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II Utsal L, Tillmann V, Zilmer M, Mäestu J, Purge P, Saar M, Lätt E, Jürimäe T, Maasalu K, Jürimäe J. Serum interferon gamma concentration is associated with bone mineral density in overweight boys. *Journal of Endocrinological Investigation* 2014; 37: 175–180.

III Utsal L, Tillmann V, Zilmer M, Mäestu J, Purge P, Saar M, Lätt E, Maasalu K, Jürimäe T, Jürimäe J. Negative correlation between serum IL-6 level and cardiorespiratory fitness in 10- to 11-year-old boys with increased BMI. *Journal of Pediatric Endocrinology and Metabolism* 2013; 26: 503–508.

In all papers, Liina Remmel (formerly Utsal) had primary responsibility for protocol development, participants'enrollment, performing measurements, data analysis, and writing the manuscripts.

ABBREVIATIONS

BA	bone area
BMAD	bone mineral apparent density
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
CRP	C-reactive protein
DXA	dual-energy X-ray absorptiometry
EGF	epidermal growth factor
FFM	fat free mass
FM	fat mass
FM %	fat mass percentage
HOMA	homeostasis model assessment
HOMA-IR	homeostasis model assessment-estimated insulin resistance
hsCRP	high sensitive C-reactive protein
IFN-γ	interferon-γ
IL-1α	interleukin-1α
IL-1β	interleukin-1β
IL-2	interleukin-2
IL-4	interleukin-4
IL-6	interleukin-6
IL-8	interleukin-8
IL-10	interleukin-10
LS	lumbar spine
MCP-1	monocyte chemotactic protein-1
NWB	normal weight boys
OWB	overweight boys
SF	skinfold
TB	total body
TNF-α	tumor necrosis factor-α
VEGF	vascular endothelial growth factor
VO_2peak	peak oxygen consumption
WC	waist circumference
WHR	waist-to-hip circumference ratio

1. INTRODUCTION

Obesity, a condition resulting from excess adipose tissue, has an adverse impact on health. In recent decades, childhood obesity has increased rapidly in Western societies, as well as in developing countries (Chen et al. 2012; Story et al. 2003; Yu et al. 2012). However, there is some data suggesting that the prevalence of childhood obesity in some European countries has levelled off (Livingstone 2001). Obesity starting from a relatively young age has been shown to have more negative effects on health than obesity starting later in life (Fontaine et al. 2003). Different studies have reported that childhood obesity is associated with increased risk of cardiovascular diseases (Meyer et al. 2006; Raj 2012). On the other hand, increased cardiorespiratory fitness is associated with reduced risk of cardiovascular diseases (Martins et al. 2010; Rodrigues et al. 2007). Hyperlipidemia and impaired glucose tolerance both increase the risk of childhood obesity and further cardiovascular diseases (Kim et al. 2010; Schwarzenberg & Sinaiko 2006). Excessive fat accumulation in childhood has also been shown to be associated with more frequent bone fractures (Dimitri et al. 2010; Ducher et al. 2011), indicating a link between adipose tissue and bone tissue. This is particularly important during puberty, when bone growth is coupled with excessive fat accumulation during this period. Obesity in prepuberty and puberty has been found to increase the risk of osteoporosis and obesity later in life (Dimitri et al. 2010; Hanks et al. 2010).

Changes in lifestyle, such as increased sedentary lifestyle, and changes in diet, such as increased caloric intake, have been considered to be key factors responsible for the continuous increse in the prevalence of childhood obesity (Berndtsson et al. 2007; Carlson et al. 2012; Cottrell et al. 2012; Limbers et al. 2008; Tremblay & Willms 2003). Increased physical activity in childhood, both in children with normal weight and in those with obesity, has been found to be associated with lower risk of developing cardiovascular diseases in adulthood (Berndtsson et al. 2007; Eliakim et al. 2006; Gaeini et al. 2009; Katzmarzyk et al. 1999; Mota et al. 2006; Rosa et al. 2011). However, the exact mechanisms as to how these changes in lifestyle and diet will lead to the accumulation of fat mass (FM) are not yet fully understood. Recent studies have shown that an inflammatory process is involved in the development of obesity (Aygun et al. 2005; Sacheck 2008; Todoric et al. 2011). It has now been established that adipose tissue is also a source of various inflammatory markers (Fried et al. 1998; Lee & Choue 2009). In adulthood, obesity has been found to be associated with systemic inflammation (Brinkley et al. 2012; Unek et al. 2010), whereas in children the link between obesity and inflammation is not so well established. Accordingly, the general aim of this dissertation was to study the associations between different serum inflammatory markers and characteristics of body composition, bone health, and cardiorespiratory fitness in boys with different body mass index (BMI) values at the beginning of puberty.

2. REVIEW OF THE LITERATURE

2.1. General overview of obesity

According to the World Health Organisation, obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue to the extent that health may be impaired (WHO 2000). Obesity is an increasing health problem all around the world and children are a major concern in this trend (Rocher et al. 2008). Obesity is especially widespread among American children, and has significantly increased in recent decades (Skelton et al. 2009; Story et al. 2003). Choi et al. (2013) recently described obesity itself or the risk of developing obesity as concerning nearly one third of all North American children and adults. Obesity is also increasing among Chinese children and adolescents (Yu et al. 2012) and in many European countries (Jarosz et al. 2007; Pizarro & Royo-Bordonada 2012; Serra 2003). There is little data about the prevalence of obesity in Estonian children and adolescents. A study of 9–11-year-old children in five European countries showed that the lowest rate of children who were overweight was found in Estonian children, compared to Belgian, Cyprian, Italian and Swedish children (Pigeot et al. 2009). The other study of children in eight European countries aged 2–9 years showed that the lowest percentage of overweight children, including those who were obese, was in Estonia, at 13.6% (Bammann et al. 2013).

It is well known that childhood obesity is associated with increased risk of metabolic syndrome (Juonala et al. 2011; Meyer et al. 2006; Raj 2012; Weiss et al. 2004). A study of the Danish population showed that higher body weight during childhood was associated with an increased risk for coronary heart disease in adulthood, and that this association was stronger in boys than in girls (Baker et al. 2007). Even a small loss in weight (5–10%) can improve lipid profiles, insulin sensitivity, and reduce risk of thrombosis (Aronne & Isoldi 2007). Larsson et al. (2011) found that overweight Swedish children (aged 10) had significantly higher waist circumference (WC), insulin levels, and homeostasis model assessment (HOMA) indexes than children of normal weight. In this study, BMI correlated positively with fasting insulin levels and HOMA index, both well-established risk factors for metabolic syndrome (Larsson et al. 2011). A study of Estonian and Swedish children showed that improvement in cardiorespiratory fitness throughout childhood and adolescence was associated with reduced risk of becoming overweight (Ortega et al. 2011). In addition, obesity also affects quality of life, which has been found to be lower in obese children than their healthy peers (Pinhas-Hamiel et al. 2006; Quaresma et al. 2009).

In conclusion, obesity among children is an increasing problem all over the world with a severe impact on long-term health. Therefore, every new aspect in the understanding about the development of obesity and its impact on health should improve the effectiveness of interventions to tackle this problem.

2.2. Obesity as an inflammatory process

2.2.1. General overview

Recent studies have shown that obesity is an inflammatory process (Gordero et al. 2012; Heredia et al. 2012; Todoric et al. 2011). Excessive adipose tissue can influence the development of inflammation in two ways: Ectopic fat storage causes an intracellular inflammatory response and altered adipokine secretion by adipocytes. The latter is closely associated with the development of insulin resistance, which in the long-term leads to the development of type 2 diabetes. A high level of inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) can predict the development of type 2 diabetes in adults (Pradham et al. 2001). Type 2 diabetes and many different cardiovascular diseases in adulthood have their roots in childhood: Obesity in childhood increases the risk of developing these diseases in adulthood (Balas-Nakash et al. 2013; De Boer et al. 2013). High serum CRP levels have been shown to be associated with higher body mass and being overweight or obese in a large population of European children, including Estonians. Children with a higher baseline serum CRP concentration showed higher increases in their BMI z-scores and central adiposity over time, and were at higher risk of becoming overweight or obese during the study (Nappo et al. 2013). Galcheva et al. (2011) found that those with increased trunk FM, as measured by WC, had significantly higher blood pressure, HOMA index, insulin, total cholesterol and triglyceride levels compared to those with normal WC. Serum CRP concentration increased proportionally with the degree of abdominal obesity, and, after controlling for adiposity, CRP was significantly correlated with systolic blood pressure (Galcheva et al. 2011). Central (truncal) adiposity in particular has been linked to systemic inflammation and further development of atherosclerosis (Festa et al. 2001; Park et al. 2005). Brooks et al. (2010) have shown that inflammation is a key factor in the development of atherosclerosis. Ridker et al. (2000) found that many inflammatory markers, such as CRP and IL-6, were risk factors in predicting future cardiovascular diseases.

Greater cardiorespiratory fitness in children and adolescents has been found to be associated with a lower risk of developing cardiovascular diseases in adulthood (Martins et al. 2010; Rodrigues et al. 2007). Ekelund et al. (2007) found that cardiorespiratory fitness and physical activity were both independently associated with metabolic risk factors in European children, including Estonians. A study of Estonian and Swedish children, aged 9–10, showed that cardiorespiratory fitness was associated with cardiovascular risk factors more strongly than physical activity (Hurtig-Wennlöf et al. 2007). A study of children from Portugal, Denmark, and Estonia showed that at least one hour of moderate intensity physical activity per day was enough to reduce the risk factors for cardiovascular disease (Andersen et al. 2006). High aerobic fitness has been found to be associated with low-grade inflammation in 9-year-old children

(Steene-Johannessen et al. 2013), as well as in adults aged 55–66 years with type 2 diabetes (Jennersjö et al. 2012).

2.2.2. Biochemical inflammatory markers

White adipose tissue is a biochemically active organ producing different hormones and peptides. White adipose tissue from lean individuals secretes predominantly anti-inflammatory adipocytokines, such as adiponectin and interleukins (interleukin-4 (IL-4), interleukin-10 (IL-10), and interleukin-13 (IL-13)), whereas adipose tissue from obese individuals produces more pro-inflammatory adipocytokines, such as tumor necrosis factor-α (TNF-α) and IL-6. Different inflammatory markers have been studied in obese adults and children. Some of them, such as CRP, IL-6, and TNF-α, have been studied more extensively than others (Aygun et al. 2005; Caballero et al. 2008; Ford et al. 2005; Sacheck 2008; Sumarac-Dumanovic et al. 2009). Inflammatory markers such as IL-6, CRP, and TNF-α have been found to be increased in the serum of obese children (Alvarez et al. 2009; Arslan et al. 2010; Aygun et al. 2005; Caballero et al. 2008; Ford et al. 2005; Herder et al. 2007; Kim et al. 2010; Sacheck 2008; Sumarac-Dumanovic et al. 2009).

Cytokine IL-6 is a pro-inflammatory cytokine that is synthesised in adipose tissue. It is also an anti-inflammatory myokine that can be derived from muscle tissue (Petersen & Pedersen 2006). This multifunctional cytokine plays a role in the inflammatory process, tissue injury, and in the pathogenesis of osteoporosis and obesity (reviewed by Papanicolaou et al. 1998). Increased serum IL-6 levels have been found in subjects with higher BMI, compared to those with normal BMI (Arslan et al. 2010; Aygun et al. 2005; Herder et al. 2007; Kim et al. 2010). Increased serum IL-6 levels are also associated with increased insulin resistance and further development of type 2 diabetes (Arslan et al. 2010).

