Journal of Musculoskeletal and Neuronal Interactions JMNI

Original Article

Lower insulin sensitivity is related to lower relative muscle cross-sectional area, lower muscle density and lower handgrip force in young and middle aged non-diabetic men

T. Gysel[1], C. Tonoli[1], S. Pardaens[1], D. Cambier[1], J-M. Kaufman[2], H-G. Zmierczak[3], S. Goemaere[3], B. Lapauw[2], P. Calders[1]

[1]Department of Rehabilitation Sciences and Physiotherapy, Ghent University, De Pintelaan 185, 1B3, Ghent, Belgium; [2]Department of Endocrinology, Ghent University Hospital, De Pintelaan 185, 9K12 Ghent, Belgium; [3]Unit for Osteoporosis and Metabolic Bone Diseases, Ghent University Hospital, De Pintelaan 185, 9K12 Ghent, Belgium

Abstract

Objectives: This study investigated whether an association between insulin resistance (IR) and muscle parameters is appreciable in young healthy men, independent of obesity. Furthermore, markers of muscle metabolism and hormones/possible determinants, were explored. **Methods:** 358 healthy young men were divided into a less and more insulin sensitive (LIS [age=33.2±5.4, BMI=23.4±2.3] and MIS [age=35.5±5.3, BMI=28.1±3.7]) group based on upper and lower quartile of HOMA-IR. Muscle cross-sectional area (CSA), -density, handgrip force, serum testosterone, estradiol, SHBG, Vitamin 25(OH)D, creatinine, IGF-1, IGFBP-3 and leptin levels were compared between these groups, correcting for differences in age, physical activity and fat mass. Correlations between HOMA-IR and these parameters, and between muscle measures and biochemical parameters, were calculated. **Results:** LIS is related to lower relative muscle CSA, muscle density, muscle/fat CSA ratio, relative handgrip force and level of physical activity. Furthermore, lower levels in SHBG, testosterone, Vitamin 25(OH)D and higher leptin, IGF-1 & IGFBP-3 levels were observed in LIS. Bio available T, FT, TE2, FE2, bioavailable E2, serum and urinary creatinine levels did not differ between groups. **Conclusion:** Differences in muscle performance are already present in healthy men with lower insulin sensitivity and could be possibly modifiable risk factors for the development of type 2 diabetes.

Keywords: Insulin Resistance, Muscle Cross-sectional Area, Handgrip Strength, Estradiol

Introduction

Type 2 diabetes mellitus (T2DM) affects several organ systems, including the muscle. Diabetics have lower relative muscle mass, lower muscle density and poorer muscle performance[1]. As both insulin and IGF-1 promote glucose transport in the muscle and have growth-promoting activities in the muscle[2], it is a crucial organ for maintaining glucose homeostasis in patients with and without T2DM. Though, insulin resistance (IR), the unresponsiveness of myocytes to an insulin stimulus, is the pathophysiological stage preceding T2DM[3].

IR is associated with increased intermuscular, intramyocellular and visceral fat. Apart from an increase in adipose tissue, an absolute increase in the lean tissue volume has been reported in T2DM but with a decrease in the density of the lean volume, which may indicate higher muscle fat infiltration[4]. Intracellular lipid metabolites induce inflammation and oxidative injuries, which consequently disturbs the insulin signaling cascade[5]. Fat infiltration thus leads to an impaired glucose uptake into muscle cells and impaired glycogen synthesis[2].

Both muscle fat infiltration, as expressed by muscle density, and IR have been associated with a loss in relative muscle strength in a healthy adult population[6]. The balance between protein synthesis and degradation is a critical determinant of muscle cross sectional area (CSA). Several authors demonstrated a positive association between HOMA-IR index and in-

The authors have no conflict of interest.

Corresponding author: Prof. dr. Patrick Calders, Ghent University, Dept. of Rehabilitation Sciences and Physiotherapy, De Pintelaan 185, 1B3, 9000 Ghent, Belgium
E-mail: patrick.calders@ugent.be

Edited by: F. Rauch
Accepted 20 July 2016

creased protein catabolism and synthesis, but with breakdown exceeding synthesis, resulting in a negative balance[7]. The higher turnover would also increase myoglobin and creatinine levels in blood and urine[8]. Furthermore, vitamin 25(OH)D has been positively associated with muscle mass[9], muscle density[9,10] and insulin sensitivity in obese populations[10].

