Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 55 (2006) 128–134

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Cardiovascular/non–insulin-dependent diabetes mellitus risk factors and intramyocellular lipid in healthy subjects: a sex comparison

Lesley J. White[a,*,1], Michael A. Ferguson[a,b,1], Sean C. McCoy[a,1], Hee-Won Kim[c,d,1], Vanessa Castellano[a,1]

[a]*Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, FL 32611, USA*
[b]*Clinical and Strategic Development, Medtronic Sofamor Danek, Memphis, TN 38132, USA*
[c]*Department of Radiology, University of Florida, Gainesville, FL 32611, USA*
[d]*Department of Radiology, VA Medical Center, Gainesville, FL 32611, USA*

Received 23 September 2004; accepted 5 August 2005

Abstract

Little is known about the relationship between intramyocellular lipid (IMCL) and coronary artery disease (CAD)/non–insulin-dependent diabetes mellitus risk factors. The aim of the study was to examine the relationship between IMCL and CAD/non–insulin-dependent diabetes mellitus risk factors in healthy male (n = 9) and female (n = 10) subjects with similar norm-based aerobic fitness and body composition. Nineteen volunteers (21-36 years) completed health and physical activity questionnaires, cardiovascular and body composition evaluation, and assessment of thigh IMCL using proton magnetic resonance spectroscopy. Outcome measures were blood pressure, total cholesterol, high-density lipoprotein cholesterol, C-reactive protein, interleukin 6, tumor necrosis factor α (TNF-α), homocysteine, insulin resistance (IR), percentage of body fat, waist-to-hip ratio, physical activity levels, and cardiovascular fitness. Analysis of variance was used for group comparisons. Correlation analyses were used to determine association between variables, and stepwise regression was used to determine predictive variables of IR. Women had 2-fold higher IMCL and greater IR than men ($P < .05$). Men had greater plasma homocysteine and interleukin 6 concentration ($P < .05$) as well as stronger correlations with CAD risk factors than female subjects. Correlation analyses using all subjects revealed a significant relationship between IMCL and waist-to-hip ratio, body weight, percentage of body fat, and IR. In the regression analysis, age, IMCL, and TNF-α were the strongest predictors of IR ($R^2 = 0.62$, $P < .01$). Our results suggest that female subjects, matched for age and fitness, have higher IMCL compared with their male counterparts. IMCL was correlated with several CAD and prediabetes markers in both male and female subjects. In the final regression model, age, IMCL, and TNF-α were the strongest predictors of IR. Future studies with larger sample sizes are warranted.

1. Introduction

Skeletal muscle has 2 lipid compartments, intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL) [1,2]. Although these storage depots consist primarily of triacylglycerol (two thirds of total fatty acids in IMCL consist of 3 fatty acids [18:0, 18:1, and 16:0]) [1], there are functional differences between the 2 compartments. IMCL functions as a storage site for fuel used at rest and during physical activity [3], whereas EMCL has a longer half-life and serves as a long-term storage depository [3]. With non–insulin-dependent diabetes mellitus (NIDDM), increased concentration of IMCL is associated with increased disease risk [4,5].

The mechanism(s) whereby elevated IMCL concentration contributes to increased disease risk remains unclear. However, the work by Shulman et al [6] suggested that increased fatty acids in skeletal muscle interferes with insulin cell signaling and increases insulin resistance (IR), potentially leading to NIDDM and increased risk for coronary artery disease (CAD). The relationship between IMCL and IR has been reported by others [4,5]. However, the relationship between IMCL and CAD risk factors

* Corresponding author. Tel.: +1 352 392 9575x1338; fax: +1 352 392 0316.
E-mail address: lwhite@hhp.ufl.edu (L.J. White).
[1] LW and MF contributed in the manuscript preparation and experimental design; SM collected and analyzed the data and contributed in the manuscript preparation; HK collected the MR data; and VC analyzed the data and contributed in the manuscript preparation.

