (psychosocial factors with RH-PAT), this is known as effect modification. The statistical significance of effect modification can be assessed using interaction testing (Bland 2000). Interaction testing estimated the difference in association between sexes, by creating an interaction term for the stratifying variable (sex) with the characteristic (psychosocial factor) and examining the association of the interaction term with the dependent variable (Bland 2000, Osika 2006). Adjustment for the main effects of the interaction term (psychosocial factors and sex) ensures that the estimate for the interaction term is independent of these and is a true indicator of variation in association by sex (IV, Bland 2000). The measure of parental education (PE) was categorized into 5 groups, where PE5 was the highest level and used as a comparison group. The scores where then added together for each parent couple. We also assessed if single parenthood was a confounding factor. Statistical significance is indicated as $p<0.05$.

We analysed the data using SPSS (SPSS base 11.0 and 14.0 users´guide. New Jersey: Prentice Hall, 2005) and Stata 9.2 (Stata statistical software: Release 9.2. College Station, TX:Stata 2006).

6. Review of results and discussion

Measurement of psychological health

BYI, SiC (I, IV)

	Girls Gothenburg (n=126-134)	Boys Gothenburg (n=104-109)	Girls Karlskoga (n=50)	Boys Karlskoga (n=34)
Age	14.5±1.0	14.5±1.0	10.0±0.6	9.8±0.5
SiC	2.03±0.40	1.98±0.38	1.98±0.42	2.15±0.37
Depression	11.8±8.6	7.5±7.8	10.8±6.2	12.2±7.0
Anger	12.3±7.7	8.7±10.3	11.1±7.0	12.5±7.0
Anxiety	14.3±8.4	8.7±6.8	12.8±7.0	13.3±8.7
Disruptive behavior	5.7±5.9	6.5±8.6	3.6±3.2	5.3±4.3
Self concept	42.3±11.0	44.6±11.2	39.8±12.0	39.9±9.4

Table 1. Mean BYI subscale and SiC scores in Karlskoga (I) and Göteborg (II).

In study I the subjects were approximately 4 years younger than the subjects in study IV. In the older subjects there was a statistically significant difference between sexes, with higher BYI subscale scores for depression, anger and anxiety in girls compared with boys, which is consistent with data from the Swedish normative study Fig 6 (Tideman 2004). Moreover, our data are also consistent with the higher prevalence of depression seen in Swedish female adolescents compared with male adolescents measured using the Beck Depression Inventory (Olsson 1997), suggesting that gender differences in "psychological health" appears stable between 1997 and 2007 in Swedish children and adolescents.

Further data by Olsson et al. have shown that Swedish high school students (16-17 year-olds) girls have a higher prevalence of depression compared with boys. The one-year prevalence of major depression was 5.8% and the lifetime prevalence was 11.4%, with female to male ratio of four to one (Olsson 1999).

The reported higher prevalence of depression in females is also consistent with a report from the Swedish National Institute of Public Health (2006) based on data collected from 11, 13 and 15-year-olds, within the framework of the cross-sectional study "Health Behaviour in School-Aged Children". At age of 11 years, children feel better, have a healthier life-style and have a more positive attitude towards school than when they are older (13-15 years).

Self-rated health, somatic and mental problems and general well-being all worsen as children grow older, and the gap widens between boys and girls. Girls of all ages are less happy with life than boys of the same age. This difference has become more marked over the period between 1985 and 2005, and the contentment of 13- and 15-year-old girls in particular has declined.

Adult men report consistently fewer symptoms of depression than women, even though little or no sex differences in the *quality* of such symptoms have been found in community studies (Dryman 1991). The reason for this difference is not fully understood. *Gender* role-related stressors to which women are more exposed than men, such as low socioeconomic status, lack of power, and sexual abuse, and associated psychological attributes such as emotion-focused coping styles, interpersonal orientation and related vulnerability, anxiety and lowered self-esteem might explain some of the difference between men and women. Sex differences in endocrine stress reactions, as shown in study (I), might influence processes leading to depression (Piccinelli 2000).
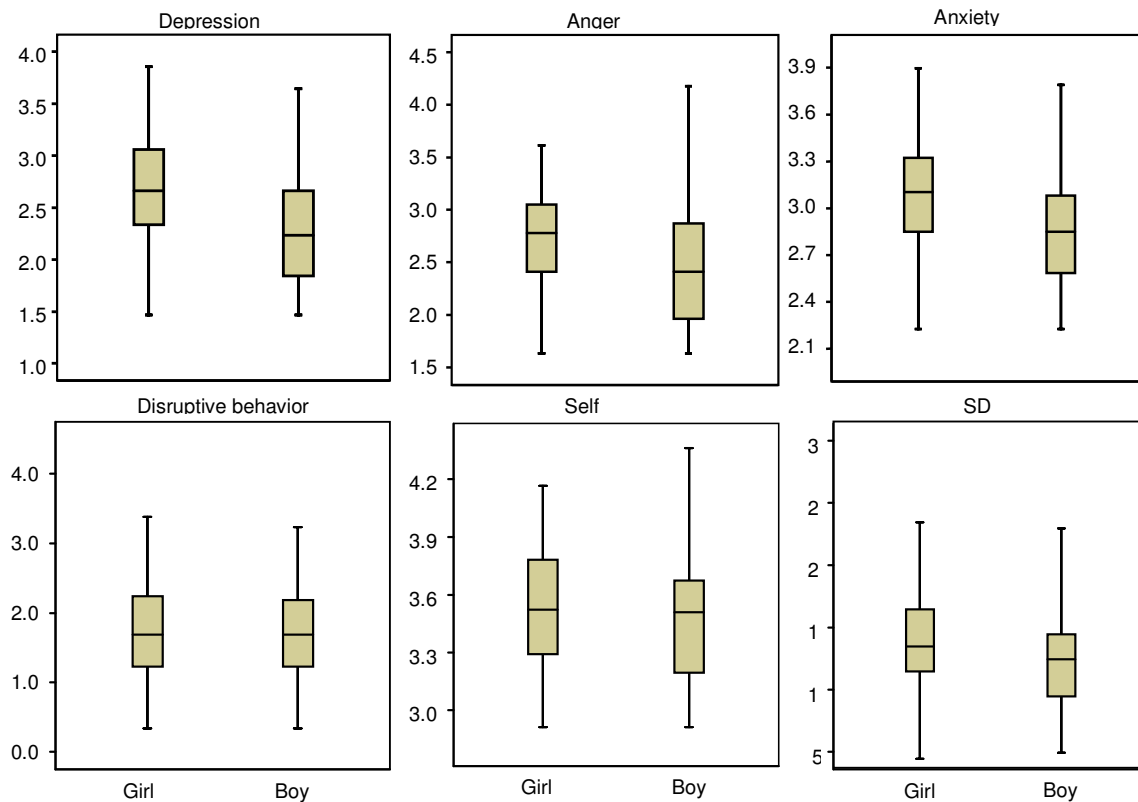
Figure 6. *Log-transformed depression-, anger-, anxiety-, disruptive behaviour-, self concept-, SDQ scores by sex (independent t-test, p<0.000 = ***). Each box shows the median and quartiles.*

