The utility of skinfold thickness for estimating insulin resistance and serum triglycerides in adolescents

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OPPONG ADDO

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Advisor: **John H. Himes**

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Dedication

To my parents; my father, Gideon Kofi Kuma and to my mother Paulina Adubea Forkuo Kuma for teaching me the value of hard work and perseverance

To my sister, Cynthia Yaa Kuma Acheampong

To all my teachers

Skinfold thickness has long been found to be highly correlated with total body fat. Yet it remains largely underutilized in comparison to other anthropometric measures. The ability of skinfold thickness to characterize adiposity-related health outcomes should contribute to its utility in studying these outcomes.

Objective: To determine comparability of skinfold thickness with whole body total fat, measured by dual energy X-ray absorptiometry (DXA), and their relationships with insulin resistance and serum triglyceride levels in a large sample of US adolescents. The utility of skinfold thickness was evaluated by determining optimum subscapular percentile-cut points for identifying adolescents who are at risk of elevated insulin resistance.

Methods: Pooled serial cross-sectional data from the continuous National Health and Nutrition Examination Survey (NHANES) cycles 2001-04 were analyzed. Primary data used included skinfold thicknesses, DXA-based total body fat (DXF), serum insulin and fasting glucose (for homeostasis model assessment of insulin resistance; HOMA-IR), and serum triglycerides. Data from a total of approximately 1500 US youths aged 12-18 years were used in this work.

Findings from manuscript one demonstrated that skinfold thickness is comparably associated with both continuous HOMA and the upper quintile of elevated insulin resistance in adolescents as total body fat weight measured with DXA. Similarly, in manuscript two skinfold thickness was comparable to DXA in associations with variation in serum triglycerides, and in predicting adolescents who have elevated serum

triglyceride levels. Additionally, subscapular skinfold was found to be better at identifying adolescent girls at risk of elevated serum triglyceride levels than DXA whole-body fat weight.

In manuscript three, subscapular skinfold thickness was found to be sufficiently correlated with HOMA-IR to justify identification of age- and sex-specific percentile cut-offs for identifying elevated insulin resistance in adolescents. These new subscapular skinfold percentile cut-offs can be used as a screening tool for identifying US adolescents at risk of elevated insulin resistance, who can then be referred for subsequent follow-up and diagnostic studies.

Findings from this dissertation have demonstrated that skinfolds are amply correlated with insulin resistance and serum triglyceride levels, thus supporting their wider use in anthropometric assessment of obesity and its related health outcomes in adolescents.

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Chapter 1

INTRODUCTION AND OVERVIEW

A. Overview

A growing body of evidence has shown that the onset of type 2 diabetes and cardiovascular disease risks are becoming increasingly prevalent in children and adolescents in countries world-wide. Screening for these conditions during childhood in an effort to preventing their future occurrence during has been a major public health challenge. Clinical tests for these conditions are often expensive to carry out on a large scale in many situations. As result, simple surrogate nutritional assessment methods such as anthropometry have long been relied upon in identifying individuals at greatest risk.

The utility of an anthropometric assessment tool lies in its ability to be correlated to pertinent health-related outcomes(1, 2). The body mass index (BMI) is an important indicator of obesity or adiposity in children and adolescents, and in screening for obesity-related health outcomes. However, if alternate anthropometric methods more specific to adiposity *per se* during childhood could be shown to be strongly associated with health-related outcomes, they would contribute greatly to research and practice, given that assessing obesity and adiposity with BMI is often fraught with conceptual challenges (3-5). One such method of measuring adiposity is the skinfold thickness. Although it has long been used in nutritional status assessments in the

undernutrition spectrum, it has been used less frequently in the assessment of obesity and its concomitant health associations.

B. Why skinfold thickness?

The measurement of subcutaneous fat thickness has a long history of recognition in nutritional studies for several reasons. One of these reasons is the high correlation with total body fatness(2, 6, 7). Due to its morphologic representation of global nutritional status, subcutaneous fat thickness provides a measure very specific to adipose tissue(7) in comparison to bulk measurements such as body weight, which is a heterogeneous mix including fat, lean mass, and bone.

With respect to pertinent health-related correlates, the understanding of the main function of subcutaneous fat has metamorphosed from an index of energy storage and general nutriture, among others, to an active endocrine site in recent years(8, 9). Endocrine activities for subcutaneous fat in adolescents range from being a cytokine production site and inflammation activity, to modulation of insulin sensitivity(8-11). There is also evidence that increased subcutaneous adipose tissue mass associated with obesity is interrelated with insulin resistance and type 2 diabetes occurrence(12, 13). This evidence of endocrine activity of subcutaneous adiposity coupled with its strong correlation with total body fat in youth motivated the quest to explore linkages between skinfold thickness and a cytokine-mediated disorder, such as insulin resistance in adolescents (Chapter 2).

Childhood adiposity is a strong predictor of subsequent obesity, insulin resistance, and abnormal lipids in adulthood(14,15). Obesity in children and adolescents and their accompanying risk factors go on to confer heightened risks for subsequent atherosclerotic cardiovascular diseases in adulthood (16, 17). US adolescents have been documented to have a high prevalence of adiposity as indicated by overweight and obesity (18). Also, adolescents have a higher proportion of total fat that is subcutaneous compared with their adult counterparts, and this probably has an effect on adolescent metabolism (19, 20).

These pieces of evidence are consistent with the findings that subcutaneous adiposity measured by skinfold thickness is strongly correlated with abnormal lipid levels (21,22). Therefore, skinfold thickness might be relied on to characterize fatness well and its concomitant associations with serum triglyceride levels. Given this rationale, manuscript two (Chapter 3) focused on the comparability of skinfold thickness with DXA total body fat and its associations with elevated serum triglyceride levels in US adolescents. The hypotheses for manuscripts 1 and 2 were:

i. Insulin resistance or serum triglycerides are related to subcutaneous fatness and adolescents with higher skinfold thickness are more likely to be insulin resistant or have higher serum triglyceride levels compared to their peers with low skinfold thicknesses. Subscapular skinfold thickness, as a measure of trunk fat, will be more highly associated with insulin resistance or serum triglycerides than triceps skinfold thickness.

ii. Skinfold thicknesses will be sufficiently correlated with insulin resistance or triglycerides to recommend their use as valid measures of fatness in studies when total body fat based on DXA are unavailable or impractical.

Having demonstrated that skinfold thicknesses are associated with two major obesity health-related outcomes, i.e., insulin resistance and serum triglyceride levels in US youths, the third manuscript (Chapter 4) examines one practical application of the findings. Optimum subscapular skinfold percentile cut-offs, based on US national reference curves were established for identifying adolescents who are at risk of elevated HOMA insulin resistance, who may then be referred for subsequent follow-up diagnosis studies and treatment, if possible. The hypotheses for manuscript 3 were:

i. Triceps and subscapular skinfold will have different strengths of associations for predicting insulin resistance. Subscapular skinfold thickness, as a measure of trunk fat, will be more strongly associated than triceps skinfold thickness and, as a result, would be a preferred screening indicator of insulin resistance in adolescents

ii. Age- and sex-specific skinfold thickness percentile cut -offs can be used to reasonably identify adolescents who are risk of elevated insulin resistance

DXA total fat weight was chosen for the criterion measure of adiposity for comparisons with skinfolds. The intent of comparing skinfolds with DXA total fat weight in their associations with insulin resistance and serum triglycerides was to compare the

measures of subcutaneous fat with an established *gold standard* of whole body adiposity. Accordingly, the rationale was that if skinfolds perform reasonably in this regard compared with total fat weight, because they are much easier and cheaper to measure they are good candidates to consider in studies interested in including a measure of body fat in estimating variation and elevated risks in insulin resistance and serum triglycerides in adolescents.

Some investigators prefer that total body fat be expressed as a percentage of body weight rather than as fat weight. Reasonable physiological models can be built supporting either total fat weight or percent body fat as the most important construct to capture adiposity-related pathology associated with obesity for the present comparisons. The fact that the two measures are inter-correlated at levels >0.9 indicate that either would be adequate to answer the research question, although percent body fat is an inappropriate proxy for body fat. The results in papers 2 and 3 were replicated using DXA percent body fat rather than total fat weight with close to identical findings and no differences in conclusions. These additional analyses using percent body fat are mentioned in the respective discussions to address concerns for readers who may prefer percent total body fat as the criterion.

Chapter 2

MANUSCRIPT 1: IS ADIPOSITY FROM SKINFOLD THICKNESS AS GOOD AS DXA WHEN ESTIMATING ELEVATED HOMA INSULIN RESISTANCE IN US ADOLESCENTS?

Skinfold thicknesses are highly correlated with total body fatness. However, there is little information to elucidate its utility at identifying adolescents at increased risk of insulin resistance (IR). We compared adiposity from triceps and subscapular skinfold thicknesses (SF) with total body fat from dual energy X-ray absorptiometry (DXA) in their relationships with HOMA insulin resistance for a large sample of US adolescents.

Methods: We analyzed cross-sectional data for 1496 adolescents from 12-18 y of age examined as part of the US National Health and Nutrition Examination Survey (NHANES) cycles 2001-04. Data collected included skinfold thicknesses, DXA-based total body fat (DXF), serum insulin and fasting glucose for homeostasis model assessment of insulin resistance (HOMA-IR). Regression models adjusting for complex survey design effects and correspondence analyses were used to study associations with HOMA-IR and concordance between the two measures of adiposity.

Results: After regression adjustments, HOMA-IR was substantially associated with SF and DXF in both sexes. There were no differences between the full SF and DXA models in the amount of variation accounted for or precision of estimation of HOMA-IR, based on bootstrap estimates of 95% confidence intervals. Subscapular skinfold was more strongly associated with HOMA-IR than triceps skinfold. The upper quintiles of predicted

HOMA-IR from both fatness models identified very high proportions of the same individuals (positive percent agreement > 0.94), and high proportions of those in the upper quintile of observed HOMA-IR (positive percent agreement >0.86).

Conclusions: Triceps and subscapular skinfold thicknesses estimate HOMA-IR and identify those at highest risk for insulin resistance as well as total body fat from DXA. Skinfold thickness provides an inexpensive and widely applicable measure of fatness that is appropriate for studies of insulin resistance, and perhaps other metabolic variables, in adolescents.

A. Introduction

The increasing prevalence of childhood obesity is a major public health concern in US. Closely related to this rapid increase obesity in recent decades(23, 24) is the increased risk for metabolic risks such as insulin resistance, among many others (25,26). As a result, there has been growing interest in the association between body fatness and insulin resistance, given the onset of type 2 diabetes is becoming increasingly prevalent at younger ages(27, 28). Anthropometric measurements of body composition specific to adiposity have long played important roles in assessing and monitoring nutritional status and changes in status. These changes range from those associated with malnutrition and intervention evaluations (2)to screening for abnormal biochemical profiles (29-31), with specific applications depending on the population and the exact question of concern.

The choice of anthropometric methods for the assessment of nutritional status in different settings has often depended on ease of use relative to availability of reference/standards, ease of measurement, non-invasiveness, accuracy and cost. Often, methods like body mass index (BMI) and weight are used to assess adiposity because of their ease of measurement and convenience, despite their known limitations. Dual energy x-ray absorptiometry (DXA) is one of the most accurate methods for measuring total body fat, bone and lean tissues. Although DXA can be considered as one of the 'gold standards' for body composition measurements, it is expensive, not portable, and logistically inapplicable in many field situations.

Comparatively, the skinfold caliper is inexpensive, portable and applicable in many situations, and the method is not associated with exposure to ionizing radiation.

Subcutaneous fat thicknesses have been found to have high correlations with total body fatness (2, 6, 7). Due to its morphologic representation of global and long-term nutritional status, subcutaneous fat thickness provides a measure very specific to adipose tissues per se (1, 7, 32). Additionally, skinfold thicknesses have been reported to correlate well with other measures of nutritional adequacy including many laboratory measures of blood and serum (1).

Authors have criticized skinfold thickness because of the difficulty in measuring them reliably (33, 34). Nevertheless, if skinfolds have sufficiently strong associations with health-related outcomes and metabolic risk, they may be very useful for screening and other research purposes, especially in field and routine clinical settings.

In this study, we compared total body fat weight (kg) from DXA with triceps and subscapular skinfold thicknesses (mm) in their associations with insulin resistance, and their utility to correctly identify US adolescents who are at elevated risk of insulin resistance. We hypothesize that skinfold thickness can identify those at high risk as well as adiposity from DXA. Also, we hypothesize that subscapular skinfold is more closely associated with insulin resistance than triceps skinfold thickness because of the established relationships between metabolic risk and trunk fat.

B. Methods

Study design and participants

The study participants included 1496 US adolescents between the ages of 12.0-18.00 years who participated in two rounds of the continuous US National Health and Nutrition Examination Survey (NHANES) between 2001-04 (35). Survey participants were non-institutionalized civilian Americans and were sampled by a stratified, multistage probability design. Data on adolescents who were sub- sampled to attend morning session blood draws and assigned non-zero sample weights were included in this study. Participants who were diagnosed with diabetes or fasted for less than 8 hours were excluded. All measurements were carried out in the mobile examination centers (MEC) by trained technicians/professionals.