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.08.026

remains unclear. Given the high prevalence of both heart disease and contributing risk factors such as obesity and diabetes, early CAD detection and intervention could influence morbidity and mortality. Therefore, the primary purpose of this study was to examine the relationship between IMCL and CAD/NIDDM risk factors in healthy active men and women. A second purpose was to provide preliminary data in healthy male and female subjects for future comparative studies examining IMCL and CAD/NIDDM risk. We hypothesized that there would be a positive relationship between IMCL content in the vastus lateralis and CAD/NIDDM risk factors.

2. Materials and methods

2.1. Subjects

Nineteen healthy men (n = 10) and women (n = 9) 21 to 36 years volunteered as study participants. Subjects were part of a larger study designed to compare between sexes their IMCL use during aerobic exercise [7]. Study inclusion criteria were (1) no family history of NIDDM, (2) current participant in an aerobic exercise program for the previous 6 months (3-5 times per week, 30-60 minutes per session), (3) lack of tobacco use, (4) normal healthy levels of body fat (men, 10%-20%; women, 15%-25%), and (5) dietary intake patterns similar to American Heart Association guidelines [8]. A 2-day food log was used to screen subjects for nutrition inclusion criteria (ie, 50%-60% carbohydrate, <30% fat, 10%-15% protein). Subjects taking lipid-altering medications or women using contraceptives were excluded. Menstrual cycle histories were assessed with questionnaires to ensure female subjects were eumenorrheic. Female subjects were tested during the early to mid follicular phase of their menstrual cycle to minimize the influence of estrogens on blood measures. All subjects signed an informed consent approved by the university's institutional review board before study entry.

2.2. Study design

Research participants made 2 laboratory visits during the study. During visit 1, subjects completed questionnaires (health risk, physical activity, etc) and were subsequently tested for body composition and cardiorespiratory fitness level (maximum oxygen consumption, or $\dot{V}O_2$max). During the second visit, 1 week later, resting blood samples were acquired and skeletal muscle IMCL content was assessed using proton magnetic resonance spectroscopy (^{1}H-MRS). Subjects reported for visit 2 in a fasted state (10 hours) and had refrained from physical activity for the previous 48 hours.

2.3. Anthropometric measures

A calibrated physician's scale was used to assess body weight and height. Body mass index (BMI) was calculated by dividing body weight (kg) by height (m^2). Body density was estimated using the 3-site skinfold method of Jackson and Pollock [9]. Percentage of body fat was estimated using the formula of Brozek et al [10].

2.4. Fitness assessment

Subject's physical activity level was assessed with the Framingham questionnaire [11]. The first and fifth Korotkoff sounds were used for blood pressure estimates after 5 minutes of rest [12]. Maximum oxygen consumption was determined using a modified Åstrand cycle ergometer protocol [13] that began at 50 W (50 rpm) and was increased by 25 W every minute until exhaustion. This test was designed to elicit $\dot{V}O_2$max in 8 to 12 minutes. Criteria used for $\dot{V}O_2$max was one or more of the following: (1) subject exhaustion; (2) a less than 2 mL/kg increase in oxygen consumption with an increase in work rate; and (3) a respiratory exchange ratio of 1.10 or higher [14]. Respiratory gas variables were measured continuously using a calibrated Truemax 2400 metabolic cart (Parvomedics, Salt Lake City, UT). Heart rate (Polar, Woodbury, NY) and blood pressure were monitored throughout exercise testing.