Androgens and estrogens[11,12], both highly bound by sex hormone binding globulin (SHBG), are thought to play a role in the regulation of fat and muscle metabolism. For example, testosterone and estradiol stimulate mitochondrial activity and fatty acid oxidation in the muscle, lower fat accumulation in muscle and thus protect against IR[11-13]. An increase in fat mass and concomitant increased aromatase activity would however decrease testosterone levels and might increase estradiol levels in obesity[14].

From the above, it is clear that several parameters related to IR are influencing skeletal muscle metabolism, mass, density and performance. Interventions reversing impaired insulin sensitivity early in its course may consequently be a key intervention to prevent long-term complications of diabetes or IR. The aim of this study was therefore to investigate differences in muscle characteristics (muscle mass, CSA and density), muscle function (handgrip force), and biochemical anabolic/catabolic parameters (testosterone, estradiol, SHBG, vitamin 25(OH)D, creatinine, IGF-1, IGFBP-3 and leptin) between more insulin sensitive and less insulin sensitive healthy young men. Additionally, associations between HOMA-IR and these parameters were investigated. **It was hypothesized** that LIS would be associated with an increase in absolute muscle CSA, but a decrease in muscle density and relative muscle strength, even in young healthy men. These differences would be related to differences in several biochemical parameters.

Materials and methods

Subjects

The population for this study consisted of a group of 358 young unrelated healthy adult men who were, in turn, randomly selected, choosing one brother out of a sibling-pair study which included 276 pairs, 17 triplets, two quartets of dizygotic brothers, and 63 single participants. Out of these 358 unrelated men, subjects with lower insulin sensitivity (LIS) and subjects with higher insulin sensitivity (MIS) were selected for further analyses, using the upper and lower quartiles of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR index). Our population was defined as a 'more insulin sensitive' and a 'less insulin sensitive' (the upper and lower quartile for HOMA-IR) group instead of an 'insulin resistant' group, since the cut-off value for defining individuals as being 'insulin resistant' varies widely.

These men, aged 24 to 45 years, were recruited from population lists of semirural communities around Ghent (Belgium). Exclusion criteria were diseases or medication use that may affect body composition, bone metabolism or sex steroid levels. Medical history was obtained using questionnaires which were reviewed with medical assistance. Subjects were not excluded based on weight, risk factors for diabetes or daily exercise level. Subjects with diabetes were excluded from the present study based on fasting glucose higher than 7 mmol/l. Blood samples were obtained after overnight fasting. Subjects were asked not to perform heavy exercise in the last 24h previous to the testing protocol.

The study was approved by the Ethical Committee of the Ghent University Hospital and was performed in accordance with the Declaration of Helsinki. All participants gave their written informed consent.

Blood sampling and analyses

Serum was obtained between 08.00h and 10.00h, after overnight fasting, and stored at -80°C until analysis. Commercial direct radioimmunoassay (RIAs) was used to determine serum levels of leptin (human leptin RIA; Linco Research Inc., St. Charles, MO), SHBG (Orion Diagnostica, Espoo, Finland), and vitamin 25(OH)D levels (DiaSorin, Stillwater, MN, USA, detection limit 5.0 nmol/l). Hormone assays were completed using a validated and highly specific liquid chromatography tandem mass spectrometry technique (as previously described[15]) to measure fasting serum levels of total testosterone (TT) and total serum estradiol (TE2) under good laboratory practices.

Serum non–SHBG-bound testosterone (bioavailable T), free testosterone (FT), non–SHBG-bound E2 (bioavailable E2), and free E2 (FE2) were calculated from serum TT, TE2, SHBG, and albumin concentrations using a previously validated equation derived from the mass action law[16]. For all measurements, intra- and interassay coefficients of variation (%CV) were less than 15%. Assays were performed at the laboratory for Hormonology at the Ghent University Hospital.

Serum creatinine was determined according to a modified Jaffé reaction in Hitachi 912 (Roche Diagnostic System, Basel, Switzerland), by an isotope dilution mass spectrometry traceable method. IGF-I & IGFBP-3 was determined by an immunoassay with extraction (DSL-5600; Diagnostic System Laboratories, Inc., Webster, TX, USA).