Data Collection

Serum glucose and insulin were analyzed by the diabetes diagnostic laboratory at the University of Missouri-Columbia for all participants using standard techniques(36). Serum insulin methods changed over the two survey cycles (Pharmacia for 2001-02 and Tosoh Method for 2003-04). To ensure consistency of insulin measures, linear regression adjustments provided by the NCHS (37)were used to adjust for the differences in methodology. Four-year examination weights for individuals to yield results representative of the US population were constructed by NCHS from each of the two-year fasting subsample weights.

Anthropometric measurements included standing height (cm), triceps (Tri) and subscapular(Sub) skinfold thickness (mm). Skinfold thicknesses were measured by trained technicians with Holtain calipers (Holtain Ltd, Cymych, UK) using standard protocols. DXA whole-body scans were conducted for all participants using a Hologic QDR 4500A fan-beam densitometer (Hologic Inc. Bedford, MA). Pregnant females and participants whose weight exceeded 300 lb were not scanned. Race/ethnicity was recorded as non Hispanic white (63.7%), non Hispanic black (14.0%), Mexican American (11.2%), other Hispanics (5.4%), and other races (including multiracial, 5.4%). Girls' menarcheal status was determined from a (yes/no) answer to a question if the girls had a "menstrual period in the last 12 months?". A total of 641 girls (91.6%) reported they were post menarcheal. Only adolescents who had complete data for laboratory and anthropometric measures were included in study analysis.

Statistical Analyses

Insulin resistance was estimated by the homeostasis assessment model (HOMA-IR) and was calculated as: [plasma fasting insulin (uIU/l) x plasma fasting glucose (mmol/l)]/22.5, first described by Mathews et al(38). HOMA-IR was transformed logarithmically to approximate normality in all parametric analysis. A very small number (4) of observations were excluded based on their HOMA-IR values (with corresponding SF and DTF values) as being outliers(39). This is a conservative non parametric approach and identifies observations approximately beyond +/- 5 SD units of a normal distribution. The aim was to exclude only observations which were extreme and as result and likely due to errors.

Generalized multivariate linear regression models accounting for the sampling design effects were used to study the associations between insulin resistance (HOMA-IR) and measures of body fatness. To accommodate the complex sampling strategy of the two NHANES cycles the Taylor linearization series method of variance estimation(40)was used. This method takes into account all design effects such as sample weighting and clustering (pseudo PSUs and strata).

In addition to the postulated main fat-related predictors, other covariates were included in the models based on theoretical evidence of their relationships with adiposity (subcutaneous and whole body) and with insulin resistance in adolescents. These covariates included, age, height, race-ethnicity as well as subscapular and triceps subcutaneous adiposity (41-45), and for girls, menarcheal status (46, 47). Linear associations between anthropometric measures and HOMA-IR were evaluated by modeling predictors as continuous terms, and departures from linearity and curvilinearity of associations were assessed with addition of quadratic or cubic terms. Statistical interaction terms among predictors were evaluated. We used standard diagnostic approaches to ascertain if all regression models meet the distributional assumptions. All models satisfied the all assumptions. Although all fat-related predictors in the current study were right hand skewed, identical results were obtained in additional analyses with normalized predictors. For ease of interpretation of results, predictors were kept untransformed. Additionally, influential points were assessed in all regression models using appropriate diagnostic procedures. One case was excluded in the boy's final model based on Cooks' D statistic (48, 49)

The amount and change in the amount of variation explained (adjusted R^2), in the regression models were evaluated after the inclusion of adiposity terms (Tri and Sub, DTF) into the basic regression models. Finally, the root mean square prediction errors (RMSE) of the models were assessed to evaluate the precision of all regression models with respect to HOMA-IR.

We addressed the validity of our final models by bootstrapping (50, 51). Optimism of overfitting was used to estimate model optimism associated with the measure of prediction precision, RMSE as well as those associated with cross-classification statistics such as the agreement (K) statistics. Model optimisms ranged from 0.001 to 0.002 and were used to correct for overfitting associated with RMSEs and K. Using the optimism-corrected performance approach of model validation described by Steyerberg(51).We corrected for optimism of overfitting by subtracting the estimate of optimism from model apparent performance. Optimism, defined as the true performance minus apparent performance was obtained from 1000 bootstrap replicates. Consequently, optimism estimates that are very low values are indicative of minimal model overfitting(52, 53) as evidenced in the current study. Accordingly, final model adjusted R^2 and root mean square errors (RMSEs) with their 95% confidence intervals were obtained from bootstrap replication of the total sample sizes within each sex.

The top quintiles of predicted values of HOMA-IR from the SF and DTF regression models were compared with each other, and also with the top quintile of the observed logHOMA-IR. Kappa statistics (κ), positive percent agreement (PPA) and negative

percent agreement (NPA) (54) were used to evaluate agreement between the two models in identifying the individuals in the highest HOMA-IR quintile within each sex. Additionally, using Fisher's z transformations, equality of correlations were tested for coefficients(r) between observed continuous HOMA and those predicted from the 2 adiposity models. Two-tailed p-values <0.05 were considered statistically significant. All analyses were conducted with PC-SAS version 9.2 (SAS Institute, Inc., Cary, NC).

C. Results

The mean age of the study participants was 15.49 years with little difference between boys and girls (**Table 2-1)**. As expected, girls had higher mean levels of fatness than boys for both skinfold and DXA measures, and slightly higher HOMA-IR.

Table 2-2 presents results from three regression models for each gender estimating HOMA-IR as a continuous variable. The basic models include the background covariates and account for fairly small proportions of the variance in HOMA-IR. After adjusting for background variables, insulin resistance was substantially and significantly associated with fatness measures, for both SF and DTF models, and in both boys and girls. Adding fatness to the models increased the variance explained from 20% to 33% compared with the basic models. The strength of association with HOMA-IR was higher for subscapular than for triceps skinfold in boys (Sub $\beta = 0.04$; Tri $\beta = 0.02$). In girls however, Tri was non- significant with Sub in the model (Tri $\beta = 0.00$, $p=0.63$).

Improvements in total variation explained after the addition of body fat variables to the basic models showed that changes in adjusted R^2 of DTF compared to SF models, were slightly higher in boys (DTF 0.326 vs. SF 0.289) and girls (DTF 0.263, SF 0.200). Even so, when 95% CI were calculated using 1000 bootstrap replications, it is clear that the differences in adjusted R^2 and RMSE between the SF and DTF models are within the mutual 95% confidence intervals and therefore should not be considered statistically different by conventional criteria. Additionally, estimates of optimism were comparable for both SF and DTF models (**Table 2-3**). The correlations between predicted values of HOMA from the DTF and SF models were 0.93 in boys and 0.90 in girls (data not shown). Even so, there was no statistical difference in correlation coefficients (from Fisher's z-transformation tests) between the observed HOMA and those predicted from the 2 models; for both sexes (Boys SF_r=0.54, DTF_r=0.59; p=0.19; Girls SF_r=0.44, DTF_r =0.52; p=0.05). **Figure 2.1** shows a scatter plot of predicted HOMA vs. observed logHOMA for both sexes. Other than an apparent linear trend, there appears to be no marked difference in the plots between SF and DTF.

The upper quintiles of HOMA-IR predicted from the SF and DTF regression models were compared to evaluate the correspondence of the two fatness models in identifying those adolescents with the highest insulin resistance. The resulting 2X2 table ($< 80^{th}$ percentile, $\geq 80^{th}$ percentile) for predicted HOMA-IR from the two fatness models yielded 92.7% exact agreement for boys and 92.1% for girls. The corresponding values for positive percent agreement (PPA) and negative percent agreement (NPA) were 95.5 and 81.5, and 94.9 and 80.8 for boys and girls, respectively. When compared to the

upper quintiles of the observed HOMA-IR, the upper quintiles of HOMA-IR predicted from the SF regression models had greater values of kappa than those from the DTF models, and slightly higher agreement statistics (**Table 2-4**). **Appendix 2-A2** has the 2 x 2 tables which formed the bases for all agreement statistics (K) and other measures of correspondence such as PPA and NPA for the top-quintiles of HOMA for both sexes and for both SF and DXA fat in table 2-4 of manuscript 1(chapter 2).

D. Discussion

This study examined the associations of measures of adiposity with insulin resistance in 12-18y old adolescents. Findings from this nationally representative sample of US adolescents are consistent with previous studies in showing that insulin resistance is significantly associated with both total body fat weight (55-57) and subcutaneous fatness (58,59) in both sexes. After adjusting for background factors, skinfolds accounted for 20-29% of additional variance in HOMA-IR beyond the basic model, and total fat weight from DXA accounted for an additional 26-33% of variance. Bootstrap estimates of adjusted $R^2$and RMSE as well as tests of equality of population correlation coefficients indicated there were no statistical differences between the skinfold thicknesses models and DXA total fat models in estimating HOMA-IR as a continuous variable. When the upper quintiles of predicted HOMA-IR from the SF and DTF models were used as categories to identify those adolescents at highest risk of insulin resistance the exact agreement exceeded 92%. The optimism-corrected performance of model validation(51) was used in this analysis because it the approach

that is based on overall prediction measures such as RMSEs and could also accommodate the complex design effects associated with the NHANES.

From these analyses, we conclude that subscapular and triceps skinfold thicknesses are acceptable estimates of adiposity in studies of insulin resistance using the HOMA model in adolescents, both in terms of estimating fatness-related contributions to HOMA values and as identifying those at most risk of insulin resistance.

Subscapular skinfold was more strongly associated with HOMA-IR than triceps in the regression models. This is consistent with other evidence that a relative distribution of subcutaneous fat on the trunk is more closely associated with insulin resistance and some other metabolic outcomes than extremity fat (22,60). We found a positive association between age and HOMA-IR in boys, but in girls this association was negative. Other studies have reported transient changes in insulin resistance during puberty (41, 58), but most of these studies are in younger pubescent children. The average decrease in HOMA-IR across this age range in girls may represent a return to prepubertal levels after a transient puberty-related rise in insulin resistance(58).

There was a significant negative interaction of height X age with HOMA-IR in the models including fatness variables in boys. We interpret this interaction as a measure of somatic maturation progress so that the boys who are relatively tall for their age are less insulin resistant. The corresponding maturational association in girls is seen in the significant associations with menarcheal status and the height X menarcheal status, such

that menarcheal girls had significantly higher insulin resistance than their non menarcheal counterparts.

Although DXA is not issue free, it is considered one of the most accurate measures of fat, lean mass and bone (18, 61). One cannot tell if DXA is the perfect standard for comparison for skinfolds with respect to adiposity. Therefore, in addition to chance-adjusted agreement (κ) and exact agreement (accuracy), we also reported positive percent agreement (PPA) and negative percent agreement (NPA)(54) for cross-classification IR risk in DTF and SF models, assuming DXA is an imperfect standard for skinfolds. The PPA was very high in both boys (95.5%) and girls (94.9%). The NPAs were also high (Table 2-4). These additional estimates (PPA and NPA) of agreement further support the comparability of SF to DTF models in identifying IR risks in adolescents.

HOMA has been considered to be an appropriate measure of IR in large epidemiologic studies (62). The test-retest reliability of HOMA in both clinical and epidemiologic studies has been reported to be around 0.55-0.66(63-65). A single serum insulin and glucose measurement was carried out in continuous NHANES data. Future investigators should be aware of this issue regarding the reliability of HOMA in assessing IR. Although, the quantitative insulin sensitivity check index (QUICKI) has been reported by some researchers and clinicians as a better IR measure than HOMA in comparison to the euglycemic clamp(65-67), it is essentially an algebraically rearranged and log transformed version of HOMA formula. In our study, HOMA values were log

transformed to approximate normality in analysis; log transformed HOMA has been reported to have almost perfect correlation with QUICKI (68).

Currently, there is no consensus on a specific insulin resistance cut-off in screening and testing for the onset of type 2 diabetes in US children and adolescents (69). Similar to previous studies involving US adolescents, which used the top quintiles of distributions for risk categorization for metabolic conditions including insulin and serum glucose (70, 71), the top quintile was selected to indicate elevated IR. Using the top quintile of the current HOMA distribution although arbitrary, represent the youth who might be at most risk for follow-up tests and intervention in a practical setting. Furthermore, follow-up IR tests could be expensive themselves and as result using the top quintile would imply follow-up for the proportion of adolescents at most risk rather than all. Practically, not many at risk adolescents will be missed using the top quintile in comparison to other quantiles in a trade-off between cost of follow-up tests and cost of not identifying all those at risk.

