Abdominal Obesity, Liver Fat and Muscle Composition in Young Adult Survivors of Childhood Acute Lymphoblastic Leukemia

By

PETER M. JANISZEWSKI

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Queen's University

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ABSTRACT

Background: Survivors of childhood acute lymphoblastic leukemia (ALL) gain excess body weight in the years following therapy. We sought to determine whether cranial radiotherapy (CRT) and/or sex are associated with elevations in total and abdominal obesity, specifically visceral adipose tissue (VAT), as well as liver and muscle fat accumulation, and altered growth hormone (GH) and leptin status in young adults ALL survivors.

Methods: Abdominal AT, VAT, abdominal subcutaneous AT (SAT) masses were quantified from the L3-L4 to the L4-L5 inter-vertebral space using computed tomography (CT) in 52 male (15 CRT treated) and 62 female (24 CRT treated) young adult ALL survivors. The ratio of mean liver to mean spleen CT attenuation was used as a qualitative measure of liver fat infiltration. Mean muscle attenuation from 11 CT images spanning from 12-18 cm above the patella was used to indicate the degree of muscle fat deposition. Total fat and lean body mass were measured using dual energy x-ray absorptiometry (DEXA). Commercial radio-immunoassays were used to measure serum insulin growth factor-1 (IGF-1) and leptin levels. IGF-1 levels were used as a surrogate measure of GH status.

Results: Controlled for age and race, CRT treated ALL survivors had higher VAT, body fat percentage, and leptin levels, but lower lean mass and IGF-1 levels as compared to non-CRT survivors ($P < 0.05$). Among female survivors, CRT was associated with a significantly higher VAT: SAT ratio ($P < 0.01$). Levels of IGF-1 were inversely associated with total adiposity, VAT and VAT: SAT ratio in both sexes ($P < 0.01$). Female ALL survivors had less lean mass and VAT but higher fat mass and SAT than

males ($P < 0.05$). Neither CRT nor sex was associated with muscle and/or liver fat content. ***Conclusion:*** Among young adult ALL survivors, CRT is a risk factor for elevated total and abdominal obesity, visceral adiposity and reduced fat-free mass in association with an altered IGF-1 and leptin levels.

Co-Authorship

Peter M. Janiszewski was solely responsible for the analysis of computed tomography imaging data, statistical analysis of all anthropometric, body composition and hormone data, interpretation of the findings and preparation of the manuscript and thesis.

Acknowledgments

Although the saying "you are what you eat" is likely more popular, I believe that the saying "you are who you meet" is much more profound. While we believe to be independent and autonomous creatures, in fact much of our personalities, beliefs, and goals are an amalgamation of the collective influences of the people we interact with. To that effect, innumerable people have played a pivotal role in shaping my life. First off, without my parents I would not be here. Aside from their obvious role in producing me, had my parents never left Poland with me in tow for a 'vacation' to Greece and then Canada – today I would likely me the proud owner of a fast-food Pierogi & Sausage franchise. Mamusiu i Tatusiu, bez was Ja nigndy bym to tego punktu w życiu doszedł. Dziękuje wam za waszom miłość, pomoc, i poświęcenie wszystkjego dla mnie – Ja jestem produktem waszej ćężkiej pracy.

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In essence, one becomes a researcher to quench their own curiosity. As a young boy in Poland, my mother would take me on walks 'into the unknown' – it did not matter where, as long as I had never been there before. My drive for exploration and knowledge was born on these walks. The excitement I got from walking through a new neighbourhood in Częstochowa is the same basic feeling I attain from learning and discovering in research. Although this marks the end of one chapter of my life, in the words of Michelangelo, "I am still learning…"

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1.0.0 INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood[1]. Fortunately, improved treatment strategies for ALL have lead to a growing population of long-term survivors [2]. Unfortunately, along with the growing population of survivors comes the recognition of many adverse health consequences of childhood cancer treatment. In particular, since the original observation of Sainsbury *et al.* in 1985[3], numerous studies have reported that ALL survivors are overweight and obese as compared to healthy age-matched controls[4-12]. Consequently, a significant proportion of ALL survivors exhibit a constellation of obesity-related cardiovascular risk factors [6, 7, 11, 13] and are at greater risk for all-cause and cardiovascular-related mortality[14]. The use of cranial radiotherapy (CRT) during ALL treatment has been implicated as a potential cause of excess weight gain among survivors[8, 15, 16]. Further, the risk of obesity following ALL treatment appears to be more pronounced in females than in males[8, 15, 17, 18].

Although increasing levels of total obesity are mirrored by increased risk for morbiditiy[19] and mortality[20], the location of excess fat, rather than its absolute amount has been shown to influence the link between obesity and health risk [21]. It is now apparent that obesity-related health risk is most prominent in those with excessive abdominal fat deposition[22-25]. Specifically, the excess accumulation of visceral adipose tissue (VAT) within the abdomen is strongly and independently associated with metabolic aberration[26], morbidity[27], and mortality[28]. Furthermore, apart from the health risks associated with abdominal obesity, emerging evidence suggests that storage of fat in non-adipose tissues such as liver and skeletal muscle also carries independent health risk[29-31].

The following review will consider current knowledge regarding ALL epidemiology, therapy-related sequelae (specifically obesity), and the mechanisms linking ALL therapy and subsequent obesity and metabolic risk. Available evidence on total, abdominal, and visceral obesity, as well as skeletal muscle and liver fat storage will also be considered with regards to their respective associations with health risk.

2.0.0 REVIEW OF LITERATURE

2.1.0 Acute Lymphoblastic Leukemia

2.1.1 Epidemiology

More than 12 000 youths are diagnosed with cancer each year in the US [1]. Cancer is the second leading cause of mortality among US children, accounting for 10% of all childhood deaths [32]. Acute lymphoblastic leukemia (ALL) is the most common type of childhood malignancy, representing approximately a quarter of all oncological diagnoses in children[1]. Between 1975 and 2002 the incidence of childhood ALL increased by approximately 1% per annum[1].

The occurrence of ALL peaks in children between the ages of 2-3 years, and during this age is nearly 10-fold greater than that for young adults [33]. Childhood ALL is approximately 20% more prevalent in males versus females, and almost twice as common in whites as in blacks [32, 33]. Although the specific causes of childhood ALL are largely unknown, potential risk factors include prenatal and postnatal exposure to ionizing radiation, and the presence of various genetic conditions such as Down syndrome, Shwachman syndrome, and Klinefelter syndrome [33, 34].

Although originally coined "acute" due to ALL's rapid progression to fatality, the event free survival rates for childhood ALL have improved significantly over past decades and are currently around 80% [1, 35, 36]. Indeed, each year in the US approximately 2000 individuals become 5-year survivors of childhood ALL [2]. By direct consequence, these improved survival rates have led to an ever-growing population of long-term survivors of childhood ALL. Survival for children with ALL is however dependent on age at diagnosis; those diagnosed between the ages of 1-9 years have significantly greater

survival rates than do infants (<1yr) or adolescents (15-19yr) [1]. Furthermore, females fare slightly better than males and children of Caucasian descent fare better than non-Caucasian children in terms of long-term survival [1].

2.1.2 Long-term sequelae of ALL therapy

2.1.2.1 General

Concurrent with the improved survival rates is the mounting recognition of various late complications of therapy for childhood ALL. The treatment strategies used in children with ALL, including cranial radiotherapy (CRT) and chemotherapy, have the potential to affect many tissues and functional systems of the body. Indeed, as summarized in Table 1, survivors of childhood ALL experience a multitude of adverse effects related to therapy, including body composition, neuro-cognitive, endocrine, musculoskeletal, cardio-respiratory, and metabolic aberrations. Along with the presence of these treatment sequelae, survivors of childhood ALL experience an excess in morbidity and all-cause mortality for many years post initial cancer diagnosis and treatment[14, 37].

2.1.2.2 Total Obesity

Although the list of ALL treatment sequelae described in Table 1 is extensive, the most commonly cited of these is the development of obesity. Over 20 years ago, Sainsbury et al. retrospectively studied medical records for a cohort of childhood ALL survivors and noted excessive weight increase during and post-treatment[3]. Multiple reports in subsequent years have echoed these initial findings demonstrating that by completion of therapy, attainment of final height, and in young adulthood

Table 1. Adverse long-term consequences of ALL therapy

Outcome	Description	Associated therapy	References
Cognitive function	Deficits in verbal and non-verbal IQ, reasoning, concentration, memory, and hindered academic performance	CRT, methotrexate	{38-40}.
Endocrine function	Disturbance in hypothalmic-pituitary axis; growth hormone deficiency, and possible hypothalamic leptin insensitivity	CRT	{7, 10, 41-43}.
Cardiorespiratory function	Late-onset cardiac myopathy (reduced ventricular dimensions/performance); pulmonary impairement (reduced lung volumes, abnormal gas exchange, exercise arterial O_2 desaturation); reduced cardio-respiratory fitness levels	CRT, anthracyclines	{7, 44-48}.
Skeletal system	Reduced age-adjusted bone mass and bone mineral density; increased risk of fracture	CRT, corticosteroids, methotrexate	{49, 50}.
Muskuloskeletal/ Gross motor function	Reduced strength, running speed, flexibility; decreased motor nerve conduction; poor balance and postural control	Vincristine, corticosteroids, methotrexate	{51, 52}.
Growth and maturation	Stunted linear growth, precocious puberty	CRT	{53-55}.
Body habitus	Increased fat mass and decreased fat-free mass; high incidence of obesity	CRT, corticosteroids	{4-12, 15, 16, 18, 50, 56-60}.
Metabolic status	Hyperglycemia, hyperinsulinemia, insulin resistance, dyslipidemia, and hypertension	CRT, corticosteroids	{6, 7, 11, 13}.

ALL survivors are at heightened risk for becoming overweight and obese as compared to age-matched healthy controls (Table 2) and/or population reference norms (Table 3). Many reports have indicated that the risk of obesity following ALL treatment is more pronounced in females than males [8, 15, 17, 18]. Further, the use of CRT during ALL treatment has been implicated as the major potential cause of excess fat gain among survivors [8, 15, 16].

As illustrated in Tables 2 and 3, prior investigations into body habitus of ALL survivors have focused almost exclusively on total obesity using indices of height and weight, dual energy x-ray absorptiometry (DEXA), skinfolds or bioelectric impedance analysis. The application of a height and weight index derived from the normal population to assess adiposity in subjects with abnormalities in growth and body composition is not ideal. Indeed, body mass index (BMI as defined by weight (kg) divided by height (m^2)) has been shown to underestimate body fat measurement in diseased individuals, with a sensitivity as low as 50% for obesity detection [61]. In fact, survivors of ALL possess a higher percentage of fat mass per given BMI unit compared to healthy controls, thus questioning the usefulness of this anthropometric technique in this specific population [58].

2.1.2.3 Total Fat Mass and Lean Body Mass

Using more direct measures of body composition, increased total fat body mass and decreased lean mass in ALL survivors as compared to matched controls has been reported in many [6, 7, 50] but not all [4, 9] previous investigations. In one of the only studies to specifically investigate the role of CRT on body composition in ALL

Table 2. Obesity in ALL survivors as compared to healthy controls

Reference	Sample	Method	Findings
Talvensaari (1996)[11]	17♀, 11♂ (Age: 11-26yr)	Skinfolds	ALL survivors absolute FM > control
Brennan (1999)[10]	15♀, 17♂ (Age: 7-28yr)	DEXA	ALL survivors FM = control
Warner (2002)[9]	21♀, 14♂ (Age: 7-18yr)	DEXA	♀ ALL survivors % FM > control, other cancers
Oeffinger (2003)[8]	885♀, 880♂ (Age: 18-42yr)	BMI	↑Risk for obesity, especially in ♀ treated young
Link (2004)[7]	21♀, 23♂ (Age: 19-32yr)	DEXA	ALL survivors BMI, FM, WC > controls
Bulow (2004)[6]	10♀, 1♂ (Age: 25-33yr)	BIA	ALL survivors % FM > controls
Jarfelt (2005)[12]	18♀, 17♂ (Age: 21-29yr)	DEXA	♂ ALL survivors BMI, FM, WC > controls
Marinovic (2005)[5]	17♀, 20♂ (Mean age: 8.9 yr)	DEXA	ALL survivors % FM = controls
Murphy (2006)[4]	13♀, 11♂ (Age: 6-13yr)	DEXA	ALL survivors BMI & FMI > controls

Abbreviations: BMI, body mass index; DEXA, dual-energy x-ray absorptiometry; FM, fat mass; CRT, cranial radiotherapy; ALL, acute lymphoblastic leukemia

Table 3. Obesity in ALL survivors as compared to population or reference norms

Reference	Sample	Method	Finding(s)
Schell (1992)[56]	58♀, 33♂ (At 18 yrs)	BMI	Obesity in 38% of survivors
Odame (1994)[15]	21♀, 19♂ (2-4 yrs post diagnosis)	BMI	Obesity in 57% of ♀ ALL survivors
Didi (1995)[18]	63♀, 51♂ (Age: 13-21yr)	BMI	Obesity in 45% ♂ and 47% ♀ ALL survivors
Van Dongen-Melman (1995)[57]	55♀, 58♂ (4 yr post treatment)	BMI	Obesity in 38% of survivors
Birkebaek & Clausen (1998)[53]	19♀, 14♂ (Age: 15-27yr)	BMI	Obesity in 36% of ALL survivors
Nysom (1999)[58]	45♀, 50♂ (Age: 3-23yr)	DEXA	ALL survivors %FM > reference
Sklar (2000)[16]	67♀, 59♂ (Mean age: 18yr)	BMI	CRT treated ALL survivors BMI > reference
Van der Sluis (2000)[59]	10♀, 13♂ (Age: 12-25yr)	DEXA	ALL survivors % FM = reference
Kimball-Dalton (2003)[54]	301♀, 317♂ (6 yrs post diagnosis)	BMI	ALL survivors BMI > reference
Davies (2004)[50]	7♀, 7♂ (Age: 3-17yr)	DEXA	ALL survivors % FM > reference
Meacham (2005)[60]	828♀, 837♂ (Age: 20-47yr)	BMI	ALL survivors BMI > reference, other cancers

Abbreviations: BMI, body mass index; DEXA, dual-energy x-ray absorptiometry; FM, fat mass; CRT, cranial radiotherapy; ALL, acute lymphoblastic leukemia

survivors, Jarfelt et al [12] report significantly higher total body fat mass and reduced lean mass in CRT versus non-CRT males, but not females. However, these results were based on a limited (n = 35), non-obese sample, and may be confounded by a significant difference in age between the CRT versus non-CRT participants.

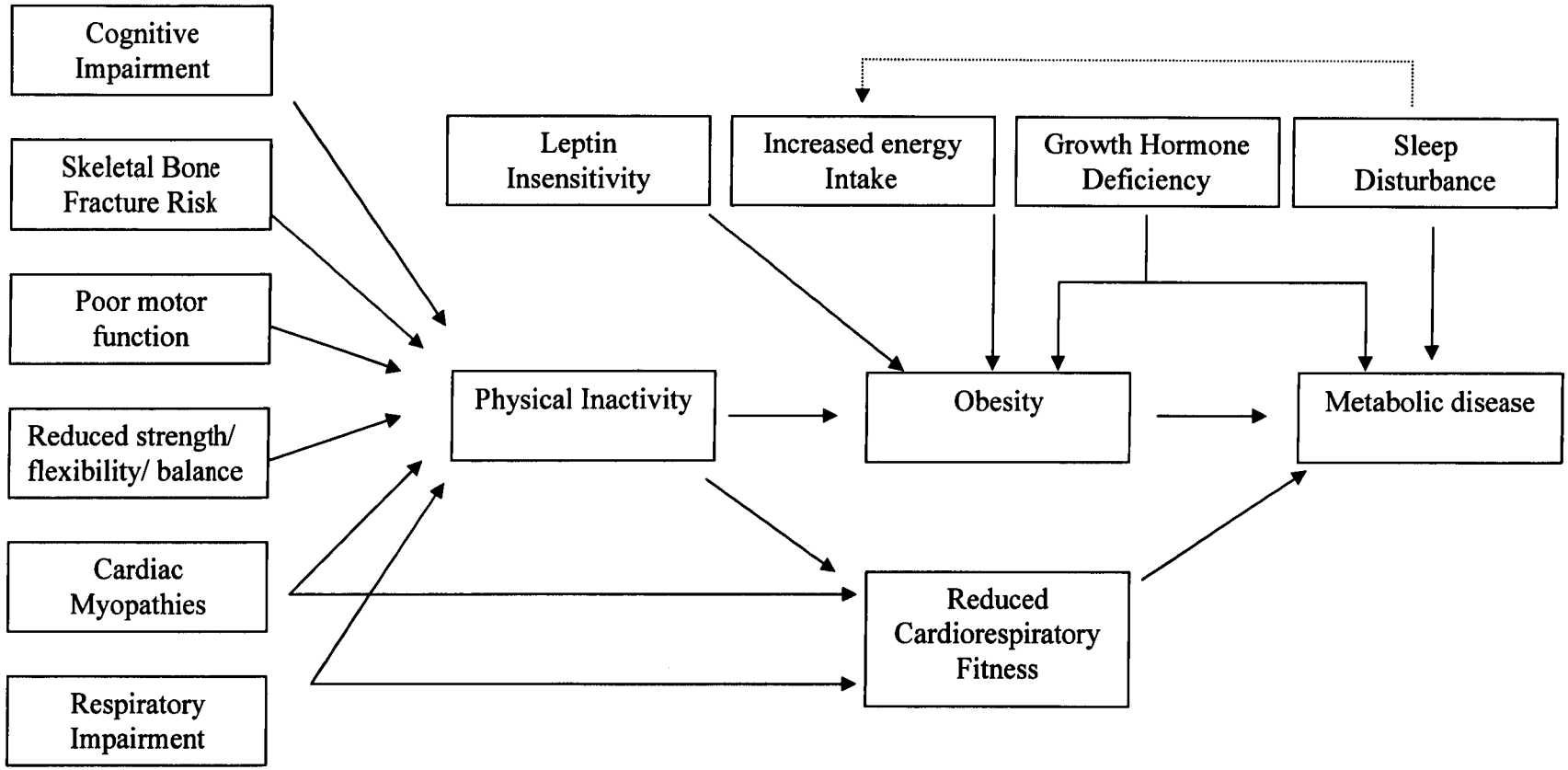
2.1.2.4 Abdominal Obesity

While numerous studies have documented elevated total obesity in survivors of ALL, current evidence on abdominal obesity in this cohort is limited and largely inconsistent. As assessed by waist circumference or waist-to-hip ratio, it is reported that abdominal obesity in ALL survivors is higher than [7] and/or equal to [6] that of healthy controls. Further, while one study reports that males, but not females treated with CRT have a greater waist circumference and waist-to-hip ratio as compared to non-CRT counterparts [12], others found no such difference [13]. Using DEXA Jarfelt et al. [12] report a higher trunk fat percentage in CRT versus non-CRT male but not female ALL survivors. One potential confounder in all these studies is the CRT dose, which is known to influence post-treatment obesity[8]. Thus, it remains unclear whether ALL survivors are at risk for abdominal obesity, and whether CRT is associated with elevations in abdominal adiposity.

2.2.0 From ALL Treatment to Obesity: The Potential Mechanisms

In a population with so many health abnormalities and a condition with numerous potential causes, the large number of putative mechanisms linking the two is not surprising. In a schematic fashion, Figure 1 illustrates a schematic representation of the plausible mechanisms by which survivors of ALL may become

Figure 1. From ALL therapy to obesity and metabolic disease: The potential pathways.



obese and potentially develop metabolic risk. These mechanisms are described in greater detail below.

2.2.1 Endocrinopathologies

The use of CRT during ALL treatment has been implicated as a potential cause of excess fat gain among survivors [8, 15, 16]. Used as prophylaxis against potential central nervous system malignancy in ALL, CRT may induce damage at the radiosensitive hypothalamus, thus leading to growth hormone deficiency and/or leptin insensitivity. Via different mechanisms, these conditions favor deposition of excess lipid and development of obesity.

2.2.1.1 Growth Hormone Deficiency

It is been reported that a large portion of adult survivors of childhood ALL treated with CRT exhibit severe growth hormone deficiency (GHD) [7, 41, 42]. In comparison to BMI-matched controls, GHD adults have greater absolute fat mass, reduced fat-free mass, and a propensity for a central fat distribution[6, 62]. Moreover, compared to healthy controls, growth hormone deficient adults also have a greater visceral (VAT): subcutaneous abdominal adipose (SAT) tissue ratio, suggestive of preferential VAT storage [63]. Growth hormone replacement in deficient individuals has been shown to reduce abdominal obesity via disproportionate mobilization of VAT, with minimal [63, 64] or no change in SAT [65]. Thus, in addition to causing excess abdominal fat deposition, GHD may also cause a redistribution of abdominal fat into the VAT depot. Although not yet tested, it is reasonable to suggest that survivors of ALL treated with CRT may have increased storage of abdominal fat, specifically VAT. GHD has also been directly linked to various metabolic disturbances including

insulin resistance and dyslipidemia [6, 7]. While GH therapy in deficient adults appears to improve body composition and lipid profiles, no beneficial effect on insulin sensitivity has been documented [6, 62].