Glucose (hexokinase method) and insulin concentrations were determined on a Modular P and E respectively using Roche Diagnostics consumables (Roche Diagnostics, Mannheim, Germany) and the HOMA-IR index was calculated as [fasting serum insulin (mU/l) * fasting plasma glucose (mmol/l)/22.5], with higher values indicating a higher degree of IR. By using lower and upper quartiles of IR, the more insulin sensitive group had HOMA-IR-indices of ≤0.96 and the less insulin sensitive group had HOMA-IR-indices of ≥2.09.

pQCT and DXA measurements

A peripheral quantitative computed tomography (pQCT) device (XCT-2000, Stratec Medizintechnik, Pforzheim, Germany) was used to scan the dominant leg (tibia) and forearm (radius). Slice thickness was set to 2 mm and voxel size was set to 0.8 mm. The muscle and fat CSA (mm^2), as well as

Table 1. Age, anthropometric data, body composition (weight, height, BMI, lean and fat mass measured with DEXA, fat cross-sectional area (CSA) measured with pQCT), physical activity and smoking habits. Comparison of these characteristics in the two subject groups. Values are means ± Standard Deviations (*p*-values result from an independent t-test), only the present smoking status is reported in % (*p*-value results from chi-square test).

	More insulin sensitive (n=89)	Less insulin sensitive (n=89)	*p*-value
HOMA	0.70 ± 0.21	3.27 ± 1.33	<0.001*
Fasting glucose (mmol/l)	4.54 ± 0.41	5.05 ± 0.59	<0.001*
Fasting insulin (mU/l)	3.48 ± 1.01	14.51 ± 5.06	<0.001*
Age (years)	33.2 ± 5.4	35.5 ± 5.3	0.006*
Height (m)	1.80 ± 0.063	1.79 ± 0.064	0.523
Body weight (kg)	76.0 ± 8.27	90.4 ± 12.56	<0.001*
Body Mass Index (kg/m²)	23.4 ± 2.3	28.1 ± 3.7	<0.001*
Lean mass (kg)	63.6 ± 6.0	69.0 ± 7.5	<0.001*
Fat mass (kg)	12.7 ± 4.5	21.7 ± 6.9	<0.001*
Fat % (of body weight)	16.4 ± 4.7	23.6 ± 5.0	<0.001*
Fat CSA (mm²) lower leg	1376.4 ± 593.6	2016.9 ± 722.8	<0.001*
Fat CSA/total CSA (%) lower leg	13.1 ± 4.9	17.3 ± 5.4	<0.001*
Fat CSA (mm²) forearm	656.16 ± 317.7	1163.36 ± 501.6	<0.001*
Fat CSA/total CSA (%) forearm	11.82 ± 5.4	18.1 ± 6.2	<0.001*
Physical activity (score/15)	8.63 ± 1.39	7.94 ± 1.4	0.002*
Pack years	4.36 ± 7.65	5.61 ± 8.87	0.324
Smoking(%) Yes	24.7	25.8	0.500
No	75.3	74.2	

**Significant at p<0.05 level.*

muscle density (mg/cm³) were measured at the mid-radius and mid-tibia, both at 66% of bone length from the distal end. The pQCT is a noninvasive reliable and valid tool to assess cross-sectional muscle size[17] and density[18]. The latter reflecting the fat content of skeletal muscle such that greater infiltration is represented by lower muscle density.

The pQCT device was calibrated and quality assurance procedures were completed daily, in order to ensure precision of measurements. The coefficient of variation for the calibration phantom was <1% as calculated from these daily phantom measurements. All scans were analysed using the Stratec software (version 6.2 C).

To determine whole body fat and lean mass, all participants underwent total-body dual energy X-ray absorptiometry (DXA) using a Hologic QDR 4500 DXA Discovery A device (Hologic Inc., Bedford, MA, USA). Whole body fat mass is given in absolute numbers (kg) and is also calculated as percentage of total body weight.

Handgrip force

A calibrated, Jamar dynamometer (Smith and Nephew, Irwington, NY 10533, USA) was used to assess hand grip force at the dominant hand. Three measurements of each grip were obtained at minimum 15s intervals (preceded by two trial efforts) and mean values were used in the analysis. The %CV for the measurement was 16,3%. Apart from the measured absolute values (in kg), relative values (the ratio of hand grip force to the corresponding forearm muscle CSA) were also calculated.

Covariates

Standing height and weight were obtained from each participant, and body mass index (BMI) was calculated. Age of participants was determined to the nearest year. The 'Baecke questionnaire' was used to determine levels of physical activity[19]. Cumulative exposure to cigarette smoking was summarized by multiplying the average number of packs smoked per day (cigarettes smoked per day divided by 20) by the number of years smoked (pack years of smoking), regardless of whether smoking status was former or current.