C-reactive protein (CRP) is an acute-phase protein and is one of the most commonly used inflammatory markers in clinical practice (De Boer 2013; Szalai et al. 1997). It can be measured as "classical" CRP (detection limit above 0.04 mg/L) and also as highly sensitive CRP (hsCRP, detection limit below 0.04 mg/L). Serum CRP is synthesised in the liver and increases during infection, inflammation, or trauma (Du Clos 2000). Elevated serum CRP levels may predict further development of type 2 diabetes (Ridker 2007). In addition, Calabro et al. (2005) showed that adipocytes can produce CRP under the stimulation of several pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-β. Furthermore, Ford et al. (2005) found that children and adolescents with metabolic syndrome have elevated CRP concentrations. Serum CRP and IL-6 levels were positively correlated with BMI and body fat percentage in children (Parrett et al. 2010). It has been found that adipose tissue is positively correlated to serum CRP levels in obese prepubertal children (Parrett et al. 2010). From the many studies of inflammatory markers, only serum CRP concentration was

positively associated with visceral adipose tissue in a group of overweight children (Maffeis et al. 2008).

Another inflammatory marker, TNF-α, is a multifunctional immunoregulatory cytokine (Okamatsu et al. 2009; Sack 2002). This pro-inflammatory cytokine has effects in a diverse array of tissues, in cellular differentiation, growth, and apoptosis (Sack 2000,Sack 2002), and also a role in the development of metabolic syndrome (Plomgaard et al. 2005). This cytokine is produced by adipocytes (Okamatsu et al. 2009; Petersen & Pedersen 2006). In addition, TNF-α stimulate osteoclast formation *in vivo* and *in vitro* (Roodman 2001). Higher serum TNF-α levels have been found in obese adult subjects compared to lean subjects (Maachi et al. 2004). In children and adolescents the studies have given contradictory results. Although Herder et al. (2007) did not find correlations between serum TNF-α level and BMI in adolescents, Breslin et al. (2012) and Rosa et al. (2011) both found that serum TNF-α levels were significantly higher in obese children compared to normal weight children. It has also been suggested that elevated serum TNF-α (and also IL-6) levels in obese children may indicate an early phase of atherosclerosis (Arslan et al. 2010).

Other inflammatory markers, such as interleukin-2 (IL-2), IL-4, interleukin-8 (IL-8), IL-10, vascular endothelial growth factor (VEGF), interferon-γ (IFN-γ), monocyte chemotactic protein-1 (MCP-1), epidermal growth factor (EGF), interleukin-1α (IL-1α), and interleukin-1β (IL-1β) have been less investigated (Sumarac-Dumanovic et al. 2009; Tam et al. 2010).

Interleukin-2 (IL-2) is an inflammatory cytokine regulating the immune system (Gaffen & Liu 2004). The major function of this marker is to promote the spread of $CD4^+$ and $CD8^+$ T-cells (Gaffen & Liu 2004). Diseases such as obesity, metabolic syndrome, and atherosclerosis are considered to be inflammatory diseases, and T-cells are involved in the pathogenesis of these conditions (Łuczynski et al. 2012). Rosa et al. (2011) found that IL-2 levels were significantly higher before and throughout a 30-minute intermittent exercise test in the overweight group compared to the control group, whereas Aygun et al. (2005) found opposite results: Serum IL-2 levels were significantly lower in obese children compared to their peers of normal weight.

Interleukin-4 (IL-4) is an anti-inflammatory cytokine derived from T-cells (Okamatsu et al. 2009; Rainsford & Reen 2002), and its main function is to activate T- and B-cells (Rainsford & Reen 2002). Rosa et al. (2011) found overweight children to show a trend towards higher IL-4 levels compared to normal weight peers, but this trend was non-significant. Okamatsu et al. (2009) could not detect any IL-4 levels in any of the 99 samples studied for overweight or normal weight children.

Interleukin-8 (IL-8) is a pro-inflammatory cytokine secreted mainly by macrophages, but also by endothelial or other epithelial cells, and has roles in inflammation, cell recruitment, and lymphoid trafficking (Bosch et al. 2002). Cytokine IL-8 can also be derived from the adipocytes (Waters et al. 2007). Its

main function is to induce chemotaxis in target cells, causing them to migrate toward the site of infection. There are very few studies that look at IL-8 levels in obesity. Breslin et al. (2012) and Rosa et al. (2011) did not find a significant difference in serum IL-8 levels between children who were overweight and those of normal weight. Interestingly, IL-8 may play a role in reducing muscle soreness after exercise training (Buford et al. 2009).

Anti-inflammatory cytokine IL-10 has a role in immunoregulation and inflammation. It regulates the release of immune mediators, antigen presentation, and phagocytosis (Sabat et al. 2010). Serum IL-10 also stimulates the maturation of B-cells and T-cells (Okamatsu et al. 2009; Sabat et al. 2010). Lower levels of serum IL-10 have been associated to states of inflammation, tissue injury, and obesity (Arslan et al. 2010). Low IL-10 has been seen in patients with obesity, metabolic syndrome, and type 2 diabetes (Arslan et al. 2010), as well as in obese children and adolescents (Glowinska & Urban 2003; Okamatsu et al. 2009).

The role of MCP-1 is as a chemical messenger (Hillenbrand et al. 2012). This protein has an important role in the development of obesity and diabetes, by affecting endothelial cells and expanding and remodelling adipose tissue (Panee et al. 2012). Higher serum MCP-1 levels have been found in overweight children compared with their healthy controls (Breslin et al. 2012), but in adolescents no correlation was found between MCP-1 and WC (Herder et al. 2007).

Vascular endothelial growth factor (VEGF) is a vascular permeability factor that increases the permeability of small blood vessels (Bates et al. 2002). Serum VEGF plays a role in the stimulation of vasculogenesis and in the formation of the lymphatic vascular system (Tammela et al. 2005). As an angiogenic polypeptide, it is involved in the development of many cells, including adipose and muscle cells (Claffey et al. 1992). In adults, serum VEGF concentration has been found to be correlated with BMI and visceral fat (Loebig et al. 2010; Miyazawa-Hoshimoto et al. 2003). In obese children, a trend toward higher VEGF levels was found compared to the non-obese subjects, but the difference was not statistically significant (Siervo et al. 2012). However, increased BMI was associated with increased serum VEGF levels (Siervo et al. 2012).

Interferon-γ (IFN-γ) is a T-cell-derived cytokine, and its main function is to fight against viral and intracellular bacterial infections (Schoenborn et al. 2007). It has been suggested that IFN-γ may also be involved in the pathogenesis of obesity (Todoric et al. 2011). Serum IFN-γ has been found to be associated with Hedgehog signalling in white adipose tissue (Todoric et al. 2011). Studies of adults (Sumarac-Dumanovic et al. 2009) and children (Rastogi et al. 2012) did not find a significant difference in serum IFN-γ concentration between obese and lean subjects. However, the percentage of IFNγ-positive T-helper cells in obese children has been positively associated with non-alcoholic steatohepatitis, insulin level, and the HOMA index (Pacifico et al. 2006).

Epidermal growth factor (EGF) is a growth factor whose role is to stimulate cell growth, proliferation, and differentiation (Barnham et al. 1998). Increased serum EGF levels have been found in obese children compared to lean children, and EGF level correlated with increased BMI (Schipper et al. 2012).

Two quite similar cytokines belonging to the IL-1 family, IL-1α and IL-1β, are involved in inflammation (Tack et al. 2012). They have similar biological characteristics, but they vary in terms of localisation, maturation, and secretion (Tack et al. 2012). These cytokines bind to IL-1 type I receptors (Tack et al. 2012). The pro-inflammatory marker IL-1α is produced as a biologically active form, while IL-1β reaches its biological activity after contacting the cysteine protease caspase-1 (Tack et al. 2012). In addition, IL-1α inhibits insulin signalling in adipocytes, whereas IL-1β affects insulin signalling and action by targeting the insulin receptor substrate (He et al. 2006; Jager et al. 2007). Studies have shown that both markers play an important role in obesity (Tack et al. 2012; Um et al. 2011). Um et al. (2011) showed that IL-1α plays a role in the development of simple obesity, whereas IL-1β plays a role in the development of obesity associated with insulin resistance (Tack et al. 2012). Jung et al. (2010) showed a trend in overweight adolescents toward higher serum IL-1α levels compared to lean subjects, but this was not statistically significant. Serum IL-1β levels have been found to be significantly higher in obese children compared to the control group (Aygun et al. 2005).

2.2.3 Interactions between different inflammatory markers

Various cytokines are related to each other and act on the body as an ensemble. Many of them are associated with adipose tissue (Coppack 2001) and the immunoregulatory system (Patel et al. 2009). Cytokines are inflammatory mediators that may have a pro-inflammatory effect, i.e. aggravate the inflammatory response (e.g. IL-6) or have anti-inflammatory properties, i.e. reduce inflammation (e.g. IL-10) (Stoner et al. 2013). Chemokines are a large group of structurally similar and chemotactically active cytokines, such as IL-8 and MCP-1 (Stoner et al. 2013). The cytokines and chemokines are released by activated macrophages at the beginning of the inflammatory process (Murphy 2012). Some cytokines express an inhibitory effect and others stimulatory effects on the synthesis of other cytokines, making the systems very complex and comprehensive. As mentioned, CRP is synthesised in the liver by hepatocytes as part of the acute response to the stimulation by IL-6, and to a lesser degree by TNF-α and IL-1β (Calabro et al. 2005; Stoner et al. 2013). For example, TNF-α stimulates the production of IL-6 and consequently CRP (Petersen & Pedersen 2006; Stephens & Pekala 1991). Cytokine IL-6 anti-inflammatory effects are demonstrated by stimulating the production of IL-10 (Petersen & Pedersen 2006). In addition, IFN-γ decreases the production of IL-4 by the Th2 cells, but it also potently blocks the effects of IL-4 on B-cells (Gattoni et al. 2006). Cytokine IL-2 is a growth factor and expansion factor for

T-helper cells, and it stimulates the synthesis and production of IL-4, IFN-γ, and TNF-α, while IL-10 has an inhibitory effect on IFN-γ, IL-2, and TNF-α (Gaffen & Liu 2004; Gattoni et al. 2006; Patel et al. 2009). Serum MCP-1 has been found to be involved in attracting monocytes in adipose tissue and to secrete pro-inflammatory cytokines such as IL-6, TNF-α and IL-1β in response to IFN-γ (Roth et al. 2011).

Various studies have investigated the associations between different biochemical markers. For example, serum IFN-γ levels correlated positively with CRP (Patel et al. 2009), IL-6 (Roth et al. 2011), TNF-α (Prondzinsky et al. 2012), and IL-1β (Roth et al. 2011). Roth et al. (2011) also found that in obese children IL-6 was significantly associated with IL-8 and IL-1β, whereas IFN-γ was also associated with IL-8 and IL-1β. Serum TNF-α was associated significantly with IL-8, MCP-1, and IL-1β levels, and IL-8 with IL-1β in obese children (Roth et al. 2011).

In conclusion, although there have been many studies looking at the role of different biochemical markers in the development of obesity, very few studies have looked at ten or more biochemical markers simultaneously. Studying a large number of different biochemical markers at the same time should give us new information about the role of inflammation in the development of obesity.