We used total fat weight from DXA instead of fat as a percentage of body weight in our analyses to avoid modeling a ratio covariate and to avoid any confounding effects of body weight. Some researchers have cited percent DXA total body fat (%BF) rather the DXA total fat weight as a more valid measure of body fat status. Although the use of %BF as a proxy for body fat is actually incorrect, the main question of this paper was to compare skinfolds with DXA total fat weight instead of %BF and to demonstrate if skinfold can be a good surrogate of total body fat**.** Even so, the results of a separate

analysis (**Appendix table 2 - A1**), found close to identical total explained variance and overall model performance estimates (adjusted R^2 or RMSE) for %BF in predicting HOMA in comparison to DXA total fat in the current study sample . The study conclusions did not change, for that matter. Furthermore, DXA %BF and total fat weight were highly inter-correlated with each other with correlation coefficient (r) of 0.91 in the study sample. This high inter-correlation should address any concerns by investigators who prefer to use %BF, although %BF is still an inappropriate proxy for total adiposity.

The large sample size weighted to approximate a representative sample of US adolescents is strength of this study. Consequently, the results should be widely generalizable. The data used are of high research quality, with well documented protocols and established measurement reliabilities. There are a number of limitations of this study. The measurement reliabilities of skinfolds have been cited as one of the drawbacks of its usage in research and clinical settings(33, 34, 72, 73, 74) . Experience, practice, and regular training are therefore required of measurers to maximize reliability. Highly trained technicians measured the NHANES skinfold measurements. The rest-retest reliability of skinfold measurements has been reported to be approximately 0.85- 0.89 for well trained observers (72)and these have been considered adequate (75) for the NHANES. The body weight limit of the DXA scan was 300 lb; as a result data on the few (n=24) adolescents heavier than 300lb were not included in this study. This may have biased some estimates of association, although in practice, the

small number of cases should not have made an appreciable difference given the overall sample size.

No data were available for sexual maturation status in boys comparable to menarcheal status in girls. Nevertheless, because a height X age interaction term was included in the boys' models, it seems unlikely that appreciable maturation-related variation in IR was left unaccounted for in the regression models. Although the cross sectional nature of the design did not allow assessment of temporal associations between adiposity and HOMA IR, the results remain valid for concurrent status and associations.

To date, no recommendations exist regarding the routine use of skinfolds for monitoring, screening, management, or surveillance of obesity in children and adolescents (3). The results of this paper demonstrate that subscapular and triceps skinfold thicknesses are valid and useful in identifying health-related risks of insulin resistance in adolescents, and that they perform as well as total body fat estimated from DXA. Certainly, the ease of measurement, costs, portability, and lack of any ionizing radiation argue in favor of much broader use of skinfolds in this context. It is unknown whether the associations between skinfolds and IR are sufficient to be used for screening purposes for those at risk of elevated IR, although this would be an important application. Also, it remains for further research to determine if the associations between skinfolds and other adiposity-related metabolic outcomes are sufficient to allow a similar recommendation.

Chapter 3

MANUSCRIPT 2: COMPARABILITY OF SKINFOLD THICKNESS TO DXA WHOLE-BODY TOTAL FAT IN THEIR ASSOCIATIONS WITH SERUM TRIGLYCERIDES IN ADOLESCENTS

Like other measures of adiposity, higher skinfold thicknesses are associated with abnormal lipid levels during childhood. It is however, unclear how subcutaneous fatness measured with skinfold thickness compares with whole body total fat weight in relation to adverse serum triglyceride (TG) levels in adolescents. We determined whether triceps and subscapular skinfold thicknesses are comparable to dual X-ray absorptiometry (DXA) whole-body total fat weight in relation to serum triglyceride levels and increased risk of elevated TG levels in a sample of adolescents.

Methods: Pooled data from US adolescents ages 12.0-18.0 years who participated in two continuous National Health and Nutrition Examination Survey (NHANES) cycles 2001-4 were used for this study. Data on triceps and subscapular skinfold thickness, DXA whole-body total fat and serum triglycerides were included in the current analysis. We used logistic regression models to study the associations of elevated TG with measures of adiposity in two models; skinfolds (SFs) and DXA (DTF) models. Model prediction accuracies SFs and DTF were evaluated with the bias corrected area under the curve (AUC).

Results: Triceps skinfold was not significantly associated with increased odds of elevated TG levels in the SFs model in both sexes, but subscapular skinfold was

significant. In general, there was an increased odd of elevated TG per unit increase in subscapular skinfold (in SFs model) just as was total fat in DTF model with an adjusted after adjusting for age and race-ethnicity both sexes. Using the AUC as metrics of model accuracy with bootstrapped 95% confidence interval, no significant difference in prediction accuracy were found between SFs and DXA models (Boys SF AUC=0.89 95% CI: 0.86- 0.93, DTF AUC= 0.88, 95% CI: 0.85 – 0.93; and for girl SF AUC=0.89 95% CI: 0.85-0.92, DTF AUC=0.87 95% CI: 0.84- 0.91) in predicting elevated triglyceride levels. Similarly, SF and DTF models had comparable precision in predicting continuous serum triglycerides using the bootstrapped RMSEs for both sexes.

Conclusion:

Despite the known measurement errors associated with skinfold measurement, skinfold thickness was comparable to DXA whole-body total fat in identifying US adolescents at risk of elevated serum triglyceride levels. Because of its high correlation with truncal adiposity, subscapular skinfold thickness maybe a more beneficial screening tool for identifying adolescent girls who are at risk of elevated serum triglyceride levels than DXA whole-body fat.

Keywords: Skinfolds, DXA whole-total body fat, prediction accuracy, correspondence, screening

A. Introduction

The presence of frank chronic conditions such as cardiovascular diseases is normally infrequent in childhood. However, when adverse cardiovascular risk factors are present in childhood, they tend to track into adulthood (76). For example, overweight children and adolescents have been shown to have significantly higher levels of serum triglycerides (77). Additionally, increased adiposity in childhood is a strong predictor of abnormal lipids in adulthood (14), among other risk factors. Obesity in children and adolescents and their accompanying risk factors go on to confer heightened risks for subsequent atherosclerotic cardiovascular diseases in adulthood (16, 17). Given the high prevalence of childhood obesity in the US population (23, 24, 78) it would not be unusual to find a high proportion of adolescents having abnormal triglyceride levels as a result. Monitoring of lipid levels in children is therefore essential to protecting against life-long CVD in the future.

Screening for elevated serum triglycerides (TG) levels at the clinical level, for example, has been recommended by the American Academy of Pediatrics (AAP) and other professional bodies like the American Heart Association(AHA) as an early modifiable risk factor in childhood (16, 79), especially for obese/overweight children . For practical reasons however, it would be very costly to recommend TG screening for all overweight or obese children and adolescents on a scale comparable to other screening programs.

Skinfold thicknesses have been shown to be closely correlated with total body fatness (21, 80) as well as other nutrition and biochemical measures of the serum such

as triglycerides(1). In many research and screening settings including serum triglycerides, the availability of a cheap, portable and non-invasive measure of body fatness, such as skinfold thickness, may be very useful to characterize fatness if they could be shown to be valid estimators of triglycerides and if the associations were sufficient to identify those at highest risks of elevated serum triglycerides.

In this study, we compared two measures of adiposity, triceps and subscapular skinfold thicknesses (SFs) and DXA whole-body total fat (DTF), in the strength of their associations with serum triglyceride levels in a nationally representative sample of US adolescents. Also, we evaluated the effectiveness of skinfold thickness and DXA total body fat to identify adolescents at risk of elevated serum triglyceride levels. Finally, we determined whether adiposity measured by skinfolds is associated with measured or elevated TG independent of adiposity measured by DXA total body fat.

B. Methods

Study design and participants

We analyzed data from US adolescents from 12.0-18.0 years who participated in the continuous National Health and Nutrition Examination Survey (NHANES)(35) .The continuous NHANES are biennial serial cross sectional surveys involving non-institutionalized civilian Americans who were sampled by a complex stratified, multistage, probability design across the different geographic locations in the US(81). The current study consists of pooled data from two NHANES cycles from 2001-2004. Adolescents who were sub- sampled to attend morning session blood draws and assigned non-zero sample weights were included in this study. Three cases described as 'borderline diabetic' were not included in the final dataset. There were no diabetics in the sample. Overall, a total of 1505 US adolescents were used for this study.

Data Collection

Serum triglycerides (TG) were measured using standard diagnostic kits. The Hitachi 704 Analyzer (Roche Diagnostics, Indianapolis, IN) was used to analyze triglycerides by a series of enzymatic reactions (82) in both surveys. Four-year MEC examination weights were constructed from each of two-year fasting sub-sample weights (35) to account for sampling weights in the combined analytic dataset from the two survey cycles. Standing height, weight, triceps and subscapular skinfold thickness were measured using standard protocols (83). Holtain calipers (Holtain Ltd, Crymych, UK), a Toledo electronic weight scale (Mettler-Toledo Inc., Columbus, OH), and the Seca

electronic stadiometer were used to measure skinfold thicknesses, weight, and standing height respectively. Whole-body DXA scans were taken using a Hologic QDR 4500A fan-beam densitometer (Hologic, Inc., Bedford, MA). DXA total body fat was measured in kg on all adolescents. Participants who exceeded 300 lb body weight and pregnant females were not scanned. Also included in the analysis was girls' menarcheal status, coded as yes/no. All five race/ethnic groups listed in NHANES documentation for the US were used, namely non Hispanic white, non Hispanic black, Mexican American, Other Hispanics, and other races-including multiracial. Only adolescents who had complete data for serum triglycerides and the other analytic variables were included in the analyses. One case with a TG value of 621 mg/dl was excluded as an outlier (39).

We used percentiles recommended for lipid screening and cardiovascular health for children by the American Academy of Pediatrics (AAP) to classify adolescents at risk of elevated triglycerides. Elevated triglyceride (mg/dL) was defined as levels above the 90^{th} percentile adjusted for age and sex (79).

Statistical Analyses

All statistical analyses were conducted using SAS version 9.2 (SAS Institute; Cary, NC, USA). A two-tailed p-value < 0.05 was considered statistically significant. Pearson correlation coefficients were used to study correlations between adiposity measures and serum triglycerides within each sex. Multiple linear regression models were used to study associations between measures of adiposity and serum triglycerides, and logistic regression was used to estimate the odds of elevated triglycerides. Serum triglycerides were transformed using logarithms to approximate normality in all linear regression

models. Unbiased variance estimation in the analyses was facilitated by the use of Taylor linearization series (40) which accommodates the complex survey design effects of the NHANES.

Age, sex, race, and menarcheal status (in girls) were adjusted for as covariates in both linear and logistic regression models. Two-way interaction terms were evaluated in all regression models to facilitate the study of associations. Linearity assumptions for associations were evaluated by including covariates as quadratic terms. The -2 log likelihood ratio test was used to test for the global significance (global Ho: $\beta=0$) of our logistic prediction models, while the local test of significance was tested with the Wald's chi square tests and their associated p-values. Odds ratios were estimated with their 95% confidence intervals.

The effect of influential points on model estimates were assessed using Pearson's standardized residuals and df betas (48). No influential data points were identified. We selected our final logistic models based on the lowest AIC. The C-statistic or the area under the curve (AUC) was used as measure of prediction accuracy of final logistic models whereas the root mean square error (RMSE) was used to measure the precision of linear models. To ascertain that our final models were the best obtainable or not over fitted ,internal validation methods which involves correcting for optimism of overfitting our models using 2500 bootstrap replicates (50, 51) was carried out . Consequently, we evaluated correspondence between skinfold and DXA models by comparing the optimism-corrected performance (AUC and RMSE) (51) along with their

95% confidence; associated with each of the final adiposity models selected. Estimates of model optimism from bootstrapping indicated that there was minimal overfitting(52). Using the predicted values from the 2 adiposity linear regression models, the Fisher's z transformations (and equality of correlations tests) was used to compare 2 population correlations coefficients(r) from observed continuous triglycerides and those predicted from both adiposity models -skinfolds and DXA.

C. Results

The descriptive characteristics of study participants are shown as mean and standard errors in **Table 3-1**. The mean age of the adolescents in this study sample was 15.5 years, with the boys being slightly older than the girls. Although boys of this adolescent sample were, on the average, heavier than their female peers by approximately 7 kg, the girls had approximately 4 kg more total body fat. There were no marked differences in mean serum triglyceride levels between boys and girls. After adjusting for age, sex and race-ethnicity, the Pearson partial correlations (r) between TG and the two measures of adiposity were higher in boys (triceps r=0.30, subscapular r= 0.35, DTF r=0.35) than girls (triceps r=0.12, subscapular r=0.11, DTF r=0.13). All correlation coefficients (r) were statistically significant ($p<.05$).

Table 3-2 shows significant associations between serum TG as a continuous outcome and measures adiposity (DTF and SFs) in both boys and girls after adjusting for age, race-ethnicity and for girls, menarcheal status, using 3 different multivariate

regression models. The basic model included only background variables, and the skinfolds and DXA fat models add the respective measures of adiposity.

While subscapular skinfold was significantly associated with serum TG; triceps skinfold was not in both sexes. Compared to their non-Hispanic (NH) white peers, non Hispanic black adolescents had significantly lower serum TG after controlling for demographic factors and menarcheal status. This race/ethnicity difference persisted after adjusting for adiposity in both boys and girls. Although we included menarcheal status in the models for girls it did not contribute significantly. Similarly, a series of 2-way interaction terms were evaluated, found non-significant and left out of the final regression models**.** The amount of variance explained for predicting TG (as adjusted R squared) showed significant increases beyond the basic model after the inclusion of fatness variables. In girls, however, this improvement was small in both SFs and DTF models (adjusted R^2 changes, SFs: = 0.03 and DTF= ~0.04).