Our results, showing a higher prevalence of psychological difficulties reported in girls than in boys, are consistent with previous studies, both for adolescents and adults. Girls are more likely than boys to internalise symptoms such as depression and anxiety. In contrast, boys have a greater tendency to externalize symptoms through processes such as "acting out" (Ostrov 1989), which may help to explain the differences between sexes.

One suggestion is that the difference in the prevalence of symptoms between the sexes which starts between 11-13 years might be influenced both by hormonal changes due to puberty, and to the different gender roles associated with adulthood. The level of oestrogen tends to increase in girls at these ages, and may interfere with the stress response (Shansky 2006).

The present SDQ results (IV) also revealed sex differences supporting the findings from BYI (IV), as the girls had higher scores than boys (14.2±4.1 vs 12.6±3.9 p=0.002). In the normative study by Smedje (1999) a younger population was studied (6-10 years), and they report a sex difference where boys had higher total score (median 7) than girls (median 6). The scores in our results are higher, and we observed a sex difference in the opposite direction compared to the normative study. However, our cohort was distinctly older. In our

study of BYI (I, IV) and in other studies (Hankin 1999, Sweeting 2003), the magnitude of symptoms tended to be more pronounced from age 13 years, and girls reported more symptoms than boys.

The SiC questionnaire did not show any statistically significant sex difference in either age group (I, IV). In order to study the psychometric properties of the new SiC questionnaire, a Rasch analysis was performed. The Rasch analysis is a member of the group of models based on latent trait analysis, focusing on the operating characteristics across the whole range of the latent trait and the properties of those characteristics. The Rasch model offers opportunities to examine latent trait constructs in a rigorous way, e.g. composite measures of subjective health (Hagquist 2001). It requires uni-dimensionality of the latent trait, and an underlying continuum from low to high for values of the trait is assumed.

The Rasch analysis indicated that the SiC questionnaire failed to measure subjective perceived stress according to the Rasch model of invariance across the latent trait, when all 21 items are categorized as in the original version. In order to find a set of items based on data that did not suffer from the problem of disordered thresholds, three items were removed: item 2 (I get headaches), 13 (I feel happy) and 17 (When I am sad I show it). The model worked consistently with respect to the response categories using the reduced set of 18 items and showed relative invariance across the latent trait in this population. Moreover, the reliability measured by Cronbach's alpha remained high (0.831) indicating no loss of precision. The new version of the SiC questionnaire will be analysed further in extended studies.

Measurement of neuroendocrine markers of stress
The saliva cortisol concentration showed significant variation between the different sampling times, (ANOVA, $p < 0.0001$) but no significant differences were found between the sexes when the analysis was limited to the subjects where all five saliva cortisol samples were valid (I). There was a statistically significant rise in saliva cortisol level between saliva cortisol at waking and after 15 minutes (mean diff 4.333 nmol/l, $p < 0.0001$) demonstrating the expected waking response (Fig 7).
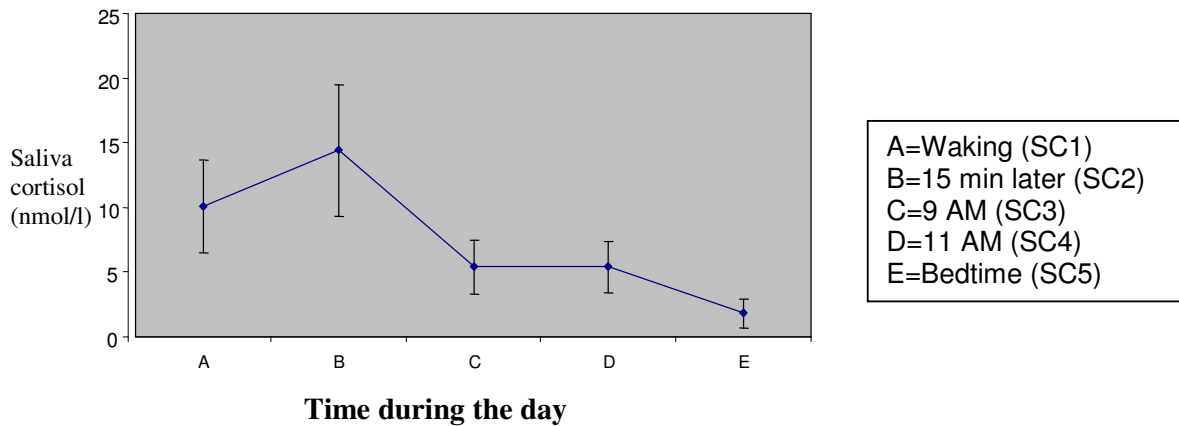
Saliva Cortisol in 10 year-old Children

Figure legend:
A=Waking (SC1)
B=15 min later (SC2)
C=9 AM (SC3)
D=11 AM (SC4)
E=Bedtime (SC5)

Figure 7. *Saliva cortisol measurements five times during a school day in children 10-12 years of age (I).*

When comparing the saliva cortisol results between the sexes with the inclusion of samples from subjects with less than all 5 saliva measures, girls had a higher level of saliva cortisol 15 min after waking (15.3 ±5.2 vs 12.6 ±4.9, p=0.037). No sex differences were seen for waking cortisol, but this may have been due to insufficient statistical power. A similar pattern was seen in a study by Rosmalen et al. (2005) who investigated 1141 children (10-12 years). This study found that the mean increase 30 minutes after waking was 3.79±6.93 nmol/l. Girls had significantly higher saliva cortisol levels for waking saliva levels and after 30 min (11.8±4.7, 16.0±6.8) compared with boys (11.2± 4.7, 14.7±6.3,), but no sex differences were seen at 08.00 PM (2.0±1.3). However, no psychological measures were investigated.