2.3. The impact of obesity on bone development in children

It is well known that the main bone mass acquisition occurs during the first two decades of life (Compston 2001). Maximal bone mass acquisition occurs during puberty, and about 85–90% of human bone mass is acquired before adulthood (Ausili et al. 2012). Body mass has been found to be one of the main determinants of bone mineral density (BMD) (Rhie et al. 2010). However, it appears that bone mineral content (BMC) is not different in overweight children compared to normal weight children, even though overweight subjects had slightly higher BMC values (Ellis et al. 2003). With the rise in obesity worldwide, there is also an ongoing debate about whether excess adiposity has a protective or detrimental effect on bone health in children and adults (Dimitri et al. 2012). Studies about the impact of body fat on bone health in children have given conflicting results (Ackerman et al. 2006; Clark et al. 2006; Dimitri et al. 2010; Wey et al. 2011). It has been found that body fat % and trunk fat % are negatively correlated with BMD (Lu et al. 2011), while a positive correlation between FM and BMC and BMD has been found in prepubertal (Clark et al. 2006; Cole et al. 2012; Pollock et al. 2010) and pubertal children (Pietrobelli et al. 2002; Wang et al. 2007), as well as in adolescents (El Hage et al. 2010; Hong et al. 2010). However, other studies have found that obese children had lower BMC and BMD after adjustment for their body size when compared to normal weight children (El Hage et al. 2010; Goulding 2000; Rocher et al.

2008). Fintini et al. (2011) showed that there was a negative impact by obesity on BMD in boys aged 12–16 years. Rocher et al. (2008) found that obesity did not have a protective effect on bone development in prepubertal children. In contrast, Clark et al. (2006) found that FM was a positive independent determinant of bone mass and bone size in prepubertal children.

Being overweight in childhood has been linked to increased risk of future bone fractures (Dimitri et al. 2012; Mobley et al. 2005). Overweight children with a previous fracture have reduced BMC for their fat free mass (FFM), suggesting that FM inhibits bone mineral accrual in children with previous fractures (Dimitri et al. 2010). It has also been shown that despite having greater bone size, overweight children tend to have reduced volumetric BMD (Cole et al. 2012; El Hage et al. 2011; Rocher et al. 2008). While increased FM correlates with increased BMC, it also correlates with lower volumetric BMD (Cole et al. 2012). This work suggests that normal weight, overweight, and obese children all have slightly different pathways in bone mass acquisition.

Many serum inflammatory markers, such as IL-6, IL-1β, CRP, and TNF-α, have been found to be elevated in obesity (Aygun et al. 2005; Caballero et al. 2008; Ford et al. 2005; Kim et al. 2010; Sacheck 2008; Sumarac-Dumanovic et al. 2009) and associated with bone health (Barbour et al. 2012; Ding et al. 2008; Hanks et al. 2010). For example, serum CRP level was found to be a significant predictor of osteoporotic fractures in elderly women (Nakamura et al. 2011). Increased serum IL-6 concentration has been found to be associated with an increased risk for hip fractures in postmenopausal women (Barbour et al. 2012). Serum IL-6 levels also predicted bone loss and bone resorption rate in 50–79-year-old adults, suggesting that targeted inflammation-modulating therapy may prevent osteoporosis (Ding et al. 2008). However, there is limited data about the role of inflammatory markers in the development of obesity and bone accumulation in children (Dimitri et al. 2012; Hanks et al. 2010). Cytokine IL-6 and tumor necrosis factor receptor-2 (TNFR-2) have been associated with low BMC in 7–12-year-old children of normal weight (Hanks et al. 2010). However, a study of overweight prepubertal children did not find a significant association between BMC and another inflammatory marker, serum CRP levels (Pollock 2010). Valuable information about the role of different inflammatory markers in bone health has been obtained from animal studies (Duque et al. 2011; Zhang et al. 2011). For example, Duque et al. (2011) described IFN-γ as being produced locally in the bone by cells of immune origin, whereas EGF binding to its receptor (EGFR) plays an important role in bone metabolism and bone development in mice (Zhang et al. 2011).

In conclusion, although there are different studies looking at the roles of various biochemical markers on bone mass acquisition, very few studies have looked at them in concert by using a panel of different biochemical markers. An approach using a panel of many markers simultaneously should provide us further information about the role of inflammation in bone health.

2.4. The impact of obesity on cardiorespiratory fitness in children

Cardiorespiratory fitness is the ability of a subject to perform aerobic exercise. Absolute peak oxygen consumption (VO_2peak), an accurate measure of aerobic capability, is a parameter characterising cardiorespiratory fitness (Gaeini et al. 2009) and is expressed in l/min. Another parameter used to characterise cardiorespiratory fitness is relative VO_2peak (VO_2peak/kg), which is calculated as VO_2peak per kilogram of body mass and expressed in ml/min/kg (Berndtsson et al. 2007). Many studies have shown that the cardiorespiratory fitness level of children is best determined by absolute VO_2peak (Gaeini et al. 2009; Nemet et al. 2003). The absolute VO_2peak increases steadily with age in children and adolescents of normal weight, as well as those who are obese (Berndtsson et al. 2007). In contrast to the normal weight group, no age differences were seen in relative VO_2peak in obese children and adolescents (Berndtsson et al. 2007). Overweight children had significantly lower relative VO_2peak than those of normal weight (Berndtsson et al. 2007; Eliakim et al. 2006; Esmaeilzadeh et al. 2012; Gaeini et al. 2009; Rosa et al. 2011). Klasson-Heggebo et al. (2006) found that relative VO_2peak was associated with WC and the sum of 4 skinfolds (SF) in 9-year-old children. In addition, Stratton et al. (2007) found that cardiorespiratory fitness level decreased over a 6-year period in both obese and normal weight children aged 9–11. However, in daily life activities, relative VO_2peak/kg is more important than absolute VO_2peak. Therefore, although obese children may have similar absolute VO_2peak values to normal weight children, their relative VO_2peak values are lower and they perform more poorly in weight-dependent physical activities (Berndtsson et al. 2007).

It is well known that a low level of both cardiorespiratory fitness and physical activity has been associated with obesity, many cardiovascular diseases, and type 2 diabetes (Llorente-Cantarero et al. 2012). Some investigators have also shown that greater cardiorespiratory fitness in children and adolescents is associated with a lower risk of cardiovascular diseases in adulthood (Martins et al. 2010; Rodrigues et al. 2007). Children with higher levels of physical activity and consequently higher levels of cardiorespiratory fitness had better body composition and biochemical markers related to cardiovascular disease risk (Cordova et al. 2012). Children and adolescents with high cardiorespiratory fitness have better health (Padilla-Moledo et al. 2012) and lower frequency of obesity (Byrd-Williams et al. 2008).

Serum IL-6 and CRP were negatively correlated with relative VO_2peak in children (Gaeini et al. 2009), as well as in adults (Kullo et al. 2007), whereas serum TNF-α levels were positively correlated with absolute VO_2peak in children (Nemet el al. 2003). Rosa et al. (2011) studied changes in a group of different inflammatory markers during an acute cycling exercise by children and found that overweight children had higher exaggerated responses of serum IL-6 and TNF-α levels; however, no correlations were found between resting serum

levels of these markers and absolute or relative VO_2peak. In 7- to 11-year-old boys, cardiorespiratory fitness decreased progressively as BMI increased (Esmaeilzadeh et al. 2012).

In conclusion, although there have been many studies looking at the impact of obesity on cardiorespiratory fitness, very few studies have looked at the role of different inflammatory markers in these associations. An approach studying the relationship between a panel of different biochemical markers and cardiorespiratory fitness in obese and normal weight children should provide us new information about the role of inflammation in cardiorespiratory fitness among obese children.

3. AIMS OF THE STUDY

The general aim of this dissertation was to study the associations between serum inflammatory markers and body composition, bone health, and cardiorespiratory fitness in boys aged 10–11 years with different BMI values.

The specific aims were:

1. to study the levels of serum inflammatory markers between overweight and normal weight boys (Study I);
2. to examine the relationships between serum levels of inflammatory markers and body composition in overweight and normal weight boys (Study I);
3. to examine the relationships between serum levels of inflammatory markers and bone mineral characteristics in overweight and normal weight boys (Study II);
4. to examine the relationships between serum levels of inflammatory markers and cardiorespiratory fitness in overweight and normal weight boys (Study III).

4. METHODS

4.1. Participants

The study included 76 Estonian schoolboys aged between 10 and 11 years, who were recruited from 11 local schools in Tartu City (n=59) and from 9 schools in Tartu County (n=17). The participants were divided into two groups. The overweight boys (OWB) group included 36 boys with BMI above the 95th centile, and 2 children with BMI between the 85th and 95th centile. The normal weight boys (NWB) group included 38 boys with BMI below the 85th centile. Each participant and their parents completed a questionnaire about current acute or chronic illnesses, and only boys who reported themselves healthy and without any acute or chronic illnesses, were recruited. Both groups of boys took part in obligatory physical education lessons twice per week at school. Pubertal development of the participants was assessed by self-report using an illustrated questionnaire according to the Tanner classification (Tanner 1962). All boys were in their ordinary everyday diet and they were also asked not to change their eating habits. The study was approved by the Medical Ethics Committee at the University of Tartu (Estonia), and study participants' parents signed an informed consent form.

4.2. Anthropometry

Body height was measured to the nearest 0.1 cm using Martin`s metal anthropometer. Body mass was measured to the nearest 0.05 kg using medical electronic scales (A & D Instruments Ltd.; Abingdon, UK). Body mass index (BMI) was also calculated (kg/m^2). Nine skinfold (SF) (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh, medial calf, and mid-axilla) thicknesses were measured on the right side of the body. All SF, waist circumference (WC), and hip circumference were measured according to the protocol recommended by the International Society for the Advancement of Kinantropometry (Marfell-Jones et al. 2006). Waist-to-hip circumference ratio (WHR) was calculated as an indicator of body fat distribution. Skinfold thickness measurements were taken with a Holtain (Crymmych, UK) skinfold caliper. All measured SF were also summarized (sum of 9 SF) as an indicator of total subcutaneous body fat.

4.3. Bone mineral density and body composition

Total body (TB) and lumbar spine (LS) bone mineral density (BMD) (g/cm^2), TB bone mineral content (BMC) (g) and TB bone area (BA) (cm^2) were measured by dual-energy X-ray absorptiometry (DXA) using the DPX-IQ densitometer (DPX-IQ; Lunar Corporation; Madison; WI, USA) and a software,

version 3.6. Bone mineral apparent density (BMAD) (g/cm^3), an estimate of volumetric BMD, was calculated as previously described (Katzman et al. 1991): 1) TB BMAD = BMC/(TB BA^2/body height); 2) LS BMAD = LS BMC/LS $BA^{3/2}$. In addition, the expression of TB BMC for height was calculated to adjust for TB bone size (El Hage et al. 2011). TB FM, TB FM percentage (FM %), trunk FM, trunk FM % and FFM were also measured by DXA. Participants were scanned in light clothing while lying flat on their backs with arms on their sides. The fast scan mode and standard subject positioning were used for TB measurements. Analysis was performed using an extended analysis option. A single examiner evaluated all DXA measurements and results. Intra-subject variations of bone mineral and body composition measurements were less than 2%.

4.4. Maximal exercise testing

Cardiorespiratory fitness was determined by a stepwise incremental exercise test until volitional exhaustion using an electrically braked bicycle ergometer (Corival V3; Lode, Netherlands). Initial work rate was 50 W and was incremented by 25 W after every 3 min until volitional exhaustion. Pedalling frequency was set to 60–70 rpm. Participants were verbally encouraged to produce maximal effort. Respiratory gas exchange variables were measured throughout the test using breath-by-breath mode with data being recorded in 10 s intervals. During all tests, the subjects breathed through a facemask. Oxygen consumption, carbon dioxide output, and minute ventilation were continuously measured using a portable open-air spirometry system (MetaMax I, Cortex, Leipzig, Germany). The analyzer was calibrated with gases of known concentration before the test according to the manufacturer's guidelines. All data were calculated by means of computer analysis using standard software (MetaMax-Analysis 3.21, Cortex). Peak oxygen consumption (VO_2peak) (l/min) was measured and VO_2peak per kilogram of body mass (VO_2peak/kg; ml/min/kg) was calculated.