The results of the logistic regressions comparing skinfolds and DXA in identifying the individuals at highest risk of elevated triglycerides are presented in **Table 3-3**. As seen in the continuous analyses, triceps skinfold thickness was not significantly associated with increased odds of elevated triglycerides. A unit increase in subscapular skinfold, however, was associated with a 22% increase in the odds of elevated serum TG levels in both sexes, after adjusting for age and race-ethnicity. Similarly, a unit increase in whole-body total fat weight was associated with significant odds of elevated TG in a separate DXA total fat. We also tested race-ethnicity –triceps interactions on the risk of

elevated TG in both boys and girls, and found no significant interactions on both omnibus and race category-specified tests in the models.

Table 3-4 shows the results of the metrics of correspondence between the two adiposity models. The correspondence between DXA and SFs were assessed by the model accuracy/precision measured by the optimism-corrected AUC and RMSE after the inclusion of fatness terms (SFs and DTF) into the basic(no adiposity) models by sex. There were minimal increases in AUC and RMSE beyond the basic models in both sexes but comparable for both adiposity measures. Additionally, model RMSEs and AUCs from both adiposity models were statistically not different from each other with overlapping confidence intervals constructed from percentile bootstrapping as shown in table 3 - 4.

This comparability of both SFs and DTF models in predicting both measured and elevated TG motivated us to consider a final model containing both SFs and DTF adiposity variables in the same regression models. This enabled us to evaluate the independent contribution of subcutaneous adiposity to elevated TG levels beyond that associated with total body fat weight from DXA. **Table 3-5** shows that subscapular skinfold was significantly associated with continuous serum TG and the odds of elevated TG in girls but not in boys, independent of DXA whole body total fat in models adjusted for age and race-ethnicity. Additionally in girls, while subscapular skinfold was significantly associated with both continuous and categorical elevated TG; no independent association remained with DTF or triceps skinfold thickness.

A scatter plot of predicted TG vs. observed TG for both sexes is shown in **figure 3.1**. There appears to be no marked difference in the plots for both SF and DTF models. There was no statistical difference in correlation coefficients (from Fisher's z-transformation tests) between the observed TG and those predicted from the 2 models; for both sexes (Boys SF_r=0.49, DTF_r=0.49; p=0.85; Girls SF_r=0.38, DTF_r =0.39; p=0.81).

D. Discussion

The results of this study show that adiposity measured by both skinfold thickness and whole-body DXA fat weight is positively associated with serum triglyceride levels in adolescents. This finding is congruent with previous studies involving children and adolescents(21, 22, 84) . Similar to the findings of Freedman(22) we found subscapular skinfold thickness was more strongly associated with serum TG than triceps skinfold. In fact, triceps was not significant in our models containing the two skinfold thicknesses after adjusting for race-ethnicity, age and sex. Whole-body total fat weight was also positively associated (from DTF models) with continuous serum triglycerides.

Using optimism-corrected AUCs and RMSEs as measure of model accuracy, no significant differences in correspondence was observed among the two measures of adiposity in predicting both continuous serum TG and the risk of elevated TG levels in these adolescents. Even though DXA total fat is not without some controversy, it remains one of the most accurate measures of whole body adiposity (18, 61) . We found no statistically significant difference between SFs and DTF models either the predicting elevated TG or measured serum TG. The optimism-corrected AUCs from logistic

regression were used as metrics of comparison for the 2 adiposity models because the binary outcome nature of elevated triglyceride risk. Also, the RMSE served a similar purpose for predicting continuous serum triglycerides between the 2 adiposity models. Optimism-correction approach was used to address the issue of optimism of overfitting which can be evidence by reduced model performance in a different population but not in the current study sample. This optimism-corrected performance approach also afforded model validations taking into account the design effects associated with the NHANES because it is based on overall metric of model performance (51) of final regression models in comparison to other methods.

Additionally, the amount of explained variance (adjusted R^2) for predicting continuous serum TG showed very comparable levels due to SFs, and DTF. Furthermore, a test of differences in population correlation coefficients (r) among SF and DTF predicted values and the observed serum TG showed no statistical difference. SFs therefore had comparable prediction ability with DXA for predicting serum triglycerides on both continuous and categorical scales in all models considered.

From our logistic regression models subscapular skinfold thickness was associated with increased odds of elevated TG levels in girls but no such association was found in boys. Further, the odds of elevated TG with DXA whole body total fat was not significant in both sexes in models containing the two measures of adiposity-SFs and DTF. We interpret this relative association of subscapular skinfold with increased odds of elevated TG but not with triceps or DXA total body fat as being attributable to

importance of subcutaneous fat distribution in the abdominal region on the risk of elevated TG levels in US adolescent girls.

Consistent results were obtained for girls in a study of Spanish adolescents where abdominal adiposity was positively associated with TG levels independent of total adiposity (84). Given that subscapular skinfold is anatomically measured on the trunk, our results support the notion that truncal or abdominal adiposity is more closely associated with circulating TG levels than whole body total fat. Some of this association may probably be attributable to the correlation of visceral fat with circulating levels of serum triglycerides which was not examined in the current analysis. The association of visceral fat with lipids in general does not however invalidate the current association between subscapular skinfold and serum triglycerides in adolescents because trunk subcutaneous fat in of itself, has been found to be strongly correlated with visceral fat (60) in adolescents. Abdominal adiposity has been found in children and adolescents to be disproportionately deposited subcutaneously (20, 60, 85) and to contain higher proportions of saturated fatty acids (86) in comparison to other anatomical sites in adolescents.

From the forgoing, not only is skinfold comparable to DXA total body fat in general, subscapular skinfold in particular, may be more appropriate at identifying US adolescent girls at risk of elevated TG levels than DXA whole-body total fat. Furthermore, it is noteworthy that while late adolescent changes in girls normally result in disproportionate adipose tissue deposition in the truncal region, DXA whole body

total fat failed to independently predict the risk of elevated TG with subscapular skinfold thickness in the model. This result confirms the additional utility of subscapular skinfold, particularly in girls.

Other studies have demonstrated positive associations between percent body fat (%BF) from DXA and skinfolds (from Slaughter equations) and cardiovascular risk factors including elevated triglyceride levels (21, 60) among children and adolescents. The current study has shown that the DXA whole-body total fat weight (kg) instead of %BF is similarly associated with the serum triglyceride levels. We chose to use total fat weight from DXA rather than fat as a percentage of body weight in our analyses to avoid using a ratio covariate and avoid any confounding effects of body weight, per se(denominator). Nonetheless, DXA %BF and total fat weight were highly inter-correlated with each other with correlation coefficient (r) of 0.91 in the study sample of adolescents. It is therefore unlikely that different conclusions would have been obtained with %BF instead of total fat in this study, even though %BF is an inappropriate proxy for total body fat. In fact, it should be assuring to investigators who prefer to percentage body fat to know that, additional analysis using %BF instead of DXA total body fat weight found identical overall model performance estimates (RMSE) and total variance explained (adjusted R^2) as did fat weight (data shown in **appendix table 3-1A**) although that analysis was not the main focus of this paper.

The intent of comparing skinfolds with DXA total fat weight instead of %BF was not to show if skinfold is a valid measure of relative fat status or not; but rather as a

good surrogate of total body fat measured by an established laboratory-based method like DXA. This rationale is built on the premise that several decades of work have consistently shown skinfolds to be highly correlated with total body fat (1, 2, 7, 87) in both malnourished and over nourished populations. The methods of total body fat measurements included in these comparisons included whole body analysis at autopsy, densitometry, and radiography.

After adjusting for adiposity and demographic factors, non Hispanic black adolescents had significantly lower measured serum triglyceride levels compared to their non Hispanic white peers. This finding is consistent with the results of another US study involving black and white children and adolescents (88). These black-white differences in serum triglycerides have been attributed to differential lipoprotein lipase activity, with non Hispanic blacks having a greater clearance of serum TG than whites(89). These differences, however, did not translate into differential risks of elevated TG in our sample of adolescents in logistic models predicting elevated TG as a categorical outcome. Although there has been a long recognized race-ethnicity differences in subcutaneous adiposity, particularly at the triceps site, in the US population(90-92), we found no significant race-ethnicity –triceps interaction and the risk of elevated TG in both sexes of our study sample.

We could not determine any temporal sequences of associations between the measures of adiposity and serum triglycerides because of the cross-sectional design of this study.

Nevertheless, these associations remain valid at a single point in time and should fairly represent the concurrent associations.

Conclusions

Skinfold thickness is as useful as DXA whole-body total fat in identifying US adolescents at risk of elevated serum triglyceride levels, despite the often cited errors associated with skinfold measurements. Because of its high correlation with truncal adiposity, subscapular skinfold thickness maybe a more beneficial screening tool for identifying adolescent girls who are at risk of elevated serum triglyceride levels than DXA whole-body fat.

Chapter 4

MANUSCRIPT 3: OPTIMAL SUBSCAPULAR SKINFOLD PERCENTILE CUT-OFFS FOR IDENTIFYING INSULIN RESISTANCE IN US ADOLESCENTS

Skinfold thickness has been found to be associated metabolic risk factors such as insulin resistance (IR) in youth. To date, there are no skinfold thickness percentile cut-offs for the identifying US adolescents at high risk for insulin resistance. We identify subscapular skinfold percentile cut-offs based on US national reference curves for optimally identifying adolescents at risk of insulin resistance.

Methods: Pooled data from two continuous National Health and Nutrition Examination Survey (NHANES) cycles 2001-4 for US adolescents ages 12.0-18.0 years were used for this study. Data on subscapular skinfold thickness, serum insulin and fasting glucose for homeostasis model assessment of insulin resistance (HOMA-IR) were included in the current analysis. The main outcome of this study, insulin resistance was defined as HOMA-IR > 3.50 mmol/L x uU/ml. Logistic regression models and the ROC curves were used to identify subscapular skinfold percentile cut-offs for identifying US adolescents who are at risk of IR.

Results: The prevalence of insulin resistance ranged from 8-25% across the different ages. All final model area under the curves (AUCs) was statistically different from chance and ranged from 0.75-0.89 for both sexes. From the ROC curves, sensitivities ranging from 74-92% and specificities 16% -75% were used to select the optimal thresholds for

subscapular skinfold. Based on published age-and sex-specific L M S parameters , percentiles ranging from the 64th to 82nd for girls and 85th to 89th for boys were identified as optimal subscapular skinfold cut-offs. The optimal percentiles converted to subscapular skinfold thicknesses of 9-19 mm. The efficiency of the final skinfold percentile cut-offs in identifying adolescents who are at risk of IR was high and at approximately 93% for current the study sample.

Conclusion

Subscapular skinfold thickness is sufficiently correlated with HOMA-IR to afford the selection of age- and sex-specific optimal percentile cut-offs for identifying US adolescents at risk of insulin resistance. These new percentile cut-offs may be used as a screening tool for identifying adolescents at risk of insulin resistance in subsequent follow-up and diagnosis, especially in research settings.

Keywords: Subscapular skinfold, optimal cut-offs, HOMA Insulin resistance, US adolescents

A. Introduction

A growing body of consensus has favored the establishment of population specific cut-offs for screening purposes, especially for chronic metabolic conditions. Consequently, studies around the world have sought to derive anthropometric cuts-offs associated with obesity because they have been shown to be associated with metabolic risk factors. Waist circumference and BMI cut-offs, for example, have been derived for predicting or screening metabolic risk factors in adolescents and adults in US and other countries(93, 94, 95).

The understanding of the function of subcutaneous fat has metamorphosed from an index of energy storage and general to an active endocrine site(8, 9) , including the modulation of insulin sensitivity in adolescents(10) . Accordingly, skinfold thickness has been shown to be correlated with HOMA-IR and or insulin levels in adolescents (44, 96) . Findings from manuscript one(chapter 2) of this dissertation work showed that skinfold thickness is strongly associated with HOMA IR, and that subscapular skinfold was more strongly correlated with HOMA than triceps in US adolescents.

The growing incidence of type 2 diabetes mellitus in US adolescents is a major health concern, and early intervention or, better yet, prevention is called for (97, 98). This is accompanied with high levels of total adiposity, overweight and obesity (18). The strong associations between skinfold thickness and HOMA insulin resistance and the noninvasive nature of skinfold measurement suggest that subcutaneous fatness measured as skinfolds may be a good screening tool for US adolescents who are at risk

of insulin resistance. Such a tool would have important applications for easy and noninvasive identification and referral for more detailed and diagnostic studies of insulin resistance.

Herein, we investigate the usefulness of subscapular skinfold thickness for identifying adolescents at risk of insulin resistance in US adolescents, and determine optimal subscapular skinfold percentile cut-offs based on US national reference curves appropriate for screening applications.