The urine adrenaline concentrations were statistically significant higher in boys than girls, whereas no sex differences were seen in noradrenalin concentrations or the metabolite 3-metoxy-4-hydroxymandelic acid. Noradrenalin in urine has a complex source, which makes the interpretation of our finding difficult (Goldstein 1995). Possibly boys had higher adrenal medullary activity, but not increased sympathetic neurotransmitter activity, that is noradrenalin, release. Caution should be used in interpretation these data, due to the imprecision of measuring cathecholamines in urine (Folkow 1983).

The relationship between psychological health and neuroendocrine markers of stress (I)

A statistically significant association between the global mean SiC questionnaire score and waking saliva cortisol was found (r = 0.302, p = 0.012).

When the analysis was stratified by sex, we found statistically significant associations between the SiC questionnaire scores with waking saliva cortisol (r = 0.477, p=0.001), saliva cortisol after 15 min (r = 0.313, p=0.038) and with the "area under the curve" (r = 0.330, p=0.049), among girls, but no statistically significant associations were observed among boys.

In additional studies, we analysed the associations between saliva cortisol measures and the five BYI subscales. The BYI self-concept scale (boys and girls together) was associated with waking saliva cortisol, (r = -0.259, p = 0.036) and with saliva cortisol 15 min later (r = -311, p=0.008). Thus a higher self-concept score, which is positively rated, is associated with lower morning saliva cortisol levels.

When the analysis was stratified by sex, statistically significant associations were seen in girls, but not in boys for self-concept with waking saliva cortisol (r = -0.528, p<0.000), and saliva cortisol after 15 minutes (r = -0.416, p=0.006). Furthermore, in girls there was a positive association between higher morning saliva cortisol measures and higher scores in anxiety, depression and anger. No statistically significant associations were seen between saliva cortisol measures and disruptive behaviour. No statistically significant associations between saliva cortisol and the BYI scales were observed among boys.

In conclusion, we observed a waking response for the cortisol release pattern and girls had statistically significantly higher saliva cortisol waking response levels than boys. These findings are consistent with the sparse earlier research in children (Rosmalen 2005).

In adults, Kunz-Ebrecht (2004) have shown that women have higher levels of waking cortisol than men on work days, and that women also had higher ratings for stress and lack of control than men. In students reporting high as opposed to low work load, those reporting greater work loads had higher average cortisol levels directly after waking. Further analysis indicated a sex difference with larger increases in chronically stressed women compared with stressed men (Schultz 1998). The sex difference shown in our study could be caused by several mechanisms. The difference in saliva cortisol levels could be due to physiological sex differences. The difference in association between saliva cortisol and psychosocial features between sexes could also be due to a physiological difference, or differences in the

perceptions and rating of psychosocial characteristics. The results could also be influenced by the relatively small sample of boys, making it more difficult to show any associations.

No notable associations were observed for the questionnaire scores with adrenalin, noradrenalin, or their metabolite 3-metoxy-4-hydroxymandelic acid. Neither was any association observed for the BYI with catecholamines and their metabolites.

Parental education (PE)

The distribution of the subject's PE was as follows: PE1 n=35, 15.7%, PE2 29, 13.0%, PE3 45, 20.2%, PE4 29, 13.0%, PE5 85, 38.1%. As mentioned in the background, the importance of socioeconomic circumstances such as PE is associated with CVD risk factors in childhood (Leino 2000, Kocaoglu 2005, Goodman 2005, Kivimäki 2006) and later mortality (Strand 2007), with lower PE conferring increased risk.

Measurement of vascular function

The RH-PAT score was 1.82 ±0.55 (range 1.07 to 5.11) for the entire study population (136 females and 112 males). The RH-PAT score underwent a *logarithmic* transformation due to its skewed distribution (0.56 ±0.27). No gender differences were seen and no statistically significant association with age could be found. Height and BMI-SDS were not associated with the RH-PAT score in either sex, and therefore cannot be a confounding factor in any of the analysis performed.

It has been demonstrated in adults that the RH-PAT score is highly correlated with measures of coronary and peripheral endothelial dysfunction (Kuvin 2003, Bonetti 2004). Although the RH-PAT is a rather new technique, the majority of data presented are for adults (Rosenthal, 1999, Kuvin 2006). Given the easy, non-invasive and user-independent approach, this technique is very suitable for use in children and adolescents. However, there are only a few results reported of studies in the young.

A lower RH-PAT score in children and adolescents with type 1 DM (1.63±0.5) compared with health controls (1.95±0.3, p=0.01) has been shown (Haller 2007). Bhangoo et al (2007, abstract) investigated 123 healthy 7th grade school children in Brooklyn, NY, (12.2 ±0.57 yrs) with BMI at least < 85th%. They did not find any association with BMI, blood pressure, waist circumference, and they found no sex differences. In general, they had a lower RH-PAT score (1.65±0.38), compared with our findings. Their lower scores could be because the entire study population had higher BMI, were younger, came from different ethnic background (approximately 70% were Hispanic) had a lower level of physical fitness or differed in other classical cardiovascular risk factors. Hence, our study providing normative data is to our knowledge the largest conducted among healthy school children.

Measurements of PWV (III) showed a clear positive association with age, SBP and DBP, but there was no difference between the sexes. That implies, that the observed sex differences in the radial and dorsal pedal artery IT, MT and IMT apparently do not affect this functional characteristic of the arterial system, at least not at this young age. Im (2007) studied the brachial-ankle PWV in healthy adolescents and demonstrated that boys had higher values than girls, suggesting somewhat less distensible arterial system in boys. However, the arterial sites investigated were not the same as in our study (we measured the radial-carotid PWV), and Im's study included subjects up to 18 years of age, where the difference between genders might be more pronounced compared with our younger subjects.