Statistical data analysis

Statistical analysis was performed with IBM SPSS Statistics (version 23; IBM Corp., Armonk, N.Y., USA) and considered significant at α = 0.05. Normality of the data was

Table 2. Comparison of muscle parameters between 89 MIS and 89 LIS subjects. Values are estimated means ± Standard Error with matching p-values.

	More insulin sensitive (n=89)	Less insulin sensitive (n=89)	*p*-value
LOWER LEG			
Muscle CSA (mm^2)	8135 ± 118.8	8707.1 ± 125.8	0.002*
Muscle CSA/ total CSA (%)	78.8 ± 1.5	75.3 ± 0.6	<0.001*
log (muscle CSA/ fat CSA)	0.82 ± 0.22	0.66 ± 0.02	< 0.001*
Muscle density (mg/cm^3)	76.02 ± 0.18	76.38 ± 0.19	0.233
FOREARM			
Muscle CSA (mm^2)	4446.6 ± 61.8	4759.7 ± 65.4	0.001*
Muscle CSA/ total CSA (%)	80.9 ± 0.6	75.7 ± 0.6	< 0.001*
log (Muscle CSA/ fat CSA)	0.885 ± 0.2	0.656 ± 0.03	< 0.001*
Muscle density (mg/cm^3)	78.39 ± 0.14	78.86 ± 0.15	0.037*
grip force (kg)	53.01 ± 0.9	51.7 ± 1.0	0.37
grip force/muscle CSA (kg/cm^2)	1.2 ± 0.1	1.1 ± 0.1	< 0.001*
**Significant at p<0.05 level.*			

Table 3. Comparison of biochemical parameters between 89 MIS and 89 LIS subjects. Values are *real* means ± Standard Deviations.

	More insulin sensitive (n=89)	Less insulin sensitive (n=89)	*p*-value
SHBG (nmol/l)	28.4 ± 1.0	21.8 ± 1.1	< 0.001*
Total Testosterone (pmol/l) (TT)	639.1 ± 18.9	558.9 ± 19.6	0.009*
Free testosterone (pg/ml) (FT)	11.16 ± 0.33	11.32 ± 0.34	0.769
Bio-av testosterone (ng/l) (bio-av T)	272.2 ± 7.8	277.63 ± 8.1	0.666
Total estradiol (pg/ml) (TE2)	23.21 ± 0.8	22.35 ± 0.8	0.470
Free estradiol (nmol/l) (FE2)	0.39 ± 0.02	0.42 ± 0.02	0.216
Bio-av estradiol (pmol/l) (bio-av E2)	15.94 ± 0.62	17.16 ± 0.63	0.181
Log Leptin	0.464 ± 0.03	0.880 ± 0.03	< 0.001*
Vitamin 25(OH)D (ng/ml)	22.36 ± 1.0	18.6 ± 1.1	0.024
Creatinine in Serum (mg/dl)	0.949 ± 0.014	0.916 ± 0.015	0.160
Creatinine in Urine (mg/dl)	182.43 ± 8.6	193.58 ± 8.9	0.423
IGF-1 (nmol/l)	35.32 ± 1.15	45.52 ± 1.16	< 0.001*
IGFBP-3 (ng/ml)	3626.26 ± 52.40	4056.07 ± 52.02	< 0.001*
*All biochemical parameters except estradiol and leptin levels were corrected for age, physical activity and fat mass. Estradiol related parameters and leptin were corrected for age and physical activity as fat mass is the main source of these parameters. *Significant at p<0.05 level.*			

tested with the Kolmogorov-Smirnov test. All parameters that did not meet the normal distribution (in the two separate groups for the comparison on the one hand, and in the whole population for the correlation on the other hand), including HOMA-IR, were logarithmically transformed. Descriptive statistics were reported as means ± standard deviation (SD), unless otherwise stated. Differences in muscle characteristics (muscle mass, CSA and density), muscle function (handgrip force), and biochemical anabolic/catabolic parameters (testosterone, estradiol, SHBG, Vitamin 25(OH)D, creatinine, IGF-1, IGFBP-3 and leptin) were assessed between 89 MIS and 89 LIS healthy young men using a univariate analysis of covariance (ANCOVA). Muscle mass, CSA, muscle density, muscle function, testosterone, SHBG, Vitamin 25(OH)D, creatinine, IGF-1 and IGFBP-3 were corrected for age, physical activity and fat mass. Estradiol and leptin were only corrected for age

and physical activity, as fat mass is the main source of these parameters. The association between muscle related parameters (muscle CSA, -density and -force), HOMA-IR and several parameters was also investigated in the cohort of 358 subjects, using Pearson correlation coefficient analyses.