2.5. Magnetic resonance procedures

^{1}H-MRS was performed using a 3.0-T whole-body scanner (SIGNA-VH2, General Electric, Milwaukee, MI). Localized ^{1}H spectra were obtained using a quadrature-driven birdcage knee coil in the transceiver mode. Subjects entered the magnet, feet first, while in a supine position. The anatomical location selected as the region of interest was one third the distance from the superior patella and iliac crest as previously reported [7]. The extremity coil was placed at midsection level of the vastus lateralis of the subject's dominant leg. Axial scout images were obtained using either a T_1-weighted spin echo (repetition time [TR], 500 milliseconds; echo time [TE], 17 milliseconds) or a T_1-weighted gradient echo (TR, 500 milliseconds; TE, 15 milliseconds) sequence. The voxel of 1.5 × 1.5 × 1.5 cm^3 was selected at the middle of the vastus lateralis. The magnetic field was homogenized on the water signal from the same voxel. The water signal was suppressed by 3 consecutive chemical shift selective pulses [15]. The water-suppressed ^{1}H signal was then obtained from the voxel using PROBE-SV PRESS (General Electric Medical System) with TE of 25 milliseconds, TR of 2 seconds, average of 128, and 2048 data points collected [16]. The spectral raw data were apodized by 1.25 Hz line broadening and zero filled after 20-Hz high-pass gaussian convolution filtering. The proton spectra were reconstructed by Fourier transformation and the zero/first-order phase correction. Each moiety contents were estimated using a Lorentzian fit in the frequency domain. IMCL was identified as the peak area at approximately 1.3 ppm $(CH_2)_n$, and EMCL at approximately 1.5 ppm $(CH_2)_n$. IMCL content was quantified using the peak area expressed in arbitrary units (AU) as well as the ratio of IMCL to muscle water content (AU/water). The coefficient of variation between repeat scans

obtained from the same leg was 6% for IMCL (AU) using the methods in this study.

Several strategies have been used to quantify IMCL. For example, creatine [5], water [2,17], and bone marrow lipid [18,19] have been used as an internal reference for reporting IMCL. Peak area, expressed in arbitrary units, has also been used [18,20]. We report our primary study findings using IMCL (AU) and additionally as IMCL (AU/water).

2.6. Blood sample analysis

Blood samples were acquired from each subject via venipuncture into EDTA tubes after a 10-hour fast. To minimize day-to-day variability in blood parameters, samples were acquired at the same time of day for each subject. Samples were inverted gently and briefly stored in a cold refrigerator until centrifugation at 4°C for 10 minutes at 1500*g*. Plasma was stored at −70°C. Analyses of plasma were completed within 6 months of collection. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and plasma glucose were determined by enzymatic colorimetric methods (Wako Chemicals USA, Richmond, VA; Eagle Diagnostics, De Soto, TX). The TC/HDL-C ratio was calculated. Plasma insulin was measured with a radioimmunoassay procedure based on the methods of Wide and Porath [21]. Sex comparisons and group means for glucose have been presented previously [22]; however, the data have been used to estimate IR. The homeostasis model assessment of IR (HOMA-IR) was determined by the formula of Matthews et al [23]. This procedure uses fasting glucose and insulin in an equation (insulin × glucose/22.5) to estimate IR. The HOMA-IR has been validated in normoglycemic subjects against IR measured directly from the euglycemic-hyperinsulinemic clamp technique [23].

C-reactive protein (CRP), homocysteine, interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) were determined by enzyme-linked immunosorbent assay techniques (Anogen, Mississauga, Ontario, Canada; Axis Biochemicals, Oslo, Norway; Linco, St. Louis, MO). Data for TNF-α have been reported previously [22], but were used in this study as a variable in the correlation analysis. All assays were performed in either duplicate or triplicate and in a single run. The average within variability for plasma variables were as follows: TC, 6.5%; HDL, 9.1%; glucose, 8.2%; insulin, 6.3%; CRP, 4.0%; homocysteine, 11.8%; and IL-6, 4.8%.

2.7. Statistical analysis

One-way analysis of variance was used to evaluate sex differences for each dependent variable. Pearson product moment correlations (R) were used as a measure of univariate association between IMCL and predictor variables (CAD/NIDDM risk factors). Multiple linear regression with stepwise variable selection in blocks was used to evaluate the associations between the variables in each block and IR. Besides demographic variables, only variables with a correlation of at least 0.40 with IR were included in the initial model. Block 1 variables included the demographic measures, age, and sex. Block 2 contained IMCL and block 3 contained CAD/NIDDM risk factors (TNF-α and physical activity levels). Significance levels for entry and exclusion from the model were set at $P = .15$. Any

Table 1
Baseline anthropometric, IMCL, risk factor, and subject descriptive statistics