2.2.1.2 Leptin Insensitivity

Leptin, a hormone derived from adipose tissue, exists in plasma in direct proportion to the size of the adipose organ [66]. Via a negative feedback mechanism at the level of the hypothalamus, leptin is thought to regulate appetite and energy expenditure. Previously, leptin deficiency and/or insensitivity has been shown to cause obesity[67, 68]. It has been proposed that CRT may damage the leptin feedback mechanism between adipose tissue and the receptors at the hypothalamus[10]. Indeed, it is reported that ALL survivors have increased plasma leptin levels when expressed per unit of fat mass as compared to matched controls or a reference population[6, 7, 10, 50]. Indeed, Ross et al reported that a polymorphism in the leptin receptor gene may lead to poor leptin binding affinity and predispose ALL survivors to obesity secondary to CRT[69]. Interestingly, although hyperleptinemia has been related to GHD, 12 months of GH replacement therapy in deficient ALL survivors was not associated with any changes in leptin/kg fat mass, thus arguing against any causal link between the two conditions [6].

2.2.2 Thermodynamics:

In its simplest form, obesity represents a perturbation of the first law of thermodynamics, where energy intake exceeds energy expenditure. Some evidence suggests that ALL survivors are especially prone to such a disturbance. Compared to control subjects, ALL survivors have a significantly reduced level of total daily

energy expenditure (TDEE) [70]. This reduction in TDEE is largely accounted by decreased physical activity levels (PAL). Furthermore, energy expenditure during sub-maximal exercise is lower in ALL survivors as compared to sibling controls[47]. A number of therapy related adverse effects (as outlined in Table 1) including risk of fractures, poor strength, flexibility, balance, and motor function, as well as impaired cardiac and respiratory function could contribute to the reduced PAL seen in survivors of ALL. It has also been proposed that parental overprotection may restrict physical activity during ALL therapy, and perhaps set future sedentary habits[3].

The reduced PAL, combined with the direct effects of impaired cardiac and pulmonary function, may lead to reduced cardiorespiratory fitness (CRF) levels. In fact, as recently reviewed by van Brussel et al, survivors of childhood ALL have significantly reduced maximal oxygen consumption levels as compared to healthy controls[48]. As CRF has been shown to be an independent predictor of morbidity and mortality[71], and may in fact mediate much of the health risk associated with obesity [72], the reported low CRF levels in ALL survivors, may independently lead to health risk in this population.

From the other side of the energy equation, it is reported that habitual energy intake increases by an average of 20% (range: 10-40%) during glucocorticoid treatment for ALL[73], possibly leading to a habit of over-consumption post-therapy. Furthermore, Sainsbury et al[3] previously proposed that in attempts to ease further suffering, parents of children with ALL allow the child to consume foods he/she prefers, which will likely be high energy snacks, thus leading to excessive calorie consumption and poor dietary habits.

2.2.3 Additional Mechanisms

2.2.3.1 Sleep Deprivation

A recent investigation into the quality of life of 161 young adult ALL survivors revealed a high prevalence of sleep disturbance as assessed by the Pittsburgh Sleep Quality Index [74]. Specifically, almost 50% of ALL survivors reported sleep problems as compared to 30% reported in the general population [75]. A number of large epidemiological studies have noted a negative relationship between sleep duration and BMI[76, 77]. Furthermore, experimentally induced sleep deprivation has been shown to elevate levels of ghrelin (appetite stimulant), reduce levels of leptin (appetite suppressant), leading to elevated perceptions of hunger and increased cravings for calorie-dense foods [78]. Cortisol levels also tend to be elevated after sleep loss [79]. Patients of Cushing's syndrome, an endocrine disorder of marked hypercortisolemia, exhibit a tendency for excessive, and specifically android obesity [80]. Therefore, hypercortisolemia, secondary to sleep deprivation may also lead to alterations in body composition in ALL survivors. Sleep debt may also lead directly to metabolic disturbance as it has been shown to reduce glucose tolerance by approximately 40% in experimental conditions[79]. In fact, based on a review of the relevant literature, Spiegel and colleagues recently proposed that sleep loss may be an important risk factor for the development of insulin resistance and Type 2 diabetes[81]. Thus, by yet unknown mechanism ALL treatment may cause a disturbance in sleep, leading to excessive caloric intake, obesity, and metabolic aberrations.

2.2.3.2 Premature and Precocious Puberty

Premature and even precocious puberty in female but not male ALL survivors has previously been noted[55, 82]. In a study by Leiper et al, the prevalence of precocious puberty in the studied females was 4 times that observed in the normal population[55]. The early onset of puberty has been associated with young age at treatment and the use of CRT in ALL therapy[55, 83]. Prior research shows that at attainment of final height (Tanner stage 5), girls who experience precocious puberty have approximately double the level of adiposity as do girls of normal maturation[84]. Further, in a sample of male and female ALL survivors, Reilly et al investigated the onset of adiposity rebound, the period in childhood when adiposity begins to increase after reaching a nadir[85]. Both male and female ALL survivors experienced a premature adiposity rebound as compared to healthy controls. As an early adiposity rebound is associated with heightened risk of obesity in adulthood[86], these results reinforce the notion that early development in ALL survivors may predispose to obesity in later life.

2.2.4 Sexual Dimorphism in Susceptibility to Late-Effects of Treatment

Although the mechanism(s) that explain the greater susceptibility of female ALL survivors to the adverse effects of therapy are unclear, several are plausible. First, there is evidence that maturation of the brain occurs considerably earlier in males than in females[87]. Thus, it has been suggested that young males may be resistant to the effects of CRT since their brain has reached a critical level of maturity before the onset of treatment[17, 88]. On the other hand, at the same age the still

developing female brain may be highly vulnerable to CRT effects. Whether or not this is indeed true is still unknown.

Recently, Ross et al reported that a polymorphism in the leptin receptor gene in ALL survivors may predispose to obesity secondary to CRT via poor leptin binding affinity[69]. However, this effect was noted in female survivors only, thus providing a possible explanation for sex differences in incidence of obesity post treatment.

2.3.0 Characterizing Obesity and Associated Health Risk

2.3.1 Total Obesity and Associated Health Risk

As evidenced by the Venus figurines found throughout Europe, human obesity as a physical state has been documented for at least 25,000 years. By the time of Hippocrates, obesity was already recognized as a condition with deleterious health consequences. Indeed, Hippocratic writings state that "Sudden death is more common in those who are naturally fat than in the lean"[89]. Since that time numerous studies have reported that overweight and obesity are associated with risk of type 2 diabetes[19], hypertension[19], cardiovascular disease (CVD)[90] and all-cause mortality[20]. As the simple index of height and weight developed by Quetelet was shown to predict total adiposity with a moderate degree of accuracy[91], BMI has become the most common, indirect measurement of obesity in large population studies and in clinical settings. Evidence-based guidelines have been developed to define overweight and obese using BMI cutpoints[92]. These guidelines identified a BMI between 25.0-29.9 kg/m^2 as overweight, and a BMI greater than or equal to 30.0 kg/m^2 as obese. The risk of mortality, cardiovascular disease, cancer, and other diseases has been shown to

increase throughout the BMI categories of overweight and obese for both men and women[20].

2.3.2 Abdominal Obesity and Associated Health Risk

In the 1950's Jean Vague was the first to note that the location of body fat rather than its absolute amount was an important indicator of obesity-related health risk[21]. Through anthropometric measurements Vague defined two unique obesity phenotypes; gynoid obesity, represented by predominantly lower body fat accumulation, and android obesity, of predominantly upper body fat accumulation. While gynoid obesity was found to be a fairly benign condition, android obesity, on the other hand, was associated with premature artheroscleoris, diabetes, gout, and uric calculous disease. In the early 1980's the laboratories of Bjorntorp et al. from Sweden and Kissebah et al. from the US expanded on Vague's work and showed that for a given degree of adiposity, the regional distribution of fat plays an important role in the development of the metabolic aberrations seen[93, 94]. More specifically, they noted that upper body[94] or abdominal obesity[93] was a stronger correlate of fasting insulin, blood glucose and triglyceride levels as well as blood pressure and glucose tolerance than was peripheral obesity. Furthermore, long-term longitudinal studies of large cohorts of Swedish men[95] and women[96] demonstrated that abdominal obesity as assessed by the WHR strongly predicted myocardial infarction, stroke, and death independent of age and total obesity (BMI). More recent investigations including a combined population of approximately 100 000 participants from over 50 countries continue to support the notion that abdominal obesity phenotype, rather than total obesity, is the best predictor of health risk[23-25]. Additionally, while many have

suggested that the measurement of abdominal obesity and total obesity in combination provides the best assessment of health risk[97-99], some argue that abdominal obesity alone sufficiently explains obesity related health risk[25]. Indeed, the prognostic importance of abdominal obesity in the identification of health risk is exemplified by its inclusion in the diagnostic criteria for the metabolic syndrome[100, 101].

While most of the earlier studies used the ratio of waist to hip circumference, the majority of recent studies utilize waist circumference alone as the index of abdominal obesity. WC has been chosen over WHR as the abdominal measurement of choice due to its closer association with VAT amount (discussed later) and cardiovascular risk[102]. Based on this evidence the National Institutes of Health published sex specific WC cut-points to define abdominal obesity[92]. Subsequently, it was reported that within each BMI strata, those with abdominal obesity (WC > 102 and 88cm in men and women, respectively) exhibited greater risk of hypertension, type 2 diabetes and dyslipidemia, than those of normal WC values[97].

2.3.3 Visceral Adipose Tissue and Health Risk

The association between WC and health risk may be explained by excess accumulation of adipose tissue in two distinct depots within the abdomen, namely abdominal visceral (VAT) and subcutaneous adipose tissue (SAT) (Figure 2). Only with the advent of imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) has the quantification of these depots become possible. In the mid 1980's, Sparrow et al. were the first to note associations between VAT as assessed by CT and health risk[103]. Since then, while some report SAT to be

Figure 2. Quantification of visceral AT and abdominal subcutaneous AT using computed tomography (CT).

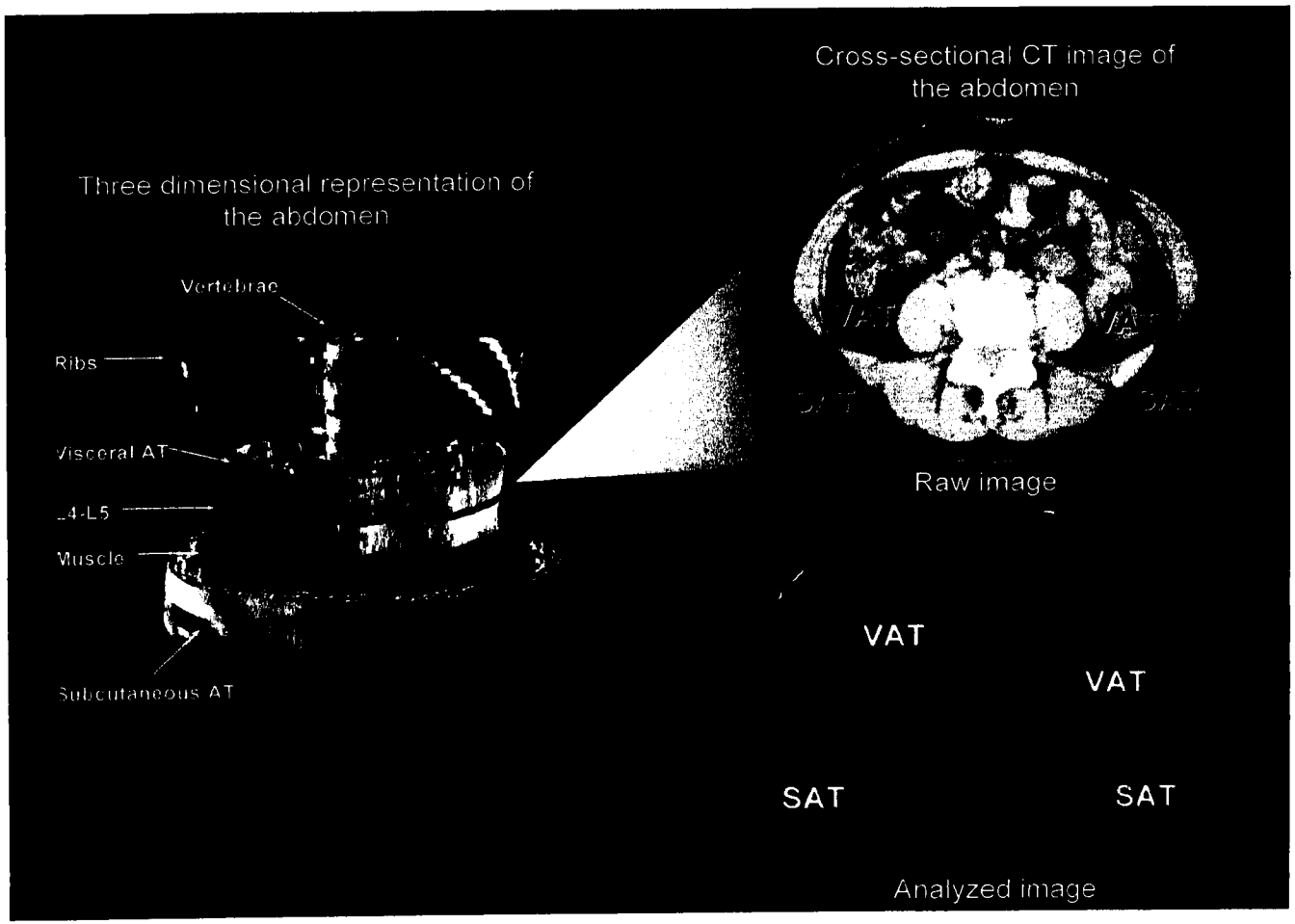


Table 4. Associations between visceral AT and health outcomes independent of abdominal subcutaneous AT.

		Outcome					
Reference	**Sample**	**Dyslipidemia**	**Glucose/Insulin dynamics**	**Inflammation**	**Hypertension**	**CVD**	**T2D**
Pouliot (1992)[104]	58 OB ♂	✓	✓				
Lemieux (1996)[105]	30 ♀		✓				
Banerji (1997)[106]	52 T2D AA ♂/♀		✓				
Fujimoto (1999)[107]	175 JA ♂	✓				✓	
Banerji (1999)[108]	20 AI ♂	✓	✓				
Boyko (2000)[27]	520 JA ♂/♀						✓
Brochu (2000)[109]	44 Post OB ♀		✓				
Forouhi (2001)[110]	113 SA/EU ♂/♀			✓			
Rendell (2001)[111]	55 Post ♀	✓	✓				
Janssen (2002)[112]	38 Pre OB ♀	✓	✓				
Ross (2002)[113]	40 Pre ♀		✓				
Nguyen-Duy (2003)[114]	161 ♂	✓					
Nieves (2003)[115]	196 ♂/♀	✓	✓				
Hayashi (2004)[116]	300 JA ♂/♀				✓		
Kuk (2006)[28]	291 ♂						

Abbreviations: CVD, cardiovascular disease; T2D, type II diabetes; OB, obese; AA, African American; JA, Japanese; AI, Asian Indian; Post, post-menopausal; Pre, pre-menopausal; SA, South Asian; EU, European.

an independent predictor of health risk[31, 117], the preponderance of literature indicates that VAT is a strong predictor of dyslipidemia, glucose and insulin dynamics (including insulin resistance), systemic inflammation, incidence of hypertension, CVD, T2D, and mortality, independent of ASAT (Table 4). The benign consequence of SAT is well exemplified in Japanese sumo wrestlers[118] and in patients with multiple symmetric lipomatosis (MSL)[119], both of whom have massively expanded SAT depots, but exhibit normal metabolic profiles. However, both of these conditions are characterized by very little VAT accumulation. In contrast, retired sumo wrestlers substantially accumulate VAT, become insulin resistant, and show an increased incidence of T2D and CVD[118]. Additionally, surgical intervention studies show that while the selective removal of VAT leads to significant improvements in metabolic profile[120], SAT liposuction confers no such benefit[121]. Lastly, exercise and/or diet intervention studies show that the reduction in VAT is independently associated with improvements in metabolic profile[122-125].

Although attempts to define a critical cut-point of VAT accumulation beyond which health risk is elevated (akin to those established for BMI and WC) have been reported, to date, no age and sex specific VAT threshold values have been firmly established. In one of the only attempts to delineate such a VAT cut-point in young Caucasian adults, Despres and Lamarche[126] found that a cross sectional area of VAT at the L4-L5 intervertebral space beyond 100cm^2 was associated with deteriorations in metabolic profile and glucose tolerance. That VAT amount is linked with adverse health outcomes is unequivocal, however, the mechanism(s) that describe this association have yet to be fully understood.

2.3.4 The Link between VAT and Health Risk: The Putative Mechanisms

2.3.4.1 Location Driven Mechanism: The Portal Theory

The most popular mechanism connecting VAT to health risk relates to the anatomical location of VAT and its venous drainage. As VAT is drained into the portal venous system, the liver is exposed to all products secreted by VAT, particularly free-fatty acids (FFA). An increased flux of FFA from an expanded VAT depot to the liver can cause enhanced secretion of low density lipoproteins[127], elevated rates of gluconeogenesis[128], and reduced rates of insulin metabolism by the liver[128]. These changes in hepatic metabolism then lead to systemic hyperlipidemia, hyperglycemia, and hyperinsulinemia – all known markers of health risk.

2.3.4.2 Metabolically Driven Mechanism

Although all adipocytes were originally considered homogenous, many reports suggests that white adipocytes from the visceral depot are metabolically different from those of the subcutaneous depot. Visceral adipocytes show elevated rates of lipolysis upon catecholamine stimulation[129], have a greater number of β-adrenergic receptors[130], and are less sensitive to the anti-lipolytic effect of insulin[131] as compared to subcutaneous adipocytes. The overall effect of these differences is a higher lipid turnover and exaggerated release of FFA from VAT. The elevated levels of FFA in the circulation may lead to disordered hepatic metabolism, deposition of fat in the liver and skeletal muscle, and resultant insulin resistance and associated metabolic disturbance[132].

2.3.4.3 Adipocytokine Driven Mechanism

In addition to its metabolic functions, adipose tissue also acts as an endocrine organ secreting many cytokines (referred to as adipokines) including adiponectin, leptin, plasminogen activator I (PAI-1), interleukins (IL), and tumour necrosis factor alpha (TNFα). In particular, VAT has been shown to secrete more PAI-1 and IL-6 than SAT[133]. As both PAI-1[134] and IL-6[135] are known to promote systemic inflammation and atherogenesis, their elevated secretion from visceral adipocytes may explain the health risk associated with excess VAT.

2.3.4.4 Non-causal Theory

Lastly, it has been proposed that VAT may not in itself be pathological, but may be a proxy for some yet unknown factor or list of factors, genetic and/or environmental which lead to metabolic disturbance and by coincidence to VAT storage as well [136, 137]. In fact, some have suggested that this common factor may be SAT, which is both correlated with VAT and health risk[138]. However, in light of weighted evidence (Table 4) showing associations between VAT and various health outcomes independent of SAT, and the dearth of evidence to the contrary this is unlikely to be the case.

2.3.5 Fat Storage in Non-adipose Tissues

It has been proposed that with excessive adiposity, there is a diversion of fat from adipose tissue to non-adipose tissues such as liver and skeletal muscle. This diversion may occur due to adipocyte resistance to the anti-lipolytic effects of insulin [139], and/or the exhaustion of storage capacity of the adipose organ [140], both of which would lead to elevated levels of FFAs in systemic circulation, and subsequent ectopic

fat storage. For example, lipodystrophy, a syndrome characterized by limited adipose tissue storage capacity, is associated with excessive fat storage in the liver and muscle, as well as insulin resistance and diabetes [141]. Furthermore, pharmacologic interventions using thiazolidinediones (TZD), a group of PPAR-γ agonists, have been shown to promote the development of new subcutaneous but not visceral adipocytes [142]. Paradoxically, the expansion of the subcutaneous adipose depot is mirrored by decreased muscle [143] and liver fat storage [144] and improved insulin sensitivity [145]. Additionally, as FFA are released into the circulation in proportion to the size of the adipose organ, by a mass effect, the large fat mass in obese individuals may lead to elevation of FFA flux to non-adipose tissues in the absence of any abnormality in adipose tissue metabolism [146].

2.3.5.1 Fat Accumulation in Skeletal Muscle and Health Risk

In 1967, Denton and Randle were the first to note that triglycerides (TG) can be stored in skeletal muscle[147]. A few decades later, Pan et al reported the inverse association between the storage of TG in the muscle and peripheral insulin sensitivity in humans[148]. Since that time, a number of studies using various methods have supported these initial findings (Table 5). Skeletal muscle of obese and T2D subjects has been shown to store more TG than that of lean, sedentary individuals[31, 149, 150]. Conversely, weight loss has been shown to induce significant reductions in the muscle TG storage[149, 151]; reductions which correlate with improvements in insulin sensitivity[151]. Further, many studies have shown that muscle lipid storage was significantly associated with insulin resistance independent of obesity[31, 148, 150, 152-155], abdominal obesity[31, 148, 152, 153, 155, 156], and VAT[31]. Paradoxically, the relationship

Table 5. Relationship between skeletal muscle triglyceride storage and insulin resistance.