4.5. Blood analysis

Blood samples were obtained from a vein before breakfast, between 8 a.m. and 9 a.m., after an overnight fast. The blood serum was separated and then frozen at –80°C for further analysis. Using Evidence® Biochip Technology (Randox Laboratories Ltd), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), vascular endothelial growth factor (VEGF), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-1α (IL-1α), interleukin-1β (IL-1β), monocyte chemotactic protein-1 (MCP-1), epidermal growth factor (EGF), and high sensitive C-reactive protein (hsCRP) were measured from fasting serum samples. There were no detection

limits or sensitivity problems with any markers measured. The Cytokine and Growth Factors High-Sensitivity Array of the Biochip system was used for simultaneous quantitative detection of multiple, related cytokine immunoassays (in parallel) from a single sample. None of the biochemical markers were below detection limits. Intra-assay precision was 5.1–8.5%, and inter-assay precision was 5.8–9.9% for all measured markers. Insulin was analysed using Immulite 2000 (DPC, Los Angeles, USA). The intra- and interassay CVs were less than 5% and 12%, respectively, at an insulin concentration of 6.6 μU/ml. Glucose was measured with a commercial kit (Boehringer, Mannheim, Germany). Insulin resistance index was calculated using homeostasis model assessment (HOMA-IR): fasting insulin (μU/ml) x fasting glucose (mmol/L)/22.5 (Wallace et al. 2004).

4.6. Statistical analysis

All statistical analyses were performed with SPSS (Chicago, IL, USA) 15.0 for Windows. Standard statistical methods were used to calculate means and standard deviations. Evaluation of normality was performed with the Shapiro-Wilks method. Variables not normally distributed were log-transformed. An unpaired, independent two-tailed t-test or Wilcoxon's (Mann-Whitney) rank-sum test were used to assess differences in measured parameters between OWB and NWB groups. Relationships between inflammatory markers and other measured variables were assessed by Pearson correlation analysis. Stepwise multiple regression analysis was performed to determine the independent effect of the 13 different measured inflammatory parameters on measured body composition, bone mineral, and cardiorespiratory fitness characteristics in OWB and NWB groups. A P value of less than 0.05 represented statistical significance.

5. RESULTS

5.1. Body composition, cardiorespiratory fitness, and serum inflammatory markers in overweight and normal weight boys

The main characteristics of the participants are presented in Table 1. As expected, OWB were taller and heavier, and more frequently in Tanner stage 2 or 3 than were NWB (34 vs. 28) (Table 1). As expected, mean VO_2peak was significantly higher and VO_2peak/kg significantly lower in OWB than in NWB (Table 1). FM and FFM values were significantly higher in OWB compared to those in NWB (Table 1). TB BMD, LS BMD, TB BMC, TB BMC for height and TB BA were significantly higher, and TB BMAD significantly lower in OWB compared to the respective values in NWB (Figure 1). In addition, the OWB group had 10 boys who had experienced at least one bone fracture, compared to 15 boys in the NWB group, but this difference was not statistically significant.

Table 1. Main characteristics of the subjects (mean ±SD).

	NWB (n=38)	OWB (n=38)	Total (n=76)
Age (yrs)	11.0±0.8	11.2±0.7	11.1±0.7
Body height (cm)	146.0±7.5	153.2±7.8*	149.5±8.4
Body mass (kg)	36.8±5.6	64.5±12.0*	50.3±16.7
BMI (kg/m^2)	17.2±1.7	27.3±3.6*	22.1±5.8
Sum of 9SF (mm)	75.7±32.5	223.4±44.4*	147.7±83.7
WC (cm)	60.3±4.9	82.2±8.7*	71.0±13.0
WHR	0.82±0.04	0.88±0.07*	0.85±0.06
TB FM %	20.4±7.0	41.9±6.0*	30.8±12.6
TB FM (kg)	7.3±3.4	26.1±8.4*	16.4±11.4
TB FFM (kg)	27.6±3.7	35.0±4.9*	31.1±5.7
Trunk FM %	17.8±7.7	41.3±6.9*	29.1±13.8
Trunk FM (kg)	2.7±1.6	11.6±4.0*	7.0±5.4
VO_2 peak (l/min)	1.8±0.3	2.1±0.3*	2.0±0.4
VO_2 peak/kg (ml/min/kg)	48.9±6.4	33.7±4.7*	41.1±9.4
Tanner stage (1|2|3|4|5)	1.88±0.61 (10|25|3|0|0)	2.00±0.46 (4|30|4|0|0)	1.94±0.54 (14|55|7|0|0)

* Significantly different between NWB and OWB; P<0.05.

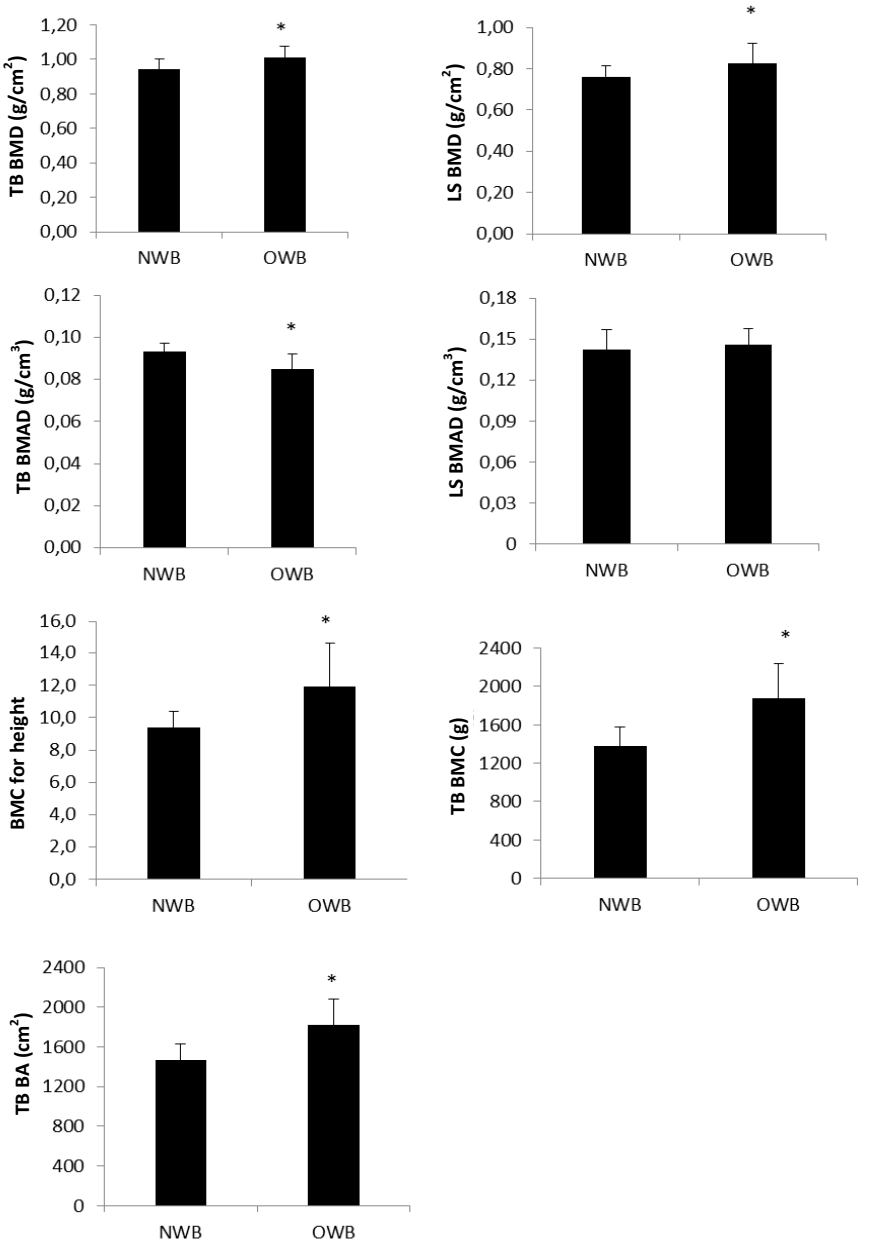

Figure 1. Bone mineral characteristics of the participants (mean ±SD). Significantly different between NWB and OWB; * $P<0.05$.

Out of the 13 inflammation-related markers, OWB had significantly higher serum IL-6, IL-8, IFN-γ, MCP-1 and hsCRP values compared to NWB (Table 2). In addition, OWB had significantly lower blood glucose, but significantly higher insulin and HOMA-IR values (Table 2).

Table 2. Inflammatory and insulin resistance parameters in the study population (mean ±SD).

	NWB (n=38)	OWB (n=38)	Total (n=76)
IL-2 (pg/ml)	1.8±0.9	1.7±0.8	1.8±0.8
IL-4 (pg/ml)	2.2±1.1	2.3±1.3	2.2±1.2
IL-6 (pg/ml)	0.8±0.3	1.1±0.6*	1.0±0.5
IL-8 (pg/ml)	7.7±2.9	9.5±3.6*	8.6±3.3
IL-10 (pg/ml)	0.8±0.4	0.7±0.2	0.8±0.3
VEGF (pg/ml)	94.0±61.1	111.6±59.7	102.6±60.6
IFN-γ (pg/ml)	1.5±1.0	2.2±1.2*	1.8±1.1
TNF-α (pg/ml)	4.3±2.0	4.8±1.8	4.6±1.9
IL-1α (pg/ml)	0.2±0.3	0.4±0.4	0.3±0.3
IL-1β (pg/ml)	1.3±0.7	1.4±0.7	1.3±0.7
MCP-1 (pg/ml)	165.8±67.9	212.8±58.0*	188.7±67.2
EGF (pg/ml)	22.8±17.4	25.4±15.0	24.1±16.2
hsCRP (mg/L)	1.0±0.2	2.3±2.1*	1.6±1.6
Insulin (mU/L)	4.0±2.2	11.9±4.8*	7.5±5.5
Glucose (mmol/L)	5.4±0.4	4.9±0.4*	5.2±0.5
HOMA-IR	0.9±0.6	2.6±1.1*	1.7±1.2

* Significantly different between NWB and OWB group; $P<0.05$.

5.2. Relationships between serum inflammatory markers and body composition characteristics

Pearson correlation coefficients between body height, body mass, BMI, WC, WHR, sum of 9 SF, Tanner stages (Table 3), TB FM %, TB FM, trunk FM %, trunk FM (Table 4), and serum inflammatory parameters and insulin-resistance markers were calculated. BMI correlated significantly with TNF-α ($r= 0.39$, $P<0.05$) and hsCRP ($r= 0.38$, $P<0.05$) in OWB. WC correlated positively with IL-6 ($r= 0.35$, $P<0.05$) in OWB and negatively with IL-4 ($r= –0.32$, $P<0.05$) in NWB. Insulin ($r= 0.35$, $P<0.05$) and HOMA-IR ($r= 0.34$, $P<0.05$) correlated with WC only in NWB. Tanner stage and WHR were not significantly correlated with any inflammatory parameters in OWB. Tanner stage was negatively correlated to insulin ($r= –0.34$, $P<0.05$) and HOMA-IR ($r= –0.38$, $P<0.05$) in OWB (Table 3).

Skinfold thickness correlated more significantly with inflammatory markers in OWB than in NWB. The sum of 9 SF thickness measurements correlated significantly only with IL-6 ($r= 0.33$, $P<0.05$) and TNF-α ($r= 0.38$, $P<0.05$) in OWB, and with IL-6 ($r= 0.32$, $P<0.05$) in NWB (Table 3).