B. Study Methods

Data included in this analysis comprised subscapular skinfold thickness, fasting serum insulin and fasting glucose and demographic factors from a sample of approximately 1404 US teens who participated in two continuous National Health and Nutrition Examination Survey (NHANES) cycles 2001-04(99). To ensure consistency of insulin measures due to change in serum insulin methods in the 2 survey cycles (Pharmacia for 2001-02 and Tosoh Method for 2003-04), linear regression adjustments provided by the NCHS (37)were used to adjust for the differences in methodology. Skinfold thickness was measured using Holtain skinfold calipers following standard protocols. Insulin was measured using the two-site immuno-enzymometric assay method (100). Glucose was measured using enzyme hexokinase(HK) method(101) . More complete details of the NHANES protocols and measurements are available elsewhere (99). Only data from participants who attended morning blood draw sessions,

who were assigned non-zero probability sampling weights, and who had subscapular skinfold values and non-diabetic were used in the current analyses. Homeostasis model assessment of Insulin resistance (HOMA-IR) was calculated as: [plasma fasting insulin (uIU/l) x plasma fasting glucose (mmol/l)]/22.5 (38).

The main outcome of this investigation, insulin resistance, was defined as HOMA-IR values >3.50 mmol/L x uU/ml. Although there are no formal recommendations defining insulin resistance in adolescents using HOMA-IR, this value is similar to that used in other studies and provides a reasonable estimate appropriate for both genders at these ages(98, 102-104). Whole-year age bins were defined such that individuals 12.00 – 12.99 years of age were considered 12 years.

Statistical Analysis

Using subscapular skinfold as the chief independent variable, and controlling for age and sex, logistic regression models were used predict to risk of IR. Final models were selected based on the lowest information criteria (AIC). The area under the curve (AUC) was used to measure overall prediction accuracy of the models. The effects of influential points on model estimates were assessed using Pearson's standardized residuals (48). No influential data points were identified. The internal validity of final regression models were tested using bootstrap optimism-corrected AUCs (from ROC), the main metric of model performance. Model optimism was minimal and thus indicative of no appreciable model overfitting(52).

The receiver operating characteristic (ROC) curves were used to identify optimal, age- and sex-adjusted subscapular skinfold thresholds that identified adolescents at risk of insulin resistance from our final logistic regression models. The criterion for selecting optimal thresholds was based on a trade-off between maximum sensitivity and specificity. Our main aim however, was to maximize test sensitivity to minimize the individuals with HOMA-IR who would be missed from the screening. For the purposes of this report 'optimal' is used to denote the process of dichotomizing a continuous predictor (subscapular skinfold) via a trade-off between sensitivity and specificity. While the term 'optimal' may be technically incorrect, it is being used in line with the conventional terminology used in studies pertaining to anthropometric screening cut-offs (70, 94).

Each of the optimal subscapular skinfolds for predicting IR were then converted to standardized z- and percentile- scores using published age-and gender-specific L M S parameters for subscapular skinfolds from US national reference curves (90).The optimal percentile cut-offs were then smoothed across ages using a resistant nonparametric compound smoother, 3RSSH, twice (105) from Tukey's family of smoothers. This smoothing approach was adopted after comparisons of results using several other smoothing algorithms. The performance of the final optimal subscapular percentile cut-offs in the study sample was evaluated with chance agreement (kappa), the efficiency and predictive values from 2 X 2 cross-classification tables. This table was based on IR status (>3.5 or <3.5) versus subscapular skinfold above (or not) the smoothed optimal percentile cut-off.

Using HOMA IR status as the validation criterion, chance agreement (kappa) was used to test for the overall agreement between the two tools. Efficiency, as a measure of diagnostic accuracy, was calculated as the ratio of the sum of true positives and true negatives to the grand total. The predictive values were also calculated as proportion of positives which were truly test positive, for the positive predictive value (PPV) and negative predictive value (NPV) as the proportion of negatives which were truly test negative. Data analysis was carried out in SAS version 9.2 (SAS, Cary NC, USA).

C. Results

The descriptive characteristics of the study population are presented in **table 4-1**. The mean age of the study participants was 15 .5 years. Boys had a slightly higher mean HOMA-IR (2.62 mmol/L x uU/ml) than girls (2.50 mmol/L x uU/ml). The mean HOMA-IR was 5.34 and 1.95 for the high and low HOMA categories respectively. Also shown in table 1 is a comparison of the current study data with national reference data. Between 22-32% and 5-13% of the adolescents were above the 85^{th} and the 95^{th} percentiles respectively of the US subscapular skinfold reference curves for both sexes. Approximately 18% of the adolescents were insulin resistant (HOMA-IR >= 3.5 mmol/L x uU/ml); with the boys having a higher prevalence (approx 3% more) than the girls.

Table 4 - 2 shows the prevalence of insulin resistance (HOMA IR > 3.5), the areas under the curves (AUC), and the sensitivities and specificities predicting optimal subscapular skinfold thresholds by age and sex. The overall prevalence of IR ranged from 8 to 25%. Girls at ages 15 to 18years had the lowest prevalence. The model AUCs(from

ROCs) ranged from 75 to 89% for both sexes. All final model AUCs were significantly greater than chance (AUC= 0.5). Sensitivities ranged from 74 to 92% and specificities from 16% to 75%.

The optimal subscapular skinfold percentile cut-offs as obtained from the ROC analyses are presented in **table 4-3**. The observed optimal percentile scores spanned from the 54th to the 92nd percentile across the half-year age bins. After resistant compound smoothing across ages, percentile cut offs ranging from the 64th to 82nd for girls and 84th to 89th for boys were obtained as the subscapular skinfold optimal cut-off for identifying IR. These percentiles are equivalent to 9-19 mm of subscapular skinfold thicknesses across the age ranges for both sexes respectively based on the US reference curves. **Figure 4-1** shows the pattern of smoothed final percentiles cut-offs relative to the US subscapular skinfold reference curves. The optimal percentile thresholds tracked closely on the 85th percentile of the reference curves until age 16 after which it increases slightly for the boys.

Table 4-4 shows the yield for the final optimal subscapular skinfold percentile cut-offs in identifying adolescents who are at risk of IR in the current study sample. The efficiency (from 2 x 2 tables) of the skinfold percentile cut-offs and the risk of IR (as validation criterion) was high; with efficiencies of approximately 93 % for both sexes. The overall chance-adjusted agreement (Κ) was 73-78%. There was perfect predictive value of a negative test (NPV=100%) while the positive predictive values were between 63-69%.

D. Discussion

This study presents optimal subscapular skinfold cut-offs for indentifying US adolescents at risk of insulin resistance using HOMA-IR. We are not aware of other published reports addressing this particular research question to which our results can be directly compared. The development of these cut-offs were highly dependent on the recent availability of national reference data for subscapular skinfold thickness yielding age-and sex-specific percentiles and Z-scores (90). Because of the nature of the sampling of the NHANES data and the reference data used, the results should be applicable to almost all US adolescents.

The final optimal subscapular skinfold percentile cut-offs, 64th to 82nd for girls may seem lower than expected, especially for the girls less than 15 years of age. The relatively lower percentile cut-offs for girls below 15yrs may be attributable to two factors: the transient changes in insulin resistance during puberty (41, 58) and the associated relatively high prevalence of IR status at these ages. Consequently, relatively lower levels of this measure of subcutaneous trunk fat are sufficient to identify girls exceeding HOMA-IR values >3.5 mmol/L x uU/ml with reasonable accuracy.

In general, the derived subscapular skinfold percentile cut-offs performed well in correctly classifying adolescents at risk of IR. The efficiency of the skinfold cut-offs (true positives + true negatives/grand total) in correctly identifying the same adolescents as would the validation criterion HOMA IR cut-off was high, yielding an approximately 7%

misclassification rate. Using the predictive values as measures of yield, the negative predictive values were perfect but the positive predictive values were modest; between 63-69%. Nevertheless, given that predictive values are functions of overall prevalence, it is unlikely that optimal percentile cut-offs are under performing in the study sample. The low misclassification rate, the substantial (106) chance-adjusted agreement (K statistic); and the yield of the new cut-off percentiles in the current study population all support the notion that the subscapular skinfold percentile cut-offs should be sufficient to be used with reasonable confidence to identify US youth at risk for IR. This is particularly remarkable given that the test-retest reliability of HOMA-IR determinations have been found to be between 0.55-0.66(63-65)in both epidemiologic and clinical studies. A single determination of serum glucose and insulin was conducted in the continuous NHANES.

There is currently no scientific consensus on a fixed cut-off value using HOMA-IR for defining insulin resistance in American youth (69). We defined insulin resistance as values >3.50 mmol/L x uU/ml, based on other results in the literature. This threshold value is greater than 3.16 mmol/L x uU/ml value used in a previous continuous NHANES (1999-2002) study of US adolescents (102). Other studies have used cut-offs ranging from 2.7 to 4.39 mmol/L x uU/ml (98, 103, 104) for the similar age ranges.

Using subscapular skinfold thickness to identify adolescents at risk is not intended to be diagnostic of insulin resistance, like many other anthropometric correlates of nutritional biochemistries(1) . Rather, application of these cut-offs may

serve as a cost-saving starting point for screening individuals for referral for other more costly diagnostic tests such as a 2-hour-glucose tolerance tests or preferably the fasting plasma glucose as recommended by the American Diabetes Association (107).

Differential truncal subcutaneous adiposity deposition in this stage of life was probably the reason that lower optimal subscapular percentile cut-offs (64^{th}- 75^{th}) in girls less than 15 years were identified. Evidence from the current study also showed a transient trend in insulin resistance status at the population level, and as such higher(percentiles)of subcutaneous adiposity were required to optimally identify insulin resistant girls after age 15. Consequently, insulin resistant adolescent girls less than 15 may potentially 'grow out' of it, although this notion can only be confirmed by longitudinal study. To improve the cost effectiveness of these new cut-offs, these adolescent girls can, instead of follow-up glucose tests, be monitored following factors aimed at preventing obesity or weight gain. Stakeholders of this effort could be parents, teachers as well as clinical practitioners.

It is also noteworthy that the identification of these subscapular cut-offs does not replace any other known tools for screening health related outcomes in US youths particularly in research settings. Hopefully, this work will invite further research to evaluate the applicability of these new subscapular skinfold cut-off in comparison to other cheap methods such as weight and BMI in various settings.

An emphasis was placed on maximizing the sensitivity rather than specificity with respect to the optimal skinfold cut-offs. The intent was to minimize false negatives

so that relatively few youth at risk would be missed by screening with subscapular skinfolds. Although some critics of skinfold thickness measurements cite relatively low measurement reliability for wide-spread use, the measurement reliability is sufficient to yield very reasonable results in a screening setting. The intent was to provide a low-cost and easily implemented screening tool that would actually improve the identification of insulin resistance and risk for type 2 diabetes because of wide-spread applicability particularly in nutrition and growth research.

The results indicate there are not fixed subscapular skinfold percentile cut-offs across this age range so that the tables or figures will probably need to be consulted rather than remembering a rule-of-thumb skinfold cut-off for IR screening. While this is not as convenient for application it reflects the biology during this age period and is typical of results for other anthropometric screening criteria (70).

Conclusion

Subscapular skinfold thickness is sufficiently correlated with HOMA-IR to afford the selection of age- and sex-specific optimal percentile cut-offs for predicting the risk of insulin resistance in adolescents. Based on published national reference curves, subscapular skinfold percentile cut-offs ranging from 64th to 82nd for girls and 85th to 89th for boys were identified to optimally identify US youth who may be insulin resistant. These percentile cut-offs could be used as a screening tool for the risk of insulin

resistance in US adolescents who should be referred for subsequent follow-up and diagnosis especially in research settings.

CHAPTER 5

SUMMARY, CONCLUSIONS AND IMPLICATIONS

A. Summary and Conclusions

Using a high quality research data from the US National Health and Nutrition Examination Surveys(NHANES), this dissertation evaluated the comparability of skinfold thickness, a routinely collected anthropometric measure; with DXA total body fat in predicting two major adiposity-related health outcomes. These health outcomes were insulin resistance and serum triglyceride levels in adolescents. These outcomes are pertinent given their strong relationships with the onset of type 2 diabetes and cardiovascular disease risks in US youth, and an interest in early intervention and disease prevention. Similar to other studies, skinfold thicknesses were highly correlated with total body fat measured with DXA in the current study sample. Crude correlations (r) ranged from 0.85 to 0.90 for triceps and subscapular skinfolds for both sexes.

Manuscript one demonstrated that skinfold thickness is comparably associated with both continuous HOMA-IR and the presence of elevated insulin resistance in a national sample of US adolescents as was total body fat weight measured with DXA. Similarly, in manuscript two, skinfold thickness was comparable with DXA total body fat in associations with variation in serum triglycerides and at predicting adolescents who are at risk of elevated serum triglyceride levels. Additionally, subscapular skinfold thickness was found to be actually better at identifying adolescent girls at of risk of

elevated serum triglyceride levels than DXA whole-body fat. This result is consistent with other findings showing that relative distribution of trunk fat is more closely associated with serum triglycerides than whole body adiposity further adding to the anthropometric utility of subscapular skinfolds.