Reference	N	Sample	Method	Correlated with insulin resistance	Independent of BMI/ % body fat	Independent of abdominal obesity
Phillips (1996)[157]	27	Women	Biopsy	✓	NR	NR
Pan (1997)[148]	28	PI Men	Biopsy	✓	✓	✓
Goodpaster (1997)[31]	26/28	OB Men/OB Women	CT	✓	✓	✓
Jacob (1999)[152]	13/13	LN-IR/LN-IS	H^1-MRS	✓	✓	✓
Krssak (1999)[158]	8/15	LN men/LN women	H^1-MRS	✓	NR	NR
Perseghin (1999)[153]	14/14	LN-T2D Off/LN-Con	H^1-MRS	✓	✓	✓
Forouhi (1999)[156]	20/20	SA/EU men	H^1-MRS	✓(EU)	X	✓
Goodpaster (2000)[159]	11/40/15	OB-T2D/OB-GT/LN-GT	CT	✓	✓	NR
Virkamaki (2001)[155]	20	LN Men	H^1-MRS	✓	✓	✓
Goodpaster (2001)[154]	9/11/8/9	LN/OB/T2D/Trained	CT	✓ (Untrained)	✓ (Untrained)	NR
Petersen (2003)[160]	13/15	Young LN/Old LN	H^1-MRS	✓	NR	NR
Thamer (2003)[161]	28/77	LN Men/ LN Women	H^1-MRS	✓ (Untrained)	X	NR

Abbreviations: OB,Obese; T2D, Type 2 Diabetic, GT, glucose tolerant; LN, lean; SA, South Asian; EU, European; PI, Pima Indians; IR, insulin resistant; IS, insulin sensitive; T2D Off, Offspring of T2D parents

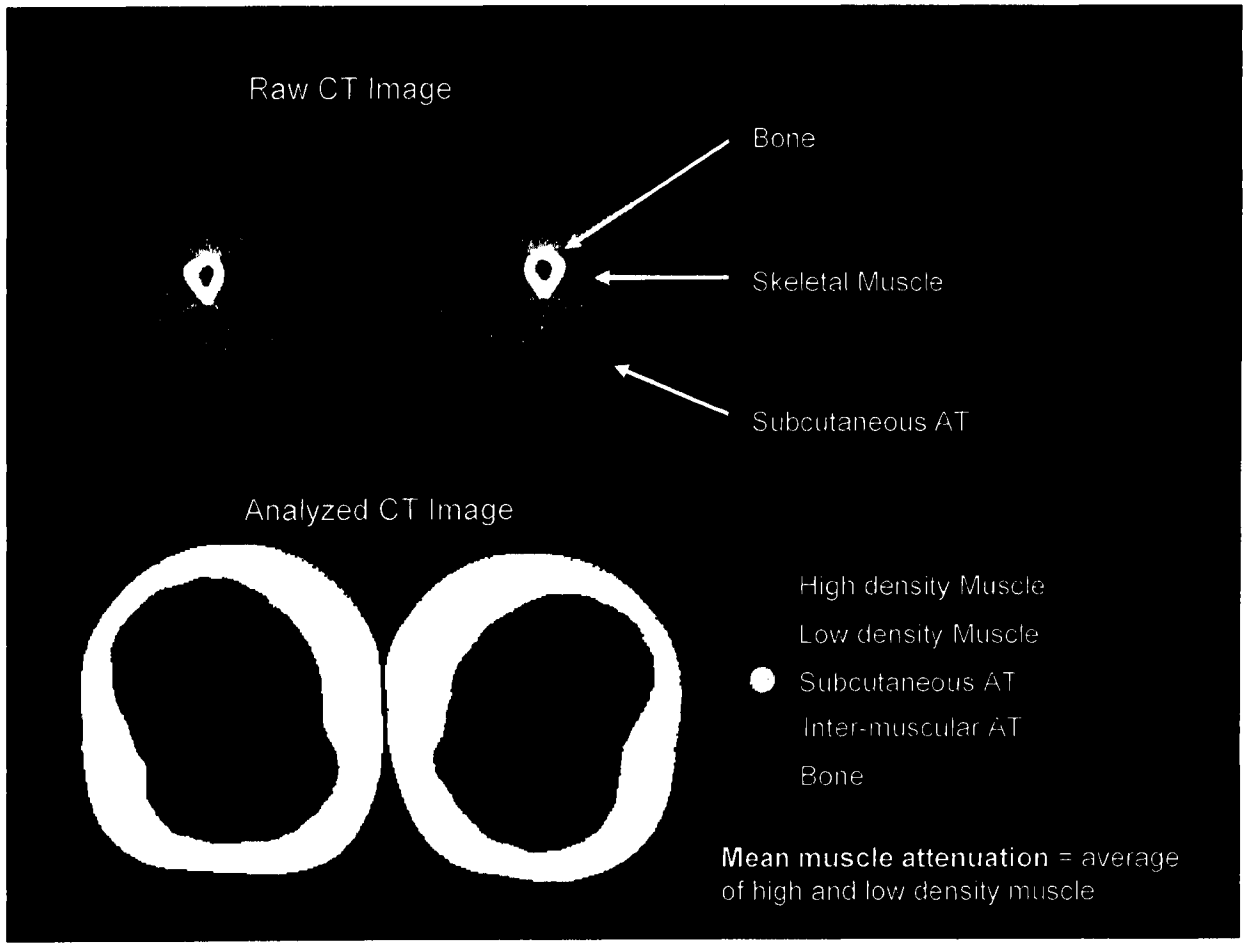
between muscle TG store and insulin resistance holds true only for sedentary individuals, as the accumulation of fat in the metabolically efficient muscle of highly trained athletes does not appear to carry adverse metabolic consequences [154, 161]. Increased muscle fat content in a sedentary obese individual may simply be a marker of a metabolically inefficient muscle, which itself is a surrogate for physical inactivity. Indeed, over a decade ago Simoneau et al noted that the skeletal muscle of obese individuals, in addition to having excess storage of TG, also had reduced oxidative capacity, which was related to the degree of peripheral insulin resistance[162]. Thus, it has been suggested that aerobic fitness, and related oxidative muscle capacity are key mediators of the relationship between muscle TG storage and insulin resistance[161].

2.3.5.2 Measurement of Muscle Fat Deposition

Originally muscle TG content was measured by biopsy and histochemical staining. Due to the invasive nature of percutaneous biopsy, more recent studies have used non-invasive radiographic imaging methods such as CT and/or proton MR spectroscopy (H^1-MRS) (Table 5). CT can differentiate tissues *in vivo* based on their attenuation characteristics, which depend on their density and chemical composition. CT attenuation values are measured in Houndsfield units (HU), and are based on a linear scale using water as the reference (0 HU). Since fat is less dense than muscle, the attenuation value of fat is lower than that of the muscle.

Based on its attenuation values, skeletal muscle can be divided into high-density (31-100 HU) and low-density (0-30 HU) muscle (Figure 3). The mean of all the pixels within the range of 0-100 HU is used as a measure of mean muscle

Figure 3. Computed tomography analysis of muscle fat content (mean muscle attenuation)



attenuation and reflects the lipid content determined by biochemical and histological analysis, such that a lower mean muscle attenuation reflects a higher lipid content[150]. Indeed, it has been determined that increasing a solution's lipid content by 1% results in a fairly consistent 1 HU decrement in attenuation. One advantage of H^1-MRS to CT lies in its ability to distinguish between intra- and extra-myocellular TG stores (IMTG and EMTG, respectively), as IMTG rather than EMTG stores appear to be more closely related to measures of insulin sensitivity[152, 153, 158]. However, a recent investigation found mean muscle attenuation by CT to be highly correlated with IMTG levels as assessed by ^{1}H magnetic resonance spectroscopy (MRS)[163].

2.3.5.3 Liver Fat and Health Risk

Although originally considered a benign finding, fatty liver, a component of non-alcoholic fatty liver disease (NAFLD) has since emerged as a novel component of the metabolic syndrome [164] and a possible predictor of T2D [165]. Fatty liver has been commonly defined as TG content exceeding 5% of total hepatic mass [166]. A number of recent studies have reported significant relationships between liver fat and derangements in metabolic profile including insulin resistance, elevated plasma glucose, insulin, TG, cholesterol and decreased HDL levels [29, 30, 114, 164, 167-174] (Table 6).

Although fatty liver is commonly associated with obesity [175], in particular VAT [114, 170, 174], others have found strong associations between liver fat and metabolic disturbance independent of total and abdominal obesity [29, 114, 168, 174]. That VAT is significantly associated with liver fat suggests that FFA flux from VAT via the portal

Table 6. Relationship between fatty liver and markers of metabolic status in various populations.

Reference	N	Sample	Method	Significant relationship with fatty liver					
				IR	Glu	Ins	TC	TG	HDL
Banerji (1995) [167]	21	AA T2D men	CT	✓	--	NS	--	✓	--
Ueno (1997) [172]	13/12	OB men/ OB women	US	--	✓	--	NS	✓	--
Marchesini (1999) [168]	46/92	NAFLD/ Con	US	✓	✓	✓	NS	✓	✓
Ryysy (2000) [169]	20	T2D	H^1-MRS	✓	--	--	--	--	--
Marchesini (2001) [164]	30/10	NFLD/Con	Biopsy	✓	NS	NS	NS	✓	✓
Tikkainen (2002) [30]	27	OB women	H^1-MRS	✓	NS	✓	--	✓	✓
Seppala-Lindroos (2002) [29]	30	Men	H^1-MRS	NS	NS	✓	NS	✓	✓
Omagari (2002) [173]	747/2685	JA NAFLD/Con	US	--	✓	--	✓	✓	--
Nguyen-Duy (2003) [114]	161	Men	CT	--	✓	--	--	✓	✓
Kelley (2003) [170]	83/12/15	T2D/ LN Con/OB Con	CT	✓	NS	NS	--	✓	✓
Weterbacka (2004) [174]	66/66	OW men/OB women	H^1-MRS	--	--	✓	--	✓	--
Adiels (2006) [171]	10/18	OB T2D/Con	H^1-MRS	✓	✓	✓	--	✓	--

Abbreviations: IR, insulin resistance; Glu, glucose; Ins, insulin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; AA African American; US, ultra sound; NAFLD, Non-alcoholic fatty liver disease; OW, overweight, JA, Japanese; OB, obese; LN, lean; Con, control

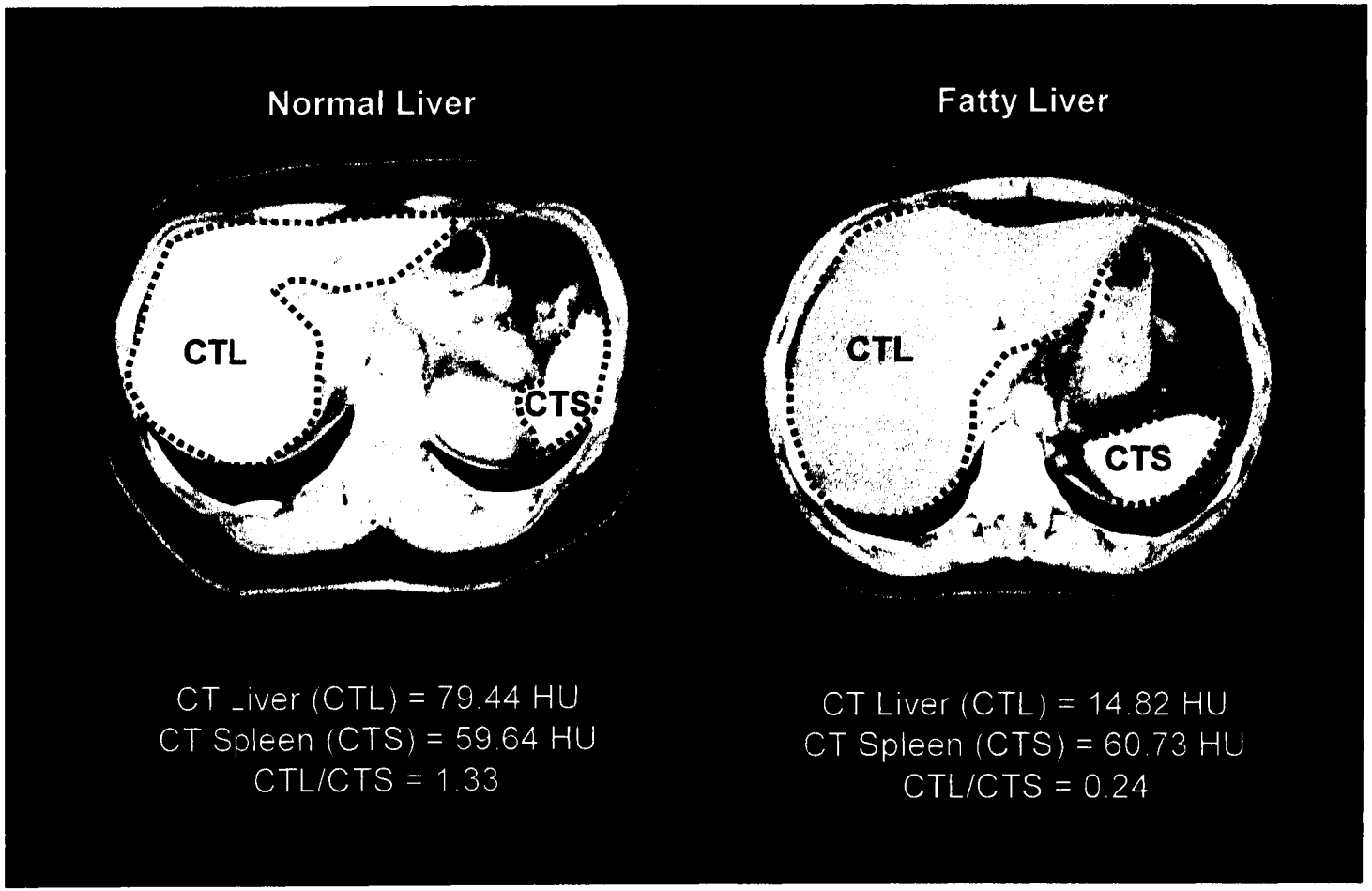
vein may lead to liver fat development, however, as this is not an unequivocal finding [29, 30], other yet unknown mechanisms may explain the development of fatty liver. One such possibility may be a high dietary consumption of saturated fats. As it has been shown that 50% of dietary fat is taken up by the liver [176], it is of little surprise that dietary fat content is related to degree of liver fat storage [177, 178].

Finally, although the associations between liver fat and metabolic risk, particularly insulin resistance are well established (Table 6), the mechanism(s) explaining these associations are largely unclear. For example, while some animal studies have shown the development of fatty liver to directly lead to hepatic insulin resistance[179], others have not [180]. A detailed overview of putative mechanisms is beyond the scope of this review, and the reader is encouraged to examine reviews on the topic[139].

2.3.5.4 Measurement of Liver Fat

Although liver biopsy is considered the most reliable method of fatty liver detection, due to the invasiveness, sampling variability, and sequelae of the procedure, there has been impetus for the development of alternative diagnostic methods. Computed tomography and H^1-MRS have emerged as safe and reliable alternatives. As with muscle lipid estimation, the CT attenuation of the liver is a function of its density, which depends on the degree of fat infiltration; thus, the higher the fat content - the lower the attenuation value (Figure 4). As the density of a healthy liver has been shown to vary between individuals [181], the density of the liver may be expressed relative to that of the spleen. A normal liver is usually denser and, consequently, has a higher attenuation value than the spleen. Using the CT image

Figure 4. Computed tomography analysis of liver fat content



analysis techniques of fatty liver index (ratio of mean liver: spleen attenuation), strong correlations were found between CT and histological measures of steatosis (r = - 0.77) [182]. A fatty liver index score below 1.0 has been chosen as the threshold for identification of moderate liver fat infiltration[182] (Figure 4).

2.4.0 Summary

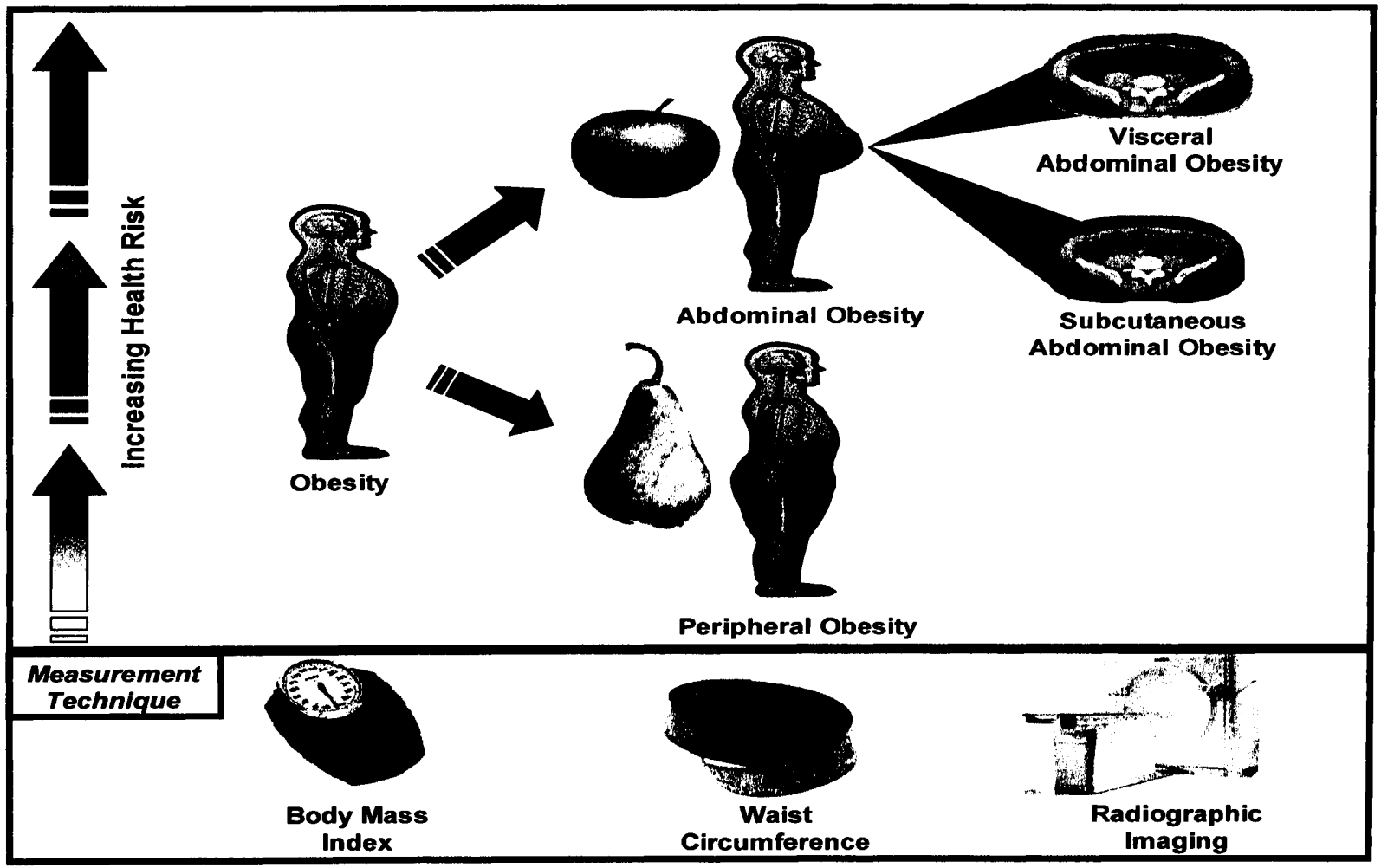
Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy; fortunately, improvements in treatment strategies have elevated survival rates for ALL, leading to a growing number of ALL survivors. Unfortunately, concurrent with the dramatically improved survival rates is the mounting recognition of various late complications of ALL therapy. In particular, multiple reports have found that by completion of therapy, attainment of final height, and in young adulthood ALL survivors are at heightened risk for becoming overweight and obese as compared to age-matched healthy controls and/or population reference norms. Specifically, the use of cranial radiotherapy (CRT) during ALL treatment [8, 15, 16] and female sex appear to be strong predictors of the subsequent development of obesity [8, 15, 17, 18]. Consequently, a significant proportion of ALL survivors exhibit a constellation of obesity-related risk factors including hyperinsulinemia[6, 7, 11], hyperglycemia[7, 11], dyslipidemia[7, 11, 13], and hypertension[13], and experience an excess in all-cause and cardiovascular-related mortality[14].

Although increasing levels of total obesity are mirrored by increased risk for morbidity[19] and mortality[20] from various causes, the location of excess fat, rather than its absolute amount has been shown to mediate the link between obesity and health risk [21]. It is now apparent that obesity-related health risk is most prominent in those

with excessive abdominal fat deposition[22-25]. Specifically, the excess accumulation of visceral adipose tissue (VAT)[26-28] within the abdomen is strongly and independently associated with metabolic aberration[26], morbidity[27], and mortality[28]. Figure 5 illustrates in summary how the health risks attributed to obesity are dependent on region and depot specific deposition of fat. Finally, recent investigations have found the ectopic storage of fat in non-adipose tissues of the liver and skeletal muscle to also carry independent health risk [29-31].

Although previous investigations have established excess total adiposity in ALL survivors through the use of body mass index (BMI)[8, 15, 16, 18, 54], dual energy x-ray absorptiometry (DEXA)[5, 7, 9, 10, 12], skinfolds[11], and bioimpedance analysis[6], to date, no assessment of regional and ectopic adiposity in this cohort has been performed. Using computed tomography (CT) and dual energy x-ray absorptiometry (DEXA), the aim of the investigation that follows was to quantify total, abdominal, and visceral adiposity as well as skeletal muscle and liver fat deposition according to sex and treatment type (CRT/ no-CRT) in a sample of young adult survivors of ALL.