Table 3. Pearson correlation coefficients between serum biochemical markers and body composition parameters in the OWB (n=38) and NWB (n=38) groups.

	Body mass		BMI		WC		WHR		Sum of 9 SF		Tanner stage	
	NWB	OWB	NWB	OWB	NWB	OWB	NWB	OWB	NWB	OWB	NWB	OWB
IL-2 (pg/ml)	–0.090	– 0.036	0.179	0.062	–0.031	0.004	–0.079	0.110	0.119	–0.057	0.081	–0.021
IL-4 (pg/ml)	–0.389*	0.296	–0.226	0.241	0.317*	0.260	–0.022	0.150	–0.238	0.159	0.310	–0.145
IL-6 (pg/ml)	0.256	0.286	0.247	0.145	0.281	0.353*	0.099	0.212	0.321*	0.334*	0.145	–0.144
IL-8 (pg/ml)	–0.008	0.077	–0.050	0.111	0.062	0.153	0.173	0.106	–0.025	0.159	0.259	–0.171
IL-10 (pg/ml)	–0.256	0.248	0.052	0.137	–0.101	0.283	0.140	0.113	0.095	0.318	0.156	–0.107
VEGF (pg/ml)	–0.075	–0.014	–0.208	0.079	–0.030	0.067	0.204	0.106	–0.154	0.100	0.044	–0.253
IFN-γ (pg/ml)	–0.069	0.274	–0.160	0.311	–0.010	0.265	0.267	0.033	–0.145	0.236	0.086	–0.117
TNF-α (pg/ml)	–0.024	0.424**	–0.017	0.387*	–0.067	0.306	–0.045	0.062	–0.046	0.375*	0.295	–0.050
IL-1α (pg/ml)	–0.204	0.007	–0.022	0.023	–0.123	0.022	0.010	0.182	0.067	–0.035	0.247	0.171
IL-1β (pg/ml)	–0.099	0.101	0.044	0.082	–0.057	0.087	0.027	0.224	0.040	0.006	0.330*	0.048
MCP-1 (pg/ml)	0.024	–0.051	–0.039	–0.045	0.067	–0.020	0.207	0.034	–0.007	0.120	0.260	–0.007
EGF (pg/ml)	0.091	–0.154	–0.098	–0.166	0.037	–0.072	0.003	0.041	–0.006	–0.095	0.071	0.224
hsCRP (mg/L)	0.216	0.327*	0.235	0.380*	0.255	0.247	0.223	–0.128	0.226	0.304	0.033	–0.269
Insulin (mU/L)	0.313*	0.236	0.118	0.208	0.352*	0.293	0.088	0.071	0.294	0.185	0.009	–0.342*
Glucose (mmol⁄L)	0.113	–0.018	–0.051	–0,053	0.101	0.068	0.002	0.206	0.008	–0.012	0.009	–0.171
HOMA-IR	0.316*	0.225	0.104	0.200	0.343*	0.297	0.071	0.095	0.279	0.184	–0.003	–0.384*

Statistically significant; *P<0.05; **P<0.01.

Table 4. Pearson correlation coefficients between serum biochemical markers and body composition parameters in the OWB (n=38) and NWB (n=38) groups.

	TB FM %		TB FM		Trunk FM %		Trunk FM	
	NWB	OWB	NWB	OWB	NWB	OWB	NWB	OWB
IL-2 (pg/ml)	0.199	–0.057	0.127	–0.079	0.174	–0.024	0.109	–0.048
IL-4 (pg/ml)	–0.167	0.165	–0.245	0.233	–0.166	0.216	–0.224	0.273
IL-6 (pg/ml)	0.244	0.396*	0.305	0.397 *	0.278	0.464**	0.338*	0.439**
IL-8 (pg/ml)	–0.004	0.099	0.041	0.094	–0.017	0.187	0.028	0.154
IL-10 (pg/ml)	0.122	0.222	–0.002	0.285	0.097	0.208	–0.006	0.253
VEGF (pg/ml)	–0.198	0.026	–0.129	0.045	–0.160	0.056	–0.080	0.052
IFN-γ (pg/ml)	–0.260	0.338*	–0.213	0.362 *	–0.236	0.237	–0.186	0.293
TNF-α (pg/ml)	–0.011	0.461*	0.007	0.460 **	–0.043	0.389*	–0.028	0.392*
IL-1α (pg/ml)	0.113	–0.107	0.024	–0.100	0.092	–0.082	0.014	–0.095
IL-1β (pg/ml)	0.079	0.014	0.046	0.033	0.059	–0.003	0.040	0.073
MCP-1 (pg/ml)	–0.042	0.122	0.015	0.042	–0.055	0.058	0.002	0.034
EGF (pg/ml)	–0.030	0.000	0.024	–0.052	–0.039	0.060	0.005	–0.010
hsCRP (mg/L)	0.207	0.348*	0.247	0.455**	0.259	0.302	0.305	0.410**
Insulin (mU/L)	0.280	0.166	0.328*	0.214	0.288	0.157	0.335*	0.261
Glucose (mmol/L)	–0.009	–0.032	0.025	–0.039	0.009	0.045	0.042	0.046
HOMA-IR	0.266	0.161	0.315*	0.205	0.276	0.161	0.324*	0.262

Statistically significant; *P<0.05; **P<0.01.

IL-6 correlated with all body composition parameters in OWB and with trunk FM in NWB (Table 4). In OWB, TNF-α correlated significantly with all four body composition parameters (TB FM %, TB FM, trunk FM %, and trunk FM). Additionally, IFN-γ significantly correlated with TB FM % ($r= 0.34$, $P<0.05$) and TB FM ($r= 0.36$, $P<0.05$) in OWB (Table 4).

There were significant positive correlations between insulin and TB FM ($r= 0.33$, $P<0.05$), and between HOMA-IR and TB FM ($r= 0.32$, $P<0.05$), between insulin and trunk FM ($r= 0.34$, $P<0.05$), between HOMA-IR and trunk FM ($r= 0.32$, $P<0.05$) in NWB (Table 4).

The results of the stepwise multiple regression analysis are shown in Table 5. 38.8% of the variability of TB FM was explained by a combination of TNF-α, IFN-γ, and hsCRP levels (Table 5).

Table 5. Results of stepwise multiple regression analysis where BMI, TB FM, TB FM %, trunk FM, trunk FM %, and sum of SF were used as dependent variables and 13 biochemical markers as independent variables.

Group		NWB	OWB	Total
BMI	TNF-α	–	12.5%	–
	TNF-α, hsCRP	–	20.3%	–
	TNF-α, hsCRP, IFN-γ	–	29.7%	–
	Insulin	–	–	50.0%
	Insulin, glucose	–	–	59.4%
	Insulin, glucose, hsCRP	–	–	65.1%
	Insulin, glucose, hsCRP, IL-8	–	–	66.6%
TB FM	Insulin	8.3%	–	–
	TNF-α	–	18.8%	–
	TNF-α, hsCRP	–	30.3%	–
	TNF-α, hsCRP, IFN-γ	–	38.8%	–
	Insulin	–	–	50.5%
	Insulin, Il-6	–	–	57.8%
	Insulin, Il-6, glucose	–	–	63.1%
	Insulin, Il-6, glucose, hsCRP		–	65.7%
TB FM %	TNF-α	–	20.1%	–
	TNF-α, IFN-γ	–	26.4%	–
	TNF-α, IFN-γ, hsCRP	–	38.2%	–
	Insulin	–	–	50.1%
	Insulin, glucose	–	–	57.2%
	Insulin, glucose, IL-6	–	–	62.1%

Table 5. Results of stepwise multiple regression analysis where BMI, TB FM, TB FM %, trunk FM, trunk FM %, and sum of SF were used as dependent variables and 13 biochemical markers as independent variables. Continuation.

Group		NWB	OWB	Total
Trunk FM	IL-6	9.0%	17.5%	–
	IL-6, insulin	17.8%	–	–
	IL-6, IFN-γ	–	25.3%	–
	Insulin	–	–	52.2%
	Insulin, IL-6	–	–	60.6%
	Insulin, IL-6, glucose	–	–	64.4%
Trunk FM %	IL-6	–	18.2%	–
	IL-6, IFN-γ	–	26.2%	–
	Insulin	–	–	49.4%
	Insulin, IL-6	–	–	56.9%
	Insulin, IL-6, glucose	–	–	62.0%
Sum of 9 SF	IL-6	7.9%	–	–
	TNF-α	–	11.4%	–
	Insulin	–	–	51.7%
	Insulin, glucose	–	–	60.1%
	Insulin, glucose, IL-6	–	–	65.8%

$R^2 \times 100$ is shown describing the percentage of variability of the dependent variables the inflammatory marker alone or together with others can explain. All values are in percentages.

5.3. Relationships between serum inflammatory markers and bone mineral characteristics

Pearson's correlation showed only a few significant correlations between inflammation markers and BMD characteristics (Table 6; Figure 2). In the OWB group, IFN-γ was significantly ($P<0.05$) correlated with TB BMD ($r= 0.36$), TB BMC ($r= 0.38$), and BMC for height ($r= 0.53$). Serum hsCRP concentration was correlated with TB BMD ($r= 0.40$), IL-10 with TB BMD ($r= 0.31$), IL-1α with BMC for height ($r= 0.33$), and EGF with BMC for height ($r= –0.33$). In the NWB group, serum IL-4 ($r= –0.32$), IL-10 ($r= –0.39$) and IL-1α ($r= –0.37$) levels were correlated with TB BMC, and serum EGF correlated with LS BMAD ($r= 0.32$). All other relationships between measured inflammatory parameters and BMD characteristics were not statistically significant.

In stepwise multiple regression analysis, 25% of the variability in BMC for height and 12.6% of the variability in LS BMD were explained by serum IFN-γ concentration in the OWB group (Table 7).

Table 6. Pearson correlation coefficients between serum inflammatory markers and body bone mineral characteristics in the OWB (n=38) and NWB (n=38) groups.

	TB BMD		LS BMD		TB BMAD		LS BMAD		TB BMC		BMC for height	
	NWB	OWB	NWB	OWB	NWB	OWB	NWB	OWB	NWB	OWB	NWB	OWB
IL-2 (pg/ml)	–0.076	0.051	0.006	0.121	0.123	0.140	0.196	–0.188	–0.232	–0.019	–0.048	0.088
IL-4 (pg/ml)	–0.154	0.184	–0.036	0.276	0.217	0.154	0.074	0.205	–0.319*	0.121	–0.225	0.181
IL-6 (pg/ml)	0.094	0.166	0.172	0.028	–0.167	–0.026	0.040	–0.121	0.231	0.185	0.219	0.016
IL-8 (pg/ml)	–0.003	0.076	0.149	0.117	0.102	–0.156	0.180	0.307	–0.025	0.128	–0.033	0.294
IL-10 (pg/ml)	–0.158	0.390*	–0.180	0.169	0.234	–0.043	0.148	–0.138	–0.387*	0.320	–0.252	0.311
VEGF (pg/ml)	–0.053	0.120	–0.089	–0.036	0.018	0.196	–0.054	–0.163	–0.008	–0.084	–0.045	–0.192
IFN-γ (pg/ml)	0.048	0.359*	0.015	0.326	–0.067	–0.185	0.158	0.250	–0.024	0.382*	–0.022	0.526**
TNF-α (pg/ml)	–0.080	0.246	0.071	–0.019	0.000	–0.002	0.087	0.052	0.015	0.286	–0.015	0.123
IL-1α (pg/ml)	–0.270	0.177	0.010	0.196	0.128	–0.086	0.141	0.126	–0.374*	0.273	–0.267	0.325*
IL-1β (pg/ml)	–0.272	0.118	–0.039	–0.053	–0.083	0.058	0.026	–0.206	–0.182	0.095	–0.091	0.252
MCP-1 (pg/ml)	0.100	0.037	0.110	0.077	0.005	–0.108	0.126	0.314	0.025	0.143	0.021	0.184
EGF (pg/ml)	0.030	–0.232	0.219	–0.154	0.189	–0.111	0.323*	–0.155	0.002	–0.129	–0.011	–0.333*
hsCRP (mg/L)	0.174	0.395*	0.109	0.244	0.059	0.119	–0.007	–0.089	0.194	0.204	0.227	–0.219

Statistically significant; *P<0.05; **P<0.01.