Validating the new very high resolution ultrasound technique (II, III)

Validation of the very high resolution ultrasound against silicone layers of different thicknesses showed a correlation coefficient of 0.98 (Fig 8). A Bland-Altman plot revealed no systematic error and a mean difference and standard deviation of 3.5±8.8 um. Thus there is good reason to believe that structures as small as in the order of 20 microns can be detected and depicted correctly by this ultrasonic image system.
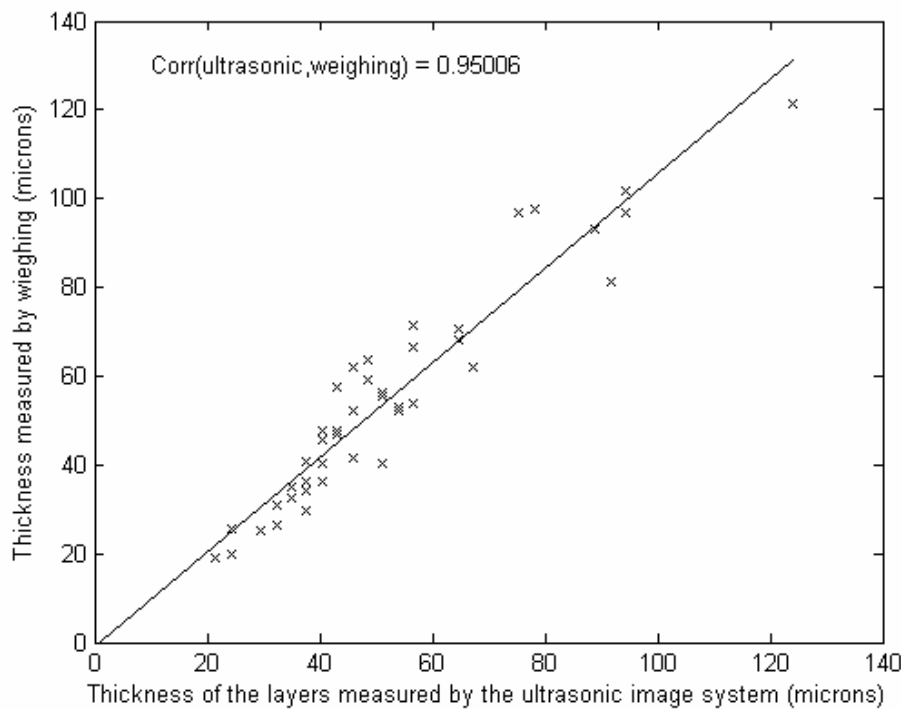

Figure 8. *Validation of the new high resolution (55 MHZ) ultrasound system; comparison with silicone layers of various thicknesses.*
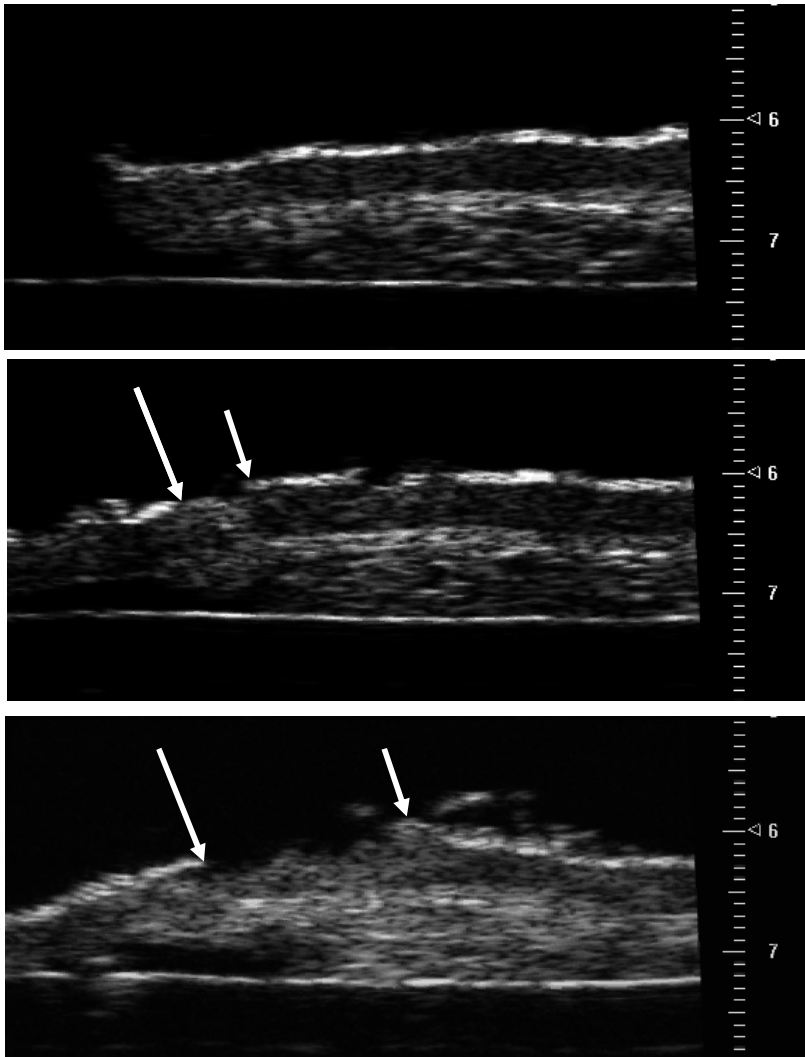
Figure 9. *Experiment showing imaging of vascular wall in vitro. The intima layer is cut, see white arrows), and the intima echo disappears (1 mm between numbers on the axis to the right).*

To further validate the characteristics of the new high resolution ultrasound using a non-fixed vascular wall specimen, a section of the superior mesenteric artery was cut obliquely and scanned along this cut (Fig 9). In the upper picture the intima is intact, whereas in the middle and bottom picture the white layer beneath the lumen disappears when the intima layer is removed. Arrows indicate were the cut begins, and where it continues through the intima and media layer.

The findings by Pesonen (2006) demonstrating coronary intima thickness of approximately 50um in the new born corroborate our estimates of intima thickness in young people. In a study by Zhdanov (2000), the mean thickness of the intima in the proximal part of the right coronary artery in males between the ages of 10–19 years was on average 72 µm (n=5, range: 9–120).