Results

The 89 subjects in the lower quartile for IR had HOMA-IR indices of ≤0.96 and were defined as the MIS group, while the 89 subjects in the upper quartile (HOMA-IR ≥2.09) were defined as the LIS group. Descriptive characteristics of both groups are presented in Table 1. The LIS subjects were slightly older, had a higher body weight, BMI, fat mass, fat CSA, and reported less physical activity than their more MIS counterparts (all p<0.05) but there was no difference in smoking habits between the two groups.

Muscle parameters: muscle CSA, muscle density, handgrip force

Table 2 presents the comparison of muscle characteristics and muscle force, corrected for age, physical activity and fat mass (except for muscle density) between the LIS and MIS subjects.

Subjects with LIS had lower relative muscle CSA (-6.5% in forearm and -4.4% in lower leg, p<0.001) and muscle/fat CSA ratio (both in forearm and lower leg, p<0.001) compared to men with MIS. Relative handgrip force was found to be significantly lower in LIS individuals compared to the MIS group (-8.3%) (p<.001). Absolute muscle CSA in the distal body parts (forearms p=0.062 and lower legs p=0.439), and absolute handgrip force did not differ between the LIS group and the MIS group. Muscle density in LIS subjects was significant lower compared to muscle density of MIS subjects (p=0.037) at the forearm, but not at the lower leg / calf.

Biochemical parameters

LIS subjects had significantly lower values for TT (-24.7%, p=0.009), SHBG (-36.2%, p<0.001), and vitamin 25(OH)D (-17.6%, p=0.024) and significantly higher IGF-1 (+17.2%, p<0.001), IGFBP-3 (+10.3%, p<0.001) and leptin levels (p<0.001). No significant differences were found in FT, bio-available T and urinary creatinine, TE2, FE2 and bio-available E2.

Correlates between muscle and biochemical parameters (Table 2 & 3)

Absolute muscle CSA was negatively correlated with muscle density (r=-0.2, p<0.001 in lower leg and r=-0.16, p<0.001 in forearm), relative handgrip force (r=-0.25, p<0.001), SHBG (r=-0.14, p=0.007 in lower leg and r=-0.19, p<0001 in forearm), TT (r=-0.15, p=0.005 in lower leg and r=-0.17, p=0.001 in forearm) and positively correlated with absolute handgrip force (r=0.56, p<0.001 in forearm), percentage of fat in lower leg (r= 0.16, p<0.016), HOMA-IR (r= 0.19, p<0.001 in lower leg and r=0.2, p<0.001 in forearm), age (r= 0.24, p<0.001 in lower leg and r=0.3, p<0.001 in forearm), weight (r= 0.56, p<0.001 in lower leg and r=0.58, p<0.001 in forearm), total E2 (r=0.1, p=0.04 in lower leg), FE2 (r=0.15, p=0.005 in lower leg and r=0.14, p=0.001 in forearm), bio-available E2 (r=0.145 p=0.007 in lower leg and r=0.13, p=0.014 in forearm), and only in lower leg with leptin (r=0.173, p=0.001).

Muscle density was positively correlated with relative hand grip force (r=0.11, p<0.041), TT (r= 0.13, p=0.012 in lower leg), serum creatinine (r= 0.12, p=0.022 in lower leg and r=0.18, p=0.001 in forearm) and negatively with absolute muscle CSA (r=-0.2, p < 0.001 in lower leg and r= -0.16, p<0.001 in forearm), HOMA-IR (r=-.16, p=0.003 in lower leg), age (r= -0.3, p>0.001 in lower leg and r=-0.2, p<0.001 in forearm), weight (r= -0.34, p<0.001 in lower leg and r=-0.3, p<0.001 in forearm), percentage of fat (r= -0.36, p<0.001 in lower leg and r=-0.28, p<0.001 in forearm) and leptin (r= -0.29, p<0.001 in lower leg and r=-0.25 p<0.001 in forearm).

In our study population, **absolute handgrip force** was positively correlated with height (r= 0.3, p<0.001 in lower leg and r=0.27, p<0.001 in forearm), weight (r= 0.38, p<0.001 in lower leg), absolute muscle CSA (r=0.24, p<0.001 in lower leg, r=0.56, p<0.001 in forearm), relative hand grip force (r=.6, p<0.001), serum creatinine (r= 0.19, p<0.001) and urinary creatinine (r=0.12, p = 0.026).