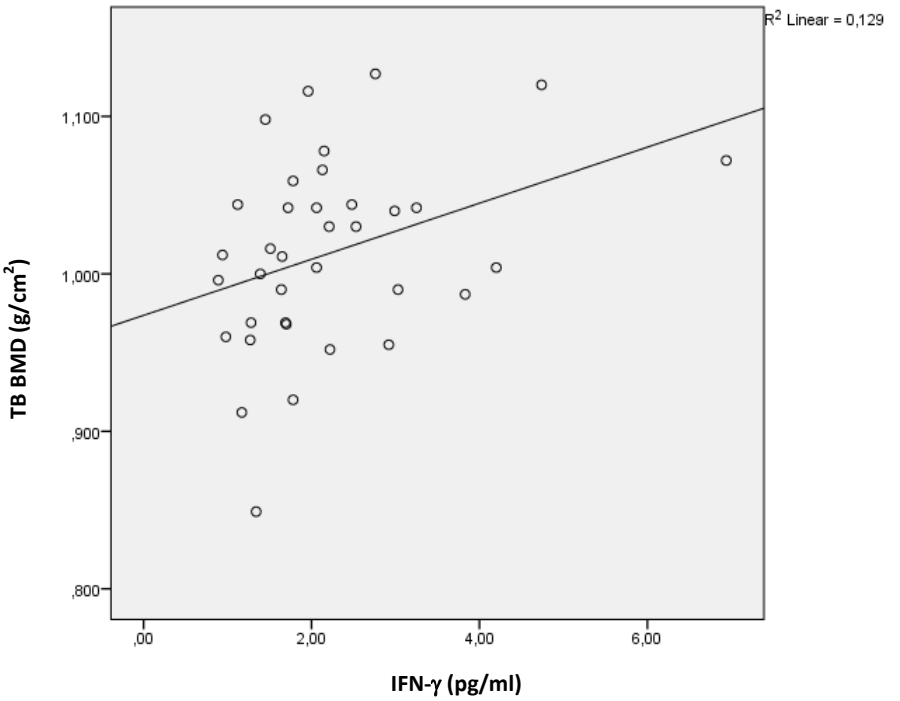

Figure 2. Correlation between TB BMD and serum IFN-γ concentration in the OWB group (r= 0.36; P< 0.05).

Table 7. Results of stepwise multiple regression analysis with TB and LS bone mineral characteristics as dependent variables and BMI and 13 serum inflammatory markers as independent variables.

Group		NWB	OWB
TB BMD	BMI	–	29.9%
	BMI, IL-10	–	38.8%
LS BMD	EGF	12.9%	–
	EGF, BMI	21.0%	–
	IFN-γ	–	12.6%
	IFN-γ, IL-4	–	20.5%
TB BMC	BMI	15.3%	14.7%
	BMI, IL-10	26.3%	–
TB BMAD	BMI	12.4%	–
LS BMAD	EGF	7.9%	–
BMC for height	BMI	24.4%	–
	BMI, IL-10	30.6%	–
	IFN-γ	–	25.0%

$R^2 \times 100$ is shown describing the percentage of variability of the dependent variables the inflammatory marker can explain. All values are in percentages.

5.4. Relationships between serum inflammatory markers and cardiorespiratory fitness parameters

The Pearson correlations between 13 inflammatory parameters and VO_2peak showed that in the OWB group only serum TNF-α concentration correlated positively with VO_2peak, while IL-6 correlated negatively ($P<0.05$) with VO_2peak/kg (Table 8; Figure 3). The other correlations are shown in Table 8.

Table 8. Pearson correlation coefficients between serum biochemical markers and VO_2peak and VO_2peak/kg in the OWB (n=38), NWB (n=38) and total (n=76) groups.

	VO_2peak			VO_2peak/kg		
	NWB	OWB	Total	NWB	OWB	Total
IL-2 (pg/ml)	0.016	0.062	0.001	–0.100	0.082	0.060
IL-4 (pg/ml)	–0.149	0.170	0.079	0.143	–0.210	–0.088
IL-6 (pg/ml)	0.046	0.105	0.242*	–0.233	–0.372*	–0.470**
IL-8 (pg/ml)	–0.075	0.163	0.181	–0.188	–0.061	–0.270*
IL-10 (pg/ml)	–0.103	0.205	0.103	0.076	–0.178	–0.130
VEGF (pg/ml)	–0.093	–0.141	–0.066	0.035	–0.116	–0.089
IFN-γ (pg/ml)	0.028	0.261	0.264*	0.180	–0.093	–0.226
TNF-α (pg/ml)	–0.163	0.405**	0.146	–0.180	–0.173	–0.229
IL-1α (pg/ml)	–0.203	0.171	0.172	–0.168	0.079	–0.288*
IL-1β (pg/ml)	–0.156	0.232	0.131	–0.184	0.110	–0.175
MCP-1 (pg/ml)	–0.064	0.054	0.147	–0.036	0.063	–0.255*
EGF (pg/ml)	0.055	0.028	0.102	–0.099	0.099	–0.026
hsCRP (mg/L)	0.169	0.221	0.368**	–0.051	–0.158	–0.450**

Statistically significant; *$P<0.05$; **$P<0.01$.

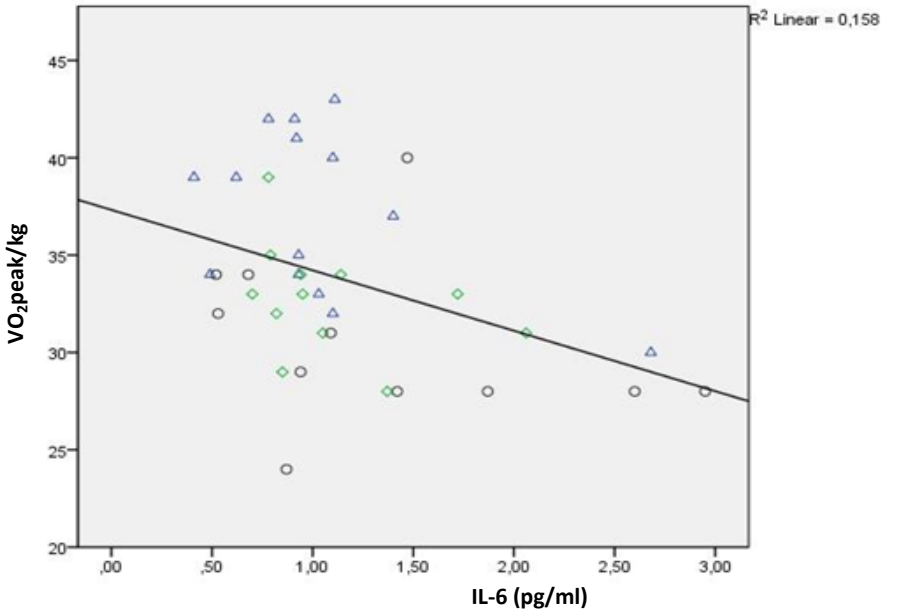

Figure 3. Correlation between VO_2peak/kg and serum IL-6 concentration in the OWB group (r= –0.37; P< 0.05). Children in the low OWB group are shown by triangles, in the middle OWB group by squares and in the high OWB group by circles.

The stepwise multiple regression analysis showed that BMI, in conjunction with IL-6, explained 44.5% of the variability in VO_2peak/kg in the OWB group (Table 9).

Table 9. Results of stepwise multiple regression analysis with VO_2peak/kg and VO_2peak as dependent variables and BMI and 13 serum inflammatory markers as independent variables.

Group		NW	OW
VO_2peak/kg	BMI	–	38%
	BMI, IL-6	–	44.5%
VO_2peak	TNF-α	–	13.8%

R^2 × 100 is shown describing the percentage of variability of the dependent variables the inflammatory marker can explain. All values are in percentages.

6. DISCUSSION

6.1. Inflammatory markers and their relationships with body composition in overweight and normal weight boys

In this study, 13 inflammatory markers and their relationships with body fat were studied in 10- to 11-year-old OWB, in comparison with NWB. As expected, OWB with similar age were taller and more advanced in their pubertal development than their NWB counterparts. It is known that obese children are taller and their pubertal maturation starts earlier (Heger et al. 2008). In OWB, serum IL-6, IL-8, MCP-1, hsCRP, and IFN-γ levels were significantly higher than in NWB.

One of the main findings of the present study was that the T cell-derived cytokine IFN-γ, whose serum concentration has not been intensively investigated in obese children, was significantly higher in OWB compared to NWB. These results are similar to the study by Lee and Choue (2009), who found that obese Korean women had significantly higher concentrations of IFN-γ compared with normal weight and overweight subjects. The percentage of IFN-γ-positive cells in obese children is positively associated with nonalcoholic steatohepatitis, insulin, and HOMA-IR values, but not with BMI SDS and TB FM (Pacifico et al. 2006). This study found a positive correlation between serum IFN-γ concentration, and TB FM % and TB FM in OWB, but not with trunk FM % and trunk FM. Thus, the prevalent Th1 pattern of secreted cytokines (i.e. IFN-γ) may be regarded as a mechanism contributing to inflammation in obesity. Todoric et al. (2011) proposed a possible mechanism by which IFN-γ may be involved in the pathogenesis of obesity. Namely, they demonstrated that IFN-γ is a potent inhibitor of Hedgehog signalling – an inhibitor in white adipocyte differentiation. To the best of our knowledge, no other studies have described a positive correlation between TB FM and serum IFN-γ levels in children. Interestingly, there was a lack of significant relationships between serum IFN-γ levels and central obesity measured as WC. Skinfold thickness on the legs had a significant relationship with IFN-γ in OWB. The findings show that IFN-γ is secreted by FM and is responsible for some peripheral complications. However, the association between IFN-γ and fat parameters does not mean that there is a causative relationship. Serum IFN-γ explained some variability of the adiposity in two models achieved from the multiple regression analysis. Additional research is required to test this assumption.

Vascular endothelial growth factor is one of the adipose tissue expressed bioactive molecules which may be involved in the development of several metabolic diseases (Claffey et al. 1992). There are no available data about the influence of obesity on VEGF concentration in children. However, increased VEGF concentrations in middle-aged obese patients were reduced as visceral fat decreased during weight loss (Miyazawa-Hoshimoto et al. 2003). In this study,

there were no significant differences in VEGF concentrations between OWB and NWB (see Table 2). Serum VEGF concentrations did not correlate with any measured anthropometric or body composition parameters. This is rather difficult to explain, due to the lack of previous investigations in children. One of the explanations may be that VEGF is involved in normal blood vessel development and vascular pathogeneses. Pathological changes in the vessels may not have developed yet in obese boys, so the role of VEGF is not seen.