	Men (n = 10)	Women (n = 9)	All subjects (n = 19)
Age (y)	27.4 ± 4.6	27.2 ± 6.9	27.3 ± 5.6
Height (cm)	174.7 ± 5.5	169.3 ± 7.1	172.2 ± 6.7
Weight (kg)	79.4 ± 8.4**	65.5 ± 9.1	72.8 ± 11.1
BF (%)	12.9 ± 2.2	20.0 ± 3.4††	16.3 ± 4.6
BMI	26.0 ± 2.0*	22.8 ± 3.3	24.5 ± 3.1
IMCL (AU)	$8.7 \times 10^6 \pm 5 \times 10^6$	$2.0 \times 10^7 \pm 1.1 \times 10^{6\dagger}$	$1.4 \times 10^7 \pm 1.0 \times 10^6$
IMCL (AU/water)	$1.0 \times 10^{-1} \pm 0.07$	$1.1 \times 10^{-1} \pm 0.04$	$1.0 \times 10^{-1} \pm 0.00$
W/H ratio	0.82 ± 0.0**	0.77 ± 0.0	0.79 ± 0.0
$\dot{V}O_2$max (mL/[kg · min])	44.9 ± 4.7	41.5 ± 8.9	43.3 ± 7.0
CAD/NIDDM risk factors			
Systolic blood pressure (mm Hg)	122 ± 12	111 ± 12	117 ± 13
Diastolic blood pressure (mm Hg)	81 ± 10	77 ± 10	79 ± 10
Physical activity score	37.5 ± 10.9	41.0 ± 8.6	39.1 ± 9.8
HOMA-IR	0.54 ± 0.3	0.90 ± 1.1††	0.79 ± 0.78
TC (mg/dL)	151 ± 31	172 ± 38	163 ± 36
HDL-C (mg/dL)	42.0 ± 2.5	41.2 ± 3.4	41.3 ± 3.5
TC/HDL ratio	2.7 ± 0.6	3.1 ± 0.7	3.0 ± 0.7
Homocysteine (mg/dL)	11.8 ± 4.1*	7.8 ± 1.7	10.2 ± 3.8
CRP (mg/L)	2.9 ± 1.1	2.2 ± 0.3	2.5 ± 0.8
IL-6 (pg/mL)	123.0 ± 114.9*	69.3 ± 60.9	96.1 ± 93.0

Values are expressed as mean ± SD. BF% indicates body fat percentage.

* $P < .05$, significantly greater than women.

† $P < .05$, women significantly greater than men.

** $P < .01$, significantly greater than women.

†† $P < .01$, significantly greater than men.

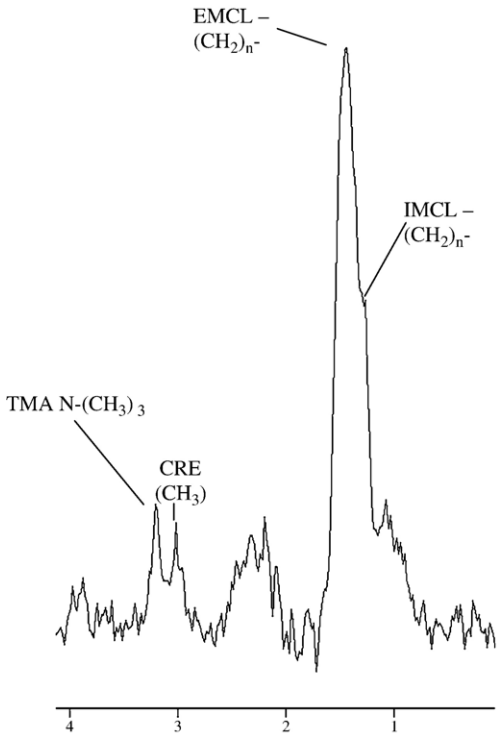

Fig. 1. Baseline ^{1}H-MRS spectral characteristics acquired from a healthy male subject's vastus lateralis. TMA indicates trimethylamine-containing compounds; CRE, creatine and/or phosphocreatine.

variable included in an earlier block was included in the model for all later blocks and in the final model. An α level of .05 was used as the criterion of statistical significance. Statistical programming was completed with SPSS software (version 11.0; SPSS, Chicago, IL).