Figure 5. Characterizing obesity and associated health risk – from total to regional to depot specific adiposity



3.0.0 MANUSCRIPT

Abdominal Obesity, Liver Fat and Muscle Composition in Young Adult Survivors of Childhood Acute Lymphoblastic Leukemia

Peter M. Janiszewski

ABSTRACT

Background: Survivors of childhood acute lymphoblastic leukemia (ALL) gain excess body weight following treatment. We sought to determine whether cranial radiotherapy (CRT) and/or sex are associated with elevations in total and abdominal obesity, specifically visceral adipose tissue (VAT), as well as liver and muscle fat accumulation, and altered growth hormone (GH) and leptin status in young adults ALL survivors.

Methods: Abdominal AT, VAT, abdominal subcutaneous AT (SAT) masses were quantified from the L3-L4 to the L4-L5 inter-vertebral space using computed tomography (CT) in 52 male (15 CRT treated) and 62 female (24 CRT treated) young adult ALL survivors. The ratio of mean liver to mean spleen CT attenuation was used as a qualitative measure of liver fat infiltration. Mean muscle attenuation from 11 CT images spanning from 12-18 cm above the patella was used to indicate the degree of muscle fat deposition. Total fat and lean body mass were measured using dual energy x-ray absorptiometry (DEXA). Commercial radio-immunoassays were used to measure serum insulin growth factor-1 (IGF-1) and leptin levels. IGF-1 levels were used as a surrogate measure of GH status.

Results: Controlled for age and race, CRT treated ALL survivors had higher VAT, body fat percentage, and leptin levels, but lower lean mass and IGF-1 levels as compared to non-CRT survivors ($P < 0.05$). Among female survivors, CRT was associated with a significantly higher VAT: SAT ratio ($P < 0.01$). Levels of IGF-1 were inversely associated with total adiposity, VAT and VAT: SAT ratio in both sexes ($P < 0.01$). Female ALL survivors had less lean mass and VAT but higher fat

mass and SAT than males ($P < 0.05$). Neither CRT nor sex was associated with muscle and/or liver fat content. ***Conclusion:*** Among young adult ALL survivors, CRT is a risk factor for elevated total and abdominal obesity, visceral adiposity and reduced fat-free mass in association with an altered IGF-1 and leptin levels.

INTRODUCTION

Improved treatment strategies for childhood acute lymphoblastic leukemia (ALL) have lead to a growing population of long-term survivors [2]. Unfortunately, along with the enhanced survival rates comes the recognition of many adverse health consequences of childhood cancer treatment [183]. Of particular concern, a number of studies have reported that ALL survivors are overweight and obese as compared to healthy age-matched controls [4-12]. Consequently, a high proportion of ALL survivors exhibit a constellation of obesity-related cardiovascular risk factors [6, 7, 11, 13] and are at elevated risk for all-cause and cardiovascular-related mortality [14]. Cranial radiotherapy (CRT) during ALL treatment has been implicated as a potential cause of excess weight gain among survivors [8, 15, 16]. Although the exact mechanism by which CRT leads to obesity is unknown, CRT may damage the radiosensitive hypothalamus leading to growth hormone (GH) deficiency and/or leptin insensitivity[7, 10]. Further, the risk of obesity following ALL treatment appears to be more pronounced in females than in males [8, 15, 17, 18].

It is well established that obesity-related health risk is greatest in those with an abdominal obesity phenotype [22-25]. Specifically, the excess accumulation of visceral adipose tissue (VAT) within the abdomen is strongly and independently associated with metabolic aberration [115], morbidity [27, 107, 116], and mortality [28]. Emerging evidence also suggests that storage of fat in non-adipose tissues such as liver and skeletal muscle may carry independent health risk [29-31].

A limited number of studies in ALL survivors have reported on abdominal obesity using indirect measurement methods including waist circumference [7, 12],

waist-to-hip ratio [6, 7, 12, 13] and DEXA [12]. The findings of these reports have been largely inconsistent [6, 7, 12, 13]. Moreover, absent from the literature are studies that have characterized survivors of ALL with respect to specific fat depots that are established independent predictors of health risk.

The primary aim of the present investigation was to assess whether among young adult ALL survivors, CRT and/or sex were associated with elevations in abdominal obesity, specifically VAT, as well as liver and muscle fat accumulation. We also determined whether the effects of CRT on body composition were related to GH status and/or leptin insensitivity. Precise characterization of the obesity phenotype in ALL survivors may help explain the elevated risk of morbidity and mortality and hence, highlight specific targets for future interventions aimed at reducing health risk in this vulnerable population.

METHODS

Project ALLIFE (Acute Leukemia Lifestyle Intervention for Everyday) was conducted in Dallas, Texas at the University of Texas Southwestern Medical Center and The Cooper Institute. Institutional Review Boards from both research organizations approved the protocol yearly. All study participants provided written informed consent for study participation and release of medical record information. Except for the body composition measures assessed by dual energy x-ray absorptiometry (DEXA) and computed tomography (CT) scans conducted at the Cooper Institute, all measures were conducted through the General Clinical Research Center (GCRC) at UT Southwestern.

Study Population

Eligibility criteria for Project ALLIFE included: 1) a diagnosis of ALL between the ages of 0-20 years of age; 2) alive and at least two years from completion of therapy for ALL, 3) 18 years of age or older at time of enrollment; 4) willingness to provide access to medical records to abstract cancer diagnosis and treatment; 5) living in the Dallas-Fort Worth area, 6) willingness to provide informed consent, and 7) able to complete testing. Because testing included radiation exposure (abdominal CT and DEXA), females who were pregnant were excluded from enrolling in the study.

A database of potentially eligible patients was created from three sources. The primary source was the cancer registry at Children's Medical Center Dallas which included all patients diagnosed as having childhood ALL from 1970 to 2000. A small group of ALL survivors were eligible who were diagnosed at Cook Children's

Hospital in Forth Worth, Texas and treated on a shared, two-institutional protocol called DFW-1. A third database included a small number of ALL survivors followed in the survivor program at Children's Medical Center Dallas and UT Southwestern (the After the Cancer Experience [ACE] Program) who were diagnosed and treated at other institutions.

We were able to identify 368 potential participants from the three sources. Of these 368 potential participants, current contact information was not available for 131 (35.6%) and they were considered lost to follow-up. A recruitment letter from the principal investigator (KO) with information about the study and contact information for the study coordinator was sent to the 237 potential participants with verifiable contact information, followed by a telephone call from the research coordinator two weeks later to verify eligibility. Forty-eight potential participants did not meet eligibility criteria (5 with severe cognitive deficits; 2 with Down syndrome; 7 with major medical problems precluding testing including paraplegia, heart disease, or uncontrolled seizure disorders; 2 were pregnant; 1 was in jail; 2 were in the military; and 29 lived out-of-state). A total of 189 potential participants were contacted who met the eligibility criteria for the study. Of this 189, 16.4% (n=31) actively refused to participate. Primary reasons for not participating included lack of interest (n=16), lack of time (n=10), previous bad experiences or fearful of having blood drawn (n=5). An additional 21.2% (40/189) were passive refusals, including eligible participants who were initially interested in the study but did not schedule an enrollment visit (n=26) and those who scheduled an enrollment visit but did not keep the appointment (n=14). The remaining 118 eligible participants enrolled in Project ALLIFE (62.4%;

118/189). Key demographic characteristics, including sex, age, race and ethnicity, age at cancer diagnosis, and interval from cancer diagnosis to present time, were not significantly different in eligible participants who did not enroll in the study (active and passive refusals; N=71) in comparison with participants (N=114). Four of the 118 participants enrolled in Project ALLIFE were excluded from this analysis due to lack of CT and/or DEXA scans.

Cancer Diagnosis and Treatment Exposures

Information regarding the cancer diagnosis and treatment exposures was abstracted from the medical records of each participant. Date of diagnosis, date of any recurrence, and interval from date of ALL diagnosis to study enrollment was recorded. Chemotherapy information (yes/no) was obtained for 11 different agents. Cumulative dose of anthracycline exposure (doxorubicin and daunorubicin) was calculated and expressed in mg/m^2. Cumulative intrathecal methotrexate was also calculated and expressed in mg/m^2. Total dose of CRT was recorded as yes/no and categorically: none, 10-19 Gy, 20-29 Gy, and $\geq$ 30 Gy.

Anthropometric and Radiographic Measures of Body Composition:

All anthropometric measurements were performed by a Nurse Coordinator of the General Clinical Research Center (GCRC) at UT Southwestern Medical. The participant's weight was measured on a digital scale in kilograms to the nearest 0.1 kg, while wearing light clothing and no footwear. Standing height was measured to the nearest 0.1 cm using a fixed stadiometer. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference was

measured at the level of superior iliac crest to the nearest 0.1 cm at the end of normal expiration.

Details of the CT analysis are included in Appendix E, and briefly described here. Computed tomography (CT) scans were performed by certified technicians at the Cooper Institute in Dallas, TX. Axial images of the abdomen and thigh region were obtained using an electron beam CT scanner (Imatron; General Electric, Milwaukee, WI) with the subject lying in a supine position. Approximately 35 contiguous images (0.6 cm thickness) were acquired from the distal iliac crest to the caudal region of the heart for the abdominal series, and approximately 70 images were acquired beginning from the superior border of the patella extending superiorly to the head of the femur for the thigh series. Images were obtained using standard procedures and analyzed using specialized image analysis software (Tomovision, Montreal, QC). A contiguous series of five to seven CT images between the L4-L5 and L3-L4 vertebral disc spaces were analyzed for determination of VAT and SAT. Adipose tissue areas (cm^2) were computed using an attenuation range of –190 to –30 Hounsfield units. Visceral fat was determined by delineating the intra-abdominal cavity at the innermost aspect of the abdominal and oblique wall musculature and the anterior aspect of the vertebral body. Abdominal subcutaneous fat area was defined as the area of adipose tissue between the skin and the outermost aspect of the abdominal muscle wall. The fat volumes were calculated by multiplying each fat area (cm^2) by the image thickness (0.6 cm) to obtain a partial volume, and subsequently adding up the partial volumes from the 5-7 contiguous images. Adipose tissue volumes (liters)

were converted to mass units (kilograms) by multiplying the volumes by the assumed constant density for fat (0.92 kg/liter).

Liver fat was determined using the ratio of the liver (CTL) to spleen (CTS) attenuation values on (CTL/CTS) [184, 185] using a single cross-sectional CT-image that clearly displayed both organs, a procedure described in detail elsewhere [186]. A lower mean liver attenuation value relative to that of the spleen is an indication of fatty infiltration of the liver [184].

Skeletal muscle composition was derived using the mean CT attenuation of thigh skeletal muscle (range 0-149 HU) averaged from 11 CT thigh images spanning a distance from 12 to 18 cm proximal to the patella. A reduced mean muscle attenuation is associated with an increased muscle lipid content [149].

The details of the DEXA scan procedure are included in Appendix F and are briefly described here. The physical concepts underlying DEXA measurement of body composition have been previously reviewed[187]. Although originally designed for the measurement of bone mineral content, DEXA is commonly used to assess total and regional fat and fat-free mass in-vivo [188, 189]. DEXA assesses body composition by measuring the attenuation of x-rays emitted at two energy levels as it traverses the body [188, 189]. Measures of total and regional skeletal muscle (coefficient of variation (CV) = 1 to 7%) [190-192] and fat mass (CV = 1 to 7%) [191] are highly repeatable. DEXA measures of appendicular or total skeletal muscle mass are highly associated with corresponding values obtained by CT or MRI (r = 0.86 to 0.98) [190, 192-195]. However, the radiation exposure associated with DEXA is less than computed tomography (CT), and is readily available at a significantly lower cost.

The DEXA scan was performed by certified technicians at the Cooper Institute in Dallas, TX. Total body fat mass, lean mass, and percent body fat were measured using a Lunar DPX DEXA scanner (MEC, Minster, OH). The DEXA scanner was calibrated daily utilizing a control vertebra provided by the manufacturer.

Insulin-like growth factor (IGF-1) and leptin:

The gold standard for diagnosing GH deficiency following cranial radiotherapy involves measuring peak GH levels following insulin provocative testing[42, 196]. Measurement of IGF-1 is not sensitive or specific enough to serve as a diagnostic test for GH deficiency[42, 196]. However, because IGF-1 levels correlate reasonably well with GH levels, it is an inexpensive and non-invasive measure of GH status[197-200].

Commercial radioimmunoassays were used to measure serum IGF-1 and leptin levels (Linco Diagnostic Services, Inc., St. Charles, MO).

Statistical Analysis:

A 2 x 2 ANCOVA for main effect of sex and CRT with an interaction term was used to assess differences between group means. Age and race/ethnicity were used as covariates in these analyses. Data not normally distributed was normalized using square root, square, and/or log transformations (See Appendix G for more details). Where an interaction term was significant, post-hoc analyses were performed using Student's t-test, with a Bonferroni adjustment for multiple comparisons. Pearson correlations and linear regression analyses were used to explore relationships among measures. All statistical analyses were performed on SPSS 13.0 (Chicago, IL).

RESULTS

Participant Details

As shown in Table 1, the mean age of the participants was 23.8 years (range, 18 - 37) and the mean interval from the date of cancer diagnosis to the date of enrollment in the study was 17.5 years (range, 4.9 – 34.0). The mean age at cancer diagnosis was 6.2 years (range 0 - 17). Fifty-four percent of participants were women and 26% were members of minority groups. CRT was included as part of therapy in 34% (39/114) of the participants. Average CRT dose did not differ between the male and female CRT participants (23 versus 23 Gy; $P > 0.1$). No differences were found in any outcomes between CRT treated participants who received less than 18 Gy as compared to all other CRT treated participants (CRT dose $\geq$ 18Gy) ($P > 0.05$).

Anthropometrics:

Results from the body composition measurements are presented in Table 2. Participants treated with CRT were significantly older and shorter than non-CRT participants ($P < 0.01$). Males were significantly taller and heavier than female participants ($P < 0.01$). Neither the presence of CRT nor sex had an effect on BMI and/or waist circumference ($P > 0.1$). Waist circumference was a significant predictor of both VAT and SAT masses in both sexes ($P < 0.001$; Figure 1).

Abdominal Adipose Tissue Distribution

Controlled for age and race/ethnicity, CRT versus non-CRT ALL survivors ($P < 0.05$) had greater abdominal fat, specifically greater VAT mass ($P < 0.01$; Figure 2). In female survivors only, CRT was associated with greater VAT mass ($P <0.01$), but similar SAT mass ($P > 0.1$), after further control for total fat mass (data not

shown). Accordingly, CRT was associated with a significantly higher VAT: SAT ratio in female but not male survivors ($P < 0.01$). Although abdominal AT did not differ between the sexes ($P > 0.1$), males had more VAT, but less SAT mass than females ($P < 0.05$), and thus, within the non-CRT category only, males had a greater VAT: SAT ratio than the females ($P < 0.01$).

Liver Fat and Skeletal Muscle Composition

Neither CRT nor sex were associated with the CTL/CTS ratio ($P > 0.1$) nor mean muscle attenuation ($P = 0.07$), indicative of liver and muscle fat content, respectively.

Total Body Fat and Lean Mass

Controlled for age and race/ethnicity, CRT survivors had a higher body fat percentage than non-CRT survivors ($P < 0.01$) which was due to both a higher total fat mass ($P = 0.06$) and a reduced total lean mass ($P < 0.01$; Figure 2). Females had a higher total fat mass ($P < 0.01$) and lower total lean mass ($P < 0.01$), and thus a higher body fat percentage compared to males ($P < 0.01$).

IGF-1 and leptin levels

Controlled for age and race/ethnicity, CRT participants had significantly lower IGF-1 levels, but higher absolute and relative (expressed per kg of fat mass) leptin levels than no-CRT counterparts ($P < 0.05$). Furthermore, females had greater absolute and relative leptin levels as compared to the males ($P < 0.01$).

Relationship between IGF-1, leptin levels and body composition

IGF-1 was significantly related to absolute and relative levels of leptin ($P < 0.05$) (Table 3). IGF-1 levels were negatively associated with total fat mass and

abdominal obesity, specifically VAT ($P < 0.05$; Figure 3). In female survivors only, low levels of IGF-1 were also associated with a higher VAT: SAT ratio, BMI and reduced height (not shown) ($P < 0.01$). Absolute leptin levels were correlated with all measures of total, regional adiposity, and muscle fat ($P < 0.05$), but not with liver fat ($P > 0.1$).

Table 1. Demographic and treatment-related characteristics of participants

	All participants N= 114 %	Females N= 62 %	Males N=52 %
Age at Study, years			
18 – 24	64.9	61.3	69.2
25 – 34	31.6	35.5	26.9
35 – 44	3.5	3.2	3.9
Race/Ethnicity			
White, NH	73.7	67.7	80.8
Black, NH	10.5	12.9	7.7
Other, NH	2.6	1.6	3.9
Hispanic/Latino	13.2	17.7	7.7
Household Income			
<$20,000	40.4	49.2	30.0
≥$20,000	59.6	50.9	70.0
Level of Education			
Did not graduate high school	11.5	14.8	7.7
Graduated from high school	21.2	19.7	23.1
Some college or technical school	40.7	36.1	46.2
Graduated from college	26.6	29.5	23.1
Current Smoker			
Yes	8.9	3.3	15.4
No	91.2	96.7	84.6
Age at Cancer Diagnosis, years			
Mean (SD)	6.2 (4.3)	5.8 (4.3)	6.7 (4.3)
Median (range)	5 (0 – 17)	4 (0 – 17)	6 (2 – 17)
Interval from diagnosis, years			
Mean (SD)	17.5 (6.0)	18.1 (6.2)	16.7 (5.7)
Median (range)	17.5 (4.9 – 34.0)	18.4(4.9– 34.0)	15.6 (5.5--30.1)
Cancer Therapy*			
Anthracycline	73.7	67.7	80.8
Cytarabine	62.3	66.1	57.7
Cyclophosphamide	43.0	40.3	46.2
Dexamethasone	11.4	14.5	7.7
Thioguanine	9.7	11.3	7.7
Etoposide	34.2	35.5	32.7
Anthracycline, any			
None	26.3	32.3	19.2
< 300 mg/m^2	54.4	48.4	61.5
≥ 300 mg/m^2	19.3	19.4	19.2
Cranial Radiation Therapy (CRT)			
None	65.8	61.3	71.2
10.0-19.9 Gy	9.7	9.7	9.6
≥ 20 Gy	24.6	29.0	19.2

Abbreviations: NH, non-Hispanic; SD, standard deviation

* Selected agents; more than 95% of participants received vincristine, methotrexate, prednisone.

Table 2: Anthropometrics, body composition and hormone status in a sample of young adult survivors of ALL.

	Men		Women		*P* value		
	No-CRT (n = 37)	CRT (n=15)	No-CRT (n=38)	CRT (n=24)	Main effect of CRT	Main effect of Sex	Interaction Effect
Anthropometrics							
Age (yr)	22.0 (3.5)	27.1 (5.7)	23.1 (4.2)	25.3 (6.0)	< 0.01	0.90	0.11
Height (cm)	175.5 (6.8)	171.6 (8.5)	162.7 (5.2)	154.8 (7.4)	< 0.01	< 0.01	0.23
Weight (kg)	80.8 (15.2)	81.9 (22.1)	71.9 (18.8)	76.3 (23.5)	0.70	< 0.05	0.29
BMI (kg/m^2)	26.2 (4.5)	27.8 (7.4)	27.2 (7.2)	31.6 (8.6)	0.16	0.35	0.08
Waist circumference (cm)	89.2 (12.1)	94.6 (16.3)	89.0 (15.3)	96.4 (16.7)	0.28	0.82	0.30
DEXA							
Total fat mass (kg)	18.0 (9.9)	23.9 (11.4)	26.0 (12.7)	32.5 (13.0)	0.06	< 0.01	0.93
Total lean mass (kg)	60.9 (8.1)	56.9 (11.1)	44.6 (6.5)	42.2 (9.5)	< 0.01	< 0.01	0.32
Percent body fat (%)	21.7 (8.7)	28.5 (6.3)	35.1 (7.8)	42.4 (4.9)	< 0.01	< 0.01	0.53
CT							
Abdominal fat (kg)	1.00 (0.64)	1.38 (0.65)	1.09 (0.59)	1.47 (0.54)	< 0.05	0.67	0.58
VAT (kg)	0.25 (0.17)	0.37 (0.22)	0.17 (0.13)	0.33 (0.17)	< 0.01	< 0.05	0.09
SAT (kg)	0.75 (0.52)	1.01 (0.51)	0.93 (0.49)	1.15 (0.41)	0.09	< 0.05	0.88
VAT @ L4-L5 (cm^2)	67.8 (42.9)	94.8 (48.6)	50.1 (34.7)	92.6 (46.3)	< 0.01	< 0.05	0.07
SAT @ L4-L5 (cm^2)	229.0 (150.0)	308.2 (178.2)	286.1 (136.7)	368.8 (134.0)	0.05	< 0.05	0.73
VAT:SAT ratio	0.31 (0.13)[a]	0.34 (0.15)	0.16 (0.07)[a,b]	0.25 (0.12)[b]	0.11	< 0.01	< 0.05
Liver fat (CTL/CTS)	1.22 (0.15)	1.17 (0.28)	1.24 (0.13)	1.23 (0.17)	0.67	0.70	0.74
Muscle attenuation (HU)	55.1 (3.2)	54.2 (3.8)	54.1 (3.3)	52.4 (4.3)	0.12	0.14	0.42
Hormone Status							
IGF-1 (µg/l)	463.1 (175.1)	315.3 (155.5)	434.3 (190.4)	254.8 (110.7)	0.01	0.49	0.16
Leptin (µg/l)	6.2 (6.9)	12.9 (15.5)	19.6 (14.3)	28.9 (14.6)	< 0.05	< 0.01	0.48
Leptin/fat mass (µg/l/kg)	0.3 (0.2)	0.5 (0.3)	0.7 (0.3)	0.8 (0.3)	< 0.05	< 0.01	0.79

Analysis based on normalized variables (age, BMI, WC, total fat mass, VAT, SAT, VAT@L4L5, SAT@L4L5, VAT: SAT ratio, liver fat, and muscle attenuation) controlled for age and race. [a,b] Significant corresponding differences at $P \leq 0.01$ in post-hoc analysis.