No backscatter signal influence on the imaging of the intima is observed, in contrast with what is described in older techniques with lower resolution. It should be emphasised that this new technique increases the resolution four to eight fold compared with older ultrasound systems (55 MHz vs. 7-15 MHz), allowing us study the pathophysiological processes affecting the intimal layer in greater detail.
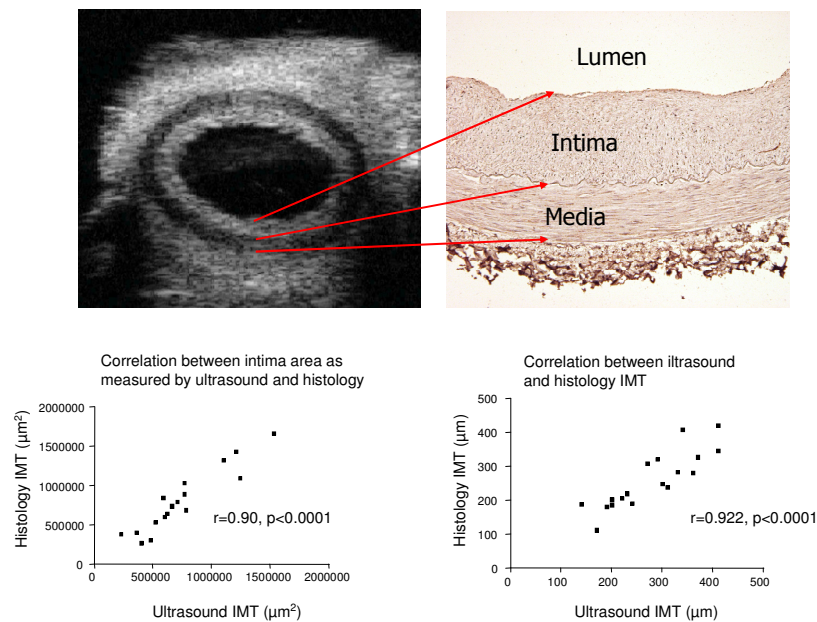

Figure 10. *Validation of a new, very high resolution image from human mesenteric artery (upper, left panel) and the same areas examined by histology (upper, right panel), clearly delineating IT and MT separate from each other. Validation experiments were performed on mesenteric artery specimens from 18 patients, and highly statistically significant correlations were found between ultrasound and histology, both calculated as IMT (lower, right panel) and IMT area (lower left panel).*

In human mesenteric vessels, we obtained sharp very high resolution ultrasound images, which were subsequently analyzed morphologically for comparison with the "gold standard". An example is shown in Figure 10, upper panels. The in vitro validation experiments performed on these arteries revealed high r values between the new ultrasound and gold standard histological measurements, in terms for both IT and intima area.

Validation of the new high resolution ultrasound measurements of peripheral arteries against carotid intima media thickness (IMT) assessment

There were positive correlations between carotid IMT and radial artery IT (r=0.53, P<0.0001) and between the carotid IMT and anterior tibial artery IT (r=0.44, P=0.0007) among material from children and adults. However, the correlation coefficients were rather modest between the arterial sites measured with different techniques, suggesting that other factors are also involved in determining IT, and that there is no fixed relationship between these measures.

The thickness of the common carotid intima-media structure as measured by ultrasound imaging represents a composite surrogate marker of atherosclerosis, even in an early phase, and predicts cardiovascular events in various populations (O´Leary 1999, Raitakari 2003,).

The atherosclerotic process starts morphologically within the intima. By examining these superficial radial and anterior tibial arteries it is possible to obtain a clearer picture of the intimal layer specifically, which may assist in the detection of changes occurring earlier than what can be observed presently through measurement of carotid IMT. Further, it makes it possible to study the relationship between different risk factors and specific pathophysiological processes in the intima, separately from processes in the media. An example of this concept is our finding of a thicker IT in the lower extremities compared with the IT in the upper limbs.

When IT and MT were combined, in the traditional way of measuring IMT, the IMT increased with age for both radial and anterior tibial arteries and no difference could be detected between the arterial sites.
When IT and MT were considered separately, it became clear that the anterior tibial artery showed a thicker intima and thinner media compared with the radial artery, particularly in the oldest age group. Furthermore, it is also relevant to consider the different luminal size of radial and anterior tibial arteries in terms of IT. Thus, we calculated the IT-to-lumen ratio. Study (II) revealed not only increasing values for this ratio with ageing, but also a consistently larger ratio for the anterior tibial artery throughout the age range studied. This suggests that IT may represent the anatomic site of atherosclerotic structural changes, whereas MT augmentation might be a hypertrophic adaptive sign of increased blood pressure load. The more pronounced IT of the anterior tibial artery in all age groups may be related to higher blood pressures in upright positions as a sum of the hydrostatic and dynamic cardiac pressure inputs. The marked difference in radial IT but not MT between the PAD subjects and the healthy age- and sex-matched controls supports this contention. Hence, the new

very high resolution ultrasound provides the possibility to conduct more detailed analyses of the different vascular wall layers and their specific features and pathophysiological processes.

IT, MT, and IMT and Age

IT and MT increased with age (from 10 to 90 years), showing high correlation coefficients with age in both the radial (IT, $r=0.74$; MT, $r=0.63$; $P<0.0001$ for both) and the anterior tibial (IT, $R=0.68$; MT, $r=0.51$; $P<0.0001$ for both) arteries.

The age-dependent intimal thickening in peripheral arteries and carotid IMT changes, and the previously shown inverse relationship between brachial FMD and carotid IMT in the young, (Jounala 2005) suggest that limited nitric oxide bioavailability may promote vessel wall thickening. Hence, as the endothelium/intima ages and is exposed to further damage, it becomes thicker as shown here and loses some of its anti-atherogenic properties, such as nitric oxide release.

PWV (III) showed a clear positive association with increasing age, SBP and DBP.

Sex differences in vascular wall features in the young

To further extend our knowledge of specific vascular wall features in the young we investigated a cohort of 12-16 year-old children (III, IV, and Fig 4).

	Girls	Boys	
Radial artery	Mean ±SD	Mean ±SD	p-value
Diameter (mm)	1.716±0.318 (n=138)	1.825±0.313 (n=113)	.007
Intimal thickness (mm)	0.054±0.008 (n=138)	0.057±0.010 (n=113)	.031
Medial thickness (mm)	0.153±0.025 (n=137)	0.176±0.033 (n=113)	.000
Intimal-medial thickness (mm)	0.207±0.026 (n=137)	0.232±0.035 (n=113)	.000
Intimal thickness /diameter (mm)	0.033±0.009 (n=138)	0.032±0.009 (n=113)	.518

Dorsal pedal artery

Diameter (mm)	1.143±0.313 (n=135)***	1.189±0.433 (n=111)***	.329
Intimal thickness (mm)	0.060±0.014 (n=135)***	0.060±0.013 (n=111)*	.714
Medial thickness (mm)	0.149±0.034 (n=131)	0.160±0.039 (n=103)***	.022
Intimal-medial thickness (mm)	0.209±0.037 (n=131)	0.222±0.041 (n=103)*	.016
Intimal thickness /diameter (mm)	0.057±0.026 (n=135)***	0.060±0.031 (n=111)***	.586

Table 2. Summary of key variables regarding arterial wall characteristics.