3. Results

Subject characteristics including anthropometric measures are presented in Table 1. Male and female subjects were similar in age, cardiorespiratory fitness (milliliter per kilogram of fat free mass) ($P > .05$), and percentage of body fat based on sex normative values. Male subjects were however heavier and had higher BMI and waist-to-hip (W/H) ratios ($P < .05$) compared with female subjects ($P < .05$). Fig. 1 illustrates a spectrum showing IMCL from a typical male subject in our study. Although the data were presented elsewhere [7], nutrient intake at study entry (percentage of fat, percentage of protein, percentage of carbohydrates) was similar between male and female subjects ($P > .05$). Total energy intake was greater for male subjects; however, when expressed relative to each subject's weight in kilograms of fat free mass, no differences between groups were observed ($P > .05$).

Evaluation of blood indexes for cardiovascular risk revealed that male subjects had greater resting plasma concentrations of homocysteine and IL-6 compared with their female counterparts ($P < .05$). For other risk factors for CAD/NIDDM (TC, HDL, blood pressure, and body fat), there were no sex differences.

The relationships among IMCL, body composition variables, and CAD risk factors for men only, women only, and collectively (men and women) are presented in Table 2. When examining data from all subjects, we found that IMCL (AU) was negatively correlated with body weight ($r = -0.66$, $P < .01$) and W/H ratio ($r = -0.52$, $P < .05$) and positively correlated with the percentage of fat ($r = 0.52$, $P < .05$) and IR ($r = 0.56$, $P < .05$). CRP was negatively correlated with HDL-C when group data were collapsed ($r = -0.52$, $P < .05$). For women only, IMCL (AU) was positively correlated with glucose, insulin, and IR ($r = 0.64$, 0.51, and 0.61, respectively; $P < .05$). For male subjects, IMCL (AU) was negatively correlated with weight ($r = -0.59$, $P < .05$) and positively with insulin, IR, and IL-6 ($r = 0.53$, 0.50, and 0.85, respectively; $P < .05$).

IMCL was correlated with CAD risk factors after normalizing IMCL to muscle water content (AU/water) in the same voxel to further examine the relationship between IMCL and CAD risk factors. The only correlation between IMCL (AU/water) and CAD risk factors during this analysis was with homocysteine ($r = 0.49$, $P < .05$). For female subjects, there was a negative correlation between IMCL (AU/water) for W/H ratio ($r = -0.47$, $P < .05$) and positive correlations with glucose and IR ($r = 0.57$ and 0.52,

Table 2
Correlations among IMCL, CAD, and NIDDM risk factors in men, women, and subject groups collapsed

Variable	Wt	BF%	W/H	BMI	SBP	DBP	PA	Glu	Ins	IR
IMCL (collapsed)	−0.66**	0.52*	−0.52*	−0.40	−0.30	−0.15	−0.04	0.30	0.45	0.56*
IMCL (M)	−0.59*	0.18	−0.36	−0.27	−0.22	−0.09	−0.22	−0.18	0.53*	0.50*
IMCL (F)	−0.35	0.09	0.33	−0.02	−0.31	−0.19	−0.11	0.64**	0.51*	0.61**

Variable	TC	HDL	TC/HDL ratio	Homocy	CRP	$\dot{V}O_2$max	IL−6	TNF−α
IMCL (collapsed)	0.27	0.41	0.27	−0.14	−0.36	−0.25	0.15	0.23
IMCL (M)	−0.43	0.14	−0.43	0.31	−0.37	−0.15	0.85**	0.26
IMCL (F)	0.35	0.35	0.35	−0.16	0.07	−0.19	0.05	−0.04

Values are expressed as mean ± SD. M indicates male; F, female; Wt, weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; PA, physical activity; Glu, glucose; Ins, insulin; Homocy, homocysteine.

* $P < .05$.