Relative handgrip force was positively correlated with forearm muscle density (r= 0.11, p=0.04), absolute hand grip force (r=0.61, p<0.001), and serum creatinine (r=0.13, p=0.017) and negatively correlated with HOMA-IR (r=-.23, p<0.001), age (r=-0.15, p = 0.005), TE2 (r=-0.16, p=0.002), FE2 (r=-0.21, p<0.001), bio-available E2 (r=-0.17, p=0.001) & leptin (r=-0.11, p=0.031).

HOMA-IR was negatively correlated with TT (r= -0.31, p<0.001), SHBG (r= -0.38, p<0.001) and 25(OH) vitamin D (r= -0.21, p<0.001). HOMA was positively correlated with leptin (r= 0.576, p<0.001), IGF-1(3 = .252; p < 0.001) and IGFBP-3 (r=0.29; p <0.001).

Discussion

This study demonstrated that LIS subjects show lower relative muscle CSA, lower muscle density, lower muscle/fat CSA ratio, lower relative handgrip force and lower level of physical activity compared to MIS healthy adult men. LIS subjects also had significantly lower levels of TT, SHBG & vitamin 25(OH)D and higher levels of IGF-1, IGFBP-3 and leptin levels. Bio available T, FT, TE2, FE2, bioavailable E2, serum and urinary creatine levels did not differ between both groups.

Skeletal muscle parameters

The lower forearm muscle density in LIS and the relation of HOMA-IR with lower relative muscle CSA, lower relative handgrip force, lower muscle density and a lower muscle/fat CSA ratio might confirm our hypothesis of higher fat and connective tissue infiltration in already in healthy subjects with

lower insulin sensitivity. These findings are in line with previous studies reporting an association between the presence of IR and a lower muscle density, both in diabetes and in healthy subjects with or without obesity[4]. A similar observation was made in T2DM patients, where adipose and lean tissue were increased with two-thirds and one-third respectively[20]. Kelley et al.[5] concluded in their study that the effects of obesity on the composition of the thigh are an increase in adipose and low density lean tissue, without an increment in normal-density lean tissue[4].

The higher absolute muscle CSA in the upper limb was not accompanied by higher absolute grip force, resulting in **lower relative grip force** in LIS subjects. This negative association of IR with relative grip force might refer to the mitochondrial dysfunction in IR, as reported in literature[6,21] and demonstrated in our previous study based on muscle mass instead of muscle CSA[22]. Furthermore, Abbatecola et al. found a negative association between BMI-adjusted handgrip force and insulin resistance, and found a positive association with muscle density in a non-diabetic male population[6]. In our study population, a significant positive correlation between forearm muscle density and handgrip force normalized to forearm muscle CSA was found, confirming the validity of muscle density as a measure of muscle force.

Biochemical parameters

Androgens, estrogens & SHBG

This study showed that young healthy subjects with IR have lower levels of SHBG, which led to lower levels of total testosterone. Higher insulin levels, as seen in IR, have an inhibitory effect on the production of SHBG by the liver[23] and thereby can explain the lower SHBG levels found in this study. Free-, bio available testosterone and estradiol levels remained unchanged. In our dataset, the level of SHBG was negatively associated with absolute muscle CSA, but had no significant correlation with (relative) grip force.

Free and bio-available estradiol serum levels were positively correlated with absolute muscle CSA and negatively correlated with absolute and relative handgrip force. The meaning of these findings is unclear and it should be noted that these analyses were not corrected for fat mass so there is possibility of residual confounding.

Insulin, IGF-1 & IGFBP-3

Previous studies reported both low- and high- IGF-I levels in subjects with IR[24]. We found higher IGF-1 and IGFBP-3 levels in LIS subjects, however as neither were related to muscle parameters and systemic IGF-1 exposure is thus unlikely to play a major role in the muscular changes associated with IR.

Leptin

As expected, leptin levels were significantly higher in LIS subjects. Both obesity and insulin resistance are known to be associated with higher leptin levels and "leptin resistance"[25]. Leptin was also positively associated with absolute muscle CSA which corroborates previous studies about leptin in mice and men. Leptin would inhibit myofibrillar protein degradation and enhance muscle cell proliferation[26]. According to Munoz & Gower (2003), leptin would also be positively associated with muscle density (it would decrease skeletal muscle lipid content, and promote lipid oxidation)[27]. In contrary, leptin was negatively correlated with muscle density and relative grip in our study population. However, as with estradiol, these negative correlations are probably caused by the negative associations between fat mass and muscle density/relative grip.