P values for the sex difference using independent *t* tests.
Statistical significant difference between the two arterial sites, using paired sample *t* tests, defined as *p <0.05 ** p <0.01 *** p <0.001

When comparing distal arterial sites at the upper and lower extremities, the radial artery had a larger lumen diameter (and a larger MT and IMT in boys), whereas IT was larger in the dorsal pedal artery. Hence, IT/lumen ratios were higher in the dorsal pedal artery than in the radial artery.

We were able to reproduce the finding from II that a separately measured intimal layer was thicker in the dorsal pedal artery than in the radial artery.

	RAD IT		RAD MT		RAD IMT		DPA IT		DPA MT		DPA IMT	
	Coeff	P-val	Coeff	P-val	Coeff	P-val	Coeff	P-val	Coeff	P-val	Coeff	P-val
SBP	0.000	0.555	0.000	0.089	0.000	0.074	0.000	0.904	0.000	0.346	0.000	0.382
DBP	0.000	0.571	0.000	0.286	0.000	0.396	0.000	0.920	0.000	0.991	0.000	0.818
Male Female	**0.003**	**0.046**	**0.020**	**0.000**	**0.023**	**0.000**	**0.001**	**0.731**	**0.013**	**0.053**	**0.012**	**0.035**
Age	0.001	0.119	0.003	0.286	0.004	0.056	0.004	0.000	0.002	0.171	0.007	0.006
BMI-z	-0.001	0.103	0.004	0.049	0.003	0.164	0.000	0.829	0.002	0.095	0.004	0.100

Table 3. Multiple linear regression analyses of the radial artery IT, MT and IMT with the independent variables.

RAD=radial artery, DPA=dorsal pedal artery, IT= intima thickness, MT=medial thickness, IMT = intima media thickness, Coeff = B, regression coefficient, p-value for association with the dependent variable. Adjustment for age, sex, SBP, DBP, BMI-z.

The adjusted associations of radial IT, MT and IMT with sex, age BMI-z and blood pressure are shown in table 3. When adjusting for sex, age, SBP, DBP and BMI-z, sex was the sole variable independently associated with radial IT and IMT, while both sex and BMI-z were associated with the radial MT. The dorsal pedal artery IT had a positive association with age, but no sex differences could be demonstrated. The sex difference for dorsal pedal artery IMT prevailed after adjustment, and the other statistically significant association was with age. For PWV, there was no difference between sexes, implying that the observed sex difference in the peripheral arteries IT, MT and IMT do not affect the functional characteristics of the arterial system.

These findings may indicate processes that help to explain why the increase in CVD risk starts at a younger age in men compared with women. When examining this healthy and representative population, we were convinced of the existence of sex differences in vascular wall structures.

Autopsy studies have demonstrated that the earliest lesions of atherosclerosis, fatty streaks, are already formed in foetal aortas, (Napoli 1997) and that the atherosclerotic process progresses throughout life. By puberty, fatty streaks appear to be gradually transformed into more advanced atherosclerotic lesions (Napoli 1999, Stary 1989, Strong 1995). Young females between ages 15-19 years in the Pathobiological Determinants of Atherosclerosis in Youth study had more extensive fatty streaks in the abdominal aorta, whereas young males had more of such fatty streaks in the thoracic aorta. Young adolescent males have more extensive raised lesions in the right coronary artery than females (Strong 1995).

In asymptomatic adults it has been demonstrated that males have thicker carotid IMT compared to women (Bhuiyan 2006) measured non-invasively, but in childhood and adolescence there are several studies with contrasting results. These studies were not able to identify sex differences in terms of carotid IMT (Sass 1998, Jourdan 2005, Tonstad 1996). In children with CVD risk factors such as overweight (Woo et al 2004), diabetes mellitus (Järvisalo, 2002) and hypertension (Litwin 2004), carotid IMT (and in the latter citation also femoral IMT) was found to be higher than normal in control groups, but without sex differences.

In conclusion, most studies have not been able to detect carotid IMT differences between the sexes in childhood and adolescence. However, our study clearly shows that such differences exist in the radial and dorsal pedal artery which can be explained either by specific properties of these vascular sites or by methodological limitations. The use of high resolution ultrasound techniques made it possible for us to obtain more detailed pictures of the wall structures in these arteries of superficial distal upper and lower extremity.

It is of course difficult to fully understand what these differences between the sexes mean in the long term, although it is tempting to speculate that the males are more prone to develop abnormal vascular changes in the medial and intimal layer, indicating earlier onset of CVD in males than females. A possible explanation is that hormonal factors influencing these vascular features. The direct effect of oestrogen on the vasculature promotes vasodilatation and inhibits the development and progression of atherosclerosis, at least in pre-menopausal women. Oestrogen receptors on vascular endothelial and smooth muscle cells bind oestrogen with high affinity (Mendelsohn 1999, Naessen 2006), which might mediate protective effects on the vascular wall with the resulting thinner intimal and medial layers observed in girls by our study. Hence, morphological ultrasound observations of the radial or dorsal pedal arteries in childhood - in the absence of autopsy data - add important in vivo information about structural vascular processes and differences between the sexes.