In manuscript three, subscapular skinfold thickness was found to be sufficiently correlated with HOMA-IR to justify identification of age- and sex-specific percentile cut-offs for identifying insulin resistant adolescents. The new subscapular skinfold percentile cut-offs can be used as a screening tool for identifying US adolescents at risk of insulin resistance, who can then be referred for subsequent follow-up and diagnostic studies. It is not intended that subscapular skinfold be used instead of other established diagnostic procedures of insulin resistance. Only that the implementation of such a screening should help target those adolescents at most risk and reduce the total number of diagnostic procedures like glucose tolerance tests that need to be preformed.

Accordingly, it is important to invite further research to evaluate the applicability of these new subscapular skinfold cut-offs in comparison to other known tools in various settings. Additionally, the overall implications to epidemiologic or clinical research of these cut-offs are yet to be evaluated. Alternate methods of assessing obesity like skinfolds thicknesses has not seen wider use in routine school, clinical and community screening programs although they are relatively accurate, non-invasive and cheap. Even though skinfold data have been collected in many national surveys like the NHANES for

decades, it is largely underutilized as an anthropometric tool for measuring adiposity/obesity in children and adolescents.

Although skinfolds have been criticized because of their anatomical specificity and their attendant measurement errors, the demonstration of appreciable significant associations with insulin resistance and serum triglycerides should support their consideration for wider use. Because skinfold measurements are routinely taken in many field nutritional status assessments at little additional cost, they may provide additional important information concerning body fatness, particularly as it relates to obesity-related health outcomes in adolescents.

To conclude, the demonstrated ability of skinfold thickness to characterize associations with insulin resistance and cardiovascular risk should present skinfold thicknesses to the scientific community as not only an economic independent assessment of subcutaneous adiposity, in addition to other indicators like BMI, but also as valid indicators of insulin resistance and serum triglycerides in US adolescents . In the absence of expensive total body fat assessment laboratory based-methods such as DXA, skinfold thickness should provide a good, non-invasive surrogate measure for total body fatness for at least these two major adiposity-related health outcomes in US youths - insulin resistance and serum triglyceride levels.

B. Study Implications

The subscapular skinfold cut-offs(chapter 4) could provide very useful tool in research pertaining to general nutrition and growth(in schools, community, and field) as well as studies on the onset of type 2 diabetes in adolescents. In a bid toward harmonizing the various methods of measuring adiposity/obesity in US children and adolescents for example, future research could address the comparability of skinfolds with other cheap methods such as BMI, waist circumference, or bioelectrical impedance. The extent to which a skinfold-for-age compares with BMI-for-age (the most widely used method) as screening tool for insulin resistance is beyond the scope of this dissertation.

Nevertheless, before such a comparison can be carried out however, there needs be a correspondence analyses study between skinfold-for-age and the BMI-for-age curves, in a nationally representative sample of US children and adolescents. While such evidence is presently unavailable, the comparison of skinfolds with BMI for their utility in screening would require a more comprehensive approach. Such an approach could consider a wide range of factors such as applicability, feasibility, acceptability, cost, accuracy and other factors. This will require inputs from expert panels and body composition working groups to make such a determination.

This dissertation sought to provide evidence in support of skinfold thickessess to be considered as a candidate tool for the assessment of adiposity/obesity in children and adolescents. Future important research questions could include the screening

ability of skinfold thicknesses, weight, BMI, waist circumference and bioelectric impedance in sequential and or in parallel combination screening tests regarding pertinent health outcomes in the US youth.

END

CHAPTER TWO - Manuscript one tables and figures

Table 2-1: Descriptive Characteristics

	Total Sample (N=1496)	**Boys(N=800)**	**Girls(N=696)**
Variables	**Mean(SE)**	**Mean(SE)**	**Mean(SE)**
Age (years)	15.49 (0.09)	15.55(0.08)	15.43(0.14)
Weight (Kg)	63.65 (0.69)	67.11(0.80)	59.92(0.93)
Height (cm)	166.15 (0.36)	170.84(0.51)	161.13(0.37)
BMI(Kg/m^2)	22.84 (0.23)	22.72(0.24)	22.98(0.32)
DXA Total Fat(Kg)	18.68 (0.45)	16.73(0.47)	20.77(0.60)
Triceps skinfold (mm)	15.99 (0.30)	13.49(0.41)	18.73(0.34)
Subscapular Skinfold (mm)	13.22 (0.24)	12.00(0.28)	14.61 (0.30)
*Gm HOMA IR (mmol/L x uU/ml)	2.22 (0.03)	2.14(0.04)	2.30(0.04)
Median HOMAIR (untransformed)	2.25 (0.07)	2.15(0.07)	2.34(0.08)

*Gm: Geometric mean of HOMA-IR back transformed from natural log units. Statistics obtained from Survey procedures adjusted for design effects

Table 2 - 2: Associations between HOMA-IR and anthropometric measures of adiposity

Model Covariates	Basic Model (Adj. R^2=0.034)		Skinfolds Model (Adj. R^2=0.323, Adj. R^2 change =0.289)		DXA fat Model (Adj. R^2=0.360, Adj. R^2 change =0.326)	
	β (SE)	**P-value**	**β (SE)**	**P-value**	**β (SE)**	**P-value**
Boys						
Age(years)	0.43(0.30)	0.17	0.60 (0.29)	0.05	0.62(0.30)	0.04
Height(cm)	0.05(0.03)	0.05	0.06 (0.03)	0.02	0.06(0.03)	0.03
Height*Age(interaction)	-.003(0.00)	0.13	-0.004(0.00)	0.04	-0.004(0.00)	0.04
Race	0.01(0.03)	0.74	0.00(0.03)	0.63	0.00(0.03)	0.63
Triceps skinfold	-		0.02(0.01)	0.03	-	-
Subscapular skinfold	-		0.04(0.02)	0.02	-	-
DXA Total fat (kg)	-		-	-	0.04 (0.00)	<.0001
Girls	Adj.R^2 =0.068		Adj.R^2=0.268,Adj. R^2change =0.200		(Adj.R^2=0.331,Adj. R^2change =0.263)	
Age(years)	-0.06(0.01)	<.0001	-0.07(0.02)	0.00	-0.07(0.02)	0.00
Height(cm)	-0.04 0.01)	<.0001	-0.04(0.01)	0.00	-0.04(0.01)	0.00
Menarcheal Status (Mstat)						
(Ref. Non Menarcheal)	5.4 (0.70)	<.0001	5.4(1.50)	0.00	5.1(1.43)	0.00
Height*Mstat(interaction)	0.030 (0.02)	<.0001	0.03(0.01)	0.00	0.03(0.01)	0.00
Race	-0.03(0.02)	0.22	0.00(0.03)	0.65	-0.03(0.02)	0.42
Triceps skinfold	-	-	0.00(0.01)	0.63	-	-
Subscapular skinfold	-	-	0.03(0.01)	0.00	-	-
DXA Total fat (kg)	-	-	-	-	0.03(0.00)	<.0001

Adjusted R^2 represents change total variance explained after the inclusion of adiposity terms to the basic model

Table 2 - 3: Adjusted coefficients of determination, prediction errors and validation for models

Model	Boys		
	Adjusted R^2 (95% CI)	**Optimism-corrected RMSE (95% CI)**	**RMSE Optimism**
Skinfolds	0.327 (0.249 - 0.399)	0.52 (0.488 - 0.573)	0.0054
DXA	0.361 (0.298 - 0.429)	0.52 (0.486 - 0.567)	0.0046
Model	**Girls**		
	Adjusted R^2 (95% CI)	**Optimism-corrected RMSE (95% CI)**	**RMSE Optimism**
Skinfolds	0.272(0.205 - 0.333)	0.44 (0.406 – 0.480)	0.0037
DXA	0.331 (0.268 - 0.396)	0.45 (0.418 - 0.488)	0.0036

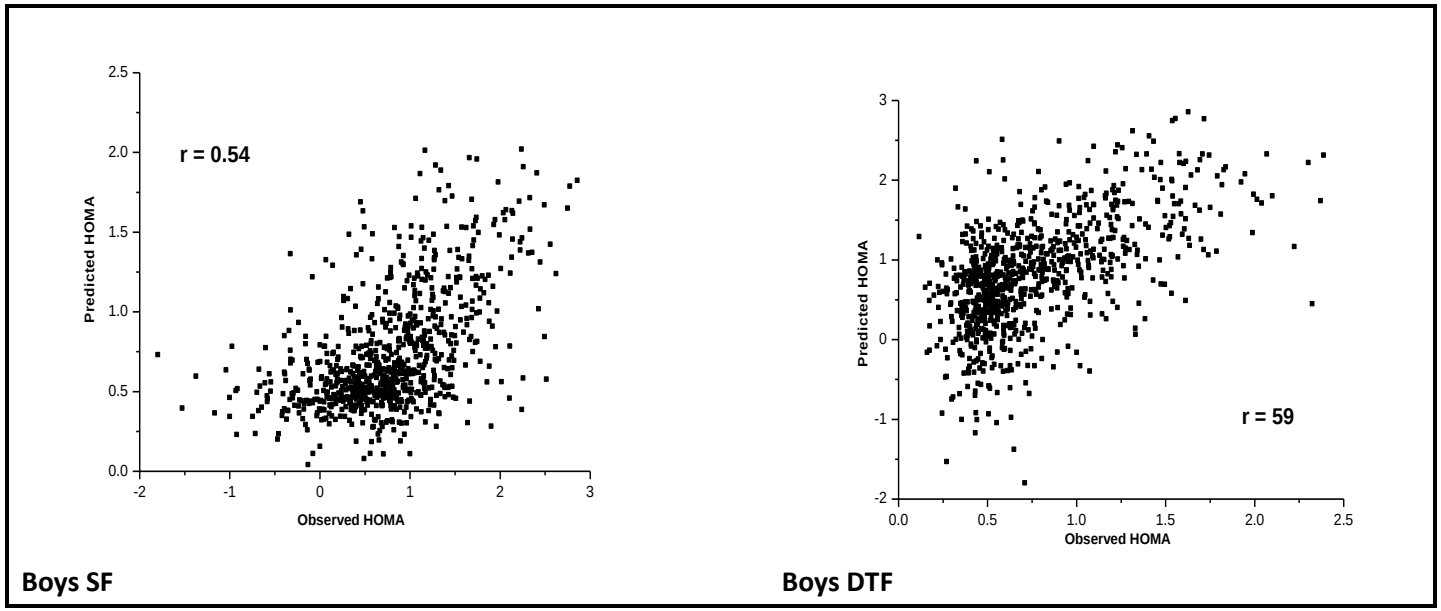
Statistics obtained from 1000 Bootstrap replications. Optimism = Bootstrap performance – apparent performance(51).

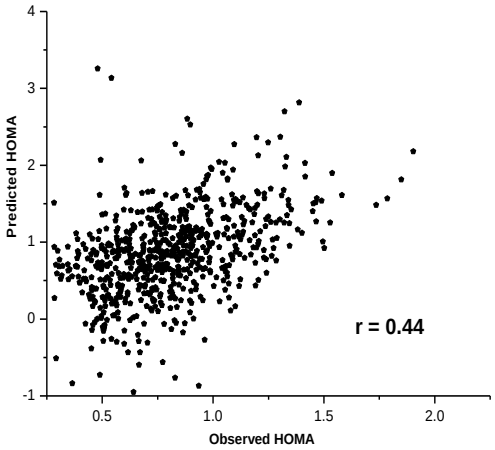
Table 2- 4: Comparison of predicted HOMA-IR values from DXA and Skinfolds models in Identifying upper quintile of IR

Observed vs. Predicted HOMA Comparisons	Top 20% at risk for Log HOMA IR				
	Optimism associated with Kappa	**Optimism-corrected Kappa (95%CI)**	**Exact Agreement (%)**	**PPA**	**NPA**
Boys					
SF model HOMA	0.0011	0.47(0.35- 0.57)	82.91	89.31	57.22
DXA model HOMA	0.0008	0.44(0.33- 0.54)	82.06	88.84	55.06
Girls					
SF model HOMA	0.0016	0.38 (0.26 - 0.49)	80.25	87.67	50.84
DXA model HOMA	0.0019	0.33(0.21 - 0.44)	78.50	86.43	46.45
Predicted HOMA Comparisons					
Boys - SF vs. DTF	0.0013	0.77 (0.70-0.84)	92.70	95.51	81.52
Girls - SF vs. DTF	0.0014	0.75 (0.67-0.82)	92.09	94.87	80.85

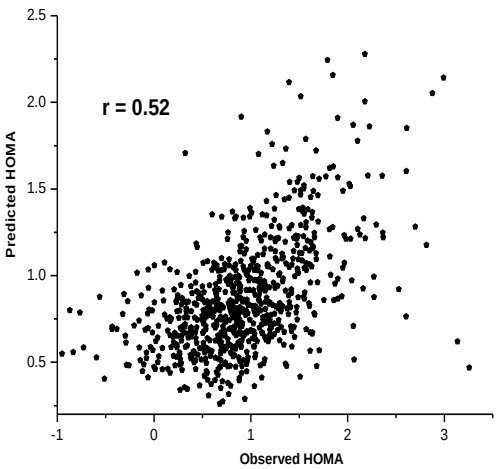
Abbreviations: EA- Exact agreement (efficiency); PPA- Positive Percent Agreement; NPA-Negative Percent Agreement