Vitamin 25(OH)D

Serum vitamin 25(OH)D levels have been positively associated with insulin sensitivity in obese adults and adolescents, but also with muscle mass and muscle density[9,10]. In our study, vitamin 25(OH)D was significantly lower in LIS subjects, independently of obesity. Although this might suggest a relation between vitamin25(OH)D levels and insulin sensitivity, residual confounding should be taken into account.

Numerous changes in skeletal muscle as a result of a reduction in vitamin 25(OH)D were previously described, with a negative impact on the actin and troponin content, an impaired calcium uptake in the sarcoplasmic reticulum, a down regulation of protein synthesis and an increase in apoptosis. But in our cohort, there was an absolute increase in muscle CSA in the LIS group and no significant correlation between vitamin 25(OH)D levels and the muscle parameters was found.

Creatinine

Despite higher absolute lean mass and muscle CSA, LIS subjects did not show higher urinary creatinine levels, which could be related to lower muscle turnover. Further, serum creatinine was positively associated with muscle density, absolute and relative hand grip strength, but urinary creatinine was only significant correlated with absolute hand grip force.

Strengths and limitations of this study

To the best of our knowledge, this is the first study to evaluate the association between IR, muscle characteristics and several biochemical parameters, and the (in)dependence of overall obesity, in a cross-sectional cohort of healthy subjects. Furthermore, this study is performed in a large cohort of men and for whom data were available to adjust our analyses for major confounders. In addition, muscle density is a valid measure of fat infiltration within the skeletal muscle18. Study limitations contain the fact that muscle parameters were only measured in the distal muscles and obviously the cross-sectional design hampers the explanation of any relationship between IR and muscle in terms of cause and effects.

In conclusion, this study demonstrated that several muscle and perhaps some biochemical anabolic / catabolic parameters are different between MIS and LIS healthy young men. The LIS young healthy men population shows higher absolute muscle CSA, but lower muscle density and relative muscle force, suggesting an increase in inter- and/or intramyocellular fat. As these were otherwise healthy men, early interven-

tions to reverse these changes in populations at risk could be a key intervention to prevent development of frank diabetes. By increasing the level of physical activity in LIS subjects, muscle force is increased (data not shown from the Baecke questionnaire). Consequently, muscle fat would decrease while muscle density could increase and have a positive impact on several biochemical parameters.

Acknowledgements

The authors are grateful to K. Toye for her excellent technical assistance. This study is supported by grants from the Fund for Scientific Research – Flanders (FWO-Vlaanderen grant #G.0662.07 and G.0867.11). This research has benefitted from a statistical consult with Ghent university FIRE (Fostering Innovative Research based on Evidence).