** $P < .01$.

respectively; $P < .05$). For men, there was a positive correlation between IMCL (AU/water) and IR, homocysteine, and TNF-α ($r = 0.53$, 0.83, and 0.58, respectively; $P < .05$).

Regression analysis using all study subjects indicated that age, IMCL (AU), and TNF-α were predictor variables explaining significant proportions of the variance of IR ($R^2 = 0.62$, $P < .01$). The final model for IR was IR = 1.22 + −0.026953 (age) + 0.00000005 (IMCL) + −0.015 (TNF-α).

4. Discussion

This study examined the relationship between IMCL and cardiovascular/NIDDM risk factors in adult male and female subjects. The major findings of this study were the significant differences in IMCL (AU) between males and female subjects, and that age, IMCL (AU), and TNF-α were the strongest predictors of IR in the final regression model.

Male and female subjects in this study were relatively matched for age and cardiovascular fitness (Table 1). In addition, both groups exercised 3 to 5 d/wk, had similar physical activity levels, as assessed by the Framingham Questionnaire, and had similar nutrient intake patterns. Although body composition was matched, based on sex normative values [24], the female subjects had significantly more body fat than male subjects. Significant sex differences were also observed for W/H ratio, BMI, and body weight. A significant study finding was that women had twice as much IMCL (AU) as men. This finding has been previously reported [19]; however, the significance remains unclear. Data from previous reports suggest that IMCL content is positively associated with aerobic fitness [25,26], whereas other studies ascribe greater IMCL with increased disease indication [18]. In our study, both male and female subjects were comparable in aerobic fitness and were apparently disease-free; however, female subjects had twice the total IMCL area as male subjects, suggesting a possible sex difference in IMCL content. IMCL serves as a reservoir of stored lipid (triacylglycerol etc) for energy metabolism [3]; thus, greater depots of IMCL in female subjects may indicate greater substrate supply and/or perhaps progression toward a metabolic state whereby normal lipid and glucose metabolism are altered.

Other CAD/NIDDM risk factors examined in our study revealed no sex differences for blood pressure, TC, HDL-C, the TC/HDL-C ratio, or CRP. HDL-C was negatively correlated with CRP concentrations when data from men and women were combined ($r = -0.52$, $P < .05$). In contrast, differences in IR, homocysteine, and IL-6 between sexes were observed. The greater IR observed for female subjects in our study is consistent with recent findings suggesting that IMCL is negatively correlated with insulin sensitivity [4,27]. However, few studies have made sex comparisons for IR when taking into account difference in IMCL between men and women. Perseghin et al [28] did not observe the differences in insulin sensitivity when comparing a group of sedentary healthy young men and women (20-40 years) with normal body fatness for their age, but did find that poor insulin sensitivity was related to increased IMCL concentration. Virkamaki et al [5] found that IMCL accumulation was associated with whole-body IR and with defective insulin signaling in skeletal muscle, independent of body weight and physical fitness. High IMCL in the vastus lateralis was associated with increased whole-body insulin in men. IR is a primary feature in the pathogenesis of NIDDM, and it may precede the clinical onset of hyperglycemia by 10 to 20 years [6].

The mechanism explaining how IMCL accumulation decreases insulin sensitivity remains speculative. Shulman et al [6] suggest that in the metabolic process of increasing depots of IMCL, increased fatty acid transport and perturbations occur within skeletal muscle, which lead to a desynthezation of the insulin receptor (through protein kinase C), which results in a reduction of insulin sensitivity and greater IR. In addition, Virkamaki et al [5] found that increased levels of IMCL blunted whole-body glucose uptake, insulin stimulation of tyrosine phosphorylation of the insulin receptor, and insulin receptor substrate 1–associated phosphatidylinositol 3-kinase activity in healthy with no family history of NIDDM. Further study of mechanisms underlying reduced insulin sensitivity in skeletal is warranted.