Figure 2: 1: Plot of predicted vs. observed HOMA (mmol/L x uU/ml) for SF and DTF models by sex





Girls SF Model



Girls DTF Model

CHAPTER 3 - Manuscript 2 tables and figures

Table 3 -1: Descriptive Characteristics

Variables	All (Unweighted N= 1505)	Boys (N=803)	Girls (N=702)
	Means(SE)	Mean(SE)	Mean(SE)
Age (years)	15.45 (0.08)	15.51(0.09)	15.38 (0.14)
Weight (Kg)	63.41 (0.71)	66.89(0.82)	59.73 (0.93)
BMI(Kg/m^2)	22.80 (0.23)	22.67 (0.25)	22.92 (0.33)
DXA Total Fat(Kg)	18.61 (0.45)	16.70 (0.48)	20.65 (0.59)
Triceps skinfold (mm)	16.00 (0.31)	13.52 (0.41)	18.67 (0.38)
Subscapular Skinfold (mm)	13.21 (0.25)	12.03 (0.32)	14.55 (0.31)
Gm TG (mg/dl)	4.51(0.02)	4.53 (0.04)	4.48 (0.03)
Median Log TG (untransformed)	4.34 (0.02)	4.36 (0.04)	4.33 (0.03)
Percent elevated TG (%)	9.53	10.64	8.40

Abbreviations: TG- triglyceride; Gm: Geometric mean of Serum triglycerides (TG) back transformed from natural log units. Elevated TG: > 90^{th} per AAP tables (Daniels et al., 2008). Estimated US adolescent population = 26.3 million. Race-ethnicity (N, %): Non Hispanic white (442, 64.2%); Non-Hispanic Black (497, 14.0%); Mexican Americans (463, 11.1%); other Hispanics (49, 5.1%) and 0ther Race-Including multiracial (56, 5.4%).

Statistics obtained from Survey procedures adjusted for design effects- weighting, cluster sampling

Table 3-2: Estimates of the associations between Serum Triglyceride and measures of body fat

Model Covariates for Boys	Basic Model (Adjusted R^2=0.050)		Skinfolds Model (Adjusted R^2=0.157)		DXA fat Model (Adjusted R^2=0.173)	
	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value
[1]Age, y	0.03(0.01)	0.03	0.01(0.01)	0.34	0.01(0.01)	0.26
Age^2	0.01(0.01)	0.05	0.01(0.01)	0.35	0.01(0.01)	0.05
Race – NH White (referent)	-	-	-	-	-	-
NH Black	-0.30(0.06)	<0.001	-0.29(0.05)	<0.001	-0.27(0.05)	<0.001
Mexican Americans	-0.07(0.05)	0.13	-0.07(0.04)	0.11	-0.07(0.04)	0.12
Other Hispanics	0.02(0.09)	0.84	0.02(0.10)	0.83	0.02(0.07)	0.76
Other Race-incl. multi racial	0.04(0.10)	0.69	0.02(0.09)	0.86	0.00(0. 09)	0.99
Triceps Skinfold			0.00(0.01)	0.51	-	
Subscapular Skinfold			0.02(0.01)	0.01	-	
DXA Total fat (kg)			-	-	0.02(0.00)	<0.001

Model Covariates for Girls	Basic Model (Adjusted R^2=0.069)		Skinfolds Model (Adjusted R^2=0.111)		DXA fat Model (Adjusted R^2=0. 0.104)	
	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value
[1]Age, y	0.00(0.01)	0.87	-0.01(0.01)	0.39	-0.01(0.01)	0.52
Age^2	0.02(0.01)	0.01	0.01(0.01)	0.01	0.02(0.01)	0.01
Menarcheal status	0.02(0.10)	0.80	0.09(0.10)	0.37	0.09(0.10)	0.37
Race – NH White (referent)	-	-	-	-	-	-
NH Black	-0.27(0.05)	<0.001	-0.34(0.06)	<0.001	-0.30(0.05)	<0.001
Mexican Americans	-0.03(0.05)	0.62	-0.07(0.05)	0.15	-0.03(0.05)	0.50
Other Hispanics	-0.11(0.05)	0.62	-0.19(0.06)	0.58	-0.10(0.05)	0.06
Other Race-incl. multi racial	0.05(0.09)	0.52	0.04(0.90)	0.70	-0.06(0.08)	0.49
Triceps Skinfold			-0.01(0.01)	0.15		
Subscapular Skinfold			0.02(0.00)	<0.001	-	
DXA Total fat (kg)			-	-	0.01(0.00)	0.00

Abbreviations: NH – Non Hispanic. [1] All continuous variables were centered; Basic model has demographic terms only

Table 3-3: The Odds of Elevated Triglyceride levels with Anthropometric measures of adiposity

Boys	Odds Ratio(95% CI)	Girls	Odds Ratio(95% CI)
Skinfolds Model (AUC change =0.034)	**AOR**	**Skinfolds Model (AUC change =0.028)**	**AOR**
[1]Age, y	2.42 (1.76 – 3.32)	[1]Age, y	2.94 (2.16 – 4.00)
Race	1.10 (0.71 – 1.70)	Race	1.47 (0.85 – 2.54)
Triceps skinfold (mm)	0.97 (0.78 – 1.21)	Triceps skinfold	1.10 (0.89 – 1.37)
Subscapular skinfold (mm)	1.22(1.06 – 1.40)	Subscapular skinfold	1.22 (1.14 – 1.31)
DXA Model (AUC change =0.052)	**AOR**	**DXA Model (AUC change =0.013)**	**AOR**
[1]Age, y	2.52 (1.90 - 3.33)	[1]Age, y	2.73 (2.03 – 3.68)
Race	1.10 (0.70 - 1.73)	Race	1.16 (0.76 – 1.75)
DXA Total fat(kg)	1.12(1.02 – 1.30)	DXA Total fat(kg)	1.16 (1.03 – 1.29)

Abbreviations: AOR- Adjusted Odds Ratio; obtained after adjusting for fatness terms (Skinfolds; and DXA fat). AUC- Area under curve; Basic model AUCs,

Boys- 0.851, girls- 0.865, AUC change: change in AUC due to adiposity; [1] All Continuous model terms were centered. Odds ratios for continuous terms represent a unit change in covariates.

Table 3-4: Model prediction accuracies, precisions and validation by sex

Models	Prediction Continuous serum triglycerides			Predicting elevated serum triglyceride		
	RMSE(95% CI)	RMSE Optimism	Optimism-corrected RMSE	**AUC(95% CI)**	AUC Optimism	Optimism-corrected AUC
Boys						
Skinfolds	0.458 (0.42 - 0.49)	0.004	0.45	0.89 (0.86- 0.93)	0.002	0.89
DXA	0.459 (0.42 - 0.49)	0.005	0.45	0.88 (0.85 – 0.93)	0.026	0.85
Girls						
Skinfolds	0.403 (0.37 - 0.43)	0.006	0.40	0.89 (0.85- 0.92)	0.001	0.89
DXA	0.416 (0.38 - 0.44)	0.005	0.41	0.87 (0.84 – 0.91)	0.002	0.87

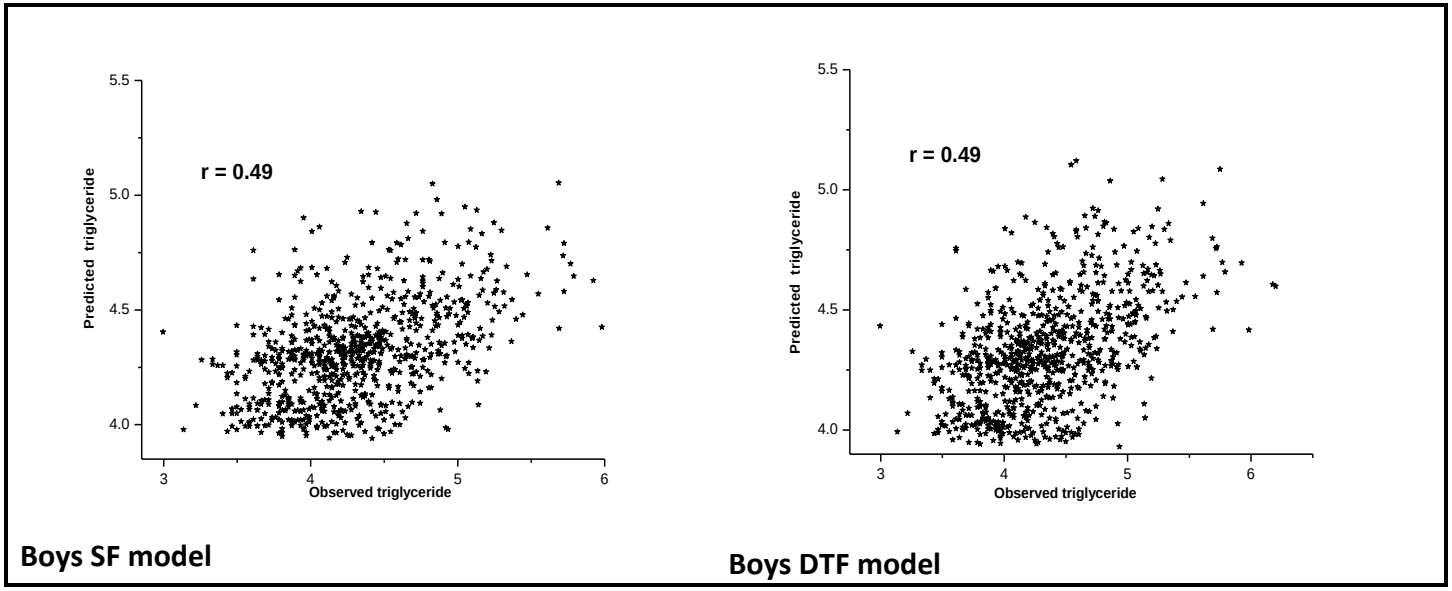
Abbreviations: AUC- Area under the Curve(from ROC). All AUC values and their 95% CIs obtained from 2500 percentile bootstrap estimates.

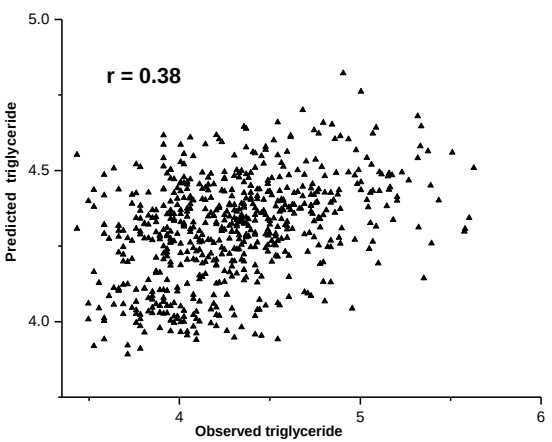
All statistics obtained from Survey regression procedures adjusted for survey complex design effects. Optimism=Bootstrap performance – apparent performance(51).