Table 3: Relationship between IGF-1, absolute and relative leptin levels and measures of body composition in men (A) and women (B) survivors of ALL.

A.

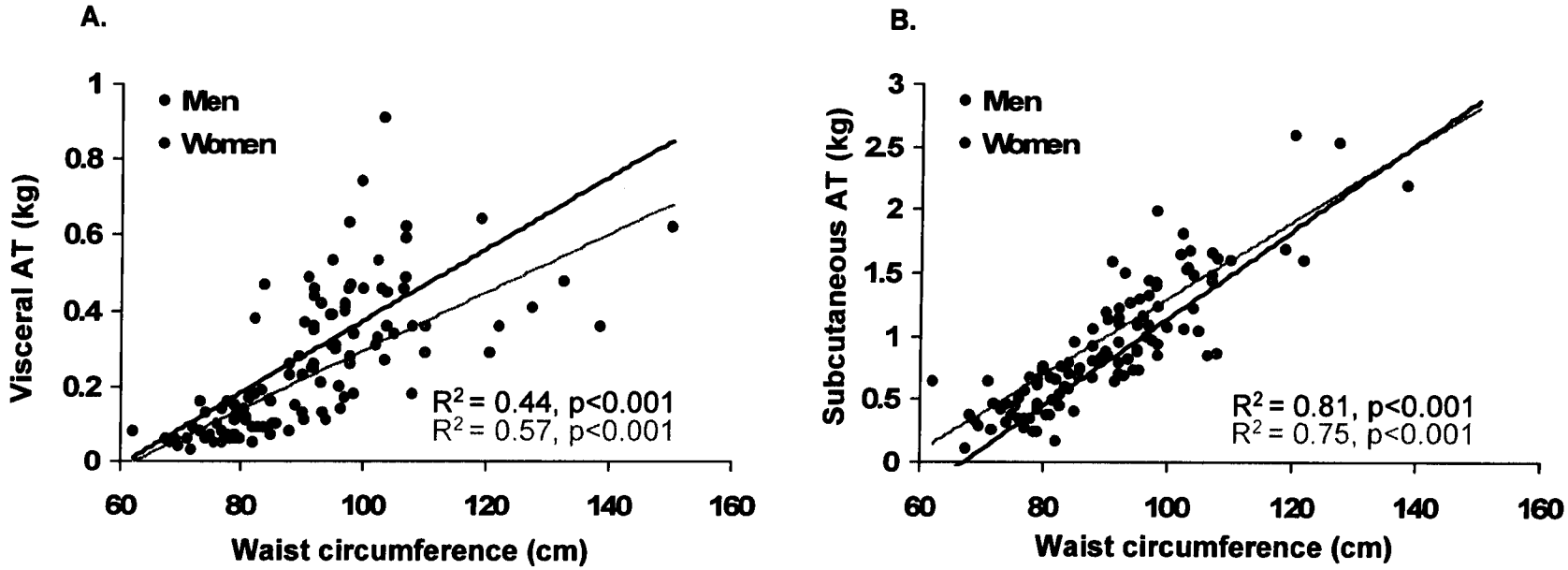
	IGF-1	Leptin	Leptin/Fat Mass	Waist Circumference	BMI	Fat Mass	Lean Mass	VAT	SAT	Muscle Fat	Liver Fat
IGF-1	1	-.31*	-.32*	-.39**	-.26	-.38**	-.04	-.40**	-.31*	.27*	.07
Leptin	-.31*	1	.86**	.64**	.65**	.72**	.21	.39**	.54**	-.45**	.05
Leptin/Fat Mass	-.32*	.86**	1	.37**	.36**	.52**	-.12	.25	.36**	-.30*	.07

B.

	IGF-1	Leptin	Leptin/Fat Mass	Waist Circumference	BMI	Fat Mass	Lean Mass	VAT	SAT	Muscle Fat	Liver Fat
IGF-1	1	-.50**	-.40**	-.39**	-.46**	-.45**	-.16	-.50**	-.37**	.24	.18
Leptin	-.50**	1	.75**	.68**	.75**	.80**	.55**	.65**	.66**	-.36**	.16
Leptin/Fat Mass	-.40**	.75**	1	.17	.23	.26*	.06	.21	.23	-.02	.15

Correlations based on transformed variables.

Figure 1: Relationship between waist circumference, visceral AT (A) and abdominal subcutaneous AT (B) in men and women ALL survivors.



R^2 based on transformed variables; raw data is presented in figure.

Figure 2: Main effects of CRT and Sex on visceral and subcutaneous abdominal AT, fat mass and lean mass in men and women ALL survivors.

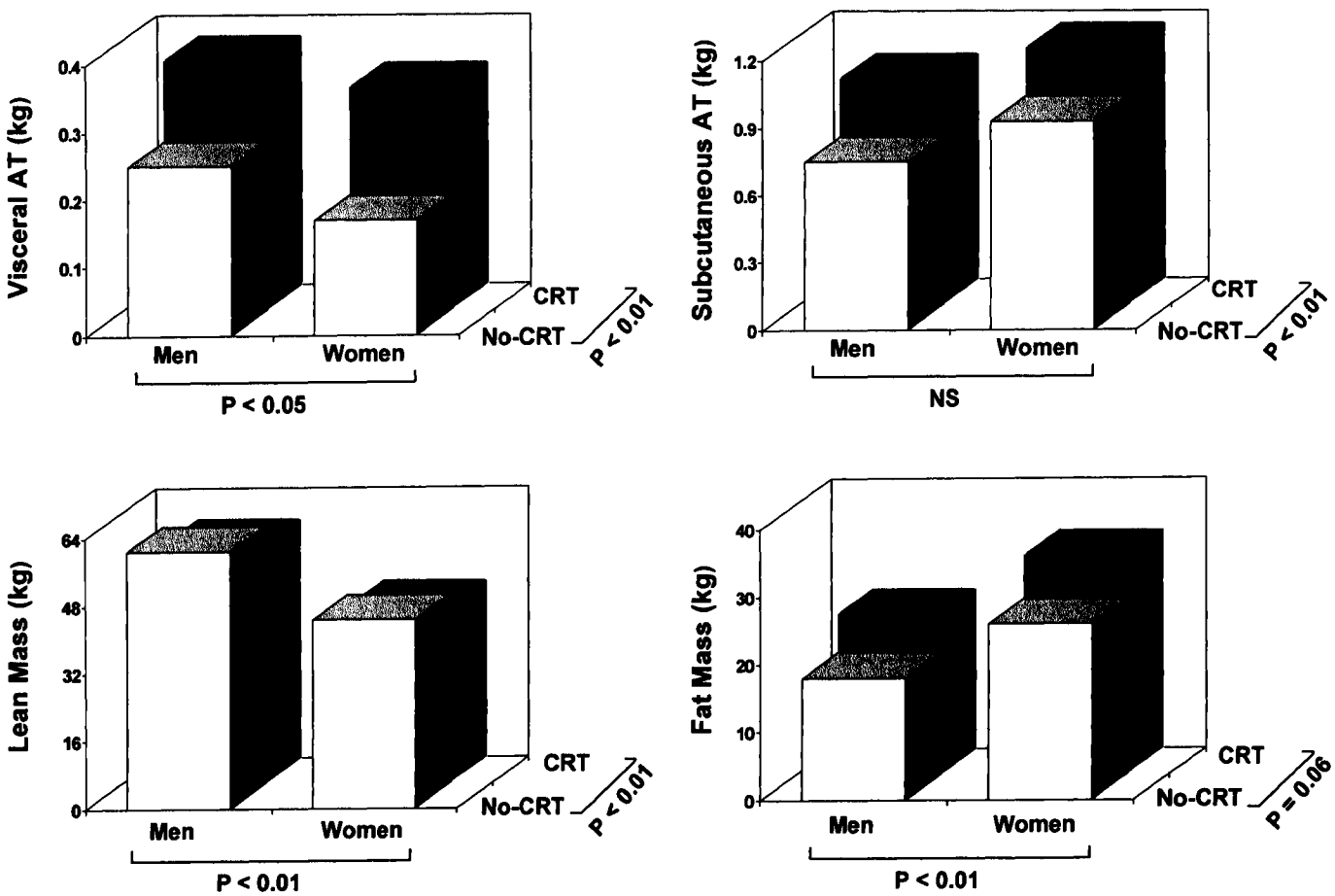
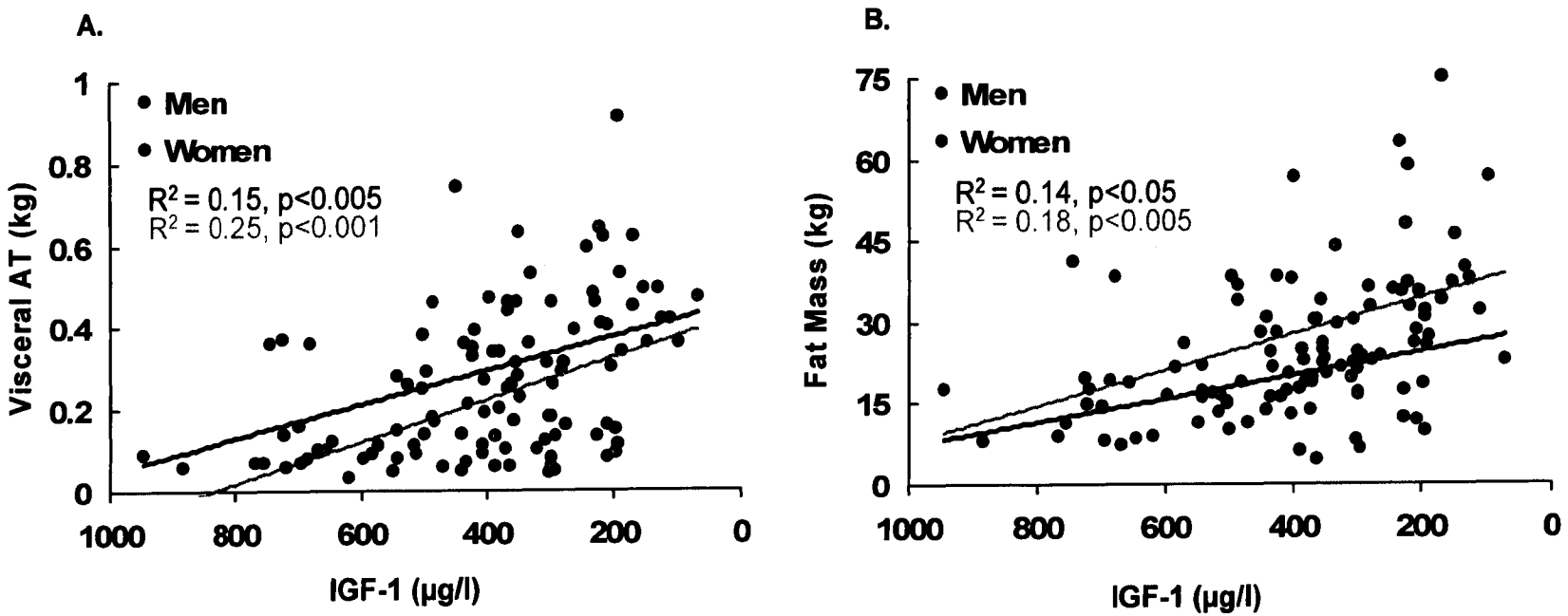


Figure 3: Relationship between levels of IGF-1, visceral AT (A) and total fat mass (B) in men and women ALL survivors.



DISCUSSION

To our knowledge this is the first study in young adult ALL survivors to quantify specific fat depots, all of which are established independent predictors of health risk. The novel finding is that abdominal adiposity, specifically VAT, is greater in CRT versus non-CRT treated ALL survivors independent of sex, age, race, and ethnicity. Among female ALL survivors, CRT is also associated with a disproportionate deposition of abdominal fat into the VAT depot. Given the strong relationship of abdominal obesity, in particular VAT with morbidity and mortality, these results support the notion that ALL survivors, especially those treated with CRT are at significantly elevated health risk. Accordingly, these findings highlight the need to monitor abdominal obesity in adjunct to total obesity in the assessment of health risk among ALL survivors.

Abdominal obesity is a strong predictor of morbidity and mortality independent of total obesity [23-25]. While some studies have shown SAT to be an independent predictor of metabolic risk [31, 117], the preponderance of literature reports VAT to be a strong predictor of dyslipidemia, insulin resistance [115], hypertension [116], cardiovascular disease [107], type II diabetes [27], and mortality [28], independent of SAT. As assessed by waist circumference or waist-to-hip ratio, abdominal obesity in ALL survivors has been reported to be higher than [7] and/or equal to [6] that of healthy controls. Also, while one study reported CRT males, but not females, to have a greater waist circumference and waist-to-hip ratio as compared to non-CRT counterparts [12], others found no such difference [13]. Using DEXA in a limited sample (n = 35), Jarfelt et al. [12] previously reported higher trunk fat percentage in CRT versus

non-CRT male but not female ALL survivors. While we did not find a difference in waist circumference between our CRT and non-CRT groups, we are the first to show that CRT treated ALL survivors have higher levels of abdominal obesity, in particular VAT, than their non-CRT counterparts, independent of sex. Further, as evidenced in the female ALL survivors, CRT is associated with a preferential deposition of fat into the VAT depot.

Our findings suggest that in the long-term follow-up of ALL survivors, clinicians should include measures of abdominal obesity in addition to measures of BMI in the assessment of patient obesity health. Indeed, many reports have suggested that the measurement of abdominal obesity and total obesity in combination provides the best assessment of obesity-related health risk [97-99]. While the routine measurement of abdominal fat by radiographic methods is impractical in clinical settings, it must be noted that waist circumference is a convenient and precise surrogate measure for VAT and SAT, as evidenced in Figure 1. In addition to its cross-sectional utility, waist circumference is also useful at tracking longitudinal changes in VAT and SAT [123, 201].

Emerging evidence has shown that the storage of fat in the liver and skeletal muscle is independently associated with metabolic disturbance [29-31]. Our results suggest that CRT is not associated with differences in either liver and/or muscle fat infiltration as assessed by CT. Although liver fat and muscle fat are both related to degree of obesity [150, 170], duration of obesity has been shown to predict health risk independent of the degree of obesity [202]. Thus, as our sample is quite young and predominantly obese (especially CRT-treated females), these results do not dismiss

the possibility of future development of liver and muscle fat infiltration, and associated metabolic disturbance.

Increased total fat body mass and decreased lean mass in ALL survivors as compared to matched controls has been shown in some [6, 7, 50] but not all [4, 9] previous investigations. In one of the only studies to compare CRT versus non-CRT treated ALL survivors, Jarfelt et al [12] report significantly higher total body fat mass and reduced lean mass in CRT versus non-CRT males, but not females. However, these results were based on a limited (n = 35) and non-obese sample, and may be confounded by a significant difference in age between the CRT versus non-CRT participants (27 versus 21 yr in the men, and 28 versus 23 yr in the females). Our data clearly show that CRT treated ALL survivors have higher total fat mass and percent body fat but lower lean body mass than non-CRT treated, independent of age and sex.

An elevated BMI in ALL survivors, especially in those treated with CRT has been previously reported [4, 7, 8, 12, 13, 15, 16, 18, 57]. That we found no significant effect of CRT on BMI despite an effect on total fat, underscores a primary limitation when using BMI to assess adiposity in subjects with abnormalities in growth and body composition [58]. Indeed, Nysom *et al.* previously observed that survivors of ALL possess a higher percentage of fat mass per given BMI unit compared to healthy controls [58].

The mechanism by which CRT treated ALL survivors accrue excess fat remains a matter of debate. It has been proposed that CRT may induce damage at the radiosensitive hypothalamus, thus leading to growth hormone deficiency and/or leptin insensitivity, either one of which could produce an obese state. A large portion of

adult survivors of childhood ALL treated with CRT exhibit either growth hormone deficiency and/or severe insufficiency [7, 41, 42]. In the present study, treatment with CRT was associated with significantly reduced levels of IGF-1, suggestive of suppressed GH status. In comparison to BMI-matched controls, growth hormone deficient adults have greater absolute fat mass, a reduced lean body mass, a tendency for a central fat deposition, particularly VAT accumulation, and a reduced adult height [6, 62, 63]. Our findings of greater total fat mass, abdominal fat mass, and VAT but reduced lean body mass and height in CRT treated survivors are all consistent with a growth hormone deficient state. Additionally, that low IGF-1 levels were significantly associated with increased levels of total fat mass, abdominal fat, and VAT in both sexes (Figure 3), and reduced height in the females, lends further support to this argument. Compared to healthy controls, growth hormone deficient adults also have a greater VAT: SAT ratio, suggestive of preferential VAT storage [63]. Growth hormone replacement in deficient individuals has been shown to reduce abdominal obesity, through reductions primarily in VAT, with minimal [63, 64] or no change in SAT [65]. Thus, in addition to causing excess abdominal fat deposition, GHD may also cause a redistribution of abdominal fat into the VAT depot. Consistent with this notion, while we observed greater levels of VAT in CRT versus non-CRT treated participants, no statistical difference in SAT was found. Among the female participants only, CRT remained associated with greater VAT mass after control for total body fat mass. Accordingly, CRT versus non-CRT females had a greater VAT: SAT ratio in association with low IGF-1 levels. This indicates that CRT treated female ALL survivors not only accumulate more fat abdominally, but also

preferentially deposit abdominal fat into the visceral depot as a likely consequence of relative GH deficiency.

It has also been proposed that CRT may damage the leptin feedback mechanism between adipose tissue and the receptors at the hypothalamus [10]. ALL survivors have significantly increased plasma leptin levels, even when expressed per unit of fat mass, as compared to matched controls or a reference population [6, 7, 10]. Our findings are in agreement with these prior investigations illustrating that CRT is associated with an absolute and relative hyperleptinemia. As leptin regulates appetite and energy expenditure via negative feedback at the level of the hypothalamus, and leptin deficiency [67] and/or leptin resistance [68] in humans has been shown to cause obesity, this may also explain obesity subsequent to CRT treatment. While leptin resistance can lead to obesity, we are unaware of a plausible mechanism by which leptin resistance may cause a disproportionate increase in visceral adiposity. This notion is reinforced by the lack of correlation between relative hyperleptinemia and VAT mass (Table 3). In addition, it has been suggested that the hyperleptinemia seen in CRT treated ALL survivors may in fact be a result of GHD, rather than leptin resistance, *per se* [10]. Our finding of a significant relationship between IGF-1 and absolute and relative leptin levels in both sexes supports a direct effect of GH status on leptin production (Table 3). Further study is required to fully elucidate the link between CRT, GHD, leptin resistance and subsequent obesity in ALL survivors.

In accordance with previous reports of a sexual dimorphism in response to CRT we found that while CRT females had a higher VAT: SAT ratio than non-CRT females, no such differences were observed in the males. There is evidence that

maturation of the brain occurs considerably earlier in males than in females [87]. Thus, it has been suggested that young males may be resistant to the effects of CRT since their brain has reached a critical level of maturity before the onset of treatment [17]. On the other hand, at the same age the still developing female brain may be highly vulnerable to CRT effects. Whether or not this is indeed true is still unknown. Recently, Ross et al reported that a polymorphism in the leptin receptor gene in ALL survivors may predispose to obesity secondary to CRT via poor leptin binding affinity [69]. This effect was noted in female survivors only, thus providing another possible explanation for sex differences in severity of late effects of CRT. The veracity of these observations remains unknown.

Limitations of our study include the lack of a healthy control group which did not permit the assessment of whether or not levels of total and abdominal obesity are elevated in non-CRT ALL survivors as compared to the general population. To further explore the relationship of GH deficiency and abdominal obesity in this population, measurement of GH peak levels following provocative testing with insulin would be preferred[42, 196]. Nevertheless, IGF-1 levels generally correlate well with GH status[197-200] and in our study were found to be strongly inversely correlated with height in females. This suggests that while provocative testing would be optimum, IGF-1 measurement in this study served as a reasonable surrogate. Lastly, due to the relatively small number of CRT treated survivors, the analysis of CRT dose or age at exposure on the primary outcomes were not possible.

The strengths of our study include the use of a multi-image CT protocol for measurement of abdominal fat that allowed us to determine subcutaneous and visceral

fat mass for a large segment of the abdomen, substantially reducing the potential limitations of measuring abdominal adiposity using a single image protocol [203]. The use of criterion methods, namely CT for measurement of liver and muscle tissue quality, and DEXA for measurement of total fat mass and lean body mass, reinforced the rigor of our study design.