Homocysteine is thought to be an early marker of future CAD risk [29]. In our study, male subjects had higher homocysteine concentrations than female subjects. Male subjects also had a stronger correlation between IMCL (AU/water) and homocysteine than female subjects. Sex differences in plasma homocysteine concentration have been previously reported [30]. Andersson et al [30] observed that differences in plasma concentration of homocysteine were correlated with differences in blood folate concentration. In our study, dietary folate intake was similar between groups ($P > .05$). Dietary folate intake may not be a sensitive marker of plasma concentration, or perhaps other mechanisms are responsible for differences in plasma homocysteine concentration in men and women. Further study is needed to determine the significance of homocysteine to evaluate the predictability of cardiac events in this relatively young subject population.

IL-6 was elevated in male subjects, nearly 2-fold, when compared with female subjects. IL-6 has been used as an independent predictor of vascular events [31] and has been positively correlated with myocardial infarction coronary death [32]. Limited information is available comparing resting IL-6 concentration in relatively matched male and female subjects. Some data suggest that resting IL-6 concentration increases with age in men, whereas no sex differences are typically observed in younger subjects [33]. Our findings of sex differences in IL-6 are in contrast to some reports [33]. Nonetheless, this finding should be interpreted cautiously given our small sample size.

Associations between IMCL and various CAD biomarkers were also examined in this study. Our data are

presented for women and men only and for a total group. The most significant finding of the group data was the positive association between IMCL (regardless of AU or AU/water) and IR. Our data suggested that increased IR was positively correlated with IMCL concentration. This type of correlation has been reported elsewhere in single-sex studies [4,5,18], but to our knowledge there have been no sex comparison studies. When comparing men and women, female subjects had more association between IMCL and IR (r = 0.61) compared with male subjects (r = 0.50). This difference was normalized when IMCL was expressed relative to muscle water content. Although our measure of IR is an estimate, this result is important, given its magnitude and because of its possible role for early detection of NIDDM. In addition to the correlation with estimated IR, IMCL (AU) was also positively correlated with the percentage of body fat. Sinha and colleagues [27] found that increases in total body fat were accompanied by higher IMCL in adolescents. IMCL represents a portion of stored lipid; thus, increasing fatness may be associated with greater concentration of IMCL [5]. However, it is possible that IMCL is less related to central adiposity than to total body weight or overall adiposity. Additional research in this area is warranted to clarify the relationship between subcutaneous adipose tissue and IMCL levels.

In conclusion, our results suggest that female subjects, matched for age and fitness, have higher IMCL (AU) compared with their male counterparts. When examining data from all subjects, IMCL (AU) was correlated with levels of body fatness and IR. IMCL (AU or AU/water) was correlated with several CAD and prediabetes markers in both male (insulin, IR, IL-6, homocysteine) and female subjects (glucose, insulin, IR) depending on how IMCL was expressed. In the final regression model, age, IMCL (AU), and TNF-α were the strongest predictors of IR. Studies designed to elucidate the cellular mechanisms associated with the accumulation/loss of IMCL and how changes in this lipid pool impact skeletal muscle metabolism may have important implications in the treatment of metabolic disorders such as NIDDM.

Acknowledgement

Funding support was provided by a University of Florida Faculty Opportunity Grant (LJW).