References

1. Sala D, Zorzano A. Differential control of muscle mass in type 1 and type 2 diabetes mellitus. Cell Mol Life Sci 2015;72:3803-17.
2. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001;414:799-806.
3. Krssak M, Petersen KF, Dresner A, DiPietro L, Vogel SM, Rothman DL, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a H-1 NMR spectroscopy study. Diabetologia 1999;42:386-.
4. Miljkovic-Gacic I, Wang XJ, Kammerer CM, Gordon CL, Bunker CH, Kuller LH, et al. Fat infiltration in muscle: New evidence for familial clustering and associations with diabetes. Obesity 2008;16:1854-60.
5. Kelley DE, Goodpaster BH. Skeletal muscle triglyceride. An aspect of regional adiposity and insulin resistance. Diabetes Care 2001;24:933-41.
6. Abbatecola AM, Ferrucci L, Ceda G, Russo CR, Lauretani F, Bandinelli S, et al. Insulin resistance and muscle strength in older persons. Journals of Gerontology Series a-Biological Sciences and Medical Sciences 2005;60:1278-82.
7. Siew ED, Pupim LB, Majchrzak KM, Shintani A, Flakoll PJ, Ikizler TA. Insulin resistance is associated with skeletal muscle protein breakdown in non-diabetic chronic hemodialysis patients. Kidney International 2007;71:146-52.
8. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clin J Am Soc Nephrol 2008;3:348-54.
9. Ceglia L, Harris SS. Vitamin D and Its Role in Skeletal Muscle. Calcified Tissue International 2013;92:151-62.
10. Gilsanz V, Kremer A, Mo AO, Wren TAL, Kremer R. Vitamin D Status and Its Relation to Muscle Mass and Muscle Fat in Young Women. Journal of Clinical Endocrinology & Metabolism 2010;95:1595-601.
11. Enns DL, Iqbal S, Tiidus PM. Oestrogen receptors mediate oestrogen-induced increases in post-exercise rat skeletal muscle satellite cells. Acta Physiol (Oxf) 2008;194:81-93.
12. Enns DL, Tiidus PM. Estrogen influences satellite cell activation and proliferation following downhill running in rats. Journal of Applied Physiology 2008;104:347-53.
13. Campbell SE, Febbraio MA. Effect of ovarian hormones on mitochondrial enzyme activity in the fat oxidation pathway of skeletal muscle. Am J Physiol Endocrinol Metab 2001;281:E803-8.
14. Lakshman KM, Kaplan B, Travison TG, Basaria S, Knapp PE, Singh AB, et al. The effects of injected testosterone dose and age on the conversion of testosterone to estradiol and dihydrotestosterone in young and older men. J Clin Endocrinol Metab 2010;95:3955-64.
15. Fiers T, Casetta B, Bernaert B, Vandersypt E, Debock M, Kaufman JM. Development of a highly sensitive method for the quantification of estrone and estradiol in serum by liquid chromatography tandem mass spectrometry without derivatization. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 2012;893:57-62.
16. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. Journal of Clinical Endocrinology & Metabolism 1999;84:3666-72.
17. Cramer JTP, Ian J. 1; Ryan, Eric D. 1; Herda, Trent J. 1; Bemben, Debra A. FACSM 1; Bemben, Michael G. FACSM 1; Stratemeier, Philip H. 2. Validity and Reliability of a Peripheral Quantitative Computed Tomography Scanner for Measuring Muscle Cross-sectional Area: 1496: Board #259 May 30 11:00 AM -12:30 PM.[Miscellaneous]. Medicine & Science in Sports & Exercise 39(5) 39:supplement 225-6.
18. Wong AK, Hummel K, Moore C, Beattie KA, Shaker S, Craven BC, et al. Improving reliability of pQCT-derived muscle area and density measures using a watershed algorithm for muscle and fat segmentation. J Clin Densitom 2015;18:93-101.
19. Pols MA, Peeters PH, Bueno-De-Mesquita HB, Ocke MC, Wentink CA, Kemper HC, et al. Validity and repeatability of a modified Baecke questionnaire on physical activity. Int J Epidemiol 1995;24:381-8.
20. Kelley DE, Slasky BS, Janosky J. Skeletal-Muscle Density - Effects of Obesity and Non-Insulin-Dependent Diabetes-Mellitus. American Journal of Clinical Nutrition 1991;54:509-15.
21. Barzilay JI, Cotsonis GA, Walston J, Schwartz AV, Satterfield S, Miljkovic I, et al. Insulin Resistance Is Associated With Decreased Quadriceps Muscle Strength in Nondiabetic Adults Aged >= 70 Years. Diabetes Care 2009;32:736-8.
22. Gysel T, Calders P, Cambier D, de Mettelinge TR, Kaufman JM, Taes Y, et al. Association between insulin resistance, lean mass and muscle torque/force in proximal versus distal body parts in healthy young men. Journal of Musculoskeletal & Neuronal Interactions 2014;14:41-9.
23. Daka B, Rosen T, Jansson PA, Rastam L, Larsson CA, Lindblad U. Inverse association between serum insulin

and sex hormone-binding globulin in a population survey in Sweden. Endocr Connect 2013;2:18-22.
24. Friedrich N, Thuesen B, Jorgensen T, Juul A, Spielhagen C, Wallaschofksi H, et al. The Association Between IGF-I and Insulin Resistance A general population study in Danish adults. Diabetes Care 2012;35:768-73.
25. Zimmet PZ, Collins VR, de Courten MP, Hodge AM, Collier GR, Dowse GK, et al. Is there a relationship between leptin and insulin sensitivity independent of obesity? A population-based study in the Indian Ocean nation of Mauritius. International Journal of Obesity 1998;22:171-7.
26. Sainz N, Rodriguez A, Catalan V, Becerril S, Ramirez B, Gomez-Ambrosi J, et al. Leptin administration favors muscle mass accretion by decreasing FoxO3a and increasing PGC-1alpha in ob/ob mice. PLoS One 2009;4:e6808.
27. Munoz J, Gower BA. Relationship between serum leptin concentration and low-density muscle in postmenopausal women. J Clin Endocrinol Metab 2003;88:1157-61.