The relationship of vascular function with self perceived psychological health, parental education and sex (III, IV).
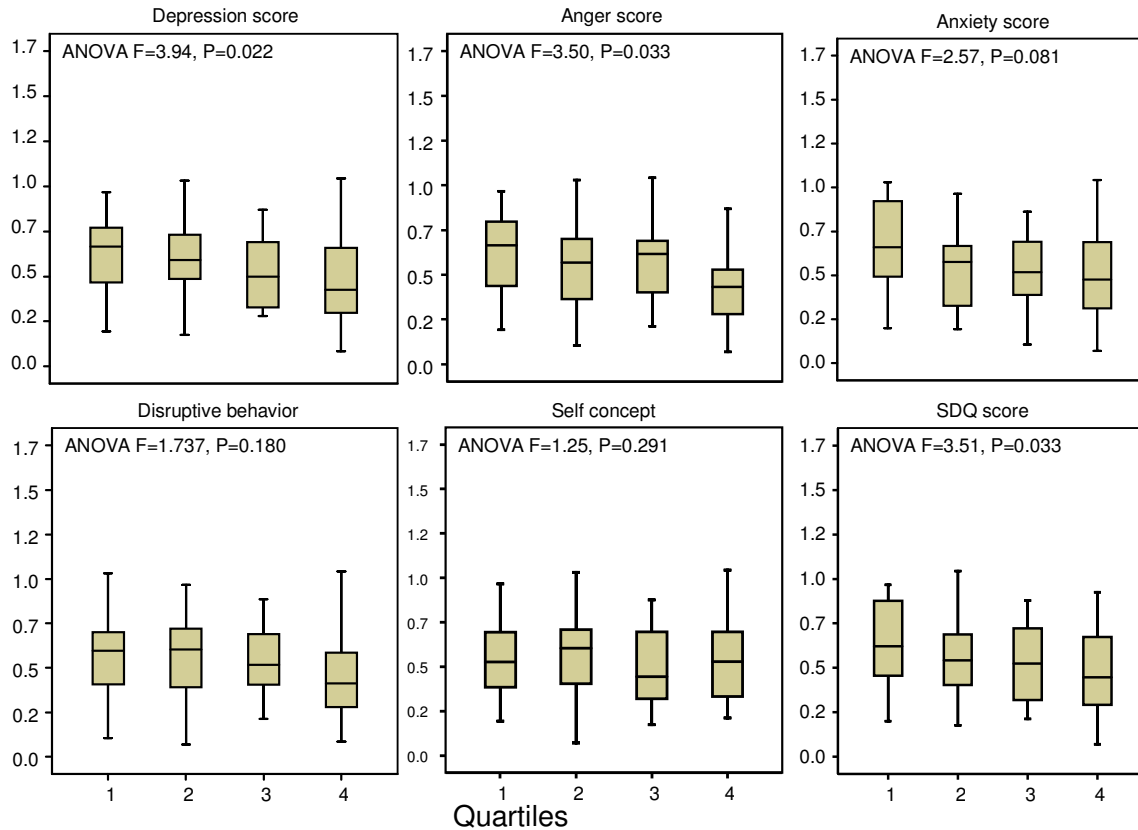

Figure 11. *Log-transformed depression-, anger-, anxiety-, disruptive behaviour-, self concept-, SDQ scores in girls, in relation to RH-PAT, adjusted for age.*

Among the entire study cohort the Strengths and Difficulties Questionnaire score has a statistically significant negative association with the RH-PAT score (Table 11), that is, more difficulties were associated with attenuated endothelial function (B -0.009, CI 95% -0.018 to 0.000). In girls the association was -0.013, (-0.024 to -0.001) between the SDQ and RH-PAT. No association was seen for boys. The test of interaction between sex and SDQ for the association with RH-PAT was not statistically significant.

The association of SiC questionnaire score with RH-PAT was only statistically significant for girls, with a coefficient of -0.113 (CI 95% -0.223 to -0.003).

We further studied the associations between RH-PAT and the BYI in boys and girls separately. In girls we found statistically significant associations between lower RH-PAT scores and higher scores for anger, depression, and anxiety but not for disruptive behaviour and self concept (Tab 3. Fig 2). After adjustment for both age and parental education in girls, the association between RH-PAT and anger was -0.091 (CI 95% -0.184 to 0.003), indicating that parental education was a confounding factor for the association. For depression the association with RH-PAT produced a coefficient of -0.089 (CI 95% -0.171 to -0.007) and for anxiety -0.118 (CI 95% -0.244 to 0.008).

The association of the SiC global mean score with RH-PAT produced a coefficient of -0.107 CI 95% (-0.220 to 0.005), and level of parental education was also a confounding factor here. In boys there was a statistically significant positive association between the RH-PAT score and the disruptive behaviour scale, but not for any of the other scales. In boys the association between disruptive behaviour and a higher RH-PAT produced a coefficient of 0.088 CI 95% (0.023 to 0.153) after adjustment for both age and parental education.

The association between the anger subscale and RH-PAT was in opposite directions for girls and boys; in girls the association was negative, but positive in boys (Tab 4).

We analysed whether sex was a true effect modifier for the association between psychosocial factors. For anger there was a statistically significant difference in association with RH-PAT, by sex as the interaction test produced a B coefficient of -0.188 (CI 95% -0.315 to -0.061). The interaction between disruptive behaviour and sex was -0.135 (-0.220 to -0.050) for the association with RH-PAT and therefore statistically significant. No statistically significant effect modification by sex was observed for anxiety, depression, self-concept, SDQ or the SiC.

These sex differences indicate the specific pattern of connections between psychosocial factors and endothelial function is different for girls and boys.

We also investigated whether PE was associated with RH-PAT, and in girls lower PE was associated with a lower RH-PAT score: PE1, B -0.077, (-0.206 to 0.052), PE2, B -0.181, (-0.330 to -0.033), PE3, B -0.158, (-0.280 to -0.036), PE4, B -0.045, (-0.193 to 0.103), compared with the highest level of education, "PE5", and adjusted for age. No such association was observed in boys.

There is consistent evidence that low socioeconomic status, as defined by occupational position, income, or education is a major risk factor for CVD and this association differes by sex. In women, the social gradient seems to be even stronger than in men (Brezinka 1995, Raine 2002). Several studies with a life-course approach to socioeconomic position found that socioeconomic disadvantage in childhood and in later life were both associated with

increased CHD risk in women (4-fold, Kuper 2006) and a twofold risk of dying from CHD in men (Wamala 2001, Lawlor 2005). Our results finding an association between lower PE and an attenuated RH-PAT seen only in girls, indicates that girls may be more susceptible to the types of exposure associated with such disadvantage.

The association between anger and coronary heart disease was described initially by investigators from the Framingham Heart Study, who reported that suppressed anger independently predicted the eight-year incidence of coronary heart disease among both men and women (Haynes 1980). Trait anger predicted the progression of IMT levels in middle-aged women over three years (Räikkönen 2004), and anger proneness in adults predicts coronary heart disease risk (Williams 2000). In a study by Shimbo et al. 2007, an anger-provoking interview compared with a neutral interview impaired endothelium-dependent and independent vasodilation.