In summary, our study is the first to demonstrate that independent of sex, age, race, and ethnicity, CRT compared to non-CRT treated ALL survivors are predisposed to abdominal obesity, primarily via excess accumulation of VAT in association with suppressed IGF-1 levels. Further, our findings corroborate previous reports of increased total fat mass, reduced lean body mass, and hyperleptinemia as a consequence of CRT. As abdominal obesity and VAT are independent predictors of morbidity and mortality, these results underscore the marked increase in health risk associated with CRT treatment in ALL survivors. In the long-term follow-up and treatment of ALL survivors, clinicians are well advised to include measures of abdominal obesity (waist circumference) in addition to measures of BMI in the assessment of patient obesity health risk. Furthermore, our findings suggest additional or alternate targets for future interventions aimed at reducing the heightened health risk among ALL survivors. In particular, as exercise is an efficacious strategy for total and abdominal fat reduction, exercise should be strongly recommended in the long-term management of young adult survivors of ALL.

4.0.0 GENERAL DISCUSSION

4.1.0 Implications of the Study

Although previous investigations have clearly established that total obesity is prevalent in ALL survivors[5-12, 15, 16, 18, 54], the evidence on regional adiposity in ALL survivors was inconsistent, and that for visceral and ectopic adiposity non-existent. Our study is the first to show that CRT compared to non-CRT treated ALL survivors are predisposed to abdominal obesity via excess accumulation of VAT as assessed directly by computed tomography. Furthermore, our findings corroborate previous reports of increased total fat mass and reduced lean body mass as a consequence of CRT. Lastly, our findings of altered IGF-1 and leptin levels in CRT ALL survivors suggest a relative GH deficiency and leptin insensitivity as potential causes of these body composition changes.

In accordance with previous reports of a sexual dimorphism in response to CRT we found that while CRT females had a higher VAT: SAT ratio than non-CRT females, no such differences were observed in the males. Interestingly, our finding of a greater VAT: SAT ratio in CRT versus non-CRT treated females, indicates that CRT treated female ALL survivors not only accumulate more fat abdominally, but also preferentially store the abdominal fat in the visceral depot. As abdominal obesity and specifically VAT are independent predictors of morbidity and mortality, these results underline yet another cause of the elevated health risk among CRT treated ALL survivors, especially females.

Although total and abdominal adiposity, specifically VAT are increased and total muscle mass is reduced in CRT ALL survivors, the degree to which these body

composition alterations predispose to disease can not be easily extrapolated. While established thresholds exist for BMI and WC, to date no established VAT or abdominal fat cut-off values are available for quantifying associated health risk. In one of the only attempts to delineate such a VAT cut-point in young Caucasian adults, Despres and Lamarche[126] found that a cross sectional area of VAT at the L4-L5 intervertebral space beyond 100cm^2 was associated with deteriorations in metabolic profile and glucose tolerance. Thus, while we may note that a greater proportion of CRT versus no-CRT ALL survivors are beyond this VAT threshold (40 versus 27%, and 50 versus 10%, in CRT and no-CRT men and women, respectively), the associated elevation in risk is difficult to quantify.

While we did not directly measure growth hormone status in our participants, the reduced IGF-1 levels along with the body composition findings in CRT treated ALL survivors (greater absolute fat mass, a reduced lean body mass, a tendency for a central fat deposition, particularly VAT accumulation, and a reduced adult height) suggest a GH deficient state. Indeed, previous research has reported that adult survivors of childhood ALL treated with CRT exhibit either GH deficiency and/or severe insufficiency [7, 41, 42]. Thus, GH deficiency likely plays a key role in the development of total and abdominal obesity post CRT treatment in survivors of ALL.

4.2.0 Measurement of Abdominal Obesity in a Clinical Setting

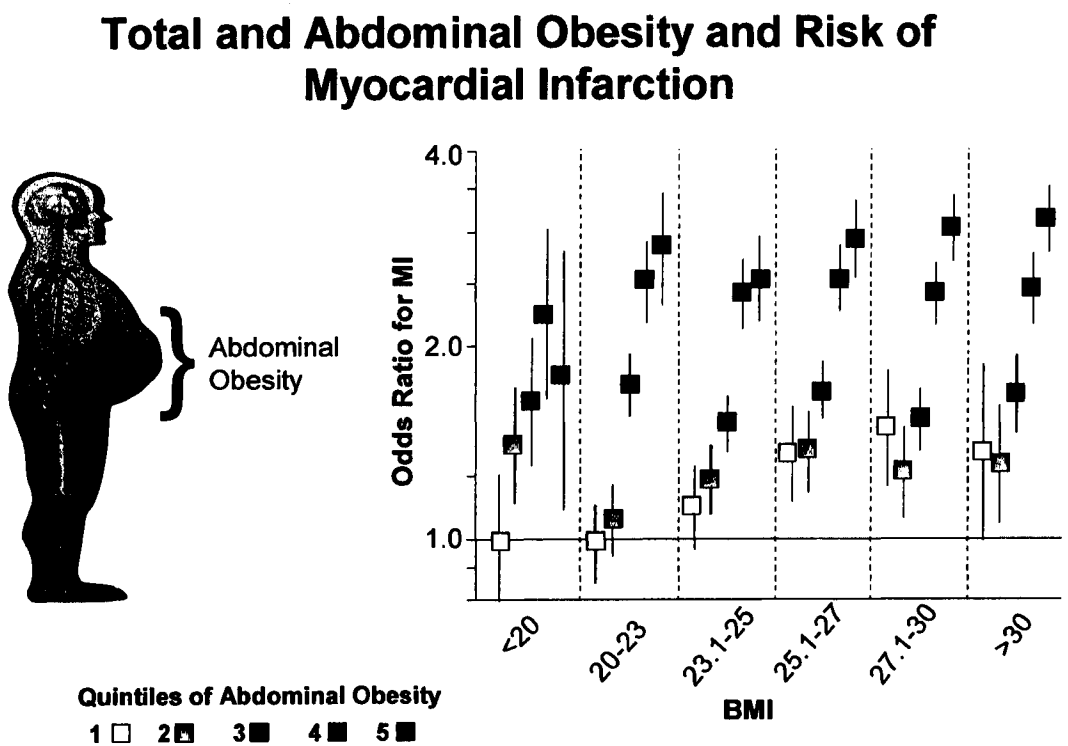
Our findings suggest that in the long-term follow-up and treatment of ALL survivors, clinicians should include measures of abdominal obesity in addition to measures of BMI in the assessment of patient obesity health risk. Indeed, many reports have suggested that the measurement of abdominal obesity and total obesity in

combination provides the best assessment of obesity-related health risk [97-99]. In fact, Figure 1 demonstrates how individuals in the lowest BMI category but in the highest quintile of abdominal obesity are at greater risk of myocardial infarction than those in the highest BMI category but lowest abdominal obesity quintile. While the routine measurement of abdominal fat as only possible via radiographic methods is impractical in clinical settings, waist circumference is a convenient surrogate measure for VAT and SAT (see 3.0.0 Manuscript; Results; Figure 1). The National Institutes of Health, National Heart, Lung and Blood Institute recommend the use of sex specific waist circumference cut-off points to predict health risk in addition to using BMI[92] (Table 1). In addition to its cross-sectional utility, waist circumference is also useful at tracking longitudinal changes in VAT and SAT [123, 201]. Thus, waist circumference is particularly important in monitoring the success of a given treatment strategy for reducing abdominal obesity.

4.3.0 Limitations of the Study

The use of a predominately Caucasian sample of men and women, limits the generalization of our findings across ethnic groups. The lack of a healthy control group did not permit the assessment of whether or not levels of total and abdominal obesity are elevated in no-CRT ALL survivors as compared to the general population. Dose of CRT treatment has previously been shown to influence the development of obesity[8, 17]. While the analysis of CRT dose on the primary outcomes was not possible, we did not find a difference in any outcomes between CRT ALL survivors treated with 18 Gy and above in comparison to those treated with less than 18 Gy.

Figure 1.



(Adapted from Yusuf et al, 2005)

Table 1. Classification of overweight and obesity by BMI, waist circumference and associated disease risk*

	BMI kg/m2	Class	Disease Risk Relative to Normal Weight and Waist Circumference		
			Men	<102 cm (< 40 in)	> 102 cm (> 40 in)
			Women	< 88 cm (< 35 in)	> 88 cm (> 35 in)
Normal range	18.5 - 24.9				
Overweight	25.0 - 29.9			Increased	High
Obesity	30.0 - 34.9	I		High	Very high
Obesity	35.0 - 39.9	II		Very high	Very high
Extreme obesity	> 40	III		Extremely high	Extremely high

* Disease risk for T2D, hypertension, and CVD

Furthermore, it is plausible that the effects on body composition we attributed to CRT *per se* may in fact be caused by the spread of malignancy from the bone marrow to the central nervous system, thus requiring the use of CRT during therapy. This of course, is impossible to ascertain. Lastly, while no prior studies have reported duration of ALL treatment to influence frequency and/or severity of treatment sequelae, this factor may be an additional confounder of our results.

4.4.0 Strengths of the Study

The use of a multi-image CT protocol for measurement of abdominal fat that allowed us to determine subcutaneous and visceral fat mass for a large segment of the abdomen, substantially reducing the potential limitations of measuring abdominal adiposity using a single image protocol [203]. The use of criterion methods, namely CT for measurement of liver and muscle tissue quality, and DEXA for measurement of total fat mass and lean body mass were additional strengths of our investigation.

4.5.0 Future Directions

As alluded to previously, although our hormone and body composition findings in CRT treated subjects suggest a GH deficient state, this was not confirmed in the present study, as GH status was not directly assessed. Thus, one important future endeavour would be to investigate whether GH deficiency, as measured by provocative testing, mediates the relationship between CRT and abdominal obesity.

Furthermore, as abdominal obesity and specifically the excess accumulation of VAT is independently associated with morbidity and mortality, efforts should be made to identify effective strategies to reduce abdominal obesity and associated co-morbidities in young adult survivors of ALL. In normal obese men and women,

regular physical exercise at moderate intensity has been shown to significantly reduce total abdominal fat, and in particular VAT[123, 201]. To that effect, our group is currently involved in a randomized, controlled trial investigating the effects of a 12-month *Lifestyle Physical Activity* intervention on reductions in total, abdominal, and ectopic fat stores and improvements in metabolic profile. The *Lifestyle Physical Activity* intervention has been tested in adults in the general population in several prospective randomized clinical trials (RCT) and found to be associated with significant increases in physical activity levels, cardiorespiratory fitness, and improvement in cardiovascular risk profiles[204, 205]. If deemed effective, the *Lifestyle Physical Activity* intervention could become a viable therapeutic strategy that could be adopted in the broader context of long-term treatment of ALL survivors.

5.0.0 SUMMARY AND CONCLUSIONS

Since the observation of Sainsbury *et al.* in 1985[3], numerous studies have reported that ALL survivors are overweight and obese as compared to healthy age-matched controls[4-12]. Consequently, a significant proportion of ALL survivors exhibit a constellation of obesity-related cardiovascular risk factors [6, 7, 11, 13] and experience an excess in all-cause and cardiovascular-related mortality[14]. To our knowledge this is the first study in young adult ALL survivors to quantify specific fat depots, all of which are established independent predictors of health risk. The novel finding of our study is that abdominal adiposity, specifically VAT mass is greater in CRT versus non-CRT treated ALL survivors. Among female ALL survivors, CRT is also associated with a preferential deposition of abdominal fat into the VAT depot. Given the strong relationship of abdominal obesity, in particular VAT with morbidity and mortality, these results support the notion that ALL survivors, especially those treated with CRT are at significantly elevated health risk. The findings of a higher VAT: SAT ratio in CRT versus non-CRT women, but not men is in accord with previous reports of a sexual dimorphism in response to CRT. Lastly, our findings of altered IGF-1 and leptin/fat mass levels suggest both a relative GH deficient and leptin insensitive state.

Increased total fat body mass and decreased lean mass in ALL survivors as compared to matched controls has been previously shown [6, 7, 50]. Our findings corroborate these previous reports and indicate that CRT plays a key causal role in these body composition changes. On the other hand, our results suggest that CRT is not associated with differences in either liver and/or muscle fat infiltration as assessed

by CT. However, as our sample is quite young and predominantly obese, these results do not dismiss the possibility of future development of liver and muscle fat infiltration and associated metabolic disturbance.

From a clinical perspective, our results suggest that in the long-term follow-up and treatment of ALL survivors, clinicians should include measures of abdominal obesity (waist circumference) in addition to measures of BMI in the assessment of patient obesity health risk. Furthermore, our findings reveal abdominal obesity as an additional or alternate target for future interventions aimed at reducing the health risk among ALL survivors. In particular, as physical exercise is an efficacious strategy for total and abdominal fat reduction, exercise should be strongly recommended in the long-term management of young adult survivors of ALL. Future endeavours are needed to develop efficacious and effective strategies to reduce the levels of total and abdominal obesity and associated co-morbidities in this vulnerable population of ALL survivors.

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Appendix A: Project ALLIFE Consent Form

The University of Texas Southwestern Medical Center at Dallas
Aston Ambulatory Care Center
General Clinical Research Center

CONSENT TO PARTICIPATE IN RESEARCH

Title of Research: PROJECT ALLIFE (Acute Leukemia Lifestyle Intervention For Everyday) - EXERCISE AND LEUKEMIA SURVIVORS

Sponsor: National Institutes of Health – National Cancer Institute

Principal Investigator:	Telephone No.	Telephone No.
Kevin C. Oeffinger, M.D.	214-648-1399 (regular office hours)	214-648-8259 (other times)

INVITATION: You are invited to participate in this research because you are a survivor of childhood leukemia.

Medical research involves offering a plan of care to a group of patients, collecting and studying information about each patient's experience, and using that information to develop the best possible care for future patients.

NUMBER OF PARTICIPANTS: The sponsor plans to include 180 young adult survivors of childhood leukemia in phase 1 of this research and 120 in phase 2.

PURPOSE: This study includes two phases. The purpose of phase 1 is to determine the risk of cardiovascular disease in survivors of childhood leukemia. The purpose of phase 2 of this research is to determine how effective two different methods are in helping you increase your level of physical activity.

This research is being done because survivors of childhood leukemia are at increased risk of developing multiple health problems, including obesity, cardiovascular disease, and osteoporosis.

PROCEDURES

Screening: The study coordinator will ask you questions about your health, medications you take for any health problems, and any surgical procedures you have had.

You will be asked the following questions to determine eligibility for phase 1 of the study:
1. Were you diagnosed with ALL between ages 0-21 years?
2. Are you 18 years or older?
3. Are you two or more years from completion of therapy for your leukemia?
4. Are you currently pregnant?

You will be asked the following questions to determine eligibility for phase 2 of the study:
1. Do you have access to the internet? If you do not know if you have internet access, we will assist you in finding a location to access the internet near your home or work through a school or a community library.
2. Do you have a history of heart attack, stroke, avascular necrosis of the hip, or any other serious medical condition that might make exercise unsafe or unwise?

3. Will you have difficulty completing a 12-month study – do you travel very frequently, do you plan to move from the area within the next year, do you plan to get pregnant in the next 12 months, do you drink more than 3 alcoholic drinks per day, have you been hospitalized for a psychological disorder in the last 5 years?

In addition to the above questions, you will complete the 7-day physical activity recall (includes questions about your activity level) during phase 1 to determine if you are eligible for phase 2 of the study.

These procedures may be done even if you do not participate in this research.

Randomization: Everyone who is eligible to participate in phase 1 of the study will complete the same questionnaires and tests. If the study doctor believes that you qualify to participate in phase 2 of this research, you will be randomly (like the flip of a coin) assigned to one of two groups. A different method to encourage physical activity will be used for each group. Upon completion of the study, all participants will be given information and access to resources for both methods.

Treatment: Two methods that are intended to help you increase your level of physical activity are being tested. If you are randomized to group 1, you will read materials in a workbook and on the internet to learn skills to become physically active, and complete short assignments to practice these skills. In addition, a health educator will schedule 10 minute phone calls about once a month to help you become more physically active. For the first six months, it should take about 1 hour per week of your time to complete the study coursework, and then about an hour per month for the second six months of the study. If you are randomized to group 2, you will receive the Berkley Wellness newsletter and have access to their website, including content for subscribers only. You will receive the newsletter once per month for a year, and can spend as much time as you want reading these materials.

Evaluations during the research: If you are eligible for the study and choose to participate you will have several tests to measure your risk for heart disease, cardiorespiratory fitness, and bone health. The questionnaires, measurements, and tests will be completed at UT Southwestern and the Cooper Institute.

Completed at UT Southwestern:

1. Questionnaires about (a) medical history and health habits, (b) your current level of physical activity, (c) your current dietary habits, and (d) your mood.
2. Measurement of your height, weight, blood pressure.
3. Blood tests (cholesterol level, blood sugar, insulin level, homocysteine level)
4. Balance and gait tests – to see if you have any problems with your balance or with your gait when you walk.
5. Treadmill VO2 max test – a test on a treadmill to measure your heart rate and your breathing.
6. You will be sent home with an accelerometer. It can be worn on your arm and will measure your activity for 8 days. A special express mail package, pre-addressed and pre-stamped, will be given to you to mail the accelerometer device back to us after you have used them.

Completed at the Cooper Institute within one month of above measurements:

1. CT scan – an exam that uses x-rays to create cross-sectional pictures of body tissues and organs.
2. Bone density test

For participants enrolled in phase 2 of the study, these same tests will be repeated at the end of the 12-month study.

Mid-way through the study, at six months, the accelerometer and some questionnaires will be mailed to your house. After completion, you will be mail them back in a pre-addressed, pre-stamped express mail package.

The tests that you will complete after enrolling in the study will take two visits. The first visit will last one half day will take place at UT Southwestern, and the second visit will take place at the Cooper Institute, and should take about 2 hours. If you participate in phase 2 of the study, you will return 1 year after your first visit for two half day visits again, one at UT Southwestern and one the Cooper Institute. For study visits at UT Southwestern, the research assistant will take you to each area of testing.

When you have blood drawn on the first day and at the end of the study, about 3-4 tablespoons of blood will be drawn.

POSSIBLE RISKS

The risks of the testing procedures and interventions planned for this study are minimal. All testing procedures have been used by our Research Team in numerous groups and for thousands of individuals.

The lifestyle questions on the various surveys generally are not sensitive items, with the possible exception of questions on alcohol intake. To prevent injury while on the treadmill, trained staff will assist you.

The methods to increase your physical activity levels are unlikely to cause major problems. We have conducted numerous exercise training studies over the past 20 years, and have never had a serious event or death. Occasionally study participants do experience minor orthopedic problems, but most are self-correcting with rest. The PI, Dr. Oeffinger, is available for medical consultation should the need arise. The emphasis of both methods is to encourage moderate intensity activity, such as walking and gardening. Because of the screening and the emphasis on moderate intensity activities, the risk of injury is low.

Radiation exposure from diagnostic tests: The DEXA scan (bone density test) is medically indicated for your condition and radiation exposure from this test is the same amount that you would receive if you were not involved in the study. The CT scan is not medically indicated for your condition and is being performed for research purposes. The risk of harm to your body from the CT scan is comparable to the everyday risk of driving 1300 miles in an automobile. There is a small risk from radiation for the bone density scan and CT scan in female participants. Radiation output is low, but remains a concern to a fetus if you should be pregnant. For females, the bone density scan and CT scan will be scheduled the first 10 days after the start of your most recent menstrual period. A urine pregnancy test will be run prior to each bone density to rule out a pregnancy.

RISKS TO AN EMBRYO, FETUS, OR BREAST-FED INFANT: A woman who is pregnant should not participate in this research.

If a woman is pregnant, radiation exposure to her reproductive organs may harm an embryo or fetus.

Pregnancy test: A pregnancy test will be performed for any woman who is able to have children and wishes to participate in this research. A pregnancy test will be repeated at the end of the study when the tests are repeated. A study doctor will ask for the date when a woman's last monthly period started.

Pregnancy during participation in this research: If you are a woman who is able to have children, and you suspect pregnancy during this research, you must tell your study doctor immediately. Your participation in the research will stop. Your study doctor can discuss new care for with you. Your study doctor will report information about your pregnancy, delivery, and the baby's first two months of life to the sponsor.

Blood samples: You will have the same amount of blood collected whether you receive standard medical care for your health problem or participate in this research. Therefore, your risk of complications from collecting the blood is the same.

You may experience discomfort, bleeding, and/or bruising. You may feel dizzy or faint. On a rare occasion, an infection could develop at the site where the blood was collected.

Unforeseen risks: A previously unknown problem could result from your participation in this research. It is not possible to estimate the chances of such problems or how serious problems could be.

How you can help reduce some of the risks: During your participation in this research, your study doctor will watch closely to determine whether there are problems that need medical care. It is your responsibility to do the following:

- Ask questions about anything you do not understand.
- Keep appointments.
- Follow the study doctor's instructions.
- Let your study doctor know if your telephone number changes.
- Tell your study doctor before you take any new medication even if it is prescribed by another doctor for a different medical problem.
- Tell your regular doctor about your participation in this research.
- Talk to a family member or friend about your participation in this research.
- Carry information about the research in your purse or wallet.

What to do if you have problems: If you have problems, such as unusual symptoms or pain, at any time during your participation in the research, your study doctor can recommend treatment. Please report the problem to your study doctor promptly. Telephone numbers where he/she may be reached are listed on the first page of this consent form.

If you suddenly have a serious problem (such as difficulty breathing) or severe pain, go to the nearest hospital emergency room, or call 911 (or the appropriate emergency telephone number in your area). Tell emergency personnel about your participation in this research. Ask them to telephone your study doctor immediately.