The inflammatory markers IL-6, TNF-α, and CRP, which are more or less synthesized in adipose tissue (except for CRP, which is mostly synthesized in hepatocytes), have been relatively well-studied in adults. Glowinska and Urban (2003) concluded that increased TNF-α and IL-6 in children may confirm the process of inflammatory progress already in the early phase of atherosclerosis. In the current study, IL-6 and hsCRP were significantly higher in OWB than in NWB (see Table 2). Alvarez et al. (2009) found similar results, where IL-6 and CRP were associated with body FM characteristics in healthy children ages 7–12. This study also found that IL-6 and hsCRP concentrations were significantly higher in overweight children than in lean children. In addition, multifunctional cytokine IL-6 was significantly higher in OWB than in NWB (see Table 2). This means that a high serum IL-6 level in OWB may be a predictor of developing type 2 diabetes, which is similar to studies in adults (Pradham et al. 2001; Voller et al. 2004). The mechanism for developing type 2 diabetes is thought to be related to reduced insulin action, which is negatively related to serum IL-6 concentrations (Vozarova et al. 2001). In other words, high serum IL-6 concentrations in obese subjects are associated with reduced insulin-stimulated glucose disposal. In addition, Ridker et al. (2000) found that elevated levels of IL-6 are associated with increased risk of future myocardial infarction.

It is known that IL-6 can be derived from muscles (Petersen & Pedersen 2006). As subjects in both groups were matched by everyday physical activity level, it is difficult to assume that increased IL-6 levels in OWB were due to increased exercise levels. If physical activity levels in these children were directly measured, it would likely be higher in the control group.

All body composition parameters correlated significantly with IL-6 in OWB. Only trunk FM correlated significantly in NWB. It is well known that IL-6 concentration is significantly enhanced by adipose tissue in obese adults (Fried et al. 1998).

There was not a significant difference between groups in TNF-α concentration, which is difficult to explain (see Table 2). Interestingly, TNF-α correlated with all body composition parameters (including BMI and Sum of 9 SF) in OWB. However, it is known that TNF-α is only secreted by omental, not subcutaneous, fat (Mohamed-Ali et al. 1997). Abdominal SF thickness significantly correlated with TNF-α in OWB ($r= 0.36$, $P<0.05$).

There was a higher mean hsCRP concentration in OWB compared to NWB. This is similar to Reinehr et al. (2005), McLaughlin et al. (2002), and Ford et al. (2001). Serum hsCRP also positively correlated with BMI in OWB, similar to

Reinehr et al. (2005) and Hiura et al. (2003). Interestingly, almost all body composition parameters (except trunk FM %) only correlated with hsCRP in OWB, indicating that hsCRP may play a role in the progression of obesity in subjects who are already overweight or obese. Serum hsCRP and TNF-α together explained 30.3% of the variability of TB FM in OWB, with no impact from age or pubertal stage.

Trunk FM was significantly correlated with IL-6, TNF-α, and hsCRP. Visceral and trunk fat are known risk factors for metabolic syndrome (McCarthy 2006; Rosito et al. 2008; Sardinha et al. 2000). This study did not directly measure visceral fat, but DXA data about trunk fat are available. Sardinha et al. (2000) found that DXA trunk fat and trunk SF were correlated with most of the metabolic variables in middle-aged men. In addition, Teixeira et al. (2001) showed that DXA- and anthropometry – based whole-body and central-fat measures were associated with cardiovascular disease risk factors in children. Kaul et al. (2012) concluded that abdominal visceral fat measured by DXA could help define diabetes and cardiovascular risk in adults. De Jongh et al. (2006) found that visceral (measured by MRI) and trunk adiposity were detrimental for capillary perfusion and that this process may start before puberty. They also concluded that these observations underline the necessity for childhood interventions to prevent trunk obesity as a risk factor for cardio-vascular diseases (De Jongh et al. 2006). In the current study, trunk FM was significantly correlated with IL-6, TNF-α, and hsCRP. In multiple regression analysis, 25–26% of the variability of trunk FM or trunk FM % in OWB was determined by IL-6 and IFN-γ. Hermsdorff et al. (2010) found that IL-6 and TNF-α were significantly higher in those with higher trunk FM. These results suggest that these two inflammatory markers may be useful to estimate the risk of developing metabolic syndrome in adulthood.

This study also found that OWB had significantly higher insulin and HOMA-IR values, similar to the study by Caballero et al. (2008). Similar results were also seen in overweight and normal weight women 38–49 years of age (Jürimäe et al. 2009).

In summary, boys with increased BMI at the beginning of puberty have significantly higher serum IL-6, IL-8, MCP-1, hsCRP, and IFN-γ levels. Serum IL-6, TNF-α, IFN-γ, and hsCRP also correlated with body composition parameters, indicating a link between proinflammatory state and obesity.

6.2. Relationships between inflammatory markers and bone development in overweight and normal weight boys

We studied a panel of 13 inflammatory markers in serum and their possible relationships with BMD characteristics in overweight 10- to 11-year-old boys in comparison with NWB. To our best of knowledge, no studies so far have looked the association between a complex of 13 different markers of inflammation and

BMD characteristics in overweight children entering puberty. We have shown that serum IL-6, IL-8, MCP-1, hsCRP and IFN-γ levels in OWB were significantly higher than in NWB (see Table 2). Most of these markers correlated significantly with body composition parameters such as TB FM, trunk FM, BMI and WC (see Tables 3 and 4), indicating that a low-grade inflammatory process is already taking place during the development of childhood obesity. For example, FM was positively correlated with serum IL-6, IFN-γ, TNF-α and hsCRP levels in OWB. In contrast, FM was not significantly correlated to any measured 13 inflammatory markers in the NWB group (see Table 4). In the present study we found higher TB and LS BMD and TB BMC, but lower TB BMAD in OWB when compared to NWB. The lower TB BMAD is most likely due to the higher BA in OWB. Indeed, OWB had significantly higher TB BA, in comparison to NWB. This reflects the results reported by Goulding et al. (2000) study with children and adolescents. We found OWB to have not only more FM, but also FFM. This also corresponds to existing literature (El Hage et al. 2011). Increased FM has been found to increase bone maturation (El Hage et al. 2009), whereby gains in bone mass size are earlier than gains in bone mineral accrual (Bass et al. 1999). Indeed, the overweight group of our study was taller, heavier, with higher BMI, and boys were more frequently in Tanner stage 2 or 3 in comparison with NWB, suggesting that they had grown or matured faster than the normal weight group. Low TB BMAD has been found to be associated with increased risk for bone fractures (Dimitri et al. 2010). However, for our surprise, the current medical history of previous bone fractures in our subjects showed a non-significant trend that obese boys have less fractures that the NWB. This is difficult to interpret in light of our small sample size. However, it may be further compounded by confounders, such as the possibility that NWB are more likely to play and behave in ways that put them at increased risk of fractures.

Unfortunately we did not ask from our subjects their everyday dietary calcium intake, an important determinant of BMD (Al-Musharaf et al. 2012; Santos et al. 2008). However, literature about the role of calcium in obese children remains controversial. For example, Al-Musharaf et al. (2012) did not find any differences in the daily intake of calcium between overweight and normal weight children aged 6 to 17, while Santos et al. (2008) found that calcium intake was significantly higher in normal weight 15–17-year-old adolescents, when compared to overweight adolescents.

Our main finding was a positive correlation between serum IFN-γ concentration and TB BMD among OWB (see Table 6; Figure 2). When excluding from the OWB group the two boys who were only overweight, i.e. BMI between 85th and 95th centile, serum IFN-γ concentration also correlated significantly to LS BMD. To the best of our knowledge, no studies have previously investigated possible relationships between circulating IFN-γ levels and TB BMD characteristics in children. Serum IFN-γ concentration was positively correlated to FM in our overweight, but not in NWB (see Table 4).

Accordingly, IFN-γ may contribute to inflammation in obesity and could be a link between increased FM and higher BMD.

Interferon-γ is a cytokine produced locally in the bone microenvironment by cells of immune origin as well as mesenchymal stem cells (Duque et al. 2011). *In vitro* IFN-γ has been shown to suppress osteoclastogenesis and *in vivo* to decrease osteoclast formation by targeting directly osteoclast precursors, but indirectly stimulates also osteoclast formation by stimulating antigen-dependent T-cell activation and T-cell secretion of the osteoclastogenic factors RANKL and TNF-α (Gao et al. 2007). Thus, IFN-γ can both inhibit and stimulate bone resorption. IFN-γ receptor knockout mice exhibited a reduction in bone volume and significant changes in cortical and trabecular bone structure typical for osteoporotic phenotype (Duque et al. 2011). Bone histomorphometry of these mice showed a low-bone-turnover pattern with a decrease in bone formation and a reduction in circulating levels of bone-formation and bone-resorption markers. Furthermore, administration of IFN-γ to wild-type ovariectomized female mice significantly improved bone mass and microarchitecture and rescued osteoporosis (Duque et al. 2011). Studies in humans have shown that the CD4+ cells taken from women with osteoporotic fractures secreted less IFN-γ than those cells taken from women with no fractures (Breuil et al. 2010). The production of IFN-γ by peripheral blood mononuclear cells has also been lower in active Crohn's disease and correlated negatively with bone resorption/formation ratio (Trebble et al. 2005), i.e. higher IFN-γ production was related to increased bone formation. Thus this literature supports the notion that IFN-γ plays a central role in stimulating bone turnover and bone accumulation. In our study, 25% of the variability of TB BMC for height and 12.6% of the variability LS BMD was determined by IFN-γ in the OWB group. These findings indicate that elevated IFN-γ in obesity may be a link between adipose and bone tissues. However, given the complexity of interplay between these three, as well as an absence of time-series data to date, the correlation we report does not give evidence for any causative relationship between IFN-γ and TB BMD.

We also found serum IL-10 and hsCRP concentrations to be positively correlated with TB BMD in OWB. In addition, we found that serum hsCRP concentration was significantly correlated with many parameters of body fat, indicating that hsCRP reflects the severity of obesity (see Table 4). This in turn may influence bone mineral acquisition in boys entering puberty. However, Pollock et al. (2010) did not find significant correlation between CRP and TB BMC in overweight 8–9-year-old children. It is known that IL-10 is an important endogenous suppressor of infection-stimulated bone resorption *in vivo* (Sasaki et al. 2000), and IL-10 has also been indicated to inhibit the differentiation of osteoclasts *in vitro* (Owens et al. 1996). To the best of our knowledge, no previous studies have investigated the possible association between IL-10 and TB BMD in children with different BMI values.

In summary, despite greater TB BMD, OWB had lower TB volumetric BMD compared with NWB and therefore may have increased risk for fractures in adulthood. The positive correlation between serum INFγ concentration and TB BMD suggest that the inflammatory process, already taking place in the early stages of obesity, may also affect bone accumulation. Further studies are needed to clarify precisely how INFγ is linked to adipose tissue and bone health in a causative manner.

6.3. Relationships between inflammatory markers and cardiorespiratory fitness in overweight and normal weight boys

The results of our study showed that absolute VO_2peak was significantly higher and relative VO_2peak per kilogram body mass significantly lower in the OWB group than in the NWB group. We also found that cardiorespiratory fitness characterized by VO_2peak (ml/min/kg) was negatively correlated with serum IL-6 concentration, while in the OWB group VO_2peak (l/min) was positively correlated with serum TNF-α level.