References

[1] Szczepaniak LS, et al. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am J Physiol 1999;276(5 Pt 1): E977-89.
[2] Boesch C, et al. In vivo determination of intra-myocellular lipids in human muscle by means of localized ^{1}H-MR-spectroscopy. Magn Reson Med 1997;37:484-93.
[3] Bessesen DH, Rupp CL, Eckel RH. Trafficking of dietary fat in lean rats. Obes Res 1995;3:191-203.
[4] Jacob S, et al. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. Diabetes 1999;48:1113-9.
[5] Virkamaki A, et al. Intramyocellular lipid is associated with resistance to in vivo insulin actions on glucose uptake, antilipolysis, and early insulin signaling pathways in human skeletal muscle. Diabetes 2001; 50:2337-43.
[6] Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest 2000;106:171-6.
[7] White LJ, et al. Intramyocellular lipid changes in men and women during aerobic exercise: a (1)H-magnetic resonance spectroscopy study. J Clin Endocrinol Metab 2003;88:5638-43.
[8] Lauber RP, Sheard NF. The American Heart Association Dietary Guidelines for 2000: a summary report. Nutr Rev 2001;59: 298-306.
[9] Jackson AS, Pollock ML. Generalized equations for predicting body density of men. Br J Nutr 1978;40:497-504.
[10] Brozek J, et al. Densitometric analysis of body composition: revision of some quantitative assumptions. Ann N Y Acad Sci 1963; 110:113-40.
[11] Kannel WB, Sorlie P. Some health benefits of physical activity. The Framingham Study. Arch Intern Med 1979;139:857-61.
[12] Joint National Committee of Prevention. The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Arch Intern Med 1997;157: 2413-45.
[13] Astrand P, Rodahl K. Textbook of work physiology. New York: McGraw-Hill; 1986.
[14] Taylor HL, Buskirk E, Henschel A. Maximal oxygen intake as an objective measure of cardio-respiratory performance. J Appl Physiol 1955;8:73-80.
[15] Galloway GJ, et al. A comparison of some gradient-encoded volume-selection techniques for in vivo NMR spectroscopy. Magn Reson Med 1987;4:393-8.
[16] Bottomley P. Selective volume method for performing localized NMR spectroscopy 1984.
[17] Weiss R, et al. Low adiponectin levels in adolescent obesity: a marker of increased intramyocellular lipid accumulation. J Clin Endocrinol Metab 2003;88:2014-8.
[18] Thamer C, et al. Intramyocellular lipids: anthropometric determinants and relationships with maximal aerobic capacity and insulin sensitivity. J Clin Endocrinol Metab 2003;88:1785-91.
[19] Larson-Meyer DE, Newcomer BR, Hunter GR. Influence of endurance running and recovery diet on intramyocellular lipid content in women: a ^{1}H NMR study. Am J Physiol Endocrinol Metab 2002; 282:E95-E106.
[20] Perseghin G, et al. Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. Am J Physiol Endocrinol Metab 2003;285:E1174-81.
[21] Wide L, Porath J. Radioimmunoassay of proteins with the use of Sephadex-coupled antibodies. Biochem Biophys Acta 1966;130: 257-60.
[22] Ferguson MA, et al. Plasma adiponectin response to acute exercise in healthy subjects. Eur J Appl Physiol 2004;91:324-9.
[23] Matthews DR, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[24] Lohman TG. Skinfolds and body density and their relation to body fatness: a review. Hum Biol 1981;53:181-225.
[25] Hurley BF, et al. Muscle triglyceride utilization during exercise: effect of training. J Appl Physiol 1986;60:562-7.
[26] Schrauwen-Hinderling VB, et al. The increase in intramyocellular lipid content is a very early response to training. J Clin Endocrinol Metab 2003;88:1610-6.
[27] Sinha R, et al. Assessment of skeletal muscle triglyceride content by (1)H nuclear magnetic resonance spectroscopy in lean and obese

adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. Diabetes 2002;51:1022-7.

[28] Perseghin G, et al. Gender factors affect fatty acids-induced insulin resistance in nonobese humans: effects of oral steroidal contraception. J Clin Endocrinol Metab 2001;86:3188-96.

[29] Ridker PM, et al. Plasma homocysteine concentration, statin therapy, and the risk of first acute coronary events. Circulation 2002;105: 1776-9.

[30] Andersson A, et al. Plasma homocysteine before and after methionine loading with regard to age, gender, and menopausal status. Eur J Clin Invest 1992;22:79-87.

[31] Pradhan AD, et al. Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. JAMA 2002;288: 980-7.

[32] Luc G, et al. C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME Study. Arterioscler Thromb Vasc Biol 2003;23:1255-61.

[33] Young DG, et al. The influence of age and gender on serum dehydroepiandrosterone sulphate (DHEA-S), IL-6, IL-6 soluble receptor (IL-6 sR) and transforming growth factor beta 1 (TGF-beta1) levels in normal healthy blood donors. Clin Exp Immunol 1999;117:476-81.