POSSIBLE BENEFITS

Benefit to you: The intervention programs used in this study are designed to help individuals become more physically active. There are numerous health benefits associated with moderate to vigorous levels of physical activity. Also, you will be provided with a copy of all of the test results from the study, including your blood pressure level, cholesterol and blood sugar levels, bone density (bone strength), CT Scan, balance and gait, and cardiorespiratory fitness level. However, your study doctor cannot guarantee that you will benefit from participation in this research.

Benefit to other ALL survivors: In the future, other survivors of childhood cancer could benefit from the results of this research. Information gained from this research could lead to improved medical care for them. However, your study doctor will not know whether there are benefits to other cancer survivors until all of the information obtained from this research has been collected and analyzed.

Benefit to others: The information gained from this study may result in new approaches to facilitate increased levels of physical activity in leukemia survivors.

ALTERNATIVES TO PARTICIPATION IN THIS RESEARCH: You do not have to participate in this research.

Please ask your study doctor as many questions as you wish. The doctor's answers to your questions could help you decide whether to participate in this research or receive the standard care that is currently available for your medical problem.

If you decide to participate in research now, and later change your mind, you may stop your participation in the research then and receive the alternative care.

THE STUDY DOCTOR'S DECISION TO STOP YOUR PARTICIPATION: Your study doctor or the sponsor may stop your participation in this research without your permission under any one of the following conditions:

- Your study doctor believes that participation in the research is no longer safe for you.
- Your study doctor believes that other treatment may be more helpful.
- The sponsor or the FDA stops the research for the safety of the participants.
- The sponsor cancels the research.

PROCEDURES AFTER STOPPING PARTICIPATION IN THIS RESEARCH: If you, the study doctor, or the sponsor stops your participation in the research, it is your responsibility to do the following:

- Let your study doctor know immediately that you wish to withdraw from the research.
- Return to the research center for tests that may be needed for your safety.
- Return any unused study materials.
- Discuss your future medical care with your study doctor and/or your regular doctor.

PAYMENT TO TAKE PART IN THIS RESEARCH: To compensate you for your time spent completing the set of tests at enrollment, you will be paid $50. Likewise, when you complete the set of tests at the end of the study, you will be paid an additional $50 to compensate for your time.

If you are an employee of UT Southwestern, tax will be deducted from the payment given to you for your participation in the research.

UT Southwestern, as a State agency, will not be able to make any payments to you for your participation in this research if the State Comptroller has issued a "hold" on all State payments to you. Such a "hold" could result from your failure to make child support payments or pay student loans, franchise taxes, etc. Should this occur, UT Southwestern will be able to pay you for your participation in this research after you have made the outstanding payments, and the State Comptroller has issued a release of the "hold."

COSTS TO YOU: The sponsor will pay the expenses for all of the tests, described above, that are part of this research study.

Expenses related to your standard follow up medical care are your responsibility (or the responsibility of your insurance provider or government program).

There are no funds available to pay for transportation to and from the research center, lost time away from work and other activities, lost wages, or child care expenses.

COMPENSATION FOR INJURY: Compensation for an injury resulting from your participation in this research is not available from the University of Texas Southwestern Medical Center at Dallas or the Cooper Institute.

You retain your legal rights during your participation in this research.

VOLUNTARY PARTICIPATION IN RESEARCH: You have the right to agree or refuse to participate in this research. If you decide to participate and later change your mind, you are free to discontinue participation in the research at any time.

Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. Refusal to participate will not affect your legal rights or the quality of health care that you receive at this center.

NEW INFORMATION: Any new information that becomes available during your participation in the research and may affect your health, safety, or willingness to continue in the research will be given to you.

RECORDS OF YOUR PARTICIPATION IN THIS RESEARCH: You have the right to privacy. Any information about you that is collected for this research will remain confidential as required by law. In addition to this consent form, you will be asked to sign an "Authorization for Use and Disclosure of Protected Health Information for Research Purposes".

YOUR QUESTIONS: Your study doctor is available to answer your questions about this research. The Chairman of the IRB is available to answer questions about your rights as a participant in research or to answer your questions about an injury or other complication resulting from your participation in this research. You may telephone the Chairman of the IRB during regular office hours at 214-648-3060.

YOU WILL HAVE A COPY OF THIS CONSENT FORM TO KEEP.

Your signature below certifies the following:

- You have read (or been read) the information provided above.
- You have received answers to all of your questions.
- You have freely decided to participate in this research.
- You understand that you are not giving up any of your legal rights.

Participant's Name (printed)	
Participant's Signature	Date
Witness' name (printed)	
Witness' signature	Date
Name (printed) of person obtaining Consent	
Signature of person obtaining consent	Date

Appendix B: Electron Beam Computerized Tomography Scan Consent Form in Accordance with the Ethical Guidelines of the Cooper Institute Institutional Review Board

The Cooper Institute

539 12330 Preston Road Dallas, Texas 75230

I understand that the information that I am providing below is for the purpose of assessing the effectiveness of measuring and using coronary artery calcium as a predictor of coronary heart disease. I also understand that my records remain confidential, as described in the letter received with this questionnaire, and that the results of this study may be published; however, my name or other identifiers will not be provided. Only group data will be presented.

If you have any questions about your rights as a research study participant, you may contact Dr. Stephen Farrell, Institutional Review Board Chair, at The Cooper Institute (972) 341-3200.

Name: (please print)_____

Signature: _____ Date: _____

INSTRUCTIONS

Please read each question carefully.

Please use a black ink pen to print your information in capital letters and avoid contact with the edge of the box.

Print letters like this: P
Not like this: p

Shade circles like this: ●
Not like this: ⊠ ⊘

Please review your name and address and make any changes below:

Last Name: **First Name:**

Middle Name: **Maiden Name, if applicable:**

Number & Street Address:

City: **State:** **Zip Code:** -

(Area Code) Home Phone Number: - -

(Area Code) Home Fax Number: - -

email

Race: ○ White ○ Black ○ Hispanic ○ Asian ○ American Indian ○ Other (Specify): _____

What is your current weight in pounds? ☐☐☐ pounds

What is your current height (feet and inches)? ☐ feet ☐☐ inches

Have you ever had or been told by a physician that you had any of the illnesses or conditions listed below? If so, please indicate the year of diagnosis.

	Yes	No	Year
Congestive heart failure	O	O	☐☐☐☐
Angina (heart pain)	O	O	☐☐☐☐
High blood pressure (hypertension)	O	O	☐☐☐☐
Heart attack	O	O	☐☐☐☐
Stroke	O	O	☐☐☐☐

Have you ever had the following procedures? If so, please indicate the year of the procedure.

	Yes	No	Year
Carotid artery surgery	O	O	☐☐☐☐
Coronary bypass surgery	O	O	☐☐☐☐
Coronary angioplasty (balloon, stent)	O	O	☐☐☐☐

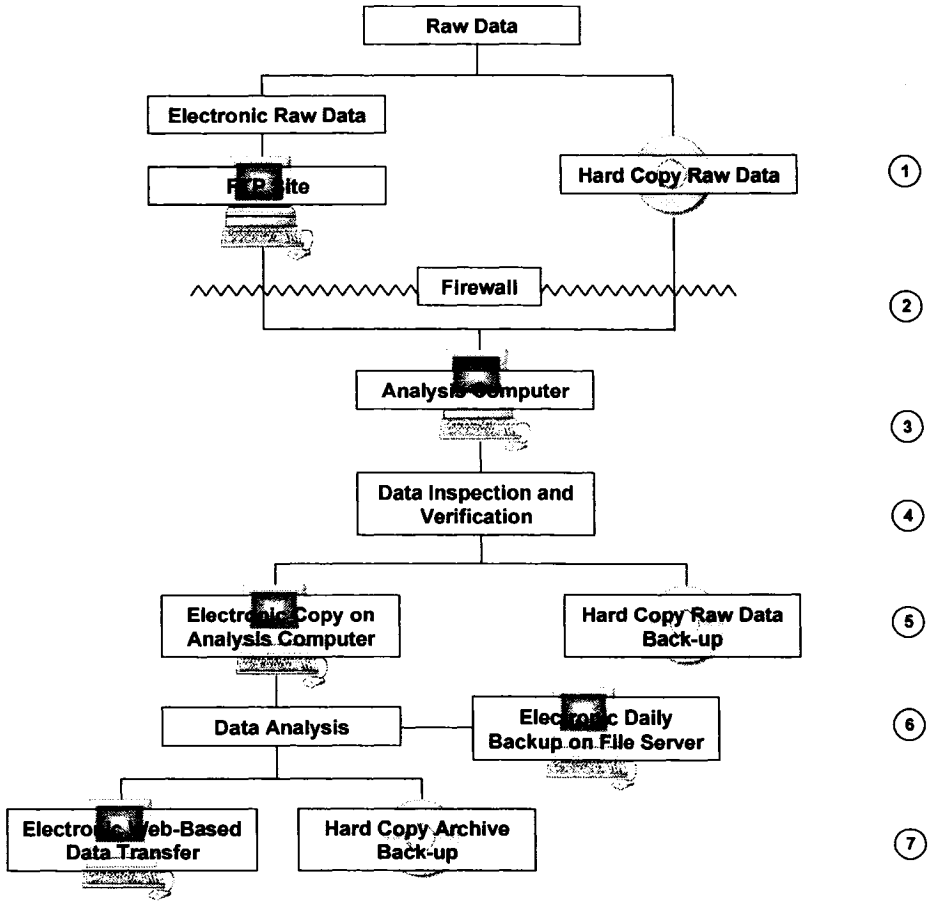
Do you use anti-hypertension medication?	O Yes	O No	What year did you start? ☐☐☐☐
Do you use cholesterol lowering medication?	O Yes	O No	What year did you start? ☐☐☐☐
Do you use aspirin regularly?	O Yes	O No	What year did you start? ☐☐☐☐
Do you use other heart medication?	O Yes	O No	What year did you start? ☐☐☐☐
Do you currently use tobacco?	O Yes	O No	If yes, what year did you start? ☐☐☐☐
Have you used tobacco in the past?	O Yes	O No	If yes, what year did you stop? ☐☐☐☐
Do you drink alcoholic beverages?	O Yes	O No	If yes, how many drinks per week? ☐☐

Do you exercise at least four days per week for at least 30 minutes? O Yes O No

How long have you been exercising regularly? ☐☐ years

Appendix C: Ross Lab Imaging Center Back-up System for ALLIFE CT Data

Ross Lab Imaging Center Back-up System for ALLIFE CT Data



1. Data is sent from Cooper Institute to Queen's University via FTP or CD.
2. The data is transferred from the FTP site is brought through the firewall by the supervising graduate student or data manager.
3. The image analysis technician/data manager/graduate student transfers the raw CT image data from either the FTP site or CD onto the hard-drive of the analysis computer (i.e. "Analyse1") in the Physical Education Center, Room 166D. The data is placed in the "Cooper" folder and into the appropriate study folder, coded sequentially (i.e. ALLIFE 1, ALLIFE 2, etc.). Within each study folder, the series of image files for each subject are saved within one folder corresponding to the

Cooper ID number (i.e. Images for Subject 12345 are saved in folder "\\Analyse 1\Cooper\ALLIFE 1\12345").

4. The image analysis technician, the supervising graduate student, or data manager unzips the raw CT image data and inspects for correct number of files, correct number of scans per sequence, and any significant artifacts.
    - A confirmation email is sent to the Cooper Institute describing the data received.
5. When the images are inspected and verified, a hard copy of the raw image data is backed up onto a CD or DVD medium and stored in a different room (Room 166C) from the analysis computers (166D) within the Physical Education Center at Queen's University, which are locked under different keys.
6. The CT raw image data are analyzed using Sliceomatic (Tomovision, Montreal) software, as described in the "CT Analysis Standard Operating Procedures" on one of four image analysis computers by a trained image analysis technician or graduate student.
    - All analysis computers are connected via a local network and are password protected.
    - The segmentation analysis creates a computer generated analysis file for each raw CT image (TAG) and a numeric database results file that are stored on the local hard-drive of the analysis computer ("Analyze 1").
    - The raw image files, TAG files and the numeric database results file are saved within each subject folder.
    - This data is backed up electronically on a daily basis. This back-up system is maintained and managed by the computer systems technician/manager.

- The supervising graduate student or the data manager audits the data on a weekly basis.

7. When all the subjects in a folder have been analyzed, 2 copies of the raw image data, TAG files, and the numeric database results file are subsequently burned onto DVDs by the supervising graduate student or data manager:

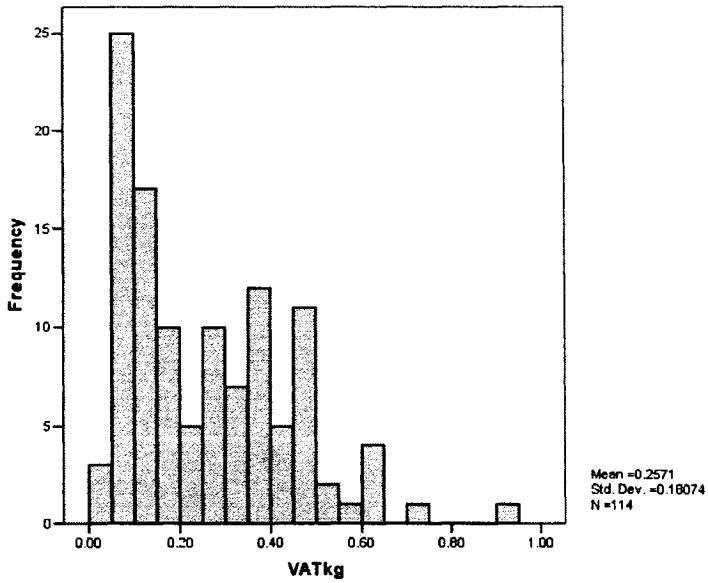
    1) Primary Copy - Archive copy

    2) Secondary Copy - Student accessible copy

   - The images are then removed from the local "Analyze 1" computer hard drive, but remain on the back-up system hard drive.
   - Primary and secondary copies of raw CT image data are stored in two separate binders, which are located in two different rooms (Room 166C and 166D) within the Physical Education Center at Queen's University. Each room is locked under a different key.
   - A summary results spreadsheet is made by cutting and pasting the individual subject results from the numeric database files into a Windows Excel spreadsheet for data manipulation by the supervising graduate student or data manager. All data is backed-up daily by the automatic system.
   - The summary results are subsequently sent to the Cooper Institute via the web-based data entry system according to subject ID numbers and exam date by the image analysis technicians/data manager/graduate student.

Appendix D: Example of Statistical Analyses Used to Derive Manuscript Results

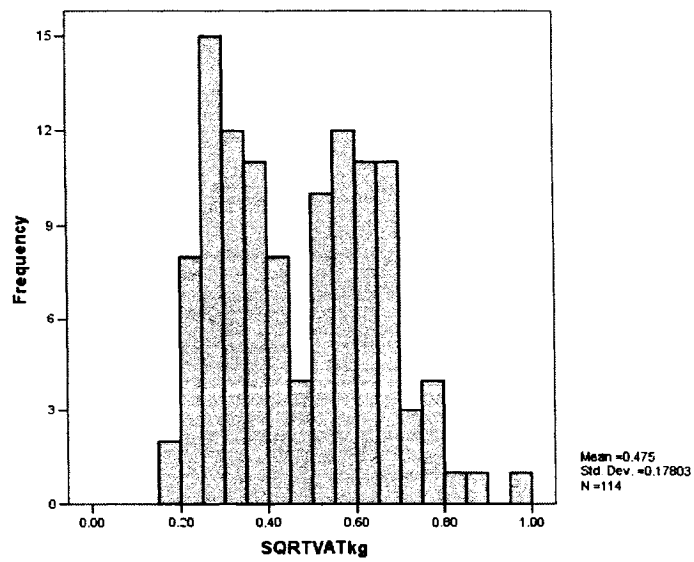
Histogram of VAT not-normalized:



VAT Normality Statistics: Skewness Present

	N	Minimum	Maximum	Mean	Std.	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
VATkg	114	.03	.91	.2571	.18074	.882	.226	.499	.449
Valid N (listwi	114								

Histogram of VAT Normalized by Square Root Transformation:



Normality Statistics: Data Normal

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std.	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
SQRTVATkg	114	.18	.96	.4750	.17803	.282	.226	-.893	.449
Valid N (listwis	114								

2 x 2 ANOVA for AGE (log transformed)

Tests of Between-Subjects Effects

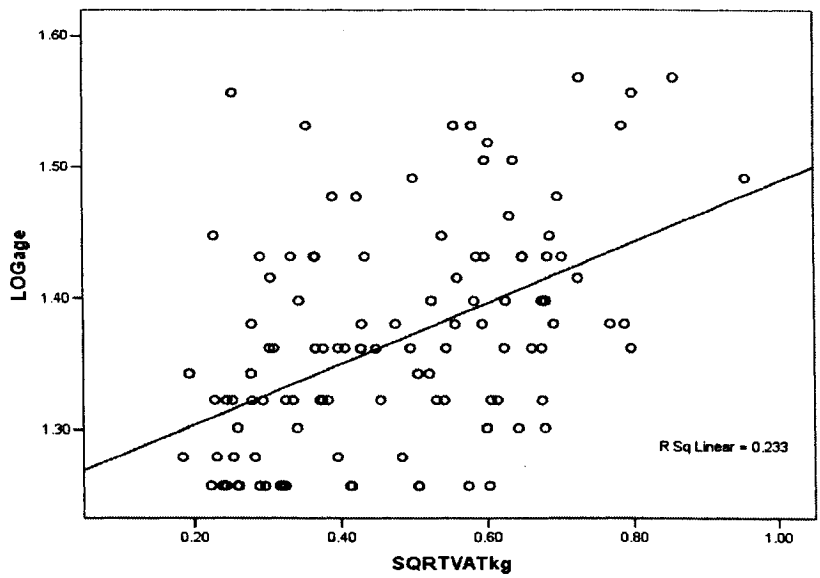
Dependent Variable: LOGage

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.097(a)	2	.048	7.377	.001
Intercept	189.824	1	189.824	29000.298	.000
sex	.000	1	.000	.025	.874
CRT	.095	1	.095	14.512	.000
Error	.727	111	.007		
Total	213.906	114			
Corrected Total	.823	113			

a R Squared = .117 (Adjusted R Squared = .101)

Relationship between AGE and VAT

Scatterplot



Pearson Correlation = 0.48 (P <0.001)

2 x 2 ANCOVA for VAT with AGE as a Covariate with Interaction Term

Tests of Between-Subjects Effects

Dependent Variable: SQRTVATkg

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.220(a)	4	.305	14.087	.000
Intercept	.181	1	.181	8.374	.005
LOGage	.539	1	.539	24.874	.000
sex	.056	1	.056	2.576	.111
CRT	.180	1	.180	8.330	.005
sex * CRT	.061	1	.061	2.798	.097
Error	2.361	109	.022		
Total	29.306	114			
Corrected Total	3.581	113			

a R Squared = .341 (Adjusted R Squared = .317)

2 x 2 ANCOVA for VAT with AGE as Covariate without Interaction Term

Tests of Between-Subjects Effects

Dependent Variable: SQRTVATkg

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.160(a)	3	.387	17.563	.000
Intercept	.157	1	.157	7.135	.009
sex	.120	1	.120	5.430	.022
CRT	.229	1	.229	10.404	.002
LOGage	.502	1	.502	22.803	.000
Error	2.422	110	.022		
Total	29.306	114			
Corrected Total	3.581	113			

a R Squared = .324 (Adjusted R Squared = .305)

Appendix E: ALLIFE CT Analysis Protocol

INTRODUCTION

Computed tomography (CT) is an imaging technique which allows for *in vivo* quantification of body composition by differentiating tissues on the basis their density and chemical composition. In a CT system, a tube emits x-rays which are variably attenuated by the tissues and detected by the receiver. Using mathematical techniques, the signals detected by the receiver are reconstructed to form an image. Each pixel of this cross-sectional image has a unique CT number, or linear attenuation coefficient, expressed in Hounsfield units (HU). Since physical density is the main determinant of attenuation, the lower the density of a tissue, the lower the HU of each pixel contained within that tissue. For example, bone and muscle tissues have a greater density than adipose tissue, and consequently have a higher attenuation value by CT. The use of CT in measurement of tissue surface area and volume has been validated, and shown to be highly accurate in comparative research utilizing human cadaver dissection.

The Cooper Institute uses electron beam CT (EBCT) to acquire series of cross-sectional images in the thigh and thorax. These images are then used for the analysis of coronary calcium, abdominal subcutaneous/visceral adipose tissue, hepatic fat infiltration and skeletal muscle mass and quality (fat content).

LIST OF MEASURED VARIABLES

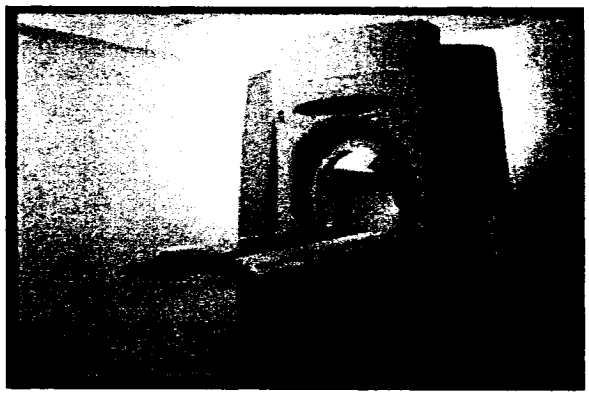
- Abdominal
  - Visceral Adipose tissue
  - Subcutaneous Adipose tissue (Deep, Superficial, Anterior and Posterior)

- Liver
  - Mean liver attenuation
  - Mean spleen attenuation
  - Liver-to-spleen ratio

- Thigh
  - Adipose tissue (Subcutaneous and Intermuscular adipose tissue)
  - Low attenuation muscle
  - Normal attenuation muscle
  - Mean attenuation muscle

MEASUREMENT DEVICE

Imatron from General Electric (Milwaukee, WI), located at Cooper Clinic, Dallas, Texas.