The low-grade systemic inflammation was associated with the body composition values, especially with the amount of FM in obese children (Alvarez et al. 2009). Proinflammatory cytokines, such as IL-6 and TNF-α, were also associated with obesity characteristics (Wärnberg et al. 2010). In our study, we found that higher IL-6 was associated with lower cardiorespiratory fitness expressed as VO_2peak/kg in the OWB group (see Figure 3). This is similar to the study by Gaeini et al. (2009), where IL-6 was negatively correlated with VO_2peak/kg in a group of boys with normal and increased BMI, whereas no such correlation was found within the OWB group. This was probably due to the fact that the number of overweight subjects (aged 11–14 years) in that study was quite small: only 10 boys. Kullo et al. (2007) found, similarly to our study, that serum IL-6 concentration was negatively associated with VO_2peak/kg in a group of men (BMI from 22.4 to 44.0 kg/m^2). Pischon et al. (2003) found that healthy men and women with lower physical activity level had higher levels of IL-6 and CRP. In the Kullo et al. (2007) study, reduced cardiorespiratory fitness was correlated with increased IL-6 and CRP. In addition, Gaeini et al. (2009) suggested that emphasis on physical activity and cardiovascular fitness in children is an important factor for prevention of atherosclerosis in adulthood. It is known that IL-6 may be a predictor for the development of type 2 diabetes (Pradham et al. 2001). In a multiple regression analysis, 44.5% of the variability of VO_2peak/kg in OWB was determined by BMI and IL-6 together. These results suggest that increased serum IL-6 concentration may be a useful biochemical marker for identifying subjects who need specific exercise formats to achieve maximal beneficial health effects from exercise and, in the long-term, can reduce their risk for the development of type

2 diabetes and atherosclerosis. The exercises that suit the overweight children with high serum IL-6 concentrations and therefore with low cardiorespiratory fitness levels are regular aerobic exercises like walking and jogging (Zorba et al. 2011). In addition, strength training with relatively low intensity is also recommended in overweight and obese subjects (McInnis et al. 2003). Whereas exercises that usually require high cardiorespiratory fitness level such as high-intensity exercises are not suitable for overweight children (Lazzer et al. 2011).

Another finding of our study was that serum TNF-α concentration was positively correlated with absolute VO_2peak (l/min) in OWB. These results are similar to the study by Nemet et al. (2003), who found that, in 12- to 14-year-old Hispanic and Asian-American normal- and overweight children, cardiorespiratory fitness measured by VO_2peak was positively correlated with TNF-α. In both studies, TNF-α level was higher in subjects with higher body fat and with higher muscle mass. Muscle mass has been found to be the major determinant of cardiorespiratory fitness (Nemet et al. 2003). In other words, subjects with increased BMI and muscle mass have also higher absolute cardiorespiratory fitness values and higher serum TNF-α level. In addition, TNF-α is produced by adipose tissue and is associated with the development of obesity complications such as diabetes and atherosclerosis (Hajer et al. 2008; Trayhurn & Beattie 2001). Thus, children with higher BMI and bigger muscle mass have a tendency to have higher serum TNF-α levels and this, in turn, results in higher absolute VO_2peak (l/min) and lower relative VO_2peak/kg (ml/min/kg) (Nemet et al. 2003). In the long-term, overweight subjects have increased risk of developing diabetes and atherosclerosis and therefore the increased levels of TNF-α may play a role in the pathogenesis of these complications (Hajer et al. 2008; Nemet et al. 2003; Trayhurn & Beattie 2001).

In our study, cardiorespiratory fitness was measured by absolute VO_2peak and relative VO_2peak/kg. The results of our study showed that VO_2peak was significantly higher and VO_2peak/kg significantly lower in the OWB group. These results are similar to the studies by Eliakim et al. (2006), Gaeini et al. (2009), and Rosa et al. (2011), where a lower VO_2peak/kg was found in overweight children compared to the children with normal weight.

Of the 13 biochemical markers measured in our study, only IL-6 was significantly correlated with cardiorespiratory fitness relative to body mass as assessed by VO_2peak/kg in the OWB group. No significant correlations were found between other inflammatory markers and VO_2peak/kg in the OWB group. To our best knowledge, no studies have previously looked at the associations between such a complex of biochemical markers and cardiorespiratory fitness in overweight children. Eliakim et al. (2006) studied the association between serum IL-1β concentration and VO_2peak in children aged 8 to 17 years, but did not find a significant correlation between these parameters. Therefore, it is likely that other measured markers of inflammation in this study are not associated with cardiorespiratory fitness, at least in children.

In summary, OWB had lower cardiorespiratory fitness level as measured by VO_2max/kg and this was negatively correlated with serum IL-6 level. Measurement of serum IL-6 level in overweight children may help to identify subjects who need specific aerobic exercise formats and levels to achieve maximal beneficial health effects and to reduce their risk for the development of type 2 diabetes and atherosclerosis later in life.

7. CONCLUSIONS

1. Significantly higher serum IL-6, IL-8, MCP-1, CRP, and IFN-γ levels were seen in overweight boys compared to those with normal BMI at the beginning of puberty;
2. Serum IL-6, TNF-α, CRP, and IFN-γ levels correlated with body fat characteristics, indicating the link between an inflammatory state and obesity;
3. Despite greater total body BMD, overweight boys had lower total body apparent (volumetric) BMD compared with normal weight boys, and therefore may have increased risk of fractures in adulthood. The positive association between serum INF-γ concentration and BMD suggests that the inflammatory process already taking place in the early stages of obesity may also affect bone accumulation;
4. Overweight boys had lower cardiorespiratory fitness levels as measured by VO_2peak/kg, and this was negatively correlated with serum IL-6 levels. Measurement of serum IL-6 levels in overweight boys may help to identify subjects who need specific exercise formats to achieve maximum beneficial health effects, and to reduce their risk of developing type 2 diabetes and atherosclerosis later in life.

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SUMMARY IN ESTONIAN

Seosed põletikumarkerite, keha koostise, luutiheduse, ja kehalise võimekuse vahel 10–11 aastastel ülekaalulistel ja normaalkaalulistel poistel

Sissejuhatus

Rasvumisel on kahjulik mõju tervisele ja see probleem on ülemaailmseks muutunud ka laste seas. Erinevad uuringud on näidanud, et ülekaalulisus lapseeas toob kaasa riski haigestuda erinevatesse südame-veresoonkonna haigustesse täiskasvanuna, samas kui suurem kehaline võimekus vähendab neid riske nii lastel kui ka täiskasvanutel. Aktiivne eluviis lapsepõlves loob eeldused paremaks terviseks täiskasvanuna. Varasemad uuringud on näidanud, et rasvunud lastel esineb sagedamini luumurde. Eriti kriitiline aeg on murdeiga, mil toimub peamine luukoemassi ülesehitamine ja selle häired võivad põhjustada osteoporoosi hilisemas elus. Rasvumise väljakujunemisel on oma kindel koht ka mitmesugustel põletikulistel protsessidel, mida saab hinnata mitmesuguste põletikunäitajate määramisega vereseerumis. Seetõttu saab neid põletikunäitajaid uurides paremini aru, kas ja kuidas põletik mõjutab rasvumise korral luu tervist ja kehalist võimekust.

Uurimustöö eesmärk ja ülesanded

Käesoleva töö eesmärgiks oli uurida 10- ja 11-aastastel poistel kolmeteist erinevat põletikumarkerit veres ja uurida seoseid nende markerite ning keha koostise, luutiheduse ja kehalise võimekuse vahel. Lähtuvalt üldeesmärgist olid ülesanneteks:

1. uurida erinevate põletikumarkerite kontsentratsioone vereseerumis ülekaalulistel ja normaalkaalulistel poistel;
2. uurida võimalikke seoseid vereseerumi põletikumarkerite kontsentratsioonide ja keha koostise vahel ülekaalulistel ja normaalkaalulistel poistel;
3. uurida seoseid vereseerumi põletikumarkerite kontsentratsioonide ja luutiheduse vahel ülekaalulistel ja normaalkaalulistel poistel;
4. uurida seoseid vereseerumi põletikumarkerite kontsentratsioonide ja kehalise võimekuse vahel ülekaalulistel ja normaalkaalulistel poistel.

Uuritavad ja metoodika

Uuringus osalesid 76 Tartu ja Tartumaa koolipoissi vanuses 10–11 aastat, kes jagati kehamassiindeksi (KMI) järgi kahte gruppi. Ülekaalulisi poisse, kelle KMI oli ülalpool 85. protsentiili, osales uuringul 38 ja kontrollgruppi kuulus 38 normaalkaalus poissi, kelle KMI oli allpool 85. protsentiili. Mitte ühelgi uuritaval ei olnud kroonilisi haigusi, samuti ei olnud neil uuringu ajal ühtegi haigestumist. Poisid võtsid osa igapäevastest kehalise kasvatuse tundidest

koolis. Uuritavad ei muutnud uuringu käigus oma igapäevaseid toitumisharjumusi.

Kõikidel uuritavatel määrati kehamass ja pikkus ning arvutati KMI. Murdeiga hindasid poisid ise 5 astmesse kasutades selleks spetsiaalseid illustreerivaid pildimaterjale Tanneri metoodika järgi. Lisaks mõõdeti 9 nahavoldi paksus ning saadud tulemused summeeriti. DXA aparaadiga määrati keha rasvamass, rasvavaba mass, rasvamassi protsent, kogu keha ja nimmepiirkonna luukoe mass, luukoe pindala ja luutihedus. Lisaks arvutati volumeetriline luutihedus, mis võtab arvesse keha suurust.

Kehalise võimekuse hindamiseks sooritasid uuritavad veloergomeetril testi kuni väsimuseni. Mõõdeti poiste keskmist hapniku tarbimist ja arvutati keskmine hapniku tarbimine kilogrammi kehakaalu kohta. Lisaks mõõdeti uuritavatel 13 erineva põletikumarkeri tase vereseerumis: interleukiin-2, interleukiin-4, interleukiin-6, interleukiin-8, interleukiin-10, vaskulaarse endoteeli kasvufaktor, interferoon-γ, tuumor nekroosis faktor-α, interleukiin 1α, interleukiin 1β, monotsüütne kemotaktiline valk-1, epidermaalne kasvufaktor ja C-reaktiivne valk.

Tulemused ja järeldused

1. Kolmeteistkümnest mõõdetud põletikumarkerist olid ülekaalulistel poistel vereseerumis oluliselt kõrgemad interleukiin-6, interleukiin-8, monotsüütne kemotaktiline valk-1, C-reaktiivne valk ja interferoon-γ;
2. Mitmed olulised seosed vereseerumi interleukiin-6, tuumor nekroosis faktor-α, C-reaktiivne valk ja interferoon-γ taseme ja keha rasvamassi erinevate näitajate vahel näitavad seost põletiku ja ülekaalulisuse vahel;
3. Vaatamata suuremale kogu keha luutihedusele oli ülekaalulistel poistel võrreldes normaalkaaluliste poistega väiksem volumeetriline luutihedus, mis kirjanduse andmetel tõstab riski luumurdude tekkeks täiskasvanuna. Positiivne seos vereseerumi interferoon-γ konsentratsiooni ja luutiheduse vahel näitab, et rasvumise põletikuline protsess, mis on käimas juba 10–11 aastaselt, võib mõjutada luutiheduse edasist suurenemist;
4. Ülekaalulistel poistel oli madalam kehalise võimekuse tase mõõdetuna maksimaalse hapnikutarbimisena kilogrammides kehamassi kohta ja see oli negatiivselt seotud vereseerumi interleukiin-6 kontsentratsiooniga. Interleukiin-6 taseme määramine vereseerumis võib aidata ülekaaluliste poiste seas avastada neid, kellel on kehaline võimekus madalam ja seetõttu vajavad spetsiaalseid treeninguid, et saavutada kehalise koormuse maksimaalne kasulik mõju tervisele ja vähendada seeläbi nende riski haigestuda 2 tüübi diabeeti ja ateroskleroosi hilisemas elus.

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