The biological mechanisms and processes by which psychosocial characteristics influence CVD risk are yet to be unravelled. Heightened sympathetic arousal and excessive circulating catecholamines are known to cause direct damage to the endothelium (Haft 1974). Sympathetic activation impairs endothelium-dependent FMD in healthy subjects (Hijmering 2002). Possibly as a result of increased sympathetic activity, or due to raised blood pressure itself, higher blood pressure tracks from childhood into adulthood, and may augment CVD risk (Bao 1995). Stress related endothelial dysfunction can be prevented by blocking cortisol production with metyrapone, demonstrating a direct or facilitative role for cortisol in the development of endothelial dysfunction (Broadley 2005).

In study I there was an association between higher saliva cortisol levels and psychosocial difficulties in girls, but not in boys, and we found an association between attenuated endothelial function and psychosocial difficulties in girls (IV).

In adults associations of vascular function with mental stress and depression have been demonstrated (Ghiadoni 2000, Rajagopalan 2001). The findings among young school children without known risk factors of ischemic heart disease makes it likely that psychological health indicated by self-assessed levels of anger, depression and anxiety and higher difficulties scores is implicated in the mechanisms responsible for lower RH-PAT scores in girls. Collectively, both chronic and acute stress are important factors, probably operating through the ANS and HPA systems, thus affecting the vasculature. In addition, local vascular receptor families like the endothelins may also be involved (Spieker 2002).

Depression has been associated with abnormalities in the hypothalamic pituitary axis, the sympathoadrenal system, and cytokine action, all of which may result in disturbances of the nitric oxide pathway, thereby influencing the endothelial function. However, our observations do not allow us to conclude which, if any, of these mechanisms are responsible for the associations between lower RH-PAT score and higher levels of anger, depression and anxiety scores in girls.

Dong (2004) found a dose-response relationship between adverse childhood experiences and adult ischemic heart disease, concluding that in childhood psychological factors appear to be more important than traditional risk factors for ischemic heart disease risk. Batten (2004) showed that childhood maltreatment was associated with an almost nine-fold increase in CVD among women but not men, in a representative sample of more than 5000 adults. Thus, a history of childhood maltreatment appears to remove the lower CVD risk usually found in women.

In Europe, about 55% of all female deaths are caused by CVD, especially coronary heart disease and stroke, compared with 44% of all male deaths (European Cardiovascular Statistics 2005).

Age-adjusted mortality for CVD has declined steadily over the last four decades, but to a lesser extent in women than in men. In fact, the temporal trend for the incidence of CVD even shows a rise among women (Tunstall-Pedoe 1999, AHA 2006). The older age onset of CVD in women (70 years) compared with men (60 years) may be influenced by oestrogen deficiency post-menopause. This may be related to an increase in mortality, as 38% of women die within one year of an initial unrecognized myocardial infarction, compared with 25% of men (Bello 2004).

The risk gradient in CHD has been ascribed to psychosocial stressors of the work environment, mainly in terms of Karasek and Theorell´s (high demand-low control) job strain model and Siegrist´s effort-reward imbalance model (Schnall 1990, Bosma 1997, Peter 2000). They reports odds ratios for CVD from 1.2 to 5.0 for job strain, and from 1.5 to 6.1 with for effort reward imbalance. Recent evidence suggests that women who are employed in male-dominated jobs (such as higher management or mechanical jobs) have a two-fold increased risk of myocardial infarction compared with those in female dominated jobs, such as nursing (Peter 2006).

The relationship between employment and CHD risk is in women is clearly complex. The protective effect of employment seems to be more pronounced in women who have

professional and managerial occupations than those with blue collar jobs (Brezinka1995). However there is evidence that employed women with children have an increased risk of CHD, perhaps because of the double load of work and family can result in anger and frustration due to lower control over their lives (La Rosa 1988). Recent results are consistent with this, as the Stockholm Female Coronary Risk Study (Wang 2007) found that women's double exposure to stress from work and family may accelerate coronary disease processes in women, whereas relative protection may be obtained from a satisfactory job and a happy marriage.

7. Summary and conclusions

What was known before the study?

I.   Psychological health is relevant to CVD in adults

II.   Imaging of early vascular changes is possible, but limitations in resolution does not allow more specific imaging of different wall layers

III.   Males have a higher coronary heart disease lifetime risk both in middle and old age and an increased magnitude of atherosclerosis at other arterial sites, compared with women

V.   HPA axis activity is related to stress and cardiovascular and metabolic activity in adults

What this study adds

I.   Psychological health is already associated with endothelial function in children and adolescents, and the association is more pronounced in girls than boys. Measuring endothelial function in an operator independent manner (RH-PAT), can result in lower variability, and makes it convenient for large-scale field studies.

II.   The new, very high resolution ultrasound produces four-fold higher resolution compared with older techniques, which makes it possible to study changes in vascular wall layers in greater detail. Measuring the intima layer separately might provide new insights in the early atherosclerotic process, and also

	contribute to the understanding of how various risk factors influence the disease process.
III.	There are sex differences in childhood and adolescence in peripheral vascular structure. Boys have thicker IT, MT and IMT in radial, and MT and IMT in dorsal pedal arteries compared with girls
VI.	HPA axis activity is related to self perceived psychosocial characteristics in 10-12 year old girls.

8. Clinical relevance and perspectives

There is strong evidence that CVD has its origins in childhood, and it is essential to detect markers of early vascular pathogenesis and investigate how and to what extent prevention strategies can be developed for use among children. Childhood cardiovascular risk factors and various forms of adversity track into adulthood. Thus, poorer psychological health and psychosocial factors are associated with CVD in adults, and this association is also likely to have its origin in childhood. The present studies display a link between psychological features and vascular function at an early age. Current guidelines for childhood prevention of adulthood CVD, do not consider psychosocial factors as risks (Kavey 2006), as the focus is on hypertension, obesity and dyslipidemia. Increased focus on psychosocial and socioeconomic factors is warranted.

There is a difference between the sexes in prevalence and incidence of CVD and risk factors, with males having a higher lifetime CVD risk, but females having a higher prevalence of depression. This paradoxical difference could be due in part to hormonal influences. Beyond genetic and biological differences, the socially constructed roles for men and women, and certainly also for boys and girls, result in different social norms and expectations. Consequently, the resulting emotions, behaviours, and attitudes are typical and desirable for each sex, and might influence the associations between vascular function and psychological features in childhood, and in adulthood between depression and CVD.

The findings of an association between self-perceived psychological health and endothelial function in childhood underlines the importance of maintaining a broad perspective on childhood and adolescence health, providing the rationale for an increased focus on children psychological health, thereby minimizing adult CVD risk.

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