MEASUREMENT PROCEDURES

Preparing the participant

- No special preparations are required.
- The patient may eat and drink before the test is performed.
- The patients are asked to remove metallic objects in their clothing before the test.
- The patients are examined in the supine position with their arms extended above their head.

Image Acquisition

Images were obtained using 130 kV and 630 mA with a 48 cm field of view and a 512 x 512 matrix. The scan takes 10 to 15 minutes and is administered by a certified technician. As for any radiological procedure, there is some health hazard associated with CT. However, the total exposure will be below 1.5 mSv, which is below the 5 mSv/year considered as the upper "safe" limit.

- Abdominal Series

Approximately 35 contiguous images (6 mm thickness) were acquired from the distal iliac crest to the caudal region of the heart during a breath hold.

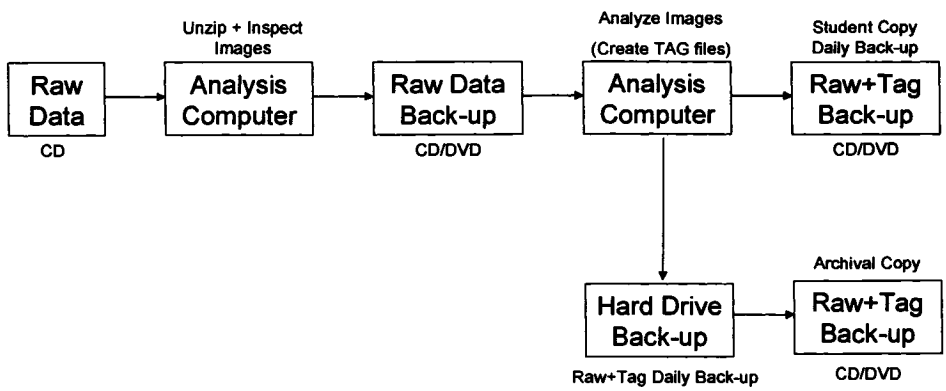
- Thigh Series

Approximately 70 contiguous images (6 mm thickness) were acquired beginning from distal to the patella extending superiorly.

DATA TRANSFER AND MANAGEMENT

- The CT data collected in Dallas is transferred to the laboratory in Kingston.
- The raw CT images are received in a condensed format.
- The files are unzipped, and stored behind a firewall, in a folder labeled by the Cooper ID number.
- The images are visually inspected using imaging software (SliceOmatic, Tomovision, Inc., Montreal, Canada) to ensure quality of acquisition.
- If the CT data are not saved to a computer behind the firewall for analysis immediately, back-up copies of the raw data is burned onto CDs or DVDs, which are stored in a fireproof safe.
- When, the CT data is analyzed, the image analysis data (.tag files) are stored with the raw CT images and are backed up daily on a hard drive by an automated system.
- After the image analysis for a patient is complete, the raw CT images, the tag images and database output files are burned together onto CDs or DVDs, which are stored in a fireproof safe.
- After the completion of ALLIFE, the data will be removed from the hard drive back up and a second copy will be burned onto DVDs and stored off site.

Back-up System



DATA ANALYSIS

Abdominal Analysis

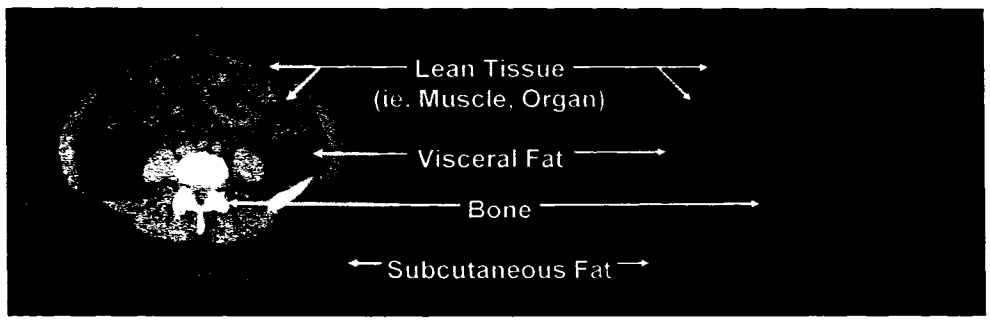
- A continuous series of 5 to 7 CT images corresponding to the L4-L5 to L3-L4 vertebral disc spaces for each subject were selected for analysis. The intervertebral spaces were identified using anatomical landmarks.

- Images are segmented using threshold values of:
  - Adipose tissue: -190 to -30 HU
  - Lean Tissue: -30 to 130 HU
  - Bone: >130 HU

- Visceral adipose tissue was determined by delineating the intra-abdominal cavity at the innermost aspect of the abdominal and oblique wall musculature and the anterior aspect of the vertebral body.

- Abdominal subcutaneous adipose tissue area was defined as the area of adipose tissue between the skin and the outermost aspect of the abdominal muscle wall.

Raw CT and Analyzed Abdominal Images at L4-L5

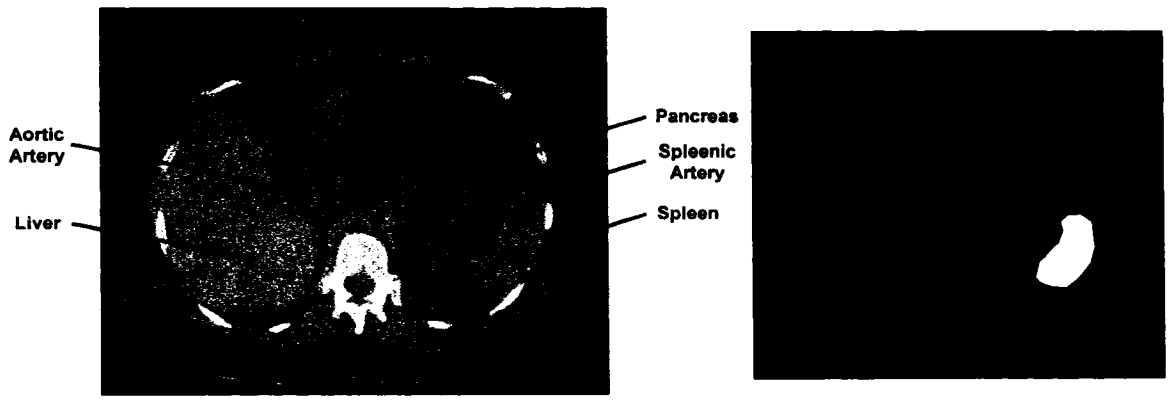


- The adipose tissue volumes were calculated using a truncated pyramid method. Adipose tissue volumes (liters) were converted to mass units (kg) by multiplying the volumes by the assumed constant density for fat (0.92 kg/L).

Liver Analysis

- Liver fat was represented as a ratio of mean liver to spleen attenuation values (CTL/CTS).
- A single image in which the spleenic artery is seen to contact the spleen is selected for the analysis (approximately T12 to L1).
- CTL and CTS were calculated using the average attenuation values of the entire liver and spleen obtained from the single CT image.

Raw CT and Analyzed Liver Image



Thigh Analysis

- Thigh adipose tissue, skeletal muscle mass and composition are determined using 11 contiguous images spanning from 12 to 18 cm proximal to the patella.

- Images are segmented using threshold values of:
  - Adipose tissue: -200 to 0 HU
  - Low Density Muscle: 1 to 30 HU
  - High Density Muscle: 31 to 150 HU
  - Bone: >150 HU

- Areas of low density within the femoral bone are edited and labeled as bone.

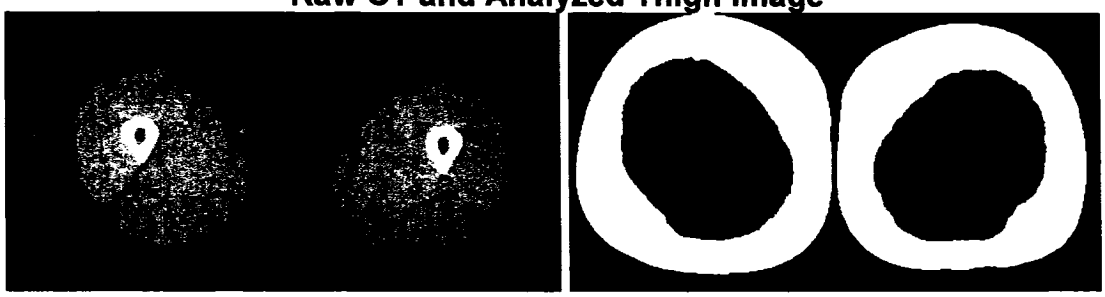
- Areas of high density within the muscle that are tagged as bone are labeled as high density muscle.

- Areas of high density on the outer edge of the thigh are labeled as subcutaneous adipose tissue.

- The subcutaneous adipose tissue is re-labeled using a different tag to differentiate it from the sub-fascial or intermuscular adipose tissue.

- The adipose tissue and muscle volumes were calculated using a truncated pyramid method as described previously. Adipose tissue and muscle volumes (liters) were converted to mass units (kg) by multiplying the volumes by the assumed constant density for fat (0.92 kg/L) and muscle (1.04 kg/L), respectively.

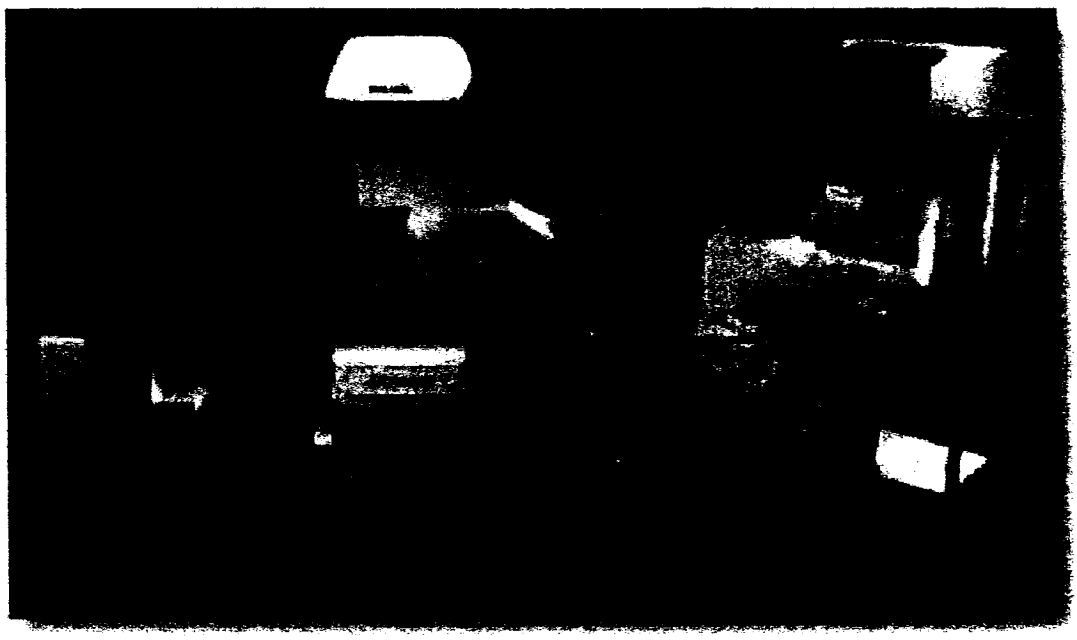
Raw CT and Analyzed Thigh Image



Appendix F: ALLIFE DEXA Protocol

Dual-energy X-ray Absorptiometry (DEXA)

- DEXA measures whole body fat mass, whole body fat-free mass, and bone density.
- The DEXA scan was performed by certified technicians at the Cooper Institute in Dallas, TX.
- Subjects were measured while wearing light clothing to minimize clothing absorption.



An example of a DEXA scanning station.

- The DEXA device measures the attenuation of the two energy X-ray beams crossing the tissue, which allows partitioning between bone vs. soft tissue and fat vs. lean tissue in pixels of the body where there is no overlying calcified tissue.
- Total body scanning area was divided into precise anatomic segments: the arms were separated from the trunk by a line passing through the humeral head and the apex of the axilla. The trunk was separated from the legs by a line passing from the iliac crest to the perineum. The head was excluded from the trunk by a horizontal line passing just below the mandible.
- Specialized computer software produces output based on the DEXA scan which includes the total fat mass, total fat-free mass, and percent body fat (See below for example).

[redacted]	Sex: Female	Height: 161.3 cm
Patient ID: 0763094	Ethnicity: White	Weight: 84.8 kg
DOB: July 09, 1931	Menopause Age: 54	Age: 72

Referring Physician: ROSS

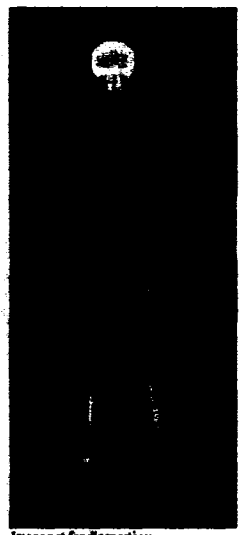
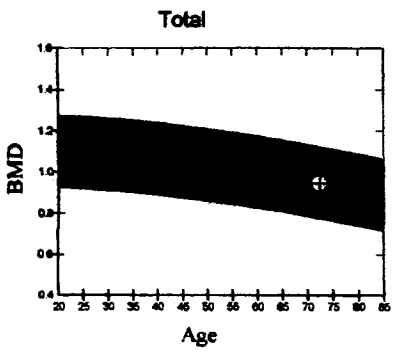


Image not for diagnostic use
k = 1.147, 40 = 46.1
327 x 150



Scan Information:

Scan Date: December 11, 2003 ID: A1211030P
Scan Type: a Whole Body
Analysis: December 11, 2003 14:45 Version 11.2.1:3
Whole Body Fan Beam
Operator: LC
Model: Delphi A (S/N 70530)
Comment:

DXA Results Summary:

Region	Area (cm²)	BMC (g)	BMD (g/cm²)	T-Score	PR (%)	Z-Score	AM (%)
L Arm	157.01	99.40	0.633				
R Arm	174.32	115.80	0.664				
L Ribs	120.81	68.81	0.570				
R Ribs	170.00	97.84	0.576				
T Spine	128.68	87.01	0.676				
L Spine	49.19	37.16	0.756				
Pelvis	133.40	144.71	1.085				
L Leg	338.42	317.20	0.937				
R Leg	330.94	318.22	0.962				
Subtotal	1602.77	1286.15	0.802				
Head	218.40	437.63	2.004				
Total	**1821.16**	**1723.78**	**0.947**	**-1.8**	**86**	**-0.0**	**100**

Total BMD CV 1.0%, ACF = 1.011, BCF = 0.987

Physician's Comment:

DXA Results Summary:

Region	BMC (g)	Fat (g)	Lean (g)	Lean + BMC (g)	Total Mass (g)	% Fat
L Arm	248.64	1546.7	3854.2	4102.9	5649.6	27.4
R Arm	239.69	1577.2	3826.6	4066.3	5643.6	27.9
Trunk	817.95	18340.0	31886.6	32704.6	51044.6	25.9
L Leg	559.97	3515.4	9484.7	10044.7	13560.1	25.9
R Leg	541.53	3348.0	9203.4	9744.9	13093.0	25.6
Subtotal	2407.79	28327.4	58255.6	60663.4	88990.7	31.8
Head	607.21	1118.1	3825.1	4432.4	5550.4	20.1
Total	**3015.00**	**29445.4**	**62080.7**	**65095.7**	**94541.2**	**31.1**

Appendix G: Manuscript Table 2 with Values of Transformed Variables

Table 2: Anthropometrics, body composition and hormone status in a sample of young adult survivors of ALL.

	Men		Women		*P* value		
	No-CRT (n = 37)	CRT (n=15)	No-CRT (n=38)	CRT (n=24)	Main effect of CRT	Main effect of Sex	Interaction Effect
Anthropometrics							
Age (yr) *LOG*	1.34 (0.06)	1.42 (0.09)	1.35 (0.07)	1.39 (0.10)	< 0.01	0.90	0.11
Height (cm)	175.5 (6.8)	171.6 (8.5)	162.7 (5.2)	154.8 (7.4)	< 0.01	< 0.01	0.23
Weight (kg) *SQRT*	8.9 (0.8)	9.0 (1.2)	8.4 (1.1)	8.6 (1.3)	0.70	< 0.05	0.29
BMI (kg/m^2) *LOG*	1.41 (0.07)	1.43 (0.11)	1.42 (1.0)	1.49 (0.11)	0.16	0.35	0.08
Waist circumference (cm) *LOG*	1.94 (0.06)	2.0 (0.07)	1.94 (0.07)	1.98 (0.07)	0.28	0.82	0.30
DEXA							
Total fat mass (kg) *LOG*	4.2 (0.3)	4.3 (0.2)	4.4 (0.2)	32.5 (13.0)	0.06	< 0.01	0.93
Total lean mass (kg)	60.9 (8.1)	56.9 (11.1)	44.6 (6.5)	42.2 (9.5)	< 0.01	< 0.01	0.32
Percent body fat (%)	21.7 (8.7)	28.5 (6.3)	35.1 (7.8)	42.4 (4.9)	< 0.01	< 0.01	0.53
CT							
Abdominal fat (kg)	1.00 (0.64)	1.38 (0.65)	1.09 (0.59)	1.47 (0.54)	< 0.05	0.67	0.58
VAT (kg) *SQRT*	0.47 (0.18)	0.59 (0.18)	0.38 (0.14)	0.56 (0.15)	< 0.01	< 0.05	0.09
SAT (kg) *LOG*	-0.22 (0.31)	-0.06 (0.24)	-0.09 (0.22)	0.03 (0.18)	0.09	< 0.05	0.88
VAT @ L4-L5 (cm^2) *SQRT*	7.8 (2.8)	9.4 (2.5)	6.7 (2.3)	9.3 (2.4)	< 0.01	< 0.05	0.07
SAT @ L4-L5 (cm^2) *SQRT*	14.4 (4.8)	17.0 (4.7)	16.5 (3.8)	18.9 (3.5)	0.05	< 0.05	0.73
VAT:SAT ratio *LOG*	-0.55 (0.20)[a]	-0.51 (0.18)	-0.82 (0.16)[a,b]	-0.64 (0.18)[b]	0.11	< 0.01	< 0.05
Liver fat (CTL/CTS) *SQRD*	1.51 (0.35)	1.57 (0.25)	1.54 (0.31)	1.56 (0.38)	0.67	0.70	0.74
Muscle attenuation (HU) *SQRD*	3045 (345)	2948 (403)	2942 (353)	2759 (440)	0.12	0.14	0.42
Hormone Status							
IGF-1 (μg/l)	463.1 (175.1)	315.3 (155.5)	434.3 (190.4)	254.8 (110.7)	0.01	0.49	0.16
Leptin (μg/l)	6.2 (6.9)	12.9 (15.5)	19.6 (14.3)	28.9 (14.6)	< 0.05	< 0.01	0.48
Leptin/fat mass (μg/l/kg)	0.3 (0.2)	0.5 (0.3)	0.7 (0.3)	0.8 (0.3)	< 0.05	< 0.01	0.79

Controlled for age and race/ethnicity.[a,b] Significant corresponding differences at $P \leq 0.01$ in post-hoc analysis. *LOG*, *SQRT* and *SQRD* represent log, square root and squared transformations, respectively, of non-normal variables.

Appendix G: The Saga of Daniel Lambert

No examination of the obese state would be complete without the mention of Daniel Lambert, the human colossus (picture below). Dando, as he was also known, typified the true Englishman – rotund and jolly. Although by today's standards Lambert was morbidly obese most of his life, he was physically active and healthy – an anomaly to today's dogma on obesity and disease. There exist stories of Dando, at 448 lbs, fighting off a bear which was attacking one of his dogs, as well as swimming with two men of ordinary size on his back. He was also known to frequently walk from Woolwich to London – a hefty distance - without showing any signs of fatigue. After Lambert's weight surpassed that of Edward Bright – the Fat Man of Essex, he became known as the heaviest man in Britain. Daniel was so proud of his accomplishment that on April 7, 1806, he began exhibiting himself in London, charging five shillings per visitor. Surprisingly, female spectators were the most frequent visitors of Daniel's, who would admire all 50 stone (approximately 700 lbs) of good old Dando, and would comment how much they appreciated "his manly and intelligent countenance." His weight notwithstanding, doctors deemed Lambert to be in perfect health: his breathing was free; he slept well, never felt pain, and was mentally alert and active. He was also considered to have a gracious and sociable personality – "with manners of a gentleman." Oddly enough, all that is associated with the obese today miraculously did not affect the fattest man in Britain in the least. Furthermore, people of that time did not see Daniel as a freak show, but as a "wonder of nature." It is unfortunate, for all the obese individuals in developed societies, that this is no longer the prevailing view.

Daniel Lambert: The Human Colossus