Table 3 - 5: Independent contribution of subcutaneous adiposity to triglyceride levels

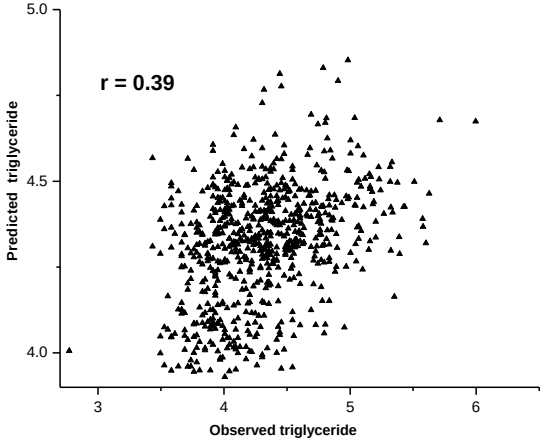
Covariates	**Linear Associations**		**Odds of elevated TG**
Boys	**β (SE)**	**p-value**	**OR (95% CI)**
Age	0.01(0.01)	0.48	2.43 (1.81 – 3.27)
Race	0.01(0.02)	0.59	1.04 (0.72 – 1.50)
DXA Total fat	0.01(0.00)	0.06	1.08(0.98 -1.21)
Triceps skinfold	0.00(0.01)	0.87	0.97 (0.87-1.08)
Subscapular skinfold	0.02(0.01)	0.06	1.03(0.95 – 1.13)
Girls	**β (SE)**	**p-value**	**OR (95% CI)**
Age	-0.01(0.01)	0.54	2.95(2.19 – 4.00)
Race	0.03(0.02)	0.19	1.33 (0.81 – 2.16)
DXA Total fat(kg)	-0.01(0.01)	0.99	1.06 (0.90 -1.24)
Triceps skinfold	-0.01(0.01)	0.42	0.83(0.73- 1.0)
Subscapular skinfold	0.02(0.01)	0.03	1.19(1.07 -1.33)

Figure 3-1: Plot of predicted vs. observed triglyceride (mg/dl) by sex





Girls SF model



Girls DTF model

CHAPTER 4- Manuscript 3 tables and figures

Table 4 - 1: Descriptive Characteristics

Variables	**Total N = 1404** **Mean(SE)**	**Boys N=775** **Mean(SE)**	**Girls N= 629** **Mean(SE)**
Age (years)	15.50 (0.09)	15.54 (0.08)	15.45(0.15)
BMI(Kg/m^2)	22.34 (0.20)	22.50 (0.23)	22.16(0.24)
Subscapular Skinfold (mm)	13.22 (0.24)	12.00 (0.30)	14.62(0.31)
[a] HOMAIR (mmol/L x uU/ml)	2.56 (0.08)	2.62 (0.12)	2.50(0.09)
High HOMA category(>=3.5)	5.34(0.18)	5.69(0.28)	4.87(0.23)
Low HOMA category (<3.5)	1.95(0.03)	1.88(0.05)	2.02(0.05)
Glucose (mmol/l)	5.08(0.02)	5.20(0.03)	4.96(0.03)
Glucose -High HOMA	5.34(0.05)	5.49(0.08)	5.14(0.04)
Glucose - Low HOMA	5.03(0.03)	5.13(0.02)	4.91(0.03)
Insulin (uU/ml)	11.17(0.32)	11.13(0.47)	11.21(0.36)
Insulin - High HOMA	22.45(0.71)	23.33(1.08)	21.27(0.93)
Insulin -Low HOMA	8.68(0.14)	8.18(0.20)	9.23(0.23)
Categorical descriptors	**Total (%)**	**Boys (%)**	**Girls (%)**
Percent elevated HOMA-IR(>3.50 mmol/L x uU/ml)	18.10	19.50	16.50
Overweight- BMI(>=85th percentile [b])	31.24	33.30	29.10
Obese - BMI (>=95th percentile)	17.10	18.36	15.74
>= 85th percentile [c]	27.61	32.10	22.82
>= 95th percentile	9.34	13.00	5.50

[a]: HOMA-IR , glucose and insulin untransformed. US Reference: [b] CDC 2000 BMI Curves; [c] 2010 Subscapular Skinfold reference curves

Table 4 -2: Prevalence, model accuracy, sensitivity and specificity

Age bins	**Elevated HOMA IR** (>3.5) Prevalence		Model Prediction Accuracy(AUC)		**Screening Validity**			
					Boys		Girls	
	Boys (%)	Girls (%)	Boys	Girls	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
12	23.1	22.4	85.0	81.0	0.91	0.60	0.76	0.72
13	18.0	25.4	89.3	76.7	0.74	0.71	0.86	0.37
14	20.9	21.1	84.6	78.6	0.75	0.70	0.88	0.45
15	24.1	14.9	75.0	80.0	0.96	0.16	0.77	0.65
16	17.5	7.9	78.2	82.1	0.84	0.75	0.83	0.40
17	18.1	13.8	78.4	77.1	0.81	0.70	0.75	0.61
18	15.9	8.1	83.2	83.5	0.79	0.71	0.80	0.64

Table 4-3: Optimal percentile cut-off and observed subscapular skinfold cut-points

Age, y	Boys			Girls		
	Crude Optimal Skinfold (mm)	Smoothed Optimal Skinfold(mm)	Smoothed optimal Percentile	Crude Optimal Skinfold(mm)	Smoothed Optimal Skinfold(mm)	Smoothed Optimal Percentile
12.00 – 12.49	10.2	10.2	85	9.1	9.8	64
12.50- 12.99	8.5	10.4	85	11.6	10.2	64
13.00 -13.49	10.7	10.7	85	9.3	10.8	65
13.50- 13.99	15.4	10.6	84	14.6	11.4	66
14.00- 14.49	12.8	10.9	84	11.7	12.4	69
14.50-14.99	10.7	11.2	84	11.2	13.5	72
15.00- 15.49	9.5	11.5	84	11.2	14.7	75
15.50- 15.99	9.1	12.2	85	17.4	16.0	78
16.00- 16.49	14.2	13.0	86	24.3	17.1	80
16.50- 16.99	20.0	14.2	88	26.1	18.0	81
17.00- 17.49	17.1	15.2	89	18.1	18.9	82
17.50- 17.99	17.7	15.8	89	19.8	19.3	82

Optimal percentile selected at median of the standardized percentile scores. Smoother 3RSSH, twice nonparametric compound smoother (105)

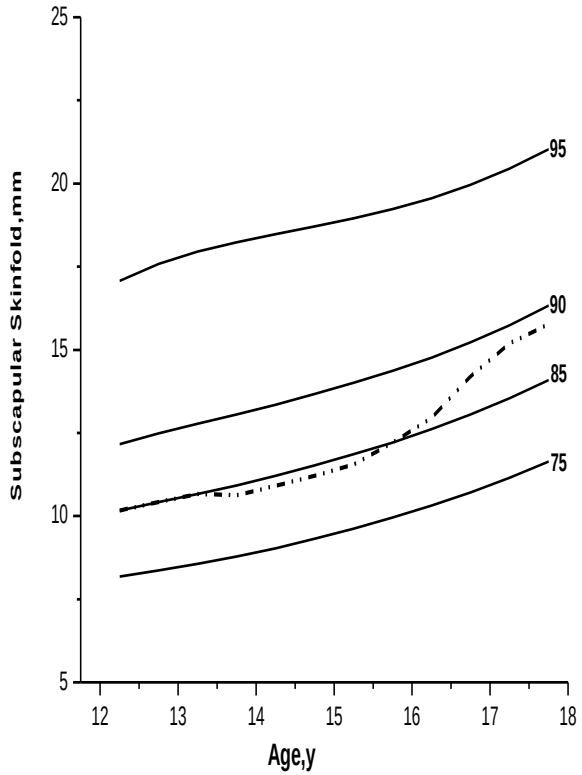
Table 4 - 4: Yield results of optimal percentile cut-offs in study sample

Metric	Kappa (%)	Efficiency (%)	PPV (%)	NPV (%)
Boys	78.4	93.8	69.5	100
Girls	73.1	92.5	63.0	100

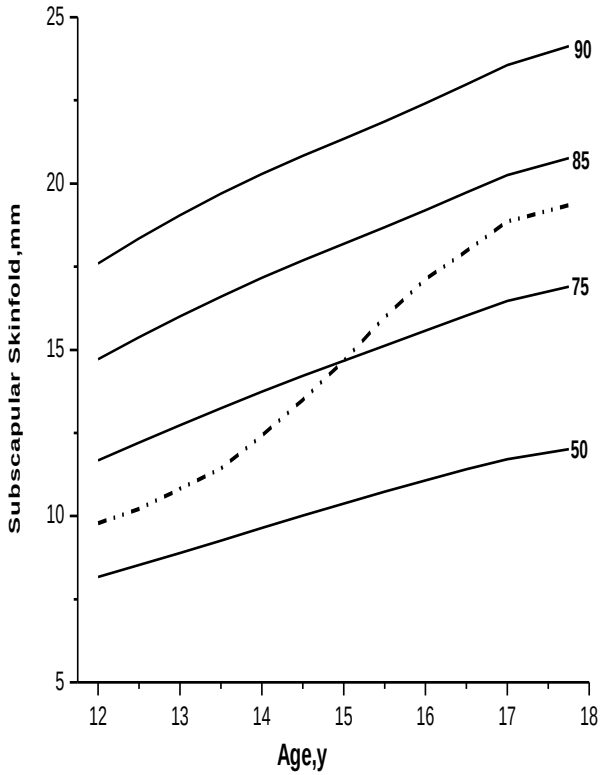
Abbreviations: PPV: Positive predictive value; NPV: Negative predictive value.

Efficiency= True positive+ True Negatives/Grand total. Statistics estimated with procedures adjusted for survey design effects. Cross-classifications tables based on elevated IR status (HOMA >3.5 or <3.50) versus subscapular skinfold above (or not) the optimal percentile cut-off. All calculations are based on the final smoothed subscapular skinfold cut-offs.

Figure 4-1: Subscapular skinfold optimal percentile cut-offs relative to US reference curves for boys and girls



Boys



Girls

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Appendix table 2 - A1: Comparison of model performance of Percent DXA Body Fat and DXA Total fat Weight (Outcome-HOMA)

Model Covariates	Basic model + %DXA BF (RMSE =0.550; Adj.R^2=0.301)		Model 3: Basic + DXA fat (RMSE =0.525; Adj.R^2=0.356)	
	β (SE)	P-value	β (SE)	P-value
Boys				
Age(years)	0.62 (0.29)	0.01	0.62(0.30)	0.04
Height(cm)	0.06 (0.03)	0.01	0.06(0.03)	0.03
Height*Age(interaction)	-0.004 (0.00)	0.01	-0.004(0.00)	0.04
Race	0.00(0.03)	0.97	0.00(0.03)	0.63
%DXA Body Fat	0.043(0.02)	<.0001	-	-
DXA Total fat (kg)	-	-	0.046 (0.00)	<.0001
Girls	(RMSE =0.469; Adj.R^2=0.283)		(RMSE =0.453; Adj.R^2=0.320)	
Age(years)	-0.07(0.02)	<.0001	-0.07(0.02)	0.00
Height(cm)	-0.04(0.01)	<.0001	-0.04(0.01)	0.00
Menarcheal Status (Mstat) (Ref. Non Menarcheal)	5.2(0.76)	<.0001	5.1(1.43)	0.00
Height*Mstat(interaction)	0.03(0.01)	<.0001	0.03(0.01)	0.00
Race	-0.02(0.03)	0.27	-0.03(0.02)	0.42
%Body Fat	0.039(0.01)	<.0001	-	-
DXA Total fat (kg)	-	-	0.032(0.00)	<.0001

Appendix table 2 – A 2: 2X2 tables; basis of statistics of chapter 2 table 2-4

All Statistics have been adjusted for complex survey design effects

Boys		**Top quintile observed logHOMA**	
		Below (%)	Above (%)
Top quintile SF model Predicted logHOMA	Below (%)	71.49	8.54
	Above (%)	8.56	11.42
Top quintile DTF model Predicted logHOMA	Below (%)	71.00	9.02
	Above (%)	8.92	11.06
		Top quintile DTF model Predicted logHOMA	
		Below (%)	Above (%)
Top quintile SF model Predicted logHOMA	Below (%)	76.33	3.71
	Above (%)	3.59	16.37

Girls		**Top quintile observed logHOMA**	
		Below (%)	Above (%)
Top quintile SF model Predicted logHOMA	Below (%)	70.00	9.91
	Above (%)	9.84	10.24
Top quintile DTF model Predicted logHOMA	Below (%)	69.29	10.62
	Above (%)	10.87	9.21
		Top quintile DTF model Predicted logHOMA	
		Below (%)	Above (%)
Top quintile SF model Predicted logHOMA	Below (%)	76.05	3.80
	Above (%)	4.11	16.04

Appendix table 3-A3: Comparison of model performance of Percent DXA Body Fat and DXA Total fat Weight

(Outcome- Serum triglyceride)

Model Covariates	%DXA BF Model (RMSE=0.459, Adj.R^2=0.166)		DXA fat Model (RMSE=0.459, Adj.R^2=0.173)	
	β (SE)	P-value	β (SE)	P-value
Boys				
[1]Age, y	0.04(0.01)	0.002	0.01(0.01)	0.26
Age^2	0.01(0.01)	0.26	0.01(0.01)	0.05
Race – NH White (referent)	-	-	-	-
NH Black	-0.26(0.05)	<0.001	-0.27(0.05)	<0.001
Mexican Americans	-0.10(0.05)	0.03	-0.07(0.04)	0.12
Other Hispanics	0.00(0.09)	0.99	0.02(0.07)	0.76
Other Race-incl. multi racial	0.02(0.09)	0.78	0.00(0. 09)	0.99
%DXA BF	0.02(0.00)	<0.001	-	
DXA Total fat (kg)	-	-	0.02(0.00)	<0.001
Model Covariates	**%DXA BF Model (RMSE=0.409;Adj. R^2=0.105)**		**DXA fat Model (RMSE=0.415, Adj.R^2=0.104)**	
Girls				
[1]Age, y	-0.00(0.01)	0.920	-0.01(0.01)	0.52
Age^2	0.02(0.01)	0.003	0.02(0.01)	0.01
Menarcheal status	0.10(0. 90)	0.27	0.09(0.10)	0.37
Race – NH White (referent)	-	-	-	-
NH Black	-0.27(0.06)	<0.001	-0.30(0.05)	<0.001
Mexican Americans	-0.07(0.05)	0.15	-0.03(0.05)	0.50
Other Hispanics	-0.12(0.05)	0.04	-0.10(0.05)	0.06
Other Race-incl. multi racial	0.06(0.08)	0.45	-0.06(0.08)	0.49
%DXA BF	0.012(0.01)	0.001		
DXA Total fat (kg)	-	-	0.01(0.00)	0.00